



Synthesis, Characterization and Environmental Behaviour of Silver Nanoparticles

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Abstract

Engineered nanoparticles are currently being emitted into the environment, mainly via aquatic pathway. The increase number of studies that relate them to toxicity effects, both in biological and in environmental systems, are raising concern throughout the scientific community. Due to the general lack of knowledge about the nanoparticles chemistry under environmental conditions it is imperative to start understanding how they behave under aquatic settings. In this context, one-step *in situ* synthesis of silver nanoparticles using silver nitrate as a starting material, trisodium citrate as stabilizer and sodium borohydride as reducing agent was prepared successfully. The nanoparticles were analyzed by UV-visible spectrophotometer and dynamic light scattering. Behaviour studies of the silver nanoparticles were done upon interaction with natural organic matter and sodium chloride. Dynamic light scattering and nanoparticles tracking analysis were used for obtaining sizes and sizes distributions, and zeta potential measurements for colloidal stability determination.

Keywords: Silver nanoparticles, Citrate method, NOM, Zeta potential, Dynamic Light Scattering, Nanoparticles Tracking Analysis

Resumo

Nanopartículas sintetizadas artificialmente são usualmente emitidas para o ambiente, principalmente via aquática O crescente número de estudos que relacionam estas a efeitos tóxicos, tanto em sistemas biológicos como em sistemas ambientais, está a preocupar, cada vez mais, a comunidade científica. Devido à geral falta de conhecimento acerca da química das nanopartículas aquando em condições ambientais, é imperativo começar a entender o seu comportamento em ambientes aquáticos. Neste contexto, nanopartículas de prata foram sintetizadas *in-situ* através do uso de nitrato de prata como material de partida, citrato trisódico como estabilizador e borohidreto de sódio como agente reductor, com sucesso. As nanopartículas foram analizadas através de espectrofotometria de UV-Vis e *dynamic light scattering*. Estudos comportamentais das nanopartículas de feitos através da interacção destas com matéria orgânica natural e cloreto de sódio. Foram utilizados o *dynamic light scattering analysis*, para obtenção de diâmetros e distribuição dos mesmos, e medições de potencial zeta para determinação da estabilização coloidal.

Palavras-chave: Nanopartículas de prata, Método do citrato, Matéria Orgânica Natural, Potencial zeta, *Dynamic Light Scattering, Nanoparticles Tracking Analysis*,

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List of Abbreviations and Acronyms

General

AgNPs – Silver Nanoparticles ENPs – Engineered Nanoparticles IACF – Intensity Autocorrelation Function MSD – Mean Square Displacement NPs - Nanoparticles NOM – Natural Organic Matter PZC – Point of Zero Charge PDI – Polydispersity Index S.O.P. – Standard Operating Procedures

Reagents

 $AgNO_3 - Silver Nitrate$ HCI - Hydrochloric Acid H₂O - Water NaBH₄ - Sodium Borohydride NaCL - Sodium Chlorohydride PEG - Poly(ethylene) glycol

Experimental techniques

- AFM Atomic Force Spectroscopy
- DLS Dynamic Light Scattering
- EM Electron Microscopy
- FFF-ICP-MS Field Flow Fractionation- Induced Coupled Plasma- Mass Spectrometry
- Flow-FFF Flow- Field-Flow Fractionation
- HPLC High Performance Liquid Chromatography
- ICP-MS Induced Coupled Plasma-Mass Spectroscopy
- ICP-OES Induced Coupled Plasma-Optical Emission Spectrometer
- IR Infrared spectroscopy
- LC-ESMS Liquid Chromatography-Electrospray Mass Spectrometry
- NTA Nanoparticle Tracking Analysis

NMR – Nuclear Magnetic Resonance SAED – Selected Area Electron Diffraction Sed-FFF – Sedimentation Field-Flow Fractionation TEM-EDX – Transmission Electron Microscopy UV-Vis – Ultraviolet-Visible XRD – X-Ray Diffraction

Units

A.U. – Arbitrary Units
cm- centimetre
Da – Dalton
M – Molar
m - Meter
mL – Millilitre
mV – Millivolt
nm – Nanometres
ppm – Part per million
s – Second
V - Volt
µm – micrometer

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1. Introduction

1.1 Importance of nanotechnology in contemporary scientific culture

The scientific community was first introduced to the concept of "nanotechnology" in 1974 by Professor Norio Taniguchi from Tokyo Science University as: "Nano-technology' mainly consists of the processing of separation, consolidation, and deformation of materials by one atom or one molecule." ^[1] Since then the investment in research and development of this specific technology has increased worldwide. Academically, in 1990 1,000 papers were published on nanomaterials against the 2004 28,000 papers. Industrially, previsions state that between 2011-2020 58,000 tons of engineered nanomaterials will be produced against the 2004 2,000 tons.^[2] This confirms that nanotechnology shows no trend of decaying in interest.

The reason for the increase of interest of this area relies on the vast uses that nanotechnology can allow. It has emerged as a revolutionary field in science that offers many novel applications, product possibilities and problem solutions. Over the past two decades it has been vastly used to greatest advantages in areas such as electronic, biomedical, pharmaceutical, cosmetic, energy, environmental, catalytical and material applications. The most various products like paints and coatings, food and food packaging, plant production products, paper manufacturing, textiles and sport items, lubricants, batteries or filters for air purification are successful results of nanotechnology.

Two of the fields where this technology shows the highest potential are in the areas of human health care and environment. Within the latter, it is already used for detection^[3] and removal of pollutants^[4], converting contaminants into less toxic chemicals^{[5],[6]} or even for creating both cleaner processes and 'greener' products. In the human health area possibilities like new formulations and routes for drug delivery, development of new vaccines, use of nano-devices for early diagnosis or even some kinds of surgeries and health monitoring are just a few of the potential uses of nanotechnology.

Although only the benefits have been stated so far, the fact remains that still little is known about the impact of human and environmental exposure to the products of this technology. In order to understand the full possible consequences of nanotechnology one has to understand the concept of nanoparticles and all it implies.

1.2 Brief introduction to the concept of nanoparticles

Nanoparticles (NPs) can be produced naturally or engineered (ENPs) and are defined as materials with at least one dimension in the size range of 1 to 100nm.^[7] They can exist in different forms, like fused, aggregated or agglomerated, and have diverse shapes, like spherical, tubular or irregular shaped. In a size-dependent classification (Figure 1), NPs can be defined as colloids as they don't always 'dissolve' in solution, forming a colloidal dispersion. They are easily found in the environment and can result of volcanic eruptions, forest fires, pollen fragments and viruses^[8] and have been found in glacial ice cores with 10,000 years old^[9].



Figure 1. Size domains of natural nanoparticles and colloids.^[10]

What makes NPs such an interesting subject of study is the high surface area to volume ratio that they possess when compared with conventional materials. This ratio allows them to react much faster and will increase dramatically as particles become smaller (Figure 2). This specific property allows them to differ from their bulk or even micro-sized material. Another relevant aspect is that the laws from quantum physics start to apply for sizes lower than 20nm. This will cause an alteration in material properties like transparency, electrical conductivity or magnetic permeability as soon as they start to dominate thermal effects.^{[11],[12]}



Figure 2. Relation between a spherical particle's size and its surface area (for a density of 1000Kg m⁻³).^[10]

Regarding their surface, NPs are never used in their initial or as-synthesized form. These materials are subjected to surface modification through the addition of surfactants, or other reactive agents, with suitable functional groups. This is done to increase its surface reactivity in order to allow a maximum nanocomposite loading since its initial surface and chemical properties seldom suit their intended purpose.^[13] This happens because a considerable percentage of NPs change their surface properties when they aggregate or precipitate from suspension.

According to the literature^[12], NPs can be divided in three layers: surface, shell and core. The first, as mentioned above, can be functionalised with metal ions or small molecules, surfactants or polymers. Materials like PEG (polyethylene glycol) or SDS (sodium dodecylsulfate) are currently widely used, but covalently bound molecules are also used such as citrate, cysteine or carbonate. The shell is defined as the outer layer that has a different composition when compared to the core of the NP. Examples of this are the quantum dots. They usually possess a reactive semiconductor core surrounded by a shell that prevents the core's oxidation. Well-known for their optical properties, they usually use elements like heterostructure core/shell CdSe/CdS, CdSe/ZnS, InAs/CdSe and CdTe/CdSe.^[14] Also iron NPs are known for developing an outer layer formed by iron oxide. The final layer is the core and is the centre of the NP. In the field of physical sciences it is, usually, where the physical-chemical properties are defined. Although, in ecotoxicology, it will also represent an important role it may not define the fate and behaviour under environmental conditions.

Regarding the examples of existing NPs, one has to first identify the type of material used. For instance, the three main materials used are metal NPs (silver, gold and iron), metal oxides (titanium dioxide, silica, zinc oxide and iron oxide) and carbon nanotubes (single, double and multi-walled). There are still other materials like sulfides, nitrides, selenides and phosphides used but in lower proportions.

The metal NPs are supposed to be the first ever prepared anthropogenically. The interest for these specific NPs has been increasing in recent years due to their optoelectronic properties. This is due to

the interaction between the wavelength of light and the free electrons in the material, resulting in a plasmon resonance, manifested by an intense absorption band. When this latter appears in the visible range it exhibits a brilliant colour. ^[15] These metal NPs have applications in many different fields specially in surface-enhanced Raman spectroscopy.^[16]

The development of cost-effective synthesis of metal NPs has influenced the large-scale production to which it is related to. Its compatibility with polymers has allowed a wider interdisciplinary field of applications.^[17]

1.3 Engineered nanoparticles in the environment

Nanotechnology is a rapidly developing area as it is the commercialization and use in consumer products of ENPs. This development implies that the release of nanomaterials to the environment is now occurring in a large scale and, although NPs have brought multiple benefits and advantages, there has been a growing concern about the human and environmental exposure to NPs. This can be supported by the high surface reactivity that makes them important binding targets for both organic and inorganic contaminants. For this reason the development and application of nanotechnology needs to be followed by risk assessment. This requirement directly implies the use of proper analytical methods for determining concentrations and characteristics of NPs (Table 1) in intricate matrices like soil, water, sediment, sewage sludge and biological matter. The need to characterize their behaviour is crucial to understand effects and creating exposure assessments.

	Instruments and methods	
Physical properties/metrics		
Diameter	EM, AFM, Flow-FFF, DLS,	
Volume	Sed-FFF	
Area	EM, AFM	
Mass	LC-ESMS	
Surface charge	$\boldsymbol{\zeta}$ -Potential, electrophoretic mobility	
Crystal structure	XRD, TEM-XRD (SAED)	
Aspect ratio or other shape factor		
Chemical composition/analytes		
Elemental composition	Bulk: ICP-MS, ICP-OES, single nanoparticle: TEM-EDX, particle population: FFF-ICP-MS	
Fluorophores	Fluorescence spectroscopy	
Fullerene ("molecules")	UV-vis, IR, NMR, MS, HPLC	
Total organic carbon	High temp chemical oxidation	
Other properties not falling within the above classes		
Aggregation state	DLS, AFM, ESEM, etc.	
Hydrophobicity	Liquid-liquid extraction chromatography	
Dissolution rate	Dialysis or voltammetry or spectrometry	
Surface chemistry, coating composition, # of proton exchanging surface sites	Optical or X-ray spectroscopic methods, acid-base titrations	

Table 1. Properties and respective methods and instruments.^[18]

Among the diverse possibilities, when studying the potential toxic effects of ENPs one should focus on processes like the adsorption of ENPs on to surfaces, aggregation, aptitude to form stable dispersions in water, influence of shape, size, surface are, surface charge on the aggregation chemistry and effect of abiotic parameters like pH, salinity, presence of certain cations and anions, water hardness and presence of humic acids.^{[19],[20]} These processes are currently the basis of all laboratory studies of potential toxicity of NPs in the environment, but are difficult to associate with aquatic colloids in natural systems because of their spatial and temporal variability, complexity and polydispersity. Even though the changes in physical-chemical properties are not that linear to interpret, toxicity studies on the uptake of NPs by biological systems have been done. These studies related damage of bacterial cell membrane integrity, protein destabilization and oxidation, damage of nucleic acid, production of reactive oxygen species, interruption of energy transduction and release of toxic components to NPs uptake.^[21] Several other studies are being performed on bacteria, freshwater invertebrates and primary producers and freshwater vertebrates.

1.4 The importance of silver nanoparticles

Silver is a natural organic metal with three known isotopes: 106.90 Ag, 108.90 Ag and 107.8 Ag, being the latter the most common one. This metal has been used since ancient times in medicine, eating utensils, food containers, jewellery, coins, clothes, building materials and disinfectant for both human infections and water treatment, revealing its high versatility. Alternatively, silver has been classified by the U.S: Environmental Protection Agency as a leading concerning pollutant in natural waters due to its persistence in the environment and high toxicity to some organisms. This was initially caused by industries like mining, smelting, photography and urban wastes.^[22] Due to various applications this starts posing a serious concern when silver NPs are considered to be the fastest growing nanomaterials. They have characteristic optical, electrical, magnetic and catalytic properties, that depend strongly on the particle size and shape^[23], which makes them the more manufactured nanomaterial for use in consumer products.

The most applied method when synthesizing silver NPs is chemical reduction as it produces stable, colloidal dispersions in water or organic solvents, with particles of several nanometres. It consists in reducing the silver ion Ag⁺ to the silver atom Ag⁰, followed by agglomeration into oligomeric clusters. These will eventually form the colloidal silver NPs. ^[24] The lack of difficulty of this synthesis and the constant demand for these NPs are ideal components for an increase in production and, consequently, in environmental pollution.

If in one hand silver has been proved to have antimicrobial activity^[25] it has also been related to the toxicity of biological systems^[26], showing the need to clarify the action mechanisms of silver NPs.

1.5 Experimental Strategy

The experimental part of this work consisted in synthesizing silver NPs using a chemical reduction process followed by their characterization and behavioural study in the presence of increasing amounts of NOM and NaCl.

1.5.1 Synthesis

The synthesis of NPs can be grouped into two techniques: top down or bottom-up^[27]. The first includes starting with a bulk solid material and, by structural decomposition, obtain the nanomaterials required.

The bottom-up implies assembling atoms, ions or molecules until the ideal dimension. This was the experimental strategy used in this work.

For the synthesis of silver NPs, two procedures were chosen using different stabilizing and reducing agents. One of the methods followed a well-established synthesis of these metal NPs using silver nitrate as the starting material, sodium borohydride as reducing agent and trisodium citrate as a stabilizer. The other method was based on a polymer-stabilized synthesis were PEG was used both as a reducer and stabilizer. The purpose was mainly to verify how different stabilizations (electrostatic or steric, respectively) could affect the behaviour of silver NPs.

1.5.2 Characterization

The synthesized batches were characterized with UV-Vis. This technique is shown to be a sensitive method for the detection of silver nanoparticles since they are characterized by a peak in the 380-400nm range (depending on the synthesis method) due to the surface plasmon excitation.^[28] The characterization study followed with size and zeta potential (colloidal stability) measurements using Dynamic Light Scattering.

1.5.3 Environmental behaviour

A considerable percentage of silver NPs (and other ENPs) is being found in wastewater treatment systems and thus is essential to understand its behaviour in natural waters. Since these nanomaterials are well known by their high surface area and reactivity, increasing their exposure to the environment, increases the importance of processes like aggregation or sedimentation. These are related to aquatic and terrestrial mobility of NPs as well as with its interaction with plants, algae and fungi

In this work was analysed the effect of NOM and NaCl on the aggregation and/or sedimentation of the synthesized silver NPs.

NOM stands for Natural Organic Matter and it represents a major component of natural colloids. It is defined as matter produced by natural occurring processes like decay and transformation of plant and microbial remains. With, at least, one dimension in the size range of 1 to 1,000 nm^[29], NOM is largely composed by humic substances (50-80%), like humic and fulvic acids, which are involved in processes like plant nutrition, pH buffering, trace metal mobility, degradation and transport of hydrophobic organic chemicals, formation of disinfection by-products during water treatment, heterotrophic production in blackwater ecosystems, toxicity and bioavailability.^[30] Although there is not direct data it is likely that NOM intervenes in the aggregation process of NPs since it plays a role in colloid stabilization through surface coating (Figure 3). The stabilization through charge^[31] and steric^[32]

mechanisms have been known to decrease aggregation but charge neutralization and bridging mechanisms caused by fibrillar attachment^[33] can cause the opposite tendency.

For these reasons Natural Organic Matter (NOM) and salt (NaCl) were added to the synthesized NPs, separately.



Figure 3. Schematic representation of NOM interaction with ENPs.^[10]

In these studies the techniques of Dynamic Light Scattering and Nanoparticles Tracking Analysis were used for size measurements of the NPs. Predictions of the dispersion's stability were based on zeta potential measurements.

• Dynamic Light Scattering (DLS)

Dynamic Light Scattering, also known as Quasi-Elastic Light Scattering or Photon Correlation Spectroscopy, is a well known technique used to calculate the diffusion coefficient of the sample.

The basis of this technique is the so-called Theory of Rayleigh Scattering and is applied to small particles with dimensions significantly smaller than the wavelength of the radiation. In this specific technique red laser light was used (λ =632.8nm).^[34]

The theory states that light, as an electromagnetic wave, interacts with matter constituted by charged atoms. When this interaction occurs the incident photon will cause a reorganization of the spatial charge distribution of the molecules inducing a dipole moment. The dipole will then be responsible for the scattering of an isotropic electromagnetic wave of the same wavelength as the incident one (Figure 4).

For larger particles the Mie Theory is applied, giving an analytical solution for the Maxwell's equations and, therefore, describing the propagation of electromagnetic radiation for spherical particles with a size equal or larger than the wavelength of the illuminating light.^[35]



Figure 4. Induced dipole caused by an incident photon and consequent light scatter.^[36]

Although light scattering has been reported as an elastic process, if the particles are suspended in a solution or gas, the intensity scattered detected at a certain scattering angle will change with time. This variation is verified because particles undergo what is called Brownian motion: a random movement caused by thermal fluctuations of the solvent.^[37] Due to this the particles mobility will vary with time causing a constant change in the local concentration by diffusion and, consequently, a change in the resulting scattered intensity detected. Due to the Brownian motion the signal read by the detector will not be constant but rather fluctuating (Figure 5).This effect can be enhanced by the increase of temperature and decrease of both viscosity and particle size.



Figure 5. Fluctuations in the scattered light caused by Brownian motion.^[38]

Using an intensity autocorrelation function (iacf), the intensity can be related to the diffusion coefficient through a variety of computational algorithms such as Cumulant analysis, double exponential sampling, nonlinear least squares, CONTIN, etc. It quantifies the non-randomness of the signal comparing the intensity of the scattered light of a particle to the same varied intensity recorded at a slightly later time (down to 10ns). Using small scales of time resolution the intensity varies slower due to the fact that the particles don't have enough time to diffuse. With time the iacf decays to zero: the comparison between the intensities in the initial and in the final position of the particle will no longer be possible since the movement grows in significance with time (Figure 6).^{[38],[39]}



Figure 6. Variation of particle position with time and its implication in iacf.^[38]

With the appropriate data analysis, the iacf also gives the mean size, or Z average, and the polydispersity index of the particle size using a single exponential fitting known as Cumulant method^[40] (Equation 1).

$$g(\tau) = \langle I(t)I(t+\tau) \rangle / \langle I(t) \rangle^2 = A + Be^{(-2q^2D\tau)}, \text{ with } q = \left(\frac{4\pi n}{\lambda_0}\right) \sin\left(\frac{\theta}{2}\right)$$

Equation 1

Where *g* is the autocorrelation function, *I* is the intensity, *t* is time, τ is the time difference of the correlator, *A* is the baseline of the correlation function, *B* is the intercept of the correlation function at infinite time, *q* is the scattering vector, *D* is the particle's translational diffusion coefficient, *n* is the refractive index, λ_0 is the wavelength of the laser and θ is the scattering angle.

For a complete size distribution then a multi-exponential fitting called the CONTIN algorithm^[42] (Equation 2) is applied, ideal for polydisperse samples.

$$g(\tau) = \langle I(t)I(t+\tau) \rangle / \langle I(t) \rangle^2 = A + \sum Be^{(-2q^2D\tau)}$$

Equation 2

The diffusion coefficient given by the iacf can then be associated to the hydrodynamic radius through the Stokes-Einstein equation (Equation 3), requiring just *a priori* knowledge of the temperature value and the viscosity of the solvent.

$$D = \frac{k_B T}{6\pi\eta r_H}$$

Equation 3

where k_B is the Boltzmann constant (1.38 x 10⁻²³ J K⁻¹), *T* is the temperature, η is the solvent viscosity, *D* is the diffusion coefficient and r_H is the hydrodynamic radius.

The advantages of using this technique rely on the fact that is a non-destructive, very fast method that requires the use of low volume of sample. It is also a non-invasive technique, as the optics are not in contact with the sample, and uses a backscattering angle of 173° (Figure 7) that prevents the detection of larger particles, like dust, that mainly scatter forward and reduces the multiple scattering effect.



Figure 7. Schematic representation of a DLS (adapted from^[41]).

Other benefits of the method are related to the sizing analysis: it is repeatable within minutes and has also new limits of sensitivity allowing measurements of biomolecules with a molecular weight < 1000 Da.

• Zeta Potential

Zeta potential was also measured and it basically gives the charge stability of a disperse system. For better understanding this concept one has to consider the different layers that are associated to the electric double layer of a charged particle (Figure 8):

- Diffuse Layer region in which non-specifically adsorbed ions are accumulated and distributed by the contrasting action of electric field and thermal motion;
- Hydrodynamic Plane of Shear (Slipping Plane) inner region of the diffuse layer where the ions and the particle act as a single entity;
- Stern Layer hypothetical boundary constituted by counter ions in immediate contact with the particle's surface;

To every single of one of these layers there is a potential associated.^[42]



Distance from negatively charged particle surface

Figure 8. Schematic illustration of the variation of potential with distance from a charged surface.^[43]

The electrical potential associated to the Slipping Plane is called the zeta potential and it plays an important role when considering interparticle interactions like aggregation or flocculation, since this happens according to the magnitude of this specific potential and not the surface charge of the particles. If a certain dispersion has a large negative or positive zeta potential (higher than +30mV or lower than -30mV) the particles will repel each other and the dispersion may be classified as stable, on the other hand if the zeta potential is low (lower than +30mV or higher than -30mV) there is no driving force that prevents aggregation, flocculation or coagulation.^[44]

The zeta potential can be measured through the application of the laser Doppler principle to electrophoresis. If an electric field is applied in a capillary cell then the charged particles of the dispersion will be attracted towards the electrode of the opposite charge with a characteristic velocity associated to them (Figure 9).



Figure 9. Capillary cell used for zeta potential measurements.^[45]

While subjected to the electric field the particles will be illuminated by a laser beam scattering light at a certain frequency. However if this frequency is compared to the one given by a reference beam that is routed outside the capillary cell, the combination between both of them will create a beam with intensity variations due to the particles mobility. In practice the pair of laser beams is derived from a single source, following similar pathways and having the same detector.

When comparing again the combined beam to the reference beam, which is modulated by an oscillating mirror, the frequency shift measured will be called the Doppler shift, being this theory designated as Electrophoretic Light Scattering (ELS).^{[46],[47]}

According to a simple equation (Equation 4) the electrophoretic mobility of the sample can then be deduced.

$$\mu = \frac{U}{E}$$

Equation 4

where μ is the electrophoretic mobility, *U* is the particle velocity and *E* is the applied electric field strength.

The zeta potential can then be related according to the Henry equation (Equation 5):

$$\mu = \frac{2}{3} \frac{\varepsilon \varepsilon_0 \zeta}{\eta} f(ka)$$

Equation 5

Where ε is the dielectric constant of the medium, ε_0 is the permittivity in vacuum (8.85 x 10⁻¹² Fm⁻¹), *f(ka)* is the Henry function, η is the viscosity and ξ is the zeta potential.

The Henry function^[48] describes how mobility is affected by the ionic surrounding of the particle and it depends strongly on the radius (*a*) of the spherical particles and its Debye length (*k*). The latter is a measure of the thickness of the electrical double layer conditioned by the ionic strength of the medium. When the electric field is applied, this thickness affects the mobility of the particles, causing a retardation effect, due to the excess of counter ions that will exert a force in an opposite direction of that of the NPs flow. This parameter can be calculated according to the Debye length equation (Equation 6).^[49]

$$\kappa = \sqrt{\frac{\sum_{i} (n_i)_0 z_i^2 e_0^2}{\varepsilon \varepsilon_0 k_B T}}$$

Equation 6

where n_i is the concentration of the ion species in units of number density, z_i is the valency (charge of the ion), e_0 is the elementary charge (1.60 x 10⁻¹⁹ C), k_B is the Boltzmann constant (1.38 x 10⁻²³ J K⁻¹) and *T* is the temperature (K).

The limits of the Henry function range from 1 (Hückel limit) to 1.5 (Smoluchowski limit), allowing to order the existing models as in Table $2^{[50]}$.

Table 2. Colloidal stability models: their limits and associated electrophoretic mobility equations.

Hückel	ka<0.1	$\mu = \frac{2}{3} \frac{\varepsilon \varepsilon_0 \varsigma}{\eta}$
Smoluchowski	ka>100	$\mu = \frac{\varepsilon \varepsilon_0 \zeta}{\eta}$
Henry (general form)	0.1 <ka<100< td=""><td>$\mu = \frac{2}{3} \frac{\varepsilon \varepsilon_0 \zeta}{\eta} f(ka)$</td></ka<100<>	$\mu = \frac{2}{3} \frac{\varepsilon \varepsilon_0 \zeta}{\eta} f(ka)$

The Hückel equation can be applied in case of a thick double layer, which is equivalent to say one is dealing with diluted concentrations of electrolyte (low ionic strength); in case of a thin double layer, or high concentrations of electrolyte (high ionic strength), the Smoluchowski equation is in order; for all the other cases, that eventually will be the majority found in colloid science (Figure 10), the Henry formula should be applied.



Figure 10. Domains of the aqueous colloidal systems.^[50]

Besides the influence of the ionic strength of the medium, the zeta potential can also be affected by the pH.^[51] This is one of the most important influences since the measurements can vary dramatically. As can be seen in Figure 11, if one adds base to a positively charged particle, both pH and the concentration of OH⁻ ions will increase in solution. These counter ions will tend to neutralize the charged surface of the particle, consequently, lowering its zeta potential value. If the addition is continued, there is the possibility the sum of all charges in the Hydrodynamic Plane of Shear will be zero as well as the electrophoretic mobility. This value that reflects the instability of the electrostatic equilibrium of the particle can be designated as isoelectric point or point of zero charge. Considering the literature ^[52] the difference between these two points is related to the surface charge. Point of zero charge is reached when the surface charge density is zero and isoelectric point will be the pH value at which net electric charge of an elementary entity is zero.

If base is continually added, the negative charges will outnumber the positive ones, being possible to invert the initial signal of the zeta potential to negative values. This, for instance, will depend on the

type of adsorption associated to the inorganic ions found in solution. It is possible to have non-specific and specific ion adsorption to the charged surface. The latter can lead to a change in the position of the isoelectric point while the first can't.

The study of the influence of pH in a colloidal system is imperative as one can detect at which pH's should the sample be in order to avoid processes like flocculation or aggregation.



Figure 11. Typical zeta potential variation with pH in a positively charged surface.^[53]

Conductivity can also affect this property, since inorganic ions can adsorb specifically or non-specifically, as well as the type of additive used (e.g. polymer, surfactant, etc.).

The use of measurements of zeta potential is only reliable when the colloidal system is stabilized according to the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, as explained so far. In agreement with the literature^[54], this theory states that at a given interparticle distance, the interaction energy (*V*) consists of two interparticle terms: the attractive potential (V_A) and the repulsive potential (V_R). All three terms (V, V_A and V_R) vary with distance between particles. Variation of *V* with interparticle distance leads to either a maximum value for *V*, in which case the colloid is stable, or no maximum, in which case coagulation occurs.

When dealing with steric stabilized colloids, zeta potential stops being a useful technique for stability prediction.^[55] Adding a polymer to a dispersion causes the reduction of the value of zeta potential to near zero due to the fact the polymer chains mask the van der Waals attraction forces and the charge of the colloids. Although the zeta potential reaches a value considered unstable for the colloid stability there is a repulsive force in action, when considering interparticle interaction, as a partial result of the polymer's steric effect. This will stabilize the dispersion in order to avoid coagulation.

Nanoparticle Tracking Analysis (NTA)

The NTA is an alternative light scattering technique that allows direct visualization and determines size of NPs in a typical size range of 10 to 1000nm. Similarly to DLS, NTA is based on the light scattered by particles while undergoing Brownian motion, but instead of depending on scatter intensity it individually tracks the NPs and analyzes its trajectories through a tracking software (Nanoparticle Tracking Analysis [NTA] 1.5 software).^[56]

This recently developed instrument is based in a conventional optical microscope that uses a laser beam in the sample chamber to illuminate the NPs present in a solution (Figure 12). These can be seen through the microscope when they cross the beam path, according to Brownian motion, as small spots of light.



Figure 12. Schematic representation of the principle of NTA.^[57]

The NTA software, which is connected to the microscope's camera (Figure 13), will allow the operator to record directly the movement of the particles. Afterwards, the video can be adjusted according to some existing settings in terms of image smoothing, background subtraction, removal of blurring, threshold detection, etc., that will then enable the operator to track and size the NPs under optimal conditions and in an individual basis.^[58] This particle-by-particle tracking will determine, for each particle, the mean squared displacement (MSD)^[59] that gives the average distance a particle travels in a certain system (Equation 7):

$$MSD = \left\langle (r(t) - r(0))^2 \right\rangle$$

Equation 7

where *r* is the position vector of the particle.

This concept can then be related to the diffusion coefficient (Equation 8) at times large enough to guarantee random motion, i.e., the particle should travel over distances larger than its the characteristic length scale.

$$D = \frac{1}{2d} \lim_{t \to \infty} \frac{d}{dt} MSD$$

Equation 8

Where d is the number of spatial dimensions and D is the diffusion coefficient.

From this latter and using the Stokes-Einstein equation (Equation 3), the hydrodynamic radius can be calculated and a particle size distribution plotted.



Figure 13. Nanosight LM10.[60]

When comparing this light scattering method with DLS there are some differences worth mentioning. One of the advantages of using NTA is the absence of bias towards large particles mainly because it makes a particle-by-particle analysis instead of DLS's average. This makes the latter a better method when using monodisperse systems and NTA more suitable for polydisperse ones. Also it allows measurements using more diluted samples (between 10⁵ to 10¹⁰ particles per mL) providing an estimation of concentration. Other benefit is the possibility of visualising the sample through the image given by the camera, validating the result. But DLS has too some advantages when compared to NTA: it can measure more concentrated samples, thus avoiding dilutions, its wide size range (0.6nm to 6000 nm) allows protein measurements unlike NTA and, as mentioned above, is a more reliable technique for monodisperse dispersions.^[61] Overall, these two methods are best used as complementary techniques.

2. Methodology

2.1 Materials

The following materials were purchased from Sigma Aldrich and used as received without any pretreatment: Silver Nitrate 204390-10G 99.9999% trace metals basis, PEG_{200} 81150, PEG_{2000} 81190, PEG_{20000} 81399, Sodium Citrate S1804-500G C₆H₅Na₃O₇.2H₂O, Sodium Borohydride 480886-25G 99.99% trace metals basis, Sodium Bicarbonate 71628. >=99.7% (T) and Sodium Chloride 71381. Assay >= 99.5% (AT). HCI (0.5M) solution was already prepared and available in the laboratory. NOM 1R101N from Suwannee River Humic Acid Standard (SRHA) was purchased for IHSS. Milli-Q water from Milli-Q Element A10 Millipore was used throughout the experiments. All the glass ware was washed previously in a HNO₃ ~8% bath for 2/3 days and rinsed vigorously with Milli-Q H₂O.

2.2 Preparation of silver nanoparticles

All batches prepared were stored at room temperature in a dark cabinet^[62]; for every synthesis a blank was done solely not using $AgNO_3$.

PEG method

AgNO₃ (400 mg, 2.4 mmol) was dissolved in 50 cm³ of PEG at room temperature. The suspension was stirred at 23°C and 3000 rpm until complete dissolution of the salt. The system was slowly heated, with a constant rate of 1°C/min up to 30 and 70°C. The reaction was allowed to proceed at the last temperature for 3h. The colloidal dispersion was left to cool down at room temperature.^[63]

Citrate Method

For solution **A**, $AgNO_3$ (1.0x10⁻³M) was heated in deionised water until it began to boil. As soon as the boiling commenced $Na_3C_3H_5O(COO)_3$ was added dropwise and, after the change of colour to greyish yellow, the solution continued to be heated for an additional 15minutes. It was then cooled down to room temperature.^[64]

For solution **B**, NaBH₄ (6 mL, 10 mM) was added to a 200 mL solution of AgNO₃ (0.25mM) and Na₃C₃H₅O(COO)₃ (0.25 mM). The reaction was stirred for 30 min, resulting in a yellow colloidal silver solution, and was then left undisturbed overnight.^[65]

For solution **C**, 1 mL of 0.01M of AgNO₃ was rapidly added to 99mL of a solution of 30 mM Na citrate and 1 mM of NaBH₄. This was done with vigorous stirring in an ice-cold bath. The solution changed to light yellow after which stirring was continued up to 10 minutes. The solution was then cooled down to room temperature.^[66]

For solution **D**, NaBH₄ (50 mM, 1 mL) was injected to a 100 mL aqueous solution of AgNO₃ (0.1 mM) in the presence of Na₃C₃H₅O(COO)₃ (0.3 mM).^[67]

2.3 Test solutions

In order to investigate the aggregation and stabilization behaviour of silver nanoparticles dispersions as a function of dilution with water, or bicarbonate buffer, and NOM, twelve aliquots (15 mL) were prepared according to the Table 5 and Table 6 (Appendix 6.2)

2.4 Characterization

• UV-Vis

Solely the synthesized nanoparticles were characterized by UV–Vis spectroscopy using an Agilent 8453 from Agilent technologies. The method was validated through the use of blanks for each synthesis batch. The UV-Vis spectra was recorded three days after synthesis and, for time variation studies, was also recorded 7 and 13 days after the nanoparticles formation. All samples were subjected to this analysis.

DLS

Dynamic light scattering measurements analysis was performed in a Malvern Zetasizer Nano ZS at 25°C using low volume polystyrene disposable cuvettes. Both the zeta average diameter and the

polydispersity index (PDI) were calculated directly by the instrument using Cumulant analysis. Only the batch of the chosen synthesis was subjected to this analysis and measurements were done only once. In between recordings, the cuvettes were washed with Milli-Q H_2O .

Zeta potential and zeta potentiometry

Zeta potential measurements were performed at 25°C using a Malvern Zetasizer Nano ZS. These values were obtained at the same time as the zeta average diameter using a disposable capillary cell. The measurements were recorded once and in between the cell was washed with Milli-Q water.

Zeta potentiometry analysis was done with a Malvern MPT-2 Multi-purpose titrator also using disposable capillary cells. This technique was used to determine de point of zero charge using HCL and for the salt titrations. The average diameter values were also obtained simultaneously as the zeta potential. Before initiating the potentiometry, the titration and sample tubes were washed with Milli-Q H_2O and HNO_3 0.01M and the pH probe was calibrated using standard buffer solutions with pHs of 4, 7 and 9. The measurements were recorded twice and only the batch from the chosen procedure was analysed.

pH measurements

For the pH measurements, a pH meter VWR Simphony SB80PC from VWR International was used. pH analysis was done to all nanoparticles synthesized through the citrate method and to all test solutions while being used for the environmental behaviour studies.

2.5 Environmental behaviour studies

For the NOM studies the test solutions, diluted in water or buffer, were analysed for four days both with the Malvern Zetasizer Nano ZS, for determining zeta average diameter and zeta potential, and NTA, for average diameter and size distribution. For the first two methods, the measurements were done as mentioned previously. For the NTA, before and in between measurements, the cell was washed vigorously with water, ethanol and dried using an air stream. All measurements done were recorded once at 25°C (DLS and zeta potential), at room temperature (NTA) and with two different recording cameras: Andor and Marlin. Overnight the solutions were stored at room temperature in a dark cabinet. These studies were validated through the use of controls.

For the salt studies the Malvern MPT-2 Multi-purpose titrator was used, using the same measurement conditions as mentioned in zeta potentiometry. The sample used was a batch of silver nanoparticles without being submitted to any type of pre-treatment.

3. Results and Discussion

3.1 Characterization of silver nanoparticles

After proceeding with the protocols for silver nanoparticles synthesis, the batches were subjected to characterization studies.

3.1.1. UV-Vis





Figure 14. UV-Vis spectra of the silver particles synthesis using PEG with different chain lengths.

As can be seen, at around 300nm and using PEG_{200} , the absorbance has negative values. This means that the corresponding blank used had higher values in that range than the solution measured since the other spectra did not manifest that behaviour. This could have been caused by contamination. It is also verified that no silver particles were synthesized using PEG_{200} .

For synthesis using PEG_{2000} and PEG_{20000} , the small band around 300nm is due to the presence of silver nitrate. The broad peak in the range of 350-650nm is due to the silver particles. The absorbance values indicate that their concentration increases with the increase of the chain length of the polymer, meaning the longer the chain the higher is the reduction power.

The broadness of the band indicates that the particles of silver in solution have different sizes and shapes (see Figure 15.).



Figure 15. List of silver with different shapes with their typical location of the surface plasmon resonance band in the visible regime.^[28]

In order to monitor the stability of the silver particles in solution, the absorbance variation in time was measured and the results are shown in Figure 16.



Figure 16. UV-Vis time variation spectra of the silver particles synthesis using PEG₂₀₀₀.

As can be seen, there is an increase in the absorbance meaning that the amount of silver particles in solution has also increased. The consistent shape of the spectra reveals that these new particles are not aggregating. Also there is a red shift of the peak, revealing that the particles are increasing in size.

As to the citrate method, the UV-Vis spectra correspondent to the four syntheses done is presented in Figure 17.



Figure 17. UV-Vis spectra of silver nanoparticles synthesized by the citrate method.

All solutions present the peak caused by the surface plasmon resonance. Solution A shows lower absorbance values and a red shift of the peak (see Table 3) when compared to all the others. This indicates that larger particles are present in solution. As to the other three solutions, absorbance maxima occur at similar wavelengths. The lower absorbance of solution B means that fewer particles are present.

Solution	Peak (nm)
Α	430
В	397
С	392
D	395

Table 3. Peak wavelengths for the citrate stabilized nanoparticles.

To decide which solution to use for further studies, the absorbance was measured with time, in order to evaluate the colloidal stability. The results obtained are shown in Figure 18.


Figure 18. UV-Vis time variation spectra of the solution C and D.

Through the analysis of the graphs, one can see that the equilibrium of the system in solution C, given by the absorbance maximum, changes over 13 days while in solution D these values are stable during the same period of time, revealing just a slight red shift. For this reason, the method used for solution D was the one used for the following studies. It consisted on injecting NaBH₄ (50 mM, 1 mL) to a 100 mL aqueous solution of AgNO₃ (0.1 mM) in the presence of Na₃C₃H₅O(COO)₃ (0.3 mM) The PEG method, for not providing single NPs as needed, was not further used.

3.1.2. Size, zeta potential and pH

For this characterization three batches were measured. Batch A was the one used throughout the characterization studies and Batch 1 and 2 were used for the NOM and salt addition studies. The measurements were recorded, in case of batch A, one week after synthesis, and in the other cases three days after syntheses.

	Size (d. nm)	zeta potential (mV)	рН
Batch A	24 ± 1	-44 ± 2	9.2
Batch 1	20.8 ± 0.1	-32 ± 1	9.8
Batch 2	20 ± 1	-31 ± 1	9.6

 Table 4. Size, zeta potential and pH values for the synthesized batches.

As shown in Table 4, the NPs of batch A presented a higher diameter when compared to batch 1 and 2. This slight increase reflects a higher stability, regarding the zeta potential values, of the colloidal dispersion. Batch 1 and 2 showed more similar results both in size and in zeta potential measurements.

3.1.3. Point of Zero Charge

The batch A used for this study was two months old. The results of the titration done to determine the point of zero charge are presented in Figure 19 and the values of pKa shown belong to citric acid. The experimental points of HCl added throughout the experiment are collected in Table 8 (Appendix 6.3).



Figure 19. Results of the titration performed for the determination of the point of zero charged of batch A.

As can be seen, both size and zeta potential are not affected significantly when the values of pKa_3 and pKa_2 of citric acid are reached. It is only when approaching the value of pKa_1 that the trend in both parameters starts to change.

Regarding the size measurements it is clear that the diameter of the particles starts to increase for pH<3.5. This happens because some NPs have started to loose citrate due to its protonation. Without the agent responsible for electrostatic repulsion, the particles tend now to aggregate. As the citrate is continuously being protonated, the silver NPs keep forming bigger aggregates.

As to the zeta potential measurements, the values slightly increase till pH around 4. This could be explained by the neutralization of the citrate's negative charges, therefore shifting the zeta potential for more positive values. This statement, however, does not reflect the rest of the behaviour. For pH<4 there is a clear shift towards negative values. For a possible interpretation one has to admit that the silver NPs are not the only entities being measured. If they were, assuming that the aggregates are in the Ag_n form having zero surface charge, the zeta potential value would also reach zero at same point. Bearing in mind that, through electrophoretic mobility, one of the parameters that zeta potential depends on is the velocity of the measured species (Equation 4), the added HCI may be causing the decrease in the potential. Considering that the concentration of chloride and hydrogen ions was increasing and that the latter is responsible for the citrate's protonation, one may attribute the highly negative values of zeta potential to the chloride ions. This may be supported by its high mobility due to the low radius and high electronegativity.

3.2 NOM additions

Regarding the NOM additions, the samples measured were done using three different concentrations of silver NPs (15, 1.5 and 0.15 ppm) and for each set three different concentrations of NOM were used (also 15, 1.5 and 0.15 ppm). For each concentration of silver NPs a control was done without adding NOM.

The batch 1 was used for the dilutions in Milli-Q H_2O and in carbonate buffer 1600 ppm, while batch 2 was used for the dilutions done in carbonate buffer 100 ppm. In order to avoid the influence of ionic strength, the measurements of size and zeta potential of the dilution made with without buffer were examined under a more thorough analysis.

3.2.1 Size

3.2.1.1 [AgNPs]=15ppm

40 35 30 [NOM]=15ppm Size (d.nm) 25 [NOM]=1.5ppm 20 [NOM]=0.15ppm 15 Control A 10 5 0 2 3 0 1 Day

The results obtained for the size mean variation according to DLS are shown in Figure 20.

Figure 20. Size mean variation with time in [AgNPs]=15ppm according to DLS.

As can be seen the mean size of each sample does not change significantly, with values in the range of 20-37nm. The slight variability in the results may indicate that the NOM has not reached equilibrium with the NPs. Nevertheless, an unexpected behaviour is the fact that all samples present an initial size lower than the control. One possible explanation is that the NPs started to form some aggregates in the presence of NOM. This will cause a more thin distribution at lower values.

The measurements showed by the NTA (Figure 21) also indicate that the mean sizes of the samples shift in a similar range: 25-40nm.



Figure 21. Size mean variation with time in [AgNPs]=15ppm according to NTA.

The addition of NOM apparently does not cause any type of destabilization, but since the mean average is not a quantitative data when working with polydisperse nanoparticles, the behaviour can be better seen through the sizes distributions.

In Figure 22 are the size distributions for NOM with 15ppm.



Figure 22. Size distributions varying in time for [NOM]=15ppm according to DLS.

As can be seen there is a similar behaviour in the first three days of measurements but in the last day the shape of the curve changes and becomes narrower. By looking at day 2 and 3 there are aggregates being formed which may cause this narrowing in the final day.

The results provided by the NTA (Figure 23), show that there is already some narrowing of the size distribution from day 0 to day 1 accompanied by an increase in concentration. This can be due to a desegregation effect that NOM may also cause regarding the bigger particles. The positive charge of the humic acids will compete with citrate causing the removal of the latter from the silver NP surface and making small NPs disaggregate from the bigger ones. The decrease in concentrations in the following days can indicate that some of the NPs may have been used for the formation of bigger particles.



Figure 23. Size distributions varying in time for [NOM]=15ppm according to NTA.

For 1.5 ppm of NOM (in Figure 24), in the second day of measurements with DLS, there might have started some aggregation as all size distributions are narrower than in the first day.



Figure 24. Size distributions varying in time for [NOM]=1.5ppm according to DLS.

The same sample in Figure 25, according to NTA, shows a little less aggregation than the observed with DLS (Figure 24), which can be related to a higher sensitivity of the NTA method.



Figure 25. Size distributions varying in time for [NOM]=1.5ppm according to NTA.

According both with DLS and NTA, samples with 1.5 ppm of NOM show a little less aggregation than the observed for 15 ppm of NOM, which can be related to the fact this concentration has lowered.

For the sample with 0.15 ppm of NOM (Figure 26), the shape of the peak is broadening along the days. As observed in the size distributions Day 0 presents some large NPs that 'disappear' in day 1. The fact that particles at higher values are not shown maybe due to the fact that when particles agglomerate, they become heavier and deposit in the bottom, in this case, of the test tube or cell. At day 2 the nanoparticles 'reappear' showing that the two size distributions are tending to move closer.



Figure 26. Size distributions varying in time for [NOM]=0.15ppm according to DLS.

The results obtained with NTA for this NOM concentration also indicates that some aggregation may be occurring (Figure 27). If so the NOM will function as a steric stabilizer that will mask the electrostatic effect (repulsive forces) that prevents aggregation.



Figure 27. Size distributions varying in time for [NOM]=0.15ppm according to NTA.

Similar measurements were done with the control samples. According to DLS (Figure 28), in the second day of measurements the size distribution is broader than in the first day, indicating a trend to aggregate. The following days, however, the distributions appear thinner, not showing particles at higher values. This particular behaviour may be an indication that the nanoparticles are already showing a trend to aggregate just by diluting.



Figure 28. Size distributions varying in time for Control A according to DLS.

Comparing the distributions obtained with NTA (Figure 29) and DLS, they show quite similar shapes in the size distribution although with different peak areas. This may be related with the analysis of the NTA data since it is performed by the operator and, consequently, is susceptible to systematic errors.



Figure 29. Size distributions varying in time for Control A according to NTA.

In summary, although the NPs with NOM show a trend to aggregate so does the control. This may indicate that the silver NPs system is aggregating due to the free ions present in solution (Na^+, NO_3^-) and BH_4^-).

3.2.1.2 [AgNPs]=1.5ppm

For the silver NPs at 1.5ppm (Figure 30), their mean size value is higher than for NPs at 15ppm indicating that they are susceptible to the presence of NOM.



Figure 30. Size mean variation in time with [AgNPs]=1.5ppm according to DLS.

The control shows again higher initial values than the samples with NOM, indicating formation of aggregates. There is no significant variation in the mean sizes of the NPs with the exception of the highest concentration of NOM. This may be caused by a competition between the citrate and the NOM for the stabilization of NPs.

With the NTA (Figure 31) the shift in the mean of the particles is more similar and shows lower values. Although this happens, the mean size values for 15ppm of NOM are still higher than those found for the other samples, supporting the competition theory between NOM and citrate.



Figure 31. Size mean variation in time with [AgNPs]=1.5ppm according to NTA.

The size distributions with the highest concentration of NOM and according to DLS (Figure 32), show a trend to narrow down with time. The loss of smaller NPs may be attributed to the formation of bigger ones. The same trend was observed for all NOM concentrations as shown in Appendix 6.7.



Figure 32. Size distributions varying in time for [NOM]=15ppm according to DLS.

The same trend can be identified in the size distributions provided by the NTA for the same NOM concentration (Figure 33). The rest of the samples indicate stable peaks at 22nm but a decrease in concentration of NPs in the last day, for all, suggesting aggregation as shown in Appendix 6.9.



Figure 33. Size distributions varying in time for [NOM]=15ppm according to NTA.

The control in DLS (Figure 34) also shows a trend to aggregate by the broadening of the band and posterior narrowing. With the NTA the control B shows a more consisting shape in the first three days and then also a decreasing in concentration in the last day.



Figure 34. Size distributions varying in time for Control B according to NTA and DLS.

These results indicate that controls and NOM samples show the same trend to aggregate the silver NPs suggesting that the NOM is not interfering with them with the exception of the sample with 15ppm of NOM. Apparently it is when the NOM is ten times more concentrated than silver NPs that it will reflect its influence in their behaviour.

3.2.1.3 [AgNPs]=0.15ppm



For the less concentrated samples, the results for size mean are shown in Figure 35.

Figure 35. Size mean variation in time with [AgNPs]=0.15ppm according to DLS.

As observed for 1.5ppm silver NPs (Figure 30), the highest concentration of NOM presents higher values in the mean size of NPs. However when observing the size distribution of this concentration (Figure 36), it is seen that the sample is contaminated or the size distribution became so polydisperse to the point of influencing the measurements in DLS.



Figure 36. Size distributions varying in time for [NOM]=15ppm according to DLS.

For this same sample the size distributions given by the NTA are displayed below (Figure 37).



Figure 37. Size distributions varying in time for [NOM]=15ppm according to NTA.

As can be seen the mean sizes are smaller than the ones provided by DLS which can indicate that the NPs reached a level of polydispersity too high for DLS to measure (Figure 38).



Figure 38. Polydispersity index values for [AgNPs]=0.15ppm provided by DLS.

As can be consulted in Appendix 6.7 and 6.9 through the size distributions, the polydispersity index increases when the concentration of silver NPs decreases. This effect is caused by the NOM as it is a mixture of hydrophobic acids and hydrophilic bases, acids and neutral components with a well-known polydispersity index above 1.^[68]

3.2.2. Zeta Potential

In order to calculate the zeta potential one has to know which equation should apply according to the three stability models for colloidal dispersions. The calculations were performed using the electrophoretic mobility value corresponding to the maximum value of intensity and are presented in Appendix 6.4.

The test solutions diluted with Milli-Q H_2O all belonged to the Hückel domain with the exception of the sample with [AgNPs]=0.15ppm and [NOM]=15ppm. The ionic strength here was higher and it was stabilized according to Henry's domain.

The calculations were also done for the buffered solutions where it was stated that in buffer 100ppm all samples were in the Henry domain, but in buffer 1600ppm some of the samples would belong the Smoluchowski, specially the ones with lower concentration of silver NPs, as expected.

The zeta potential measurements for the all samples (Figure 39, Figure 40 and Figure 41) showed no real trend, in terms of stabilization.



Figure 39. Zeta Potential variation in time with [AgNPs]=15ppm according to DLS.

Regarding the test solutions with NOM in the higher concentration of silver NPs (Figure 39), the shift in values maybe due to the electrostatic stabilization being masked by the steric one provided by the NOM and, as stated in the introduction, the measurements of zeta potential are no more reliable when this happens, since it does not neutralize or amplify the charge of the particles. Other possible interpretation relies in the fact that the zeta potential is not measuring solely the nanoparticles, since Na⁺, NO₃⁻ and BH₄⁻ are present in solution. This can be supported by the fact that the size mean and mode of these NPs do not shift significantly to the point of explaining these results.

The control shows also variability in the results, which can indicate that the equilibrium of silver NPs is not stable even when diluted in a small ratio.



Figure 40. Zeta potential variation in time with [AgNPs]=1.5ppm according to DLS.

For the [AgNPs]=1.5ppm (Figure 40) the values of zeta potential are no better considering that the variation of the results is not consisting. Again the control is around negative values which indicate instability and therefore possibility of aggregation.



Figure 41. Zeta Potential variation in time with [AgNPs]=0.15ppm according to DLS.

The lower concentration of silver NPs (Figure 41) showed that both the lower concentration of NOM and the control, the zeta values shift more inconsistently. Like suggested during the size analysis the presence of dust or any other contamination may be influencing this kind of behaviour.

3.3 Ionic Strength

The importance of the ionic strength of the medium in the aggregation processes was verified trough the addition of salt (NaCl) and dilution in buffer of the silver NPs.

3.3.1 Salt Additions

For the salt additions, the batch 1 was used and the first titration was done until a limit of 0.5 M of NaCl, considering it represents the value of salinity concentration in sea water.^[69] The values of size and zeta potential obtained from that titration are presented in Figure 42.



Figure 42. Salt titration performed up to 0.5M NaCl in a silver dispersion.

According to the DLVO theory, the electrostatic repulsion of particles decreases with increasing ionic strength of the medium. The salt, as an electrolyte, will cause this effect. The free ions (Na⁺ and CI) will shield the nanoparticles repulsion, compressing the double layer and promoting aggregation. This is the so-called screening effect.

Observing the size values obtained one can confirm this effect as the diameter increases with the increase of added salt. As the size increases so does the standard deviation associated to them. This is due to the fact that the size of the aggregates is approaching the limit for DLS's detection.

When observing the zeta potential slope one could state that there are two trends: a negative, only verified from the first to the second point, and a positive one, generally dominant throughout the rest of the values. Regarding the first behaviour, it was observed that, between those two points, the bright yellow colour typical of the colloidal dispersion disappeared to a seemingly colourless solution. This

means that the NPs became aggregates, which is confirmed by the slope of the sizes. The zeta potential shifts to more negative values in the first addition due to the fact that the electric double layer is being compressed by the increasing ionic strength in the medium.^[70] This is predictable considering zeta potential is the difference between the bulk and the Slipping Plane of Shear.

The second slope of the zeta potential shows a positive trend, shifting the zeta potential to values less negative. This could be explained when the dispersion reaches salt saturation. In this case the attractive forces between NPs become more dominant over the repulsive forces creating a highly agglomerated and unstable dispersion.

This overall behaviour of zeta potential where first it shifts to lower values until it reaches a plateau and then reverses the trend to higher values is a phenomenon known as 'salting-out' colloids. It is usually verified in continuously additions of electrolytes, as in this case.

Another titration was performed in a more specific range, in order to better analyse the nanoparticles behaviour. The results are presented in Figure 43.



Figure 43. Salt titration performed up to 0.04M NaCl in a silver dispersion.

In the second titration the loss of colour was verified in between the measurements highlighted in green. From this, one could state that the nanoparticles loose their quantic proprieties at around 200nm when NaCl added reaches 0.03 M. As expected the zeta potential decreases.

3.3.2 Buffer Effect

Dispersions of silver NPs were diluted in carbonate buffer according to Table 6 and Table 7. There were used two concentrations of buffer: 100 and 1600ppm.

In Figure 44, Figure 45 and Figure 46 values from the first day for samples with decreasing concentrations of silver NPs and NOM at 15ppm were compared.



Figure 44. Size distributions for sample [AgNPs]=15ppm and [NOM]=15ppm diluted in different solutions.

For the higher concentration of silver NPs (Figure 44), unlike the distribution shown by the solution with no buffer, all the others samples present aggregation in the first day. The more concentrated buffer was used, the bigger the aggregates were.



Figure 45. Size distributions for sample [AgNPs]=1.5ppm and [NOM]=15ppm diluted in different solutions.

For a lower NPs concentration (Figure 45), the curve given by the test solution without buffer shows a defined band lacking any signs of aggregation. In the same day, both buffered samples also show broad bands at higher values of size and, consequently, thinner distributions at lower values.



Figure 46. Size distributions for sample [AgNPs]=0.15ppm and [NOM]=15ppm diluted in different solutions.

In the more diluted set of solutions, although there were some problems regarding their measurements, the same trend can be detected. In this case the sample with no buffer presents large aggregates but still smaller than the ones detected in the buffered solutions.

4. Final Considerations

The aim of the first part of this work was to synthesize silver NPs according to two different methods. The method where solely PEG was used did not provide satisfactory results as the UV-Vis showed the existence of a wide range of silver particles and not only NPs. The functional group (-OH) of the PEG may not be sufficient to stabilize the silver ions when the reduction occurs.

The well-established citrate method formed stable nanopartículas in the range of the 20 nm with a surface plasmon resonance band at around 400nm in the UV-Vis. The characterization of these NPs showed an absence of point of zero charge when titrated with HCl even after reaching the last pKa of the citric acid even though the size slope increases considerably around it. The zeta potential, until pKa₁, did not shift much, indicating stability at negative values. After this it decreased to lower values of zeta potential instead of the increase expected. Since this property measures every charged particle in solution, and that the concentration of HCl kept rising, this can be due to protonation of citrate by H⁺ and consequent measurement of the Cl⁻ and other negative species present in solution

Regarding the size analysis it was observed, by the use of controls, that the effect of NOM was not clear. It was indicated that, at higher concentrations organic matter seems not to interfere in the size of the NPs, while at lower concentrations the polydispersity associated to NOM seems to overthrown the effect of silver. At intermediate values, particularly at [AgNPs]=1.5ppm and [NOM]=15ppm, it was observed that there was a competition between the citrate and NOM for the stabilization of the molecule. This suggests that there is both an optimal concentration of silver NPs and NOM in order to analyse the influence of the latter.

The general trend for aggregation verified through the controls can be caused by the presence of the free ions, as reaction products, in solution (Na⁺, BH_4^- and NO_3^-). They can be responsible for the masking of the electrostatic forces, therefore promoting aggregation.

The results of zeta potential in all samples dictate that they have not reached stability since the NOM addition. The fact that the controls presented equal disparity in results, showed that the citrate stabilized silver NPs can be susceptible to the dilution factor or that the small solute ions are interfering with the results. As reported in literature^[17] these organic colloids are known for stabilizing NPs but the effect is annulled when in the presence of high ionic strength due to its charge screening effect. This indicates that this method is not the most suitable for understanding the effect of exterior elements like NOM under these synthesis conditions.

The ionic strength influence in the aggregation behaviour was studied through the use of buffers and by addition of salt. In this last case, increasing the concentration of NaCl in a dispersion of NPs, decreased the electrostatic repulsion between the particles, promoting aggregation. This was due to the screening effect of these free ions by masking the repulsion forces. Zeta potential was also measured while the titration was being performed and until the first addition, where [NaCl]=0.0357M, it declined for lower values. The cause is related to the thinning of the electric double layer by the increase in ionic strength of the medium, decreasing the potential associated to the Slipping Plane of Shear.

After this salt concentration the zeta potential values started to increase in value. It was verified that the colloids had been 'salted-out'. This phenomenon is not fully understood but is known to cause shifts n the zeta potential: first the shift to lower values, followed by a plateau and reversal of charge. It can be explained by the compression of the double layer by Cl⁻ ions (decreasing the electrostatic repulsion) up to their penetration into the inner shell of the silver-stabilized NPs. This will lead to the disappearance of the stabilizing system of these particles.

In a second titration it was verified that the loss of colour of these synthesized NPs, and consequently loss of quantic properties like the surface plasmon resonance, happened at around 200nm in the presence of these electrolytes.

With the use of different concentrations of buffer, it was also verified that it influenced the NPs trend to aggregate. Like in the previous study, the screening effect is also responsible for this behaviour.

In the future, particularly in the NOM effect in size and zeta potential, it could be interesting to better optimize the conditions regarding the methodology applied and to do a time variation study more extended. Also, the citrate-stabilized NPs could be purified with dialysis in order to better control the possible variables of this study. Finally, it could also be of interest to study the influence of the synthesis method as the different origins and structures may have a role in the NPs behaviour.

The environmental studies of NPs have great interest since their aggregation process influences aspects like residence time in surface waters and their transport to ground water. These will determine aquatic pollution, influencing the ecotoxicity of NPs in biological systems as they have the ability to uptake metal ions or cross cell membranes. For these reasons environmental risk assessment is a growing concern in the scientific community.

5. References

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6. Appendix

6.1. NOM Composition

NOM 1R101N	%w/w	pKa₁	pKa ₂
С	48.8		
Н	3.9		
0	39.7	3.94 9.74	9.74
Ν	1.02		
S	0.6		
Р	0.02		

Table 5. NOM composition

6.2 Dilution Series

Stock of NaHCO ₃ (ppm)	1600						Batch 1
Stock of NOM (ppm)	500						
Stock of AgNP (ppm)	16.68						
	Ratio (AgNP:NOM)	AgNP (mpm)	(mqq)	V _t (mL)	V _{Ag} (mL)	V _{NOM} (mL)	V _{Milli-Q} / _{buffer} (mL)
+#4	100:100	15	15	15	13.489	0.450	1.061
#2	100:10	15	1.5	15	13.489	0.045	1.466
#3	100:1	15	0.15	15	13.489	0.005	1.506
Control A	100:0	15	0	15	13.489	D.000.0	1.511
**	10:100	1.5	15	15	1.349	D.450	13.201
#2	10:10	1.5	1.5	15	1.349	D.045	13.606
9#	10:1	1.5	0.15	15	1.349	D.005	13.647
Control B	10:0	1.5	0	15	1.349	0,000 0	13.651
L#	1:100	0.15	15	15	0.135	0.450	14.415
8#	1:10	0.15	1.5	15	0.135	D.045	14.820
6#		0.15	0.15	15	0.135	D.005	14.861
Control C	0: 1:0	0.15	0	15	0.135	0.000	14.865

Table 6. Data for the synthesis of the test solutions with Batch 1.

Stock of AgNP (ppm)	16.67						
	Ratio (AgNP:NOM)	AgNP (ppm)	(mon	۷ [٬] (mL)	(mr) ^{by}	V _{NOM} (TTL)	стано Манто Л
# #	100:100	15	15	15	13.497	0.450	1.0
7#	100:10	15	1.5	15	13.497	0.045	1.4
#3	100:1	15	0.15	15	13.497	0.005	1.4
Control A	100:0	15	0	15	13.497	0.00	1.5
#4	10:100	1.5	15	15	1.350	0.450	13.2
#5	10:10	1.5	1.5	15	1.350	0.045	13.6
9#	10:1	1.5	0.15	15	1.350	0.005	13.6
Control B	10:0	1.5	0	15	1.350	0.00	13.6
L#	1:100	0.15	15	15	0.135	0.450	14.4
8#	1:10	0.15	1.5	15	0.135	0.045	14.8
6#	1:1	0.15	0.15	15	0.135	0.005	14.8
Control C	0.1	0.15	0	<u>5</u>	0.135	0.000	14.8

Batch 2

6

Stock of NaHCO₃ (ppm)

500

Stock of NOM (ppm)

Table 7. Data for the synthesis of the test solutions with Batch 2.

6.3 Point of Zero Charge

рН	Volume injected (mL)	[HCI] added (M)
7.53	1.00E-04	2.95E-08
7.01	5.49E-05	9.77E-08
6.74	2.07E-05	1.82E-07
6.44	5.21E-05	3.63E-07
6.08	6.83E-05	8.32E-07
5.73	6.75E-05	1.86E-06
5.14	5.74E-05	7.24E-06
5.01	3.22E-05	9.77E-06
4.56	4.37E-05	2.75E-05
4.28	4.96E-05	5.25E-05
3.92	1.04E-04	1.20E-04
3.53	2.15E-04	2.95E-04
3.15	1.34E-05	7.08E-04
2.57	6.24E-05	2.69E-03
2.35	5.01E-05	4.47E-03
2.07	1.10E-04	8.51E-03

Table 8. Experimental data for the determination of the point of zero charge of Batch A.

6.4 pH measurements for NOM additions

For the dilutions with 15, 1.5 and 0.15ppm of silver NPs made with Milli-Q H_2O , the results of the variation in time are presented in Figure 47, Figure 48 and Figure 49, respectively.



Figure 47. pH variation with time in the series of 15ppm



Figure 48. pH variation with time in the series of 1.5ppm



Figure 49. pH variation with time in the series of 0.15ppm

6.5 Polydispersity Index

The polydispersity index was measured through DLS for the test solutions performed in Milli-Q H_2O and the results are displayed in Figure 50, Figure 51 and Figure 52.



Figure 50. Polydispersity Index for [AgNPs]=15ppm



Figure 51. Polydispersity Index for [AgNPs]=1.5ppm



Figure 52. Polydispersity Index for [AgNPs]=0.15ppm

6.6 Comparison between the cameras Marlin and Andor

In order to decide which camera recordings should be used in the discussion of results, three random samples were chosen and the graphs compared.



Figure 53. Camera comparisons using the Control C day 2 with no buffer



Figure 54. Camera comparisons using the sample [AgNPs]=1.5ppm and [NOM]=1.5ppm day 1 with carbonate buffer 100 ppm



Figure 55. Camera comparisons using the sample [AgNPs]= 15ppm and [NOM]=1.5ppm day 3 with carbonate buffer 100 ppm

As observed in Figure 53, Figure 54 and Figure 55 the Andor camera reveals higher sensitivity to smaller nanoparticles than the Marlin camera.



6.7.1 [AgNPs]=15ppm

Figure 56. Size mean variation in time with [AgNPs]=15ppm according to DLS.



Figure 57. Size mean variation in time with [AgNPs]= 15ppm according to NTA.



Figure 58. Size mean variation in time with [AgNPs]= 1.5ppm according to DLS.



Figure 59. Size mean variation in time with [AgNPs]=1.5ppm according to NTA


Figure 60. Size mean variation in time with [AgNPs]=0.15ppm according to DLS.



Figure 61. Size mean variation in time with [AgNPs]=0.15ppm according to NTA.

6.8 Size distributions (DLS)

6.8.1 [AgNPs]=15ppm



Figure 62. Size distributions varying with time for [NOM]=15ppm



Figure 63. Size distributions varying with time for [NOM]=1.5ppm



Figure 64. Size distributions varying with time for [NOM]=0.15ppm



Figure 65. Size distributions varying with time for Control A

6.8.2 [AgNPs]=1.5ppm



Figure 66. Size distributions varying with time for [NOM]=15ppm



Figure 67. Size distributions varying with time for [NOM]=1.5ppm



Figure 68. Size distributions varying with time for [NOM]=0.15ppm



Figure 69. Size distributions varying with time for Control B



Figure 70. Size distributions varying with time for [NOM]=15ppm



Figure 71. Size distributions varying with time for [NOM]=1.5ppm



Figure 72. Size distributions varying with time for [NOM]=0.15ppm



Figure 73. Size distributions varying with time for Control C

6.9 Size distributions (NTA)

Some recordings done with the NTA demanded the dilution of samples. The final results were not *converted* to undiluted as other measured samples since the necessary data was not provided.

6.9.1 [AgNPs]=15ppm diluted 1:100



Figure 74. Size distributions varying with time for [NOM]=15ppm



Figure 75. Size distributions varying with time for [NOM]=1.5ppm







Figure 77. Size distributions varying with time for Control A





Figure 78. Size distributions varying with time for [NOM]=15ppm



Figure 79. Size distributions varying with time for [NOM]=1.5ppm



Figure 80. Size distributions varying with time for [NOM]=0.15ppm



Figure 81. Size distributions varying with time for Control B



Figure 82. Size distributions varying with time for [NOM]=15ppm



Figure 83. Size distributions varying with time for [NOM]=1.5ppm



Figure 84. Size distributions varying with time for [NOM]=0.15ppm



Figure 85. Size distributions varying with time for Control C

6.10 Electrophoretic Mobility

The graphs recorded for the electrophoretic mobility of the test solutions diluted in Milli-Q H_2O are displayed below and are shown from the highest concentration of NOM to the lowest.

6.10.1 [AgNPs]=15ppm



Figure 86. Electrophoretic mobility variation in time with [NOM]=15ppm



Figure 87. Electrophoretic mobility variation in time with [NOM]=1.5ppm



Figure 88. Electrophoretic mobility variation in time with [NOM]=0.15ppm



Figure 89. Electrophoretic mobility variation in time with Control A

6.10.2 [AgNPs]=1.5ppm



Figure 90. Electrophoretic mobility variation in time with [NOM]015ppm



Figure 91. Electrophoretic mobility variation in time with [NOM]=1.5ppm



Figure 92. Electrophoretic mobility variation in time with [NOM]=0.15ppm



Figure 93. Electrophoretic mobility variation in time with Control B



Figure 94. Electrophoretic mobility variation in time with [NOM]=15ppm



Figure 95. Electrophoretic mobility variation in time with Control B



Figure 96. Electrophoretic mobility variation in time with [NOM]=0.15ppm



Figure 97. Electrophoretic mobility variation in time with Control C

6.11 Colloidal Stability Model Calculations

The results for the dilution series performed in Milli-Q H_2O are presented in Table 9.

Day 0	Ionic Strength	a (m)	k	ka
1	1.888E-03	1.165E-08	4.512E+06	0.686
2	1.888E-03	1.608E-08	4.512E+06	0.692
3	1.888E-03	1.264E-08	4.512E+06	0.687
Control A	1.888E-03	1.701E-08	4.512E+06	0.693
4	1.888E-04	2.664E-08	1.427E+06	0.681
5	1.888E-04	2.108E-08	1.427E+06	0.678
6	1.888E-04	1.533E-08	1.427E+06	0.675
Control B	1.888E-04	4.104E-08	1.427E+06	0.688
7	1.888E-05	2.900E-07	4.512E+05	0.709
8	1.888E-05	2.119E-07	4.512E+05	0.699
9	1.888E-05	1.862E-07	4.512E+05	0.696
Control C	1.888E-05	1.933E-08	4.512E+05	0.670

Table 9. Data of the calculation of *ka* for test solutions diluted in Milli-Q H₂O in Day 0.

where *a* is the radius of the nanoparticles.

According to the results of *ka* presented, to calculate the zeta potential the Hückel model was used according to the corresponding equation in Table 2. The values are presented in Table 10.

Table 10. Data of the calculation of zeta potential for test solutions diluted in carbonate buffer 100	opm in
Day 0.	-

Day 0	Electrophoretic Mobility (µmcm/vs)	Zeta Potential (mV)
1	-3.63	-69.76
2	-4.38	-84.16
3	0.57	11.00
Control A	-1.73	-33.27
4	-2.13	-40.93
5	-0.38	-7.25
6	-5.04	-96.80
Control B	-0.37	-7.08
7	-3.40	-65.21
8	-0.29	-5.59
9	-4.74	-91.00
Control C	-0.09	-1.71

For the following the days the same values of ionic strength and decay parameter were used for the calculations of the stability model. The values are presented in Table 11 were can be observed that all samples are stable according to Hückel, but sample 7 shifts to the Henry stabilization model.

	Day 1		Day 2		Day 3	
	a (m)	ka	a (m)	ka	a (m)	ka
1	9.650E-09	0.044	1.222E-08	0.055	1.123E-08	0.051
2	1.025E-08	0.046	1.468E-08	0.066	1.063E-08	0.048
3	1.206E-08	0.054	1.813E-08	0.082	1.368E-08	0.062
Control A	1.715E-08	0.077	1.403E-08	0.063	1.509E-08	0.068
4	4.221E-08	0.060	4.526E-08	0.065	3.690E-08	0.053
5	1.302E-08	0.019	1.897E-08	0.027	1.498E-08	0.021
6	1.077E-08	0.015	2.164E-08	0.031	1.426E-08	0.020
Control B	2.041E-08	0.029	2.095E-08	0.030	2.351E-08	0.034
7	3.913E-07	0.177	3.421E-07	0.154	2.595E-07	0.117
8	2.939E-08	0.013	1.709E-07	0.077	9.495E-08	0.043
9	3.215E-08	0.015	3.789E-08	0.017	8.895E-08	0.040
Control C	2.684E-07	0.121	9.750E-08	0.044	6.830E-08	0.031

Table 11. Data of the calculation of ka for test solutions diluted in Milli-Q H₂O in Day 1, 2 and 3.

In order to calculate the zeta potential for samples belonging to the Henry domain, it is needed to apply the Henry function. For dilute spherical particles the equation used is presented below.^[71]

$$f(ka) = \frac{2}{3} \left[1 + \frac{1}{2\left(1 + \frac{2.5}{ka\{1 + 2\exp(-ka)\}}\right)^3} \right]$$

Equation 9

The corresponding values of zeta potential for days 1, 2 and 3 are presented in Table 12.

	Day 1		Day 2		Day 3	
	Electrophoretic Mobility (µmcm/vs)	Zeta Potential (mV)	Electrophoretic Mobility (µmcm/vs)	Zeta Potential (mV)	Electrophoretic Mobility (µmcm/vs)	Zeta Potential (mV)
1	-1.85	-35.53	0.09	1.81	-3.72	-71.39
2	-1.38	-26.50	-0.76	-14.57	-4.38	-84.14
3	-0.43	-8.21	-1.65	-31.65	-0.42	-8.06
Control A	-3.09	-59.33	-0.59	-11.42	-3.78	-72.64
4	0.02	0.39	-2.68	-51.49	-0.90	-17.35
5	0.02	0.31	-6.30	-120.98	-2.81	-54.03
6	-0.51	-9.71	-0.51	-9.73	-3.95	-75.85
Control B	-0.35	-6.73	0.00	-0.08	0.18	3.39
7	-0.57	-7.93	-0.67	-9.13	-0.69	-9.34
8	-4.23	-81.19	-3.61	-69.37	-1.28	-24.64
9	4.12	79.15	-6.24	-119.79	5.62	107.98
Control C	7.38	141.67	-0.41	-7.85	-2.59	-49.65

Table 12. Data of the calculation of Zeta Potential for test solutions diluted in Milli-Q H₂O in Day 1,2 and 3.

Analogue calculations were performed for the test solution diluted in carbonate buffer.

The results for the carbonate buffer with a concentration of 100ppm for day 0 are shown in Table 13 and Table 14.

Day 0	Ionic Strength	a (m)	k	ka
1	8.522E-02	1.194E-08	3.611E+07	0.497
2	1.174E-01	1.346E-08	1.081E+08	2.123
3	1.206E-01	1.063E-08	1.128E+08	2.843
Control A	1.209E-01	1.378E-08	3.031E+07	0.362
4	1.048E+00	9.415E-08	3.557E+07	0.479
5	1.080E+00	1.966E-08	3.606E+07	0.383
6	1.083E+00	1.881E-08	1.063E+08	10.008
Control B	1.084E+00	1.964E-08	1.079E+08	2.121
7	1.144E+00	4.243E-07	1.081E+08	2.032
8	1.176E+00	4.058E-07	1.111E+08	47.121
9	1.180E+00	3.416E-08	1.126E+08	45.695
Control C	1.180E+00	2.521E-08	1.128E+08	3.852

Table 13. Data of the calculation of *ka* for test solutions diluted in carbonate buffer 100ppm in Day 0.

As shown through the values of *ka* the samples fall in the Henry domain. The corresponding values of zeta potential are presented in Table 14.

Day 0 Electrophoretic Mobil (µmcm/vs)		Zeta Potential (mV)
1	-0.71	-10.24
2	-0.37	-5.47
3	-1.14	-16.54
Control A	-0.02	-0.36
4	-0.32	-5.69
5	-1.52	-24.37
6	-7.26	-116.53
Control B	-4.82	-77.60
7	-1.59	-30.03
8	-5.68	-107.15
9	-5.28	-88.47
Control C	-6.89	-112.85

Table 14. Data of the calculation of zeta potential for test solutions diluted in carbonate buffer 100ppm inDay 0.

The rest of the days also fit in the Henry stabilization (Table 15).

Table 15. Data of the calculation of ka for test solutions diluted in carbonate buffer 100ppm in Day 1, 2and 3.

	Day 1		Day 2		Day 3	
	a (m)	ka	a (m)	ka	a (m)	ka
1	1.593E-08	0.483	1.216E-08	0.368	1.120E-08	0.339
2	1.684E-08	0.599	1.263E-08	0.449	1.090E-08	0.388
3	1.975E-08	0.712	1.147E-08	0.414	1.427E-08	0.515
Control A	1.372E-08	0.495	9.660E-09	0.349	1.441E-08	0.520
4	6.920E-08	7.356	5.700E-08	6.059	7.230E-08	7.685
5	1.890E-08	2.040	1.792E-08	1.933	1.999E-08	2.157
6	2.011E-08	2.173	2.003E-08	2.165	2.096E-08	2.265
Control B	1.815E-08	1.961	2.285E-08	2.470	2.173E-08	2.349
7	5.915E-07	65.697	2.964E-07	32.921	3.565E-07	39.596
8	2.369E-07	26.674	2.072E-07	23.335	1.296E-07	14.590
9	1.829E-07	20.626	9.865E-08	11.125	9.315E-08	10.505
Control C	3.842E-08	4.333	9.895E-08	11.160	5.360E-08	6.045

The zeta potential values are shown in Table 16.

	Day 1		Day 2		Day 3	
	Electrophoretic Mobility (µmcm/vs)	Zeta Potential (mV)	Electrophoretic Mobility (µmcm/vs)	Zeta Potential (mV)	Electrophoretic Mobility (µmcm/vs)	Zeta Potential (mV)
1	-0.95	-13.99	-0.14	-2.07	-0.24	-3.41
2	-1.06	-15.80	-0.32	-4.70	-0.02	-0.26
3	-1.40	-21.10	0.61	8.95	-2.20	-32.54
Control A	-2.28	-33.64	-0.33	-4.70	-1.81	-26.74
4	-1.85	-32.53	-0.22	-3.81	0.13	2.29
5	-0.31	-4.99	0.67	10.71	-0.48	-7.80
6	6.43	103.50	-0.65	-10.48	-1.02	-16.41
Control B	-0.26	-4.14	-0.23	-3.66	-1.14	-18.48
7	-1.11	-21.00	-0.34	-6.37	-0.01	-0.19
8	-0.77	-14.45	-5.73	-106.47	-5.03	-91.84
9	-0.07	-1.32	-5.68	-102.38	-6.87	-123.49
Control C	6.23	105.23	-0.19	-3.50	-1.55	-26.95

Table 16. Data of the calculation of zeta potential for test solutions diluted in carbonate buffer 100ppm inDay 1, 2 and 3.

For the carbonate buffer with a concentration of 1600ppm, the values for the day 0 are listed in Table 17

Table 17. Data of the calculation of ka for test solutions diluted in carbonate buffer 1600ppm in Day 0.

Day 0	Ionic Strength	a (m)	k	ka
1	1.349E+00	4.173E-08	1.206E+08	5.032
2	1.863E+00	2.053E-08	1.417E+08	2.909
3	1.915E+00	4.796E-08	1.437E+08	6.890
Control A	1.920E+00	1.727E-08	1.439E+08	2.485
4	1.676E+01	3.210E-07	4.251E+08	136.440
5	1.728E+01	1.138E-07	4.316E+08	49.114
6	1.733E+01	3.765E-08	4.322E+08	16.273
Control B	1.733E+01	1.881E-08	4.323E+08	8.131
7	1.830E+01	6.300E-07	4.442E+08	279.863
8	1.882E+01	4.092E-07	4.504E+08	184.314
9	1.887E+01	1.747E-07	4.510E+08	78.774
Control C	1.887E+01	8.205E-08	4.511E+08	37.013

As can be seen, there are some samples that belong to the Smoluchowski domain. For the calculations of zeta potential, the equation shown in Table 2 will be used. The results are displayed in Table 18

Day 0	Electrophoretic Mobility (µmcm/vs)	Zeta Potential (mV)
1	-2.31	-37.57
2	-1.79	-31.76
3	-1.32	-24.78
Control A	-3.63	-62.04
4	-0.41	-6.68
5	-5.59	-97.90
6	-0.31	-3.94
Control B	-2.25	-42.57
7	-2.22	-40.73
8	-2.73	-34.94
9	-0.51	-6.58
Control C	-1.35	-25.57

Table 18. Data of the calculation of zet apotential for test solutions diluted in carbonate buffer 1600ppm in
Day 0.

The calculations to verify what models to use in days 1, 2 and 3 are presented in Table 19.

Table 19. Data of the calculation of ka for test solutions diluted in carbonate buffer 1600ppm in Day 1, 2and 3.

	Day 1		Da	Day 2		Day 3	
	a (m)	ka	a (m)	ka	a (m)	ka	
1	2.187E-08	2.637	3.202E-08	3.861	2.149E-08	2.592	
2	1.982E-08	2.808	2.621E-08	3.715	1.935E-08	2.742	
3	1.544E-08	2.218	1.813E-08	2.604	2.508E-08	3.603	
Control A	3.068E-08	4.414	1.795E-08	2.582	2.967E-08	4.268	
4	6.615E-08	28.121	5.340E-08	22.701	6.310E-08	26.825	
5	2.479E-08	10.699	1.685E-08	7.272	9.300E-08	40.137	
6	1.910E-08	8.253	1.895E-08	8.191	2.631E-08	11.370	
Control B	2.705E-08	11.694	2.528E-08	10.928	3.595E-08	15.539	
7	4.266E-07	189.507	6.370E-07	282.973	4.064E-07	180.512	
8	1.410E-07	63.510	1.879E-07	84.635	1.440E-07	64.839	
9	3.079E-08	13.885	1.338E-07	60.326	1.257E-07	56.673	
Control C	9.940E-08	44.840	2.291E-07	103.349	2.545E-07	114.784	

The zeta potential calculated for each day is displayed in Table 20.

	Day 1		Day 2		Day 3	
	Electrophoretic Mobility (µmcm/vs)	Zeta Potential (mV)	Electrophoretic Mobility (µmcm/vs)	Zeta Potential (mV)	Electrophoretic Mobility (µmcm/vs)	Zeta Potential (mV)
1	-2.94	-47.90	-2.13	-35.60	-2.45	-39.87
2	-0.19	-3.15	-0.82	-13.75	-0.41	-6.65
3	-3.46	-55.82	-1.82	-29.70	-1.98	-32.93
Control A	-3.07	-51.98	-2.30	-37.43	-1.96	-33.11
4	0.45	8.49	-0.56	-10.42	0.20	3.80
5	-1.86	-33.40	-0.86	-15.16	-3.03	-57.08
6	-1.12	-19.92	-1.88	-33.35	-2.38	-42.93
Control B	-1.99	-35.89	-0.81	-14.54	0.53	9.74
7	-2.70	-34.53	0.85	10.91	-2.25	-28.85
8	-0.94	-17.83	-2.25	-42.76	0.49	9.38
9	2.00	36.44	-1.59	-30.06	-0.85	-16.01
Control C	-0.38	-7.16	-4.20	-53.71	-1.16	-14.83

Table 20. Data of the calculation of zeta potential for test solutions diluted in carbonate buffer 1600ppmin Day 1, 2 and 3.