

Settling and aggregation of colloidal silica and engineered DNA tagged silica-iron particles in surface water

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Settling and aggregation of colloidal silica and engineered DNA tagged silica-iron particles in surface water

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Abstract

Recently, engineered DNA tagged silica-iron microparticles (SiDNAFe MPs) tracer were designed. We aim to understand the effect of the suspended solids present in natural water on silica colloids and SiDNAFe MP's settling behaviour.

Hereto, we set up a series of sedimentation experiments with SiDNAFe MPs and silica colloids (as the proxy particle) in three types of river water. And the sedimentation experiments with SiDNAFe MPs or silica colloids in Milli-Q water were experimental control groups. The sedimentation periods is 30 hours.

The most important results from the sedimentation experiments were that the sedimentations of SiDNAFe MPs and silica colloids in Milli-Q water were single particle settling, in which settling particles have no interaction, the so-called type I sedimentation. The observed settling behaviour of SiDNAFe MPs in three types of natural river water was similar to Milli-Q water behaviour. However, the observed settling behaviour of silica colloids in three types of river water is different from Milli-Q water behaviours.

The settling experiments were simulated using a sedimentation model. This is the simulation of a Type I sedimentation behaviour. The simulations show that there is no significant difference between the simulated sedimentation of SiDNAFe MPs in Milli-Q water and the observed in three types of natural waters. As for the silica colloids, there is a significant difference between the simulated settling in Milli-Q water and the observed in three types of river water, and the values of residuals were positive.

Overall, the mass loss of SiDNAFe in natural water was due to type I sedimentation, and the settling of SiDNAFe MPs have no interaction in natural water. Differently, the mass loss of silica colloids in natural water was due to sedimentation and aggregation.

Keywords: SiDNAFe MPs; Silica colloids; Sedimentation; Aggregation; Natural water

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Abbreviations

- BOE - Buffered Oxide Etch solution
BTCs - Breakthrough Curves
Cq - quantification Cycle
DNA - Deoxyribonucleic Acid
DOC - Dissolved Organic Carbon
DLS - Dynamic Light Scattering
EC - Electrical Conductivity
ENPs - Engineered Nanoparticles
F-EEM - Fluorescence Excitation - Emission technique
F0.45MK - 0.45 µm filtered Merkske river water sample
F1.2MK - 1.2 µm filtered Merkske river water sample
F0.45Mu - 0.45 µm filtered Meuse river water sample
F1.2Mu - 1.2 µm filtered Meuse river water sample
F0.45Strij -0.45 µm filtered Strijbeek river water sample
F1.2Strij -1.2 µm filtered Strijbeek river water sample
GO - graphene oxide nanosheets
HemNPs - Hematite nanoparticles
IC - Ion chromatography
ICB - Institute for Chemical and Bioengineering
ICP-MS -Inductively coupled plasma mass spectrometry
NOM - Nature Organic Matter
NPs - Nanoparticles
TiO₂ - Titanium Dioxide
TEOS - Tetraethoxysilane
PSD - Particle size distribution
qPCR - quantitative Polymerase Chain Reaction
SiDNFe MPs - Engineered DNA tagged silica-iron microparticles
UFMK - unfiltered Merkske river water sample
UFMu - unfiltered Meuse river water sample
UFStrij - unfiltered Strijbeek river water sample

Chapter 1 Introduction

1.1 Background

Tracer testing is a powerful method for source tracking and characterising flow pathways. However, traditional tracers (e.g. fluorescence dyes, salt tracers, environmental isotopes and so on) have drawbacks, such as limited tracer types. Recently, engineered DNA tagged silica-iron microparticles (SiDNAFe MPs) tracer were designed(Anuvansh Sharma et al., 2021). Theoretically, it could develop to unlimited kinds of DNA tracers with different sequences. The project, called the Water tagging project, wants to apply this microparticle tracer to determine multiple advective-dispersive transport processes of flows within one experiment. However, a new study shown that the low mass recovery of encapsulated DNA tracers was observed when tracking in natural water in a field(Pang et al., 2020).

We speculated that sedimentation and aggregation are the causes of mass loss of encapsulated DNA tracer during the tracing process in surface water. Because sedimentation is a process of particles removal from a liquid phase, and particle flocculation or aggregation might play an important role during settling. Small suspended particles can aggregate to larger sizes, whereby the settling velocity increases, leading to mass removal. Therefore, we designed a series of experiments to explore the sedimentation and aggregation of SiDNAFe MPs. Additionally, silica colloids were used as the proxy to conduct settling experiments in natural waters due to the limited number of SiDNAFe MPs.

Aggregation includes homo-aggregation and hetero-aggregation. The former is the aggregation of similar particles, while the latter is the aggregation of different particles. In recent years, several studies have found that nanoparticles aggregated with other particles in the aquatic environment. For example, the aggregation of CeO₂ nanoparticles (NPs) with natural colloids (NCs) were main mechanism causing CeO₂ NPs' sedimentation, and the engineered nanoparticles (ENPs) hetero-aggregated with suspended sediments resulting in the rapidly settling (Quik et al., 2012; Velzeboer et al., 2014).

1.2 Hypothesis

Sedimentation of particles in a suspension is a critical process for particles' mass removal. It may lead to the low mass recovery of SiDNAFe MPs in tracer experiments. Our H₀ hypothesis is that in natural water, a significant amount of SiDNAFe MPs mass is removed due to hetero-aggregation as a result of the formation of aggregates of SiDNAFe MPs and naturally occurring suspended solids and natural colloids. Our H₁ hypothesis is that there is mass removal, but not necessarily due to aggregation.

1.3 Objective

The main objective was to understand and quantify the sedimentation mechanism of SiDNAFe MPs and the effect of the suspended solids, present in natural water, on the settling behaviour of SiDNAFe MP. Sub-objectives were:

- To conduct the sedimentation experiments for colloidal silica and SiDNAFe in Milli-Q water and various filtered natural waters (0.45 um filter and 1.2 um filter)
- To quantify the sedimentation of colloidal silica and SiDNAFe in Milli-Q water
- To identify the aggregation of colloidal silica and SiDNAFe in various filtered natural waters

1.4 Innovation and Practical Value

In present study, I will explore and understand sedimentation and aggregation of SiDNAFe MPs in natural waters. And I will create a model to simulate the particles' sedimentation.

This will increase our understanding of the behaviour and fate of SiDNAFe MPs in tracer experiments in natural waters.

Chapter 2 Literature review

2.1 Development of DNA tracer and encapsulated DNA tracer in tracer testing for hydrological process

In 1999 and 2000, Sabir and co-workers first applied synthetic DNA to determine groundwater flow paths qualitatively by detecting the presence/absence of the tracers in water samples (Sabir et al., 1999, 2000). It proved the possibility of multi-tracing the origins and flow patterns within one experiment by unlimited DNA tracers with different sequences. In contrast, the maximum number of traditional tracers used simultaneously within one experiment would be at most 8 to 10 (Ptak et al., 2004). Until 2004, DNA concentration could be quantitatively determined by one molecular biology technique-quantitative Polymerase Chain Reaction (qPCR), and the DNA sequences could be amplified exponentially for the DNA concentration measurement and readout (Ptak et al., 2004). This technique is good for the measurement of extremely low DNA concentrations. However, the analytical tools for measuring the traditional tracers' concentrations (i.e. mainly different types of salt tracers and fluorescence dyes (Leibundgut et al., 2009)) are different, dramatically increasing multi-tracing experiments' efforts and costs. In 2011, Foppen et al. (2011) successfully performed breakthrough curves (BTCs) of DNA tracers in natural streams. Meanwhile, they made a rigorous assessment of this novel tracer's behaviour in 2013, compared its performance with sodium chloride (Foppen et al., 2013). Overall, during two decades of development, DNA tracer has been applied in many field experiments to characterise the hydrological processes. These include the identification of groundwater flow paths in fractured rock aquifer (Sabir et al., 2000), karst groundwater system (Aquilanti et al., 2013, 2016; Bovolin et al., 2014) and alluvial sand and gravel aquifer (Pang et al., 2017; Ptak et al., 2004; Sabir et al., 1999, 2000), as well as the surface flow paths in natural streams (Foppen et al., 2011, 2013) and glacial streams (Dahlke et al., 2015).

Previous studies employed 'free' DNA for tracer testing (Aquilanti et al., 2013, 2016; Bovolin et al., 2014; Dahlke et al., 2015; Foppen et al., 2011, 2013; Pang et al., 2017, 2020; Ptak et al., 2004; Sabir et al., 2000, 1999). However, Foppen et al. (2011, 2013) and Dahlke et al. (2015) observed the low mass recoveries of 'free' DNA (i.e. no encapsulated) in natural waters in tracer testing. The reason is that the 'free' DNAs without protection are sensitive and unstable to oxidation, alkylation (Baust, 2008) and hydrolysis (Willerslev et al., 2005) in complex natural aquatic environmental conditions. Therefore, several studies developed encapsulated DNA tracers to increase the DNAs' stability in the natural environment, such as the DNAs sealed with the polyvinyl acetate (PVA) (Dahlke et al., 2015; Asha Sharma et al., 2012), clay (Mahler et al., 1998), alginate and chitosan (Pang et al., 2020) or silica (Foppen et al., 2015; Grass et al., 2014). Nevertheless, the encapsulated DNA tracer for tracer testing in the surface water is not commonly. Dahlke et al. (2015) worked to PVA-encapsulated DNA tracer with iron oxide core in glacial streams for tracing flow path and got low mass recoveries from 1% to 66%. Pang et al. (2020) injected 'free' DNA and alginate-chitosan coated DNA tracers into a stream next to agricultural land to tracking fast-flowing water, and the mass loss of coated DNA tracers were 1-3 orders of magnitude lower than those of 'free' DNA tracers.

2.2 Sedimentation and settling velocity

The suspended particles' sedimentation is an essential process for the particles' mass removal from the fluid phase—the sediment transport driven by gravity and fluid drag. When a particle settles through a fluid, gravity accelerates the particle, and its inertia reduces due to fluid drag. Finally, the particle falls at a constant settling velocity in the viscous fluid. Early in 1851, Sir George Gabriel Stokes(1851) mathematically estimated the fluid drag, known as Stokes' Law. The Stokes' Law describes the fluid drag (F_d) on a sphere of diameter d [m]with a small Reynolds number (<1) in a fluid:

$$F_d = 3\pi\mu Ud \quad (2-1)$$

Where μ [Pa·s] - the medium's dynamic viscosity; U [m/s] - the free stream velocity. This equation is the critical prerequisites to understanding the sedimentation of suspended particles in water, and the terminal (settling) velocity (v) of a sphere in a fluid is reached when the balance of fluid drag and excess force (the difference between the weight and buoyancy of spherical particle)(Lamb, 1993). The velocity of v given by:

$$v = \frac{gd^2(\rho_p - \rho_m)}{18\mu} \quad (2-2)$$

Where ρ_p [kg/m³] - the particle's density; ρ_m - [kg/m³]the medium's density; g [m/s²] - gravitational constant. It is the central parameter to drive sedimentation's critical process (Dey et al., 2019). However, Stokes' law and the impact formula could only describe spherical particles' sedimentation when Reynold's number lower than 1. It neglected the inertia term in equation (2-2) which becomes significant when particles size larger than 0.14 mm (Rubey, 1933). In 1933, Rubey published a paper to describe settling velocities of gravel, sand and silt particle (composition: silica). The maximum value of particle sizes adapting Rubey's equation is more extensive than those for Stokes' law, and the particle's drag force is neglected at Reynold number (Re) < 10. The sedimentation of spherical particles in a fluid can be described, given by:

$$F_g - F_b = F_v + F_i \quad (2-3)$$

F_g [N] is the gravitational force; F_b [N] is the buoyancy; F_v [N] is the viscous force; F_i [N] is the inertial force. The conceptual representation is shown in figure 2-1.

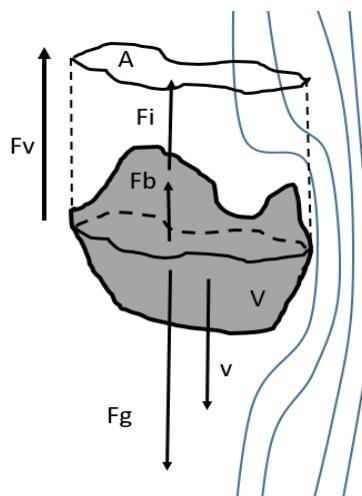


Figure 2-1 Conceptual representation of forces (F_g , F_b , F_v and F_i) on a settling particle in liquid.

Thus, the terminal settling velocity of the particle could be written by solving equation (2-3) algebraically as follows:

$$v = \frac{-18\mu + \sqrt{18^2\mu^2 + 6gd^3\rho_m(\rho_p - \rho_m)}}{3d\rho_m} \quad (2-4)$$

The Stokes' law and Rubey equation has solved the hydrodynamic problem of one particle fall through a fluid but did not give the formula to calculate particles' concentration in suspension. In 1951, Kynch proposed a theory of sedimentation that the change of particles' concentration was described by a mass continuity equation, and the rate of change of particle concentration- sedimentation rate- could be derived from this equation. The concentration means the number of particles per unit volume of the dispersion. Einstein and Hans (1968) applied and extended Kynch's theory to describe the suspended silts particles' sedimentation in a gravel bed. The sedimentation characteristic of Fluorescent particles was explored according to this theory (Newman et al., 1990).

Moreover, Based on Einstein and Hans' theory, Kretzschmar et al. (1997) estimated colloid sedimentation rates in natural porous media. In the recent two decades, Petosa et al. (2010) characterised the deposition of engineered nanomaterials (fullerenes (C60) and carbon nanotubes (CNTs)). Quik et al. worked on the nanoscale particles' sedimentation, and the sedimentations of different nanoparticles (C60, CeO₂, SiO₂-Ag, PVP-Ag) in natural water were characterised according to previous theories(Quik et al., 2012, 2014). Moreover, the sedimentation process of non-cohesive particles was modelled by computational fluid dynamics and discrete element method (CFD-DEM)(Xu et al., 2018). After the development of sedimentation theory, there are four types of particles' sedimentation in a suspension(J. G. Speight, 2017):

- Type I suspension contains the particles with a virtually continuous distribution of settling velocities, and the sedimentation of each particle is independent without any flocculation.
- Type II sedimentation is the sedimentation with accompanying flocculation. Under the flocculation role, the small particles are removed primarily from the size distribution at a particular location and the number of the large particles increase. The large particles are still removed primarily by sedimentation.
- In concentrated suspensions, the zone settling and hindered settling of particles occur, called type III sedimentation.
- Particles compress in type IV suspension.

All four types of sedimentations depend on the size and density of particles and the medium's physical properties. To sum up, the settling velocity, given by Rubey and Stokes' law, is always the central parameter to drive the key process of particle's sedimentation no matter how the sedimentation theory develop.

2.3 Silica settling and flocculation

Silica settling is one of the most common processes in the aquatic environment. The sedimentation mechanism of silica in natural water has been studied. Twenhofel (1950) discovered that most of the silica particles settle physically in seawater, while electrolytes flocculated some silica particles. It classified as type II sedimentation, as mentioned before. Therefore, it could be accepted that the sedimentation of colloidal silica particles in natural water (e.g. river water and steam) is type II. Seven years later, Okamoto et al. (1957) elaborated the property of silica in water. He indicated that a trace amount of aluminium influences silica colloids' precipitation, and the optimum pH for the sedimentation is 4.5. Those two papers implied that flocculation (aggregation) play an important role on silica colloids settling in

natural water. In recent decades, several studies focused on the flocculation of silica colloids. The silica colloids exhibits aggregation features in suspension similar to predictions of the Derjaguin, Landau, Verwey, and Overbeek (DLVO) theory(Kobayashi et al., 2005). Abe et al.(2011) discovered that the aggregation of colloidal silica particles with natural organic matter (NOM), and the enhancement of hetero-aggregation rate was due to the bridging of silica colloids by the Ca-mediated bridging of alginate.

However, it is not easy to estimate the sedimentation rate of aggregated silica. An approach for the estimation of irreversible flocculation was proposed by Smoluchowski (Chandrasekhar, 1943; Von Smoluchowski, 1916). The equation could describe the flocculation of silica particles in natural water. After that, a combination of the Smoluchowski equation to model flocculation and Stokes equation to describe particle settling was given from Benjamin and Lawler (2013):

$$\frac{\partial n_{k,z}}{\partial t} = \left[\frac{1}{2} \alpha_{emp} \sum_{i+j=k} \alpha_{ij} \beta_{ij} n_{i,z} n_{j,z} \right] - \left[\alpha_{emp} n_{k,z} \sum_{\text{All } i} \alpha_{ik} \beta_{ik} n_{i,z} \right] - \left[v_k \frac{\partial n_k}{\partial z} \right] \quad (2-5)$$

Where $n_{j,z}$ [-] - the particle number of size class j at z depth; $n_{i,z}$ [-] - the particle number of size class i at z depth; α_{ij} [-] - the attachment efficiency between particle aggregate i and j ; α_{ik} [-] - the attachment efficiency between particle aggregate i and k ; β_{ij} [m^3/s] - the collision efficiency between particle aggregate i and j ; β_{ik} [m^3/s] - the collision efficiency between particle i and other particle k ; α_{emp} [-] - the empirical fitting factor. The first two terms in Eq. 2-5 are the gain and loss by collision and attachment, and the last term represents the variation for the particles settling. Notably, the sedimentation of colloidal silica particle in type II suspension can be mathematically characterised by this combined Smoluchowski-Stokes equation.

2.4 Homo-aggregation of micro-particles in natural water

Few studies studied the homo-aggregation of nano- or micro-particles and found it is not the main aggregation mechanism in natural water. In 2014, Quik et.al (2014) calculated the homo-aggregation and hetero-aggregation rates for CeO₂ NPs in the Rhine and Meuse river water. He concluded that homo-aggregation rates is much less than hetero-aggregation rates. Similarly, Fang et.al (2017) investigated that the hetero-aggregation between ZnO and TiO₂ nanomaterials in natural water is more obvious than the homo-aggregation. Furthermore, Suwannee River humic acid humid acids (SRHA) reduce homo-aggregation of Sliver NPs in Rhine river water samples(Metreveli et al., 2014).

2.5 Hetero-aggregation of micro-particles in natural water

By the contract, so many studies focused on the hetero-aggregation of nano- or micro-particles in the aquatic environment. For instances, Labille et al. (2015) investigated that, with increasing the surface area ratio of NPs to clay, the aggregated particle (Titanium Dioxide (TiO₂) NPs and natural clay colloids) aggregated with natural clay colloids again in the aquatic environment. It proved a possibility that the secondary aggregation of aggregated colloids with other particles in natural water. Moreover, the nanoplastic formed small hetero-aggregation with alginate at low concentration when the surface charge changed from positive to negative(Oriekhova & Stoll, 2018). It means the importance of NPs' surface charge neutralisation for the particles' hetero-aggregation. Another evidence is that negatively charged graphene oxide nanosheets (GO) hetero-aggregated with positively charged hematite nanoparticles (HemNPs) in deionised water, leading to GO settling(Feng et al., 2019). In natural water, NCs play a crucial role in the hetero-aggregation mechanism of nanoparticles. For examples, four different NPs (C60, CeO₂,

$\text{SiO}_2\text{-Ag}$, PVP-Ag) hetero-aggregated with NCs in quiescent natural water samples causing sedimentation (Quik et al., 2014). Lv et al. (2020) explored that NCs have no effect on the sedimentation of CeO_2 NPs in seawater but aggregated with CeO_2 NPs at a high initial CeO_2 NPs' concentration in lake waters.

Chapter 3 Methodology

3.1 General workflow

We designed a series of sedimentation experiments for SiDNAFe MPs and silica colloids in three natural water samples (Meuse, Strijbeek and Merkske) and Milli-Q water. The natural waters were filtered by 0.45 um filter and 1.2 um filter to discover the effect of different size particles in natural water on the sedimentation and aggregation of SiDNAFe MPs and silica colloids. Furthermore, the experimental results are modelled to quantify the sedimentation aggregation of SiDNAFe MPs and silica colloids, and identify the aggregation behaviours. The general workflow includes experiment process and data analysis, as shown in figure 3-1.

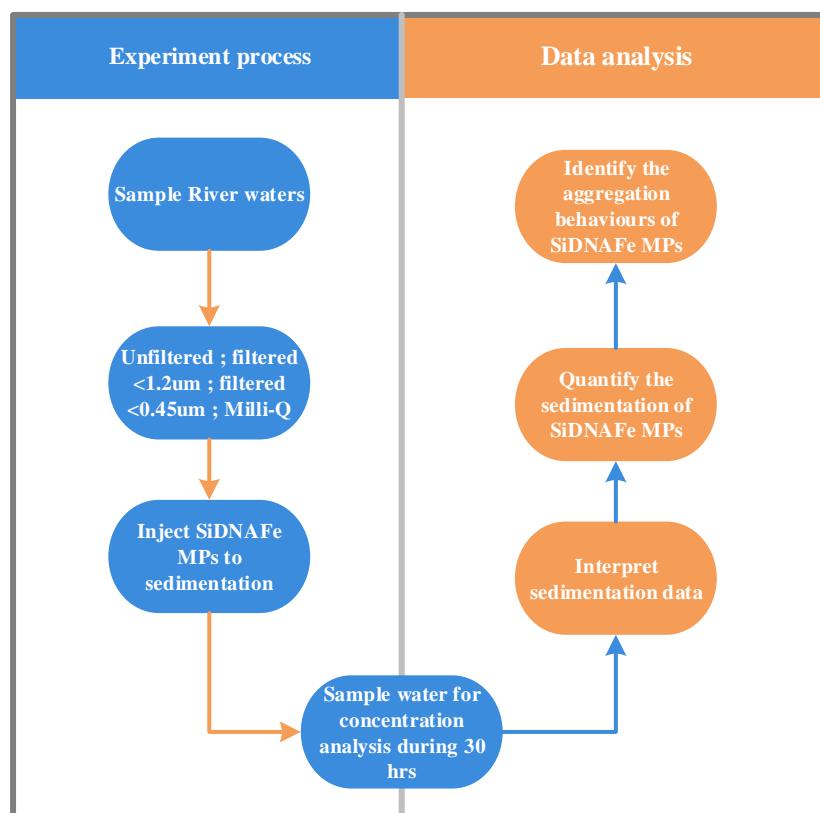


Figure 3-1 A general workflow for the experiment process and data analysis.

3.2 Silica colloids experiments

Due to the limited number of SiDNAFe MPs, the silica colloids were used as a proxy to conduct settling experiments in natural waters to familiarise the whole experimental procedure and compare the aggregation sedimentation behaviours of those two micro-particles.

3.2.1 Preparation of silica colloids suspension

The particle size of SiDNAFe MPs is in the sub-micron scale, so we prepared silica colloids suspension with particle size less than 1000 nm. Thereto, silica powder was purchased from Sigma-Aldrich, Germany (product number: 85356), with a particle size of 0.2 - 0.7mm. We manually milled 15 gram using a mortar and pestle, and then suspended the milled silica in a 1L plastic bottle (height of 206mm) with Milli-Q water. Using Stoke's settling law, we determined the distance of settling of silica particles larger than 1000nm after 24 hours. The top 4.8 cm of water was pipetted from the bottle and used for further experiments.

The zeta potentials of silica colloids in suspension were measured by Zetasizer Nano ZS (Malvern Panalytical, Malvern, United Kingdom). A partner, from the Norwegian University of Science and Technology (NTNU, Trondheim, Norway), measured the particles size distributions (PSD) of silica colloids in Milli-Q water using a LUMisizer (LUM GmbH, Berlin, Germany).

3.2.2 Measurement for the relative concentration of silica suspension

The relative concentration of the silica suspension were determined by measuring the optical density (OD) of silica colloids in suspension. We used a Lambda 365 UV/Vis Spectrophotometer (Perkin Elmer, Waltham, Massachusetts, USA) to measure the OD at a wavelength of 420 nm.

To measure concentration of suspended sediments in the experiments we made use of relative OD. The relative concentration (C/C_0) of suspended particles is the current concentration (C) over the initial concentration before settling (C_0). This can be linearly related to the relative OD. Hereto, a series of dilution curves were plotted, and dilution factors used were 2, 5, 10, 20, 50, and 100. For an example, figure 3-2 presented a dilution curve of silica colloids in Milli-Q water. The dilution curves of silica colloids in different natural waters as well as more details were shown in the annex B.

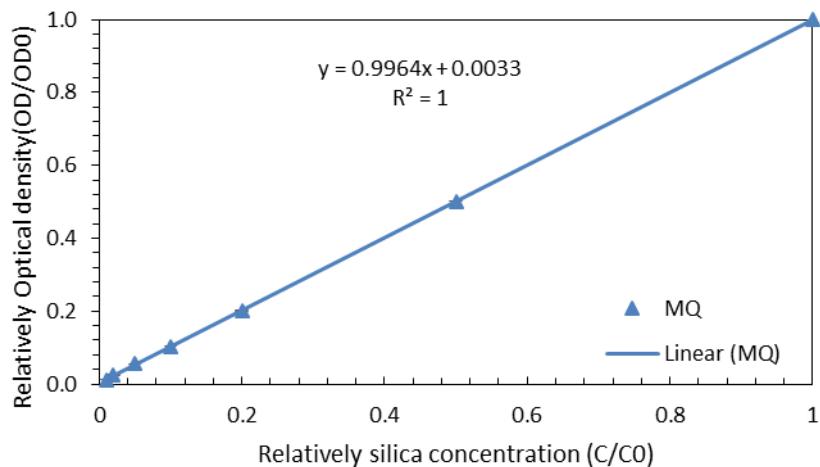


Figure 3-2 A dilution curve between the relative optical density and relative concentration of silica colloids in Milli-Q water.

Based on the dilution curve and UV/Vis spectrophotometer, the relative concentrations of silica colloids in sedimentation experiments could be measured.

3.3 Preparation of SiDNAFe MPs and qPCR analysis

3.3.1 Preparation of SiDNAFe MPs

Engineered DNA tagged silica-iron microparticles (SiDNAFe MPs) containing magnetic cores (iron oxide) are kindly offered by the Norwegian University of Science and Technology (NTNU, Trondheim, Norway), and the synthesis of the particles was described in a paper written by Sharma(2021). The initial concentration of injected SiDNAFe MPs in the natural water was 10^{-5} mg/mL.

3.3.2 qPCR analysis

In order to separate SiDNAFe MPs from a natural water sample, a 1 ml sample from a sedimentation experiment placed in a magnetic rack for magnetic separation for 30 mins. Then, 0.85ml natural water was taken out, and 0.85 ml Milli-Q water was dosed to remove 'noise' particles from natural water. The magnetic separation step repeated to remove the 'noise' particles thoroughly. Before amplified in qPCR, the free DNAs was released from MagSi-DNA MPs by adding a buffered oxide etch solution (BOE) [fluoride buffer, adding 2.3g (NH4) HF + 1.3mg NH4F in 10 ml water](Anuvansh Sharma et al., 2021). The mixed solution was allowed to stand for 10 mins. Finally, the released DNA could be amplified and quantified using qPCR for determining the relative concentration of DNA.

3.4 Preparation of natural water sample

3.4.1 Natural waters

60 L River waters were sampled from each location (i.e. Meuse water, Strijbeek water, and Merkske water) utilising six 10 L plastic bottles (Figure 3-3) and stored at 4 °C in a refrigerator. The shelf life of river water samples is two months.



Figure 3-3 Sampling Meuse water

The Electrical Conductivity (EC), pH and dissolved organic matter (DOC) of original river waters were analysed by 940 Professional Ion chromatography (IC) (Metrohm AG, Herisau, Switzerland), as shown in table 3-1

Table 3-1 DOC, EC, and pH values in three river water samples

Water type	DOC (mg/l)	EC(µS/cm)	pH
Meuse	6.0 ± 0.26	371.3 ± 0.58	7.9 ± 0.01
Merkske	17.5 ± 0.37	543.3 ± 0.58	7.3 ± 0.17
Strijbeek	17.0 ± 0.03	491.3 ± 2.51	7.1 ± 0.08

Note: Measurement was triplicated and data displayed as average ± standard deviation.

3.4.2 Filtration

The presence of suspended solids in natural water is key in hetero-aggregation of NPs (Quik et al., 2012, 2014; Velzeboer et al., 2014). Therefore, we hypothesised that suspended solids aggregated with silica colloids or SiDNAFe MPs in natural water.

For exploring the effect of the particle size of suspended solids on silica colloids and SiDNAFe MP settling, the natural water was filtered sequentially by 1.2 µm glass fibre filter and 0.45 µm cellulose filter to obtain three types of natural water, in terms of 0.45 µm filtered natural water, the 1.2 µm filtered and the unfiltered. The first type of water contains particles less than 0.45 µm. The second contains particles less than 1.2 µm, and the last is the original natural water.

The types of natural waters included 0.45 µm filtered Merkske river water sample (F0.45MK), 1.2 µm filtered Merkske river water sample (F1.2MK), unfiltered Merkske river water sample (UFMK), 0.45 µm filtered Meuse river water sample (F0.45Mu), 1.2 µm filtered Meuse river water sample (F1.2Mu), unfiltered Meuse river water sample (UFMu), 0.45 µm filtered Strijbeek river water sample (F0.45Strij), 1.2 µm filtered Strijbeek river water sample (F1.2Strij) and unfiltered Strijbeek river water sample (UFStrij).

3.5 Details for sedimentation experiments

We allowed SiDNAFe MPs to quiescently settle in various filtered and unfiltered Milli-Q, Meuse, Strijbeek and Merkske water in 15ml tubes during 30 hours at 4 C° in a refrigerator. The low temperature was to inhibit growth of microorganisms (figure 3-4). To determine if the refrigerator is at a constant temperature, real-time temperature in the refrigerator were measured by a Van Essen diver (Van Essen, Delft, Netherland), and the diver measured temperature every half hour. Moreover, in order to minimise vibration during the experiments, 85.1kg of iron dishes were placed on the fridge's top.



Figure 3-4 Field photos of the experimental setup.

Before conducting a quiescently settling experiment, each combination solution (natural water sample and introduced SiDNAFe MPs) was aliquot to seven subsamples, as shown in figure 3-6. The water samples of 1mL used for measuring SiDNAFe MPs' relative concentration were taken respectively from those seven subsamples by pipette at 5.66 mm below the water surface after 0, 2, 4, 6, 22, 24 and 30 hrs. The separately sampling operation is to eliminate the disturbing effect of the immersion of a pipet tip.

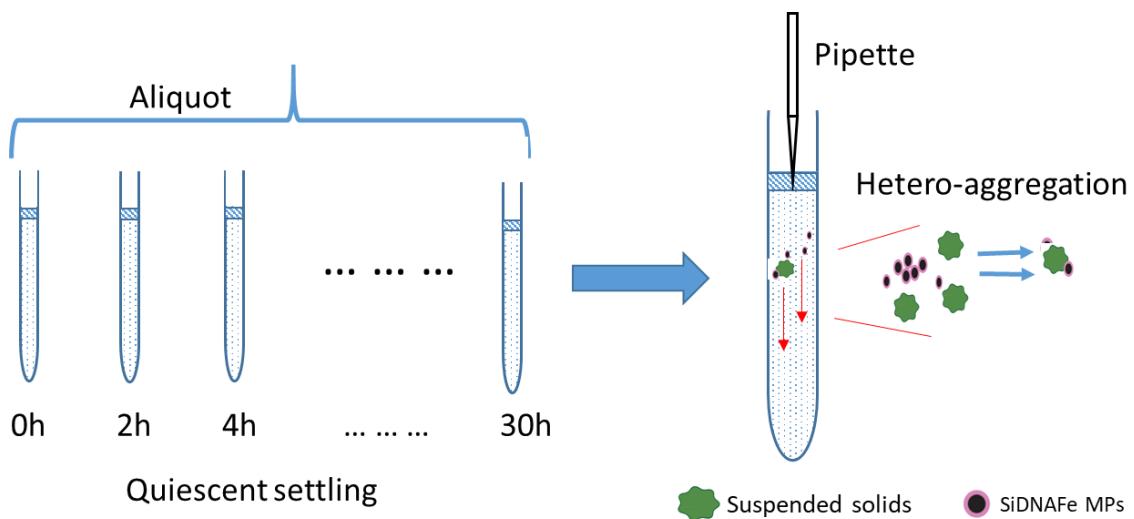


Figure 3-5 Cartoon of a quiescent sedimentation experiment.

The right pattern is SiDNAFe MPs quiescent settling with simultaneously hetero-aggregation in natural water matrix, and the left pattern is the aliquot process for a combination solution with the natural water sample and introduced SiDNAFe MPs.

The sedimentation experiment in each type of natural water was duplicated, and Data displayed as average.

3.6 Modelling type I sedimentation

In type I suspension, every particle settles independently in a suspension without collusion and flocculation, and the particles' concentration only depends on the settling velocities (Dey et al., 2019). Following Benjamin and Lawler (2013), we use a fundamental partial differential equation (Stokes' law) to describe the decrease of spherical particles for each size class at z depth in type I suspension:

$$\frac{\partial n_i}{\partial t} = -v \frac{\partial n_i}{\partial z} \quad (3-1)$$

,where n_i [−] is the number of particles of size class i .

However, the concentrations of silica colloids and SiDNAFe MPs were measured from water samples I sampled, called 'sampled water layer', in sedimentation experiments, and the concentration in a sampled water layer with a certain thickness should be simulated based on the solution of equation (3-1). For this situation, the solution of equation 3-1 in a sampled water layer was Heaviside function times the initial particle number (n [−]).

For initial and boundary condition:

$N = 0$ where no particle in the sampled water layer at $t=0$

$N = n$ where n number of particles in the sampled water layer at $t = 0$

$N = \text{constant}$ at boundary at all $t > 0$

The process is complex. Let us first modelled a given size spherical particle at z_1 [mm] depth settling through the sampled water layer with a certain thickness (h [mm]) at z_2 [mm] depth ($z_2 > z_1$), as shown in figure 3-6.

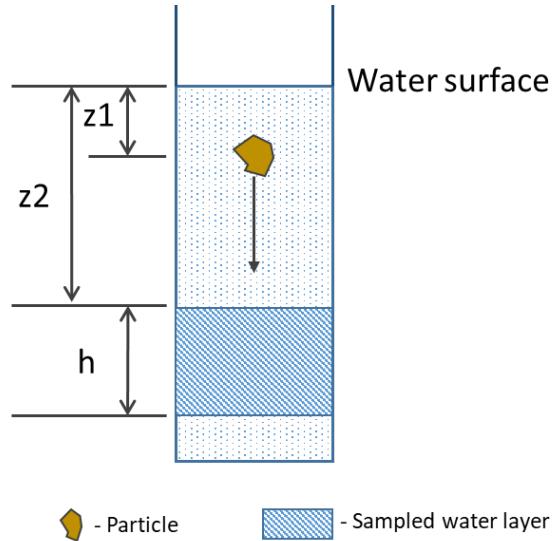


Figure 3-6 A given size particle at z_1 [mm] depth settling through the sampled water layer with a certain thickness (h [mm]) at z_2 [mm] depth ($z_2 > z_1$)

The water sample located in z_1 depth means the up boundary is at z_2 depth and the bottom boundary is at $z_2 + h$ depth. We get:

$$N(v, t) = \begin{cases} 0, & \text{for } z_2 - z_1 \geq vt \\ 1, & \text{for } z_2 - z_1 \leq vt \leq z_2 - z_1 + h \\ 0, & \text{for } z_2 - z_1 + h < vt \end{cases} \quad (3-2)$$

The settling velocity (v [mm/h]) was calculated using Eq.2-4, and it depends on the densities of location particle and medium (Rubey, 1933).

We assumed the distribution of given size particles from z_1 depth to $z_2 + h$ depth was homogenous. The depths of the particles placed (between z_1 depth and $z_2 + h$ depth) were discrete. The averaged depth (\bar{z}_i) and the number (n_i) of the particles in the finite element i at a certain depth could be calculated. Then, the particle number in the sampled water layer was given by number (n_i) times equation (3-2):

$$N_{v,t} = \sum_{\text{all } i} n_i N_i|_{z_2 - \bar{z}_i \leq vt \leq z_2 - \bar{z}_i + h} \quad (3-3)$$

Final, the spherical particles with a continuous particle size distribution (PSD) placed in between z_1 depth and $z_2 + h$ depth could be simulated by finite element model, and the final particle number ($TN[-]$) in the sampled water layer was got:

$$TN_t = \sum_{\text{all } j} N_{v,j,t} \quad (3-4)$$

, where $N_{v,j,t}$ [-] - the particle number of particle for size class j in the sampled water layer.

We assumed that the concentration of a microscale particle in suspension is the same as other microscale particles. Based on this assumption, the relative concentration (C/C_0) - the current concentration (C_t) divided by initial concentration (C_0) - can be replaced by the current particles' number (TN_t [-]) divided by the total number of initial particles (TN_0 [-]) equivalently. Thus, a combination equation (equation 3-3 and 3-4) calculated the relative concentration of the particles in the sampled water layer:

$$\frac{C_t}{C_0} = \frac{TN_t}{TN_0} = \frac{\sum_{\text{all } j} N_{v,j,t}}{TN_0} = \frac{\sum_{\text{all } i} n_i N_i|_{z_2 - \bar{z}_i \leq vt \leq z_2 - \bar{z}_i + h}}{TN_0} \quad (3-5)$$

The equation 3-5 can simulate the sedimentation of SiDNA MPs and silica colloids in type I suspension. At any time (t), the sampled water layer would contain particles with settling distance (vt) greater than $z_2 - z_1$ and lesser than $z_2 - z_1 + h$ at their original concentrations. It is vital that the equation (3-1) is valid for particles settling independently in type I suspension, which do not change in shape and weight. This model fits the observation data to derive the particle size distribution and the settling velocity distribution, and we will simulate it utilising Python, as shown in annex C.

I used root-mean-square error (RMSE) and the coefficient of determination (R^2) as objective functions to indicate the goodness of a fitting curve for observation data. RMSE is a measure of the difference between the observation and simulation data. Thus, the smaller the MSE, the smaller the difference between the observed and simulated values. R^2 is the proportion of the variance in simulation data that is predictable from the observation.

Chapter 4 Results

4.1 Settling and aggregation of silica colloids in Milli-Q and natural waters

4.1.1 Type I sedimentation in Milli-Q water

To highlight and contrast the settling behaviour of silica colloids in river water samples, I set up a control group of silica colloids settling in Milli-Q water (Figure 4-1). The figure presents the relative concentrations of silica colloids settling in Milli-Q water sample after 0, 2, 4, 5.3, 9.7, 24.3 and 29.3 hours. The relative concentration of silica colloids dropped directly from the start to reach a minimum of 0.19 at 24.3 hours, and the experiment reached a final relative concentration of around 0.25 of 29.3hrs. Besides, the relative concentration of silica colloids settling after 9.73 hours is slightly higher than the value after 4 hours. The outlier after 9.73 hrs is likely related to a human sampling error.

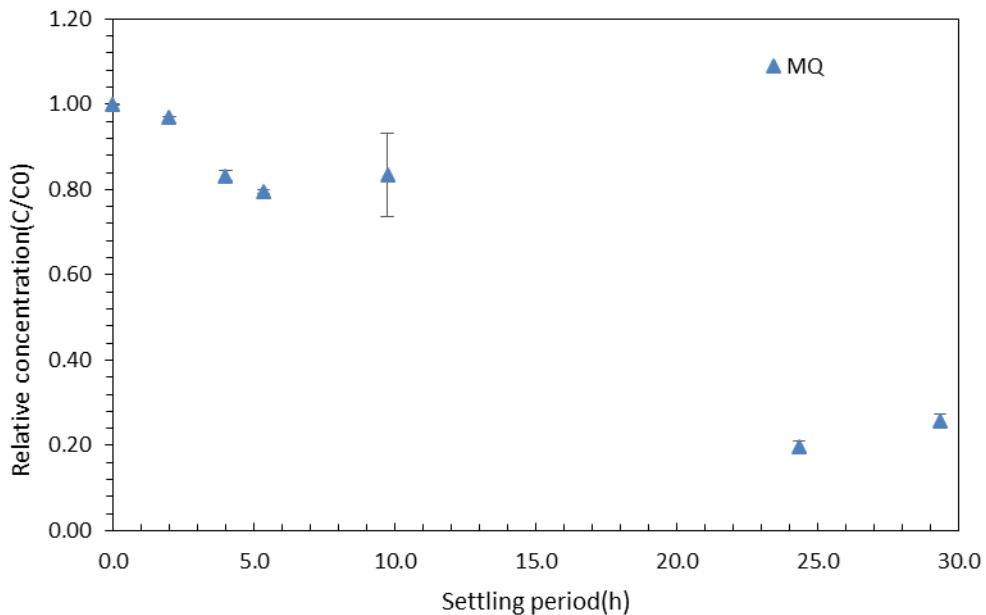


Figure 4-1 The relative concentrations of silica colloids settling in Milli-Q water sample after 0, 2, 4, 5.3, 9.7, 24.3 and 29.3 hours.

The averaged zeta potential of silica colloids in Milli-Q water is -40.2 mV (Appendix B-1). For sub-micron particles, a high negatively zeta potential confer stability, and the dispersion resists aggregation. In other words, silica colloids did not homo-aggregate in Milli-Q water during settling, known as type I sedimentation.

4.1.1 Particle size distribution (PSD) of silica colloids in Milli-Q water

To estimate PSD Of silica colloids in Milli-Q water, the sedimentation model fitted the relative concentrations of silica colloids settling in Milli-Q water sample during 30 hours. The thickness

of sampled water layer is 8.42 mm and the sampling location is at 5.5 mm depth below the water surface.

The PSD of silica colloids could be first considered as essentially a continuous function for fitting observed sedimentation data. (Benjamin & Lawler, 2013). The function we chose was a normal distribution function. The PSD varied with the two parameters (mean and standard deviation) of the normal distribution function, and the range of the PSD was 100 nm to 1000 nm. The simulated relative concentrations of silica colloids in Milli-Q water during 30 hours changed owing to different PSD (Appendix C-7). The increase of mean value leads to a rapid decrease in relative concentration in the fitting curve's initial part, comparing plots on the vertical columns in Appendix C-7. It means that particle settling accelerates as particle size increases. However, the value at the tail of the fitting curve reduces to zero with the decrease of standard deviation, which represents the more concentrated the particle size distribution, the faster the reduction in the relative concentration.

Thereto, the observed silica colloids sedimentation in Milli-Q water was best modelled with a normal distribution of particles between 100 and 1000nm, a mean of 1.5, a standard deviation of 4 and a skewness factor of -0.855 (Figure 4-2). Note that the observation at hour 9.73 was considered an outlier.

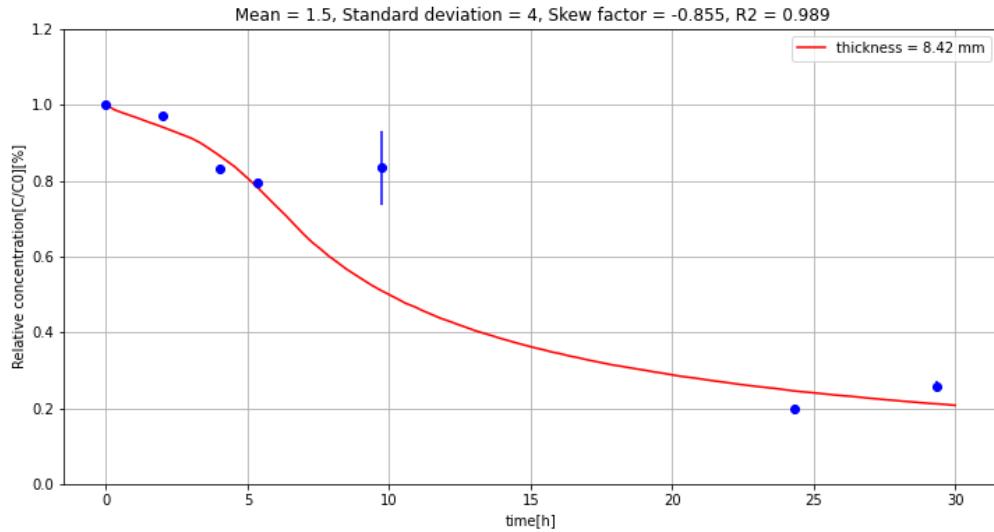


Figure 4-2 the best fitting curve for the relative concentration of silica colloids during settling in Milli-Q water

Figure 4-3 presents the scatter plots of the observed and beset fitted data and its fitting curve with a slope of 1.0029, an intercept of 0.0012 and R square of 0.989. The closer the slope and R^2 approach 1 and the intercept approach 0, the better the sedimentation model fits the observed data.

The skew factor represents the asymmetry of silica colloids' PSD. If the skew factor is between -1 and -0.5 or between 0.5 and 1, the distribution is moderately skewed. If the skew factor is between -0.5 and 0.5, the distribution is approximately symmetric. Therefore, the PSD of silica colloids in Milli-Q water is moderately skewed (Figure 4-4).

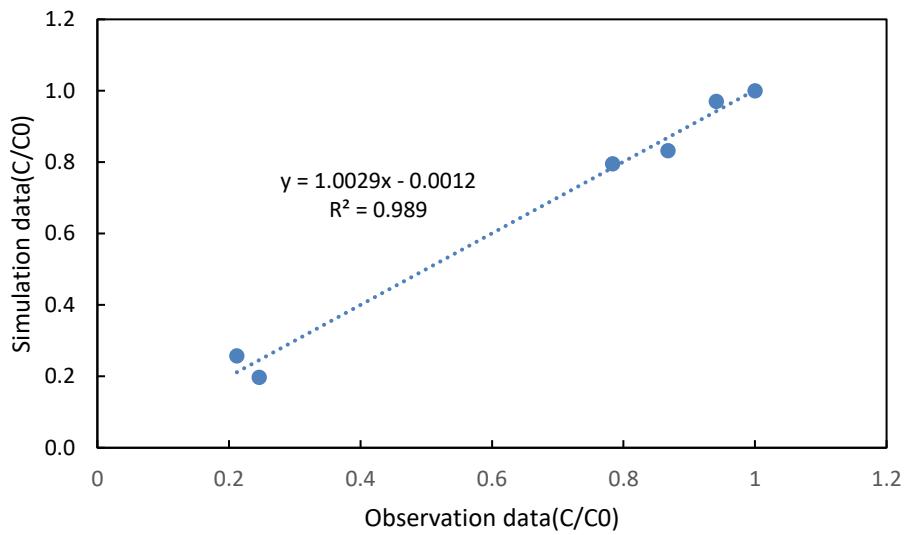


Figure 4-3 Scatter plot of the observed relative concentrations and the modelled data based on best fitted PSD of silica colloids

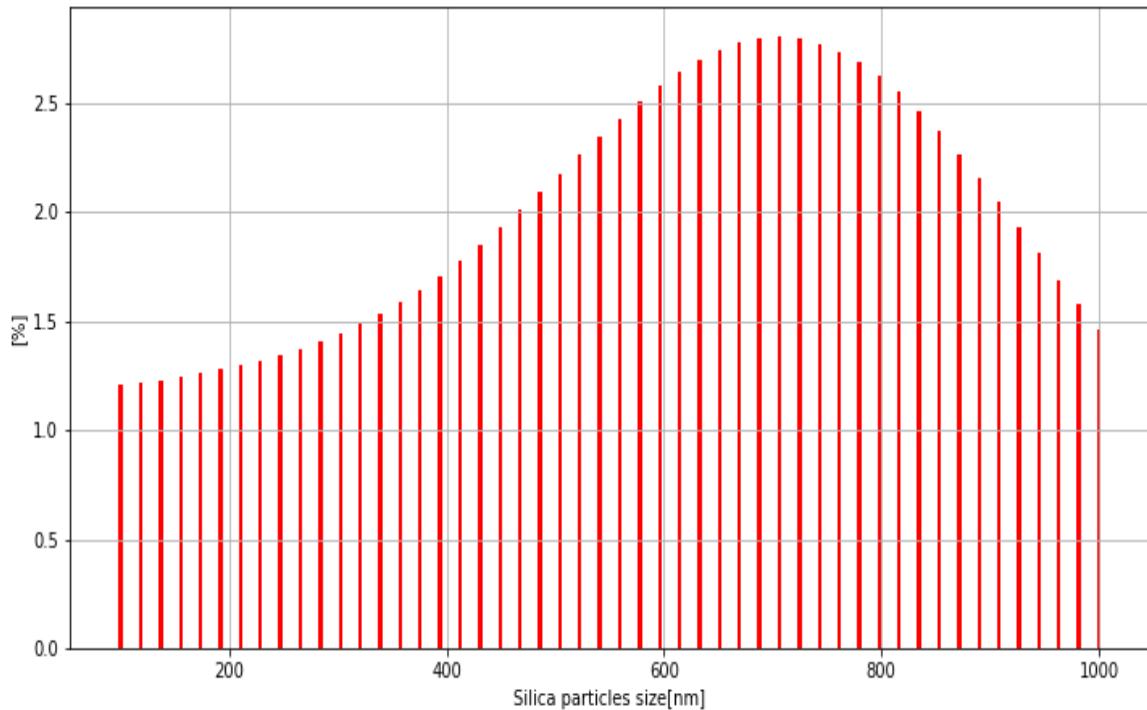


Figure 4-4 Simulated PSD of silica colloids in Milli-Q water by normal distribution function with mean of 1.5 and standard deviation of 4

The parameters in the sedimentation model for the best fitting curve included a mean of 1.5, a standard deviation of 4, skew factor of -0.855, RMSE of 0.033, R squared of 0.989, sampling location of 5.5 mm and column thickness of 8.42 mm, as shown in table 4-1.

Table 4-1 The parameters in the sedimentation model for the best fitting curve of the relative concentration of silica colloids settling in Milli-Q water

Parameters	Values
Mean	1.5 [-]
Standard deviation	4 [-]
Skew factor	-0.855 [-]
RMSE	0.033 [-]
R squared	0.989 [-]
Sampling location	5.5mm
Column thickness	8.42mm

Independently, the PSD of silica colloids in Milli-Q water was measured using a LUMisizer at NTNU. Figure 4-5 shows the volume-weighted cumulative distribution of the silica colloids (blue line and blue dots). The red line is the number cumulative distribution of simulated particle size data of silica colloids in Milli-Q water. Observed values from 300nm to 500nm and >900nm are slightly smaller than simulated values, and the rest of the fit is good.

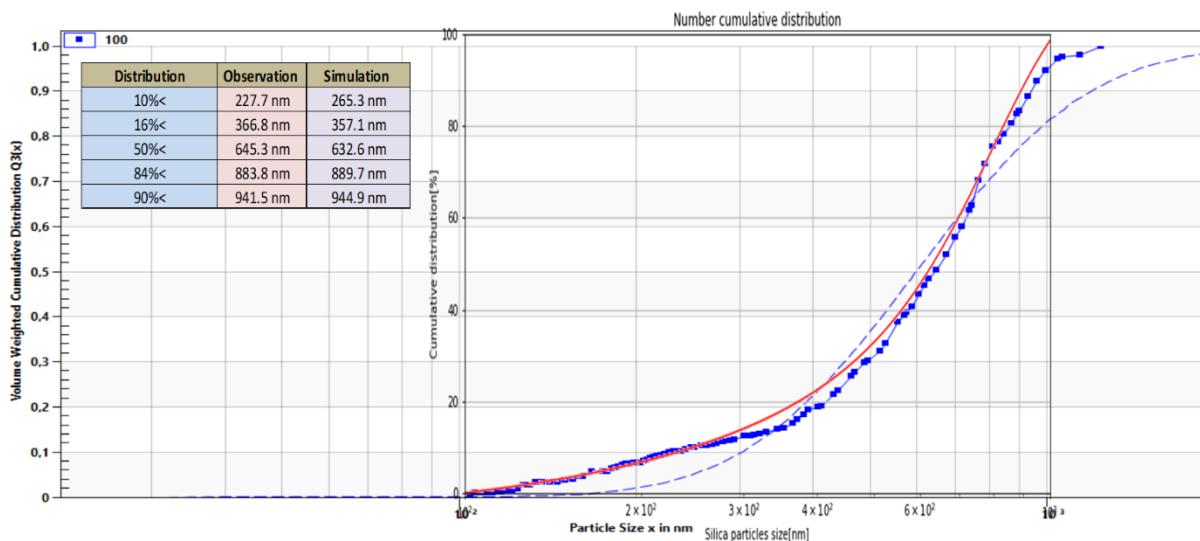


Figure 4-5 Volume weighted Cumulative distribution of silica colloids (blue dots) measured by a LUMisizer and its fitting curve (red line) simulated in Python

I compared the maximum particle sizes of modelled data and the observations in the different percentages of the cumulative distribution. The percentages of the distribution were given from the LUMisizer report with 10%, 16%, 50%, 84%, and 90%, respectively. The differences between the simulated and observed values is a maximum of 37.6 nm and a minimum of 3.4 nm, and they are not large compared with particle size.

All in all, my simulated PSD was generally consistent with the distribution of the actual silica colloids in the Milli-Q water based on double-checking the LUMisizer results and observed sedimentation data.

4.1.2 Sedimentation and aggregation of silica colloids in natural waters

To investigate silica colloids' settling behaviours in natural water we conducted a series of silica colloids settling experiments in natural waters. As described in the method section (see 3.4.2), we are interested to study the effect of particulates we prepared 3 version of the natural water; unfiltered, 1.2 mu filtered, 0.45mu filtered.

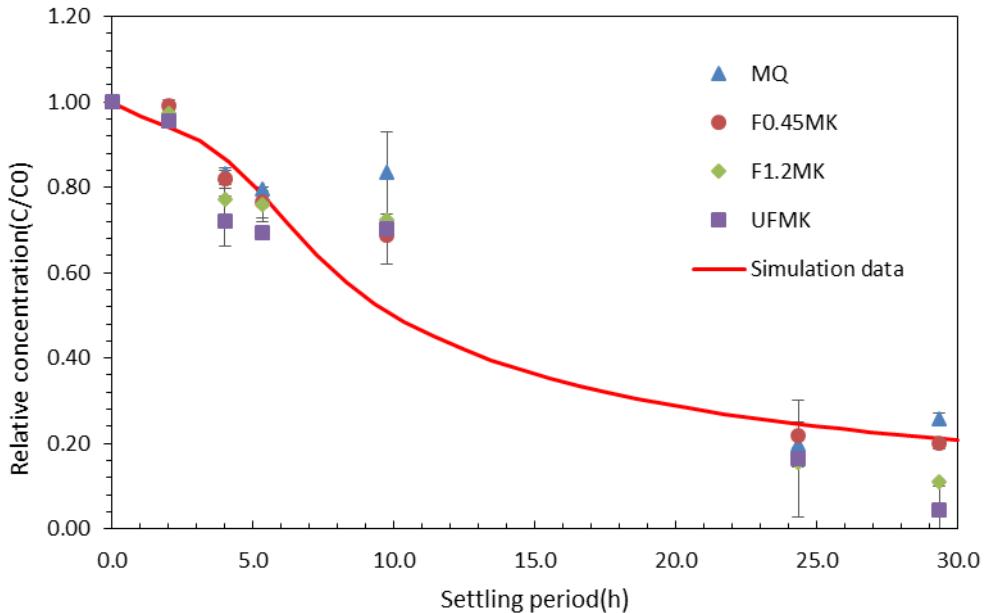


Figure 4-6 Silica particles settling experiment of results in Merkske water (a) F0.45MK (red dots); (b) F1.2MK (green solids); (c) UFMK (purple square), and the observed (blue triangle) the simulated (red line) relative concentrations of silica colloids in Milli-Q water.

Figure 4-6 presents silica colloids' sedimentation in F0.45MK, F1.2MK and UFMK and the observed (blue triangle) the simulated (red line) relative concentrations of silica colloids in Milli-Q water. Note that the observations at hour 9.73 were considered outliers. The observed data from F0.45MK are distributed on both sides of the simulation curve with low deviations except the outlier, and the relative concentration of silica colloids decline from 1 at 0 hours to 0.2 at 29.3 hours. As for observed data in F1.2MK, there is a decrease in the relative concentration from 1 at 0 hours to 0.11 at 29.3 hours. The two values in the tail deviate obviously from the simulated values shown in (b) plot of figure 6. The relative concentration at 29.3 hours from UFMK is much lower than the simulated data.

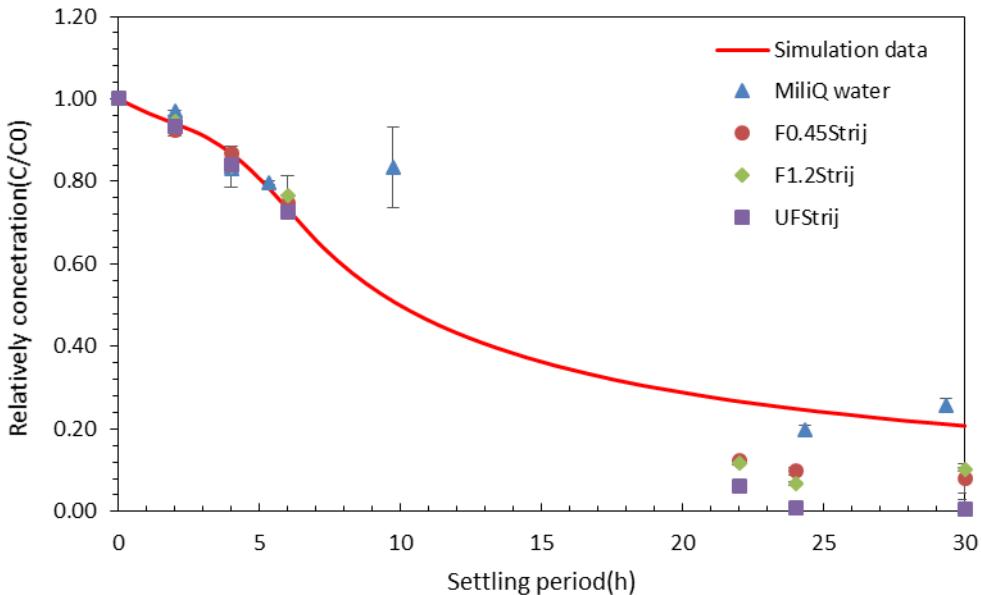


Figure 4-7 Silica particles settling experiment of results in Meuse river water (a)F0.45Mu(red dots); (b)F1.2Mu(green solids); (c)UFMu (purple square) and the observed (blue triangle) the simulated (red line) relative concentrations of silica colloids in Milli-Q water.

The sedimentation of silica colloids during 30 hours in F0.45Mu, F1.2Mu and UFMu were shown in figure 4-7. The (a) plot in the figure presents that the relative concentrations of silica colloids in F0.45Mu are higher than the simulation data in Milli-Q water, except the initial value of 1. The observed data from F1.2Mu (green solids) are distributed on both sides of the fitting curve (red line) with low deviations. They decrease from 1 at 0 hours to 0.21 at 30 hours, as shown in (b) plot of figure 4-7. Moreover, after 4 hours, the relative concentrations from UFMu are lower than the simulated data, and the difference between the observed data (purple square) and the modelled (red line) increase over time.

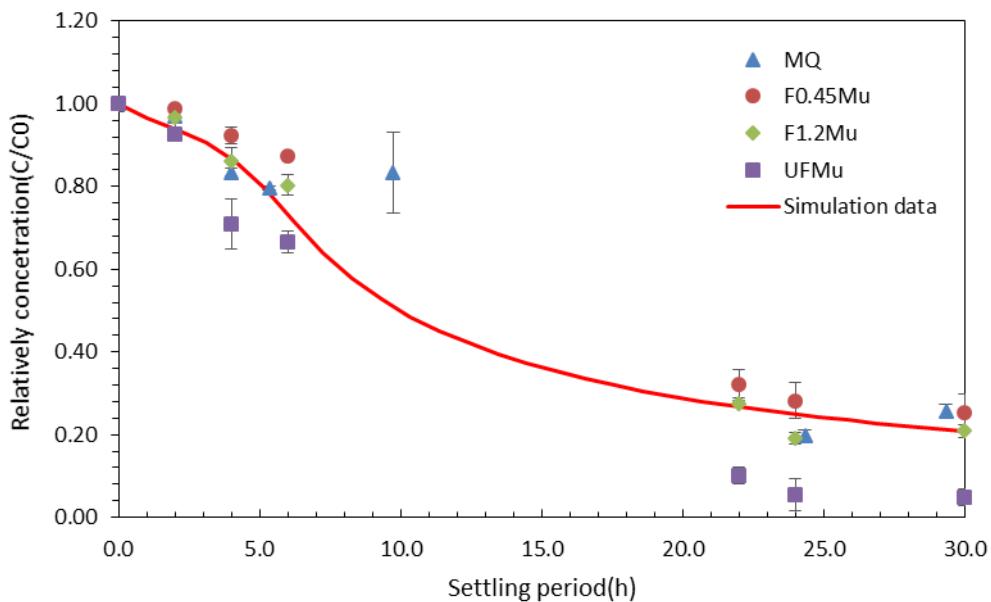


Figure 4-8 Silica particles settling experiment of results in Strijbeek river water (a)F0.45Strij(red dots); (b)F1.2Strij(green solids); (c)UFStrij(purple square), and the observed (blue triangle) the simulated (red line) relative concentrations of silica colloids in Milli-Q water.

The relative concentrations of colloidal silica particles over time in Strijbeek river water with 3 filtered types were shown in Figure 4-8. At the period of the initial 6 hours, observed data from F0.45Strij, F1.2Strij and UFStrij were distributed on the red line, as shown in the (d) plot of Figure 4-8. The red line is the simulated relative concentrations of silica colloids in Milli-Q water. Then, observed data are lower than the simulated after 22 hours. The overall trend of observed data is a decrease in all three types of Strijbeek water.

To determine whether the settling of silica colloids in natural waters behaves as type I sedimentation, we checked the statistical difference between the observed results of silica colloids settling in natural waters and the simulated data in Milli-Q water (red line in figure), and used paired two-sample t-tests. The t-test statistic follows a student's t distribution under the null hypothesis, and the significant level (α) defaults as a 0.05. The null hypothesis is that the observed data and the simulated are not significantly different from each other. The alternative hypothesis is that the observed data and the simulated are significantly different.

The p-values derived by comparing the simulated data from Milli-Q water with observed data from different types of natural waters, as shown in table 4-2.

Since the p-values of F0.45MK and F1.2Mu exceed 0.05, and the null hypothesis is accepted. The observed data are not significantly different from simulated data. It represents that the sedimentations of silica colloids in F0.45MK and F1.2Mu follow type I sedimentation.

On the contrary, the p-values of other natural waters are lower than 0.05, and the null hypothesis is rejected. There is a significant statistical difference between the observed data and the simulated. Therefore, silica colloids settling do not follow Type I sedimentation in other natural waters except F0.45MK and F1.2Mu.

Table 4-2 P-values derived by comparing the simulated relative concentration of silica colloids in Milli-Q water with observed data in natural waters with different filtered types.

Water type	P-values
F0.45MK	0.272
F1.2MK	0.0498
UFMK	0.024
F0.45Mu	0.008
F1.2Mu	0.357
UFMu	0.006
F0.45Strij	0.040
F1.2Strij	0.049
UFStrij	0.028

We got the observed values in natural waters that are significantly different from Milli-Q water's simulated values. Figure 4-9 presents the differences between simulated values in Milli-Q with the observed relative concentrations in F1.2MK, UFMK, F0.45Mu, UFMu, F0.45Strij, F1.2Strij and UFStrij, called Residuals.

In F0.45Mu, the differences are negative, which means the observed relative concentration values are higher than simulated values, as shown in Figure 4-9. However, aggregation's role leads to accelerated settling of particles in suspension(Benjamin & Lawler, 2013). Therefore, the sedimentations of silica colloids in F0.45Mu are not affected by aggregation.

In other natural waters, most differences are positive, and the slopes of the linear trend lines of the differences are positive. However, the values of R^2 of the linear trend lines from

differences in F1.2MK, UFMK and UFMK are lower than 0.8, and the upward trend of the differences over time does not quite follow a linear trend. Since the values of R^2 of the linear trend lines exceed 0.8, the upward trend of the differences in F0.45Strij, F1.2Strij and UFStrij over time follow a linear trend.

In any case, the aggregation of silica colloids in these water samples during the settling occurred. And silica colloids bound to particulate matter ($>0.45\text{um}$) present in Merkske river water, and in Meuse water, the silica colloids aggregated with the suspended solids ($>1.2 \text{ um}$). Moreover, the silica colloids aggregated with the matter present in Strijbeek river water.

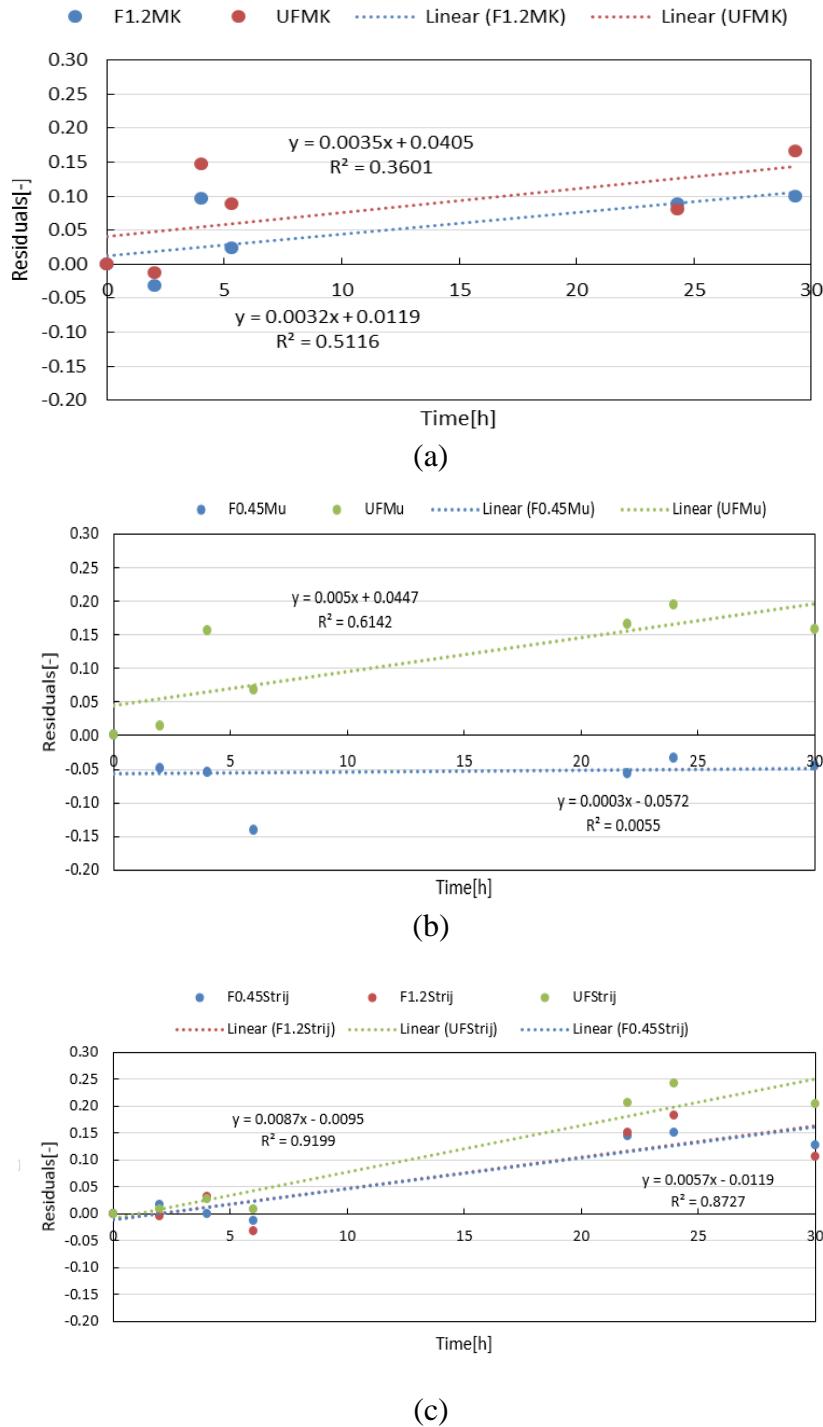


Figure 4-9 The differences between simulated values in Milli-Q with the observed relative concentrations in different natural waters (a)F1.2MK (red dots) and UFMK(blue dots);(b) F0.45Mu(blue dots) and UFMu(green dots); (c) F0.45Strij(blue dots), F1.2Strij(red dots) and UFStrij(green dots).

4.2 SiDNAFe MPs settling in river water samples

4.2.1 SiDNAFe settling in Milli-Q water and its PSD

Similarly, SiDNAFe MPs first quiescent settled in Milli-Q water as a control group. The relative concentrations were measured from water samples of 1 ml (sampled water layer). The samples were taken at 5.66 mm depth below the water surface in a 15 ml plastic narrow neck tube. The thickness of a sampled water layer is 5.66 mm. The zeta potential of SiDNAFe MPs in Milli-Q water is -23.6 mV, and it indicates SiDNAFe settling without any aggregation in Milli-Q water.

The results are presented in figure 4-10, and the overall trend is a gradual decrease over time. The relative concentration first drops from 1 to 0.48 after 2 hours settling and then slightly increases to 0.57 at 4 hours. After 22 hours, the relative concentration reaches the bottom with 0.23. The experiment reached a final relative concentration of around 0.2 of 30 hours.

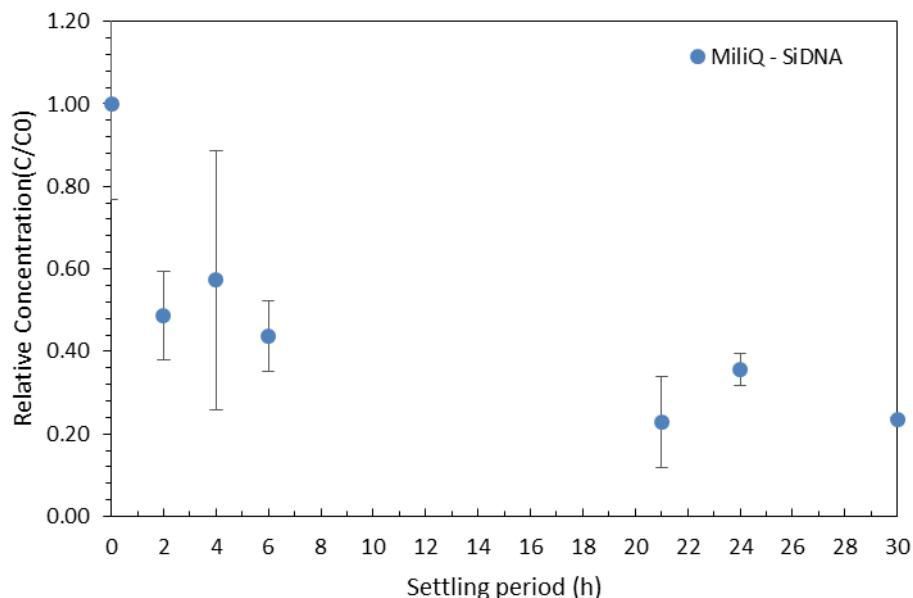


Figure 4-10 SiDNAFe MPs settling experiment of results in in Milli-Q water

For obtaining the particle size distribution of SiDNAFe MPs, observed data (figure 4-10) were simulated using the sedimentation model. However, based on the continuous function - normal distribution function, the best fitting curve for the observed data visually fitted poorly with low R square value and high RMSE value. Thus, I used the superimposed normal distribution function, and the function was given by adding two normal distributions together. It has four parameters (two mean values and two standard deviation values), and the parameters can be changed individually.

Figure 4-11 presents the observed (blue dots) and simulated (red line) relative concentrations of SiDNAFe MPs settling in Milli-Q water during 30 hours base on the optimum solution of superimposed normal distribution function. The observations are distributed on both sides of the red line, except the values at 2 and 24 hours which have large deviations

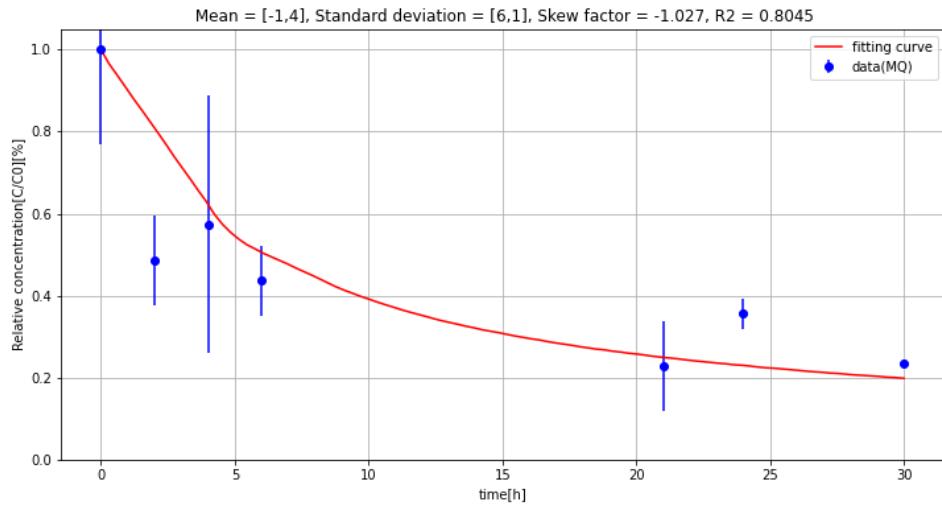


Figure 4-11 The observed (blue dots) and simulated (red line) relative concentrations of SiDNAFe MPs settling in Milli-Q water during 30 hours based on the optimum solution of superimposed normal distribution function.

The objective parameters for the optimal solution of superimposed normal distribution function include R^2 of 0.7781 and RMSE of 0.136. The slope of the trend line and R^2 derived from the scatter plot of observed data and the simulations, with 0.8795 and 0.7781, respectively (Figure 4-12). The intercept of the trend line was set to 0 and the slope was larger than 0.8. Thus, the simulate data is fit well with the observed data.

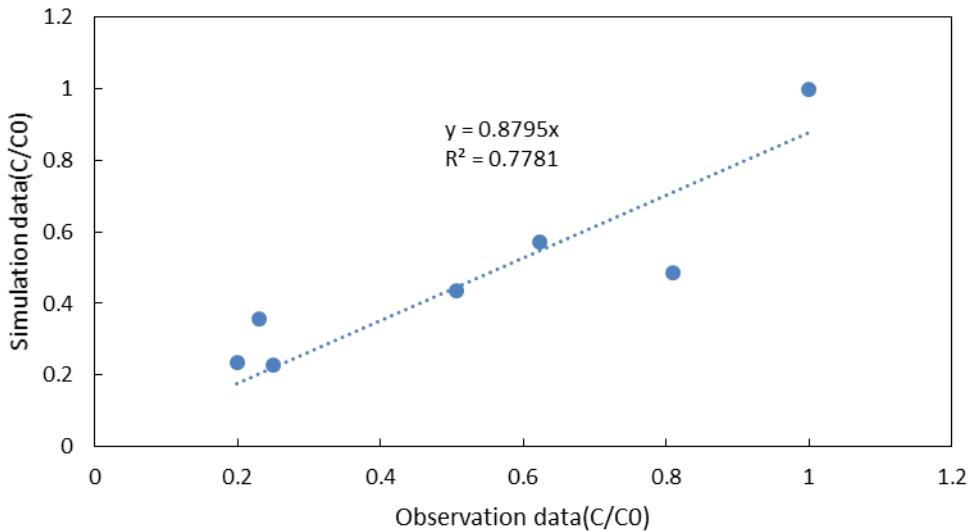


Figure 4-12 Scatter plot of the observed relative concentrations and the modelled data based on best fitted PSD of SiDNAFe MPs

All parameters in the sedimentation model for the best fitting curve were shown in table 4-3. The Skew factor with 1.78 represents the PSD of SiDNAFe MPs is right-skewed. Figure 4-13 shows the modelled PSD of SiDNAFe MPS in Milli-Q water from the sedimentation model's

optimal solution. The particle sizes are mainly concentrated between 800 and 1000 nm. The ratios of particle number for the other particle sizes are small, with 1%

Table 4-3 The parameters in sedimentation model for the best fitting curve of the relative concentration of SiDNAFe MPs settling in Milli-Q water

Parameters	Values
Mean	-1, 4 [-]
Standard deviation	6, 1 [-]
Skew factor	1.78 [-]
RMSE	0.136 [-]
R squared	0.778 [-]
Sampling location	5.66mm
Column thickness	5.66mm

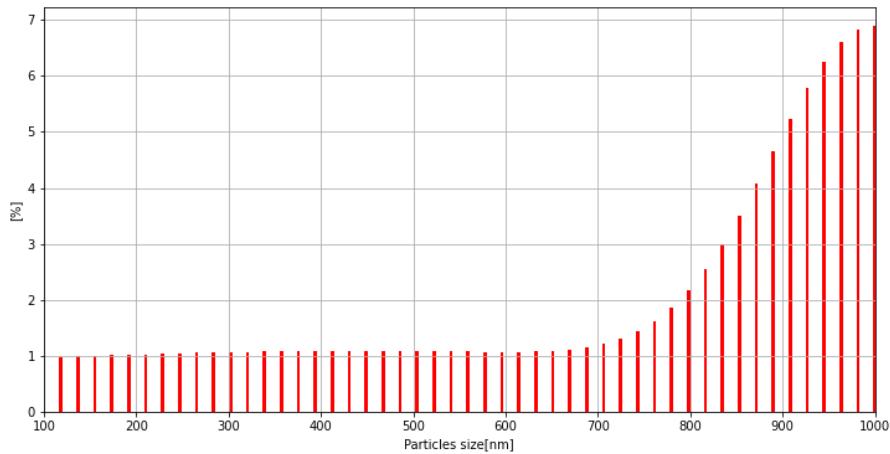


Figure 4-13 Particle size distribution of SiDNAFe MPs from the optimal solution of the sedimentation model

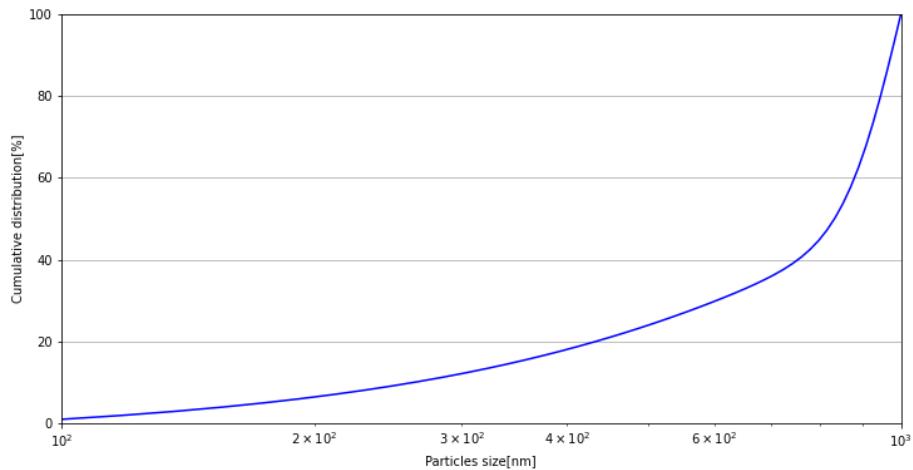


Figure 4-14 Number cumulative distribution of the simulated data

The curve of number cumulative distribution for the simulated data rises gently at first and then dramatically accelerates after 800nm, as shown in figure 4-14.

Therefore, through a series of simulations, we got the PSD of SiDNAFe MPs and simulated SiDNAFe MPs settling in Milli-Q water for 30hrs.

4.2.2 SiDNAFe MPs settling in natural waters

To investigate sedimentation behaviour of SiDNAFe MPs in natural water, we conducted the sedimentation of SiDNAFe MPs in different types of natural waters. Figure 4-15 presents the change of relative concentration of SiDNAFe MPs over time in Merkske water of three filtered types (F0.45MK, F1.2MK and UFMK) and the simulation data in Milli-Q water (red line). The overall trend in relative concentrations of SiDNAFe MPs over time is a reduction. During the initial 6 hour period, the observed values in UFMK were significantly smaller than the simulated values. After 21 hours of settling, the observed values in F0.45MK and F1.2 water were significantly higher than the simulated values.

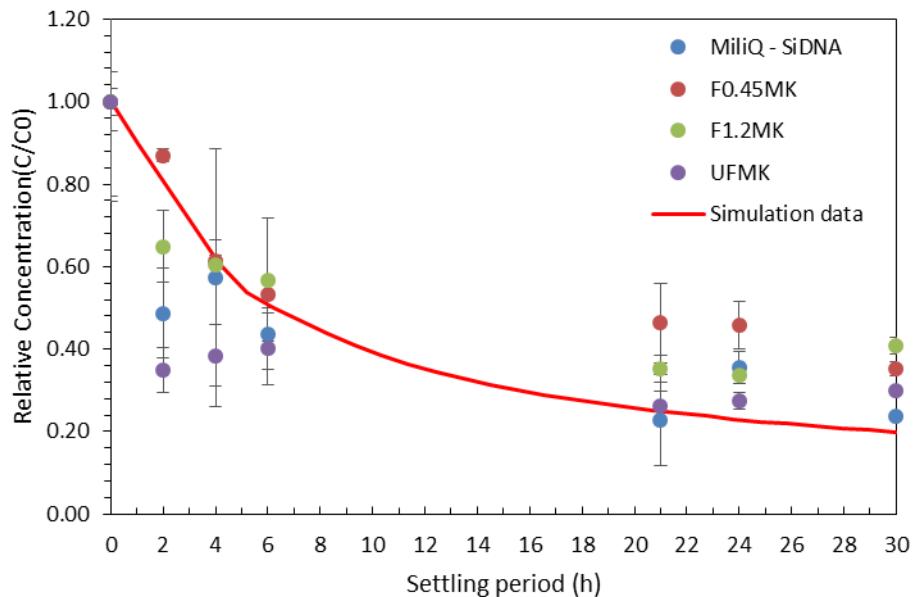


Figure 4-15 SiDNAFe MPs settling experiment of results in Merkske water (a)F0.45MK (red dots); (b)F1.2MK (green dots); (c)UFMK (purple dots), and the observed (blue dots) the simulated (red line) relative concentrations of SiDNAFe MPs in Milli-Q water.

SiDNAFe MPs' sedimentations in Meuse water with three filtered types (F0.45Mu, F1.2Mu and UFMu) were shown in Figure 4-16. The relative concentrations of SiDNAFe MPs decrease in all three Meuse water. The observed data in F0.45Mu and F1.2Mu are distributed around the simulation curve with low deviations. All observations obtained in F1.2Mu are higher than the simulated data except for the initial value.

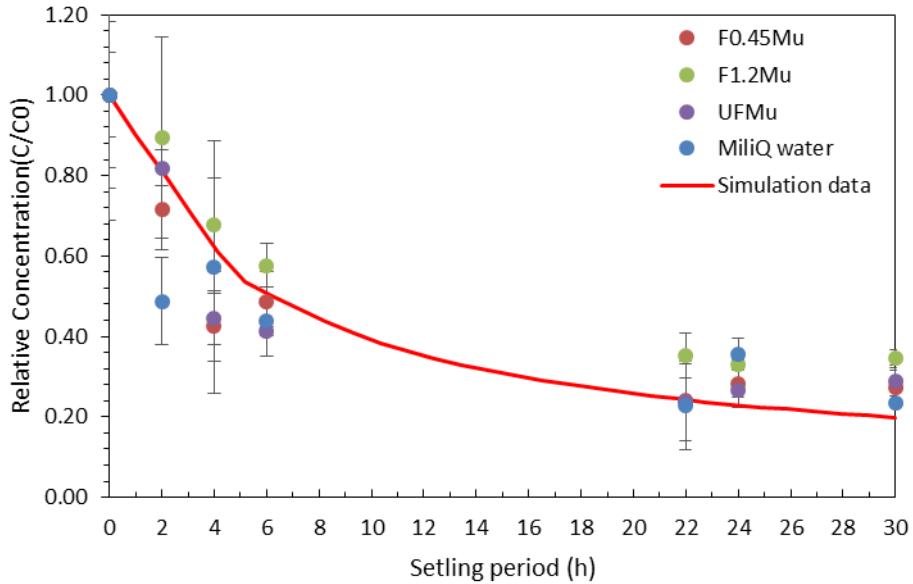


Figure 4-16 SiDNAFe MPs settling experiment of results in Meuse water (a)F0.45Mu (red dots); (b)F1.2Mu (green dots); (c)UFMu (purple dots), and the observed (blue dots) the simulated (red line) relative concentrations of SiDNAFe MPs in Milli-Q water.

The relative concentrations of SiDNAFe MPs in Strijbeek river waters with three filtered types (F0.45Strij, F1.2Strij and UFStrij) during 30 hours were presented in Figure 4-17. The overall trend of the relative concentrations is a slight decrease. However, the observed values in UFStrij in water first rises to 1.05 at 2 hours, then falls to 0.32 at 30 hours. The values are higher than the simulation value in Milli-Q water. Moreover, the observed data in F0.45Strij and F1.2Strij water are closer to simulated data, except the value in F0.45strij at 2 hours which is much lower than simulated data.

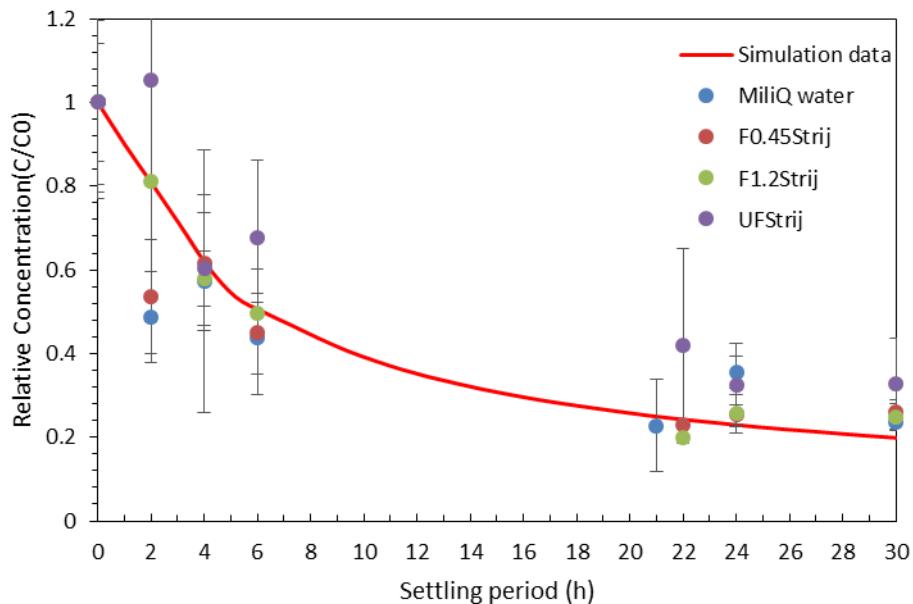


Figure 4-17 SiDNAFe MPs settling experiment of results in Strijbeek water (a)F0.45Strij (red dots); (b)F1.2Strij (green dots); (c)UFStrij (purple dots), and the observed (blue dots) the simulated (red line) relative concentrations of SiDNAFe MPs in Milli-Q water.

To determine whether the settling of SiDNAFe in natural waters behaves as type I sedimentation, we checked the statistical difference between the observed results of SiDNAFe MPs settling in natural waters and the simulated data in Milli-Q water (red line in figure), and used paired two-sample t-tests. The t-test statistic follows a student's t distribution under the null hypothesis, and the significant level (α) defaults as a 0.05. The null hypothesis is that the observed data and the simulated are not significantly different from each other. The alternative hypothesis is that the observed data and the simulated are significantly different.

The p-values derived by comparing the simulated data in Milli-Q water with observed data from natural waters with different filtered types, as shown in table 4-4.

Since the p-values of F0.45MK, F1.2Mu and UFStrij are lower than 0.05, the null hypothesis is rejected. There is a significant statistical difference between the observed data and the simulated. Therefore, SiDNAFe MPs settling in F0.45MK, F1.2Mu and UFStrij do not follow type I sedimentation.

On the contrary, the p-values of other natural waters are larger than 0.05, and the null hypothesis is accepted. The observed data are significantly different from simulated data. It represents that the sedimentations of SiDNAFe MPs in other natural waters follow type I sedimentation.

Table 4-4 P-values derived by comparing the simulated relative concentration of SiDNAFe MPs in Milli-Q water with observed data in natural waters with different filtered types

Water type	P-values
F0.45MK	0.02
F1.2MK	0.18
UFMK	0.13
F0.45Mu	0.24
F1.2Mu	0.004
UFMu	0.28
F0.45Strij	0.2
F1.2Strij	0.43
UFStrij	0.01

It is clear from the hypothesis test that the observed values in UFStrij, F1.2Mu and F0.45MK are significantly different from the simulated values in Milli-Q water. Figure 4-18 shows the difference between the observed and simulated values, called Residuals. Most of the residuals are negative except for the values in UFStrij and F0.45MK at 4 hours. It means the observed values are large than simulated values. However, aggregation's role leads to accelerated settling of particles in suspension(Benjamin & Lawler, 2013). Therefore, the sedimentations of SiDNAFe in UFStrij, F1.2Mu and F0.45MK are not affected by aggregation.

After significance tests and residual analysis, we conclude that the major mass losses of SiDNAFe in natural waters were due to type I sedimentation, and aggregation did not play a role.

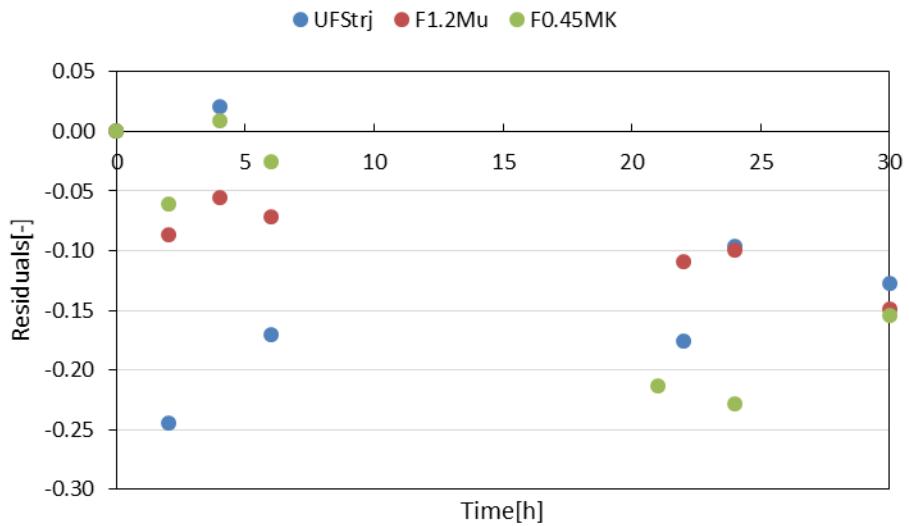


Figure 4-18 The differences between simulated values in Milli-Q with the observed relative concentrations in different natural waters (F1.2Mu (red dots), UFStrij (blue dots) and F0.45MK (green dots)).

Chapter 5 Conclusion

We aim to investigate the sedimentation behaviours of SiDNAFe MPs and silica colloids (as the proxy particle) in natural waters and the effect of the suspended solids present in natural water on the settling behaviour of silica colloids and SiDNAFe MP.

I designed a series of sedimentation experiments with SiDNAFe MPs and silica colloids (as the proxy particle) in three types of river water (Merkske, Meuse and Strijbeek). Each type of river water was divided into 3 versions of river water samples (unfiltered, 1.2 mu filtered, 0.45mu filtered). The sedimentation experiments with SiDNAFe MPs or silica colloids in Milli-Q water were experimental control groups. The sedimentation periods is 30 hours.

The sedimentation of colloidal silica and SiDNAFe in Milli-Q water were quantified by the sedimentation model. This is the simulation of a Type I sedimentation behaviour. Statistical significance tests on the observed values in natural waters and the simulated data in Milli-Q were done.

The sedimentation experiments' results are that that the sedimentations of SiDNAFe MPs and silica colloids in Milli-Q water were single particle settling, in which settling particles have no interaction, the so-called type I sedimentation. The observed settling behaviour of SiDNAFe MPs in three types of natural river water was similar to Milli-Q water behaviour. However, the observed settling behaviours of silica colloids are different from Milli-Q water behaviours, except the behaviours in F0.45MK, F0.45Mu and F1.2Mu.

The results from the simulation and significance tests are that the there is no significant difference between the simulated sedimentation of SiDNAFe MPs in Milli-Q water and the observed in three types of natural waters, except the difference in F0.45Mk, F1.2Mu and UFStrij. However, the residuals' values in those three water samples are negative, and the aggregation of SiDNAFe MPs in those three water samples did not occur. As for silica colloids, there is a significant difference between the simulated sedimentation of silica colloids in Milli-Q water and the observed in three types of natural waters, except the difference in F0.45MK and F1.2Mu. The values of residuals in other water samples are positive, except the residuals in F0.45Mu.

From the experiments and analysis, the most important conclusions were that the mass loss of SiDNAFe in natural water was due to type I sedimentation, and the settling of SiDNAFe MPs have no interaction in natural water. On the contrary, the mass loss of silica colloids in natural water was due to sedimentation and aggregation.

Compared to the behaviours of silica colloids and SiDNAFe MPs in natural water, silica colloids and SiDNAFe MPs settled in natural water. However, the silica colloids aggregated with suspended solids during settling, but SiDNAFe MPs did not.

In 3 versions of river water (unfiltered, 1.2 mu filtered, 0.45mu filtered), silica colloids' aggregation behaviours in three types of river water were different. Silica colloids bound to particulate matter (>0.45um) present in Merkske river water, and in Meuse water, the silica colloids aggregated with the suspended solids (>1.2 um). Moreover, the silica colloids

aggregated with the matter present in Strijbeek river water. Consequently, the aggregation of silica colloids is not related to suspended solids' particle size in natural water.

Overall, the sedimentation behaviour of SiDNAFe MPs in natural water is only mono-dispersed settling. And this behaviour is quite different from the sedimentation behaviours of other NPs which are type II sedimentation (Kobayashi et al., 2005; Quik et al., 2014; Velzeboer et al., 2014). Moreover, the hetero-aggregation of the silica colloids with suspended solids in natural water occurred, and the behaviour has been also mentioned in literatures(Abe et al., 2011; Okamoto et al., 1957). Abe et.al (2011) indicated that natural organic matter (part of suspended solids) aggregates with silica colloids in water.

In our experiments, we only observed changes in the relative concentration of SiDNAFe MPs in three river water samples, and the sedimentation occurred in an environment of a low-temperature dependence, anti-vibration, and high photolytic stability. Thus, there are limitations to the applicability of our conclusions.

Our results showed that, based on a dilution curve of silica colloids, the relative concentration relates linearly to the OD (light absorbance) of sampled water layer at a certain wavelength by UV/vis spectrophotometer (Appendix B-2 to B-10). However, in natural waters, there are some suspended substances present. Light absorbance of a water sample will be the result of the presence of both silica colloids and natural suspended particles. Therefore, the light absorbance of natural pollutant impacts the slope of the dilution curve and the relative concentration of silica colloids. However, the light absorbance of particles at different wavelengths of the spectrophotometer are different. We should find a certain wavelengths to make the silica colloids' light absorbance is much larger than the values of suspended substances to reduce the influence of the suspended substances for experimental results. The slope of a dilution curve closet to 1 is doing that the best and it means that suspended substances are less influence.

The qPCR analysis detected the DNAs in SiDNAFe to obtain the quantification cycle (Cq.). According to a dilution curve of SiDNAFe, the values of Cq. relates linearly to the logarithm value of the concentrations (Appendix B-11 to B-113). However, the relative concentrations of SiDNAFe MPs present in linear scale and the logarithm of the concentration should be transformed to the linear. The error between the linear-scale concentrations in duplicate water samples are logarithmically amplified because of the transformation. This is the reason that the high variance of each relative concentration of SiDNAFe MPs in natural waters (Figure 4-15 to 4-17).

From my sedimentation model, it is clear that the PSD can simulate particle settling in the water. However, this leads to that we cannot quantify the sedimentation of particles. It is vital to find a model that can numerically characterise the sedimentation of particles.

Significance tests demonstrated that silica colloids aggregated in different natural water types, but the difference between observed and simulated values could not be well modelled linearly.

In future experiments, we will likely investigate the settlement behaviour of SiDNAFe MPs in natural water under vibrational conditions. Because SiDNAFe MPs act as a tracer in the tracing experiment and are constantly moving with the water. The main idea is to shake the experimental setup on a shake table and measure the change in concentration over 30 hours.

Secondly, the aggregation of silica colloids in natural water is related to matter present in natural water but not significantly to the matter's size. Therefore, we need to investigate what matters hetero-aggregate with silica colloids in natural water. I think that the rate of aggregation of silica colloids is related to the composition of the particles and each component's concentration.

Thirdly, the relative concentrations of SiDNAFe MPs in F0.45MK, F1.2Mu and UFStrij and silica colloids in F1.2Mu increased compared to the simulated values. I have not yet found a reasonable explanation for this.

Based on our experiments, we have demonstrated that the mass loss of SiDNAFe MPs in river water is mainly due to sedimentation, and therefore the sedimentation should be taken into account when calculating the mass recovery of SiDNAFe MPs in a tracing experiment.

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Appendices

Appendix A. - Research Ethics Declaration Form



United Nations
Educational, Scientific and
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IHE
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Institute for
Water Education
under the auspices
of UNESCO

Research Ethics Committee
IHE Delft Institute for
Water Education
E ResearchEthicsCommittee@un-ihe.org

Date: 10 March 2021
To: Zhang Fengbo
MSc Programme: WSE-HWR
Approval Number: IHE-RECO 2020-081

Subject: Research Ethics approval

Dear Zhang Fengbo,

Based on your application for Ethical Approval, the Research Ethics Committee (RECO), IHE Delft RECO has been approved ethical clearance for your research proposal - Understanding Hetero-aggregation of Silica-encapsulated-DNA-based Microparticles with Natural Organic Matter in surface water injection experiments.

This approval valid until April 16, 2021. You need to notify the RECO of any modifications to your research protocol. If you do not complete your research by the specified date, you should contact RECO to request an extension.

Please keep this letter for your records and include a copy in the final version of MSc. Thesis, together with your personal reflection. Additional information is available at <https://ecampusxl.un-ihe.org/course/view.php?id=1555§ion=2>.

On behalf of the Research Ethics Committee, I wish you success in the completion of your research.

Yours sincerely,

Dr. Angeles Mendoza Sammet
Acting Ethics Coordinator

Copy to: Archive.

Personal Declaration

The research based on five principles of honesty, scrupulousness, transparency and independence. And the personal declaration disclosed in next five parts: (1) Design research, (2) Conduct Research, (3) Report Results, (4) Assessment & Peer Review (5) Communication of Research.

(1) Design Research

In my thesis, I did not took ideas or methods from other authors. The SiDNAFe MPs was design by Dr. Foppen and Sharma from NTNU. The SiDNAFe MPs had never be tested the sedimentation behaviour before. Therefore, it is impossible took ideas from other authors. I explained the methods with enough detail to all other person do the same and validate my results. For example, I presented the formulas and python codes of sedimentation model. The name, brand, manufacture, and origin of devices for analysis data were indicated in paper. And the details of the sedimentation experiments were introduced in the methodology (chapter 3). I did not bias in the design of research. The design of research is based on the scientific logic. The funding of research were received from the water Tagging project, TUD. My research designed to be objective and neutral. It did not influence by non-scientific factors or interests. My research designed to benefit society. The SiDNAFe tracer is a new tracer that greatly improves the efficiency of tracer tracing water flow. The study of the sedimentation behaviour of this tracer will greatly accelerate its application in field experiment.

(2) Conduct Research

I tested alternative hypothesis and considered uncertainty. The null and alternative hypotheses are that 'SiDNAFe settling behaviours in natural water and it aggregates with natural organic matter' and 'SiDNAFe settling behaviours in natural water and it aggregates with suspended solids'. The alternative hypothesis was accepted. For reducing the uncertainty, all the sedimentation experiments were duplicate. The research followed established protocols and methods. The silica suspension with particles smaller than 1000 nm were prepared following the manual milled silica particles and silica settling protocol. The sedimentation model created in Jupyter Notebook followed the formulas in methodology (see 3.6 in thesis). I reported errors or uncertainty that could influence my results and reported any changes in methods. The OD measurement and qPCR analysis influence on the experimental results. They were reported in discussion part. I had not conduct experiment before this experiment, and I have less knowledge in particle settling. Therefore, the research update my knowledge and expertise. I considered people and the environment when conducting my research, and the one of advantage of SiDNAFe tracer has a lower environmental than the traditional tracer (e.g. salt tracer and fluorescence dye).

(3) Report Results

I considered contradictory results or information, and discusses its implications, not manipulating data. The 'bad' results were also presented. For instance, I reported the outliers in the silica colloids settling experiment in Merkske water and Milli-Q water. And the outliers are likely related to a human sampling error. I explained methods and analyses with enough detail to allow other person repeat what I did and validate my results. The method I validate data was statistical hypothesis testing and the distribution is student's t-distribution. The more details I explained in paper. I detailed how I acquired and used data. The data were measured by myself from the sedimentation experiments. The other people can acquired the data by following the protocol of sedimentation experiment provided in methodology. I declare that I have no relevant or material financial interests that relate to the research described in this paper. Because, before

this research, I had not worked in this knowledge area. My research did not potentially affect to the legitimate interest of people supporting my research. My study focused only on the sedimentation of SiDNAFe MPs in surface water. The only thing that will be affected is whether SiDNAFe MPs can be traced in surface water.

(4) Assessment & Peer Review

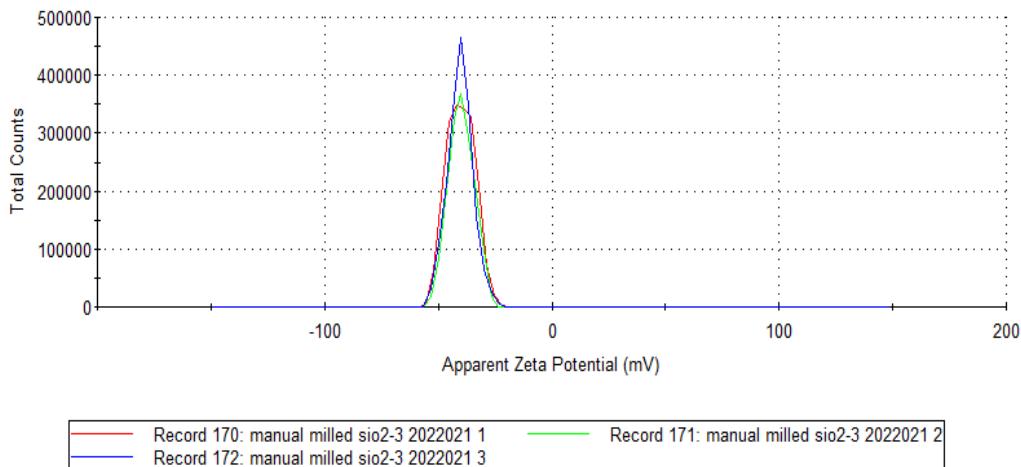
I submitted the real data for peer review. The real data includes the results of sedimentation experiments of silica colloids in Milli-Q water, Merkske river water, Meuse river water and Strijbeek river water. Also, the results of sedimentation experiments of SiDNAFe MPs in the in Milli-Q water, Merkske river water, Meuse river water and Strijbeek river water were submitted. The dilution curves for transforming from the original data to the relative concentration of SiDNAFe MPs and silica colloids were presented in annex B. The review works within my area of expertise. The external reviewer is Dr. ir. C. (Claire) Chassagen. She is interested in linking the large-scale properties of clay suspensions and sediment beds, as monitored in-situ and measured in the laboratory, to their micro-scale properties, and in particular to the clay surface charge. In 2006 she became an assistant professor at NTNU and since 2008 she is an assistant professor at Department of Environmental Fluid Mechanics of the Delft University of Technology (TU Delft). I gave information for reviews to verify sources of data. I gave credit to reviewers if I used their feedback in my report. The reviewers acted neutrally and professionally. I declare that they have no relevant or material financial interests that relate to the research described in this paper.

(5) Communication of Research

I avoided discussing or making conclusions without supporting evidence. Each conclusion is based on my results. I paid attention to write my report based on my standards for academic writing, using good English. I disclosed my assumptions. One is in natural water, a significant amount of SiDNAFe MPs mass is removed due to hetero-aggregation as a result of the formation of aggregates of SiDNAFe MPs and naturally occurring suspended solids and natural colloids. Another is that there is mass removal, but not necessarily due to aggregation. I reported results accurately and I did not omit relevant results to please someone. The outliers in sedimentation experiment of silica colloids in Milli-Q water and Merkske water were still plotted. And I mentioned the reason of the outliers. I paid attention to write my report in a way that lay people or information can understand the results and what they mean for them.

Appendix B. - The dilution curves in natural waters and the zeta potential of silica colloids in Milli-Q water

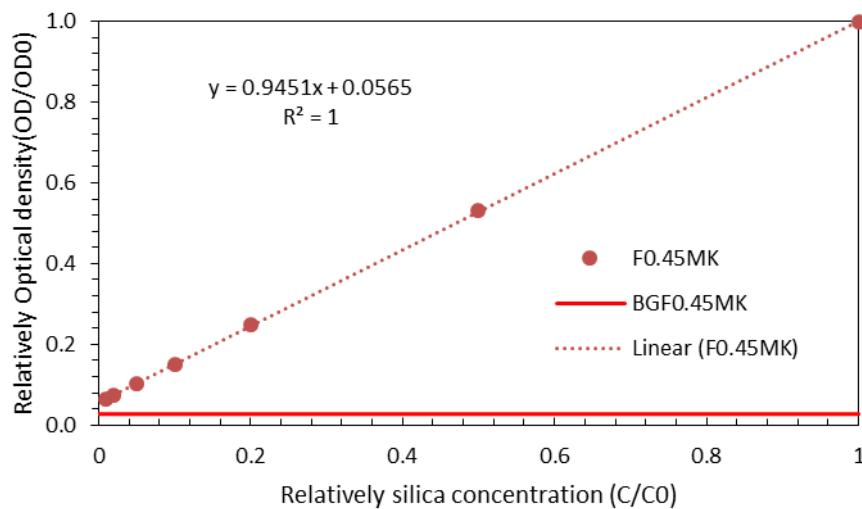
1. The zeta potential of silica colloids in Milli-Q water



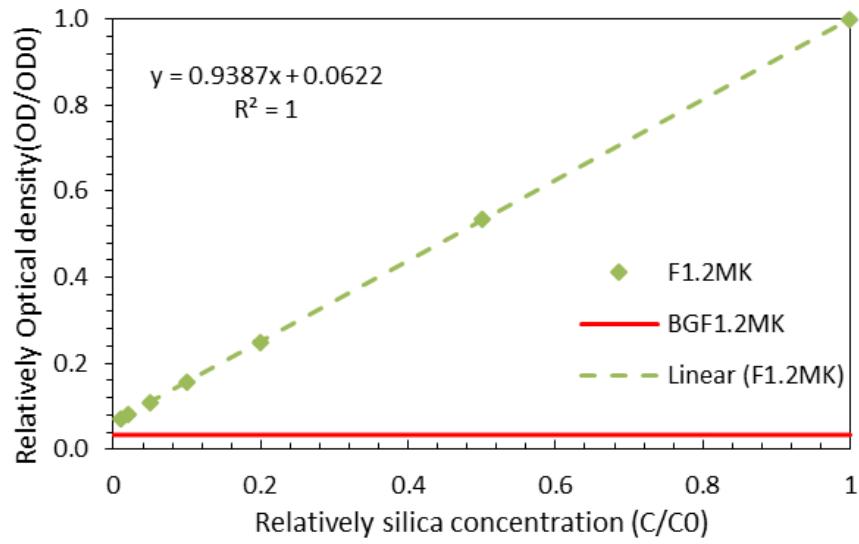
Appendix B-1 The apparent zeta potential of silica colloids in Milli-Q water

Appendix B-1 represents that the averaged zeta potential of silica colloids in Milli-Q water is -40.2 mV.

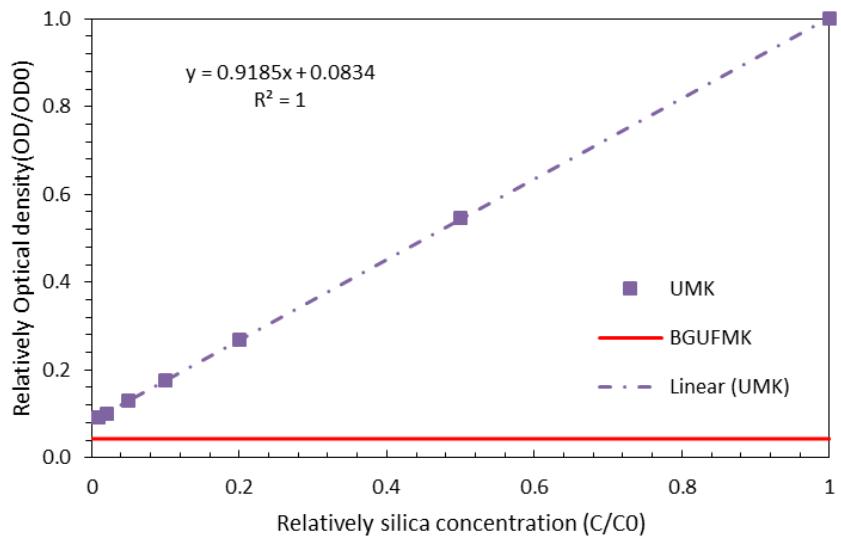
2. Dilution curves of silica colloids in Milli-Q water and natural waters



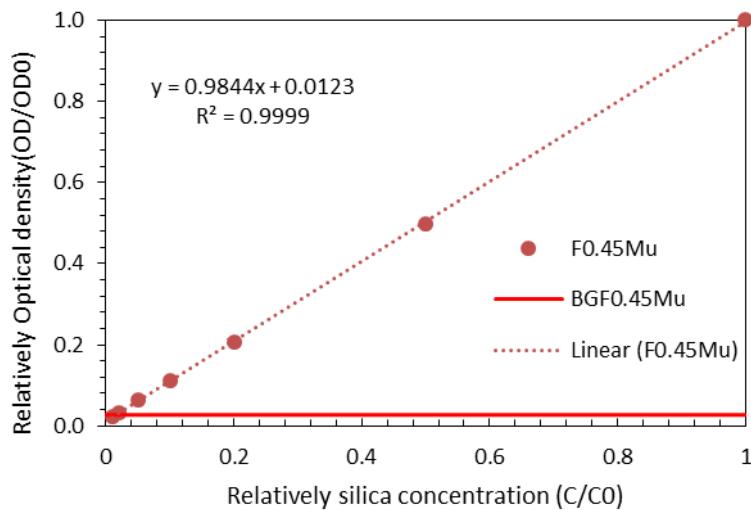
Appendix B-2 The dilution curve of silica colloids in F0.45MK.



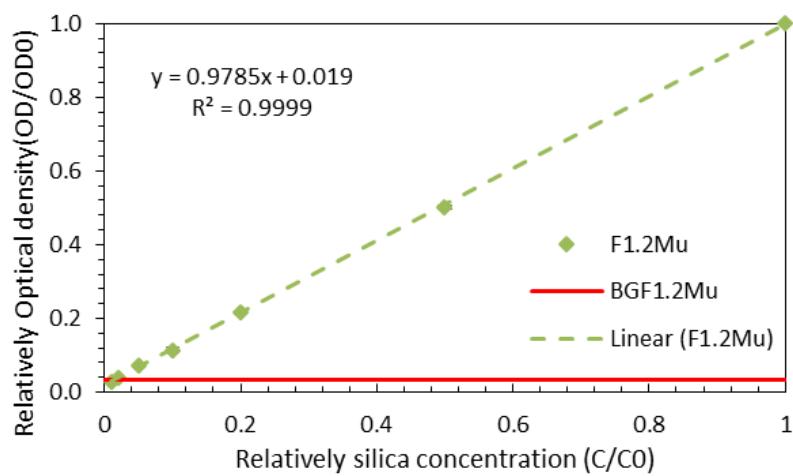
Appendix B-3 The dilution curve of silica colloids in F1.2MK.



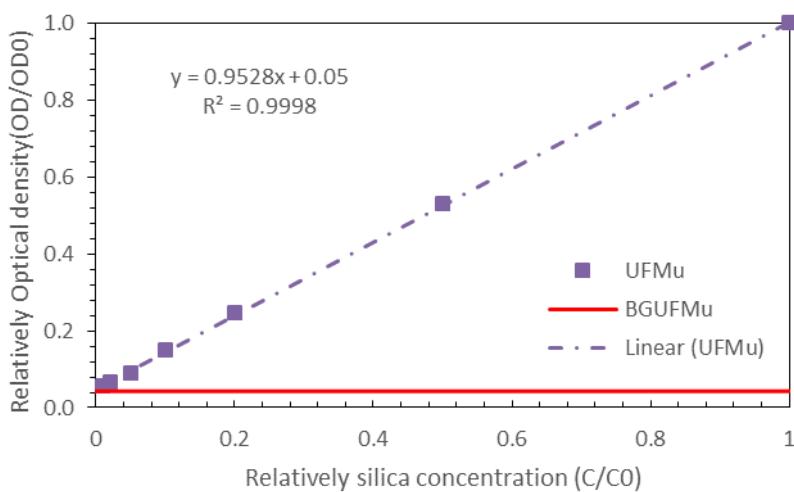
Appendix B-4 The dilution curve of silica colloids in UFMK.



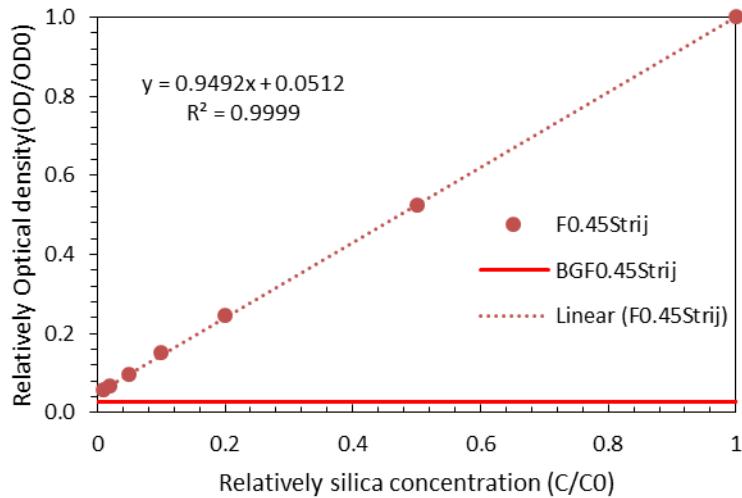
Appendix B-5 The dilution curve of silica colloids in F0.45Mu.



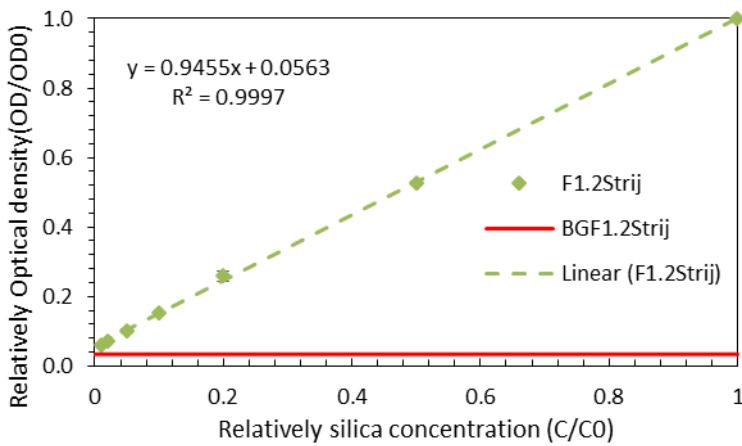
Appendix B-6 The dilution curve of silica colloids in F1.2Mu.



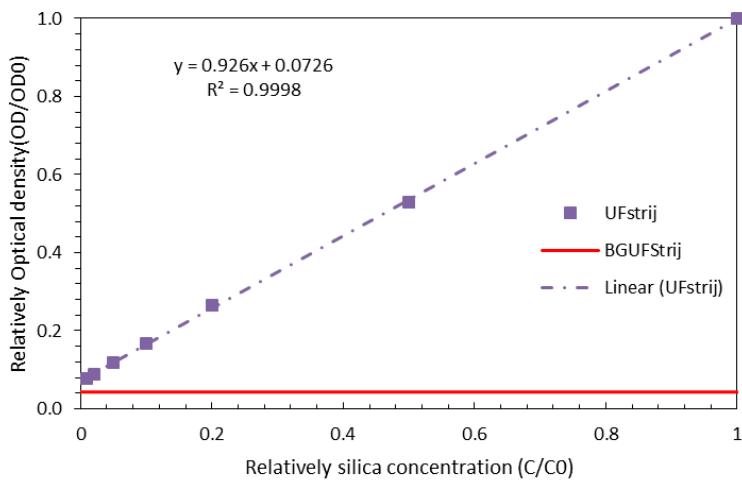
Appendix B-7 The dilution curve of silica colloids in UFMu.



Appendix B-8 The dilution curve of silica colloids in F0.45Strij.

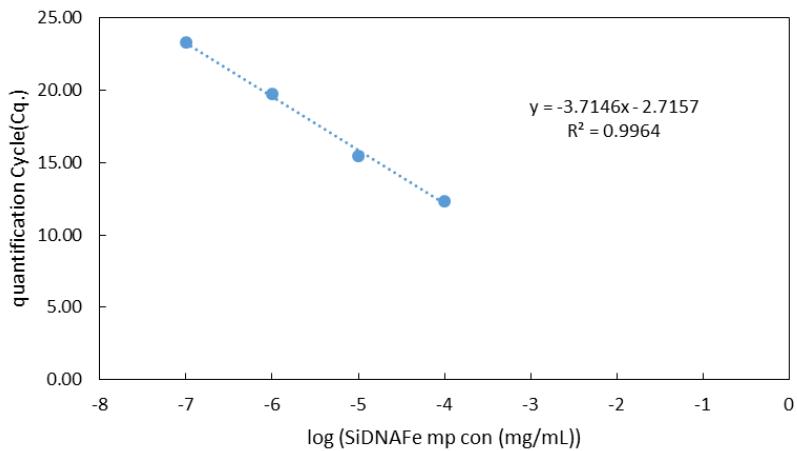


Appendix B-9 The dilution curve of silica colloids in F1.2Strij.

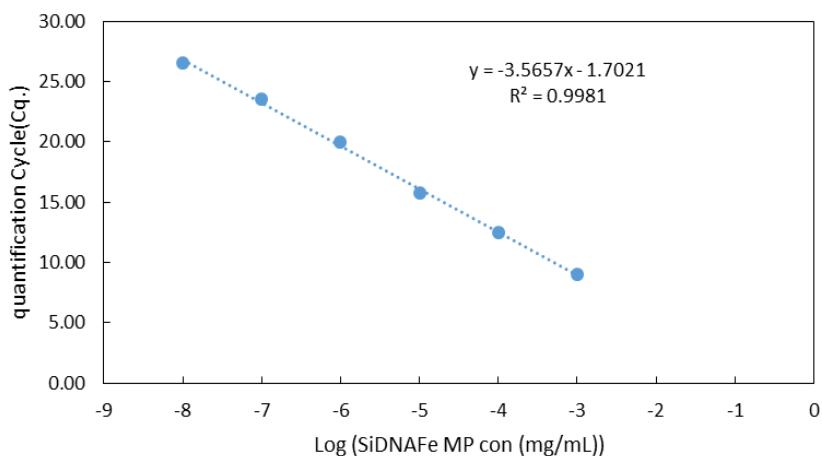


Appendix B-10 The dilution curve of silica colloids in UFStrij.

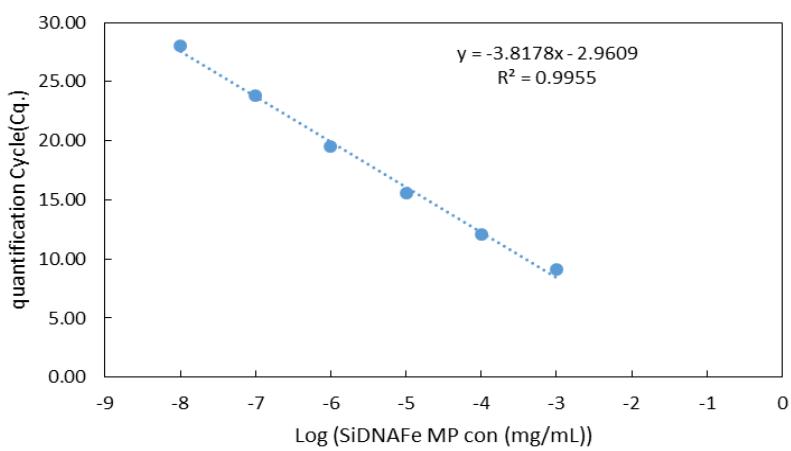
3. Dilution curves of SiDNAFe MPs in Milli-Q water and natural waters



Appendix B-11 The dilution curve of SiDNAFe in Milli-Q and Merkske water.



Appendix B-12 The dilution curve of SiDNAFe in Meuse water.

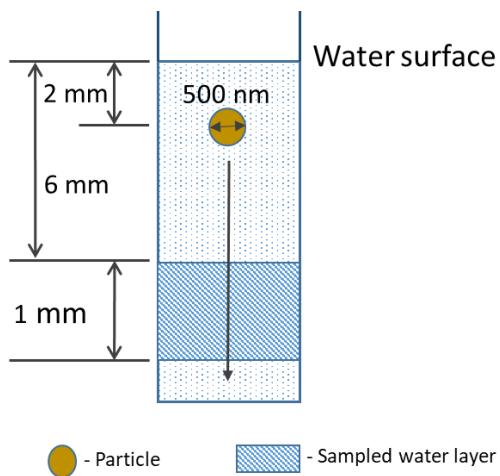


Appendix B-13 The dilution curve of SiDNAFe in Strijbeek water.

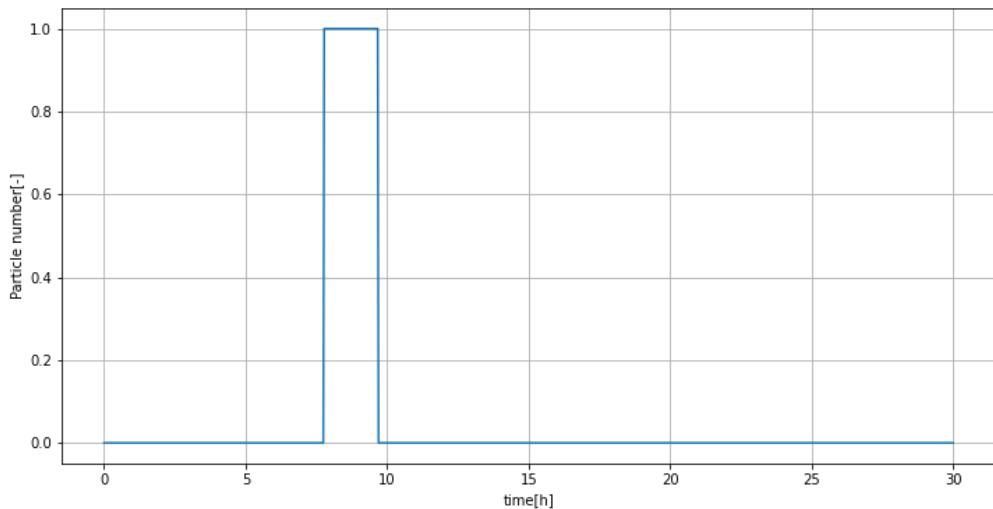
Appendix C. - Sedimentation model

1. Validation of the model in Python

In the sedimentation experiment, the relative concentration data was obtained by reading and converting the average optical density of sampled water layer with a particular thickness pipetted in a test tube. Therefore, the sedimentation model simulated the change in relative concentration in a certain thickness of a sampled water layer over time based on the mass balance equation (3-12) in Python. The sedimentation processes are complex. The simulation of the model started from a given size spherical particle settling without aggregation through a sampled water layer.



Appendix C-1 A sketch of a given size spherical particle(500nm) at 2mm depth settling through a sampled water layer(1mm thickness) at 6 mm depth

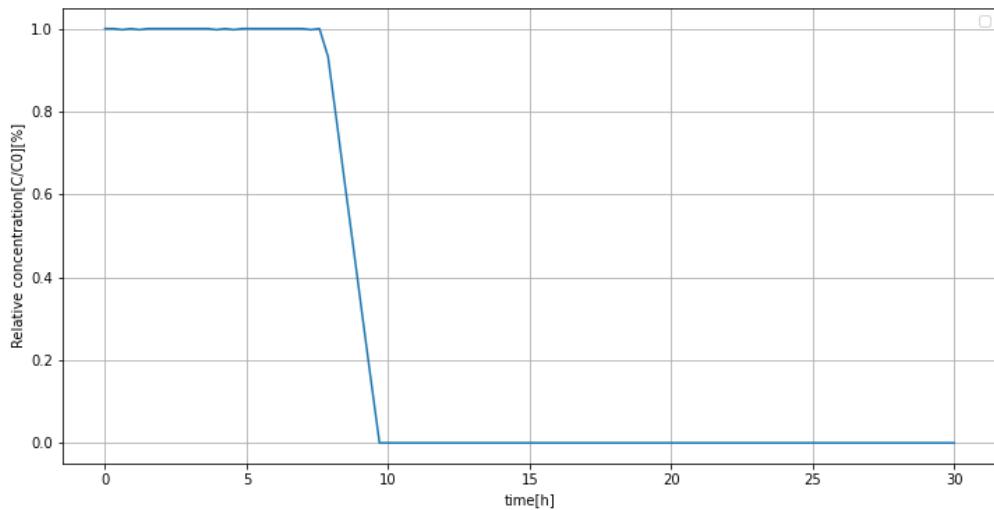


Appendix C-2 The particle number in the sampled water layer at 5 mm depth over time during the particle settling

For this case, the solution of the mass balance equation (3-1) was the Heaviside function with the initial and boundary conditions mentioned in Methodology (Chapter 3). A 500 nm particle at 2 mm depth settling through a sampled water layer at 6 mm depth with the thickness of 1mm was presented in figure 4-1 and Figure 4-2 shows the particle number in sample water layer

during the particle settling. The particle number keeps stable at 0 for the first 7.7 hours, reaches 1 at 7.7 hours, remains there for two hours, and then returns to 0. It represents that the particle settles into the sampled water layer from above after 7.7 hours and settles out of the layer at 9.7 hours. The model successfully simulated a particle settling at a location through a sampled water layer.

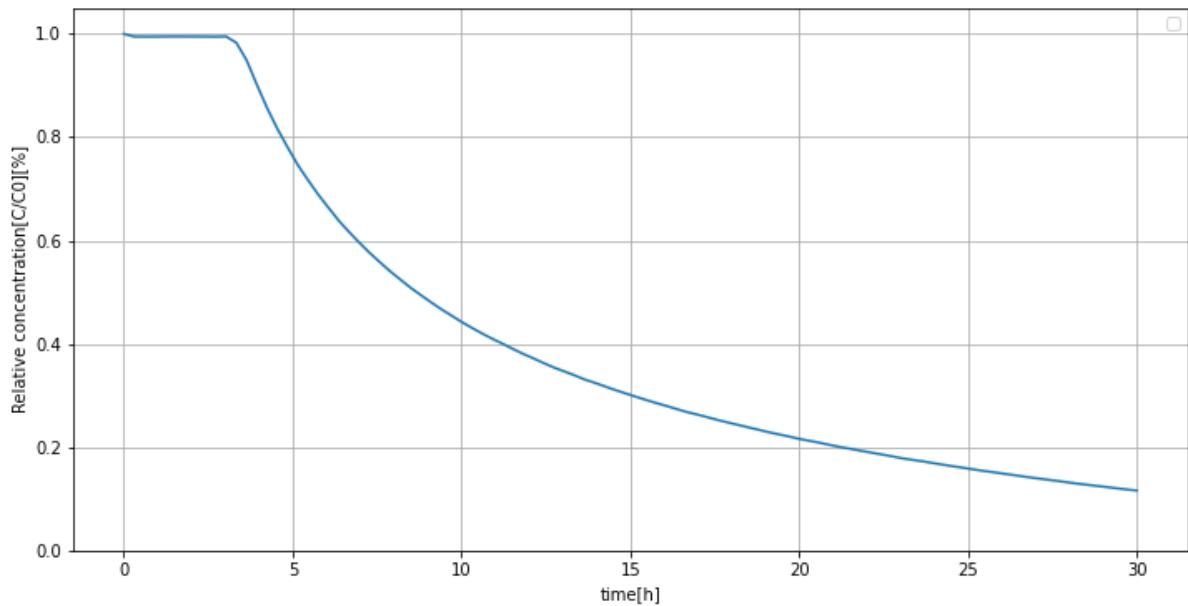
Thereto, the settling of numerous particles at different locations through the same sampled water layer can be modelled based on a large number of Heaviside functions - equation (3-3). Figure 4-2 presents the mono-disperse settling through a sampled water layer in homogenous suspension (blue line). The particles of 500 nm in size settled through the sampled water layer with 1 mm in thickness at 4 mm depth.



Appendix C-3 Mono-disperse settling with particles in size of 500 nm through a sampled water layer at 4mm depth in homogenous suspension.

The relative concentration (non-aggregation, blue line) decreases after 7.7 hours and reaches 0 after 9.7 hours. It means that no more particles enter the sampled water layer from the layer's upper boundary after 7.7 hours, and all particles settle out of the sampled water layer after 9.7 hours.

The model then simulated the sedimentation of spherical particle with a range of particle sizes in homogenous suspension based on equation 3-4.



Appendix C-4 Poly-disperse settling of particles with a particle size distribution in a range of 200 nm to 800nm.

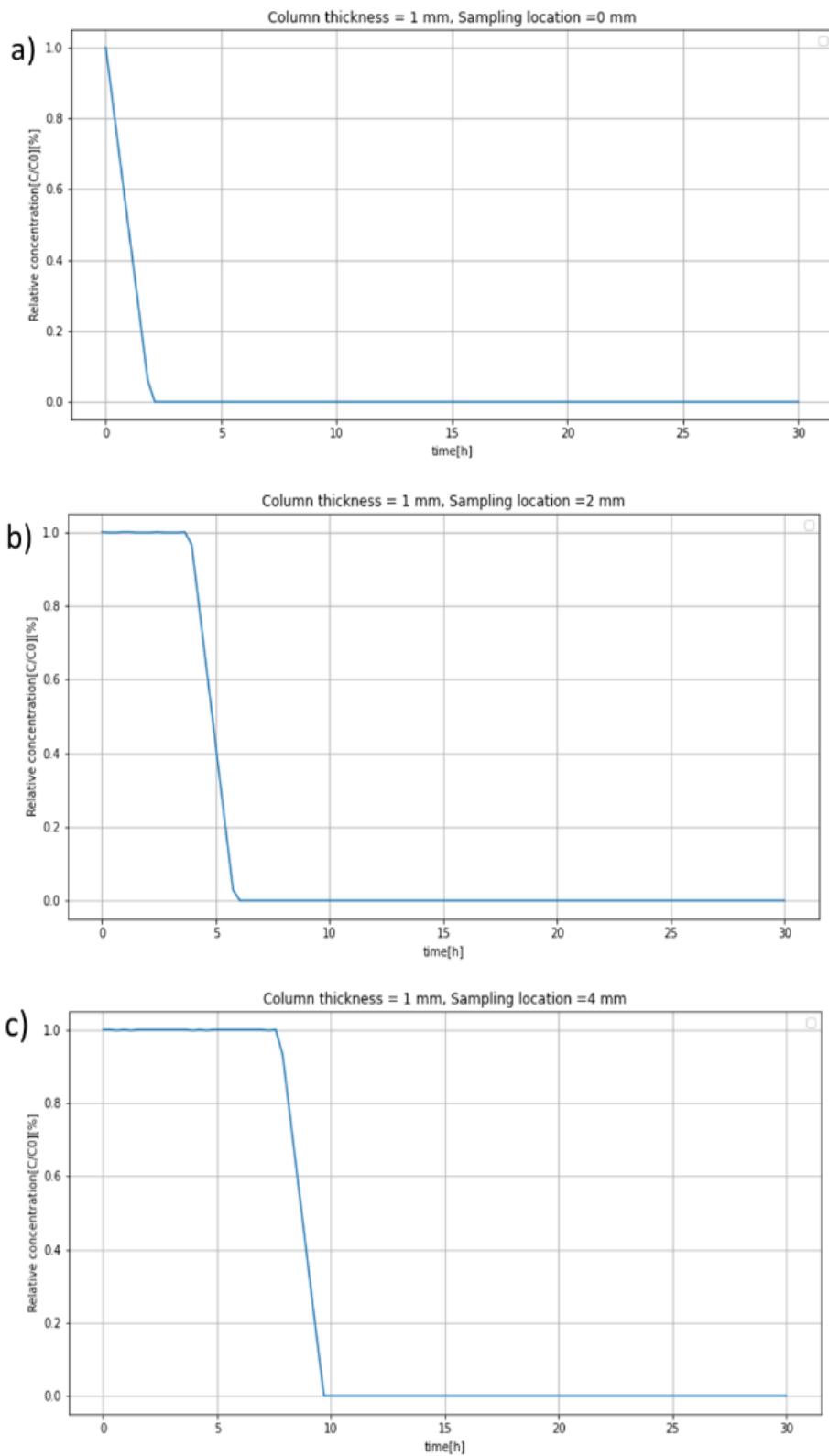
Particles in the range of 200 nm to 800 nm settling through a sampled water layer with 1 mm in thickness at 4 mm depth in homogeneous suspensions were modelled, as shown in figure 4-4. The blue line in figure 4-4 represents particles settling without aggregation. The relative concentrations start to decrease after 3.9 hours. The rate of decrease in relative concentration decrease over time and is greater for settling with aggregation than settling without. The relative concentration in the orange line tended to zero after 20.3 hours.

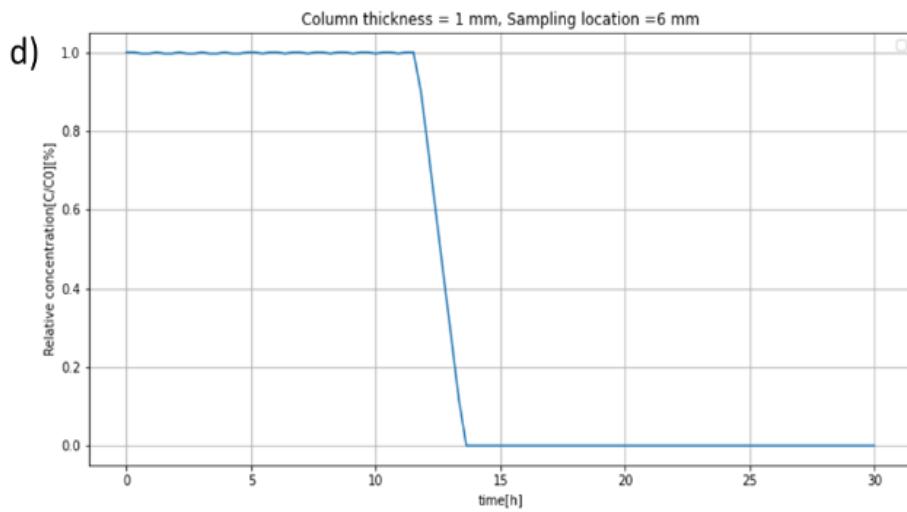
To sum up, we proved that the sedimentation model could simulate that particles with a particle size distribution settling through a sampled water layer with a given thickness at a given depth, and the model could calculate the relative concentration in the layer at any time t.

2. The effect of layer thickness and sampling location on the results

We need to determine the volume of sampled water (layer thickness) and sampling location during the sampling process. The effect of layer thickness and sampling location on the relative concentration results was simulated using the sedimentation model.

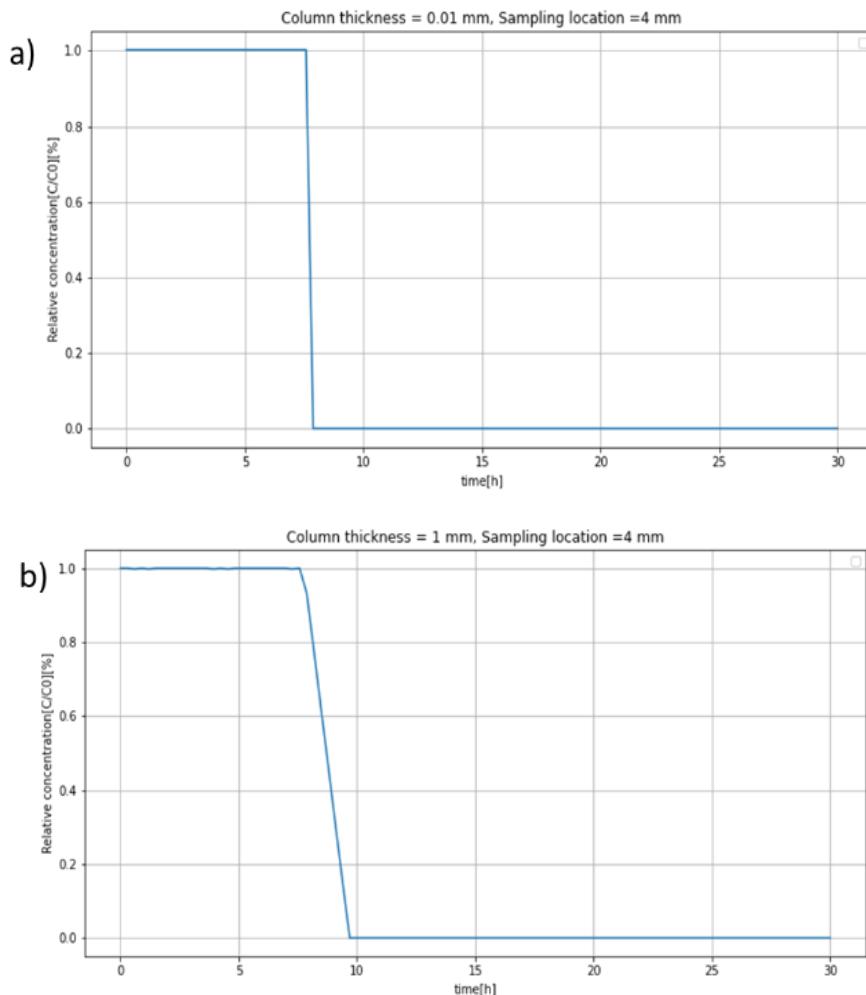
Figure 4-5 represents the variation in the relative concentration of monodisperse settled particles in sampled water layers at different depths (0, 2, 4 and 6 mm). The particles size was 500nm, and the thickness of the sampled water layer was 1mm. Comparing the four plots in figure 4-5 shows that the curve's drop delay as the sampling location gets deeper. It means that, with increasing the sampling depth, the period of the particles settling into the sampled water layer from above goes longer.

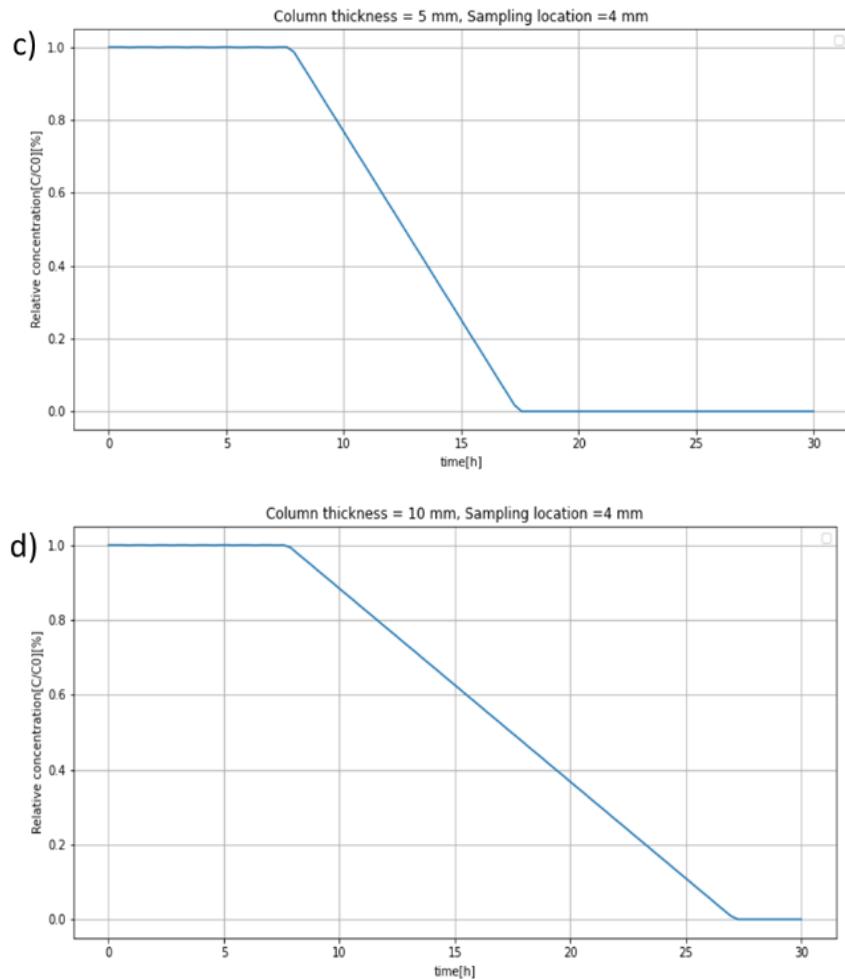




Appendix C-5 Transient behaviour of relative concentration of suspended particles at different sampling location (z) (a) $z=0$ mm; (b) $z=2$ mm; (c) $z=4$ mm and (d) $z=6$ mm following constant input at surface column.

The volume of water pipetted (layer thickness) also affected the results, as shown in figure 4-6. Four plots in figure 4-6 present that it takes longer for the relative concentration to converge from 1 to 0 with increasing layer thickness. In other words, the smaller the thickness, the shorter the time for the particles settling out of the sampled water layer.



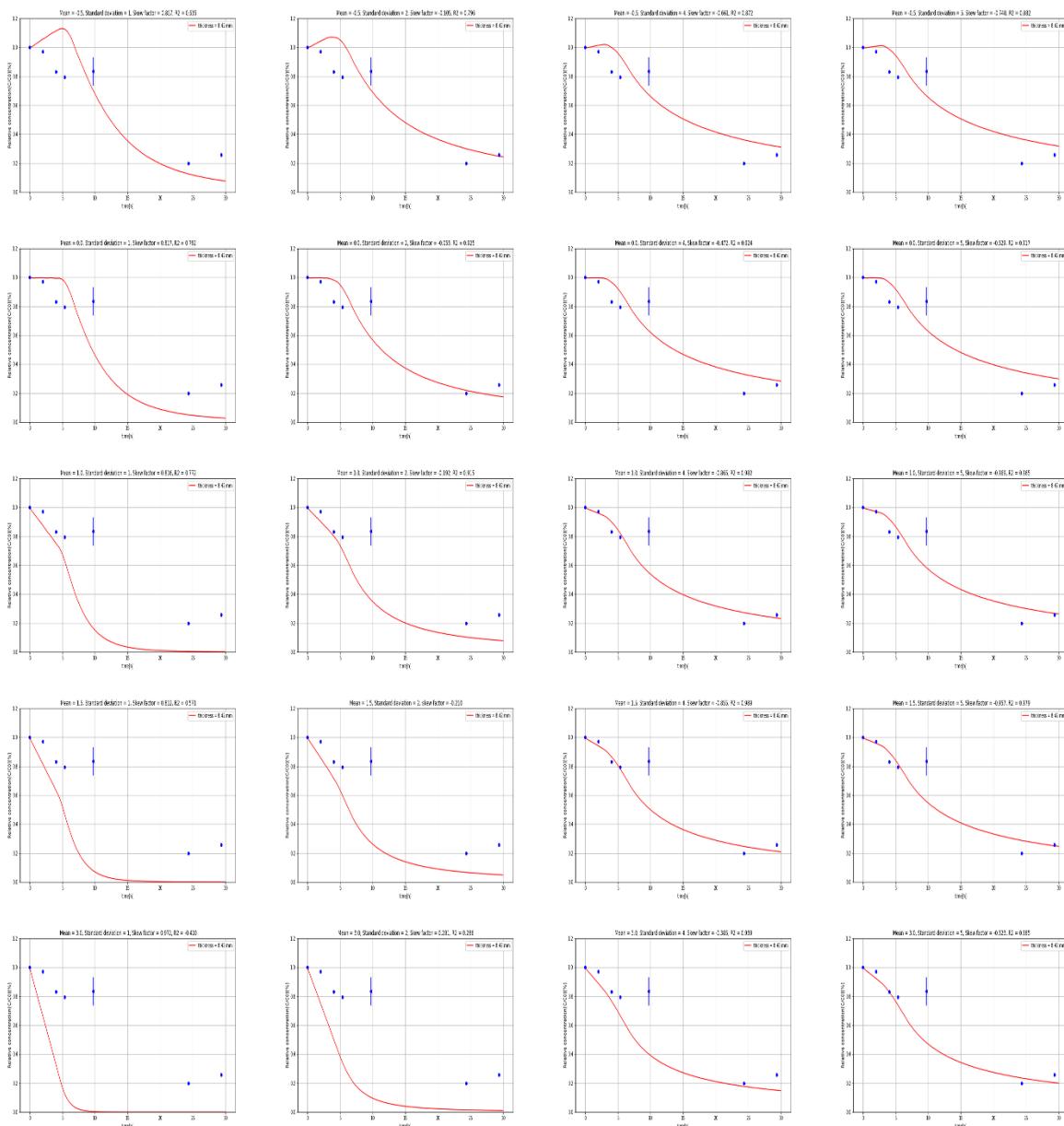


Appendix C-6 Transient behaviour of relative concentration in sampled water layers with different thickness (h)
(a) $h=0.01$ mm; (b) $h=1$ mm; (c) $h=5$ mm and (d) $h=10$ mm following constant input at surface column.

In summary, the sampling location determined the starting position of the relative concentration drop fraction, and the layer thickness could change the width of the relative concentration drop. Therefore, it is necessary to ensure that the same amount of water is taken and the same sampling locations are used for all sampling processes in the same type of particle settling experiment.

In the silica colloids settling experiments, the layer thickness was 8.42mm and the sampling position was 5.5mm. In the sedimentation experiments of SiDNAFe MPs, the layer thickness was 5.66mm and the sampling position was 5.66mm.

3. Simulating the PSD of silica colloids in Milli-Q water



Appendix C-7 Simulation curves with different mean and standard deviation parameters (1) Mean: -0.5, 0, 1, 1.5, 3 ;(2) Standard deviation: 1,2,4,5.

Appendix C-8 he values of skew factor, RMSE and R² at different means and standard deviations

No.	Mean	Standard deviation	Skewness factor	RMSE	Rsquared
1	-0.5	1	0.817	0.196	0.635
2	-0.5	2	-0.105	0.147	0.796
3	-0.5	4	-0.661	0.116	0.872
4	-0.5	5	-0.748	0.111	0.882
5	0	1	0.817	0.148	0.792
6	0	2	-0.055	0.089	0.925
7	0	4	-0.472	0.09	0.924
8	0	5	-0.529	0.094	0.917
9	1	1	0.816	0.155	0.772
10	1	2	-0.192	0.095	0.915
11	1	4	-0.865	0.044	0.982
12	1	5	-0.969	0.061	0.965
13	1.5	1	0.812	0.213	0.571
14	1.5	2	-0.21	0.143	0.806
15	1.5	4	-0.855	0.033	0.989
16	1.5	5	-0.957	0.047	0.979
17	3	1	0.972	0.383	0.041
18	3	2	0.281	0.273	0.286
19	3	4	-0.385	0.08	0.939
20	3	5	-0.523	0.04	0.985

Appendix C-8 presents the values of skew factor, RMSE and R² at different means and standard deviations. Of all the mean and standard deviation combinations, combination number fifteen has the smallest RMSE and the largest R² with 0.033 and 0.989, respectively. The PSD of silica colloids derived from these parameters of combination number fifteen best match the PSD in Milli-Q water.

Python codes for sedimentation model

Sedimentation model

March 29, 2021

1 Sedimentation model

Zhang Fengbo

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```
[7]: import numpy as np
import matplotlib.pyplot as plt
import pandas as pd
from scipy.stats import norm, skew, sem, t
import scipy.stats
from scipy.optimize import least_squares # nonlinear least squares
import math
from sklearn.metrics import r2_score
```

```
[8]: # define the framework of figure

def newfig(title='', xlabel='forgot xlabel?', ylabel='forgot ylabel?', □
           xlim=None, ylim=None,
           xscale='linear', yscale='linear', size_inches=(12, 6)):
    fig, ax = plt.subplots()
    fig.set_size_inches(size_inches)
    ax.set_title(title)
    ax.set_xlabel(xlabel)
    ax.set_ylabel(ylabel)
    ax.set_xscale(xscale)
    ax.set_yscale(yscale)
    if not xlim is None: ax.set_xlim(xlim)
    if not ylim is None: ax.set_ylim(ylim)
    ax.grid()
    return ax
```

```
[9]: # Rubey equation

def RubeyEq (PDi=None):      # particle diameter [nm]
    g = 9.8 # gravity [m/s2]
    MVi = 0.001569 # dynamic viscosity in 4 degree[Pas]
    MDe = 997 # medium density [kg/m3]
```

```

PDe = 2650 # silica particle density [kg/m3]
TVe = (-18*MVi + np.sqrt(np.square(18)*np.square(MVi)+ 6*g*np.square(PDi/
→1000000000)*(PDi/1000000000)*MDe*
(PDe-MDe)))/(3*(PDi/1000000000)*MDe)*3600*1000
→#terminal velocity [mm/h]
return TVe

```

```

[10]: # input the silica settling data in milli-g water
obsdata = pd.read_excel('Observation data_ silica.xlsx', sheet_name='MQ',
→usecols='B:D:F')
ts = []
MQCt = []
STD = []
for iw in range(len(obsdata)):
    time0, con0, STD0= obsdata.iloc[iw][['time(h)', 'observation data(mg/l)',
→'STD']]
    if not np.isnan(time0):
        ts.append(time0)
    if not np.isnan(con0):
        MQCt.append(con0)
    if not np.isnan(STD0):
        STD.append(STD0)

```

1.1 A given size particle at z1 depth settling through a sample column(1mm thickness) at z2 depth (z2>z1)

```

[11]: P1 = 500 # particle size of silica [nm]
z1 = 2 # the location of the particle [mm]
z2 = 6 # boundary [mm]
h = 1 # the column thickness [mm]

t = np.linspace(0,30, num= 1000) # settling periods [h]
v = RubeyEq(P1)
print('Settling velocity:',v)

# Draw the map
subttl = f'{z1:.0f} mm '
subttl1 = f'{z2:.0f} mm'

ax1 = newfig(title ='A given size particle (500 nm) at '+ subttl +'depth'
→settling through \n a sample column at '+subttl1+' depth (z2>z1) with the
→thickness of 1 mm',
xlabel='time[h]', ylabel='Particle number[-]', )
# model the sedimentation following RubeyEq' lawM

```

```

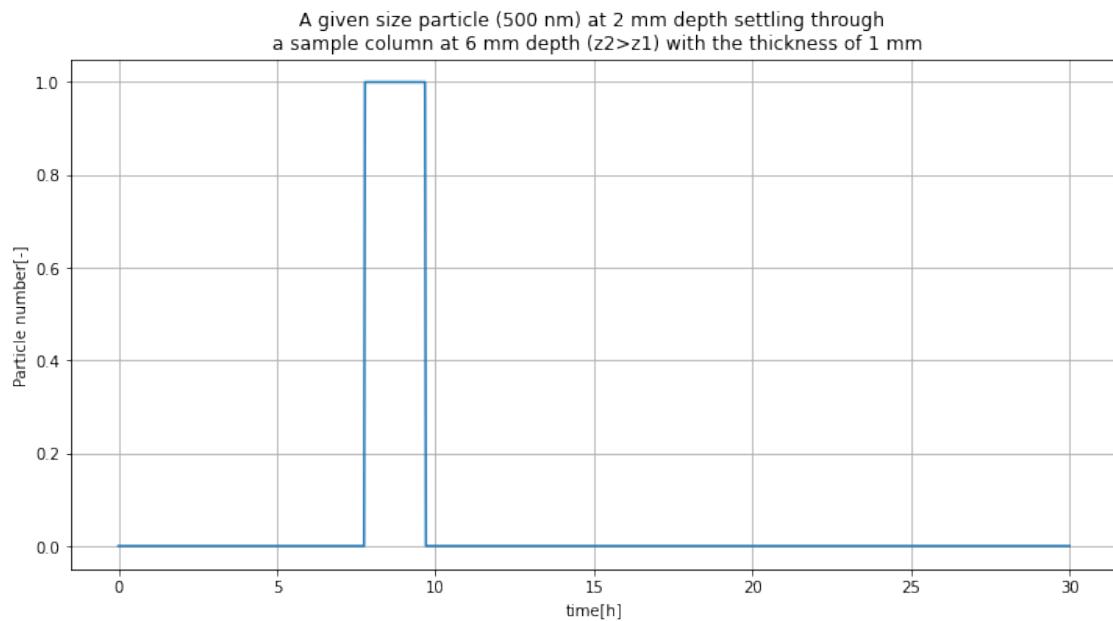
c = 0
Con = 0
Scon = []

for i in t:
    if z2-z1+h >= i * v >= z2-z1:
        Con = 1
    else:
        Con = 0
    Scon.append(Con)

ax1.plot(t, Scon)
plt.savefig('A given size particle at z1 depth settling.png')

```

Settling velocity: 0.5162332614208762



1.2 Mono-disperse settling through a sample column with a certian thickness(h)

```

[12]: P1 = 500 # particle size of silica [nm]
Pnum = 2000 # particle number [-]
Dp = 5 # the distance from z1 depth to z2+h depth [mm]
h = 1 # the thickness of the sample cloumn [mm]

# the distances from the water surface to the locations of silica particles
LPs = np.linspace(Dp,0,num=Pnum)

```

```

t = np.linspace(0,30, num= 100) # settling periods [h]
v = RubeyEq(P1) # the velocity of silica particle [mm/h]

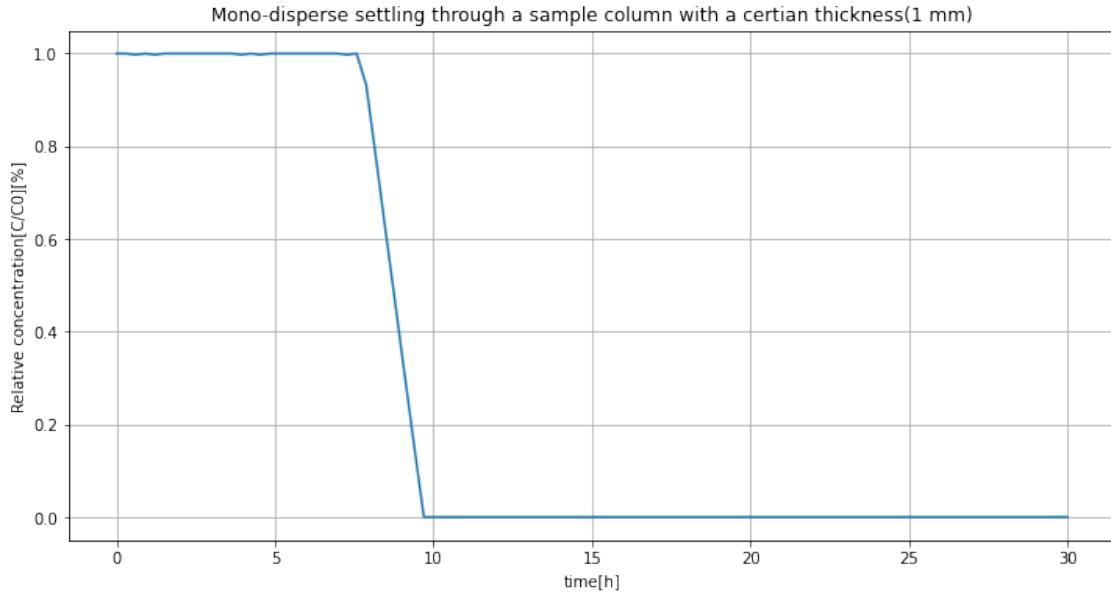
# Draw the map
subttl = f' in size of {P1:.0f} nm'

ax1 = newfig(title ='Mono-disperse settling through a sample column with a\u2192certian thickness(1 mm)', xlabel='time[h]', ylabel='Relative concentration[C/C0] [%]')
# Heavisde function
c = 0
Con = 0
ConT = 0
Scon = []

for i in t:
    for d in LPs:
        if i * v < Dp- d- h:
            Con = 0
        elif i * v > Dp- d- h and i * v <= Dp- d:
            Con = 1
        else:
            Con = 0
        ConT = ConT + Con
    Scon.append(ConT/(Pnum*(h/Dp)))
    ConT = 0

ax1.plot(t, Scon)
plt.savefig('Mono-disperse settling without aggreagtion.png')

```



1.3 Poly-dispersed settling in a sample column

```
[13]: # the list of silica particle sizes following the normal distribution and their
      ↪ratios
Num =500
SizeMax = 200 #[nm] the size of largest particle in the cell
SizeMin = 800 #[nm] the size of smallest particle in the cell
org = np.linspace(-4,4, num = Num)
org2 = np.linspace (SizeMin,SizeMax, num = Num) # the size range of silica
      ↪particles

def normal_dist(x, mean, sd):
    prob_density = (1/(2*np.pi*sd**2) ) * np.exp(-0.5*((x-mean)/sd)**2)
    return prob_density

#Apply function to the data.
pre4org = [1] * len(org2)
orgPre = pre4org/ np.sum(pre4org)

OrgWpre = (org2,orgPre)

ax0 = newfig(title = 'Particle size and distribution',
             xlabel='Particles size[nm]', ylabel='[%]')
ax0.bar(org2, orgPre*100, color='r', width=3.5)

lyrnum = 200 # discrete layer number
```

```

DZ = 1 # the thickness of sample column [mm]
uD = 4 # the distance from the particle location to the up boundary of the element [mm]

t = np.linspace(0,30, num= 100) # settling periods [h]
siTVe = RubeyEq(PDi = org2)
Vmean = np.mean(siTVe)

# the distances from the water surface to the locations of silica particles
LPs = np.linspace(uD+DZ,0,lyrnum)
Lyr_more_than_DZ = LPs <= DZ
totalLyr = Lyr_more_than_DZ.sum()

# Draw the map
ax1 = newfig(title ='Poly-disperse settling of particle in size range of 200 nm to 800 nm',
              xlabel='time[h]', ylabel='Relative concentration[C/CO] [%]', ylim=[0,1.05])

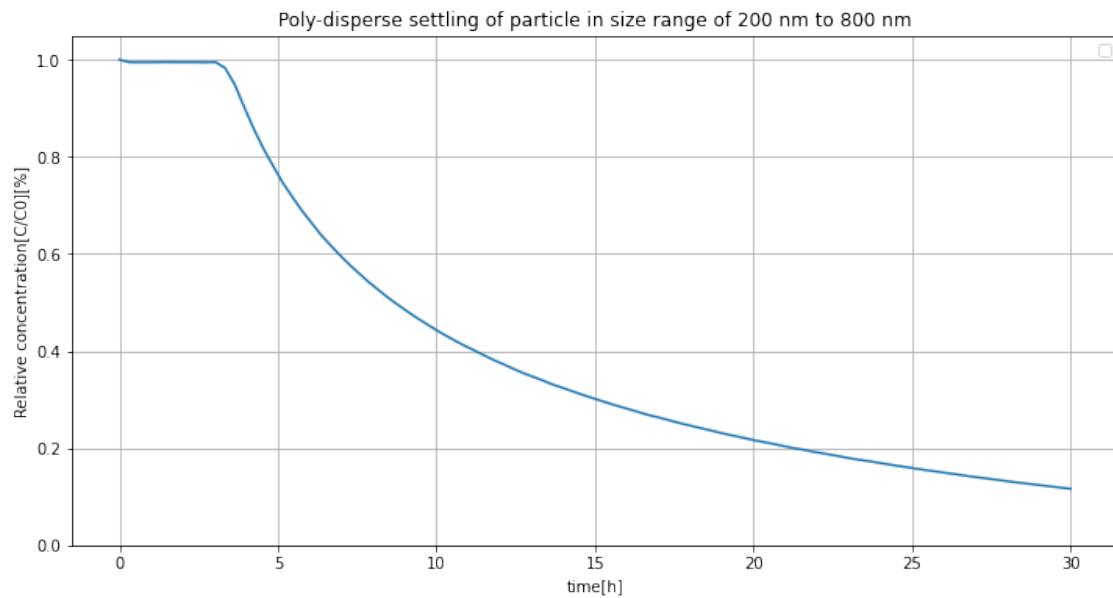
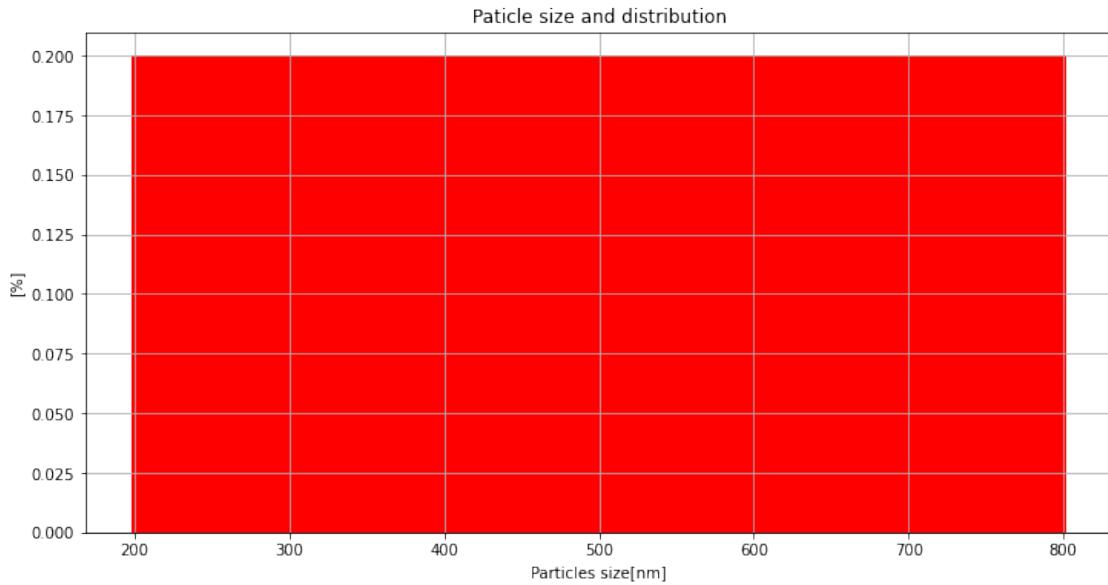
# the sedimentations of multi-particles through a differential element are modeled
c = 0
Con = 0
Scon1 = []

for i in t:
    for h in LPs:
        for v in siTVe:
            if i * v >= uD - h and i * v <= DZ + uD - h:
                c += 1
            Con = Con + np.sum(orgPre[:c])
            c = 0
        Con = Con/totalLyr
        Scon1.append(Con)
        Con = 0

ax1.plot(t, Scon1)
ax1.legend()
plt.savefig('poly-disperse settling.png')

```

No handles with labels found to put in legend.



1.4 Simulate particle size distribution of silica particles by normal distribution with two parameters(mean and standard deviation)

[14] : *### To optimize parameters of mean and standard deviation by fitting sedimentation data*
#####

```

# the list of silica particle sizes following the normal distribution and their ratios
Num =50
SizeMax = 1000 #[nm]
SizeMin = 100 #[nm]
org = np.linspace(-4,4, num = Num)
org2 = np.linspace (100,1000, num = Num) # the size range of silica particles

def normal_dist(x, mean, sd):
    prob_density = (1/(2*np.pi*sd**2) ) * np.exp(-0.5*((x-mean)/sd)**2)
    return prob_density

# the average particle size and standard deviation of the silica - the speard of the silica
Mean = 3
Std_Dev = 5

#Apply function to the data.
pre4org = normal_dist(org,Mean,Std_Dev)
print(f'Skew factor:{skew(pre4org): .3f}')
orgPre = pre4org/ np.sum(pre4org)
OrgWpre = (org2,orgPre)

lyrnum = 200 # layer number
DZ = 8.42 # the thickness of sampled column [mm]
uD = 5.5 # the distance from the particle location to the up boundary of the element [mm]

t = np.linspace(0,30, num= 100) # settling periods [h]
siTVe = RubeyEq(PDi = org2)

# the distances from the water surface to the locations of silica particles
LPs = np.linspace(uD+DZ,0,lyrnum)
Lyr_more_than_DZ = LPs <= DZ
totalLyr = Lyr_more_than_DZ.sum()

#####
##### simulate the sedimentations of silcia colloids and to estiamte the concentration at time t
c = 0
Con = 0
Scon = []

for i in t:
    for h in LPs:
        for v in siTVe:

```

```

        if i * v >= uD - h and i * v <= DZ + uD - h:
            c += 1
        Con = Con + np.sum(orgPre[:c])
        c = 0
    Con = Con/totalLyr
    Scon.append(Con)
    Con = 0
#####
#####

# objective function-R square
c = 0
Con = 0
MQScon = []

for i in ts:
    for h in LPs:
        for v in siTVe:
            if i * v >= uD - h and i * v <= DZ + uD - h:
                c += 1
            Con = Con + np.sum(orgPre[:c])
            c = 0
        Con = Con/totalLyr
        MQScon.append(Con)
        Con = 0

MQCt1 = np.delete(MQCt,4)
MQScon1 = np.delete(MQScon,4)

Rsquared = r2_score(MQCt1,MQScon1)
mse = ((MQScon1-MQCt1 )** 2).mean()
Rmse = np.sqrt(mse)
print(f'MSE in this case : {mse: .3f}')
print(f'RMSE in this case : {Rmse: .3f}')
print(f'R2 in this case : {Rsquared: .3f}')

tval, pval = scipy.stats.ttest_1samp(MQScon1-MQCt1,0)
print(f't-value:{tval:0.2f}, P-value:{pval:0.2f}')


# Draw the map
ax0 = newfig(title = 'Particle size distribution of silica colloids',
             xlabel='Silica particles size[nm]', ylabel='[%]')
ax0.bar(org2, orgPre*100, color='r', width=3.5)

ax1 = newfig(title =f' Mean = {Mean:.1f}, Standard deviation = {Std_Dev:.0f},\n Skew factor = {skew(pre4org):.3f}, R2 = {Rsquared:.3f}',


```

```

        xlabel='time[h]', ylabel='Relative concentration[C/CO] [%]',  

        ylim=[0.0,1.2])  

ax1.plot(t, Scon, 'r',label = f'thickness = {DZ:.2f} mm')  

ax1.errorbar(ts, MQCt, yerr= STD, fmt= 'o',color='b' )  

ax1.legend()  

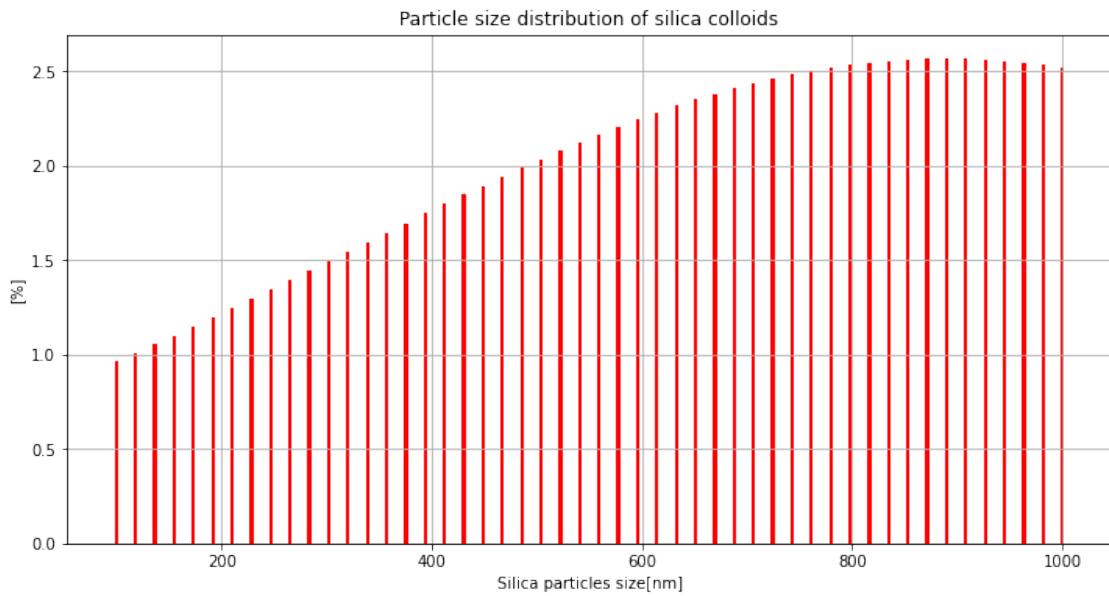
  

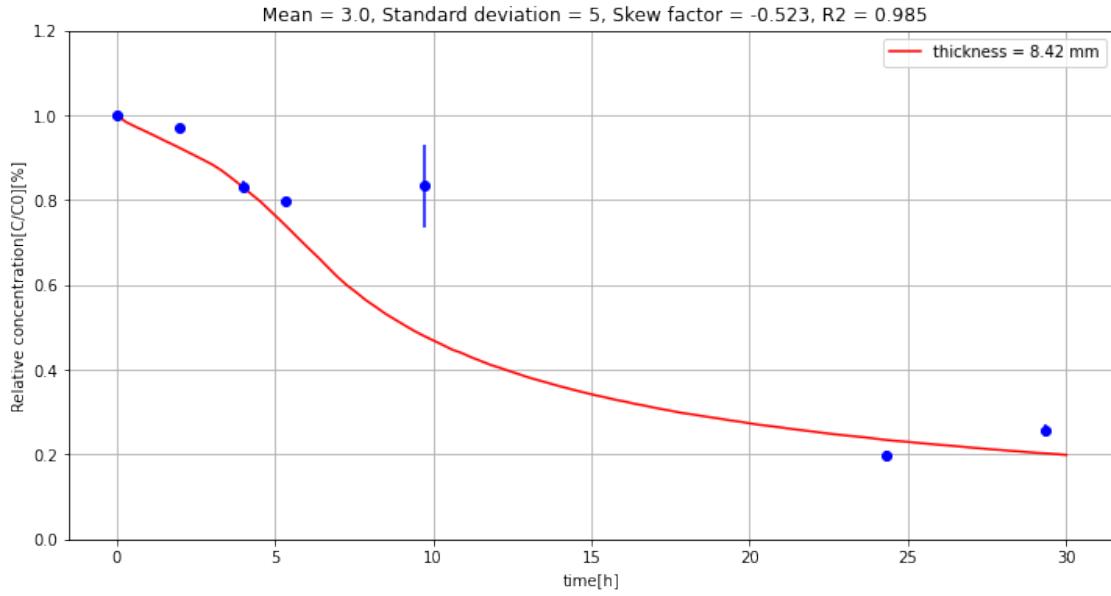
plt.savefig(f' Mean = {Mean:.1f}, Standard deviation = {Std_Dev:.0f}, Skew  

        ↪factor = {skew(pre4org):.3f}.png')

```

Skew factor:-0.523
 MSE in this case : 0.002
 RMSE in this case : 0.040
 R2 in this case : 0.985
 t-value:-1.31, P-value:0.25





1.4.1 View graphs into one graph

```
[15]: from IPython.display import Image as mage
import PIL.Image as Image
import os

IMAGES_PATH = 'D:\Jupyter notebook\Rubeys function\PSD fitting\\' # image path
IMAGE_WIDTH = 2000 # the width of images
IMAGE_HEIGHT = 1000 # the height of images
IMAGE_ROW = 5 # Row
IMAGE_COLUMN = 4 # Column
IMAGES_FORMAT = ['.jpg', '.JPG', '.png'] # Format of image
IMAGE_SAVE_PATH = 'final_silica_mean_stddev.png' # address for saving final image

# obtain the image name from the image path
image_names = [name for name in os.listdir(IMAGES_PATH) for item in IMAGES_FORMAT if os.path.splitext(name)[1] == item]

# Simple quantitative judgement on the setting of parameters and the size of the actual set of images
if len(image_names) != IMAGE_ROW * IMAGE_COLUMN:
    raise ValueError("The parameters of the composite image and the requested number do not match!")

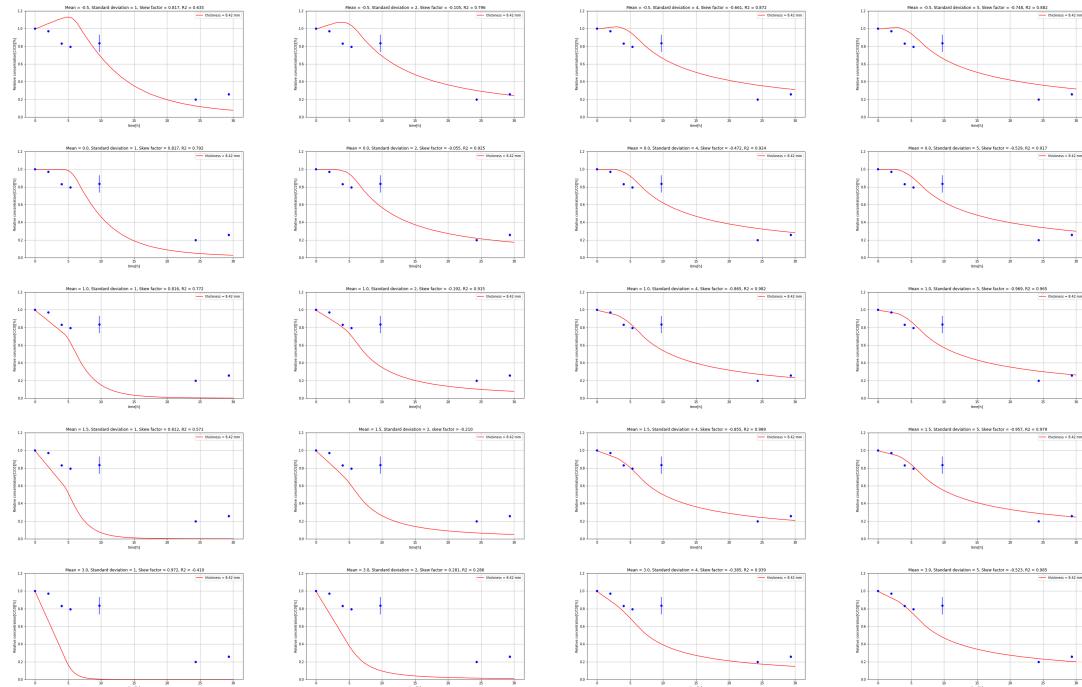
# definition of paste the image together
```

```

def image_compose():
    to_image = Image.new('RGB', (IMAGE_COLUMN * IMAGE_WIDTH, IMAGE_ROW * IMAGE_HEIGHT)) #create a new image
    # Iterate through the loop, pasting each image in order to the corresponding position
    for y in range(1, IMAGE_ROW + 1):
        for x in range(1, IMAGE_COLUMN + 1):
            from_image = Image.open(IMAGES_PATH + image_names[IMAGE_COLUMN * (y - 1) + x - 1]).resize(
                (IMAGE_WIDTH, IMAGE_HEIGHT), Image.ANTIALIAS)
            to_image.paste(from_image, ((x - 1) * IMAGE_WIDTH, (y - 1) * IMAGE_HEIGHT))
    return to_image.save(IMAGE_SAVE_PATH) # save new image
image_compose() #Calling functions
image("final_silica_mean_stddev.png")

```

[15]:



1.5 Simulation results of particle size distribution under optimal parameters

[16]: # the average particle size and standard deviation of the silica - the speard of the silica

```
# the list of silica particle sizes following the normal distribution and their ratios
```

```

Num =50
SizeMax = 1000 #[nm]
SizeMin = 100 #[nm]
org = np.linspace(-4,4, num = Num)
org2 = np.linspace (100,1000, num = Num) # the size range of silica particles

def normal_dist(x, mean, sd):
    prob_density = (1/(2*np.pi*sd**2)) * np.exp(-0.5*((x-mean)/sd)**2)
    return prob_density

#Apply function to the data.
Mean = 1.5
Std_Dev = 4
pre4org = normal_dist(org,Mean,Std_Dev)
orgPre = pre4org/ np.sum(pre4org)
OrgWpre = (org2,orgPre)
Skew_factor =skew(pre4org)

print(f'skew factor ={skew(pre4org):.3f}', 'Mean =' ,Mean, 'Standard deviaton=' , Std_Dev)
# measure the 10%, 16%, 50%, 86%, 90% size distribution of silica colloids
CulDis =0;CulDisT =[]
for i in orgPre:
    CulDis=CulDis+ i*100
    CulDisT.append(CulDis)

n = 0;CulDis =0;Meansize =0;Pre10size = 0;Pre90size=0
for i in orgPre:
    CulDis=CulDis+ i*100
    n = n +1
    if CulDis > 10:
        Pre10size =org2[n]
        break
for i in orgPre:
    CulDis=CulDis+ i*100
    n = n +1
    if CulDis > 16:
        Pre16size =org2[n]
        break
n = 0;CulDis =0
for i in orgPre:
    CulDis=CulDis+ i*100
    n = n +1
    if CulDis > 50:
        Meansize =org2[n]
        break
n = 0;CulDis =0

```

```

for i in orgPre:
    CulDis=CulDis+ i*100
    n = n +1
    if CulDis > 84:
        Pre84size =org2[n]
        break
n = 0;CulDis =0
for i in orgPre:
    CulDis=CulDis+ i*100
    n = n +1
    if CulDis > 90:
        Pre90size =org2[n]
        break
print('10% of Distribution:',Pre10size)
print('16% of Distribution:',Pre16size)
print('50% of Distribution silica colloids:',Meansize)
print('84% of Distribution:',Pre84size)
print('90% of Distribution:',Pre90size)

ax0 = newfig(title = 'Particle size distribution of silica colloids',
             xlabel='Silica particles size[nm]', ylabel='[%]')
ax0.bar(org2, orgPre*100, color='r', width=3.5)
#plt.savefig('Particle size distribution of silica colloids.png')
ax1 = newfig(title = 'Number cumulative distribution',
             xlabel='Silica particles size[nm]', ylabel='Cumulative distribution[%]')
             ,ylim =[0,100],xlim=[100,1000], xscale='log')
ax1.plot(org2, CulDisT, color='r')
plt.savefig('Number cumulative distribution.png')

lyrnum = 200 # layer number
DZ = 8.42 # the thickness of sampled column [mm]
uD = 5.5 # the distance from the particle location to the up boundary of the element [mm]

t = np.linspace(0,30, num= 100) # settling periods [h]
sITVe = RubeyEq(PDi = org2)

# the distances from the water surface to the locations of silica particles
LPs = np.linspace(uD+DZ,0,lyrnum)
Lyr_more_than_DZ = LPs <= DZ
totalLyr = Lyr_more_than_DZ.sum()

# Draw the map
subttl = f' in size range of {SizeMax:.0f} to {SizeMin:.0f} nm'

```

```

ax1 = newfig(title ='silica colloids settling in milliq water sample and its fitting curve',
             xlabel='time[h]', ylabel='Relative concentration[C/C0] [%]', ylim=[0.0,1.05])

#####
# simulate the sedimentations of silcia colloids and to estimate the concentration at time t
####

c = 0
Con = 0
Scon = []

for i in t:
    for h in LPs:
        for v in siTVe:
            if i * v >= uD - h and i * v <= DZ + uD - h:
                c += 1
            Con = Con + np.sum(orgPre[:c])
            c = 0
        Con = Con/totalLyr
        Scon.append(Con)
        Con = 0
####

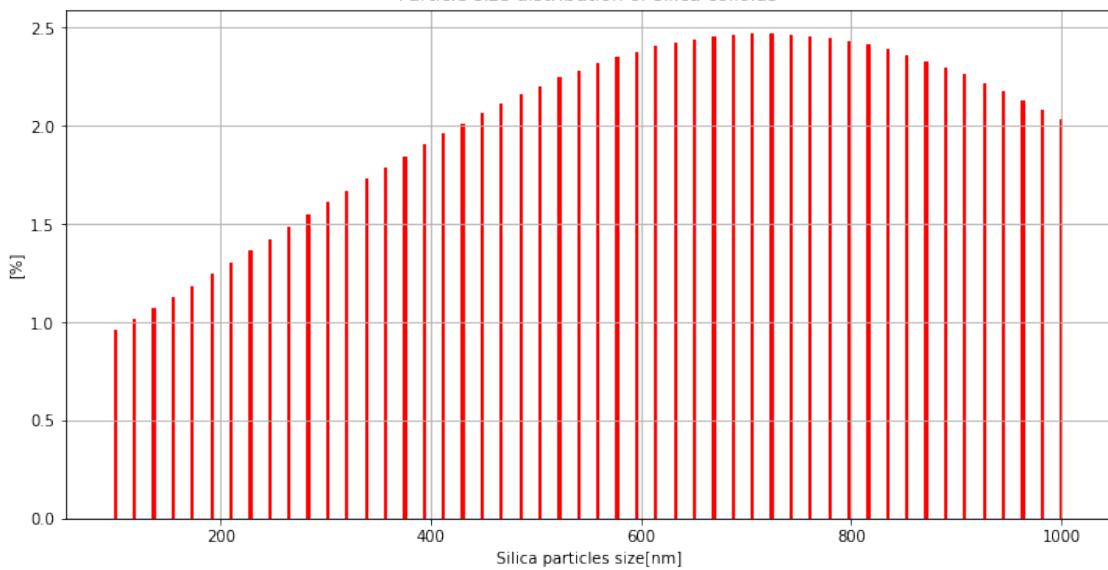
ax1.plot(t, Scon,'r',label = 'fitting curve')
ax1.errorbar(ts, MQCt, yerr= STD, fmt= 'o',color='b',label = 'data(MQ)')
ax1.legend()

plt.savefig('Silica colloids settling in milliq water sample and its fitting curve.png')

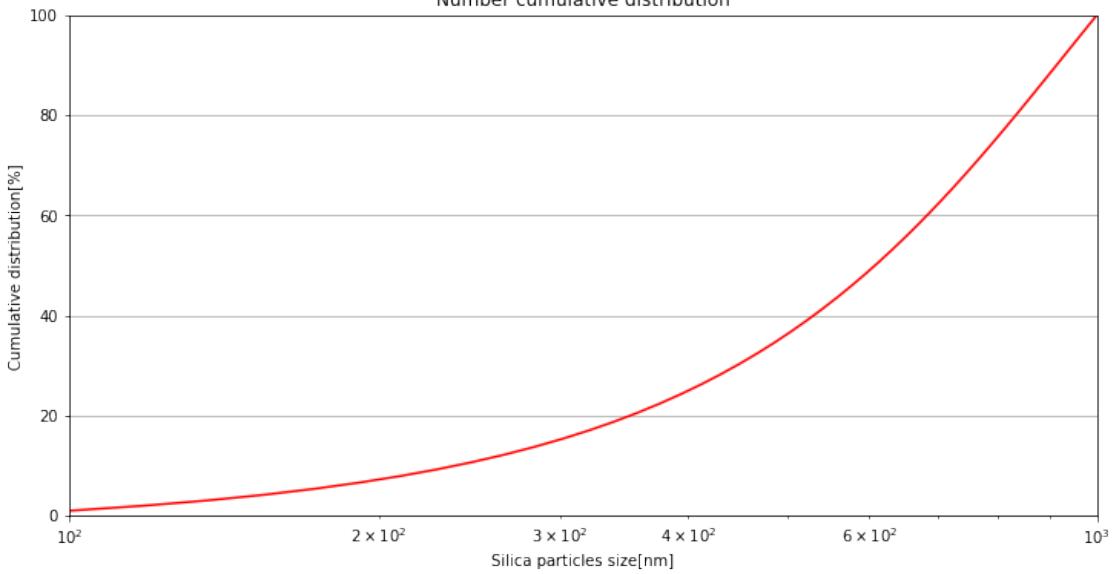
```

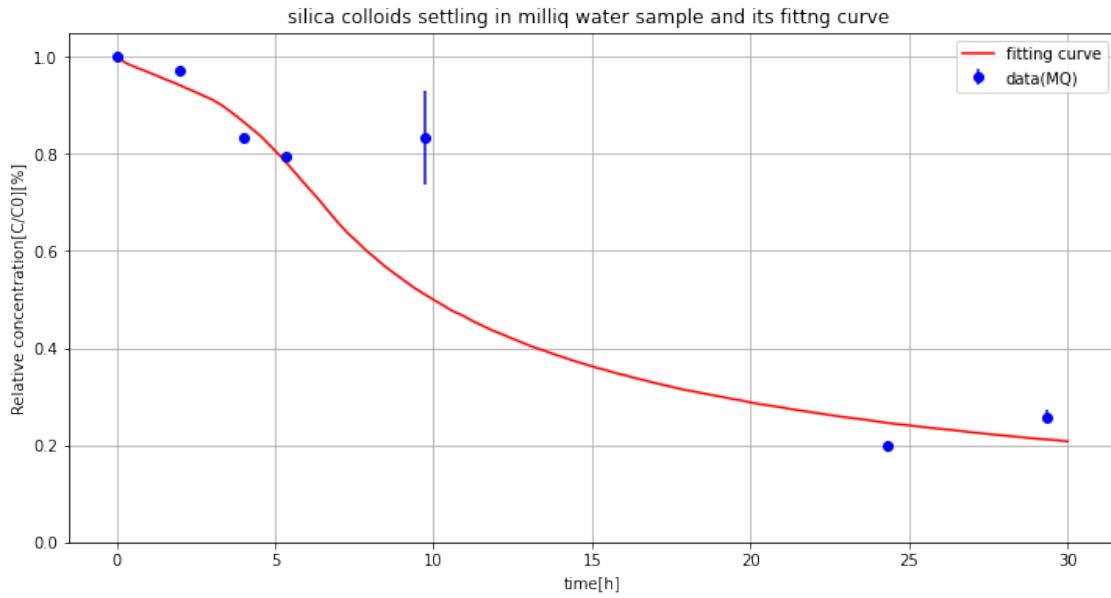
skew factor ==-0.855 Mean = 1.5 Standard deviaton = 4
10% of Distribution: 265.30612244897964
16% of Distribution: 357.14285714285717
50% of Distribution silica colloids: 632.6530612244899
84% of Distribution: 889.795918367347
90% of Distribution: 944.8979591836735

Particle size distribution of silica colloids



Number cumulative distribution





1.6 Settling and aggregation of SiDNAMag MPS

1.6.1 Rubey's equation

```
[19]: # Rubey equation

def RubeyEq (PDi=None):      # particle diameter [nm]
    g = 9.8 # gravity [m/s2]
    MVi = 0.001569 # dynamic viscosity in 4 degree[Pas]
    MDe = 997 # medium density [kg/m3]
    PDe = 2100 # silica particle density [kg/m3]
    TVe = (-18*MVi + np.sqrt(np.square(18)*np.square(MVi)+ 6*g*np.square(PDi/
    ↪1000000000)*(PDi/1000000000)*MDe*
        (PDe-MDe)))/(3*(PDi/1000000000)*MDe)*3600*1000
    ↪#terminal velocity [mm/h]
    return TVe

# input the parameters and observation data
obsdata = pd.read_excel('Observation data_ SiDNAMag.xlsx', sheet_name='MQ',
    ↪usecols=('B,D:F'))
ts = []
MQCt_SiDNAFe = []
STD_SiDNAFe = []
for iw in range(len(obsdata)):
    time0, con0, STD0= obsdata.iloc[iw][['time(h)', 'observation data(mg/l)',
    ↪'STD']]
    if not np.isnan(time0):
```

```

        ts.append(time0)
if not np.isnan(con0):
    MQCt_SiDNAFe.append(con0)
if not np.isnan(STD0):
    STD_SiDNAFe.append(STD0)

```

1.6.2 Paritice size distribution of SiDNAFe MPs

```
[21]: # the list of silica particle sizes following the normal distribution and their
      ↪ratios
Num =50
org = np.linspace(-4,4, num = Num)
org2 = np.linspace (100,1000, num = Num) # the size range of silica particles

def normal_dist(x, mean, sd):
    prob_density = (1/(2*np.pi*sd**2) ) * np.exp(-0.5*((x-mean)/sd)**2)
    return prob_density

#Apply function to the data.
pre4org = normal_dist(org,-1,6)
pre4org1 = normal_dist(org,4,1)
orgPre = (pre4org/ np.sum(pre4org) + pre4org1/np.sum(pre4org1))/2
OrgWpre = (org2,orgPre)

print(f'skew factor ={skew(orgPre):.3f}')
# measure the 10%, 16%, 50%, 86%, 90% size distribution of silica colloids
CulDis =0;CulDisT =[]
for i in orgPre:
    CulDis=CulDis+ i*100
    CulDisT.append(CulDis)

n = 0;CulDis =0;Meansize =0;Pre10size = 0;Pre90size=0
for i in orgPre:
    CulDis=CulDis+ i*100
    n = n +1
    if CulDis > 10:
        Pre10size =org2[n]
        break
for i in orgPre:
    CulDis=CulDis+ i*100
    n = n +1
    if CulDis > 16:
        Pre16size =org2[n]
        break
n = 0;CulDis =0
for i in orgPre:
    CulDis=CulDis+ i*100
```

```

n = n +1
if CulDis > 50:
    Meansize =org2[n]
    break
n = 0;CulDis =0
for i in orgPre:
    CulDis=CulDis+ i*100
    n = n +1
    if CulDis > 84:
        Pre84size =org2[n]
        break
n = 0;CulDis =0
for i in orgPre:
    CulDis=CulDis+ i*100
    n = n +1
    if CulDis > 90:
        Pre90size =org2[n]
        break
print('10% of Distribution:',Pre10size)
print('16% of Distribution:',Pre16size)
print('50% of Distribution:',Meansize)
print('84% of Distribution:',Pre84size)
print('90% of Distribution:',Pre90size)

ax0 = newfig(title = 'Particle size and distribution of SiDNAFe MPs',
             xlabel='Particles size[nm]', xlim=[100,1000],ylabel='[%]')
ax0.bar(org2, orgPre*100, color='r', width=3.5)
plt.savefig('PSDFe.png')

ax1 = newfig(title = 'Number cumulative distribution of SiDNAFe MPs',
             xlabel='Particles size[nm]', ylabel='Cumulative distribution[%]',
             ylim =[0,100],xlim=[100,1000], xscale='log')
ax1.plot(org2, CulDisT, color='b')
plt.savefig('cumulativeFe.png')

lyrnum = 200 # layer number
DZ = 5.66 # the thickness of sampled column [mm]
uD = 5.66 # the distance from the particle location to the up boundary of the
           ↴ element [mm]

t = np.linspace(0,30, num= 100) # settling periods [h]
siTVe = RubeyEq(PDi = org2)

# the distances from the water surface to the locations of silica particles
LPs = np.linspace(uD+DZ,0,lyrnum)
Lyr_more_than_DZ = LPs <= DZ
totalLyr = Lyr_more_than_DZ.sum()

```

```

#####
# the sedimentations of multi-particles through a differential
# element are modeled
####

c = 0
Con = 0
Scon = []

for i in t:
    for h in LPs:
        for v in siTVe:
            if i * v >= uD - h and i * v <= DZ + uD - h:
                c += 1
            Con = Con + np.sum(orgPre[:c])
            c = 0
        Con = Con/totalLyr
        Scon.append(Con)
        Con = 0
#####

# objective function-R square
c = 0
Con = 0
MQScon = []

for i in ts:
    for h in LPs:
        for v in siTVe:
            if i * v >= uD - h and i * v <= DZ + uD - h:
                c += 1
            Con = Con + np.sum(orgPre[:c])
            c = 0
        Con = Con/totalLyr
        MQScon.append(Con)
        Con = 0

s = np.subtract(MQScon,MQCT_SiDNAFe)
mse = (s** 2).mean()
Rmse = np.sqrt(mse)
print(f'MSE in this case : {mse: .3f}')
print(f'RMSE in this case : {Rmse: .3f}')

Rsquared = r2_score(MQCT_SiDNAFe,MQScon)
print(f'R2 in this case : {Rsquared: 3f}')

#Confidence interval
n= len(MQCT_SiDNAFe)
s =np.subtract(MQCT_SiDNAFe,MQScon)

```

```

se = scipy.stats.sem(s)
h = se * scipy.stats.t.ppf(0.975, n-1)

C05= np.add(Scon, h)
C95= np.subtract(Scon, h)

# Draw figures
subttl = f' in size range of {SizeMax:.0f} to {SizeMin:.0f} nm'

ax1 = newfig(title =f' Mean = [-1,4], Standard deviation = [6,1], Skew factor =  

→{skew(pre4org):.3f}, R2 = 0.8045',  

    xlabel='time[h]', ylabel='Relative concentration[C/C0] [%]',  

    ylim=[0,1.05])
#ax1.plot(t, C05, '--')
#ax1.plot(t, C95, '--')
#ax1.fill_between(t, C05, C95, alpha = 0.15, color = 'gray', label =  

→'Confidence level = 95%')

ax1.plot(t, Scon,'r',label = 'fitting curve')
ax1.errorbar(ts, MQCt_SiDNAFe, yerr= STD_SiDNAFe, fmt= 'o',color='b',label =  

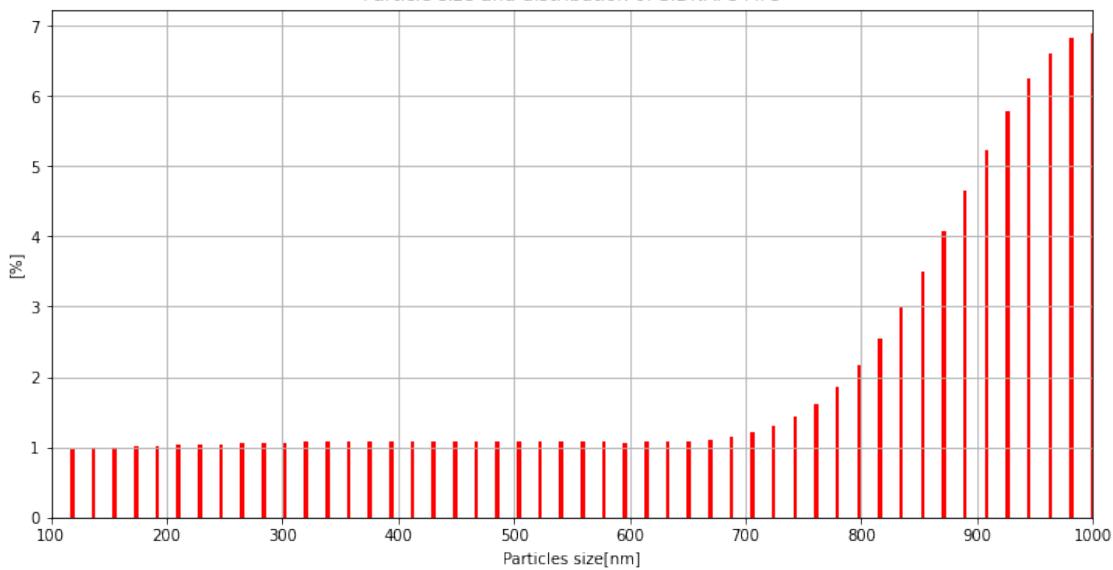
→'data(MQ)')
ax1.legend()
plt.savefig('MQFe.png')

t, pval = scipy.stats.ttest_1samp(s,0)
print(f'T-value:{t:0.2f},P-value:{pval:0.2f}')

```

skew factor =1.783
10% of Distribution: 283.6734693877551
16% of Distribution: 393.8775510204082
50% of Distribution: 853.061224489796
84% of Distribution: 981.6326530612246
90% of Distribution: 1000.0
MSE in this case : 0.018
RMSE in this case : 0.136
R2 in this case : 0.693151
T-value:-0.82,P-value:0.45

Particle size and distribution of SiDNAFe MPs



Number cumulative distribution of SiDNAFe MPs

