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Aggregation and charge reversal of humic substances in the presence of hydrophobic monovalent counter-ions: effect of hydrophobicity of humic substances

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Abstract

To investigate the effect of hydrophobicity of humic substances (HSs) on their charging and aggregation, we studied the electrophoretic mobility and aggregation-dispersion of HSs in the presence of hydrophobic monovalent cations, namely, tetraphenylphosponium TPP^+. The used HSs were standard Suwannee river fulvic acid (SRFA), Suwannee river humic acid (SRHA), and Leonardite humic acid (LHA) with different contents of aromatic carbons. All of the HSs in the presence of TPP^+ showed charger reversal. The charge reversal pH or iso-electric point (IEP) of LHA was higher than that of SRFA and SRHA in every concentrations of TPPCl, demonstrating the strong hydrophobic interaction between HSs, especially LHA, and TPP^+. We also found that the formation of large visible aggregates of all the HSs at lower pH in the presence of TPP^+. Large HS aggregates were markedly formed for LHA, manifesting the existence of stronger hydrophobic attraction among LHAs with TPP^+. The appearance of aggregates of all the HSs with TPP^+ was confirmed by microscopic observation and the size determination by dynamic light scattering. The HS aggregates showed fractal structure. The values of fractal dimension D_f of HS aggregates were 2-2.2 in quiescent conditions, indicating that the HS aggregates with TPP^+ were formed via cluster-cluster aggregation with restructuring. The D_f increased to 2.8-2.9 in stirring conditions, implying that the compact aggregates were formed through the continuous aggregate breakage and the regrowth between smaller aggregates and larger aggregates.

Keywords: humic substances, charge reversal, aggregation-dispersion, hydrophobicity

Introduction

Humic substances (HSs) are considered as one of the most important sources or sink of organic carbons in soil and water environments [1] and play a vital role in nutrient cycling, and the fate and transport of pollutants [2]. The surface active binding sites and highly reactive nature of HSs [3] influence the binding of organic and inorganic contaminants and also affect the bioavailability of metal ions and mobility in soil environment [4]. Some previous studies specially highlight on the structural heterogeneity and the molecular conformation such as supramolecular and self-assembly [5-8], fractal aggregate [9-13], and spherocolloids, [14]. Some studies on the adsorption of humic substances on minerals surfaces [15] and aggregates formation [16] were carried out. The charging and aggregation behaviors of minerals particles and nanoparticles [17-20] with humic substances were also investigated. Nevertheless, there are scanty data on the structure of humic substances aggregates and the charge reversal of humic substances itself as natural colloids. A recent study on humic acid co-precipitation with ferrihydrite discussed about the unlikeliness of the humic acid used as a model colloid, though the use of humic acid as network of linear macromolecules and large colloids is more consistent [21].

Humic substances with Ca^{2+}, lysozyme, surfactants, and polymer can be aggregated to form large visible and settleable aggregates [4, 22, 23], which are crucially important for the fate and separation of HSs with chemicals. Generally aggregation and dispersion of colloidal particles is discussed on the basis of Derjaguin, Landau, Verwey and Overbeek (DLVO) theory, which assumes that the interparticle interaction is given as the sum of van der Waals and electrostatic interactions. The latter interaction is influenced by the electric charge of particles. The aggregation usually occurs at the condition where the net charge becomes zero. Such condition is called charge neutralization detected as an iso-electric point obtained from electrophoresis. The charge neutralization is often realized by the adsorption of appropriate amount of oppositely charged ionic substances such as polymers, surfactants, and proteins. The bindings and adsorption of metal ions depend on different factors such as pH, humic concentration, types of humic substances, ionic strength, temperature, ternary complexes formation, etc [24]. The binding of cationic surfactants to humic acid was observed due to the combined effect of electrostatic and hydrophobic interactions, whereas no notable binding was observed between anionic surfactants and humic acid [25]. In some cases the overdose of absorbable ionic substances induces charge reversal. Screening of electrostatic interaction and charge reversal are observed by the addition of various amounts of polyelectrolyte and oppositely charged ion as influenced by the amount of adsorption. Several recent researches report that the hydrophobic interaction plays an important role in charge reversal and/or charge neutralization for different natural and synthetic colloids and biomaterials [26-31].

In addition to the DLVO interaction, some other forces and interactions such as the hydrophobic interactions [22, 23, 26-31], hydrogen bonding [32, 33], hydration forces [34, 35], and depletion interactions [36] are recognized to play an important role in aggregation-dispersion of colloid particles in different environmental systems. Humic substances from different sources show the aggregation and sedimentation behaviors in the presence of inorganic salts, organic surfactants, proteins and enzymes [22, 23, 27, 37]. Nevertheless, the mechanisms behind the aggregation and charging behaviors of humic substances in the presence of adsorbing ions are still vague. Therefore, the research on the fundamental colloidal behavior of humic substances is still important in the field of natural colloids.

Big hydrophobic ions, such as tetraphenylboron, tetraphenylarsonium, and tetraphenylphosphonium, are used to modify the interfacial properties of clay colloids [38, 39] and to monitor permeability and interaction of the lipid membranes and biological cells [40, 41]. Recently, big hydrophobic tetraphenylboron anions and tetraphenylarsonium cations are also selected as tracers to study the extent of hydrophobicity of typical proteins [42]. While it is inferred that the hydrophobicity of humic substances plays crucial roles in the adsorption and aggregation, the interaction of humic substances with such big hydrophobic ions have never been reported.

In this context, we came up with performing the study on the influence of hydrophobic tetraphenylphosphonium (TPP) cations on the behaviors of humic substances. We found that TPP significantly affects the aggregation and charge reversal of three different humic substances with different hydrophobicity. In this paper, we report the result of the study on the electrophoretic mobility and aggregation behaviors of the humic substances with TPP in different concentration and pH of the solution. To the best of the authors' knowledge, our results are the first mobility data demonstrating the charge reversal of humic substances itself and thus are of novelty. We expect that the results obtained in this research can be generalized to the study of the removal of hydrophobic pollutants and aggregation-dispersion of HSs with hydrophobic organic pollutants such as dyes and drugs.

Experimental

Materials

Three humic substances (Suwannee river fulvic acid, Suwannee river humic acid and Leonardite humic acid) from International Humic Substances Society (IHSS) were used as natural organic matters in this study. These are referred to as SRFA, SRHA, and LHA hereafter. The primary stock solutions of the humic substances were prepared by dissolving solid samples to KOH solution (Wako Pure Chemical Industries) containing base equivalent or more than the amount of carboxylic acid groups of each humic substance. Before dissolving the solid sample to KOH solutions, the supplied samples were oven-dried at 65˚C overnight to reduce moisture. After dissolving solid humic substances to KOH solutions, the suspension (wt. %) were stirred overnight, then the secondary standard solutions were prepared by dilution with deionized water (Elix, Millipore) to a concentration 500 mg/L. The pH values of the secondary standard solutions of humic substances were around neutral (6.5-7.2). The concentration of humic substances (HSs) was maintained at 50 mg/L in every measurement in this study. Some of the parameters of the HSs from IHSS are listed in Table 1. The HSs used in this research were chosen depending on their charge groups and hydrophobicity differences based on their carbon content and aromaticity.

The electrolytes of simple KCl (JIS special grade, Wako Pure Chemical Industries) and hydrophobic tetraphenylphosphonium chloride (TPPCl) (EP grade, Tokyo Chemical Industry Co.) were used to examine the effect of hydrophobic ions. The salt concentrations were from 10 mM to 100 mM. The pH was controlled by using HCl (Wako Pure Chemical Industries) or KOH. Carbonate free KOH solutions were prepared by following the method described in ref. [43]. The salt solutions were filtered (DISMIC 25HP 0.2 μm, ADVANTEC) in every new preparations. All the solutions of this study were degassed under reduced pressure (GCD-051X, ULVAC) to avoid the CO_2 contamination prior to our experiments.

Methods

Electrophoretic mobility measurements

The electrophoretic mobilities of the three humic substances (HSs) were measured in the presence of both KCl and tetraphenylphosphonium chloride (TPPCl) at 20 °C with a Zetasizer Nano ZS apparatus (Malvern Instruments). The electrophoretic mobility measurements were carried out as a function of salt concentration of KCl and TPPCl. The KCl of 10 mM and 50 mM and the TPPCl of 10 mM, 50 mM, and 100 mM were used as a function of pH (3-10). For each condition of salt concentration, the measurements were reproduced and every experiment was done at least two times and we got similar data. For the measurements of electrophoretic mobility, all secondary standard solutions of humic substances were sonicated one-time for 20 minutes before mixing with water, salts (TPPCl) and KOH/HCl. The concentration of humic substances (SRFA, SRHA, and LHA) was maintained at 50 mg/L. The pH values of the suspension of each measurement were checked by a combination electrode (ELP-035, TOA-DKK).

Macroscopic and microscopic observation of aggregation and dispersion

Visual observations of aggregates of humic substances (SRHA, SRFA, and LHA) in TPPCl solutions were performed at 50 mM of TPPCl with 50 mg/L of humic substances of each type as a function of pH for 24 hours. A series of 5 mL suspensions of 50 mg/L humic substances in 50 mM of TPPCl at pH 3-10 was prepared in the prewashed and screw-capped polystyrene bottles and left stand for 24 hours. The pH was controlled by the addition of KOH and HCl. It was hard to definitely control the sharp pH value at each cases of the experimental measurement because of the protonation and de-protonation of humic substances at various pH. The macroscopic pictures and videos (see supporting information) of the suspensions after 24 hours were taken for visual confirmation of the formation of aggregates.

After 24 hours later, the aggregated and dispersed suspensions of humic substances in TPPCl were observed under a microscope for a range of pH from 3 to 9. This microscopic study was performed to evaluate the tentative size and arrangements of aggregates of humic substances with TPPCl salts. The microscope used in this study was Shimadzu BA210E, Moticam 580INT.

Dynamic light scattering

The size of the particles and/or aggregates of humic substances in TPPCl was determined by dynamic light scattering (DLS) technique applying backscattering detection (173° detection optics) at 20 °C with a Zetasizer Nano ZS apparatus (Malvern Instruments). Cleaned disposable cuvettes containing 1 mL of sample were used in every measurement of DLS. The sample suspensions of humic substances with TPPCl in the cuvettes were briefly sonicated for 2 minutes after mixing, and then the measurements for DLS just started after 5 minutes from the mixing. After measurements started, it took times for temperature equilibrium at 20˚C and for the optimization of optical condition. Each measurement consisted of 5 runs and the duration of each run was 10 s.

Aggregate structure analysis

For the aggregates of humic substances (50 mg/L) formed with 50 mM TPPCl, a structural analysis was performed. For the purpose of aggregates structure analysis, the determination of fractal dimension of aggregates was carried out by small angle light scattering using SALD 2300 (SHIMDZU), by which the relation of scattered light intensity I with scattering angle, is obtained. In the medium of suspension, the magnitude of the incident and scattered wave vectors difference of light, a scattering vector, is denoted as Q, and the Q is expressed as the following equation (1)

$$Q = \frac{4\pi n \sin\left(\frac{\theta}{2}\right)}{\lambda} \qquad (1)$$

where n is the refractive index of the suspending medium, θ is the scattering angle, and λ is the wavelength of the laser light in a vacuum. The fractal dimension D_f is determined as the relation of I and Q for scattering aggregates. That is, relation among I, Q and D_f is expressed as (2)

$$I \propto Q^{-Df} \qquad (2)$$

Thus, the log-log scale plot of I against Q, with the power law relationships yields a straight line in a fractal regime. The slope of this straight line is used as fractal dimension D_f in this study. Measurements were carried out after mixing of the suspension at pH 3 as a function of different time intervals. The experiment for aggregates structure analysis was carried out no stirring and upon stirring conditions in a batch cell of SALD 2300 (SHIMDZU) using 12 mL of solutions in every set-up.

Table 1. Some selected composition of the three humic substances reported by IHSS.

IHSS samples	Carbon content % (w/w)	Carboxylic groups (meq/g-C)	Phenolic groups (meq/g-C)	Aromatic carbon (peak area percentages) (165-110 ppm)
SRFA II (Suwannee river fulvic acid)	52.34	11.17	2.84	22
SRHA II (Suwannee river humic acid)	52.63	9.13	3.72	31
LHA (Leonardite humic acid)	63.81	7. 46	2.31	58

Results and Discussion

Electrophoretic mobility in KCl

The electrophoretic mobility of three HSs (SRFA, SRHA, and LHA) in the presence of KCl at various pH values are presented in the supporting information. Electrophoretic mobility is presented with the difference of hydrophobicity (Table 1) of these three humic substances at 10 mM and 50 mM KCl concentrations (see supporting information). All the values of mobility of HS in KCl solution are negative and thus we observe no charge reversal of HSs in the KCl solution.

Electrophoretic mobility of HSs in the presence of TPPCl

The electrophoretic mobility of humic substances in the presence of hydrophobic tetraphenyl phosponium chloride (TPPCl) at the pH range 3-10 in 10 mM, 50 mM and 100 mM of TPPCl concentrations is presented in Fig. 1. We find the obvious charge reversal for all three humic substances (SRFA, SRHA, and LHA) at every concentrations of TPPCl (10 mM, 50 mM, and 100 mM) as demonstrated in Fig. 1. The charge reversal is probably induced by hydrophobic interactions between TPP^+ and HSs as inferred from the result in earlier literature for well-characterized colloidal particles; hydrophobic ions give rise to the charge reversal of hydrophobic colloids [26, 29, 30, 44, and 46]. The results indicate that all the HSs are hydrophobic in nature, while FA is well-dissolved in water at all pH. This is because hydrophobic resin is used to collect FA, meaning that rather hydrophobic fraction of dissolved natural organic matter at all pH can be collected as fulvic acid.

The pH where the charge reversal occurs or the iso-electric point (IEP) of these HSs increases with the increase in TPPCl concentration (Fig. 1). The IEP also shifts towards higher pH values with the increase of hydrophobicity or aromaticity (hydrophobicity: LHA>SRHA>SRFA, see Table 1). At low TPPCl concentration (10 mM), the IEP for SRFA and SRHA is around pH 3 and it is around pH 4 for LHA. The higher the hydrophobicity is, the higher the pH of the charge reversal point is. The result indicates more hydrophobic interaction prevails in the case of LHA with TPPCl than that of SRFA and/or SRHA with TPPCl.

In the presence of TPPCl, the pH range of charge reversal of LHA is more than that of SRFA and SRHA (Fig. 1 A- Fig. 1 C). The IEPs or charge reversal points for LHA are around pH 4, pH 6 and pH 7, which are higher than those of SRFA and/or SRHA at pH around 3, 5 and 6 in 10 mM, 50 mM and 100 mM TPPCl, respectively. Hyuang and Kim [45] reported that HSs with higher content of aromatic groups adsorb more on hydrophobic carbon nanotubes. Thus,

Figure 1 provides the evidence of strong hydrophobicity of LHA. Hakim et al. [26] and Sugimoto et al. [46] suggest that hydrophobic interaction is strong for weakly charged surface. This suggestion is supported by the data in Figure 1. That is, the charge reversal occurs at low pH, where HSs are weakly charged. An investigation on the interaction of humate and cetyltrimethylammonium (CTAB) confirmed the formation of humate-CTAB complex and the occurrence of charge neutralization and charge reversal by electrostatic and hydrophobic interactions [47]. These results also support our study.

Figure 1 shows that the absolute value of electrophoretic mobility decreases with the increase of TPPCl concentrations (10 mM-100 mM) at the pH range (7-10). This reduction could be explained by the compensation of surface charge by the adsorption of TPPCl on humic substances. The charge reversal points, where the positive electrophoretic mobility of HSs reverses to negative mobility increases with the increase in TPPCl concentration from 10 mM to 50 mM. At pH 7-10 at 10 mM and 50 mM of TPPCl, however, the magnitude of mobility decreases. While this reduction is probably related to the change in conformation of HSs and ion distribution around HSs, the quantitative description is still difficult. Many studies reported the zeta potential and electrophoretic mobility of humic substances specifying no inversion of charges in different mono and divalent electrolytes solutions [4, 33, and 48]. The electrophoretic mobility of these three humic substances in KCl solutions measured in this study show no reversal of charges of humic substances (see supporting information), supporting the previous literature. It should be noted that, to the best of the authors’ knowledge, this paper reports the first data on electrophoretic mobility showing the charge reversal of humic substances and concurrent relations with aggregation as a factor of hydrophobic interactions.

Macroscopic and microscopic observations of HSs aggregates formation with TPPCl

We performed the visual observation of aggregation and dispersion of humic substances (SRFA, SRHA, and LHA) in tetraphenylphosphonium chloride (TPPCl) solutions at 50 mM as a function of pH after 24 hours (Photograph 1). We observed large ramified and interlinked aggregates of the HSs with TPPCl (Photograph 1 A, B, C) at pH around 3-5 for SRFA and SRHA and pH around 3-7 for LHA. The stronger network formation of large aggregates of LHAs at low pH can allow aggregates of LHAs withstand sedimentation. The videos of macroscopic observation at low pH confirm the strong interconnections and ramification of the HSs aggregates (see supporting information videos S1 –S5). Smaller size aggregates were also observed in the pH around 6 in the case of SRFA and SRHA. These smaller and mostly deposited aggregates, that were not large and ramified like the pH around 3-5, were more obvious in the case of SRHA. In the case of LHA at higher pH (7-10), smaller settled aggregates were also observed and those were not large ramified and strongly interconnected. Therefore, the sediment is clearly seen. We see clear views showing that the interconnection and size of aggregates decrease with the increase in pH for the HSs-TPPCl suspension. At higher pH values (7-10), the SRFA and SRHA were more dispersed probably due to weak hydrogen bond and weak hydrophobic interaction or the domination of electrostatic repulsion over the attractive interactions with TPPCl and/or humic substances itself. This qualitative observation of aggregates formation has some limitations due to manual handling and observation by naked eye.

These complex and interlinked aggregates are related to the change of electrophoretic mobility with pH as described in the previous section. Avena and Wilkinson [49] pointed out the importance of the hydrophobic interaction and hydrogen bond for the interaction of humic substances. The probable causes behind the formation of large aggregates are due to the existence of hydrophobic interactions [50]. This rather strong connection at low pH (3-5) makes it possible that the aggregates withstand the force of gravity in the medium and the sedimentation is inhibited [51].

The microscopic observation was also performed for the confirmation of behavior and nature of aggregates. In photographs 2, 3, and 4, the structural arrangements and tentative size of the aggregates can be seen. The photographs 2, 3, and 4 show the aggregates formed at different pH from 3-9 in 50 mM TPPCl after 24 hours from the mixing. These microscopic photographs depict the verification of the existence of aggregates with interconnection among the particles in low pH of SRFA and SRHA. The LHA shows aggregation at all pH with a decreasing trend of size with pH, although there were some limitations in the microscopic study. With the increase of hydrophobicity (SRFA<SRHA<LHA), larger and concentrated aggregates appeared from photographs 2 to 4. The photographs 2, 3, and 4 of optical microscopy show some smaller units and some large units at pH around 3, and the large aggregates units look to be formed through the repetition and self-assembly of smaller units. At higher pH value, the SRFA and SRHA show no larger aggregate units in the photographs 2 and 3 even though at higher magnification, although LHA shows large aggregates in the higher range of pH. This result indicates the higher hydrophobicity triggered the aggregation. The repetition and rearrangements of the aggregates manifest the hierarchical architecture of the aggregates reported in the previous study [4, 9]. Some authors found similar structural natures and arrangements of humic acid aggregates with $CaCl_2$ in different magnification of optical microscope [52] and transmission electron microscopy studies. During the microscopic observation of the aggregates in this research, we observed the motion of some aggregates as a moving unit by Brownian motion of the aggregates. Such motion was also reported in the earlier study [4]. The motion of the aggregates can also be due to fluid flow and sedimentation due to gravity in the medium.

Dynamic light scattering (DLS) and aggregates size

To characterize the pH dependence of the aggregation, the DLS measurements of HSs aggregates in TPPCl solutions were carried out. Figure 2 shows that the hydrodynamic Z-average diameter of HSs is around 1200-1600 nm at pH 3-4 for SRFA and SRHA with a single discrete value of Z-average diameter higher than this and at pH 3-5 for LHA, indicating that the formation of HS aggregates in the presence of TPPCl. The DLS results are consistent with those by the visual observation described above in the presence of TPPCl. Generally, the aggregation of colloidal particles occurs around IEP. Our results, however, deviate from this general rule. Probably, hydrophobic interaction is the driving mechanism of TPP-induced aggregation. That is, the presence of TPP^+ ions induces the aggregation of HSs by increasing the hydrophobicity of HSs, and thus enhances the formation of HS aggregates through the increased tendency of HSs escaping from the water. The largest aggregates size around 2300 nm was found for LHA at pH 3-4. At every pH values, the sizes of aggregates of LHA with TPPCl are larger than that of SRFA and SRHA with TPPCl. The DLS results manifest the highest hydrophobicity and the evidence of stronger hydrophobic interaction for LHA. On the contrary, the aggregates size of HSs with 50 mM TPPCl decreases with increasing pH in every type of HSs (SRFA, SRHA, and LHA) as expected from the visual observation. At low pH, less ionization of surface charging groups, the COOH and –OH causes more hydrophobic interaction between HSs and TPPCl. Furthermore, at low pH, the HSs aggregates are formed by hydrophobic interaction, hydrogen bonding, and probably some charge-patch attraction. At high pH, the electrostatic repulsion and weak hydrophobic interaction among HSs predominate and thus hinder the formation of the larger size of aggregates. In addition we focused on the formation of aggregates and concentrated the effect of hydrophobicity which provoke us to use different HSs with varying hydrophobicity regardless the size of primary humic substances. It was difficult to maintain the same size of primary particles varying with hydrophobicity in this investigation. The decrease of the sizes of aggregates with pH indicating the electrostatic repulsion at higher negative charge with increasing pH, lower aggregates size and less hydrophobic interactions. The Z- average diameters of different humic substances with pH variation in hydrophilic salts were studied in the previous researches conducted by different groups. They found aggregates of humic acids larger than 1000 nm of Z-average diameter at low pH [33, 53]. Another group found aggregates of humic acids larger than 1000 nm of average hydrodynamic radius in NaCl and $MgCl_2$ at different concentration and time intervals in pH around 3.6 and 7.1 [54]. The previous study [33] shows the decreasing tendency of size at high pH due to the hindering of the H-bond and increasing of the electrostatic repulsion. The larger size at low pH is probably due to the reduction of repulsion and the intermolecular H-bonding. All our results confirm that the hydrophobic counter-ions TPP^+ induce the aggregation of humic substances and the degree of hydrophobicity influences the degree of aggregation of HSs, LHA > SRHA > SRFA. It should be noted here that the Z-average diameter of aggregates at low pH in this investigation shows higher values in DLS other than the previous investigation preformed in monovalent hydrophilic electrolytes solutions refer to a hydrophobic interaction for larger aggregation.

Fractal dimension and aggregates structure analysis

The temporal change in fractal dimension D_f of the aggregates of HSs in 50 mM TPPCl at pH 3 is shown in Fig. 3, where the effect of stirring is also demonstrated. In every cases, the fractal dimension with stirring shows higher values than that of non-stirring condition. Clear dependence of D_f on the type of HSs is not found. The values of fractal dimension of HSs are 2-2.31 at no stirring. On the contrary, the values of D_f of SRFA, SRHA and LHA are raised to 2.8, 2.88, and 2.87 at stirring condition (Fig. 3). The D_f values without stirring are reasonable compared to the previous study. The fractal dimension of simulated flocs formed through diffusion limited cluster-cluster aggregation is around 1.8, while the D_f around 2.1 can be obtained by reaction limited cluster-cluster aggregation with restructuring [55]. From the comparison of the simulation results with our data, HSs flocs with TPP are formed through weaker contact forces allowing restructuring even at most aggregated state at low pH.

The values of fractal dimension with stirring are relatively higher than that by previous studies for aggregates such as kaolin with alum [56], natural organic matters such as humic acid with chitosan coagulants and Al species [57]. The Fe precipitates flocs showed a D_f value 2.6 after breakage [58], a modelling of viscosity for coagulated suspension showed D_f values around 2.2 - 2.6 [59], and a comparative study of fractal dimension of humic acid flocs and clay flocs showed higher fractal dimension of humic acid flocs (D_f around 2.7) than that of clay flocs [60]. In this study, we found the higher value of D_f, indicating the more compact structure of aggregates due to the stirring. Studies revealed that the polydispersity of primary particles and aggregates cluster of TiO_2 aerosols, polystyrene latex, some polymers, and hematite particles may have an impact on the compactness and subsequent fractal structure [61-63]. From this situation in our case we measured the fractals at a fixed pH around 3, though HSs are not totally monodisperse and this has some effect on fractals structure by influencing the power law relation in a largely overlapping scattering exponent regimes between scattering wave vector and scattering intensity. Considering this effect of polydispersity the fractals has somehow influenced and had a higher value observed due to the presences of small and large aggregates and particles in stirring condition. Hoekstra et al. [64] reported that D_f of 2.7 was obtained for flocs formed in reaction limited regime under shear. Torres et al. [65] showed that D_f increased to 2.9 through the collision between a single particle and a cluster in a shear flow. Some recent researches reported high D_f value around 2.75 of chitosan coagulant for different aluminium species in humic acid treatment [57], and D_f value almost 3.0 for polystyrene latex floc induced by $FeCl_3$, NaCl and $Al_2(SO_4)_3$ and found the increase of fractal dimension with salt and particle concentrations [66]. The comparison of our data with previous studies suggests that the repeated collision between the smaller HS aggregates and larger HS aggregates during the breakage and re-growth under stirring produces TPP-induced HSs flocs with higher values of the fractal dimension. This formation of aggregates may be due to the cluster-cluster aggregation [56] with restructuring and/or penetration of small cluster or broken aggregates fraction into the large aggregates structure.

The aggregation between a small cluster and large cluster, which can often happen in sheared suspension at high particle concentration, might result in higher D_f. This is one of the possible mechanisms of the present high D_f value. The penetration is also stimulated by soft permeable structure [48] of humic substances. The aggregation of humic substances in stirring condition caused by hydrophobic interaction and/or by charge neutralization with TPPCl undergoes continuous collisions by fluid flow. Also the flow causes frequent collision and irreversible penetration of some smaller aggregates in the permeable porous structure of HSs aggregates. These processes make the HSs aggregates more compact in structure, and thus flocs formed in shear flow have the compact structure [64, 67].

Conclusion

We found that the obvious charge reversal of all the humic substances (HSs) occurs in the presence of hydrophobic cation TPP^+ and the iso-electric point (IEP) depends on the hydrophobicity of HSs and TPP^+ concentration. The HSs form large visible aggregates in TPPCl solution at low pH for SRFA and SRHA, and in all pH for LHA. The size of LHA aggregates is also larger than those of SRFA and SRHA. The HS aggregates formed with TPP^+ have large size and fractal structure. This new findings of charge reversal and formation of large HS aggregates in the presence of hydrophobic counter ion will definitely help us to partly understand and may unfold a new arena of future research of humic substances. Our findings are available to predict and depict the fate of various hydrophobic ions such as dyes and medicines in soil and water environment.

Conflict of interest

The authors declare that they have no conflict of interest associated with this article.

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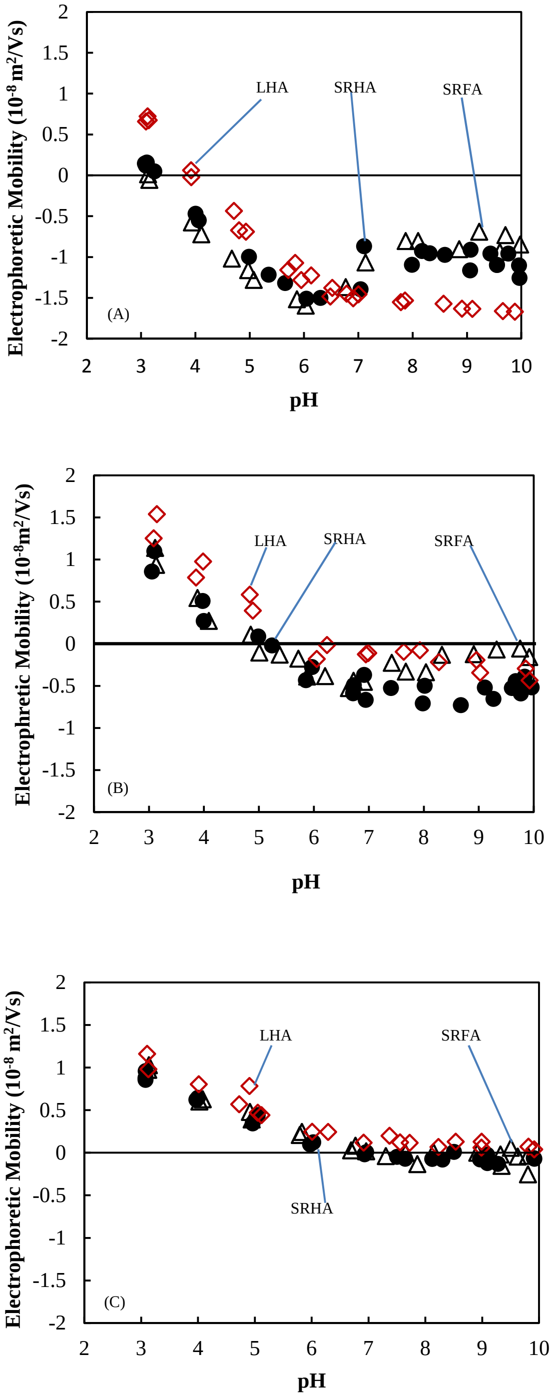
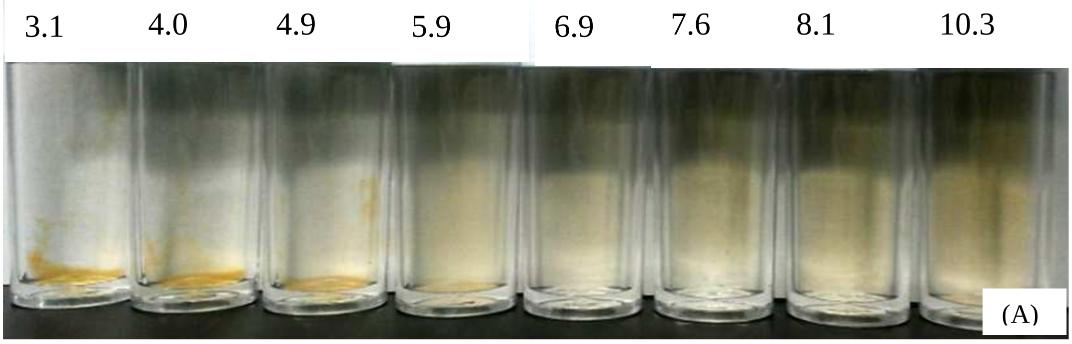
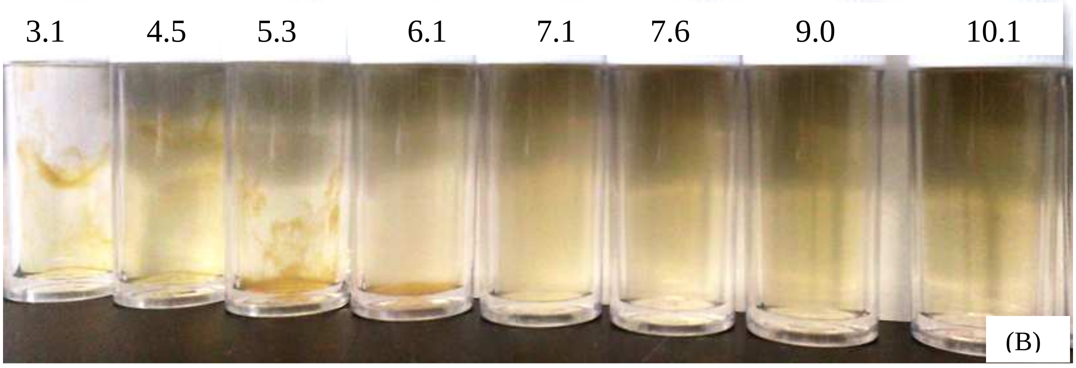


Figure 1. Electrophoretic mobility of Suwanne river fulvic acid (SRFA), Suwannee river humic acid (SRHA) and Leonardite humic acid (LHA) as a function of pH with tetraphenylphosphonium chloride (TPPCl) 10 mM (A), 50 mM (B), and 100 mM (C). Concentration of humic substances (SRFA, SRHA and LHA) is 50 mg/L. Symbols: SRFA (Δ), SRHA (●), and LHA (◇).

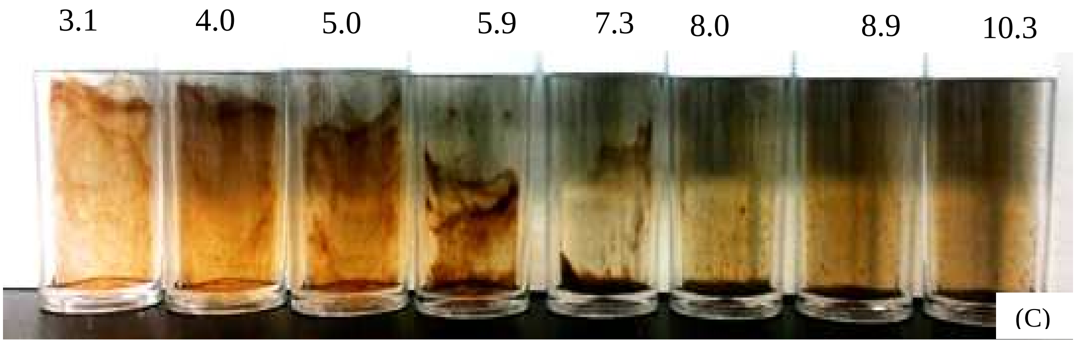
SRFA_TPPCl_50mM (24 hours)



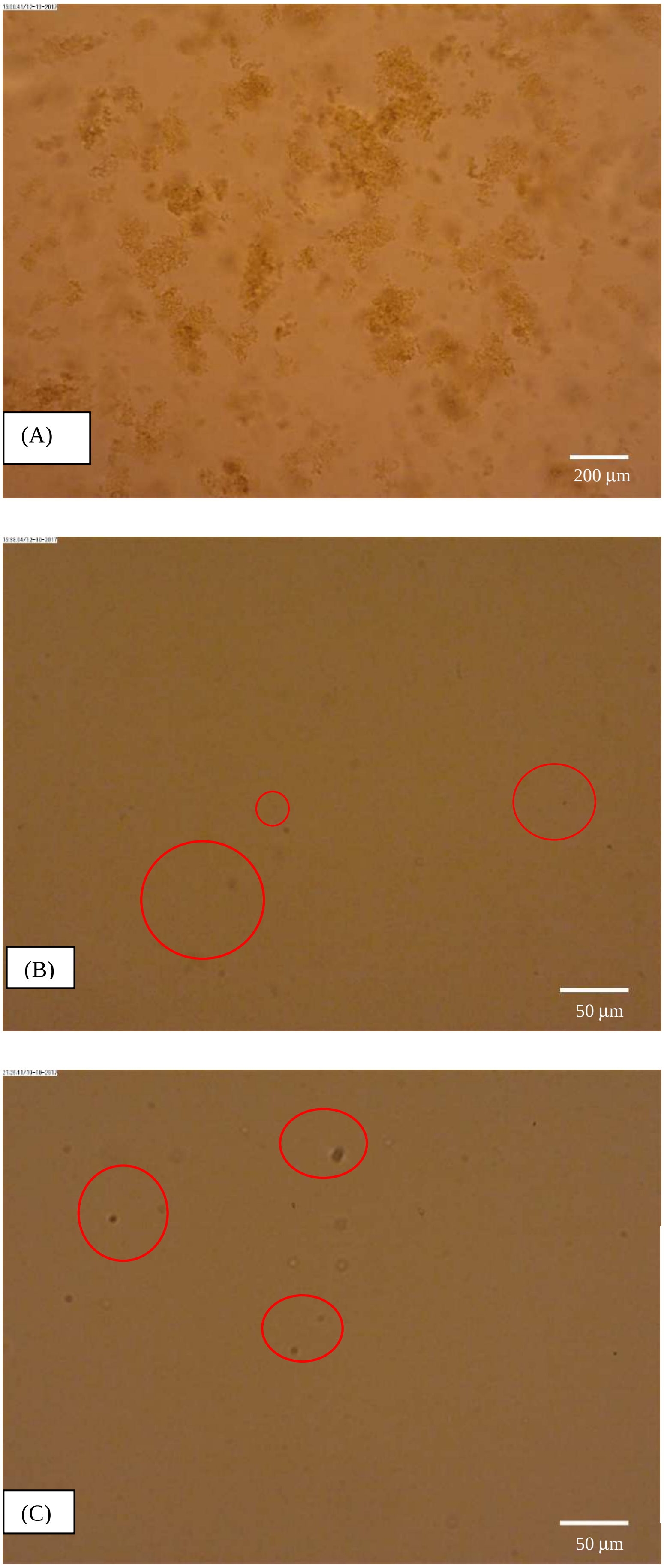
SRHA_TPPCl_50mM (24 hours)



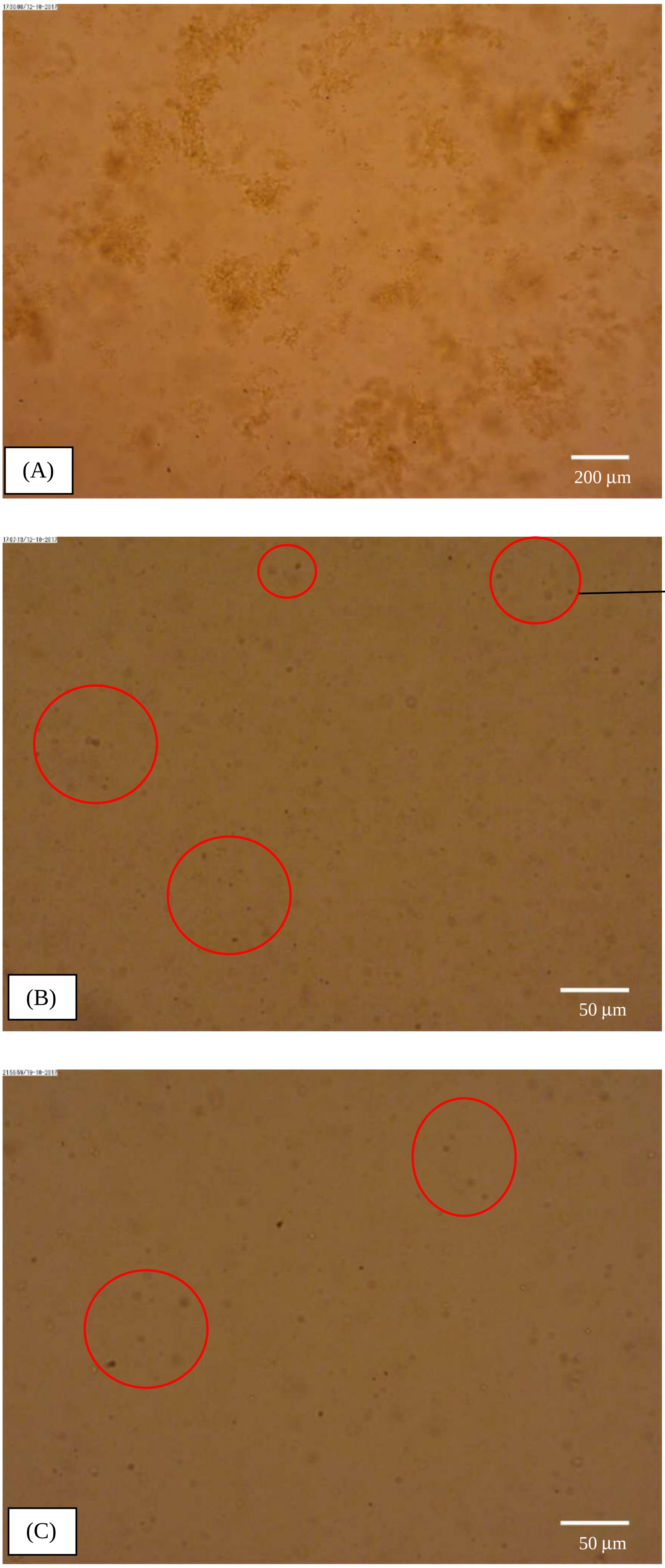
LHA_TPPCl_50mM (24 hours)



Photograph 1. Macroscopic view of aggregation and re-dispersion for humic substances (SRFA, SRHA, and LHA) in tetraphenylphosphonium chloride (TPPCl) at 50 mM as a function of pH after 24 hours. Photos representing Suwannee river fulvic acid (SRFA) with TPPCl (A), Suwannee river humic acid (SRHA) with TPPCl (B), and Leonardite humic acid (LHA) TPPCl (C). Photo colour was adjusted by using GIMP 2.8.22

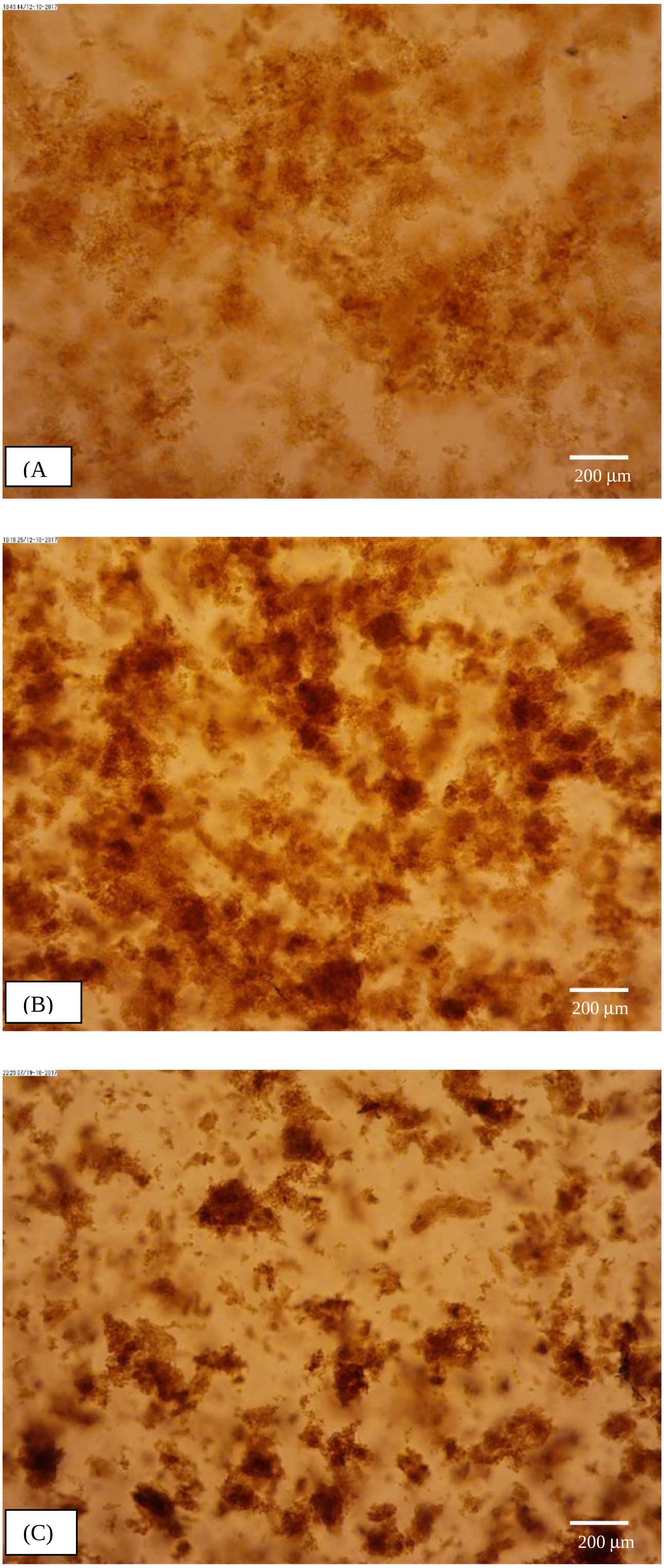


Photograph 2. Microscopic photographs of flocs in Suwannee river fulvic acid (SRFA) with tetraphenylphosphonium chloride (TPPCl) 50 mM at pH 3.1 (A), pH 6.9 (B), and pH 8.9 (C). Scale bar is 200 µm (A), 50 µm (B), and 50 µm (C).



Some small aggregates and particles are disperse in the suspension. Which indicate the aggregation is induced by tetraphenylphosphonium chloride (TPPCl) at high pH but those are very small in size compared to low pH

Photograph 3. Microscopic photographs of flocs in Suwannee river humic acid (SRHA) with tetraphenylphosphonium chloride (TPPCl) 50 mM at pH 3.2 (A), pH 7.1 (B), and pH 8.8 (C). Scale bar is 200 µm (A), 50 µm (B), and 50 µm (C).



Photograph 4. Microscopic photographs of flocs in Leonardite humic acid (LHA) with tetraphenylphosphonium chloride (TPPCl) 50 mM at pH 3.2 (A), pH 6.8 (B), and pH 8.9 (C). Scale bar is 200 µm.

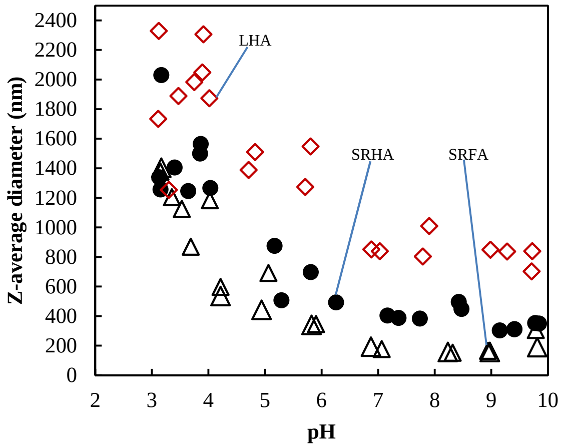


Figure 2. Z average diameter as a function of pH for Suwanne river fulvic acid (SRFA), Suwannee river humic acid (SRHA) and Leonardite humic acid (LHA) in 50 mM tetraphenylphosphonium chloride (TPPCl). Concentration of humic substances (SRFA, SRHA and LHA) is 50 mg/L. Symbols: SRFA (∆), SRHA (●), and LHA (♢).

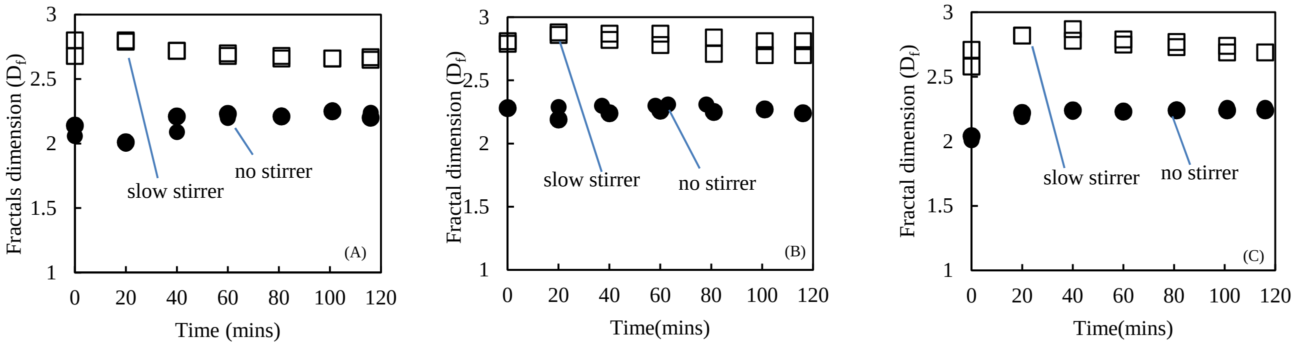


Figure 3. Change in fractal dimension with time in slow stirrer and no stirrer condition of Suwannee river fulvic acid (SRFA) flocs (A), Suwannee river humic acid (SRHA) flocs (B), and Leonardite humic acid (LHA) flocs (C) in 50 mM tetraphenylphosphonium chloride (TPPCl) at pH around 3. Concentration of humic substances (SRFA, SRHA and LHA) is 50 mg/L. Symbols: (□) slow stirrer, and (●) no stirrer