**Effect of water chemistry on the aggregation and photoluminescence behavior of carbon dots**

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**ABSTRACT**

Carbon dots are rapidly emerging carbon-based nanomaterials that, due to their growing applications, will inevitably find their way to natural waters; however, their environmental fate is mostly unknown. Carbon dots with different surface functionality were fabricated and characterized by TEM and FT-IR. Their surface charge, given by the zeta potential, and their hydrodynamic diameter in suspension were investigated under a variety of environmentally relevant conditions. The effect of ionic strength was studied in the presence of monovalent (NaCl) and divalent (CaCl₂) cations, for pH levels from 3 to 11; humic acid was used as a model for dissolved natural organic matter. Total potential energies of interactions were modeled by classical DLVO theory. The experimental results showed that water chemistry altered the surface charge of the nanomaterials, but their hydrodynamic size could not be correlated to those changes. Evidence of specific interactions was found for the amino functionalized particles in most cases, as well as the plain carbon dots in the presence of Ca²⁺ and humic acid. Nanoparticles remained largely stable in suspension, with some exception at the highest ionic strength considered. DLVO theory did not adequately capture the aggregation behavior of the system. Moreover, cation and/or humic acid adsorption negatively affected the emission intensity of the particles, suggesting limitations to their use in natural water sensing applications. The particular stability shown by the carbon dots results in exposure to organisms in the water column and the possibility of contamination transported to significant distances from their source.

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**Introduction**

Carbon dots (CDs) constitute a class of quasi-spherical, photoluminescence (PL) carbon nanoparticles with sizes below 10 nm (Baker, and Baker, 2010). Their easy fabrication and presumably negligible environmental impact make them an attractive alternative to inorganic quantum dots (Li and Kaner, 2008; Rao et al., 2009). Early studies have shown that CDs are biocompatible and less cytotoxic than Cd-based quantum dots and may even inhibit cancer cells (Qu et al., 2012; Song et al., 2013; Hsu et al., 2013). These fluorescent nanoparticles have been successfully utilized in biomedicine, optoelectronics, catalysis, and chemical sensors (Wang and Hu, 2014). Their application in the environmental field is particularly promising. Semiconductor quantum dots (SQDs) and graphene quantum dots (GQDs) can be distinguished within the large group of carbon-based nanoparticles since SQDs and GQDs are perfect spherical nanocrystals of metallic...
atoms and nanosheets of sp² carbons, respectively, while the other types of CDs have an undefined carbogenic structure, being in some situations a combination of crystalline structures resembling graphene layers combined with amorphous carbon. Carbon particles exhibiting a crystalline core based on a mixture of sp² and sp³ carbons are termed carbon quantum dots (CQDs) while those composed of a mixture of primarily sp³ carbons in a disordered structure carbon nanodots (CNDs) (Cayuela et al., 2016).

The PL properties of CDs vary sensitively with size, and emissions are red-shifted with increasing size (Wang and Hu, 2014). At present, the PL mechanism of CDs is still under debate (Zhu et al., 2015), although different researchers have attributed their emission properties to quantum confinement effect (Li et al., 2010), surface states determined by hybridization of the carbon backbone and the connected chemical groups (Fang et al., 2011; Hsu et al., 2009), fluorophores with different degrees of p-conjugation (Mao et al., 2010; Bourlinos et al., 2012), and the recombination of electron–hole pairs localized within small sp² carbon clusters embedded within a sp³ matrix (Srivistava and Gajbhiye, 2011). Furthermore, the PL can be tuned via surface modification or via electron/energy transfer, and numerous capping strategies have been reported (Wang and Hu, 2014).

CDs characteristics can be tuned by the choice of precursors during synthesis, surfac passivation and functionalization. Two types of synthesis methods, top-down and bottom-up, have been reported (Esteves da Silva and Gonçalves, 2011). The top-down methods involve cutting carbon resources, most commonly using concentrated oxidizing acid (HNO₃ or H₂SO₄/HNO₃ mixture) (Tao et al., 2012). The bottom-up synthesis methods consist of thermal carbonization and acid dehydration of suitable molecular precursors (Liu et al., 2012); passivation and/or the functionalization may be required to create highly PL CDs with tunable fluorescent features (Cayuela et al., 2015). CDs possess numerous surface reactive groups, that offer the chance for surface modifications. Passivation can enhance the quantum yields (QYs) of the CDs, changing the PL emission and meeting the requirements of selected applications (Zhu et al., 2015). Different strategies have been used to functionalize CDs with different types of molecules; for instance, macromolecules, chelating ligands, specific oligonucleotides, and metal ion or metal-mediated DNA binding for heavy metal detection (Vázquez-González and Carrillo-Carrión, 2014; Cayuela et al., 2016). Acid oxidative treatment, typically using HNO₃, can introduce carbonyl functionalities at several carbon surfaces. These groups enhance the CDs water solubility, which is very important for biologically applications. Furthermore, the PL can be increased significantly by surface passivation agents, as for example, amino-terminated reagents (Baker, and Baker, 2010).

As interest for both fundamental research and applications has surged dramatically in the last few years, it is inevitable that CDs will find their way to natural water sources where, unlike the more well studied fullerenes and carbon nanotubes, their environmental impact and fate is mostly unknown. The presumed negligible environmental impact of CDs can only be confirmed if their behavior in natural systems is fully understood. However, the colloidal stability of CDs in environmentally relevant aqueous suspensions has not been investigated, and neither the effect of those conditions on their PL properties.

Due to their small size and large surface area, adsorption processes are expected to play a key role in determining the physicochemical properties of CDs (Chowdhury et al., 2013). Adsorption may alter the surface characteristics of the nanoparticles so that they become dispersed and thus, mobile in aqueous media (Lanphere et al., 2013). Natural organic matter, e.g., humic acid (HA), and exopolymeric substances, e.g., proteins and polysaccharides, have been shown to adsorb onto carbon nanotubes and act as stabilization agents (Saleh et al., 2010); these macromolecules can potentially interact with CDs surfaces by π–π bonding and hydrophobic effects, due to the presence of C=C double bonds as well as aromatic rings (Dimiev et al., 2013).

CDs are well known for their colloidal stability in water suspensions at low ionic strength (IS); their PL is stable under those conditions but generally expected to decline if aggregation occurs. Other matrix chemistry effects have been reported. For example, their fluorescence intensities were shown to be pH-dependent, decreasing with increasing pH (Jelinek, 2017). Furthermore, a study showed no significant change in fluorescence quenching of CDs by HA up to a concentration of 10 ppm (Cayuela et al., 2015), while another study reported that the quenched fluorescence intensity of surface functionalized QDs was linearly proportional to the concentration of Hg²⁺ under optimum conditions (Vázquez-González and Carrillo-Carrión, 2014).

In summary, there is extensive literature on inorganic nanoparticle stability in natural waters, as well as for some common carbon-based particles such as carbon nanotubes, fullerenes and a few studies on graphene oxides, but the aggregation and surface properties of CDs under environmental conditions have not been investigated. In view of the increasing amount of research devoted to CDs applications for sensing and imaging, an understanding of the colloidal stability is needed as a first step towards the prediction of environmental fate, transport and potential toxicity of these nanomaterials. It has been recognized that our knowledge on nanoparticle environmental behavior needs to be continuously revised and extended to account for new materials or novel commercial applications as they emerge, in order to accurately assess the associated risks (Troester et al., 2016).

The objective of this work is to investigate for the first time the colloidal stability of CDs, an emerging carbon nanomaterial, in natural water conditions (i.e., variable salinity, pH, dissolved organic matter) and the effect of the transformations they undergo under such circumstances on their fluorescence properties. Two types of CDs were considered: as obtained from thermal treatment of organic molecules, and surface functionalized, to represent differences in their surface chemistry. The particles were synthesized and characterized with respect to size and surface chemistry. Aggregation was investigated under environmental relevant conditions and DLVO theory was applied to the modeling of particle stability.

1. Materials and methods

1.1. Synthesis

Unfunctionalized or plain CDs were synthesized following a modification of a previously published method (Wang et al.,
A 70% (W/W) glycerol solution (Sigma, > 99.5%) was mixed with a 20% (W/W) solution of NaH₂PO₄ (Fisher Scientific) in a 10:1 volume ratio. The solution was heated for 20 min at 900 W power in a domestic microwave. The product was allowed to cool to room temperature and the CDs resuspended by addition of water and continuous stirring until a homogenous brown colored suspension was obtained.

Amine capped CDs (a-CDs) were fabricated following a procedure described by Zheng et al. (2014). Briefly, 2.10 g of a 10 mmol solution of citric acid (Sigma-Aldrich, ACS reagent, ≥99.5%) was added to 10 mL of branched polyethyleneimine ethylenediamine (BPEI) (Sigma-Aldrich) with an average molecular weight of 800 Da, as reported by the manufacturer. The reaction was heated to 170°C in a silicone oil bath (Fisher) for 2 days for purification. Water was replaced every 6 hr. Finally, CDs and a-CDs were freeze-dried.

1.0 kDa) against ultrapure water (specific resistance 18 MΩ-cm) cooled to room temperature followed by addition of deionized water.

The reaction was heated to 170°C in a silicone oil bath (Fisher) for 2 days for purification. Water was replaced every 6 hr. The yellow colored products were naturally cooled to room temperature followed by addition of deionized water.

The resulting solutions of both CDs and a-CDs were dialyzed with a Biotech CE dialysis membrane (Spectrum Labs, MWCO 1.0 kDa) against ultrapure water (specific resistance 18 MΩ-cm) for 2 days for purification. Water was replaced every 6 hr. Finally, CDs and a-CDs were freeze-dried.

1.2. Characterization

The surface functional groups of the synthesized materials were investigated by Fourier-transform infrared spectroscopy (FT-IR) in a Nicolet 4700 FT-IR spectrophotometer (ThermoScientific) and the spectra collected over a wavenumber range from 400 to 4000/cm.

CDs and a-CDs were imaged by transmission electron microscopy (TEM) in a Tecnai F30 Twin transmission electron microscope (FEI). TEM images were then analyzed using the software ImageJ to obtain the particle size distribution (Schneider et al., 2012). In brief, the TEM images were selected and imported to ImageJ, then the particles on the images were segmented by adjusting the threshold value and sizes were analyzed by the software. At least 400 particles were measured for each type of nanoparticle.

Hydrodynamic diameter (Dh) and zeta potential were measured with a ZetaSizer Nano ZS (Malvern Instruments, Worcestershire, UK), using a monochromatic coherent He–Ne laser with a fixed wavelength of 633 nm. Laser Doppler velocimetry was applied to characterize the electrophoretic mobility (EMF) of the nanomaterials in the various electrolyte solutions. Measured EMFs were converted to zeta potential using the Smoluchowski equation (Elimelech et al., 1995).

\[ U = \frac{\eta \varepsilon}{\mu} \]  

where U is the EPM, ε is the dielectric constant of the solution, μ is its viscosity, and ζ is the zeta potential. Disposable folded capillary cells were employed.

The hydrodynamic properties of CDs were measured under a wide range of solution compositions, including monovalent and divalent electrolytes, represented by NaCl (reagent grade, Acros Organics) and CaCl₂ (reagent grade, J.T.Baker) respectively. HA (technical grade, Acros Organics) was introduced as a model of natural dissolved organic matter (NOM). The pH of the solution was adjusted by adding HCl, (10 mmol/L, Fisher Scientific) or NaOH (10 mmol/L, Fisher Scientific) solutions.

UV-visible adsorption spectra for CDs and a-CDs were recorded using UV-vis spectrophotometer (Lab Tech, UV 8100B, USA) with scanning range 200–600 nm. PL was measured on a Hitachi F-4500 Fluorescence Spectrophotometer, equipped with a 150 W Xenon lamp. Emission scans were performed at an excitation wavelength of 350 nm. The slit width was 5 nm and scans were conducted in an emission wavelength range from 300 to 700 nm at a speed of 1200 nm/min. Concentration of a-CDs were kept constant at 200 mg/L for all absorption and fluorescence experiments; CDs concentrations were 1600 mg/L for adsorption and 52,000 mg/L for fluorescence experiments.

1.3. DLVO modeling

The total interaction energy between particles as they approach each other (V_TOTAL) was calculated to determine the aggregation process, as described by the traditional DLVO theory. According to this theory, the stability of colloidal particles in aqueous environments is determined by the net effect of Van der Waals interactions (V_dW) and electrical double layer interactions (V_EMF):

\[ V_{TOTAL} = V_{EDL} + V_{vdw} \]  

where a (nm) is the particle radius; h (nm) is the surface-to-surface distance between two particles; \( A_{vdw} \) is the Hamaker constant, \( 6.26 \times 10^{-20} \text{ J} \) was employed in this study (Feriancikova and Xu, 2012), and λ is the characteristic wavelength of the dielectric and usually assumed to be 100 nm.

Electrostatic double layer interactions were calculated using the following equations (Eqs. (4)–(6)):

\[ V_{EDL} = 2\pi a_0 \epsilon_0 \psi^2 \ln[1 + \exp(-kh)] \]  

\[ n_s = 1000 \times N_A \times C_s \]  

\[ \gamma = \frac{Z e \psi}{4 k T} \]  

where \( a_0 \) (nm) is the radius; h (nm) is the surface-to-surface distance between two particles; \( n_s \) is bulk number density of ions which is calculated according to Eq. (5) where \( C_s \) (mol/dm³) is the electrolyte molar concentration, and \( N_A \) (atoms/mol) is Avogadro’s constant; \( k \) (J/K) is Boltzmann constant; and \( T \) (K) is absolute temperature.

The parameter \( \gamma \) is calculated according to Eq. (6), where Z is the charge of the ions; \( \psi \) (V) is the experimental zeta potential; \( e \) (Coulombs) is the electron charge; and \( K \) (1/m) is the Debye length as calculated below:

\[ k = 2.32 \times 10^9 \sqrt{\sum q^2} \]
where $C_i$ (mol/m$^3$) is the ion concentration; and $z_i$ is the valence of ion $i$ including sign of charge. The application of Eq. (5) is limited to for $h \ll a_p$ and $K_{ap} > 5$.

2. Results and discussion

The size, morphology and structure of the nanoparticles were investigated by TEM; the corresponding images of a-CDs and CDs are shown in Fig. 1a and c. The image analysis (Fig. 1b and d) revealed an average particles size of 5.1 nm for a-CDs and 1.8 nm for CDs with size ranges between 1 to 9 and 1 to 3 nm respectively, which is in good agreement with previous reports in the literature (Wang et al., 2011). Fig. 2a and b represents High Resolution Transmission Electron Microscopy (HRTEM) images for CDs and a-CDs respectively. The images reveal the lattice spacing of 0.32 nm for both type of CDs, which agree with (002) spacing of graphitic carbon (Tang et al., 2013).

The surface chemistry was investigated by FT-IR (Fig. 3). The FT-IR spectrum of CDs (Fig. 3b) showed the existence O–H, C–C, epoxy groups at the positions of 3223, 1450 and 1058, respectively. In contrast, inspection of the a-CDs spectrum suggests abundant presence of amino groups on the surface (Fig. 3a). The stretching vibrations of N–H at 3286/cm, C–H at 2958/cm and 2819/cm were observed in the high wavenumber region. Two strong peaks at 1658 and 1570/cm contributed to the amide linkage formed. The adsorption bands of C=C, C=N, C==O and epoxy groups were identified at 1466, 1362, 1300, 1111/cm, respectively.

As prepared, both nanoparticles formed stable colored suspensions in ultrapure water due to their highly hydrophilic nature. The resulting pH was 5.7 and 9.8 for the CDs and a-CDs suspensions, respectively. This result indicates that the basic functional groups were exposed on the surface of the nanoparticles. Under these conditions, their hydrodynamic diameter was determined to be $1.1 \pm 0.4$ nm (CDs) and $1.9 \pm 0.1$ nm (a-CDs) and their surface charge, given by the zeta potential, was $-11.4 \pm 3.5$ (CDs) and $6.9 \pm 0.6$ mV (a-CDs). It is important to note that the mean particle size measured by dynamic light scattering (DLS) in solution was smaller than the TEM based determination. DLS size measurements are usually expected to

![Fig. 1](image-url)

Fig. 1 – (a), (c) Representative transmission electron microscopy image of amine capped carbon dots and plain carbon dots: insets represent the segmented particles after subtracting the background using ImageJ software. (b), (d) Size distribution of amine capped carbon dots (a-CDs) and carbon dots (CDs), as percent number of particles.
yield larger values than those from microscopy images, given that they represent hydrodynamic diameters, which led to the conclusion that there was some degree of aggregation during the drying stage in the preparation of TEM samples, even though extreme care was exercised to avoid it with highly diluted particle suspensions and different evaporation methods. As sample drops were deposited on the carbon mesh, CDs possibly accumulated in one spot, producing an image compatible with larger particle size.

The combined effect of small particle size and surface charge was responsible for the easy dispersion and remarkable stability of CDs suspensions. Moreover, suspensions prepared in the laboratory remained free of sediments for as long as 2 months.

2.1. Influence of IS

Fig. 4 summarizes the effect of IS and electrolyte type (NaCl, CaCl2) on zeta potential of CDs and a-CDs. CDs were negatively charged for all NaCl and CaCl2 concentrations considered; in contrast, a-CDs exhibited positively charged surfaces. Fig. 4a shows that zeta potential values of CDs became less negative from −11.4 ± 3.5 to −3.9 ± 0.5 mV, as IS of the NaCl solution increased from 1 to 100 mmol/L. This less negative surface charge can be attributed to the electrical double layer compression, as predicted by classical colloidal theory (Verwey, 1947; Elimelech et al., 1995). As IS increases and the thickness of the electrical double layer diminishes, a faster decay of the potential energy of interaction with distance from the surface occurs, placing the shear plane, where zeta potential is measured, at a location of lower absolute potential value. In contrast, IS variation caused a moderate zeta potential increase for the already positively charged a-CDs, followed by a decrease at higher electrolyte concentrations. The zeta potential values of a-CDs increased from 5.4 ± 0.6 to 15.4 ± 0.6 mV, as IS (NaCl) increased from 1 to 10 mmol/L and decreased to 8.64 ± 0.6 mV at 50 mmol/L level. The effect was more noticeable for Na+ (monovalent) than Ca2+ (divalent) cations. This observation suggests the occurrence of specific interactions between the dissolved electrolytes and the nanoparticles, that gave rise to an apparent higher surface charge; however, this phenomenon

Fig. 2 – (a), (b) Representative high resolution transmission electron microscopy images of a-CDs and CDs respectively with lattice parameters of 0.32 nm.

Fig. 3 – Fourier-transform infrared spectroscopy spectra of: (a) a-CDs, (b) CDs.
was overcome by the double layer compression at higher IS conditions, leading to a reduced magnitude of the zeta potential.

Fig. 5a shows a significant increase of CDs hydrophilic diameter ($D_h$) from 1.1 ± 0.5 to 533.2 ± 23.1 nm suspended in NaCl solutions. The high $D_h$ at 100 mmol/L is in coincidence with the low absolute zeta potential value. The presence of NaCl did not affect the $D_h$ of a-CDs appreciably as shown in Fig. 5b and the $D_h$ remained approximately constant around 2 nm.

In most natural freshwater bodies, IS is below 10 mmol/L (Chowdhury et al., 2013), which indicates that aggregation is not expected for CDs in those aquatic environments; however, seawater and groundwater conditions may lead to substantial size increase.

The zeta potential and hydrodynamic characterization of CDs, and a-CDs in the presence of a divalent cation (Ca$^{2+}$) are shown in Figs. 4b and 5b, respectively. Ca$^{2+}$ influenced both zeta potential and hydrodynamic diameter more noticeably than the monovalent Na$^+$. Fig. 4b shows that zeta potential values of CDs are significantly altered by Ca$^{2+}$, as they became less negative from $-14.8 \pm 0.7$ to $-0.6 \pm 0.04$ mV, as IS (CaCl$_2$) increased from 1 to 100 mmol/L. The almost null zeta potential observed may be attributed to specific adsorption of Ca$^{2+}$ on the negatively charged surface groups of the CDs. On the other hand, the zeta potential of a-CDs increased from $5.9 \pm 0.3$ to $17.9 \pm 1.6$ mV, as the IS (CaCl$_2$) increased from 1 to 10 mmol/L but remained virtually constant from 10 to 50 mmol/L.

Fig. 5b shows that the $D_h$ of CDs increased to 19.3 ± 1.0, 20.8 ± 1.7, and 8.6 ± 5.3 nm in the presence of 1, 10, and 100 mmol/L IS given by CaCl$_2$ respectively. The stability behavior does not appear to be affected by the change in surface charge of the nanoparticles under those conditions. In comparison to the small diameters of the nanoparticles, hydrated Ca$^{2+}$ ions have a non-negligible size of 1.2 nm (Dubin, 2003), the specific adsorption on CDs may increase the size of nanoparticles and cause moderate aggregation among them at low concentrations, while tend to repulse each other at higher concentrations. Similar to Na$^+$, no effects of Ca$^{2+}$ on a-CDs were observed, and $D_h$ remained constant around 3 nm. The observed behavior of the BPEI coated a-CDs

![Graph](image1.png)

**Fig. 4** – Zeta potential of CDs (pH = 5.7) and a-CDs (pH = 9.7) as a function of ionic strength (NaCl and CaCl$_2$).

![Graph](image2.png)

**Fig. 5** – Hydrodynamic diameter of CDs (pH = 5.7), a-CDs (pH = 9.7) as a function of ionic strength (NaCl and CaCl$_2$).
were strikingly different from CDs under these conditions, probably due to radical differences in surface chemistry given by the branched amino groups on the surface. a-CDs exhibit a very good stability in the presence of dissolved salts for all the conditions investigated.

### 2.2. Influence of pH

Our measurements indicated that zeta potential values of CDs were pH sensitive and they decreased with increasing pH. Zeta potential of CDs remained negatively charged over a pH range from 2 to 11 in the presence of NaCl and CaCl₂, and positively charged over the pH range from 2 to 10 for a-CDs (Fig. 6).

In the presence of 1 mmol/L IS (NaCl), CDs' zeta potential decreased from $-1.4 \pm 0.7$ to $-35.1 \pm 2.2$ mV, as pH increased from 3 to 11 respectively. The zeta potential decline can be related to the deprotonation of surface functional group, such as carboxyl and hydroxyl groups. Wang showed that CDs also contain hydroxyl and carboxyl functional groups, which agrees with our FT-IR results (Wang et al., 2011). DLS results (Fig. 7a) show that the pH did not have a noticeable effect on CDs stability from pH 4 to 11 in the presence of NaCl. The $D_h$ of CDs was around 1 nm, and then increased only slightly as pH decreased from 4 to 2.

When CDs were suspended with a background IS of 1 mmol/L given by CaCl₂, the zeta potential decreased from $-3.3 \pm 0.3$ to $-20.1 \pm 4.1$ mV as a pH increased from 3 to 11 (Fig. 6b). The CDs were mostly well dispersed at pH 9, as evidenced by a measured $D_h$ of 1 nm, but showed slight aggregation for other pHs considered, when the $D_h$ was higher but under 40 nm.

a-CDs zeta potentials decreased from $+40.73 \pm 6.2$ to $+6.88 \pm 0.6$ mV in the presence of 1 mmol/L NaCl as pH increased from 2 to 10 (Fig. 6a); on the other hand, the $D_h$ showed little to no variation (Fig. 7a). The a-CDs suspensions in 1 mM IS CaCl₂ showed a decreasing zeta potential from 44.1 to 1.4 mV when pH increased from 3 to 9, and remained almost constant at higher pH values. The presence of amino groups indicated that pH significantly changed the state of charge and the properties of CDs. Amino groups have a pKₐ value that usually varies between 9 and 10 (Stark, 1965; Wang et al., 2011). The basic groups resulted in a higher positively charged surface due to protonation of amino groups for all pH range investigated. The surface charge increased with decreasing pH, as a result of further protonation of the surface.

The measured $D_h$ was small at pHs between 7 to 9, an indication of stability, but increased at lower pHs, where nanosized aggregates were formed, mostly below 30 nm. As discussed, divalent cations have greater influence on the aggregation of negatively charge nanomaterials compared to monovalent ions (Wagner et al., 2014). In this situation, pH did not appear to be a key factor affecting hydrodynamic size, but it significantly changed the surface charge due to protonation or deprotonation of surface functional groups on CDs and a-CDs (Jia et al., 2012). Moreover, size and surface charge values for both particles in ultrapure water suspensions were found to be similar to those under 1 mmol/L NaCl, within the experimental error, for the pH range between 2 and 11 (data not shown). Given the potential inaccuracy of pH determinations for low IS solutions, i.e., ultrapure water, the 1 mmol/L NaCl condition was used as the indifferent electrolyte condition for comparisons in this study (Davison and Woof, 1985).

### 2.3. Influence of natural organic matter

The influence of dissolved natural organic matter on the stability of CDs and a-CDs was investigated in the absence of added electrolytes at circumneutral pH. NOM has been shown to stabilize nanoparticles by inhibiting the formation of aggregates by adsorption on the surface of the nanomaterials, imparting a negative charge, and through steric effects, preventing particles to approach each other to close distances at which Van der Waals interaction become significant to overcome repulsion forces. Whether NOM results in stabilization or destabilization depends on several factors, including chemical nature of the organic molecules, concentration, and solution chemistry.
Stabilization usually results from NOM forming a charged layer outside the particle, while destabilization results from particles being bridged by larger NOM molecules and/or Ca\(^{2+}\) which dominate interactions between nanoparticles (Wagner et al., 2014).

The \(D_h\) of CDs was increased from 1.1 to 50–200 nm range in the presence of HA (as shown in Fig. 8a); the \(D_h\) of a-CDs was also increased with existing NOM, and reached 491 nm at the concentration of 50 mg/L HA. Surprisingly, particle sizes decreased at higher HA concentrations. This phenomenon, observed for both CDs and a-CDs, can be explained by the initial bridging effect at relatively low concentration when a single HA molecule may interact with multiple nanoparticles. At increasing HA concentrations, HA molecules are present in excess and competition for surface sites resulted in HA molecules being associated with individual nanoparticle surfaces. Results are also compatible with higher adsorption affinity for a-CDs, which had a more noticeable size change. The functionalized a-CDs have abundant amine groups on the surface, which may form complexes with HA (Tang et al., 2012). Some studies have utilized this property to improve the performance of HA absorbents by functionalizing with polyethylenimine (PEI) (Deng et al., 2006; Tao et al., 2010). The absorption of HA on a-CDs may result in agglomeration and produce the size growth. Zeta potential of CDs and a-CDs as a function of HA concentration is shown in Fig. 8b. CDs zeta potential decreased from \(-16.8 \pm 1.1\) mV to as low as \(-58 \pm 2.7\) mV, at a concentration of 50 mg/L HA. At neutral pH values, the anionic HA was adsorbed onto the surface, yielding a rather low negative zeta potential. However, a-CDs zeta potential was positive and even higher with existence of HA, reaching values above 45 mV for all concentrations. Zhang and Bai (2003) studied HA adsorption on chitosan-coated granules by X-ray photoelectron spectroscopy and showed the formation of \(-\text{NH}_3^+–\text{OOC-R}\) or \(-\text{NH}_3^+–\text{O–C}_6\text{H}_4–\text{R}\) surface complexes, which involved the generation of \(-\text{NH}_3^+\) as a result of interaction between the amino groups and the absorbed HA. We postulate that this HA induced
protonation of the amino groups may be the reason for the increased positive charge observed in our experiments for a-CDs in the presence of HA.

2.4. DLVO modeling

DLVO theory was used to model CDs and a-CDs behavior. The ratio of total interaction energies to thermal energy $kT$, was calculated as a function of separation distance and plotted for pH ranging from 3 to 10, and IS levels of 1, 10 and 100 mmol/L for CDs (Fig. 9) and a-CDs (Fig. 10). Both CDs and a-CDs exhibited very low interaction energies for all IS and pH conditions, with values in the same order of magnitude as the thermal energy of the system. Such a system is expected to be unstable; the extremely weak repulsion forces present will allow particles to approach to very close separation distances, where attraction predominates. Thus, particles may aggregate irreversibly in the deep primary energy minimum.

Pelley and Tufenkji (2008) demonstrated that particle interaction energies are very sensitive to particle size changes. The height of repulsive energy barrier and the depth of secondary energy well increased dramatically with increasing particle diameter. The small particle size of CDs had contributed to the low calculated energies barrier, leading to instability predictions for most of the modeled conditions. However, experiments revealed the remarkable stability of CDs, except for the highest IS level conditions. The modeling equations contemplate two effects of the addition of salts on repulsion: a decrease in zeta potential and an increase in Debye length ($\kappa$); both will decrease the repulsion at a given separation distance, and hence promote aggregation.

Even if relatively accurate predictions were obtained for some conditions (i.e., CDs suspended in NaCl, highest IS conditions), the DLVO modeling was largely in disagreement with experimental results, especially for a-CDs. Classic DLVO relies exclusively in the interplay of electrostatic and Van der Waals interactions to describe colloidal stability. However, our experimental work evidenced a lack of correspondence between surface charge and size variations, which suggests a negligible influence of electrostatic repulsion in the colloidal stability of the system. In addition, the very small particle size, comparable to the Debye length and the hydrated cation diameters, further contests the applicability of the theory.

2.5. Fluorescence

We investigated the fluorescence emission intensity of both nanomaterials at three levels of IS, given by two electrolytes:

![Fig. 9](image-url)  
Fig. 9 – Calculated DLVO interaction energy plotted as a function of separation distance between CDs particles; effect of ionic strength and pH variations. (a) CDs with different NaCl concentrations. (b) CDs with different CaCl$_2$ concentrations. (c) CDs in 1 mmol/L NaCl with different pH. (d) CDs in 1 mmol/L CaCl$_2$ with different pH.
NaCl and CaCl₂. The experiments were conducted at a constant particle concentration for each type of CD: 200 mg/L for a-CDs and 52,000 mg/L for CDs. Those concentrations were chosen in order to achieve a high initial fluorescence emission intensity. Analysis of emission intensity changes with particle concentration showed that the height of the emission peak increased linearly with concentration up to the chosen particle concentrations, ruling out the potential interference of interparticle interactions, such as aggregation, in the fluorescence determinations (Fig. 11).

The IS results are presented in Fig. 12. Since aggregations negatively affect the emission, we focused our work on those IS conditions in which we did not observe significant particle size growth in our DLS determinations.

Fluorescence emission intensity increased with IS for both CDs and a-CDs in the presence of an indifferent electrolyte. The IS PL behavior implies that the surface defects may serve as capturing centers for carriers, thereby resulting in correlated luminescence (Jia et al., 2012). However, different effects were observed for the suspensions prepared in CaCl₂. Lower emission intensities were observed for CDs with increasing concentration of Ca²⁺ in comparison with the same IS level given by the monovalent ion. However, the presence of calcium resulted in a negligible effect on the a-CDs. The specific adsorption of Ca²⁺ was much more noticeable on the negatively charged CDs than on the a-CDs; there was, nevertheless some adsorption as indicated by the differences in zeta potential detected for the latter, but it was not strong enough to have an influence on the fluorescence properties of the CDs.

We studied the influence of natural organic matter on fluorescence emission intensity of CDs and a-CDs, using HA as a model compound in concentrations from 1 to 100 mg/L. The PL intensity of pure HA at the concentrations used in the experiment, shown in Fig. 13, was substantially lower than that of the CDs. In the presence of HA, the emission intensity of CDs and a-CDs decreased with increasing concentration of the model compound. Compared to CDs, a-CDs were affected more noticeably. As discussed, HA can be adsorbed on both nanomaterials and the degree of adsorption is dependent on their surface characteristics. Therefore, functionalized a-CDs had higher affinity for adsorption of HA because of the positively charged amino groups on the surface. The better adsorption due to the
formation of surface complexes between protonated amino groups and HA resulted in a larger change in emission intensity (Wagner et al., 2014). Our results suggested that PL from CDs is related to the surface states of the nanoparticles, since adsorption played a major role in controlling emission intensity. However, HA adsorption at the wavelength of emission peak for both CDs became significant at high HA concentration (50 and 100 mg/L), which would further contribute to the observed quenching. The negative impact by dissolved NOM on CDs and a-CDs has implications for sensing, potentially limiting the applications of CDs in high BOD streams or wastewaters (Part et al., 2016).

3. Conclusions

As prepared, CDs and a-CDs formed highly stable water suspensions and yielded a circumneutral pH for CDs and basic conditions for a-CDs. Negatively charged CDs suspended in NaCl solutions exhibited behavior compatible with classical colloidal stability principles, but aggregation was inhibited in the presence of CaCl₂, even for very low surface charge conditions. a-CDs remained positively charged for all conditions investigated and showed higher zeta potentials at intermediate IS levels followed by a moderate charge decrease, but remained stable in suspension. This is in contrast to the general behavior observed for nanoparticles (lower surface charge and aggregation with increasing IS), and hints to the importance of specific adsorption of divalent cations on CDs and even monovalent cations for the amino-coated particles. The surface charge of both CDs varies with pH as expected from the acidity constants of their surface functional groups. However, particle size remains fairly constant, always below 50 nm. Although nanoparticles tend to aggregate because of their high surface energies, more strongly so for smaller particles as in the case of CDs, coatings (either engineered or adsorbed from the suspension media, e.g., NOM) may hinder aggregation.

Fig. 11 – Fluorescence spectra for (a) CDs, and (b) a-CDs, at different concentrations.

Fig. 12 – Fluorescence spectra for (a) CDs, and (b) a-CDs, at various levels of ionic strength and electrolytes.
Aggregation is expected as a result of most typical concentrations of Ca$^{2+}$ in surface waters regardless of the presence of NOM and under groundwater conditions, Ca$^{2+}$ and NOM being key factors in nanoparticle stability, in contrast with our findings. If nanomaterials aggregate fast under natural water conditions and settle, they will impact mostly sediments and benthic organisms. We have shown in this work that CDs exhibit an opposite behavior, as they will most likely remain suspended in natural waters and will impact animals that inhabit the water column such as planktonic species, fish, and marine mammals. Moreover, high stability translates into excellent transport properties, which may lead to impacted ecosystems far from source of contamination.

The presence of HA also led to a unique response, inducing aggregation due to bridging for low to moderate concentrations and settle, they will impact mostly sediments and benthic organisms. We have shown in this work that CDs exhibit an opposite behavior, as they will most likely remain suspended in natural waters and will impact animals that inhabit the water column such as planktonic species, fish, and marine mammals. Moreover, high stability translates into excellent transport properties, which may lead to impacted ecosystems far from source of contamination.

The presence of HA also led to a unique response, inducing aggregation due to bridging for low to moderate concentrations and followed by stabilization at higher levels, independently of surface charge modifications. Interestingly, both positively and negatively charged particles presented an increase in the magnitude of zeta potentials with HA concentration.

Fluorescence emission intensity declines were observed for water chemistry conditions at which specific adsorption of ions or HA was suspected, suggesting surface states as the origin of the PL. However, the adsorption of HA at the peak emission wavelength for the CDs may have also contributed to this observation. This phenomenon may limit the application of CDs in sensing of natural waters.

REFERENCES


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