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# Resolving humic and fulvic acids in binary systems influenced by adsorptive fractionation to Fe-(hydr)oxide with focus on UV–Vis analysis

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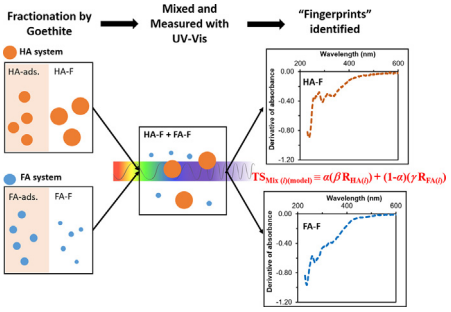
## HIGHLIGHTS

- The UV–Vis spectroscopic method developed can identify the fingerprints of humic and fulvic acids in their mixtures.
- Preferential adsorption of acidic polar moieties decreased the specific UV–Vis absorbance of humic and fulvic acids.
- The shape of UV–Vis spectra of humic and fulvic acids was little affected by adsorptive fractionation.
- The UV–Vis method performs well in quantifying fractionated samples with the absolute error of 5 mgC $L^{-1}$.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Humic acid (HA) and fulvic acid (FA) are two operationally defined classes of natural organic matter. In the environment, both materials are present simultaneously and bind in a competitive manner to Fe-(hydr)oxides and other minerals, but their quantification in mixtures is a challenge. In this study, an UV–Vis method was developed to quantify concentrations of HA and FA without and after adsorptive fractionation by an iron oxide (goethite, α-FeOOH). In addition, the performance of the UV–Vis method was compared to that of acid precipitation and size exclusion chromatography (SEC). Among the three methodologies (UV–Vis, acid precipitation, SEC), the UV–Vis method is the most successful in quantifying the ratio of HA to FA subject to fractionation. The UV–Vis method is based on distinct differences in the UV–Vis spectra of HA and FA, including fingerprints in both the spectra shape and intensity. Adsorption to goethite decreased the specific light absorbance of HA and FA, but the changes in spectral shape were not significant enough to cover their differences. The acid precipitation method can also quantify the HA to FA ratio. But to minimize the influence of incomplete HA precipitation or co-precipitation of FA, the concentration of both HA and FA needs to be at least ~20 mgC $L^{-1}$. The SEC method is not suitable to measure HA and FA after adsorption, because preferential adsorption significantly affects the shape of SEC chromatograms.

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## 1. Introduction

In environmental systems such as soil, water, and sediments, humified natural organic matter (NOM), i.e. humic substances, are widely present. Based on differences in solubility, humic substances are commonly divided into fulvic acid (FA), humic acid (HA), and humin. Because of their high reactivity, the HA and FA fractions are of great interest for researchers to gain insight into the environmental fate and geochemical cycling of many elements (e.g. heavy metals).

Despite recent discussions about the essential difference between HA and FA [1,2], the purified HA and FA show distinct characteristics concerning solubility, molecular size distribution, light absorption, as well as charge density and specific ion adsorption [3–6]. The solubility is lower for HA than for FA because HA particles are generally larger, contain more carbon, and have less reactive groups and charge per unit mass [7]. Since there are differences in chromophores between FA and HA, the absorbance of ultraviolet–visible (UV–Vis) light varies, resulting in distinctive spectra of FA and HA [8,9].

The operational definition of HA and FA (i.e. HA is soluble under alkaline conditions, but not under acidic conditions, whereas FA is soluble under both alkaline and acidic conditions) indicates that in a mixture, HA and FA can be quantified by TOC analysis before and after acid precipitation of HA [10]. One may also discriminate HA and FA based on their differences in light absorbance and particle size distribution. Examples of analytical methods are ultraviolet–visible light spectroscopy (UV–Vis) [11–13] and size exclusion chromatography (SEC) [12,14–17], as well as Fourier transformed infrared spectroscopy (FT-IR) [18,19]. These analytical methods have been mainly used to characterize HA and FA, and in limited number of studies [10–12,15] also for separation and quantification purposes.

Both HA and FA adsorb strongly to minerals such as iron (hydr) oxides, creating highly reactive materials of interest for both the environment and chemical engineering [20–23]. In soil, this adsorption process is important in controlling the bioavailability of nutrients such as phosphate [24], and the mobility of pollutants such as pesticides and heavy metals [4]. In engineering, the process may affect for instance the transport of substances in iron pipe water distribution systems [23]. For HA as well as FA, the adsorption on minerals has been studied extensively [7,11,13,24–30] and models have been developed to describe the adsorption behavior [7,24,28,31].

HA and FA are often present simultaneously, and they bind to minerals in a competitive manner. Because of their difference in particle size, charge density, and hydrophobicity, mineral particles covered by HA or FA may behave differently. However, little research is available regarding the competition between these two NOM fractions (HA and FA) in their adsorption to minerals, partly due to a lack of analytical methodologies that can effectively quantify the HA and FA fractions remaining in equilibrium solution after adsorption to minerals. An important issue is the polydispersity and chemical heterogeneity of HA and FA, which can lead to preferential adsorption of certain fractions of HA and FA to minerals [21,27–30,32]. These fraction changes through adsorption making their determination in a mixture even more intricate.

The aim of the present study is to develop a suitable analytical methodology for quantifying the concentrations of HA and FA when present in a mixture. As the properties of FA and HA may change due to fractionation by adsorption to metal oxide surfaces, the present challenge is to find a robust method for quantification irrespective of changes in size and composition of the molecules as a result of the interaction with mineral surfaces. Our intention is to apply the methodology in future studies for measuring the competitive binding of HA and FA to minerals. In present study, three principally different methodologies will be considered, comprising acid precipitation, UV–Vis spectroscopy, and size exclusion chromatography (SEC). We will put emphases on the development of the UV–Vis method that is fast and easy to use as we will show. The potentials of these techniques will be evaluated by comparing the analytical concentrations to known values for getting insight in the possibilities, limitations, and reliability of these methodologies. The development and evaluation of the methodology will be done using mixtures of unfractionated as well as fractionated HA and FA materials.

## 2. Material and methods

### 2.1. Primary materials

Fulvic acid (FA) and humic acids (HAs) were extracted following the protocols of the International Humic Substances Society (IHSS). The fulvic acid (FA) was purified from a soil sample collected from the Bh-horizon of a podzol soil at Telefoonweg, Renkum, The Netherlands (N 52°00′, E 5°45′). Three humic acids (HAs) were extracted from different soils. One HA (TVHA) was purified from a sample collected from the B-horizon of a podzol soil in Tongbersven forest close to Oisterwijk, Tilburg, The Netherlands (N 51°20′, E 5°08′). Another HA (JLHA) was purified from a soil sample collected from the top layer of a brown soil (Alfisols) in Tonghua, Jilin province, China (N 41°30′, E 125°55′). A third HA (JGHA) was purified from a sample collected from the top layer of a mountainous meadow soil (semi-hydromorphic soil) of Jiugong Mountains, Hubei Province, China (N 29°27′, E 114°42′). After the purification of TVHA, an extra step was applied, in which the TVHA obtained was re-dissolved using NaOH and ultrapure water and then re-precipitated by acidifying to pH 1.5 for removal of readily soluble TVHA molecules. The purified FA and HAs were freeze-dried and stored in a refrigerator at 4 °C.

The carbon content of the FA and TVHA is 48% and 58% respectively, determined by measuring the TOC concentration in solutions of known FA or TVHA content (Sievers 900, GE, USA). The weight average molar mass ($M_w$) is 1.9 kDa for FA and 17 kDa for TVHA, which were measured with SEC in combination with UV–Vis spectrometry. Both JLHA and JGHA have been characterized in previous studies [33,34]. The carbon content of JLHA and JGHA is 58% and 56%, whereas the weight average molar mass of JLHA and JGHA is 137 kDa and 38 kDa respectively. The color of the HA solutions was dark brown, and the intensity follows the order of TVHA < JAHA < JLHA, in line with their order of molar mass.

A stock solution of FA (2.0 g $L^{-1}$) was prepared by dissolving the freeze-dried FA material in ultrapure water. The solution was equilibrated for 20 h, and then filtered through a pre-washed 0.45 μm hydrophilic filter to remove any particulate material. Stock solutions of HA (2.0 g $L^{-1}$) were prepared by adding 0.10 M NaOH (under purified $N_2$) until pH > 10, followed by dilution with ultrapure water. The pH was adjusted back to ~6.0 with HCl before filtering with 0.45 μm filter.

HA and FA were fractionated (Section 2.2) using goethite prepared according to Hiemstra et al. [35]. The BET-$N_2$ specific surface area of this goethite is 99 $m^2$ $g^{-1}$ and the pristine point of zero charge (PZC) is pH = 9.3 [26].

### 2.2. Fractionation of HA and FA

In order to study the effect of adsorptive fractionation on the quantification of HA or FA, goethite suspensions with either HA or FA were prepared from the original stocks. For TVHA, the initial concentrations were 300, 350, 400, 450, and 500 mg $L^{-1}$ with 3.0 g $L^{-1}$ goethite. These concentrations were chosen to ensure that the HA concentrations remaining in solution after adsorption to goethite were mostly higher than the detection limits of all three methods (e.g., UV–Vis, SEC and acid precipitation), especially the acid precipitation method which required relatively high concentration; For FA, the initial concentrations were 100, 150, 200, 250, and 300 mg $L^{-1}$ with 3.0 g $L^{-1}$ goethite, and 100 mg $L^{-1}$ with 1.0 g $L^{-1}$ goethite. The involvement of JLHA and JGHA in the analysis was to develop and validate the UV–Vis method, which enabled measurement of relatively

low concentrations. For both JLHA and JGHA, the initial concentrations were 75, 100, 150, 200, 250 mg $L^{-1}$ with 1.0 g $L^{-1}$ goethite. All suspensions were prepared in a background electrolyte of 0.01 M NaCl. 0.01 M HCl and 0.01 M NaOH were added to adjust the pH to 6.0.

After shaking for 7 days at 20 °C in dark, the suspensions were centrifuged (18,000*g*, 30 min) and the supernatant was filtered through a 0.45 μm membrane filters. Control samples without goethite were included to detect any loss of HA and FA upon centrifugation and filtration. From each filtrate, a subsample was taken and the dissolved organic carbon (DOC) concentration was measured with a TOC analyzer (Sievers 900, GE, USA), which was regarded as the actual equilibrium concentration of HA or FA after adsorption. The percentages of TVHA, JGHA, JLHA and FA adsorbed to goethite were between 48 and 76%, 34–90%, 37–95% and 45–94% respectively (Table S1). The fractionated HA or FA in the equilibrium solutions were used for preparing series of test solutions.

### 2.3. Preparing test solutions

From both the original HA and FA stock solutions and fractionated equilibrium solutions, test solutions were prepared. Mixtures of HA and FA, either original or fractionated, were prepared in different HA to FA ratios. Solutions with only HA or FA were prepared as well and used as references. For comparing the three methods, FA and TVHA were used. For developing and testing the UV–Vis method, FA, TVHA, JLHA and JGHA were used.

#### 2.3.1. Mixtures of the original HA and FA

The original HA and FA were mixed at various total (HA + FA) concentrations of 25, 50, 100, or 150 mg $L^{-1}$, and the mass ratios of HA and FA were HA:FA = 1:9, 1:3, 1:1, 3:1, and 9:1. For all treatments, the pH was adjusted to 6.0 with 0.01 M HCl and 0.01 M NaOH, and the ionic strength was kept at 0.01 M with NaCl. The samples prepared were shaken for 7 days before quantifying the concentrations of HA and FA.

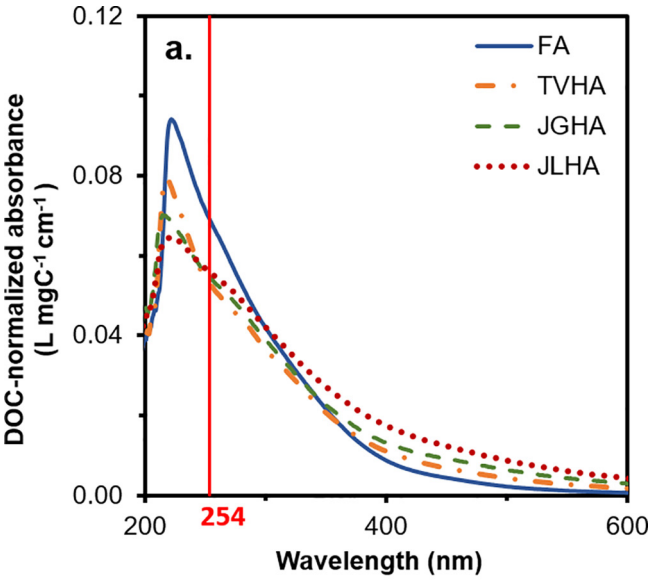
#### 2.3.2. Mixtures of fractionated HA and FA

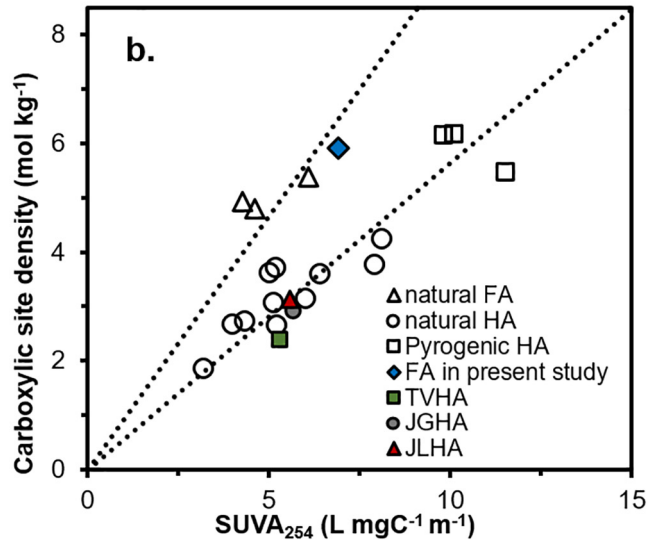
Mixtures of fractionated TVHA and FA samples were prepared from the fractionated equilibrium solutions of the 350 mg $L^{-1}$ TVHA with 3.0 g $L^{-1}$ goethite treatment (TVHA350-F) and of 300 mg $L^{-1}$ FA treatment (FA300-F) obtained as described above. These treatments were chosen because at these loadings, a substantial amount of HA and FA was adsorbed, resulting in significant adsorptive fractionation, while the concentrations in the collected equilibrium solutions after adsorption were high enough to be detected. Mixtures of JLHA or JGHA and FA samples after adsorption to goethite were prepared from the equilibrium solutions of the 200 mg $L^{-1}$ JLHA or JGHA treatment with 1.0 g $L^{-1}$ goethite (JLHA200-F, JGHA200-F) and 100 mg $L^{-1}$ FA with 1.0 g $L^{-1}$ goethite (FA100-F). To evaluate the ability of the UV–Vis method together with the SEC and acid precipitation methods in resolving mixtures of fractionated HA and FA, mixtures at volumetric ratios of HA-F: FA-F = 1:9, 1:3, 1:1, 3:1, and 9:1 were prepared. These ratios were chosen to create mixtures in which either HA dominates (9:1) or FA dominates (1:9) and situations in between. The mixtures of HA-F and FA-F thus prepared were shaken for 7 days before further measurement.

### 2.4. HA and FA quantification

#### 2.4.1. UV–Vis spectroscopy

The pH of each test solution (including blank) was adjusted to ~6.9 with 0.1 M phosphate buffer containing $Na_2HPO_4$ and $NaH_2PO_4$ (1:1) to eliminate any effect of pH on the UV–Vis spectroscopic measurements. Samples with a high absorbance were diluted to a maximum absorbance of 1 unit. All the sample solutions were filtered with 0.45 μm filter before UV–Vis measurements. The spectra were recorded with a



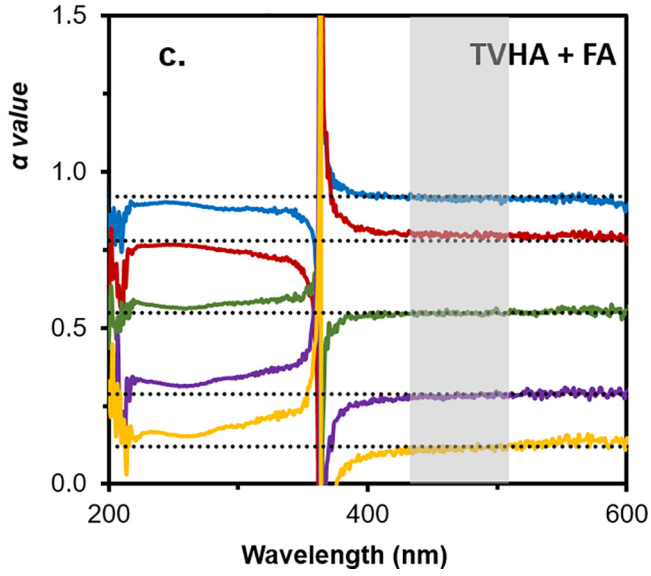




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**Fig. 1.** Carbon normalized UV–Vis spectra of original HA and FA (a), site density of weak acidic (carboxylic) groups of HA and FA as a function of the specific UV absorption at 254 nm ($SUVA_{254}$) (b), and the $\alpha$ value derived using Eq. (2) in unfractionated mixture solutions of TVHA and FA (c). In Fig. 1a, the spectra were measured in 0.01 M NaCl and 0.1 M phosphate buffer at pH = 6.9 at a concentration of 20 mg $L^{-1}$. In Fig. 1b, data of natural FA (△), natural (○) and pyrogenic (□) HA refer to Hiemstra et al. [24], Janot et al. [28], Wang et al. [37], Deng et al. [38], Milne et al. [39], Hur et al., [40]). The presently used TVHA (■), JGHA (●), JLHA (▲) and FA (◆) are also indicated. In Fig. 1c, the dotted horizontal lines give the nominal $\alpha$ *values* (HA fractions), and the grey shadowed indicates the optimal wavelength window (430–510 nm).

UV–Vis spectrophotometer (PN3212 UV Detector, Postnova Analytics, Germany) over the wavelength range of 200 and 600 nm with a resolution of 1 nm (example spectra can be found in Fig. 1a). The first-order derivatives of the absorption spectra were calculated and smoothed according to Hur et al. [12] using an interval of 17-data points with the software Origin 9.0. A fitting method was developed to derive the HA and FA concentrations in the test solutions (see Section 3).

#### 2.4.2. SEC chromatography

Size exclusion chromatograms of HA and FA were obtained using a Biosep-SEC S 2000 column (pore size 150 Å, Phenomenex, USA), connected with a HPLC pump. A volume of 100 µL sample solution was injected. The carrier used was a 1:1 mixture of 0.1 M $NaH_2PO_4$ and 0.1 M $Na_2HPO_4$ (pH 6.9), flowing at a rate of 1.0 mL $min^{-1}$. The UV–Vis light absorbance of the effluent was detected with a DAD detector (diode array detector) (PN3241 UV Detector, Postnova Analytics, Germany) over the wavelength range of 200–800 nm, from which the absorbance at 254 nm was used for further data processing (examples of SEC chromatograms can be found in Fig. S1). A fitting method similar to that of the UV–Vis method (see Section 3) was developed to derive the HA and FA concentrations in the test solutions (see Appendices. 2 SEC Methodology Development).

#### 2.4.3. Acid precipitation

Acid precipitation was chosen as one of the methods because it is the classical method to discriminate HA and FA and preliminary experiments showed promising results for samples containing only HA at higher concentrations [10]. About 10 mL of a test solution was acidified to pH 1.0 with 6 M HCl. After standing for 20 h to achieve aggregation and primary settling, high-speed centrifugation (20 min, 18,000*g*) was used for the final separation. The supernatant was filtered through a 0.45 µm membrane filter, and the filtrate was sampled for TOC analysis ($TOC_{filtrate}$) after raising the pH of the filtrate to around 6.0 using NaOH. Since the mixture was only composed of HA and FA, after acidification the HA was precipitated and the solution phase ($TOC_{filtrate}$) contained only FA ($C_{FA} = TOC_{filtrate}$) based on the operational definition of HA and FA. Therefore, the difference between the original TOC content ($TOC_{total}$) and $TOC_{filtrate}$ will be equal to the carbon concentration of HA ($C_{HA} = TOC_{total} - TOC_{filtrate}$). The TOC concentration was measured with a TOC analyzer (Sievers 900, GE, USA).

## 3. Results and discussion

### 3.1. UV–Vis method development

#### 3.1.1. Mixtures of unfractionated HA and FA

The UV–Vis spectra of the original HAs and FA showed distinct differences (Fig. 1a). There was one peak in the spectra around 230 nm for both HAs and FA, but the decrease of absorbance at > 230 nm was steeper for FA than for HAs. The specific UV absorbance (SUVA) measured in the vicinity of 250 nm (e.g., 254, 280 nm) has been regarded as a measure of aromaticity of natural organic molecules [36]. A clear relation between $SUVA_{254}$ and carboxylic site density of HS was observed (Fig. 1b), suggesting that HA and FA were different in charge density.

For the mixtures of the original HAs and FA, if one assumes no interaction of HA and FA upon mixing, the spectrum of a mixture can be treated as a simple superposition of the independent spectra of HA and FA based on the Beer-Lambert law as expressed in Eq. (1).

$$TS_{Mix(i)} = \alpha R_{HA(i)} + (1 - \alpha) R_{FA(i)} \quad (1)$$

in which $R_{HA(i)}$ and $R_{FA(i)}$ represent the specific light absorbance of HA or FA (L $mgC^{-1}$ $cm^{-1}$) in the reference spectra at a certain wavelength *i* nm. The reference spectra were obtained by measuring the original HA or FA samples with a known concentration. $TS_{Mix(i)}$ (L $mgC^{-1}$ $cm^{-1}$) is the specific absorbance of the mixture sample at the same wavelength (*i*), and $\alpha$ value is HA carbon mass fraction in the mixture, whereas 1- $\alpha$ is the FA carbon mass fraction in the mixture. By transforming Eq. (1), the HA fraction ($\alpha$) can be calculated from

$$\alpha = \frac{TS_{mix(i)} - R_{FA(i)}}{R_{HA(i)} - R_{FA(i)}} \quad (2)$$

Ideally, one may derive with Eq. (2) the HA and FA concentrations in mgC $L^{-1}$ ($C_{HA}$ and $C_{FA}$) in an unknown mixture using the value of $\alpha$ based on measurement at a certain wavelength in combination with the TOC analysis of the mixture ($TOC_{mix}$), according to:

$$C_{HA} = \alpha \times TOC_{mix} \quad (3a)$$

$$C_{FA} = (1 - \alpha) \times TOC_{mix} \quad (3b)$$

To minimize the error, it is better to choose a range of wavelength and calculate the average value of $\alpha$ instead of using one single wavelength. To select the optimal range of wavelength that may result in the appropriate value of $\alpha$, $\alpha$ values were calculated for different wavelengths for the HA and FA mixtures at different total carbon concentrations.

For TVHA, the results are shown in Fig. 1c (colored lines) together with the known values of $\alpha$ according to the mixing ratios (dotted horizontal lines). It is obvious that around 350 nm very large deviations exist. In the UV range (200–400 nm), the calculated $\alpha$ values deviate from the expected values. This was also found for JGHA and JLHA (Fig. S2). Over the wavelength range of 430–510 nm (grey shadowed), the relative difference in the absorbance between HA and FA was the largest (Fig. 1a), which explained why results based on measurements in this wavelength range were the most reliable ones. Restricting us to this range, the recovery (estimated $\alpha$/known $\alpha$) for TVHA was improved to 99% ± 5%, while the error was bigger (recovery of 94% ± 16%) if the entire wavelength range was considered (Fig. S3).

With the $\alpha$ values based on the selected wavelength range (430–510 nm) and TOC concentration of the samples, the HA and FA concentrations were derived. These concentrations were in excellent agreement with the added amounts of HA and FA if the absolute scale was considered (Fig. S4a, b), i.e. ± 2.8, ± 2.0, and ± 1.0 mgC $L^{-1}$ for respectively TVHA, JGHA and JLHA (Fig. S4c, d). If the concentrations are high enough (e.g., HA or FA > 12 mgC $L^{-1}$), the relative error is within 25%. (Fig. S4e, f).

Overall, our results demonstrate that the above UV–Vis data treatment with selected spectral windows (430–510 nm) may perform well in HA and FA quantification for unfractionated mixtures. The question arises how the methodology performs in systems with fractionated HA and FA, which will be discussed next.

#### 3.1.2. Mixtures of fractionated HA and FA

For studying the role of fractionation, fractionated HA and FA solutions were produced by interaction with goethite with a series of either HA or FA addition. These fractionated solutions were mixed at different volume ratios (1:9, 1:3, 1:1, 3:1, 9:1) (see Section 2). Evaluation of the collected data showed that the amount of fractionated HA

(HA-F) or fractionated FA (FA-F) could not be retrieved as above for unfractionated samples with Eq. (2) (Fig. S5), and further development is needed.

For both HA and FA, adsorption to goethite has resulted in a decrease of the DOC-normalized light absorption over the entire wavelength range (200–600 nm) (Fig. S6). As shown by the decrease of $SUVA_{254}$, the degree of decrease is related to the percentage adsorbed (Table S1). The solutions with fractioned HA and FA contain less aromatic structures and have a lower charge density, which has been found by other researchers [13,28,32,41]. It implies that the concentrations of HA and FA cannot be derived from the experimental UV–Vis spectrum using the spectra of the unfractionated HA and FA as reference in Eqs. (1) and (2).

To derive the concentrations of fractionated HA and FA in their mixtures using the original unfractionated materials as reference, we adapted Eq. (1) considering the adsorptive fractionation effect. Two additional terms were introduced that were meant to account for decrease of specific light absorbance due to fractionation, leading to:

$$TS_{Mix(i)(model)} \equiv \alpha(\beta R_{HA(i)}) + (1-\alpha)(\gamma R_{FA(i)}) \quad (4a)$$

in which $R_{HA(i)}$ and $R_{FA(i)}$ are the carbon normalized light absorbance of original HA or FA (L $mgC^{-1}$ $cm^{-1}$) at a certain wavelength $i$ nm (same as in Eqs. (1) and (2)). $TS_{Mix(i)(model)}$ is the calculated value of the carbon normalized light absorbance of the mixture sample of HA and FA at the same wavelength ($i$). $\beta$ and $\gamma$ are fractionation factors describing the change of specific light absorbance (intensity) caused by adsorptive fractionation. Rewriting leads to:

$$TS_{Mix(i)(model)} = AR_{HA(i)} + BR_{FA(i)} \quad (4b)$$

in which $A \equiv \alpha\beta$ and $B \equiv (1-\alpha)\gamma$. The values of $A$ and $B$ can be found by fitting the spectra of the mixture solution, minimizing the sum of squared differences between the measured and calculated total absorbance ($\Sigma(TS_{Mix(i)} - TS_{Mix(i)(model)})^2$) over the selected range of wavelength. With the fitted values of $A$ and $B$, one can calculate fractionated HA fraction in the mixture, $f_{HA\text{-}F} \equiv A/(A+B)$. This fraction can be combined with the TOC analysis of the mixture ($TOC_{mix}$), resulting in the concentrations of fractionated HA ($C_{HA\text{-}F}$) and FA ($C_{FA\text{-}F}$) in the mixture:

$$C_{HA\text{-}F} = f_{HA\text{-}F} \times TOC_{mix} \quad (5a)$$

$$C_{FA\text{-}F} = (1 - f_{HA\text{-}F}) \times TOC_{mix} \quad (5b)$$

This approach assumes that the fractionation factor for HA ($\beta$) and for FA ($\gamma$) can be different, but the value of $\beta$ or $\gamma$ remains the same over the wavelength range considered. The latter assumption means that the shape of the spectra does not change after adsorptive fractionation. This approach distinguishes HA and FA not only by the ratios in light absorption at a certain wavelength, but more importantly by their difference in spectral shape.

To verify the assumption that the spectral shape of the original and fractionated HA and FA are similar, the first-order derivatives of the DOC-normalized spectra were calculated, similar to that of Hur et al. [12]. This compelling data processing method can improve detection of subtle spectral features [12,42]. As shown in Fig. 2, the derivative spectra of HAs reveal several well-resolved peaks, especially in the UV region, which differ from the derivative spectra of FA. These differences are identifiable signatures in spectral shape of HA and FA. Furthermore, compared to the original samples, the derivative spectra of both HA and FA show little changes after adsorptive fractionation. These changes in derivative spectral became larger at a low equilibrium concentration (< 5 mgC $L^{-1}$) when most (> 90%) of HA or FA was adsorbed (not shown). Compared to the decrease of the specific absorbance, the changes in the spectral shape were much smaller (Figs. 2 and S6), and could not cover up the difference in spectral shape between HA and FA. The fingerprints in the spectral shape still can be used as identifiable characteristics to differentiate HA and FA in fractionated samples.

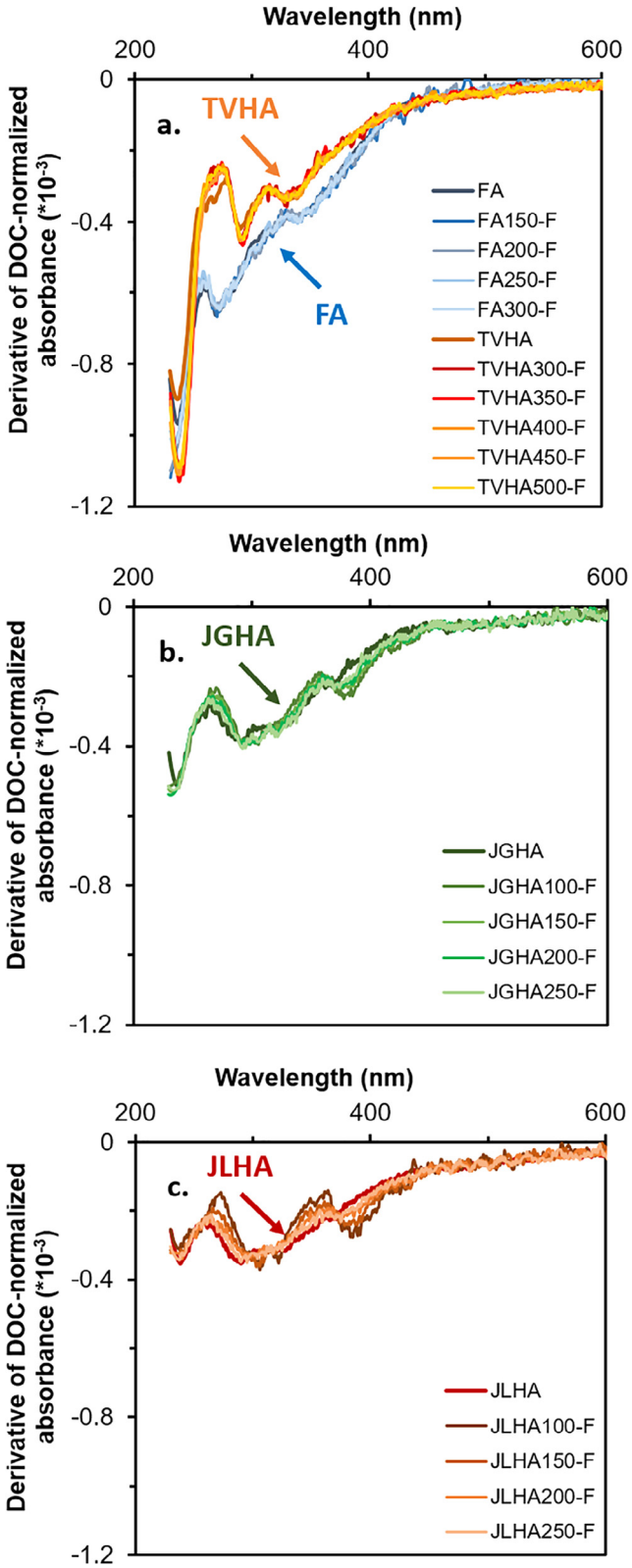


**Fig. 2.** Derivatives of the DOC-normalized UV–Vis spectra of FA and TVHA (a), JGHA (b), JLHA (c) in their original and fractionated solutions.

We tested the fitting method (Eq. (4b)) described above for the mixtures of fractionated samples using two sub-ranges (UV range of 250–330 nm and visible range of 430–510 nm) as well as the entire range of wavelength (230–600 nm). These two subranges were chosen because it was found that the slopes of the spectra of HA and FA

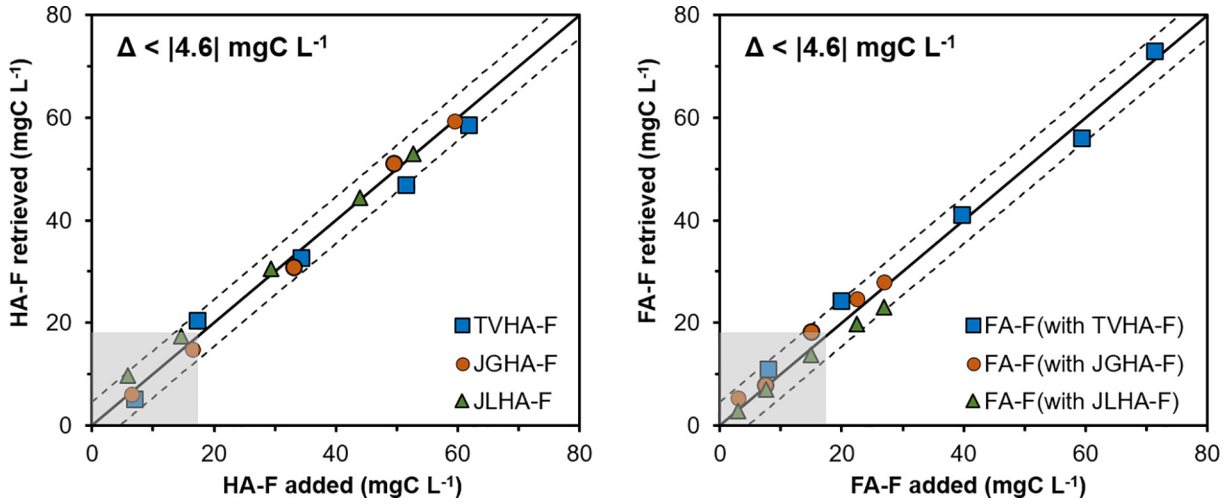


**Fig. 3.** Comparison of the retrieved and added concentrations of HA-F and FA-F in their mixtures measured with the UV–Vis approach proposed using spectra covering the range of 230–600 nm. The solid line indicates the 1:1 line, and the dashed lines indicate boarders with an uncertainty (Δ) of ± 4.6 mgC $L^{-1}$. The grey shadowed area indicates that larger deviations (> 25%) can be found at lower concentrations (< 18 mgC $L^{-1}$). Details are shown in Fig. S7.

differed the most in the first wavelength region (250–310 nm), whereas the intensity (specific absorption) differed the most in the second wavelength region (430–510 nm) (Figs. 1 and 2). The results (Table S2) showed that for fractionated HA (HA-F), the visible light range (79–114% recovery) was more suitable than the UV wavelength range (78–253% recovery). The visible light range could also be used successfully (within a recovery of 100% ± 25%) to derive the concentration of FA-F when the FA-F concentration was higher than 18 mgC $L^{-1}$. As shown in Fig. S6, the absorbance of FA is relatively low at long wavelengths (> 400 nm) compared to that of HA. When combined with low FA concentrations, the signal becomes very weak at the visible light region and is more sensitive to the spectral modification caused by the adsorptive fractionation. The recoveries of FA-F with data over the entire wavelength range was improved to 86–178% in comparison to those with only the UV range (0–374%) or visible light range (0–202%).

Generally, the retrieved concentrations of HA-F and FA-F agreed well with the added ones when the spectra of entire wavelength range 230–600 nm were used (Table S2 and Fig. 3). For mixtures of FA-F with TVHA-F, JGHA-F and JLHA-F, the absolute errors were ± 4.6, ± 2.4 and ± 3.9 mgC $L^{-1}$ respectively, when compared to the known values (the added concentrations of the HA-F or FA-F which had been measured with TOC analyzer). Similar to the results in the original samples, when the concentration of HA or FA in solution is higher than about 18 mgC $L^{-1}$, the results are acceptable (with a deviation less than 25%). In competitive HA and FA adsorption studies, a high percentage of adsorption may lead to a relatively low solution concentration and the distinction between HA and FA may become less certain. Nevertheless, the method is able to derive the corresponding adsorption with a rather high accuracy because adsorbed amount is usually dominant in the overall mass balance.

The above analysis shows that the accuracy of the method depends on HA material used and the degree of fractionation. As has been discussed above, the difference in the specific absorbance between HA and FA over the visible light wavelength range was used in the analysis for unfractionated samples. For fractionated samples, differences in the UV region together with the visible region were included for HA and FA quantification. We noticed that the difference of HA from FA over the visible light region increased in the order of TVHA < JAHA < JLHA. The same order is found for the molar mass of HAs (Section 2) and for the intensity of solution color. HA with a larger molar mass differs more from FA, and can be better assessed in a mixture with FA. This finding can be used to screen and select HA materials prior to use in HA/FA competition experiments.

For fractionated samples, the accuracy depends also on the fractionation degree after contacting with mineral (e.g., goethite). A larger degree of fractionation led to a more significant change in spectral shape, resulting in a larger deviation from the defined values.

To summarize, the proposed method that combines UV–Vis spectroscopy and TOC analysis performs well (within an error of 25%) in quantifying HA and FA concentrations in both unfractionated and fractionated mixtures, providing that the absolute concentrations are sufficiently high, preferable > 18 mgC $L^{-1}$.

### 3.2. Comparison of methodologies

The original and fractionated TVHA and FA solutions were used to prepare mixtures for comparing the performance of the UV–Vis method with SEC and acid precipitation method.

For the SEC method, an approach similar to that of the UV–Vis method described above was followed. The difference is that the UV–Vis method uses spectra, whereas the SEC method uses chromatograms. In the approach, the most characteristic part of the SEC chromatograms was selected to optimize the determination. Details are given in Appendices (2. SEC Methodology Development).

With the acid precipitation method, the assessment of the FA and HA concentrations in mixtures is straightforward as described in Section 2.

#### 3.2.1. Unfractionated HA and FA in mixtures

The performance in determining the original TVHA and FA using the three methodologies was compared in Fig. S8. The acid precipitation method performed less well, especially at lower added concentrations (< 20 mgC $L^{-1}$), than the other two methods. The deviation from the known amount could be as high as around 100%. With acid precipitation, recovery substantially higher than 100% was found for TVHA (Fig. S8e), which points to simultaneous precipitation of some FA that was, by definition, interpreted as TVHA in the measurement. On the other hand, an overestimation of FA was observed at low concentrations (Fig. S8f). This can be explained by incomplete precipitation of TVHA. Such an incomplete precipitation of HA has also been found by Van Zomeren and Comans [10]. At lower total amounts and smaller FA fractions in the mixture solution, the relative importance of the incomplete precipitation of HA will increase. In case of unfractionated materials, both the UV–Vis and SEC method performed satisfactorily in all treatments in quantifying TVHA and FA (Fig. S8).

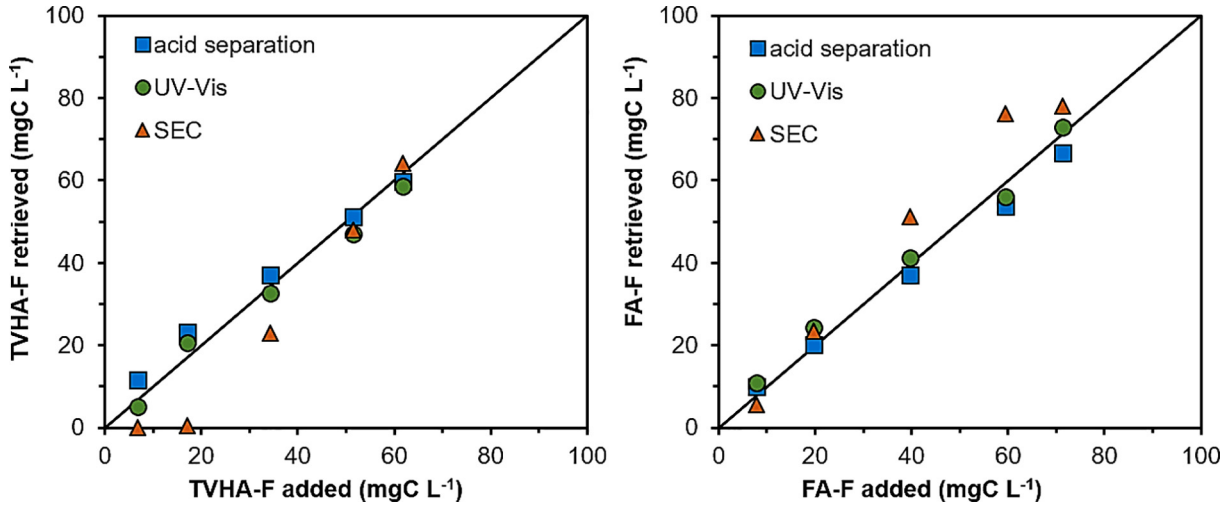


**Fig. 4.** Comparison of the three methods for quantifying fractionated FA-F and TVHA-F in their mixtures at different mixing ratios.

The absolute error for both methods was within ± 2.8 mgC $L^{-1}$ for the UV–Vis method and ± 1.5 mgC $L^{-1}$ for the SEC method. However, the situation can be different for fractionated ones.

### 3.2.2. *Fractionated HA and FA in mixtures*

In Fig. 4, the concentrations of fractioned HA and FA measured in their mixtures with the three methods are compared to the known values.

As discussed in Section 3.1.2, the UV–Vis methodology proposed in the current study improved the accuracy in quantifying fractionated HA and FA in comparison to traditional UV approaches with the use of a single wavelength (e.g., 254 nm, 260 nm) for deriving HS concentrations [18]. As shown in Fig. 4, the deviations of the TVHA-F and FA-F measured from the 1:1 line are small (< ± 4.6 mgC $L^{-1}$).

The SEC method performed poorly in quantifying TVHA-F and FA-F in their mixtures (Fig. 4). An important reason is the change in the molecular weight distribution of HA and FA left in solution after adsorption (Fig. S9), which is consistent with previous studies showing that fractions with a certain size were preferred in the adsorption to oxides [23,25]. In addition, the characteristic peak for TVHA cannot be resolved and distinguished (Fig. S9) from that of FA at a relatively low amount of TVHA in the mixture. This leads to an underestimation of the TVHA-F and simultaneously to an overestimation of FA-F (Fig. 4).

With the acid precipitation method, the measurement of fractionated HA (TVHA-F) was satisfactory (recovery 97–108%) when the relative amount of TVHA in the mixture was high (> 50%), as was also found for unfractionated TVHA. At a low percentage of TVHA-F (< 50%) in the mixture, co-precipitation of FA-F has led to overestimation (34–70% more) of TVHA-F (Fig. 4). For FA-F, the acid precipitation measurements were reasonable (recovery 91–102%) as long as the concentration in the samples was relatively high (> 20 mgC $L^{-1}$). The performance of acid precipitation in measuring FA in mixtures without (Fig. S8) and with (Fig. 4) fractionation shows an improved accuracy in the latter one, particular at lower FA-F fractions. As discussed in Fig. S6, the improvement can be attributed to changes in the molecular composition due to fractionation, making that the HA-F molecules more easily precipitated.

Based on the above discussion, the advantages and disadvantages of the three methodologies (i.e., UV–Vis, SEC, acid precipitation) were compared in Table S3. The UV–Vis and SEC method are rapid, require a small volume of sample and minimal pretreatment for analysis. The acid precipitation method is the classical separation method, but it is more time-consuming, and requires larger sample volume to discriminate HA and FA. Since the UV–Vis or SEC analysis is non-destructive, samples can be re-used for other analyses (e.g. on-line TOC analysis coupled to SEC). In addition, the UV–Vis method gives the best measurement of HA and FA with and without adsorptive fractionation by goethite.

## 4. Conclusions

In previous studies, HA or FA have been separately used as a proxy for NOM to study their adsorption behavior. Only a limited number of studies focused on their competitive adsorption to minerals due to a lack of methodology to distinguish and quantify HA and FA in mixtures after interacting with minerals. For HA and FA, their competitive interaction at mineral surfaces can only be quantitatively studied, providing that one has a reliable method to distinguish both types of molecules in a quantitative manner.

In the present study, a UV–Vis spectroscopic method was developed to quantify in original and fractionated HA and FA in their mixtures. Compared to the other two methods tested (i.e. the acid precipitation, and the SEC method), the UV–Vis methodology is based on the analysis of the UV–Vis spectrum in combination with TOC analysis. This approach is fast, convenient and can be accessible to many labs. It provides possibilities for HA and FA quantification without complex lab work, and more importantly, the UV–Vis method gives the best accuracy for fractionated samples. The accuracy varies with the type of HAs involved, and error varies between 1 and 3 mgC $L^{-1}$. From this perspective, the method developed is highly suitable for future competitive adsorption studies to iron oxides, and can be optimized by screening and selecting the best UV–Vis performing HA materials.

Within limitations, the classical acid precipitation method can also be applied to measure the concentrations of HA and FA in mixtures when their concentrations are not too low. The SEC method performs very well in resolving unfractionated HA and FA in their mixtures, but cannot be recommended in quantifying the concentrations of fractionated HA and FA.

The methodology developed here will facilitate future research to improve the insight in the interaction of NOM with mineral surfaces. This is highly valuable to improve our understanding of the reactivity of humic-oxide materials that are used in chemical engineering.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cej.2020.124380.

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