Influence of DOM characteristics on the flocculation removal of trace pharmaceuticals in surface water by the successive dosing of alum and moderately hydrophobic chitosan

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Abstract: Hydrophobically-modified chitosan (HC) has emerged as a promising flocculant for trace pharmaceutical removal from surface water. However, the variation in the characteristics of dissolved organic matter (DOM) in different water sources influences the efficacy of HC in removing pharmaceutical compounds. In this work, the flocculation performance of sequentially dosing alum and HC (alum+HC) for the treatment of five water types (three synthetic waters, and samples of two real waters collected from the Yangtze River and the Thames River), having different DOM and five representative pharmaceuticals (initial concentration: 100 ng/L), was assessed by bench-scale jar tests. The DOM characteristics were correlated quantitatively with the removal efficiencies (REs) of the pharmaceuticals. Density functional theory computations were performed to illuminate the interfacial interactions in the flocculation. Alum+HC exhibited a remarkably higher RE of all five pharmaceuticals (maximum RE: 73%-95%) from all waters compared to a conventional coagulant or flocculant (alum or polyacrylamide, respectively). In contrast to using HC alone, alum+HC also achieved a higher RE of pharmaceuticals with nearly half the HC dosage, thereby enhancing the cost-effectiveness of the alum+HC dosing system. Among the different key DOM characteristics, the surface charge and molecular weight of DOM had no evident correlation with RE(pharmaceutical), but the hydrophobic/hydrophilic nature and functional group composition of organic carbon of DOM were strongly correlated: Strongly hydrophobic fractions, with C-C & C=C functional groups (binding pharmaceuticals via hydrophobic association), were beneficial, while hydrophilic fractions with C-OH groups were less effective, for pharmaceutical removal. This work showed the enhanced performance of the alum+HC dosing combination in the removal of different pharmaceutical compounds from different waters, and filled the knowledge gap regarding the performance of hydrophobically-modified chitosan.
modified flocculants in the treatment of different surface water sources.

**Keywords:** Hydrophobically-modified flocculant; Successive alum and flocculant dosing; Dissolved organic matter; Fractionation; Pharmaceuticals; Surface water treatment
1. Introduction

With the rapid growth of population and human activities, increasing amounts of anthropogenic pharmaceutical compounds, among other emerging pollutants of concern, are continuously entering surface water sources from urban runoff, municipal sewage effluents, medical industrial and livestock farming discharges, etc. (Wilkinson et al., 2017). Although pharmaceuticals are typically present in trace concentrations (ppt to ppb levels) in surface water, the potential risk of negative human health effects produced by them after long-term exposure, has drawn considerable attention (Richardson and Kimura, 2020). Previously, the removal of pharmaceutical compounds has not been considered as a particular treatment objective in the design of many existing drinking water treatment works (DWTWs), but this may change in the future as these contaminants can pose a potential threat to drinking water safety.

Flocculation, with its merits of cost-effectiveness and facile operation, has been one of the central technologies in many DWTWs worldwide for many years (Barbosa et al., 2018; Zhang et al., 2021). By dosing coagulants (e.g. inorganic metal salts) and/or flocculants (e.g. organic polymers) to enhance contaminant aggregation and floc development, flocculation is well known for its high performance in the removal of particulate and colloidal contaminants (Liu et al., 2019). However, for the removal of soluble trace organic compounds (TrOCs, involving most pharmaceuticals), conventional coagulants/flocculants have been shown to be ineffective (Wang et al., 2020), and therefore treatment needs to be enhanced in order to meet the additional requirements of reducing pharmaceutical concentrations. Research concerned with developing new functionalized flocculants to provide enhanced treatment has been undertaken in recent years, including the modification of conventional coagulants (e.g. metal salts) and flocculants (e.g. chitosan) using hydrophobic organic segments, which
has shown promising results in several cases (Du et al., 2018; Liu et al., 2019; Ng et al., 2019; Ren et al., 2017; Yang et al., 2020b; Yang et al., 2021; Zheng and Taylor, 2020).

It has been shown that introducing a moderate amount of hydrophobic segments strengthens pair-wise binding interactions among flocculants, TrOCs and coexisting particles/collloids, which promotes contaminant aggregation (floc formation) and notably improves the removal of trace pharmaceutical (TrPs) (Liu et al., 2019; Ren et al., 2017; Yang et al., 2020b; Yang et al., 2021).

Despite these encouraging developments, the diversity in the characteristics of dissolved organic matter (DOM) in different surface water sources presents a challenge to achieving higher treatment performance. Commonly present in raw water, DOM is widely recognized as a key factor determining water treatability (Gonzalez-Torres et al., 2019; Rutlidge et al., 2021; Zhang et al., 2019). The removal of DOM (important for the control of disinfection by-products (Okoro et al., 2021)) is associated with the characteristics of its different components/fractions, which include their charge density, molecular weight, hydrophobicity/hydrophilicity, and functional group compositions (Collins et al., 1986; Su et al., 2021; Zang et al., 2020), as these influence the interactions between flocculants and the DOM (Nystrom et al., 2020; Zhang et al., 2021). Similarly, the complexity of the DOM characteristics may also change the pair-wise interactions among flocculants, TrOCs and DOM in the flocculation process (Lapointe et al., 2020), which further influences the removal of TrPs. Thus, in simple terms, a hydrophobically-modified flocculant that is effective in removing a pharmaceutical compound in one source water, may not be effective in another source. Therefore, to establish the likely effectiveness of a hydrophobically-modified flocculant for the removal of TrPs from waters with different DOM compositions, it is necessary to study the influence of the different DOM characteristics, in terms of
quantitative relationships between the DOM characteristics and pharmaceutical removal efficiencies, and the relevant underlying mechanisms, which are unknown at present.

An important factor relating to the potential application of hydrophobically-modified flocculants is their cost. However, sequentially dosing inorganic coagulants and organic flocculants has achieved success in many environmental engineering applications (Gao et al., 2016; Murujew et al., 2020), which has not only reduced the dosage of higher-priced organic flocculants, but also achieved an improved flocculation performance with higher removal efficiencies of a wider spectrum of contaminants. In view of this, the potential cost-effectiveness of sequentially dosing low-priced conventional metal salt coagulants and hydrophobically-modified flocculants, is worth investigating, given that hydrophobically-modified flocculants can be developed to target soluble TrOCs, and inorganic metal salts are good at coagulating particulate/colloidal contaminants.

Consequently, the objectives of this study were as follows: (1) to evaluate the effectiveness of a hydrophobically-modified flocculant for the removal of a wide spectrum of TrPs from different water sources (three synthetic waters prepared in the laboratory, and two real waters collected from the Yangtze River and the Thames River) having different DOM; (2) to evaluate the effectiveness of sequentially dosing inorganic metal salt coagulant and hydrophobically-modified flocculant for TrP removal; (3) to study the influence of different DOM characteristics on TrP removal by correlating the DOM characteristics with pharmaceutical removal efficiencies. The flocculation tests involved the following: five representative pharmaceutical compounds (norfloxacin (NOR), sulfamethoxazole (SMX), roxithromycin (ROX), sodium diclofenac (DCF), and tetracycline (TC)) were selected as target contaminants; Jar tests, to determine
contaminant flocculation performance, were conducted using different coagulants/flocculants (alum, polyacrylamide (PAM), hydrophobically-modified chitosan (HC), alum coupled with subsequent PAM or HC (denoted as alum+PAM or alum+HC)); DOM in each type of water was fractionated according to molecular weight, hydrophobicity/hydrophilicity, and functional group composition of organic carbon. The results of the tests were expected to enhance the practicability of hydrophobically-modified flocculants in the removal of various TrPs from different surface water sources, and to clarify the mechanism of the influence of DOM characteristics on pharmaceutical removal in the hydrophobically-modified-flocculant-based flocculation system.

2. Materials and methods

2.1. Raw water

Five types of raw water (three synthetic and two real samples) having different DOM characteristics were tested in this work, to better support the application of the findings from quantitative correlation between DOM characteristics and TrPs flocculation removal. Real water samples were taken from the Yangtze River (Ma’anshan City, Anhui Province, China; 31°38’49”N 118°27’06”E; the sampling point is shown in the Supporting Information (SI), Fig. S1a) and the Thames River (London, UK; 51°25’25”N, 0°18’24”W; the sampling point is shown in the SI, Fig. S1b), two well-known rivers representing important surface water sources, and were denoted as YR and TR, respectively. For the preparation of synthetic waters, kaolin powder (Sinopharm; to produce a turbidity of 20 NTU), alternative types of DOM (to generate a dissolved organic carbon (DOC) concentration of 5 mg/L), NaHCO₃ (Sinopharm; 1 mmol/L), and NaCl (Sinopharm; 1 mmol/L) were added into tap water which had been
left to stand for 1 day to ensure the decay of residual chlorine. The alternative types of
DOM were humic acid (HA), obtained from two sources (HA-1 from Aladdin Co., and
HA-2 from Sigma-Aldrich LLC.; used in previous studies as representative DOM in
synthetic surface water (Feng et al., 2015; Li et al., 2020; Peydayesh et al., 2021; Yang
et al., 2020a)), and organic extract (OE) from an upland (Scar House) reservoir; the
method of extraction has been described elsewhere (Lambert and Graham, 1991; Xiong
and Graham, 1992). Prior to conducting jar tests, five target pharmaceutical compounds
(Dalian Meilun Biotechnol. Co. Ltd.; physicochemical properties and species
distribution with pH are given in the SI, Fig. S2) were pipetted from stock solutions
into the test water to obtain a concentration of 100 ng/L of each compound. The test
water was continuously mixed at room temperature for 1 day before use, and the quality
parameters of the five types of test water are listed in Table 1.

-Table 1-

2.2. Coagulant and flocculants

KAl(SO₄)₂·12H₂O (alum) was purchased from Aladdin Co. Polyacrylamide (PAM)
with a weight-average molecular weight of 3,000 kDa was purchased from Sinopharm
Co. Hydrophobic chitosan (HC), with previously optimized structural parameters (SI,
Fig. S3), was synthesized by grafting relatively hydrophobic poly(N-n-
propylacrylamide) segments onto hydrophilic chitosan (chitosan purchased from
Shandong Aokang Biotechnol. Co., with a viscosity-average molecular weight of 820
kDa and a deacetylation degree of 86%), according to a previously reported method
(Ren et al., 2017; Yang et al., 2020b). Stock solutions of each coagulant/flocculant (1
g/L) were freshly prepared for each jar test.
2.3. Jar tests

Flocculation performance using either a single coagulant/flocculant, or a combination of alum and an organic flocculant, was determined by use of jar tests. For the combined use of alum and a flocculant, a known volume of alum stock solution was dosed firstly into the raw water. After stirring the solution at 200 rpm for 1 min, a known volume of organic flocculant (PAM or HC) stock solution was then added. The alum dosage in the alum+flocculant system was fixed at the half the optimal dosage when alum alone was used, according to preliminary experiments and relevant literature (Liang et al., 2014; Ma et al., 2019). After the coagulant/flocculant was dosed, the procedure of the jar tests, employing a six-place flocculation stirrer, consisted of rapid stirring (200 rpm for 5 min), slow stirring (50 rpm for 15 min), and floc sedimentation (for 30 min) steps. Then, samples of the supernatant water in the jars were withdrawn for turbidity determination (Shanghai Xinrui WGZ-200 turbidimeter). The zeta potential (ZP) of water samples was measured by a Malvern Nano-Z Zetasizer. After filtration by 0.45-μm filter paper, water samples were tested for UV absorbance at 254 nm (UV254; Shimadzu UV-2401PC UV-vis spectrophotometer) and DOC (Shimadzu WV-S TOC analyzer) concentrations. The specific ultraviolet absorbance (SUVA) was obtained as the UV254 divided by DOC. After solid phase extraction, the concentrations of target pharmaceutical compounds were determined by an Ultra-High Performance Liquid Chromatograph (UHPLC) coupled to an electrospray ionization quadruple-time-of-flight (ESI-Q-TOF) high-definition mass spectrometry (MS; Waters Synapt G2-Si), using an Acquity UPLC BEH C18 column (1.7 μm, 2.1 mm × 5 mm). The compound removal efficiencies (RE) were obtained, as follows:

\[ \text{RE} (%) = \left[ \frac{(C_{\text{before}} - C_{\text{after}})}{C_{\text{before}}} \right] \times 100 \quad \text{(Eq.1)} \]

where \( C_{\text{before}} \) and \( C_{\text{after}} \) were the average contaminant concentrations from three parallel
tests, before and after flocculation treatment, respectively.

2.4. DOM fractionation

Different components of DOM in raw and treated water were fractionated according to molecular weight, hydrophobicity/hydrophilicity, and chemical compositions of organic carbon, respectively. The contents of different fractions/components were represented in terms of DOC percentage of the corresponding components. For molecular weight fractionation, a MSC3000 ultrafiltration cup (Shanghai Mosu Chem. Co., Ltd.) equipped with a series of flat sheet membranes (with cutoff molecular weights of 100, 10, and 1 kDa) was used under pressures of 0.1 MPa (for 100 kDa and 10 kDa membranes) or 0.2 MPa (for 1 kDa membrane) to separate the DOM into four categories. The membranes were replaced (with a new membrane) after each 300 mL (for 100 kDa and 10 kDa membranes) or 100 mL (for 1 kDa membrane) of water filtered. For hydrophobicity/hydrophilicity fractionation, every 200 mL of water sample was firstly pumped into an adsorption column filled with Superlite DAX-8 resin (8 g) at a rate of 5 mL/min, and the effluent was then fed into a second column filled with Amberlite XAD-4 resin (8 g) at 15 mL/min. Three components of DOM were thus obtained, comprising strongly hydrophobic (adsorbed by DAX-8), weakly hydrophobic (or transphilic) (adsorbed by XAD-4), and hydrophilic (in the final effluent) species (Aiken et al., 1992; Leenheer, 1981). Before use in the separation process, both DAX-8 and XAD-4 were washed using methanol and then deionized water until the DOC of the used water was undetectable. DOC values of the three different components (DOC_{hydrophobic}, DOC_{transphilic}, and DOC_{hydrophilic}) were obtained based on mass balance calculation according to the following equations:
$\text{DOC}_{\text{hydrophobic}} = \text{DOC}_{\text{initial}} - \text{DOC}_{\text{intermediate}}$ (Eq. 2)

$\text{DOC}_{\text{transphilic}} = \text{DOC}_{\text{intermediate}} - \text{DOC}_{\text{hydrophilic}}$ (Eq. 3)

where $\text{DOC}_{\text{initial}}$, $\text{DOC}_{\text{intermediate}}$ and $\text{DOC}_{\text{hydrophilic}}$ were the DOC values before adsorption, after adsorption by DAX-8, and after adsorption by XAD-4, respectively.

Quantities of different functional group compositions of organic carbon, divided into C-O-C, C-OH, C=O, C-N and C-C & C=C, were calculated according to C1s X-ray photoelectron spectra (XPS) recorded by a Thermo Fisher ESCALAB Xi+ XPS spectrometer.

### 2.5. Theoretical computation

Density functional theory (DFT) computations of complexes of flocculant-DOM, flocculant-pharmaceutical, and DOM-pharmaceutical, as well as molecules (or typical fragments of molecules) of flocculant, DOM and pharmaceuticals, were conducted using Gaussian 09 software (Frisch et al., 2016). Optimized geometries and corresponding binding energies (BEs) were obtained at the M06-2x/6-31 + G(d) level.

### 3. Results and discussion

#### 3.1. Flocculation performance

The flocculation performance of dosing a single coagulant or flocculant (alum, PAM or HC), as well as the successively dosing both inorganic coagulant and organic flocculant, was evaluated according to the variation of REs of different parameters (turbidity, DOC, and UV$_{254}$) with coagulant/flocculant dosage, as depicted in the SI (Figs. S4-S6).

For turbidity removal (SI, Fig. S4), although optimal dosages (OD) corresponding to the maximum RE varied, most RE-dosage profiles exhibited a sharp rise and then a
gentle decline with dose, indicating the typical destabilization (at dosage < OD) and re-
stabilization (at dosage > OD) effects of flocculants on particulate contaminants,
respectively, as widely reported before (Divakaran and Pillai, 2002; Wang et al., 2018).
When a single flocculant was dosed, the maximum RE(turbidity) for PAM was
relatively low due to the nonionic nature of PAM; in contrast, both alum and HC were
effective, with maximum RE(turbidity) values greater than 85%. When both alum (10
mg/L) and HC were dosed sequentially, the maximum RE(turbidity) (> 90%;
summarized in Fig. 1a) for all five types of water further increased, with a
corresponding treated water turbidity lower than 1 NTU.

The DOC and UV$_{254}$ of treated water were measured (SI Figs. S5 and S6) to
indicate the removal of aromatic, and total, DOM (Okoro et al., 2021). When no
flocculant was added (dosage = 0), the REs of DOC, mainly caused by DOM adsorption
onto kaolin particles, which were removed by water sample filtration before the DOC
determination, were slight (<2.2%). Dosing PAM resulted in the increase, instead of the
decrease, of DOC, which was likely due to the presence of residual PAM, with relatively
low-molecular-weight fractions in treated water. Similar to the trend of results for
turbidity removal, as aforementioned, dosing alum or HC alone enabled both UV$_{254}$ and
DOC to be substantially reduced, and the successive dosing of alum and HC further
improved the performance, with maximum REs of UV$_{254}$ and DOC higher than 80% in
most situations. For the two parameters, the maximum RE(UV$_{254}$) was generally greater
than the corresponding maximum RE(DOC) for the same water, as summarized in Fig.
1a, leading to the decrease of SUVA after treatment for most of the raw waters (SI, Fig.
S7). This implied that the aromatic composition was more readily removed than other
components of the DOM by the combined use of alum and HC.
RE-dosage profiles for the removal of target TrPs from the five types of raw water are illustrated in the SI, Figs. S8-S12. Similar to DOM adsorption onto kaolin, a small proportion (10-18%) of the pharmaceuticals were removed when no coagulant/flocculant was added, due to the adsorption of pharmaceuticals onto kaolin. Compared to the commercial flocculant PAM and coagulant alum, HC demonstrated a notably improved performance for the removal of all selected pharmaceuticals in this work, which demonstrated the broad-spectrum TrOC removal activity of hydrophobically-modified flocculants. Furthermore, the combined (sequential) use of alum and HC not only further enhanced the RE(pharmaceutical), but also nearly halved the required optimal dosages of relatively expensive HC, which should enhance the cost-effectiveness of the flocculant in full-scale applications. Compared to the small REs of pharmaceuticals when no coagulant/flocculant was dosed (due to adsorption onto kaolin as aforementioned) or only the coagulant alum was dosed, the REs of pharmaceuticals (in the range of 73%-95%) at the optimal flocculant dosages in the alum+HC system were much larger, indicating the predominant role of HC in pharmaceutical removal. The improved pharmaceutical removal and lower optimal HC dosage can be explained as follows: when single HC was dosed, although some HC molecules were utilized for binding pharmaceutical molecules, the other large parts of the HC molecules were consumed in the destabilization and binding of particulate and colloidal contaminants; by comparison, in the flocculation system of successive dosing of alum and HC, alum firstly destabilized the particulate and colloidal contaminants before the HC was dosed. In this way, HC was used where it was needed most and the required dosage of HC was reduced. Considering all the above results for the removal of turbidity, DOC, UV\textsubscript{254}, and the five pharmaceuticals, it was evident that the combined, sequential dosing of alum and HC was highly effective.
According to results given in the SI (Figs. S8-S12), the maximum REs of all pharmaceuticals when alum and HC were combined are summarized in Fig. 1b. When the five pharmaceuticals were compared in the treatment of the same water, it was always found that the maximum RE followed the sequence of \( \text{RE(SMX)} > \text{RE(ROX)} > \text{RE(NOR)} > \text{RE(TC)} > \text{RE(DCF)} \). The sequence corresponded well with the calculated BEs of optimized complex geometries formed by hydrophobic poly(N-n-propylacrylamide) segments of HC and pharmaceuticals (obtained from DFT computations; as shown in the SI, Fig. S13), indicating the indispensable role of HC in binding and aggregating pharmaceuticals into flocs. The binding energy of \( \text{Al(OH)}_3 \) (or PAM) with each pharmaceutical (given in the SI, Fig. S14) was much lower than the corresponding value between HC and pharmaceutical, which agreed with the best flocculation performance of HC in pharmaceutical removal when a single coagulant/flocculant was dosed. In addition, the dose of alum gave rise to a slight decrease of pH of the treated water (as listed in the SI, Table S1) due to the consumption of \( \text{OH}^- \) by the hydrolysis of \( \text{Al}^{3+} \); the slightly reduced pH might promote the flocculation of kaolin particles and DOM, through charge attraction between coagulant/flocculant and kaolin/DOM, as reported in the literature (Hussain et al., 2013; Slavik et al., 2012; Yang et al., 2020b). However, the small pH change did not affect the dominant pharmaceutical species as shown in the SI, Fig. S2. Since hydrophobic association and H-bonding, rather than charge attraction, between HC and pharmaceuticals predominated in the efficient pharmaceutical removal (SI, Fig. S13), the slight pH decrease made little contribution to pharmaceutical removal. On the other hand, as coexisting DOM could be one of the factors determining pairwise interference interactions in the floc formation process (Jia et al., 2016; Lapointe et al., 2020; Yang et al., 2016; Zhang et al., 2021), as stated in the introduction, the sequence might also
be affected by DOM-pharmaceutical interactions, which will be discussed in detail in Section 3.4. In addition to the comparison of pharmaceuticals in the same water, the REs of the same pharmaceutical in different types of water were compared, and the sequence of maximum RE of each pharmaceutical was in the order of “TR > HA-2 > YR > OE > HA-1”. The intrinsic reason for this sequence of compound removal was likely related to the DOM characteristics of the different waters, as will be discussed in detail later.

3.2. Effects of DOM characteristics on DOM removal

The effects of DOM characteristics on the removal of DOM in flocculation systems using conventional coagulants/flocculants have been studied extensively and shown their importance in flocculation process optimization (Collins et al., 1986; Matilainen et al., 2002; Xu et al., 2011; Yuan et al., 2017). However, it was unclear whether similar or different effects were present in the flocculation system using the combined, sequential dosing of alum and HC, and these were considered in this study in order to provide a comprehensive understanding of the new flocculation system.

The negatively charged nature of the DOM was indicated by the negative ZP values of the different DOM solution as given in the SI (Table S2). As alum (with Al-containing cationic species) and HC (with cationic amino groups on the chitosan backbones) were both positively charged (Yang et al., 2020b), charge attraction between the coagulant/flocculant and the DOM should be one of the principal mechanisms enabling DOM removal. This was validated by ZP-dosage changes in the flocculation system (SI, Fig. S15), whereby an increasing dosage of HC led to a continuous increase of ZP (less negative / more positive) in the flocculation of all test waters. However, the ZP did not reach zero when the dosage was in the optimal dosage ranges, implying that
other interaction(s)/mechanisms were contributing in the flocculation (Yang et al., 2020b). The optimized HC-DOM complexes (obtained from DFT computations; SI, Fig. S16) showed that hydrophobic association and H-bonding also contributed to DOM removal, as has been reported previously (Hu et al., 2021; Yang et al., 2020b).

Further insight into the flocculation mechanisms was provided by measured changes in three other characteristics of the DOM, these being the molecular weight, hydrophobicity/hydrophilicity, and functional group composition of organic carbon. Among these, methods for the former two characteristics (i.e. MW, hydrophobicity) have been well established (Aiken et al., 1992; Leenheer, 1981; Su et al., 2017; Xu et al., 2011), while results for the functional group composition were obtained according to C1s XPS spectra of the DOM in untreated and treated water, as given in the SI (Fig. S17). A summary of the maximum RE values of the different characteristics for the different raw waters are shown in Fig. 2. Higher molecular weight (Fig. 2a) and more hydrophobic (Fig. 2b) DOM was more readily removed in the alum+HC system for all waters. This behaviour was consistent with that obtained previously in conventional coagulant/flocculant systems (Collins et al., 1986; Golea et al., 2020; Yuan et al., 2017), where large-sized contaminants more readily formed large floc nuclei, and hydrophobic contaminants were thermodynamically spontaneous in aggregating via hydrophobic association in water. Both effects were important in promoting the growth of flocs. Among the different functional group compositions of organic carbon (Fig. 2c), for each water, the C-C & C=C fractions with nonpolar covalent bonds had the largest maximum RE than any other types of functional groups with polar bonds. This provided a molecular-scale explanation for the facile removal of hydrophobic DOM as aforementioned.
3.3. Correlation of pharmaceutical removal with DOM characteristics

As indicated previously in the introduction, the influence of DOM on TrP removal was considered by evaluating correlations between DOM characteristics and pharmaceutical removal. Linear correlation, the most commonly used type of correlation in statistics (Guillossou et al., 2021; Huang et al., 2021; Sgroi et al., 2018; Xu et al., 2018), was used to correlate the maximum REs of each pharmaceutical in the alum+HC system with the different DOM characteristics, specifically the surface charge, molecular weight, hydrophobic/hydrophilic nature, and functional groups.

With regard to the surface charge properties of the DOM, no evident correlation with pharmaceutical removal was found (SI, Fig. S18), with small $R^2$ values ranging from 0.053 to 0.312 for the five data sets. Although the five selected pharmaceuticals in this work had differently charged species at the near-neutral pH of the raw waters (predominant species were neutral for NOR, neutral or anionic for TC, cationic for ROX, and anionic for SMX and DCF, as illustrated in SI, Fig. S2), the relatively low $R^2$ values (given in the SI, Fig. S18) clearly indicated that charge interaction between DOM and pharmaceuticals was not a decisive factor affecting pharmaceutical removal.

The proportion (%) of different molecular-weight fractions of DOM in the different test waters are depicted in Fig. 3a, on the basis of four molecular weight ranges. Linear correlations between the maximum RE of each pharmaceutical and the proportion of each molecular-weight fraction, and associated $R^2$ values of the fitted lines, were calculated and are shown in Fig. 3b-3f. The results showed that, while the molecular weight of DOM notably affected the DOM removal, as reported in Section 3.2, its influence on pharmaceutical removal was not so significant, since the $R^2$ values (given in Fig. 3b-3f) were less than 0.47 (most of them less than 0.2).
The influence of hydrophobicity/hydrophilicity of DOM was different (Fig. 4) from that of molecular weight. Here, DOM was divided into three compositions, namely, strongly hydrophobic, weakly hydrophobic, and hydrophilic, with the proportion of each composition illustrated in Fig. 4a. The maximum RE of each pharmaceutical was correlated with the proportion of each composition as demonstrated in Fig. 4b-4f. Among the three compositions, the percentages of strongly hydrophobic and hydrophilic fractions were strongly and moderately correlated with pharmaceutical removal, with $R^2$ values in the ranges of 0.739-0.879 and 0.520-0.739, respectively, which made it meaningful to further analyze the slope values of the corresponding fitted lines. In contrast, the influence of the weakly hydrophobic composition was insignificant with $R^2$ values lower than 0.23. Of the five $R^2$ values of the strongly hydrophobic fraction (0.739-0.879), and the five $R^2$ values of the hydrophilic fraction (0.520-0.739), the lowest value (0.739 or 0.520) was for the removal of DCF (Fig. 4f), which might be related to DCF having the lowest RE among the five pharmaceuticals in each type of water, as reported above. According to the slope values, the effects of strongly hydrophobic (slope $> 0$) and hydrophilic (slope $< 0$) fractions on pharmaceuticals removal were opposite, so that a water with a DOM with a greater content of more strongly hydrophobic, and less hydrophilic, composition, was favorable for the flocculation removal of TrPs. It is speculated that strong hydrophobicity might originate from the dominant composition of alkyl and/or aryl groups in DOM, whereas hydrophilicity could be associated with DOM having large amounts of certain oxygen- (such as -OH) and/or nitrogen-containing (such as -NH$_2$) functional groups. Therefore, the maximum REs of pharmaceuticals were further correlated with the proportion of functional groups of organic carbon in DOM, as
depicted in Fig. 5, in order to extract more information of the DOM properties at a molecular scale.

The functional group composition of organic carbon in various DOM (Fig. 5a) was obtained from C1s XPS spectra (SI, Fig. S17) as mentioned previously. The results of correlation analysis are depicted in Fig. 5b-5f, which showed that, among the five groups, the proportions of C-C & C=C and C-OH had a strong correlation with the removal of all five pharmaceuticals, with correlation coefficients in the ranges of 0.975-0.994 and 0.761-0.942, respectively, which were much greater than the $R^2$ values corresponding to the other three groups (C-N, C-O-C and C=O). Interestingly, the lowest values of the two ranges, 0.975 and 0.761, were found for the removal of DCF (Fig. 5f), which was a similar finding to the $R^2$ values corresponding to strongly hydrophobic and hydrophilic fractions in Fig. 4. According to the slope values in Fig. 5b-5f, a greater proportion of C-C & C=C groups, and less proportion of C-OH groups, could enhance the maximum RE of pharmaceuticals. This phenomenon corresponded to the effects of strongly hydrophobic and hydrophilic fractions in Fig. 4. Thus, the results implied that the promotion role of strongly hydrophobic DOM in pharmaceutical removal was ascribed to C-C & C=C functional groups, while the inhibition role of hydrophilic DOM could be attributed to C-OH groups. The proportions of C-C & C=C groups of DOM (Fig. 5a) followed the same order of magnitude of “TR > HA-2 > YR > OE > HA-1” as that of the maximum REs of each pharmaceutical in different types of water (Fig. 1b). However, according to previous studies of the adsorption of organic contaminants, both C-C & C=C and C-OH groups can promote the binding of contaminants through hydrophobic association and H-bonding, respectively (Chen et
Thus, the previous studies did not support the opposite roles of C-C & C==C and C-OH found in our tests (Fig. 5). Considering the effect of functional group composition of organic carbon on DOM removal (C-C & C==C fraction of DOM was the easiest to be removed from water, as discussed in Section 3.2), one reason to explain the results was that pharmaceuticals bound with the C-C & C==C groups could be transferred to flocs more easily during the flocculation of DOM. One of the possible C-C & C==C groups binding with pharmaceuticals might be aromatic. However, according to correlation analysis results of maximum REs of each pharmaceutical to SUVA values (the latter representing the portion of aromatic DOM in the total DOM) as shown in the SI, Fig. S19, there was no evident correlation between the two parameters. Hence, the role of aromatic groups of DOM did not predominate in pharmaceutical removal in the alum+HC system. To further investigate the mechanisms from the aspect of interfacial interactions between the pharmaceuticals and DOM, DFT computations were performed and described in section 3.4, in addition to the above numerical analysis of experimental data in this section.

3.4. Interfacial interactions between pharmaceuticals and DOM

Optimized geometries and corresponding BEs of complexes formed by typical fragments of DOM and pharmaceuticals, according to two different priorities (given to H-bonding and hydrophobic association, respectively), are illustrated in Fig. 6. Although both H-bonding of C-OH and hydrophobic association of C-C & C==C in DOM could bind with pharmaceuticals, as shown in the Fig. 6 the BE of complexes with a preference to hydrophobic association (Fig. 6a-6e) was larger than that with a preference to H-bonding (Fig. 6f-6j), for the binding of the same pharmaceutical molecule onto DOM. Therefore, C-C & C==C groups were superior to C-OH groups for
binding with pharmaceuticals when in a competitive situation. This is another reason that can explain the positive role of C-C & C=C groups in the removal of pharmaceutical compounds as shown in Fig. 5. With respect to the binding of different pharmaceuticals, the BE values of complexes with a preference to hydrophobic association were in the order of SMX > ROX > NOR > TC > DCF, which was similar to the order of maximum RE of different pharmaceuticals shown in Fig. 1b, indicating that interfacial interactions between C-C & C=C groups in the DOM and pharmaceuticals were also a key factor in determining the removability of pharmaceuticals. In addition, binding energies of coexisting Al(OH)₃ (or kaolin) with pharmaceutical (or DOM) (shown in the SI, Figs. S14, S20 and S21) were much lower than those of DOM-pharmaceutical through hydrophobic association (Fig. 6a-6e), indicating that coexisting Al(OH)₃ and kaolin in the alum+HC flocculation system had little impact on DOM-pharmaceutical interactions.

- Fig. 6 -

4. Conclusions

The main conclusions in this work can be listed as follows:

(i) Successive dosing of alum and HC exhibited a greatly enhanced removal of five commonly found TrPs (initial concentration of 100 ng/L; maximum RE in the range of 73%-95%) from five water sources (three synthetic waters and samples of two real waters, having different DOM qualities and quantities), compared to common commercial coagulant and flocculant chemicals (alum, PAM, and the combination of alum and PAM).

(ii) The combined alum+HC flocculation arrangement, having a higher maximum RE of pharmaceuticals than HC alone, nearly halved the required optimal dosage of
HC, which considerably enhanced the practical cost-effectiveness of HC for the
treatment of surface waters.

(iii) In the alum+HC flocculation arrangement, large-molecular-weight and
hydrophobic DOM, with more C-C & C=C groups, was more easily removed.

(iv) Among different DOM characteristics, the surface charge and molecular
weight of DOM had no evident correlation with pharmaceutical removal. In contrast,
the hydrophobic/hydrophilic nature and functional group composition of organic
carbon of the DOM had a strong correlation with, and led to differences in, the
RE(pharmaceutical) in various water sources. Strongly hydrophobic fractions, with C-
C & C=C functional groups (binding pharmaceuticals via hydrophobic association and
promoting the transfer of pharmaceuticals into flocs) were beneficial, whereas
hydrophilic fractions, with C-OH groups, were less effective for the removal of
pharmaceuticals.

(v) For different types of pharmaceutical compounds, those having stronger
interfacial interactions with the C-C & C=C groups of DOM and hydrophobic segments
of HC, were more readily removed.

In summary, this work has confirmed the enhanced performance of successively
dosing alum coagulant and HC flocculant for the removal of common pharmaceutical
compounds from different water types, and demonstrated the influence of key DOM
characteristics on their removal during flocculation.

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Captions

Table 1. Basic parameters of waters used in the study.

Fig. 1. Maximum removal efficiencies of (a) turbidity, DOC, UV$_{254}$, and (b) different pharmaceutical compounds from various water types with successive dosing of alum and HC.

Fig. 2. Removal efficiencies of different fractions of DOM from various water types (fractionation according to (a) molecular weight, (b) hydrophobic/hydrophilic nature, and (c) functional group compositions of organic carbon).

Fig. 3. (a) Proportion (%) of different molecular-weight fractions of DOM; (b-f) Correlations between maximum RE of pharmaceutical compounds ((b) SMX, (c) ROX, (d) NOR, (e) TC, and (f) DCF) and percentages of different molecular-weight fractions of DOM in the alum+HC flocculation system.

Fig. 4. (a) Proportion (%) of different hydrophobic/hydrophilic fractions of DOM; (b-f) Correlations between maximum RE of pharmaceutical compounds ((b) SMX, (c) ROX, (d) NOR, (e) TC, and (f) DCF) and percentages of different hydrophobic/hydrophilic fractions of DOM in the alum+HC flocculation system.

Fig. 5. (a) Proportion (%) of different functional-group fractions of DOM; (b-f) Correlations between maximum RE of pharmaceutical compounds ((b) SMX, (c) ROX, (d) NOR, (e) TC, and (f) DCF) and percentages of different functional-group fractions of DOM in the alum+HC flocculation system.

Fig. 6. Optimized complex geometries of DOM (characteristic segment) - pharmaceutical according to different priorities given to (a-e) hydrophobic association and (f-j) H-bonding, respectively: (a and f) DOM-SMX, (b and g) DOM-ROX (characteristic segment), (c and h) DOM-NOR, (d and i) DOM-TC, and (e and j) DOM-DCF.
Table 1. Basic parameters of waters used in the study.

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<th>Water</th>
<th>pH</th>
<th>Turbidity (NTU)</th>
<th>UV&lt;sub&gt;254&lt;/sub&gt; (cm&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>DOC (mg/L)</th>
<th>SUVA (L/(mg·m))</th>
<th>Each antibiotic (ng/L)</th>
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<td>3.41</td>
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</table>
**Fig. 1.** Maximum removal efficiencies of (a) turbidity, DOC, UV$_{254}$, and (b) different pharmaceutical compounds, from various water types with successive dosing of alum and HC.
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