Absorption spectroscopy of colored dissolved organic carbon in Georgia (USA) rivers: the impact of molecular size distribution

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ABSTRACT

Dissolved organic carbon (DOC) was collected in six rivers that transect the coastal plain of Georgia in July 1999 and February 2000. DOC concentrations ranged from 4.9 to 40.7 g m^{-3} and from 7.1 to 40.5 g m^{-3}, respectively. The absorption coefficient at 440 nm was highly correlated with DOC concentration, suggesting that the optical parameter may be utilized for rapid estimation of DOC in these waters. The isolated DOC was separated into fractions of operationally defined molecular size, using an ultrafiltration technique that yielded three fractions: <10 ("small"), 10-50 ("medium") and >50 ("large") kilodalton. The smallest fraction was the most abundant (>50%) in 4 rivers in July and in all rivers in February, and considerably more abundant than in previous years. The wavelength-dependent absorption of the total DOC and its fractions showed approximately uniform shape of a curve declining exponentially with the increase of wavelength. The average slope of logarithmically transformed curves was 0.0151 and 0.0159 nm^{-1}, for the material collected in July and February, respectively and showed a dependence on DOC molecular size. In unfractionated DOC samples, the mass-specific light absorption determined at 440 nm was on average 0.33 m^2 g^{-1} in July, and 0.26 m^2 g^{-1} in February. The mass-specific absorption coefficient in all fractions ranged between 0.085 and 1.347 m^2 g^{-1} in July and between 0.085 and 1.877 m^2 g^{-1} in February, and was positively correlated with the molecular size of the measured samples. The results of the reported study clearly suggest that the specific absorption coefficient of the yellow substance is an outcome of the relative contribution of its different size fractions.

Key words: absorption coefficient of DOC, humic substances, size fractions of DOC

1. INTRODUCTION

Most organic molecules dissolved in water lack optical activity in the visible range, and therefore appear colorless to the human eye. A prominent exception is that of humic substances, which strongly absorb in the blue range of the spectrum, and impart a yellow-brownish hue to water. Considering their color, those substances were called Gelbstoff (Kalle 1937 cf. Kalle 1966), yellow substance (Bricaud *et al*. 1981; Davis-Colley & Vant 1987), aquatic humus (Zepp & Schlotz-hauer 1981) and gilvin (Kirk 1994). Humic substances strongly absorb solar energy in the UV range, therefore the concentration of those compounds, and their composition have a high potential of influence on the aquatic environment, either as direct moderators of the in-water light climate, or sources of carbon for the aquatic biota (e.g., Obernosterer *et al*. 2001). Humic substances also display chelating activity (e.g., Alberts *et al*. 1984), and thus are capable of altering the bioavailability and/or the toxicity of elements, with a potential to influence the development of organisms.

The decline of light absorption by colored dissolved organic carbon (DOC) in water from the UV wavelength range towards longer wavelengths in the visible range is best described by an exponential function.

Logarithmic transformation yields, therefore, almost a linear line, and at any point is linearly correlated with all other points (Bricaud *et al*. 1981). DOC absorption is often defined quantitatively as the value measured at 440 nm, since that wavelength is located in the midpoint of the blue range peak of most plant pigments (Kirk 1994). The measured variability of DOC absorption at 440 nm varies over 3 orders of magnitude, with high values found in inland waters and the lowest in oligotrophic oceans (Kirk 1994). Attempts to translate the optical characteristic to concentrations were done only on a few occasions, and those attempts show that the specific absorption of different DOC sources varies considerably (Carder *et al*. 1989).

Humic substances are products of decomposition of plant material and comprise a large number of different molecules. A distinction is made between humic acid, and fulvic acid. The elemental composition of both groups is fairly similar, but they differ in their molecular weight range; humic acid is comprised of larger molecules than fulvic acid. The specific light absorption of humic acid is higher than that of fulvic acid, and the slope of the line characterizing a decline of absorption with increasing wavelength, is larger for fulvic than for humic acid (Carder *et al*. 1989). However, in aquatic systems the DOC contains a wide range of molecular

sizes, even in acidic waters, so the operational definition is less reliable.

The rivers that transect the coastal plain of Georgia, and discharge water to the Atlantic Ocean drain 89,000 km^2 of forested and agricultural uplands (NOAA 1985; Alexander *et al.* 1986). The concentration of DOC in those rivers ranges between approximately 5-40 g m^{-3}, which is ten times the concentration of particulate organic carbon (POC) (Alberts *et al.* 1990). For the most part, the rivers are colored by DOC, which is dominated by high molecular weight fractions (Alberts & Filip 1994). In this study we sampled six rivers (five draining into the Atlantic Ocean and one which drains into the Gulf of Mexico). The DOC was isolated and separated into fractions of defined molecular size. The goal of this study was to define the optical characteristics of those fractions in terms of spectral behavior and their impact on the optical characteristics of the bulk colored DOC.

2. MATERIAL AND METHODS

Water samples (approximately 10 l) were collected in July 1999 and once again in February 2000, from the Savannah, Ogeechee, Altamaha, Satilla, and St. Marys rivers, which drain into the Atlantic Ocean and the Suwannee River at Fargo, Georgia, near its origin in the Okeefenokee Swamp. The Suwannee River drains into the Gulf of Mexico. The sampling locations of the rivers have previously been described (Alberts *et al.* 1984, Alberts & Griffin 1996), as has the method of separation by ultrafiltration (Alberts & Griffin 1996). Briefly, the samples were transported (within less than 6 hours) in opaque, plastic carboys to the University of Georgia Marine Institute (UGAMI), and stored for maximally 4 days in the dark at 4 °C. Samples were filtered through 0.7 μm glass fiber filters (Gelman GF/F, precombusted at 450 °C for 4 hours). The product obtained after the filtration is called "origin" samples throughout this paper. The DOC of the origin samples was fractionated

by means of differential ultrafiltration in a stirred cell (Model 401, Amicon Corp.).

For ultrafiltration the cell was filled up to 400 ml, then the volume of the cell was reduced to 200 ml. We added 200 ml of deionized water to fill the cell , and again reduced the volume to half. This procedure, of deionized water additions, was repeated three times for each of two ultrafilters (Amicon Corp., XM-50 and YM10). Ultrafiltration resulted in three molecular size fractions: <10 ("small"), 10-50 ("medium") and >50 ("large") kilodalton nominal molecular weight (NMW).

The concentration of DOC in the filtered origin water sample and in the fractions was determined by high temperature combustion (Model 500 TOC Analyzer, Shimadzu Corp.) following the method by Benner & Hedges (1993).

Light attenuation of the samples was measured with a Perkin-Elmer Lambda 40 spectrophotometer, in a 1-cm-path-length quartz-glass cuvette over the range from 200 to 700 nm. Considering the short light pathway, and the fact that samples were filtered through 0.7 μm glass fiber filters, we assumed that scattering may be neglected as a factor causing light attenuation, and defined measurements as absorption. Readings were done against distilled water, and the absorption coefficient, a, was multiplied ×100, to get the absorption value expressed as m^{-1}. Absorption coefficient per mass unit of particles, specific absorption coefficient, a_{440}*, was determined from the ratio of the calculated absorption coefficient at 440 nm, (m^{-1}), to the DOC concentration (g m^{-3}), following the procedure used previously by Carder *et al.* (1989), and its unit is m$^2 \cdot$g^{-1}.

3. RESULTS

In July 1999 DOC concentrations ranged from 4.9 to 40.7 g m^{-3}, and from 7.1 to 40.5 g m^{-3}, in February 2000. Percentage recovery of DOC after ultrafiltration ranged from 90-112%, and 88-104%, respectively (Tab. 1). This

Tab. 1. Dissolved organic carbon (DOC) concentrations in Georgia Rivers from July 1999 and February 2000. Absorption coefficient at 440 nm (a_{440}) of the origin samples and the percentage contributed by the 3 size class fractions (see Material and Methods for size class definition).

	DOC (g m^{-3})	a_{440} (m^{-1})	Percent of fraction in total			Recovery (%)
			large	medium	small	
July 1999						
Suwannee	40.7	10.05	25.3	31.2	37.4	93.9
Ogeechee	35.0	10.19	24.0	24.1	41.5	90.0
Satilla	24.2	7.04	16.7	30.2	59.5	106.4
St Marys	20.0	6.65	14.2	35.2	50.4	99.8
Altamaha	6.8	3.48	18.6	31.0	62.7	112.3
Savannah	4.9	1.61	17.6	22.4	70.2	110.2
February 2000						
Suwannee	40.5	8.61	33.2	8.4	61.5	103.1
Ogeechee	13.5	3.27	19.0	11.0	67.0	96.9
Satilla	17.6	4.86	20.4	9.3	68.9	98.6
St Marys	25.7	6.67	14.3	19.2	55.2	88.7
Altamaha	7.1	2.60	12.8	10.1	70.6	93.5
Savannah	7.8	1.87	10.4	9.8	83.8	104.0

range is acceptable given the number of manipulations involved in the ultrafiltration process. In most cases in July 1999, and in all cases in February 2000, the majority of the DOC (>50%) was in the smallest size fraction (Tab. 1). In all comparisons between July samples and February samples, the proportion of the small class was greater in February, by a factor that ranged from 1.10 to 1.64. In July the medium fraction contributed higher proportion to DOC than the large fraction in 5 out of 6 cases. In February, on the other hand, the contribution of the large fraction was higher than that of the medium fraction in 5 out of 6 cases.
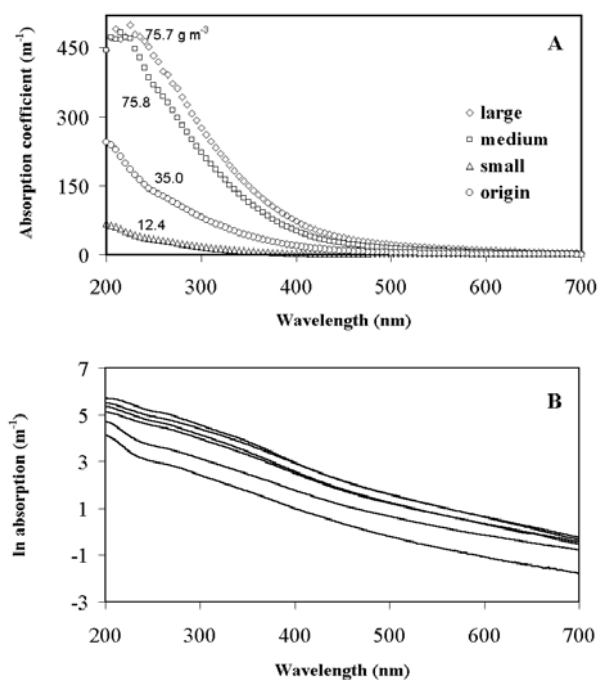

Fig. 1. Absorption coefficient spectra of dissolved organic carbon (DOC). A) Comparison of the origin and three molecular size fractions isolated from Ogeechee River samples in July 1999. The concentration of DOC (g m^{-3}) is indicated in each case; B) Ln transformed absorption coefficient spectra of the origin samples of the six rivers in the study. Measurements of the samples taken in July 1999. The order of presentation, from top to bottom, is: Suwannee (40.7), Ogeechee (35.0), Satilla (24.2), St Marys (20.0), Altamaha (6.8), Savannah (4.9). The number in parentheses indicates the concentration of DOC (g m^{-3}) in each sample.

The spectra of light absorption by DOC assumed the same basic pattern of monotonous decline with increase of wavelength, with a small exception in the range from 200 to approximately 230 nm regardless of the molecular size fraction examined (Fig. 1A). There were also exceptions in the small fraction from two rivers, with a slight increase of the absorption coefficient in the domain from 650 to 700 nm (data not shown). Otherwise, the similarity among rivers was high. The absorption values of the entire measured range were ln trans-

formed. The resulting line was not perfectly linear (Fig. 1B), therefore, a first derivative of the ln transformed data was calculated, to pinpoint the spectral range with minimal change of slope, for computation of the line parameters. It showed that in most samples, the slope in the range from 300 throughout 450 nm showed minimal variation. That was, therefore, the interval used for the calculation of the slope coefficient, S, of the total DOC and its fractions. The slope coefficient of the small fraction was larger than the slope coefficient of the medium fraction, and the slope coefficient of the medium fraction was on average larger than that of the large fraction (Tab. 2). In July, the variability of the large and small fraction was lower than that of the medium fraction, while in February the medium fraction showed the lowest variability. The correlation between DOC concentration and S in the different fractions was very low, and insignificant.

Light absorption coefficient at 440 nm (a_{440}) in the origin samples ranged from 1.61 to 10.19 m^{-1} in July, and from 1.87 to 8.61 m^{-1} in February (Tab. 1). The linear correlation between DOC and a_{440} was positive and high (Fig. 2). The mass-specific absorption coefficient, a^*_{440} spanned between 0.085 and 1.347 m^2 g^{-1} in different fractions and was highest in the largest DOC fraction. The average a^*_{440} value was smallest in the small fraction, and the a^*_{440} value of the medium fraction was between those extremes, both in July 1999 and February 2000 (Tab. 3). In the origin samples the average of a^*_{440} in July was 0.33 m^2 g^{-1}, and the only prominent exception was the value found for the Altamaha sample, i.e., 0.51 m^2 g^{-1}. In February, the average a^*_{440} was 0.26 m^2 g^{-1}, and once again, an exceptionally high value was found for the Altamaha sample (0.37 m^2 g^{-1}). The variability of a^*_{440} in the medium and small fractions was comparable to that found in the origin, but in the large fraction the variability of specific absorption was much higher than in the origin samples (Tab. 3).

4. DISCUSSION

The dissolved component of aquatic environments is composed, by definition, of any molecule capable of passing through a given pore size. The cutoff size is mostly determined by the filter used, which enables the passage of many types of colloids and ions, obviously with variable size and inherent optical characteristics. The current study clearly demonstrated a relationship between molecular size of colored DOC and optical characteristics, and moreover showed that the optical traits revealed by the colored DOC reflect the additive effect of the different molecular structures it is composed of. A comparison of the measured specific absorption coefficient in the origin filtrate (>0.7 μm), with the weighed average of the fractions showed a strong similarity (Fig. 3). The weighed average was 87-93% of the measured values in July 1999, and 98-116% in February 2000.

Tab. 2. Slope coefficients (S) of ln transformed data of light absorption in samples taken from coastal rivers in Georgia, in July 1999 and February 2000. S was calculated for the wavelength range from 300 to 450 nm in samples of the bulk DOC (origin), and 3 size class fractions (see Material and Methods for size class definition). In all cases, the correlation coefficient, r^2, of the calculated slope was >0.99. (av = average; std = standard deviation; cv = coefficient of variation).

Fraction	Large	Medium	Small	Origin
	(S, nm^{-1})			
JULY 1999				
Suwannee	0.0146	0.0175	0.0209	0.0166
Ogeechee	0.0139	0.0152	0.0186	0.0152
Satilla	0.0150	0.0142	0.0188	0.0160
St Marys	0.0149	0.0136	0.0178	0.0151
Altamaha	0.0149	0.0114	0.0189	0.0135
Savannah	0.0115	0.0139	0.0163	0.0140
av	0.0141	0.0143	0.0186	0.0151
std	0.00135	0.00201	0.00151	0.00117
cv	9.6	14.0	8.1	7.8
February 2000				
Suwannee	0.0154	0.0175	0.0201	0.0172
Ogeechee	0.0136	0.0164	0.0169	0.0160
Satilla	0.0130	0.0157	0.0175	0.0162
St Marys	0.0140	0.0156	0.0181	0.0163
Altamaha	0.0108	0.0151	0.0148	0.0142
Savannah	0.0116	0.0159	0.0169	0.0156
av	0.0131	0.0160	0.0174	0.0159
std	0.00167	0.00083	0.00173	0.00099
cv	12.8	5.2	10.0	6.2

Comparison of the light absorption coefficient at 440 nm from this study with data from other sources (Dekker 1993; Kirk 1994; Laurion *et al*. 2000) showed that the optical activity of yellow substance in Georgia rivers is among the highest in world water bodies. In the current study most of the DOC was found was in the small fractions (<10 kilodalton). The relatively low proportion in the large and medium fractions (Tab. 1) was in contest to previous studies in Georgia coastal rivers, which showed that the larger fractions dominated the bulk DOC (Alberts *et al*. 1984; Alberts & Filip 1994; Alberts & Griffin 1996).
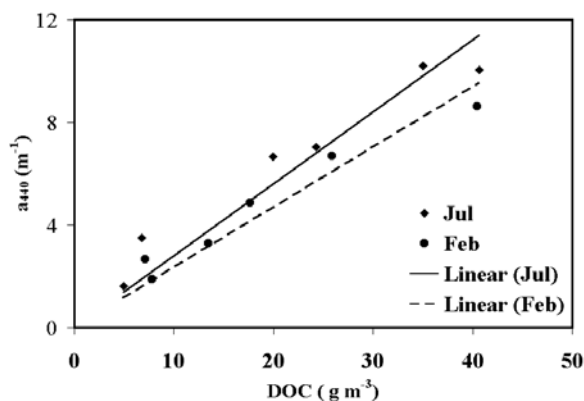

Fig. 2. A plot of the absorption coefficient at 440 nm *versus* DOC concentration in the origin samples. The relationship between the two parameters was: y = 0.2796x (r^2 =0.9051, n = 6, p <0.0007) in July 1999, and y = 0.2352x (r^2 =0.9210, n = 6, p <0.0005) in February 2000.

The logarithmic transformation of the absorption coefficient function of colored DOM did not result in a perfectly linear line in this study, as it was not absolutely linear in previous studies (Bricaud *et al*. 1981; Davis-Colley & Vant 1987; Carder *et al*. 1989; Dekker 1993; Mopper *et al*. 1996). The assumption that the deviation from linearity is caused by variability of the constituents of yellow substance (Dekker 1993) hardly applies, as the curvature seems to follow a similar pattern in all fractions (Fig. 1A). The deviation from linearity underlines the need of cautious choice of the portion taken for the calculation of S – the slope coefficient of the function. Our value calculated for the interval from 300 to 450 nm, 0.015 – 0.016 m^{-1}, was within the range previously reported for other freshwater and estuarine DOC (see compilation in Dekker 1993; Blough *et al*. 1993; Laurion *et al*. 2000), but were lower than the values found in the opened sea (Green & Blough 1994; Ferrari & Dowell 1998; Stedmon *et al*. 2000). The average S value found in our study is approximately an average between S values calculated by Carder *et al*. (1989) for fulvic acid and humic acid, isolated from sea-water in the Gulf of Mexico.

Carder *et al*. (1989) and Green & Blough (1994) found that fulvic acid has higher S than humic acid. Given the domination of the small fraction in our samples, we assume that fulvic acid constituted a higher proportion of yellow substance than humic acid. Fulvic acid was also found to be more abundant than humic acid in yellow substance in productive freshwater bod-

Tab. 3. Mass-specific absorption at 440 nm ($a*_{440}$, m^2 g^{-1}) in samples in the bulk DOC (origin), and 3 size class fractions (see Material and Methods for size class definition) taken from coastal rivers in Georgia, in July 1999 and February 2000. (av = average; std = standard deviation; cv = coefficient of variation).

Fraction	Large	Medium	Small	Origin
July 1999				
St Marys	0.612	0.400	0.152	0.333
Ogeechee	0.550	0.369	0.112	0.291
Satilla	0.500	0.369	0.128	0.291
Savannah	0.811	0.311	0.124	0.324
Suwannee	0.438	0.246	0.085	0.247
Altamaha	1.347	0.400	0.112	0.513
av	0.710	0.349	0.119	0.333
std	0.337	0.060	0.022	0.093
cv	47.5	7.2	18.7	28.0
February 2000				
St Marys	0.541	0.402	0.152	0.258
Ogeechee	0.525	0.265	0.112	0.243
Satilla	0.581	0.310	0.128	0.276
Savannah	1.396	0.244	0.124	0.238
Suwannee	0.391	0.217	0.085	0.213
Altamaha	1.877	0.289	0.112	0.374
av	0.885	0.288	0.119	0.262
std	0.605	0.065	0.022	0.056
cv	68.3	22.5	26.9	21.1

ies (Zepp & Schlotzhauer 1981; Visser 1984; de Haan & de Boer 1986), as it is in the opened sea. In the marine environment, bacterial activity was assumed to be the factor for the decrease in S (Blough *et al*. 1993), and it is highly probable that bacterial activity in freshwater is causing decrease in the proportion of large molecules of DOC in inland productive water.
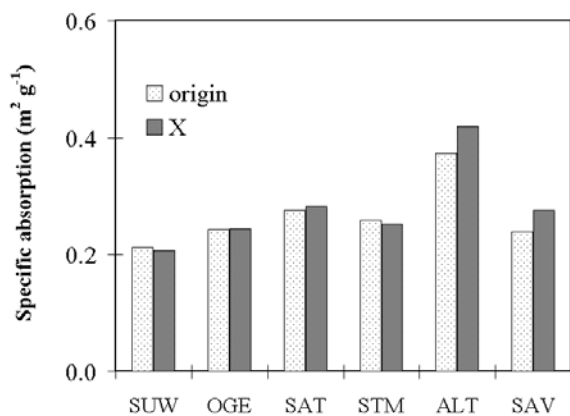
440 nm, $a*_{440}$, of the origin samples in our study, were within the range of 0.162 and 0.407 m^2 g^{-1} found for lakes in the Netherlands (Dekker 1993). The only exception was the extreme value of 0.513 m^2 g^{-1} found in Altamaha, in July. In July the average $a*_{440}$ was higher than in February (Tab. 3) and the slope of a_{440} *versus* DOC, was also higher (Fig. 2).

Fig. 3. Comparison of measured specific absorption coefficient of DOC of origin samples (filtrates passing 0.7 μm), with the weighed average of the fractions, in each sample (x). The presented data are from February 2000. SUW = Suwannee; OGE = Ogeechee; SAT = Satilla; STM = St Marys; ALT = Altamaha; SAV = Savannah.
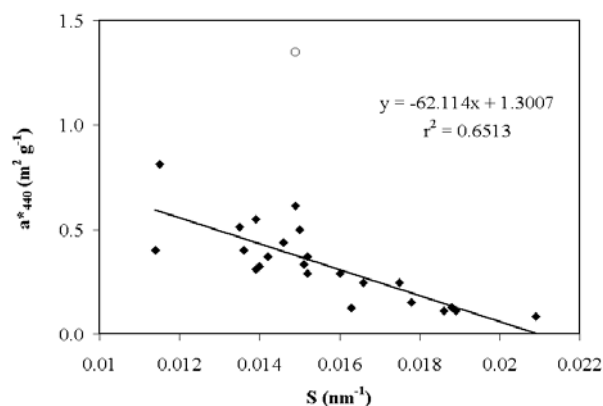

Fig. 4. A plot of slope coefficient, S, *versus* specific attenuation coefficient, $a*_{440}$. All samples and fractions of the July 1999 survey are included. The variables measured on the Altamaha large fraction sample (O) were excluded from the calculation of the regression line. The relationship of the 2 parameters in February 2000 was: $a*_{440} = -69.034*S+1.3$, $r^2 = 0.72$, n = 22, p <0.001.

Despite the similarity of spectral characteristics of all fractions studied, the mass-specific optical activity widely ranged. The mass-specific absorption values at

The higher average $a*_{440}$ in July is apparently the result of the higher proportion of the large and medium fractions in the colored DOM than in February (Tab. 1).

The high correlation between DOC concentration and specific absorption was seen in two separate surveys in this study, and is also known from other studies where DOC was dominated by colored substances (Vodacek *et al.* 1995; Laurion *et al.* 2000). Using measurements of the origin samples and all resulting fractions, we found a moderately high correlation between S and a*$_{440}$ (Fig. 4). The relationship between those two parameters was nearly identical, in two separate surveys. It indicates that both the pattern of light absorption and its magnitude is dependent on molecular size distribution of DOC, and underlines that the optical characteristics of the yellow substance are an outcome of the additive influence of DOC components.

ACKNOWLEDGMENTS

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