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3   **Freshwater DOM Quantity and Quality from a Two-Component Model of UV**
4   **Absorbance**

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17 **Highlights**

18 • UV optical absorbance by DOM can be explained by two end-member components (A
19 & B).

20 • We analysed a data set of $c.$ 1700 samples to derive the spectrum of each
21 component.

22 • Fractions of A and B can be obtained from optical absorbance at two wavelengths.

23 • The results permit DOC concentration to be estimated accurately and without bias.

24 • The fractional contributions provide information on DOM quality.

25

26 **Keywords**

27 Dissolved organic carbon, dissolved organic matter, model, UV absorbance

28 **ABSTRACT**

29 We present a model that considers UV-absorbing dissolved organic matter (DOM) to consist

30 of two components (A and B), each with a distinct and constant spectrum.   Component A

31 absorbs UV light strongly, and is therefore presumed to possess aromatic chromophores and

32 hydrophobic character, whereas B absorbs weakly and can be assumed hydrophilic.   We

33 parameterised the model with dissolved organic carbon concentrations, [DOC], and

34 corresponding UV spectra for c. 1700 filtered surface water samples from North America

35 and the United Kingdom, by optimising extinction coefficients for A and B, together with a

36 small constant concentration of non-absorbing DOM (0.80 mg DOC L^{-1}).   Good unbiased

37 predictions of [DOC] from absorbance data at 270 and 350 nm were obtained (r^2=0.98), the

38 sum of squared residuals in [DOC] being reduced by 66% compared to a regression model

39 fitted to absorbance at 270 nm alone.   The parameterised model can use measured optical

40 absorbance values at any pair of suitable wavelengths to calculate both [DOC] and the

41 relative amounts of A and B in a water sample, i.e. measures of quantity and quality.   Blind

42 prediction of [DOC] was satisfactory for 9 of 11 independent data sets (181 of 213

43 individual samples).

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46 **1. Introduction**

47 Dissolved organic matter (DOM), comprising the partial decomposition products of plant and
48 other biological materials, is ubiquitous in surface, soil and ground waters (Perdue and
49 Gjessing, 1990; Kullberg et al., 1993; Hessen and Tranvik, 1998). It has numerous
50 ecological and geochemical functions, including light absorption, pH buffering, interactions
51 with metals and organic contaminants, adsorption to surfaces and photochemical activity. It
52 plays a role in the terrestrial and aquatic carbon cycles, so that monitoring its
53 concentrations and fluxes can aid in understanding the effects of land use change,
54 acidification reversal and climatic warming (Pastor et al., 2003; Worrall et al., 2004;
55 Monteith et al., 2007;). Because differences in source material, rate and extent of
56 decomposition, and fractionation processes, are likely to generate substantial variability in
57 DOM concentrations and properties, monitoring studies often require measurements not
58 only of concentration, but also one or more indicators of quality. Currently this is most
59 often performed by measuring the concentration of dissolved organic carbon - [DOC] -
60 together with spectroscopic properties.

61 The main techniques for measuring [DOC] are persulphate oxidation or high temperature
62 combustion methods (Menzel and Vaccaro, 1964; Chen et al., 2002), including either the
63 removal or measurement of inorganic carbon. Both methods are fairly time-consuming and
64 costly, and require the transfer of samples to a laboratory. Qualitative information about
65 DOM is commonly sought from UV-visible absorption and fluorescence spectroscopy. For
66 example specific UV absorbance (SUVA), measured at 254 or 280 nm, is a measure of
67 aromaticity (Chin et al., 1994; Weishaar et al., 2003), absorbance slopes and slope ratios
68 provide information about DOM sources and properties (Helms et al., 2008; Loiselle et al.,
69 2009). Fluorescence spectra are widely and increasingly used to compare DOM in time and
70 space (Cabaniss and Shuman, 1987; Coble et al., 1990; Chen et al., 2003; Cory and
71 McKnight, 2005).

72 Since DOM varies in spectroscopic properties, it follows that, in general, measurement of
73 absorbance at a single wavelength cannot give accurate estimates of [DOC] (Tipping et al.,
74 2009). However within an individual environmental system (stream, lakewater) it is often
75 the case that temporal variability is sufficiently small for absorbance to provide a useful
76 estimate of DOC concentration (Mattson et al., 1974; Lewis and Canfield, 1977; Grieve,
77 1984; Moore, 1987) Furthermore, data for more than one wavelength have been utilised in
78 several studies to improve conversions from absorbance to concentration (Downing et al.,

79   2009; Fichot and Benner, 2011). A variant on this approach calibrates a multi-wavelength

80   detector against a DOM standard formulated in the laboratory (Sandford et al., 2010).

81   In the multiple wavelength methods (Mattson et al., 1974; Downing et al., 2009), empirical

82   approaches were taken to extract [DOC] from the spectra. However, Tipping et al. (2009)

83   showed in a preliminary study that a formal two-component (A and B) model could account

84   for absorbance data at two wavelengths (254 and 340 nm), and thereby provide accurate

85   estimates of [DOC]. Using a modest dataset of 48 samples from unpolluted surface- and

86   ground-waters, extinction coefficients for the two components were optimised and an

87   excellent model fit was obtained, with r^2=0.997 and root-mean-squared deviation (*rmsd*) of

88   0.7 mg L^{-1} in [DOC]. The much greater absorption of UV light by component A indicates

89   greater aromaticity and hydrophobicity (Thacker et al., 2005, 2008), so that knowing its

90   fractional amount provides a simple measure of DOM quality.

91   However, deviations from model predictions were evident in samples from waters draining

92   urban and industrial areas, and it was speculated that these arose from the presence of

93   anthropogenic non-UV-absorbing DOM.

94   The aim of the present study was to test and parameterise the two-component approach

95   more thoroughly, by making new parallel measurements of [DOC] and optical absorbance

96   on UK surface waters, and combining the results with data already obtained for sites in

97   Canada (Koprivnjak et al., 2010) and the USA (Miller and McKnight, 2010). The UK

98   sampling was designed to cover a wide range of water types, in an attempt to maximise

99   variability in DOM. We also tested the parameterised model on independent data sets.

100

101 **2. Methods**

102 In all, 427 samples were collected from UK sites, mainly from the North of England, but also
103 from East Anglia and Scotland. Most samples were from streams or rivers, but one
104 sampling site was a eutrophic lake. About one-third were repeat samples from 6 sites, and
105 the rest were single samples from different waters. The land-use types in the drainage
106 areas included upland and lowland peats, arable farmland, intensive pasture, urban and
107 industrial areas, coniferous and broadleaved forest. Samples for analyses of [DOC],
108 absorbance, conductivity and iron were collected in acid-washed polyethylene sampling
109 vessels of 500 cm^3 capacity. A separate sample was collected in a 100 cm^3 Pyrex bottle,
110 fully filled, for pH determination. The reported results refer to measurements made within 5
111 days (usually two days) of collection. To test for stability, in some cases a second 500 cm^3
112 sample was taken and stored in a refrigerator (5°C) before analysis.

113 The UK samples were passed through GF/F glass-fibre filters with a nominal size cut-off of
114 0.7 μm. Filtered samples were analysed for conductivity (Jenway 4510 instrument) and
115 total iron using ferrozine (Lofts et al., 2008), and for [DOC] by combustion to CO_2 using a
116 Shimadzu TOC-VCPH instrument, after acidification and purging with nitrogen to remove
117 dissolved inorganic carbon (DIC). The instrument was calibrated with phthalic acid solutions
118 (0 - 40 mg C L^{-1}). Suwannee River Fulvic Acid (SRFA) and sodium bicarbonate solutions
119 were used for quality control, the latter to check that the acidification-purge procedure
120 completely eliminated DIC. Measurements were made in triplicate, with random ordering
121 within each batch.

122 Absorption spectra in the UV-visible wavelength range (200-900 nm) were recorded first for
123 unfiltered surface water samples (without settling of particulates), and then for the same
124 samples after filtration. Measurements were also made on blanks and Suwannee River
125 Fulvic Acid as a quality control. An Agilent 8453 diode array instrument was used, with 10
126 mm quartz cuvettes, which were washed with 18.2 mΩ water and then rinsed with sample
127 before each measurement. All samples were allowed to reach room temperature before
128 recording spectra, to avoid condensation on the cuvette. Spectra were recorded in triplicate
129 in random order with quality standards at regular intervals. Sample pH was measured with
130 a glass electrode using a Radiometer instrument. The pH electrodes and the conductivity
131 meter were calibrated at the start of each set of samples, the spectrophotometer calibration
132 was checked monthly. To obtain absorbance values in the UV range for modelling, we
133 routinely subtracted the value of A_{700} from the measured values.

134   In Colorado, 116 samples were collected from May-September 2006 from oligotrophic alpine
135   and subalpine stream and lake sites in the Green Lakes Valley and adjacent Como Creek
136   watershed. The Green Lakes Valley is part of the Niwot Ridge Long-term Ecological Research
137   (NWTLTER) site and is not influenced by direct human impacts. The system is characterized
138   by a pulse of DOC during snowmelt in late May or early June, followed by a gradual return
139   to lower concentrations during baseflow (Miller & McKnight, 2010). Samples were filtered
140   with GF/F glassfibre filters of 0.7-μm nominal pore size (Whatman, c/o GE Healthcare Bio-
141   Sciences Corporation, Piscataway, NJ, USA), DOC was measured by high temperature
142   catalytic oxidation with a Shimadzu 5050A TOC Analyzer (Columbia, MD, USA), and
143   absorption spectra were measured on an Agilent 8453 UV-visible spectroscopy system
144   (Santa Clara, CA, USA).

145   Trent University collected stream and lake samples from a forested region of the
146   Precambrian Shield in Ontario from November 2007 to October 2008. The lakes are
147   oligotrophic to mesotrophic and are all headwater lakes, with one exception. Wetlands,
148   primarily Sphagnum-conifer swamps or beaver ponds, comprise from 0 to 25% of the
149   catchment areas. All streams were sampled near the mouth and all lakes at the outflow.
150   Samples were filtered with Millipore 0.45-μm membrane filters, and analysed for DOC
151   (Shimadzu TOC-VPH, Columbia, MD, USA) and optical absorbance (Cary 59 UV/Vis
152   spectrophotometer, Varian, Palo Alto, CA, USA). We assume that the different filters used
153   at Trent University and in the other two laboratories produce negligible differences in both
154   [DOC] and UV spectra.

155

156 **3. Modelling**

157 In our previous work (Tipping et al., 2009), we described a two-component model of DOM,
158 which accounted for optical absorbance in terms of the linear sum of two components (A
159 and B) each with its own fixed absorbance spectrum. We adopt the same approach here,
160 with one modification, which is the inclusion of a third component (C) that does not absorb
161 light and is present at the same concentration in all water samples. Thus, the DOC
162 concentration in a given sample is given by

163 $$[DOC] = [DOC_{AB}] + [DOC_C] \tag{1}$$

164 where DOC_{AB} refers to the light-absorbing components. Therefore

165 $$[DOC] = \frac{A_\lambda}{E_{AB,\lambda}} + [DOC_C] \tag{2}$$

166 where A_λ is the absorbance of the sample at wavelength λ (nm) in the UV range, and $E_{AB,\lambda}$ is
167 the extinction coefficient (absorbance cm^{-1} $[DOC_{AB}]^{-1}$) of the light-absorbing DOM. If the
168 DOM comprises two components, the extinction coefficient is given by;

169 $$E_{AB,\lambda} = f_A E_{A,\lambda} + f_B E_{B,\lambda}$$

170 $$= f_A E_{A,\lambda} + (1 - f_A) E_{B,\lambda} \tag{3}$$

171 where f_A and f_B are the fractions of components A and B that comprise the light-absorbing
172 DOM ($f_A + f_B = 1$), and $E_{A,\lambda}$ and $E_{B,\lambda}$ are the extinction coefficients at wavelength λ.
173 Equation (3) can be written for two different wavelengths, $\lambda1$ and $\lambda2$, and then a ratio R
174 defined by;

175 $$R = \frac{E_{AB,\lambda1}}{E_{AB,\lambda2}} = \frac{f_A E_{A,\lambda1} + (1 - f_A) E_{B,\lambda1}}{f_A E_{A,\lambda2} + (1 - f_A) E_{B,\lambda2}} = \frac{A_{\lambda1}}{A_{\lambda2}} \tag{4}$$

176 The value of R can thus be obtained simply from the measured absorbances at the two
177 wavelengths. Its combination with the extinction coefficients of components A and B yields;

178 $$f_A = \frac{E_{B,\lambda1} - R E_{B,\lambda2}}{R (E_{A,\lambda2} - E_{B,\lambda2}) + (E_{B,\lambda1} - E_{A,\lambda1})} \tag{5}$$

179 Therefore, if the values of $E_{A,\lambda1}$ $E_{A,\lambda2}$, $E_{B,\lambda1}$ and $E_{B,\lambda2}$ are known, f_A can be calculated and then
180 substituted back into equation (3) to obtain E_{AB} at either of the two wavelengths. Then if
181 $[DOC_C]$ is known, [DOC] can be obtained from equation (2).

182   For a chosen pair of wavelengths, measurements of [DOC], $A_{\lambda 1}$ and $A_{\lambda 2}$ for sufficient

183   samples allow estimation of the model parameters, i.e. the four extinction coefficients $E_{A,\lambda 1}$

184    $E_{A,\lambda 2}$, $E_{B,\lambda 1}$ and $E_{B,\lambda 2}$, together with [DOC$_C$]. This is done by adopting an initial trial

185   parameter set, calculating [DOC] for each sample and then computing the sum of squared

186   residuals between measured and calculated [DOC]. Improvement of the parameter values

187   (we used Microsoft Excel Solver to do this) then leads to their optimisation by minimising

188   the sum of squared residuals.

189   In principle, any pair of wavelengths could be used, but practicalities impose some

190   restrictions. While specific UV absorbance (SUVA) at 254 nm is widely employed in DOM

191   research, this wavelength has two disadvantages. Firstly there is possible interference due

192   to nitrate in systems with high levels (Edwards et al., 2001; Thomas and Burgess, 2007).

193   Secondly, when [DOC] is high (> 50 mg L^{-1}), the absorbance may be too high for reliable

194   determination. Therefore we favour 270 nm as λ1. Wavelength 2 (λ2) needs to be as

195   different from λ1 as possible, while still permitting measurable absorbance values even on

196   dilute samples, and we chose 350 nm. We propose these wavelengths for routine general

197   use, and employed them in the main analysis presented here. However, application of the

198   model is not restricted to absorbance values at 270 and 350 nm; we also fitted results for

199   other wavelengths in order to be able to analyse published data (See Sections 4.1 and 4.5).

200

201

202 **4. Results**

203 The 1698 samples studied cover wide ranges of [DOC], pH and conductivity (Table 1), and

204 originate from many different source environments, as described in Methods. Samples

205 stored for one week before processing gave indistinguishable results from those analysed

206 immediately, but there were decreases of c. 5% in both [DOC] and optical absorbance in

207 samples stored for periods between 50 and 120 days.

208 We previously justified the use of a two-component model on the basis of a plot presented

209 by Thacker et al. (2008) showing that for 23 concentrated DOM samples the ratio (E_{340}/E_{254})

210 varied monotonically with E_{340}, i.e. the values fell approximately on a single curve. The

211 same behaviour was evident in the present data (Figure S1). Simple modelling with three

212 components confirms that, if these were to occur randomly and have sufficiently different

213 UV spectra, monotonic behaviour would not be observed (Figure S2). The wide observed

214 range in E_{350} (Figure S2) confirms the high degree of variability in the UV absorption

215 properties of DOM.

216 *4.1. Parameterisation*

217 The five model parameters ($E_{A,\lambda1}$, $E_{A,\lambda2}$, $E_{B,\lambda1}$, $E_{B,\lambda2}$ and [DOC$_C$]) could be optimised to fit the

218 whole data set with good precision (*rmsd* = 1.2 mg L^{-1}, r^2 = 0.979), but as was found

219 previously (Tipping et al., 2009), a unique combination of parameters does not exist, i.e. an

220 infinite number of parameter sets can be found that provide identical results. It is even

221 possible for some of the extinction coefficients to have negative values, and for values of f_A

222 to be negative or greater than unity, all of which are physically meaningless. Therefore we

223 introduced a constraint, based on evidence from Thacker et al. (2001) who showed that

224 some physico-chemical properties of different samples of isolated DOM are strongly

225 correlated with their extinction coefficients. In particular, the fractions of the total DOM

226 sorbing to XAD8 resin or to alumina were positively, and approximately linearly, related to

227 E_{340}. Although in neither case is precise extrapolation to 100% sorption possible, the plots

228 given by Thacker et al. (2008) indicate that the limiting value of E_{340} is between 25 and 35 L

229 g^{-1} cm^{-1}. We interpreted this to approximate the extinction coefficient of DOM fraction A,

230 which has greater aromaticity, and therefore higher optical absorbance, hydrophobicity and

231 propensity to sorb to surfaces. Since the extinction coefficients at 340 nm and 350 nm

232 cannot differ greatly, we assigned a value of 30 L g^{-1} cm^{-1} to $E_{A,\lambda2}$. However, a unique

233 parameter set was still not obtained, and therefore a further constraint was imposed, based

234 on the finding that $E_{B,\lambda2}$ always took on low values (< 10 L g^{-1} cm^{-1}), suggesting that simply

235   setting $E_{B,\lambda2}$ to zero would be justifiable.   This means that our idealised component B

236   absorbs UV light at wavelengths < 350 nm, but is assumed to be fully transparent at higher

237   wavelengths. Refitting with these constraints did not affect either *rmsd* or r^2. The resulting

238   parameter set is unique, still gives the best fit, and provides a physically realistic picture.

239   Other constraints could be applied but they would differ only slightly from those we have

240   employed, and would produce the same results.   Setting $E_{B,\lambda2}$ to zero is only for

241   convenience, and equally good results would be obtained were it set to some low positive

242   value.

243   Inspection of the derived values of f_A showed that for 18 of the 1698 samples (0.11% of the

244   total) the ratio of measured to calculated [DOC] was <0.5 or >1.5, and these were treated

245   as outliers (we presume the discrepancies reflect analytical errors). After their removal, the

246   fit was slightly improved (*rmsd* = 1.1, r^2 = 0.989). The final model parameters are given in

247   Table 2, which also lists extinction coefficients at other wavelengths, used in the analysis of

248   independent data (Section 4.5). The measured and modelled [DOC] values are compared in

249   Figure 1. Figure 2 shows the full UV spectra of components A and B, derived by applying

250   the parameterised model to observed spectra in the range 250-400 nm.

251   Residuals between modelled and measured [DOC] tend to increase with [DOC], while the

252   relative difference tends to decline (Figure 1). Use of the model to estimate [DOC] will give

253   95% confidence limits of ± 0.9 mg L^{-1} for 0 < [DOC] < 5 mg L^{-1}, ± 2 mg L^{-1} for 5 < [DOC]

254   < 20 mg L^{-1}, and ± 4 mg L^{-1} for 20 < [DOC] < 80 mg L^{-1}. For the UK data set, the

255   corresponding 95% confidence limits for ranges of [DOC] determined by combustion were ±

256   0.2, ± 0.4 and ± 2 mg L^{-1} respectively. Note however, that the errors in the spectroscopic

257   method must result partially from inevitable differences in analytical results among the

258   three contributing laboratories.

259   *4.2.   Comparison with single wavelength approaches*

260   The results obtained with the model are superior to the global use of absorbance at a single

261   wavelength to estimate [DOC]. Fitting the whole data set for 270nm gave r^2 = 0.964, and

262   for 350 nm the agreement was worse, with r^2 = 0.932. Moreover, as shown by Figure S3,

263   there are biases, with problems at low concentrations, especially shown up by the log-log

264   plot. If the single wavelength relationships are used to construct predictive models, i.e. the

265   regression equations are used to predict [DOC] simply from absorbance at one wavelength,

266   the *rmsd* is 1.9 mg L^{-1} for 270 nm and 2.6 mg L^{-1} for 340 nm (the value for the two-

267   component model is 1.1 mg L^{-1}). These values mean that the two-component model using

268 both 270 and 350 nm reduces the sum of squared deviations between observed and
269 predicted [DOC] values by 66 and 82% respectively for the two wavelengths. If the single
270 wavelength regression at 270 nm were used to estimate [DOC], 95% confidence limits
271 would be ± 1.2 mg L^{-1} for 0 < [DOC] < 5 mg L^{-1}, ± 3.5 mg L^{-1} for 5 < [DOC] < 20 mg L^{-1},
272 and ± 6.6 mg L^{-1} for 20 < [DOC] < 80 mg L^{-1}.

273 We also compared the results obtained using the two-component model with those obtained
274 with single-wavelength modelling for individual UK sites that had been sampled repeatedly.
275 For each site, a linear regression analysis was performed for [DOC] vs A_{270}, and the
276 regression parameters were used to predict [DOC]. The results of these site-specific
277 calibrations were compared with the modelled values of [DOC] obtained from the global fits
278 of both the single wavelength and two-component models (which included the data for the
279 sites in question, but overwhelmingly more data for other sites). As shown in Table 3, even
280 when the single wavelength model is calibrated to an individual site, the two-component
281 global model (i.e. without site-specific calibration) gave higher values of r^2 in five of the six
282 cases, with no overall difference in *rmsd*. Overall the single wavelength global model gave
283 appreciably poorer results, although when the DOM characteristics are similar to the global
284 average, and relatively unvarying, which was found for the River Gowan (Table 3), good
285 results are of course obtained.

286 *4.3.   DOM quality*

287 By mathematically fractionating DOM into components A and B, i.e. deriving the value of f_A
288 for each sample, the model provides a simple measure of quality; the greater the proportion
289 of component A, the more light-absorbing and hydrophobic is the material. To display the
290 results, we plotted f_A against [DOC], although in principle there is no reason to expect any
291 relationships because f_A depends upon ratios of absorbance to [DOC] not [DOC] itself.
292 Indeed, for the UK samples, no relationships were evident (Figure 3), and the broad range
293 of f_A therefore reveals considerable natural variability in DOM quality, when steps have been
294 taken to sample disparate waters. The Canadian data set, although larger, refers to
295 samples from more similar surface waters, and the values of f_A tend to increase with [DOC].
296 Samples from the Colorado data set with [DOC] > 2 mg L^{-1} had f_A values of around 0.3,
297 while for more dilute samples, f_A fell in the range 0.1 to 1.0.

298 Another widely-used simple measure of DOM quality is SUVA$_{254}$ which also provides a
299 measure of aromaticity and hydrophobicity (Chin et al., 1994; Weishaar et al., 2003). As
300 might be expected, f_A and SUVA$_{254}$ are correlated. For UK samples with [DOC] > 3 mg L^{-1}

301 (results for lower concentrations were excluded because of noise in the data associated with

302 taking ratios of low values), linear regression gave r^2 = 0.66 (Figure S4). A closer

303 relationship between f_A and SUVA$_{254}$ is not found because values of f_A are derived after

304 factoring out the non-UV-absorbing component C, which would otherwise contribute to the

305 calculation of SUVA$_{254}$. Fraction B also contributes to SUVA$_{254}$. Note that if absorbance at

306 254 nm is known, the model-derived [DOC] can be used to estimate SUVA.

4.4.   Aspects of practical application

308 In our preliminary study (Tipping et al., 2009) we reported that [DOC] in surface water

309 samples from areas of industry and high human populations was underestimated by the

310 model (fitted to results from relatively unpolluted sites). During the present research, we

311 discovered that the measured [DOC] values for the non-conforming sites had been

312 overestimated, due to inadequate sparging of acidified samples, and the consequent

313 presence and inadvertent determination of DIC. This artefact has been reported in the

314 literature (Findlay et al., 2010). To obtain the results reported here, we took care to ensure

315 that all DIC was removed before the DOC analysis, and found that water samples from the

316 locations that had previously appeared anomalous now fully conformed to the model.

317 For the UK samples, we examined possible dependences of model output on pH and Fe,

318 both of which have been shown to influence spectra (Bloom and Leenheer, 1989; Maloney

319 et al., 2005). The ratio of calculated to measured [DOC] showed a slight pH dependence,

320 falling by 0.018 per pH unit (p < 0.001). There was no trend with total Fe concentration.

321 For filtered samples, the subtraction of the absorbance at 700 nm when processing the UK

322 data was intended to account for instrumental drift (cf. Hernes et al., 2008), but in practice

323 the correction had negligible effects. For unfiltered samples, the subtraction was explored

324 as a possible means of correcting for minor turbidity, none of these samples being

325 noticeably cloudy. The model was fitted to both the filtered and unfiltered UK samples,

326 maintaining the constraints on E_{A2} and E_{B2} at 30 and 0 L g^{-1} cm^{-1}. For the filtered samples

327 we obtained E_{A1} = 69.3 and E_{B1} = 15.4 L g^{-1} cm^{-1}, while for unfiltered samples the values

328 were 67.7 and 20.7 L g^{-1} cm^{-1}. The values of [DOC$_C$] were 0.80 (filtered) and 1.23

329 (unfiltered) mg L^{-1}. Values of r^2 were 0.989 (filtered) and 0.986 (unfiltered), and *rmsd* was

330 1.1 mg L^{-1} for the filtered samples and 1.4 mg L^{-1} for the unfiltered samples

4.5.   Testing on independent data

332   We tested the parameterised model by using it to predict [DOC] in water samples analysed
333   in 11 independent studies (213 data points) from a range of locations, for which appropriate
334   [DOC] and UV absorbance data were available. In most cases, absorbance data were not
335   available at our favoured wavelengths (270 and 350 nm), and so we modified the model
336   parameters using the spectra of Figure 2. As summarised in Table 4, we obtained
337   satisfactory agreement between predicted and observed [DOC] in 9 cases (181 data points).
338   For these data, 92% of the predictions fell within the 95% confidence limits presented
339   above, which can be considered satisfactory performance given that both the modelled and
340   directly-measured [DOC] values will have been subject to errors in each of the laboratories
341   that produced the independent results. Furthermore, linear and logarithmic plots of
342   predicted vs observed [DOC] (Figure S5) show no overall bias.

343   In two cases (32 data points) agreements were unsatisfactory. Data for Lake Pitkjärv in
344   Estonia (Selberg et al., 2011) gave an overall ratio of predicted to observed [DOC]
345   reasonably close to unity, but a high scatter in the results. For shallow lakes of the Yangzte
346   basin (Zhang et al., 2005), the model appreciably underestimated [DOC], by an average
347   factor of 2.1. The DOM in these lakes absorbs UV light extremely weakly, with an average
348   extinction coefficient at 280 nm of only 6.5 L g^{-1} cm^{-1}, about half of the values estimated by
349   Gondar et al. (2008) for autochthonous DOM produced in a UK eutrophic lake and in Lake
350   Fryxell, Antarctica (Aiken et al., 1996).

351   We also used the parameterised model to predict our own data, separated according to their
352   laboratories of origin.   The UK and Canada data gave ratios close to unity and values of
353   *rmsd* close to the overall average for all the satisfactory data sets. The Colorado data set
354   gave an average ratio of 1.19, but the smallest *rmsd*, reflecting the generally low [DOC] for
355   the Colorado samples, and consequently greater "noise" and more outliers. Nonetheless,
356   these data make an important contribution to the parameterisation by defining low
357   concentration conditions.

358   Selected independent data sets were used to test further the applicability of single
359   wavelength predictive models. Following the approach described in Section 4.2, the Table 4
360   data sets for the California agricultural stream, Congo River, North Wales streams, Scottish
361   & Welsh upland lakes, UK groundwaters and Yukon River (i.e. the larger collections for
362   which the two-component model predictions were reasonably good) were each fitted to
363   single wavelength models, using both the shorter and longer wavelength in each case
364   (Table S1). For the shorter wavelength (254 nm in all cases) the site-specific optimised
365   single wavelength model gave better fits (as judged by the *rmsd* in [DOC]) than the

366   globally-parameterised two-component model in four of the six cases, while the opposite

367   was the case for fits at the longer wavelengths (340 or 350 nm). Thus blind predictions

368   with the two-component model are at least as good as the optimised fitted values. When all

369   the data were combined and fitted to a single wavelength (254 nm) model, the *rmsd* in

370   [DOC] was 1.25 mg L^{-1}, greater than the value of 1.01 mg L^{-1} obtained with two-component

371   blind predictions, and the r^2 value was lower (0.93 vs 0.96). As found for the UK sites

372   referred to in Table 3, the parameter values for the single wavelength models varied

373   appreciably among the data sets (Table S1). Furthermore, the single wavelength model for

374   the combined data showed bias with overestimation of [DOC] at low and high

375   concentrations, and underestimation at intermediate concentrations, whereas the two-

376   component blind predictions fell centrally within the data.

377

378   **5. Discussion**

379   The results suggest that [DOC] and DOM quality in freshwaters can usefully be estimated

380   simultaneously simply from optical absorbance data at two suitable wavelengths.   The

381   estimation of [DOC] by this approach is markedly superior to the use of a single

382   wavelength, since the model is able to take into account variations in DOM extinction

383   coefficients among samples.   This is shown by the ranges of parameter values obtained

384   when single wavelength models are derived for individual locations or collections of similar

385   sites (Tables 3 and S1).   Such variation means that, although good fits can often be

386   obtained under restricted circumstances, a general single-wavelength model will always be

387   imprecise. The use of data at two wavelengths takes advantage of the wavelength variation

388   of the extinction coefficient range, thereby providing more accurate estimates of [DOC].

389   Equally important, the two-component model predictions are unbiased (Figure 1), in

390   contrast to single wavelength fits (Sections 4.2 and 4.5, Figures 1 and S3).   Even if the

391   single wavelength model is calibrated to a specific site, the two-component approach,

392   globally parameterised, gives results that are generally just as good (Tables 3 and S1,

393   Sections 4.2 and 4.5).   An important finding of the present work is that the previous

394   apparent discrepancies for some UK waters strongly impacted by human activities (Tipping

395   et al., 2009) were artefacts caused by incomplete removal of DIC during analysis. Although

396   we deliberately sampled a wide range of water types in the UK work, it could still be argued

397   that the data used for parameterisation refer to a somewhat limited range of environments,

398   restricted to mid-latitudes of the northern hemisphere. However, the satisfactory testing on

399   independent data sets (Table 4) covering a variety of sites from the tropics to the Arctic

400   suggests wide applicability.

401   Our primary aim in this paper was to explore the performance of the two-component model,

402   rather than to find the most precise and accurate means of predicting [DOC] from

403   spectroscopic data.   But we can mention comparative tests using the logarithmic multiple

404   regression model of Fichot and Benner (2011), which they used to predict [DOC] in coastal

405   seawater from absorbance values at 275 and 295 nm. We fitted their equation to data

406   from the UK (i.e. the data set with the highest variability in DOM sources), and obtained r^2

407   = 0.944. When used to predict linear [DOC] values, the r^2 was 0.946 and the *rmsd* 1.9 mg

408   L^{-1}, i.e. a poorer fit than provided by the two-component model (r^2 = 0.979; *rmsd* 1.2 mg

409   L^{-1}).   Moreover, the fitted Fichot-Benner model gave biased predictions, with

410   underestimation at low and high [DOC] and overestimation at intermediate values.

411   Therefore the two-component model can be considered superior, at least when applied to

412   wide-ranging freshwater data. This is perhaps not surprising, given that Fichot and Benner

413 (2011) found it necessary to calibrate their model separately for different locations and also
414 for different ranges of [DOC]. Of course, other multiple regression models involving
415 absorbance values at different wavelengths might yield better results, and this is certainly
416 worth exploring.

417 The two-component model accounts very well for the UV-absorbing components of DOM,
418 which often dominate, but the analysis requires the assumption that there is a constant
419 concentration of non-absorbing DOM, component C, with $[DOC_C] = 0.80$ mg L^{-1}. By
420 definition, this is not accessible to spectroscopic study, and the value of 0.80 mg DOC L^{-1}
421 must be considered an overall average. It is likely that some of the deviations between
422 measured and modelled [DOC] are due to variations in $[DOC_C]$. In further systematic
423 studies, efforts might be made to estimate how $[DOC_C]$ depends upon site characteristics for
424 example.

425 As explained in Section 4.3, the inclusion of $[DOC_C]$ in the model is one reason for the only
426 approximate relationship between f_A and SUVA (Figure S4), since the calculation of SUVA
427 would include $[DOC_C]$ as part of the total DOC. Thus while both f_A and SUVA reflect DOM
428 aromaticity and hydrophobicity they are not directly related. Both can be regarded as
429 empirical indices of hydrophobicity, derived from spectroscopic data, and they might be
430 used in combination for interpretative purposes, along with other spectroscopic indicators
431 such as spectral slopes and absorbance ratios (Zhang et al., 2005; Helms et al., 2008;
432 Hernes et al., 2008; Spencer et al., 2009; Spencer et al., 2010; Fichot and Benner, 2011).

433 The wide range of f_A found for the UK samples (Figure 3) can be attributed to the
434 deliberately wide range of water types chosen for sampling. For example, low f_A values
435 were obtained for samples from a eutrophic lake during summer, dominated by
436 autochthonous DOM, while peat drainage gave high values of f_A. Although the Canadian
437 data set is more than twice the size of the UK one, the sampled water bodies were less
438 variable, being mainly in boreal forested areas, within which systematic underlying control
439 processes on DOC quality and quantity may cause f_A to be correlated with [DOC] (Figure 3),
440 and explain why high f_A values are not found. The wide range of f_A values in the Colorado
441 data set represents the high spatial and temporal variability in the chemical quality of the
442 DOM in the alpine and subalpine watersheds. Average f_A values found for the independent
443 data (Table 4) vary, the highest values being found for the sites in Wales and the southern
444 USA, the lowest for groundwaters and the two Australian reservoirs. Obvious next steps to
445 improve understanding of variations in f_A (and other spectroscopic variables) are more
446 systematic studies on freshwaters with respect to land-use, soil and vegetation type, water

447   residence time etc, and application of the model to coastal seawater data (cf. Fichot and
448   Benner, 2011).

449   We do not propose the two-component model as a replacement of conventional
450   determinations of [DOC], but it could find significant applications in biogeochemical survey
451   and monitoring work, and in screening the quality of supply water destined for treatment
452   (Rosario-Ortiz et al., 2007). Combined with conventional analyses for quality control, it
453   offers the possibility to increase greatly the number of samples analysed, while multi-
454   wavelength monitoring would yield simultaneous derivation of DOM quality indicators
455   through f_A, derived SUVA, and other spectroscopic indicators mentioned above.
456   Furthermore, the approach might permit *in situ* monitoring of [DOC] and DOM quality in real
457   time, if practical difficulties such as turbidity effects and sensor fouling can be overcome.
458   Our results for the UK samples suggest that corrections using long-wavelength
459   absorbance/scattering values might be feasible at least for waters of relatively low turbidity.
460   The model might be improved by taking into account the minor pH dependence of estimated
461   [DOC]. There is also a need to standardise the gathering of matched [DOC] and
462   spectroscopic data, to minimise analytical differences among contributing laboratories.

463   The apparent two-component behaviour demonstrated here extends the observations made
464   in work on the functional properties of DOM (Thacker et al., 2008; Gondar et al., 2008) in
465   which variations in UV absorption and hydrophobicity were accounted for using mixing
466   models with two end-members. Given the accepted high degree of complexity of DOM
467   (Leenheer and Croué, 2003), this clearly cannot mean that there are actually only two
468   discrete chemical compounds in DOM, but there might possibly be two sufficiently similar
469   collections of molecules for two-component behaviour to emerge. Another possibility is a
470   regular continuum of DOM spectra, since models with any number of components could
471   equally well fit the data presented here, provided the extinction coefficients vary linearly
472   between those of the end-members (see Figure S2). To understand how the two-
473   component situation could come about, we need to know about the UV spectroscopic
474   properties of DOM as initially formed (e.g. from terrestrial plant litter, via soil organic matter
475   turnover, or from freshwater algae), and how the processes of fractionation (e.g. sorption
476   by mineral soils) and modification (e.g. photolysis in lake waters) affect those spectra.
477   Interpretation may depend upon how the UV spectra of DOM come about, either from the
478   linear superposition of the spectra of many different chromophores (Bloom and Leenheer,
479   1989; Korshin et al., 1997), or, as proposed by DelVecchio & Blough (2004), from
480   "intramolecular charge-transfer interactions between hydroxy-aromatic donors and quinoid
481   acceptors formed by the partial oxidation of lignin precursors". In a more general sense,

482   the two-component behaviour of DOM UV spectra may help to constrain models of DOM

483   production, transport and modification in soil-water systems.

484

485 **6. Conclusions**

486 The UV optical absorbance spectra of a large number of freshwater samples varying in origin

487 could be resolved into contributions from two components. Thus, the spectra appear to be

488 simple combinations of the strongly-absorbing component A, and the weakly-absorbing

489 component B, the former showing relatively more absorbance at longer wavelengths. The

490 different spectral shapes of A and B permit their resolution in a given sample, simply from

491 measurements of absorbance at two suitable wavelengths (e.g. 270 and 350 nm). Knowing

492 the fraction of each component and their individual extinction coefficients (L g^{-1} cm^{-1}), and

493 assuming a constant small (0.80 mg DOC L^{-1}) background level of non-absorbing DOM, then

494 allows [DOC] to be calculated. The fraction of one or the other of A and B is an indicator of

495 DOM quality; thus, the greater is fraction A, the more light-absorbing and hydrophobic is

496 the DOM. The results offer the prospect of rapid and inexpensive determination of [DOC]

497 and DOM quality, including *in situ* field monitoring. They also raise questions about the

498 origins of DOM spectra.

499

513

514

515   **Supplementary Data**

516   Table S1 shows results of single-wavelength modelling of independent data sets.   Figures

517   S1–S5 comprise plots of relationships among spectroscopic variables, results from single-

518   wavelength modelling, relationships between SUVA and f_A, and predicted and observed

519   [DOC] from independent data sets.

520

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653

654   Table 1. Summary of sample chemistries.

	UK	Canada	US	All
No. of samples	427	1154	117	1698
DOC (mg L^{-1})	0.9 - 54.6	1.2 - 74.5	0.6 - 10.1	0.6 - 74.5
pH	3.0 - 8.5	3.4 - 7.4	5.9 - 7.4	3.0 - 8.5
Conductivity(µS cm^{-1})	17 - 1959	8 - 316	7 - 98	7 - 1959

655

656

657

658 Table 2. Summary of model parameters. The extinction coefficients (E) have units of L g^{-1}
659 cm^{-1}, [DOC$_C$] is in mg L^{-1}.

660

	Component A	Component B
E_{270nm}	77.1	21.3
E_{350nm}	69.3	15.4
E_{254nm}	63.9	12.0
E_{280nm}	61.1	10.6
E_{285nm}	47.6	4.7
E_{310nm}	34.1	0.7
E_{340nm}	30.0	0.0
E_{355nm}	27.9	0.0
[DOC$_C$]	0.80	

661

662

663   Table 3. Comparison of predictions of [DOC] (mg L^{-1}) using single wavelength (270 nm)

664   and two-component models. For site-specific single wavelength fitting, linear regression

665   (slope m, intercept c) was used to relate [DOC] to Abs_{270} at each site, then the relationship

666   was used to back-calculate [DOC]. The single wavelength global predictions were made

667   using the linear regression fits of all 1698 data points. Numbers of samples are indicated by

668   n.

669

| | | | single wavelength models | | | | | | two-component | |
| | | | site-specific fit | | | | global prediction | | global prediction | |
Site	n	mean [DOC]	m	c	r^2	$rmsd$	r^2	$rmsd$	r^2	$rmsd$
River Conder	28	5.9	20.3	1.6	0.94	0.6	0.94	0.8	0.97	0.6
Cottage Hill Syke	23	29.3	18.5	3.8	0.70	2.9	0.70	4.9	0.88	3.0
Esthwaite Water	12	3.7	12.1	2.6	0.40	0.3	0.40	0.4	0.58	0.3
River Gowan	25	3.4	21.7	1.3	0.94	0.4	0.94	0.5	0.94	0.4
Rough Sike	30	14.5	19.0	0.8	0.96	1.3	0.96	4.0	0.99	1.5
Troutbeck	24	12.2	17.8	2.0	0.95	1.3	0.95	3.3	0.98	1.0

670

671

672   Table 4. Comparison of [DOC] predicted with the parameterised model and determined by conventional analysis.

Sample location and type	n^a	[DOC] range mg L^{-1}	λ_1 nm	λ_2 nm	mean ratio^b	*rmsd* mg L^{-1}	$f_A{}^c$ mean	ref.
Australian reservoirs	2	3.2 - 11.6	270	350	1.00	0.71	0.18	Liu et al., 2010
California agricultural stream	29	2.1 - 7.1	254	350	0.96	0.70	0.34	Hernes et al., 2008
California urban stream	4	4.4 - 10.6	254	310	0.79	1.37	0.33	Izbicki et al., 2007
Congo River	28	5.2 - 9.0	254	350	0.91	0.69	0.38	Spencer et al., 2010
North Wales streams^d	30	1.1 - 28.0	254	340	1.14	1.64	0.41	Evans et al., 2007
Scottish & Welsh upland lakes	31	0.6 - 8.1	254	340	1.14	0.57	0.51	Yang et al., 2009
SE USA rivers^i	6	1.8 – 9.8	270	340	1.00	0.53	0.42	Koprivnjak, 2007
UK groundwaters	12	1.1 - 11.0	254	340	0.96	0.82	0.19	Tipping et al., 2009
Yukon River	39	2.6 - 17.0	254	350	0.95	1.22	0.31	Spencer et al., 2009
All the above	*181*	*0.6 - 28.0*	*-*	*-*	*1.01*	*1.01*	*0.37*	*-*
Lake Pitkjärv, Estonia^e	10	16.5 - 45.6	254	285	1.10	3.93	0.44	Selberg et al., 2011
Yangtze River lakes	22	2.7 - 10.1	280	355	0.47	3.27	0.17	Zhang et al., 2005
UK data^f	426	0.9 - 54.6	270	350	1.03	1.37	0.50	this study
Canada data^g	1148	1.2 - 74.5	270	350	0.99	0.98	0.39	this study
Colorado data^h	106	0.6 - 10.1	270	350	1.11	0.25	0.36	this study

673   [a] no. of data points   [b] [DOC]$_{pred}$/ DOC]$_{obs}$ [c] for [DOC]$_{obs}$ > 2 mg L^{-1} Outliers omitted: [d]1, [e]3, [f]1, [g]6, [h]11

674   [i] includes two data points determined on samples concentrated by reverse osmosis

675

676 **Figure captions**

677 Figure 1. Modelled *vs* observed [DOC] after full optimisation (filtered samples); (a) all data

678 except for 18 omitted outliers (see text); (b) data for [DOC] $\leq$ 10 mg L^{-1}; (c) data plotted

679 logarithmically, with one point omitted because the calculated [DOC] was < 0. Solid lines

680 indicate 1:1 correspondence.

681

682 Figure 2. UV spectra of DOM components A and B. The extinction coefficients and

683 wavelengths derived from the parameterisation are indicated.

684

685 Figure 3. Variation of f_A with log [DOC]; (a) United Kingdom data, (b) Ontario data, (c)

686 Colorado data. Only values for [DOC] > 1.5 mg L^{-1} are plotted, because of the high degree

687 of uncertainty at lower concentrations. Three UK values with calculated f_A > 1 are omitted.

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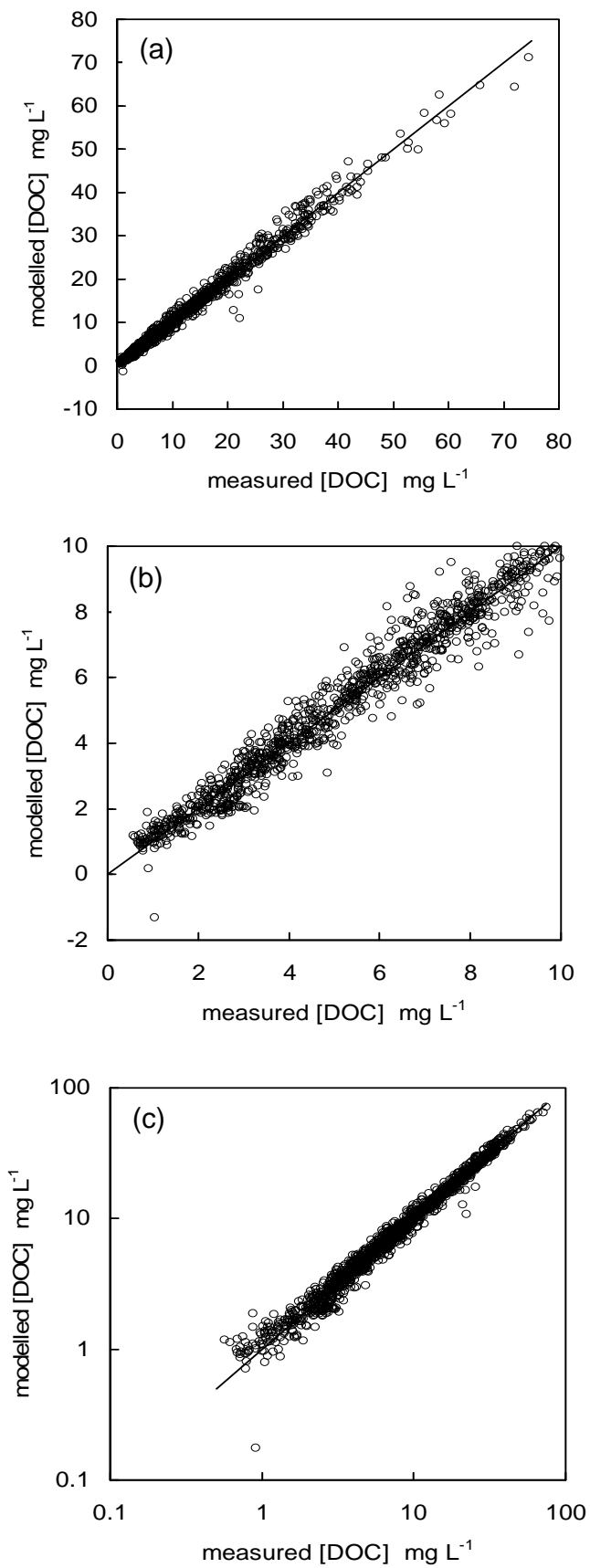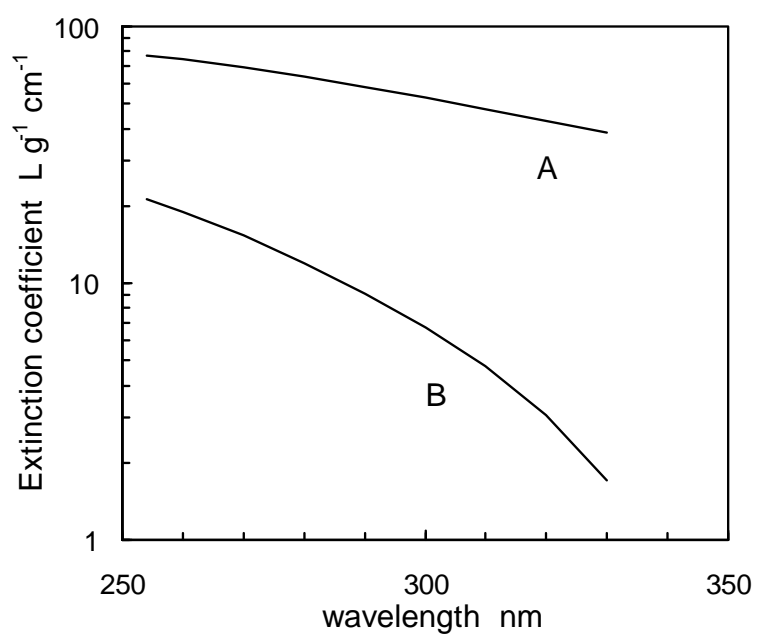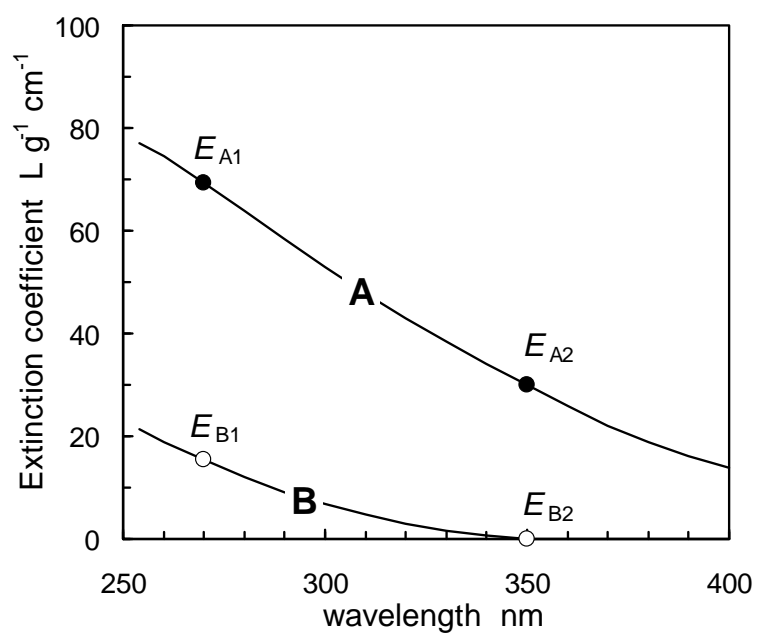
736   Figure 2

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Figure 3