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Improved automation of dissolved organic carbon sampling for organic-rich surface waters

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Abstract

In-situ UV-Vis spectrophotometers offer the potential for improved estimates of dissolved organic carbon (DOC) fluxes for organic-rich systems such as peatlands because they are able to sample and log DOC proxies automatically through time at low cost. In turn, this could enable improved total carbon budget estimates for peatlands. The ability of such instruments to accurately measure DOC depends on a number of factors, not least of which is how absorbance measurements relate to DOC and the environmental conditions. Here we test the ability of a Scan SpectrolyserTM for measuring DOC in peatland streams with routinely high DOC concentrations. Through analysis of the spectral response data collected by the instrument we have been able to accurately measure DOC up to 66 mg L^{-1} , which is more than double the original upper calibration limit for this particular instrument. A linear regression modelling approach resulted in an accuracy $> 95\%$. The greatest accuracy was achieved when absorbance values for several different wavelengths were used at the same time in the model. However, an accuracy $> 90\%$ was achieved using absorbance values for a single wavelength to predict DOC concentration. Our calculations indicated that, for organic-rich systems, in-situ measurement with a scanning spectrophotometer can improve fluvial DOC flux estimates by 6 to 8% compared with traditional sampling methods. Thus, our techniques pave the way for improved long-term carbon budget calculations from organic-rich systems such as peatlands.

Keywords: absorbance, peatlands, DOC, spectrophotometer, carbon fluxes

1 Introduction

Aquatic carbon fluxes from organic-rich systems such as peatlands are thought to be very important. For peatlands, estimates suggest that carbon released into river waters is equivalent to between a third to over half of the annual net ecosystem exchange (Dinsmore et al., 2010; Nilsson et al., 2008; Roulet et al., 2007). Traditionally, DOC flux measurements, particularly in peatlands, are based on occasional (e.g. weekly) grab sampling of river water (Strack et al., 2015; Tipping et al., 2010). However, this can be highly problematic. For example, in ombrotrophic peatland systems, river flow regimes can be very flashy (Holden and Burt, 2003; Price, 1992) and the greatest fluxes of dissolved organic carbon (DOC) may occur during short intervals at high flow (Holden et al., 2012b). Therefore, sampling DOC at monthly or weekly intervals in rivers draining ombrotrophic peatlands is likely to be inadequate for deriving annual flux estimates because crucial phases of flow will probably be under-represented by the samples. For this reason some researchers also supplement their weekly (or less frequent) sampling regime with storm event sampling for a handful of events throughout the year (Clark et al., 2007; Worrall et al., 2012). While this additional storm sampling may provide improved estimates of DOC flux there will still be large uncertainties because the relationship between discharge and DOC concentration is non-linear and may vary strongly depending on antecedent conditions. There is therefore an urgent need for a system to provide high resolution DOC sampling over time for organic-rich aquatic systems.

Samples collected from rivers and lakes which require DOC analysis in the laboratory are often directly measured using thermal catalytic oxidation (Billett et al., 2012; Clark et al., 2007). The method is well established and thought to have good accuracy and precision but can be expensive due to the relatively high cost of TOC analysers and consumables. A widely accepted alternative, for cases where it is neither possible nor practical to directly measure DOC using thermal oxidation, involves the use of UV-Vis absorbance as a proxy measure of

DOC. Humic substances strongly absorb light over the UV-Vis spectrum and a spectrophotometer approach offers both lower instrument and consumable costs. Most such proxy analysis has been conducted in the laboratory, on samples previously collected in the field. As such this technique merely reduces cost and does not deal with the need for a high resolution sampling frequency of river waters in organic-rich systems. However, the advent of logging UV-Vis spectrophotometers which can be left *in situ* in the field offers a potential opportunity to significantly improve the quantification of aquatic DOC fluxes and our understanding of how types of aquatic DOC change through time (Grayson and Holden, 2012). Nevertheless, these devices have not been fully tested under field situations for use as a DOC proxy.

Logging UV-Vis spectrophotometers were developed to provide 'on-line' water quality measurements within water and sewage treatment works. Their apparent ability to 'measure' DOC relies on the fact that humic acids strongly absorb light over the UV-Vis range (Chen et al., 2002). Thus these spectrophotometers require effective calibrations using laboratory-based DOC measurements and this may be constrained by local conditions and spatial variability in the relationship between absorbance and DOC. Absorbance measurements at numerous wavelengths have been proposed as the most effective proxy for DOC including 254 (Spencer et al., 2007), 272 (Spencer et al., 2007), 285 (Kalbitz et al., 1999), 340 (Baker et al., 2008; Spencer et al., 2007; Tipping et al., 1988; Tipping et al., 1999), 360 (Kalbitz et al., 1999), 365 (Baker et al., 2008; Spencer et al., 2007), 400 (Clay et al., 2009; Worrall et al., 2003; Worrall and Burt, 2007), 410 (Baker et al., 2008; Spencer et al., 2007), 465 (Hautala et al., 2000; Wallage and Holden, 2010) and 470 nm (Kalbitz et al., 1999). The use of a single wavelength for a calibration to DOC has been questioned (Wallage and Holden, 2010). Indeed, the exact make up and composition of DOC can vary between sites, typically being made up of a combination of humic and fulvic acids (Thurman, 1985). Indices such as E4/E6

(absorbance at 465 nm divided by absorbance at 665 nm) and specific UV absorbance (SUVA; ratio of UV absorbance at 254 nm to DOC concentration), have been used to characterise the composition of DOC. The very fact that scientists use SUVA as a variable, shows that it is expected that the relationship between DOC concentration and absorbance at 254 nm is not stable. As a result Tipping et al. (2009) developed a 'universal' two-wavelength model that uses absorbance at 254 and 340 nm as a proxy for DOM. This model was further developed by Carter et al. (2012) who suggested that the relationship between absorbance at 254 nm and DOC concentration might not be reliable in samples with high DOC concentrations ($> 50 \text{ mg L}^{-1}$) and hence proposed using 270 and 350 nm. The use of multiple wavelengths may allow measurement errors associated with a single wavelength to be minimised. This is particularly important for in-situ measurements where sampling conditions are outside of the user's control. For example, in peatland systems, particulate organic carbon (POC) may make up a significant proportion of suspended sediment concentrations (SSC) and it remains unclear how effective optical instruments are in quantifying POC due to differences in the properties of POC compared with mineral dominated SSC which affects how particles scatter and absorb light (Evans and Warburton, 2007).

If the above absorbance proxy issues can be overcome, then the development of submersible in-situ UV-Vis spectrophotometers offers the potential for hitherto unobtainable high-resolution aquatic DOC concentration change measurements and flux calculations. Grayson and Holden (2012) previously explored the use of an in-situ UV-Vis spectrophotometer to measure absorbance across the UV-Vis spectrum within a UK peatland stream. However, their study was focussed on the response of absorbance to flow conditions and did not include a DOC calibration. As yet, the use of in-situ UV-Vis spectrophotometers to measure DOC in aquatic systems remains relatively low, particularly in peatland systems (Grayson and

Holden, 2012; Koehler et al., 2009) where DOC concentrations can routinely exceed 20 mg L⁻¹ (Clark et al., 2007). The pathlength of submersible, *in situ*, UV-Vis samplers determines the concentration range over which they can measure, as with lab based UV-Vis methods narrow pathlengths are required where concentrations, and hence absorption, are high.

The overall aim of our study was, for the first time to produce high resolution aquatic DOC flux measurements in organic-rich environmental waters with routinely high DOC concentrations. In order to convert high resolution absorbance data to DOC concentrations we use data across the UV-Vis spectrum to determine the most appropriate calibrations. We also establish whether these calibrations can be used to extend instrument use well beyond the narrow manufacturer quoted instrumental measurement range. In doing so we demonstrate that wider pathlength devices, which are designed to measure lower concentrations but are less susceptible to biofouling, can in fact be used over a much greater measurement range which is suitable for capturing natural variability in streamwater DOC concentrations.

2 Materials and methods

2.1 Sites

Two blanket peatland catchments were used to conduct field trials in the study. Cottage Hill Sike (CHS) is located in the Moor House World Biosphere Reserve in upper Teesdale (54°41'41.0"N, 2°22'57.7"W), northern England (Figure 1). This site has been extensively used for carbon flux measurements (Billett et al., 2012; Clark et al., 2005; Holden et al., 2012b), with weekly regular sampling under strict protocols as part of the UK's Environmental Change Network programme (Sykes and Lane, 1996). Of the 7.4 ha catchment (Smart et al., 2013) blanket bog covers approximately 98 % (Adamson et al., 1998; Miller et al., 2001), with peat depth varying across the site, being typically 3 to 4 m

thick and extending up to 8 m in places (Holden et al., 2012a). The stream is gauged with a rated flume and water level sensor. The channel, although down to bedrock in places, is dominated by peat and so turbidity remains low. A pump sampler (ISCO 6712C) was installed to collect water samples for DOC calibrations every 8 hours or at hourly or 30-min intervals when triggered by high flow events.

Foulstone Beck (FB), South Yorkshire ($53^{\circ}24'57.2''\text{N}$, $1^{\circ}40'43.5''\text{W}$) is part of a legally designated Site of Special Scientific Interest and is located in blanket peatland within a privately owned estate that is managed as a grouse moor (for gun sports). Peat depths tend to be relatively shallow, normally no more than 1 m in depth, but our previous extensive sampling in northern England had identified the catchment as one with typically high DOC concentrations. The site was instrumented with a level sensor to measure discharge, a multiparameter sonde measuring conductivity and turbidity (Eureka Environmental Manta 2 with McVan Analite turbidity sensor) and a pump sampler (ISCO 3700C) to collect samples during storm events; the sampler was triggered based on rates of change in stream height on both the rising and falling limb of storm events. The stream sampling point was located in an incised reach, with the channel having cut down to bedrock and being characterised by a relatively high mineral bed load. As such it has a higher mineral turbidity load than that at Cottage Hill Sike.

2.2 Measurements

A Scan SpectrolyserTM (Scan Messtechnik GmbH) (hereafter referred to as the 'spectrolyser') with a 35 mm pathlength was installed into Cottage Hill Sike between July 2009 and March 2012. In April 2012 it was relocated to Foulstone Beck, with data being collected to August 2012. The spectrolyser measures and logs a 'fingerprint' of absorbance at 2.5 nm intervals between 200 and 750 nm during each individual sampling. The spectrolyser

software provided by the manufacturer can retrospectively calculate a range of parameters including total suspended solids, turbidity, $\text{NO}_3\text{-N}$, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total organic carbon (TOC), dissolved organic carbon (DOC), UV254, colour, Benzene, Toluene and Xylene (BTX), O_3 , H_2S and assimilable organic carbon (AOC) using the measured 'fingerprint' and a manufacturer provided 'global calibration' algorithm. The spectrolyser has been developed so that absorbance at a number of wavelengths is used to calculate DOC and then a correction for turbidity is applied where necessary. However, the propriety nature of the technology means it remains unclear exactly which wavelengths are used to either determine DOC or to correct for turbidity. Peatland stream systems are relatively unique due to their high organic content (both dissolved and particulate) and their acidic nature. Generic algorithms designed to calculate DOC from absorbance in rivers may not, therefore, be as effective in peatland streams where DOC concentrations are routinely much higher. The ISO 7027 approach for measuring turbidity uses scatter at 860 nm which is within the near infrared range. The spectrolyser is not capable of measuring at 860 nm but is capable of measuring absorbance at a number of wavelengths at the upper end of the visible range and lower end of the infrared range where organic substances do not absorb.

At both study sites the instrument was installed horizontally within the stream and was connected to a controller and a compressed air cleaning system. The system was powered using a 38 Ah sealed lead acid battery attached to a 10 W solar panel. Given the remote location of both sites a cylinder system was chosen to provide the compressed air for the cleaning system. An oxygen free nitrogen cylinder was used due to its inert, non-toxic nature and the fact that it does not support combustion. Due to the experimental nature of the field deployment in peatland waters, data loss often occurred due to biofouling. It took some time to establish the optimal cleaning setup, particularly in relation to how often cleaning took

place. Occasional siltation of the cleaning ducts also caused the system to fail and these had to be manually cleaned out before the system could operate correctly. To reduce the impact of vegetation entrained in streamwater fouling the probe, netting was placed within the channel to capture any vegetation and prevent it catching on the probe. The trapped vegetation was then removed from the netting during each site visit.

Site visits were carried out every two to three weeks to download data, check the power and compressed gas supplies, carry out manual cleaning of the probe and retrieve water samples collected by the pump samplers to support calibration. Measurement intervals for the spectrolyser ranged from 15 to 20 minutes, with the latter being the most commonly used. Where a 20-minute measurement interval was used, the instrument was capable of continually running for approximately 21 days before the available memory was filled. Stream water samples were collected during site visits and from the pump samplers. In total, both DOC and accompanying spectrolyser data are available for 85 samples from Cottage Hill Sike and 53 samples from Foulstone Beck. However, *in situ* spectrolyser data from river water at 15 or 20-min intervals were available for long periods between 2009 and 2012.

In order to improve the number of combined DOC and spectrolyser samples available to create and test a wavelength model to predict DOC, the spectrolyser was also setup in the laboratory. This allowed samples from a number of different peatlands to be included in the calibration without the requirement of moving and installing the spectrolyser at different sites, which can be both time consuming and expensive. In total 154 additional samples from six peatland sites across North and West Yorkshire (Woo Gill, High West Moor, Keighley Moor, Watersheddles, Marsden Moor and Close Moss) (Figure 1) were processed. Spectrolyser measurements were performed on unfiltered samples using the manual mode, with the manufacturer's multifunctional slide used to hold the sample; the slide and

instrument were rinsed with deionised water between samples. Therefore, the total calibration dataset includes 292 samples.

For all 292 samples DOC was determined using an Analytic Instruments Thermalox TOC analyser, or an Analytik Jena multi N/C 2100 TOC analyser; checks were carried out to ensure both instruments produced comparable results. In addition to the calibration dataset of 292 samples a further 107 stream water samples were filtered at 0.45 μm and measured in the laboratory for absorbance at 254, 340, 350, 400, 410, 450, 465 and 665 nm using a benchtop Jasco V360 dual beam spectrophotometer.

A series of calibration steps were undertaken to determine the accuracy of the spectrolyser. First, comparison of DOC calculated by the inbuilt algorithm and the DOC measured in the laboratory was conducted covering the natural concentration range observed. The second calibration stage involved comparing absorbance measurements made by the spectrolyser with measurements made in the laboratory by the Jasco V630. The third stage involved comparing individual wavelengths and groups of wavelengths as measured by the spectrolyser with lab based DOC measurements to establish whether predictive models of DOC using groups of wavelengths offer an improvement over single wavelength calibrations. Two approaches to the third stage were used: using groups of wavelengths that have, on an individual basis been widely adopted in the literature; and the use of linear regression modelling to identify key wavelengths capable of improving DOC prediction.

3 Results and discussion

3.1 Sensor calibration

Laboratory measured DOC in streamwater ranged from 0.51 to 66.8 mg L^{-1} . When the DOC concentrations calculated using the manufacturer's inbuilt algorithm within the local

calibration dataset, are plotted against DOC measured within the laboratory using a TOC analyser, two interesting findings emerge. First, there is a relatively strong linear relationship between DOC measured in the laboratory and that determined by the manufacturer's software within the spectrolyser where laboratory measured DOC was below 10 mg L⁻¹. However, between 10 and 34 (maximum returned DOC measurement by the spectrolyser) mg L⁻¹ the local calibration performs poorly (Figure 2). Second, the spectrolyser generally overestimates DOC in comparison to the TOC analyser (a series of standards are measured by the TOC analyser to ensure accuracy).

While the long term (1992-2007) ECN average DOC concentration for Cottage Hill Sike is 9.6 mg L⁻¹ the average for the data included in our wider calibration dataset was higher at 18.6 mg L⁻¹. At Foulstone Beck the average DOC concentration from routine stream water samples collected between 2011 and 2012 was 23.7 mg L⁻¹. Therefore, DOC concentrations were often in excess of 10 mg L⁻¹ at both Cottage Hill Sike and Foulstone Beck. As such, a large number of samples were unsuitable for inclusion within this local calibration as the inbuilt algorithm was unable to accurately calculate DOC. This also meant that the instrument was unable to calculate DOC concentrations for a large proportion of the continuous time series of spectrophotometric measurements taken at both sites, as they were in excess of the upper limit; at Cottage Hill Sike the instrument was only able to calculate DOC for c. 30% of the full fingerprint measurements made.

The relationship between absorbance measured in-situ using the spectrolyser and absorbance in the laboratory using a standard Jasco UV-Vis spectrophotometer indicates that for the majority of wavelengths included in our analysis, strong positive relationships exist between field and laboratory data (Figure S1). The two wavelengths where the relationships are weak are at either end of the spectrum (254 nm and 665 nm).

Data for 254 nm indicate that while it was possible to measure a wide range of absorbance within the laboratory using bench top spectrophotometers, the 35 mm pathlength spectrolyser is only capable of measuring over a narrow range and is, therefore, incapable of accurately measuring absorbance at 254 nm above approximately 100 Au m^{-1} . Within the laboratory, a narrower cuvette (2 or 5 mm) is often used to ensure absorbance saturation does not occur. Grayson and Holden (2012) previously identified that a high level of variability was observed between wavelengths below 300 nm when measured by the 35 mm pathlength spectrolyser; as such these wavelengths are unlikely to be useful for calculating DOC where concentrations are high. A shorter pathlength would result in less variability in absorbance for wavelengths shorter than 300 nm allowing wavelengths in this region to be included in any algorithm to calculate DOC. However, this would then mean the spectrolyser was less reliable for lower DOC concentrations.

In general, the laboratory-based measurements of absorbance at 665 nm were much lower than those measured by the spectrolyser due to the fact that laboratory measurements were made on filtered samples whereas in-situ measurements were made on unfiltered samples in streams. The presence of suspended material increased absorbance at the upper end of the visible spectrum even at relatively low concentrations.

The relationship between DOC measured using thermal catalytic oxidation and absorbance at all wavelengths measured by the spectrolyser varies by wavelength, being strongest between 340 and 435 nm (Figure 3). The relationships between DOC and absorbance for all wavelengths within the 320-442.5 nm range were found to have r^2 values in excess of 0.85. In comparison, the relationship between absorbance at 254 nm and DOC is lower ($r^2 = 0.638$). Although absorbance at 400 nm is often used as a proxy for DOC it is apparent from the field and laboratory measurements that the relationship between absorbance and DOC is stronger at other wavelengths. The strongest relationship between absorbance and DOC was observed

at 345 nm with an r^2 of 0.914 compared to an r^2 of 0.887 for 400 nm. This indicates that if only a single wavelength were to be used as a proxy for DOC when using the 35 mm pathlength spectrolyser in waters with high DOC concentrations, wavelengths around 345-360 nm may be a better proxy for DOC concentration in peatland stream waters. The poor quality of the spectrolyser data at wavelengths below 300 nm unfortunately also means that neither the Tipping et al. (2009) nor Carter et al. (2012) models can be used to calculate DOC from the continuous timeseries of spectrophotometric stream data.

3.2 Turbidity correction

The spectrolyser did not return any absorbance values in peat waters for wavelengths between 732.5 and 750 nm. At Foulstone Beck, mean absorbance at wavelengths between 710 and 730 nm exhibited similar temporal variability to the turbidity record from the Sonde, despite the turbidity record being noisy (Figure 4). This suggests that mean absorbance at these wavelengths may offer the potential to provide a turbidity correction. There are periods when gradual increases in the spectrolyser turbidity record are not mirrored in the turbidity record collected using a separate turbidity probe in the field (Figure 4). This is evidence that biofouling impacts absorbance measured by the spectrolyser at this upper end of the visible range, and must be dealt with in the field and accounted for when analysing data.

3.3 Wavelength models for predicting DOC

Of the 292 DOC samples available, 248 were included in a training dataset for model development, with the remainder being used to test the models. To explore the effectiveness of groups of wavelengths for predicting DOC, directly measured DOC data for the training dataset were plotted against seven groups of different wavelengths measured by the spectrolyser (Figure 5, Table 1). It is apparent that for the available dataset the use of groups of wavelengths offers marginal or no improvement over the use of a single wavelength,

increasing from an r^2 of 0.914 when just 345 nm is used to an r^2 of 0.918 for Group 7. No improvement was observed when applying the simple turbidity correction.

A linear regression model using IBM SPSS 20 was developed to predict DOC, with absorbance at all wavelengths being included as independent variables. Given that data are available every 2.5 nm there is a danger of multicollinearity. Using a forward stepwise approach with adjusted r^2 as the selection criteria, the model was capable of a high accuracy, with an r^2 of 0.953 (Figure 5). This model included nine statistically significant ($p < 0.05$) wavelengths (in order of importance 525, 400, 712.5, 685, 690, 727.5, 632.5, 575 and 392.5 nm) (Table S1).

The automatic linear modelling (ALM) approach (forward stepwise) in SPSS was also used to produce a model with adjusted r^2 as the selection criteria. This model included 18 wavelengths (in order of importance 400, 712.5, 685, 232.5, 335, 632.5, 237.5, 290, 575, 690, 707.5, 727.5, 525, 332.5, 677.5, 227.5, 285 and 287.5 nm) and automatically excluded two outlier DOC measurements. This helped improve the accuracy of the DOC prediction, with the r^2 increasing to 0.968 (Figure 5; Table 1). However, it is apparent that there is a high degree of multicollinearity within the dataset; for the first model all variance inflation factors were > 5 . Therefore, small changes in the input values are likely to result in changes in the variables included in the models and the coefficients used. Overall, the inclusion of additional wavelengths only resulted in a small improvement in the r^2 achieved by both models (Figure S2). The multiple regression approach only offers at best, an improvement of 5.4% over the use of absorbance at 345 nm on its own as a proxy for DOC (Table 1).

The ability of the models to predict DOC was tested using the 44 samples not included in model construction (Figure 5). The models appeared capable of accurately predicting DOC for these additional samples (Figure 5; Table 1). However, while the model using absorbance

at 345 nm as a predictor produces the highest r^2 it appears to over predict a set of 24 samples collected from a single site over a 12 hour period (Figure 5). This over prediction also occurred for the models using the seven groups of wavelengths (Figure 5). Therefore, we conclude that the regression modelling approach using multiple wavelengths appears to offer a more stable predictor of DOC which is better suited to a wider range of samples.

DOC flux estimates for peatland streams have often been calculated using the Walling and Webb (1985) flux calculation method, which uses routine grab sample data, discharge at the time of sampling and average discharge over the measured period to calculate DOC flux. Weekly (10/08/2009 to 08/11/2009) and monthly (August, September and October 2009) DOC fluxes (kg ha^{-1}) were calculated for Cottage Hill Sike using the linear regression, 345 nm and ALM models and the Walling and Webb method (Table 2). The weekly flux estimates show considerable variability between the different methods. In general the 345 nm model produced the lowest flux estimates with the linear regression and ALM models producing the highest. The weekly flux estimates show considerable inter-week variability, and it is also apparent that while the Webb and Walling method typically estimates lower DOC flux compared to the linear regression and ALM models, this was not always the case; in weeks 40, 41 and 42 the Walling and Webb method estimated the highest weekly DOC flux.

As the spectrolyser measures continuously it was possible use the ALM, linear regression and 345 nm models to produce continuous records of stream water DOC concentration and, when combined with the discharge data, DOC flux (Figure 6). It is clear that both the ALM and linear regression models produce noisier instantaneous DOC concentration records compared to the model that uses the single wavelength at 345 nm.

4 Discussion

This paper tested the use of an *in situ* scanning UV-Vis spectrophotometer for use in continuously measuring DOC concentrations for organic-rich stream waters. Despite the use of the wider pathlength instrument a number of biofouling issues were observed that had to be overcome when setting up these devices in the field, even with the use of pressurised gas cleaning systems. Hence careful quality control checks are necessary when using these devices in peatlands along with regular maintenance. With a routine programme of cleaning and prevention of vegetation entrapment, the instrumental drift was minimal.

A strong linear relationship existed between DOC calculated by the spectrolyser using its inbuilt proprietary algorithm and DOC measured in the laboratory up to 10 mg L^{-1} . However, for samples with DOC concentrations above this value, which is common for peatland streams, the relationship broke down indicating that the proprietary software was not suitable for measuring DOC above 10 mg L^{-1} in peatlands. Direct use of the software readout values for DOC from the device also meant that the spectrolyser overestimated DOC relative to laboratory measurements. Thus users of such devices in peatlands should be wary of applying previously developed algorithms taken from other types of environment. However, the benefit of using the spectrolyser was that the full absorbance spectrum was recorded making it possible to create a bespoke peatland stream DOC calibration for such instruments. We were successfully able to extend the upper limit of the 35 mm pathlength spectrolyser from 10 mg L^{-1} to at least 66 mg L^{-1} DOC. While use of shorter pathlengths would allow higher concentrations to be measured, they would be highly problematic in peatland streams as their use would greatly reduce the accuracy of *in situ* measurements for times when the streams are at low DOC concentrations and they would also be subject to much greater biofouling problems in natural peat systems.

The fact that the calibration dataset included data from eight geographically distinct peatlands, and yet the relationship was also strong for the two spectrolyser deployment sites,

is encouraging. The strongest relationship between absorbance at a single wavelength and DOC was found at 345 nm rather than at other wavelengths more traditionally used as proxies for DOC. Reliable absorbance data at 254 or 270 nm would have allowed us to apply the Tipping et al. (2009) and Carter et al. (2012) absorbance to DOC conversion equations to the data. Unfortunately the wide pathlength of the instrument tested prevented accurate measurement of absorbance at these wavelengths due to saturation. The use of groups of wavelengths based on previous literature did not substantially improve the DOC predictor models beyond that possible using a single wavelength. If such groups of wavelengths are used it was apparent that mean absorbance between 345 and 375 nm offered the best potential proxy. Linear regression modelling indicated that the use of multiple wavelengths in a stepwise model offered some improvements over the use of single wavelengths for predicting DOC. The two multiple wavelength models developed helped improve accuracy by 3.9 and 5.4%. However, an examination of the time-series records produced for instantaneous DOC concentration indicates that the linear regression and ALM models produce much noisier records compared to the 345 nm model. This reflects the fact that these two models include multiple wavelengths. While the 345 nm model produces a less noisy record compared with the other two models it also underestimates DOC compared to the other models. Likewise when weekly and monthly DOC flux estimates are calculated the 345 nm model routinely underestimates DOC compared with the linear regression and ALM models and the Walling and Webb (1985) method.

The spectrolyser was capable of accurate in-situ measurements of absorbance that are comparable with laboratory-based measurements. However, there was a discrepancy between the laboratory and field absorbance measurements at both 254 and 665 nm which is disappointing given the historic use of these wavelengths as diagnostic tools in determining the dominance of mature humic acids over immature fulvic acids as components of DOC. In

any case, however, the influence of SSC on absorbance at 665 nm limits its use as a suitable DOC proxy for *in situ* measurements in unfiltered natural stream waters. Nevertheless, the case for using a turbidity correction in peatland streams remains uncertain particularly where the catchment is dominated by blanket bog and any input of mineral material to the SSC load might be low. Where a simple turbidity correction factor was included in the predictive groups they offered a marginal improvement and further research is required to understand whether absorbance at UV wavelengths are affected by sediment to the same proportion as those at the top end of the visible range.

We recognise that there are fractions of DOC that do not absorb at any of the wavelengths typically used as proxies for DOC. However, without the development of an in-situ combustion method, UV-Vis absorbance is currently the most effective and promising method for carrying out high resolution in-situ DOC flux measurements within streams.

5. Conclusions

We presented high temporal resolution DOC flux estimates from upland peatland streams with routinely high DOC concentrations. Such high resolution DOC concentration and flux measurements have previously been unobtainable using standard approaches for calculating DOC fluxes. Our calculations indicated that in-situ measurement with a scanning spectrophotometer can influence DOC flux estimates by an average of 6 to 8% compared with traditional grab sampling methods. This suggests that the use of in-situ spectrolysers can help improve the accuracy of total carbon budget calculations from peatland catchments even though those instruments do not directly measure the carbon concentrations. This paves the way for using multiple wavelength *in situ* absorbance probes for estimating DOC fluxes in peatland streams and rivers over longer periods. We recommend the wider adoption of multiple wavelength *in situ* absorbance probes for long-term carbon flux studies of peatlands.

In order to ensure good quality data when using such devices we recommend i) that peat-based calibrations are developed for the instruments and users must not rely on previous calibrations or algorithms developed for other types of aquatic environments, these should be periodically checked through time; ii) compressed air is regularly used to clean the instrument, along with routine manual cleaning and blank measurements; iii) screens/netting are used to prevent entrapment of vegetation by the instrument.

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Table 1: Accuracy in prediction of DOC (r^2) for the different wavelength groups (including which wavelengths were included and whether a turbidity correction was included), the linear regression model, the automatic linear model and absorbance at 345 nm using both the training dataset and the full dataset.

	Training set	Test set
Group 1 (340-350, 400-410, 450)	0.906	0.815
Group 2 (340-350, 400-410, 450, turb)	0.904	0.801
Group 3 (340, 360, 365, 400, 410, 465, 475, turb)	0.895	0.773
Group 4 (340, 350, 400, 410, 450, 465)	0.910	0.806
Group 5 (340, 350, 400, 410, 450, 465, turb)	0.908	0.799
Group 6 (350-400, turb)	0.908	0.823
Group 7 (345-375)	0.918	0.850
Linear regression	0.953	0.832
Automatic linear model	0.968	0.834
345 nm	0.914	0.854

Table 2: Weekly and monthly DOC fluxes (kg ha^{-1}) from Cottage Hill Sike between August and November 2009 calculated using the Walling and Webb (1985) flux calculation method, the spectrolyser 345 nm data the spectrolyser ALM model, there is no November monthly flux as the monthly data were incomplete

	DOC Flux (kg ha^{-1})			
	Walling and Webb	Linear Regression	345 nm	ALM
week 33	3.91	5.06	3.81	5.69
week 34	1.95	2.12	1.87	2.13
week 35	5.12	5.75	4.96	5.81
week 36	9.60	11.32	9.84	11.49
week 37	1.09	1.26	1.15	1.26
week 38	0.06	0.06	0.05	0.06
week 39	0.06	0.06	0.06	0.06
week 40	4.25	3.85	3.57	4.02
week 41	2.30	2.18	1.89	2.24
week 42	0.35	0.29	0.26	0.29
week 43	4.60	5.06	4.51	5.17
week 44	4.89	4.94	4.23	4.77
week 45	7.59	8.79	6.81	9.02
Aug	17.6	20.9	15.1	15.6
Sept	9.1	9.1	7.7	7.8
Oct	15.1	16.3	13.7	14.0

Table S1: Variables and coefficients used in the linear regression model and the automatic linear model

	Linear Regression			Automatic Linear Model	
	Wavelength	Coefficient		Wavelength	Coefficient
intercept		-1.974			-0.73
Variable 1	525	-6.379		400	2.397
Variable 2	400	5.733		712.5	-39.424
Variable 3	712.5	-42.194		685	31.504
Variable 4	685	28.579		232.5	0.21
Variable 5	690	-11.788		335	0.276
Variable 6	727.5	11.920		632.5	36.167
Variable 7	632.5	34.018		237.5	-0.166
Variable 8	575	-17.329		290	-0.194
Variable 9	392.5	-2.737		575	-17.093
Variable 10				690	-12.926
Variable 11				707.5	-16.227
Variable 12				727.5	12.005
Variable 13				525	-7.682
Variable 14				332.5	-0.116
Variable 15				677.5	10.907
Variable 16				227.5	-0.078
Variable 17				285	0.067
Variable 18				287.5	0.081

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Figure 6: Discharge from Cottage Hill Sike between 10/08/2009 and 05/11/2009 plotted along with modelled DOC concentrations and fluxes calculated by the ALM, linear regression and 345 nm models.

Figure S1: Absorbance measured in-situ by the spectrolyser versus measurements made in the laboratory using the Jasco UV-Vis spectrophotometer. The black line shows the fit between the two.

Figure S2: Changes in the r^2 value for each step of both the linear regression and the automatic linear model.

Figure 1

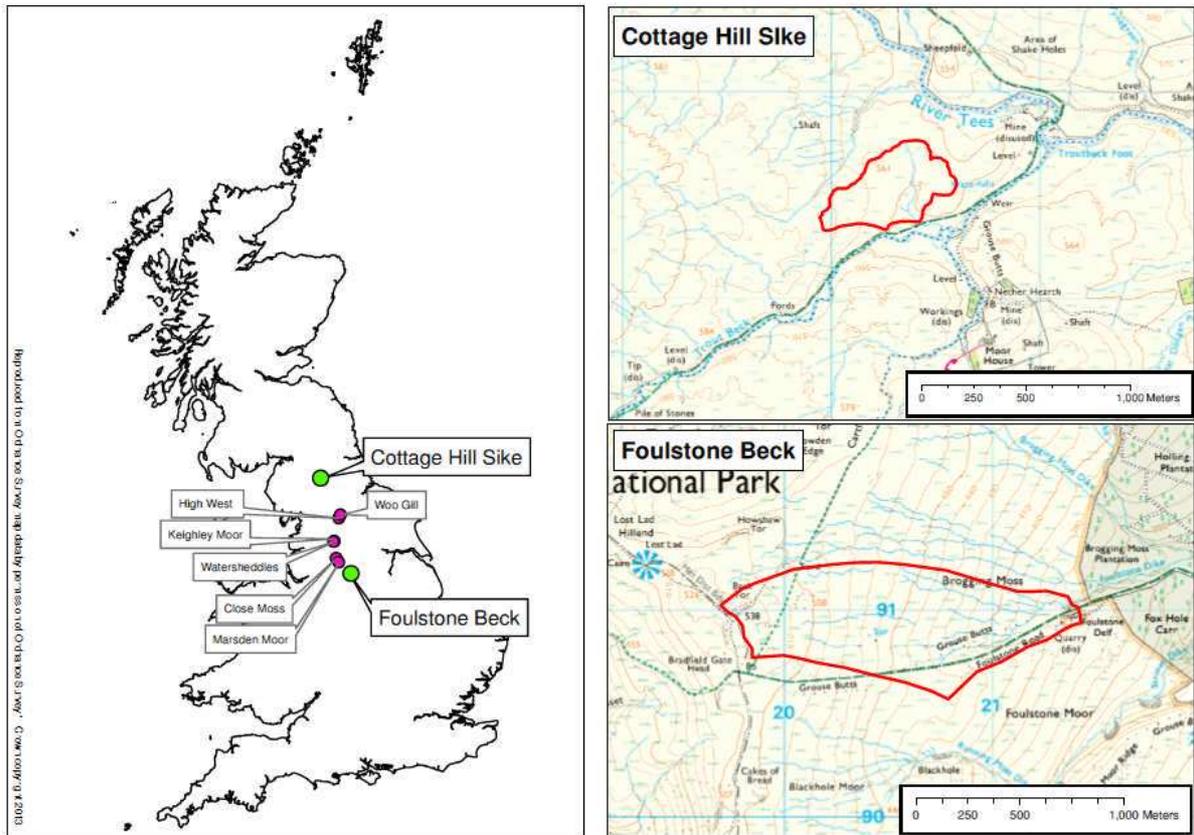


Figure 2

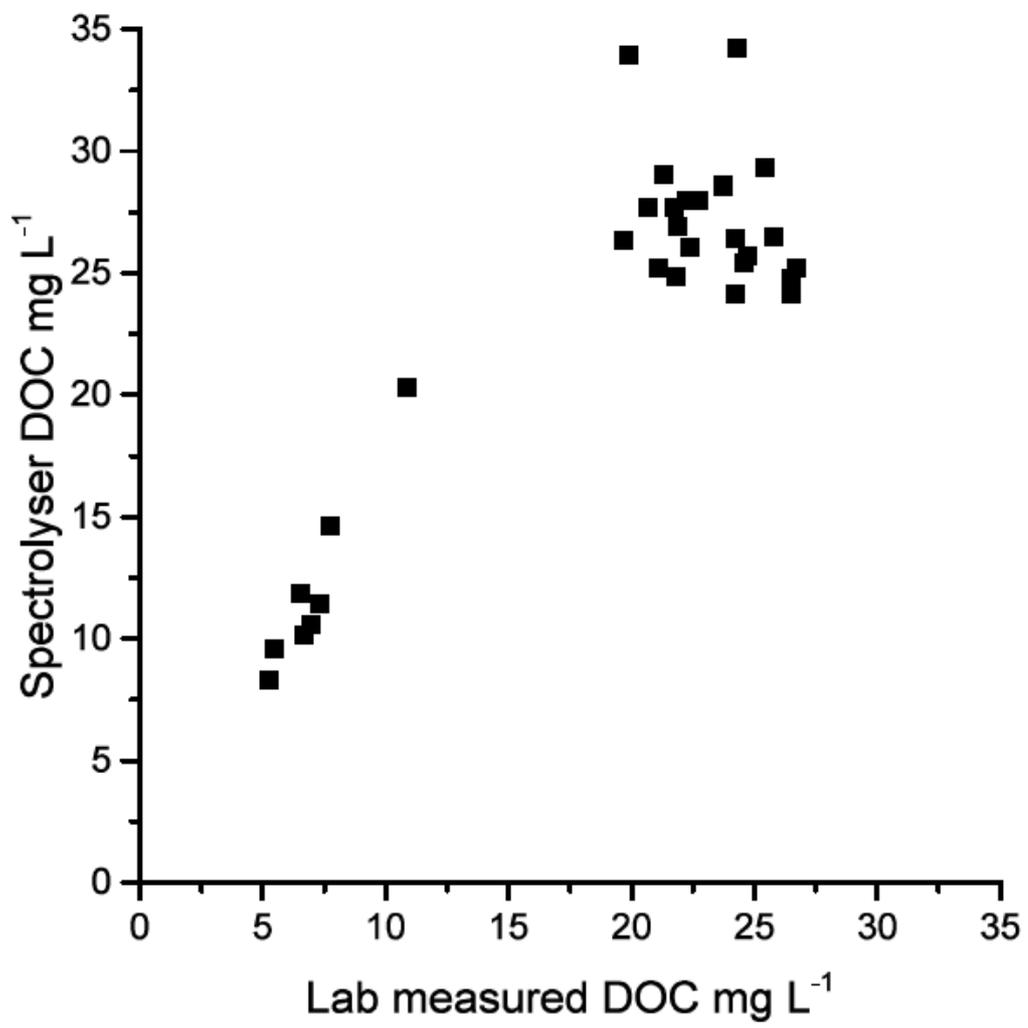


Figure 3

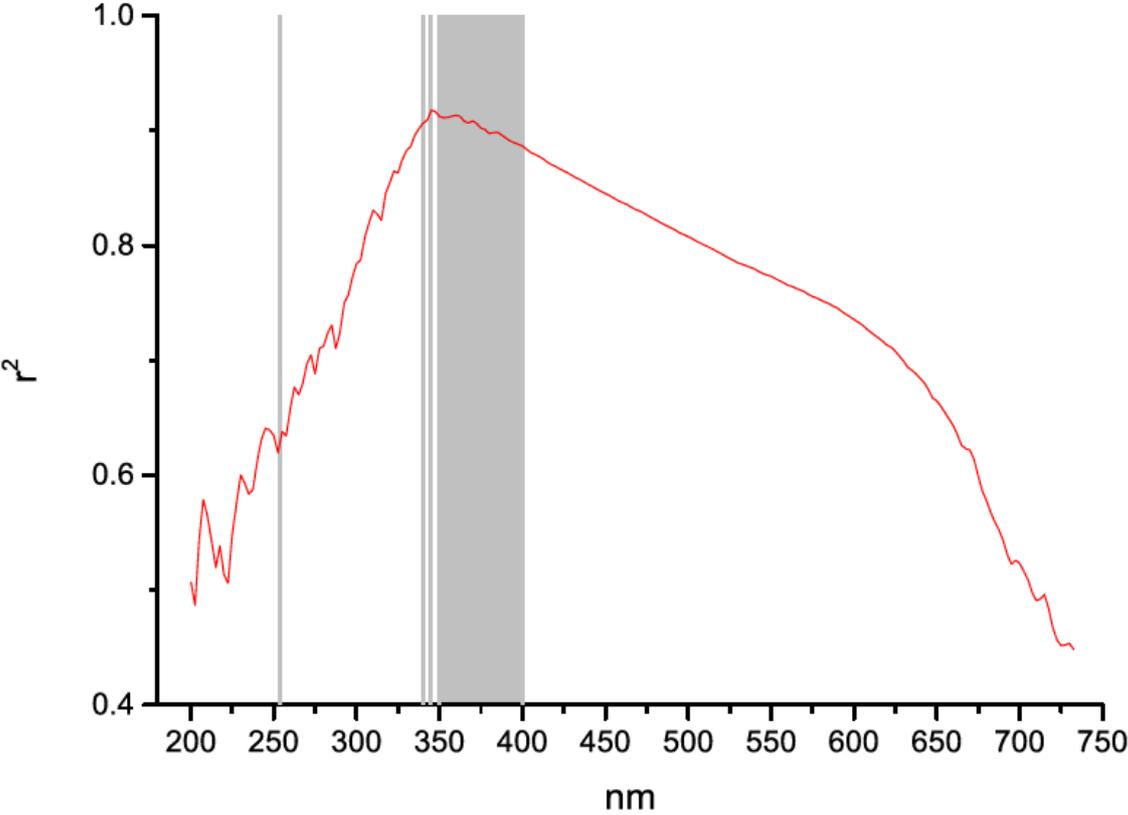


Figure 4

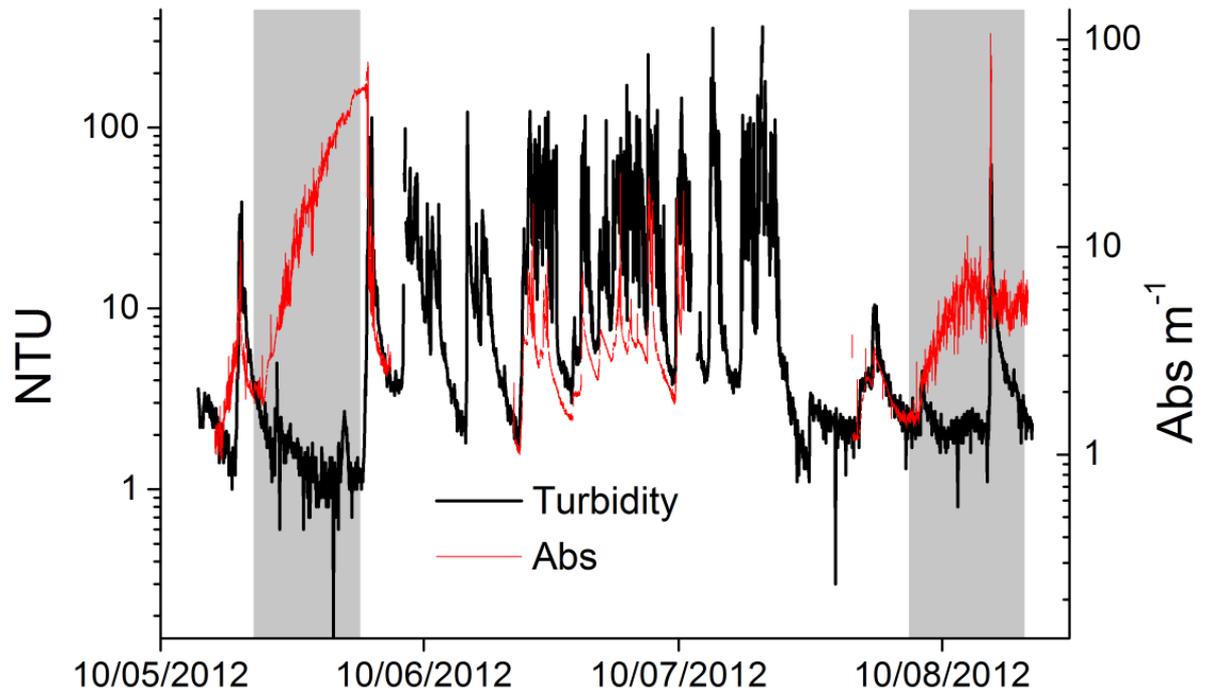


Figure 5

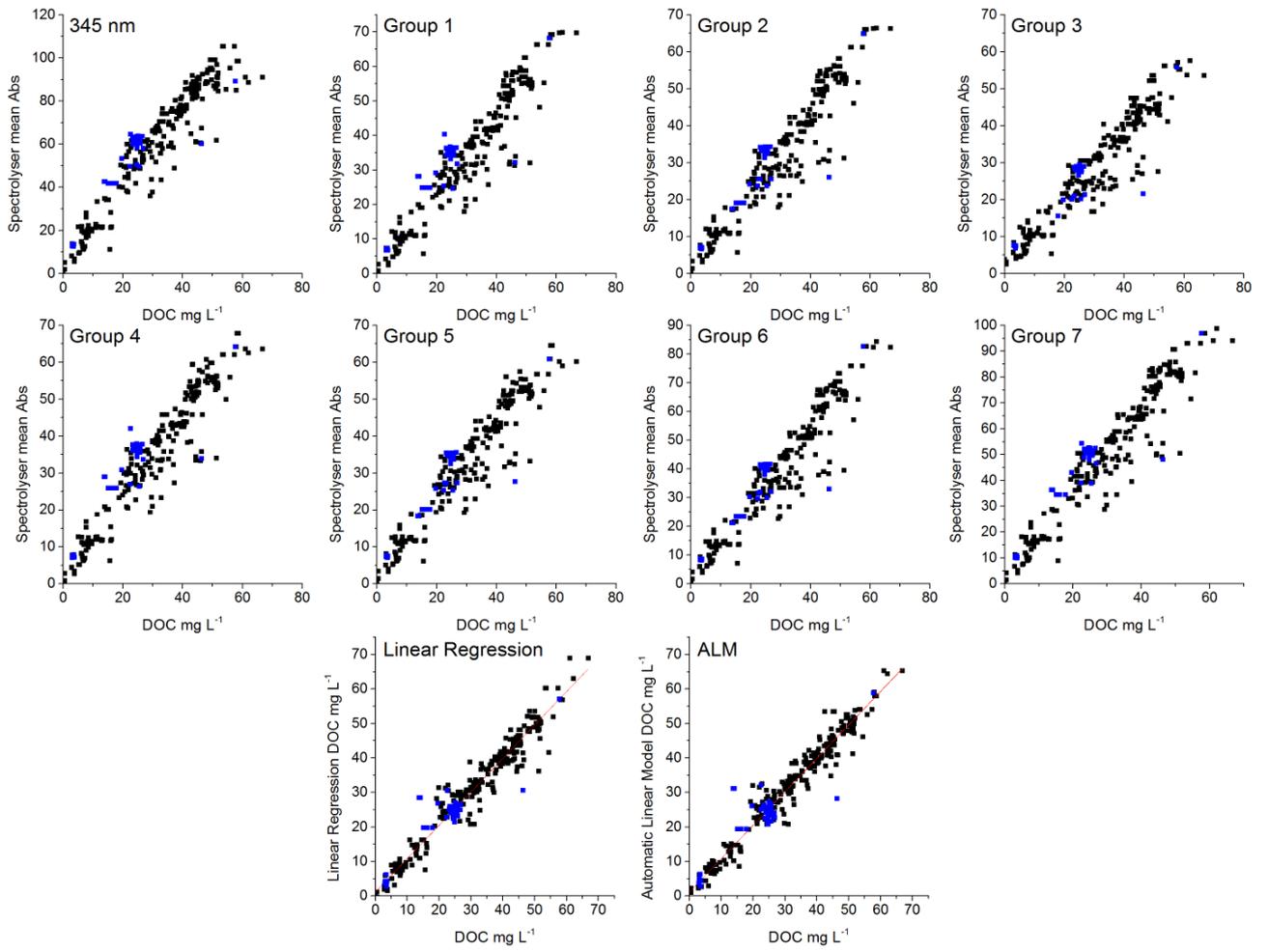


Figure S1

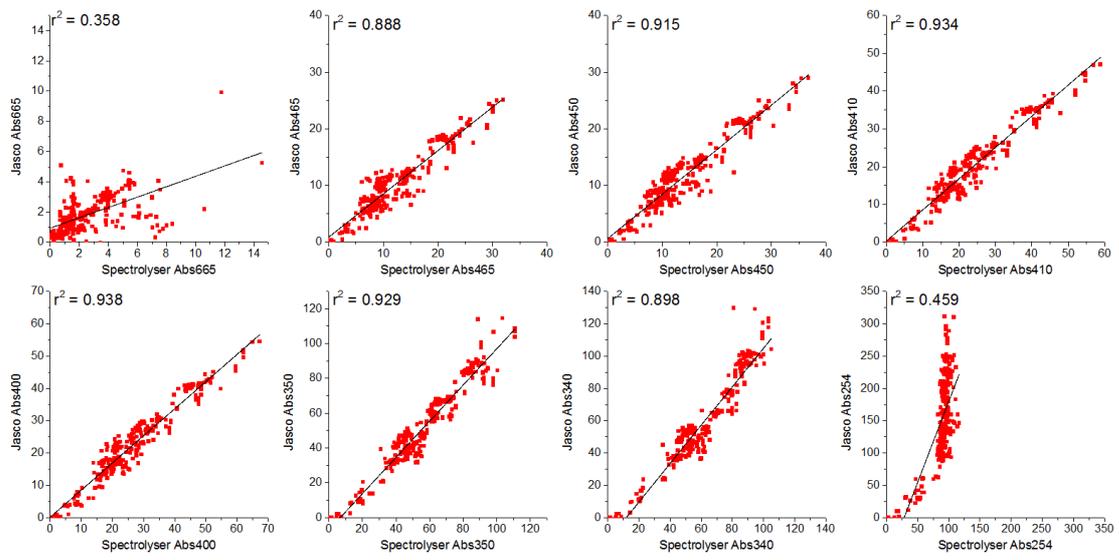


Figure S2

