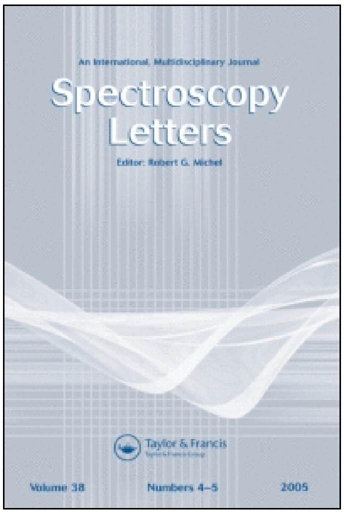

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Limitations of the Quantification of Organic Carbon in Sediment from C-H Stretching Vibrations in DRIFT Spectra

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Limitations of the Quantification of Organic Carbon in Sediment from C–H Stretching Vibrations in DRIFT Spectra

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Abstract: The limitations of quantifying organic carbon (OC) or various organic substances in sediment from the integration of infrared C–H stretching bands using diffuse reflectance infrared Fourier transform (DRIFT) spectra are pointed out, both from theoretical arguments and by presenting experimental data. Such determinations are inaccurate and imprecise because (i) the band at 2930 cm^{-1} is not exclusively due to CH_2 groups; (ii) there is a spectral interference from CO_3^{2-} absorption; and (iii) the proportion of CH_2 groups in organic matter varies for different sediments. The measurement of aliphatic C–H stretching band areas only provides an approximate measurement of aliphatic carbon bonded to hydrogen, which turns out to be a factor of about 3 to 3.5 times smaller than the OC content for Hong Kong marine sediments, depending on their nature and origin.

Keywords: Carbonate, C–H vibration, DRIFT, IR, marine sediment, organic carbon, organic matter

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INTRODUCTION

The quantification of organic carbon (OC) in sediment is important in estimating anthropogenic inputs from domestic sewage, livestock waste discharges, trade effluent, and mariculture activities. Furthermore, dissolved metal ions are complexed by films of organic matter coating most inorganic particles. Because the surface area to volume ratio is greatest for the smallest particles, the trace metals and OC are more concentrated there.

A simple method based on diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy has been put forward for the analysis of OC in dried sediment.[1] The method is based on the construction of a calibration curve from humic acid (HA) standards in a KCl matrix, of the peak height at 2930 cm^{-1} in the infrared (IR) spectrum (using a baseline crossing from 2800 to 3010 cm^{-1}) plotted against HA concentration. Then the concentration of HA in a homogenized, ball-milled sediment sample in the same matrix was determined from the peak height at 2930 cm^{-1}, by reference to the calibration curve. Furthermore, from the *same* sample measurement, it was proposed that the distribution of fulvic acids, humin, and total OC in the sediment could also be obtained. The theory of DRIFT spectroscopy has been given elsewhere,[2] so that only a brief, simple summary of the principle follows. IR radiation incident upon the top surface of the sample in a cup penetrates and undergoes absorption, reflection, transmission, and scattering so that part of the radiation reemerges at various angles from the surface. This radiation is collected by a hemispherical mirror and passed on to the IR detector. Using a mathematical transformation of the radiation intensity at the detector into Kulbelka–Munk (KM) units, then this transformed signal at the relevant vibration energy is linearly proportional to the concentration of the species involving this vibration in the sediment. In the use of DRIFT spectroscopy, particular attention should be given to possible artifacts from particle-size and bulk refractivity effects,[3] packing density,[4] and water content.[5]

OC has been related to OM (organic matter) by the factors of 2.5,[1] 2.0,[6] between 1.9 to 4.6,[7] or 1.7 as used by a referee of this work, which are all approximations. In principle, the former can be accurately determined,[8] but the latter term, OM, is vague. Accurate measurement of OC in marine sediment by a CHN analyzer *alone* requires first the removal of inorganic carbon by a suitable acid.[9] This determination then not only requires the quantification of the dissolved OC removed from the sediment by the acid but also the mass correction (from before and after the drying) of the sediment, which is partly changed to hygroscopic salts upon aqueous acid treatment.[10]

It is the purpose of the current work to show the limitations and scope of measurements of C–H stretching vibration band areas in quantifying organic substances. We do this by providing a theoretical background and also by giving some experimental results. The latter concern the spectral interferences and baseline errors in the DRIFT spectra. The choice of a unique fingerprint for OC (at 2930 cm^{-1}) in the DRIFT spectra is then experimentally investigated from the correlation with independent reference measurements of OC and alternatively by setting up a reference calibration graph. Our conclusions are that the simple measurement of one IR C–H stretching vibration band does not permit an accurate determination of OC in sediments but may be of use in screening if carbonate absorptions are corrected for or removed by chemical treatment.

MATERIALS AND METHODS

Sediment Collection and Pretreatment

Bulk wet surficial sediment was collected from two locations in Hong Kong with a Ponar grab from Aberdeen Typhoon Shelter (subsequently referred to as ATS), and with a plastic scoop from Shuen Wan (SW). Bulk surface marine sediment was also provided by the Hong Kong Environmental Protection Department from Green Island (GI). ATS sediment was situated >10 m beneath the seawater, highly anoxic, finely particle-sized, and polluted by domestic and industrial discharges. Sediment collected from the SW near-coastal region was oxic, subjected to tidal flushing and currents, coarsely particle-sized, and less prone to direct pollution sources. In the laboratory, the silt-clay fractions (<63 μm) were wet-sieved from the bulk sediment using seawater collected *in situ*, in order to remove coarse particles (mostly consisting of inorganic carbon) and grain size effects in the subsequent analyses. The silt-clay fractions were then collected in a beaker, mixed thoroughly with a glass rod, and subjected to freeze-drying or oven drying at 105°C. The dried samples were ground to fine powder using a mortar and pestle and stored in air-tight glass bottles. Subsequent analyses of these samples were performed separately in two sets of 5-replicates. The results listed herein refer to the pooled 10-replicate sets, but we were also able to assess the variability of the analyses from the two datasets.

CHN Dry Combustion Analysis

A CHN analyzer (Leco CHN-900) was employed for the reference determination of OC in sediment, after removal of inorganic carbonates.[9] Approximately 2 mg of dried, decarbonated sediment was accurately weighed into a tin capsule using a Leco 650 microbalance. Cystine (29.99% carbon content by weight), weighed in the same manner as the samples, was used for calibrating the CHN analyzer. Control samples were analyzed at every 20th measurement to check that the instrumental conditions were maintained, and if not, recalibration was performed.

The reference values for the wt% OC contents of the sediment samples in the current study were taken as 2.42 ± 0.04 ($N = 20$; ATS) and 2.08 ± 0.07 ($N = 20$; SW). The inorganic carbon contents of the sediment were $<1\%$.

Sample Preparation for DRIFT Spectra

The above sediment samples were mixed to $\sim$4 wt% with IR-grade NaCl or KBr (Riedel-de-Haën), and the mixture was hand-ground for 2 min. A Specamill (Graseby Specac Ltd.) ball-mill vibrator was then employed for 30 min homogenization and particle size standardization of the sediment samples (and also the NaCl or KBr) prior to IR measurements. The mixed sediment sample was placed in the cup of a SpectraTech DRIFT accessory incorporated into a Bomen 1020 FTIR spectrometer or in the cup of a Perkin-Elmer DRIFT accessory incorporated into a Perkin-Elmer 1600 FTIR spectrometer, scanning in the energy range from 4000 to 400 cm^{-1}, using 100 scans at resolution 2–4 cm^{-1}, after N_2 purging. The spectra were collected in the "transmittance" mode, and converted to Kulbelka–Munk (KM) presentation using NaCl or KBr background spectra. In each case, the detector signal was adjusted to its maximum for the position of the sample cup in the accessory. Quantitative analysis was also performed by external calibration using sodium dodecyl sulfate (SDS) diluted to various concentrations with NaCl and homogenized by ball-milling. In this case, the peak area between 2810 and 3020 cm^{-1} was integrated. The use of standard addition was also investigated using SDS, but the precision was poor. DRIFT spectra of calcium carbonate mixed with KBr (in the range from 0.1% to 20% w/w) were also recorded.

RESULTS AND DISCUSSION

Correspondence of DRIFT C–H Stretch Vibration Measurement with Concentration of OC in Sediment

We now point out from theoretical arguments that there is not a proportional relationship between the concentration of OC in a sample and a DRIFT C–H stretching vibration absorption band. The IR spectra of organic species, such as humic acids (HA), present a peak at 2930 cm^{-1}, which is superimposed upon a broad band from $\sim$2600 to 3700 cm^{-1} due to various aqua and hydroxy species.[11–13] Because this 2930 cm^{-1} band is due to aliphatic C–H stretching,[11–13] we take it (as others have previously done[1]) as a model candidate for quantitative analysis. However, there are implicit assumptions if we use this band to quantify OC or HA that: (i) the amount of OC (or, for example, HA) present in different samples is proportional to the aliphatic carbon bonded to hydrogen, and that if this is so, (ii) this unique frequency reflects the amount of carbon bonded to hydrogen (as CH_2).

It is useful to provide some generalizations and background concerning the compositions of OC from the use of other spectroscopic probes of the nature and structure of OC in sediment. The components of soil OC are decomposing residues, living biota, and resistant organic matter. The residues are mainly made up of cellulose, hemicelluloses, and lignin,[14] and the referee has also included "black carbon." Cellulose contains 46% carbon, of which roughly 83% is in CH_2 groups and 17% in CH. Typically, lignin contains roughly 9% of carbon as CH_3, 10% as CH_2, and 19% as aliphatic CH.[15] The structures of the other complex components are variable but have been extensively investigated by techniques such as ^{13}C CPMAS and ^{1}H NMR (for example, see Ref. 15). There is general agreement that soil OC is composed of at least 50% humic substances (HS),[14,16] but the proportion in marine and lacustrine sediments is less well characterized. In some lake sediments, HA only contributes between 1% to 8% of the total OC in the sediment.[17] Humin or HA in sediment may comprise ca. 50% C by mass,[18] and for the latter (HA) the carbon make-up from various postulated structures is about 2% in CH_3, 8% in CH_2, and 7% in aliphatic CH;[15] and from NMR measurements 6% CH_3, 22% CH_2, 4% CH_2OH, 12% CHOH.[18] Fulvic acid has a lower carbon content and a higher oxygen content than HA, and the carbon comprises 19–37% aliphatic CH, 6–24% CH_2 and 3–8% CH_3 from postulated structures.[19] The average C:H mole ratio differs considerably for lignin (0.9 : 1[6]), humin (0.5 : 1;[6] 1.1 : 1;[20] 0.7–0.9 : 1[21]), HA (0.8 : 1;[6,14] 1.2 : 1;[20] 0.7–1.0 : 1;[21] 0.8–1.2 : 1;[22] 0.5 : 1[23]), fulvic acids (0.64 : 1;[23] 0.5–0.8 : 1;[24] 0.6–1.2 : 1[25]), and HS (0.7–1.4 : 1;[26] 0.8–0.9 : 1[27]). We conclude that the use of carbon–hydrogen bonding in accurate quantification of OC, HA, or other substances does not appear to be tractable in view of the *low* and *variable* abundance of carbon in various aliphatic C–H groups. This is particularly so when sediments are characterized by high percentages of lignin to total sedimentary OC,[28] so that only a small portion of the carbon exists as aliphatic CH_2. Statement (i) should therefore not be generally accepted as true.

Regarding the statement (ii), the intensities, energies, and number of spectral bands for C–H stretching vibrations in the IR spectrum differ for simple CH, CH_2, and CH_3 groups. The degeneracy of a vibration indicates the number of symmetry-equivalent vibrational modes that have the same energy. A simple CH_3–X type molecule has one nondegenerate C–H symmetric stretching mode near 2965 cm^{-1}, $\nu_s(CH_3)$; and a doubly degenerate C–H stretching mode near 3040 cm^{-1}, $\nu_d(CH_3)$. The normal vibrations of a simple CH_2X_2 type molecule in this spectral region are the C–H symmetric stretch near 2985 cm^{-1}, $\nu_s(CH_2)$, and antisymmetric stretch near 3085 cm^{-1}, $\nu_{as}(CH_2)$, both of which are nondegenerate. Finally, simple CHX_3 type molecules exhibit a nondegenerate C–H stretching mode near 3020 cm^{-1}. These bands, due to aliphatic carbon bound to hydrogen, are usually stronger than the weak bands due to CH vibrations of aromatic and unsaturated carbon, which lie in the range 3000–3100 cm^{-1}. All of these energies vary, depending on factors such as the nature of the group(s) X, the phase, and temperature. There is no simple relationship between the intensities of bands due to these different vibrations. The C–H stretch region is further complicated by the presence of overtone and combination bands of bending vibrations, which may be enhanced by Fermi resonance. In sediment, it is not possible to assign bands to these three individual types of groups. This is because of the fact that in complex matrices, or polymers, the symmetry coordinates of C–H stretching vibrations of CH_n groups ($n = 1, 2, 3$) mix, and several unresolved bands are observed in the C–H stretch spectral region. Thus, the maximum near 2920 cm^{-1} is then usually assigned not only to CH_2 stretching, but to CH_n stretching,[12,29] and, for example, at least 5 bands between 2956 to 2849 cm^{-1} were curve-resolved in the IR spectrum of a coal sample.[29] We have found[8] that the use of low temperatures, down to 10 K, does not result in a complete resolution of spectral features in this region. Moreover, the energy of the band maximum is not constant and shifts depending on the molecular weight fraction: for example, for HA in peat to an energy between 2930 and 2966 cm^{-1},[11] or between 2934 and 2950 cm^{-1} for Laurentian fulvic acids.[30] In conclusion, the statement (ii) that a unique frequency (such as at 2930 cm^{-1}) represents the amount of carbon bonded to hydrogen (as CH_2) appears problematic, as different CH_n groups do not give spectral features with intensities proportional to n, and the band energies are variable. If spectral interferences are absent, it may be possible that the integrated C–H stretching spectral region represents some sort of "average" measure of carbon bonded in aliphatic CH_n groups, as we investigate herein.

Spectral Interferences upon the Analyte Signal

We now present experimental data showing the importance of the spectral interference of carbonate within the C–H stretching vibration region. Figure 1 shows DRIFT spectra, in the range between 2400 and 3500 cm^{-1}, of $CaCO_3$ diluted in KBr. Peaks are evident between 2847 cm^{-1} to 2978 cm^{-1}, which are due to combination and overtone bands of CO_3^{2-} stretching vibrations. If the sediment sample contains carbonates, these bands will therefore overlap and interfere with the C–H stretch band quantification. This is normally the case for marine sediments, as the remains of marine organisms contain $CaCO_3$. Figure 1 illustrates this overlap of C–H stretch and CO_3^{2-} bands for freeze-dried near-coastal marine sediment sample from Green Island, Hong Kong. In particular, the detrital material from shells and so forth is generally of coarse particle size. Especially if sediments are not sieved to the silt-clay fraction, such material would be copious and would interfere with measurements in the C–H stretch spectral region. The peak area from 2800 to 3010 cm^{-1} for 6% w/w CO_3^{2-} in a KBr matrix (i.e., 10% w/w $CaCO_3$, as in Fig. 1) is 29 KM cm^{-1}. Typically, the peak area within this wavenumber range for standard sediment (e.g., in Fig. 2 of Ref. 1) is ~16 KM cm^{-1}. Thus, the presence of CO_3^{2-} in the sediment can lead to a considerable overestimation of peak area for C–H stretching.

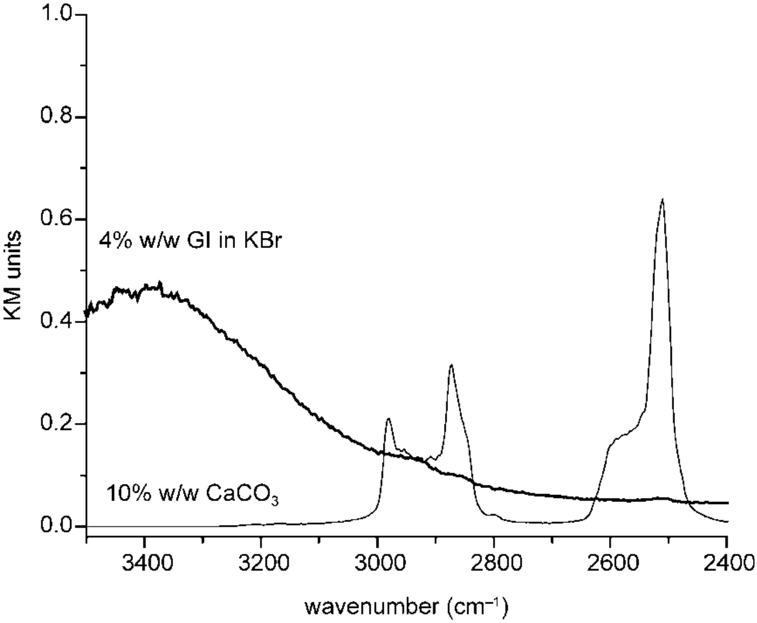

Figure 1. DRIFT spectra, in the range between 2400 and 3500 cm^{-1}, of CaCO$_3$ and freeze-dried near-coastal Hong Kong marine sediment from Green Island (GI) mixed with KBr.

Baseline Inaccuracies

Figures 2a and 2b show the DRIFT spectra of sediment samples SW and ATS from the current study. The integration of the C–H stretch bands between 2800 and 3010 cm^{-1} presents an appreciable baseline error, due to the small peak size on a high, sloping baseline. The total OC content of marine sediments generally ranges from $<0.5\%$ in open ocean areas to 2.5 w/w% in polluted marine areas such as typhoon shelters. This range is rather narrow, but an accurate determination to $\pm 0.1\%$ is desirable. Besides the other factors discussed above, the DRIFT quantitation from band integration at 2930 cm^{-1} is barely capable of such accuracy.

Comparison of TC and OC Contents with Peak Height at 2930 cm^{-1}

The reference values of TC and OC contents obtained by CHN combustion in seven field samples and three SRMs are plotted against peak height (above baseline) at 2930 cm^{-1}, using the DRIFT spectra (Fig. 3). The coefficient of determination (R^2) is improved from 0.08 (for TC, Fig. 3a) to 0.31 (for OC,

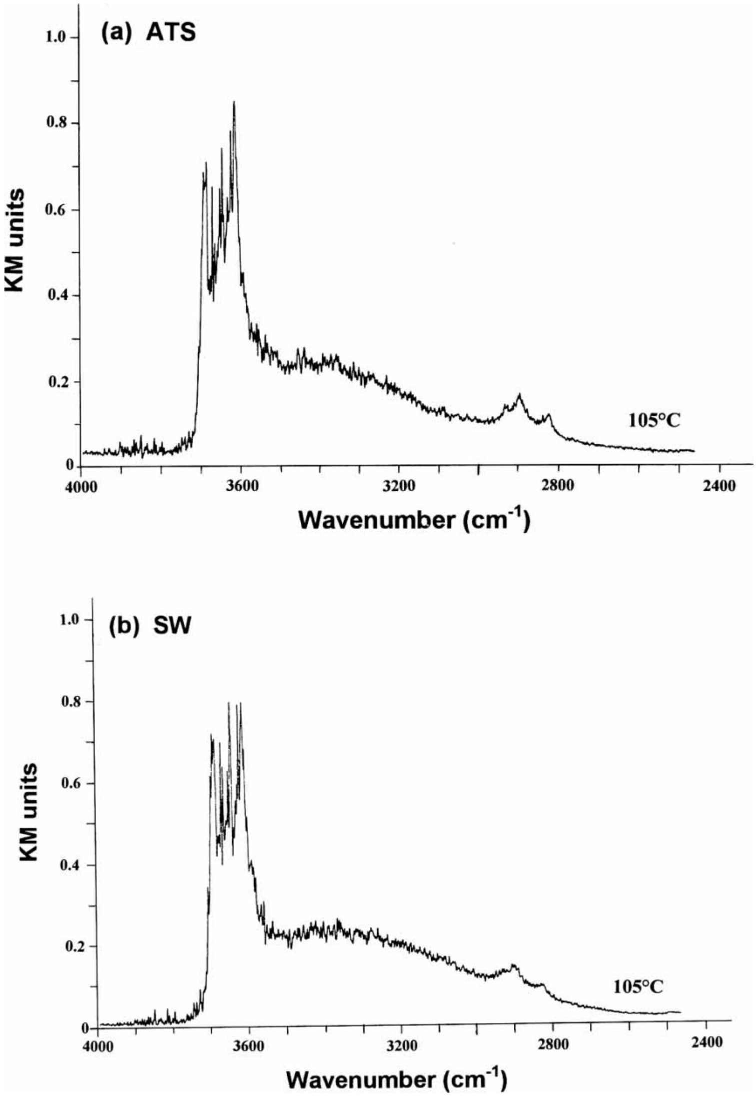

Figure 2. DRIFT spectra of marine sediment (dried at 105°C and mixed with NaCl) from (a) Aberdeen Typhoon Shelter (ATS) and (b) Shuen Wan (SW) sites, in the range from 2400 to 4000 cm^{-1}.

Fig. 3b) after decarbonation of the same samples. This again shows that the interferences from the carbonate bands in marine sediments are significant. The poor coefficient of determination (even for the decarbonated samples) indicates that this method is only applicable for a rough screening of OC in marine sediment.

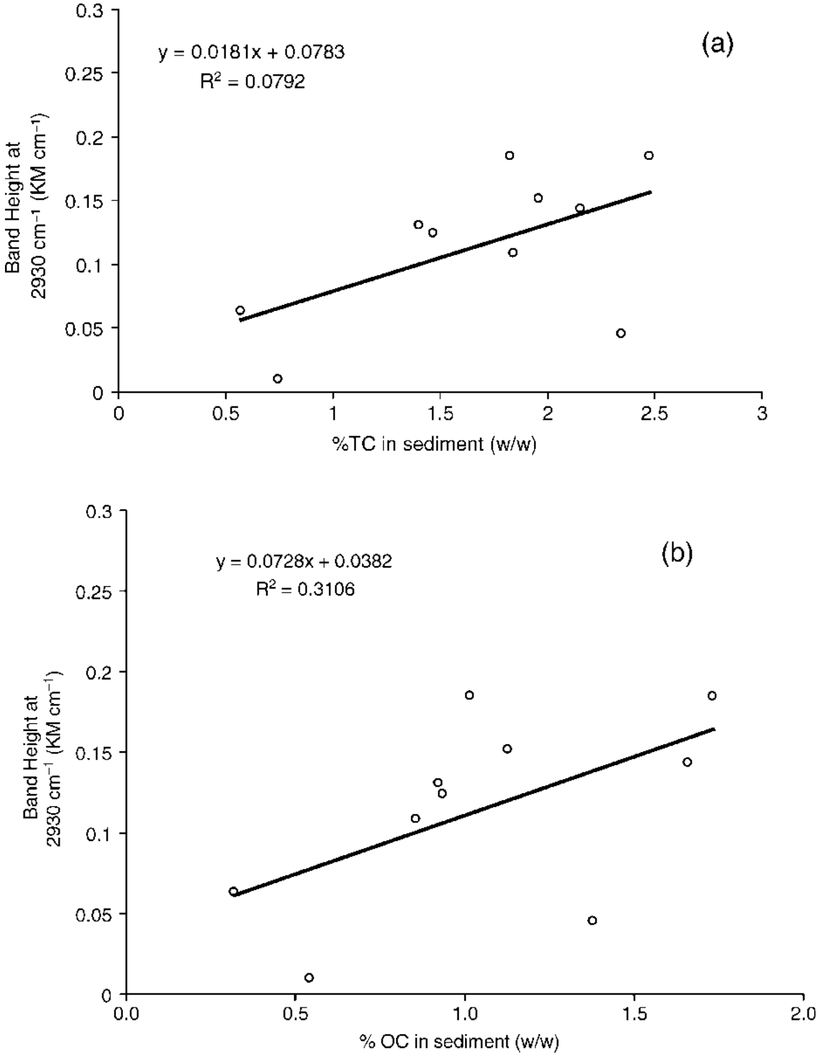

Figure 3. Scatter graphs of the band height at 2930 cm^{-1} and reference concentrations of total carbon (a) and organic carbon (b) in seven field samples and three standard reference materials of sediments. The regression lines are drawn.

Calibration Graph for Aliphatic Organic Carbon: Sample Heterogeneity

An external calibration graph was constructed from 7 sets of 10-replicates, which plotted the integrated area of the aliphatic C–H stretching bands in sodium dodecyl sulfate (SDS) mixed in different concentrations with NaCl against the %OC from the CHN analyses of the SDS standards (identified briefly as aliphatic carbon from CH_2, %ACH_2). The choice of this calibrant (as most of the carbon is present as CH_2) was solely for illustrative purposes. The linearity of the calibration was reasonable up to 7% ACH_2 ($R^2 > 0.98$), but the precision was poor (coefficient of variation ranging between 6% and 25%). Using this calibration graph, the "%ACH_2" results were deduced for two sets of Hong Kong sediment samples: ATS and SW. The values were greater for typhoon-shelter sediments (ATS), but the %ACH_2 values were smaller by factors of 3 and 3.5 from the "%OC" reference values measured for the sediments by the CHN analyzer. In fact, this may be expected because only between about 15% and 40% of carbon atoms are bonded as aliphatic CH_n in the various components of OC, and the measurement at 2930 cm^{-1} reflects an "average" of the OC with carbon bonded in aliphatic CH_n groups. Note that this proportionality factor is not the same for the two different sediment types. This is due to the heterogeneity of the organic matter composition and to the different inorganic carbon content, which ranged from 0.3% to 0.6% w/w.

CONCLUSIONS

We have pointed out the limitations, accuracy, and precision of DRIFT measurements of C–H stretching vibration bands, which are used to quantify organic substances in sediment. From theoretical arguments, it has been shown that there is not a direct proportionality between the analytical signal and the particular organic determinand, due to low, variable abundance of carbon in various aliphatic C–H groups and the absence of a unique frequency for quantifying OC. From experimental observations, the CO_3^{2-} spectral interference has been shown to be problematic. Furthermore, the C–H stretching bands are observed very weak on a high, sloping background so that intensity measurements are not accurate. In principle, the spectral interference by CO_3^{2-} bands in the C–H stretch region can be accounted for by simultaneously measuring the CO_3^{2-} peak near 2510 cm^{-1} and applying a correction or by chemical removal of carbonates. Finally, we have shown that OC is heterogeneous in sediment and that only a small part is present as $-CH_2$.

The advantages of DRIFT spectroscopic measurements are well-known: it is a rapid, nondestructive, and cheap-to-use technique. Other previous approaches to the measurement of OC by DRIFT spectra have focused on using multivariate analytical techniques in the near-IR region, where band congestion and overlapping is not as severe, with some success at screening soil and sediment samples, but not in accurate and precise determinations. The combination of DRIFT spectral data with that from other techniques such as NMR in a combined multivariate approach is the route to provide more accurate measurements of complex organic species in sediment. Finally, it is pointed out that the most appropriate, fully instrumental method for the determination of total OC uses the measurement of TC and IC[8] and is not based on measurements of C–H stretching vibrations.

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