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Determination of Low Molecular Weight Dicarboxylic and Ketocarboxylic Acids in Seawater Samples

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We report a new method developed for the isolation and determination of low molecular weight dicarboxylic acids and related polar compounds (C₂–C₉) from seawater samples. The seawater sample was first acidified and then passed through an activated charcoal column to adsorb the dicarboxylic acids. They are then desorbed with NH₄OH/methanol/water mixture and derivatized with BF₃/1-butanol to dibutyl esters, which are determined using a capillary GC/FID. We tested different pH (0.5, 1, 1.5) and different amounts of charcoal (100, 200, 300, 500 mg) to maximize the recovery of dicarboxylic acids. Using 300 mg of activated charcoal for 100 mL of sodium chloride solution acidified at pH 1, the recoveries for C₂, C₃, C₄, C₅, C₆, C₇, C₈, and C₉ were 61, 49, 96, 84, 77, 67, 57, and 53%, respectively. We applied this method to real seawater samples collected from the northwestern Mediterranean Sea. Here, we report, for the first time, concentrations of a homologous series of C₂–C₉ dicarboxylic acids as well as ketocarboxylic acids including glyoxylic and 4-oxobutanoic acids in surface water and deep seawater samples, with glyoxylic acid being the dominant species (8–40 μg L⁻¹). This method allows the detection of dicarbonyls such as glyoxal in the samples although its quantification is not possible.

Low molecular weight (LMW) dicarboxylic acids and related polar compounds (DCAs) (C₂–C₉) are ubiquitous water-soluble organic compounds that have been detected in a variety of environmental samples including atmospheric aerosols,^{1,2} rainwaters,³ snowpacks,⁴ ice cores,⁵ meteorites,⁶ marine sediments,^{7,8}

hypersaline brines,⁹ and freshwaters.^{10–12} In the atmosphere, DCAs originate from incomplete combustion of fossil fuels,¹³ biomass burning,¹⁴ direct biogenic emission,¹⁵ and ozonolysis and photo-oxidation of organic compounds.^{16,17} Because of their hygroscopic properties, DCAs can act as cloud condensation nuclei and have an impact on the radiative forcing at the Earth's surface.¹⁸ DCAs also participate in many biological processes. They are important intermediates in the tricarboxylic acid and glyoxylate cycles and the catabolism and anabolism of amino acids.¹⁹

In seawater, DCAs may be produced by phytoplankton photorespiration,²⁰ photochemical degradation of dissolved organic matter (DOM),^{21,22} and microbial degradation of long-chain lipids.²³ Other possible sources of DCAs in seawater include diffusion from surface sediments (especially for oxalic acid),⁷ inputs of terrigenous material from rivers,²² and wet and dry depositions.¹⁶ Therefore, DCAs could represent a non-negligible fraction of LMW DOM and have a potential role in the organic carbon cycle in the marine environment. However, only a few studies have reported concentrations of DCAs or related compounds in seawater.

In the Pacific Ocean and coastal waters off California and Peru, one dicarboxylic acid, i.e., oxalic acid, and two ketocarboxylic acids (glyoxylic and pyruvic) were detected by reversed-phase and size exclusion HPLC using derivatization with o-phenylene diamine followed by an acetonitrile extraction.^{19,20} In the Sargasso Sea and coastal waters off Florida, ketocarboxylic acids (glyoxylic and pyruvic) and dicarbonyls (methylglyoxal and glyoxal) were

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detected by reversed-phase HPLC using derivatization with 2,4-dinitrophenylhydrazine.^{24–26} However, there is no report dealing with a complete distribution of C₂–C₉ DCAs in seawater. This paucity of data on DCAs in seawater results in large part from a lack of suitable chromatography methods with sufficient sensitivity, combined with the high volatility of the compounds and the large amount of sea salts, which can interfere with the absorption of DCAs during the procedure leading to low recoveries. Consequently, the accurate concentrations of DCAs in the ocean are still unknown. The methods for isolation of DOM from seawater include mainly electrodialysis, solvent extraction, ultrafiltration, and solid-phase extraction.²⁷ The latter involves adsorption on activated charcoal and adsorption on ion exchange and Amberlite XAD resins.^{27,28} Difficulties of the methods are the evaporation of large volumes of water, selectivity for specific classes of compounds, low recoveries of extraction, and high blank.²⁸

In this study, we chose activated charcoal to isolate DCAs (C₂–C₉) from seawater samples. Indeed, charcoal is easily pretreated, blank values can be minimized using large sample volumes, the major portion of DOM is adsorbed, and small volumes of eluting solvents are required to remove the adsorbed DOM.²⁹ Our technique consists of the adsorption of DCAs from acidified seawater onto activated, pretreated charcoal followed by desorption using different eluents. DCAs are then derivatized with BF₃/1-butanol to dibutyl esters and detected by GC/FID-MS. The derivatization technique has been reported as an efficient method for a simultaneous detection of C₂–C₉ dicarboxylic acids and C₂–C₉ ketocarboxylic acids and dicarbonyls (glyoxal and methylglyoxal) in rainwater samples.³⁰ The method we report here was first tested in sodium chloride solutions amended with authentic DCAs and then applied to seawater samples from the northwestern Mediterranean Sea.

EXPERIMENTAL SECTION

Material and Reagents. Activated charcoal (powdered, pro analysis for CG, 100 g, particle size 0.3–0.5 mm, bulk density 0.41 g cm⁻³) and acetonitrile (GC gradient grade) were purchased from Fluka (Saint-Quentin Fallavier, France). Methanol, *n*-hexane, and dichloromethane (Supra Solv) were from Merck (Strasbourg, France), ammonia (25%, Normapur) was from Prolabo (Strasbourg, France), 14% BF₃/1-butanol was from Alltech Associates (Templemars, France), and hydrochloric acid (20%, double-distilled, PPB/Teflon grade), sodium hydroxide (pellets), and humic acid were from Sigma-Aldrich (Saint-Quentin Fallavier, France). Sodium chloride (Acros Organics, Strasbourg, France) was heated at 450 °C for 6 h before use. Authentic α,ω -dicarboxylic acids (C₂–C₉) including maleic (M) and fumaric (F) acids, phthalic (Ph) acid and ketocarboxylic acids (glyoxylic (ω C₂), 4-oxobutanoic (ω C₄) acids, and dicarbonyl (glyoxal), were obtained from Sigma-Aldrich. All reagent solutions were prepared in Milli-Q water obtained from a Millipore unit with a UV organic oxidation attachment. Glassware was heated at 450 °C for 6 h and rinsed

successively with Milli-Q water, methanol, and dichloromethane before use.

Principle of Solid-Phase Extraction. Water samples were acidified before solid-phase extraction of DCAs. Acidification releases DCAs as free protonated species in solution and increases the efficiency of DCA extraction because hydrophobic interactions are the primary mechanism for adsorption onto activated charcoal. During the passage of the sample through the column onto activated charcoal, DCAs are continually adsorbed from the mobile phase to the stationary phase while salts are eliminated. DCAs are then desorbed from charcoal and collected in a salt-free solution using a mixture of alkaline solutions and organic solvents.^{28,31}

Pretreatment of Activated Charcoal. Before use, activated charcoal was heated at 700 °C for 4 h in the presence of air in a porcelain flask, transferred in a 250-mL glass flask, and extracted with different solvents to remove contaminants. Charcoal was stirred vigorously for 1 h (20–25 °C) in 1 N NaOH, methanol, 6 N HCl, 7 N NH₄OH in water, 6 N HCl, methanol, and Milli-Q water. This procedure was repeated twice. After each solvent extraction, charcoal was rinsed several times with Milli-Q water whereas small particles were removed by decantation. Finally, charcoal was dried using a rotary evaporator under a vacuum, heated at 450 °C for 5 h in the presence of air, and stored at –18 °C.

Isolation of Dicarboxylic Acids. In a precombusted glass column (20-cm length, 0.7-cm internal diameter), a pretreated activated charcoal (100, 200, 300, or 500 mg) was packed with quartz wool (rinsed several times with methanol). After passing Milli-Q water (30 mL), 100 mL of sample was passed through the column. The sample was a solution of sodium chloride (35 g L⁻¹ NaCl in Milli-Q water) acidified to pH 0.5, 1, or 1.5 by addition of 6 N HCl and spiked with authentic DCAs (50 μ g L⁻¹ final concentration). The flow rate of sample into the column was ~1 mL min⁻¹. After passing the sample, DCAs were desorbed from charcoal with successively 7 N NH₄OH in water (20 mL), 7 N NH₄OH in water/methanol (50/50, 20 mL), methanol (30 mL), and Milli-Q water (30 mL). The flow rate of eluents into the column was ~2 mL min⁻¹. The temperature was constant during the procedure (22 ± 2 °C). The 100-mL solution obtained was stored at 4 °C in the dark for 15–20 h before BF₃/1-butanol derivatization.

Butyl Ester Derivatization of Dicarboxylic Acids. The sample was first concentrated down to 3–4 mL using a rotary evaporator under a vacuum, then transferred into a 25-mL pear-shaped flask, further concentrated by the rotary evaporator, and finally concentrated to almost dryness under a nitrogen stream. A total of 0.25 mL of 14% BF₃/1-butanol was immediately added to the sample, and the flask was sealed with a glass stopper, Teflon tape, and clamp. The organic acids and reagent were mixed with ultrasonication for 1 min and then heated at 100 °C for 30 min to derive dibutyl esters for the carboxyl group and dibutoxy acetal for the aldehyde group. The derivatives were extracted with 5 mL of *n*-hexane after adding 3 mL of Milli-Q water and 0.2 mL of acetonitrile. The hexane layer was further washed with Milli-Q water (3 × 3 mL). The derivatives were dried by using a rotary evaporator, and then a nitrogen stream and were finally dissolved

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Table 1. Recoveries of DCAs Spiked into Sodium Chloride Solutions Acidified to pH 0.5, 1, and 1.5 Using 100, 200, 300, and 500 mg of Activated Charcoal^a

compounds	recoveries (%)											
	100 mg			200 mg			300 mg			500 mg		
	pH 0.5	pH 1	pH 1.5	pH 0.5	pH 1	pH 1.5	pH 0.5	pH 1	pH 1.5	pH 0.5	pH 1	pH 1.5
oxalic acid, C ₂	31	24 ± 3	11	65	42 ± 9	23	53	61 ± 8	20	60	59 ± 10	34
malonic acid, C ₃	9	24 ± 3	14	16	45 ± 1	27	35	49 ± 10	17	44	55 ± 5	28
succinic acid, C ₄	64	67 ± 7	46	59	88 ± 9	56	80	96 ± 6	43	85	96 ± 11	56
glutaric acid, C ₅	64	66 ± 6	51	55	81 ± 7	64	74	84 ± 7	41	81	83 ± 9	54
adipic acid, C ₆	65	61 ± 6	52	54	76 ± 6	68	75	77 ± 8	40	81	76 ± 8	55
pimelic acid, C ₇	nd ^b	53 ± 5	nd	nd	68 ± 8	nd	nd	67 ± 8	nd	nd	66 ± 8	nd
suberic acid, C ₈	nd	43 ± 5	nd	nd	57 ± 4	nd	nd	57 ± 8	nd	nd	56 ± 10	nd
azelaic acid, C ₉	54	40 ± 4	41	43	53 ± 4	57	61	53 ± 10	34	68	52 ± 8	44
maleic acid, M	nd	38 ± 6	nd	nd	41 ± 5	nd	nd	44 ± 4	nd	nd	39 ± 2	nd
fumaric acid, F	nd	95 ± 7	nd	nd	119 ± 6	nd	nd	115 ± 8	nd	nd	122 ± 15	nd
phthalic acid, Ph	nd	41 ± 5	nd	nd	55 ± 1	nd	nd	41 ± 8	nd	nd	48 ± 8	nd
glyoxylic acid, ωC ₂	nd	0	nd	nd	1 ± 0	nd	nd	1 ± 0	nd	nd	1 ± 2	nd
4-oxobutanoic acid, ωC ₄	nd	12 ± 1	nd	nd	19 ± 1	nd	nd	17 ± 3	nd	nd	16 ± 4	nd
glyoxal	nd	0	nd	nd	0	nd	nd	0	nd	nd	2 ± 1	nd

^a Averaged percentages are given with a standard deviation based on triplicate analyses. Recoveries were corrected for the blank values. The sodium chloride solutions (100 mL) were acidified, amended with DCAs (50 μg L⁻¹ final concentration), passed through charcoal, eluted, and derivatized. ^b nd, not determined.

in 100 μL of *n*-hexane before gas chromatography-FID determination.^{1,17}

Gas Chromatography Conditions. The dibutyl esters were determined with a Perkin-Elmer autosystem XL gas chromatograph equipped with a split/splitless injector, a fused-silica capillary column (HP-5, 25 m × 0.32 mm × 0.52 μm, Hewlett-Packard), and an FID detector. GC conditions were as follows: injection temperature of 250 °C; column oven temperature of 50 °C (2 min) programmed to 120 °C at 30 °C min⁻¹, then to 300 °C at 8 °C min⁻¹, and maintained for 10 min; FID temperature of 315 °C.¹ Peak areas were calculated with a Turbochrom v4 integrator. Peak identification was performed by a comparison of GC retention times with those of dibutyl ester standards. For seawater samples, the identification of DCAs was also confirmed with a GC/MS (ThermoQuest Trace MS system) under similar GC conditions.¹⁷

Application to Seawater Samples. Seawater samples were collected in the northwestern Mediterranean Sea (Bay of Marseille (43°18'N, 05°22'E) and at Dyfamed station (43°25'N, 07°52'E)) in September and October 2005, respectively. In the Bay of Marseille, samples were collected on the surface using 2-L glass bottles. At the Dyfamed station, samples were collected at 5-, 100-, and 1500-m depth using 10-L Niskin bottles equipped with Viton O-rings and silicone tubing. Samples were immediately filtrated through precombusted (450 °C for 6 h) GFF filters (47-mm filter diameter, rinsed with 2 L of Milli-Q water and 150 mL of sample prior to filtration) and then through 0.2-μm polycarbonate filters (Nuclepore 47-mm filter diameter, rinsed with 10% HCl, 2 L of Milli-Q water, and 150 mL of sample prior to filtration) in a precombusted glass apparatus. After filtration, duplicate subsamples (10 mL) were acidified with 85% phosphoric acid and stored in the dark in combusted, Teflon-capped glass vials for dissolved organic carbon (DOC) analyses. DOC concentrations were measured using a Shimadzu TOC-5000 carbon analyzer.³² Samples for DCAs analy-

ses were transferred into 500-mL precombusted bottles, poisoned with HgCl₂ (10 mg L⁻¹ final concentration) to avoid any biological activity and stored at 4 °C in the dark until solid-phase extraction. Some of seawater samples (Bay of Marseille) were amended with authentic DCAs (from 0.1 to 9.5 μg L⁻¹ final concentration) to perform spike experiments. All the seawater analyses were conducted in duplicate in the laboratory in Marseille.

RESULTS AND DISCUSSION

Extraction of Dicarboxylic Acids in Sodium Chloride Solutions. Table 1 present recoveries of extraction in the spiked sodium chloride solutions (100 mL of 35 g L⁻¹ NaCl amended with 50 μg L⁻¹ DCAs) acidified at pH 0.5, 1, and 1.5 for 100, 200, 300, and 500 mg of activated charcoal. Recoveries were corrected for the procedural blanks, in which no serious contamination peaks were observed, except for C₂, ωC₂, and glyoxal.

(a) pH Effect. Whatever the amount of charcoal, recoveries for C₂, C₃, C₄, C₅, C₆, and C₉ were systematically lower at pH 1.5 than at pH 1 (Table 1). This means that, at pH 1.5, adsorption onto charcoal is less efficient due probably to a partial protonation of DCAs. Whatever the amount of charcoal, recoveries at pH 0.5 were equal or higher (C₂), systematically lower (C₃), or equal or lower (C₄, C₅, C₆, C₉) than at pH 1 (Table 1). These results suggest that the decrease in pH from 1 to 0.5 does not lead to a significant increase in adsorption, even for C₂, which presents the lowest pK_a (1.2). The decrease in C₃ recovery is most likely due to an increase in the decarboxylation process (see below) and rather difficult to limit at low pH. Therefore, pH 1 was selected as the best pH condition since higher or lower pH limits the adsorption efficiency of the rest of the DCAs. Consequently, only pH 1 was considered in the rest of this report.

(b) Amount of Charcoal Effect. For C₂, the blank value for 100, 200, 300, and 500 mg represented 18, 19, 16, and 26% of the value measured in the spiked samples, respectively. For ωC₂ and glyoxal, the blank values for 100, 200, 300, and 500 mg represented 61, 34, 34, and 77% and 51, 48, 63, and 69% of the values measured in the spiked samples, respectively. Whatever the amount of

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charcoal, recoveries increase from C₂ to C₄, without any significant difference between C₂ and C₃, and decrease from C₄ to C₉ (Table 1). They remain low for M (~40%) and very high for F (more than 100%). The lowest recoveries, observed for ω C₂ and glyoxal, explain the relative high percentage of blank values for these compounds (Table 1).

When charcoal increases from 100 to 200 mg, recoveries increase on average for C₂–C₄ and C₅–C₉ by 20 and 19%, respectively. However, when charcoal increases from 200 to 300 mg, recoveries increase only for C₂ (19%), C₃ (4%), and C₄ (8%) and remain similar for C₅–C₉. When charcoal increases from 300 to 500 mg, there is no significant increase in recoveries for C₂–C₄ (Table 1) whereas contamination levels were higher. Therefore, there is a positive mass charcoal effect on the recoveries for C₂–C₄ and C₅–C₉ in the ranges 100–300 and 100–200 mg, respectively. The amount of charcoal influences the efficiency of extraction and the level of contamination. Consequently, a mass of 300 mg of activated charcoal with a 100-mL solution acidified at pH 1 was selected as the most valuable conditions since it represents the best compromise between recoveries, blank levels and reproducibility (Table 1).

Decarboxylation and Isomerization Processes. The relative low recoveries of C₃ (Table 1) may be explained by the presence of HCl since C₃ is very unstable and can be easily decarboxylated.^{33,34} Our laboratory experiments showed that, in the presence of HCl for few hours, C₃ concentration systematically decreases by 30% compared to nonacidified solutions. Clearly, these results show that, when acid is added, 30% of the initial amount of C₃ is lost by decarboxylation.

For 300 mg of charcoal and pH 1, recoveries of M and F are 44 and 115%, respectively (Table 1), although no contamination was found for F in the procedural blanks. M and F are two isomers of unsaturated C₄ dicarboxylic acid that differ from each other in their cis and trans configurations, respectively. F can be produced from M by catalytic isomerization with mineral acids (HCl),³⁵ but such an isomerization does not occur during BF₃/1-butanol derivatization. Consequently, the excess of F could come from a partial isomerization of M due to the acidification of the samples before solid-phase extraction. This hypothesis was confirmed by performing experiments with acidified and nonacidified solutions. When a solution containing only M (50 μ g L⁻¹) is acidified to pH 1 for few hours, its concentration decreases by 18 μ g L⁻¹ (37%) whereas F is produced in the same range. Moreover, when a solution containing only F is passed through 300 mg of charcoal, its recovery of extraction is about 75–80%.

Sorption Capacity of Dicarboxylic Acids onto Charcoal.

To assess the sorption capacity of DCAs onto activated charcoal, we performed adsorption and desorption isotherms. A 100-mL aliquot of Milli-Q water (without NaCl) was acidified to pH 1, amended in DCAs (10, 50, 100, 200, and 500 μ g L⁻¹ final concentration), and passed through 300 mg of activated charcoal ($T = 22 \pm 2$ °C). Milli-Q water after passing through the charcoal (after neutralization by addition of a strong base) and the regular eluent solution were both analyzed for DCAs. Adsorption isotherm, which represents the relationship between the concentration of

the compound in the liquid solution and the concentration of the compound adsorbed onto charcoal, was linear in the range 10–500 μ g L⁻¹ for all the compounds (R^2 ranging from 0.95 to 0.99, $n = 5$), except for glyoxal (none adsorption) and ω C₂ (slight adsorption in the range 100–500 μ g L⁻¹) (data not shown). Desorption isotherm, which represents the relationship between the concentration of the compound in the liquid solution and the concentration of the compound desorbed from charcoal, was linear in the range 10–500 μ g L⁻¹ for all the compounds (R^2 ranging from 0.86 to 0.99, $n = 5$), except for glyoxal (none desorption) and ω C₂ (slight desorption in the range 100–500 μ g L⁻¹) (data not shown).

To better explain the recoveries of extraction observed, we calculated the percentages of DCAs adsorbed, desorbed, and extracted when using 300 mg of activated charcoal. Results are reported in Table 2 along with the logarithmic octanol/water partition coefficients ($\log K_{ow}$) calculated using Environmental Protection Agency (EPA)'s Estimation Program Interface (EPI) suite. $\log K_{ow}$ represents an index of the water solubility; i.e., compounds with low $\log K_{ow}$ values (<1) may be considered relatively hydrophilic whereas compounds with high $\log K_{ow}$ (>4) are generally considered as very hydrophobic. Percentages were corrected for the blank values as well as for the decarboxylation (for C₃) and isomerization (for M and F) processes (Table 2). Whatever the initial concentration of DCAs, glyoxal is not adsorbed onto charcoal and thus is not extracted from the initial solution (Table 2). Whatever the initial concentration of DCAs, adsorption capacity is close to 100% for C₄–C₉, M, F, and Ph and are around 80–90% for C₂ and C₃. Glyoxylic acid is adsorbed onto charcoal from 100 μ g L⁻¹ with a lower efficiency (~25–60%) whereas ω C₄ is adsorbed from 10 μ g L⁻¹ with a higher efficiency (~60–80%) (Table 2). When the initial concentration reaches 500 μ g L⁻¹, desorption capacity of DCAs decreases, particularly for C₄–C₉, M, F, and Ph, resulting in a substantial decrease in the percentage of extraction (Table 2). The desorption capacity of ω C₄ (~20–30%) and Ph (~30–50%) remains very low for any concentration. Consequently, these percentages show clearly that the adsorption and desorption capacity of DCAs onto 300 mg of activated charcoal is constant up to an initial concentration of 200 μ g L⁻¹ (a slight decrease in desorption capacity was observed for 500 μ g L⁻¹) (Table 2).

In the range of initial concentrations 10–200 μ g L⁻¹, except for ω C₄ and Ph, we observe that, for $\log K_{ow}$ values ranging from -1.66 (glyoxal) to 0 (M and F), the percentages of adsorption and desorption tend to increase, which leads to an increase in the percentage of extraction (Table 2). For $\log K_{ow}$ values ranging from 0 to 2.67 (C₉), the percentage of adsorption remains constant close to 100% whereas the percentage of desorption tends to decrease, which leads to a decrease in the percentage of extraction (Table 2). Clearly, in the range of initial concentrations 10–200 μ g L⁻¹, the solid-phase extraction of DCAs is driven by both adsorption and desorption in the range of $\log K_{ow}$ -1.66 to 0 and only by desorption in the range of $\log K_{ow}$ 0–2.67. The best agreement between efficient adsorption and desorption is found for compounds with $\log K_{ow}$ around 0 (M, F and C₄) (Table 2). Note that (i) the desorption capacity starts decreasing at 500 μ g L⁻¹ and that (ii) ω C₄ and Ph do not follow the same trends since

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Table 2. Percentages of DCAs Adsorbed onto and Desorbed from 300 mg of Activated Charcoal and Extracted from the Initial 100-mL Liquid Solution for Different Initial Concentrations (10, 50, 100, 200, and 500 $\mu\text{g L}^{-1}$)^a along with the Logarithmic Octanol/Water Partition Coefficients (log K_{ow})^b

comps	Log K_{ow}	initial concentrations ($\mu\text{g L}^{-1}$)														
		% adsorbed onto charcoal					% desorbed from charcoal					% extracted from initial solution				
		10	50	100	200	500	10	50	100	200	500	10	50	100	200	500
C ₂	-0.77	83	64	93	95	85	73	100	74	61	58	60	64	69	58	49
C ₃	-0.28	87	92	91	91	87	75	54	65	64	63	66	50	60	59	55
C ₄	0.21	92	95	95	99	96	102	96	96	91	81	94	91	91	90	77
C ₅	0.7	98	99	96	100	99	89	88	89	86	68	87	87	86	86	68
C ₆	1.2	98	98	96	100	99	88	86	85	82	62	86	84	82	82	61
C ₇	1.69	97	100	96	100	99	79	78	76	73	53	77	78	74	73	53
C ₈	2.18	98	100	97	100	100	88	66	61	52	64	86	66	59	51	64
C ₉	2.67	97	99	97	100	99	71	72	69	64	44	70	71	67	64	44
M	0	96	100	97	100	99	103	71	93	103	75	98	71	89	103	75
F	0	99	100	96	100	100	77	88	80	70	62	77	87	77	70	62
Ph	1.22	98	99	94	100	99	41	51	43	40	29	40	50	40	40	29
ωC_2	-1.07	0	0	40	24	56	0	0	4	5	1	1	3	1	1	1
ωC_4	-0.4	59	83	80	83	69	31	22	21	29	18	18	18	17	24	13
glyoxal	-1.66	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0

^a 100 mL Milli-Q water solutions were acidified to pH 1, amended with 10, 50, 100, 200, and 500 $\mu\text{g L}^{-1}$ of DCAs (initial solutions) and passed through 300 mg of charcoal. The acidified Milli-Q water solutions passing directly through charcoal were collected and neutralized (intermediate solutions). The regular solutions of eluents were then passed through charcoal to desorb DCAs (final solutions). From the concentrations of DCAs observed in the initial, intermediate, and final solutions, we assessed the percentage of DCA adsorbed onto charcoal ($= (([\text{DCA}]_{\text{initial solution}} - [\text{DCA}]_{\text{intermediate solution}})/[\text{DCA}]_{\text{initial solution}} \times 100)$), the percentage of DCA desorbed from charcoal ($= ([\text{DCA}]_{\text{final solution}}/([\text{DCA}]_{\text{initial solution}} - [\text{DCA}]_{\text{intermediate solution}}) \times 100)$), and the percentage of DCA extracted from the initial solution ($= ([\text{DCA}]_{\text{final solution}}/[\text{DCA}]_{\text{initial solution}} \times 100)$). All the percentages were corrected for the blank values as well as for the decarboxylation (for C₃) and isomerization (for M and F) processes. ^b Log K_{ow} were estimated using EPA's EPI suite.

their adsorption (ωC_4) and desorption (ωC_4 and Ph) capacities are lower in regard to their log K_{ow} (Table 2).

Concentrations of Dicarboxylic Acids in Seawater Samples.

The activated charcoal method was applied to seawater samples. To minimize the blank values, 500-mL samples were acidified to pH 1 and passed through 300 mg of charcoal. A typical gas chromatogram of the derivatives of DCAs isolated from the seawater sample collected in the Bay of Marseille is presented in Figure 1. The identification of DCAs was confirmed by GC/MS.

To ensure that the method is consistent with DCA levels in seawater, spike experiments were conducted on surface samples collected in the Bay of Marseille. Table 3 shows the statistical data of the linear regression between the spiked concentration of DCA (from 0.5 to 9.5 $\mu\text{g L}^{-1}$ final concentration) and the measured peak areas corrected for the blank values (in $\mu\text{V s}$). Different ranges of spiked concentrations were used depending on the initial concentration of the compound in seawater. The method responds linearly to the increasing concentrations of DCAs in seawater for all the compounds including ketocarboxylic acids ($R^2 = 0.88$, $n = 5$ and $R^2 = 0.97$, $n = 4$ for ωC_2 and ωC_4 , respectively, with slopes significantly different from zero), except for glyoxal, which is not adsorbed onto charcoal ($R^2 = 0.30$, $n = 5$ and slope not significantly different from zero) (Table 3).

Averaged recoveries of DCAs spiked into seawater samples (Table 3) are not significantly different from those measured in the sodium chloride solutions for 300 mg of charcoal (Table 1), except for C₂ (31 ± 5 and $61 \pm 8\%$) and C₃ (13 ± 1 and $49 \pm 10\%$). A hypothesis to explain these lower recoveries found in seawater was a competition with other dissolved organic compounds such as humic substances for the adsorption onto activated charcoal. However, spike experiments conducted on Milli-Q water, Milli-Q water amended in humic acid (2 mg L^{-1} final concentration), and

natural river waters (Rhône River, 2.4 mg of C L^{-1}) showed that DCA recoveries are not significantly different ($\text{CV} \pm 8\%$ for each DCA, data not shown). Consequently, the decrease in C₃ could be essentially due to a higher decarboxylation rate according to the higher amount of acid added in the seawater samples. Indeed, we observed experimentally that the rate of decarboxylation was proportional to the amount of acid added in the sample. For C₂, the formation of calcium oxalate could occur, leading to a less efficient derivatization. According to the statistical data of the spike experiments in seawater, the activated charcoal method allows a reliable quantification of DCAs in different seawater samples, including ketocarboxylic acids (ωC_2 and ωC_4), except glyoxal.

Table 4 presents the averaged concentrations of DCAs (in $\mu\text{g L}^{-1}$) in the northwestern Mediterranean Sea (Bay of Marseille and Dyfamed station), based on duplicate analyses. Concentrations were corrected for procedural blanks. No serious contamination was detected from the procedural blanks, except for C₂, C₃, C₄, and ωC_2 for which the blank values represented 14 ± 6 , 11 ± 6 , 14 ± 8 , and $17 \pm 10\%$ of the values measured in the different seawater samples, respectively. In all the samples, ωC_2 was found as a dominant species (~ 60 – 70% of total DCAs) followed by C₂ (~ 20 – 30% of total DCAs) and then C₃. Total concentrations of DCAs measured in the surface samples are similar: 50 ± 9 and $58 \pm 8 \mu\text{g L}^{-1}$ in the Bay of Marseille and Dyfamed station, respectively. At the Dyfamed station, total concentration of DCAs decreases substantially from 5 to 100 m (58 ± 8 and $21 \pm 8 \mu\text{g L}^{-1}$, respectively). However, the decrease is lower from 100 to 1500 m (21 ± 8 and $16 \pm 1 \mu\text{g L}^{-1}$, respectively). Total concentrations of DCAs at 0, 5, 100, and 1500 m represent 1.9, 2.4, 0.9, and 1.1% of DOC concentration, respectively (Table 4).

The distribution of DCAs into the water column (highest values in surface and diminution with depth) suggests that they are

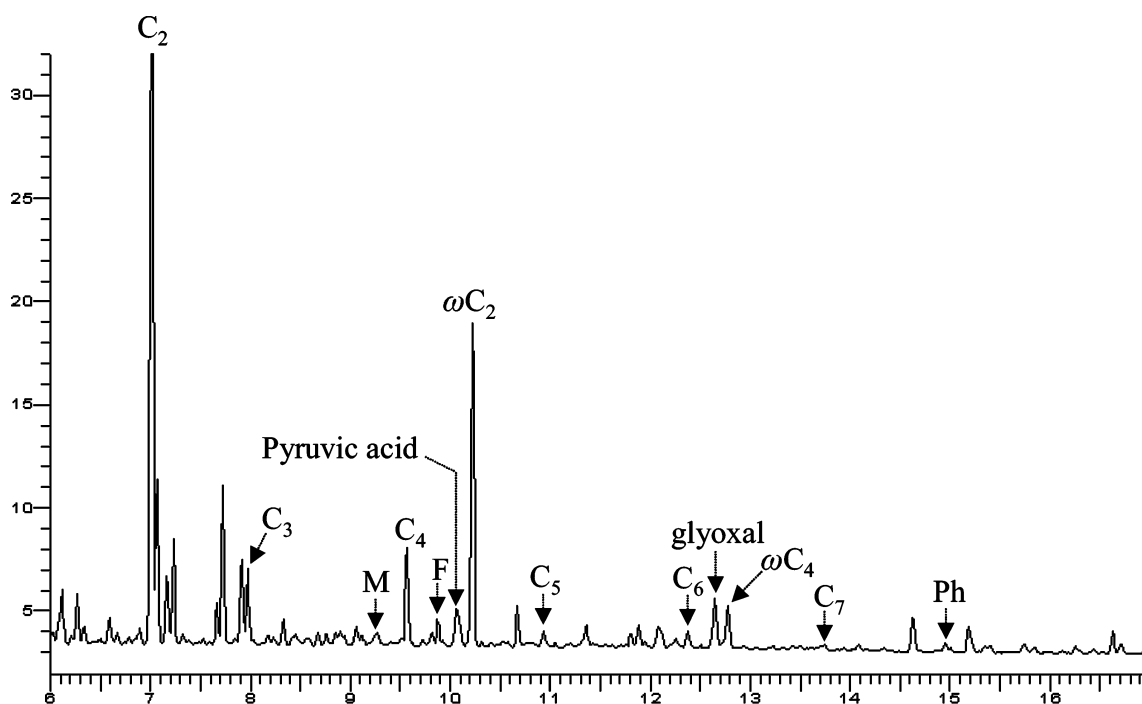


Figure 1. Typical capillary gas chromatogram of the derivatives of dicarboxylic acids (C_2 – C_9), ketocarboxylic acids (ωC_2 , ωC_4 , pyruvic acid), and dicarbonyl (glyoxal) isolated from the seawater sample collected in the Bay of Marseille (northwestern Mediterranean Sea) in September 2005. The identification of DCAs was confirmed by GC/MS. Pyruvic acid was tentatively identified. Mass spectra of the organic acid butyl esters (not presented here) are equal to those published in the literature.³⁰ See Table 1 for the abbreviation of compounds.

Table 3. Statistical Data of the Linear Regression between the Spiked Concentration of DCA in Seawater (0.1–9.5 $\mu\text{g L}^{-1}$ Final Concentration) and the Measured Peak Area (in $\mu\text{V s}$)^a

comps	spiked concn ($\mu\text{g L}^{-1}$)	<i>n</i>	R^2	slope ($\mu\text{V s}/\mu\text{g L}^{-1}$)	y-intercept ($\mu\text{V s}$)	recovery (%)
C_2	0, 2, 4.5, 7, 9.5	5	0.94	$7763 \pm 1171^{**}$	$85055 \pm 6701^{**}$	31 ± 5
C_3	0, 2, 4.5, 7, 9.5	5	0.99	$2735 \pm 114^{***}$	$5415 \pm 655^{**}$	13 ± 1
C_4	0, 0.5, 1, 1.5	4	0.98	$15387 \pm 1426^{**}$	$10126 \pm 1334^{**}$	77 ± 8
C_5	0, 0.1, 0.2, 0.5	4	0.99	$14228 \pm 1238^{**}$	$2516 \pm 339^*$	83 ± 12
C_6	0, 0.5, 1, 1.5	4	0.99	$13075 \pm 258^{***}$	$3226 \pm 241^{**}$	79 ± 2
C_7	0, 0.1, 0.2, 0.5	4	0.99	$12395 \pm 157^{***}$	$1059 \pm 43^{**}$	66 ± 2
C_8	0, 0.5, 1, 1.5	4	0.99	$11786 \pm 391^{***}$	$676 \pm 366^{\text{ns}}$	64 ± 2
C_9	0, 0.5, 1, 1.5	4	0.99	$12431 \pm 228^{***}$	$899 \pm 213^{\text{ns}}$	71 ± 1
M	0, 0.1, 0.2, 0.5	4	0.99	$6862 \pm 432^{**}$	$2609 \pm 118^{**}$	36 ± 3
F	0, 0.1, 0.2, 0.5	4	0.99	$22976 \pm 1518^{**}$	$1670 \pm 415^{\text{ns}}$	111 ± 14
Ph	0, 0.1, 0.2, 0.5	4	0.99	$7829 \pm 351^{**}$	$1761 \pm 96^{**}$	41 ± 7
ωC_2	0, 2, 4.5, 7, 9.5	5	0.88	$855 \pm 181^*$	$24006 \pm 1035^{***}$	2 ± 1
ωC_4	0, 0.5, 1, 1.5	4	0.97	$4969 \pm 666^*$	$4036 \pm 623^*$	15 ± 2
glyoxal	0, 2, 4.5, 7, 9.5	5	0.30	$107 \pm 95^{\text{ns}}$	$3262 \pm 544^{**}$	nd

^a Measured peak areas used for the linear regressions were corrected for the blank values. Averaged recoveries of DCAs in seawater samples were determined from the different spiked concentrations and are given with a standard deviation based on $n - 1$ analyses. Spike experiments were conducted on surface seawater samples collected in the Bay of Marseille (northwestern Mediterranean Sea) in September 2005. Samples (500 mL) were acidified to pH 1, amended with DCAs, passed through 300 mg of charcoal, eluted, and derivatized. Slopes and y-intercepts are very highly significantly (*** , $p < 0.001$), highly significantly (** , $p < 0.01$), significantly (* , $p < 0.05$), or not significantly ($^{\text{ns}}$, $p < 0.1$) different from zero (t -test). nd, not determined.

produced mainly into the euphotic zone by phytoplankton activities and probably by the photochemical degradation of DOM, whereas they would be consumed mainly by bacterial activities in the whole water column. In seawater, some DCAs (particularly oxalic acid) can be complexed with metals such as iron (Fe^{3+}) to form strong iron-binding ligands.³⁶ But when the seawater sample is acidified at pH 1 before solid-phase extraction, a major part of these ligands are reduced (Fe^{2+}) and release oxalic acid. Therefore, using the

activated charcoal method, we may analyze as well as complex forms of DCAs, particularly for oxalic acid.

At the Dyfamed station, glyoxylic and oxalic acids concentrations represent 39, 12, and 8 and 14, 6.5, and 5 $\mu\text{g L}^{-1}$ at 5, 100, and 1500 m, respectively (Table 4). These concentrations are in the same range as those previously reported in the Pacific Ocean with 22.2, 5.2, and 7.4 and 9, 3.6 and 20 $\mu\text{g L}^{-1}$ for glyoxylic and oxalic acids at 75, 100, and 1800 m, respectively.²⁰ Therefore, according to the concentrations of DCAs measured in this study,

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Table 4. Concentrations of DCAs (in $\mu\text{g L}^{-1}$) in Natural Seawater Samples (Bay of Marseille and Dyfamed Station, Northwestern Mediterranean Sea)^a

compds	Bay of Marseille		Dyfamed station	
	0 m	5 m	100 m	1500 m
C ₂	12.55 ± 2.25	13.71 ± 2.03	6.48 ± 2.45	5.39 ± 0.37
C ₃	2.40 ± 0.59	2.25 ± 0.02	0.71 ± 0.31	0.60 ± 0.07
C ₄	0.74 ± 0.11	0.44 ± 0.08	0.12 ± 0.04	0.17 ± 0.06
C ₅	0.15 ± 0.04	0.16 ± 0.03	0.04 ± 0.02	0.08 ± 0.05
C ₆	0.21 ± 0.05	0.34 ± 0.00	0.17 ± 0.08	0.29 ± 0.15
C ₇	0.08 ± 0.00	0.00	0.00	0.00
C ₈	nd	0.00	0.00	0.00
C ₉	nd	0.16 ± 0.02	nd	nd
M	0.36 ± 0.02	0.79 ± 0.13	0.43 ± 0.18	0.34 ± 0.13
F	nd	nd	nd	nd
Ph	0.24 ± 0.02	0.46 ± 0.10	0.12 ± 0.03	0.27 ± 0.12
ωC_2	32.31 ± 5.99	38.96 ± 5.60	12.41 ± 4.50	8.45 ± 1.05
ωC_4	0.95 ± 0.19	1.01 ± 0.16	0.27 ± 0.09	0.31 ± 0.08
glyoxal	nd	nd	nd	nd
pyruvic	nd	nd	nd	nd
total	50.07 ± 9.05	58.28 ± 7.59	20.78 ± 7.70	15.93 ± 0.99
% DOC	1.9 ± 0.3	2.4 ± 0.3	0.9 ± 0.3	1.1 ± 0.05

^a Averaged concentrations are corrected for the blank values and are given with a standard deviation based on duplicate analysis. For the Bay of Marseille, concentrations were determined by dividing the y -intercept of the linear regression (corrected by the blank value) by the slope of the linear regression (values reported in Table 3). For the Dyfamed station, the same slopes were used with the measured peak area (also corrected by the blank value and analyzed with t -test). Seawater samples (500 mL) were collected in September and October 2005, acidified to pH 1, passed through 300 mg of charcoal, eluted, and derivatized. nd, not determined, used when a peak is detected but the compound cannot be quantified because of an unknown slope (pyruvic acid) or a slope (glyoxal) or a y -intercept/measured peak area (C₈, C₉, F) not significantly different from zero. A concentration of 0.00 $\mu\text{g L}^{-1}$ means that the peak is not detected.

glyoxylic acid (8–40 $\mu\text{g L}^{-1}$) could be, with glycolic acid (24–89 $\mu\text{g L}^{-1}$),³⁷ one of the major low molecular weight dissolved organic compounds in seawater.

CONCLUSION

We have developed a new analytical technique to measure low molecular weight DCAs (C₂–C₉) in natural seawater samples. This technique consists of adsorption of DCAs from acidified seawater onto activated, pretreated charcoal following by desorption using different eluents. DCAs are then derivatized with BF₃/1-butanol to dibutyl esters and determined with GC/FID-MS. The method was first tested in sodium chloride solutions using different pH and different amounts of charcoal and then applied to real seawater samples. The recoveries of DCAs from the spiked sodium chloride solutions acidified at pH 1 for 300 mg of charcoal were found to be 49–96% with standard deviation of 3–10% for different DCAs. We observed that glyoxylic acid was the dominant species in the

seawater samples with a concentration range of 8–40 $\mu\text{g L}^{-1}$. Those concentrations suggest that glyoxylic acid could be one of the major low molecular weight dissolved organic compounds in seawater. Total DCAs and related water-soluble compounds account for 0.9–2.4% DOC in the seawater samples.

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