in IPD. As the molar absorptivity for the additive increases, detection limit becomes more favorable (*13*) providing the chromophoric II additive concentration in the mobile phase is adjusted so that its absorbance is <1.0. A second factor that is limiting in IFD is the fluorescence detector. Base-line noise in a fluorescence detector is greater due to source flicker. When the electronic offset is used to compensate for the background fluorescence, base-line noise remains and is magnified as detector sensitivity is increased since the difference between signal and background is detected. Improvement in the source quality should decrease the flickering, improve the base-line noise, and, therefore, improve IFD detection limits.

Registry No. F^-, 16984-48-8; Cl^-, 16887-00-6; NO_2^-, 14797-65-0; Br^-, 24959-67-9; NO_3^-, 14797-55-8; ClO_3^-, 14866-68-3; I^-, 20461-54-5; BF_4^-, 14874-70-5; SCN^-, 302-04-5; HPO_4^{2-}, 14066-19-4; $H_2AsO_4^-$, 16518-47-1; SO_4^{2-}, 14808-79-8; CrO_4^{2-}, 13907-45-4; CN^-, 57-12-5; $Ru(bpy)_3^{2+}$, 15158-62-0; $Ru(phen)_3^{2+}$, 22873-66-1; $Ru(bpy)_3Cl_2$, 14323-06-9; $Ru(bpy)_3(ClO_4)_2$, 15635-95-7; $Ru(phen)_3(ClO_4)_2$, 14767-24-9; glycolic acid, 79-14-1; acetic acid, 64-19-7; lactic acid, 50-21-5; propionic acid, 79-09-4; acetoacetic acid, 541-50-4; α-hydroxybutyric acid, 565-70-8; chloroacetic acid, 79-11-8; isobutyric acid, 79-31-2; butyric acid, 107-92-6.

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Determination of Formate in Natural Waters by a Coupled Enzymatic/High-Performance Liquid Chromatographic Technique

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An enzymatic method was developed to quantify formic acid in natural water samples at submicromolar concentrations. The method is based on the oxidation of formate by formate dehydrogenase with corresponding reduction of β-nicotinamide adenine dinucleotide (β-NAD^+) to reduced β-NAD^+ (β-NADH); β-NADH is quantified by reversed-phase high-performance liquid chromatography with fluorometric detection. An important feature of this method is that the enzymatic reaction occurs directly in aqueous media, even seawater, and does not require sample pretreatment other than sample filtration. The reaction proceeds at room temperature at a slightly alkaline pH (7.5–8.5) and is specific for formate with a detection limit of 0.5 μM (S/N = 4) for a 200-μL injection. The precision of the method was 4.6% relative standard deviation (n = 6) for a 0.6 μM standard addition of formate to Sargasso seawater. Average recoveries of 2 μM additions of formate to seawater, porewater, or rain were 103, 103, and 87%, respectively. Intercalibration with a Dionex ion chromatographic system showed an excellent agreement of 98%. Concentrations of formate present in natural samples ranged from 0.2 to 0.8 μM for Biscayne Bay seawater, 0.4 to 10.0 μM for Miami rain, and 0.9 to 8.4 μM for Biscayne Bay sediment porewater.

There is considerable interest in the role of formic acid and other volatile fatty acids in the early diagenesis of organic matter in lacustrine and marine sediments (*1*, *2*). Formic acid is an important fermentation product or substrate for many aerobic and anaerobic bacteria and for some yeasts (*3*). In the atmosphere, formic acid is an important product in the photochemical oxidation of organic matter (*4*).

Despite its potential importance, formic acid has proven difficult to quantify at submicromolar levels in natural water samples. Formidable analytical difficulties are associated with its detection in highly saline samples (*5*). Ion exclusion, anion exchange, and reversed-phase high-performance liquid chromatography (RP-HPLC) techniques based on the direct detection of formic acid in aqueous samples are prone to interferences (especially from inorganic salts) that ultimately limit the sensitivity of these methods.

A potentially more sensitive and selective approach involves reaction of formic acid with a reagent to form a chromophore or fluorophore, followed by chromatographic analysis. A wide variety of alkylating and silylating reagents have been used for this purpose (*6*). Two serious drawbacks to this approach are that inorganic salts and/or water interfere with the derivatization reaction, and these reactions are generally not specific for formic acid or other carboxylic acids. These techniques are prone to errors from adsorption losses, contamination, and decomposition of the components of interest (*7*). Enzymatic techniques, in contrast, are ideal for the analysis of natural water samples, since they are compatible with aqueous media and involve little or no chemical or physical alterations of the sample (e.g., pH, temperature) that

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introduce errors in the analysis.

In this study, we adapted a formate dehydrogenase assay for the determination of formic acid in natural waters. The method is based on the oxidation of formic acid by formate dehydrogenase with corresponding reduction of β-nicotinamide adenine dinucleotide (β-NAD^+) to reduced β-NAD^+ (β-NADH); the β-NADH that is formed is quantified by RP-HPLC with fluorometric detection. Optimization of the method is described, with special emphasis on the analysis of seawater, marine sediment porewater, and rain.

EXPERIMENTAL SECTION

Apparatus. The HPLC system consisted of a Gilson Model 302 reciprocating pump (Gilson Medical Electronics, Middleton, WI) connected to a Gilson Model 811 dynamic mixer and a Gilson Model 802 manometric module. Samples were injected into the chromatograph with a Valco Model CV-6-UHPa-N60 manual injection valve (Valco Instrument Co., Houston, TX) fitted with either an 11-, 200-, or 500-μL sample loop. Chromatographic separations were carried out with an RCM-100 Waters radial compression module (Millipore Corp., Milford, MA) containing a 0.5 × 10 cm Nova-PAK column with 4-μm reversed-phase (C_{18}) packing. A 0.2 × 5.5 cm stainless steel precolumn (Upchurch Scientific, Oak Harbor, WA) with 40-μm C_{18} packing was placed directly before the analytical column. Detection was accomplished with a Gilson Model 121 filter fluorometer with excitation at 305–395 nm and emission at 435–650 nm. Results were recorded on a Hewlett-Packard (Avondale, PA) 3390A reporting integrator.

Chemicals. All chemicals were of either chromatographic or reagent grade. Formate dehydrogenase purified from the yeast *Candida boidinii* and the coenzyme β-nicotinamide adenine dinucleotide (β-NAD^+, lithium salt) were purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN). Water was obtained by passing distilled water through a Milli-Q system containing an Organex-Q attachment (Milli Q water, Millipore, Bedford, MA). This water was assayed for formate contamination by injection into a Dionex Model 10 ion chromatograph fitted with an AG3 guard column, AS3 analytical column, a hollow fiber suppressor, and a conductivity detector (*8*). The concentration of formate in Milli Q water was generally less than 0.1 μM.

Stock solutions of formic acid (10 mM) in MilliQ water were prepared daily and stored at 4 °C. Dilute standards were prepared by additions of the stock solution to seawater, rain, or sediment porewater. The enzyme solution was prepared by dissolving 20 units of lyophilized formate dehydrogenase in 500 μL of Milli Q water; this solution was stable for 3 weeks at 4 °C (*9*). However, the reagent blank gradually increased over this period; therefore, for determinations of submicromolar concentrations of formate in natural water samples, it was important to prepare the enzyme solution daily.

HPLC Conditions. All chromatographic separations were performed isocratically at room temperature and at a flow rate of 1.0 mL min^{-1}. The mobile phase consisted of 10% methanol in 0.25 M sodium sulfate and 20 mM phosphate buffer (pH 6.0) and was filtered through a 0.22-μm filter. It was necessary to use a high concentration of salt in the eluant to elute the β-NADH as a sharp, single peak. The analytical column was flushed daily with water/methanol or water/dimethyl sulfoxide (60/40) since the β-NADH peak shape slowly degraded with time, presumably due to protein adsorption on the guard and analytical columns.

Sampling Procedures. Rain and seawater samples were gravity-filtered through a precombusted (400 °C, 8 h) Whatman GF/C filter (Clifton, NJ) with a Gelman polycarbonate support (Gelman, Ann Arbor, MI). Sediment samples collected from Biscayne Bay mud flats in either a glass jar or from an aluminum coring device were centrifuged to extract the porewater. Sargasso seawater (0.22 μm filtered) was used in methods development.

Samples were usually analyzed within 1 h after collection. If sample storage was required, samples were frozen or stored at 4 °C after addition of a biocide (e.g., chloroform (*10*)). Sample storage is not recommended for seawater samples due to potential contamination problems at submicromolar levels (*5*).

Enzymatic Assay. The reaction mixture consisted of 20 μL of enzyme (40 units of enzyme/mL of Milli Q water), 40 μL of β-NAD^+ (1 mg of β-NAD^+/mL of Milli Q water), 20 μL of borate buffer (0.4 M, pH 8.6), and 250 μL of sample in a 5-mL all-Teflon vial. The pH of the assay mixture should be between 7.5 and 8.5 (*9*). No borate buffer was added to open ocean seawater samples, since they were naturally buffered (i.e., carbonate) at approximately pH 8.2. Addition of buffer to sediment porewater or estuarine water was essential. A pH 9.4 borate buffer (0.5 M) was used for the analysis of rain samples. The reaction time at 20 °C was 1 h for seawater, 2 h for marine sediment porewater, 20 min for rain, and 5 min for Milli Q water. When the reaction was complete, 30 μL of ammonium sulfate (3 M, pH 4.0) was added to the mixture and an aliquot was injected into the chromatograph. After chromatographic separation, β-NADH was detected by its fluorescence. At the excitation bandwidth (305–395 nm), there was no β-NAD^+ fluorescence due to its poor absorbance at these wavelengths (Figure 1).

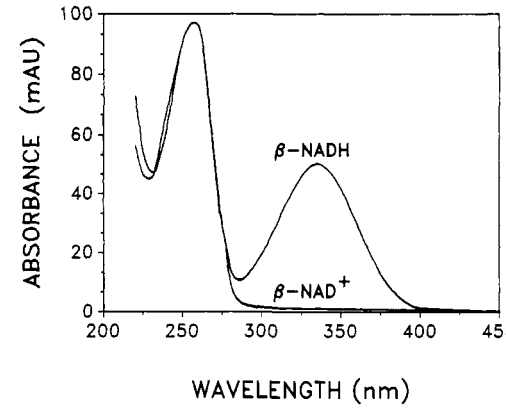

Figure 1. Absorbance spectra of β-NAD^+ and β-NADH as a function of wavelength between 200 and 450 nm. Spectra were taken of β-NAD^+ and β-NADH standards in 10% methanol and 90% 0.25 M Na_2SO_4 and 0.02 M phosphate buffer (pH 6.0).

Blanks. Two types of blanks were routinely performed for the analysis of natural samples. Unreacted, filtered rain, seawater, or sediment porewater was injected to correct for naturally occurring fluorescent interferences. A reagent blank was evaluated by adding the reagents to Milli Q water and reacting this mixture for 10 min, followed by addition of ammonium sulfate. This blank corrected for formate or β-NADH contamination in the enzyme, β-NAD^+, or borate buffer solutions.

Periodically, formate contamination in the reagents was also assessed by doubling the concentration of each reagent, one at a time, in the reaction mixture. In a few instances, formate dehydrogenase was not added to a sample to determine if β-NADH formed nonenzymatically from β-NAD^+ and to determine if β-NAD^+ was contaminated with β-NADH.

Quantification and Peak Identification. The concentration of formate in natural samples was quantified by linear regression analysis or by the peak area ratio of sample to standard; calibration plots were generated by additions of standards to seawater, rain, or sediment porewater.

A Hewlett-Packard Model 1040A diode array detector (Hewlett-Packard, Avondale, PA) was used to obtain the absorption spectrum of β-NADH as it eluted from the chromatograph. β-NADH was confirmed by its co-elution with an authentic β-NADH standard when the chromatographic selectivity was altered (e.g., change from a C_{18} to a CN column).

RESULTS

β-NAD^+ Kinetic Parameters. When β-NAD^+ or formate is present at a saturating concentration with respect to formate dehydrogenase, the reaction may be treated kinetically as a one-substrate reaction with respect to the nonsaturating substrate. This approach was used to determine the kinetic parameters by Michaelis–Menton enzyme kinetics.

The kinetic parameters for β-NAD^+ were determined by examining the effect of the β-NAD^+ concentration on the initial rate of the reaction of 0.22 μm filtered Sargasso seawater at 20 °C and pH 7.5. The β-NAD^+ concentration range tested was 75–600 μM; the reaction was saturated with respect to formate at 1.6 mM. The concentrations of borate buffer (pH

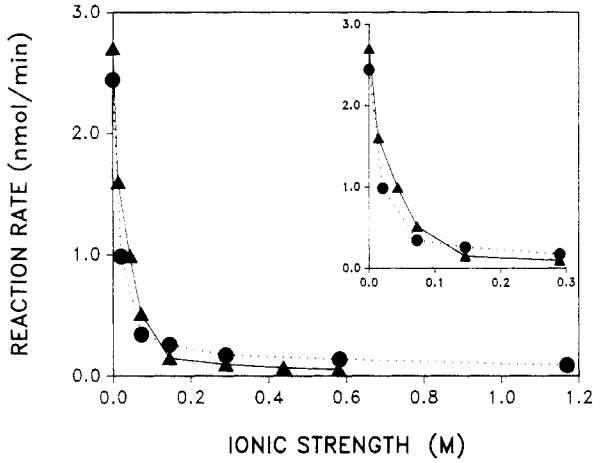

Figure 2. Plot showing the effect of ionic strength of the reaction mixture on the initial rate of the formate dehydrogenase reaction. Both seawater (▲) and sodium chloride (●) solutions were tested. Insert panel shows an expanded view of this plot for the region between 0 and 0.3 M ionic strength.

8.6) and formate dehydrogenase were held constant in the reaction mixture at 25 mM and 2.6 units mL^{-1}, respectively.

The Michaelis constant (K_M) and the maximum reaction velocity (V_{max}) that were calculated from a Lineweaver–Burk plot were 470 μM and 21 nmol min^{-1}, respectively. The K_M for β-NAD^+ that was determined for seawater was substantially higher than the K_M for β-NAD^+ that was determined for a dilute phosphate buffer (K_M = 90 μM (*11*); K_M = 100 μM (*12*)).

For the determination of formate concentrations in natural samples, β-NAD^+ was added to samples at a saturating concentration greater than 1 mM.

Formate Kinetic Parameters. K_M and V_{max} were determined for formate in 0.22 μm filtered Sargasso seawater at 20 °C and at pH 7.5. The effect of formate concentration on the initial reaction velocity was tested over the range 7–350 μM; the concentrations of formate dehydrogenase, borate buffer (pH 8.6), and β-NAD^+ in the reaction mixture was held constant at 1.9 units mL^{-1}, 25 mM, and 2.7 mM, respectively. A strong phosphate buffer (100 mM) was not used, since it caused precipitation of β-NAD^+ in seawater.

The K_M and V_{max} values for formate calculated from a Lineweaver–Burk plot were 500 μM and 0.17 nmol min^{-1}, respectively. The Michaelis constant determined for seawater was significantly lower than that previously determined in a pH 7.5 phosphate buffer at 30 °C (K_M = 13 mM (*11*); K_M = 1.5 mM (*12*)).

Ionic Strength. The effect of ionic strength on the reaction rate was determined with sodium chloride at 20 °C and pH 7.5. The ionic strength of the reaction mixture was varied from 0.016 to 0.6 M. Formate, β-NAD^+, borate buffer (pH 8.6), and enzyme concentrations were held constant at 10 μM, 1.6 mM, 12 mM, and 2.1 units mL^{-1}, respectively. The ionic strength effect was also examined by using 0.22 μm filtered Sargasso seawater that was diluted with Milli Q water; the ionic strength was adjusted from 0.015 to 0.58 M.

As shown in Figure 2, there is a strong ionic strength dependence on the velocity of the reaction for both seawater and a sodium chloride solution. This result is not surprising, since it is well-known that the salt concentration affects the structure and surface charge of enzymes (*13*). Changes in chemistry of the enzyme, in turn, affect its properties (e.g. specific activity, V_{max}, K_M, etc.).

It will be important to consider the effect of ionic strength on the reaction rate when the ionic strength is expected to vary substantially, such as in an estuary. However, in this case, it is not recommended to adjust the ionic strength of the samples, since this may introduce formate contamination or may dilute the formate concentration below the detection limit. An alternate solution is to increase all reaction times to that needed for the most saline sample.

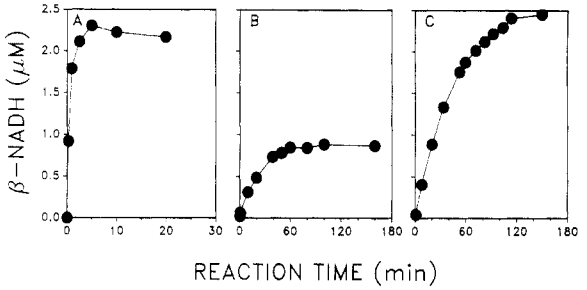

Figure 3. Effect of reaction time on the formation of β-NADH in (A) Miami rain, (B) Biscayne Bay seawater, and (C) Biscayne Bay sediment porewater. Assay conditions are as in text, except for rainwater, where 3 times the reported enzyme concentration was used.

Reaction Time. The effect of the reaction time on the formation of β-NADH was determined for rainwater, sediment porewater, and seawater. The concentration of β-NADH formed was calculated from standard additions of β-NADH to the samples. The β-NADH formed in the Milli Q water blank was subtracted from all natural water sample concentrations of β-NADH.

The reaction time was determined to vary, depending on the sample type. The reaction was complete for rainwater samples (collected in Miami) after 5 min (Figure 3A). In contrast, the reaction was complete after 1 h for Biscayne Bay seawater and 2 h for sediment porewater (Figure 3B, C). Differences in reaction time are attributed to differences in the ionic strength of these samples (e.g., seawater, $I \approx 0.7$ M; rain, $I < 0.01$ M).

The enzymatic reaction was performed at 20 °C in this study, even though the reaction rate is known to increase quite steeply with temperature up to 55 °C (*11*). Although elevated temperatures shorten the reaction time, lower reaction temperatures are preferred for the analysis of natural samples since high temperatures may induce release of formate from organisms in the sample (*5*).

Enzyme Concentration. The effect of the enzyme concentration on the reaction rate in 0.22 μm filtered Sargasso seawater was examined at 20 °C and at pH 7.5. The enzyme concentration was varied from 0.3 to 5 units mL^{-1}; formate, β-NAD^+ (free acid), and borate buffer (pH 8.6) concentrations were held constant in the reaction mixture at 5, 1.6, and 10 mM, respectively. With an increase in enzyme concentration, the initital reaction rate increased linearly from 0.005 to 0.038 nmol/min, while the β-NADH concentration in the enzyme blank increased by a factor of 16. As a compromise between an increase in reaction rate and increase in the blank, an enzyme concentration of 2.5 units mL^{-1} was used for the analysis of natural samples.

Enzyme Specificity. Results of this study and that of previous investigators indicate that the enzymatic reaction is quite specific for formate as the substrate. Micromolar to millimolar concentrations of acetate, methanol, formaldehyde, pyruvate, malate, oxalate, lactate, or succinate did not reduce β-NAD^+ to β-NADH in the presence of formate dehydrogenase (*9*, *11*, *12*). When formate dehydrogenase was not added to rain, seawater, or porewater, β-NAD^+ was not reduced to β-NADH. This result demonstrated that β-NAD^+ reduction did not result from oxidation–reduction processes involving other compounds present in the sample. In addition, β-nicotinamide adenine dinucleotide phosphate (β-$NADP^+$) or other electron acceptors cannot replace β-NAD^+ in the reaction (*9*). Nitrite, nitrate, Hg^{2+}, and Cu^{2+} inhibit the reaction at concentrations ≥1 mM; halogens at 10 mM or Ca^{2+}, Mg^{2+},

Zn^{2+}, Mn^{2+}, Cd^{2+}, and Sn^{2+} at 1 mM had no effect on enzyme activity (*11*). No activators of the reaction are known (*9*).

Enzyme Purification. At a high detector sensitivity, a fluorescent interference (presumably NADH) in the enzyme is observed to co-elute with an authentic β-NADH standard. We attempted to purify the enzyme with a Centriflo ultrafiltration membrane with a 50000 molecular weight cutoff (Amicon Corp., Lexington, MA) to remove the interference; the molecular weight of formate dehydrogenase from *Candida boidinii* is 74000 (*11*).

A concentrated solution of enzyme (160 mg mL^{-1}) was washed four to six times with 3 mL-aliquots of either Milli Q water, a strong salt solution (0.25 M sodium sulfate), or 0.2 M β-NAD^+. After concomitant washing and centrifugation of the enzyme solution in the ultrafiltration cone, aliquots of the retentate and the eluate were assayed to determine the enzyme activity in each fraction. In all cases, the enzyme was completely retained by the ultrafiltration membrane and showed greater than 90% of its original activity. At the same time, the fluorescent contamination was also retained, indicating that it was strongly associated with the enzyme. Since chromatographic evidence indicated that the contamination was β-NADH (i.e., absorption spectra for the standard and unknown were identical, and the unknown co-eluted with β-NADH), an experiment was performed to determine if β-NADH was retained by the membrane. Without enzyme present, greater than 99% of 1 mg mL^{-1} β-NADH was not retained by the membrane; however, when 160 mg mL^{-1} of enzyme was added, greater than 30% of the β-NADH was found in the retentate after repeated washes with Milli Q water, indicating that β-NADH adsorbed to the enzyme. These results demonstrated that it would not be possible to purify the enzyme by size exclusion techniques. If the contamination is from β-NADH, then it may be possible to purify the enzyme by selectively oxidizing the enzyme-bound β-NADH with subsequent removal of the oxidant; however, this was not tested in this study.

Precision and Linearity. The precision of the procedure was determined for a 0.6 μM addition of formate to seawater corresponding to 120 pmol for a 200-μL injection; at this concentration, the percent relative standard deviation was 4.6% ($n = 6$). The linearity of the fluorescent response was determined for standard additions of formate to seawater in the concentration range 0.2–1.0 μM. The fluorescent response was linear within this concentration range ($r^2 = 0.964$; $n = 16$; y-int ± SE = 1.8 ± 0.8 IU; slope ± SE = 8.8 ± 0.5 IU/μM); the y axis of all regression plots is given as unitless integration units (IU).

The precision and linearity of the method were also determined for the analysis of rain and sediment porewater. The precision was less than 5% relative standard deviation ($n = 5$) at the 100–500 pmol level (200-μL injection), and fluorescent responses were linear within the concentration range tested: 0.2–50.0 μM for porewater ($r^2 = 0.941$, $n = 8$, y-int ± SE = 2.7 ± 0.7 IU, slope ± SE = 9.5 ± 0.6 IU/μM) and rain ($r^2 = 0.996$; $n = 10$; y-in ± SE = 8.7 ± 7.8 IU; slope ± SE = 1.52 ± 0.04 IU/μM).

The linear range of the method was determined for concentration ranges of environmental interest. Consequently, these results do not indicate the dynamic range of the method; this should be tested for particular applications when appropriate. It should also be noted that the linear range of the method is a function of the enzyme concentration and reaction time. If high concentrations of formate are expected, it will be necessary to either dilute the sample, increase the enzyme concentration, or lengthen the reaction time.

Detection Limit. The detection limit of the method is controlled by contamination in the enzyme and reagents. The detection limit that can be expected for the routine analysis of natural samples is 0.5 μM with a signal-to-noise ratio of 4. If blanks are evaluated rigorously and reagents are prepared carefully to minimize formate contamination, then the detection limit can be as low as 0.1 μM, as shown in Figure 4 for seawater.

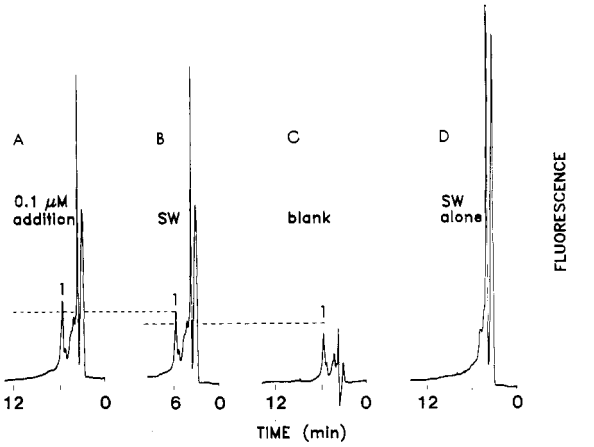

Figure 4. Chromatograms of Biscayne Bay seawater: (A) 0.1 μM addition of formate to seawater with enzyme reagents added (0.1 μM addition); (B) seawater with reagents added (SW); (C) Milli Q water with reagents (blank); and (D) seawater injected directly into the chromatograph without addition of enzyme reagents (SW alone). Peak 1 corresponds to β-NADH. Dashed lines were drawn to emphasize differnces in the β-NADH peak height between the Milli Q water blank, the seawater sample, and a 0.1 μM standard addition of formate to seawater; dashed lines accounted for differences between base lines.

Intercalibration. The concentration of formate in Miami rain determined by the enzymatic technique was compared to the formate concentration determined by ion chromatography. The two methods gave excellent agreement. The concentrations of formate determined by the enzymatic technique and the ion chromatographic technique were 5.9 ± 0.3 μM ($\bar{X}$ ± SD, $n = 3$) and 5.8 ± 0.2 μM, respectively; results of a two-sample t test showed no significant differences between mean concentrations ($p(t = 0.46) < 0.5$). Intercalibration for seawater or sediment porewater was not possible, since high salt concentrations interfered with ion chromatographic analyses.

Recovery. The recovery of 2 μM additions of formate to seawater, sediment porewater, or rain was determined relative to 2 μM additions of an authentic β-NADH standard to these samples. Recoveries were 103.2 ± 5.2% ($\bar{X}$ ± SD, $n = 4$), 103.0 ± 3.6%, and 86.8 ± 2.8% for seawater, porewater, and rain, respectively. Since recoveries were relative to authentic standards, these results demonstrated that the formate dehydrogenase reaction proceeds to completion with the equilibrium of the reaction shifted far to the right in the direction of CO_2 and β-NADH production. These results support the findings of Schutte and co-workers (*11*), who determined that the formate dehydrogenase reaction was essentially irreversible; the forward reaction rate leading to the formation of CO_2 and β-NADH was approximately 5000 times greater than the reverse reaction rate.

Natural Samples. Applications of the enzymatic method to the analysis of rain water, seawater, and sediment porewater are shown in Figures 4, 5, and 6. A summary of the analysis of selected samples is given in Table I.

Typical chromatograms of seawater are shown in Figure 4. No β-NADH was detected in seawater that was injected directly into the chromatograph without added reagents (chromatogram D). The detection limit of this method for the analysis of seawater is controlled by the formate or β-NADH contamination in the reagents; this contamination was estimated by the Milli Q water blank shown in chromatogram

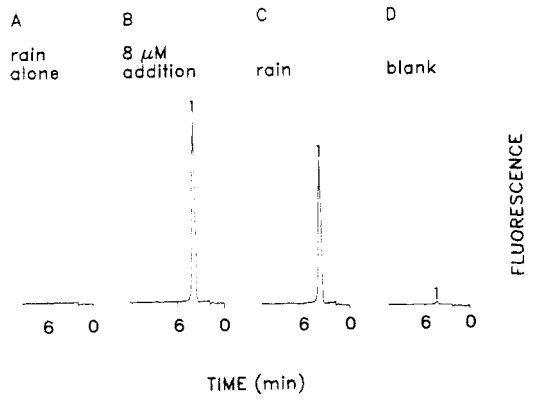

Figure 5. Chromatograms of Miami rain water: (A) rain injected directly into the chromatograph without added enzyme reagents (rain alone); (B) 8 μM addition of formate to rain with reagents added (8 μM addition); (C) rain with reagents added (rain); and (D) Milli Q water with reagents added (blank). Peak 1 corresponds to β-NADH.

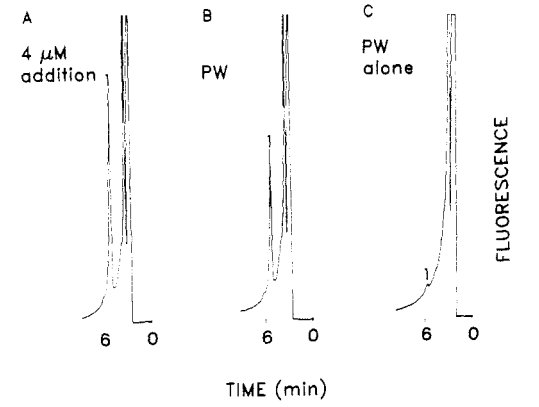

Figure 6. Chromatograms of Biscayne Bay sediment porewater: (A) 4.0 μM addition of formate to filtered porewater with enzyme reagents added (4 μM addition); (B) porewater with reagents (PW); and (C) porewater injected directly into the chromatogram without addition of enzyme reagents (PW alone). Peak 1 corresponds to β-NADH. The Milli Q blank (not shown) is nearly identical with that shown in Figure 5.

C. On the basis of this blank alone, it was difficult to determine the reagent blank, since the water blank would overestimate the seawater blank if the contamination were in the Milli Q water itself and not in the reagents. This result emphasizes the importance of evaluating different blanks discussed in the Experimental Section to arrive at the best estimate of the seawater blank. In general, the blank was not critical for rain or marine sediment porewater because of the relatively high formate concentrations (>1.0 μM) in these samples.

Analysis of rain samples resulted in simple chromatograms as in Figure 5, with one major peak at approximately 4 min corresponding to β-NADH. Chromatogram D to the far right of this figure depicts a Milli Q water blank with a trace of β-NADH relative to the rain sample (chromatogram C). Chromatogram B shows a 8 μM standard addition of formate to this rain. No β-NADH was detected in rain that was injected directly into the chromatograph without added enzyme or other reagents (chromatogram A).

In contrast to seawater, the concentration of formate in Biscayne Bay sediment porewater was generally much higher than the detection limit (Figure 6, chromatogram B). However, β-NADH was periodically detected in unreacted anoxic porewater that was injected directly into the chromatograph (chromatogram C). Therefore, for the analysis of porewater, both the reagent blank and porewater blank (with no added reagents) should be evaluated.

Table I. Concentrations of Formic Acid in Natural Water Samples as Determined by the Formate Dehydrogenase Technique[a]

date	sample type	n	$\bar{X}$ of formate, μM
	Miami Rain		
11-08-86		2	1.2
11-09-86		2	2.2
02-05-87		2	0.4
02-18-87		2	10.0
02-25-87		3	2.21 ± 0.13[b]
03-24-87		2	1.6
03-25-87		3	7.90 ± 0.42[b]
05-12-87		2	4.2
05-14-87		2	1.6
	Biscayne Bay Seawater		
03-30-87		2	0.8
03-31-87		2	0.4
04-22-87		2	0.3
11-04-87		3	0.19 ± 0.02[b]
	Biscayne Bay Sediment Porewater		
04-22-87	0–5 cm[c]	1	0.9
04-22-87	5–10 cm	1	7.2
04-22-87	10–15 cm	1	7.2
04-24-87	anoxic porewater	2	8.4
04-24-87	oxic porewater	2	1.0
	Milli Q Water		
11-04-87	reagent blank	3	0.21 ± 0.01[b]

[a] Concentrations were calculated from peak area ratio of the sample to an external standard except when noted; n = number of sample replicates. [b] Reported values are given as $\bar{X}$ ± RSD ($\bar{X}$ = average concentration; RSD = relative standard deviation). Concentrations were determined from standard addition plots. [c] Ranges given represent the depth intervals in the sediment core from which porewater was extracted.

DISCUSSION

Coupling the formate dehydrogenase assay with RP-HPLC permitted the determination of formic acid in seawater at submicromolar concentrations. Concentrations of formic acid that were determined in the present study (Table I) indicate that this organic acid may be a major component of dissolved organic matter in coastal seawater (*14*). Results of an earlier study (*15*), using a continuous extraction technique, showed that formate was present in seawater at micromolar concentrations. However, these results are suspect, since that study lacked controls. Although it was possible to detect formate in coastal seawater samples, our method in its present form was not adequately sensitive to detect formate at low concentrations (<0.1 μM) in open ocean seawater samples. Modifications of this method to improve the detection limit are currently being examined.

In the analysis of natural water samples, especially seawater or sediment porewater, the concentration that is determined chemically may not reflect the concentration of that solute that is biologically available (*16*). Clearly, the chemical and physical harshness of a technique will affect the measured concentration. In this respect, enzymatic methods, in contrast to classical chemical techniques, should provide a better estimate of the biologically available fraction of a solute. Although many enzymatic techniques are readily available, these techniques have not been applied to the determination of organic solutes in natural waters except in a few instances (*17*, *18*).

Since the method developed in this study is based on the formation of a common biochemical redox compound, this chromatographic method should be compatible with other enzymatic techniques based on the formation of reduced NAD. However, the usefulness of a particular enzymatic method for the analysis of natural water samples will depend, in a large

part, on the availability, specificity, and thermal stability of the enzyme.

The formate dehydrogenase reaction was adapted to a chromatographic method to allow for the determination of formate in natural water samples at submicromolar concentrations in the presence of fluorescent interferences; these interferences limit the sensitivity of batch techniques. However, if the concentrations of formate are fairly high (≥1 μM) and fluorescent interferences are relatively low, then the method could easily be adapted to a simple batch method (9).

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Registry No. Formate, 71-47-6; water, 7732-18-5; formate dehydrogenase, 9028-85-7.

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Preparation and Evaluation of Dry-Packed Capillary Columns for High-Performance Liquid Chromatography

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A dry-packing method to prepare fused-silica columns of 0.250-mm i.d. packed with 5-μm particles is presented. Several C_{18} packing materials (Spherisorb ODS-1, Spherisorb ODS-2, Hypersil ODS) are studied, and the evaluation of column performance is carried out by means of the reduced plate height/velocity equation and the separation impedance. A comparison between dry-packed and analogous slurry-packed columns is also carried out. It is shown that the dry-packing method yields columns of analogous or better efficiency and requires a simpler apparatus and a shorter packing time.

Over the past several years much effort has been addressed toward increasing the speed of analysis and efficiency in high-performance liquid chromatography (HPLC), and recent trends have been mostly directed toward the use of narrow-bore columns (*1–12*). In fact, microcolumns, as compared to conventional ones, are essentially characterized by higher efficiencies and lower flow rates and require minimum sample sizes. Thus, they are particularly suitable for the analysis of very complex mixtures and for direct interfacing to mass spectrometers (*13*, *14*) and flame-based chromatographic detectors (*15–17*), while providing a great potential for the analysis of biological fluids where the manipulation of very small sample volumes is often required. In addition, the routine use of hazardous or "exotic" solvents is feasible. HPLC microcolumns can be conventionally classified in three categories (*18*): open tubular (*19–24*), packed capillaries where the adsorbent is partially embedded into the column walls (*3*, *9*, *15*, *16*, *25*), and narrow-bore slurry-packed columns where the adsorbent is tightly packed as in conventional HPLC columns (*4*, *5*, *8*, *10*, *11*). Though the first two types, in particular the open tubular columns, yield higher efficiencies and shorter analysis time, severe limitations to the full exploitation of their capabilities arise from the stringent requirements imposed on the experimental apparatus. Further, they are difficult to prepare and offer both limited sample capacity and column selectivity (*5*).

Narrow-bore (capillary) columns suffer much less from these restrictions and seem to be at the moment the best compromise between open tubular and short microparticle-packed conventional columns. Fused-silica capillary columns of inner diameters ranging between 0.300 and 0.150 mm and of various lengths, packed with 5- and 3-μm particles, have been succesfully prepared by several groups (*6*, *7*, *10–12*). In all cases a slurry-packing technique has been employed. In fact, the dry-packing technique is usually confined to the preparation of columns containing particles larger than 20 μm, since

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