CO-PRECIPITATION OF DISSOLVED ORGANIC MATTER BY CALCIUM CARBONATE IN PYRAMID LAKE, NEVADA

Jerry A. Leenheer* and Michael M. Reddy

[1]U.S. Geological Survey, PO Box 25046, Denver Federal Center, Denver, CO, 80225, USA

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ABSTRACT

Our previous research has demonstrated that dissolved organic matter (DOM) influences calcium carbonate mineral formation in surface and ground water. To better understand DOM mediation of carbonate precipitation and DOM co-precipitation and/or incorporation with carbonate minerals, we characterized the content and speciation of DOM in carbonate minerals and in the lake water of Pyramid Lake, Nevada, USA. A 400-gram block of precipitated calcium carbonate from the Pyramid Lake shore was dissolved in 8 liters of 10% acetic acid. Particulate matter not dissolved by acetic acid was removed by centrifugation. DOM from the carbonate rock was fractionated into nine portions using evaporation, dialysis, resin adsorption, and selective precipitations to remove acetic acid and inorganic constituents. The calcium carbonate rock contained 0.23% DOM by weight. This DOM was enriched in polycarboxylic proteinaceous acids and hydroxy-acids in comparison with the present lake water. DOM in lake water was composed of aliphatic, alicyclic polycarboxylic acids. These compound classes were found in previous studies to inhibit calcium carbonate precipitation. DOM fractions from the carbonate rock were ^{14}C-age dated at about 3,100 to 3,500 years before present. The mechanism of DOM co-precipitation and/or physical incorporation in the calcium carbonate is believed to be due to formation of insoluble calcium complexes with polycarboxylic proteinaceous acids and hydroxy-acids that have moderately large stability constants at the alkaline pH of the lake. DOM co-precipitation with calcium carbonate and incorporation in precipitated carbonate minerals removes proteinaceous DOM, but nearly equivalent concentrations of neutral and acidic forms of organic nitrogen in DOM remain in solution. Calcium carbonate precipitation during lime softening pretreatment of drinking water may have practical applications for removal of proteinaceous disinfection by-product precursors.

Keywords: Dissolved organic matter, organic matter fractionation, calcium carbonate precipitation, organic matter co-precipitation, infrared spectrometry, ^{14}C-age, Pyramid Lake, Nevada, USA

1. INTRODUCTION

Limestone has variable amounts of natural organic matter (NOM) incorporated into the rock matrix as kerogen, including the Green River Formation oil shale, which is well known as one the world's largest potential reserves of hydrocarbon fuels [1]. The process of kerogen incorporation into limestone is complex and involves different mechanisms such as coral growth, sedimentation and burial of phytoplankton, input of allochthonous NOM from riverine and atmospheric sources, and co-precipitation and/or physical incorporation of dissolved organic matter (DOM) during calcium carbonate precipitation. DOM incorporated in limestone as kerogen is fossilized and does not degrade until the limestone itself is decomposed; thus, NOM incorporated during limestone formation is sequestered from the global carbon cycle for long periods of time. To the best of our knowledge there are no studies that have comprehensively isolated and fractionated DOM from limestone because of the difficulty in removing calcium salts from polar DOM fractions in acid solutions used to dissolve limestone [2]. The objective of this study is to characterize the content and compound-class speciation of DOM in carbonate minerals and in lake water of Pyramid Lake, Nevada to determine the mechanisms of DOM co-precipitation with calcium carbonate. This study, performed on only two samples, was intended to be an initial assessment of compositional differences in natural organic matter composition and to suggest processes that may account for these differences. The results may not be representative of Pyramid Lake as a

* Corresponding Author: U.S. Geological Survey, PO Box 25046, MS 408, Denver Federal Center, Denver, CO, 80225, USA. Phone: 303-236-3977, fax: 303-236-3934, e-mail: <leenheer@usgs.gov>

whole, and more representative and comprehensive studies are required to confirm the hypotheses of this report.

Pyramid Lake was selected for the study because of a well-documented history of carbonate mineral formation in past and present-day environments and because of past research at that location [3,4]. Pyramid Lake is a terminal lake, but has relatively fresh water (specific conductance of 7,900 microSiemens at 25°C) and is alkaline (pH near 9). The lake surface water is supersaturated with respect to several calcium carbonate minerals (for example, calcite and aragonite), which precipitate at springs and seeps located along the lake shore and occasionally as a lake-wide surface water precipitation event during summer algal blooms [5]. Calcium carbonate precipitation events associated with algal blooms are called "whitings" [3].

Dissolved organic matter may reduce calcite crystal growth rates [6-8] and previous investigations have quantified calcite crystal growth rate reduction caused by natural organic acid isolates [9]. Calcite crystal growth rate studies have demonstrated that organic acid molar mass was correlated with growth rate reduction as was aromaticity, aliphatic carbon content and heteroatom content. Moreover, observed calcite growth rate reductions for natural organic acids isolated from the Florida Everglades are consistent with a Langmuir adsorption mechanism in which DOM blocks growth sites on the growing calcite crystal surface [7].

2. MATERIALS AND METHODS

2.1. Sample Location and Site Description

Pyramid Lake, Nevada is a closed basin lake (latitude 40 N, longitude 119.5 W), with a surface area in 1995 of 430 km^2, and a maximum depth in 1995 of 100 m situated in the Great Basin of the western United States [10]. Pyramid Lake inflow is from the Truckee River. Recent basin ecological and hydrological changes (deforestation, agricultural diversions of the Truckee River, and consumptive water use associated with settlement of the Reno, Nevada area through which the Truckee River flows, have generally caused significant decreases in the level of Pyramid Lake [4], although the lake level fluctuates depending on the yearly precipitation. The lake is ice free year round with one period of overturn and is located in the high desert east of the Sierra Nevada Mountains. The climate of Pyramid Lake is arid with hot summers and cold winters [10].

Carbonate rock was obtained in August 1995 from the shore of Pyramid Lake at Wizard Cove near an actively precipitating hot spring/geothermal well (Figure 1). A carbonate rock formed by an active precipitation process formerly in contact with lake water when the lake level was higher was selected to study co-precipitation of lake-water DOM rather than a tufa rock sample that forms by more complex and periodic precipitation processes. The rock was formed between 3,000 and 4,000 years ago and had a slight rind of freshly precipitated calcium carbonate on the surface. The hot spring was in the Needles area (Wizard Cove) at the north end of the lake (Figure 1). Because of current (2008) higher lake levels than during sample collection in 1995, this site is now under water. A dip sample of surface water was collected on April 6, 2006 at the marina boat dock near the Crosby Inn, Sutcliff, Nevada by the USGS Nevada Water Science Center staff.

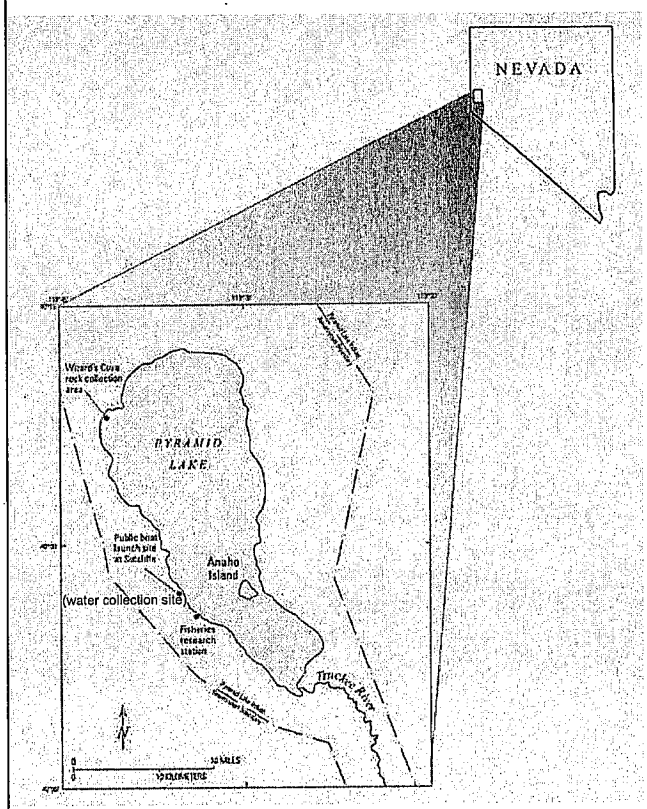

Figure 1 Map of Pyramid Lake, Nevada showing rock and water collection sites

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2.2. Laboratory Equipment

Glass chromatography columns with Teflon end caps were used for separations. Column dimensions for 1 L resin-bed volume were 5 cm ID X 60 cm long; 500 mL resin-bed volume were 5 cm ID X 30 cm long; 80 mL resin-bed volumes were 1.5 cm ID X 30 cm long; and 20 mL resin-bed volumes were 1 cm ID X 28.5 cm long. Excess volume in each column was left vacant to allow for resin bed expansion during different chemical treatments. A reciprocating, ceramic piston pump was used to pump water and reagents through the 1 L and 500 mL resin-bed columns connected with 3 mm-ID, 6 mm OD fluorinated ethylene propylene (FEP) Teflon tubing at a flow rate of 250 mL/min. A smaller pump (FMI Lab Pump Model RP-SY) was used to pump water and reagents through the 80 mL resin-bed column at a flow rate of 20 mL/min and through the 20 mL resin-bed column at a flow rate of 10 mL/min. These smaller columns were connected to the pump with 1.5 mm ID, 3 mm OD FEP Teflon tubing. Selection, preparation, regeneration, capacities, and packing of resin adsorbents have been discussed previously [2, 11, 12].

Spectra/Por 3 regenerated cellulose dialysis membranes with a molar mass cutoff of 3,500 Daltons were used. Prior to use, an appropriate length of membrane was cut to accommodate the volume to be dialyzed, and the membrane was washed by soaking it in 4 L of deionized water overnight. For dialysis, the bottom of the dialysis tubing was closed with a plastic snap closure, the solution was poured into the resulting bag using a funnel, and the top of the bag was closed with another closure. Dialysis was then conducted in 4-L Teflon beakers and the solution was stirred with a magnetic stirrer.

A freeze-dryer and two vacuum rotary evaporators were used for evaporations. The large Buchi Rotavapor 150 had 10 L evaporation and condensation flasks employed in the fractionation procedure and had an evaporation rate for water of about 2 L per hour for a water-bath temperature of 60°C at 750 mm mercury vacuum. A small Buchi Rotavapor R had 1 L evaporation and condensation flasks, and its evaporation rate for water was about 0.5 L per hour for a water-bath temperature of 60°C at 750 mm mercury vacuum. The freeze dryer was a Labconco Lyph-Lock 6 Model. Centrifuge separations used a Sorvall Superspeed RC-2B refrigerated centrifuge (with a fixed-angle head) [2] for the hydrophilic acid plus neutral fraction. Centrifuge bottles were 250 mL high-density polyethylene that were spun at 7,000 revolutions per minute for 30 min.

2.3. Isolation and Fractionation of DOM from Calcium Carbonate Rock

The isolation and fractionation procedure details for the calcium carbonate rock sample from Pyramid Lake (Figure 2) are given in previously published comprehensive DOM fractionation procedures [2]. The rock sample was broken up with a hammer and the fragments (about 400 grams) placed in two 4 L plastic beakers with 10 percent acetic acid. It took about 2 weeks for the fragments to dissolve. Acetic acid was selected to dissolve the carbonate rock because the calcium acetate reaction product has low solubility in glacial acetic acid [13] and can be removed by crystallization and filtration during zeotropic distillation of acetic acid from water. A 2 L Buckner glass filtration funnel with a 12 cm diameter fritted glass filter was fitted to a 2 L glass Erlenmeyer vacuum filtration flask, and a water aspirator was used generate the vacuum needed to filter the large masses of calcium acetate from suspension. The calcium acetate filter cake was rinsed with glacial acetic acid until the filtrate was colorless, which indicated complete removal of DOM from the salt.

Carbonate rock material insoluble in acetic acid was removed by sieving and centrifugation. These insoluble mineral components were discarded because FT-IR spectral analyses detected no peaks that could be ascribed to NOM. Organic colloids were separated from DOM by dialysis against water at pH 4.9 (near the pK_a of acetic acid, 4.7) and against 0.1 M HCl at pH 1. Brown colloids in the dialysis bag after dialysis against pH 4.9 water indicated the possible presence of insoluble DOM metal complexes that could be brought into solution at low pH. A substantial amount of DOM incorporated in the insoluble material after dialysis against water at pH 4.9 was brought into solution at pH 1 and permeated through the dialysis membrane.

Three subfractions of hydrophobic and amphiphilic DOM fractions were obtained: the subfractions that adsorbed on XAD-8 and XAD-4 resins at pH 4.9, the subfractions that adsorbed on these resins at pH 1, and the subfractions from the dialysis permeate at pH 1 that adsorbed on these resins.

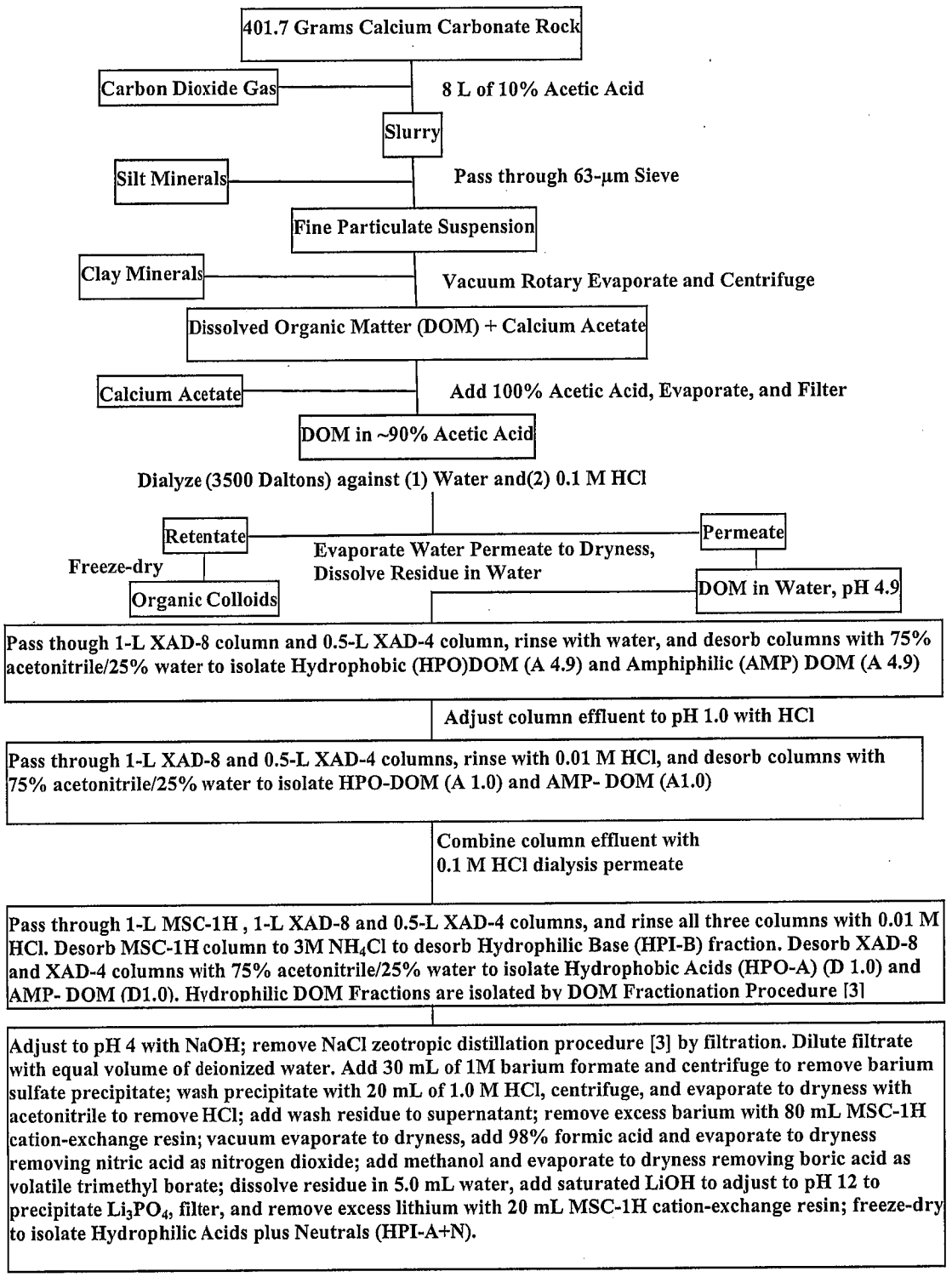

Figure 2 Flow chart for isolation and fractionation of DOM from calcium carbonate rock from Pyramid Lake

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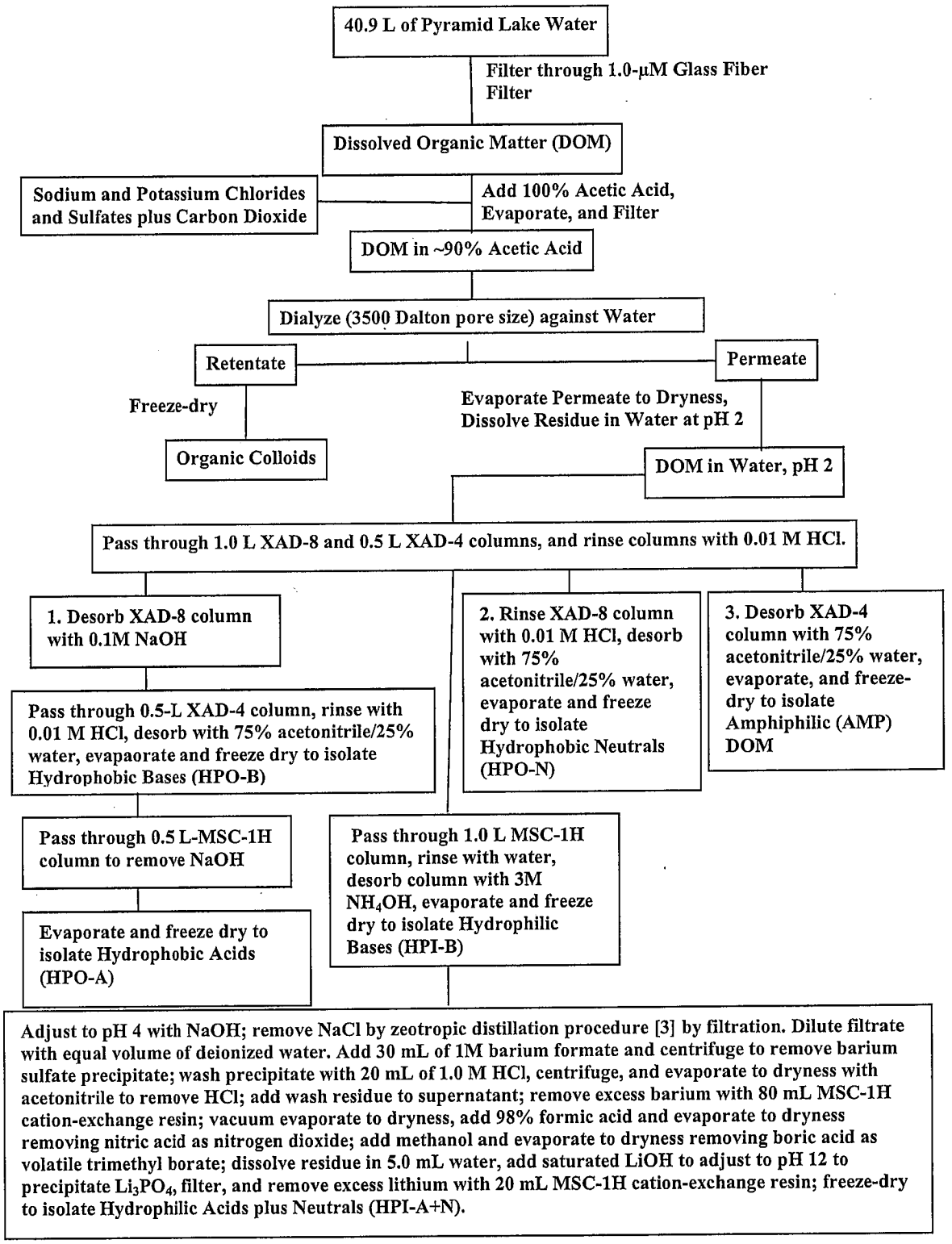

Figure 3 Flow chart for isolation and fractionation of dissolved organic matter from water sample from Pyramid Lake

2.4. Isolation and Fractionation of DOM from Pyramid Lake water

The isolation and fractionation procedure for the water sample from Pyramid Lake is presented in Figure 3.

Specific instructions for reagent and rinse volumes are given in previously published comprehensive DOM fractionation procedures [2]. The DOM fractionation procedures used here differ from previous approaches in that the zeotropic distillation of acetic acid from water was conducted first to remove inorganic salts instead of after isolation of the hydrophobic and amphiphilic DOM fractions. This procedural modification was made to make the water fractionation procedure more comparable to the rock fractionation procedure. A second modification of the previously published DOM fractionation procedure was to fractionate hydrophobic DOM into hydrophobic acid, base, and neutral fractions by the recently published dissolved organic nitrogen fractionation procedure [14]. This procedural modification was made to detect and characterize nitrogen-rich DOM fractions in lake water; in particular this modification will enhance understanding of nitrogen-rich DOM interaction with precipitating calcium carbonate minerals [14-16].

2.5. Fourier Transform-Infrared (FT-IR) Spectrometry

Fourier transform-infrared spectra were collected using 2 to 5 mg of NOM fraction isolates in KBr pellets. The Perkin Elmer System 2000 FT-IR used a ceramic glower source aligned with a laser and a deuterated triglycine sulfate detector. The instrument was set up to scan from 4,000 to 400 cm^{-1} averaging 10 scans at 1.0 cm^{-1} intervals with a resolution of 4.0 cm^{-1}. All spectra were normalized after acquisition to a maximum absorbance of 1.0 for comparative purposes.

Comprehensive interpretation of FT-IR spectra of specific compounds is difficult because of the abundance of absorption bands associated with complex organic mixtures such as DOM isolates. However, interpretation of fractionated-NOM spectra is simplified because only the strongest bands can be identified and associated with the predominant structures. For interpretation of the FT-IR spectra of specific compounds, the reader is referred to Pouchert [17], and for the analyses of complex biomolecular structures and humic substances to Bellamy [18] and Stevenson [19], respectively. Table 1 lists characteristic FT-IR frequency bands for possible natural organic matter structures found in fraction isolates for this study.

Table 1 FT-IR frequency bands for various possible natural organic matter structures in fraction isolates [6-8]. (ϕ = aromatic carbon)

Compound Class	Frequencies (cm^{-1}) and Structure
Carbohydrates	3400-3300 (O-H), 1100-1000 (C-O)
Lignin	1510 (C=C), 1460 (CH_2), 1420 (C=C), 1268 (ϕ-O), 1225 (C-O), 1127 (OCH_3), 1030 (ϕ-H)
Condensed Tannins	1610 (C=C), 1515 (C=C), 1280-1230 (ϕ-O), 1180-1140 (C-O), 1020 (ϕ-H)
Fulvic and Humic Acids	3400-3300 (O-H), 2700-2500 (COOH), 1760 (COOR), 1720 (COOH), 1660-1630 (ϕ-C=O), 1630-1600 (H-bonded enols and quinones, C=C), 1280-1150 (ϕ-O, COOH)
Hydrocarbons: Aliphatic chain	2960 (CH_3), 2940 (CH_2), 1460 (CH_2), 1380 (CH_3)
Hydrocarbons: Aliphatic ring	Broad peak from 2980 to 2900 (CH_3 and ring CH_2 and CH)
Proteins	1660 (Amide I band, N-C=O), 1540 (Amide II band, N=C-O)
N-Acetyl Amino Sugars	1660 (Amide I band, N-C=O), 1550 (Amide II band, N=C-O) 1380 (CH_3)
Lactams	1680 (Amide I band, N-C=O)
Cyclic Imides	1700 (Amide I band, O=C-N-C=O)

2.6. Elemental and Isotopic Analyses

Elemental analyses were performed by Huffman Laboratories, Wheatridge, Colorado. Carbon, hydrogen, nitrogen (CHN) analyses were performed using a C, H, N analyzer. Calcium carbonate rock samples were prepared for Accelerator Mass Spectrometry (AMS) measurement for ^{13}C and ^{14}C in the following manner. Ten to 20 mg of powdered rock was placed in a hydrolysis vessel and evacuated. After pumping for 1 hour, the sample was dissolved in 70% H_3PO_4. Carbon dioxide produced by the reaction was passed through two cold traps at -80°C to remove water. The dried gas was reduced to graphite using the method of Slota et al. [20]. Organic matter fractions were prepared for AMS measurements by oxidation to carbon dioxide. Error analysis of the AMS procedure has been described recently [21].

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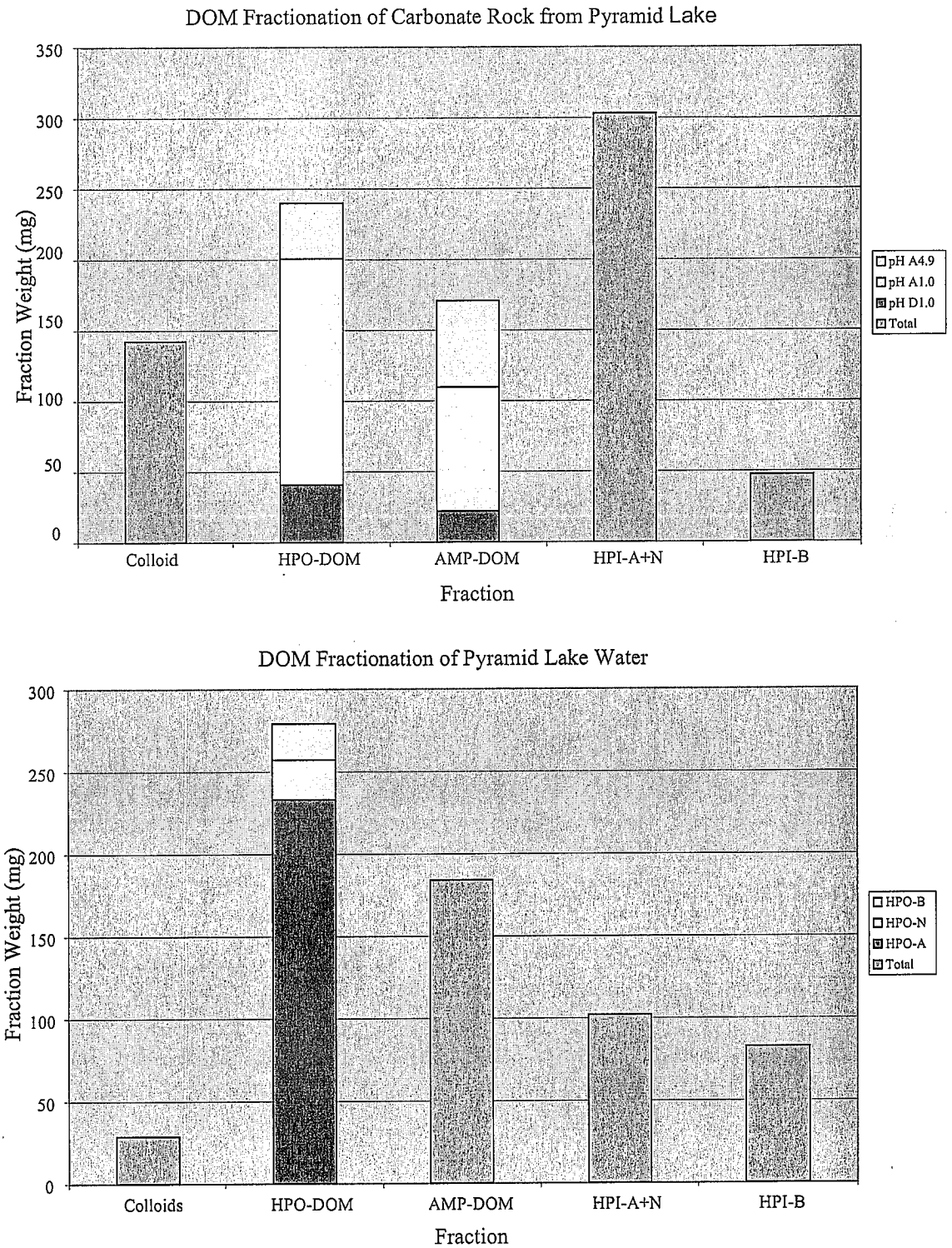

Figure 4 Bar diagrams showing DOM fractionations of Pyramid Lake carbonate and water samples. Fraction abbreviations are defined in Figures 2 and 3

3. RESULTS AND DISCUSSION

3.1. DOM Fractionations

Fractionation and characterization of Pyramid Lake carbonate rock and lake water DOM are presented together for ease of comparison and discussion. Bar diagrams presenting DOM fractionations of Pyramid Lake carbonate and water samples are presented in Figure 4. The calcium carbonate rock contained 0.23% DOM by weight. The Pyramid Lake water sample colloid and hydrophilic acid plus neutral fractions are depleted relative to the hydrophobic and amphiphilic DOM fractions in an Anaheim Lake water sample, suggesting that carbonate precipitation may affect the DOM fractionation component ratios.

In contrast to the Pyramid Lake water sample DOM fractionation, the DOM fractionation of the carbonate rock sample exhibited enrichment of the colloid and hydrophilic acid plus neutral fractions relative to the hydrophobic and amphiphilic DOM fractions. The difference in DOM constituent ratios of the water and carbonate rock samples suggests that co-precipitation and/or incorporation of components from the lake water in the precipitating calcium carbonate occurs in the colloid and hydrophilic acid plus neutral fractions.

Isolation of colloid fractions with high recovery in both the water and rock samples was an unexpected finding of both isolation procedures. Significant colloids loss was expected in the fractionation scheme shown in Figures 2 and 3. However, initial salt separation by zeotropic distillation did not remove sample colloids. Thus, water and carbonate rock colloid recovery with the revised DOM isolation procedures presented here (Figures 2 and 3) is well suited for efficient DOM isolation procedures in saline water samples.

3.2. Carbon, Hydrogen, and Nitrogen Analysis

Carbon, hydrogen, and nitrogen analyses of Pyramid Lake water and carbonate rock are presented in Table 2. Atomic C:N ratios are also presented in the bar diagrams of Figure 5. The C:N ratios of the hydrophobic and amphiphilic DOM fractions are the composite weight adjusted C:N ratios of the subfractions presented in Figure 4. The C:N ratios of the colloid and hydrophilic acid plus neutral fractions of the rock sample are much greater than these respective fractions in the water sample, indicating that during carbonate mineral formation these DOM constituents were preferentially incorporated from lake water. In contrast, the C:N ratios of the hydrophobic DOM, amphiphilic DOM, and hydrophilic base fractions of the carbonate rock sample are lower than these respective fractions in the water sample. These C:N ratio data suggest a selective (based upon functional-group chemistry) DOM fractionation resulting from co-precipitation and incorporation within calcium carbonate formed in the lake water. About 94% of the organic carbon delivered to modern Pyramid Lake was estimated to be derived from aquatic production [22]. Assuming that the Redfield C:N ratio near 6.6 [23] applies to most of the organic production in Pyramid Lake, deviations from the Redfield ratio in DOM likely result from selective degradations, selective uptake, and co-precipitation of DOM with carbonates.

Table 2 Elemental analyses of isolated organic fractions (NA = not analyzed; Fraction acronyms are defined in Figures 2 and 3)

Source	Fraction	Isolated Mass (mg)	Percent Carbon	Percent Hydrogen	Percent Nitrogen	Atomic C:N Ratio
Rock	Colloids	142.4	36.56	5.34	1.30	32.8
Rock	HPO-DOM (A 4.9)	39.1	56.18	6.33	4.00	16.3
Rock	HPO-DOM (A 1.0)	160.0	44.04	4.54	1.95	26.4
Rock	HPO-DOM (D 1.0)	40.8	50.09	4.91	3.26	17.9
Rock	AMP-DOM (A 4.9)	61.0	49.33	5.76	5.37	10.7
Rock	AMP-DOM (A 1)	87.8	NA	NA	NA	NA
Rock	AMP-DOM (D 1.0)	22.1	NA	NA	NA	NA
Rock	HPI-A+N	303.3	38.9	4.25	1.41	32.2
Rock	HPI-B	47.5	30.46	5.66	12.59	2.8
Water	Colloids	28.9	25.53	5.07	2.72	11.0
Water	HPO-N	24.2	47.22	5.79	2.11	26.1
Water	HPO-B	21.9	50.66	6.02	2.08	28.4
Water	HPO-A	233.1	47.05	5.53	1.51	36.4
Water	AMP-DOM	184.1	41.52	5.44	3.27	14.8
Water	HPI-A+N	102.0	31.24	4.36	4.00	9.1
Water	HPI-B	67.7	16.2	1.82	0.86	22.0

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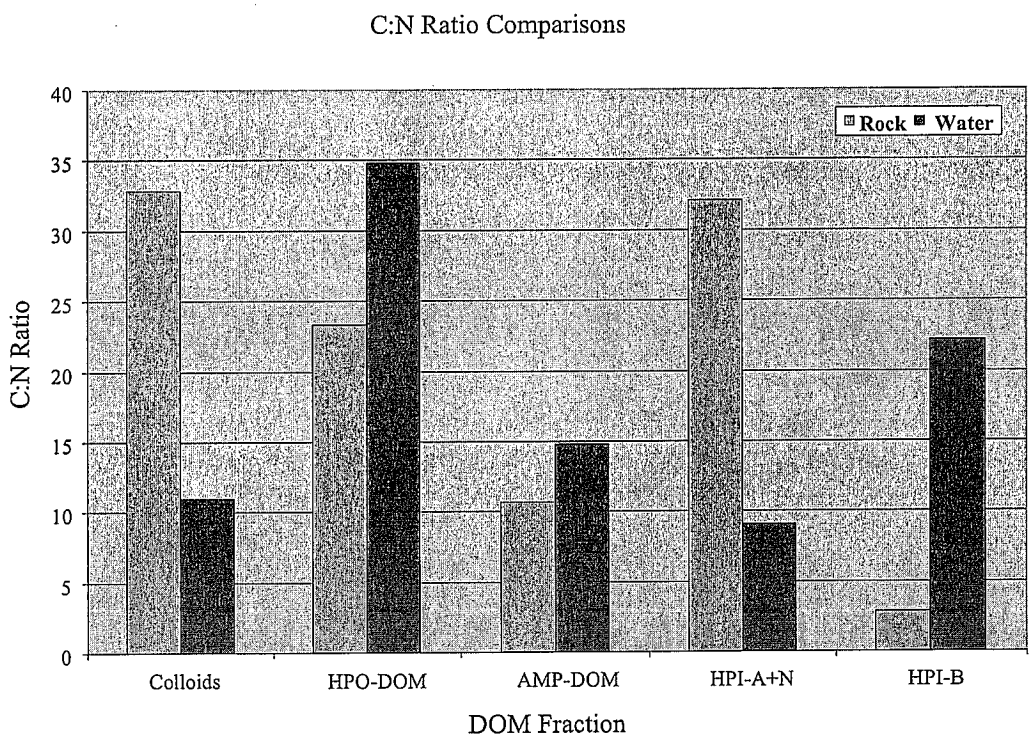

Figure 5 Bar diagrams showing atomic C:N ratios of Pyramid Lake carbonate and water samples. Fraction abbreviations are defined in Figures 2 and 3

3.3. Fourier-Transform-Infrared (FT-IR) Spectrometry

Fourier-transform-infrared spectra of colloid fractions from Pyramid Lake water and rock samples are presented in Figure 6. The colloid fraction from the water sample had a spectrum typical for N-acetyl amino sugars derived from degraded bacterial cell walls except that the carboxylic acid peak near 1720 cm^{-1} was diminished in intensity in comparison to other colloids found in fresh-water samples [2]. For the colloid spectrum isolated from the rock sample, the 1720 cm^{-1} carboxylic acid peak was enhanced in intensity, and the amide I and II peaks (1660 and 1550 cm^{-1}) and the N-acetyl methyl peak at 1380 cm^{-1} were diminished in intensity relative to the carbohydrate peak near 1050 cm^{-1}. These results indicate that acidic carbohydrates and basic amino sugar colloids are co-precipitated and/or incorporated during calcium carbonate formation in lake water. Neutral N-acetyl amino sugar colloids are not incorporated into the calcium carbonate precipitate and remain in solution in lake water.

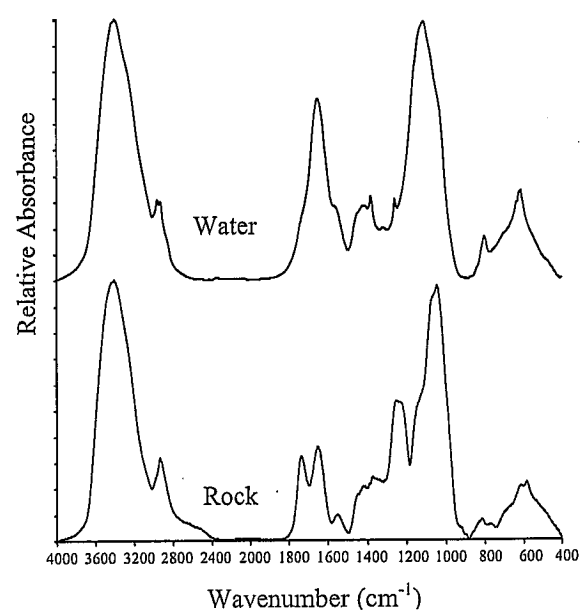

Figure 6 FT-IR spectra of colloid fractions from Pyramid Lake water and rock samples

The FT-IR spectra of the three hydrophobic DOM subfractions from the carbonate rock sample are presented in Figure 7. Protein peaks (amide I and II peaks without the 1380 cm^{-1} N-acetyl peak) are found in all three subfractions with the HPO-DOM fraction isolated at pH 4.9 being especially rich in protein constituents. The HPO-DOM fraction isolated at pH 1.0 is associated with a small amount of silica (peaks at 1050, 960, 800, and 460 cm^{-1}) that may be involved in the co-precipitation/incorporation process. The comparative C:N ratio data of Figure 5 discussed previously support co-precipitation of nitrogen-rich proteinaceous hydrophobic DOM in the carbonate rock.

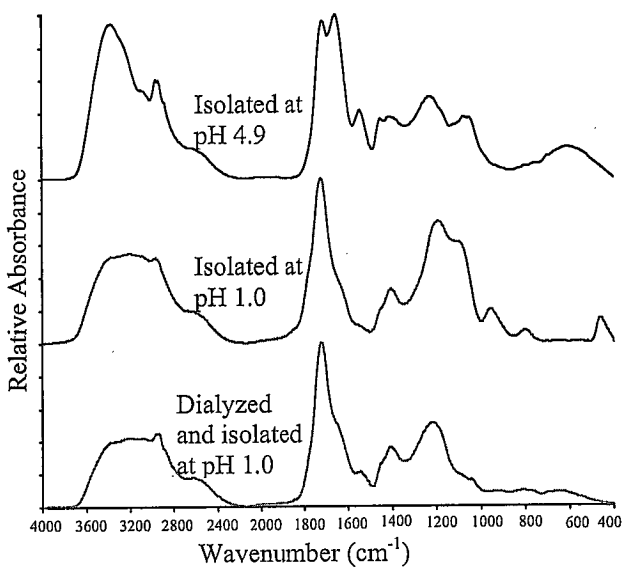

Figure 7 FT-IR spectra of HPO-DOM fractions from Pyramid Lake rock sample

Comparison of the FT-IR spectra of hydrophobic acid DOM fractions (Figure 8) and amphiphilic DOM fractions (Figure 9) indicates that protein components indicated by the amide II peak near 1540 cm^{-1} are found almost entirely in the carbonate rock samples. Trace amounts of protein were found in the FT-IR spectra (not shown) of the hydrophobic base and neutral fractions, but these fractions are minor constituents of hydrophobic DOM in the Pyramid Lake water sample (Figure 4). Lake water hydrophobic acid and amphiphilic DOM FT-IR spectra are characteristic [23] of aquatic fulvic acids derived from terpenoid source material [24]. Aquatic fulvic acids derived from tannin and lignins [25] have a broad peak centered at 1620 cm^{-1} caused by conjugated ketone and aromatic ring structures, and the absence of this peak in all of the DOM fractions of the water samples indicates terrestrial DOM sources of tannins and lignins are below the method detection limit in Pyramid Lake water. Therefore, the predominant DOM source must be autochthonous as independently estimated by Lebo et al [22].

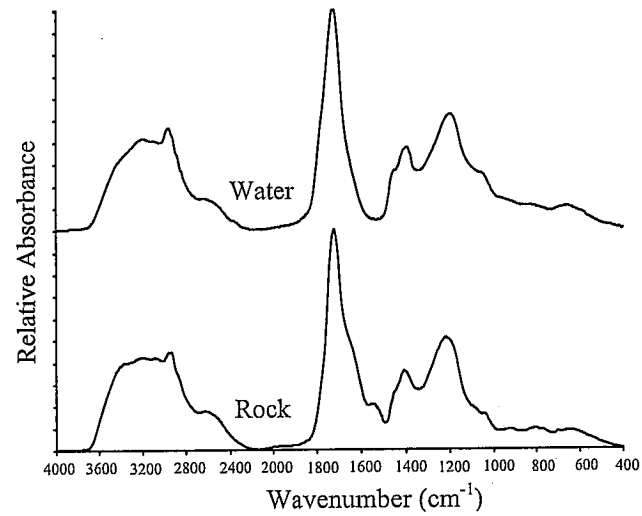

Figure 8 FT-IR spectra of hydrophobic acid (HPO-A) fraction from Pyramid Lake water and hydrophobic DOM fraction dialyzed at pH 1 (HPO-DOM D 1.0) from Pyramid Lake rock sample

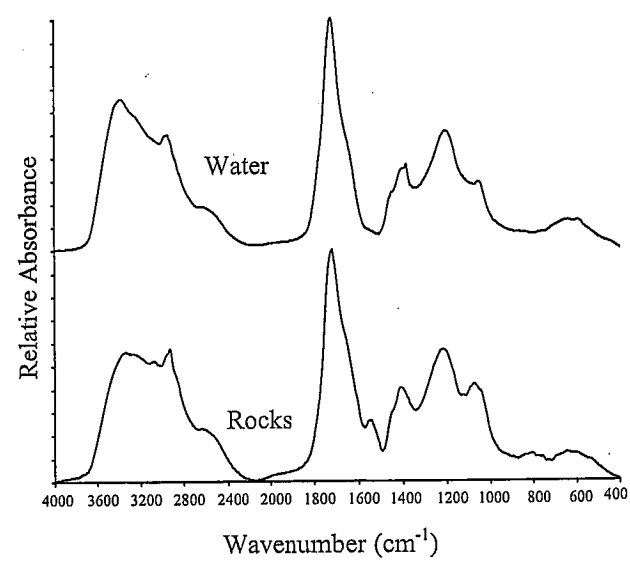

Figure 9 FT-IR spectra of amphiphilic DOM fraction (AMP-DOM) from Pyramid Lake water and amphiphilic DOM fraction adsorbed at pH 1 (AMP-DOM A 1.0) from Pyramid Lake rock sample

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Hydrophilic acid plus neutral fraction FT-IR spectra of both water and rock samples from Pyramid Lake (Figure 10) exhibit similar hydroxy carboxylic acid absorption peaks. However, subtle spectral differences between water and isolate fractionation samples, coupled with major differences in the C:N ratios of this fraction, indicate major compositional differences between the hydroxy acids in the carbonate rock and the lake water sample. The hydrophilic acid plus neutral fraction of the rock sample contains nitrogen-deficient hydroxyl-carboxylic acids (supported by the large C:N ratio of 32), but the hydrophilic acid plus neutral fraction of the water sample has a low C:N ratio of 9.1 and the FT-IR spectrum (Figure 10) does not have nitrogenous protein and amino sugar peaks. The presence of nitrogen in the water sample is indicated by the broad hydrogen-bonded nitrogen N-H stretch peak near 3100 cm^{-1} that is not as intense in the hydrophilic acid plus neutral fraction of the rock sample. Organic nitrogen in the hydrophilic acid plus neutral fraction in the water sample probably exist as lactams and cyclic imides that give carbonyl stretch peaks near 1700 cm^{-1} [17] superimposed on the carboxyl stretch peaks of carboxylic acids. Lactams and cyclic imides are derived from algae-porphyrin pigment (that is, pyrrole ring) oxidation and/or from degradation of nucleic acids.

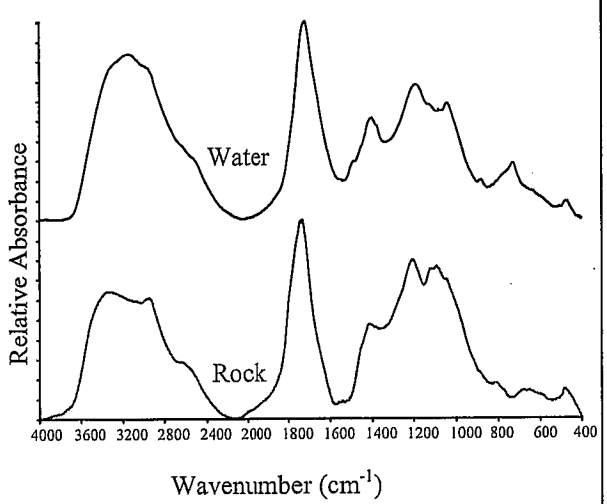

Figure 10 FT-IR spectra of hydrophilic acid plus neutral fractions (HPI-A+N) from Pyramid Lake water and rock samples

The hydrophilic base fraction FT-IR spectra of the water and rock samples (Figure 11) possibly indicate proteins and carbohydrates, although the relatively large C:N ratio of 22 in the water sample indicates a low percentage of proteins. The carbonate rock sample hydrophilic base spectrum is unusual in that while a large broad N-H stretch peak is seen near 3100 cm^{-1}, the carbonyl stretch peaks typical for amides are not seen at their usual position near 1660 and 1550 cm^{-1}. A small peak for carboxylic acids is seen at 1630 cm^{-1}, and small peaks near 1620 and 1500 cm^{-1} may be caused by C-N stretch of protonated amino groups [17]. The very low C:N ratio of 2.8 of the hydrophilic base fraction from the rock sample indicates the possibility of basic nucleic acids as precursors of this fraction.

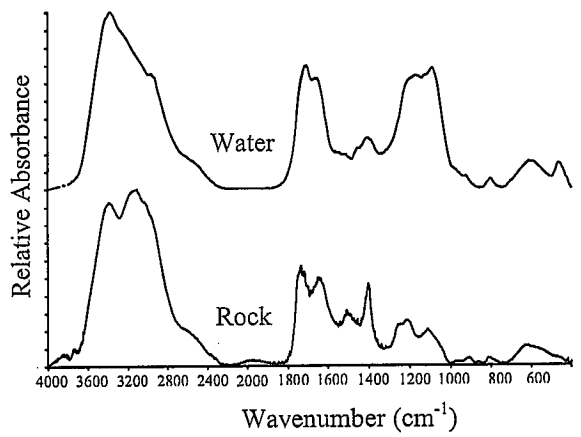

Figure 11 FT-IR spectra of hydrophilic base fractions (HPI-B) from Pyramid Lake water and rock samples

3.4. Comparison of DOM in Pyramid Lake and the Great Salt Lake

DOM composition in calcium carbonate formed in Pyramid Lake gives important insights into processes controlling carbon stabilization and reactivity during mineralization reactions in natural environments. The Great Salt Lake is comparable to Pyramid Lake in that DOM results from autothonous production and it is also a closed basin lake, but is different in that the salinity is much greater and it is density-stratified as a result of its high salinity. Carbonate precipitation does not occur in the lower-salinity surface waters, but many salts including carbonates precipitate in the high salinity waters below the halocline.

DOM was comprehensively isolated and fractionated from a surface-water sample from the Great Salt Lake collected in April 2002 [26]. The DOC of this sample was 40.5 mg/L and large fraction percentages of colloid (24 percent) and hydrophilic acids plus

neutrals (39 percent) were compared to the Pyramid Lake DOM fractionation of Figure 4.

Pyramid Lake water has smaller fraction percentages for the colloid (4.3 percent) and hydrophilic acid plus neutral (15.1 per cent) fractions. The colloid fraction in the Great Salt Lake was characterized as N-acetyl amino sugars typically found in fresh water samples [2], and the hydrophilic acid plus neutral fraction was characterized as N-acetyl hydroxyl acids resulting from degradation of the colloid fractions. The large colloid and hydrophilic acid percentages and their fraction compositions indicate that calcium carbonate precipitation does not act as a solubility and compositional control for DOM in this surface-water sample collected taken from the Great Salt Lake, whereas DOM composition in Pyramid Lake is consistent with calcium carbonate precipitation acting as a compositional and solubility control on DOM..

3.5. Isotopic Analyses

Carbon isotopic data for selected fractions isolated from the carbonate rock sample are presented in Table 3. The $\delta^{13}C$ value of the carbonate rock sample (-2.9 ‰) is near the $\delta^{13}C$ value of -3.1 ‰ reported for the Needles hot springs water [27] close to where the carbonate rock sample was collected. The $\delta^{13}C$ values for DOM fractions are heavier (lower negative values) than typically observed for terrestrial plant components [28]. Possible DOM component $\delta^{13}C$ values are: monocotyledon plants such as grasses = -12 to -19‰, dicotyledon plants = -23 to -32‰, and petroleum hydrocarbons = -28 to -32‰. Within the dicot plants, carbohydrates are isotopically heaviest ($\delta^{13}C$ = -17 to -19‰), followed by proteins ($\delta^{13}C$ = -18 to -22‰), lipids ($\delta^{13}C$ = -28 to -32‰), and lignins and tannins ($\delta^{13}C$ = -28 to -32). The heavier $\delta^{13}C$ values for the DOM fractions in the rock sample result from the heavy $\delta^{13}C$ carbonate carbon source for autochthonous DOM production in Pyramid Lake. The low $\delta^{13}C$ value for the colloid fraction indicates that this sample is dominated by carbohydrate components, which is consistent with the FT-IR spectrum of this colloid fraction.

The reported ^{14}C-ages do not likely report the actual ages during which DOM fractions were formed because of incorporation of autochthonous inorganic and organic carbon of old age. The ^{14}C-age of the carbonate rock is the oldest at 3886 years before present, and the ages of the co-precipitated DOM fractions vary from 3097 to 3480 years before present. It is possible that all DOM fractions were incorporated simultaneously during carbonate mineral formation in Pyramid Lake based upon the relatively small differences between DOM fractions for the ^{14}C-age data of Table 3.

Table 3 Carbon isotope data of co-precipitated DOM fractions

Sample	$\delta^{13}C$ (‰)	^{14}C age before present (years)
Calcium carbonate rock	-2.9	3886 ± 42
Colloids	-8.4	3148 ± 38
Hydrophobic DOM adsorbed at pH 1.0	-20.9	3441 ± 37
Hydrophobic DOM dialyzed at pH 1.0	-20.9	3097 ± 37
Amphiphilic DOM adsorbed at pH 4.9	-20.6	3480 ± 37

3.6. Mechanisms of DOM Co-precipitation and Organic Matter Incorporation during Calcium Carbonate Precipitation

The calcium ion forms intermediate to strong complexes with amino acids, hydroxy acids, and polycarboxylic acids [29]. Some calcium-organic anion ion pairs have low solubility (such as calcium oxalate and calcium tartrate) and readily form precipitates. Other complexing organic anions, such as citrate and ethylenediamine tetraacetic acid (EDTA) are very soluble and can dissolve calcium carbonate precipitates and inhibit precipitation. Relatively small changes in the structure of the organic ligand can determine whether a calcium complex is soluble or co-precipitates with calcium carbonate. For example, lime treatment of acidic wastes at the Stringfellow Superfund Site in southern Calfornia co-precipitated nitrilotriacetic acid (NTA), but did not remove EDTA from solution [30]. Other water chemistry factors such as ionic strength, ionic composition, and pH determine whether organic-ligands will co-precipitate with calcium carbonate.

Arp and co-workers [5] have stressed the critical importance of exocellular polymers on calcium carbonate precipitation in highly supersaturated natural environments. Calcium ion precipitates with complex organic anions (for example, pectate anion) that do not have a well-defined crystal structure.

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Often, these precipitates occur as a gel or colloidal material that may be incorporated in a more crystalline material [5, 31, 32]. Tufa formation may incorporate living and dead organic material into the carbonate rock matrix. A review [33] of tufa and travertine deposits of the world considered that both physico-chemical processes, such as co-precipitation and adsorption, as well as biological processes, often acting together give rise to calcium carbonate precipitation within tufa systems, such as the carbonate rocks formed in Pyramid Lake.

Interaction of organic ligands with a calcite surface is best quantified by DOM complexation reactions in solution, and on the calcite surface. Marinsky and co-workers [34, 35] have presented approaches for the description of metal ion binding to fulvic acid in aqueous solution. A calcite surface complexation reaction model was first proposed by van Cappellen et al. [36] and later modified by Pokrovsky and Schott [37]. Employing this model, Lin and Singer [38] identified a surface complex of $CaHPO_4$ as a calcite growth rate inhibiting species. Also, Lin et al. [39] employed surface complexation and a solution natural organic matter (NOM)-calcium speciation model to identify NOM-calcium surface complex species formed on the calcite surface. This surface complexation model described the solution-composition dependence of calcite growth rate inhibition in the presence of several fulvic acid samples. Unfortunately, binding-constant information for calcium ion solution and calcite-surface complexation is too limited to apply this approach to the Pyramid Lake carbonate sediments.

In the absence of a calcium-fulvic acid binding model for Pyramid Lake NOM, binding constant (log K = [ML/[M][L]) data for a number of alkaline earth cations and model organic ligands were taken from the literature [40] and are presented in Table 4. The magnitude of the binding constants is not large compared to trivalent aluminum and iron complexes known to act as solubility controls on DOM in water [41], but the calcium ion concentrations in Pyramid Lake are sufficiently large to form organic ligand complexes that co-precipitate with calcium carbonate during the whiting events. The magnitude of the binding constants for glycine and citric acid increase as the charge density on the unhydrated divalent cation increases, so there may be increasing inner-sphere complexation in going from barium to magnesium for the alkaline earth cations. For the amino acid series in Table 4, calcium ion binding constants increase as the ratio of carboxylic acid to amino-groups increase; therefore, protein in DOM that is rich in the acidic amino acids such as aspartic and glutamic acids are more likely to bind with calcium than proteins with neutral amino acids and DOM with neutral N-acetyl amino sugars.

Table 4 Log K =[ML/[M][L] Binding Constant Data [15], M = metal cation; L = ligand

M	Glycine	Citric Acid	Amino Acid	Log K_{Ca}
Mg^{2+}	2.22	3.25	Glycine	1.39
Ca^{2+}	1.39	3.18	Glutamic Acid	2.06
Sr^{2+}	0.91	2.81	Nitrilotriacetic Acid	6.39
Ba^{2+}	0.77	2.55	Ethylenediaminetetra acetic	10.61

The binding constant data for model compounds in Table 4 confirm the strong interaction of DOM with divalent ions. Nearly equivalent amounts of polycarboxylic proteinaceous acids (in the hydrophobic and amphiphilic DOM fractions) and hydroxy-acids (in the colloid and hydrophilic acid plus neutral fractions) are incorporated with calcium carbonate formed in Pyramid Lake (Figure 4). DOM functional group ligands whose structures do not bind with calcium, such as neutral N-acetyl amino sugars (colloid fraction in lake water), terpenoid fulvic acids (hydrophobic and amphiphilic DOM fractions in lake water), and possibly lactam and cyclic imides (in the hydrophilic acid fraction in lake water) are not incorportated in precipitated calcium carbonate (Figure 4).

4. CONCLUSIONS

The findings of this study suggest practical application of calcium carbonate precipitation to remove protein-rich DOM during drinking water treatment processes. This application will remove DOM precursors of drinking-water disinfection by-products. DOM fractions enriched in proteins are known to produce mutagenic haloacetonitrile disinfection by-products [42]. The common practice of lime softening of drinking water will likely remove substantial amounts of proteinaceous DOM precursors.

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