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Calcite crystal growth inhibition by humic substances with emphasis on hydrophobic acids from the Florida Everglades

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Abstract—The crystallization of calcium carbonate minerals plays an integral role in the water chemistry of terrestrial ecosystems. Humic substances, which are ubiquitous in natural waters, have been shown to reduce or inhibit calcite crystal growth in experiments. The purpose of this study is to quantify and understand the kinetic effects of hydrophobic organic acids isolated from the Florida Everglades and a fulvic acid from Lake Fryxell, Antarctica, on the crystal growth of calcite ($CaCO_3$). Highly reproducible calcite growth experiments were performed in a sealed reactor at constant pH, temperature, supersaturation ($\Omega = 4.5$), P_{CO_2} ($10^{-3.5}$atm), and ionic strength (0.1 M) with various concentrations of organic acids. Higher plant-derived aquatic hydrophobic acids from the Everglades were more effective growth inhibitors than microbially derived fulvic acid from Lake Fryxell. Organic acid aromaticity correlated strongly with growth inhibition. Molecular weight and heteroatom content correlated well with growth inhibition, whereas carboxyl content and aliphatic nature did not. *Copyright © 1999 Elsevier Science Ltd*

1. INTRODUCTION

Calcite and aragonite are ubiquitous solid phases in the environment. Fresh and ocean waters are commonly supersaturated with respect to these $CaCO_3$ phases with no observed mineral precipitation (Berner, 1975; Reynolds, 1978). Because inorganic carbonate precipitation is thermodynamically favored in oceans, rivers, lakes, and soils, natural kinetic inhibitors probably operate to inhibit crystallization and growth (Chave and Seuss, 1970; Suarez, 1983; Reynolds, 1978, Inskeep and Bloom, 1986a). It is known that naturally occurring dissolved organic matter (DOM) acts as a growth inhibitor to calcium carbonate crystal growth, but, because of the complexity and variability of chemistry, size, and structure of natural DOM, we still do not know which chemical and physical properties are most important.

Much effort has gone to understanding mechanisms and kinetics of calcite dissolution and growth in simple experimental systems (see reviews by Morse, 1983 and Shiraki and Brantley, 1995), and it is generally agreed that calcite grows by a surface reaction mechanism in stirred solutions (Gratz et al., 1993; Dove and Hochella, 1993). Calcium carbonate growth inhibition has been demonstrated in the presence of common inorganic ions, such as Mg^{2+} (Berner, 1975; Reddy, 1986), PO_4^{3-} (Reddy, 1977; Dove and Hochella, 1993), Fe^{3+} (Takasaki et al., 1994), and simple organic compounds of various sizes and composition (Kitano and Hood, 1965; Otsuki and Wetzel, 1973; Reynolds, 1978; Berner et al., 1978; Reddy, 1978). Inhibition of calcite growth is achieved by adsorption of these species to the calcite surface, which blocks crystal growth sites (Inskeep and Bloom, 1986b). Both inorganic and organic growth inhibitors are effective at relatively low concentrations (<5 mg/L dissolved organic matter).

Systematic studies with model compound inhibitors to determine the relationship between sorbate chemistry and inhibition effectiveness have yielded interesting results in $CaCO_3$ and other Ca salt precipitation reactions. Miyahara (1964) studied effects of various condensed phosphates on $CaCO_3$ crystallization rates. All compounds adsorbed strongly to the solid, but pyrophosphate and triphosphate were better inhibitors than orthophosphate and trimetaphosphate, illustrating the importance of sorbate length and stereochemistry in crystal growth inhibition. Kuntze (1966) studied the effects of a series of aliphatic polycarboxylic acids on the growth of calcium sulfate dihydrate and found that there must be at least two carboxyl groups present for a molecule to act as an inhibitor, and optimal inhibition is achieved when there is a 2 to 5 carbon distance between the carboxyls in the chain. The aragonite growth inhibition study by Berner et al. (1978) also concluded that the inhibiting mechanism must lie within the polymeric and stereochemical character of model compounds and natural organic acids, particularly in the presence of benzenecarboxyl groups. Amjad (1987) studied calcite growth inhibition in the presence of benzenepolycarboxylic acid and found that more carboxylated benzenes are better inhibitors than less carboxylated benzenes. A morphological study by Addadi and Weiner (1985) showed the selectivity of acidic protein sorption for certain crystal faces and further illustrated the subtleties required for sorbate–surface interactions.

Humic substances, including fulvic acid, humic acid, and humin, are a general class of biogenic, refractory dissolved organic matter that are ubiquitous in terrestrial and aquatic environments (Aiken et al. 1985). These natural organic materials have been shown to be effective crystal growth inhibitors (Reddy, 1978; Berner et al., 1979; Inskeep and Bloom, 1986b). The research that has led to the general recognition of humic substances as inhibitors of the precipitation and growth of sparingly soluble Ca salts often has focused on comparing inhibition effects of model compounds and one or two poorly characterized or uncharacterized humic substance samples

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Table 1. Inorganic compositions of solutions used in all experiments.

	Working solutions (M)			Titrant solutions (M)		
Reagent	A. calcium stock solution	B. carbonate stock solution	A + B reactor working solution	A. calcium titrant solution	B. carbonate titrant solution	A + B net composition of titrant added
$CaCl_2 \cdot 2H_2O$	0.004		0.002	0.053		0.026
$NaHCO_3$		0.004	0.002		0.004	0.002
Na_2CO_3					0.049	0.024
KNO_3		0.185	0.093	0.038	0.046	0.042

(Reddy, 1978; Berner et al., 1979; Grossl and Inskeep, 1992) or comparing the effects of a few unrelated and less well characterized soil humic extracts (Inskeep and Bloom, 1986b). The chemistry of aquatic humic substances differs significantly from humic materials isolated from soils. In addition, the chemistry of aquatic humic substances within a single water column or across ecosystem boundaries may be quite variable with regard to molecular weight, functional group content, and acidity. The role of DOM as an inhibitor of calcite growth in an aquatic environment was reported by Reynolds (1978), who attributed differences in the behavior of calcite in Lake Powell to differences in DOM composition. However, no detailed studies of the effect of well characterized aquatic organic acids on calcite precipitation have been reported.

It is the goal of this study to examine the effectiveness of well characterized DOM from the Florida Everglades and Lake Fryxell, Antarctica, in inhibiting calcite growth in well constrained experiments. Given these results, we can begin to ascribe calcite growth behavior to particular characteristics of the natural DOM and learn something about relative chemical reactivity of DOM of different origins in natural systems.

2. MATERIALS AND METHODS

2.1. Solutions

All solutions were prepared using doubly distilled water and American Chemical Society (chemical purity grade) [ACS] research grade chemical reagents, filtered through 0.1 μm Whatman cellulose nitrate filters (the use of trade names is for identification purposes only and does not constitute endorsement by the United States Geological Survey). Type A glassware was used throughout. In working and titrant solutions, it was critical that equal molar concentrations of calcium and carbonate were mixed. Because the Ca^{2+} salt ($CaCl_2 \cdot 2H_2O$) is hydrophilic, the calcium solutions were prepared first and analyzed for actual Ca^{2+} concentration by the ethylenediaminetetraacetic acid (EDTA)–calcein complexometric method of Schwartzenbach (1959). Base solutions then were prepared at concentrations equal to the corresponding calcium solutions. A summary of solution compositions used is given in Table 1. Experimental solutions were analyzed before and after crystal growth experiments for Ca^{2+} by complexometric titration. Alkalinity was determined before and after experiments using a Radiometer model ABU 93 automatic titrator. Solution speciation for inorganic experiments was calculated using the WATEQ4F code of Ball and Nordstrom (1991).

Stock solutions containing organic acid isolates (200 mg DOM/L) were prepared in solutions identical to base working solutions. DOM-containing solutions were stored in dark glass bottles that were previously baked for 3 h at 450°C. Prior to individual experiments, stock solutions were added to base working solutions to obtain the desired DOM concentrations and were filtered through 0.1-μm filters. The

solutions were used within 36 h. Experimental concentrations of DOM are reported in mg DOM/l; 1 mg DOM contains roughly 0.5 mg C.

2.2. Calcite Seed

Experiments were initiated by adding seed crystals to metastable, supersaturated solutions. The seed material used was Baker ACS reagent grade calcium carbonate (Lot 26832). Analysis by X-ray diffraction showed calcite as the only phase present. The specific surface area of the seed material was 0.256 ± 0.008 m²/g, as determined by a three-point nitrogen adsorption technique (Brunauer et al., 1938) on eight replicate samples. Morphologies of reacted and unreacted calcite crystals were examined using the JEOL scanning electron microscope (SEM) at the USGS Geologic Division in Lakewood, CO. Calcite seed crystal size (mean = 2.95 μm) was determined by measuring sizes of a population of grains using a petrographic microscope equipped with a filar micrometer.

2.3. Natural Organic Inhibitors

Two types of aquatic DOM isolates (fulvic acid and unfractionated hydrophobic acid) were used in the experiments. The fulvic acid isolated from a 5.5-m depth of Lake Fryxell (sample LF), Antarctica (Aiken et al. 1996) is the product of decomposed aquatic algae and bacteria. Hydrophobic organic acids obtained along a 40-mile transect in the Florida Everglades near West Palm Beach (samples F1, U3, and 2BS) were derived from decomposition of aquatic plants such as cattail and sawgrass (Aiken and Reddy, 1997). The Everglades hydrophobic DOM fraction (Aiken et al., 1992) is operationally defined as that which sorbs to Amberlite XAD-8 resin at pH 2 and can be eluted with 0.1 N NaOH. Everglades hydrophobic acid isolates are comprised of 96 to 97% fulvic acid with the balance being humic acid (Ravichandran, 1999). Preliminary constant composition calcite growth experiments indicate that fulvic and humic acid fractions of Everglades DOM have similar growth inhibiting properties to each other and to the unfractionated hydrophobic acid to which they were derived (unpublished data). Equivalent quantities of acid functional groups (Table 2) in hydrogen-saturated organic acids were determined by alkaline titration of samples (Ravichandran, 1999). Weight-average and number-average molecular weights (Table 2) were determined using high pressure size exclusion chromatography (HPSEC) (Chin et al., 1994). M_n, the number average molecular weight, reflects the total number of molecules present, regardless of size. M_w, the weight average molecular weight, is more dependent on particle mass and tends to favor higher molecular weights in polydisperse systems, resulting in higher molecular weight values for M_w than M_n (see Wershaw and Aiken, 1985 for a detailed discussion of the terms M_n and M_w). The relative standard deviations for the HPSEC measurements ranged from 10 to 12%. Quantitative data for organic acid functional groups (Table 3) were determined by liquid- and solid-state ^{13}C nuclear magnetic resonance (NMR) at the USGS in Arvada, CO. Elemental compositions (Table 4) of organic matter were determined at Huffman Laboratories (Golden, CO) by methods discussed in detail by Huffman and Stuber (1985).

Table 2. Acidity and molecular weight data for organic acids.

Sample/date collected	Description	Molecular weight (daltons)[a]			Acidity[b]			
		M_w	M_n	M_w/M_n	COOH (meq/g)	mol_C/mol_{COOH}	OH (meq/g)	mol_C/mol_{OH}
Everglades U3, 3/95	Hydrophobic fraction	1747	1180	1.48	5.99	7.61	0.735	62.02
Everglades F1, 4/96	Hydrophobic fraction	1907	1238	1.54	5.14	8.43	1.35	32.10
Everglades 2BS, 4/96	Hydrophobic fraction	1519	1080	1.41	4.74	9.19	1.03	42.31
Lake Fryxell − 5.5 m	Fulvic acid	1080	713	1.51	5.26	8.70	1.06	43.16

[a] Everglades high pressure size exclusion chromatography (HPSEC) molecular weight data from Aiken and Reddy (1997); Lake Fryxell HPSEC data is from Chin et al. (1994).
[b] All acidity data are from Ravichandran (1999).

2.4. Constant Composition Experiments

To study the inhibitory effects of added substances on calcite growth, we utilized the constant composition experimental apparatus of Tomson and Nancollas (1978). With this technique, solution pH and composition are kept constant during the course of the experiments to maintain a constant level of supersaturation with respect to a given solid phase.

Experiments were performed in a double-walled glass vessel with thermostated circulating water keeping experimental temperature constant at $25.0 \pm 0.2°C$. Constant chemical composition was maintained using a Brinkmann pH-stat titration system with a glass combination pH electrode. The electrode was calibrated before each experiment using premixed pH 7 and 10 buffers. Reactor solutions were stirred by a magnetic stir bar at a rate sufficient for good mixing (~400 rpm). Grinding from the stir bar should have been negligible during the relatively short experimental duration (Ogino et al., 1987); this was verified by SEM imaging.

Metastable working solutions were prepared by first adding 200 ml of bicarbonate stock solution to the reactor, then adding 200 ml of calcium stock solution dropwise to reduce the possibility of spontaneous nucleation. The pH was then adjusted to 8.50 using a few drops of 0.05 M KOH, and metastability was verified by observing constant pH for at least an hour prior to the experiments. One hundred milligrams of dry seed material was added to initiate experiments (for a 250 mg/L seed concentration). After addition of seed material, calcite grew according to the reaction $Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+$. The double-burette, pH stat system was adjusted so that titrants would be added when the pH dropped below 8.50 as a result of calcite growth. Thus, upon calcite growth the burette system responded by adding equal volumes of equimolar $CaCl_2$ and Na_2CO_3 titrant solutions (Table 1) to replace Ca^{2+} and CO_3^{2-} removed and maintain the pH. In addition, titrant solutions contained $CaCl_2$, $NaHCO_3$, and KNO_3 in the same concentration as the working solutions, to maintain ionic strength. The volumes of titrants added over time were recorded along with pH on a Brinkmann dual pen chart recorder; moles of titrant added were equal to moles of calcite precipitated. All experiments were run for 100 min. Longer reaction times yielded increasing apparent rates, due to increasing surface area (Kazmierczak, et al., 1982); rates were calculated using

the initial surface area value. In test experiments, titrant solutions were added dropwise to supersaturated working solutions to verify the absence of homogeneous nucleation. Thus, all crystal growth occurred on the seed crystal surfaces in the experiments. Actual volume of titrant added was checked against the amount recorded on the chart recorder between suites of experiments using 10-ml volumetric flasks and was found to be within 0.2% agreement.

After completion of an experiment, the solution and crystal mixtures were filtered through 0.1-μm filters. Solutions were collected for $[Ca^{2+}]$, alkalinity, and DOC analysis as described above. Rapidly, crystals were rinsed with distilled water, which then was decanted and the solids were dried at 60°C (for about 15 min) and stored in a dessicator for future SEM viewing.

Exchange of atmospheric CO_2 with reactor solutions was minimized by keeping the reactor lid closed and all ports sealed during the experiments. Seed addition and reactor sampling were performed as rapidly as possible. The effective isolation of the system was verified by the constancy of pH of supersaturated solutions for over 24 h in the absence of seed crystals. System pH response to opening the system was minimal and was very slow compared to the time it took to add seed or sample solutions.

2.5. Calculation of Crystal Growth Rates and Reduced Rates

Titrant addition as a function of time was recorded by a strip-chart recorder during experiments. Values for milliliters of titrant added in 10-min intervals was entered into a spreadsheet with 0.02-mL precision. The slope of the line in mL/min is a direct measure of calcite growth and is converted to crystal growth rates by the following equation:

$$\text{Rate (mol/m}^2\text{/min)} = \text{slope (L/min)} \cdot M_{titrant}\text{(mol/L)} / $$
$$(\text{mass}_{seed} \text{ (g)} \cdot SA_{seed}\text{(m}^2\text{/g))} \quad (1)$$

Most experiments yielded straight lines for titrant addition over time ($r^2 > 0.995$) with the exception of experiments with higher concentrations of DOC (Appendix). High DOC experiments started off at higher rates

Table 3. Functional character of organic acids as determined by ^{13}C NMR analysis.

Sample/date collected	Description	^{13}C NMR-percent C in functional groups (ppm chemical shift)					
		Aliphatic I (0–62)	Aliphatic II (62–90)	Acetal (90–110)	Aromatic (110–169)	Carboxyl (160–190)	Ketone (190–230)
Everglades U3, 3/95[a]	Hydrophobic fraction	42.3	15.0	7.4	19.0	14.4	4.1
Everglades F1, 4/96	Hydrophobic fraction	45.0	17.0	5.3	18.2	11.6	2.7
Everglades 2BS, 4/96	Hydrophobic fraction	48.5	14.9	4.1	15.4	15.4	1.8
Lake Fryxell − 5.5 m[b]	Fulvic acid	46.4	14.5	4.4	15.2	19.6	0.0

[a] Everglades NMR data from Aiken ad Reddy, 1997.
[b] Lake Fryxell NMR data from Aiken et al., 1996.

Table 4. Chemical composition of organic acids as weight percent constituent; standard deviations are given in parentheses.

Sample	C ($\pm$ 0.5)	H ($\pm$ 0.05)	O ($\pm$ 0.6)	N ($\pm$ 0.04)	S ($\pm$ 0.05)	Ash ($\pm$ 0.2)
F1	52.2	4.64	39.9	1.53	1.73	5.8
U3	54.7	4.79	37.5	1.88	1.15	4.6
2BS	52.3	4.79	40.2	1.58	1.23	7.3
LF$-$5.5 m	54.9	5.5	34.9	3.3	1.2	2.3

but quickly (<5 min) slowed; for these, rates were determined using the slope starting at 10 min and not forcing the line through the origin.

For the study of growth inhibition, we have calculated reduced rates R/R_o (Berner et al., 1978), which is the growth rate in the presence of an inhibitor (R) divided by the growth rate in the absence of an inhibitor (control rate, R_o). Thus, a smaller reduced rate indicates greater growth inhibition.

2.6. Complications Associated with Measuring Organic Carbon–Calcite Interactions

Water from an all-glass distillation system contained $\approx$0.2 mg C/L, which was the detection limit on the carbon analyzer. Reactor solutions made from distilled water and ACS grade dry chemicals consistently contained 0.9 $\pm$ 0.1 mg C/L. Furthermore, the calcite seed used in the experiments had organic carbon dispersed in the mineral (determined by dissolving the mineral and measuring the solution for organic carbon) and concentrated on the mineral surface (determined by soaking the mineral in distilled water and measuring the solution for organic carbon). Before and after dissolved organic carbon (DOC) concentrations were measured in calcite growth experiments using untreated calcite seed with no added organic acids; results ranged from a 130% increase in solution DOC to a 25% decrease in solution DOC, indicating that organic carbon is not uniformly dispersed on the calcite seed. To test whether residual organic carbon associated with the calcite seed affected calcite growth kinetics, we baked a portion of the seed material at 450°C for 3 h, successfully removing the surface organic carbon. The "baked seed" experiments yielded rates identical to those utilizing unbaked seed, both with and without added natural DOM.

3. RESULTS

3.1. Chemical Speciation, Ca–Organic Ligand Complexation, and Supersaturation States

Speciation calculations were performed for experiments with no added DOM. Complexes of calcium were insignificant compared to the Ca^{2+} ion and therefore did not compete for growth sites on the mineral surfaces. Complexes of K^+, Na^+, NO_3^-, and Cl^- with calcite lattice ions were not significant and did not affect calcite solubility by competing for ions in solution.

DOM might inhibit calcite growth by complexing Ca^{2+} in solution, thereby lowering the state of saturation. Phenolic and carboxylic acid functional groups are likely sites for Ca^{2+} complexation. At our pH of interest (8.5), carboxylic sites are mostly deprotonated, whereas phenolic sites should be mostly protonated and unavailable for Ca^{2+} complexation (Stumm and Morgan, 1996). If all carboxyl sites were saturated with calcium in monodentate Ca^+–OOC complexes (a maximum assumption) in experiments using the most acidic organic acid (F1) at the highest experimental DOM concentration (5 mg/L DOM), less than 2% of the free calcium would be bound to the DOM, an effect that should only lower $\Omega_{25°C}$ (ion activity product/equilibrium solubility) by about 0.08. This lowering of supersaturation would have an insignificant effect on reaction rate compared with that caused by the direct effect of dissolved

organic materials interacting with the mineral surfaces. These results are similar to those discussed by Amjad (1989) for calcium phosphate growth in the presence of acrylic acids and Reddy (1978) for calcite growth in the presence of phosphate anions.

3.2. Rate Reduction as a Function of DOM Concentration

For each DOM isolate studied, a series of experiments was performed at concentrations of 0.2 to 5.0 mg/L DOM. Examples of data for experiments using hydrophobic organic acids from site F1 are shown in Figure 1 (see Appendix for actual rates). Measurable rate reduction was observed at the lowest experimental DOM concentrations, and calcite growth was almost completely inhibited at DOM concentrations of 5.0 mg/L.

3.3. Rate Reduction as a Function of DOM Type

Different DOM isolates had different effects on calcite crystal growth rates. Rate results from all experiments are presented in Figure 2 and in the Appendix. Hydrophobic acid from the F1 site was the strongest inhibitor at all concentrations, followed by U3 hydrophobic acid and 2BS hydrophobic acid. Fulvic acid from Lake Fryxell was the weakest growth inhibitor of all compounds studied. The shape of the rate reduction vs. DOM concentration curves may be indicative of the mechanism of adsorption of organic material to the mineral surfaces, as will be discussed later in this report.

3.4. Growth Morphology of Crystals

Reacted and unreacted crystals were examined by SEM to see morphological changes that occurred during growth with and without added DOM (Fig. 3). Unreacted crystals (Fig. 3A) were well formed, sharp-edged rhombohedra. Crystals that grew for 100 min in the absence of organic inhibitors (Fig. 3B) increased their mass by about 25% and exhibited smooth planes of growth with step features on the face perimeters. Crystal morphology produced by growth in the presence of DOM is quite different than in the control experiments. For example, in the 0.5 mg/L F1 DOM experiments, the growth rate was reduced by 50% compared to control experiments; crystal mass increased by only 12%, and crystal surfaces did not exhibit smooth planes of growth but planes in which growth had been interrupted, resulting in a broken or discontinuous appearance (Fig. 3C). Crystals reacted at higher DOM concentrations showed even smaller "islands" of growth or no growth at all.
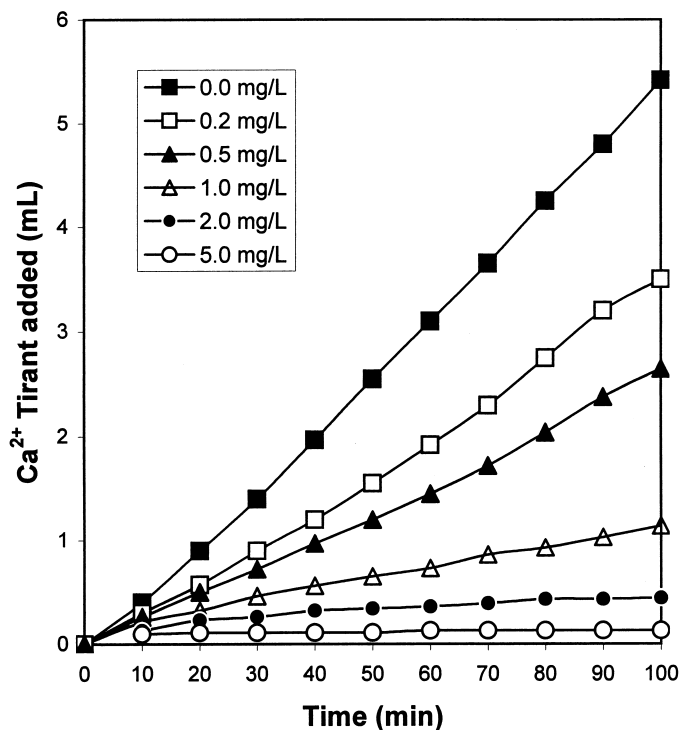
Fig. 1. Typical results for a suite of experiments performed by using various concentrations of the F1 hydrophobic organic acid. Titrants were added to experiments to replace Ca^{2+} and CO_3^{2-} removed by crystal growth. Rates were calculated from slopes that vary with DOM concentrations. Each line represents one crystal growth experiment in the presence of the organic inhibitor. The legend indicates concentration of organic acids in mg/L.

4. DISCUSSION

4.1. Molecular Weight of Humic Substances and Calcite Growth Inhibition

Weight average molecular weight (M_w) and number average molecular weight (M_n) for the organic acids vary by as much as 65% over the DOM samples in this study, so it is not surprising that the inhibitory effects also vary. Perez and Zidovec (1995) reported that calcium carbonate growth inhibition in the presence of low molecular weight polyacrylic acids (PAA) (2000–5000 daltons) is proportional to molecular weight. In this study, experiments using higher molecular weight DOM also produced greater rate reduction than the lighter organic acids at all DOM concentrations (Fig. 4). Our DOM is much more chemically complex than the PAAs of Perez and Zidovec, thus the mechanistic reasons for greater inhibition at greater DOM concentrations may not be the same. Chin et al. (1994) observed that there is a direct relationship between DOM molecular weight and degree of aromaticity in aquatic fulvic acids. The relation between aromaticity and rate reduction in calcite growth is addressed in the next section.

4.2. Functional Group Character of Humic Substances and Calcite Growth Inhibition

Because humic substances from different localities are variable in chemistry and structure (Aiken et al., 1985), there are a number of possible chemical reasons for one sample producing stronger growth inhibition effects than another. The ^{13}C NMR data we presented in Table 3 are interpretations of spectra that have been divided into chemical shift regions associated with various bonding environments of the ^{13}C isotope. The "percent functional group" notation used in Table 3 is actually the percentage of the total area of the spectrum that lies in a given signal region (interpreted to represent certain carbon-based chemical moieties) and is a relative number for a given sample (Thorn et al., 1989). The percentage functional groups (actually percentage carbon atoms in a given functional group) must be considered within the context of molecular weight. For example, the 2BS and LF DOM samples have the same percentage aromatic carbon but very different molecular weights and different absolute aromatic C content. To avoid confusion introduced by discussing relative (percentage) functional groups in a suite of organic acids of varying molecular weight, we used the ^{13}C NMR data to estimate absolute amounts of carbon atoms in given bonding environments per DOM molecule in our experiments. This was achieved as follows:

$$C_i \text{ (daltons)} = M.W.\text{(daltons)} \cdot f_c \cdot f_i \qquad (2)$$

where C_i is the average total mass of carbon in functional group i in a given DOM molecule, $M.W.$ is the weight-averaged or number-averaged molecular weight of a given DOM molecule, f_C is the fraction of carbon in a given DOM molecule (Table 4), and f_i is the fraction of carbon in a given DOM molecule that belongs to functional group i (Table 3).
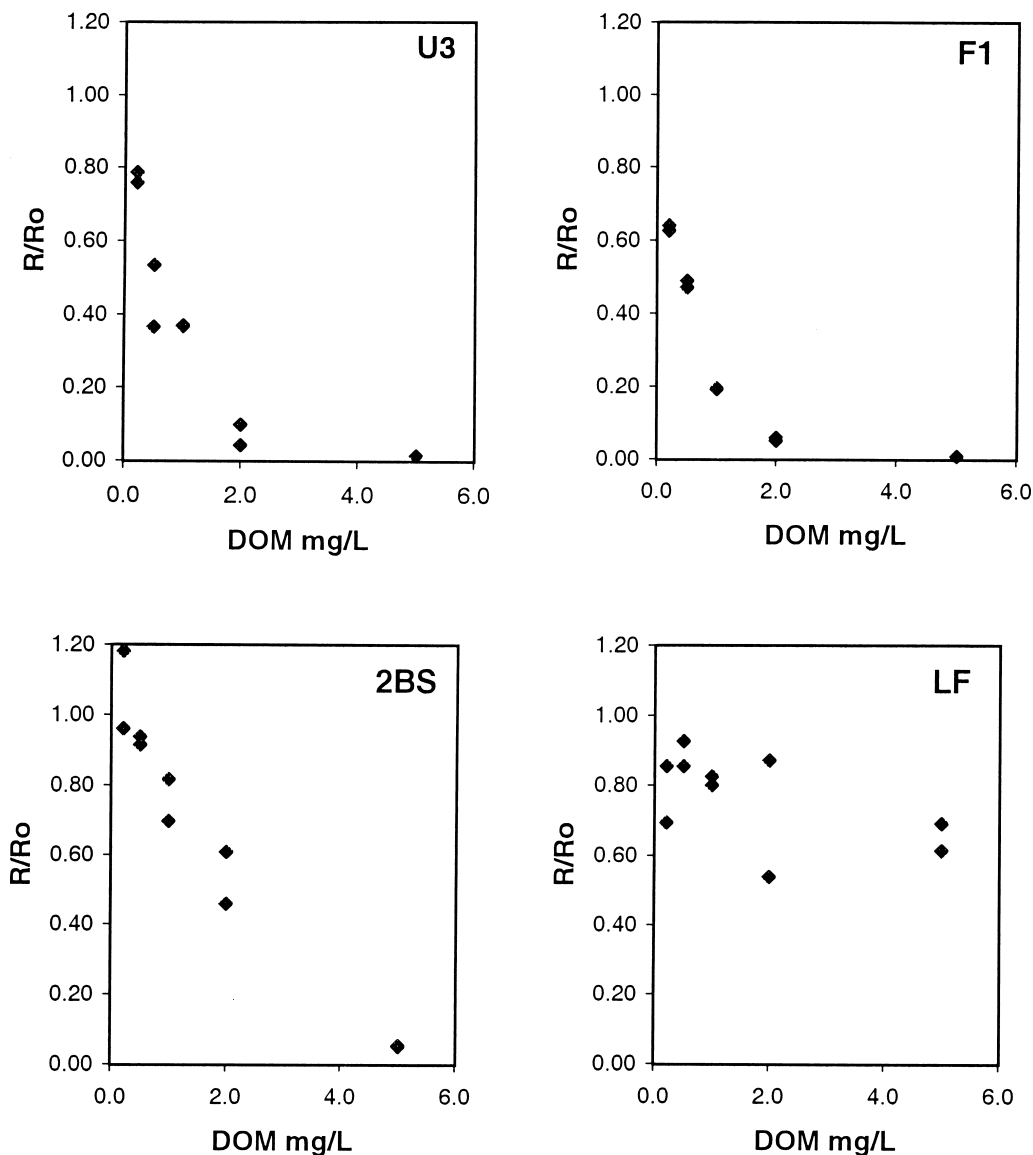
Fig. 2. Results expressed as reduced rates (R/R_o) obtained for all experiments at various concentrations of DOM. Each point represents one experiment. $R/R_o = 1$ indicates no crystal growth inhibition. F1 organic acids exhibited the strongest crystal growth inhibition at the lowest concentration, whereas LF organic acids were the least effective inhibitors.

Aliphatic I carbons (carbon–carbon single bond) are the most abundant functional groups in all organic substances studied in this report (Table 3). Figure 5A shows the relationship between rate reduction and absolute aliphatic I carbon content. Zullig and Morse (1988) found that fatty acids with longer carbon chains (which would be represented by aliphatic I carbon signal) adsorbed better to calcite surfaces, but their work is not analogous to this DOM study. Long-chain fatty acids are much more hydrophobic compared to the organic acids in our experiments and would tend to adsorb to calcite surfaces because of the "hydrophobic effect" (Stumm, 1992). Despite the notation "hydrophobic fraction" for the Everglades DOM, these substances are quite soluble in water at pH 8.5.

The aliphatic II region signal represents C–O, C–N, and C–S heteroatom single bonds. The aliphatic II content of the DOM

studied is predominantly from C–O bonds (Table 4). Aliphatic II moieties contribute to the hydrophilic nature of the DOM and are expected to have some chemical affinity to calcite surfaces. This affinity may play a role in DOM–calcite surface adsorption and crystal growth inhibition. Greater aliphatic II carbon content coincides with greater rate reduction (Fig. 5B).

The strongest growth inhibitors (FI and U3) contained more aromatic carbon/total carbon than the weaker inhibitors (Fig. 5C). This result is consistent with results of Reynolds (1978), who observed that calcite precipitation rated decreased with increasing polyphenol concentration in lake waters. Inskeep and Bloom (1986b) found the aromatic polymeric constituents from soil fulvic acid samples to be more effective inhibitors per mol C than nonpolymeric or less aromatic DOC in a soil–water extract. Berner et al. (1978) studied the effects of four sets of
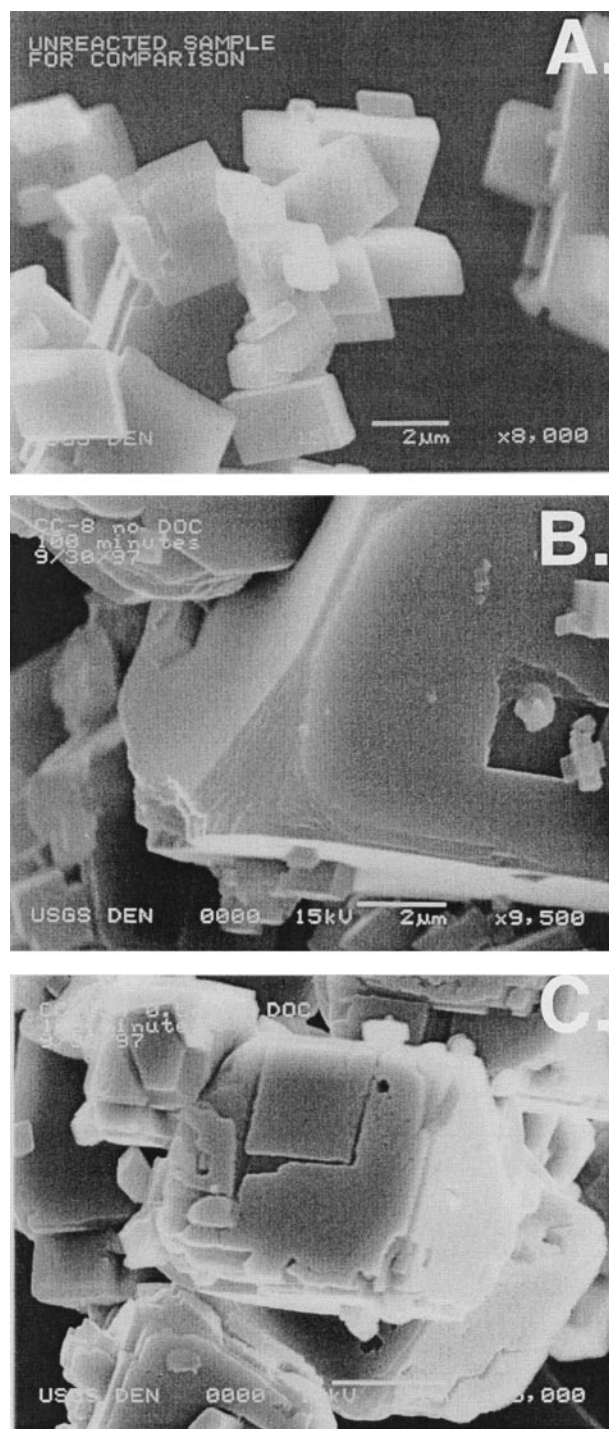
Fig. 3. SEM photomicrographs of calcite seed material before and after crystal growth experiments. Unreacted Baker calcite seed with straight edges and smooth surfaces (A). Calcite seed after 100 min growth in an experiment with no added organic materials (B). Note laterally continuous planes of growth. Calcite seed after growing for 100 min in a solution containing 0.5 mg/L F1 hydrophobic acid (C); growth rate and added mass were about 50% of that observed in control experiment (B). Note laterally discontinuous planes of growth in C.

model compounds (amino acids/proteins, fatty acids, aliphatic/polycarboxylic acids and aromatic acids) on aragonite (an

orthorhombic $CaCO_3$ polymorph) precipitation. Of these four classes of compounds, only the aromatic acids were found to be strong inhibitors (R/Ro $<$ 0.2) at 0.4 to 9.1 mg C/L. They invoked stereochemical reasons for the strong interaction of the more aromatic acids with the active growth sites on carbonate surfaces, noting that certain benzenepolycarboxylic acids were good inhibitors and some were not.

Carboxyl functional groups are among the most abundant and chemically reactive components in humic substances (Drever, 1997), and in contrast to phenolic acid groups are likely dissociated at pH 8.5 (Stumm and Morgan, 1996). Deprotonated carboxyl groups have a high affinity for aqueous Ca^{2+} (Martell and Smith, 1977) and should have an affinity for Ca on the calcite surface. However, the affinity for Ca alone does not make a functional group a strong sorbent on a carbonate mineral surface. For example, EDTA is a very strong complexing agent with aqueous Ca^{2+} but a poor inhibitor of aragonite growth (Berner, et al., 1978). We would not expect higher relative carboxyl content in humic substances to correlate strongly with rate reduction, based on these arguments. In fact, we observe that carboxyl content of the organic acids relates least systematically to rate reduction of all moieties examined in this study (Fig. 5D). We should point out, however, that the DOM sample with the highest carboxyl carbon mass/molecule (Lake Fryxell fulvic acid) also contains the lowest mass/molecule of all other functional groups discussed (Fig. 5). Thus, effects due to carboxyl groups may be masked by interactions between calcite and other more abundant functional groups in the suite of Everglades DOM.

4.3. Surface Adsorption of Humic Substances and Calcite Growth Inhibition

It has been shown by Scanning Force Microscopy imaging that calcite growth on seed crystals under ambient temperature and moderate supersaturation is initiated by multiple surface nucleation events, followed by spiral growth (Dove and Hochella, 1993). In inhibitor-free solutions, the smaller growth spirals coalesce and form smooth mineral surfaces that grow in relatively straight steps. In solutions containing solutes that sorb to mineral surfaces, the smooth steps are variably blocked, producing a jagged, discontinuous appearance as shown in Figure 3C.

We may semiquantitatively consider surface adsorption of DOM on calcite by comparing the total number of surface sites available on 100 mg of calcite seed and the amount of DOM in solution. If we assume 5 sites/nm^2 on the calcite surface (Davis and Kent, 1990), the total sites would be about 1.3×10^{17}, and the number of molecules of organic acid in the lowest concentration F1 experiments, 0.2 mg/L DOM, to be about 0.38×10^{17} (assuming molecular weight of 1200), we see that the F1 hydrophobic acid is quite an effective growth inhibitor. This is probably due to the fact that the relatively large DOM molecules are capable of adsorbing to more than one growth site on a mineral surface (Ochs et al., 1994). Accordingly, F1 DOM at 5 mg/L should almost entirely inhibit calcite growth, as we observed.
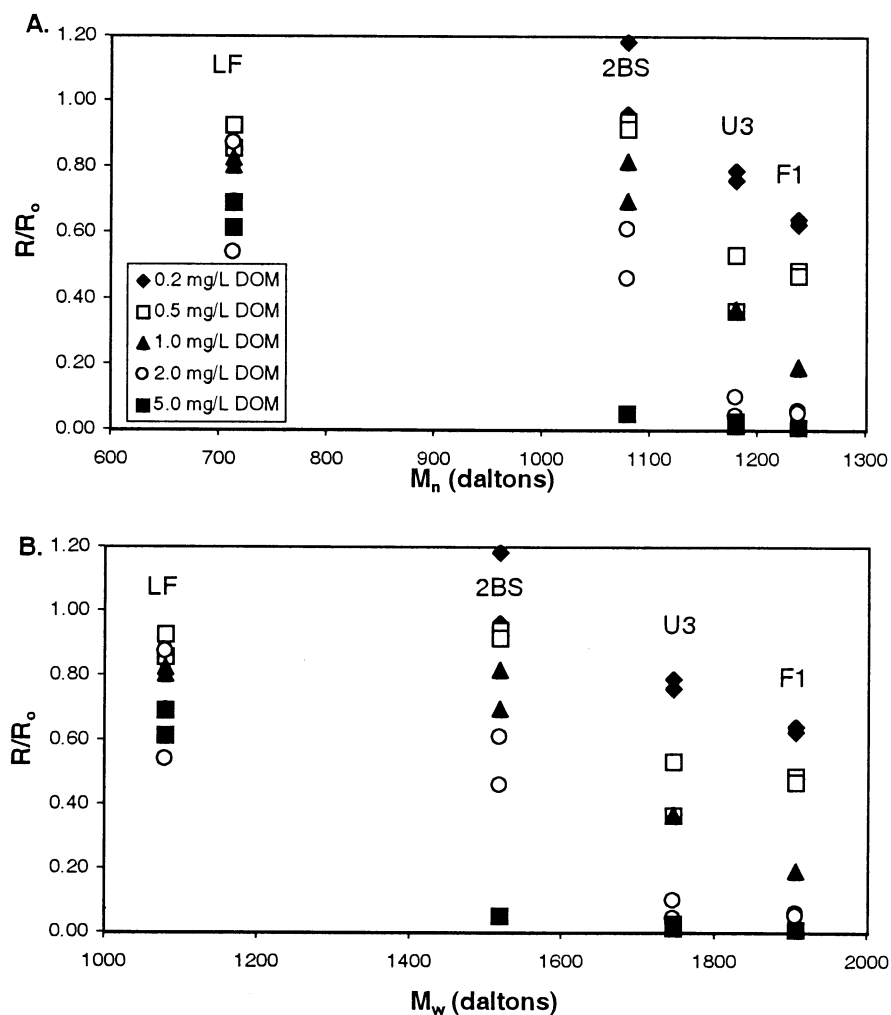
Fig. 4. Plots of all experimentally determined reduced rates R/R$_o$ vs. number-average molecular weights for DOM used in experiments (A) and weight-average molecular weight for DOM used in experiments (B). Each symbol type represents a given concentration of organic acid. Sets of symbols at given molecular weights represent suite of experiments run in the presence of a given organic acid, noted on the figure. Higher molecular weight organic acids are generally better growth inhibitors for a given DOM concentration.

5. CONCLUSIONS

Aquatic hydrophobic acids derived from higher plants in the Florida Everglades were more effective growth inhibitors than microbially derived aquatic fulvic acid from Lake Fryxell, Antarctica. The stronger growth inhibitors studied reduced calcite growth rates by 50% at concentrations as low as 0.5 mg/L DOM. DOM molecular weight and aromaticity, in particular, correlated well with growth inhibition, probably due to enhanced stereochemical effects on blocking active crystal growth sites. Aliphatic C–C chain content and carboxyl content appeared to be less important in whether an organic acid was an effective inhibitor. The morphology of crystals grown in the presence of organic acids suggests that surface growth sites are blocked by adsorbing ligands, as demonstrated in other studies using force microscopy. Our results quantitatively show the significance and variability of the inhibitory effects of natural DOM when considering kinetics of calcite growth and its

control on natural water chemistry. In natural systems, DOM concentrations may be an order of magnitude greater than those examined in this study, indicating the importance of time and/or localized areas of increased pH (such as in algal mats) for achieving significant inorganic calcite mineralization.

In a general geochemical sense, we conclude that different aquatic DOM has different effects on calcite growth. Thus, simple modeling of mineral saturation states and chemical reactions in natural waters without considering the quantity and quality of DOM may produce inconsistent or inaccurate results.
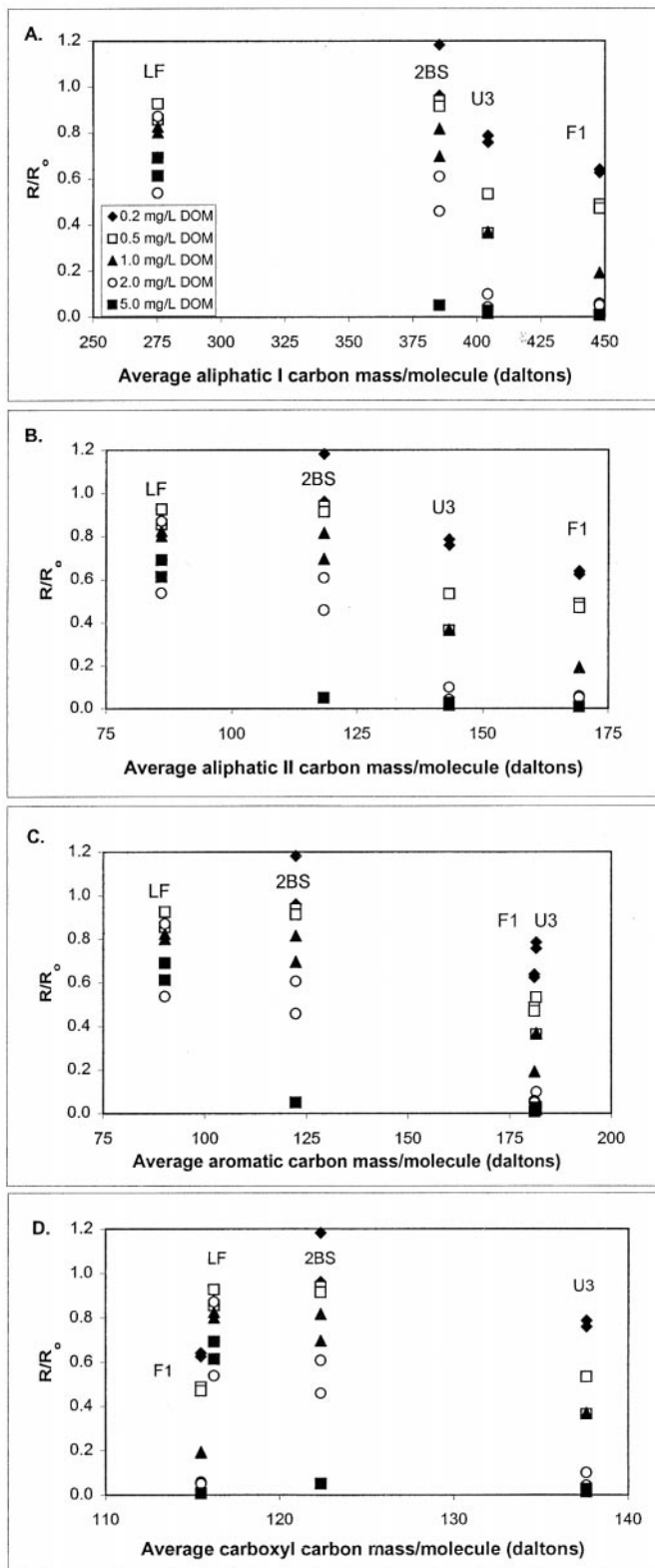
Fig. 5. Plots of all experimentally determined reduced rates R/R$_o$ vs. functional group: DOM aliphatic I carbon (C–C single bond; A); DOM aliphatic II (primarily C–O single bond; B); DOM aromatic carbon (C); and DOM carboxyl carbon (D).

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APPENDIX

Appendix A. Summary of control experiments, inhibitor experiments and reduced rates (R/R_o). Reduced rates are calculated by dividing the rate of an individual experiment by the rate of the average control experiment.

EXP ID	date	C_{DOM} (mg/L)	slope (mL/min)	error (mL/min)	error (%)	R^2	rate (mol/m^2/min)	error (mol/m^2/min)	R/R_o
Control experiments for F1 and U3 experiments									
CC-8	7/22/97	0.0	0.055	0.00072	1.31%	0.999	1.01E-04	1.32E-06	
CC-9	7/24/97	0.0	0.052	0.00112	2.16%	0.996	9.57E-05	2.07E-06	
CC-11	9/9/97	0.0	0.064	0.00112	1.76%	0.997	1.21E-04	2.13E-06	
						Ave. (R_o)	1.06E-04		
CCU3-1	7/31/97	0.2	0.044	0.00121	2.76%	0.993	8.33E-05	2.30E-06	0.79
CCU3-2	8/1/97	0.2	0.042	0.00160	3.79%	0.987	8.03E-05	3.04E-06	0.76
CCU3-3	8/5/97	0.5	0.020	0.00028	1.40%	0.998	3.86E-05	5.40E-07	0.36
CCU3-4	8/6/97	0.5	0.030	0.00050	1.67%	0.998	5.65E-05	9.44E-07	0.53
CCU3-5	8/8/97	1.0	0.019	0.00042	2.26%	0.995	3.91E-05	8.84E-07	0.37
CCU3-6	8/12/97	1.0	0.020	0.00034	1.67%	0.998	3.88E-05	6.49E-07	0.37
CCU3-7	8/26/97	2.0	0.002	0.00008	3.54%	0.990	4.38E-06	1.55E-07	0.04
CCU3-8	8/27/97	2.0	0.006	0.00028	5.05%	0.980	1.05E-05	5.31E-07	0.10
CCU3-9	9/2/97	5.0	0.001	0.00019	24.81%	0.670	1.52E-06	3.77E-07	0.01
CCU3-11	9/5/97	5.0	0.002	0.00013	8.60%	0.995	2.85E-06	2.45E-07	0.03
CCF1-1	9/10/97	0.2	0.036	0.00087	2.46%	0.995	6.77E-05	1.66E-06	0.64
CCF1-2	9/11/97	0.2	0.035	0.00082	2.35%	0.995	6.62E-05	1.56E-06	0.63
CCF1-3	9/15/97	0.5	0.028	0.00063	2.23%	0.995	5.16E-05	1.15E-06	0.49
CCF1-4	9/16/97	0.5	0.026	0.00056	2.13%	0.996	4.98E-05	1.06E-06	0.47
CCF1-5	9/22/97	1.0	0.011	0.00043	4.01%	0.986	2.02E-05	8.10E-07	0.19
CCF1-6	9/24/97	1.0	0.011	0.00039	3.63%	0.988	2.04E-05	7.40E-07	0.19
CCF1-7	9/25/97	2.0	0.003	0.00034	10.50%	0.919	6.09E-06	6.39E-07	0.06
CCF1-8	9/30/97	2.0	0.003	0.00046	16.43%	0.880	5.33E-06	8.76E-07	0.05
CCF1-9	10/7/97	5.0	0.000	0.00009	19.01%	0.776	9.51E-07	1.81E-07	0.01
CCF1-10	10/7/97	5.0	0.000	0.00007	16.94%	0.813	7.61E-07	1.29E-07	0.01

Control for 2BS experiments, using other experimental apparatus								
CC-16	1/7/98	0.0	0.044	0.00066	1.50%	0.998	8.41E-05	1.26E-06
CC-17	1/13/98	0.0	0.047	0.00069	1.48%	0.998	8.85E-05	1.31E-06
						Ave. (R$_o$)	**8.63E-05**	

CC2BS-4	1/15/98	1.0	0.037	0.00083	2.25%	0.996	7.04E-05	1.58E-06	0.82
CC2BS-5	1/16/98	1.0	0.032	0.00049	1.54%	0.998	6.01E-05	9.28E-07	0.70
CC2BS-6	1/21/98	2.0	0.028	0.00047	1.70%	0.997	5.25E-05	8.90E-07	0.61
CC2BS-7	1/21/98	2.0	0.021	0.00063	3.00%	0.991	3.96E-05	1.19E-06	0.46
CC2BS-8	1/24/98	5.0	0.002	0.00053	23.13%	0.702	4.38E-06	1.01E-06	0.05
CC2BS-9	1/28/98	5.0	0.002	0.00043	17.83%	0.795	4.57E-06	8.15E-07	0.05
CC2BS-10	1/29/98	0.5	0.043	0.00149	3.50%	0.989	8.10E-05	2.83E-06	0.94
CC2BS-11	1/30/98	0.5	0.042	0.00088	2.11%	0.996	7.89E-05	1.66E-06	0.91
CC2BS-12	1/31/98	0.2	0.044	0.00118	2.69%	0.994	8.29E-05	2.23E-06	0.96
CC2BS-13	1/31/98	0.2	0.054	0.00129	2.42%	0.995	1.02E-04	2.47E-06	1.18

Control for LF 5.5m experiments, using newest experimental apparatus								
CC-18	2/9/98	0.0	0.073	0.00089	1.23%	0.999	1.38E-04	1.69E-06
CC-19	2/20/98	0.0	0.063	0.00075	1.20%	0.999	1.19E-04	1.43E-06
						Ave. (R$_o$)	**1.29E-04**	

CCLF-1	2/10/98	0.2	0.058	0.00080	1.38%	0.998	1.10E-04	1.51E-06	0.86
CCLF-2	2/10/98	0.2	0.047	0.00065	1.38%	0.998	8.90E-05	1.23E-06	0.69
CCLF-3	2/11/98	0.5	0.058	0.00121	2.10%	0.996	1.10E-04	2.31E-06	0.86
CCLF-4	2/11/98	0.5	0.063	0.00109	1.74%	0.997	1.19E-04	2.07E-06	0.93
CCLF-5	2/12/98	1.0	0.056	0.00085	1.54%	0.998	1.06E-04	1.63E-06	0.82
CCLF-6	2/12/98	1.0	0.054	0.00095	1.75%	0.997	1.03E-04	1.80E-06	0.80
CCLF-7	2/17/98	2.0	0.036	0.00025	0.68%	1.000	6.92E-05	4.71E-07	0.54
CCLF-8	2/18/98	2.0	0.059	0.00135	2.30%	0.995	1.12E-04	2.57E-06	0.87
CCLF-9	2/19/98	5.0	0.041	0.00080	1.94%	0.997	7.88E-05	1.53E-06	0.61
CCLF-10	2/19/98	5.0	0.047	0.00097	2.07%	0.996	8.88E-05	1.84E-06	0.69