

# Kinetics of the Demineralization Reaction of Deproteinized Lobster Shells Using CO<sub>2</sub>

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Received June 17, 2014; Accepted August 4, 2014

**ABSTRACT:** The demineralization kinetics of deproteinized lobster shells using CO<sub>2</sub> were studied. Demineralization reaction proceeds until the concentration of Ca<sup>2+</sup> in solution reaches an equilibrium value. The introduction of a cation exchange resin (cationite), in an open system for CO<sub>2</sub>, allows replacement of the solution Ca<sup>2+</sup> ions by Na<sup>+</sup> ions, whereby the equilibrium shifts and an effective dissolution of the exoskeletons' calcite is achieved. The mathematical relationships between the conductivity of the solution and the concentrations of major ions, the rate constants and kinetic parameters of the reaction in the absence and presence of the resin were obtained. It was found that the reaction follows pseudo-first-order kinetics, and the experimental results were in good agreement with the proposed mathematical model.

**KEYWORDS:** Carbon dioxide, chitin, demineralization, lobster shells

## 1 INTRODUCTION

Chitin is a natural polysaccharide that has many applications in agriculture, medicine and industry [1–3]. Chitin is widely distributed in nature, both in the animal kingdom and in plants. In fact it is recognized as the second most abundant natural polysaccharide, only behind cellulose, constituting an important renewable resource. Chitin is a linear polymer composed of *N*-acetyl-2-amino-2-deoxy-D-glucose units linked together by β(1→4) glycosidic links. In native chitin some of these units are deacetylated (Figure 1).

The main sources of current commercial chitin are the shells of crabs, shrimps, prawns and lobsters discarded at seafood processing plants. The reported extraction techniques are diverse as they are largely dependent on the composition of the source, which varies greatly from one species to another [4]. These methods generally use large quantities of water and energy and often lead to corrosive waste. Currently enzymatic treatments are being investigated as a

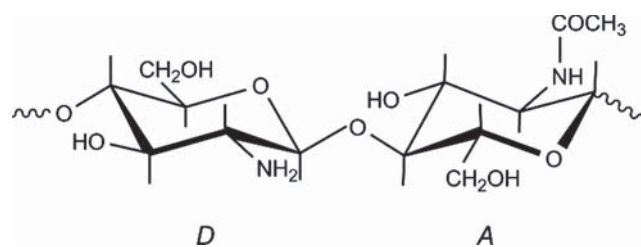
promising alternative. For this purpose, processes have been reported using enzyme extracts or isolated enzymes and microbial fermentation, but still without the efficiency of chemical methods, in particular as regards the removal of the inorganic material [5].

The Cuban fishing industry generates large amounts of waste at its spiny lobster (*Panulirus argus*) processing plants. This waste is a protein- and chitin-rich pollutant that can be valued if a method of extracting these shell components is designed and implemented for later use in various fields of activity.

Spiny lobster exoskeleton has a composition on a wet basis of 14% protein, 16% chitin and 50% minerals, mainly calcium carbonate (calcite) and magnesium, and in a much smaller proportion phosphates and metal ions. The calcium carbonate is primarily in the form of calcite, although it has been suggested that part of it is amorphous [6-7]. The exoskeleton of the spiny lobster has a complex three-dimensional structure, in which chitin chains are conveniently protected from chemical attack and physical agents, ensuring their biological function. This differentiates it from other carbonate materials, especially the inorganic ones [8].

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DOI: 10.7569/JRM.2014.634116



**Figure 1** Repeating units of  $\beta(1 \rightarrow 4)$  linked monosaccharides: (A) N-acetyl-2-amino-2-deoxy-D-glucose in the fully acetylated chitin and (D) 2-amino-2-deoxy-D-glucose in totally N-deacetylated chitin.

The isolation of chitin from shellfish waste involves separating the associated proteins (deproteinization step), the separation of minerals (demineralization step), and optional removal of pigments and lipids. This sequence can be inverted depending on the chemical composition of the starting raw material and the interest in recovering some products as collateral [9]. The importance of the particular process used is that it can bring about substantial differences in the chitin obtained from the same species [10].

In the demineralization step, crustacean exoskeletons have been mainly treated with inorganic or organic acids. Treatment with dilute solutions of hydrochloric acid is most commonly used, although it can cause modifications in the chitin chains. Therefore less aggressive methods such as smooth decalcification with EDTA [10] or ionic liquids [11] have also been employed.

In recent years there has been a considerable increase in the use of carbon dioxide in chemical reactions due to its properties as an antisolvent, its low cost and low toxicity [2,12]. Currently studies are being carried out that show the feasibility of using CO<sub>2</sub> in combination with a cation exchange resin (cationite) for the demineralization of crustacean exoskeletons for chitin obtained using mild reaction conditions. However, until now the kinetics of this process have not been studied.

CO<sub>2</sub> has been used for the dissolution of calcite present in rock minerals and the studies developed have allowed predicting the dissolution mechanism of calcite in them [13]. In these studies it has been demonstrated that the time evolution of the concentration of major ions in solution can be described according to the exponential equation

$$C = C_{\text{eq}}(1 - e^{-kt^n}) \quad (1)$$

where  $C$  is the concentration of Ca<sup>2+</sup>, Mg<sup>2+</sup> or HCO<sub>3</sub><sup>-</sup> ions at time  $t$ ;  $k$  is the rate constant;  $C_{\text{eq}}$  is the concentration of each of these ions at equilibrium; and  $n$  is an experimental coefficient which generally takes values

between 0 and 1 [14]. As shown in Equation 1, in the dissolution of calcite, with increasing concentrations of the various ionic species in solution a chemical equilibrium is eventually reached that prevents the dissolution of more calcite. The equilibrium is attained when the solution becomes saturated in Ca<sup>2+</sup> ions because of the low solubility of Ca(OH)<sub>2</sub>. One approach to avoid reaching equilibrium is to use a cation exchange resin to remove Ca<sup>2+</sup> ions from the solution. This way a more complete demineralization of the material can be achieved.

The aim of this work is to study the kinetics of spiny lobster exoskeleton demineralization using CO<sub>2</sub> in the absence and presence of a cation exchange resin, on the assumption that the solution behavior of the ions resulting from the treatment of the organic matrix should be similar to that reported for inorganic rocks.

## 2 EXPERIMENTAL

### 2.1 Materials

Spiny lobster (*Panulirus argus*) exoskeletons were obtained from the seafood processing plant at La Coloma (Pinar del Rio, Cuba). The cation exchange resin (hereinafter also called cationite) used was DOWEX 50X8, Na<sup>+</sup>-form (BDH, England). CO<sub>2</sub> gas cylinder with 99% purity and 10 MPa was supplied by the CO<sub>2</sub> Plant (Guanabacoa, Cuba). All other reagents used were analytical grade.

### 2.2 Lobster Shell Processing before Demineralization

Lobster shells were washed thoroughly with abundant water to remove adherent proteins, soluble organics and other impurities, and dried in air for 24 hours. The dry shells were ground to particle sizes between 200 and 400  $\mu\text{m}$ . Afterwards, they were deproteinized by three successive 1 hour treatments with 0.5 N NaOH at 80°C. Next they were washed with tap water and distilled water until neutral, dried in air and stored at 4°C until use.

### 2.3 Reaction Conditions and Kinetics Data Processing

Experiments were conducted in a 3½ L laboratory Teflon type double-jacketed batch reactor with agitator, suitably equipped with pH and temperature measuring facilities, a system for liquid sampling and a gas input/output system. CO<sub>2</sub> at a flow rate of 5 L·min<sup>-1</sup>

was continuously supplied (open system condition relative to CO<sub>2</sub>) and mechanical stirring was set at 100 rpm. Distilled water (2 L) with a specific conductivity of 10 μS·cm<sup>-1</sup> was added to the reactor, and was saturated by bubbling CO<sub>2</sub> for 10 minutes before adding the lobster shells. In all experiments 20g of deproteinized exoskeleton were used. In some experiments the demineralization process was followed in the presence of the cation exchange resin Dowex 50X8 (40g). Temperature was maintained constant at 25 ± 0.1°C. At the beginning of each experiment the pH, temperature and electrical conductivity of the solution were measured.

The reaction kinetics were monitored according to the method previously established for the dissolution of rock minerals using CO<sub>2</sub> [13], which essentially consists of executing two types of experiments: the first one, called REL, in which the reaction was sampled at different times, for determining the concentrations of major ions (Ca<sup>2+</sup>, Mg<sup>2+</sup>, HCO<sub>3</sub><sup>-</sup>, Na<sup>+</sup>) and the electric conductivity of the solution; and the second experiment, performed under similar conditions, called CINET, where the reaction was followed by measuring only the variation in time of the electrical conductivity and pH.

From the REL experiment, the correlations of the various concentrations of ions with the electrical conductivity of the solution were obtained. These correlations were used in the CINET experiment to calculate the values of the concentrations of these ions at different times from the measured solution conductivities. Then, considering that Equation 1 holds for this reaction, the rate constant *k* was determined. It was first assumed that *n*=1, and on this basis the rate constants *k* for different times were evaluated and the average *k* obtained. The average value of *k* and the condition *n* = 1 were then used as initial values for the nonlinear least squares fit of the kinetic data to Equation 1 for obtaining the adjusted values of *k* and *n*.

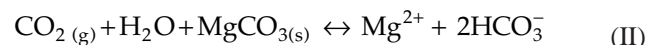
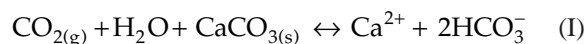
## 2.4 Measurement of Physical-Chemical Parameters

Measurements of different physical-chemical parameters were performed with a digital inoLab<sup>®</sup> pH 7110 pHmeter (WTW, Germany) and a 524-Crison conductivity meter (Barcelona, Spain) calibrated with certified conductivity standards. The HCO<sub>3</sub><sup>-</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> ion concentrations were estimated following recommended standard volumetric analytical methods established for their determination in water [15]. The concentration of Na<sup>+</sup> ions was determined by flame photometry on a Jenway flame

photometer (Bibby Scientific Ltd., Staffordshire, UK) with a sensitivity of 0.2 ppm. The Scientific Graphing and Analysis Software Origin 7.0 was used for data processing.

## 3 RESULTS AND DISCUSSION

The dissolution of calcite and magnesium carbonate of lobster shells using CO<sub>2</sub> can be represented by the following equations:



Under the conditions employed in this study the CO<sub>2</sub> concentration remains constant. Therefore, it is expected that the dissolution kinetics of the exoskeleton carbonates behave as a heterogeneous pseudo-first-order reaction, in which the electrical conductivity resultant of the contribution of Ca<sup>2+</sup>, Mg<sup>2+</sup> and HCO<sub>3</sub><sup>-</sup> ions is expressed by the following equation [16]:

$$\text{EC} = \sum_{i=1}^n (\text{C}_i \text{S}_i)^n \quad (2)$$

where EC is the electrical conductivity; C<sub>*i*</sub> is the concentration of the corresponding dissolved ions; S<sub>*i*</sub> is their equivalent specific conductivity; and *n* is an empirical exponent that depends primarily on the concentration and type of water. Therefore, the EC value at each point depends on the concentration of Ca<sup>2+</sup>, Mg<sup>2+</sup> and HCO<sub>3</sub><sup>-</sup> ions dissolved during the demineralization reaction; and to a lesser extent, on the ions acquired from the atmosphere.

It has already been stated that because the variation in time of EC and the concentration of ions present in the equilibrium system of carbonates is governed by exponential equations (Equation 1)—where *k* and *n* take similar values for each ionic species and for the electrical conductivity—the relationship between the concentration of each ion and the conductivity of the solution adjust to linear equations passing through the origin of coordinates [16].

In Table 1 the values of the solution conductivity and the corresponding concentrations of Ca<sup>2+</sup>, HCO<sub>3</sub><sup>-</sup>, Mg<sup>2+</sup> and Na<sup>+</sup> ions analytically determined for each conductivity value, taken at various times of CO<sub>2</sub> reaction with the lobster shells, are shown. As expected, the concentration increase of Ca<sup>2+</sup>, Mg<sup>2+</sup> and HCO<sub>3</sub><sup>-</sup> ions is accompanied by an increase in the conductivity of the solution. In the case of Na<sup>+</sup> ion, the concentration values found were very small and

**Table 1** Experimental values of the electric conductivity of the solution and the corresponding Ca<sup>2+</sup>, Mg<sup>2+</sup>, HCO<sub>3</sub><sup>-</sup> and Na<sup>+</sup> ions concentrations in experiment REL-1.

EC (μS·cm <sup>-1</sup> )	Ca <sup>2+</sup> (mg·L <sup>-1</sup> )	Mg <sup>2+</sup> (mg·L <sup>-1</sup> )	Na <sup>+</sup> (mg·L <sup>-1</sup> )	HCO <sub>3</sub> <sup>-</sup> (mg·L <sup>-1</sup> )
160	1.4	0.1	0.001	1.5
220	1.75	0.15	0.001	---
300	2.4	0.2	0.001	3.0
370	3.1	0.3	0.001	---
420	---	---	---	5.0
430	3.6	0.3	0.001	---
540	4.7	0.4	0.001	6.0
640	5.7	0.6	0.001	7.0
730	6.5	0.7	0.001	---
740	---	---	---	8.0
840	---	---	---	9.0
900	7.9	1.0	0.001	---
960	---	---	---	11.0
1000	8.8	1.2	0.001	---
1100	9.6	1.4	0.001	12.0

lied within experimental error. This is consistent with results obtained previously [7], showing that the Na<sup>+</sup> ion is not a major element of the lobster exoskeleton. Therefore, in the case of Na<sup>+</sup> ion the values reported later in the experiment, CINET-1 correspond to the direct determination of its concentration.

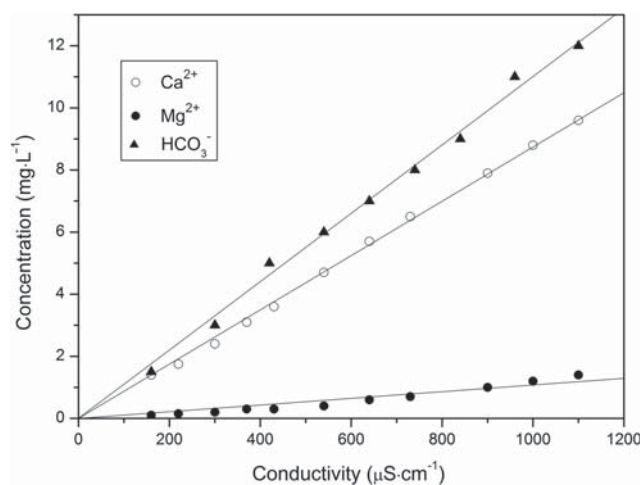
Figure 2 shows the fitting of the functional relationship between the concentration of the various species and the solution conductivity to a straight line in the form:

$$C_i = m \cdot EC \quad (3)$$

In Equation 3 EC represents the conductivity of the solution and C<sub>i</sub> is the concentration of the ionic species under consideration. The fitting results are shown in Table 2, where the goodness of fit can be appreciated, which is excellent especially for major Ca<sup>2+</sup> and HCO<sub>3</sub><sup>-</sup> ions.

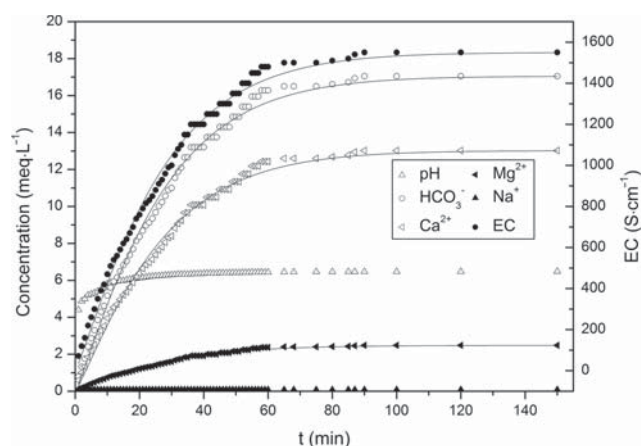
### 3.1 Reaction Kinetics Behavior

The reaction kinetics were investigated by determining only the time evolution of the solution conductivity and pH. From the results of experiment REL-1 (Table 2) it was possible to know the concentrations of Ca<sup>2+</sup>, Mg<sup>2+</sup> and HCO<sub>3</sub><sup>-</sup> ions at the corresponding times. This experiment, conducted in the absence of exchange resin, will be referred to as CINET-1, and the results are shown in Figure 3.

**Figure 2** Concentration of major ionic species (Ca<sup>2+</sup>, HCO<sub>3</sub><sup>-</sup> and Mg<sup>2+</sup>) represented as function of the solution conductivity during CO<sub>2</sub> demineralization.**Table 2** Value of the slope (*m*) and the determination coefficient (R<sup>2</sup>) resulting from the fitting of the data in Table 1 to Equation 3.

Ionic species	<i>m</i> × 10 <sup>3</sup> (mg·L <sup>-1</sup> / μS·cm <sup>-1</sup> )	R <sup>2</sup>
Ca <sup>2+</sup>	8.74 ± 0.06	0.9995
Mg <sup>2+</sup>	1.08 ± 0.06	0.9850
HCO <sub>3</sub> <sup>-</sup>	11.0 ± 0.1	0.9972





**Figure 3** Time evolution of Ca<sup>2+</sup>, HCO<sub>3</sub><sup>-</sup> and Mg<sup>2+</sup> ions concentrations calculated from the relationships found in experiment REL-1-, Na<sup>+</sup> ion concentration, electric conductivity and pH of the solution in experiment CINET-1. The curves drawn correspond to the fitting of data to Equation 1.

**Table 3** Specific rate constants evaluated for the experimental data of experiment CINET-1 using Equation 4 with  $n = 1$ .  $k_{\text{Calc}}$ ,  $n_{\text{Calc}}$  and  $R^2$  are obtained by the nonlinear least squares fit of data to Equation 1.

Values	$k_1(\text{HCO}_3^-) \text{ min}^{-1}$	$k_2(\text{Ca}^{2+}) \text{ min}^{-1}$	$k_3(\text{Mg}^{2+}) \text{ min}^{-1}$	$k_4(\text{EC}) \text{ min}^{-1}$
Minimum	0.033	0.033	0.033	0.033
Maximum	0.058	0.059	0.055	0.058
Average	0.039	0.040	0.040	0.040
Standard Dev.	0.006	0.006	0.006	0.006
$k_{\text{Calc}} \times 10^2 \text{ min}^{-1.14}$	$3.71 \pm 0.03$	$3.71 \pm 0.03$	$3.70 \pm 0.03$	$3.71 \pm 0.03$
$n_{\text{Calc}}$	$1.14 \pm 0.02$	$1.14 \pm 0.02$	$1.14 \pm 0.02$	$1.14 \pm 0.02$
$R^2$	0.9936	0.9936	0.9935	0.9936

From these results it is possible to determine whether Equation 1 holds for the demineralization of lobster exoskeleton with CO<sub>2</sub>, and if so, to evaluate the kinetic parameters of the reaction. To this end we proceeded as follows. First the values of the concentrations of Ca<sup>2+</sup>, HCO<sub>3</sub><sup>-</sup> and Mg<sup>2+</sup> ions, and the conductivity of the solution (EC) obtained at different times in the CINET-1 experiment were substituted, in each case, in Equation 4 to evaluate the reaction rate constant assuming  $n = 1$ . This assumption is based on the consideration that lobster shell demineralization using CO<sub>2</sub> follows the same pseudo-first-order heterogeneous kinetics as the dissolution of calcite in rocks does [17].

$$k = \frac{1}{t^n} \ln \frac{(C)_{\text{eq}}}{(C)_{\text{eq}} - C} \quad (4)$$

Table 3 shows the maximum, minimum and average values of each of the pseudo-first-order rate constants with their corresponding standard deviations. As can be seen, the assumption of a pseudo-first-order behavior is a good approximation for the interpretation of

the kinetics of lobster shell demineralization using CO<sub>2</sub>, the average first-order rate constants being practically the same for the three ionic species and the solution electric conductivity. These average rate constants were taken as the initial value for the nonlinear least squares fit of the concentration data (Ca<sup>2+</sup>, HCO<sub>3</sub><sup>-</sup> and Mg<sup>2+</sup>) and the conductivity of the solution, EC, at various times to Equation 1. This way it is possible to determine the proper experimental coefficient and the real rate constant, which are represented as  $n_{\text{Calc}}$  and  $k_{\text{Calc}}$ , respectively. These values are reported in Table 3, together with the coefficient of determination  $R^2$ . Given the goodness of the fit, reflected in  $R^2$  values close to unity, it can be concluded that the system studied has a kinetic behavior described by Equation 1. The experimental coefficient is  $n = 1.14 \pm 0.02$  and the reaction rate constant is  $k = (3.71 \pm 0.03) \times 10^{-2} \text{ min}^{1.14}$ . The regression curves obtained from these values shown in Figure 3, evidence good agreement with the experimental behavior.

As shown in Figure 3, both the concentrations of Ca<sup>2+</sup>, Mg<sup>2+</sup>, HCO<sub>3</sub><sup>-</sup> ions and EC increase with time during the first 80–90 minutes of reaction. From then on

their values remain constant, indicating that an equilibrium state has been reached, and under these circumstances further demineralization of the material cannot be achieved.

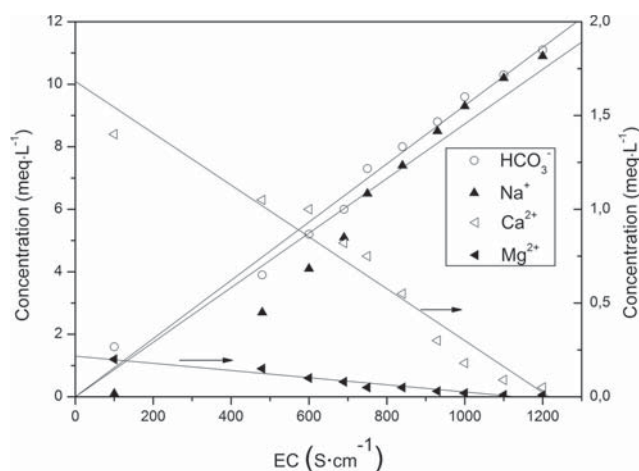
As to the kinetic processes involved in the dissolution of calcite in CO<sub>2</sub> solution, the prevailing criterion of some authors is that the chemical reactions occurring at the surface are the most influential ones on the reaction rate [13].

The exponential kinetic behavior seen in the curves can be explained by taking into account that there are three basic stages in the dissolution process. In the first stage, which takes place during the first 30 minutes, the solution is saturated with CO<sub>2</sub> and the rate of reaction depends mainly on the activity of H<sub>3</sub>O<sup>+</sup> ions and on ΔpH. With the addition of the lobster shells to the solution, very rapid and vigorous transformations of the inorganic material occurs, which are evidenced in the sudden changes of conductivity and ion concentrations produced in very short time. After that, in region 2, which runs approximately between 30 and 50 minutes (Figure 3), the pH is nearly constant and the changes are not as pronounced and rapid, while the ionic concentrations gradually increase. In region 3, which occurs between 50 and 80 minutes of the reaction, it can be seen that, as the solution approaches saturation, the reaction rate gradually decreases and the ionic concentrations move toward the equilibrium values.

### 3.2 Effect of the Cation Exchange Resin on Reaction Kinetics

With the addition of a cation exchange resin, such as Dowex 50X8 (cationite), for capturing the Ca<sup>2+</sup> ions released to the solution and exchanging them for Na<sup>+</sup> ions, reaching equilibrium is avoided and further demineralization of the chitinous material can be achieved. As before, an experiment to evaluate the correspondence between the conductivity of the solution and the concentration of major ionic species in solution, but now in the presence of cationite (experiment REL-2), was performed. In Figure 4 it can be seen that HCO<sub>3</sub><sup>-</sup> and Na<sup>+</sup> ions are now the ones responsible for the increase of the conductivity of the solution, since the exchange resin removes Ca<sup>2+</sup> and Mg<sup>2+</sup> ions produced by the reaction with CO<sub>2</sub>.

The concentrations of HCO<sub>3</sub><sup>-</sup> and Na<sup>+</sup> ions and the conductivity of the solution are again related according to Equation 3, while the relationships between the concentrations of Ca<sup>2+</sup> and Mg<sup>2+</sup> ions with conductivity can now best be interpreted in terms of the linear equation  $y = A + Bx$ . Table 4 shows the results of the fit for each ionic species.



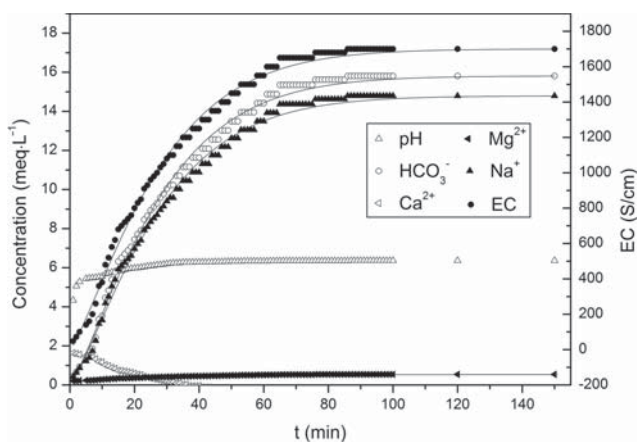
**Figure 4** Concentration of major ionic species (Ca<sup>2+</sup>, HCO<sub>3</sub><sup>-</sup>, Mg<sup>2+</sup> and Na<sup>+</sup>) represented as function of the solution conductivity during CO<sub>2</sub> demineralization in the presence of a cation exchange resin.

**Table 4** Linear relationships between HCO<sub>3</sub><sup>-</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> and Na<sup>+</sup> concentrations and solution conductivity for demineralization in presence of cationite (data in Figure 4). The parameter values and determination coefficients (R<sup>2</sup>) resulting from the fitting of the data for HCO<sub>3</sub><sup>-</sup> and Na<sup>+</sup> ions to Equation 3 and Ca<sup>2+</sup>, Mg<sup>2+</sup> ions to equation  $y = A + Bx$  are shown.

Parameters	HCO <sub>3</sub> <sup>-</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>
$m \times 10^3$	$9.3 \pm 0.4$	Not fitted	Not fitted	$8.7 \pm 0.3$
R <sup>2</sup>	0.9918			0.9906
$Y = A + Bx$		Ca <sup>2+</sup>	Mg <sup>2+</sup>	
A		$1.68 \pm 0.09$	$0.21 \pm 0.01$	
$B \times 10^3$		$-1.4 \pm 0.1$	$-0.19 \pm 0.02$	
R <sup>2</sup>		-0.9752	-0.9738	

Taking into account the above results, it was possible to calculate the concentrations of the various ionic species from the values of the conductivity of the solution at different times in a new demineralization experiment using CO<sub>2</sub>, now in the presence of cationite (CINET-2). They are shown in Figure 5, together with the values of the solution pH.

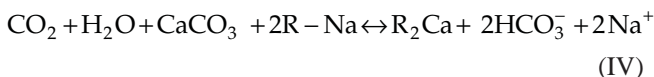
The HCO<sub>3</sub><sup>-</sup> concentration and the electrical conductivity increased in a similar way as in the absence of cationite (Figure 3), but now the concentration of Ca<sup>2+</sup> ions decreased to very low levels during the first 20 minutes of reaction. However, the concentration of Na<sup>+</sup> ions that was practically negligible in the absence of cationite, in the presence of the resin underwent a considerable increase, evidencing the ion exchange process, which occurs as represented in the following equation:



**Figure 5** Time evolution of Ca<sup>2+</sup>, HCO<sub>3</sub><sup>-</sup>, Mg<sup>2+</sup> and Na<sup>+</sup> ions concentrations calculated from the relationships found in experiment REL-2-, electric conductivity and pH of the solution in the demineralization experiment in presence of cationite (CINET-2). The curves drawn correspond to the fitting of data to Equation 1.



where R-Na stands for the cation exchange resin. Considering the calcium dissolution reaction represented in Equation I, the overall process in the presence of cationite can be written as:



The outcome of this ionic exchange is that the solution will now have NaHCO<sub>3</sub>, instead of Ca(HCO<sub>3</sub>)<sub>2</sub>, and since the first salt has a solubility product constant (K<sub>sp</sub>) much larger than the second one, calcite dissolution continues generating much higher exoskeleton demineralization levels than those obtained in the absence of cationite. We have observed (results to be published) that in the absence of cationite demineralization, CO<sub>2</sub> only reduces the exoskeleton ash contents to about 40%, whereas on using the cation exchange resin, ash contents as low as 1.3 % were achieved.

Applying the same calculation procedure used above for the reaction in the absence of the exchange resin, the kinetic parameters of the reaction were obtained. The minimum, maximum and average rate constants  $k_i$  corresponding to the time evolution of the electrical conductivity and HCO<sub>3</sub><sup>-</sup> and Na<sup>+</sup> ions are presented in Table 5.

The same as for experiment CINET-1, the values of  $k_{\text{Calc}}$  and  $n_{\text{Calc}}$  in Table 4 were obtained by the nonlinear least squares fitting of experimental data to Equation 1, taking as initial values the average  $k_i$  and  $n = 1$ . Given the goodness of fit, reflected in R<sup>2</sup> values close to unity, it can be concluded that in the presence of

**Table 5** Specific rate constants evaluated for the experimental data of experiment in presence of cationite CINET-2 using Equation 4 with  $n = 1$ .  $k_{\text{Calc}}$ ,  $n_{\text{Calc}}$  y R<sup>2</sup> are obtained by the nonlinear least squares fit of data to Equation 1.

Values	$k_1(\text{HCO}_3^-)$ $\times 10^2 \text{ min}^{-1}$	$k_3(\text{Na}^+)$ $\times 10^2 \text{ min}^{-1}$	$k_4(\text{EC})$ $\times 10^2 \text{ min}^{-1}$
Minimum	0.017	0.017	0.017
Maximum	0.058	0.059	0.059
Average	0.038	0.038	0.038
Standard Dev.	0.001	0.001	0.001
$k_{\text{Calc}} \times 10^2 \text{ min}^{-1.24}$	$3.41 \pm 0.02$	$3.41 \pm 0.02$	$3.41 \pm 0.02$
$n$	$1.24 \pm 0.02$	$1.24 \pm 0.02$	$1.24 \pm 0.02$
R <sup>2</sup>	0.9950	0.9950	0.9951

the exchange resin the studied system also follows the kinetic behavior described by Equation 1. The value of the empirical exponent is now  $n = 1.24 \pm 0.02$  and the reaction rate constant is  $k = (3.41 \pm 0.02) \times 10^{-2} \text{ min}^{-1.24}$ . The regression curves obtained from these values are shown in Figure 5 where good agreement with the experimental behavior is also noticeable.

## 4 CONCLUSIONS

It was shown that the kinetics of calcite demineralization of deproteinized lobster exoskeleton using CO<sub>2</sub> can be represented to a first approximation by a pseudo-first-order kinetics expression. The best fit of the experimental data was obtained using the equation:

$$C = C_{\text{eq}} (1 - e^{-kt^n})$$

where  $n = 1.14 \pm 0.02$  and the reaction rate constant  $k = (3.71 \pm 0.03) \times 10^{-2} \text{ min}^{-1.14}$ . This reaction proceeds to a state of equilibrium that prevents achieving complete demineralization of the material. The equilibrium can be disrupted by employing a cation exchange resin, allowing further decalcification of the material. The use of the resin does not substantially modify the reaction kinetics, which can also be expressed by a pseudo-first-order equation. The values of the kinetic parameters in the presence cationite are  $n = 1.24 \pm 0.02$  and  $k = (3.41 \pm 0.02) \times 10^{-2} \text{ min}^{-1.24}$ .

## REFERENCES

1. R.A.A. Muzzarelli, J. Boudrant, D. Meyer, N. Manno, M. DeMarchis, and M.G. Paoletti, Current views on fungal chitin/chitosan, human chitinases, food preservation, glucans, pectins and inulin: A tribute to Henri Braconnot, precursor of the carbohydrate polymers science, on the chitin bicentennial. *Carbohydr. Polym.* **87**, 992 (2012).

2. S.S. Silva, A.R.C. Duarte, A.P. Carvalho, J.F. Mano, and R.L. Reis, Green processing of porous chitin structures for biomedical applications combining ionic liquids and supercritical fluid technology. *Acta Biomater.* **7**, 1166 (2011).
3. M.A. Ramírez, A.T. Rodríguez, L. Alfonso, and C. Peniche, Chitin and its derivatives as biopolymers with potential agricultural applications. *Biotechnol. Appl.* **27**, 270 (2010).
4. K. Kurita, Chitin and chitosan: Functional biopolymers from marine crustaceans. *Mar. Biotechnol.* **8**, 203 (2006).
5. W. Arbia, L. Arbia, L. Adour, and A. Amrane, Chitin extraction from crustacean shells using biological methods. *Food Technol. Biotechnol.* **51**, 12 (2013).
6. F. Boßelmann, P. Romano, H. Fabritius, D. Raabeb, and M. Epple, The composition of the exoskeleton of two crustacea: The American lobster *Homarus americanus* and the edible crab *Cancer pagurus*. *Thermochim. Acta* **463**, 65 (2007).
7. M.A. Ramírez, A.T. Rodríguez, J.A. Alfonso, J.A. Azocar, Y. Vázquez, L. Alfonso, and C. Peniche, Composición química y elementos trazas en subproductos de exoesqueletos de langosta *Panulirus argus* con posible uso agrícola. *Rev. CENIC Cienc. Quím.* **41**, 99 (2010).
8. P. Romano, H. Fabritius, and D. Raabe, The exoskeleton of the lobster *Homarus americanus* as an example of a smart anisotropic biological material. *Acta Biomater.* **3**, 301 (2007).
9. H. No and S. Meyer, Preparation and characterisation of chitin and chitosan a review. *J. Aquat. Food. Prod. Technol.* **14**, 27 (1995).
10. C.J. Brine and R. Austin., Chitin variability related with species and methods of preparation. *Comp. Biochem. Physiol.* **69B**, 283 (1981).
11. Y. Qin, X.M. Lu, N. Sun, and R.D. Rogers, Dissolution or extraction of crustacean shells using ionic liquids to obtain high molecular weight purified chitin and direct production of chitin films and fibers. *Green Chem.* **12**, 968 (2010).
12. M. Martins, R. Craveiro, A. Paiva, A.R.C. Duarte, and R.L. Reis, Supercritical fluid processing of natural based polymers doped with ionic liquids. *Chem. Eng. J.* **241**, 122 (2014).
13. J.R. Fagundo, Química del Agua Kárstica, in *Hidroquímica del Karst*, J.R. Fagundo, J.J. Valdés, and J.E. Rodríguez (Eds.), pp. 14–119, Ediciones Osuna, Granada (1996).
14. E. Alvarez, J.R. Fagundo, P. González, V. Ferrera, and G. Benítez, Aplicación de métodos de simulación química y matemática al estudio de la disolución de carbonatos en diferentes condiciones experimentales, in *Contribuciones a la hidrogeología y medio ambiente en Cuba*, J.R. Fagundo, D. Pérez, A. Alvarez, J.M. García, and I. Morell (Eds.), pp. 91–103, Universidad de Castellón, Castellón (1996).
15. Standard Methods for the Examination of Water and Wastewater, APHA, AWWA, WPCF (1992).
16. R. Miller, W.L. Bradford, and N.E. Peters, Specific conductance: Theoretical considerations and application to analytical quantity control, in *Geological Survey Water-Supply Paper 2311*, U.S.G.P. OFFICE (Ed.), pp. 21, U. S Geological Survey, Federal Center, Denver, CO (1988).
17. E. Alvarez and F. J.R., SIMUCIN: Sistema para el estudio cinético y la modelación de las reacciones de disolución de minerales, in *El Karst y los acuíferos Kársticos*, ejemplos y métodos de estudio J. Fagundo, A. Pulido-Bosch, J. Rodríguez, and I. Morell (Eds.), pp. 209–213, Univ. Granada, Spain (1995).