Lactic acid demineralization of green crab (Carcinus maenas) shells: effect of reaction conditions and isolation of an unusual calcium complex

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ABSTRACT

Chitin and chitosan are potentially useful and environmentally friendly biopolymers with a wide range of value-added applications. Effective and green technologies for isolation of these materials are potentially important. Here, we report the use of lactic acid for the demineralization of green crab shells. Green crab shells and lactic acid, produced during cheese making, are two waste streams that could be tapped for large-scale chitin and chitosan processing. We have studied the effect of concentration and temperature on the demineralization of green crab shells. An unusual calcium lactate/lactic acid complex was also isolated and crystallographically characterized. The results have implications not only for the use of weak acids in the isolation of chitin and chitosan but also for the use of lactic acid as a solvent in green chemistry.

1. Introduction

There has been considerable interest recently in the development and commercialization of usable products derived from waste materials. Abundant and typically low in cost, waste matter and its chemical conversion to useful materials are key components in the broad field of green chemistry. Waste materials from paper production are already being used to generate lignin (1), pyrolysis of high-density polyethylene is being used to generate fuel (2), and fertilizer and other bio-degradable products are being prepared from food wastes (3). Conversion of waste materials into value-added products can have significant local impact through reduction of waste and waste treatment processes and can also add valuable revenue-generating streams through bulk materials production.

Two materials that are currently being utilized after isolation from waste products are chitin and chitosan. Figure 1 shows the chemical structures of these two compounds, chitin (R=C(O)CH₃) and chitosan (R=H). After cellulose, chitin is the most abundant naturally occurring polymer (4–6). It is composed of linear chains of poly(β-(1→4)-N-acetyl-D-glucosamine) and is present in crustacean shells as ordered crystalline microfibrils forming a complex structure with associated proteins, minerals and lipids. There are three polymorphic forms of chitin (α, β and λ). The α-form is the most common and is the form found in crab shells. α-Chitin is tightly compacted due to its crystalline structure in which alternate sheets of parallel and antiparallel chains allow the formation of strong hydrogen bonds. Complete deacetylation of chitin yields chitosan, a process requiring strong base and high temperature (100–150°C) for complete conversion. In general, chitosans have lower molecular weights, and are less crystalline than their chitin precursors. Under acidic conditions, the NH₂ groups of chitosan are protonated increasing its solubility in aqueous media relative to chitin.

Chitin occurs in large quantities in fungal and yeast cell walls, in lobster, crab and shrimp shells and also in insect cuticles. In some crustaceans, it makes up 30% of the body weight of the animal (7). Crustacean waste generated through commercial fisheries such as those for lobster, crab and shrimp is the most practical and accessible chitin source. However, in the coastal province of Nova Scotia, Canada, the invasive European green crab (Carcinus maenas) has been out-competing native species for food, destroying oyster and mussel populations and eating eelgrass beds, all of which are important stressors on the local marine ecosystem (8). These crabs are often ‘by-catch’ of other fisheries and are virtually valueless as a source of food. Once caught, government regulation prevents returning them to the environment and thus the whole green crab is wasted.
Low-cost isolation of chitin from these crabs would potentially be a large volume source of chitin and, subsequently, chitosan. This could become another significant revenue stream for the fishers and the local economies of their rural communities.

In the exoskeleton of the crab, chitin is found in association with calcium carbonate, proteins and lipids. An efficient method is needed to remove these associated materials from the crab shell. There are two broadly defined ways to do this, using either chemically or biologically based processes. A review of biological methods has recently been published by Arbia et al. (7). Typically, the chemical purification of chitin and chitosan requires several steps, grouped into demineralization and deproteinization procedures. In the chemical method, the shell is first treated with hydrochloric acid (HCl) for demineralization. The acid reacts with calcium carbonate in the shell to evolve carbon dioxide and leaves aqueous calcium chloride as a potentially useful by-product. The residue is then treated with a mild solution of sodium hydroxide, NaOH (1–4%), at temperatures of 60–70°C in order to separate proteins from the chitin. This process is very important because the highest value chitin and chitosan products are those used in biomedical applications (9). One of the most common allergies in Western civilization is to shellfish and seafood. Total removal of all the shellfish protein is required to avoid triggering this allergy in those sensitive to it. Care must also be taken at this point because in the presence of excess base, the chitin can be partially deacetylated. Complete deacetylation of chitin does yield chitosan, but this requires concentrated NaOH (50%) and correspondingly high temperatures (100–150°C). Degradation of the original polymer is generally to be avoided during the isolation process.

Purified chitin and chitosan have attracted interest due to their bio-degradable and bio-renewable properties (4). Although not normally utilized in large quantities, vis-à-vis bulk applications, chitin and chitosan have many value-added applications including medical, agricultural and industrial uses. For example, in medicine, chitin has been used as a biocide due to its antibacterial and antifungal actions. It has immune-stimulatory activity, and it promotes wound healing after surgeries. It has been used as a plant antivirus in agriculture, as an additive to liquid multicomponent fertilizer and as a metal-recovery agent. Chitosan is also applied as a film-forming agent in cosmetics, as a dye-binder in textiles and as a strengthening additive in paper. In the pharmaceutical industry, it has uses as a carrier material for drug delivery (9).

Recognizing the potential value of chitin, chitosan and products derived from them, cost-effective methods for purifying chitin are important. Typically, using crab shells as an example, chitin is isolated by the chemical treatment of the shells using aqueous hydrochloric acid. Hydrochloric acid can be handled safely but in general is challenging to work with. For example, large amounts of HCl in a fish waste-processing facility would be difficult to handle since it is highly corrosive. Care must also be taken during the actual demineralization of the shells to prevent their decomposition through direct reaction of the acid with the ether linkages in chitin. Most importantly, however, prolonged exposure to HCl can result in significant modification of the physiochemical properties of chitin: decrease in molecular weight can result from cleavage reactions and significant deacetylation of the chitin can also occur. Both of these negatively affect the fundamental properties of the chitin isolated and this, in turn affects the quality and value of the final products (10). So, while effective when used with extreme care, HCl can be viewed as the Achilles’ heel of chitin isolation and purification.

We became interested in the demineralization of crab shells for two reasons. First, as alluded to above, invasive green crabs are a problem in Nova Scotian waters (8) and there is significant interest in converting this waste stream into value-added materials. Furthermore, our interest in carbon dioxide chemistry links nicely with the chemistry required to convert the minerals found in crab shells, primarily calcium carbonate, from a sequestered form of carbon dioxide (CO$_3^{2-}$) to free carbon dioxide (CO$_2$). We recognized early on that the ‘greenness’ of chitin extraction would be enhanced by replacement of concentrated HCl by a more benign alternative.

We decided to begin our explorations using simple, readily available organic acids. Selection of an appropriate acid hinged upon two criteria. First, the acid needed to be readily available. And second, the acid had to be part of an underutilized waste stream. Mild, food waste acids, such as acetic acid and lactic acid, had been previously investigated for the
demineralization step in chitin production (11). From this study, it appeared that lactic acid was the more promising choice. Lactic acid is derived from milk processing an already viable industry in Nova Scotia. Fermentation of carbohydrates such as glucose and sucrose by *Lactobacillus*, important in cheese making, results in lactic acid formation (12). Fermentation of cheese whey, a by-product in cheese production, can produce lactic acid in large-scale quantities, making it a potentially inexpensive source of this material. If whey could be used in the isolation of chitin, either as a feedstock in the production of lactic acid for the chemical demineralization of chitin, or directly as a substrate for the bacteria utilized in the biological fermentation of the shells, then it truly would be a sustainable green process.

Lactic acid is bio-degradable and bio-compatible, features important in the manufacture of many advanced materials (12). It has found many useful applications, mainly as a precursor to bio-degradable polymers which are used in the food and beverage industries. Global production of lactic acid is between 200,000 and 400,000 tonnes per year and is growing at a rate of roughly 8% per year (13). Lactic acid is already relatively inexpensive, and the goal is to drop production costs to 0.8 USD per kilogram in the near future (13). While it is more expensive than HCl, the detrimental effects that arise from using strong acid for digestion of the polymer may be avoided by using a weaker acid. Furthermore, its use would convert a waste stream into a valuable reagent (13–15).

The *in situ* generation of lactic acid is a cornerstone in the biological fermentation of crustacean shells for demineralization. Again, the review by Arbia et al. serves as a good introductory reference (7). Although much less common than biological processing, chemical demineralization of shells using lactic acid has also been investigated by a number of groups (11, 16–19). Most of these have investigated the isolation of chitin/chitosan from shrimp shells, including Mahmoud et al. who used lactic acid to demineralize Nova Scotian shrimp shells (11). One early report does investigate the use of lactic acid for the chemical demineralization of red crab shells from Korea (16).

This paper describes the most effective conditions, including concentration and temperature, identified for the demineralization of green crab shells using lactic acid. We have also isolated a crystalline material from the reaction between lactic acid and the crab shell. It proved to be a calcium lactate/lactic acid complex, which was characterized using X-ray crystallography. Isolation of this compound has significant implications for the use of lactic acid to demineralize chitin. It also has implications in green chemistry where lactic acid is increasingly being used as a solvent for organic and catalytic reactions (20). The interaction of lactic acid with metal ions is becoming a topic of greater interest and there is little information on the subject currently available in the literature.

2. Materials and methods

2.1. Materials

2.1.1. Preparation of crab shells

Crabs shells were obtained from BioMer Innovations, Halifax, Nova Scotia, Canada. To keep the experiments consistent, only the carapace of the shell was used. These were de-fleshed and cleaned with water. Each carapace was then cut into three pieces of roughly equal weight and oven dried at 40°C until a constant dry weight was achieved. Shells were then soaked in water to a constant wet weight so that the change in weight could be monitored throughout a reaction without having to re-dry the shell.

2.1.2. Preparation of lactic acid solutions

DL-Lactic acid, 11.4 M, 85% (w/w), was purchased from Sigma-Aldrich. Samples were diluted with deionized water with ratios of 1:1, 1:4, 1:9, 1:11, 1:13, 1:16 and 1:20 (acid to water, by volume) being prepared.

2.2. Instrumentation

Conductivity was measured using a Vernier Conductivity Probe CON-BTA and pH was measured using a Vernier pH Sensor PH-BTA. LoggerPro 3.6.0 by Vernier Software & Technology was used to record the data collected. Infrared spectra were recorded as KBr pellets using a Nicolet 6700 FT-IR spectrometer. 

\(^{1}\)H NMR and \(^{13}\)C NMR data were collected using a Bruker Advance III 300 spectrometer and are referenced to residual solvent. Elemental analysis was performed at the Centre for Environmental Remediation (CEAR) at Saint Mary's University.

2.3. Determination of ideal conditions for the demineralization of crab shells

2.3.1. Measurement of the conductivity and pH of lactic acid in aqueous solution

A volume of 20 mL of 11.4 M, 85% DL-lactic acid solution was placed in a large test tube. A conductivity probe and a pH probe, both attached to a computer running Logger Pro, were placed into the solution and an initial reading taken. The solution was then diluted a specific amount and a new reading recorded. This process was repeated
in small increments until a dilution ratio of 1:30 was reached. The concentration of H\(^+\), [H\(^+\)], was calculated from the measured pH.

2.3.2. Concentration of lactic acid
All solutions were added to 50-mL test tubes along with a single piece of crab shell of uniform mass. Initial and final dry weights were recorded to determine the percentage of mass loss, which is directly correlated to the amount of demineralization of the shell. All tests were done with 20 mL of solution, at room temperature and at constant atmospheric pressure. The tests were performed for 180 minutes and the initial dry weights of the shells were approximately 0.30 (±0.05) g. After reaction, the shells were washed in tap water and oven dried at 40°C until a constant dry weight was achieved.

2.3.3. Effect of temperature on the extent of demineralization
Lactic acid dilutions of 1:1, 1:4 and 1:9 were utilized. Trials were run at temperatures of 0–1°C, 20°C and 60°C. Test tubes containing shells and solutions were placed in baths until the required temperature was reached. All tests were run in 20 mL of solution and the initial dry weights of the shells were 0.25 (±0.05) g. The tests run at 0–1°C and 20°C were allowed to proceed for 180 minutes; the tests run at a temperature of 60°C were left for 90 minutes. After reaction, the shells were washed in tap water and oven dried at 40°C until a constant dry weight was achieved.

2.3.4. Variation in the extent of the demineralization reaction with time
Shells were dried and soaked in water until a constant moist weight was reached. Moist weights of the shells were 0.73 (±0.05) g. The moist shells were submerged in 40 mL of 1.14 M DL-lactic acid solution. The test was run in triplicate at 20°C and was allowed to proceed for 360 minutes. Moist weights of the shell were recorded periodically.

2.3.5. Combining lactic acid and HCl for demineralization
The lactic acid solution was made up in a 1:4 acid to water ratio. A portion of this was diluted in a 1:1 molar ratio with 5.00 M HCl to make the second test mixture. Shells were dried and then soaked in water until a constant moist weight was reached. They were placed in the test solutions (40.00 mL lactic acid or 45.12 mL of the mixed acid solution). The test was done at 24.7°C and allowed to run for 60 minutes. The shells were weighed at 15-minute intervals and at the end of the experiment after drying.

2.4. Isolation and characterization of crystals
Aqueous DL-lactic acid was used to demineralize a crab shell containing 80–85% calcium carbonate. After the demineralization process was carried out (not to absolute completion), the shell was removed from the solution and allowed to air dry. Crystals were observed to form on the shell. These were collected and used for characterization, including single-crystal X-ray analysis. Mp: 153°C. Spectroscopic data: \(^1\)H NMR (D\(_2\)O, 75 MHz): \(\delta\) 1.36 (d, 3H, \(\text{CH}_3\)), 4.27 (q, 1H, \(\text{J}_{\text{HH}} = 7.0\) Hz, \(\text{CH}_2\)), 6.7 (s, br, \(\text{COO}^-\)) ppm. IR: 3372 (s, br), 2990 (m), 1733 (vs), 1640 (m), 1593 (m), 1459 (m), 1377 (s), 1233 (s), 1126 (vs), 1095 (m), 1046 (m), 828 (m), 653 (m). Elemental Analysis: Calc. for C\(_{12}\)H\(_{22}\)O\(_7\): C, 36.18; H, 5.58; N, 0.18%. Found: C, 36.34; H, 5.55; N, 0.18%.

3. Results and discussion
3.1. Concentration of lactic acid
The effect of acid concentration on the degree of demineralization was studied for a series of lactic acid solutions diluted with water. Before beginning, it must be noted that in the experiments carried out for this study, only the mass loss of the crab shell was measured (i.e. the demineralization of the shell). There was no attempt made to determine the calcium (carbonate) content/loss or to identify the other metal ions present in the shell of this crab species. Similarly, no measurements were made to determine the chitin content of the shells, either initially or after the demineralization experiments were carried out. In addition, the experiments were carried out on relatively large pieces of crab shell, all of about the same size. In the real-world, grinding the shell would require time and equipment. This would be offset by an increased rate of reaction though not by different final results. Grinding the shells to a powder could also degrade the chitin polymer, which is more valuable when recovered at higher molecular masses. For these reasons, the crab shells were used as provided.

As observed for most acid-base reactions, increasing the concentration of the lactic acid was expected to increase the rate of the demineralization reaction on a linear scale. However, this was not the case with lactic acid. Demineralization did not follow the expected trend of increasing with decreased pH (or increased hydrogen ion concentration). Instead, the rate of demineralization was found to have a positive correlation with the conductivity of the solution. Conductivity measures the mobility of the H\(^+\) ion in solution and
because lactic acid is very viscous, a more concentrated solution will lead to a decrease in mobility (activity) of the ions. This decrease in activity results in a decrease in the ability of the H\(^+\) ions to attack the shell and cause demineralization. It can be seen from Figure 2 that the conductivity does not increase infinitely as the concentration decreases. This results in a ‘sweet spot’ on the conductivity curve, an ideal conductivity and hence, an ideal concentration to use for the demineralization. The dilution ratio of 1:5 (acid to water), or a concentration of 1.90 M, gave the largest measured conductivity.

As shown in Figure 3, the use of the undiluted lactic acid solution (11.4 M) resulted in removal of only 5% of the calcium carbonate after 180 minutes at room temperature, compared to the 61% removal achieved with 1.14 M and the 53% removal found using 2.28 M lactic acid. The most effective lactic acid concentration for demineralization was found to be 1.14 M (room temperature and 180 minutes), but since lactic acid is consumed in the reaction, there is a range of effective concentrations. From Figures 2 and 3, it is clear that for demineralization of crab shells, the best concentrations lie in the range of 2.28 M (high conductivity) to 1.14 M (high rate of demineralization). There were some time variances observed, depending on the temperature and the shell to solution ratio, but they were insignificant enough to disregard.

Mahmoud et al. (11) used a concentration of 0.839 M lactic acid for their study on the demineralization of shrimp cells. With this, they achieved approximately 70% removal in 6 hours at room temperature. From Figure 3, it can be seen that we removed 44% of the body mass at 0.81 M in 3 hours at room temperature, which is comparable to their results. Our results are also similar to the ideal concentration for maximum chitosan yield of 1.5 M reported by Ameh et al. (18). These concentrations result in a good balance between the rate of the reaction and the extent of the reaction.

3.2. Effect of temperature on the extent of demineralization

Many chemical reactions proceed more quickly with an increase in temperature. This was found to be true for
the reaction of lactic acid with crab shells resulting in
demineralization, as shown in Figure 4. At 60°C, the
extent of the observed demineralization was relatively
high for all three concentrations (67–79%). Even at
room temperature, demineralization with either 1.14 or
2.28 M lactic acid was quite high and comparable to
that observed at the higher temperature (60°C). This indi-
cates that even though the rate of the reaction is pro-
moted at higher temperature, an extended reaction
near room temperature can produce similar results.
These results are comparable to those described by
Mahmoud et al. in their study on using lactic acid for
the demineralization of shrimp shells (11). They observed
a 64.2% weight loss for reaction at 100°C over 1 hour and
a 62.9% body weight loss over 6 hours at 24°C. All of
these results suggest that energy savings can be
achieved just by letting the room temperature reaction
run a bit longer.

3.3. Variation in the extent of the
demineralization reaction with time
The shell to acid ratio will also affect the extent of the
demineralization reaction. As the reaction proceeds,
the concentration of the H+ ion in solution decreases.
Since a larger shell holds more CaCO3, more H+ will be
needed for complete demineralization. This effect can
be observed by recording the pH of solution as the reac-
tion proceeds. A shell to solution ratio of 1:70 (w/v) was
used in all of our experiments.

Ideal conditions resulted in approximately ~90–95% removal of calcium carbonate and other minerals, in 90
minutes. Removal of the remaining ~5–10% took a sig-
nificantly longer time, upwards of 350–400 minutes.
This can be explained using the ‘shrinking core model’
that the reaction mimics. This was touched upon by
Ameh et al. (17). In essence, the reaction slows over
time as the organic framework of the shell remains the
same and the reactive site of the calcium carbonate
(and other minerals) moves deeper into the interior of
the shell. Our results are illustrated in Figure 5.

3.4. Combining lactic acid and HCl for
demineralization
A test was also conducted to determine the effect of
demineralizing a shell with a combination of lactic acid
and HCl. It was assumed that this approach would maxi-
mize the beneficial features of both individual methods.
Using a combination method, the amount of HCl needed
would be reduced (when compared to using only HCl for
demineralization) and the rate would be kept relatively
high (compared to using only lactic acid for deminerali-
zation). The results are presented in Table 1. The
mixture resulted in 77.8% body mass removal, as com-
pared to 37.5% removal with lactic acid alone, under
the same conditions. The results confirm that the combi-
nation method does provide advantages relative to
using either acid individually for the demineralization
of crab shells. We have not thoroughly investigated the
possibility that the two acids (lactic and hydrochloric) act together in a synergistic fashion, although the results suggest that this may in fact be occurring. Further investigation is required.

3.5. X-ray crystallography

Crystals were first observed growing on a crab shell left in solution at the end of a lactic acid demineralization experiment. The crystals were very small, at the limit of what could be handled with our X-ray diffraction system. This resulted in a data set of rather poor quality and final results that were merely adequate. However, they were good enough to unambiguously establish the nature of the complex isolated and to determine some of its other interesting features.

The original crystals were isolated from an aqueous solution of DL-lactic acid. Only one single crystal large enough for diffraction was ever obtained and this crystal was found to contain Ca-coordinated lactate and lactic acid ligands, all with only S-configurations. One possibility is that this compound crystallizes as a mixture of single crystals of both enantiomers and we have just isolated that with the $S,S$-configuration. It is also possible that the crab shell itself provides a substrate for asymmetric crystallization. This would explain why it proved so difficult to repeat the original crystallization reaction and isolate more of the solid product (in fact, better quality crystals were never obtained). Finally, while the crystal structure results and absolute structure parameters suggest that the chosen crystal is enantiomerically pure, the rather modest quality of the available crystals and the data do not allow complete exclusion of the possibility that the crystal is an inversion twin.

Once the original product had been characterized, the synthesis was re-attempted using L-lactic acid as the ligand source, and much larger crystals grew quite readily. They proved to be different from the original product, but only in that they were solvated with lactic acid. The central complex was still of the same formula, $\text{Ca(CH}_3\text{CHOHCOO)}_2\text{(CH}_3\text{CHOHCOOH)}_2$, with the all $S$ configuration, substantiating the original results. A more detailed discussion of our exploration of the crystallization products from lactic acid solutions will appear in a

![Figure 5. The per cent weight loss in green crab shells when reacted with 40 mL of 1.14 M DL-lactic acid solution. The data are fitted to the model $y = A + B\exp(-Ct)$, where $y$ is the per cent mass loss, $t$ is the time in minutes and $A$, $B$ and $C$ are fitting parameters. Note: The tests were run at 20°C and were allowed to proceed for 360 minutes.](image)

| Table 1. Demineralization using a lactic acid–HCl mixture compared to using lactic acid alone. |
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| **Solution** | **Wet Weights** | **Final dry weight (g)** | **Per cent weight loss (%)** |
| Lactic acid | Initial dry weight (g) | Initial wet weight (g) | Weight at 15 minutes (g) | Weight at 30 minutes (g) | Weight at 45 minutes (g) | Weight at 60 minutes (g) | 0.3252 | 37.5 |
| Lactic acid + HCl | 0.4974 | 0.7084 | 0.4705 | 0.4099 | 0.3948 | 0.3796 | 0.1104 | 77.8 |

Note: The lactic acid solution was made in a 1:4 acid to water ratio. It was then diluted in a 1:1 molar ratio with HCl. The test was carried out at 24.7°C for 60 minutes.
subsequent publication. This is a field worthy of further study, since lactic acid is finding considerable use as a solvent in green chemical applications and its interactions with metal ions will be of considerable interest in this regard (20).

The $^1$H and $^{13}$C NMR spectra of the crystals were recorded in D$_2$O and are included in the Supplemental Content. Only one signal was observed to arise for each carbon or non-exchangeable proton in solution, that is, the coordinated and free ligands all exchange readily on the NMR time scale. This behaviour was also observed by Kondoh and Oi who studied the interaction of alkaline earth metal ions (magnesium, calcium, strontium and barium) with acetic and lactic acid in aqueous solutions by $^{13}$C NMR spectroscopy (21). The authors found no distinct signals corresponding to bound and free lactate ions due to the fast interchange between the two. The chemical shifts reported for the carbon atoms in their aqueous calcium lactate solution (δ = 21 (CH$_3$), ~70 (CH(OH)) and ~183 (COO) ppm) are in very close agreement with those observed for our complex. The authors speculate on the structure of the species in solution, but their results suggest that the actual complex is likely the same as, or closely related to, that isolated in this work.

This crystal structure was solved in the tetragonal system (Laue symmetry 4/m) in the non-centrosymmetric, chiral space group P4$_2$ (#77). The asymmetric unit was found to contain one calcium atom of half-occupancy on a special position and two complete ligands each bonded to the calcium in a bidentate fashion through oxygen atoms. Growing the structure gives two complete molecules in the unit cell, each composed of one calcium centre and four bidentate coordinated ligands.

The question of the ligands’ identities was the first to be addressed. They were both similar, with the same distribution of heavy atoms, and both obviously arising from the lactic acid present in the reaction solution. The heavy atom backbone of each ligand was identical, Ca(1)–O–C(O)–C(C)–O–Ca(1), indicating that each contained some form of lactate/lactic acid, coordinated to calcium through the oxygen atoms of both the alcohol group (OH) and the carboxylic acid group (COOH) of the original lactic acid. On chemical grounds, it would be expected that the calcium would be present as the cation, Ca$^{2+}$, meaning that the four ligands should collectively have a charge of $2^-$. Based on the chemical evidence, one would suspect a distribution of two neutral lactic acid ligands and two anionic lactate ligands with uninegative charges would be the most probable.

Values for the C(1)–O(2) and C(4)–O(5) bond lengths in the complex are significantly different when ligands 1 and 2 are compared. C(1)–O(2) and C(4)–O(5) are both the equivalent bond in a carboxylate group, involving the oxygen atom of the group not coordinating to calcium (i.e. both originate from the C=O of the original lactic acid and not C–O–H). In lactic acid, this bond is 1.208(1) Å, while in the free lactate anion, it is on average 1.251 Å (see a complete discussion in the Supplemental Content, and here too, these values are significantly different (if you assume equal esds on the bonds). In ligand 1, the equivalent bond is 1.201(16) Å, while in ligand 2, it is 1.281(19) Å. Comparison of these four values suggests that ligand 1 is derived from lactic acid while ligand 2 has a bond length consistent with it being a coordinated lactate anion. If you then extend the comparison of all the bonds in ligand 1 to the equivalent bonds in lactic acid, and you do the same for the ligand 2 bonds to the lactate values, then the agreement is quite good. It seems reasonable to assign ligand 1 as coordinated lactic acid and ligand 2 as coordinated lactate anion. As such, ligand 1 should be protonated on O(1), and it is the O–H group of the acid which coordinates to the metal, while ligand 2 is not protonated at O(4).

A search of the Fourier difference map at this point of the structure determination (complete except for the identification of the final proton) located a peak roughly equidistant between O(1) and O(4) in symmetry related molecules and in the correct geometry to be the missing proton. Based on the arguments provided above, a proton was placed at the position of the Fourier peak but restrained to be bonded to O(1) rather than O(4). The final refinement proceeded without problem to give the final structure with ligand 1 being formally lactic acid and ligand 2 being a lactate anion. It is still possible that the strong O(1)–H···O(4)$^-$ hydrogen bond could be better described by a more equal sharing of the proton between the two oxygen atoms, but the data quality was not sufficient to determine which model was ultimately better. The assignment based on the overall geometry of the ligands was chosen as being the most likely.

The structure of Ca(CH$_3$CHOHCOO)$_2$(CH$_3$CHOHCOOH)$_2$ is shown in Figure 6. In the final structure, both ligands contain a chiral carbon centre, C(2) in the lactic acid and C(5) in the coordinated lactate. These carbon atoms have bonds to (a) the carbon atom of the carboxyl group, (b) the oxygen atom of the OH group, (c) the methyl group carbon atom and (d) one proton. There is no ambiguity in the structure when it comes to the determination of the absolute configuration at these centres, both of which were determined to have S configurations in the crystallographic analysis. Considering that the starting material was DL-lactic acid, only (or predominantly)
one isomer must have been selectively incorporated into the final structure of the crystal chosen for analysis.

There is little in the literature with which to compare our current structure. There are no previous reports of bidentate coordinated lactic acid to any metal and there are no structures in the Cambridge Database (CSD) (23) that have lactic acid or lactate coordinated to calcium in a bidentate fashion. The closest analogue to the current structure is diaquabis(L-lactato)magnesium described by Hong-lin Zhu and Ling Jin in 2012 (24). In the asymmetric unit of Mg(H2O)2(C3H4O3)2, the Mg2+ cation is chelated by two lactate anions bound through the carboxylate and hydroxyl groups. The other two sites are occupied by water molecules giving MgO6 units with distorted octahedral geometries. The authors prepared their compound from L-lactic acid and it is thus unsurprising that they obtained a product with the S conformation at both chiral centres in the lactate ligands. Looking at the three-dimensional structure deposited with the CSD, the configuration of both chiral centres in Mg(H2O)2(C3H4O3)2 is S, the same as observed in our structure. This conformation must be favoured for some reason, although why is unknown as of yet. The bond lengths and angles for the bidentate coordinated lactate ligands in the magnesium complex compare quite well with those found in our complex for the lactate. The Mg-O bond lengths are, of course, shorter (average 2.064 Å) than the average Ca-O bond length (2.398 Å) in our compound. The larger size of the central calcium ion relative to magnesium also produces the fundamental difference in the two complexes, a coordination number of 8 in the former compared to 6 in the latter. This is also reflected in the bite angle of the ligands in the two complexes. The magnesium lactate compound has bite angles of 76.70(7)° and 76.23(7)° for the two unique chelating rings, while in the calcium complex, the bite angles for both the lactate ligand (64.3(3)°) and the lactic acid ligand (65.4(3)°) are smaller, again due to the larger coordination number around the central atom in the latter.

There is significant hydrogen bonding in the calcium complex isolated composed as it is of lactic acid/lactate ligands (Figure 7). The presumably strongest bond, previously discussed above, connects the carboxylic acid H(O) donor of the lactic acid ligand to the acceptor, the bound C–O− oxygen of the lactate ligand,
O – H···O. In addition, there are two other unique O – H···O hydrogen bonds in the structure. Both of these involve the OH hydrogen of the original alcohol group (O(3) in the lactic acid ligand and O(6) in the lactate ligand) hydrogen bonding to the free (unbound C=O) oxygen atom in a ligand of the opposite type (O(5) in the lactate ligand and O(2) in the lactic acid ligand). These hydrogen bonds are presumed to be somewhat weaker than the first since they are characterized by longer H···O distances (see table S10 in the Supplemental Content). Figure 6 shows how the six (3 × 2) strong hydrogen bonds formed by one central molecule connect it to two adjacent molecules forming extended chains through the crystal.

There are also a number of weaker interactions located in the solid state structure of Ca(CH$_3$CHOHCOO)$_2$(CH$_3$CHOHCOOH)$_2$. These fall generally into two broad categories, weak C – H···O hydrogen bonds and the even weaker C – H···H – C contacts. Based on the distances recorded and the atoms involved, all of these interactions are far weaker than the O – H···O hydrogen bonds just discussed. Most importantly, nearly every one of these interactions occurs in the plane perpendicular to that defined by the strong hydrogen bonds. These weaker interactions thus link the chains of strongly bound molecules together.

The isolation of the solid product, Ca(CH$_3$CHOHCOO)$_2$(CH$_3$CHOHCOOH)$_2$, created an interesting dilemma within our original experimental concept. Obviously, from the chemical formula of the isolated complex, the demineralization of crab shells with lactic acid could require up to four moles of lactic acid to release one mole of calcium. This is in contrast to HCl where two moles of acid are required to liberate one mole of calcium and form one mole of CaCl$_2$. The use of lactic acid could thus require up to twice as much acid as the use of HCl (in terms of molar ratio) not to mention that it is also much less efficient in terms of chemical activity. It is unlikely that a system based on lactic acid alone will ever be useful for the demineralization of crab (or other) shells on an industrial scale. While it still may be possible to implement a bacterial system of demineralization based on the in situ generation of lactic acid, for chemical systems, HCl will be superior, though a mixed lactic acid–HCl system might also still prove to be feasible.

4. Conclusion

The use of lactic acid as an alternative to HCl in the demineralization of green crab shells was found to be between 1.14 and 2.28 M. As the temperature of the reaction increased, the rate of demineralization also increased. However, with a small increase in the time of reaction, similar results could be achieved at room temperature as were found at 60°C. Industrially, this could result in substantial energy savings.

For the first time, a well-characterized by-product of the reaction between lactic acid and crab shells was isolated and characterized. X-ray crystallography showed it to be Ca(CH$_3$CHOHCOO)$_2$(CH$_3$CHOHCOOH)$_2$, a calcium lactate–lactic acid complex.

The use of waste products in industrial processes is an important concept that must be implemented as the need for greener materials increases. The use of an organic acid (lactic acid) was investigated as a promising alternative to strong acids (HCl) for the demineralization step in the production of chitosan from green crab shells. However, our final results do not bode well for the use of lactic acid in this demineralization. The possible requirement of a higher molar ratio of lactic acid to calcium carbonate, a ratio of 4:1 is needed to synthesize Ca(CH$_3$CHOHCOO)$_2$(CH$_3$CHOHCOOH)$_2$, may be too high to make this process commercially viable. However, it is possible that a mixed acid system (lactic acid and HCl) could overcome this problem.

Disclosure statement

No potential conflict of interest was reported by the authors.

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