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Effect of functionalized multiwalled CNTs on the selective formation of calcium oxalate crystals by electrocrystallization

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ABSTRACT: The electrocrystallization (EC) of calcium oxalate (CaOx) crystals in the presence of multiwalled CNTs (MWCNT) functionalized with itaconic acid (IA) and the monoester derivatives mono-methylitaconate (MMI) or mono-octadecylitaconate (MODI), which were used as new IA-ester templates supported on indium tin oxide (ITO) glass substrate as working electrode, were performed by applying a 9-mA current at 37 °C and 60 °C for 5 min. Under the above EC reaction conditions, a broad variety of CaOx morphologies and crystal forms was

found. The morphology control and coexistence of CaOx monohydrate (COM) and CaOx dihydrate (COD) was achieved through *in vitro* EC according to XRD spectra. We found that all the functionalized MWCNTs were more efficient inhibitors of CaOx crystallization than the typical citrate model, where MWCNT-IA was the most effective stabilizing template of COM crystals due to the fact that carboxylic acid groups of IA moieties in MWCNT-IA would be better Ca^{2+} ions-binding sites than IA ester groups.

KEYWORDS: Multiwalled carbon nanotube (MWCNT), itaconic acid (IA), calcium oxalate, electrocrystallization, CaOx monohydrate (COM), CaOx dihydrate (COD)

1. INTRODUCTION

Biomineralization is the formation of biogenic minerals with biological origins, such as vertebrate bone and teeth, invertebrate shells and exoskeletons and even small particles secreted by plants and bacteria.[1,2] Kidney stones (KS) are the result of pathological biomineralization caused by the uncontrolled nucleation of CaOx.[3] KS can occur in different animal species; CaOx is one of the most copious minerals present in plants and is the most abundant mineral found in urinary calculi produced by mammals, including humans.[4,5]

CaOx is known to have three structural main forms: the monoclinic monohydrate (COM, whewellite), tetragonal dihydrate (COD, weddellite) and triclinic trihydrate (COT).[6,7] These hydrates differ in their size, morphology and stability, and COM is the most stable.[8] COD and COM are often found in urinary calculi, inducing crystal agglomeration, and COM strongly adheres to the epithelium of renal cells.[9] Instead, COD avoids the formation of KS by adsorbing urinary molecules, thus decreasing urolithiasis risk.[10] Therefore, studying the nucleation process

of KS has led to a renewed interest in evaluating the effects exerted by different additives on CaOx crystallization. The controlled crystallization of CaOx has been performed in organic media using synthetic molecules and natural biomolecules as inhibitors.[11,12]

A method rarely used to evaluate the role of additives on the mineralization of oxides and inorganic materials is the electrocrystallization (EC) technique known as electrodeposition method, in which that electrochemical deposition is a general method of fabrication of oxide or mineral coating. [13-15] This method can be used to direct oriented crystallization or selectively control the crystal morphology. EC seems to be an appropriate method for understanding the different aspects of biomineralization due to there are few instances of the study of polymorphism of inorganic mineral by electrochemistry, parameters such as nucleation and crystal growth could be addressed, and could even be utilized to study the stabilization of metastable forms.[16] Joseph and Kamath found that CaOx nucleation was affected by changing the pH (9 and 11), current density (3 mA and 6 mA) and temperature (23 °C and 65 °C). The research to date has established that the morphology, size and type of CaOx crystals are controlled by not only the crystallization method but also the variation of experimental parameters such as the concentration, molar ratio of the reactants, pH, time and effect of temperature has been studied in the *in vitro* CaOx crystallization.[17]

On the other hand, multiwalled CNTs (MWCNTs) have been used for diverse high-tech applications, such as catalysis, hydrogen storage, nanoelectronics, optics, solar cells, medicine, among other application areas. Carbon-based nanomaterials and CNTs have been extensively investigated as sensitive rheological additives for a range of nanocomposites. In spite of its long success in these diverse applications, MWCNTs have been sparsely used as template for the mineralization of calcium-salts, which is probably due to their hydrophobic character and

chemical inertness.[18-20] However, combination of carbon-based nanomaterials with calcium-salts such as calcium carbonate, hydroxyapatite, carbides, silica - titania oxide materials, among others have been utilized, since the combination of these materials offers great functional advantages over conventional materials currently employed in construction.[21] Moreover, carbon allotropes such as fullerenes and single walled CNTs also influence the synthesis of $CaCO_3$ materials resulting in amorphous phases with unique and interesting morphologies.[22] In case of CaOx, abundant natural urinary macromolecules and plant extract have been also tested as modifier acting as inhibitor of COM crystallization kinetics *in vitro*.[23,24] The precise interactions of these macromolecules with COM are yet unknown. The type of CNTs functionalization *e.g.* covalent and non-covalent reported by Tasis *et al*. demonstrated that the interface between the carbon nanomaterial and the growth medium of the inorganic mineral are extremely important as well as tubular structure of CNTs providing more favorable morphology for inorganic materials as reported for hydroxyapatite when compared to other carbon-base materials.[25,26] Therefore, the grafting of hydrophilic molecules such as IA to the highly hydrophobic CNT allows obtaining new template with potential Ca^{2+} ions-binding capability. The strategy used here may contribute to broaden the use of functionalized MWCNTs with different hydrophilic molecules allowing to control selective *in vitro* crystallization of variety of inorganic minerals. With this background in mind, we believe that the selective chemical modification of MWCNTs may allow the control of the nucleation and growth of inorganic materials to achieve nanostructures with defined sizes, shapes, and compositions.[27,28] On the other hand, the kinetic aspects of *in vitro* CaOx crystallization using active modifier molecules at different concentrations and temperatures is the basis for *in vivo* assays and is the first approach for the urolithiasis medication. Indeed, for *in vivo* delivery of drugs using implantable peristaltic mini pumps allows to foresee the classic

problems such as of solubility, concentration, control of small-volume of release, dose-response behavior to achieve a greater effect of pathological crystal inhibition using urolithiasis drugs.

The aim of this paper is to report the *in vitro* EC of CaOx in the presence of MWCNTs modified with itaconic acid (IA) and monoester derivatives, namely, monomethyl itaconate (MMI) or monooctadecyl itaconate (MODI), and supported on an indium tin oxide (ITO) glass substrate, which is used as the working electrode by applying a 9-mA current for 5 min at 37 °C and 60 °C. Chrono-potentiometric measurements of CaOx were successfully performed with all the functionalized MWCNTs, and these results were compared with citrate. These conditions were selected by first considering the potential value, where the reduction of molecular oxygen (O_2 saturation) reached its highest activity and secondly this optimized potential was applied in order to assure a stable electrochemical response, controlled size and morphology of CaOx crystals.

The experimental setup and procedure used for the measurement of the EC of CaOx are shown in Figure 1.

Figure 1.

2. EXPERIMENTAL SECTION

2.1 Materials and Chemicals.

A detailed description of the materials, chemical reagents, oxidation and functionalization of the MWCNTs is available in the Supporting Information.

2.2. Electrocrystallization of CaOx.

The *in vitro* EC of CaOx was performed for 5 min on ITO substrates heated to either 37 °C or 60 °C, and a 9-mA current was applied in the absence or presence 0.03 mg/mL MWCNT in an electrochemical cell (ECC). The negative and positive controls were prepared by performing the

EC of CaOx in the absence of the additive and in the presence of sodium citrate, respectively. Briefly, for the EC of CaOx assays, 50 mM calcium nitrate tetrahydrate solutions and 75 mM ethylenediaminetetraacetic acid were mixed and sonicated for 5 min, and the pH was adjusted to 10.5 by adding 1 M NaOH dropwise. This solution was mixed with a 50 mM sodium oxalate solution containing 0.75 mg of each MWCNTs and sonicated for 5 min. Then, 25 mL of the resulting electrolytic solution was poured into the glass ECC. To test the effect of all MWCNTs as templates on the CaOx ECC a chrono-potentiometry was performed. The applied potential range was +/- 10 V with a sample interval of 2 sec with the applied current of 9-mA. The ECC measurements were conducted using ITO substrates as the working electrodes (WE), a coiled platinum (Pt) wire as the auxiliary electrode (AE) and a silver chloride electrode (Ag/AgCl) as the reference electrode (RE). The washing and sonication protocols are provided in the Supporting Information.

2.3 Characterization.

The surface morphology and microanalysis of the resultant CaOx crystals were observed by scanning electron microscopy (SEM) coupled with energy dispersive X-ray (EDX) analysis; the elemental analysis was performed with computer-controlled software. The SEM-EDX analysis was carried out by using a JEOL JSM-IT300LV microscope (JEOL USA Inc., USA) and an Aztec EDX system (Oxford Instruments, Abingdon, UK). A Hitachi TM3000 tabletop microscope (Hitachi High Tech) coupled with Quantax 70 EDX spectrometer (Bruker, Germany) and equipped with a high-sensitivity BSE semiconductor detector was also used. The FTIR analyses of the pristine, oxidized and functionalized MWCNTs were performed by Fourier transform infrared spectroscopy/Attenuated total reflection (FTIR-ATR) using an Interspectrum Interspec p/n 200-X instrument. TEM images of MWCNTs used as templates were obtained with

a Philips Tecnai 12 Bio Twin 120 K transmission electron microscope. Chrono-potentiometry was carried out during the CaOx EC using a Potentiostat/Galvanostat BASi Epsilon (USA) instrument. Powder X-ray diffraction (PXRD) of the CaOx crystals was performed by using a Siemens D-5000X X-ray diffractometer with Cu-Kα radiation (graphite monochromator) and an ENRAF Nonius FR 590 X-ray generator. The crystal structure of CaOx was determined by using Cu-Kα radiation (40 kV), step sizes of 0.2°, and the geometric Bragg-Brentano (θ-θ) scanning mode with an angle (2θ) in the range of 5-70°. The DiffracPlus program was used as the data control software.

3. RESULTS AND DISCUSSION

To study the effect of MWCNTs functionalized with IA and IA derivatives on CaOx crystallization, a preliminary set of CaOx crystals prepared by EC were examined using two experimental setups, which consisted of an open or a closed EC system in the absence of MWCNTs template and with a current applied from 1- 24 mA for 5 min at 23 °C, 37 °C and 60 °C (Table 1).

Table 1.

In addition, the chrono-potentiometric measurements were obtained during the EC of CaOx (Figure S1), and an optical and electron microscopy analyses of the CaOx crystals in both the EC systems, where a 1 - 24 mA current was applied for 5 min at 23 °C, 37 °C and 60 °C, were carried out (Figure S2). The potentiometric response in the ECC during the CaOx EC was larger

and more stable in the closed system, and the same electrochemical behavior was observed at all the abovementioned temperatures. Therefore, after analyzing the results obtained at different temperatures and applied currents as well as the optical and electron microscopy and chrono-potentiometry results, we focused this study to evaluate the effect of the morphology and polymorphism modulation capability of the pristine and functionalized MWCNTs on the *in vitro* EC of CaOx by applying a 9-mA current at 37 °C and 60 °C. Moreover, the elemental chemical composition of the CaOx crystals obtained in the open and closed EC systems was confirmed by EDX (Table S1). Figure 2 shows the chrono-potentiometric measurements obtained during the EC of CaOx by using both the open and closed EC systems via applying a 9-mA current for 5 min at 37 °C and 60 °C. The same electrochemical behavior was observed for both of the systems at 37 °C and 60 °C. Figure 2A shows that the potentiometric response curves for the open system begins at a lower potential value of 1.73 V with respect to the closed system, which starts at 1.84 V, and the final potential values were 2.37 V and 2.09 V at 37 °C and 60 °C, respectively. We found that the starting and final potentiometric response curves recorded during the open EC system were lower than those recorded for the closed system. Figure 2 B shows the potentiometric response curves realized in the closed EC system, where the initial potential values started at 2.06 V and 2.62 V, and the final potential values were 2.35 V and 2.94 V at 37 °C and 60 °C, respectively. Comparing the two experimental results, it can be seen that the closed EC system of CaOx was more stable and exhibited an independent potentiometric response at 37 °C and 60 °C.

Figure 2.

The experimental procedures of the *in vitro* EC of CaOx and the physicochemical characterization of the MWCNTs can be found in the Supporting Information. The Fourier

transform infrared (FTIR) spectra of pristine MWCNT, MWCNT-IA, MWCNT-MMI and MWCNT-MODI are shown in Figure S3. The presence of O-H groups of adsorbed water in pristine MWCNT, the stretching vibrations of the vinyl C=C bonds and carboxylic groups of IA in MWCNT-IA, as well as absorption bands corresponding to C=C vinyl double bond, C=O of carboxyl and ester groups of MMI in MWCNT-MMI and MODI in MWCNT-MODI were confirmed by FTIR spectroscopy. All absorption bands designation of MWCNTs functionalized with IA, MMI and MODI were discussed in detail in the section 1.4 of the SI. The morphology of the MWCNTs was analyzed by transmission electron microscopy (TEM). The TEM images of pristine MWCNT and all functionalized MWCNT-IA, MWCNT-MMI and MWCNT-MODI templates are shown in Figure S4 in the Supporting Information. Similar TEM images of MWCNTs have already been reported by our group.[29] The polar surface of MWCNTs may attract Ca^{2+} ions, indicating a preferred interaction site on specific crystal surface faces during the nucleation and crystal growth of CaOx in a manner similar to biomolecules in biogenic minerals. Then, crystal habit is determined by the relative rates of growth of different crystal faces, with the slow growing surfaces dominating the final form. It is believed that these faces may be stabilised via stereoselective adsorption of the carboxylic acid groups via bidentate caroboxylate binding. Figure 3 shows the chrono-potentiometric measurements obtained during the EC of CaOx using 0.03 mg/mL functionalized MWCNTs and by applying a 9-mA current in a closed system at 37 °C or 60 °C for 5 min. This short amount of time has been reported by us to be a sufficient amount of time to induce the formation of CaOx crystals via the EC assays.[30]

Figure 3.

It is seen that both citrate and the functionalized MCWNTs inhibit the formation of CaOx crystals by EC at 37 °C and 60 °C, and MWCNT-IA is the most efficient inhibitor in both the EC

systems. The order in which the functionalized MCWNTs templates inhibit crystal formation was found to be MWCNT-IA > MWCNT-MMI > MWCNT-MODI > citrate. The more noticeable inhibition effect of MWCNT-IA was recorded at 60 °C. The higher temperature allows for a more homogeneous suspension of the CNTs in the electrolytic solution, facilitating the adsorption/inclusion of MWCNT-IA in the crystalline lattice. This is probably associated with a better disentanglement of the functionalized MWCNTs.

The inhibition effect of citrate and all the MWCNTs templates during the EC of CaOx by applying a 9-mA current in a closed system at 37 °C or 60 °C was compared with the control experiment, although more CaOx crystals precipitated under these conditions (Figure S5). We found that when the EC was performed at a higher temperature, the potential value increased for each applied current at 37 °C and 60 °C. It was also found that the potentiometric response curves begin to stabilize by increasing the temperature, and the effect of the system decreases the variation between each point measured in the EC of CaOx. In addition, we have reported that anionic MWCNTs also acted as efficient inhibitors in the *in vitro* $CaCO_3$ crystallization.[31]

Herein, MWCNT-IA was a better inhibitor template than the well-known organic citrate, which was used to inhibit the EC of CaOx at 37 °C and 60 °C. The morphology (Figure 4) and hydrated crystal forms (Figure 5) of CaOx obtained via EC using citrate and all the MWCNTs by applying a 9-mA current for 5 min in a closed system at 37 °C or 60 °C were analyzed by SEM and XRD, respectively. Figure 4 shows the SEM images of representative CaOx crystals obtained in the presence of all the functionalized MWCNTs and citrate via EC by applying a 9-mA current at 37 °C. The SEM analysis of CaOx under the same experimental conditions but at 60 °C was also performed (Figure S6). In general, Figure 4 shows that when citrate was used as the additive at 37 °C, few undifferentiated round CaOx crystals (4 μm) were obtained (Figure

4A-B). When MWCNT-IA was used, abundant round COM crystals (5 μm) with porous surfaces were found at 37 °C (Figure 4C-D). When MWCNT-MMI was used, scarce COD crystals (5 μm) and small undifferentiated pieces were observed at 37 °C (Figure 4E-F). Rough COM and COD crystals (40 μm) were observed at 37 °C when MWCNT-MODI was utilized (Figure 4G-H).

Figure 4.

The optical and SEM analyses of the CaOx crystals obtained when MWCNTs were used as templates and by using the chrono-potentiometry method via EC are summarized in Table 2.

Table 2.

In addition, an SEM-EDX analysis of the surface of the ITO substrates was also performed to determine the elemental composition of the deposited CaOx crystals. In the absence of additives, the chemical composition of CaOx did not differ from theoretical predictions. As is known, XRD is a more suitable technique than SEM-EDX to identify inorganic constituents in KS by their unique diffraction patterns, which allows the identification of unknown crystalline substances.[14,32-34] Therefore, XRD was performed on the CaOx crystals formed on the surface of the ITO substrates via the EC experiments by applying 3, 9, 15, and 18 mA currents at 37 °C and 60 °C (Figure S7). In general, the XRD patterns of the CaOx crystals electrodeposited on ITO were quite similar to each other. Figure 5 (A-D) shows the XRD patterns of the CaOx crystals grown in the presence of citrate and all the functionalized MWCNTs when a 9-mA current was applied at both 37 °C and 60 °C. The XRD patterns showed diffraction peaks at 2θ = 15 °, 23.2 °, 24.2 °, 25.8 ° 30 °, 35 °, 50 ° and 53 °, confirming the coexistence of COD and COM crystals. It was found that the intensities of the crystallographic peaks of COM and COD were small, and both hydrates were promoted at these temperatures. Surprisingly, the diffraction peak

corresponding to the (200) plane of COM at 15° did not appear when MWCNT-IA was utilized as template at both temperatures.

Figure 5.

The differences between the diffractograms of the CaOx crystals obtained using control samples and all the MWCNTs templates are also presented (Tables S2, S3, S4 and S5). The XRD analysis demonstrated that the sampled obtained using functionalized MWCNTs are capable of modulating the nucleation and growth of CaOx crystals obtained via EC.[30]

Prior EC studies on CaOx obtained by the cathodic reduction of acidic aqueous solutions containing calcium and oxalate ions have highlighted the importance of electrodeposition to the polymorphic modification of CaOx crystals.[15] These authors found that the stabilization of the COD was favored when an electric current density of 6 mA/cm^2 was applied at 23 °C, whereas the formation of COM crystals was promoted when an electric current density of 3 and 6 mA/cm^2 were applied at 65 °C. Both hydrates showed oriented growth with respect to the substrate under different deposition conditions. Therefore, COD is formed first, and then, it transforms to COM in a manner similar to that occurs in nature, namely, by dissolution and reprecipitation mechanisms. The COM crystals were stabilized at a pH of 11 and with an electric current density of 6 mA/cm^2 applied at 65 °C. The current study demonstrated that when functionalized MWCNTs are used as template via the EC of CaOx, the selective control of the morphology and polymorphism of CaOx can be achieved, and thus, the deposition of COM and COD crystals on ITO is promoted, which act as effective inhibitors of the pathological COM polymorph. It is known that the inorganic mineral deposition occurs only in the immediacy of the conducting ITO´s surface and not in the bulk of electrolytic solution.[15,30] Therefore, the mechanism of the modulation of CaOx during the EC process occurs by the adsorbed MWCNTs

used as template onto ITO surface via electrostatic interaction (Figure 6 A-B). In general, Figure 6 illustrates how carboxylic acid (COOH) groups of adsorbed MWCNT-IA onto ITO surface (Figure 6A) are more prone than ester groups of adsorbed MWCNTs functionalized either with MMI (Figure 6B) or MODI to form electrostatic interaction with Ca^{2+} ions. However, the presence of unreacted COOH groups on the surface of functionalized MWCNTs could also interact with Ca^{2+} ions. The aforementioned mechanism is backed by our experimental data, where the zeta potential presented negative values for functionalized MWCNTs at pH 4.0, in which the *in vitro* EC of CaOx occurs and positive values for pristine MWCNT.[29] In addition, we found that the concentration of unreacted –COOH groups, determined by an automated acid-base titration, are still present in the range of 12.0 - 15.0 mmol/g for these functionalized MWCNTs templates instead of *ca.* 1.8 mmol/g for the pristine MWCNTs.[29] Therefore, the crystal habit in both cases of functionalized MWCNTs template is the resulting of all electrostatic interactions determined by the relative rates of growth of different crystal faces, with the slow growing surfaces dominating the final form.

Figure 6.

Moreover, we believe that by varying the electrical current during chrono-potentiometry, it is possible to detect the precise moment when the ITO substrate is covered with CaOx crystals or to assess an electrochemical approach to determine when the transformation of COD to COM occurs. We found that the potentiometric response curves of the CaOx crystals obtained using a closed system for the *in vitro* EC showed a significant and constant increase in the potential values at both temperatures, which were maintained over time. The electrochemical approach for the deposition of inorganic minerals under laboratory conditions is relatively cheap, fast and simple.[35] We identified the experimental conditions necessary to carry out the *in vitro* EC of

CaOx to evaluate the effect of different functionalized MWCNTs, which can also be used to generate natural or synthetic molecules and nanomaterials. The formation of CaOx crystals was reproducible, as shown by the statistical analysis (Figures S8, S9 and S10). In this regard, the GLM model was adjusted according to its theoretical quantiles (Figure S8), treatments (Figure S9) and temperatures (Figure S10). We used citrate as the positive control in the EC of CaOx, because this molecule is widely used in the symptomatic treatment of human and mammalian urolithiasis.[12,36] The applied statistical model allowed for the prediction of the data analysis in an optimum manner. The tests of the marginal hypotheses indicated that there were treatment and temperature effects. The Bonferroni correction was used due to the large amount of data (Table S6). The treatment effect between citrate and the rest of the treatments showed that MWCNT-IA has a lower average (Table S7) and that there was a temperature effect on the means of the treatments, which were all more efficient at a higher temperature (Table S8). These values are similar to each other for treatments at 37 °C and 60 °C but are different than the control, and thus, this was deemed to be an appropriate comparison with the functionalized MWCNTs.[11,36] The EDX results obtained via EC were similar to those reported by Kumar et al.[32] and Didenko et al.[37] The XRD patterns of CaOx allowed us to compare the obtained diffractograms with those known in the literature.[11,35-37] A mixed crystalline XRD pattern was identified, where COM corresponded to the predominant crystallographic peaks, but COD was also found.

Our results indicate that the functionalized MWCNTs induce morphological changes of CaOx and could control the polymorphism of CaOx by the direct interaction of MWCNTs containing carboxylic or ester groups with specific facets of CaOx crystals in a manner similar to how urinary proteins induce effects on CaOx.[38] Citrate and MWCNT-IA, which are both anionic compounds, can bind Ca^{2+} ions during the EC of CaOx in a better way than MWCNT-MMI and

MWCNT-MODI. This is why it is very important to analyze the electrochemical solution to determine the amount of Ca^{2+} in solution and to understand how urinary molecules prevent the formation of KS.[39] We state that the experimental conditions are indicators of the modulation of the EC of CaOx. MWCNTs induces changes in the morphology and acted in an active way to control the nucleation and growth of CaOx crystals. In fact, MWCNT-IA inhibits the (200) plane of COM, which corresponds to the peak at 15° in the XRD pattern, in a similar manner as biomolecules behave in mammalian calculi. Oxalate ions may attract Ca^{2+} ions, causing CaOx crystals to form; however, if the Ca^{2+} ions are not available in the electrolytic solution due to their interactions with MWCNTs, the Ca^{2+} ions may not bind oxalate. Carboxylic acid groups are better than ester groups for binding Ca^{2+} ions and thus inhibit nucleation and control the growth of CaOx crystals.[40]

4. CONCLUSIONS

The ability of MWCNTs functionalized with itaconic acid (IA) and IA esters (MMI and MODI), which are used as template, on the nucleation, growth and the formation of different CaOx hydrate crystals via *in vitro* EC by applying a 9-mA current at 37 °C and 60 °C for 5 min was demonstrated. The order in which the templates inhibit the formation of different hydrate forms of CaOx was found to be MWCNT-IA > MWCNT-MMI > MWCNT-MODI > citrate. The mechanism of the modulation of CaOx during the EC can be explained by the adsorbed MWCNTs template on ITO glass substrate. It was found that the electrostatic interaction of COOH groups of IA moieties in MWCNT-IA would be better Ca^{2+} ions-binding sites than IA ester groups in MWCNT-MMI and MWCNT-MODI. In addition, types and morphologies of

CaOx crystals including the COM and COD hydrates was stabilized by the EC of CaOx applying a 9-mA current at 37 °C and 60 °C for 5 min.

ASSOCIATED CONTENT

Supporting Information.

Detailed synthesis and experimental procedure. Figures S1-10 and Tables S1-9. Electronic Supplementary Information (ESI) available: The experimental procedures for the functionalization of MWCNTs, the EC of CaOx, the statistical strategy used to study the electrochemical response obtained by EC, the spectroscopic, optical, and SEM-EDX analyses of CaOx and the TEM analyses of the MWCNTs are given in the Supporting Information.

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Author Contributions

The manuscript was written using contributions of all the authors. All the authors have approved the final version of this manuscript.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

EC, electrocrystallization; CaOx, calcium oxalate; CNT, carbon nanotube; MWCNT, multiwalled CNT; IA, itaconic acid; MMI, monomethyl itaconate; MODI, monooctadecyl itaconate; COM, CaOx monohydrate; COD, CaOx dihydrate; KS, kidney stones; ITO, indium tin oxide.

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FIGURE CAPTIONS

Figure 1. The experimental setup and procedure used to study the EC of CaOx.

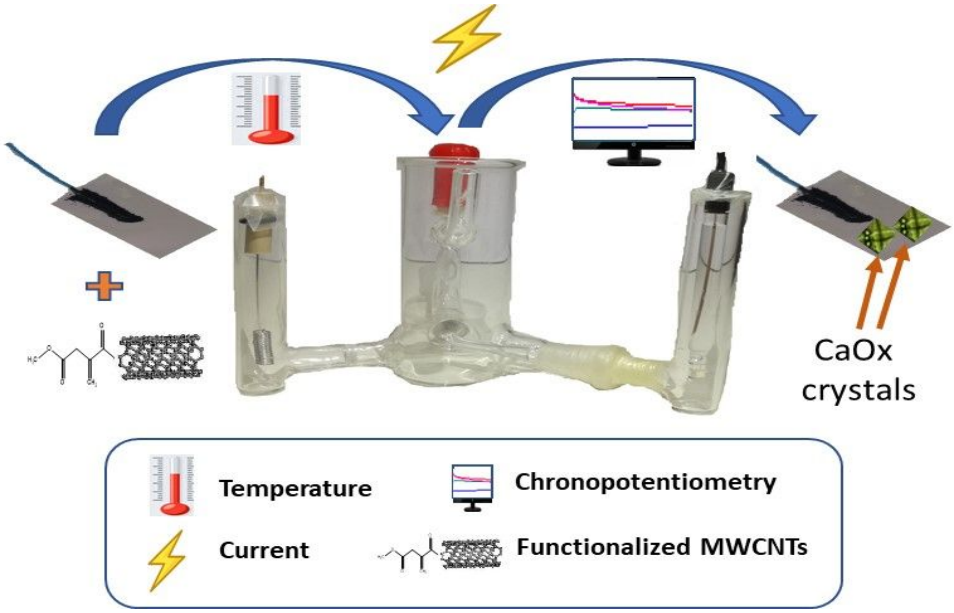
Figure 2. Chrono-potentiometric measurements obtained during the EC of CaOx in (A) an open system and (B) a closed system by applying a 9-mA current for 5 min at 37 °C or 60 °C.

Figure 3. Chrono-potentiometric measurements obtained during the EC of CaOx with 0.03 mg/mL functionalized MWCNTs and by applying a 9-mA current in the closed system for 5 min at (A) 37 °C and (B) 60 °C.

Figure 4. SEM of the CaOx crystals obtained via EC by applying a 9-mA current at 37 °C with (A,B) citrate, (C,D) MWCNT-IA, (E,F) MWCNT-MMI and (G,H) MWCNT-MODI.

Figure 5. XRD patterns of the CaOx crystals obtained via EC and grown in the presence of (A) citrate, (B) MWCNT-IA, (C) MWCNT-MMI and (D) MWCNT-MODI on ITO substrates with a 9-mA current applied at 37 °C and 60 °C. The COM and COD crystals are marked with the letters M and D, respectively.

Figure 6. Schematic representation of electrostatic interaction between adsorbed MWCNTs used as template onto ITO surface and CaOx during EC. (A) MWCNT-IA and (B) MWCNT-MMI.



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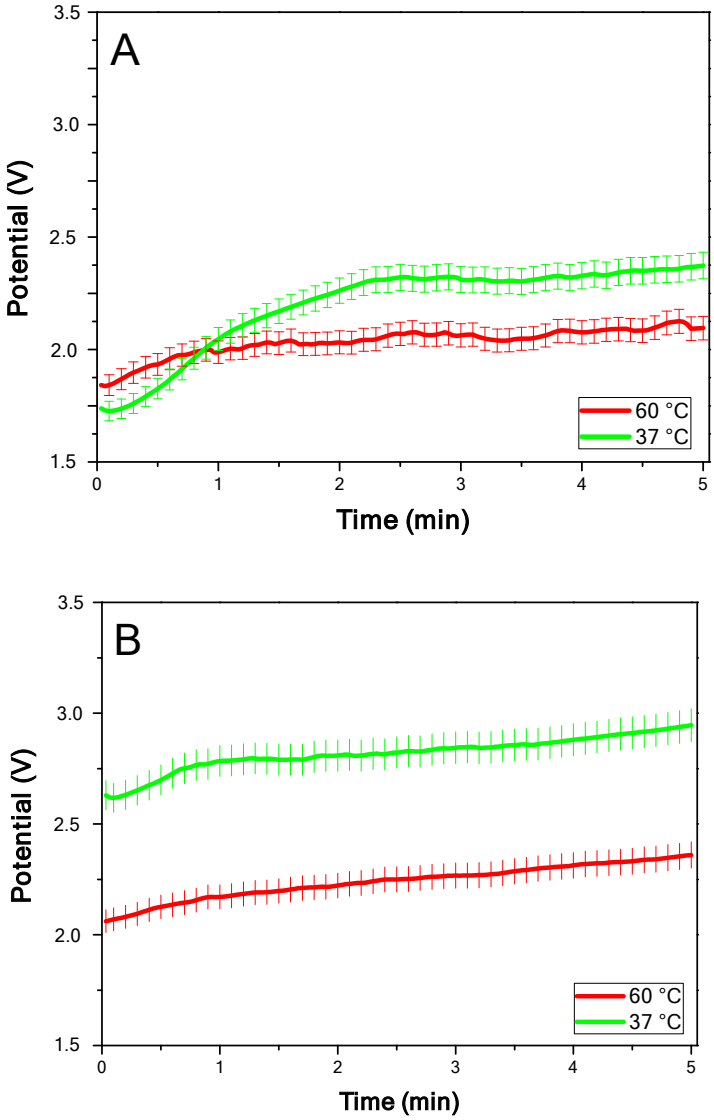

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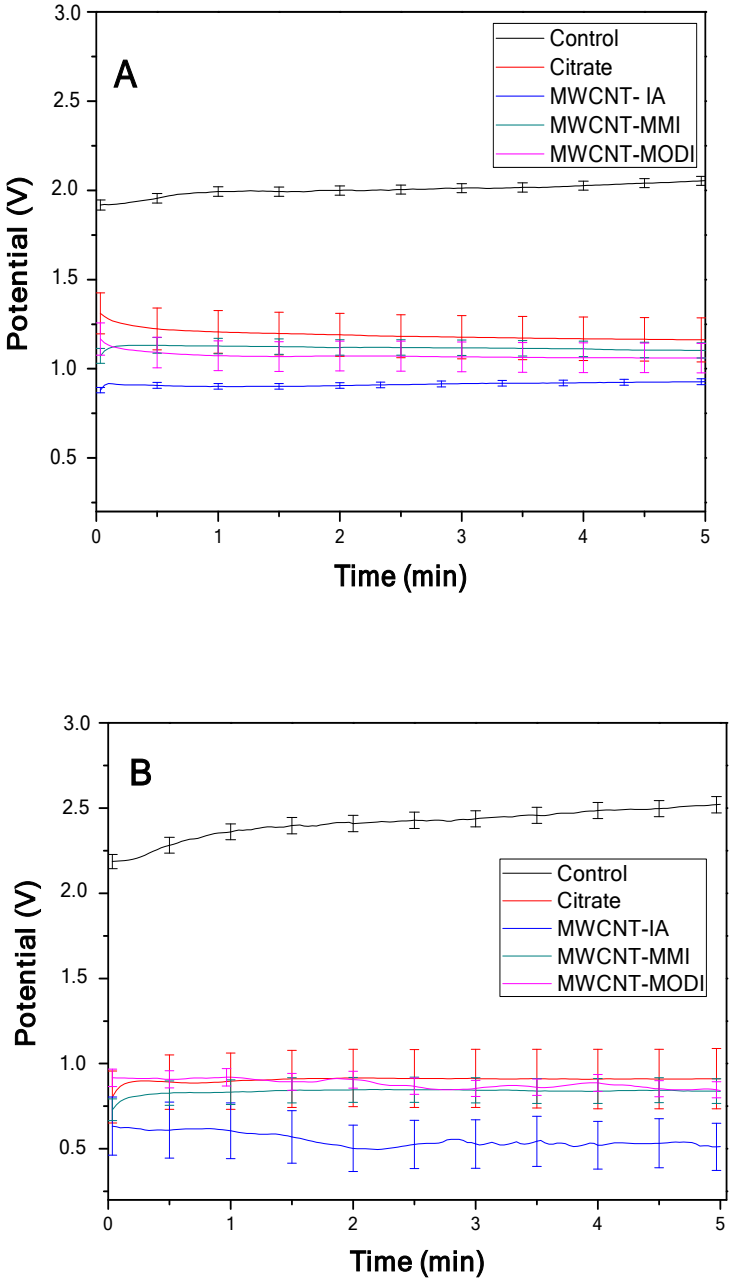

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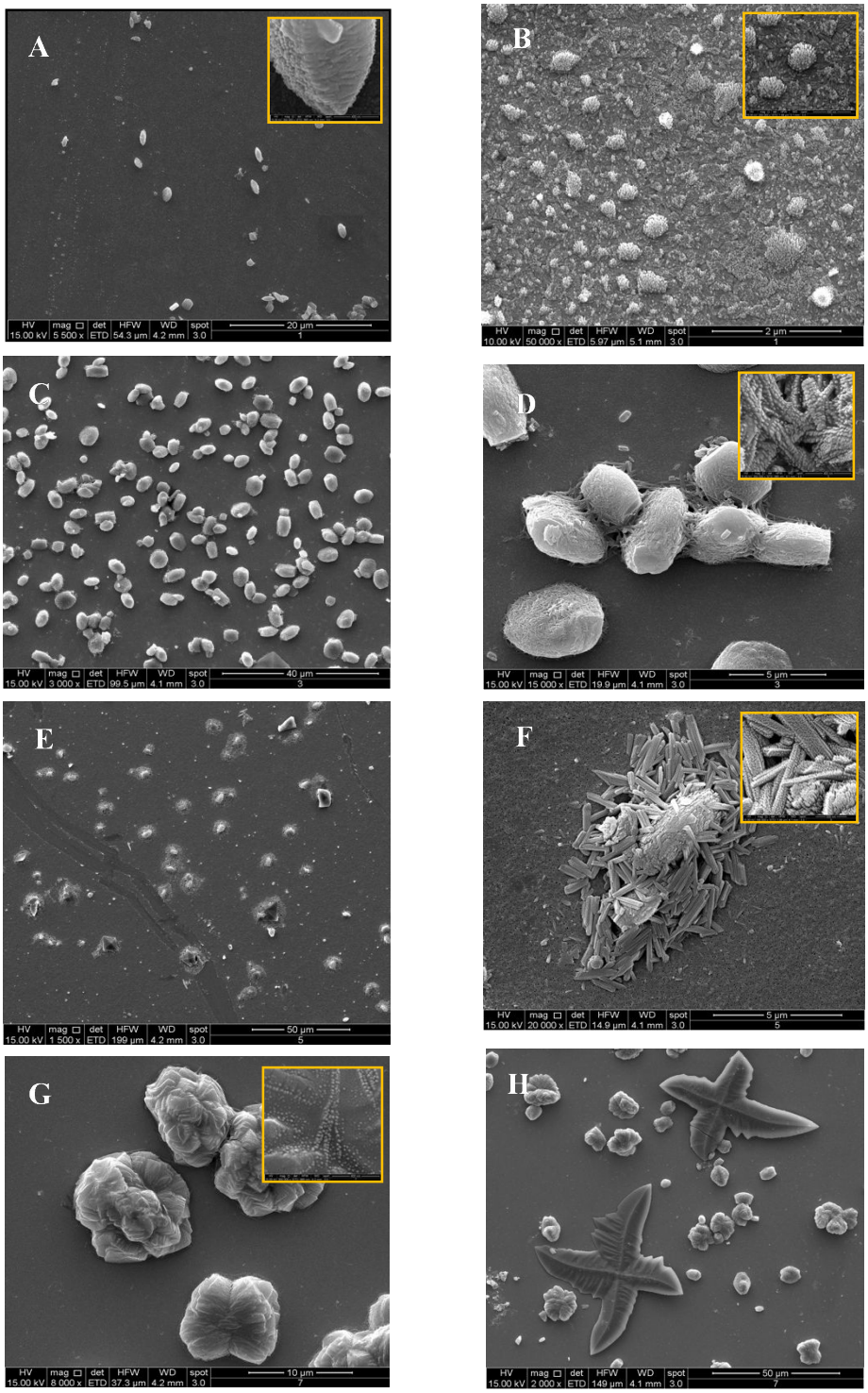

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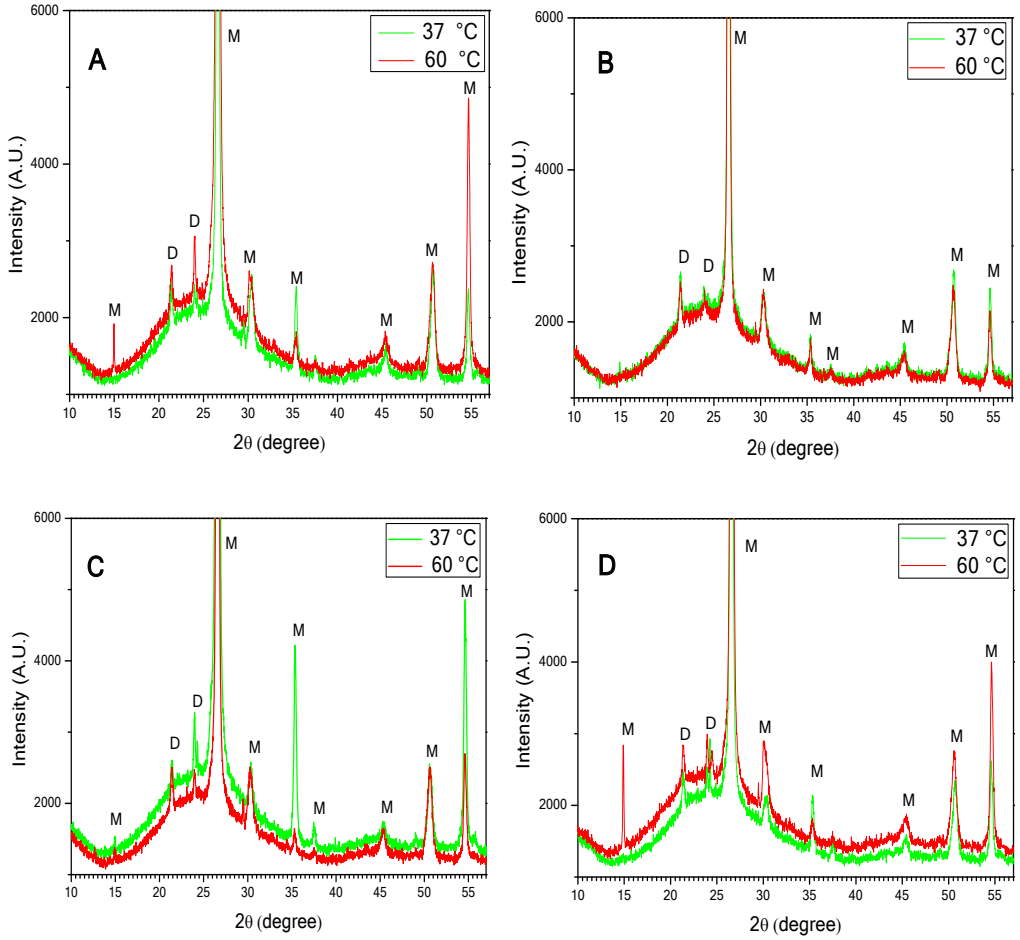

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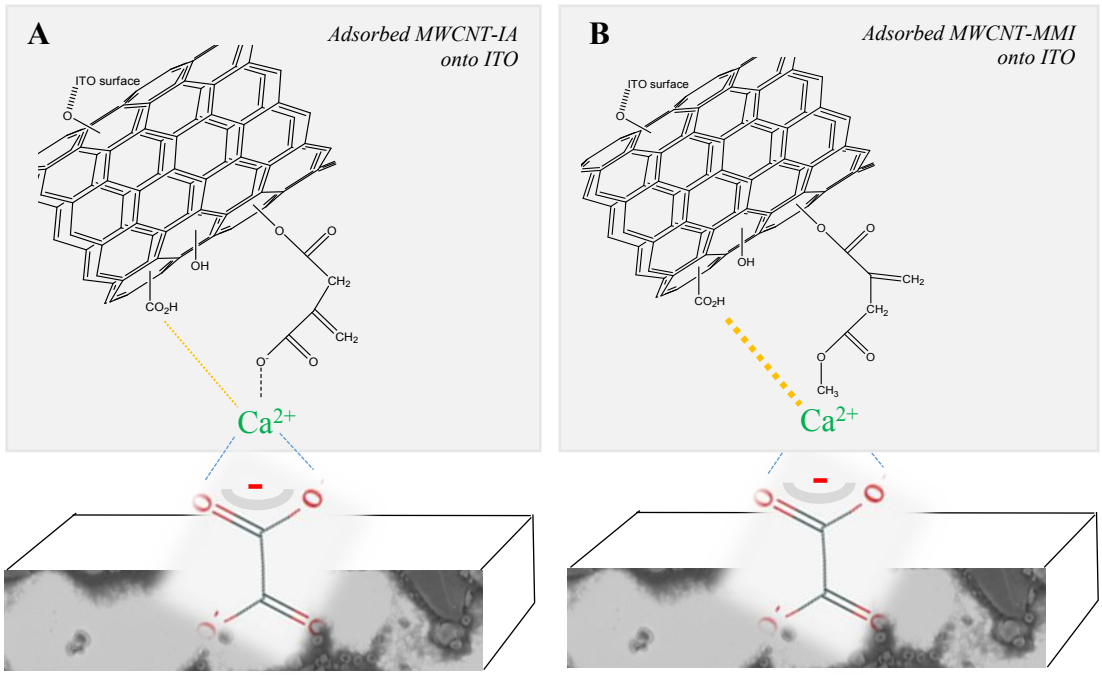

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TABLE CAPTIONS

Table 1. Optical and scanning electron analyses of the CaOx crystals obtained in the absence of templates via EC by using the chrono-potentiometry method.

Table 2. Optical and scanning electron analyses of the CaOx crystals obtained in the presence of templates via EC by using the chrono-potentiometry method.

Table 1. Optical and scanning electron analyses of the CaOx crystals obtained in the absence of templates via EC by using the chrono-potentiometry method.

System	Current (mA)	Temperature (°C)	Main features
Closed	1	37	Abundant crystals
Open	1	60	Large crystals, 120 μm
Closed	1	37	Abundant crystals
Open	3	60	Aggregated COD crystals
Closed	3	60	Typical COM crystals
Open	6	60	Large crystals, 100 μm
Closed	6	60	Aggregated typical COM crystals
Open	9	60	Large crystals of COD, 60 μm
Closed	9	60	Aggregated crystals.
Open	12	60	Large crystals, 80 μm
Closed	12	60	Aggregated crystals
Open	15	60	Large crystals, 60 μm
Closed	15	60	Aggregated COD crystals
Open	18	37	Filamentous crystals
Closed	18	37	Aggregated crystals
Open	21	37	Aggregated crystals
Closed	21	60	Abundant aggregated crystals
Open	24	60	Crystals with undefined shapes

Table 2. Optical and scanning electron analyses of the CaOx crystals obtained in the presence of templates via EC by using the chrono-potentiometry method.

Templates	Current (mA)	Temperature (°C)	Main features
Control	9	37	Undifferentiated small crystals
Control	9	60	Abundant and small COM crystals, 4 μm
Citrate	9	37	Few round crystals, 4 μm. Additionally, COD and COM crystals
Citrate	9	60	Abundant COM, 4 μm, and COD, 10 μm crystals
MWCNT-IA	9	37	Abundant round porous COM crystals, 5 μm
MWCNT-IA	9	60	Abundant COD and COM crystals
MWCNT-MMI	9	37	Few COD crystals, 5 μm and COM crystals with undifferentiated shapes
MWCNT-MMI	9	60	Agglomerated of COD and COM crystals, 40 μm
MWCNT-MODI	9	37	COD crystals, 40 μm, and rough COM crystals
MWCNT-MODI	9	60	Abundant COD crystals, 4 μm and COM crystals, 2 μm

Effect of functionalized multiwalled CNTs on the selective formation of calcium oxalate crystals by electrocrystallization

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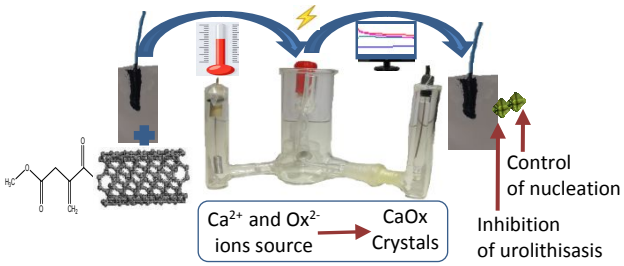

We report the use of inhibitor templates of CaOx crystallization that are more efficient than citrate by using multiwalled CNTs functionalized with itaconic acid and itaconic acid esters via an EC technique at 37 °C and 60 °C.