Anal. Bioanal. Electrochem., Vol. 16, No. 9, 2024, 809-816
https://www.doi.org/10.22034/abec.2024.716094

Analytical &
Bioanalytical
Electrochemistry

2024 by CEE
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Full Paper

Electrochemical Evaluation of Lemon Juice as a Chelating Agent for Calcium Oxalate Crystal Prevention in Crystalluria

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Received: 11 March 2024 / Received in revised form: 1 September 2024 /
Accepted: 9 September 2024 / Published online: 30 September 2024

Abstract- Crystalluria, characterized by the formation of calcium oxalate crystals, is a common urinary disorder. While increasing urinary volume is a traditional approach to manage this condition, novel therapeutic strategies are being explored. Lemon juice (LJ), rich in citric acid, is of particular interest due to its calcium ion chelating properties. This study evaluated the in vitro potential of LJ to inhibit calcium oxalate crystal formation. Using specialized electrodes (CPE-GC) and various electrochemical techniques (SWV, CV, LV, EIS), we characterized the interactions between LJ and calcium ions. Our findings, obtained from a simplified model using calcium carbonate, demonstrate the ability of LJ to effectively complex calcium ions, thereby reducing their availability for crystal formation. Although these results are promising, it's important to note that the experimental conditions do not fully replicate the complexity of physiological urine. In vivo studies are therefore necessary to confirm the efficacy of LJ in a clinical setting. In conclusion, lemon juice appears to be a promising approach for preventing calcium oxalate crystal formation. Its mechanism of action, based on calcium ion chelation, offers an alternative to conventional treatments. However, further research is required to evaluate its clinical efficacy and optimize its usage.

Keywords- Crystalluria; Calcium oxalate; Lemon juice; Chelation; Electrochemical methods

1. INTRODUCTION

Urine is a complex solution containing a variety of dissolved substances. However, its composition can become unstable, leading to the dissociation of calcium oxalate (CaC_2O_4) into calcium (Ca^{2+}) and oxalate ($C_2O_4^{2-}$) ions, which can then aggregate and form crystals [1,2]. The detection of these crystals in urine (crystalluria) is a common occurrence, especially in individuals with urolithiasis (urinary stone disease) compared to healthy people [3-10].

Analyzing urinary sediment plays a vital role in diagnosing and monitoring urolithiasis, both inherited and acquired [11-13]. The presence of specific crystal types in the urine provides valuable clues for doctors to diagnose the underlying condition and determine the most appropriate treatment. Additionally, monitoring changes in crystal types over time helps assess the effectiveness of implemented therapies [14].

Crystalluria often involves the presence of calcium oxalate crystals, and it can be caused by various factors such as metabolic disorders, medications, or even physiological changes [15,16,17]. These crystals are major contributors to nephrolithiasis (kidney stones), a painful condition [18-20].

This study aims to evaluate the effect of lemon juice (LJ) on calcium ions (Ca^{2+}), focusing on its potential to inhibit the formation of these crystals. To better understand this effect, we used calcium carbonate ($CaCO_3$) as a source of Ca^{2+}. Lemon juice is rich in citric acid, a natural chelating agent capable of binding to calcium ions and preventing them from forming crystals. In our approach, Carbon Paste Electrode Modified by Green Clay (CPE-GC) were used to selectively detect Ca^{2+} ions and to study the interaction between these ions and the components of lemon juice. The evaluation was performed using various electrochemical techniques, including Cyclic Voltammetry (CV), Linear Voltammetry (LV), Square Wave Voltammetry (SWV), and Electrochemical Impedance Spectroscopy (EIS). These techniques allowed for the characterization of the chelation process of Ca^{2+} ions by lemon juice and to determine its effectiveness in preventing the formation of CaC_2O_4.

2. EXPERIMENTAL SECTION

In this section, the details of the electrochemical measurements conducted during this study are presented, focusing on the characterization of LJ chelation effect on Ca^{2+} ions using CPE-GC sensors.

2.1. Reagents and chemicals

All reagents were of analytical grade and were used in this study without further purification. Carbon graphite powder, paraffin oil, and green clay (100% natural) were purchased from Merck Chemicals. All solutions were prepared using deionized water. Lemon juice was freshly squeezed from lemons.

2.2. Electrochemical apparatus

Electrochemical measurements were carried out in three-electrode cells using a potentiostat/electrochemical analyzer driven by general electrochemical systems software for data processing. The working electrode was a Carbon Paste Electrode Modified by Green Clay (CPE-GC). A Saturated Calomel Electrode (SCE) served as the reference electrode, and a platinum wire Counter Electrode (CE) completed the three-electrode system.

2.3. Electrode preparation

CPE-GC was prepared by meticulously hand-mixing paraffin oil with specific amounts of carbon powder and green clay powder in a mortar and pestle to achieve a uniform consistency. The resulting paste was then packed and smoothed onto its respective electrode [21].

The modification of the carbon paste electrode with green clay was undertaken to enhance its electrochemical performance. Green clay, rich in minerals such as magnesium, calcium, and iron, possesses a layered structure that increases the active surface area of the electrode. This structure facilitates enhanced electrostatic interactions with calcium ions (Ca^{2+}), thereby improving the sensitivity and selectivity of the electrode for detecting these ions. The inclusion of green clay not only optimizes the interaction with Ca^{2+} ions but also ensures the electrode's stability and biocompatibility, making it well-suited for electrochemical applications involving biological samples. This modification is crucial for accurately assessing the chelation effect of lemon juice on Ca^{2+} ions, as it maximizes the efficiency of ion detection and interaction in the analytical process.

2.4. Electrochemical measurement and characterization

To assess the electrochemical behavior of the modified electrodes and investigate their Ca^{2+} ion chelation capacity with LJ, a suite of electrochemical techniques was employed. These techniques included Cyclic Voltammetry (CV), Linear Voltammetry (LV), Square Wave Voltammetry (SWV), and Electrochemical Impedance Spectroscopy (EIS). These methods provided valuable insights into the interaction and potential redox reactions between Ca^{2+} ions, LJ, and the modified electrode surfaces.

The combined application of these techniques allows for a comprehensive and detailed analysis of the electrochemical interactions between Ca^{2+} ions and LJ. CV provides a broad view of the redox processes of Ca^{2+} ions over a wide potential range, offering an overview of electrochemical reactions. LV gives insights into peak currents as a function of potential variations, useful for observing changes in Ca^{2+} ion concentration. SWV, with its high sensitivity, is crucial for detecting fine variations in electrochemical signals. Lastly, EIS is used to assess electron transfer resistance at the electrode/solution interface, helping us understand the impact of LJ on the availability of Ca^{2+} ions for electrochemical reactions. By integrating these techniques, we obtain a multidimensional and robust evaluation of LJ's chelation capacity.

3. RESULTS AND DISCUSSION

3.1. Behavior of CPE-GC electrode towards Ca^{2+} ions

The cyclic voltammetry (CV) of a modified electrode, denoted as CPE-GC, was investigated in the presence and absence of Ca^{2+} ions. The electrolyte solution consisted of 0.3 M NaCl with 0.03 g Ca^{2+} ions for the experiment involving Ca^{2+} (Figure 1).

The presence of Ca^{2+} ions significantly impact the CPE-GC electrode's CV response. As observed in Figure 1, the voltammogram displays two distinct redox peaks:

✓ A well-defined cathodic peak at around -1 V during the negative scan (cathodic scan) indicates the reduction of Ca2+ ions to metallic Ca.

✓ A broad anodic peak at 1 V during the positive scan (anodic scan) corresponds to the oxidation of the deposited metallic Ca. This broadness likely originates from the oxidation of Ca unevenly distributed within the natural green clay of the CPE-GC electrode.

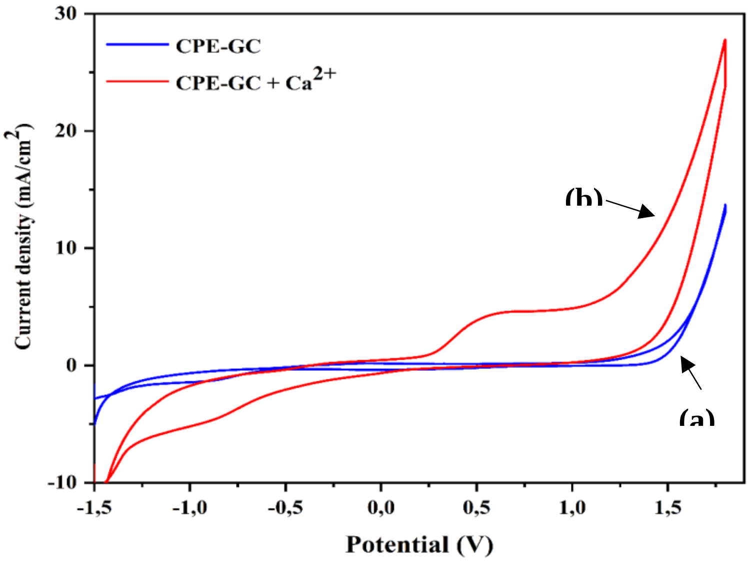

Figure 1. CV recorded at CPE-GC at a scan rate of 50 mV/s in 0.3 M NaCl solution, (a) without Ca^{2+} ions, (b) with Ca^{2+} ions

3.2. Lemon juice chelation effect on Ca^{2+} ions

Figure 2 presents the impact of lemon juice (LJ) volume on the corresponding linear voltammetry (LV) profiles. The investigation employs three distinct LJ volume levels. Analysis of the LV data unveils a significant reduction in Ca^{2+} oxidation peak currents with increasing LJ volume (Figure 2). This intriguing observation suggests the potential of LJ to effectively chelate Ca^{2+} ions, thereby mitigating calcium oxalate formation and potentially reducing the risk of crystalluria.

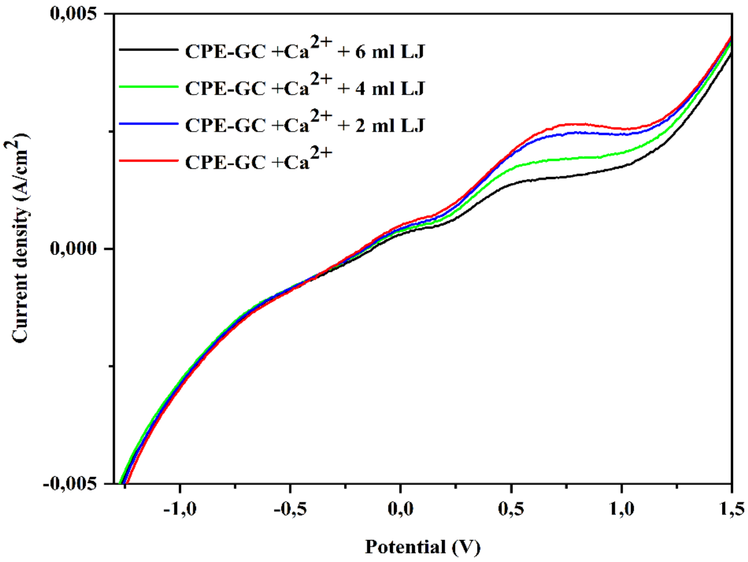

Figure 2. LV recorded at CPE-GC at a scan rate of 50 mV/s in 0.3 M NaCl solution, with Ca^{2+} ions and lemon juice (LJ).

SWV confirms the effect of lemon juice on Ca^{2+} ions. Figure 3(a) shows a distinct peak at around 1.25 V, corresponding to the redox reaction of Ca^{2+} ions. This peak almost completely disappears with the addition of lemon juice (Figure 3(b)). This observation is important because it indicates that lemon juice affects the electrochemical reactions involving Ca^{2+} ions.

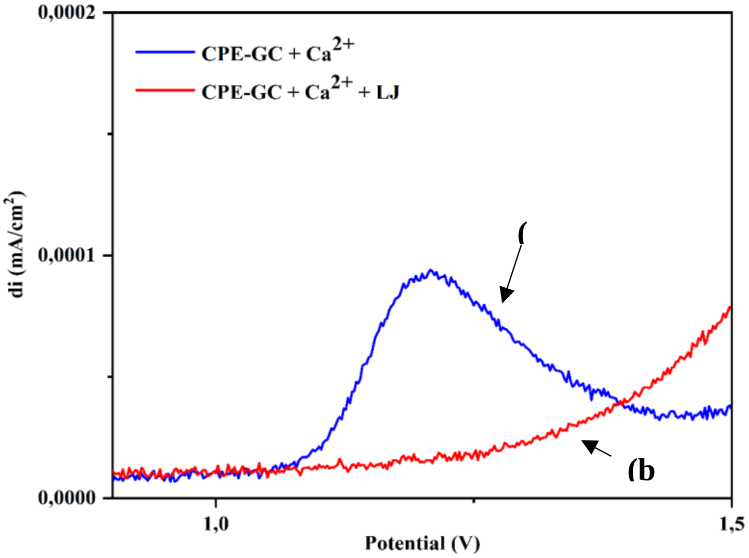

Figure 3. SWV recorded at CPE-GC at a scan rate of 50 mV/s in 0.3 M NaCl solution, (a) with Ca^{2+} ions, (b) with Ca^{2+} ions and lemon juice (LJ)

Figure 4 illustrates the effect of Ca^{2+} ion chelation by lemon juice on electron transfer resistance, as measured by electrochemical impedance spectroscopy (EIS). In the absence of lemon juice, the semicircle exhibits a smaller diameter, indicating a low resistance to electron transfer. This suggests the presence of Ca^{2+} ions that facilitate this transfer at the electrode/solution interface. In contrast, with lemon juice, the diameter of the semicircle increases considerably, indicating a high resistance to electron transfer. This suggests that Ca^{2+} ions have been chelated by lemon juice, reducing their available concentration for electron transfer. The increase in electron transfer resistance in the presence of lemon juice confirms the chelation of Ca^{2+} ions by lemon juice. This chelation could have important implications for electrochemical processes involving Ca^{2+} ions, such as the detection of calcium oxalate, which is responsible for crystalluria.

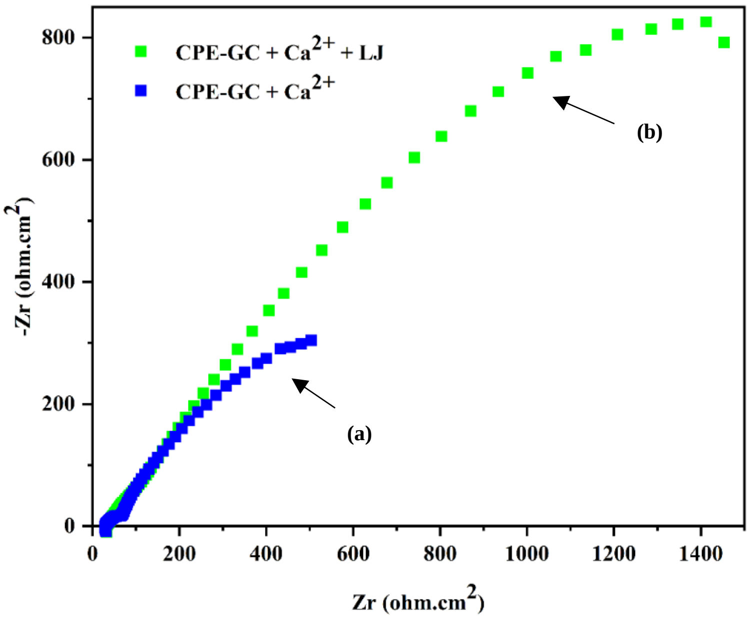

Figure 4. EIS recorded at CPE-GC at a scan rate of 50 mV/s in 0.3 M NaCl solution, (a) with Ca^{2+} ions, (b) with Ca^{2+} ions and lemon juice (LJ)

Figure 5 shows the effect of lemon juice on the electrochemical impedance spectroscopy (EIS) diagrams over time, from 5 to 15 minutes. Analysis of the EIS data reveals a notable trend: the electron transfer resistance increases with time. The increase in electron transfer resistance can be explained by the presence of citric acid in lemon juice. Citric acid has the ability to chelate calcium ions (Ca^{2+}), which play a crucial role in electron transfer at the interface. The chelation of Ca^{2+} ions by citric acid reduces their available concentration for electron transfer, hindering the process and explaining the observed increase in resistance.

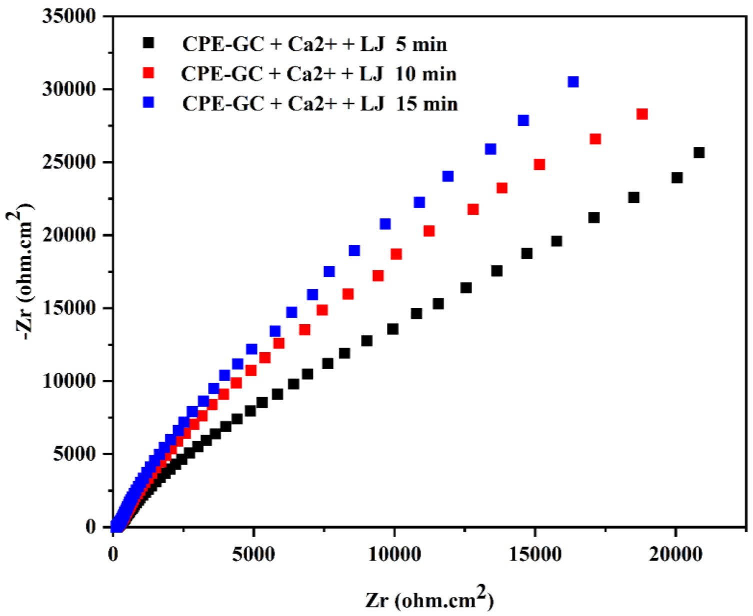

Figure 5. EIS recorded at CPE-GC at a scan rate of 50 mV/s in 0.3 M NaCl solution, with Ca^{2+} ions and lemon juice (LJ)

4. CONCLUSION

This study demonstrates the efficacy of lemon juice as a calcium ion chelator, attributed to its high citric acid content. Advanced electrochemical techniques, combined with the use of calcium carbonate as a calcium ion source, reveal that lemon juice significantly inhibits the formation of calcium oxalate crystals by forming stable complexes with calcium ions. While this study was conducted under controlled laboratory conditions, it establishes a strong foundation for understanding the chelation mechanism and the potential benefits of lemon juice in preventing crystallization.

To validate the clinical relevance of these findings, further research is imperative. Future studies should focus on analyzing real urine samples with varying compositions, considering factors like pH and oxalate levels. Such investigations will provide a more comprehensive understanding of lemon juice's impact on crystal formation in a physiological setting. Despite the controlled nature of this study, the encouraging results suggest that lemon juice could be a promising complementary therapy for preventing crystalluria. Additional research is warranted to explore its potential clinical applications and optimize its use.

Declarations of interest

The authors declare no conflict of interest in this reported work.

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