IN VITRO STUDY OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF OXALIC ACID-DERIVED BIOACTIVE CHELATING AGENT

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ABSTRACT

Objective: The aim of this study is to evaluate the biological potentials of sodium cadmium oxalate dihydrate complex prepared by a single diffusion method in the silica gel medium.

Methods: The present complex was derived by the oxalic acid using a single diffusion method in gel medium and tested for single-crystal X-ray diffraction, ultraviolet-visible (UV) spectrum (190–500 nm), and solubility (distilled water at 20–29°C) studies. Antioxidant activity was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay.

Results: The present chelating complex was successfully synthesized by gel technique. The solubility of sodium cadmium oxalate dihydrate was moderately good for deionized warm water. The UV spectral studies confirmed the chelating O-H, Na-O, and C-O bonds of the newly synthesized complex for the optical and biological properties.

Conclusions: The metal-oxygen and oxygen-hydrogen bonds of the present newly synthesized sample much improved its optical, antimicrobial, and antioxidant activities and find its applications in the field of pharmaceutical and biomedical applications.

Keywords: Sodium cadmium oxalate, Single-crystal X-ray diffraction, UV, Solubility, Biological activities.

INTRODUCTION

Oxalic acid is a natural chemotherapeutic void of troubling side effects and is a normal element in human blood and must be available to the immune system to fight against the diseases of cancer, viral, bacterial, and vascular conditions. It has a mean value of 288 mg of anhydrous oxalic acid/100 ml of blood. When it drops below an effective level, the immune system can no longer protect the body from various diseases [1]. In the body, oxalic acid is joined with divalent cadmium (Cd²⁺) metal to form crystals of the corresponding oxalates, which are then expelled in urine as minute crystals. The use of an oxalate to combine with a metal (as Fe, Na, Cd, Zn, and Cu) in the body to form a chelate so that the metal loses its toxic effect or physiological activity is known as the chelating agents which are found to be potential candidates for the removal of heavy metals in the series of trisodium citrate > disodium oxalate > sodium sulfate has been reported [2]. The adequate chelating efficiency of sodium or potassium mixed metal complexes of oxalate has been reported [3] due to their better solubility in water. In addition to that, metal chelates have a tendency to increase the biological activities through the charge carrier distribution process by the presence of intermolecular hydrogen O-H…O bonds [4]. Cd-O, and Na-O asymmetric stretching bonds [5]. Hence, the chelating O-H…O bonds are liable to increase the hydrophilic and lipophilic properties of the central metal ions, probably leading to liposolubility and permeability through the lipid layer of cell membranes [6,7]. Recently, an octahedral cadmium complex having a tetradentate acyclic ligand with antimicrobial potency has been reported [8]. Some cadmium complexes have also been exposed to biological properties in vitro [9]. The significance of the present study mainly focuses the biological activities of the sodium cadmium oxalate by studying the better optical conductivity of the as-grown crystal to find its usefulness in the field of pharmaceutical and biomedical applications.

METHODS

The high-purity essentials such as cadmium chloride (LOBA Chemie, 99.95% assay), sodium chloride (LOBA Chemie, 99.95% assay), oxalic acid (LOBA Chemie, 99.99% assay), and sodium metasilicate (LOBA Chemie, 99.9% assay) with AR grade were used as the starting materials in the single diffusion chemical reaction method at a temperature of 29°C. ENRAF NONIUS CAD4 X-ray diffractometer (XRD) equipped with Mo Kα radiation was worked to estimate the unit cell parameters of the as-grown crystals. The various analyses were carried out at ambient temperature. The diffuse reflectance spectral analysis was carried out with the help of a LAMBDA 35 UV-visible spectrophotometer. The reflection spectrum was traced to the range of 190–1100 nm. The antimicrobial activity was assayed against Escherichia coli (Gram-negative), Staphylococcus (Gram-positive), Aspergillus niger, and Candida albicans (fungus) using the agar well diffusion method [10]. Nutrient agar (Merck, Germany) was used as solid media for preparing the nutrient plates while the Mueller-Hinton broth was applied as liquid culture media in biological tests. Bacterial cultures such as Escherichia coli (Gram-negative), Staphylococcus (Gram-positive), Aspergillus niger, and Candida albicans (fungus) were bought from the Eumic Analytical Lab and Research Institute, Tiruchirappalli, Tamil Nadu, South India. Bacterial strains were maintained on nutrient agar slants (HiMedia) at 4±0.2°C. Microbial cultures were subcultured in liquid medium (nutrient broth) at 37±0.2°C for 8h and further used for the test (10⁻¹⁰CFU/mL). These suspensions were prepared immediately before the test was carried out. The antioxidant activity of sodium cadmium oxalate dihydrate was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay.

Synthesis of sodium cadmium oxalate material

The sodium cadmium oxalate crystal was grown by single diffusion technique in the silica gel medium. The gel was prepared to set in a glass tube (length 200 mm and diameter 25 mm) using sodium metasilicate (specific gravity 1.04 g-cm⁻³) and oxalic acid (1 M) in 10:9 volume ratio. The observed pH of gel solution was 4.75. Gel formation took about a week. A mixture of cadmium and sodium chloride solutions was then added slowly along the walls of the glass tube. The chloride solution diffused deep into the gel as indicated by
the Liesegang rings and their movements. The supernatant solution of the cadmium chloride was diffused into the gel column and reacted with the electrolyte sodium hydroxide mixed oxalic acid solution lead to the making of sodium cadmium oxalate crystal is given in the following reactions (1) and (2).

\[ 2Na_2SiO_3 + 6H_2O \rightarrow 2H_2SiO_4 + 4NaOH \]  
(1)

\[ 4NaOH + 4H_2C_2O_4 \rightarrow Na_2[Ca(C_2O_4)_4]_2H_2O + 4HCl \]  
(2)

Subsequent diffusion of a supernatant solution to room temperature was improved that the structure of thick, transparent, and well-formed rectangular shaped colorless sodium cadmium oxalate dihydrate material is formed into the gel.

Particle size

The particle size of the sodium cadmium oxalate dihydrate was measured and refined by 0.30 mm × 0.25 mm × 0.20 mm\(^3\) using the full matrix least square technique employing the SHELXL program of single crystal XRD analysis method.

RESULTS AND DISCUSSION

Single-crystal XRD analysis

In the structure of sodium cadmium oxalate [Na\(_2\)Cd\(_2\)(C\(_2\)O\(_4\))\(_4\)H\(_2\)O]\(_n\) complex as exhibited in Fig. 1, the repeat unit has two oxalate groups, one cadmium (II) atom, two sodium atoms, and four water molecules. The present compound crystallized in monoclinic, space group P\(_{2_1}/c\), with a=12.83, b=11.43, c=14.11, \(\beta=113.02^\circ\), \(V=1908.86\)\(A^3\), \(M=1481.84\), and \(R=0.0234\) based on 3752 observed reflections was calculated by single-crystal XRD method using SHELXL program. The result of the structural analysis is shown in Table 1 inform that the structure of the present complex is formed by the Na-O and Cd-O bonds in the two-dimensional network is further linked by the O-H-O hydrogen bonds to stretch into the three-dimensional structure. The oxalate ligand has an inversion center of the midpoint of the C-C bond. Intermolecular hydrogen bonds [0-H...O] improve the optical conductivity of the as-grown crystals. The eight oxygen atoms from the oxalate units and the water molecules bind the cadmium atom of the (Cd-O), (O-Cd), (Na-O), and (O-Na) bond distances are in the range of 2.3075\(\pm\)0.24627 A\(^2\) and 2.3633\(\pm\)0.23930 A\(^2\) build the repeated binding units of the sodium cadmium oxalate crystal into the polycrystalline material [11]. The centrosymmetric dimers of carboxylic acids are attributed to intermolecular resonance-assisted hydrogen bonds for the greater optical activities [12]. In the polymeric packing of sodium cadmium oxalate crystal, the framework stability is maintained by an extensive hydrogen bond network (Table 2) involving all 0 atoms of cadmium oxalate anion, sodium, and water molecules. The H\(_2\)O molecules (Fig. 2) of the polymeric [Na\(_2\)Cd\(_2\)(C\(_2\)O\(_4\))\(_2\)H\(_2\)O]\(_n\) crystal form the O-H...O hydrogen bonds in which the resonance-assisted strong hydrogen bond (O-H) is attributed to the optimized optical activities of the bimetallic crystal have been reported [13].

![Fig 1: Atom numbering arrangement of the sodium cadmium oxalate structure](image)

| Selected oxygen-hydrogen (O-H), cadmium-oxygen (Cd-O), and sodium-oxygen (Na-O) bond lengths of sodium cadmium oxalate crystal |
| --- |
| O (17)-H (17A) | 0.835 (16)±0.03 |
| O (17)-H (17B) | 0.830 (17)±0.02 |
| O (18)-H (18A) | 0.837 (17)±0.01 |
| O (18)-H (18B) | 0.825 (17)±0.02 |
| O (19)-H (19A) | 0.841 (17)±0.04 |
| O (19)-H (19B) | 0.835 (17)±0.02 |
| O (20)-H (20A) | 0.821 (18)±0.03 |
| O (20)-H (20B) | 0.825 (18)±0.01 |

| Selected sodium-oxygen and cadmium-oxygen bond lengths (Å)* |
| --- |
| Na (1)-O (6) | 2.2379 (16)±0.02 |
| Na (1)-O (6) #10 | 2.2379 (16)±0.02 |
| O (6)-Na (1) | 2.2006 (15)±0.03 |
| O (6)-Na (1) #10 | 2.2006 (15)±0.03 |
| 0 (6)-Na (3) | 2.3446 (18)±0.04 |
| 0 (6)-Cd (1) | 2.3840 (17)±0.01 |
| O (3)-Cd (1) | 2.3619 (16)±0.02 |
| Cd (1)-O (14) #7 | 2.3101 (15)±0.01 |
| Cd (1)-O (16) #7 | 2.4475 (15)±0.03 |

*Mean±SD

Ultrasound-visible-near infrared (UV) diffuse reflectance and absorption spectral studies

The diffuse reflectance from the absorbance spectrum of as-grown crystal in the UV-visible-NIR region was recorded in Fig. 3a. The optical band gap is determined by plotting absorbance versus energy of the material as shown in Fig. 3b. The linear fit of the curve on the energy axis is yielded the band gap as 4.2±0.3 eV reveals that the high optical conductivity of the as-grown crystal leads to the better biological activities. The UV-Vis reflectance spectrum of the present complex shows two peaks at 245.94 nm and 310.34 nm. The sharp absorption peaks observed in the UV spectrum are confirmed the electronic transitions to the Na\(^+\) ion in the sodium cadmium oxalate complex. The first peak at 245.94 nm can be attributed to \(\pi-\pi^*\) transition to the metal-oxygen (M-O) bonds within the sodium cadmium oxalate unit, while the peak at 310.34 nm would be due to \(\pi-\pi^*\) transition to the oxygen-hydrogen (O-H) group of intermolecular hydrogen bonds of the water molecules [14]. On coordination complex, \(\pi-\pi\) transition to ligand shifts to a longer wavelength. This indicates the coordination of ligand to metal. The cadmium ion has an oxidation number equal to +2 and coordination number equal to "8" due to the coordination of oxalate anion to a metal as a bidentate chelate. The cadmium ion has ground state \(2\)D and the electronic spectra of the sodium cadmium oxalate dihydrate crystal are exhibited maximum at 245.94 nm move to ligand-to-metal charge transfer [15]. On the coordination complex about the strong intermolecular bond lengths (>2.3±0.03 Å), the maximum optical activity is perceived by the \(\pi-\pi^*\) transition to ligand shifts to a longer wavelength increase the chelating effect of the as-grown sample favors for the biological applications [16].

Solubility study

Solubility is one of the features for better biological studies of the as-grown sample has been reported [17]. Hence, the solubility of sodium cadmium oxalate was determined in the temperature range of 19°C to 32°C using double distilled deionized warm water as a solvent. Solubility studies were carried out in a constant temperature bath with the cryostat facility with an accuracy of ±0.03 K.

The solution was stirred constantly for an hour to achieve the stabilization. After attaining the saturation, the equilibrium concentration...
of the solute was estimated gravimetrically. The same process was continual and the solubility curves were observed to 0.04 g/100 ml for sodium cadmium oxalate dihydrate at different temperatures (Fig. 4). It is observed from the curve that the solubility is found to increase within the rise in the temperature for sodium cadmium oxalate was due to the soluble nature of sodium oxalate in water. This result revealed that the improved solubility demands the sodium cadmium oxalate dihydrate complex is for further pharmaceutical research and applications.

**Antimicrobial screening studies**

**Kirby–Bauer agar well diffusion assay**

The nutrient agar medium was prepared and sterilized by autoclaving at 121°C and 15 lbs pressure for 15 min, then poured the agar medium into the sterile Petri plates and allowed to solidify. Then, the bacterial broth culture was cleansed on each Petri plate using sterile buds. Finally, the wells were made by the good cutter. The organic solvent extracts of leaves were added aseptically. This procedure was repeated for each Petri plate and they were incubated at 37°C for 24 h. After incubation, the plates were observed in the zone of inhibition. The antimicrobial activity of metal complexes about the ligand of the as-grown title compound sodium cadmium oxalate dihydrate (Fig. 5) was tested by the disc diffusion technique. Results were compared with standard drug gentamycin at the same concentration. However, the antibacterial screening activity varied for different strains [18]. The increased bond length of the structure-activity relationships between Na-O, Cd-O, C-O, and O-H...O in sodium cadmium oxalate dihydrate improved the biological properties than the mononuclear complex [19,20] has been explained by the Overtone’s concept and Tweedy’s chelation theory [21,22]. According to Overtone’s concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials since the liposolubility is an important factor that reamins the antimicrobial activity. The increasing lipid solubility character of the metal chelate influences its permeation through the lipid layer of the microorganism which possibly leads to the breakdown of permeability barrier of cell process. During this process, increase to the lipophilic nature of the central metal (II) ions which favors its permeation more efficiently through the lipid layer of the microorganisms, thus building the chelate compounds is more antimicrobial activity [23]. It has also been observed that the concentration plays a key role in increasing the degree of inhibition so that when the concentration is increased, the antimicrobial activity also enhanced (Table 3). The quantitative assays gave minimum inhibitory concentration values of the range of 25–100 µg/ml are confirmed that the results are superior to the previously published values [24,25]. Coordination complex about antimicrobial active C=O part of metal(II) ions decreases the polarity of metal ions significantly due to the partial sharing of its positive charge with donor groups and delocalization of π electrons [26] over the whole chelate ring resulting in high degree of inhibition [27] is due to the more antimicrobial activity of the present sample.

**Table 2: Selected hydrogen bonds and bond angles of the studied crystals**

| S. No. | Hydrogen bond formation [O-H...O] | Bond length [O-H] (Å)* | Bond length [H...O] (Å)* | Bond length [O...O] (Å)* | Bond angle (OHO) (deg)* |
|--------|---------------------------------|------------------------|------------------------|------------------------|------------------------|
| 1      | O (1)-H (18)...O (7)#4           | 0.87 (3)±0.02          | 1.89 (3)±0.03          | 2.726 (2)±0.04         | 160 (3)±0.1            |
| 2      | O (2)-H (2B)...O (6)#1           | 0.83 (3)±0.01          | 1.88 (3)±0.04          | 2.6818 (15)±0.03       | 162 (3)±0.1            |
| 1      | O (7)-H (7A)...O (7)#6           | 0.87 (3)±0.02          | 1.89 (3)±0.05          | 2.726 (2)±0.02         | 160 (3)±0.1            |
| 2      | O (2)-H (2A)...O (5)#8           | 1.00 (3)±0.03          | 1.70 (3)±0.1           | 2.6918 (15)±0.01       | 168 (3)±0.1            |

*Symmetric stretching mode of oxygen-hydrogen atoms, *asymmetric stretching mode of hydrogen-oxygen atoms, *asymmetric stretching mode of oxygen-oxygen atoms, *Angle between OHO water molecules. *Mean±SD
Antioxidant analysis

DPPH radical scavenging activity

DPPH assay was performed following standard protocol [28]. The present synthesized sample at various concentrations (25–100 µg/ml) was added to a 100 µM solution of DPPH in methanol. The mixture was allowed to stand for 30 min to perform the complete reaction in the dark. The absorbance was measured at 517 nm using a UV spectrophotometer. The reduction in the absorbance of the DPPH solution indicates the free radical scavenging activity of the test sample. Methanol without the sample should be used as a control. DPPH radical scavenging activity will be calculated according to the following expression (1):

\[
\text{Radical scavenging activity (\%)} = \left(1 - \frac{A_s}{A_o}\right) \times 100
\]

Where, \(A_o\) = Absorbance of control and \(A_s\) = Absorbance of sample. Control was the test solution without sample. IC50 is defined as the amount of antioxidant required to inhibit 50% of DPPH free radical under the experimental conditions. Ascorbic acid was taken as a standard solution. The antioxidant activity of the synthesized sodium cadmium oxalate dihydrate was studied by DPPH radical scavenging assay and compared with ascorbic acid (AA) as standard. Free radicals of reactive oxygen species and nitrogen species generations of cells are normal physiological activity. The excessive formation of these radicals in the human body causes cell damage by combining with the biomolecules such as protein, carbohydrates, and lipids, and finally leading to various diseases such as cancer, coronary heart disease, aging, neuro-degenerative disorders, and diabetes [29]. Antioxidants play a significant role to control such oxidative stress situation by neutralizing these reactive radicals. Table 4 illustrates that DPPH scavenging activity is directly proportional to increase in the concentration of sodium cadmium oxalate dihydrate material. Sodium cadmium oxalate dihydrate showed an effective free radical scavenging activity of 84±0.13% at the concentration of 100 µg/ml, whereas the standard L-Ascorbic acid showed 75±0.2% scavenging activity of the same concentration. Due to the presence of the inter-molecular and intra-molecular O-H···O hydrogen bonds are one of the key parameters to the higher optical conductivity of the as-grown sample. The more reactive oxygen atoms of these hydrogen molecules are responsible for the higher antioxidant activity of the present sample than the previously reported transition metal oxalate crystals [30,31].

Table 3: Antimicrobial activity of sodium cadmium oxalate (II) complex

| Organism        | Minimum zone of Inhibition (mm)* | Control |
|-----------------|----------------------------------|---------|
|                 | Concentration (µg/ml)            |         |
|                 | 25  | 50  | 75  | 100 |
| **Escherichia coli** | 26±0.2 | 30±0.1 | 34±0.3 | 40±0.2 | 25±0.2 |
| **Staphylococcus** | 22±0.3 | 25±0.1 | 28±0.2 | 30±0.3 | 15±0.1 |
| **Candida albicans** | 20±0.3 | 22±0.2 | 24±0.2 | 26±0.1 | 18±0.3 |
| **Aspergillus niger** | 22±0.3 | 24±0.1 | 26±0.2 | 28±0.3 | 18±0.1 |

*Mean±SD

Table 4: Assessment of in vitro antioxidant activity of sodium cadmium oxalate complex and L-ascorbic acid in various concentrations

| Name of the compound | Concentration (µg/ml) | Calculated antioxidant activity (a.u.)* | IC50* |
|----------------------|-----------------------|----------------------------------------|-------|
| Sodium cadmium oxalate | 25 | 0.34±0.04 | 16±0.8 |
|                       | 50 | 0.28±0.02 |       |
|                       | 75 | 0.24±0.04 |       |
|                       | 100 | 0.14±0.02 |       |
| L-ascorbic acid       | 05 | 0.46±0.03 |       |
|                       | 10 | 0.43±0.02 |       |
|                       | 15 | 0.39±0.04 | 8±0.7 |
|                       | 20 | 0.36±0.03 |       |

*Mean±SD

CONCLUSIONS

Optically active single-crystal sodium cadmium oxalate has been obtained by single diffusion silica gel method. The results of the structural analysis were indicating that the complex formed by O-Na-O and O-Cd-O coordination bonds was a two-dimensional network structure which was further linked by O-H-O hydrogen bonds to give a three-dimensional structure. On adding sodium, the triclinic structure of cadmium oxalate transformed into a monoclinic structure was confirmed by single-crystal XRD studies. The optical behavior of sodium cadmium oxalate crystal was studied by using UV-visible-near infrared spectrum and found to be 98.4% of the transmittance. The wide transparency was due to the strong intermolecular O-Na, O-Cd, and O-H···O bonds for having an efficient optical conductivity and biological activity of the sodium cadmium oxalate complex. The sodium cadmium oxalate complex showed good antimicrobial activity.
against *E. coli* (Gram-negative), *Staphylococcus* (G-positive), *A. niger*, and *C. albicans* (fungus) was attributable to the significant properties of its O-H…O resonance-assisted bonds, optical conductivity, and semi-polarity and liposolubility mechanisms. Further, the remarkable antioxidant activity of the present oxalic acid-derived complex proved that it may favor its application in the field of pharmaceutical and biomedical instrumentation.

ACKNOWLEDGMENTS

The authors are also thankful to University Grants Commission, Grant no.FNO: MRP-5808/15 (SERO/UGC) for providing financial support under Minor Research Project (MRP) scheme.

AUTHORS' CONTRIBUTION

D.S. performed the study, experimental synthesis, and the computational analysis. All authors equally contributed to writing the manuscript and approved the final description.

CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

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