TASR Trends in Applied Sciences Research

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Solution Growth and Characterization of 4-Carboxyanilinium Tartrate Single Crystal

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ABSTRACT

Background and Objective: The organic compound of 4-carboxyaniline has UV absorption and antifibrotic properties. It was naturally found in *Streptomyces griseus, Synechococcus elongatus* and other organisms. In this study, an effort was made to the growth of the 4-carboxyaniline derivative crystal with tartaric acid using the solution growth technique. **Materials and Methods:** The 4-carboxyanilinium tartrate crystal was grown from a slow evaporation technique in an aqueous ethanol solution at room temperature. The grown crystal was characterized by single crystal XRD, powder XRD, FT-IR, FT-Raman, UV-visible spectroscopy, SEM with EDX and antibacterial activity studies. **Result:** The monoclinic crystal system and $P2_1$ space group was confirmed from the single crystal XRD study. The diffraction peaks were indexed using INDX software. The average crystalline size was determined as 55 nm. All the wavenumber assignments were exactly matched with recently reported corresponding compounds. From the optical absorbance data, the band gap was determined as 3.75 eV using Tauc's plot. The well-defined shape of the crystal was confirmed by the SEM micrographs. *Bacillus subtilis* and *Acinetobacter baumannii* exhibited the highest zone of inhibition towards the grown crystal than *Escherichia coli*. **Conclusion:** The finding result of this study supports the previous knowledge of the structural and biological assortment of 4-carboxyanilium tartrate complex crystals.

KEYWORDS

PABA, vitamin B-complex, optical study, 4-carboxyanilium, tartrate complex

INTRODUCTION

The 4-carboxyaniline also known as 4-aminobenzoic acid or p-ABA, in which the amino and carboxylic acid groups are attached to the benzene ring in para-position[1,2]. It is a vitamin-like substance, particularly vitamin B-complex and is considered a non-essential nutrient in humans[3]. The 4-carboxyaniline is used in the synthesis of the vitamin folic acid and is found in several foods including eggs, grains, meat and milk[4,5]. It is also used to darken grey hair, prevent hair loss, make skin look younger and prevent sunburn and therefore known as an ultraviolet sun screen that is applied to the skin as sun lotions[6]. It is widely used as a therapeutic agent such as an antioxidant, antibacterial, antimutagenic, anticoagulant and fibrinolytic[7,8]. The 4-carboxyaniline is considered a protective drug against UV-irradiation which could donate and also accept hydrogen through both the amine and carboxylic acid functional groups[9]. The complexes of 4-carboxyaniline had already been studied by many authors to understand their structure extension properties through their hydrogen bonding associations[10-15]. The crystal structure of 4-carboxyanilinium

ISSN: 2151-7908 (Online)
ISSN: 1819-3579 (Print)
https://doi.org/10.3923/tasr.2022.180.190

Received: 10 Apr. 2022
Accepted: 14 Jul. 2022
Published: 01 Oct. 2022

tartrate has already been reported by Athimoolam *et al.*[16]. Keeping in view, the structural and biological assortment of the 4-carboxyaniline compound, here report the growth by a slow evaporation method, single crystal XRD, PXRD, FT-IR, FT-Raman, UV-Visible-NIR spectroscopy, SEM with EDX and antibacterial activity studies.

MATERIALS AND METHODS

Study area: The title crystal was developed at the Research Department of Physics, Devanga Arts College, Aruppukottai, Tamilnadu, India in November, 2021. The data were composed between December, 2021 and February, 2022.

Crystal growth: The growing preliminary materials for 4-carboxyanilium tartrate single crystal were 4-carboxyaniline, tartaric acid and ethanol which were purchased from the Modern Scientific Company, a Laboratory equipment supplier in Madurai, Tamil Nadu. According to the slow evaporation method, a single crystal of 4-carboxyanilinium tartrate was obtained. First, 1.37 g of 4-carboxyanilne was dissolved in the aqueous ethanol solution (50 mL water+50 mL ethanol) and 1.5 g of tartaric acid was dissolved in water with a molar ratio of 1:1. Then these solutions were mixed and stirred well for 1 hr at room temperature. The mixed solutions were poured into the petri dish after filtration. Finally, the title crystals were grown within 10 days. The quality crystals were collected and kept in the zip lock cover. The photographic view and the chemical structure of 4-carboxyanilium tartrate are shown in Fig. 1 and 2, respectively.

Experimental details: The single crystal X-ray diffraction was performed using Bruker SMART APEX CCD diffractometer with Mo Kα radiation (λ = 0.71073 Å). The powder XRD analysis was carried out by XPERT-PRO X-ray diffractometer with Cu Kα radiation (1.54060 Å) in the 2θ range 20-80°. Using SHIMADZU FT-IR spectrometer in the wavenumber range 400-4000 cm^{-1} with the KBr disc method, the FT-IR spectrum was obtained. The FT-Raman spectrum was prepared with BRUKER: RFS 27 Raman

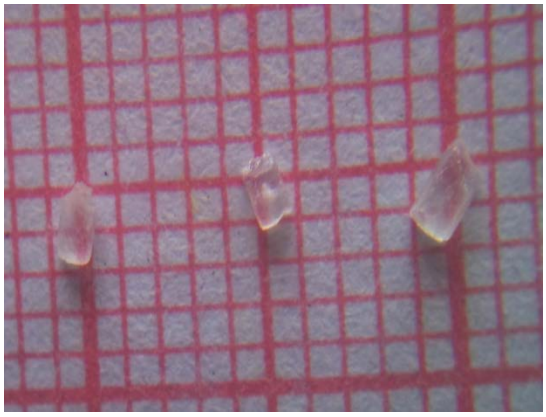

Fig. 1: Grown crystal of 4-carboxyanilinium tartrate

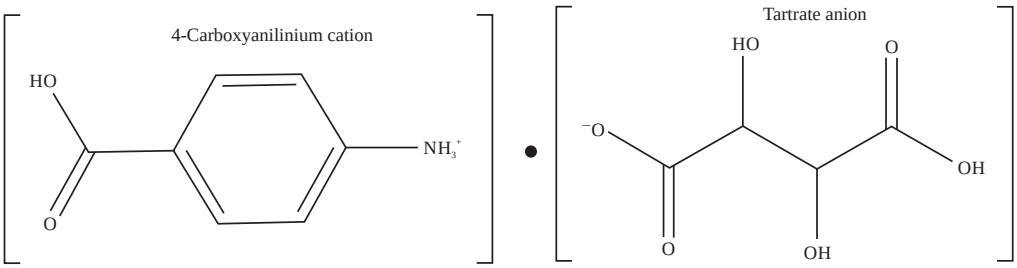

Fig. 2: Chemical structure of 4-carboxyanilinium tartrate crystal

spectrometer in the wavenumber range 4000-400 cm^{-1}. The optical absorbance spectrum had been recorded with SHIMADZU-UV 1601 double beam spectrometer in the wavelength range 190-1100 nm. The SEM with EDX analysis was performed using CARLZEISS EVO18 scanning electron microscope. The antibacterial activity study was performed by agar well diffusion method with the three different microorganisms.

RESULTS AND DISCUSSION

Single crystal XRD analysis: The unit cell dimension was determined as a = 6.021(4) Å, b = 28.789 (11) Å, c = 7.426 (5) Å, α = 90°, β = 107.89 (4)°, γ = 90° and volume V = 1225.0 (12) $Å^3$ from the single crystal XRD study and other crystallographic data of title crystal is shown in Table 1. The molecular structure of 4-carboxyanilinium tartrate crystal is depicted in Fig. 3. The molecular structure consists of discrete protonated 4-carboxyanilinium cation and deprotonated tartrate anion which are linked together through N-H...O hydrogen bonding to form the molecular complex. The title crystal belongs to the monoclinic crystal system with space group $P2_1$. All the unit cell parameters are exactly matched with the already reported values of the same compound by Athimoolam *et al.*[16].

Table 1: Lattice parameters of 4-carboxyanilinium tartrate crystal

Lattice parameters	Current work	Reported work[16]
Compound names	4-Carboxyanilinium tartrate	4-Carboxyanilinium tartrate
Empirical formula	$C_7H_8NO_2^+ \cdot C_4H_5O_6^-$	$C_7H_8NO_2^+ \cdot C_4H_5O_6^-$
Molecular weight	287.22	287.22
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1$	$P2_1$
Unit cell dimensions	a = 6.025 (6) Å	a = 6.021 (4) Å
	b = 28.790 (12) Å	b = 28.789 (11) Å
	c = 7.430 (7) Å	c = 7.426 (5) Å
	α = 90°	α = 90°
	β = 107.92 (5)°	β = 107.89 (4)°
	γ = 90°	γ = 90°
Volume	1227.0 (14) $Å^3$	1225.0 (12) $Å^3$

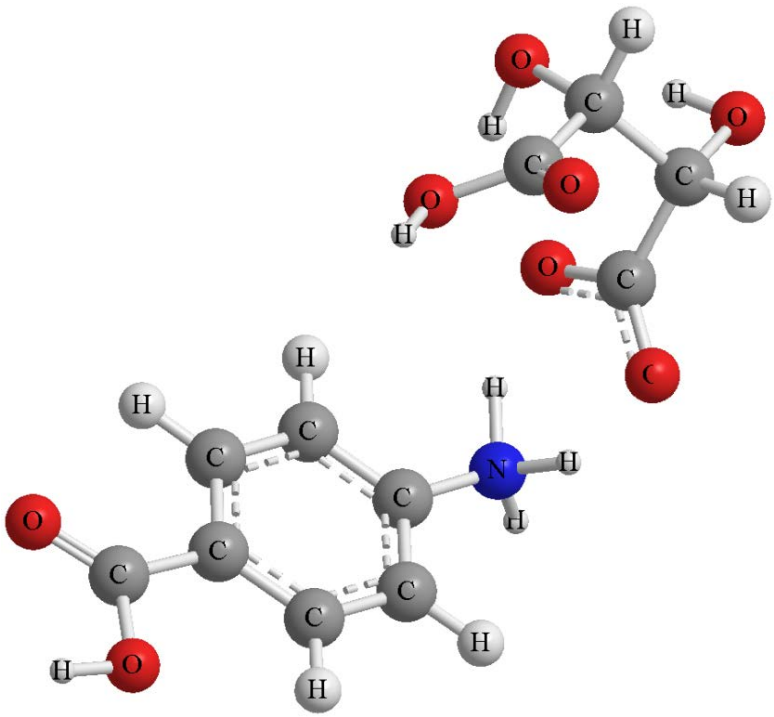

Fig. 3: Molecular structure of 4-carboxyanilinium tartrate crystal

Determination of crystalline size: The 4-carboxyanilinium tartrate crystal was finely ground, homogenized and the diffraction pattern was recorded. The experimentally recorded powder diffraction data is shown in Fig. 4. The one-dimensional distribution in diffraction intensities shows that every substance in the grown material produces its diffraction pattern. The highest peak appeared at 24.62°. Indexing of peaks was carried out using INDX software. This PXRD analysis reveals the good crystalline nature of the title crystal. The average crystalline size was determined as 55 nm using the Debye-Scherrer formula and also the dislocation density was calculated as 3.306×10^{14} m^2 using the formula:

$$\delta = \frac{1}{D^2} m^2$$

FT-IR and FT-Raman spectroscopy analysis: The 4-carboxyanilinium tartrate crystal has a di-substituted benzene ring in the para position, -$[NH_3]^+$, -COOH, -C(OH) and COO^- functional groups. The vibrational IR and Raman spectra are shown in Fig. 5 and 6, respectively. The detailed wavenumber and functional group assignment are given accordingly in Table 2.

Vibrations of 4-carboxyanilinum cation

Para substituted benzene ring: The C = C and C-C ring stretching modes normally appear in the region 1650-1430 cm^{-1} and 1400-1300 cm^{-1}, respectively[17]. The ν (C = C) mode was attributed at 1607, 1584 and

Table 2: Wavenumber and functional group assignments for 4-carboxyanilinium tartrate crystal in FT-IR and FT-Raman spectra

FT-IR ($\bar{\nu}$ cm^{-1})	FT-Raman ($\bar{\nu}$ cm^{-1})	Assignment
3422 (s, br)	-	ν_{as} $(O\text{-}H)_{water}$
3406 (s, br)	-	ν_{as} $(O\text{-}H)_{water}$
3321 (sh)	-	ν_s $(O\text{-}H)_{water}$
3265 (m)	-	ν_s $(O\text{-}H)_{water}$
3179 (m)	-	ν_{as} $[\text{-}NH_3]^+$
3150 (m)	-	ν_{as} $[\text{-}NH_3]^+$
-	3084 (s)	ν (C-H), ν $(\text{-}OH)_{acid}$
-	3015 (m)	ν (C-H), ν $(\text{-}OH)_{acid}$
2970 (m)	2985 (m)	ν_s $[\text{-}NH_3]^+$, ν_{as} $(C\text{-}H)_{tartrate}$, ν $(\text{-}OH)_{acid}$
2922 (m)	2940 (m)	ν_s $[\text{-}NH_3]^+$, ν_s $(C\text{-}H)_{tartrate}$, ν $(\text{-}OH)_{acid}$
1726 (s, br)	1714 (s)	ν_{as} $(C{=}O)_{acid}$
1636 (m)	-	ν_s $(C{=}O)_{acid}$, $\beta(O\text{-}H)_{water}$
1607 (m)	1615 (s)	ν (C=C)
1584 (m)	-	ν (C=C), ν_{as} (COO^-) ρ $[\text{-}NH_3]^+$
1510 (m)	-	ν (C=C)
1422 (m)	-	ν_{as} (COO^-), β $(\text{-}OH)_{acid}$
1337 (m)	1337 (m)	ν_s (COO^-), ν (C-C), β $(\text{-}OH)_{acid}$
1308 (s)	-	ν (C-N), $\beta(C\text{-}H)_{tartrate}$, ν (C-C), ν (C-O)
1240 (s)	1252 (w)	ν (C-O)
-	1210 (m)	ν (C-O)
1177 (m)	-	τ $[\text{-}NH_3]^+$, β(C-H)
1130 (s)	1136 (m)	β (C-H)
1099 (m)	1112 (m)	β (C-H)
1074 (s)	-	ν (-O CH)
1018 (m)	-	ν (-O CH)
986 (m)	-	γ $(\text{-}OH)_{acid}$
897 (m)	897 (m)	γ $(\text{-}OH)_{acid}$
837 (m)	835 (s)	Benzene ring breathing mode
783 (w)	-	γ (C-H)
758 (m)	-	γ (C-H)
729 (w)	-	γ (C-H)
683 (m)	639 (m)	γ $(C\text{-}H)_{tartrate}$, ρ (O-C=O), ρ (COO^-)
611 (m)	-	ω (COO^-), t $(\text{-}OH)_{acid}$, ω (O-C=O), γ $(C\text{-}H)_{tartrate}$
600 (m)	-	t $[\text{-}NH_3]^+$, τ (O-C=O),
548 (m)	-	τ (COO^-), t $(\text{-}OH)_{acid}$
420 (s)	-	t $(\text{-}OH)_{acid}$

s: Strong, m: Medium, w: Weak, sh: Shoulder, u: Stretching, u_s: Symmetric stretching, u_{as}: Antisymmetric stretching, β-in-plane bending, γ- out-of-plane bending, ρ: Scissoring, ω: Wagging, τ: Rocking and t: Twisting

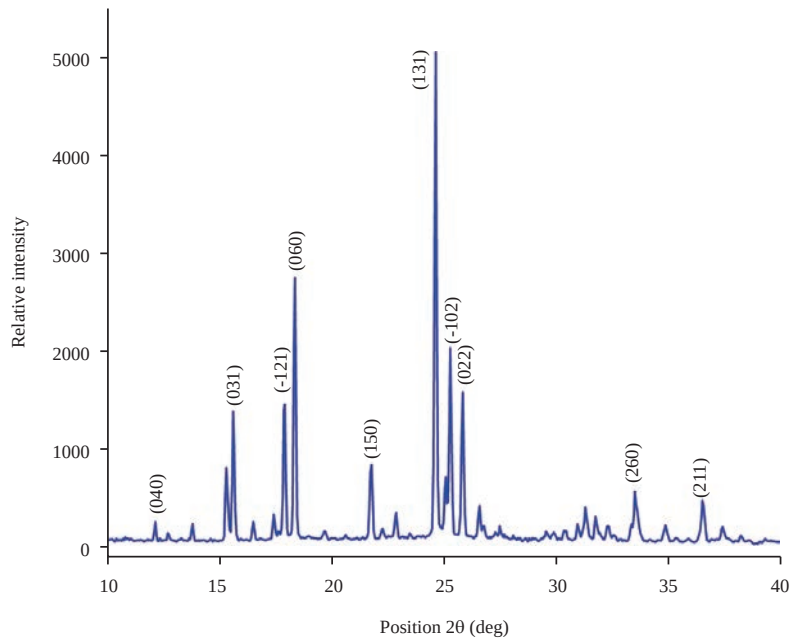

Fig. 4: Diffraction peaks for 4-carboxyanilinium tartrate crystal

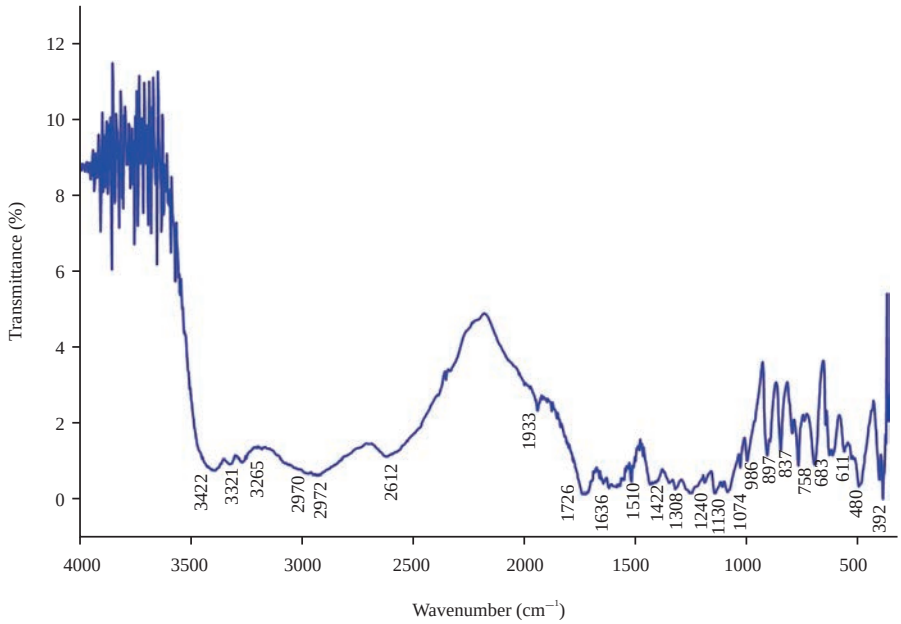

Fig. 5: FT-IR spectrum for 4-carboxyanilinium tartrate crystal

1510 cm^{-1} in infrared and at 1615 cm^{-1} in Raman spectra. Also, ν (C-C) mode was identified at 1337 cm^{-1} and 1308 cm^{-1} in the IR spectrum. Also, the C-N stretching mode was identified at 1308 cm^{-1}. The ring breathing mode was identified at 837 cm^{-1} in IR and 835 cm^{-1} in Raman spectra. The C-H stretching mode of the para-substituted benzene ring is expected in the region 3115-3005 cm^{-1} [18]. In the present study, the bands observed at 3084 cm^{-1} and 3015 cm^{-1} in the Raman spectrum were assigned to the ν (C-H) mode. The in- and out-of-plane bending vibrations of C-H appear in the range 1250-1000 and 900-690 cm^{-1}, respectively[18]. The in-plane bending mode β (C-H) was identified at 1240, 1177, 1130, 1099 cm^{-1} in FT-IR and at 1252, 1136, 1112 cm^{-1} in FT-Raman spectra, respectively. The out-of-plane bending mode γ (C-H) was pragmatic at 783, 758 and 729 cm^{-1} in the IR spectrum only.

Carboxylic acid group: The bands recorded at 2970, 2922 cm^{-1} in IR and at 3084, 3015, 2985 and 2940 cm^{-1} in Raman spectra were assigned to the O-H stretch from CO-OH group. The in-plane and

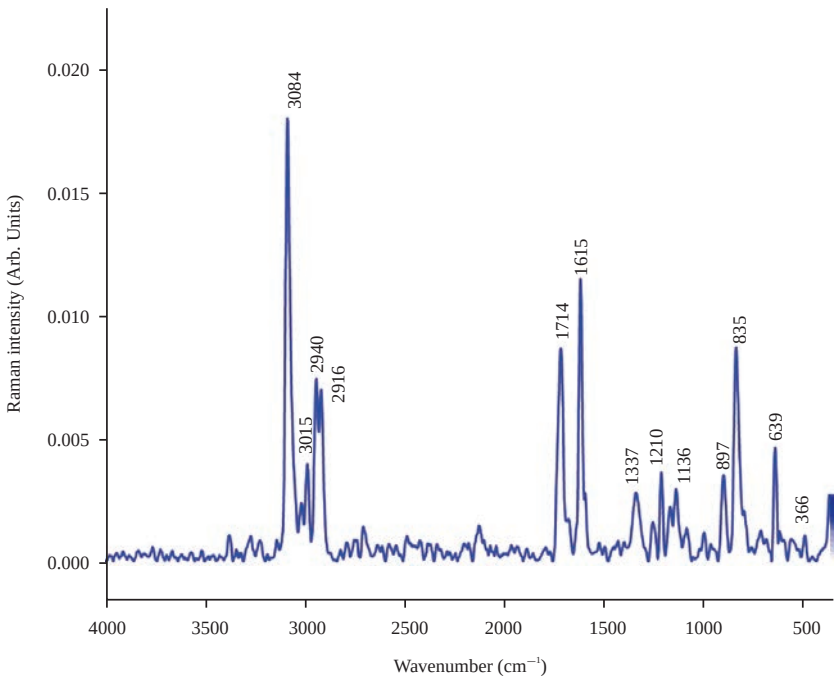

Fig. 6: FT-Raman spectrum for 4-carboxyanilinium tartrate crystal

out-of-plane bending wavenumbers of the O-H group occur between 1440-1395 and 960-875 cm^{-1}, respectively[19]. In the present work, β (O-H) and γ (O-H) modes were observed at 1422, 1337 cm^{-1} and 986, 897 cm^{-1}, respectively. The O-H twisting mode was expected to appear at 611, 548, 420 cm^{-1}. The ν_{as} (C = O) mode has the absorbance bands at 1726 and 1714 cm^{-1} in both spectra. Also, bands at 1636 cm^{-1} in IR were assigned to the ν_s (C = O) mode. The ν (C-O) mode occurs in the characteristic region of 1320-1210 cm^{-1} [17,19]. The title compound has the wavenumbers at 1308, 1240 cm^{-1} in IR and 1252, 1210 cm^{-1} in Raman spectra was attributed to ν (C-O) mode. The scissoring, wagging and rocking modes of the O-C=O group were familiar at 683, 611 and 600 cm^{-1}, respectively.

Anilinium group: For anilinium -$[NH_3]^+$group, the antisymmetric and symmetric stretching mode is normally identified in the region of 3200 and 2800 cm^{-1} [19,20]. For the title compound, the bands attributed at 3179, 3150 cm^{-1} for ν_{as}-$[NH_3]^+$ mode. Also, ν_s-$[NH_3]^+$ mode was observed at 2970, 2922 cm^{-1} in IR and 2985, 2940 cm^{-1} in Raman spectra. The scissoring, rocking and twisting modes of the -$[NH_3]^+$ group were identified at 1584, 1177 and 600 cm^{-1} in the IR spectrum, respectively.

Vibrations of tartrate anion: The antisymmetric and symmetric stretching modes of the C-H group were attributed at 2970 and 2922 cm^{-1}. The C-H out-of-plane bending vibration was identified at 683 cm^{-1} and 611 cm^{-1} in the IR spectrum. The C-H in-plane bending mode was observed at 1308 cm^{-1} in the IR spectrum. The stretching -OCH mode of tartrate is seen in the range 1068 cm^{-1}[21]. The peak that appeared at 1074 and 1018 cm^{-1} in the FT-IR spectrum was assigned to the ν (-OCH) mode for the title crystal. The vibration band was identified at 1584 cm^{-1} in IR for the antisymmetric mode of the COO^- group. Also, the symmetric stretching mode for the same group was identified at 1337 cm^{-1} in both spectra. The scissoring, wagging and rocking deformation modes were recorded at 683, 611 and 548 cm^{-1}, respectively. All these wavenumber assignments are exactly in concurrence with the recently reported values of related compounds[22]. The wavenumber assignments for carboxylic group vibrations present in tartrate anion are seen in Table 2.

Vibrations of the water molecule: Water was trapped between the Kbr particles during pellet preparation and so the additional peaks were observed in the FT-IR spectrum. The water molecule has two

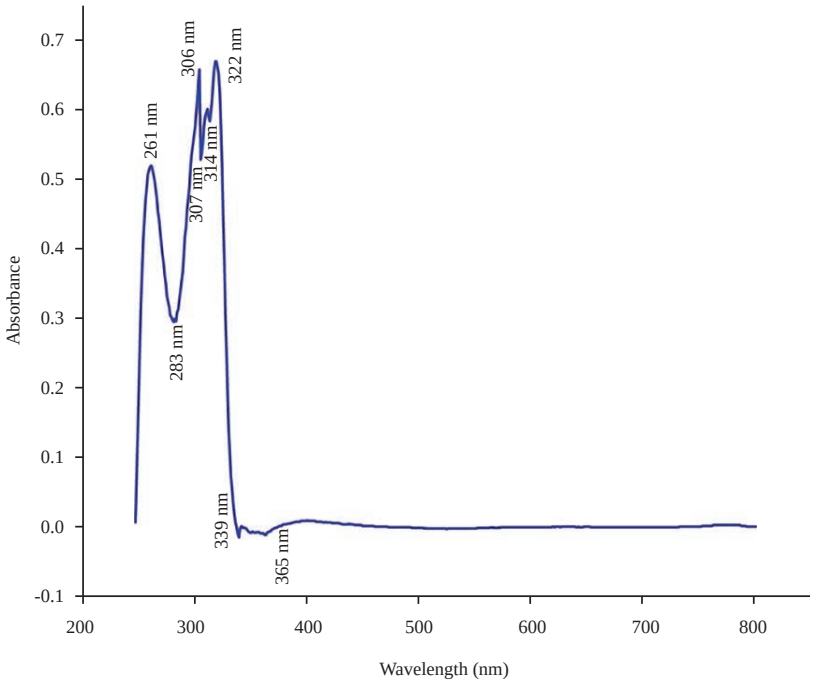

Fig. 7: Optical absorbance spectrum for 4-carboxyanilinium tartrate crystal

Table 3: Composition of (%) elements in 4-carboxyanilinium tartrate crystal

Elements	Weight (%)	Atomic (%)	Error (%)	K ratio
C	43.0	49.8	5.7	0.2558
N	6.2	6.2	16.8	0.0080
O	50.8	44.1	9.7	0.1002

OH stretching modes around 3600 cm^{-1} and one bending mode around 1630 cm^{-1} [20]. Strong infrared bands located at 3422, 3406 and 3321, 3265 cm^{-1} were attributed, respectively, to the antisymmetric and symmetric stretching vibration of a water molecule. A medium infrared band at 1636 cm^{-1} was assigned to the in-plane deformation mode of a water molecule.

Optical absorbance spectroscopy analysis: The recorded optical absorbance spectrum of 4-carboxyanilinium tartrate crystal is shown in Fig. 7. The crystal absorbs maximum ultraviolet radiation in the wavelength of 261, 306 and 322 nm. The lower cut-off wavelength was found to be at 283, 307, 314, 339 and 365 nm. The title crystal has 100% transmittance in the entire visible region. The optical band gap E_g is determined using Tauc's relation $(\alpha h\nu)^2 = A(h\nu - E_g)$. The calculations were performed using origin software. Then the Tauc plot was drawn by plotting the $(\alpha h\nu)^2$ Vs photon energy and extrapolating the linear portion of $(\alpha h\nu)^2$ to the photon energy axis directly giving the optical band gap value. From Fig. 8, the optical band gap was determined as 3.75 eV for a 4-carboxyanilinium tartrate crystal.

Scanning electron microscopy (SEM) with energy dispersive X-ray spectroscopy (EDX) analysis: SEM/EDX study was used to analyze the surface morphology as well as the presence of elements in a 4-carboxyanilinium tartrate single crystal. The captured SEM micrographs are shown in Fig. 9a and b with 500x and 2.5kx magnifications. The SEM micrographs revealed that the grown crystal has a well-defined shape throughout the surface. The recorded EDX pattern is shown in Fig. 10 which reveals that the carbon atom has the highest counts than the oxygen and nitrogen compounds. The C, N and O elements have the atomic percentage of 49.8, 6.2 and 44.1%, respectively. The element, weight (%), atomic (%), error (%) and K ratio of all the elements present in the 4-carboxyanilinium tartrate crystal are presented in Table 3. The EDX study confirms that tartaric acid was incorporated with the 4-carboxyaniline compound.

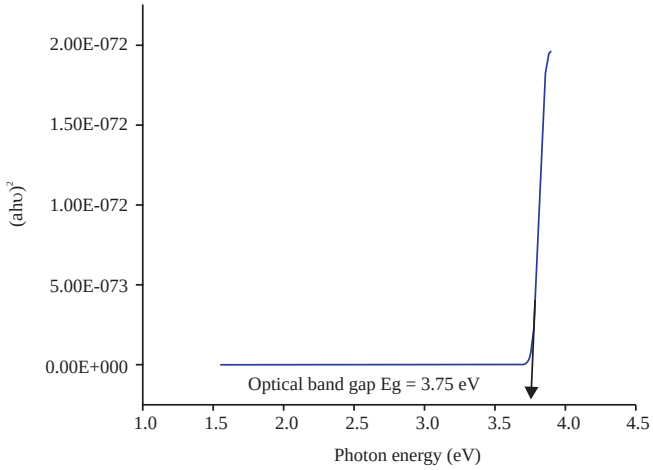

Fig. 8: Tauc plot for 4-carboxyanilinium tartrate crystal

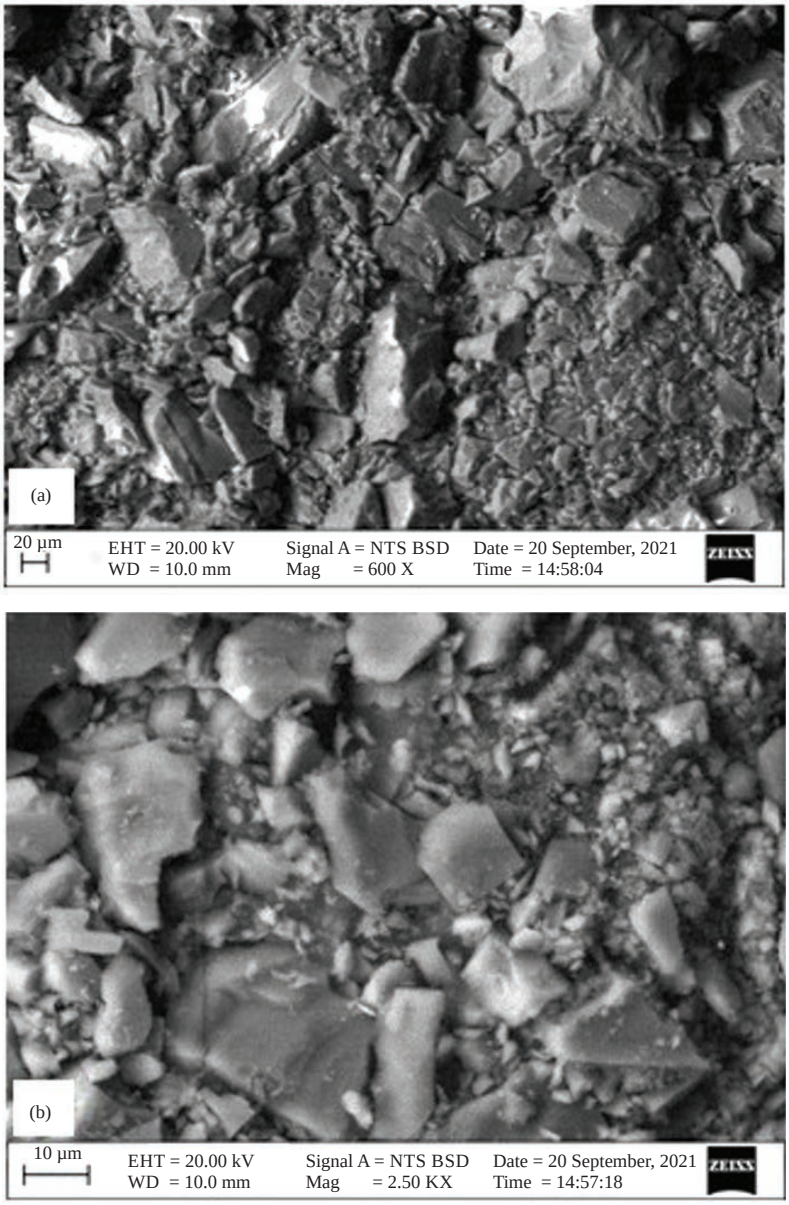

Fig. 9(a-b): SEM micrographs of 4-carboxyanilinium tartrate crystal, (a) 500X magnification and (b) 2.5 kX magnification

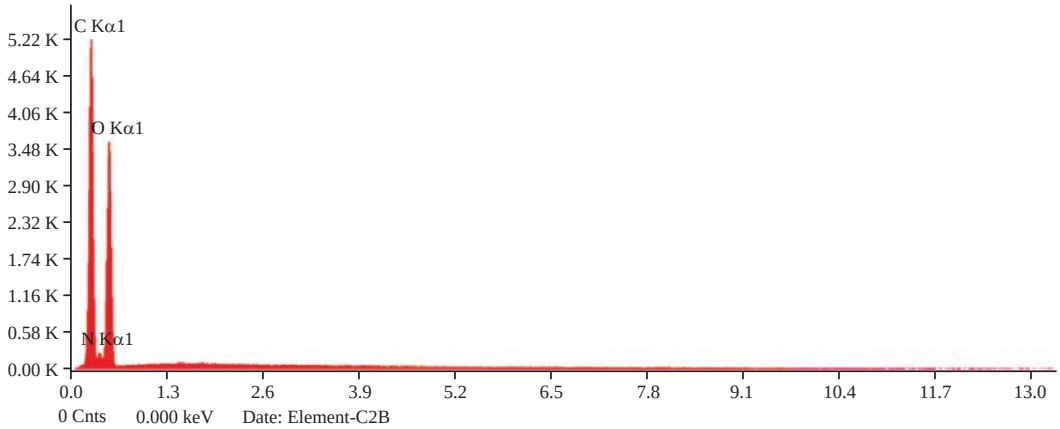

Fig. 10: EDX spectrum of 4-carboxyanilinium tartrate crystal

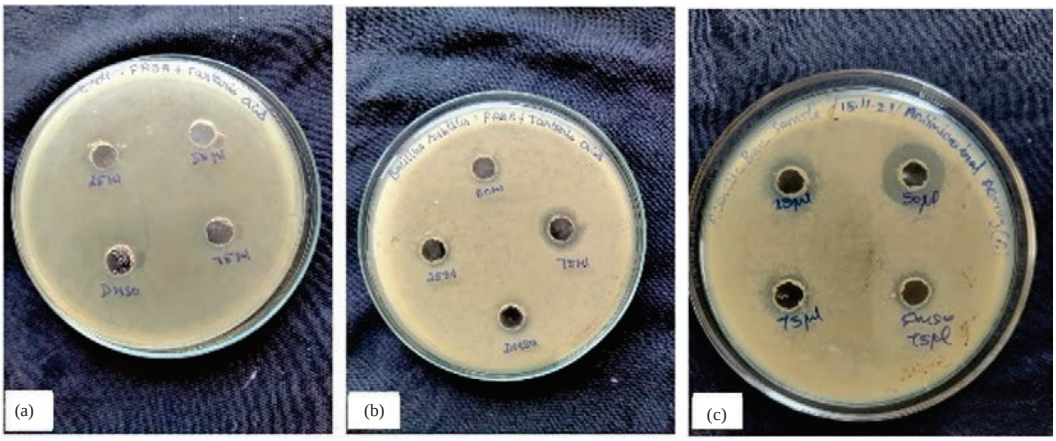

Fig. 11(a-b): Zone of inhibition of 4-carboxyanilinium tartrate crystal in, (a) *Escherichia coli* bacterial strains, (b) *Bacillus subtilis* bacterial strains and (c) *Acinetobacter baumannii* bacterial strains

Table 4: Diameter of zones of inhibition (mm) of 4-carboxyanilinium tartrate crystal against microorganisms

	Plant concentration (μg mL^{-1})		
Bacteria	25	50	75
Escherichia coli	NZ	NZ	NZ
Bacillus subtilis	6 mm	8 mm	9 mm
Acinetobacter baumannii	7 mm	9 mm	7 mm

*NZ: No zone of inhibition

Antibacterial analysis: The 4-carboxyanilinium tartrate crystal sample was tested for antibacterial activity against *Escherichia coli*, *Bacillus subtilis* and *Acinetobacter baumannii* bacterial strains by agar well diffusion method with different concentrations of 25, 50 and 75 μL. Plant extracts of 50 mg mL^{-1} concentration were prepared in Dimethyl Sulfoxide (DMSO) as a positive control. The plates are incubated at the temperature of 37°C for 18-24 hrs. The progress of the inhibition zone in the well was measured and noted. The antibacterial behaviour of three different bacterial strains is shown in Fig. 11a-c and the results are reported in Table 4. According to the zone of inhibition, *Bacillus subtilis* and *Acinetobacter baumannii* exhibit the highest sensitivity towards 4-carboxyanilinium tartrate crystal while, *Escherichia coli* shows no sensitivity among the tested microorganisms.

CONCLUSION

The single crystal of 4-carboxyanilinium tartrate was grown by slow evaporation technique in an aqueous ethanol solution at room temperature. Crystal structure and lattice parameters were determined using

single crystal X-ray diffraction and it showed the monoclinic system which was found in close covenant with the earlier report's experimental findings. The good crystalline nature of grown crystals was confirmed by the powder XRD study. The average particle size was determined as 55 nm and the diffraction peaks were indexed using INDX software. The presence of various functional groups was confirmed by FT-IR and FT-Raman spectroscopy. The optical band gap was calculated from absorbance data using Tauc relation and found to be 3.75 eV. The surface morphology and elemental composition were determined by SEM/EDX studies, which showed that the grown crystal was of good quality. The antibacterial activity was performed against three kinds of bacterial strains by the ager well diffusion method for various concentrations. *Bacillus subtilis* and *Acinetobacter baumannii* exhibited the highest sensitivity towards the grown crystal while, *Escherichia coli* showed no warmth among the tested microbes.

SIGNIFICANCE STATEMENT

This study discovered the drug complex crystal with the organic acid combination that can be beneficial for the biological and pharmacological field. This study will help the researchers to uncover the critical areas of complex crystal synthesis that many researchers were not able to explore. Thus a new theory on the formation of drug complex crystal with organic acid combination may be arrived at.

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