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Single Diffusion Gel Growth, Spectral and Antimicrobial Activity Studies on 4-Carboxyaniline Crystal

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ABSTRACT

In this work, the single crystal of 4-carboxyaniline was successfully grown by single diffusion gel technique. It was characterized by single crystal XRD, powder XRD, FT-IR, FT-Raman, UV-Visible spectroscopy, SEM with EDX and antimicrobial activity studies. The crystal lattice parameters and space group was obtained from the single crystal XRD study. The powder X-ray diffraction analysis was done to determine the particle size and dislocation density of the crystal. The FT-IR and FT-Raman spectra of 4-carboxyaniline crystal have been recorded at room temperature to elucidate the presence of functional groups. The percentage of transmittance and band gap was calculated using UV-Visible spectroscopy. The morphology and elemental composition was investigated by SEM with EDX technique. The antibacterial activities of the title compound were performed by disc diffusion method against staphylococcus epidermidis, serratia marcescens, methicillin-resistant staphylococcus aureus and polyphosphate-accumulating organisms.

Keywords: 4-carboxyaniline**,** XRD, FT-IR, FT-Raman, SEM, EDX and Antimicrobial activity.

1. INTRODUCTION

The 4-carboxyaniline (PABA) is historically referred to as vitamin B_x which acts as a bacterial cofactor to synthesis the folic acid [1]. Even though, it is not fully recognized as vitamin because most people have colon bacteria that generate PABA from chorismate by the combined action of the enzymes [2]. It is naturally found in foods such as wheat, rice, eggs, and molasses [3]. It is used in cosmetic products to protect an excess ultra-light exposure against skin cancer and skin burn [4]. In pharmaceutical field, it is play an important role to relieve the pain from headaches, nervous states [5, 6]. It has two different polymorphs, which were analyzed by many authors [7-12]. The α- polymorph appears as long, fibrous needles and this form has been investigated recently by Athimoolam et al. [7]. The β- polymorph appears in the form of prisms and it has been reported by Gracin et al. [8]. The crystal structure of 4-carboxyaniline was already reported by slow evaporation method [7]. But to the best of our knowledge, there is no report on spectroscopic and antimicrobial activity studies for this crystal. So, an attempt was made to grow single crystal of 4-carboxyaniline by single diffusion gel method for the first time in the present work and the grown crystal is characterized by single crystal XRD, powder XRD, FT-IR, FT-Raman, UV-Visible spectroscopy, SEM with EDX and antimicrobial activity studies. These results are discussed and summarized in this present work.

2. MATERIALS AND METHODS

Materials

4−aminobenzoic acid, sodium meta silicate, ethanol and acetic acid (99. 5 %) were used as the raw materials in this crystal growth process and they were purchased from the Merck company, India.

Single Diffusion Gel Method

The 4-carboxyaniline single crystal was crystallized by the single diffusion gel method. In this method, silica gel was created by mixing an aqueous solution of 1 M sodium meta silicate with 1M acetic acid. These solutions are stirred continuously by the magnetic stirrer to avoid the pre local gel formation. Then the mixture was transferred into the test tube of length 15 cm and 3 cm diameter. The mouth of the test tube was covered by the cork to keep the solution free from dust and impurities. The gel was set within 2 or 3 days and leaves it for another 24 hours for aging. Then the aqueous ethanol solution of 4-aminobenzoic acid (1M) was poured slowly over the set gel without disturbance. The crystals were appeared within two days in the gel medium which are harvested after 3−5weeks and washed with the distilled water. The crystals were collected and dried crystals were stored in the clean container. The photographic view of the grown crystals is shown in fig.1. The optimum conditions used in the crystal growth of this method are given in Table 1.

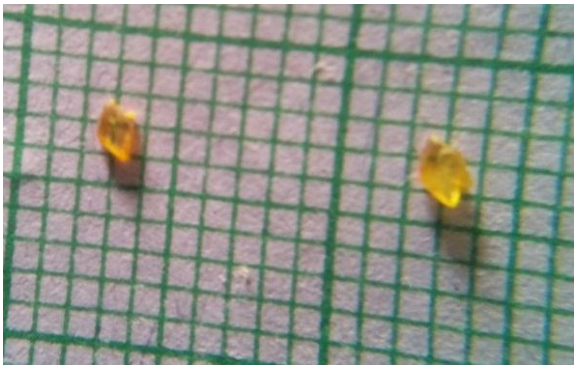

Fig.1: Photographic view of 4-carboxyaniline crystals

Table 1: The optimum conditions for 4-carboxyaniline crystal

Parameters	**Optimum Conditions**
Density of Na_2SiO_3	1.05 g/cm^3
Concentration of acetic acid	1 M
Concentration of 4-carboxyaniline	1 M
pH of the gel	4.6
Gel setting period	2 days
Gel aging	24 hours
Period of growth	3−5 weeks
Temperature	Room temperature

Experimental details

The single crystal Bruker SMART APEX CCD X-ray diffractometer with Mo Kα radiation (λ = 0.71073 Å) was used to carry out the unit cell dimension and space group of the title compound. The XPERT–PRO X–ray

diffractometer with Cu Kα (λ = 1.54060 Å) radiation was used to record the powder diffraction pattern. The FT−IR vibrational spectrum was recorded using SHIMADZU FT−IR spectrometer in the range 4000−400 cm^{-1}. Also, the FT−Raman spectrum was recorded using the BRUKER: RFS 27 Raman spectrometer in the wavenumber range 4000−400 cm^{-1}. The optical absorption spectrum of 4-carboxyaniline crystal has been recorded with SHIMADZU−UV1800 double beam spectrometer in the wavelength range 200−1100 nm insteps of 1nm. The surface morphology and elemental analysis has been carried out by CARLZEISS EVO18 scanning electron microscope. Using the disc diffusion method, the antimicrobial activity of title crystal was tested against four different kinds of micro-organisms.

3. RESULTS AND DISCUSSION

Single crystal XRD

The lattice parameter values of 4-carboxyaniline crystal were obtained from the SMART APEX CCD area-detector diffractometer. The preliminary crystallographic data of 4- carboxyaniline crystal is shown in Table 1 and is compared with already reported values [7]. The molecular structure of title crystal is illustrated in Fig.2. The single crystal XRD study reveals that the grown crystal belongs to monoclinic crystal system with space group $P2_1/n$.

Fig. 2: Molecular structure of 4- carboxyaniline crystal

Table 1: Crystallographic data of 4- carboxyaniline crystal

Parameters	**Present study**	**Already reported [7]**
Compound Name	4- Carboxyaniline	4- Carboxyaniline
Empirical formula	$C_7H_7NO_2$	$C_7H_7NO_2$
Molecular formula weight	137.14	137.14
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/n$	$P2_1/n$
Unit cell Dimensions	a= 18.55 (5) Å	a= 18.57 (8) Å
	b= 3.84 (7) Å	b= 3.84 (3) Å

	c= 18.67 (3) Å α=90 ° β=93.60 (2)° γ=90 °	c= 18.63 (9) Å α=90 ° β=93.67(11)° γ=90 °
Volume	1329 (2) Å^3	1327 (13) Å^3

Powder XRD Analysis

The powder XRD patterns of 4-carboxyaniline crystal was recorded using XPERT–PRO X–ray diffractometer with Cu Kα (λ = 1.54060 Å) radiation from 10° to 80° at 10°/s scan step time. The 2θ and d−spacing values of 4−carboxyaniline crystal are shown in Table 2 which is compared with JCPDS values of the same compound [JCPDS card no: (49-2187)]. The experimentally recorded XRD pattern of title crystal is shown in Fig.3 and the peaks are indexed using INDX software. The sharp intense peaks are emerged out from the XRD pattern due to the every substance in the grown material produces its own diffraction pattern.

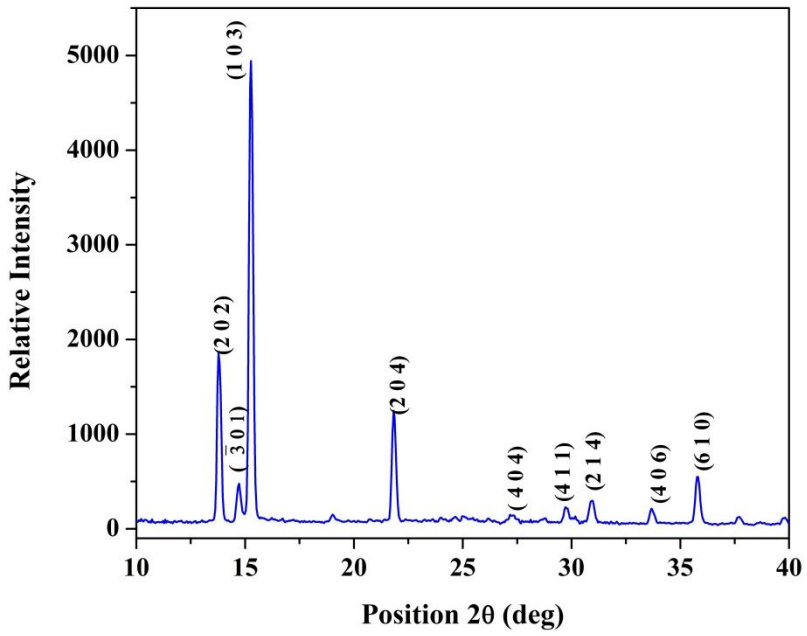

Fig. 3: Diffraction patterns for 4-carboxyaniline crystal

The sharp intensity peaks found in PXRD pattern shows good crystalline nature and purity of the grown crystal. The average crystalline size of 4- carboxyaniline crystal was determined by using the Debye-Scherrer equation, which can be written as,

$$D = \frac{K\lambda}{\beta \cos\theta}.$$

Where,

D = average crystallite size

K= dimensionless shape factor (0.94)

λ = wavelength of X-ray radiation (Cu Kα = 1.54060 Å)

θ = diffraction angle

β= Full width at half maximum intensity

The Dislocation density can be calculated from,

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

Where,

δ Dislocation density,

D is the crystallite size

The average crystalline size is found to be as 45 nm for 4-carboxyaniline crystal. Also, the dislocation density is determined as 4.92 $X10^{14}$ m^2.

Table 2: Powder XRD data of 4-carboxyaniline

Present Work		**JCPDS file (49-2187)**	
Position [°2θ]	**d–spacing [Å]**	**Position [°2θ]**	**d–spacing [Å]**
13.80	6.42	13.77	6.43
15.27	5.80	15.25	5.80
19.02	4.67	19.04	4.66
21.83	4.07	21.84	4.07
25.09	3.55	24.94	3.57
27.26	3.27	27.27	3.27
28.77	3.10	28.70	3.11
29.76	3.00	30.09	2.97
30.95	2.89	30.94	2.89
33.68	2.66	33.69	2.66
35.79	2.51	35.77	2.51
37.68	2.39	37.31	2.41
39.76	2.27	39.88	2.26
40.46	2.23	40.45	2.23

This result suggests that experimentally observed powder XRD data of 4-carboxyaniline crystal is exactly coinciding with the JCPDS values of the same compound.

Vibrational Analyzes

The 4-carboxyaniline molecule consists of para substituted aromatic ring, NH_2 and carboxylic acid functional groups which were analyzed using the FT-IR and FT-Raman spectrometer in the wavenumber range 4000−400 cm^{-1}. The experimentally recorded IR and Raman spectra is shown in fig.4 and fig.5 respectively and their corresponding IR and Raman wavenumber assignments are listed in Table 3.

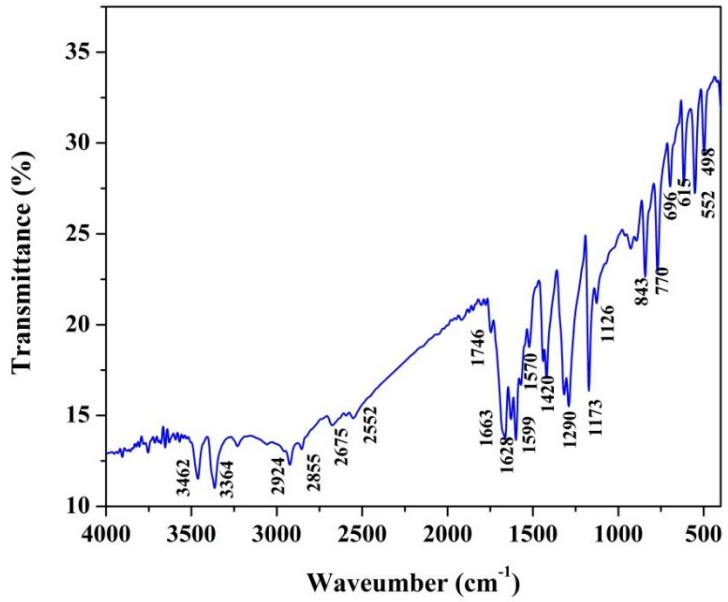

Fig. 4: FT-IR spectrum for 4-carboxyaniline crystal

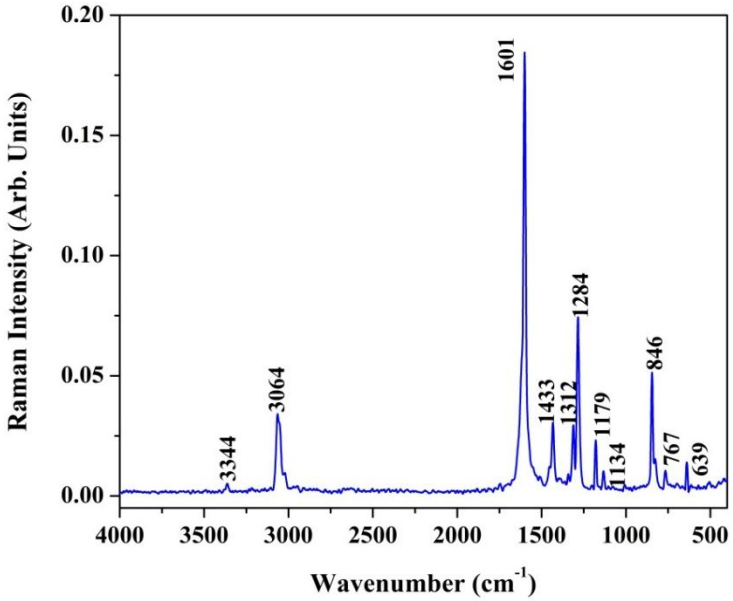

Fig. 5: FT-Raman spectrum for 4-carboxyaniline crystal

Table 3: Wavenumber assignments for 4-carboxyaniline crystal in FT – IR and FT – Raman spectra

FT – IR ($\bar{\upsilon}$ / cm^{-1})	**FT− Raman ($\bar{\upsilon}$ / cm^{-1})**	**Assignment**
3462 (s)	-	$\nu_{as}(NH_2)$
3364(s)	3361(w)	$\nu_s(NH_2)$
3057 (w)	3044(m)	ν (O-H); ν (C-H)
2924 (s)	-	ν (O-H)
2855(s)	-	ν (O-H)
1663(s)	-	ν_{as} (C=O)
1628(s)	-	ν_s (C=O); $\rho(NH_2)$
1599(m)	1601(s)	ν (C=C)
1570(m)	-	ν (C=C)
1522(m)	1525(w)	ν (C=C)
1441(w)	1433 (w)	β(O-H)
1420 (m)	-	β(O-H)
1317(s)	1312(m)	ν (C-O); ν (C-C)
1290(s)	1284(m)	ν (C-O) ; ν (C-N)
1211 (w)	-	β(C-H)
1173(m)	1179(m)	β(C-H)
1126(m)	1134(s)	β(C-H)
928(w)	-	γ (O-H)
893(w)	-	γ (O-H); γ(C-H)
843(m)	846 (m)	Ring breathing
770(w)	767 (m)	γ(C-H)
696 (w)	639(w)	γ(C-H)
552(w)	-	$\omega(NH_2)$

w–weak; s– strong; m– medium;υ– stretching; υ_s– symmetric stretching; υ_{as}–antisymmetric stretching; γ– out–of–plane bending; β– in–plane bending; ρ-rocking; ω-wagging

Carboxylic group vibration

The carboxylic group C=O antisymmetric and symmetric stretching vibrational mode have wavenumbers in the region 1720-1680 cm^{-1} and 1680-1640 cm^{-1} respectively [13]. In the present study, the bands occur at 1663 cm^{-1} in IR spectrum is assigned to ν_{as} (C=O) mode. Also, bands observed in IR spectrum at 1628 cm^{-1} is assigned to ν_s(C=O) mode for the title crystal. The ν (C-O) mode of carboxylic group normally occurs in the vibrational region of 1320–1210 cm^{-1} [14]. The title compound has wavenumbers at 1317 cm^{-1}, 1290 cm^{-1} in IR and at 1312 cm^{-1}, 1284 cm^{-1} in

Raman spectra is attributed to ν (C-O) mode. The O–H stretch from CO–OH group is identified at 3065–2826 cm^{-1}[15, 16]. This mode is attributed at 3057, 2924, 2855 cm^{-1} in IR and at 3064 cm^{-1}in Raman spectra respectively for title compound. The O–H group in-plane and out-of plane bending wavenumbers appear in the region between 1440–1395 cm^{-1} and 960–875 cm^{-1} respectively [15, 16]. In the present work, β (O–H) mode is identified as medium intensity bands at 1441 cm^{-1}, 1420 cm^{-1} in IR and at 1433 cm^{-1} in Raman spectra. Also for the title compound, γ (O–H) mode is attributed only at 928 cm^{-1}, 893 cm^{-1} in IR spectrum in the present study.

Para substituted benzene ring vibration

The para substituted benzene ring C-H stretching mode is expected in the region 3115 – 3005 cm^{-1} [12, 17]. In this case, the bands observed at 3057 cm^{-1} and at 3064 cm^{-1}in both spectra is assigned to ν (C–H) mode. The C–H in–plane and out –of –plane bending vibrations are seen in the range 1250 –1000 $cm^{-1}$and 900 – 690 cm^{-1} respectively [17, 18]. For the title compound, the β(C–H) mode is identified at 1211, 1173, 1126 cm^{-1} in FT-IR and at 1179, 1134 cm^{-1} in FT-Raman spectra respectively. The γ(C–H) mode is identified at 893, 770, 696 cm^{-1} in IR spectrum and at 767, 639 cm^{-1} in Raman spectrum for the title compound. The ν(C=C) and ν(C-C) modes occur in the region 1650−1430 cm^{-1} and 1400−1300 cm^{-1} respectively [13, 19]. For the title compound ν (C=C) mode is assigned at 1599, 1570, 1522 cm^{-1} in IR and 1601, 1525 cm^{-1} in Raman spectra. Also ν (C-C) mode is observed at 1317 cm^{-1}, 1312 cm^{-1} in both spectra for the title compound. The C-N stretching mode is at 1290 cm^{-1} in IR and at 1284 cm^{-1} in Raman spectrum respectively. The ring breathing mode is identified at 843 cm^{-1} in IR and 846 cm^{-1} in Raman spectra for the title compound.

Aniline group (NH_2) vibration

The NH_2 group gives rise to the six internal modes of vibrations such as the antisymmetric stretching (ν_{as}), symmetric stretching (ν_s), the symmetric planar deformation (scissoring), the antisymmetric planar deformation (rocking), the symmetric non-planar deformation (wagging) and the anti-symmetric non-planar deformation (torsion). The aniline (NH_2) stretching mode is normally expected in the region 3480-3250 cm^{-1} [19, 20]. For the title crystal, this mode is observed as strong bands at 3462 cm^{-1} for ν_{as}(NH_2) mode, 3364 cm^{-1} for ν_s(NH_2) mode cm^{-1} in IR spectrum and as a weak band at 3344 cm^{-1} for ν_s(NH_2) mode in Raman spectrum. The scissoring mode of (NH_2) group appears in the region 1650-1615 cm^{-1} [21]. In the present work, this mode is identified as strong peak at 1628 cm^{-1}in IR spectrum only. The wagging mode of aniline is expected in the range 620 ± 100 cm^{-1}[21]. For the 4-carboxyaniline crystal ω (NH_2) mode is attributed at 552 cm^{-1} in IR and no counterpart in Raman spectrum for this mode.

Optical analysis

The SHIMADZU−UV1800 double beam spectrometer is used to record the absorbance spectrum of 4-carboxyaniline crystal in the wavelength range 200−1100 nm. The experimentally recorded absorbance spectrum is shown in Fig.6.

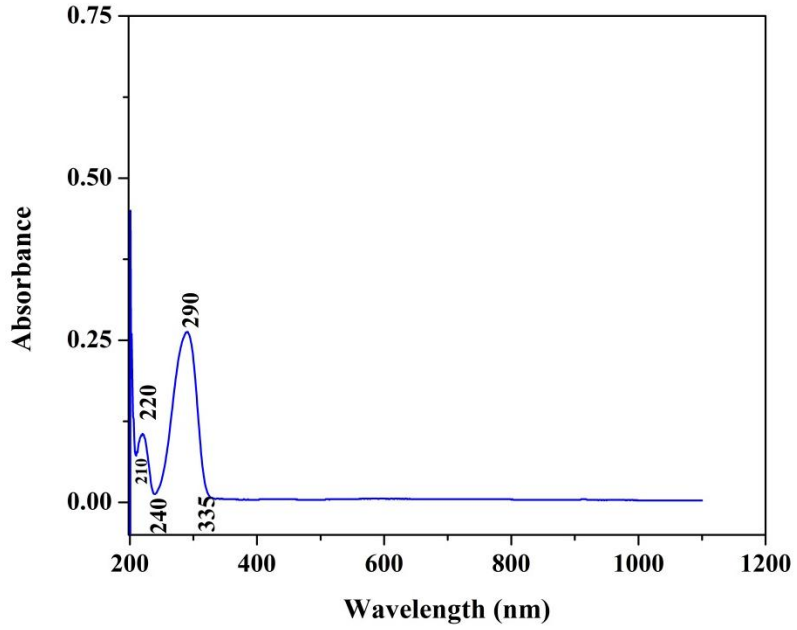

Fig. 6: Absorbance spectrum for 4-carboxyaniline crystal

The 4-carboxyaniline crystal absorbs the maximum wavelengths at 220 nm and 290 nm and its lower cut-off wavelength is found to be at 210 nm, 240 nm and 335 nm. Also, it has 100% transmittance in the entire visible region which makes usefulness of this material in optical application.

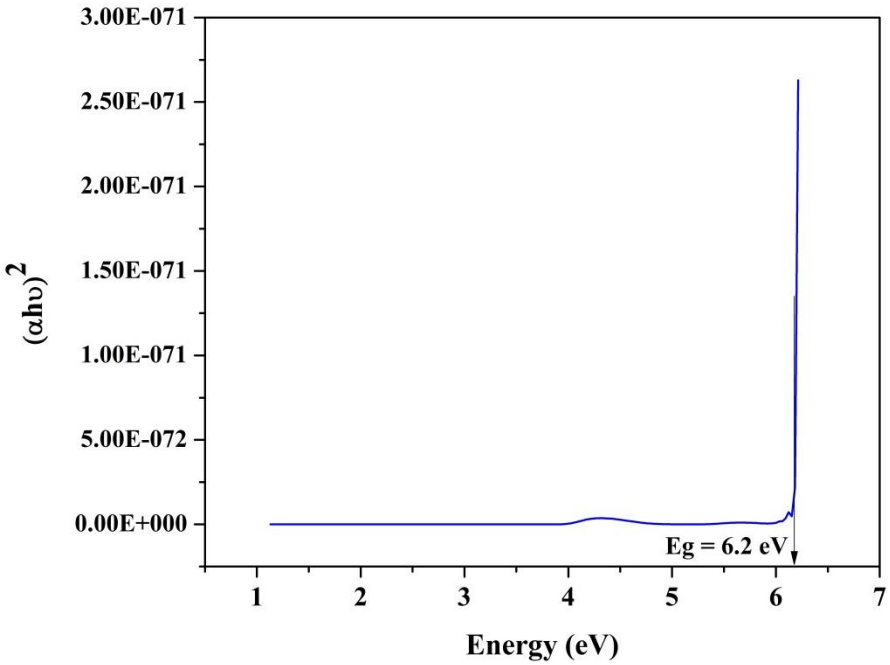

Fig.7: Optical band gap for 4-carboxyaniline crystal

The optical energy band gap E_g is determined by using the Tauc's relation $(\alpha h\nu)^2 = A(h\nu - E_g)$ by plotting the $(\alpha h\nu)^2$ Vs photon energy and extrapolate the linear portion of $(\alpha h\nu)^2$ to the photon energy axis gives the energy gap value of title crystal. It is found at 6.2 eV from the Fig.7.

SEM with EDX Analyzes

The microphotograph of title crystal is depicted in Fig.8 which was recorded using CARLZEISS EVO18 scanning electron microscope. This image shows that the grown crystal has a smooth surface and well defined shape. The EDX spectrum for 4-carboxyaniline crystal is shown in Fig.9. The composition of element present in crystal is shown in Table 4.

Table 4: Elemental composition for 4- carboxyaniline crystal

Elements	**4-caboxyaniline**	
	Atomic%	Weight %
C	44.19	14.59
N	37.21	35.48
O	17.85	48.13

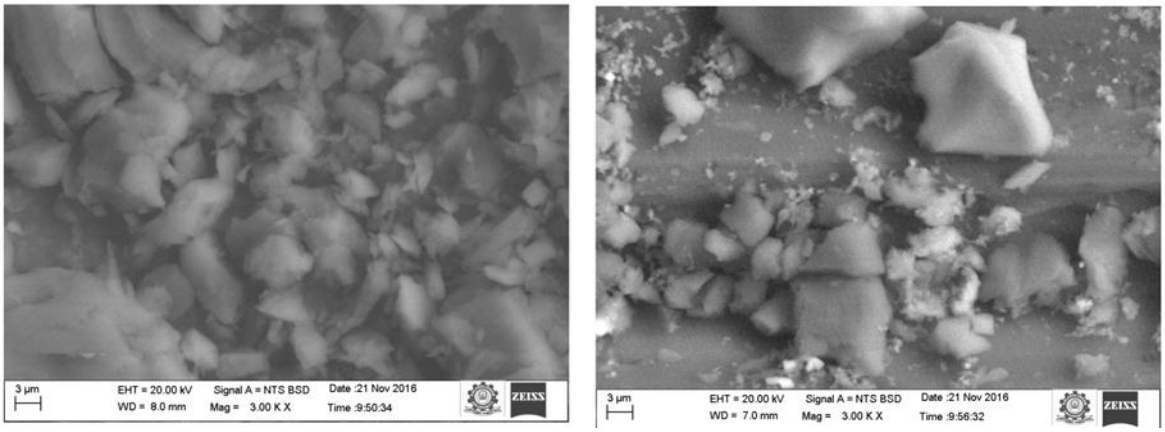

Fig. 8: SEM photograph for 4-carboxyaniline crystal

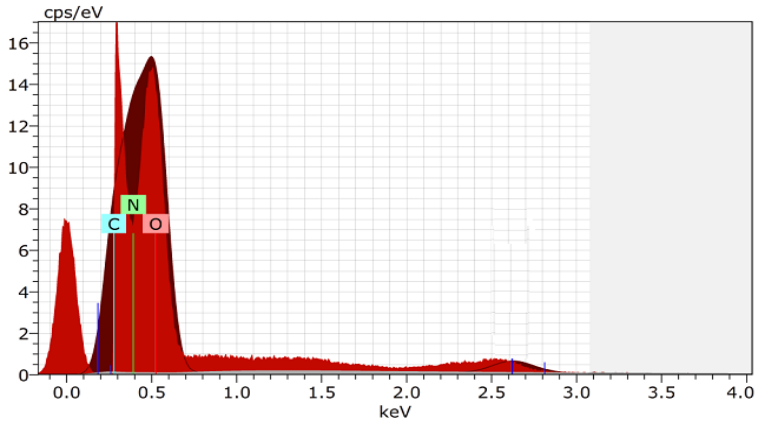

Fig. 9: EDX spectrum for4-carboxyaniline crystal

The C, N and O elements were identified from the EDX spectrum and their atomic and weight percentage has been calculated. This study confirms the presence of all elements in the title crystal.

Antimicrobial Activity Study

The biological important drug compound of 4-carboxyaniline crystal was tested against Staphylococcus epidermidis (S. epi), Serratia marcescens (S. mar), Methicillin-resistant Staphylococcus aureus (MRSA) and Polyphosphate-accumulating organisms (PAO-1) to analyze the antimicrobial activity by disc diffusion method. The photographic view of bacterial screening for title crystal is shown in fig.10. The above mentioned bacterial species were prepared at 50µg/ml and 100 µg/l concentrations. The measured diameter zone of inhibition of these micro-organisms is shown in Table 5.

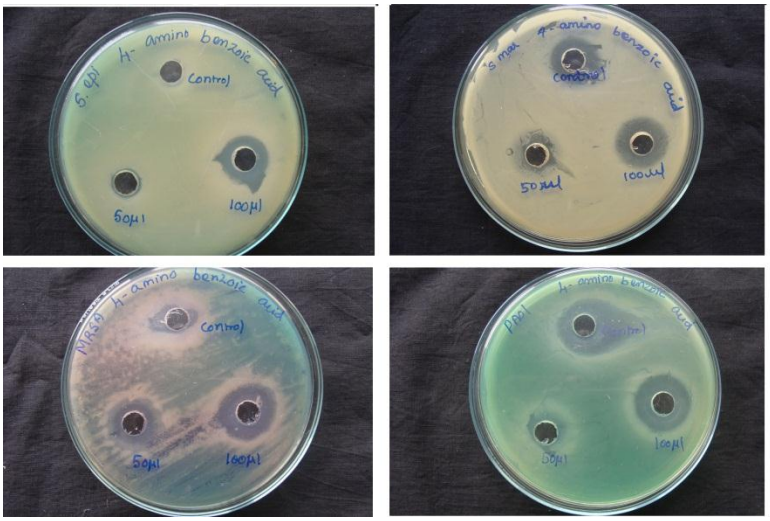

Fig. 10: Photographic view showing inhibition region of four different micro-organisms at 50µg/ml and 100 µg/l concentrations against the 4-carboxyaniline crystal

Table 5: Effective values of inhibited zone for 4- carboxyaniline crystal

S.NO.	Micro-organisms	Zone of inhibition for 4-Carboxyaniline	
		50 µl (mm)	100 µl (mm)
1.	S. epi	1	4
2.	S. mar	6	10
3.	MRSA	8	9
4.	PAO - I	4	10

The 4-carboxyaniline crystal has an effective zone of inhibition if the concentration was increased. Especially, it was very dominant against the S. epi, S. mar and PAO - I bacteria.

4. CONCLUSION

The 4-carboxyaniline crystal was successfully grown using single diffusion gel method for the first time. The single crystal XRD study reveals that the title compound belongs to monoclinic crystal system with the space group $P2_1/n$. The sharp peaks observed in powder XRD pattern is due to the good quality of crystalline nature of title compound and all the observed reflections were indexed using INDX software. Further, the presence of functional groups and elements were verified using FT-IR, FT-Raman spectroscopy and energy dispersive X-ray studies respectively. The SEM analyzes shows that the title crystal has smooth surface and well defined morphology. The optical property of this crystal was analyzed using UV- Visible spectroscopy technique and the optical band gap is determined as 6.2 eV. The grown crystal was involved in an antibacterial activity against certain potentially threatening microbes such as Staphylococcus epidermidis, Serratia marcescens, Methicillin-resistant Staphylococcus aureus and Polyphosphate-accumulating organisms by disc diffusion method which shows that the title crystal screened the bacteria if the concentration was increased.

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REFERENCES

[1] Para-amino benzoic acid, *Medline Plus Medical Encyclopedia*, United States National Institutes of Health, (2014).

[2] T. Maki and K. Takeda, Benzoic Acid and Derivatives, *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH, (2000).

[3] Elson M. Hass, *Staying Healthy with Nutrition*, (2003).

[4] H.Thune and P. Eeg Larsen, *Arch. Dermatol. Res.*, 282 (1990) 38 – 41.

[5] *http://www.raysahelian.com/paba.html*

[6] *http://www.naturalpedia.com/PABA.html*

[7] S. Athimoolam and N. Natarajan, *Acta Cryst.*, C63 (2007) o514-o517.

[8] S. Gracin and A. Fischer, *Acta Cryst.*, E61(2005): 1242-1244.

[9] M. Prasad, M. R. Kapadia and V. C. Thakar, *J. Univ. Bombay Sci.*, 8 (1939) 667- 669.

[10] R. C. G. Killean, P. Tollin, D. G. Watson and D. W.Young, *Acta Crystallogr.*, 19(1965) 482 - 483.

[11] M. Kuhnert-Brandstaetter and H. Grimm, *Mikrochim. Acta*, 6 (1969) 1208 - 1209.

[12] M. Alleaume and G. Salas-Ciminago, *J. Compt. Rend.,Ser. C*, 262 (1966) 416- 417.

[13] N. B. Colthup, L. H. Daly and S. E. Willely, *Introduction to Infrared and Raman Spectroscopy*, Academic Press, New York, (1990).

[14] L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, Chapman and Hall, London, **(**1975).

[15] R. Anitha, S. Athimoolam, M. Gunasekaran and K. Anitha, *Journal of Molecular Structure*, 1076 (2014) 115 125.

[16] K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Co-ordination Compounds*, Wiley, New York, (1986).

[17] C. Muthuselvi, S. Pandiarajan and R. V. Krishnakumar, *Elixir Vib. Spec.*, 95 (2016) 40673- 40678.

[18] C. Muthuselvi, M. Dhavachitra and S. Pandiarajan, *Journal of Chemical and Pharmaceutical Research*, 8(5) (2016) 804-814.

[19] Hema Tresa Varghese, C. Yohannan Panicker and Daizy Philip, *Spectrochimica Acta Part A*, 6(2006) 155-158.

[20] Abdullah G Al-Sehemi. *JKAU: Sci.,* 23 (2011) 63-70.

[21] U. O. Ozdemir, P. Guvenç, E. Sahin, F. Hamurcu. *Inorganica Chimica Acta*, 362 (2009): 2613- 2618.