Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2017, 9(5):122-130

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Growth, Characterization and Antibacterial Study of Sulfanilamide Doped by 4-Aminobenzoic Acid

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ABSTRACT

To enhance the antibacterial activity of toxic drug compound sulfanilamide, it is doped by non-toxic compound 4-aminobenzoic acid by slow evaporation method. The powder XRD analysis shows that the doped crystal has both parent phases. The FT-IR and FT-Raman spectra of doped compound reveals the presence of functional groups of sulfanilamide and 4-aminobenzoic acid. The optical property of doped crystal is analyzed by UV-Vis-NIR spectroscopy. The morphology and elemental composition study confirms the presence of 4-aminobenzoic acid in the crystal lattice of sulfanilamide crystal. The antimicrobial activities of pure and doped crystals were tested against the five different micro-organisms by disc diffusion method. This result reveals that the doped crystal has more antibacterial activity than the pure sulfanilamide crystal.

Keywords: Sulfanilamide; 4-aminobenzoic acid; Antibacterial activity; Vibrational spectra; Optical analyzes; SEM with EDX

INTRODUCTION

The antibacterial drug compound of sulfanilamide is used as an active agent to breakdown the prontosil in the human body [1-3]. This prontosil is inhibiting the growth of streptococci in mice [4-7]. The nontoxic drug of 4-aminobenzoic acid (PABA) is also called vitamin B_x which is also used to make various anesthetics drugs. In micro-organism the sulfanilamide's activity is overcome by PABA due to their structural similarity [8]. The sulfa drug is toxic to all cells which undergo the rapid cancer cell division. To improve the medicinal effect and reducing toxicity of the drug, it is used only their derivatives as a potential drug [8,9]. The chemical modification of sulfanilamide drug gives the broader antibacterial activity and different pharmacological actions [10]. Due to these interesting reports, attempt was made to grow the single crystals of sulfanilamide doped by PABA by slow evaporation method. The grown crystal is characterized by the powder XRD, FT-IR, FT-Raman, UV-Visible spectroscopy and SEM with EDX. These studies and antimicrobial activity techniques lead to confirm the incorporation of PABA into sulfanilamide.

MATERIALS AND METHODS

Materials

The raw materials used for this crystallization are sulfanilamide, ethanol, 4-aminobenzoic acid, double distilled water purchased from Merck, India.

Crystal Growth by Slow Evaporation Method

The 1:1 stoichiometric ratio of sulfanilamide and 4-aminobenzoic acid were mixed in the aqueous solution of ethanol. This solution was stirred for 1 hour and filtered using grade A1quality Whatman paper. Then it was allowed to evaporate under room temperature. After 15 days the grown crystals of sulfanilamide doped by PABA were harvested from the petri dish. The same procedure is used to grow the parent sulfanilamide crystal. The molecular structure of sulfanilamide and 4-aminobenzoic acid depicted in Figure 1. It also shows that he photographic view of pure and doped crystals.

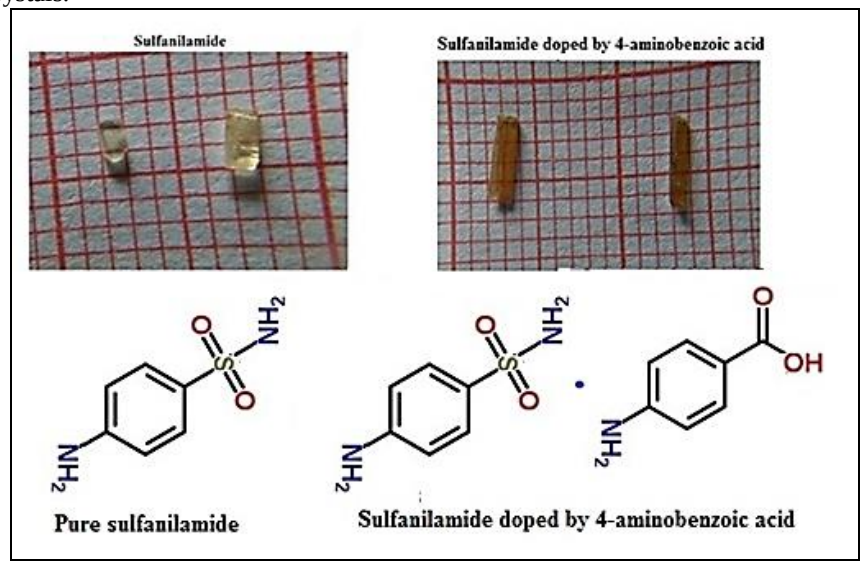


Figure 1: Photographic view of pure and doped crystals and their structures

Characterization Techniques

The powder X-ray diffraction patterns were collected for pure sulfanilamide, 4-aminobenzoic acid and sulfanilamide doped by 4-aminobenzoic acid crystals using a XPERT–PRO X–ray diffractometer with Cu Kα (λ = 1.54060 Å) radiation which is used to identify crystalline phases. The FT−IR spectra were recorded by using SHIMADZU FT−IR spectrometer in the range 4000−400 cm^{-1}. The FT−Raman spectra were recorded by using the BRUKER: RFS 27 Raman spectrometer in the range 4000−400 cm^{-1}. The optical absorption spectra of grown crystals have been recorded with SHIMADZU−UV1800 double beam spectrometer in the wavelength range 200−1100 nm insteps of 1 nm. Morphologies of pure and doped crystals were investigated by using SEM analysis. The surface morphology and elemental analysis of pure and doped sulfanilamide have been analyzed by CARLZEISS EVO18 scanning electron microscope. The antimicrobial activity of pure and doped sulfanilamide crystals were tested against five different kinds of micro-organisms by disc diffusion method.

RESULTS AND DISCUSSION

Powder XRD Analysis

The powder X-ray diffraction patterns of pure sulfanilamide, 4-aminobenzoic acid and sulfanilamide doped by 4-aminobenzoic acid crystals were recorded using the Cu Kα (λ = 1.54060 Å) radiation from 10° to 80° at 10°/s scan step time. The experimental diffraction patterns of pure and doped crystals are shown in Figure 2. The experimentally observed diffraction peaks of doped crystal are at angles (2θ) of 13.82°, 15.27°, 21.86°, 35.79°, and 38.81° and at 19.04°, 28.77°, 30.94°. The corresponding indexed planes are (2 0 2), (1 0 3), (2 0 4), (4 0 6), (6 1 0) and (0 0 2), (2 2 0), (2 1 -3) which are reflected from 4-aminobenzoic acid and sulfanilamide crystals respectively.

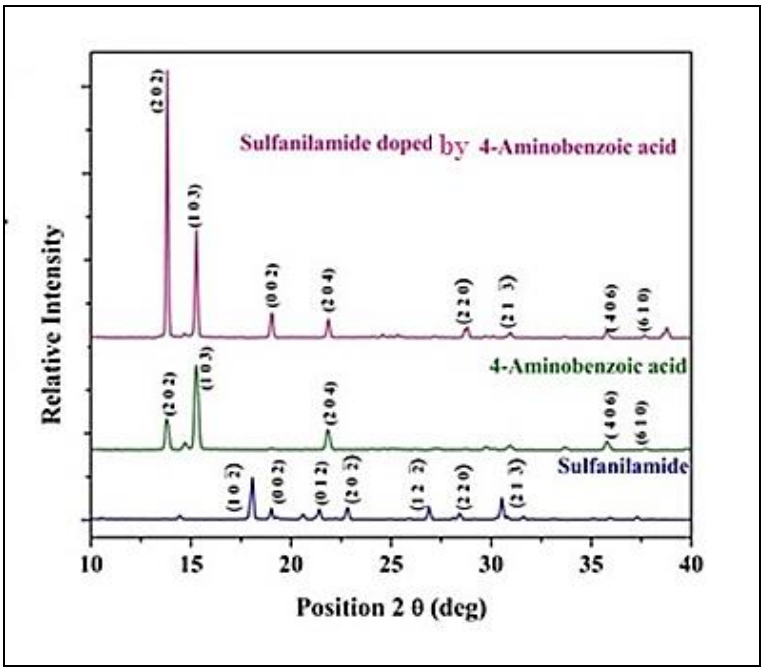


Figure 2: Diffraction patterns for pure and doped crystals

The peaks are indexed using INDX software. This result suggests that both parent phases have emerged out from doped crystal XRD pattern confirming the incorporation of 4-aminobenzoic acid into the crystal lattice of sulfanilamide.

Vibrational Analyzes

The IR and Raman spectra of pure and doped sulfanilamide crystals are shown in Figures 3 and 4 respectively. The detailed wavenumber assignments of absorption bands in both spectra of pure and doped sulfanilamide are depicted in Table 1.

Aniline Group (NH_2) Vibrations

Generally, the aniline (NH_2) stretching mode is expected in the region 3480-3250 cm^{-1} [11,12]. It is observed for pure sulfanilamide crystal at 3476 cm^{-1} in IR spectrum. But it is shifted to 3464 cm^{-1} for doped crystal due to the involvement of hydrogen bond. The bands identified at 1620 ± 20 cm^{-1} corresponds to bending vibrational mode of aniline [13]. In the present work, this mode is confirmed by the strong peaks at 1630 cm^{-1} for pure and doped crystals. The wagging mode of aniline is expected in the range 620 ± 100 cm^{-1} [13]. In IR and Raman spectra of pure sulfanilamide crystal ω (NH_2) of aniline is attributed at 540 cm^{-1} as a medium band. This mode is not identified in the doped crystal.

Sulfonamide Group Vibrations

Normally, the NH_2 stretching and bending modes of sulfonamide are predictable in 3390 - 3230 cm^{-1} and 1565 ± 15 cm^{-1} region respectively [14,15]. The experimentally observed bands of pure sulfanilamide crystal at 3374 cm^{-1} and 1553 cm^{-1} in IR and Raman spectra respectively are designated to the stretching and bending modes of NH_2 group of amide. Due to the presence of hydrogen bond this mode is slightly deviated at 3364 cm^{-1} and 1570 cm^{-1} in both spectra for doped crystal. The wagging mode of NH_2 group of sulfonamide occurs in the range 690 ± 40 cm^{-1} [16]. It is observed at 686 cm^{-1} and 696 cm^{-1} for pure and doped crystals respectively in IR and Raman spectra. The antisymmetric and symmetric stretching vibrations of SO_2group appear in the region 1330 ± 30 cm^{-1} and 1160 ± 30 cm^{-1} [14,15]. In the present work, $\nu_{as}(SO_2)$ mode is attributed at 1314 cm^{-1} in both spectra for pure and doped crystals and $\nu_s(SO_2)$mode is found at 1148(IR), 1156(Raman) for the pure crystal only. The scissoring and wagging modes of SO_2 group are assigned in the region 570 ± 60 cm^{-1} and 520 ± 40 cm^{-1} respectively [16]. They are observed at 621 cm^{-1} and 563 cm^{-1} for sulfanilamide crystal respectively in IR spectrum only and their counterparts are not observed in the Raman spectrum. But the $\omega(SO_2)$ mode of doped crystal is shifted to 550 cm^{-1}. The stretching mode

of SN provides a band in the region 905 ±70 cm^{-1} [17]. The pure and doped crystals exhibit a medium intensity band in the both spectra at 899 cm^{-1} and 893 cm^{-1} respectively.

Carboxylic Acid Group Vibrations

The ν (C=O) mode of carboxylic acid group has a characteristic band in the region 1800-1680 cm^{-1} [18]. In the doped crystal, it is identified in the IR spectrum as a broad strong band at 1668 cm^{-1}. This wavenumber down shifting may be due to the involvement of carbonyl group in the hydrogen bonding. Normally, the C–O stretching of carboxylic acid appears in the region 1320–1210 cm^{-1} of vibrational spectra [18,19]. The observed strong peaks in both spectra for doped crystal at 1290 cm^{-1} and 1284 cm^{-1} are assigned to the ν (C–O) mode. The O–H stretch from CO–OH vibration is observed at 3065–2826 cm^{-1} [20]. In the present study, these wavenumbers are observed at 3051, 2913, 2826 cm^{-1} in the FT–IR and 3065 cm^{-1} in the FT–Raman spectra for doped crystal only. The in- plane and out -of plane bending wavenumbers of O–H group normally appear in the region between 1440–1395 cm^{-1} and 960–875 cm^{-1} respectively [20,21].

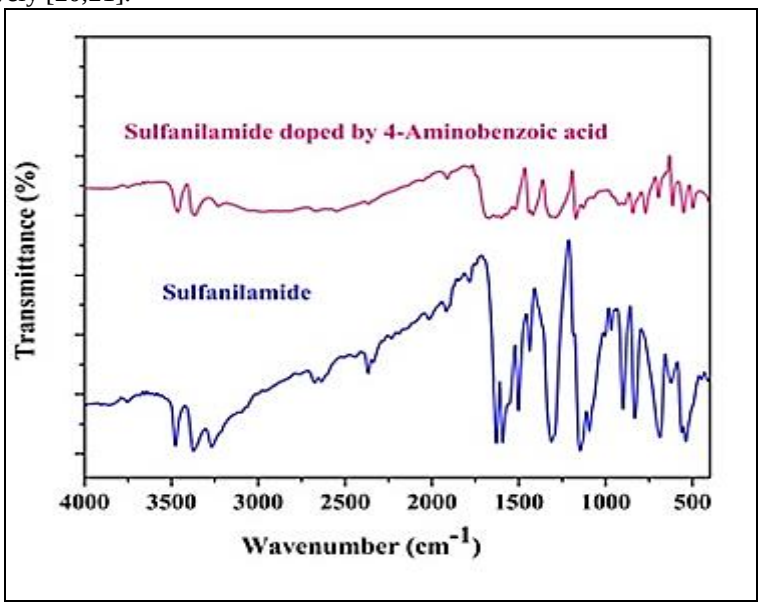


Figure 3: FT-IR spectra for pure and doped sulfanilamide crystals

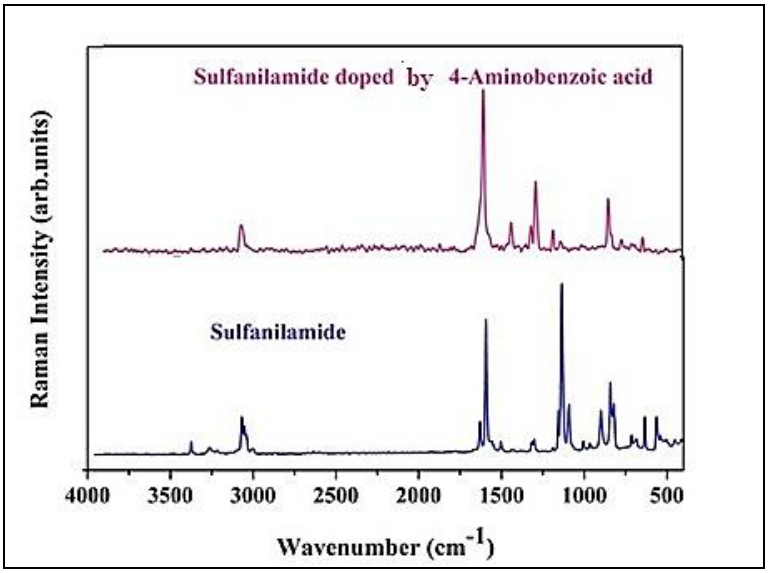


Figure 4: FT-Raman spectra for pure and doped sulfanilamide crystals

The doped crystal has strong bands at 1420 cm^{-1} and 923 cm^{-1} which are attributed to O–H in plane and out–of–plane deformation modes respectively in IR spectrum. This carboxylic group vibrations are identified only for the doped crystal spectra. This confirms the inclusion of 4-aminobenzoic acid with the sulfanilamide through the –COOH group by the formation of hydrogen bonds. The appearance of broad band centered around 3000 cm^{-1} in IR spectrum of doped crystal supports the presence of hydrogen bonding network in the doped crystal.

Table 1: Wavenumber assignment for pure and doped sulfanilamide crystals in FT – IR and FT – Raman spectra

Sulfanilamide		Sulfanilamide doped by 4-Aminobenzoic acid		Assignment
FT – IR ($\bar{\upsilon}$ / cm^{-1})	FT– Raman ($\bar{\upsilon}$ / cm^{-1})	FT – IR ($\bar{\upsilon}$ / cm^{-1})	FT – Raman ($\bar{\upsilon}$ / cm^{-1})	
3476 (s)	–	3464 (s)	–	ν_{as} $NH_{2\ aniline}$
3374 (s)	3374 (m)	3364 (s)	3363 (w)	ν_{s} $NH_{2\ amide}$
3267 (s)	3262(w)	3231 (m)	–	ν_{s} $NH_{2\ amide}$
–	3068 (m)	–	3065 (m)	υ X□H;υ O□H
–	–	3051 (s, br)	–	υ X□H;υ O□H
–	–	2913(s, br)	–	υ O□H
–	–	2826(s, br)	–	υ O□H
–	–	1668(s, br)	–	ν C=O
1630 (s)	1629 (w)	1630(s)	–	δ_s (NH_2) ; ν (C-C)
1593 (s)	1594(w)	1599(s)	–	ν C=C + δ NH_2; ν (C-C)
1553(w)	1554(s)	1570(w)		δ $(NH_2)_{amide}$
1437 (m)	–	1441(m)	1433(m)	ν C=C
–	–	1420(s)	–	β (O-H)
1314 (s)	1314(w)	1317(s)	1312(m)	ν CN ; ν_{as} (SO_2)
–	–	1290(s)	1284(s)	ν C-O
1148(s)	1156(s)	–	–	ν_s (SO_2); β (C-H)
1094 (s)	1091(w)	–	–	β (C-H)
1003(w)	1005(s)	–	–	β (C-H)
968(w)	967(w)	–	–	γ (C-H)
–	–	923(m)	–	γ O–H+β C–H
899(m)	899(sh)	893(m)	–	γ (C-H); ν (S-N)
831(m)	841(m)	843 (s)	846(m)	γ (C-H); Ring breathing
–	821(m)	–	–	γ (C-H)
686(s)	685(w)	696(s)	–	ω $(NH_2)_{sulfonamide}$
	634(m)	–	640(w)	ν (C-S)
621(m)	–	–	–	δ_s(SO_2)
563(m)	563(m)	550(s)	–	ω (SO_2)
538(m)	540(w)	–	–	ω $(NH_2)_{aniline}$

w: weak; vs: very strong; m: medium; sh: shoulder; u: stretching; us: sym. stretching; uas: asym. stretching; γ: out–of–plane bending; β: in–plane bending; ρ: rocking; ω: wagging; δ: scissoring; t: twisting

Para Substituted Benzene Ring Vibrations

The para substituted benzene ring C–H stretching modes vibration normally occurs in the region 3115–3005 cm^{-1}. By the nature of the substituents, bands are not affected appreciably in this region [22]. The Raman bands observed at 3068 cm^{-1} and 3065 $cm^{-1}$are assigned to the C–H stretching vibrations for pure and doped crystals. The in–plane bending vibrations of C–H are seen in the range 1315 –1225 cm^{-1} and 1190 – 995 cm^{-1} [23]. Also, the out–of–plane C–H deformation bands γ(C–H) are expected in the range 990 – 790 cm^{-1} [22,23]. The IR peaks at 1314, 1148, 1094, 1003 $cm^{-1}$and Raman peaks at 1314, 1156, 1091, 1005 cm^{-1} are assigned to this mode. The γ(C–H) mode is observed in both vibrational spectra at 968, 899, 831cm^{-1} and 967, 899, 841, 821 cm^{-1} respectively for pure sulfanilamide crystal. The above modes are also observed also for doped crystal. The C–C and C=C vibrations are ordinary to appear in the regions 1620–1585 cm^{-1} and 1590–1565 cm^{-1} respectively [24]. In spectra, these bands are known at 1630 cm^{-1}, 1593 cm^{-1} as strong peaks in IR spectrum and at 1629, 1593 cm^{-1} as weak peaks in Raman spectrum correspond to these C–C vibrations for both compounds. Also C=C modes are observed as medium bands at 1437, 1441 cm^{-1} in FT–IR for pure and doped crystals respectively. The para substituted benzene ring breathing mode is observed at 831 cm^{-1} and 841 cm^{-1} in FT–IR and FT-Raman spectra for pure crystal. But this mode is identified at 843 cm^{-1} and 846 cm^{-1} in both spectra for doped crystal.

Optical Analyzes

The optical analyzes of pure and doped sulfanilamide crystals were performed by using the UV-Visible-NIR spectroscopy technique. The absorbance spectra of both crystals are recorded in the wavelength range from 200 nm-1100 nm which is shown in Figure 5.

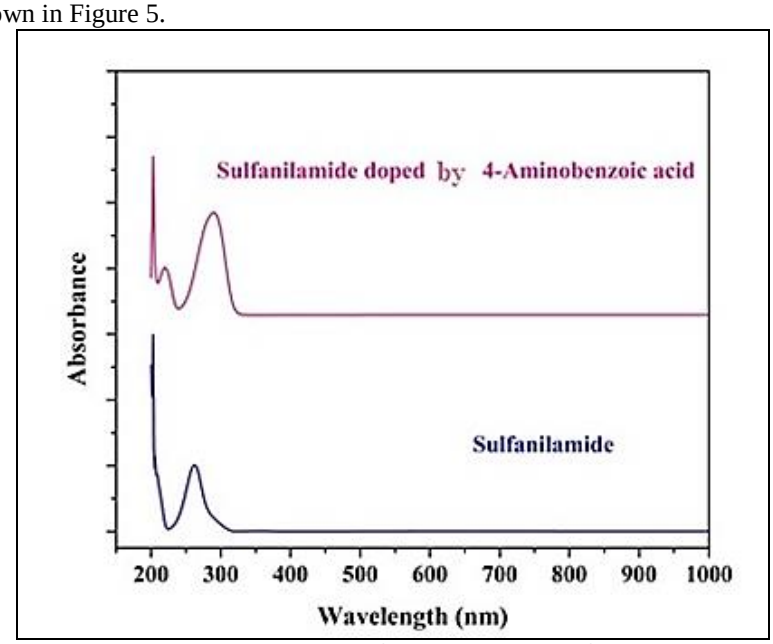
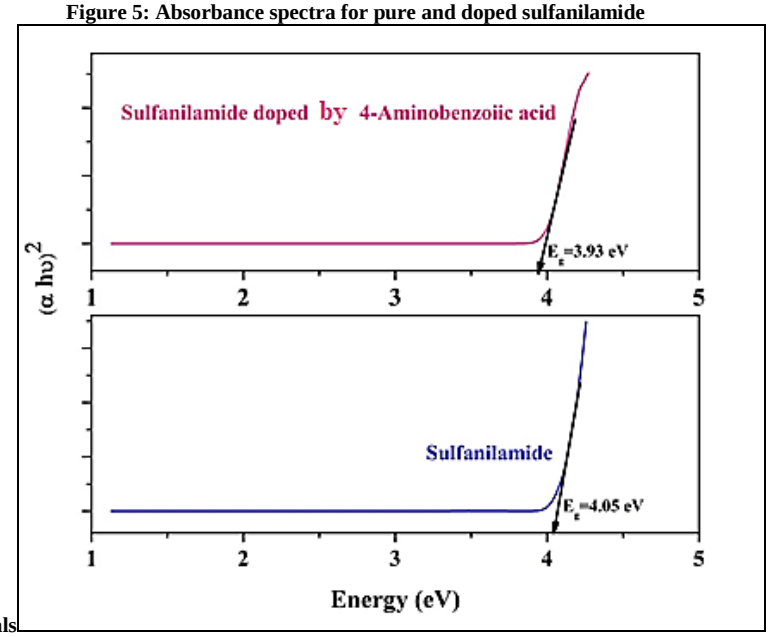


Figure 5: Absorbance spectra for pure and doped sulfanilamide



crystals

Figure 6: Optical band gap for pure and doped sulfanilamide crystals

The pure sulfanilamide crystal has a maximum absorbance peak at 262 nm. But the two peaks are observed at 291 nm and 220 nm for the doped crystal. The lower cut-off wavelengths are found at 324 nm, 225 nm and 341 nm, 240 nm, 211 nm for pure and doped crystals respectively. This result suggests that both crystals exhibit almost similar transparency in the entire visible region. The Tauc's relation $(\alpha h\nu)^2 = A(h\nu - E_g)$ is used to determine the energy gap value E_g of both crystals by plotting $(\alpha h\nu)^2$ Vs photon energy. The linear portion of $(\alpha h\nu)^2$ is extrapolated to the photon energy axis to give the energy gap values of both crystals. From the Figure 6, the energy gap values are determined as 4.05 eV and 3.93 eV for pure and doped sulfanilamide crystals respectively.

SEM with EDX Analyzes

The pure and doped crystals SEM photograph is shown in Figure 7 which reveals that the both crystals have well defined face formation. The morphology of the doped crystal is entirely different from that of pure crystal.

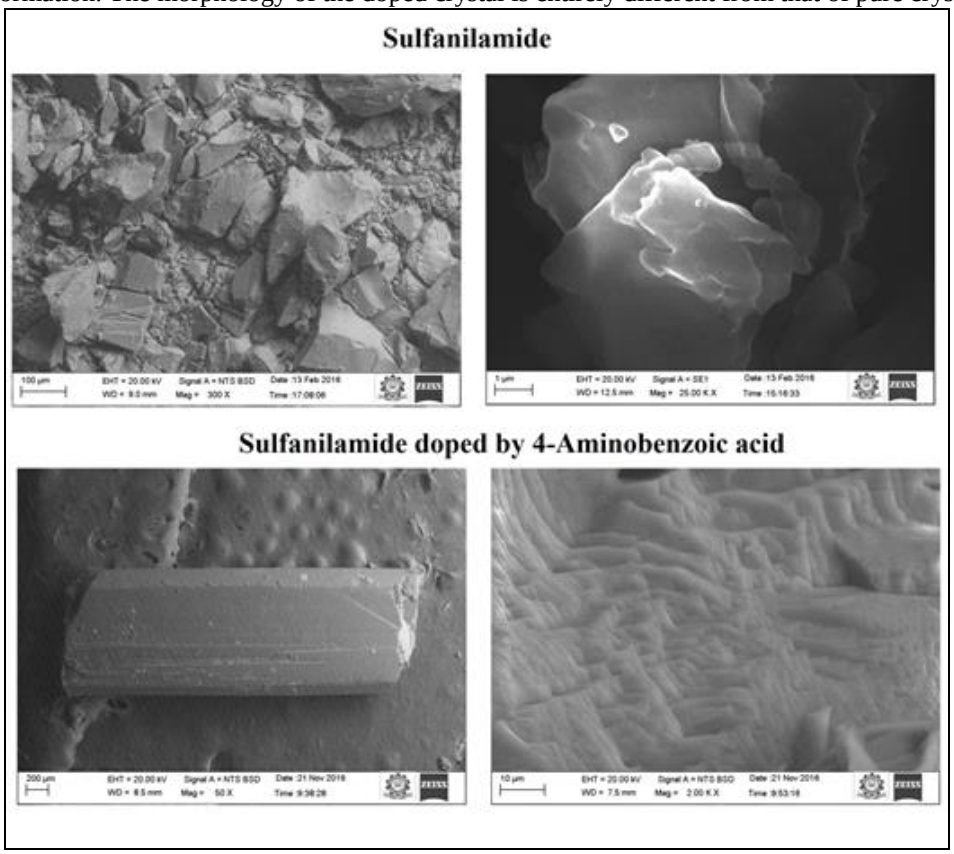


Figure 7: SEM photograph for pure and doped sulfanilamide crystals

The EDX spectra of the both crystals are shown in Figure 8 and their elemental composition are shown in Table 2.

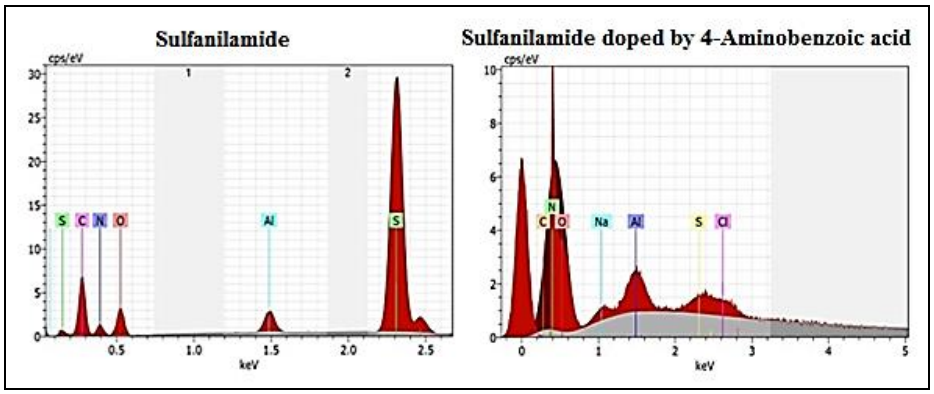


Figure 8: EDX spectra for pure and doped sulfanilamide crystals

The atomic and weight percentage of C, N, O increased and S is decreased when the dopant of 4-aminobenzoic acid is added to the sulfanilamide crystal. This result suggests the dopant has been incorporated into the crystal lattice of sulfanilamide.

Antimicrobial Activity Study

The antimicrobial activity of sulfanilamide and sulfanilamide doped by 4-aminobenzoic acid crystals were analyzed by disc diffusion method against *Staphylococcus aureus*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Bacillus subtilis*

and *Escherichia coli* microorganisms. The photographic view of bacterial screening for pure and doped sulfanilamide crystals are shown in Figure 9.

Table 2: Elemental composition for pure and doped sulfanilamide crystals

Elements	Sulfanilamide		Sulfanilamide doped by4-amino benzoic acid	
	Atomic%	Weight %	Atomic%	Weight %
C	3.9	1.18	5.92	2.38
N	34.67	6.07	42.51	14
O	43.53	8.21	47.39	18.03
S	16.21	1.94	0.61	0.22

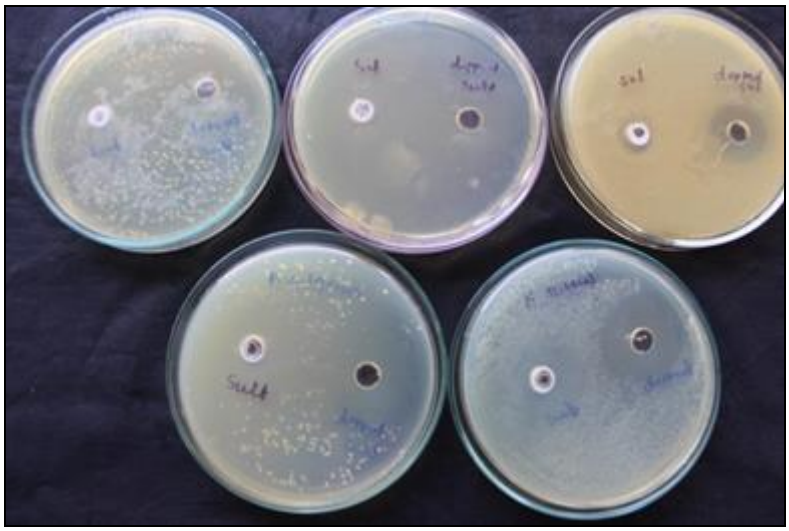


Figure 9: Photographic view showing inhibition region of five different microorganisms against pure and doped sulfanilamide samples

The measured values of zone of inhibition for five different microorganisms of pure and doped crystals are shown in Table 3

Table 3: Effective values of inhibited zone for pure and doped sulfanilamide crystals

S.NO.	Microorganism	Zone of Inhibition in mm (50µl)	
		Sulfanilamide	Sulfanilamide doped by 4-Aminobenzoic acid
1	*Staphylococcus aureus*	NIL	20
2	*Proteus vulgaris*	NIL	13
3	*Klebsiella pneumoniae*	NIL	15
4	*Bacillus subtilis*	NIL	NIL
5	*Escherichia coli*	10	14

The antimicrobial study of pure sulfanilamide compound has little effect only on *E. coli* than other microorganisms. But in contrast, the doped sulfanilamide crystal by PABA shows the greater effect on all test bacterias except *Bacillus subtilis*. At lower concentration, the doped crystal has higher antimicrobial activity on *Staphylococcus aureus* microorganism. The effect of doped compound on tested microorganisms from higher to lower order is given as follows *Staphylococcus aureus> Klebsiella pneumonia> Escherichia coli> Proteus vulgaris*. This study shows that antibacterial activity of sulfanilamide is enhanced by doping of PABA.

CONCLUSION

By using the slow evaporation method, the sulfanilamide is doped successfully by the 4-aminobenzoic acid and crystals are obtained after 15 days duration. The PXRD study confirms the doping of 4-aminobenzoic acid with sulfanilamide. The vibrational wavenumber assignments for title compound are performed using IR and Raman spectroscopy analyzes. The optical band gap and transparency of the pure and doped crystals are found by UV-Visible spectroscopy study. It reveals that band gap of sulfanilamide crystal is decreased in the presence of 4-aminobenzoic acid. The SEM study shows that the morphology of doped crystal is different from the pure crystal.

The elements present in the pure and dope crystals are identified from the EDX. The antimicrobial activity of sulfanilamide and doped crystals are analyzed by disc diffusion method which suggests that 4-aminobenzoic acid can use as an active substance to enhance the antibacterial activity of sulfanilamide.

ACKNOWLEDGEMENT

The authors sincerely acknowledge their thanks to the Management and Principal of Devanga Arts College, Aruppukottai for their permission and encouragement during their research work.

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