International Journal of Research Instinct
(Peer-reviewed Journal)

ISSN ONLINE 2348-2095
Research Article

Powder x-ray diffraction pattern and antimicrobial activity of butyl para ben crystals

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ABSTRACT

The butyl para ben crystals were recrystallize by the slow evaporation method with ethanol as the solvent and the powder x-ray diffraction pattern confirms that the grown crystals have hexagonal crystal system with cell parameters a= b = 9.9545 Å, c= 4.0643 Å, α=β=90° and γ=120°. The measured crystal size tabulated. The Williamson hall plot shows that the crystals crystallized in (001) direction. The increased concentration of butyl para ben shows better antimicrobial activity against *Escherichia coli* organism. The zone of inhibition was greater in 0.01 molarity of concentration of butyl para ben crystals when comparing it with 0.001molarity of concentration.

KEYWORDS: Butyl para ben, powder x-ray diffraction, hexagonal crystal system, antimicrobial activity

1. INTRODUCTION

Butyl para ben a naturally derived preservative, is widely used in pharmaceutical products and cosmetics (Charnock & Finsrud, 2007), and generally considered to be safe (Hossaini et al., 2000). The solubility of BPN has been reported in various solvents (Yang & Rasmuson, 2010; 2012; 2013). The earlier report shows that butyl para ben was crystallized in monoclinic system. But he present work report that the recrystallized butyl para ben crystal has hexagonal crystal system and have antimicrobial activity against *Escherichia coli* organism.

2 MATERIALS AND METHODS

2.1 PREPARATION

Butyl para ben was dissolved in ethanol. The solution was stirred for 30 minutes and allowed to form crystals at room temperature. After one week colourless crystals were harvested.

2.2 POWDER X-RAY DIFFRACTION

Powder x-ray diffraction pattern were collected from diffracto-meter XPERT-PRO, with starting position 2θ = 10.0231°, end position 2θ = 80.9231°, step size 2θ = 0.0500°, specimen length = 10.00 mm, measurement temperature = 25°C, Cu as anode material and K-Alpha = 1.54060 Å.

2.3 MICROORGANISM

Gram-negative bacteria *Escherichia coli* (ATCC 25922) selected for antimicrobial studies. The organism obtained from the Department of Biotechnology, Srimad Andavan Arts and Science College (Autonomous), Trichy-5. The bacterial isolate was first sub cultured in a nutrient broth and incubated at 37°C for 18h.

2.4 PREPARATION OF STANDARD BACTERIAL SUSPENSIONS

Bacterial culture was sub cultured in liquid medium (Nutrient broth) at 37°C for 8h and further used for the test (10^5-10^6CFU/ml). Bacterial strains were maintained on Nutrient agar slants (Hi media) at 4°C. These suspensions were prepared immediately before screening.

2.5 ANTIBACTERIAL SUSCEPTIBILITY TESTING USING KIRBY BAUER AGAR WELL DIFFUSION ASSAY

To assess the antibacterial activity of the prepared crystals, 20ml of nutrient agar were distributed into sterile Petri dishes. The agar was left to set. Then 0.1ml of *Escherichia coli* culture was spread over the agar. Wells (6mm diameter) were made with the help of cutter. Crystal solution at two different concentrations namely 0.01 and 0.001 molarity, were placed on agar well and were allowed to stand for 1 h at room temperature so as to allow the crystals to diffuse into medium and then incubated at 37°C for 24 hours. Well diffusion tests were performed for the *Escherichia coli* strain and the antibacterial activity was expressed as average mean of inhibition diameter (mm) produced by as grown crystals

2.6 NUTRIENT AGAR MEDIUM

Nutrient agar medium is one of the most commonly used medium for several routine bacteriological purposes:

Ingredients	:	Grams/Liter
Peptone	:	5gm
Beef extract	:	3gm

After adding all the ingredients into the distilled water it is boiled to dissolve the medium completely and sterilized by autoclaving at 15 pounds pressure (121°C) for 15 minutes.

3. RESULT AND DISCUSSION

The X-ray crystallographic pattern of the butyl para ben crystal shown in the figure 1. The complexes, belongs to the hexagonal crystal system. The values for each peak have been calculated with help of cell parameters and the corresponding h, k, l in all cases are in good agreement with observed values as in tables I. The lattice constants a, b, c for each unit cell have been found out and are tabulated in table II.

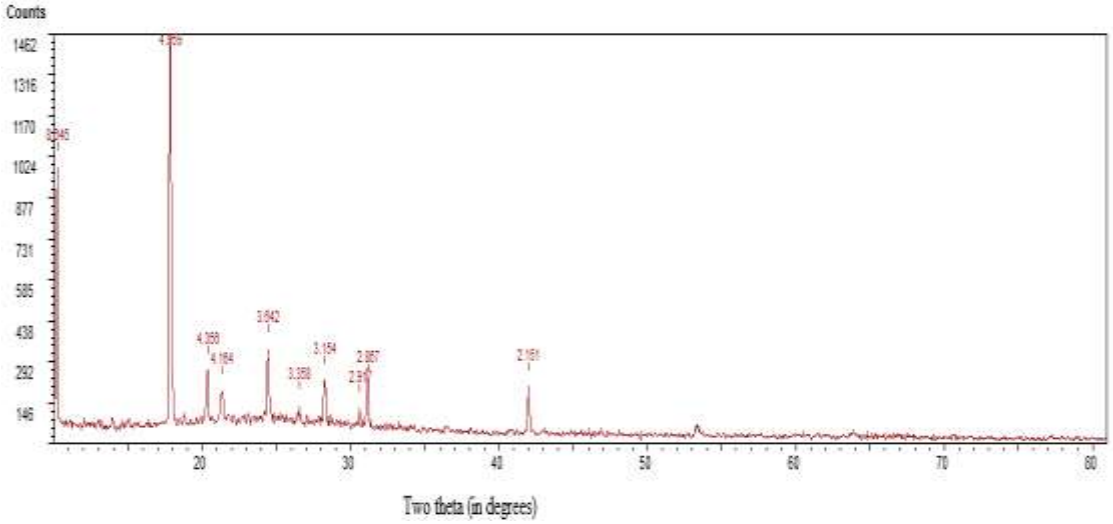

Figure 1 Powder x-ray diffraction pattern of butyl para ben crystals

S.No	Position 2θ(°)	d-spacing (Å)	hkl
1	10.12	8.646	010
2	17.82	4.959	110
3	20.414	4.356	020
4	21.277	4.164	001
5	24.464	3.642	011
6	28.248	3.154	111
7	30.639	2.917	021
8	31.037	2.867	030
9	41.926	2.151	040

TABLE I: INDEXED VALUE OF THE POWDER X-RAY DIFFRACTION PATTERN

a = b = 9.9545 Å, c = 4.0643 Å
α = β = 90°, γ =120°
Crystal system: Hexagonal

TABLE II: CELL PARAMETERS FOR BUTYL PARA BEN

Figure 2 shows the full width half maximum value for the peak having hkl (001). The Scherrer formula ($t = 0.9 \lambda / B \cos \theta_B$) is used to estimate the particle size of very small crystals from the measured width of their diffraction curves.

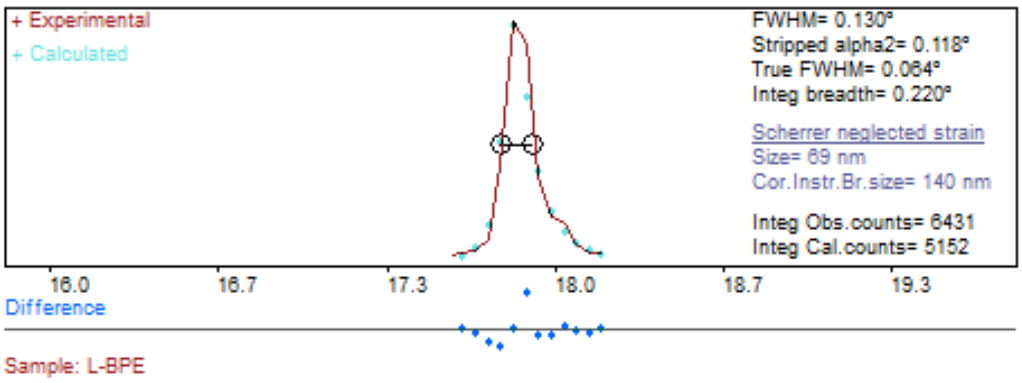

Figure 2 The FWHM plot for one peak

The calculated breadth B of 2θ° due to small crystal effect alone of powder pattern line of particle is shown in table III. From this table we could confirm that the breadth decreases with increase in particle size (t) with mosaic defect.

S.No	**Calculated t (Å)**	**B ° = θ1- θ2**	**Θ_B°**
1	113.46	1.3274	23
2	145.97	1.12	32
3	180.29	0.8	16
4	227.14	0.6538	21
5	421.55	0.35	20

TABLE III: CALCULATED (B) AND (t) VALUES:

Williamson-Hall plot is shown in figure 4 and has illustrated that line broadening was basically isotropic. Due to micro strain contribution the diffracting domains were isotropic. Size-strain parameters can be obtained from the "size-strain plot" (SSP). This has a benefit that less importance is given to data from reflections at high angles. In this estimation, it is assumed that profile is illustrated by "strain profile" by a Gaussian function and the "crystallite size" by Lorentzian function. Pseudo-Voigt profile coefficients were as parameterized in Thompson et al. (1987) with profile coefficients for Simpson's rule integration of the pseudo-Voigt function according to Howard (1982). The asymmetry correction of Finger et al. (1994) was applied, and micro strain broadening by Stephens (1999). This Williamson hall plot confirms that the crystals were grown in (001) direction.

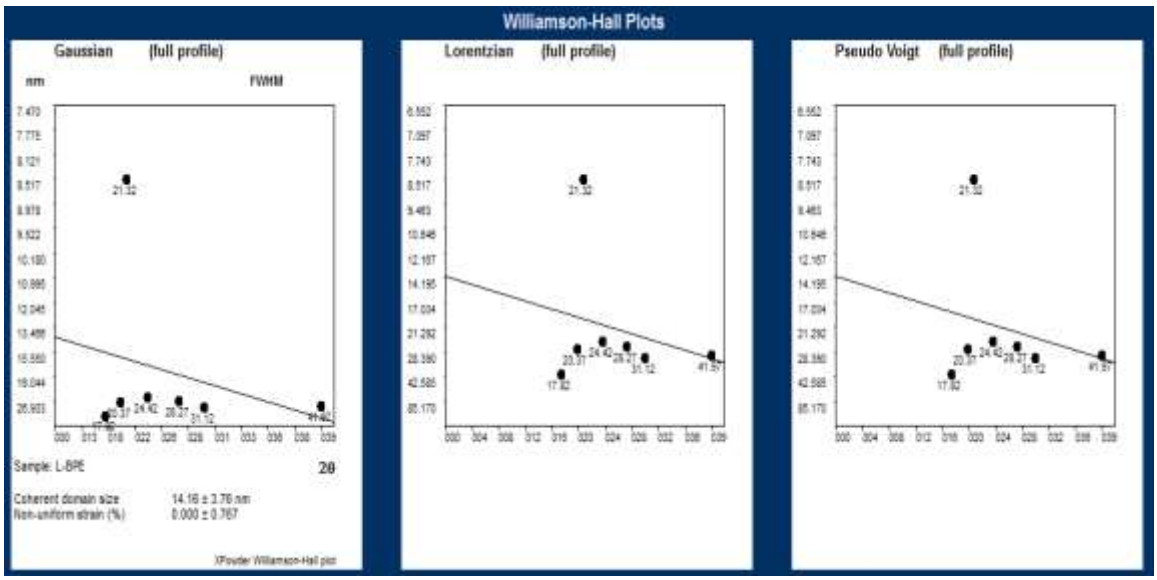

Figure 4 Williamson hall plot for butyl para ben crystals

3.1 ANTI-MICROBIAL ACTIVITY

Crystals prepared by slow evaporation method was screened for its antimicrobial efficacy against *Escherichia coli* by employing well diffusion method. The antimicrobial activity of butyl para ben depends on the concentration. For 0.01 molarity of concentration of butyl para ben form inhibition zone against *Escherichia coli* with diameter 1.8 cm, for 0.001 molarity of concentration of butyl para ben form inhibition zone against *Escherichia coli* with diameter 0.2 cm and are shown in figure 5. The zone of inhibition was greater in 0.01 molarity of concentration of butyl para ben crystals when comparing it with 0.001 molarity of concentration.

Antimicrobial zone in 0.01 molarity concentration

Antimicrobial zone in 0.001 molarity concentration

Figure 5 Antimicrobial activity of butyl para ben

3.2 CONCLUSION

As grown crystals were harvested using slow evaporation method and powder x-ray diffraction pattern was used to find the crystal system and to find the crystal sizes. The antimicrobial activity was identified for butyl para ben against *Escherichia coli.*

ACKNOWLEDGEMENT

The authors thank the first year Post graduate students S. Abirami, G. Srivethi and V. Keerthana of Department of Biotechnology, Srimad Andavan Arts and Science College (Autonomous), for helping to identify the antimicrobial activity of the grown crystals. And also the authors thank the Management of Srimad Andavan Arts and Science College (Autonomous) for entire support given in completing this research work.

REFERENCE

[1.] Charnock, C. & Finsrud, T. *J. Clin. Pharm. Ther.* **32**, 567–572 (2007).

[2.] Hossaini, A., Larsen, J. J. & Larsen, J. C. *Food Chem. Toxicol.* **38**, 319–323 (2000).

[3.] Yang, H. Y. & Rasmuson, A. C. *J. Chem. Eng. Data*, **55**, 5091–5093 (2010).

[4.] Finger, L. W., Cox, D. E. & Jephcoat, A. P. *J. Appl. Cryst.* **27**, 892–900 (1994).

[5.] Thompson, P., Cox, D. E. & Hastings, J. B. *J. Appl. Cryst.* **20**, 79–83 (1987).

[6.] Howard, C. J. *J. Appl. Cryst.* **15**, 615–620 (1982).

[7.] Stephens, P. W. *J. Appl. Cryst.* **32**, 281–289 (1999).