



## Mechanochemical Synthesis and Hirshfeld Surface Analysis of Benzylidene Based Pharmaceutical Co-crystals

<sup>1</sup>Jeevan Dontulwar\*, <sup>1</sup>Piyush R. Yadav

<sup>1</sup>Professor

<sup>1</sup>Department Of Chemistry,

<sup>1</sup>Shri Mathuradas Mohota College of Science, Nagpur, India

**Abstract:** The simple condensation process were used to synthesized the benzylidene based derivatives and which are analysed using different spectroscopic techniques. Further, the solvent assisted grinding approaches were utilized to synthesized the benzylidene based pharmaceutically active co-crystal. The synthesized compounds were screened using FT-IR and PXRD methods. The Cambridge Crystallographic Database Survey were done for the above compound where we have observed no hit for the synthesized compound. However, similar type of structural moiety were noticed and CIF file are used to explain the various structural and possibility of interactions present in the synthesized molecule. The Hirshfeld surface were developed on reported CIF molecule and explain the type of interactions and its percentage contribution present in the benzylidene based molecule. Where it is observed that O-H interactions play very important role for the molecular recognition which contribute 47%.

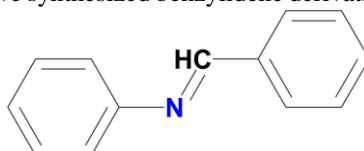
**Index Terms -** Pharmaceutical cocrystals, co-crystallization, solubility, stability, bioavailability, Schiff base, Benzylidene.

### I. INTRODUCTION

The solubility are the main factor for the development of a new pharmaceutical based compounds. In addition, the poor solubility and low oral bioavailability of an active pharmacological component has been decreasing the pharmacological effect. Various ways have been explored to improve the solubility of poorly water-soluble drug, but their effectiveness is dependent on the physical and chemical composition of the molecules being created. Co-crystallization of drug substances provides an excellent opportunity for the development of new drug products with superior physicochemical properties such as melting point, solubility, stability, bioavailability, and permeability while preserving the active pharmaceutical ingredient's pharmacological properties.<sup>[1,2,3]</sup>

Co-crystals are multi-component systems in which two components, an active medicinal ingredient and a co-former, are present in a stoichiometric ratio and linked together in the crystal lattice via non-covalent interactions. Depending on the nature of coformers, Duggirala and colleagues divided the cocrystals into molecular and ionic types. The majority of known cocrystals are classified as molecular cocrystals, which contain neutral or non-ionized coformers in a stoichiometric ratio.<sup>[4,5]</sup> In a stoichiometric ratio, ionic cocrystals were produced by charge-assisted hydrogen bonds and/or coordination bonds and contained ionised coformers. Pharmaceutical cocrystals were described as having an API as one component and another component acting as a coformer in a stoichiometric ratio. Cocrystals should have the benefit of being in a stable crystalline state and not requiring additional excipients or additives in formulations among all of these. The characteristics of APIs and coformers, the type of molecular interaction that occurs between them, and the synthetic techniques used all have a significant impact on the physicochemical attributes. The main benefit of creating cocrystals is that, while maintaining their pharmacological qualities, the APIs' physicochemical properties will be improved due to the inclusion of a conformer. In the current work we have synthesized Benzylidene based Schiff based compounds using condensation reactions and used it for co-crystallisations.<sup>[6,7]</sup>

The Schiff bases are the condensation by products of primary amines, and in the modern environment, carbonyl compounds are becoming more and more significant. Schiff bases are substances with an imine or azomethine (-C=N-) functional group and are discovered to be a flexible pharmacophore for the design and development of numerous bioactive lead compounds. Schiff bases display beneficial biological properties such as anti-inflammatory, analgesic, antibacterial, anticonvulsant, antitubercular, anticancer, antioxidant, anthelmintic, antiglycation, and antidepressant actions. The current study emphasizes the several Schiff bases that have recently been synthesized as possible bioactive cores while summarizing data on a range of biological functions.<sup>[8,9,10,11,12]</sup> In view importance of schiff base compound, here we have synthesized benzylidene derivative and co-crystallized it with 4-nitrobenzoic acid.



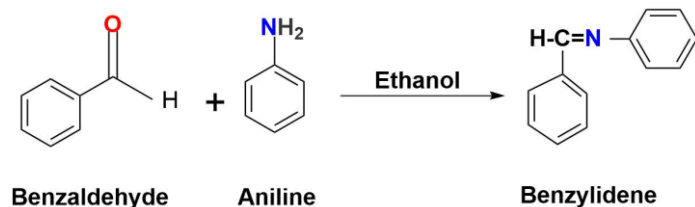
Scheme :1 Structure of benzylidene compound

## II. MATERIAL AND METHODS

### 2.1 General Experimental method for synthesis of Benzylidene

The benzylidene is synthesized from benzaldehyde and aniline by simple condensation reactions the details of chemical reactions were explained below.

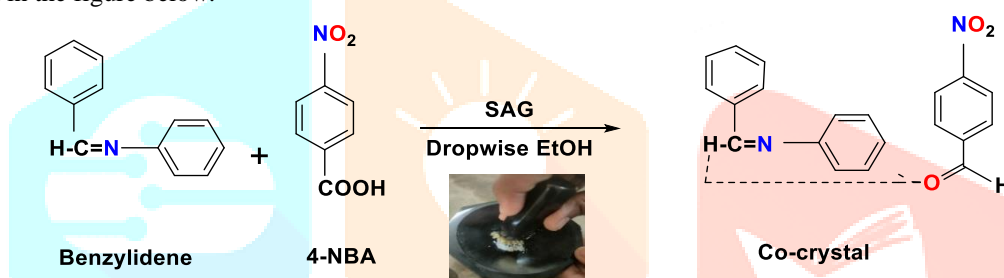
0.05 mole of Benzaldehyde 5.3 ml and 0.05 mole of aniline 4.6 ml is added with rapid stirring. After few second a reaction occurs with evolution of heat and separation of water mix is allowed to stand for 15 minutes and is then poured with vigorous stirring into 10% ethyl alcohol in a beaker. Crystallization begins in about 5 minutes. Mix is allowed to stand, first 10 min at room temperature then 30 minutes in ice water. The almost solid mass is then transferred to large Buckner funnel, filtered by suction pressed out and air dried. Observations after the synthesis were-Yield of synthesized Benzylidene = 1.65 g and Melting point of Benzylidene was found to be 54°C.



Scheme 2: Synthesis of Benzylidene based compounds

### 2.2 Co-crystal of Benzylidene and 4-NBA acid

Weight about 0.16g i.e., 0.001 Moles of 4-NBA and 0.15g i.e., 0.001 Moles of synthesized Benzylidene. Put both the compound in Mortar pestle as shown in the figure above. Start grinding the solids gently in a monotonous continuous manner for about 30-45 minutes by adding drop wise ethanol to it after regular intervals. Grinding should be uniform and ethanol should not be added in excess amount. Using Mercury software, hydrogen bonding between Citric acid and Benzylidene is predicted which may or may not form shown in the figure below.



Scheme 3- Schematic representation of expected hydrogen bonding in co-crystals

After grinding is completed, the grinded composed yield was found to be 0.13g. The melting point of 1:1 cocrystal of benzylidene and 4- NBA was taken which was found to be 230°C.

### 2.3 Cambridge Crystallographic Data Centre (CCDC)

The study of crystals is the focus of the nonprofit Cambridge Crystallographic Data Centre (CCDC). In order to make crystal structure information systematically available to everyone, Dr. Olga Kennard OBE FRS and her team started collecting published bibliographies for all tiny compounds studied by X-RAY and neutron diffraction techniques in 1965. This information has to be manually retyped after being printed out in the form of published journals. Later, as technology advanced, it changed into an electronic format that is now referred to as a cif file. For organic, metal-organic, and organometallic compounds, the Cambridge Structural Database (CSD) is a three-dimensional structural data repository and curated resource.

### 2.4 Hirshfeld Surface Analysis

The Hirshfeld surface was created in an effort to categorize the area occupied by a molecule within a crystal in order to separate the crystal's electron density into molecular portions. It has been demonstrated that Hirshfeld surfaces, which represent the electrostatic potential of a surface, are an easy-to-use visualization tool for analysing interactions between molecules in crystal structures. To create a Hirshfeld surface, open the relevant CIF in Crystal Explorer and select the desired molecule. The Hirshfeld surface generation command is used to produce a colorful graphic of electrostatic potentials projected across the surface after the molecule has been chosen. Red indicated stronger electrostatic contact, blue indicated weaker electrostatic interaction, and light blue or white indicated a medium or intermediate kind of electrostatic interaction in a Hirshfeld surface diagram. The research of the interactions between molecules in crystals is now being approached in an innovative and visually appealing way thanks to Hirshfeld surface analysis. The examination of the interactions between molecules in crystals came before this study.

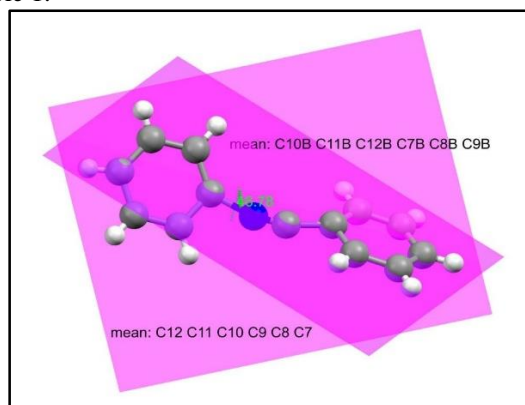
## 3. RESULT AND DISCUSSION:

The Cambridge Structural Database (CSD) (Conquest version 3.7; CCDC version 2.0.0) [1] shows that the crystal structures of the title molecules (Scheme 1) were reported by G. C. Rovnyaket al., [2]. The crystal structure and crystallographic details are explained here.

### 3.1 Crystallographic analysis

The title compound benzylidene based derivative were crystallize in monoclinic crystal system with one molecule in asymmetric unit and four molecules in the unit cell. The space group of the molecule is observed C2/c. The tetrahedral angle between two

phenyl ring is observed as  $76.78^\circ$  shown in figure 1. The torsional angle between C13-C12-C10-C9 and C13-C12-C10-C11 are  $162.9^\circ$  and  $31.1^\circ$  respectively. The unit cell volume of title compounds is shown as  $2733\text{\AA}^3$ . The cell parameter and other crystallographic details are shown in table 1.



**Figure-1:** Tetrahedral angle between two different phenyl rings of synthesized compounds.

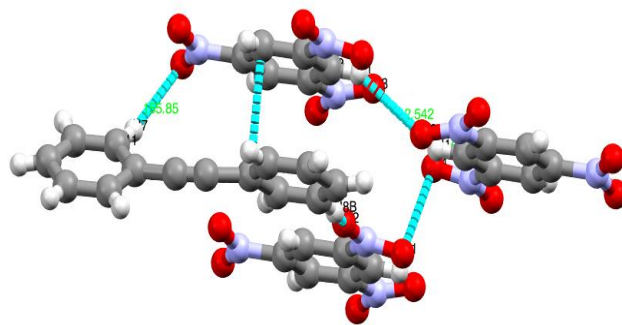
**Table 1:** Cell parameters and crystallographic details of the compound

Crystal Data	Compound code
Formula	$C_{13}H_{11}N_2(C_6H_3N_3O_6)$
Molecular weight	194.08
a(Å)	11.034(3)
b(Å)	9.973(1)
c(Å)	24.940(8)
$\alpha$ (°)	90
$\beta$ (°)	95.17(2)
$\gamma$ (°)	90
Z'	1
Z	4
Cell volume	2733.28
Density( $g\text{ cm}^{-3}$ )	1.46
Space group	C 2/c
Crystal System	Monoclinic

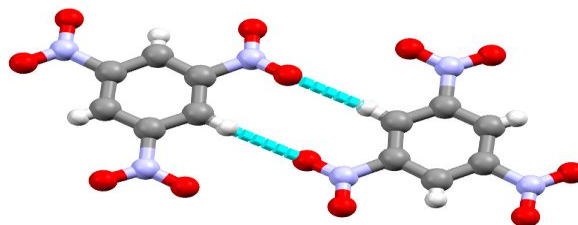
In the crystal structure the strong hydrogen bonds are formed and responsible for the well packing. The  $C_{11}B-H_7B...O_5(2.507\text{\AA}, 155.85^\circ)$  hydrogen bonds form strong packing in the crystal. The various intermolecular hydrogen bonding present in the compounds are shown in figure 2. The hydrogen bonding details and its analysis are listed in table 2. A ring motif is formed in crystal structure. To generate intermolecular hydrogen bonding, it is made up of twelve atoms ( $H_1C_2C_3N_2O_4O_3H_1C_2C_3N_2O_4O_3$ ). The ring contains two acceptor and two donor atoms (acceptor-oxygen and donor-hydrogen). The number of hydrogen bond donor and acceptor present in a crystal structure determines the ring patterns. The ring motif structure is shown in figure 3. It is depicted as follows.

**Table 2:** Prominent hydrogen bonding interactions present in the compounds

D-X...A	D-X(Å)	X...A(Å)	D...A(Å)	$\angle D-X...A$ (°)
$C_{11}-H_7B...O_5$	0.967	2.507	3.41	155.85
$C_{12}-H_8...O_2$	0.898	2.588	3.359	144.37
$C_2-H_1...O_3$	0.928	2.542	3.378	150.12



**Figure-2** The molecule forms various hydrogen bonding in their crystal packing structure.



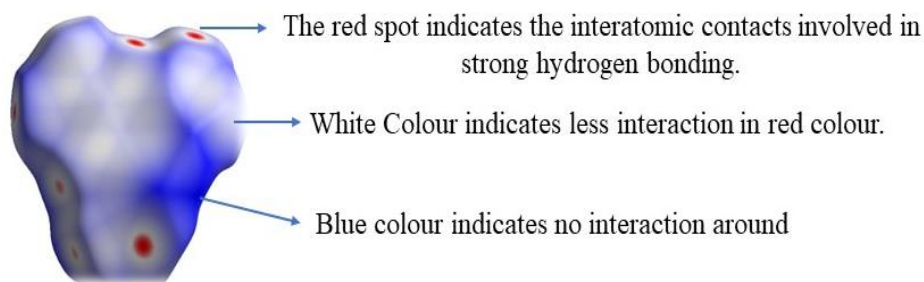
**Figure-3** Ring motif structure shown in crystal structure.

### 3.3 Hirshfeld Surface Analysis

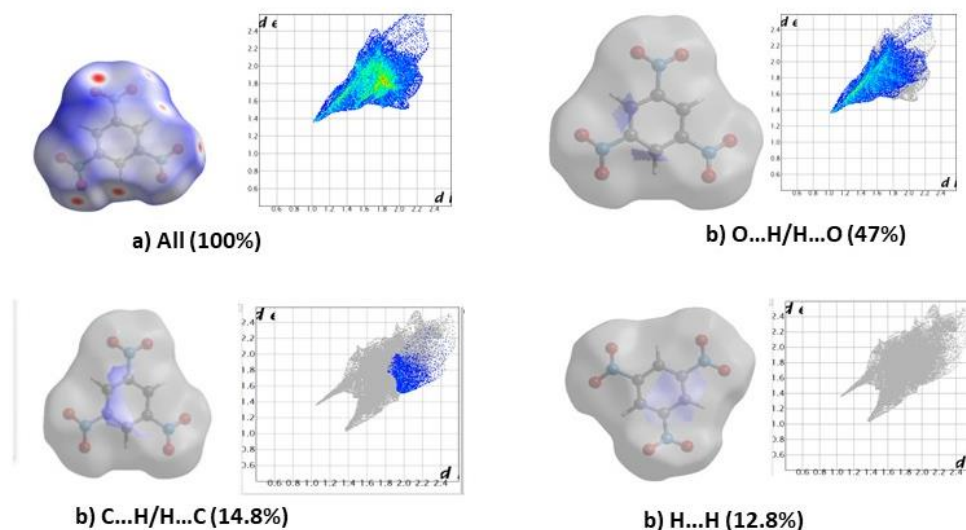
Hirshfeld surface is a visualizing tool which quantifies the intermolecular interactions using a two-dimensional finger-print plot. The Hirshfeld surface of the molecule is mapped over  $d_{\text{norm}}$  which comprises two values,  $d_i$  which represents the distance of the surface nearest to the exterior atoms and  $d_e$  which represents a distance of the surface from nearest to the internal atoms. Here, the Hirshfeld surface and two-dimensional finger-print plots using **crystal software 17.5** have been developed as shown in Figure 4. The intermolecular interaction of the compound is strongly evidenced by the two-dimensional fingerprint plot. The red spot on the Hirshfeld surface shows the presence of strong intermolecular interactions and blue color shows free from contacts. The title compound shows a red spot due to strong  $\text{-C}\cdots\text{H}$  interaction shown in Figure 5. The finger-print plots shown in figure-5. show the  $\text{O}\cdots\text{H}$  intermolecular contacts contribute relatively high (47%) compared to the other intermolecular interactions. The percentage contribution of other intermolecular interactions in the title compound is as follows:

**Table 3** Types of intermolecular interaction and its percentage contribution

Sr. No	Type of intermolecular interaction	Percentage contribution(%)
1.	Total	100%
2.	$\text{O}\cdots\text{H}$	47% %
3.	$\text{C}\cdots\text{H}$	14 %
4.	$\text{H}\cdots\text{O}$	12%
5.	$\text{C}\cdots\text{N}$	0 %



**Figure-4** The  $d_{\text{norm}}$  Hirshfeld surface generated on the molecule for title compound.

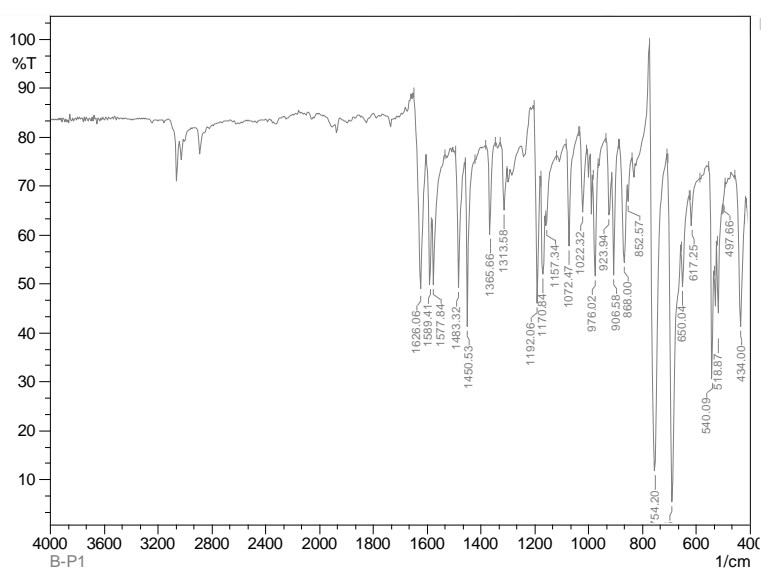


**Figure:5** Two-dimensional (2d) fingerprint plot with percentage contribution of interaction presents in the compound

Above diagram shows that 2D-fingerprint of the Hirshfeld surface represents a novel method for summarizing the complex information contained in a molecular crystal structure into a single, unique full colour plot, which provides a fingerprint of the intermolecular interaction in the crystal. Derived from the Hirshfeld surface, these 2D-fingerprint plot provides a visual summary of the frequency of each combination of  $d_e$  and  $d_i$  across the surface of the molecules, so they not only indicate which intermolecular interaction are present, but also the relative area of the surface corresponding to each kind of interaction. For each point on the Hirshfeld surface, we determine both  $d_e$  and  $d_i$ . Each point on the 2D fingerprint plot corresponds to a unique  $(d_e, d_i)$  pair, and the colour of each point corresponds to the relative area of the surface with that  $(d_e, d_i)$  pair. points on the plot with no contribution on the surface are coloured blue for a small contribution through green to red for points with the greatest contribution. All fingerprint plot are coloured on the same relative scale, so some fingerprint plots do not have any red points. Because Hirshfeld surface nearly fill the available space, so voids spaces are small these 2D-fingerprint plots are pseudo-mirrored along the  $d_e$ - $d_i$  diagonal. Feature along the diagonal occur due to like contacts while the wings on the plot are due to the C-H and pi interaction.

### 3.4 FT-IR Pattern for Benzylidene and 4- NBA

There is a significant dipole moment associated with the C=N bond which leads to a significant change when it interacts with infrared radiation usually leading to an intense sharp peak at 2200-2280  $\text{cm}^{-1}$ . Very few other groups absorb at this region with this intensity. The hydroxyl stretch is similar to the N-H stretch in that its hydrogen bonds but does so more strongly. As a result, it is often broader than the N-H group. In those rare instances when it is not possible to hydrogen bond, the stretch is found as a relative weak to moderate absorption at 3600-3650  $\text{cm}^{-1}$ . One exception is the N-H bend which occurs at about 1600  $\text{cm}^{-1}$ . This band is generally very broad and relatively weak. Most other functional groups absorbing in this region are either sharper or more intense. The terminal carbon triple bond (C-C≡H) is the most reliable and easiest to identify. The discussed the C-H stretching frequency; coupled with a band at 3300  $\text{cm}^{-1}$ , the presence of a band at approximately 2100  $\text{cm}^{-1}$  is a strong indication of the -C-C≡H group. (Figure 6,7)



**Figure 6** FT-IR spectra for Benzylidene

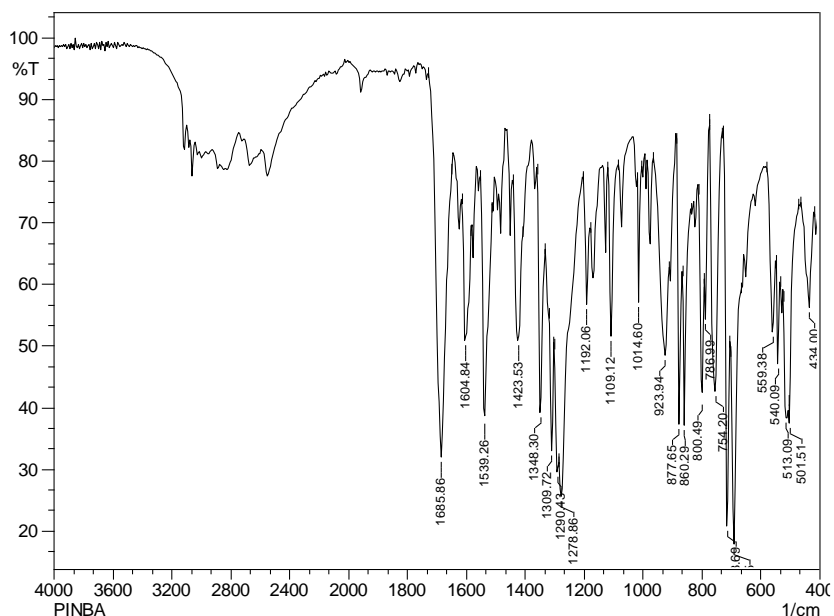


Figure 7: FT-IR Pattern for Benzylidene-NBA co-crystal

### 3.5 Powder X-ray Diffraction Spectra

The PXRD diffractogram of Benzylidene showed crystalline pattern with characteristic intense diffraction peaks at varying 2 theta values. The co-crystal displayed varying degrees of crystallinity. The cocrystal's PXRD pattern was distinct from that of Benzylidene, and several new diffraction peaks that weren't present in the benzylidene compound. Therefore, the emergence of fresh diffraction peaks denotes the development of a fresh crystalline phase. It is well established that crystallization is dependent on the PXRD pattern. Cocrystal PXRD was utilized to confirm the production of new co-crystals because it differed from the PXRD of the pure drug.(Figure 8,9)

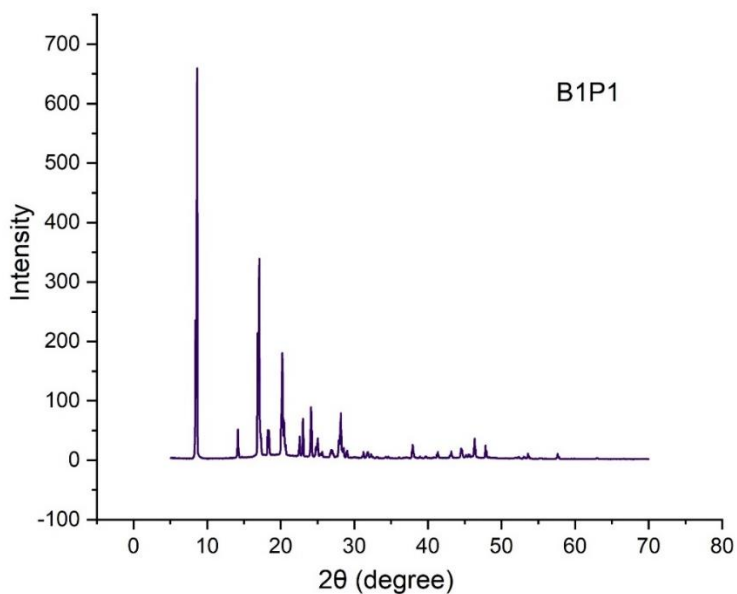
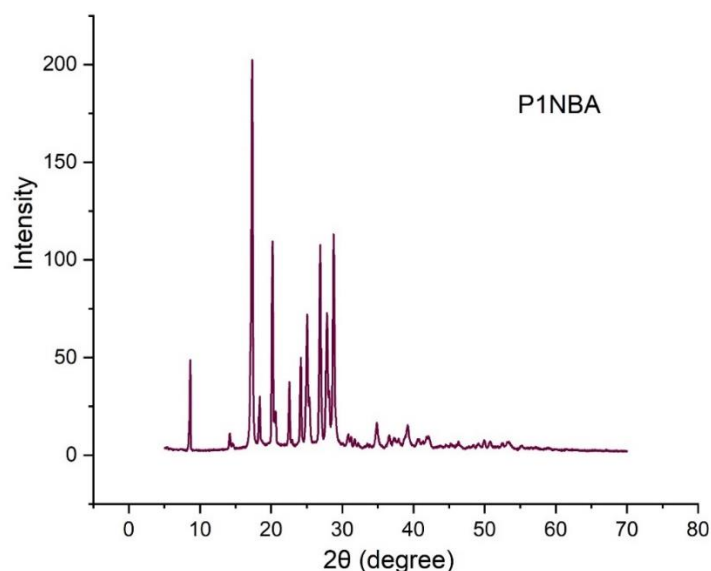


Figure 8 Powder X-ray diffraction Pattern for Benzylidene



**Figure 9** Powder X-ray diffraction Pattern for Benzylidene-NBA Co-crystal(P1NBA)

#### IV. CONCLUSION

The benzylidene based compound and its cocrystal were synthesized successfully. The product obtained and recrystallized by ethanol, has been characterized by infrared spectroscopy, which gave characteristic bands at frequencies of 3057 and 1630  $\text{cm}^{-1}$ . This synthesis was conducted without solvent to obtain N- Benzylidene which is in the form of yellowish crystals in 80% The cocrystal definition, its significance, and this are summarized. From the review of the literature, it can be inferred that employing co-crystallization to enhance the performance characteristics of APIs is a promising strategy. Although there are some restrictions, utilizing knowledge from the real world can alleviate problems with co-crystal development of an API. This technique's ability to be used with all APIs that have poor water solubility is a key feature. A patent for a co-crystal medicinal product intermediate may be granted if a molecule meets all requirements from the standpoint of intellectual property rights. Last but not least, we draw the conclusion that methodical structural investigation of molecules and their potential hydrogen bonding pattern can result in the production of highly good co-crystals.

#### V. REFERENCES

- [1] Babu NJ and Nangia A. Solubility advantage of amorphous drugs and pharmaceutical cocrystals. *Cryst Growth Des* 2011;11:2662-79.
- [2] Fong SYK, Ibisogly A, Bauer-Brandl A. Solubility enhancement of BCS class-II drug by solid phospholipid dispersions: Spray drying versus freeze-drying. *Int J Pharm* 2015;496:382-91.
- [3] Yuvaraja K, Khanam J. Enhancement of carvedilol solubility by solid dispersion technique using cyclodextrins, water soluble polymers and hydroxyl acid. *J Pharm Biomed Anal* 2014;96:10-20.
- [4] Hisada N, Takano R, Takata N, Shiraki K, Ueto T, Tanida S, *et al.* Characterizing the dissolution profile of supersaturable salts, cocrystals and solvates to enhance *in vivo* oral absorption. *Eur J Pharm Biopharm* 2016;103:192-9.
- [5] Savjani KT, Gajjar AK, Savjani JK. Drug solubility: Importance and enhancement techniques. *ISRN Pharm* 2012;2012:195727.
- [6] Z. Cimerman, S. Miljanić, and N. Galić, "Schiff bases derived from aminopyridines as spectrofluorimetric analytical reagents," *Croatica Chemica Acta*, vol. 73, no. 1, pp. 81–95, 2000.
- [7] H. Schiff, "Mittheilungen aus dem Universitätslaboratorium in Pisa eine neue reihe organischer Basen," *Justus Liebigs Annalender Chemie*, vol. 131, no. 1, pp. 118–119, 1864.
- [8] D. N. Dhar and C. L. Taploo, "Schiff bases and their applications," *Journal of Scientific and Industrial Research*, vol. 41, no. 8, pp. 501–506, 1982.
- [9] B. S. Sathe, E. Jaychandran, V. A. Jagtap, and G. M. Sreenivasa, "Synthesis characterization and anti-inflammatory evaluation of new fluorobenzothiazole Schiff's bases," *International Journal of Pharmaceutical Research and Development*, vol. 3, no. 3, pp. 164–169, 2011.
- [10] S. M. Sondhi, N. Singh, A. Kumar, O. Lozach, and L. Meijer, "Synthesis, anti-inflammatory, analgesic and kinase (CDK-1, CDK-5 and GSK-3) inhibition activity evaluation of benzimidazole/benzoxazole derivatives and some Schiff's bases," *Bioorganic and Medicinal Chemistry*, vol. 14, no. 11, pp. 3758–3765, 2006.
- [11] Bairagi, K. M.; Younis, N. S.; Emeka, P. M.; Venugopala, K. N.; Alwassil, O. I.; Khalil, H. E.; Sangtani, E.; Gonnade, R. G.; Mohanlall, V.; Nayak, S. K., (2020) Anti-diabetic activity and structural analysis of substituted dihydropyrimidine analogues.
- [12] Journal of Molecular Structure, 129412 Bairagi, K. M.; Venugopala, K. N.; Mondal, P. K.; Gleiser, R. M.; Chopra, D.; García, D.; Odhav, B.; Nayak, S. K., Larvicidal (2018) Study of tetrahydropyrimidine scaffolds against *Anopheles arabiensis* and structural insight by single crystal X-ray studies. *Chemical biology & drug design*, 92 (6), 1924-1932. Clare F.
- [13] Macrae, Ioana Sovago, Simon J. Cottrell, Peter T. A. Galek, Patrick McCabe, Elna Pidcock, Michael Platings, Greg P. Shields, Joanna S. Stevens, Matthew Towler and Peter A. Wood, (2019)