IJBPAS, October, Special Issue, 2021, 10(10): 470-478
ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)
'A Bridge Between Laboratory and Reader'
www.ijbpas.com

AN OUTLOOK FOR SAMPLE PREPARATION IN XRD ANALYSIS OF PHARMACEUTICAL SOLIDS

MUNDE AS*[1], HOLE RJ[1], LOHAKANE VA[1] AND JAYBHAYE SV[2]

1: Department of Chemistry, Milind College of Science, Nagsen Vana, University Road, Cantonment, Aurangabad, MS, India-431001

2: Department of Chemistry, B. K. Birla College of Science (Autonomous), Bhiwandi-Murbad Highway, Kalyan (West)-421304, MS, India

***Corresponding Author: E Mail:** as_munde@yahoo.com

Received 27th April 2021; Revised 25th June 2021; Accepted 1st Aug. 2021; Available online 1st Oct. 2021

https://doi.org/10.31032/IJBPAS/2021/10.10.1043

ABSTRACT

Evaluation of polymorphic forms of sample specimens has secured an inevitable importance across the pharmaceutical industry as it is scientifically proven that, the alteration of small change in polymorphic form of drug substance and/or drug product may end up with significant changes in physico-chemical properties of solids. The aforesaid polymorphic evaluation can be achieved by powder XRD technique which is primary analytical tool globally used by crystallographers for the determination of polymorphic forms. However sample preparation is key attribute while using powder XRD tool for said evaluation. The illegitimate sample preparation may lead to ambiguous data and falsification of conclusion. As sample specimens meant for polymorphic assessment may have different physical nature, a practical and enriched skill-set is required to reach to the desired goal. In this paper, we focused on various sample types, their physical nature, polymorphic targets and road map for sample preparations. Also we covered expectations of regulatory agencies from pharmaceutical industries as far polymorphic data is concerned.

Keywords: Powder XRD, Regulatory agency, Ointments, DPIs, Suspensions

1.0. INTRODUCTION

Sample preparation is vital step in almost all the analytical techniques because the techniques are often not responsive to the analyte in its in-situ form or the results are distorted by interfering species **[1]**. Sometimes pre-treatment is done to prepare the sample into a form ready for analysis by specified analytical equipment. Pharmaceutical industries are performing polymorphic evaluations of various drug substances and drug products and generate an inevitable data which is required during product filing.

The polymorphic data is the requirement of various regulatory agencies like USFDA (USA), MHRA (UK), TGA (Australia), CDSCO (India), HEALTH CANADA (CANADA), MCC (South Africa), ANVISA (Brazil), EMEA (European Union), SFDA (China), NAFDAC (Nigeria), MEDSAFE (New Zeland), MHLW (Japan), MCAZ (Zimbabwe), SWISSMEDIC (Switzerland), KFDA (Korea), MoH (Sri Lanka) **[2]**.

The physical nature of drug substances which are subjected to polymorphic evaluations may be either solids or powders and semi-solids also. However the nature of drug products may vary from solids/powders to suspensions, nasal sprays, ointments, creams, gels and foams. It becomes challenging task when the sample nature varies from analysis to analysis.

Powder X-ray diffraction (XRPD or p-XRD) technique is the gold standard for the evaluation of polymorphic form of any drug substance and drug product **[3]**.

The sample preparation is the most crucial part involved in the aforesaid evaluation. Since powder XRD is the surface phenomenon, the selection of appropriate sample holder and sampler accessories is immensely important and it relies with expertise of concerned analytical scientist.

In this paper, we focused on the various types of samples and relevant sampler accessories.

2.0.MATERIALS USED IN THE STUDY:

The materials i.e. the types of sample holders used in the proposed study are basically of two types of available for XRD analysis as **[1]**,

i. Back Loading Holder and
ii. Top Loading Holder or Zero Background Holder.
iii. Back loading holder is primarily used for the solid samples provided adequate sample quantity must be available whereas the top loading holder or zero background holder is used in the case when less sample quantity is available like in nasal

spray samples and when the targeted sample bears hydroscopic nature.

Semi-solid nature of samples specifically creams, ointments, suspensions, gels etc.

Figure 1 and **Figure 2** respectively are the representative figures of back loading and top loading holders.

Figure1: Pictorial Presentation of Backloading Holder (Courtesy: Malvern-Panalytical X-ray diffraction manual and X'Press magazine)

Figure 2: Pictorial Presentation Toploading Holder

3.0. SAMPLE PREPARATION ETHICS:

As XRD is surface phenomena, the surface of sample must be smooth enough so as to obtain pretty intense 2-theta reflections. The word 'smooth' herewith refers to uniform. If sample surface is not uniform then expected XRD profile may not obtain and data may end up with misleading conclusion.

To make the sample surface uniform, analyst must use either a 'glass slide' or 'metallic spatula' to avoid distortion of peaks **[4]**.

Figure 3a indicate the practical way of sample preparation methodology to achieve the desired goal and Figure 3b indicates the back loading holder with uniform sample surface.

For the hygroscopic samples, cut silicon single crystal zero background holder provided with kapton film is preferred. The kapton film is nothing but a poly-imide film which is used to cover the sample throughout analysis in order to prevent the sample from atmospheric conditions.

This activity enables the crystallographer to gather the key knowledge about the hydrate polymorphic form of sample, if any **[5]**.

Figure 4 shows representative silicon cut silicon single crystal zero background holder with kapton film.

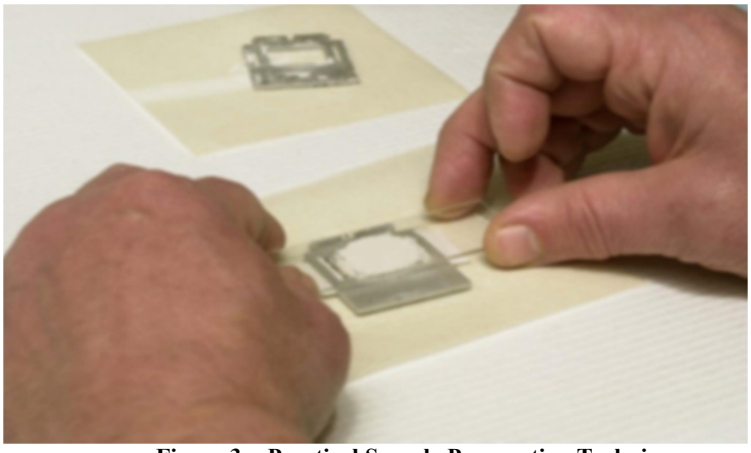

Figure 3a: Practical Sample Preparation Technique

Figure 3b: Uniform Sample Surface in back loading holder

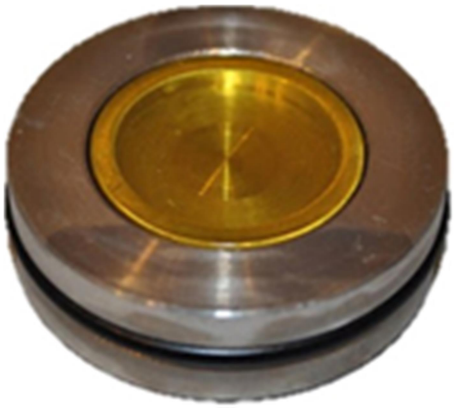

Figure 4: Silicon Cut Silicon Single Crystal Zero Background holder with Kapton film

If hygroscopic sample are being analyzed without kapton film then there will be conversion of solid nature of sample into semi-solid and may be liquid also if the analysis time is longer. The outcome of such analysis leads to generation of additional 2-theta peaks in XRD profile which may not be the part of actual crystal lattice of solid

sample but due to exposure of sample to atmospheric moisture during analysis. Thus scientist will obtain false crystallographic data **[6]**.

4.0.SAMPLE PREPARATION IN DRUG PRODUCTS:

To analyze the samples like creams, ointments and suspensions, the zero back ground holder with kapton film is again suitable way forward. The semi-solid samples can be gently spread inside the groove of zero back ground holder and covered with kapton film to avoid spillage of sample due to rotation mode of XRD configuration. The crucial operation during preparation of such samples is uniform distribution of sample with smoothening of its surface. Broadening of peaks can be overcomes by better smoothened surface of these samples. Due to broadened peaks there may be shifting in 2-theta values from lot to lot of same product or may be shifting in comparison with reference data base **[1, 7]**.

5.0.SAMPLE NATURE AND SAMPLE PREPARATION HURDLES:

Every times it is not mandatory that, analyst will get powder sample for XRD analysis. Since the sample nature solely depends on the route of synthesis adapted in particular. It is quite possible, the sample may be mixture of lumps or may have plate like shape or maybe needle shaped **[8]**.

If samples of above said nature are used for XRD analysis without pre-treatment then sample will not be appropriately fitted into holder and lead to broadening of XRD bands resulting into amorphicity of sample **[9, 10]**. Figure 5shows different nature of solid samples for XRD analysis **[11]**.

Figure 6 shows amorphous voids and broadening of XRD bands and due to non-uniformity of samples.

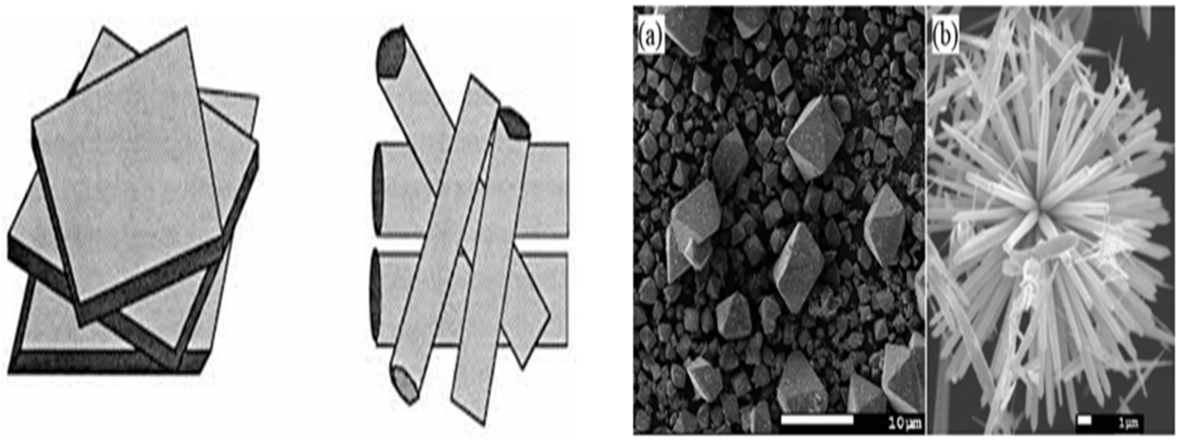

Figure 5: Different Nature of Solid Samples for XRD analysis

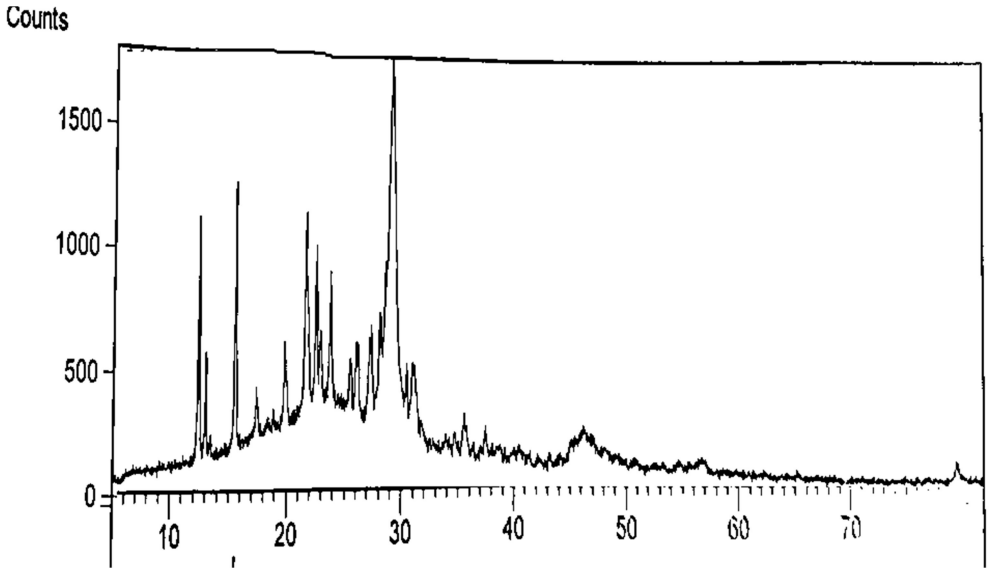

Figure 6: Broadening of XRD Peak Pattern

The broadening of XRD bands also result in absence of some 2-theta peaks which may be the characteristic of polymorphic form in particular **[12, 13]**.

6.0.SCIENTIFIC APPROACH FOR XRD SAMPLE PREPARATION:

Analytical scientist cannot restrict the sample nature receiving from process development team. However he can establish the approach of sample pre-treatment before XRD analysis so that to avoid peak broadening followed by generation of amorphicity in XRD pattern and absence of characteristic 2-theta bands.One way is the trituration of sample with the help of mortar-pestle before applying to XRD exposure. The term 'trituration' herewith refers to gentle operation so that to make the sample uniform and not grinding the sample which may end with alteration in polymorphic form **[8, 9]**.

The triturated sample must be filled into the cavity of sample holder in such way that the sample surface sould be closely packed and smooth. The trituration followed by closed packing of sample into the holder overcomes the preferred orientation effect in XRD pattern. Figure 7, 7a and 7b shows the sequential process of sample trituration, filling of sample into holder and sharpening of XRD peaks due to this process **[1, 7]**. In Figure 7b comparison of XRD patterns before (above graph) and after (below graph) following the suggested scientific approach is shown **[14, 15, 16]**.

Figure 7: Sample Trituration in Mortar-Pestle

Figure 7a: Sample Filling in Holder and Surface Smoothening

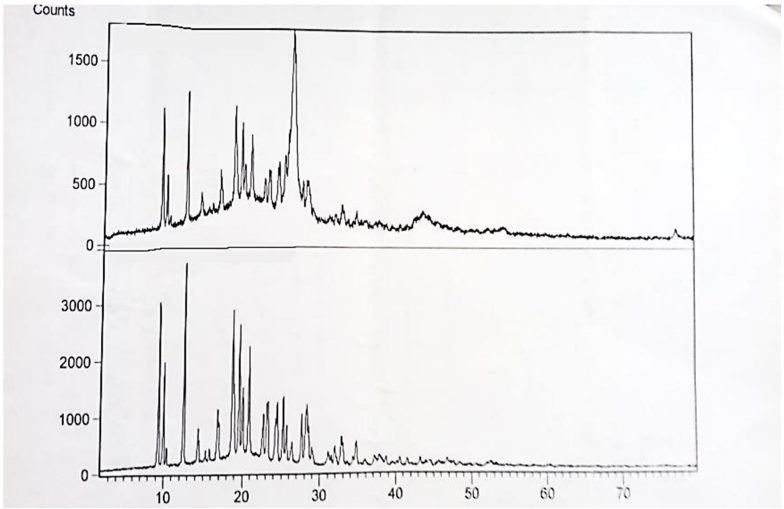

Figure 7b: Comparative XRD Peak Profile showing impact of Sample Trituration and appropriate Sample Preparation on Peak broadening (above graph) and peak sharpening (below graph)

7.0. DISCUSSION

The scientific approach suggested by us is an inevitable practice to obtain more precise and rugged XRD data. Figure 7 and 7a indicates the procedure to overcome the problems arises due to varying sample nature. Figure 7b clearly shows the difference in XRD peak profile obtained before and after implementing the recommended approach.

Figure 3a and 3b exhibits the accurate methodology for sample loading into cavity of holder followed by surface smoothening to get reproducible results. The regulatory bodies expect the precise and robust data from pharmaceutical industries which shall be reproducible during product life cycle. The sample preparation approach attempted in this article will surely add values to the crystallographer to achieve the desired goal.

8.0. CONCLUSION

The scientific approaches defined in these experiments are the road maps to get improved XRD profiles of different types of drug substances and drug products. The sample nature will not be the hurdle for analytical scientist to get more robust and precise XRD data if recommended approach has been followed. A special precaution has to be taken while analyzing hygroscopic samples to maintain exactness of conclusion about hydrate form. The expertise of end-user or crystallographer plays an important role during selection of appropriate sample holder and sampler accessory depending on the nature of sample meant for polymorphic assessment. Finally, perfection of data leads to minimize the queries of regulatory bodies.

9.0. ACKNOWLEDGMENT

The authors are thankful to Nanotech Research Laboratory of B. K. Birla College (Autonomous) Kalyan for XRD analysis data and Department of Chemistry, Milind College of Science, Aurangabad for giving inputs to shape up the presented research article.

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