IJPSR (2025), Volume 16, Issue 6 **(Review Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 16 December 2024; received in revised form, 04 January 2025; accepted, 07 January 2025; published 01 June 2025

HARNESSING THE POTENTIAL OF PAT TOOLS IN PHARMACEUTICAL CRYSTALLIZATION

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Keywords:

Crystal growth, Crystal size distribution, Crystallization, Nucleation, Polymorphism, Process Analytical Technology Tools

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ABSTRACT: Controlling the crystal growth in a crystallization set up is a complex and time taking process. In the domain of pharmaceutical set up, crystal differences will largely affect the drug performance and stability. Therefore, it is essential to control the crystallization process and optimize the experimental set up to get desired particle characteristics. Process Analytical Technology (PAT) tools are becoming popular for real-time monitoring and control of crystallization process. These tools are endorsed by regulatory bodies such as Food and Drug Administration in pharmaceutical manufacturing enabling consistent product quality and regulatory compliances. This review is not very exhaustive in nature but provides an overview of the crystallization processes and implications on crystal nucleation and growth. Various available PAT tools and their role in optimizing crystallization process have been discussed. Representative case studies employing PAT tools for monitoring pharmaceutical crystallization have been reviewed and their panoramic view depicted in tabular form. Besides, the challenges associated with the PAT tools in large scale manufacturing have also been touched. PAT in crystallization appears to have a bright future ahead.

INTRODUCTION: Crystallization is a long-standing method used in many industries, particularly the pharmaceutical industry for the separation and purification of compounds. It significantly affects the critical quality attributes such as purity of final product, polymorphs, crystal shape, and crystal size distribution (CSD) which directly impact the drug's performance and stability [1].

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DOI: 10.13040/IJPSR.0975-8232.16(6).1465-79

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.16(6).1465-79

The CSD has a significant impact on downstream processes such as filtration and drying, as well as product quality attributes such as solubility, dissolution and biopharmaceutical performance [2]. Active pharmaceutical ingredients (API) are mostly manufactured in crystalline form and crystallization control is essential to achieve pharmaceutical products with the necessary physical attributes and chemical purity [3].

The crystallization process involves a variety of techniques including cooling, antisolvent, reaction crystallization, and slow evaporation method. Various factors affect the outcome of the crystallization experiments which are not limited to supersaturation, temperature, choice of solvents and presence of additives [4].

Among several available crystallization techniques, cooling crystallization is the most widely employed approach because of less complexity, easy optimization and scalable. However, in the absence of suitable solvent for cooling crystallization, antisolvent crystallization can be good alternative by employing liquid (antisolvent) to get desired supersaturation [5]. Overall, crystallization plays critical role in pharmaceutical industry as a separation process, and production of desired solid form of optimum physicochemical attributes [6].

The FDA has defined regulatory framework for Process Analytical Technology (PAT) tools to encourage the pharmaceutical industries which sparked interest in developing control approaches to optimize product manufacturing and to achieve desired attributes. PAT promotes continuous improvement in manufacturing process by utilizing real-time data to minimize variation and enhance efficiency. It requires a deep understanding of all the processes involved in the operation to achieve consistent product quality. Recently, several PAT tools have been developed and used for real time monitoring and controlling crystallization process through various control strategies, such as, near infrared spectroscopy (NIR), raman spectroscopy, attenuated total reflectance ultraviolet-visible (ATR-UV/Vis) spectroscopy, attenuated total reflectance fourier transform infrared (ATR-FTIR) spectroscopy, focused beam reflectance measurement (FBRM), particle vision and measurement (PVM). These advanced control tactics and intelligent systems based on PAT tools are becoming increasingly significant in both laboratory research and industrial applications [6].

This present review encapsulates the different available PAT tools and their applications in controlling the pharmaceutical crystallization process. Various case studies pertaining to monitoring of crystal nucleation, supersaturation, polymorphic transformation etc have been reviewed briefly and are summarized in tabular form for quick understanding. Besides, the challenges associated with these tools and futuristic scope have also been covered.

Crystal Growth Mechanism: The basic steps involved in crystallization are nucleation, growth, agglomeration, and breakage. Nucleation is the critical step in crystallization that establishes the final structure and characteristics of crystal. It is a one-step process where a crucial crystal nucleus is created by clusters aggregates spontaneously [7]. According to crystal nucleation theory, crystals that are smaller than the nuclei dissolve slowly, whereas the ones exceeding the critical size will continue to grow. As depicted in **Fig. 1**, the surface free energy (ΔG_s) increases with the development of the new interface in the nucleation process, whereas there is a gradual reduction in the free energy of the bulk phase (ΔG_v). The radius of the nucleus is depicted by *r*, while γ depicts the surface free energy per unit area in the new phase. It is demonstrated that when the size of the nucleus is lesser than the critical size, surface free energy (ΔGs) takes the leading role, causing the total free energy (ΔG) to rise significantly while the chances of nucleus dissolution are greater. Beyond the maximum free energy at the critical radius (r_c), the total free energy (ΔG) progressively decreases, and the bulk free energy (ΔG_v) starts to dominate as the crystal slowly forms [8].

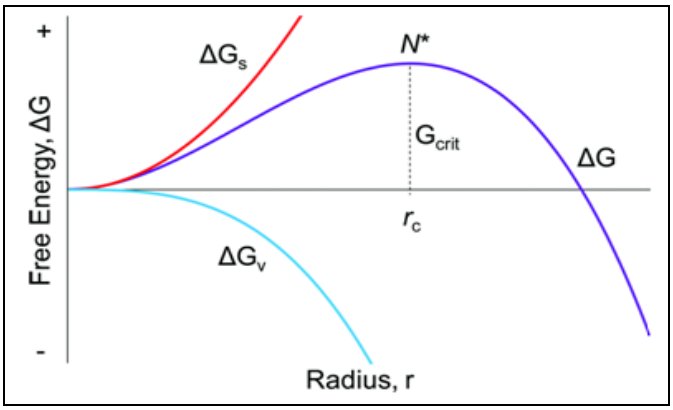

FIG. 1: FREE ENERGY DIAGRAM IN NUCLEATION PROCESS (ADAPTED FROM REF. [8])

Crystal growth is a fundamental phenomenon in which the atoms, ions or molecules added to the pre-existing crystal lattice cause the crystal to grow. Various methods such as evaporation, cooling and reaction crystallization are employed for the crystal growth in the solution phase. The rate at which solute molecules diffuse to the crystal surface and the rate at which they are integrated into the lattice frequently regulates the process. Factors such as temperature, supersaturation and the presence of impurities or additives influence the process that can inhibit or promote growth at crystal faces.

While, in the vapor phase, vaporized material condenses onto a substrate during the crystal growth where it goes through a series of reactions to form a solid crystal. The kinetics of this process are affected by the substrate temperature, gas flow dynamics and chemical reactions occurring at the surface [9, 10]. The kinetics of the crystal growth largely affect the crystal size, orientations as well as morphologies of the resulting crystal [11]. The growth of nuclei can occur through different mechanisms viz., normal growth, Layer-by-layer growth (2D growth), and spiral growth [12].

In the case of normal growth, rough and uneven crystal surface can arise under high substantial supersaturation conditions because of three dimensional nuclei on the crystal surface. In layer-by-layer growth mechanisms, the formation of smooth and flat surface occurs when particle such as atoms, ions or molecules attach themselves to the edge of a preexisting crystal layer. On the other hand, spiral growth occurs at screw dislocation - crystal structure flaws that create a continuous, step-like surface **Fig. 2.** These dislocations give particles a constant source of new steps to adhere and eventually continuous growth can happen even under low supersaturation conditions [10]. Better control over crystal size, shape and purity is now possible because of the development of advanced computational models and experimental techniques that can improve the understanding of crystal growth mechanisms.

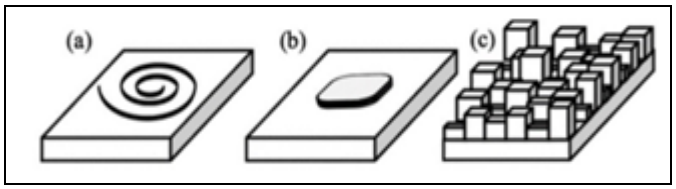

FIG. 2: ILLUSTRATION OF VARIOUS CRYSTAL GROWTH MECHANISMS (A) SPIRAL GROWTH (B) 2D NUCLEATION & GROWTH (C) ROUGH GROWTH (ADAPTED FROM REF. [11]

Challenges Prevailing in Crystal Growth and Characterization: Crystal growth faces many challenges during the process that make it more difficult to produce and maintain high-quality crystalline material. One of the main issues is to achieve precise regulation of nucleation because it is essential for determining the initial size and distribution of crystal. The overall characteristics and performance of the product might be impacted by differences in crystal size and quality caused by inconsistent nucleation [13]. Another major issue is to control the growth environment to prevent the formation of defects such as dislocations, stacking faults, and twin boundaries which affect the optical, mechanical, and electrical features of the crystal. Advanced and complex techniques like high-resolution transmission electron microscopy (HR-TEM) and atomic force microscopy (AFM) are needed to characterize these defects and evaluate their influence on growth dynamics. These methods demand not only sophisticated equipment but also significant expertise, making them less accessible for routine analysis. Additionally, the integration of computational models with experimental data remains a challenge because of the difficulty in accurately simulating the infinite variables involved in crystal growth dynamics [14].

Environmental factors, such as temperature, pressure, and the presence of impurities, further complicate the growth dynamics. Achieving uniform and defect-free crystals, especially at the nanoscale, requires careful control over these parameters, which is difficult to maintain consistently. Moreover, the characterization of crystals often involves advanced and expensive equipment, such as scanning electron microscopy (SEM), X-ray diffraction (XRD), and nuclear magnetic resonance (NMR) spectroscopy. These techniques require specialized knowledge to operate and interpret the results accurately. Additionally, the resolution and sensitivity of these methods can be limited, making it challenging to detect subtle defects or variations within the crystal structure. Another significant challenge is the development of standardized protocols for crystal characterization. The lack of universally accepted methods can lead to discrepancies in the data and make it difficult to compare results across different studies.

This is particularly problematic in industrial settings where reproducibility and consistency are crucial. Overcoming these challenges is essential for advancing the application of crystalline materials in pharmaceutical industries. In summary, the challenges in crystal characterization include the complexity of growth processes, the requirement for advanced equipment and expertise, together with the absence of standardized

protocols, hinder the ability to achieve consistent and reliable results.

Process Analytical Technology (PAT) Tools: PAT tools are a collection of technologies, and methods employed to monitor, analyze, and control manufacturing processes. PAT tools aim to confirm the product quality by measuring critical process parameters (CPPs) and critical quality attributes (CQAs) during production instead of depending solely on the testing of end product [15]. Food and drug administration (FDA) defines PAT tools as "a set of approaches for designing, assessing, and managing manufacturing by taking timely measurement of essential quality and performance features of raw and in-process materials and processes during production, aiming to ensure the quality of final product" [16].

PAT tools are crucial in modern manufacturing, especially in the biopharmaceutical and pharmaceutical industries. The primary importance of PAT tools lies in their ability to ensure product quality, enhance process efficiency, and support regulatory compliance [17]. These are some key points highlighting the significance of PAT tools:

Enhanced Product Quality: PAT tools allow for real-time tracking and management of manufacturing processes, helping to ensure that products meet predefined quality specifications. By continuously measuring CQAs and CPPs, PAT tools help to maintain product consistency and quality.

Process Understanding and Control: Using PAT tools, manufacturers achieve a better understanding of the processes, which allows for better control and optimization. This leads to more robust processes that can adapt to variations in raw materials and environmental conditions.

Efficiency and Cost Reduction: Implementing PAT tools reduces the need for end-product testing, which can be time-consuming and costly. Real-time monitoring can identify deviations and correct them promptly, minimizing waste and rework. This results in increased efficiency and lower production costs.

Regulatory Compliance: Regulatory agencies, such as the FDA, encourage the adoption of PAT as it aligns with quality-by-design (QbD) principles. PAT tools help ensure that manufacturing processes are well understood, controlled, and capable of consistently producing quality products, thereby meeting regulatory requirements.

Innovation and Flexibility: PAT facilitates the development of innovative manufacturing techniques, such as continuous manufacturing, which can be more efficient and flexible than traditional batch processes. This flexibility can accelerate product development and market launch.

Risk Management: By giving real-time data and insights into the manufacturing process, PAT tools help to identify potential risks early and implement corrective actions. These tactics minimizes the likelihood of product recalls and enhances overall product safety.

Importance of PAT Tools in Crystal Growth: Crystal growth is a critical step in industrial processes, particularly pharmaceuticals and chemicals industries. The properties and quality of the end crystalline product rely primarily on precise control of the crystal growth. PAT tools are essential for optimizing and controlling crystal growth. It enables continuous monitoring of the crystal growth process that ensures the final product meets the predefined quality specifications ensures consistent crystal quality and reduces the risk of defects. It provides real-time data on crystal size distribution, morphology, and polymorphism. Some key applications of PAT tools (Figure 3) in crystal growth dynamics are listed below:

1. It helps in monitoring the nucleation and growth of crystal during the crystallization process.
2. Monitor the supersaturation level of the solution, which is a key factor in crystal nucleation and growth.
3. It helps in the monitoring and detection of the generation of polymorphs during crystallization of APIs.
4. Particle size and shape analysis real-time visualization helps in identifying any abnormalities in crystal morphology.

5. Monitoring of crystallization kinetics by providing information on molecular dynamics and interaction in solution.
6. Provides real-time monitoring of temperature and helps in maintaining temperature control.
7. It helps in monitoring the purity and impurity profile during the crystallization process.
8. In continuous crystallization process, helps in monitoring and control of crystallization dynamics.
9. It helps in the optimization of cooling and antisolvent crystallization processes.
10. Real-time analysis of solvent and additives impacts on the crystal properties.

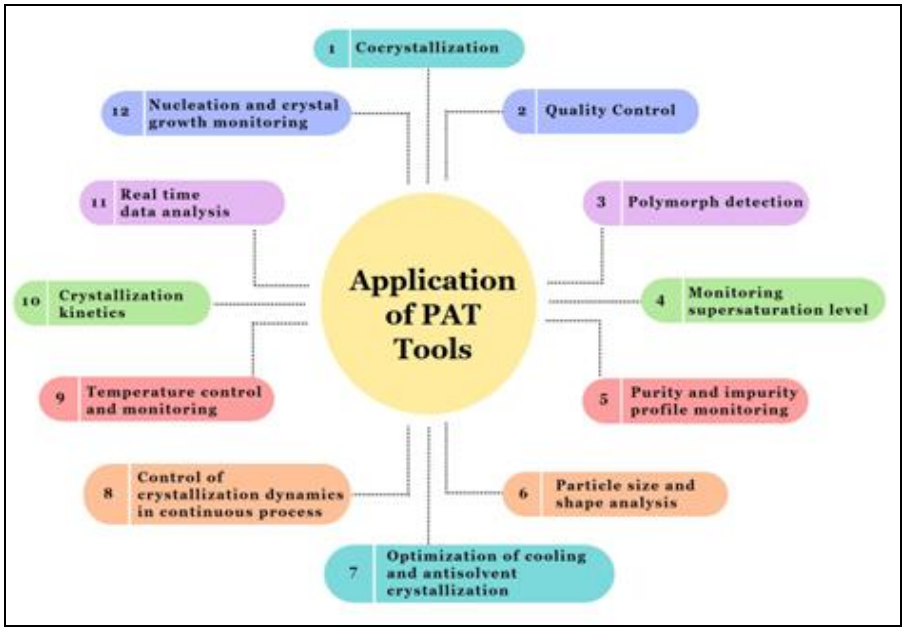

FIG. 3: ROLE OF PAT TOOLS IN CRYSTALLIZATION PROCESS

Types of PAT Tools: Several PAT tools are available which are used widely in the pharmaceutical industries. These tools are compact in size and portable and can be easily handled during crystallization set-up. They are primarily based on spectroscopic and imaging technologies and are summarized in **Fig. 4.**

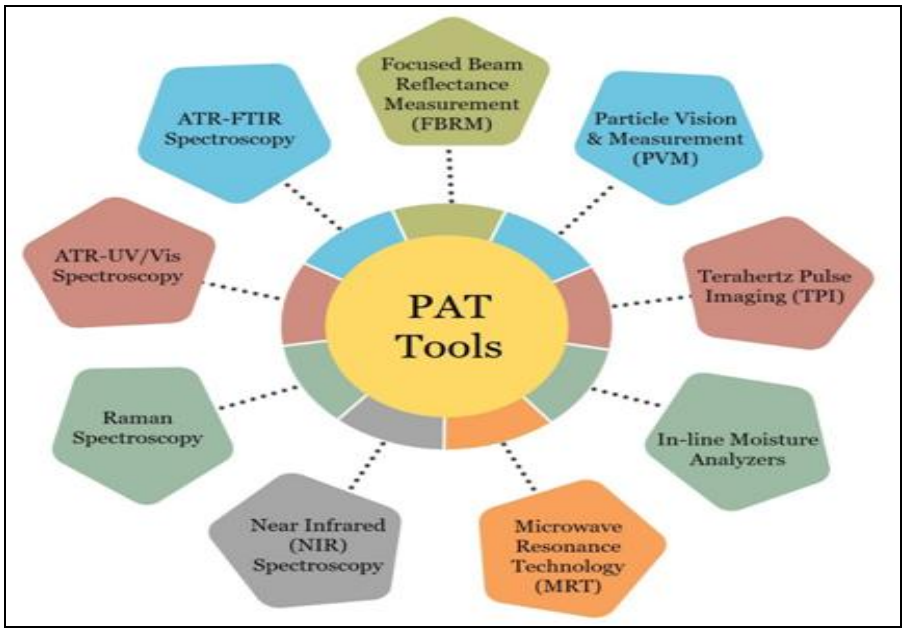

FIG. 4: VARIOUS PAT TOOLS EMPLOYED IN CRYSTALLIZATION SET-UP

Near Infrared Spectroscopy (NIR): NIR spectroscopy is a powerful analytical technique used as a PAT tool to observe and manage the manufacturing process in real-time. NIR spectroscopy operates by measuring absorption in the near-infrared region (700-2500 nm) of the electromagnetic spectrum, which lies between the visible and mid-infrared regions. In the mid-infrared region, basic absorption bands of functional groups are very strong. The NIR spectrum characteristic features are overtones and combination bands of CH, OH, and NH functionalities [18].

NIR spectroscopy measures the light absorption of near-infrared range caused by molecular vibration. It is performed in reflectance mode to analyze the chemical properties of solid samples. This technique is sensitive to crystal lattice and the change in hydrogen band, making it suitable for analyzing solid pharmaceutical powders. Based on the physical properties of the sample, different measurement modes are available, such as transmittance mode, diffuse reflection mode, and transflection mode. NIR techniques are fast because they require little to no sample pretreatment [19]. These methods allow for rapid, consistent, nondestructive analysis of solid samples and can evaluate both the chemical and physical features of molecules. These capabilities make NIR suitable as a process analytical technology integral to current good manufacturing practices. It is useful in monitoring various parameters like nucleation, polymorphic transformation, and solute concentration.

Raman Spectroscopy: Raman spectroscopy is an analytical tool for molecular characterization with diverse applications across many scientific fields. This technique is used to identify and characterize materials by observing rotational, vibrational, and other low-frequency modes in a system. It relies on the inelastic scattering of monochromatic light, typically from a laser. When light communicates with molecular vibrations, it results in energy shifts that provide a molecular fingerprint unique to each substance. Raman spectroscopy has emerged as a powerful PAT tool due to its ability to deliver real-time evaluation of chemical and physical properties. Its non-damaging nature, high specificity, and capability to analyze samples in various states make it an invaluable technique [20]. This technique helps in tracking the progress of chemical reactions, including polymorphic transition and crystallization.

Attenuated Total Reflectance Ultraviolet-Visible (ATR-UV/Vis) Spectroscopy: ATR-UV/Vis spectroscopy combines the principles of UV/Visible spectroscopy and the Attenuated Total Reflectance (ATR) sampling technique to enable direct and non-destructive analysis. It operates by directing ultraviolet and visible light through an ATR crystal in contact with the sample. The light undergoes multiple internal reflections within the crystal, creating an evanescent wave that penetrates the sample. The interaction of this wave with the sample provides detailed spectral information on absorbance, which can be used to determine concentration, monitor reaction progress, and assess phase transitions [21]. By offering real-time, in-line data, ATR-UV/Vis spectroscopy enhances process control, ensures product quality, and supports regulatory compliance, making it a valuable tool. This technique is used to monitor polymorphic transformation.

Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) Spectroscopy: ATR-FTIR spectroscopy involves infrared light which is directed into an ATR crystal, where it experiences several internal reflections, resulting in the creation of a fleeting wave that penetrate the sample. In-depth spectrum information regarding chemical bonds and molecular vibration is provided by this interaction [22]. This technique is useful to ensure product quality by detecting contaminants and confirming the chemical composition of the finished product. It also helps in monitoring the chemical reactions and identifying phases and polymorphs. Its capacity to acquire data in real-time facilitates accurate process management, regulatory compliance, and optimization.

Terahertz Pulse Imaging (TPI): Terahertz Pulse Imaging (TPI) is an emerging non-destructive, real-time analytical technique that utilizes terahertz (THz) radiation to probe materials. Operating in the terahertz frequency range (0.1 to 10 THz), this technique provides unique insights into the chemical constituent, structure, and dynamics of materials, making it highly suitable for PAT

applications in the pharmaceutical industry. In the electromagnetic spectrum, THz radiation falls between the microwave and infrared regions. It can penetrate a variety of materials, including polymers, without causing damage. THz pulses are typically generated using ultrafast laser systems that produce short optical pulses, which are then converted into THz pulses using photoconductive antennas or nonlinear optical crystals. Detected using similar photoconductive antennas or electro-optic sampling techniques. TPI operates in the time domain, capturing the time delay and amplitude of the reflected or transmitted THz pulses [23].

Focused Beam Reflectance Measurement (FBRM): Focused Beam Reflectance Measurement (FBRM) is an advanced PAT tool used extensively in industries for real-time observation of particle and crystal characteristics in suspensions and emulsions. FBRM provides valuable insights into particle shape, size, and distribution, which are critical for ensuring product quality and optimizing manufacturing processes. FBRM uses a rotating laser beam that is focused on a process stream. As the laser scans across particles, it captures the backscattered light from particle surfaces. This technique measures the chord length distribution of particles. A chord is a linear line connecting two points on the boundaries of a particle, effectively providing information on particle size and shape. FBRM provides continuous, real-time data, allowing for immediate analysis and process adjustments. The measurement is conducted directly in the process stream without the need for sample extraction or preparation [24]. This technique is used in monitoring crystal growth, nucleation, and dissolution in real time to control crystal size distribution and optimize yield. Additionally, it can identify the onset of polymorphic transitions and ensure consistent product quality. It also enhances understanding of particle dynamics and process behavior, facilitating robust process development and optimization.

Particle Vision and Measurement (PVM): Particle Vision and Measurement (PVM) is a PAT tool that uses imaging technique for real-time observing and analysis of particle systems in various industrial processes. PVM provides direct visual and quantitative information on particle size, shape, and behavior in situ, making it invaluable for optimizing and controlling manufacturing processes in the pharmaceutical, chemical, and food industries. PVM employs high-resolution digital cameras and advanced image analysis software to capture and analyze images of particles in real time. Unlike other particle characterization techniques, PVM provides direct visual information, allowing for immediate assessment of particle morphology and behavior. PVM systems are designed to be integrated directly into the process stream, enabling continuous, real-time monitoring without the need for sample extraction or preparation [6]. This technique is employed to monitor crystal growth, nucleation, and dissolution in real time to control crystal size distribution and optimize yield.

In-line Moisture Analyzers: In-line moisture analyzers are integral instruments in the field of process analytical technology, used to continuously monitor and control moisture content in various manufacturing processes. During crystallization, precise moisture control is vital to achieve the desired crystal form, size, and purity and to prevent polymorphic transformations that can affect bioavailability and stability [25]. Techniques such as NIR spectroscopy and microwave resonance are commonly used in these analyzers. NIR spectroscopy measures the absorption of near-infrared light by molecule vibration, particularly those involving water molecules. The near-infrared light penetrates the crystallizing solution or slurry, and the transmitted or reflected light is collected and analyzed to determine the moisture content [26].

Microwave Resonance Technology: Microwave resonance technology is an advanced PAT tool used extensively for in-line moisture analysis during the crystallization process. This technique operates based on the contact between microwave signals and water molecules within a sample. Water molecules have a high dielectric constant compared to most dry materials. Microwave resonance technology measures the dielectric properties of the material, which vary with moisture content. When microwaves interact with the water molecules in the sample, they cause a shift in the resonance frequency. This shift is directly proportional to the amount of water present in the sample. By analyzing these frequency changes, the moisture

content can be accurately determined in real-time [27].

Integration of Chemometric Approaches with PAT Tools: Chemometrics is a process that uses mathematical techniques and multivariate data analysis to extract information from chemical data. The integration of chemometric approaches with PAT tools significantly enhances the monitoring and managing of crystallization processes [28]. By giving real-time insights and predictive capabilities, this combination ensures optimal crystal quality and process efficiency. Chemometric models facilitate real-time interpretation of data from PAT tools, enabling immediate adjustments to the crystallization process to maintain optimal conditions [15].

Multivariate Data Analysis (MVDA): MVDA is a statistical approach employed to analyze data that arises from more than one variable. In the context of PAT technologies, MVDA is essential for interpreting complex datasets generated during manufacturing processes. MVDA is applied to monitor CQAs and CPPs in real time, enabling immediate detection of deviations and corrective actions. This analysis method helps in understanding the relationships between multiple variables, monitoring processes in real-time, and ensuring product quality and consistency across various industries, including pharmaceuticals, chemicals, and food processing [29]. Techniques like Partial Least Squares (PLS) regression and Principal Component Analysis (PCA) analyze complex datasets obtained from PAT tools, extracting significant patterns and correlations within the data. PCA is employed to convert the original variables into a new set of uncorrelated variables (principal components) that capture the highest variance in the data. This helps in visualizing and interpreting complex data structures. PLS regression models the relationship between dependent and independent variables by finding the latent structures in both sets. It is particularly useful for predicting outcomes and understanding variable interactions [30].

Quality by Design (QbD): QbD is a methodological approach to pharmaceutical development and manufacturing that emphasizes designing quality into products and processes from the outset [31]. It is a key aspect of PAT tools designed to reduce inconsistency in product quality by maintaining process parameters within acceptable range. The QbD principle endorsed by regulatory agencies like the FDA and EMA and uplift the use of QbD principles in pharmaceutical manufacturing where consistent quality is of key significance [16]. This systematic approach involves a thorough understanding of CQAs and CPPs, risk management, and the establishment of a design space that ensures consistent product quality. By employing real-time monitoring and control strategies using PAT tools such as NIR spectroscopy and MVDA, QbD allows for proactive adjustments to maintain process stability and product consistency. This methodology not only enhances product quality and reduces risks but also offers regulatory flexibility and cost efficiencies by allowing manufacturers to make changes within the approved design space without additional regulatory submissions [32]. The continuous improvement framework inherent in QbD further supports the ongoing optimization of both product quality and manufacturing processes.

Applications of PAT tools in Pharmaceutical Crystallization: The pharmaceutical industry enormously employed PAT tools for real-time monitoring of several manufacturing processes. PAT tools have been applied for in-situ monitoring and characteristics of liquid and solid phases during the crystallization process **Fig. 5.**

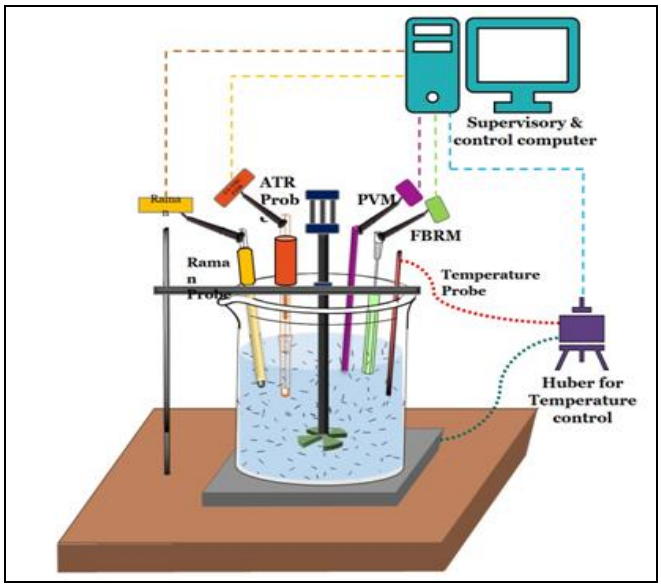

FIG. 5: SCHEMATIC REPRESENTATION OF PAT TOOLS IN REAL-TIME MONITORING

This section highlights the application of PAT tools in the crystallization of various drugs and excipients in the pharmaceutical industry. Only representative case studies dealing with crystal growth, particle size distribution, polymorphism etc. have been briefly reviewed while all the reported studies in the literature wherein PAT tools have been used are summarized in **Table 1.**

TABLE 1: SUMMARY OF THE PAT TOOLS EMPLOYED IN THE CRYSTALLIZATION OF DIFFERENT API/ ORGANIC COMPOUNDS

S. no.	API / Compound	Investigation Parameters	Process	Solvent used	PAT Tools	Remarks
1	Ceritinib[33]	Monitoring crystallization	Batch crystallization	Water, acetone and tetrahydrofuran	Fiber optic raman spectroscopy	Raman spectroscopy measures solute concentration by analyzing the intensity of characteristic Raman peaks. Partial least square regression (PLSR) and Artificial neural network (ANN) are used to develop calibration models. ANN model estimated the solute concentration with high accuracy
2	Succinic acid[34]	Polymeric additive crystallization	Batch cooling crystallization	Water	FBRM, PVM	FBRM is used to monitor particle counts and size distribution, help to identify nucleation and dissolution processes during temperature cycling. PVM identified the shape transition from irregular plate to diamond-like structure. Pluronic P123 was used as additive which affect the shape of crystal without incorporated in it.
3	Anthranilic acid[35]	Polymorphic transformation	-	Water and isopropyl alcohol	Fiber optic raman, NIR, ATR-UV/Vis spectroscopy, FBRM, PVM	Raman, ATR-UV/Vis and NIR spectra were employed to distinguish the polymorphic forms by analyzing the intensity of characteristic peak. FBRM is used to detect the amount of solid in the solution and crystal size distribution. PVM detected the transformation and captured the image of from I as prismatic crystal.
4	Vitamin B12[36]	Crystallization in the presence of impurities	Linear cooling crystallization	Water and ethanol	Fiber optic raman spectroscopy, ATR-UV/Vis, FBRM	Raman spectroscopy was implemented to detect the existence and change in impurity level. UV/Vis spectroscopy monitors the concentration of vitamin B12 and provides insights into purity and growth dynamics. FBRM detected the crystal size & particle count and higher value of total count indicates longer crystal size. PCA was performed on Raman spectroscopic data to determine a relation between purity of analyzed crystal and

						their Raman spectra.
5	Carbamazepine[37]	Polymorphic transformation	Batch and continuous crystallization	Ethanol	Fiber optic raman spectroscopy	Raman spectroscopy was used to differentiate the stable and metastable form by monitoring the characteristic peak associated with these forms. Polymorphic transformation from form III to form II was monitored by adding form II seed crystals to slurry of form III.
6	Piroxicam[38]	Polymorphic transformation	Batch cooling crystallization	Deionized water and acetone	Fiber optic raman spectroscopy, ATR-UV/Vis spectroscopy, FBRM, PVM	Raman spectroscopy was used to monitor polymorphic transformation by analyzing the characteristic peak intensity. ATR-UV/Vis and FBRM were applied to measure the changes in solute concentration and control nucleation and growth rate. Images captured by PVM indicated that form II might occasionally crystallize during process.
7	p-toluenesul fonamide and triphenylphosphine oxide cocrystal[39]	Monitoring cocrystallization	Batch and semi-batch cocrystallization	Acetonitrile	In situ raman spectroscopy, ATR-UV/Vis spectroscopy, ATR-FTIR, FBRM, PVM	Raman spectroscopy was implemented to control crystallization and to obtain more stable cocrystal. FBRM and PVM were used for nucleation and dissolution detection and to track the evolution of crystal properties.
8	Urea barbituric acid cocrystal[40]	Monitor cocrystal polymorphism	Batch and continuous crystallization	Methanol	Fiber optic raman spectroscopy, ATR-UV/Vis spectroscopy, FBRM, PVM	Raman spectra identify the desired form by providing characteristic peaks in the spectra. ATR-UV/Vis spectra show spectral distortion in the presence of form III, confirms the polymorphic. Transformation.
9	L-Aspartic acid[41]	Process variables optimization	Antisolvent crystallization	Water/formic acid - Isopropanol	FBRM	FBRM was used to optimize the process variables such as storage temperature, stirrer velocity, storage time and solvent ration during crystallization.

FBRM: Focused Beam Reflectance Measurement, **PVM:** Particle Vision and Measurement, **NIR**: Near Infrared Spectroscopy, **ATR-UV/Vis:** Attenuated Total Reflectance Ultraviolet-Visible Spectroscopy, **ATR-FTIR:** Attenuated Total Reflectance Fourier Transform Infrared, **PCA:** Principal Component Analysis

Gavran *et al*. [33] developed a Raman-based calibration model for estimating the ceritinib concentration during the crystallization process in a tetrahydrofuran solvent. During the crystallization process, Raman spectroscopy was used to collect data on the Raman spectrum, temperature, turbidity, and chord length distribution of ceritinib suspensions with varying solute and solid concentrations. The data was then used to develop a calibration model using partial least squares regression (PLSR) and artificial neural network (ANN) models. The results showed that the PLSR model overestimates and underestimates solute concentration, indicating a non-linear relationship in the dataset. The ANN model, on the other hand, estimates the solute concentration with higher accuracy, with a prediction error not exceeding 1% of the normal solute concentration. This work

highlights that the developed calibration model was found suitable for estimating the solute concentration of ceritinib in a crystallization process, and can be employed for in-line monitoring and control of the solution crystallization process. Another interesting study was conducted with FBRM and PVM to investigated the crystal size and shape of succinic acid [34]. The study demonstrated the impact of polymeric additives (pluronic P123) and temperature cycling (heating and cooling cycle) on crystal morphology **Fig. 6.**

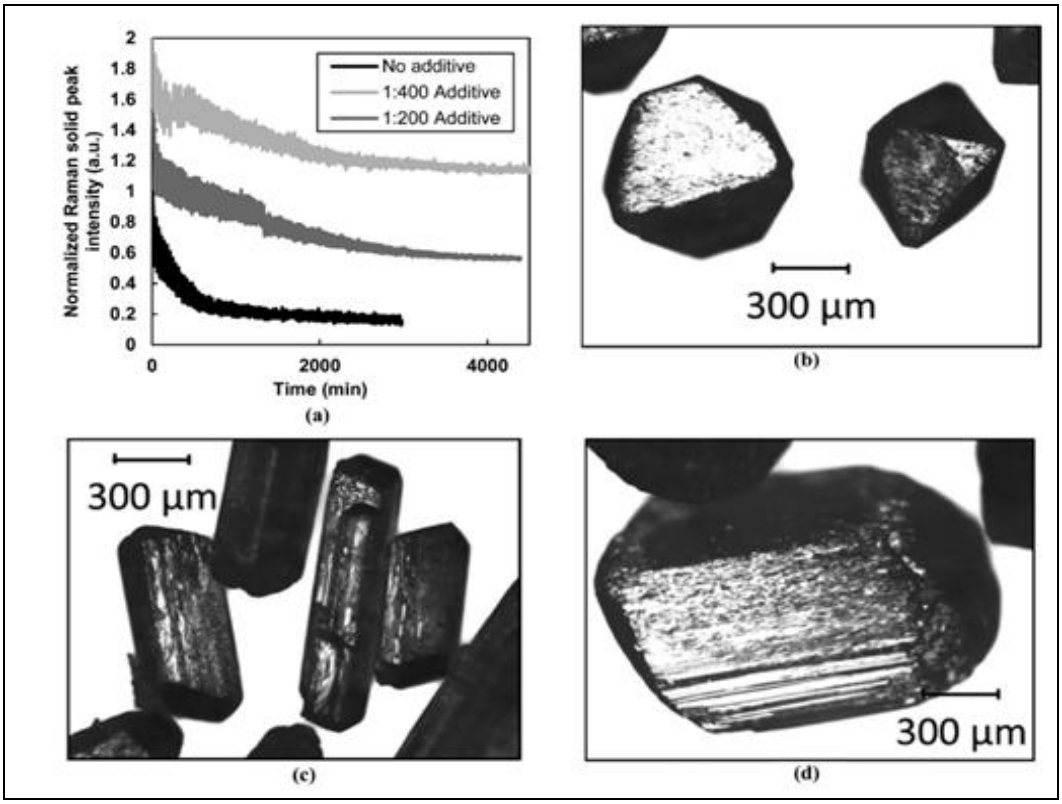

FIG. 6: (A) RAMAN PEAK TRENDS ASSOCIATED WITH SOLID SUCCINIC ACID FOR CYCLING EXPERIMENTS AT DIFFERENT ADDITIVE CONCENTRATIONS; CRYSTALS FORMED AFTER THE CYCLING EXPERIMENT (B) WITHOUT ADDITIVE AND (C) & (D) WITH ADDITIVES (ADAPTED FROM REF. [34])

Faster heating/cooling rates have shown more irregular diamond shapes compared to those obtained at a slower rate. A considerable change in the crystal shape was observed with a change in the PVM and FBRM statistics while in the presence of polymeric additives, no change was seen in shape, but the size of the crystal was increased.

In-situ monitoring of polymorphic transformation of anthranilic acid was conducted by Simone *et al.*[35] using multiple PAT tools like ATR-UV/Vis, Raman, NIR spectroscopy, PVM and FBRM. The study focused on the transformation from metastable form II to the stable form I of anthranilic acid in isopropyl alcohol and water solution. Raman & NIR spectra distinguished the forms I and II in a mixture based on the intensity of signature peaks **Fig. 7.** ATR-UV/Vis probe was used to monitor the changes in the solute concentration during the process. The transformation in the crystal was observed by the PVM and the conversion of the needle into a prismatic shape crystal was ascribed to the polymorphic transformation into form I.

The data obtained from all the techniques were merged into a single matrix using a chemometric technique i.e., PCA and used this method to automatically identify the polymorphic transformation. Overall, the study eloquently describes that the Raman spectra gave the most information regarding crystal growth, nucleation, and polymorphic transformation. The water present in the system restrict the application of NIR but transformation was possible to detect by its baseline effect.

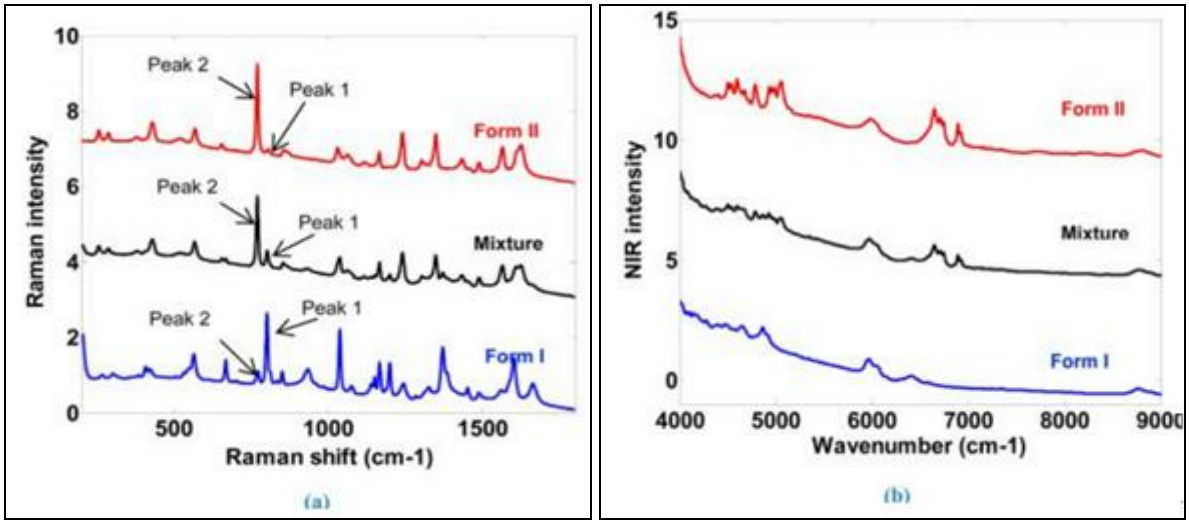

FIG. 7: (A) RAMAN SPECTRA AND (B) NIR SPECTRA FOR SOLID ANTHRANILIC ACID SAMPLES: PURE FORM I (BLUE), PURE FORM II (RED), AND MIXTURE (BLACK) OF THE TWO FORMS (ADAPTED FROM REF. [35])

Simon *et al.* [36] carried out a study to understand the crystallization process of vitamin B12 produced by fermentation. This study shows how impurities affect the growth rate and how PAT tools can be applied to improve both crystal size distribution and purity.

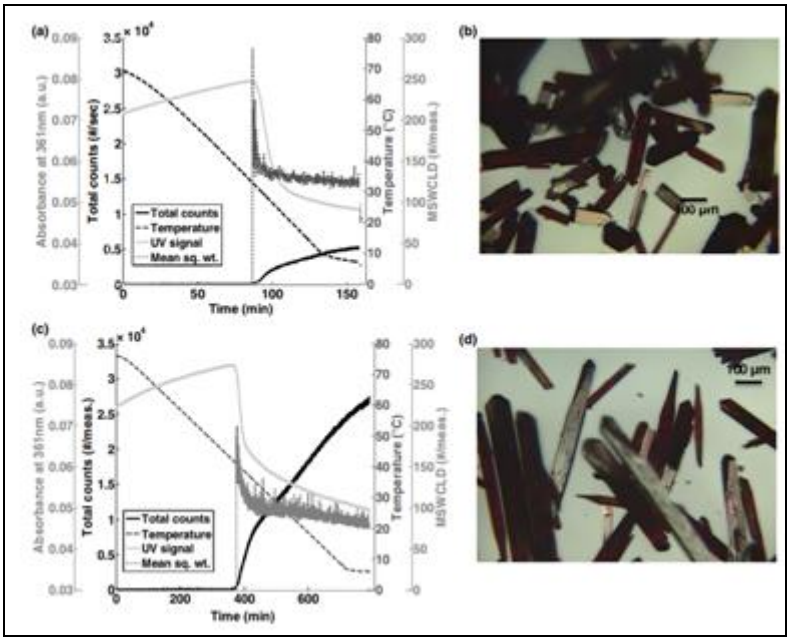

FIG. 8: FBRM TOTAL COUNT AND CHORD LENGTH DISTRIBUTION FOR FAST COOLING (A & B) AND SLOW COOLING CRYSTALLIZATION (C & D) OF VITAMIN B12 (ADAPTED FROM REF. [36])

Linear cooling crystallization were carried out under various conditions including different solvents, cooling rate, and seeding. A set of PAT tools like Raman, ATR-UV/Vis spectroscopy and FBRM were employed to monitor and analyze the crystallization process. Raman spectroscopy was used to detect the existence and change in impurity level by monitoring the amount of fluorescence in the Raman spectra. ATR- UV/Vis spectroscopy was employed to monitors the concentration of vitamin B12 and provide insights into purity and growth dynamics. FBRM detected the crystal size and particle count, higher value of total count showed the longer crystal size. The result showed that impurities significantly hinder the growth of vitamin B12 crystal, leading to increased nucleation and resulting in poor crystal size distribution. In fast cooling experiments, fewer but larger crystals were formed while slow cooling produced smaller crystals with better purity.

However, crystal size distribution was found to be broader due to secondary nucleation **Fig. 8.** Seeding was also found to improve crystal purity but change in the solvent composition did not notably affect the crystal size distribution or purity. In addition to these, several studies are also reported in the literature, in which PAT tools are applied to detect and real time monitoring of nucleation process, cocrystallization and polymorphic transformation [37-41].

Challenges and Future Directions: PAT tools have many potential benefits in the crystallization processes but there are also several drawbacks. PAT tools are effective at a laboratory scale but may encounter difficulties when scaled up to a large scale. The variability in process conditions at a larger scale can reduce the effectiveness of PAT tool monitoring and control strategies. This technique is used for real-time process monitoring but many times lag between data collection, analysis, and the implementation of process adjustment can limit the effectiveness of real-time control. Sensors used in PAT tools require frequent calibration to maintain accuracy which can be time-consuming and can disrupt production. Management of data is also an issue because PAT tool generates vast number of data which can be difficult to manage and interpret, requiring trained personnel with the necessary skills to interpret data. However, the future of PAT tools in the industry looks promising. Advances in sensor technologies can improve monitoring capabilities and integration with machine learning and artificial intelligence are expected to improve data analysis and process optimization. There should be a collaboration between industry, academia, and regulatory bodies to overcome these challenges and fully unlock the potential of PAT tools in pharmaceutical manufacturing, particularly crystallization experiments.

CONCLUSION: This work summarizes the importance of PAT tools to efficiently monitor and control the crystallization process. The integration of PAT tools in the pharmaceutical industry can provide significant advantages by enhancing the product quality and faster process optimization. The focus of the review is to outline the use of PAT tools in the crystallization of active pharmaceutical ingredients. Various case studies have been compiled and the role of PAT tools in real time monitoring of nucleation, crystal growth, polymorphic control, solute concentration, particle size, shape etc. have been discussed. Overall, PAT tools have a significant role in ensuring product quality, enhancing process efficiency, and support regulatory compliance

AKNOWLWDGEMENT: JH would like to thank ICMR, New Delhi, India for providing financial support in the form of an Adhoc Research Project (EM/Dev/SG/215/6200/2023).

CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

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How to cite this article:
Haneef J and Khan MD: Harnessing the potential of pat tools in pharmaceutical crystallization. Int J Pharm Sci & Res 2025; 16(6): 1465-79. doi: 10.13040/IJPSR.0975-8232.16(6).1465-79.