ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 16, Issue 11, 2023

Online - 2455-3891
Print - 0974-2441

Review Article

BIOAVAILABILITY AND POLYMORPHIC STABILITY CHALLENGES AFFECTING DRUG PRODUCT'S POTENTIAL: A CRITICAL EVALUATION AND PERTINENT SOLUTION

RAMAKANT PANDA*, SRINIVAS LANKALAPALLI

Department of Pharmaceutics, GITAM School of Pharmacy, GITAM Deemed University, Rushikonda, Visakhapatnam, Andhra Pradesh, India.
***Corresponding author: Ramakant Panda; Email: 121965201519@gitam.in**

Received: 25 April 2023, Revised and Accepted: 04 June 2023

ABSTRACT

Clinical failure remains an ongoing challenge in pharmaceutical drug product development. Solubility and permeability therefore play a very critical role in achieving desired bioavailability and pharmacological response, which in turns affects clinical safety and efficacy significantly. The situation becomes more critical when the drug candidate exhibits polymorphism and undergoes polymorphic transformation due to its meta-stable nature. This review article outlines the available technologies, pertinent regulations, the concepts involved in the enhancement of bioavailability and polymorphic stability to overcome the clinical failures. Various available technologies for bioavailability enhancement such as salification, micronization, complexation, microemulsification, nano emulsification, cocrystal formation, and amorphous solid dispersion with their advantage and disadvantage in formulating a stable drug product containing a polymorphic and meta-stable drug substance. Thermodynamic and kinetic aspects of polymorphic transformation are discussed to understand different excipient and process-induced transformation during manufacturing and shelf life of the drug product. Selecting the right instrument from the analytical toolbox is equally important to understand the diverse nature of polymorphic transformation. This review provides state-of-the-art information available on advanced analytical tools along with their capabilities, advantages, and disadvantage with respect to physical/structural analysis of polymorphs and polymorphic transformation.

Keywords: Polymorphism, Metastable polymorph, Polymorphic stability, Solubility, permeability, Bio-availability enhancement, Polymorphic transformation.

 DOI: http://dx.doi.org/10.22159/ajpcr.2023v16i11.48177. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Clinical failure remains an on going challenge in pharmaceutical drug product development. Solubility and permeability therefore play a very critical role in achieving desired bio-availability and pharmacological response which, in turn, affects clinical safety and efficacy significantly. The situation becomes more critical when the drug exhibits meta-stable polymorphism.

Poor solubility restrains drug from getting absorbed through membranes and reaches the site of action regardless of drug delivery technology and route of drug delivery. Many methods, including size reduction, the use of lipids, and amorphous solid dispersion, are available to address poor solubility. However, each technique has some limitations and poses new formulation challenges [1].

Stable polymorph with desired solubility is usually preferred for New drug Application (NDA) by the innovator companies and is patent protected. The patent protection by the innovators restricts other generic players to explore the same polymorph with same route of synthesis for abbreviated NDA. In such cases, exploring meta-stable polymorphs other than the patented polymorph becomes the preferred option for generic drug development and subsequent approval. However, it is essential to select and explore suitable formulation stabilization techniques for the meta-stable polymorph.

Strategies such as selection of suitable polymorph, salification, and cocrystal formation mayhelp in increasing the drug solubility, However, there has been limited success in improving bioavailability. Whereas techniques such as solid dispersion, lipid microemulsion, nanoemulsion may help to increase both solubility and permeability. In many case despite having good solubility and permeability, clinical success are limited due to poor polymorphic stability involving polymorphic transformation.

Inadequate polymorph screening process, polymorphic transformation during manufacturing, stability, and shelf life of the drug product have a significant impact on *in vitro* and *in vivo* dissolution leading to a variable oral bioavailability and clinical responses, thereby posing a big challenge for pharmaceutical industry. In the past, pharmaceutical industry has encountered serious concern related to polymorphism and polymorphic transformation leading to clinical failure and subsequent product recall of Norvir® (Ritonavir Capsule), Coumadin® (Warfarin sodium 2-propanol solvate), Neupro® (Rotigotine Transdermal patch), Avalide® (Hydrochlorothiazide and Irbesartan), and Tegretol® (Carbamazepine Tablets) (Lee *et al.*). Compromised drug safety and efficacy with increasing product recalls has steered the emergence of many guidance documents on pharmaceutical solid polymorphism and cocrystal by global drug authorities United States Food and Drug Administration, World Health Organization, and International Conference on Harmonization [2-6].

With increasing concern of polymorphism on drug product efficacy and safety, it is required to have a more rational approach to understand about the polymorphic transformation occurring during manufacturing, *in vivo* precipitation, or polymorphic transformation at the time of administration and during shelf life of the drug product.

In general, exploring combinatorial chemistry and high-throughput screening studies in identifying suitable polymorphs, understanding thermodynamic aspects and transformation kinetics during polymorphic drug processing, understanding the effects of excipients and manufacturing processes on polymorphic transformation and exploring suitable advanced analytical tools contributes to the development of a stable and clinically effective pharmaceutical drug products.

FACTORS AFFECTING POLYMORPHIC STABILITY AND BIO-AVAILABILITY

Polymorphism must be considered from the pre-clinical development. The impact of polymorphisms should be assessed and monitored

during preclinical development, pre-formulation and formulation development, process development, manufacturing, stability, and storage throughout the drug products life cycle.

The stability and bioavailability of any drug substance are strongly influenced by polymorphism. The drug substance can be a stable polymorph, pseudopolymorph (hydrate, solvate, and cocrystal), or a meta-stable polymorph. Meta-stable polymorphs are known to be vulnerable to the environmental and processing condition and undergo polymorphic transformation. It is reported that 85% of new chemical entities (NCEs) exhibit pseudopolymorphism and 50% having real polymorphism [7,8].

Retaining the polymorphic form of the meta-stable polymorph in the formulation throughout the stability study and shelf life has been a major challenge for pharmaceutical formulation scientists for a long time. Exploring various novel solubilization techniques helps in improving solubility and dissolution of the selected polymorph but ensuring the polymorphic stability (retaining the original polymorphic form) for the same polymorph in the formulation sometimes poses a big challenge. Hence, it becomes very important to understand the factors affecting the polymorphic transformation, thereby affecting the stability and bioavailability of the drug.

To overcome the above challenges, following factors are to be considered and studied extensively.

- Physicochemical and crystal nature of the polymorphic drug substance:

Crystal structure
Lattice energy
Solubility
Solvation Enthalpy

→ Thermodynamic Aspect

Seed growth rate
Rate of Nucleation and cross-nucleation
Competition kinetics (between seed growth and cross -nucleus formation)
Relaxation Time
Rate of Transformation conversion

→ Kinetic Aspect

- Nature of processing:

Thermal, mechanical, pressure, compression force, process kinetics, Transformation rate of transformation of inetic energy into heat energy during conversion of meta-stable polymorphic form to stable form or vice versa.

Example of unit operation for a tablet manufacturing process includes direct compression, aqueous or non-aqueous wet granulation, dry granulation, hot melt extrusion (HME), sizing, thermal drying, lyophilization, spray drying, tablet compression, film coating, and functional film coating.

- Nature of additives:

For example, presence of moisture or water in the excipients, type of solvents, and other excipients which can induce/favour a polymorphic transformation through thermodynamic process.

The underlying mechanism of phase transformation during various unit operation is listed in Table 1.

One schematic presentation showing polymorphism affecting solubility, permeability, and bioavailability is depicted in Fig. 1.

APPROACHES TO OVERCOME SOLUBILITY AND BIOAVAILABILITY CHALLENGES FOR LOW SOLUBLE AND LOW PERMEABLE DRUGS

At present, many available techniques such as solid dispersion, polymeric amorphization, complexation, Microemulsifying drug delivery System (SMEDDS) Nanoemulsifying drug delivery system (SNEDDS), cocrystal approach, and use of surfactants are explored for the enhancement of solubility and bioavailability of low soluble drugs. However, each of the available techniques bears advantages and disadvantages with respect to drug loading, drug stability, and *in vivo* permeation. The advantages and disadvantages of the above technologies are summarized in Tables 2 and 3.

Apart from solubility, permeability plays an important role in bioavailability. Through the selection of suitable polymorph/NCE with respect to solubility and permeability is usually done during pre-clinical screening selection of suitable excipient, pre-clinical formulation development is also of great importance in facilitating intestinal permeability.

As per the guidance documents from USFDA and EMA on BCS based Bio waiver of generic drug products, *in vivo* bioequivalence studies can be exempted if the generic drug product with a BCS Class I/III active

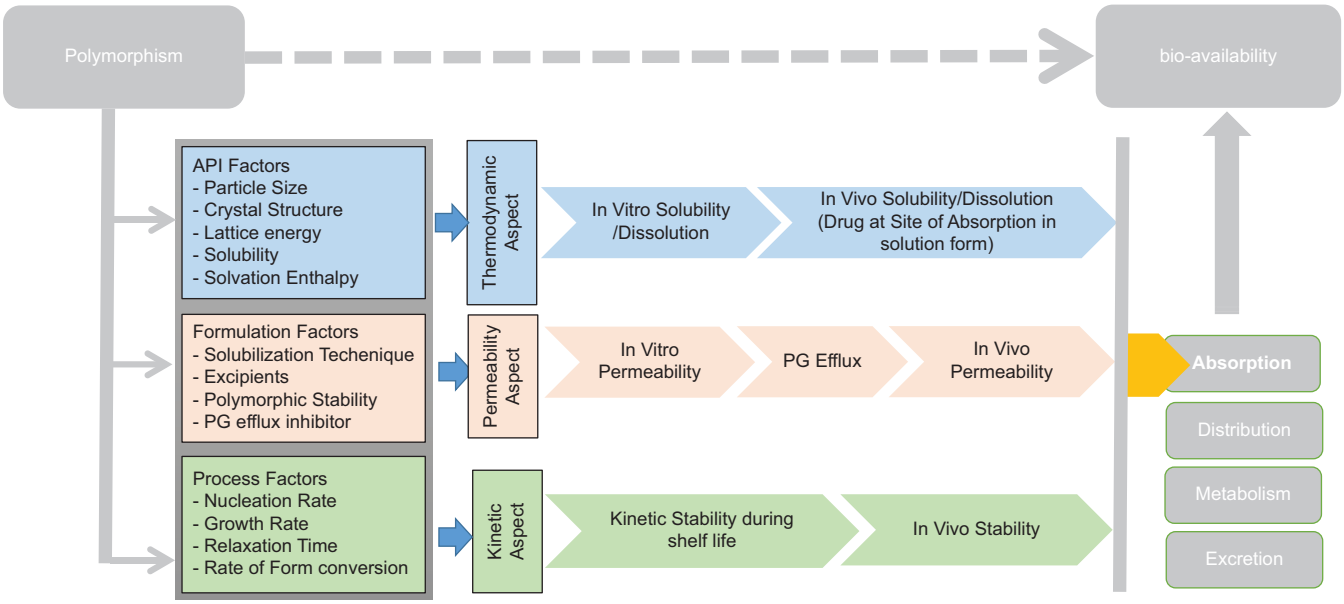

Fig. 1: Diagrammatic representation of polymorphism affecting solubility, permeability, and bioavailability

Table 1: Formulation/excipient-induced stability challenges

S. No.	Metastable API/ Formulation	Formulation	Stability issue	Stabilization approaches	Analytical tools for stability evaluation	References
01	Indomethacin (α form)	Nanocrystal	Interconversion of α form of Indomethacin to the stable γ form in the presence of a solvent, heat, pH, or exposure to the stable form seed crystal	stabilized in cellulose Nanocrystal Aerogel Scaffolds	DSC, Raman	[48]
02	Fluconazole (Metastable form II)	ASD	Polymorphic Transformation to stable form I	Amorphization with Soluplus® (SOL) by solid dispersion using spray drying and fusion methods	pXRD, FTIR, DSC,TGA, HPLC, SEM	[28]
03	Piracetam (metastable Form I)		Polymorphic Transformation to stable form II & III	Preparing thin Film Piracetam on silicon oxide substrates using Spin coating	sXRD, GIXD	[53]
04	Carbamazepine/ Polyvinylpyrrolidone ASDs	ASD	Metastable crystallization	Use of Low molecular weight Crystalline excipients like saccharin or Tryptophan explored in the formulation	X-ray diffraction (PXRD)	[19]
05	Nitrofurantoin anhydrate		Metastable Hydrate formation	Hygroscopic partially crystalline excipients explored	X-ray diffraction (PXRD)	[25]
06	Theophylline anhydrous wet granulation	SR Granules	Psudopolymorphic changes altering dissolution rate (Hydrate formation)	Exploring SMCC to prevent process-induced transformation i.e. Hydrate formation during the wet granulation process	PXRD, NIR, SEM, Raman	[35]
07	Rifaximin (α form)	Tablet	Dissolution kinetics issue due to Polymorphic transformation from α form to β form in DC process	Ethanol granulation process adopted to prevent kinetics of polymorphic transformation	FTIR, PXRD	[54]
08	Olanzapine anhydrous (Form I)		Metastable Hydrate formation	Exploring PVP & HPC to prevent Hydrate formation	pXRD, FTIR, DSC, Thermo gravimetric water vvapor sorption	[36]
09	Tenofovir Disoproxil Fumarate (Form I)	Tablet	Conversion of Form I to Form A during formulation	pH Adjustment	PXRD, Raman	[55]
10	Ritonavir ASD	ASD	Stability Issue	Stabilization through increasing glass transition Temperature (Tg), formulating ASD with PEG 8000	TMDSC, XRPD	[29]
11	Gabapentin Anhydrous (Form II)	Co-milled excipient mixture	Instability due to Lactamization of Gabapentin (Gabapentin Lactam)	Selecting suitable excipients retarding polymorphic transformation due to competitive hydrogen bonding. i.e HPC Mantaining RH above 31 %	13C CP/MAS NMR and HPLC, AMASTK (Advanced Modeling and Simulation Tool Kit, UI Copyright 2012)	[49,56]
12	Etoricoxib amorphous form	Tablet	Instability due to conversion to crystalline form during compression of Melt granule of Amorphous etoricoxib (MG AET)	Stabilization of Amorphous form exploring Polyglycolized glycerides in the formation of hydrogen bonding	DSC, XRPD	[57]
13	Felodipine (Form I) ASD	Amorphous solid dispersion	Re-crystallization to metastable form II and Form III	Reducing drug loading to 10 % with ASD prepared with polyvinyl Acetate (PVAC)	DSC, XRPD, FTIR, TEM, Conical DF imaging	[30,31]

ASD: Amorphous solid dispersion, pXRD: Powder X-ray diffraction, FTIR: Fourier transform infrared, DSC: Differential scanning calorimetry, TGA: Thermogravimetric analysis, HPLC: High performance liquid chromatograph, SEM: Scanning electron microscopy

pharmaceutical ingredient (API) satisfies the solubility permeability, dissolution criteria along with the possible effect of excipient. There are excipients which can have possible effects on *in vivo* absorption. Such excipients can affect drug solubility, permeability gastric motility, gastrointestinal transit time, and P-gp efflux system. Hence, for a bio-waiver application, the possible effect of excipients needs to be considered and needs to be justified that the proposed excipients in the generic product will not affect the absorption profile of the drug substance under consideration [9,10].

Examples of excipients affecting drug absorption include mannitol, sorbitol, and sodium lauryl sulfate.

Table 2: Available technologies for overcoming solubility challenges

S. No.	Technology	Advantages	Disadvantages
01	Particle size reduction (milling, micronization, nano-milling)	• Little API required	• Poor control of Particle size distribution • Poor flow characteristics • Polymorphic Transformation
02	Complexation	• Enhanced API stability	• Highly modified Pharmacokinetics (PK) • Poor IVIVR • Drug Loading Capacity • Drug stability
03	Solid dispersion	• High API load possible • Amorphous state	• Risk of recrystallization • Drug Stability Issue • *In vivo* absorption
04	Salification	• Easy to obtain	• Ionisable API only • Highly modified Pharmacokinetics (PK)
05	Use of Solubilizers	• Easy to use	• Chemical stability issue • Wet Granulation
06	Use of Lipid (SMEDDS, SNEDDS)	• Efficient	• Stability issue • Poor IVIVR • Polymorphic Transformation during processing

API: Active pharmaceutical ingredient

Table 3: Excipients preferred in different solubilization techniques

S. No.	Technology	Recommended Excipients/polymers/Carrier
01	Amorphous Solid Dispersion (ASD)	Povidone, Copovidone, Hypromellose (HPMC), Hydroxypropyl cellulose (HPC),Polyethylene Glycol (PEG), polymethacrylates (Eudragit), polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer (PCL-PVAc-PEG) (Soluplus),Hypromellose Acetate Succinate, Hypromellose Phthalate, Sugars/Polyols
02	Use of Lipid (SMEDDS, SNEDDS)	Medium Chain mono and Tri glycerides, Soybean oil, Fractionated coconut oil, Corn oil, Olive Oil, Sesame Oil, Hydrogenated Castor oil, Hydrogenated Vegetable oil, Beeswax, Phosphatidylcholine, mono- and di-glycerides, soy fatty acids, ascorbyl palmitate Mono-/di-glycerides of caprylic/capric acids, Capryl caproyl Polyoxyglycerides
03	Complexation	Cyclodextrin (Hydroxy Propyl β-Cyclodextrin, Methyl β-Cyclodextrin), Carboxylic acid (eg., Citric acid, Tartaric acid), Water soluble polymers (i.e., povidone, soluplus), Amino acids (e.g., Arginine, tryptophan, leucine, phenylalanine, methionine, and isoleucine, Sugar alcohols (e.g., sorbitol, mannitol),Caffeine, etc.
04	Co-Crystal	Coformers : Nicotinamide, Isonicotinamide, Formaldehyde, Glutaric acid, Fumaric acid, Succinic Acid, p-Coumaric acid, Picolinamide, p- Amino Benzoic Acid (PABA), 4-Hydroxy benzoic acid, Citric acid, Orotic acid, Adipic acid, Saccharin, Urea, Acetamide, etc.
05	Use of Solubilizers	Water Soluble: Vitamin E TPGS, Polysorbate 20/40/80, Polyoxyl 35 castor oil (Cremephor EL), Polyoxyl 40 hydrogenated castor oil (Cremephor RH) Polyglycolized glyceride (Gelucire 44/14,50/13), Poloxamer (188,407), SDS Water In-soluble : Linoleic Macro glycerides, semi-synthetic triglycerides of C8-C18 saturated fatty acids (Gelucire 33/01), Oleic acid, Glyceryl monostearate, Sorbitan monooleate, Lecithin

P-glycoprotein, an Efflux transporter, plays an important role in drug transport. It pumps drugs into the lumen, reducing their absorption. Many drugs are transported by P-glycoprotein and are also metabolized by cytochrome P450 3A4. There are examples of drugs and excipients which can induce or inhibit P-gp efflux and have a significant impact on bio-availability. List of excipients considered to be p-gp inhibitors is presented in Table 4. These excipients are explored in pharmaceutical formulations to enhance drug solubility, absorption, and bioavailability.

Polymers, surfactant, and lipid-based excipients which are known for P-gp inhibitory effects. The P-gp inhibitory activity of different pharmaceutical formulations, namely, emulsions, liposomes, solid lipid nanoparticles, micelles, polymeric nanoparticles, micro-spheres, dendrimers, and solid dispersion is shown in Fig. 2 [11,12].

Poloxamers are explored in the preparation of polymeric micelles, nanoparticles, and liposomes. They tend to modify membrane fluidization through P-gp inhibition achieved by depleting ATP and inhibiting ATPase [20]. Similarly, methylated cyclodextrin can interact with lipid components altering membrane fluidity and permeability, leading to p-gp inhibition [21]. Soluplus is an amphiphilic graft copolymer with a low critical micelle concentration explored as a component of liposomes [22], micelles [23], and solid dispersions [24].

APPROACHES TO OVERCOME STABILITY CHALLENGES FOR POORLY SOLUBLE META-STABLE POLYMORPH

Formulation/excipient-induced stability challenges

Amorphization of a low soluble crystalline API through polymeric solid dispersion or coamorphous formulation (with excipients) poses frequent stability challenges. Although amorphization helps in enhancing the solubility and bioavailability to a great extent, its crystallization driving force (CDF) leads to a polymorphic transformation thereby favouring one thermodynamically stable crystalline form during storage and *in vivo* dissolution. Furthermore, the increased molecular mobility leads to kinetic instability. Hence to have a kinetically stabilized state, the molecular mobility must be reduced and the glass transition temperature (Tg) increased. Low molecular weight crystallization excipients are known to lower CDF

Table 4: Examples of excipients explored as p-gp inhibitors

S. No.	Category	Examples	References
01	Surfactant	Polyoxyethylene (80) sorbitan monooleate (Tween 80) Polyoxyethylene(20) sorbitan monolaureate (Tween 20) Sucrose fatty acid esters (e.g., sucrose mono/distearate, sucrose mono/dilaurate, sucrose palmitate), D-α-tocopheryl polyethylene glycol succinate (Vitamin E TPGS or TPGS) PEG-35 castor oi(CremophorEL) PEG-40 castor oi(Cremophor RH) PEG-15-hydoxystearate (Solutol HS-15) Polyoxylglycerides (Labrasol, Labrafil,Softigen 767) PEG stearate (Myrj) Polyglycolized glyceride (Gelucire 44/14,50/13) polyethylene glycol (20) cetyl ether Cetyltrimethylammonium bromide (CTAB)	[12-14]
02	Polymers	Natural polymers: Dextrans, agar, gellan gum, gum arabic, gum tragacanth, guar gum, carrageenan gum, xanthan gum, alginates, chitosan), PEG, Amphiphilic copolymers: MethoxyPEG-block-polycaprolactone, Polyvinyl caprolactam-polyvinyl acetate-PEG graft co-polymer (Soluplus), Pluronic block copolymers (poloxamer): Poloxamer 407 (PEO101-PPO56-PEO101) Poloxamer 188 (PEO75-85-PPO25-30-PEO75-85)	[15,16]
03	lipid-based excipients	Glycerides (e.g., monoolein (PeceolTM) and monostearin) Phospholipids (e.g., 1,2-dioctanoylsn-glycero-3-phosphocholine (8:0 PC) and 1,2-didecanoyl-sn-glycero-3-phosphocholine (10:0 PC)) Methylated beta-cyclodextrin	[17-19]

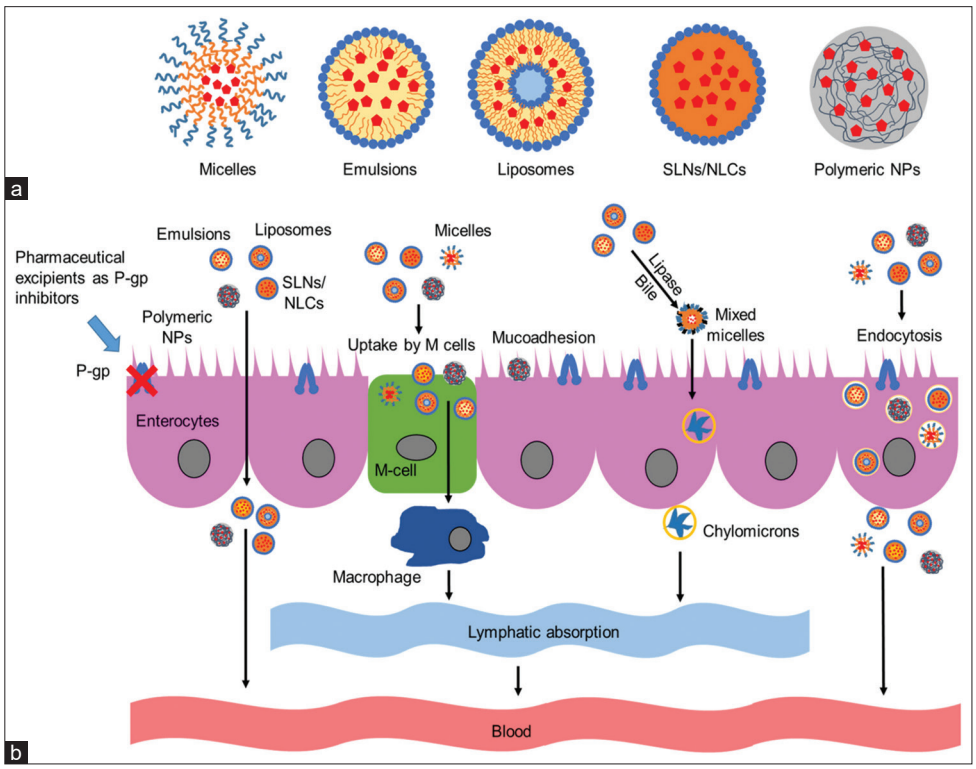

Fig. 2: P-gp efflux inhibition from different drug delivery system (containing P-gp efflux inhibitory excipients) (a) Example of drug delivery system incorporating P-gp inhibitory excipients. (b) Formulation components of various drug delivery systems contributing in P-gp efflux inhibition (reprinted with permission) [11]

and elevate glass transition temperature (Tg). Moisture also plays a critical role in the stability of ASD and can lower the glass transition RH (RH_g). The glass transition relative humidity is the transition point between surface adsorption and bulk adsorption and hence leads to molecular mobility and drug crystallization [25-27].

Stabilization of Carbamazepine/Polyvinylpyrrolidone ASD with low molecular weight crystalline excipients saccharin or Tryptophan [25], Solid dispersion of Fluconazole (metastable form II) with Soluplus® (SOL) [28], ASD of Ritonavir with PEG 8000 [29], and felodipine-polyvinyl acetate (PVAC) solid dispersion [30,31] are the examples to understand excipient induced polymorphic transformation.

Another successful approach to improve solubility is developing a stable nucleus and subsequent growth of a metastable drug polymorph. This could be achieved through the crystallization of metastable drug polymorphs with a different drug-polymer ratio. However, polymorphic stability challenges still remain as the metastable drug polymorph is susceptible to environmental condition, excipients explored and manufacturing process adopted during drug product development and manufacturing [28].

Although excipients are considered to be inert, they can have different water/vapor sorption-desorption properties. The water/vapor sorption-desorption properties of the excipients contribute to the

physicochemical, biopharmaceutical, and processing properties of the drug product, leading to phase transformation and instability Further the moisture sorption-desorption properties of crystalline and amorphous drug substance are quite different. The crystalline drugs can interact with water or moisture through adsorption and can lead to crystal hydrate formation, deliquescent, and capillary condensation whereas bulk properties of the amorphous material are altered significantly through absorption of water/moisture [32].

It has been observed that the formation of nitrofurantoin monohydrate from nitrofurantoin was more in a formulation containing amorphous excipients (i.e., silicified microcrystalline cellulose [SMCC) and α-lactose monohydrate [LMH]) compared to a formulation containing partially amorphous excipients (i.e., Pregelatinized starch and low-substituted hydroxypropylcellulose (L-HPC)] under high-humidity conditions [32-34].

In another example, it was observed that hydrate formation of theophylline was faster in a formulation containing SMCC compared to α-LMH indicating that SMCC has a better protective capability against pseudo polymorphic transformation during wet granulation [35].

Polymer type and polymer amount in the formulation also have a significant impact on the hydrate formation of many polymorphic drug substances. Example includes the transformation hydrate formation of anhydrous olanzapine Form I in the presence of polymer in humid environments It is observed that formulation containing polyethylene glycol (PEG) and hydroxy propyl cellulose (HPC) accelerates hydrate formation of Olanzapine Form I, whereas formulation containing both polyethylene glycol (PEG) and polyvinyl pyrrolidone (PVP) could able to protect hydrate transformation [36].

Stabilization of meta-stable polymorphs against inter-conversion has been an emerging research area and poses a major challenge to the field of pharmaceutical formulation research Although a number of techniques, namely, surface templating [37–39], solid dispersions [40,28], and confinement [41–43] for obtaining meta-stable forms have been reported so far stabilizing those meta-stable polymorphs are still challenging. Nanoconfinement [44] or additives [44-47] to stabilize the meta-stable forms are emerging. Cellulose nanocrystal (CNC) aerogels used as scaffolds are one such example for crystallization and stabilization of meta-stable α-form of indomethacin where interconversion to γ form in the presence of certain stimuli, that is, solvent heat, pH, and seed crystal are restricted [48].

Lactamization (intramolecular cyclization) of Gabapentin Anhydrous (Form II) to lactam (GABA-L) accompanying water loss are known. The impact of miling process, comilling with excipient, and humidity levels on lactamization have been reported. It is reported that the intramolecular cyclization in milled gabapentin samples was highest in the presence of the lowest humidity conditions in compared to the intramolecular cyclization at higher humidity (greater than 31% RH). Further comilling with hydroxy propyl cellulose (HPC) retarded the transformation due to competitive hydrogen bonding [49].

Relevant examples of formulation/excipients induced transformation are summarized in Table 1.

Process and environment-induced stability challenges

The process-induced transformation (PIT) can either be driven thermodynamically or kinetically [4]. The reconstructive mechanism and topotactic/epitactic mechanism are two important mechanisms mainly involved in polymorphic transformation. The reconstructive mechanism involves nucleation and crystal growth, which is facilitated due to the structural similarity between reactant and product, as structural similarity reduces the activation energy required for nucleation. On the other hand, topotactic/epitactic involves orientation reaction and transformation. Epitactic reaction requires only a 2D structure, similar at the crystal interphase whereas topotactic reactions require structural similarities not only at interphase but also in the bulk of both crystalline phases and results in single crystal-to-single crystal (SCSC) polymorphism. Examples of single crystal-to-single crystal (SCSC) polymorphism include the polymorphic transformation of Fingolimod hydrochloride (Form I to Form II at elevated temperature.[50,51]

The exposure of a mixture of polymorphic drug substances and different crystalline excipients to different processing conditions during manufacturing may lead to a phase transformation among polymorphs, solvates/hydrates, and the amorphous form. The transformation mechanism, factor involved, and related unit operations are enumerated in Table 5 [52].

Table 5: Underlying mechanism for phase transformation

Stages	Mechanism	Factors influencing kinetics of phase transition	Related pharmaceutical unit operations
Solid-state	• Polymorphic transformation • Sorption/de-sorption • Glass transition • Crystallization	• Environmental factors (Temperature, Relative Humidity) •% Crystallinity particle size distribution • Hygroscopicity • Impurities.	• Blending • Grinding/Milling/cryo milling • Tablet Compression and coating • Dry Granulation (RC Process) • High shear RMG granulation • FBP Granulation • Supercritical-fluid process
Melt	• Polymorphic transformation • Vitrification	• Nucleation rate, crystal growth, and cooling	• HME process • Melt granulation
Solution (solvent removal)	• Polymorphic transition • Sorption/de-sorption • Crystallization (Stable to the metastable phase and vice versa)	• Solvent evaporation rate, • Processing conditions on ease of nucleation and crystal growth	• Drying /Solvent removal in RMG/FBP • Process • Lyophilization • Spray drying
Solution-mediated (during manufacturing process)	• Polymorphic transformation • Solvation/desolvation • Amorphous crystallization • Only from the metastable phases to the stable phases (transition from a metastable phase to the stable phase, driven by the difference in solubility between the two phases)	• The initial dissolution rate of the metastable phase to reach the stable phase • Rate of Nucleation of stable phase • Rate of Crystal growth of the stable phase from metastable phase	• Wet granulation (aqueous/Non-aquous) with crystalline drug and binder/ polymer dispersed in (aqueous /Non-aquous) solvent • ASD • Microemulsion /Nano emulsion • Nanosuspension

ASD: Amorphous solid dispersion

Table 6: Process-induced stability challenges

S. No.	Molecule Name	Polymorphic form	Processing parameters	Polymorphic transformation	Reason of transformation	References
1	Ribavirin	Enantiotropic R-II stable form at room temperature (above Tg)	Milling for 15 min	Meta-stable form R-I	Milling Induced Transformation (with temperature effect)	[8,58]
			Cryomilling at −196°C, (below Tg) for 120 min	Amorphous Ribavirin	Milling Induced Transformation (without temperature effect)	
		Meta-stable form R-I	Kept at 70°C (above transition temperature) for 7 days	No Transformation	No temperature-induced transformation	
2	Piracetam	Form III (Stable form)	Wet Granulation with different quantities of granulating fluid	Meta-stable form II crystals (Hydrate formation)	Wet granulation-induced Hydrate formation	[8,59]
3	Chlorpromazine hydrochloride	Metastable form II	Aqueous wet granulation	De-hydration of Form I (heme-hydrate) to Form I (Stable) under room temperature	Wet granulation induced “meta-stable – hydrate – stable" transformation	[8,60]
4	Caffeine	Form II	Formulation with a high level of Soluplus and HME Process	Form II to Form I	HME Process and Execipient induced transformation.HME Process along with drug–soluplus interaction with high level of soluplus impacted the degree of transition	[8,61]
5	Artemisinin	Orthorhombic (meta-stable) polymorph	HME Process	high-temperature stable triclinic polymorph	Mechanical and thermal stresses induced transformation during HME Process	[8,62]
6	Theophylline	Mono-hydrate Form I	Tablet Compression at 50–196 MPa	Meta-stable Anhydrous form II	Thermal stress-induced transformation during Tablet compression process	[8,63]
7	Chlorpropamide	α form	High Pressure up to 4.2 GPa	γ phase	Pressure-induced Transformation	[64-636]
8	Fluconazole	Form I	High Pressure up to 0.8 GPa	Form VIII	Pressure-induced Transformation	[8,67]
			Higher pressure up to 3.2 GPa	Form IX	Pressure-induced Transformation	[8,67]

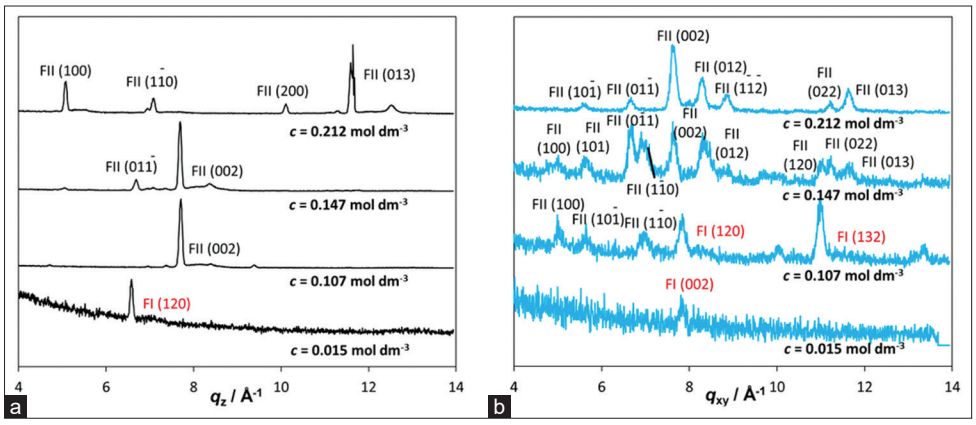

Fig. 3: Results of spin-coated sample of piracetam solution (a) s-X-ray diffraction and (b) GIWAXS reproduced with permission from Louer *et al.* [72] for form I and [53,73] for Form II

Various pharmaceutical unit operations leading to process-induced transformations include grinding (milling), granulation (Wet granulation, dry granulation, and hot melt granulation), tablet compression, lyophilization, spray drying, supercritical fluid, blending, compression, and film coating or functional coating [8].

Examples of process-induced polymorphic transformation include form conversion of caffine (From form II to I) with a high level of soluplus and HME process, formation of the metastable form of Ribavirin (R-I) after milling, crystal hydrate formation of piracetam through aqueous wet granulation, and conversion of α form of chlorpropamide to γ form in presence of high pressure [8].

Various examples of process-induced transformation are enumerated in Table 6.

EXPLORING ANALYTICAL TOOLBOX FOR EVALUATION OF POLYMORPHISM AND POLYMORPHIC TRANSFORMATIONS

Drug substances can exist in multiple polymorphic forms. There may be situations where crystalline form, amorphous form, solvates, and

Table 7: Summary of analytical techniques for physical and structural analysis of polymorphs and polymorphic transformation

Methods	Analytical tools	Advantage	Disadvantage
XRD	Single crystal XRD (SC XRD)	• The preferred technique for the determination of crystal structures • Enable characterization of enantiomers • It can rule out pseudopolymorphism. • Enable transformations of single crystal to single-crystal by altering temperature and pressure	• The preparation of a single crystal may be difficult
	Powder XRD (pXRD)	• Determining structure in the absence of single-crystal samples • Fingerprinting solid-solid transformations possible with variations in temperature and RH • Estimation of % crystallinity in amorphous/crystalline mixture samples	• Interference from crystalline excipients • Preferred Orientation
	Grazing Incidence X-ray and Neutron diffraction (GIXD)	• Can be used to determine the crystalline characteristics and in-plane order of thin films. • Local amplification of the electric field at the critical angle possible to by a factor of four to make the signal stronger • Scattering experiments are possible on thin films of very low scattering volume	• In-plane spatial resolution limited • Wave penetration is limited,hence can be explored to study surfaces and layers
Spectroscopy	Mid FT-IR	• No need of sample preparation, direct analysis possible. • Ability to show different state of water • Fingerprinting poly-crystalline samples possible using characteristic absorption spectrum for individual polymorphs • Mixture quantification possible	• Interference from moisture and excipients
	Raman	• Polymorphs fingerprinting from characteristic Raman spectrum • Reliable Polymorph differentiation • Raman microscopy and image analysis of tablet samples can be used to determine the distribution of a particular polymorph and to detect impurities. • Analysis of wet (aqueous) samples possible • Polymorphic transformation can be studied when exposed to high temperature or pressure	• Interference from excipients
Spectroscopy	Solid-state-NMR (SS NMR)	• Quantification of the amount of each form possible without the need for a standard • Fingerprint polymorphs from chemical shifts • Polymorphic form conversion can be monitored during API scale-up with the identification of different crystalline and/or amorphous forms • Capable of detecting low amounts of other forms (e.g. crystalline in amorphous), • Capable to predict physicochemical stability by measuring relaxation times. • No interference of excipient with analysis even at low level of drug substance in the drug product	• Relatively long data acquisition time; experimental artifacts • High running costs and time-consuming measurements
	Time domain -NMR (TD NMR)	• Rapid and easy measurement of relaxation times for both solid and liquid samples • Evaluation of molecular mobility of hydration water in drug substance. • Crystalline and amorphous forms of drug substances incorporated into solid dosage forms can be distinguished • Allows the evaluation miscibility of crystalline drug substances with polymers	• Analysis of precise molecular structures
	Terahertz pulsed spectroscopy (TPS)	• A potential ideal tool for studying long-range crystal lattice vibrations and low-energy torsions, and for studying crystallinity and polymorphism • Being a timed technique, it is immune to background thermal radiation (detection at room temperature is possible) The low energy used in TPS minimizes the risk of sample degradation • Minimized risk of sample degradation due to low energy exposure	• THz time-domain spectroscopy has better SNR at frequency below 3 THz only
Thermal analysis	DSC (differential scanning calorimetry)	• Requirement of a small sample size, meaningful information on phase transition, and excipient interference • Meaningful information on temperatures of de-solvation, crystallization, glass transition (Tg), and melting • Gives adequate information on Heat capacity(Cp), Heat of fusion/transition, ΔH	• Limited or no information on the nature of transition and interference of both crystalline and amorphous excipient

(*Contd...*)

Table 7: (*Continued*)

Methods	Analytical tools	Advantage	Disadvantage
	Thermal gravimetric analysis	• Stoichiometric information on solvates/hydrates • Quantify weight loss associated with de-solvation processes	• Limited to solvates hydrates, and interference from water-containing excipients
	ITC (Isothermal calorimetry)	• Can provide important information about the thermodynamic stability relationship between polymorph and kinetic information on the interconversion • Normally make iso thermal measurements as a function of time	• Cannot be used to screen for polymorphs
Thermal analysis	Melting point	• Relatively quick, easy, and inexpensive preliminary analysis • Polymorphs finger-printing from the melting point • Requirement of a small sample for analysis	• Applicable only for solid sample • Analysis is destructive • Quantitative analysis of a mixture of samples for purity not possible.
	Hot-stage microscopy	• Meaningful information on phase transition • Visualization of melting point and Structural transformations possible	• Interference from excipient
Other	Polarized Microscopy	• Qualitative information on • Crystallinity and crystal morphology • Meaningful information on phase transition	• Interference from excipient
	DVS (dynamic vapor sorption)	• Highly sensitive. Capable of detecting of low level of the amorphous phase, defining the liability of hydrates • Moisture/Solvent uptake at constant Temperature as a function of %R.H. • Visualization of polymorphic transformation possible • Identifying hydrate formation and dehydration possible	• Interference from amorphous • Excipient, large hysteresis loop possible
	Solubility / Dissolution	• Impact of Polymorphic transformation on solubility/dissolution can be studied • Equilibrium saturation solubility of polymorphs possible • Influence of crystal structure on solubility and dissolution rate can be studied	• Can not be explored for qualitative/quantitative determination of polymorphs
	SEM (scanning electron microscopy)	• Information on surface morphology of polymorphs • Observation of Crystal forms differing in size and shape possible • Influence of processing on particles: agglomeration/ breakage can be studied	• Interference from excipient

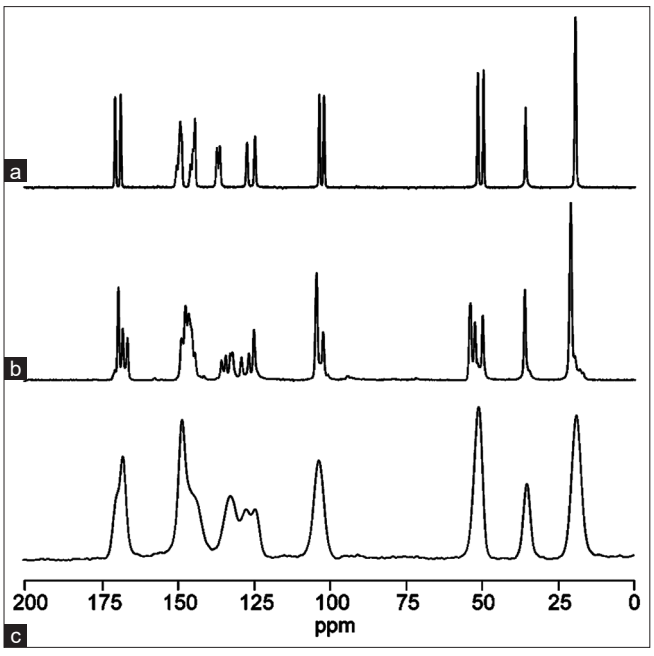

Fig. 4: Comparative ^{13}C SSNMR spectra of the different polymorphic form of nifedipine, (a) α-crystalline form (b) β-crystalline form, and (c) amorphous form, reproduced with permission from Yuan *et al.* [77]

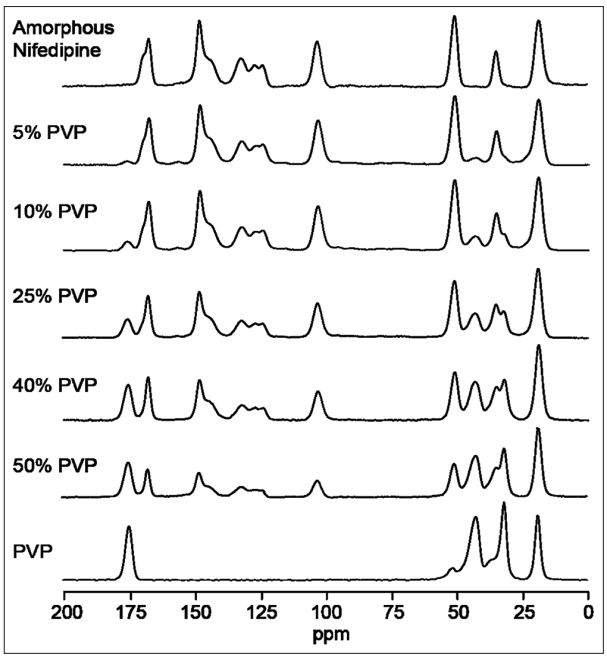

Fig. 5: Comparative ^{13}C SSNMR spectra of NIF-PVP amorphous solid dispersions prepared with 0, 5, 10, 25, 40, 50, and 100% PVP reproduced with permission from Yuan *et al.* [77]

hydrates coexist in a particular drug sample or drug product. Hence, the determination of polymorphic form becomes technologically challenging in such cases. In drug product development, drug substance along with multifunctional excipients (having different properties)

included in the formulation forming a complex mixture makes it even more challenging for solid state analysis [68].

There are various emerging amorphization techniques where the amorphous phase is kinetically trapped with varying kinetic stability. However, in such conditions, there is every possibility of crystalline transformation of the amorphous phase depending on kinetic stability. This kind of transformation poses unique analytical challenges in detecting the low levels of crystalline material in the presence of its amorphous form. In many instances, the polymorphic transformation to other forms is relatively quick, where the speed of analysis becomes very important to address the intermediates. Hence, Detection limit, excipient interference, and speed of analysis are the major challenges in selecting a right analytical tool for the evaluation of polymorphism and monitoring of polymorphic transformation. Factors such as selectivity, discriminating ability, sensitivity (LOD), and influence of sample preparation need to be considered while developing a robust analytical method [69].

Various techniques have been used to characterize solid-state pharmaceutical forms. Each method has its own merits and demerits. Common analytical tools with their advantages and disadvantages are listed in Table 7.

Powder X-ray diffraction (PXRD) is considered the "gold standard" for polymorphic phase determination. This technique has the advantage of being non-destructive for the analysis of samples of solid samples as well as samples of prepared tablets. However, due to preferential orientation effects and potential difficulties in separating single-component diffraction peaks, quantitative analysis is often degraded [52,70].

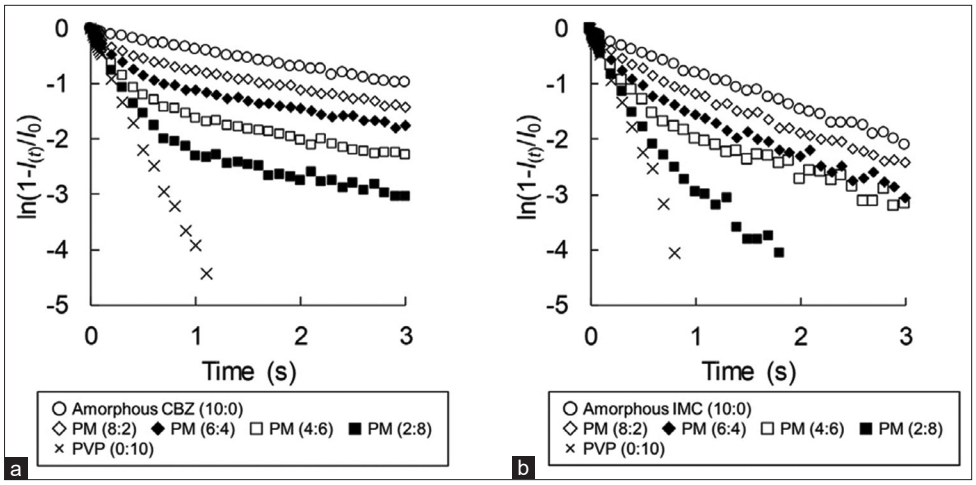

Fig. 6: Relaxation behavior of solid dispersions respect to time (a) Amorphous carbamazepine (CBZ) and (b) Amorphous indomethacin (IMC) Reproduced with permission from Okada *et al.* [78]

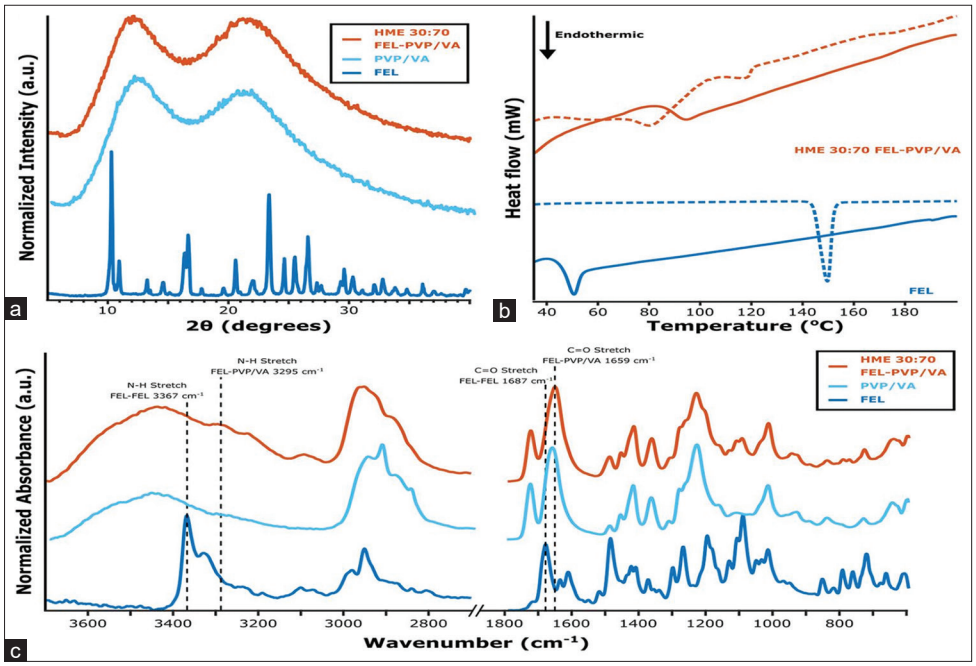

Fig. 7: Comparative characterization of amorphous solid dispersion of Nifedipine with hot melt extrusion process (a) powder X-ray diffraction results with no signs of crystallinity (b) differential scanning calorimetry thermogram for the first cycle (dotted line) and the second cycle (solid line); and (c) Fourier transform infrared results for the N–H bond region and C·O bond region. Reproduced with permission from S'ari *et al.* [79]

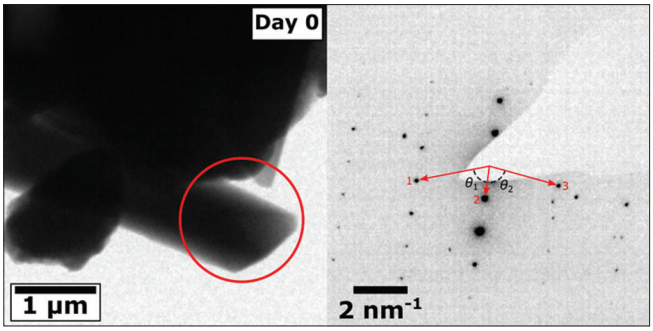

Fig. 8: Transmission electron microscopy results for amorphous solid dispersion of Nifedipine with visible large Nifedipine crystal on day 0 of analysis showing may be either form I or II. Reproduced with permission from S'ari *et al.* [79]

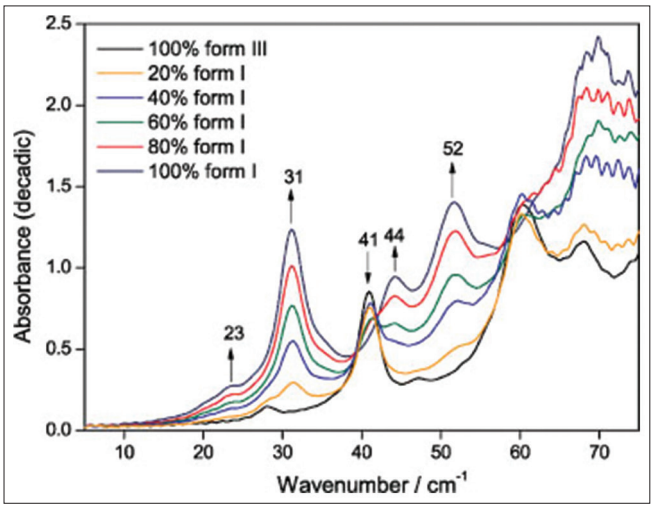

Fig. 9: Terahertz pulsed spectra of binary mixtures of forms I and III (20% intervals), reproduced with permission from Strachan *et al.* [80]

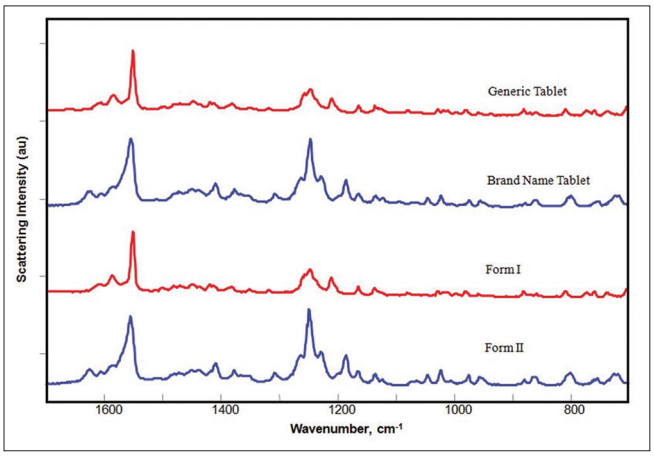

Fig. 10: Raman spectra reproduced with permission from Donahue *et al.* [81]

Single crystal X-ray diffraction (SC-XRD) and X-ray diffraction (XRD) are both suitable for crystal structure determination. SC-XRD allows for final polymorphic phase recognition. Furthermore, it allows the determination of absolute structures, making it the tool of choice for the characterization of enantiomers. However, difficulties in preparing a crystal sometimes limit its wide application [41].

Grazing incidence in-plane XRD (GIXD) is a very useful technique explored to overcome the orientation effects of p-XRD and helps to

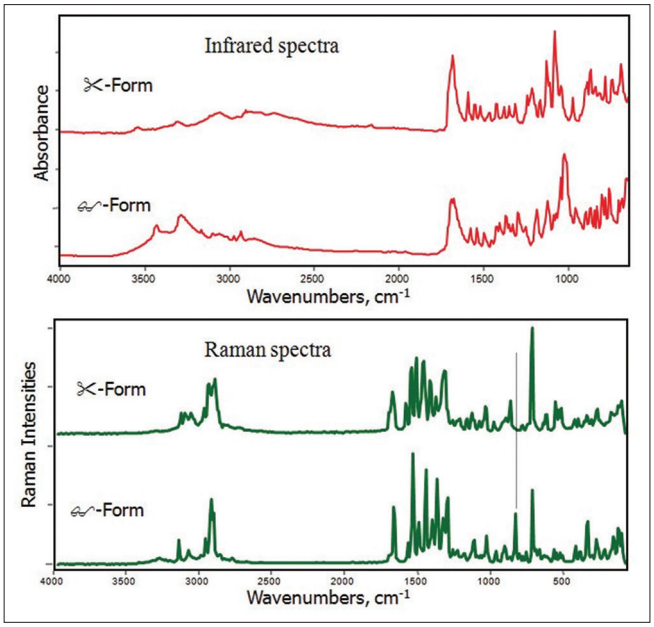

Fig. 11: Infrared and Raman spectra of two polymorphs of an active pharmaceutical ingredient, reproduced with permission from Donahue *et al.* [81]

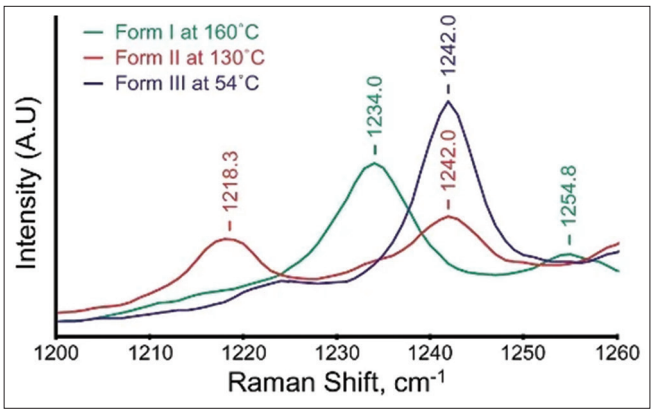

Fig. 12: Raman spectra of three polymorphs of paracetamol reproduced with permission from Kaiser Optical Systems, Inc, Raman microscopy

determine the in-plane order and crystalline properties of thin films. However, its scope is limited to in-plane spatial resolution (beam footprint) [11]. The evaluation of the polymorphic transformation of piracetam form I in thin films using grazing-incidence XRD (GIXD) (Fig. 3) reported by Simões *et al.* [53,71].

Although XRPD has been the most common analytical technique for polymorph screening identification and quantification. In many cases, Raman spectroscopy is explored as the primary screening method However, for screening of polymorph and cocrystal combinatorial screening with both XRD and Raman spectroscopy is more useful. Frampton *et al.* developed a combinatorial screening system with XRD and Raman spectroscopy in collaboration with Bruker AXS to screen for polymorphisms and cocrystals. The above composite screening technology is protected by US Patent US 2006/0023837 A1, Feb. 2, 2006 [74].

Thermal methods such as thermal analysis and calorimetry are explored to understand the thermodynamic and kinetic stability of polymorph and polymorphic transformation. Thermal analysis such as differential scanning calorimetry (DSC) and thermogravimetry (TG) performs

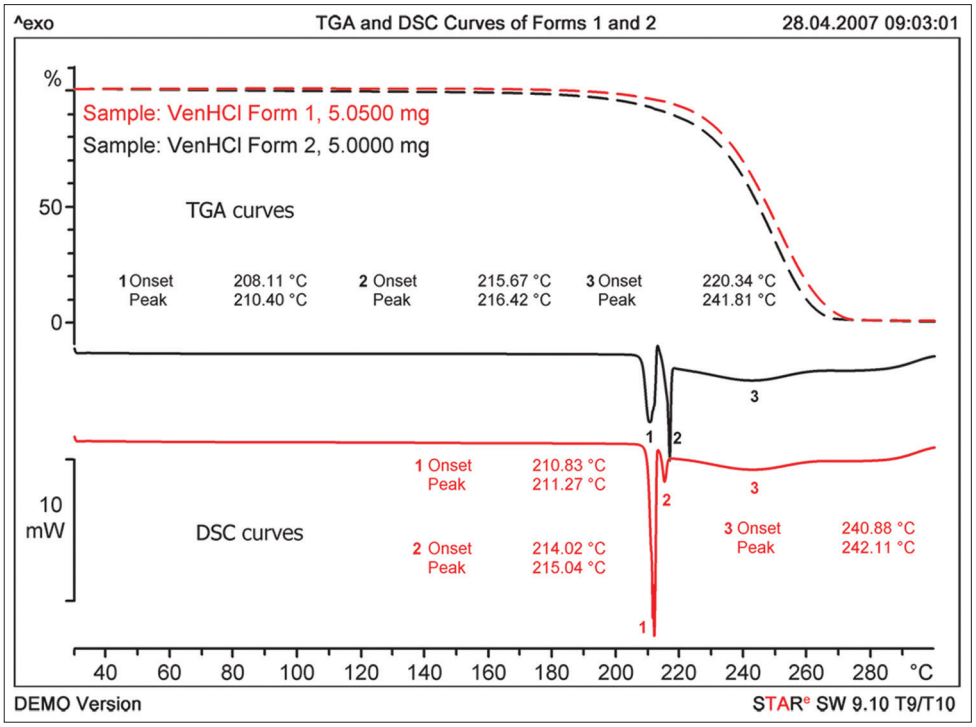

Fig. 13: Thermal gravimetric analysis (above) and differential scanning calorimetry (below) of Venlafaxine hydrochloride Form 1 and Form 2 reproduced with permission from Mettler Toledo

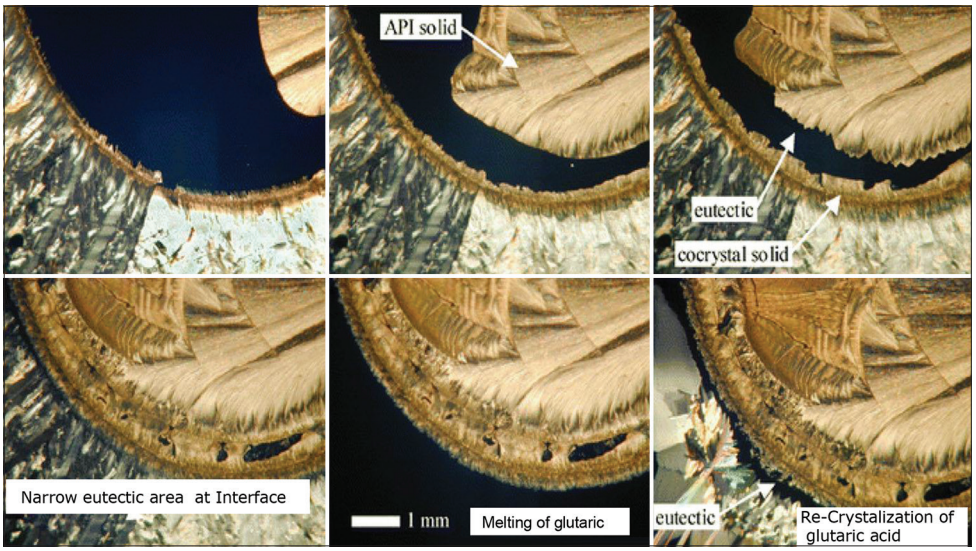

Fig. 14: HSM micrographs for glutaric acid cocrystal reproduced with permission from McNamara *et al.* 2006 and permission from the publisher [82]

measurement as a function of temperature, while calorimetry performs isothermal measurement as a function of temperature time [75].

DSC have many advantage due to its small sample size requirement and capability to provide valuable information on phase transition and excipient interaction but have potential drawbacks in many aspects, especially in quantitative estimation of polymorphic content in drug products containing excipients and small amount of impurities. The presence of a small number of impurities may interfere with the final result leading to an unreliable quantification of the polymorphic content. The impurity produces unpredictable changes to the phase transformation process or thermal events and may interfere or overlap with the phase transformation peak. Overcoming such concurrent re-crystallization with the use of high-speed DSC (Hyper-DSC) has been reported by Mc Gregor *et al.*, 2004 [70].

Various spectroscopic methods, namely, Fourier transform infrared (FTIR) absorption spectroscopy, Fourier transform Raman (FT Raman) spectroscopy, and solid-state nuclear magnetic resonance (NMR) spectroscopy, are also available and can be employed as complementary tools to pXRD and DSC [75].

Solid state NMR (SSNMR) spectroscopy has relatively low limits of detection and quantitation compared to other analytical techniques. It can quantify the amount of each polymorphic form without any need for a standard. Furthermore, quantification of both crystalline and

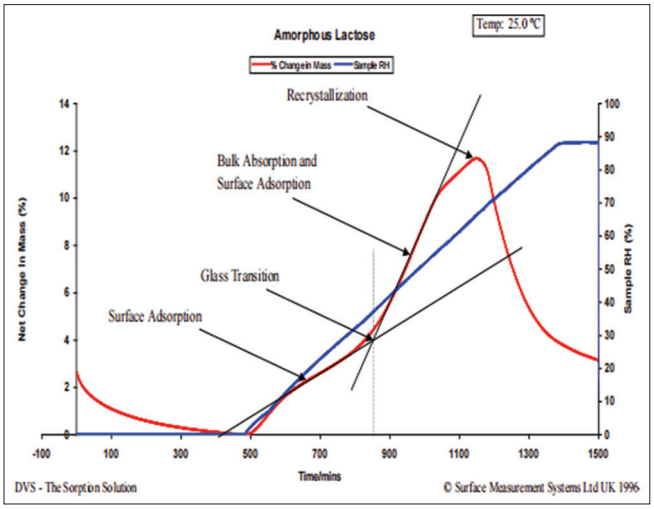

Fig. 15: A typical net percent change in mass versus time plot for an amorphous lactose, showing glass transition and recrystallization events analyzed in dynamic vapor sorption (reproduced with permission from surface management system. Red line – % change in mass, blue line – sample relative humidity

amorphous content in a drug substance as well as in a drug product is possible with this technique. Relatively long data acquisition time and high running cost are some of the limitations of solid-state NMR (SSNMR) [76].

SSNMR spectra of crystalline and amorphous nifedipine are presented in Figs. 4 and 5, respectively [77].

Time-domain nuclear magnetic resonance (TD-NMR) methods are helpful in measuring 1 H NMR relaxation, whereas SS NMR is mainly explored to study precise molecular structures and also quantification. Crystalline and amorphous forms can be differentiated in the physical mixture based on relaxation behavior with respect to time as depicted in Fig. 6. [78].

ASDs are increasingly being explored to improve the solubility and bioavailability of oral drugs. The amorphous phase is kinetically stabilized with a suitable polymer. However, as a result of incomplete pharmaceutical processing or during storage, the amorphous phase may contain a low level of crystalline drug substance which can have a negative impact on the stability and dissolution properties of the drug product. Analytical tools such as powder XRD (pXRD), DSC, and FTIR spectroscopy can be explored to characterize and measure the % crystallinity within the ASD. However, these tools may not be able to detect low level of crystalline drugs due to their low limit of detection (LOD) in the range of 1–5%. Transmission electron microscopy (TEM) can be explored successfully to identify sign of crystallization with a better detection limit [79].

The comparative characterization of an ASD of felodipine (FEL) and polyvinylpyrrolidone/vinyl acetate copolymer (PVP/VA) prepared with a HME has been reported. Comparative pXRD, DSC, FTIR, and TEM results are depicted in Figs. 7 and 8, respectively [79].

Vibrational spectroscopic techniques are also getting explored increasingly as complementary techniques to study polymorphism. A novel method called terahertz pulsed spectroscopy (TPS) can provide greater details on hydrogen bonding and crystalline lattice vibration [79,80].

Evaluation of binary mixtures of carbamazepine (CBZ) forms I and III (0 to 100% of form I with 20% intervals) with terahertz pulsed spectroscopy (TPS) are depicted in Fig. 9 [81].

Typical outputs from different analytical instruments to evaluate Polymorphism and polymorphic transformation Figs. 10-15.

CONCLUSION

Solubility, permeability, and polymorphic stability have a significant impact on the physicochemical and mechanical properties of a drug substance and drug formulation which in turn affect *in vivo* performance of the dosage form.

Screening of polymorph with desired physicochemical and mechanical properties is very much essential for the performance of dosage form with respect to clinical safety and efficacy. For the selected polymorph, screening of suitable excipients becomes very much essential. Right excipients can either stop or delay the polymorphic transformation of the selected drug polymorph in the dosage form. Apart from the selection of suitable drug polymorph and excipient selecting one suitable and viable process technology, considering thermodynamic and kinetic aspect of polymorphic transformation into account can help in manufacturing a stable, safe, and clinically efficacious drug product with optimum drug potential. Further, judicious use of analytical toolbox during polymorph screening, monitoring of excipient, and process-induced polymorphic transformation have great importance and enable the drug formulator to meet the objective of achieving optimized drug's potential with respect to *in vivo* performance.

ACKNOWLEDGMENTS

I would like to acknowledge GITAM School of Pharmacy, GITAM Deemed University, for giving me this opportunity to perform the review work.

AUTHORS' CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The author declares no conflict of interest.

REFERENCES

1. Siew A. Solving poor solubility to unlock a drug's potential. Pharm Technol 2015;39:20-7.
2. Censi R, Di Martino P. Polymorph impact on the bio-availability and stability of poorly soluble drugs. Molecules 2015;20:18759-76.
3. U.S. Food and Drug Administration. Guidance for Industry-ANDAs: Pharmaceutical Solid Polymorphism; 2007. Available from: fda.gov/media/71375/download [Last accessed on 2022 Jul 07].
4. World Health Organization. Polymorphism Draft Chapter for the International Pharmacopoeia. Geneva: World Health Organization; 2018.
5. U.S. Food and Drug Administration. Guidance for Industry-Regulatory Classification of Pharmaceutical Co-Crystals; 2018. Available from: fda.www.fda.gov/files/drugs/published/Regulatory-Classification-of-Pharmaceutical-Co-Crystals.pdf [Last accessed on 2022 Jul 07].
6. U.S. Food and Drug Administration. H.H.S. International Conference on Harmonisation. Guidance on Q6A specifications: Test procedures and acceptance criteria for new drug substances and new drug products: Chemical substances; 2000. Available from: www.fda.gov/regulatory-information/search-fda-guidance-documents/q6a-specifications-test-procedures-and-acceptance-criteria-new-drug-substances-and-new-drug-products [Last accessed on 2022 Jul 07].
7. Kwokal A. Preparation, stabilisation and advantages of metastable polymorphs, NATO science for peace and security series A: chemistry and biology. Roberts KJ, Docherty R, Tamura R, editors. Engineering Crystallography: From Molecule to Crystal to Functional Form. Berlin: Springer; 2017. p. 247-59.
8. Alsirawan M, Paradkar A. Impact of the polymorphic form of drugs/NCEs on preformulation and formulation development. In: Innovative Dosage Forms: Design and Development at Early Stage. 1st ed. United States: John Wiley and Sons; 2020. p. 1-47.
9. U.S. Food and Drug Administration. Guidance for Industry-Biopharmaceutics Classification System Based Biowaivers; 2021. Available from: www.fda.gov/media/148472/download [Last accessed on 2022 Jul 07].

10. European Medicines Agency. Guideline on the Investigation of Bioequivalence; 2010. Available from: www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-bioequivalence-rev1_en.pdf [Last accessed on 2022 Jul 07].
11. Nguyen TT, Duong VA, Maeng HJ. Pharmaceutical formulations with P-glycoprotein inhibitory effect as promising approaches for enhancing oral drug absorption and bio-availability. Pharmaceutics 2021;13:1103.
12. Constantinides PP, Wasan KM. Lipid formulation strategies for enhancing intestinal transport and absorption of P-glycoprotein (P-gp) substrate drugs: *In vitro*/*in vivo* case studies. J Pharm Sci 2007;96:235-48.
13. Kou L, Sun R, Bhutia YD, Yao Q, Chen R. Emerging advances in P-glycoprotein inhibitory nanomaterials for drug delivery. Expert Opin Drug Deliv 2018;15:869-79.
14. Guan Y, Wang LY, Wang B, Ding MH, Bao YL, Tan SW. Recent advances of D-α-tocopherol polyethylene glycol 1000 succinate based stimuli-responsive nano-medicine for cancer treatment. Curr Med Sci 2020;40:218-31.
15. Ahmad J, Amin S, Rahman M, Rub RA, Singhal M, Ahmad MZ, *et al.* Solid matrix based lipidic nanoparticles in oral cancer chemotherapy: Applications and pharmacokinetics. Curr Drug Metab 2015;16:633-44.
16. Werle M. Natural and synthetic polymers as inhibitors of drug efflux pumps. Pharm Res 2008;25:500-11.
35. Airaksinen S, Luukkonen P, Jørgensen A, Karjalainen M, Rantanen J, Yliruusi J. Effects of excipients on hydrate formation in wet masses containing theophylline. J Pharm Sci 2003;92:516-28.
36. Paisana MC, Wahl MA, Pinto JF. Effect of polymers in moisture sorption and physical stability of polymorphic olanzapine. Eur J Pharm Sci 2017;97:257-68.
37. Srirambhatla VK, Guo R, Price SL, Florence AJ. Isomorphous template induced crystallisation: A robust method for the targeted crystallisation of computationally predicted metastable polymorphs. Chem Commun (Camb) 2016;52:7384-6.
38. Telford R, Seaton CC, Clout A, Buanz A, Gaisford S, Williams GR, *et al.* Stabilisation of metastable polymorphs: the case of paracetamol form III. Chem Commun (Camb) 2016;52:12028-31.
39. Beckmann W, Nickisch K, Budde U. Development of a seeding technique for the crystallization of the metastable a modifification of abecarnil. Org Process Res Dev 1998;2:298-304.
40. Van Duong T, Lüdeker D, Van Bockstal PJ, De Beer T, Van Humbeeck J, Van den Mooter G. Polymorphism of indomethacin in semi-crystalline dispersions: Formation, transformation, and segregation. Mol Pharm 2018;15:1037-51.
41. Chyall LJ, Tower JM, Coates DA, Houston TL, Childs SL. Polymorph generation in capillary spaces: The preparation and structural analysis of a metastable polymorph of nabumetone. Cryst Growth Des 2002;2:505-10.
42. Al-Ani AJ, Herdes C, Wilson CC, Castro-Dominguez B. Engineering a new access route to metastable polymorphs with electrical confifinement. Cryst Growth Des 2020;20:1451-7.
43. Joseph S, Rappolt M, Schoenitz M, Huzhalska V, Augustin W, Scholl S, *et al.* Stability of the metastable α-polymorph in solid triglyceride drug-carrier nanoparticles. Langmuir 2015;31:6663-74.
44. Nartowski KP, Tedder J, Braun DE, Fábián L, Khimyak YZ. Building solids inside nano-space: From confined amorphous through confined solvate to confined 'metastable' polymorph. Phys Chem Chem Phys 2015;17:24761-73.
45. Gu CH, Chatterjee K, Young V, Grant DJ. Stabilization of a metastable polymorph of sulfamerazine by structurally related additives. J Cryst Growth 2002;235:471-81.
46. Ehmann HM, Werzer O. Surface mediated structures: Stabilization of metastable polymorphs on the example of paracetamol. Cryst Growth Des 2014;14:3680-4.
47. Davey RJ, Blagden N, Potts GD, Docherty R. Polymorphism in molecular crystals: Stabilization of a metastable form by conformational mimicry. J Am Chem Soc 1997;119:1767-72.
48. Banerjee M, Brettmann B. Stabilization of metastable indomethacin α in cellulose nanocrystal aerogel scaffolds. Pharmaceutics 2021;13:441.
49. Zong Z, Desai SD, Kaushal AM, Barich DH, Huang HS, Munson EJ, *et al.* The stabilizing effect of moisture on the solid-state degradation of gabapentin. AAPS PharmSciTech 2011;12:924-31.
50. Krishnan BP, Sureshan KM. A spontaneous single-crystal-to-single-crystal polymorphic transition involving major packing changes. J Am Chem Soc 2015;137:1692-6. doi: 10.1021/ja512697g
51. Wang JR, Li S, Zhu B, Mei X. Insight into the conformational polymorph transformation of a block-buster multiple sclerosis drug fingolimod hydrochloride (FTY 720). J Pharm Biomed Anal 2015;109:45-51. doi: 10.1016/j.jpba.2015.02.018
52. Zhang GG, Law D, Schmitt EA, Qiu Y. Phase transformation considerations during process development and manufacture of solid oral dosage forms. Adv Drug Deliv Rev 2004;56:371-90. doi: 10.1016/j.addr.2003.10.009, PMID: 14962587
53. Simões RG, Salzmann I, Resel R, Röthel C, Geerts YH. Stabilization of the metastable Form I of piracetam by crystallization on silicon oxide surfaces. Cryst Growth Des 2018;18:4123-9.
54. Dharani S, Barakh Ali SF, Afrooz H, Khan MA, Rahman Z. Studying effect of glyceryl palmitostearate amount, manufacturing method and stability on polymorphic transformation and dissolution of rifaximin tablets. Int J Pharm 2020;589:119785.
55. Sládková V, Dammer O, Kratochvíl B. Solid forms of tenofovir disoproxil fumarate, their mutual conversion, and stabilization of Form I in formulation. J Pharm Sci 2016;105:3136-42.
56. Tinmanee R, Stamatis SD, Ueyama E, Morris KR, Kirsch LE. Polymorphic and covalent transformations of gabapentin in binary excipient mixtures after milling-induced stress. Pharm Res 2018;35:39.
57. Shimpi S, Mahadik K, Takada K, Paradkar A. Application of polyglycolized glycerides in protection of amorphous form of etoricoxib during compression. Chem Pharm Bull (Tokyo) 2007;55:1448-51.
58. Vasa DM, Wildfong PL. Solid-state transformations of ribavirin as a result of high-shear mechanical processing. Int J Pharm 2017;524:339-50. doi: 10.1016/j.ijpharm.2017.04.002
59. Potter CB, Kollamaram G, Zeglinski J, Whitaker DA, Croker DM, Walker GM. Investigation of polymorphic transitions of piracetam induced during wet granulation. Eur J Pharm Biopharm 2017;119:36-46. doi: 10.1016/j.ejpb.2017.05.012
60. Otsuka M, Matsumoto T, Kaneniwa N. Effects of the mechanical energy of multi-tableting compression on the polymorphic transformations of chlorpropamide. J Pharm Pharmacol 1989;41:665-9. doi: 10.1111/j.2042-7158.1989.tb06337.x
61. Gorkovenko EA, Kichanov SE, Kozlenko DP, Belushkin AV, Wąsicki J, Nawrocik W, *et al.* The pressure-induced polymorphic transformations in fluconazole. J Pharm Sci 2015;104:4164-9. doi: 10.1002/jps.24644
62. Neuschitzer M, Moser A, Neuhold A, Kraxner J, Stadlober B, OehzeltM, *et al.* Grazing incidence in-plane X-ray diffraction on ultra-thin organic films using standard laboratory equipment. J Appl Crystallogr 2012;45:367-70.
63. Zhang GG, Law D, Schmitt EA, Qiu Y. Phase transformation considerations during process development and manufacture of solid oral dosage forms. Adv Drug Deliv Rev 2004;56:371-90.
64. Li Y, Chow PS, Tan RB. Quantification of polymorphic impurity in an enantiotropic polymorph system using differential scanning calorimetry, X-ray powder diffraction and Raman spectroscopy. Int J Pharm 2011;415:110-8. doi: 10.1016/j.ijpharm.2011.05.058, PMID: 21645601
65. S'ari M, Blade H, Cosgrove S, Drummond-Brydson R, Hondow N, Hughes LP, *et al.* Characterization of amorphous solid dispersions and identification of low levels of crystallinity by transmission electron microscopy. Mol Pharm 2021;18:1905-19. doi: 10.1021/acs.molpharmaceut.0c00918, PMID: 33797925
66. Bugay DE. Characterization of the solid-state: Spectroscopic techniques. Adv Drug Deliv Rev 2001;48:43-65. doi: 10.1016/s0169-409x(01)00101-6, PMID: 11325476
67. Urakami K. Characterization of pharmaceutical polymorphs by isothermal calorimetry. Curr Pharm Biotechnol 2005;6:193-203. doi: 10.2174/1389201054022904, PMID: 15974974
68. Offerdahl TJ, Munson EJ. Solid-State NMR Spectroscopy. United States: McGraw-Hill Education; 2004.
69. Yuan X, Sperger D, Munson EJ. Investigating miscibility and molecular mobility of nifedipine-PVP amorphous solid dispersions using solid-state NMR spectroscopy. Mol Pharm 2014;11:329-37. doi: 10.1021/mp400498n
70. Yi Li, Chow PS, Tan RB. Quantification of polymorphic impurity in an enantiotropic polymorph system using differential scanning calorimetry, X-ray powder diffraction and Raman spectroscopy. Int J Pharm 2011;415:110-8. doi: 10.1016/j.ijpharm.2011.05.058
71. Neuschitzer M, Moser A, Neuhold A, Kraxner J, Stadlober B, OehzeltM, *et al.* Grazing incidence in-plane X-ray diffraction on ultra-thin organic films using standard laboratory equipment. J Appl Crystallogr 2012;45:367-70.
72. Louer D, Louer M, Dzyabchenko V, Agafonov V, Ceolin R. Structure of a metastable phase of piracetami from X-ray powder diffraction using the atom-atompotential method. Acta Crystallogr Sect B Struct Sci 1995;51:182-7.
73. Kuhnert-Brandstatter M, Burger A, Vollenkee R. Stability behaviour of piracetam polymorphs. Sci Pharm 1994;62:307-16.

74. Van Arnum P. Advancing approaches in detecting polymorphism. Pharm Technol 2007;2007:4.
75. Urakami K. Characterization of pharmaceutical polymorphs by isothermal calorimetry. Curr Pharm Biotechnol 2005;6:193-203. doi: 10.2174/1389201054022904, PMID: 15974974
76. Offerdahl TJ, Munson EJ. Solid-State NMR Spectroscopy. United States: McGraw-Hill Education; 2004.
77. Yuan X, Sperger D, Munson EJ. Investigating miscibility and molecular mobility of nifedipine-PVP amorphous solid dispersions using solid-state NMR spectroscopy. Mol Pharm 2014;11:329-37. doi: 10.1021/mp400498n
78. Okada K, Hirai D, Kumada S, Kosugi A, Hayashi Y, Onuki Y. ^{1}H NMR relaxation study to evaluate the crystalline state of active pharmaceutical ingredients containing solid dosage forms using time domain NMR. J Pharm Sci 2019;108:451-6.
79. Bugay DE. Characterization of the solid-state: Spectroscopic techniques. Adv Drug Deliv Rev 2001;48:43-65. doi: 10.1016/s0169-409x(01)00101-6, PMID: 11325476
80. Strachan CJ, Taday PF, Newnham DA, Gordon KC, Zeitler JA, Pepper M, *et al.* Using terahertz pulsed spectroscopy to quantify pharmaceutical polymorphism and crystallinity. J Pharm Sci 2005;94:837-46. doi: 10.1002/jps.20281. PMID: 15736195
81. Donahue MM, Botonjic-Sehic E, Wells D, Brown CW. Understanding infrared and Raman spectra of pharmaceutical polymorphs. Am Pharm Rev 2011;14:1-12.
82. McNamara DP, Childs SL, Giordano J, Iarriccio A, Cassidy J, Shet MS, *et al.* Use of a glutaric acid cocrystal to improve oral bio-availability of a low solubility API. Pharm Res 2006;23:1888-97. doi: 10.1007/s11095-006-9032-3