



Surface characterization of pharmaceutical solids

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ABSTRACT

Surface properties alter solid solid and solid liquid interfacial interactions that profoundly impact manufacturability, processability, dissolution kinetics and stability of pharmaceutical solids. Surface properties may alter due to differential chemistry at the surface of the solid. Surface heterogeneity is driven by exposure of different crystalline facets and molecular arrangement, crystal habit, disordered lattice structure, surface functionalization and surface energy.

A pre requisite in surface characterization is to access topmost layer of a solid with extreme sensitivity and resolution. The surface characterization can be performed by a variety of qualitative and quantitative analytical tools operating on principles of vapor surface interaction, liquid surface interaction, micro scopic imaging using electron beam or mechanical probe, spectroscopy, mass spectrometry and calorimetry.

The current review introduces each technique from fundamental principle, instrumentation, methodologies and sample preparation to specific applications in relation to surface characterization. A compilation of surface properties has also been presented to enable selection of an appropriate analytical tool for their characterization.

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1. Introduction

Most of the active pharmaceutical ingredients (APIs) and excipients exist as solids. The mono and multi component solid forms have been extensively studied due to their profound influence on processing, manufacturing, biopharmaceutical performance and stability. The arrangement of molecules in the solid form and intermolecular interactions govern thermodynamic and kinetic properties of these solids [1]. Interestingly, a solid having same molecular arrangement in bulk of the solid, can provide variety of arrangement of molecules at the surface [1,2]. The outer or topmost surface layer of a solid can potentially react with surrounding vapor, liquid or solid phases. A discontinuity in interactions between atoms at the surface makes it more reactive than the bulk. In their pioneering publication in the field, Buckton et al., also emphasized on the fact that materials act, react and interact at surfaces and it is important to study the surface properties to understand the material completely [3].

In case of solids, several events of pharmaceutical interest are interfacial in nature such as flow, adhesion, wettability, hygroscopicity, stability, surface crystallization and surface energy. The surface properties are related to ease of production (for example, blending, granulation, tableting, inhalation products, coating), stability (surface mediated chemical interactions and reactions) and performance (dissolution) [1]. A differential behavior at the interface of the solid can have serious implications. This could occur due to differences in crystal facets, crystal habit, crystal defects or disordered domains, altered surface chemistry and surface functionalization [4]. Variations in intra and inter molecular interactions give rise to anisotropic surface properties of crystalline solids. They display facet dependent properties relative to orientation of crystal structure [5]. A subtle variation in surface composition, in terms of the chemical nature and location of chemical groups, causes batch to batch variations in raw materials in pharmaceutical systems. Processing of pharmaceutical solids during milling, spray drying, tablet compaction, mixing and lyophilization can disrupt the crystal structure leading to varying degree of disorder, surface amorphization and/or phase transformation [6,7]. As molecular arrangement is altered, the solid shall behave differently than rest of the ordered structure or the initial solid form [3,8]. The factors responsible for differential surface

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properties of pharmaceutical solids are compiled in Fig. 1 [3,5,9]. The knowledge of surface interactions can aid in comprehensive understanding of the surface mediated events and rationalization of the formulation approaches.

Various analytical tools are available for characterization of pharmaceutical solids such as conventional differential scanning calorimetry (DSC), modulated temperature DSC (MTDSC), high speed DSC (hyper DSC), powder X ray diffraction (PXRD), dynamic vapor sorption (DVS), Raman spectroscopy (RS), near infrared spectroscopy (NIRS), solid state nuclear magnetic resonance (ssNMR), isothermal calorimetry, inverse gas chromatography (IGC). Detailed information, strengths and limitations of these solid state analytical techniques are covered in numerous publications. Several techniques like DSC, PXRD, NIRS, RS, ssNMR are not sensitive towards surface [2,6,10]. An appropriate method is selected depending on specific property under investigation. In surface characterization, non destructive techniques that are sensitive towards topmost layer of solid are desired.

In the current review, the analytical techniques have been divided into five categories based on their principle of surface investigation, and include (i) use of vapor or liquid probes that interact with the surface, (ii) imaging the sample surface at microscopic and atomic levels, (iii) surface sensitive spectroscopic techniques, (iv) hyphenated mass spectrometry and (v) calorimetry based technique. Every section captures information on instrumentation, working principle, theory, sample requirements, modes and methods of analysis. Examples and case studies have been presented to provide insights on applications of these techniques in surface characterization. At the end, tabulation has been presented to suggest suitable analytical techniques for studying diverse surface properties of pharmaceutical solids.

2. Vapor or liquid probe based analytical techniques for surface characterization

2.1. Specific surface area (SSA)

2.1.1. Introduction

Specific surface area (SSA) of solids is defined as the total surface area of a material per unit of mass with units of m^2/g . The measurement can also be expressed as total surface area per unit of bulk volume, i.e. m^2/m^3 or m^{-1} . The total specific surface area encompasses the sum of external area (external surface of particles) and pores area (pore present in the interior of particles). Hence, the SSA of sample is function of particle characteristics such as porosity, pore size distribution, shape, size and roughness. The surface area of pharmaceutical sample is measured by four methods gas adsorption, mercury porosimetry, gravimetric vapor sorption and

gas permeation. Amongst these, a precise measurement of SSA is made using gas adsorption method by employing Brunauer, Emmett and Teller (BET) adsorption theory. Considering wide applicability of BET gas adsorption method, instrumentation details of BET specific surface area analyzer are briefly discussed below. Other methods are incorporated here by Ref. [11].

2.1.2. Instrument and parameters

Dynamic flow gas adsorption and volumetric gas adsorption methods of measurement are described in both European and United States Pharmacopoeia. Diagrammatic presentation of a typical surface area analyzer is shown in Fig. 2. Further, readers can refer 'IUPAC Technical Report' by M. Thommes et al., that thoroughly discusses technical details of the instrument [12].

The non destructive nature of the BET method is a major advantage. It is recommended to use N_2 as adsorbent for materials with larger SSA ($\geq 1 \text{ m}^2/\text{g}$), while krypton gas is employed to measure lower surface area ($< 0.2 \text{ m}^2/\text{g}$). The impurity in gases can cause error in the results, thus, moisture free analytical grade gas must be used [13]. The quantity of material required may vary from 0.4 to 2.0 g, depending on its specific surface area. However, the miniaturized flow meter (e.g. Monosorb®, from Quantachrome Instrument Co.) requires only 50–100 mg sample since it can measure the SSA of less than 0.1 m^2 . Monosorb is direct reading dynamic flow surface area analyzer, utilizing single point BET method for rapid determination of SSA regardless of the type of gas used (N_2 or Kr) [14].

The surface area of a sample may be variable or reduced when the surface is covered with molecules of previously adsorbed gases or vapors. Therefore, a critical aspect in sample preparation is

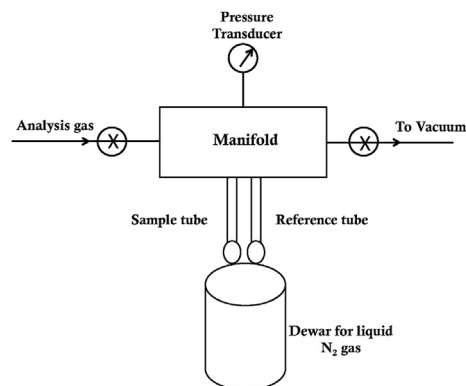


Fig. 2. Schematic diagram for a typical surface area analyzer, showing key components of the instrument.

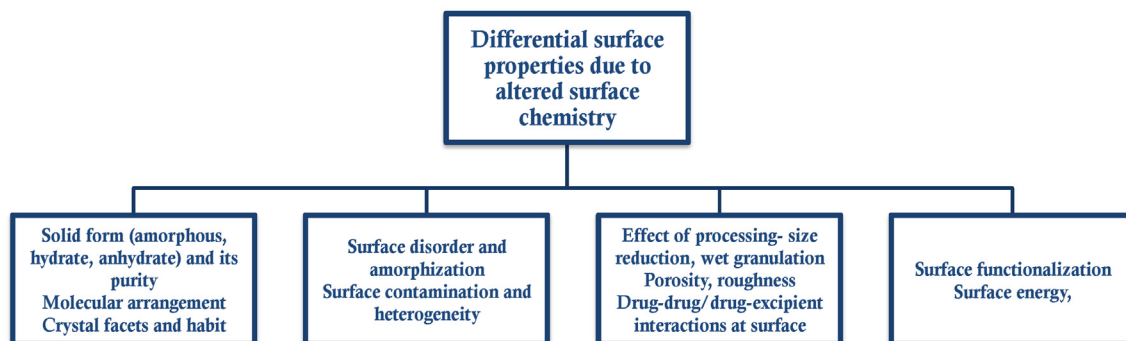


Fig. 1. Factors responsible for differential surface properties of pharmaceutical solids.

'outgassing', i.e., removal of the surface adsorbed gases or vapors from the sample, prior to measurement. The suggested methods for outgassing include (i) use of minimal effective elevated temperature, (ii) application of vacuum, purging under a stream of inert dry gas or (iii) desorption adsorption cycling. Outgassing should be carried out at a minimum possible temperature for a shorter period of time. USP recommends defining the temperature, pressure and time used for outgassing. When a material is outgassing sensitive, an alternative degassing approach such as desorption adsorption cycling should be employed [13].

The surface area of dry particle determined by conventional methods may not provide information about the particles when dispersed in a liquid, because the drying and degassing steps may induce surface or textural changes. Therefore, the actual surface area of wetted particles in a dispersion medium can be different than the solid particles in the dry state. Recently, *NMR relaxometry* has been employed to measure the surface area of wetted particles dispersed in a liquid, without the requirement of drying or degassing steps. This technique is based on the principle of differential NMR relaxation time of probe liquid alone and when in contact with particle surface [15].

2.1.3. Working principle

The working principle of SSA analyzer is based on determination of the volume of adsorbate gas (V_m) on surface of a sample by assessing relative pressure changes due to adsorption and desorption of adsorbate gas on the sample surface. In this, Nitrogen (N_2) gas is physically adsorbed on the surface of solid at the temperature of liquid N_2 (77.4 K), then desorption is triggered by heating the sample at room temperature. Physical adsorption results from relatively weak forces (such as van der Waals forces) between the gas molecules (adsorbate) and surface area of the test powder (the adsorbent). The volume of N_2 adsorbed by the solid corresponding to monolayer formation is measured and converted to a number of adsorbed molecules using ideal gas law. By multiplying the known surface area of an N_2 molecule ($16.2 \text{ \AA}^2$) and the number of adsorbed N_2 molecules, the surface area of pre weighted sample is computed as m^2/g . The amount of gas adsorbed can be measured by either volumetric or dynamic flow procedure, both methods are recognized in USP general chapter <846> [13].

Gas adsorption analyzer does not directly compute SSA value, while it just records the relative pressures of gas in samples cell due to adsorption and desorption. The classical BET Eq. (1) enables transformation of the recorded pressure data into volume (V_m) of gas adsorbed by the sample [16].

Eq. (1): BET equation

$$\frac{P}{V(P_0 - P)} = \frac{C}{V_m C} \times \frac{P}{P_0} + \frac{1}{V_m C} \quad (1)$$

Where

V volume of gas adsorbed at standard and temperature and pressure (STP), in mL; P partial vapor pressure of adsorbate gas in equilibrium with the surface at 77.4 K (Boiling point of liquid nitrogen), in Pa; V_m volume of gas adsorbed at STP to produce an apparent monolayer on the sample surface, in mL; P_0 saturation pressure of adsorbate gas at the experimental temperature, in Pa; C dimensionless constant relating the heats of adsorption and condensation of the adsorbate gas.

Therefore, using various concentrations of an adsorbate, a graph of $P/V(P_0 - P)$ is plotted against P/P_0 to yields a straight line. The relative pressure value (P/P_0) in a range of 0.05–0.3 usually yields a straight line. A minimum of 3 linear data points is required for multi point measurement. The data are considered acceptable if

the correlation coefficient (r) of the linear regression is ≥ 0.9975 . From the resulting linear plot, the slope $(C - 1)/V_m C$, and the intercept, $1/V_m C$, are evaluated by linear regression analysis. From these values, V_m is calculated as $1/(\text{slope} + \text{intercept})$, while C is calculated as $(\text{slope}/\text{intercept}) + 1$. The total surface area value is calculated by Eq. (2).

Eq. (2): Total Surface area

$$S_T = \frac{V_m N_0 A}{M_W} \quad (2)$$

where, S_T total surface area; N_0 Avogadro's number ($6.022 \times 10^{23} \text{ mol}^{-1}$); A cross sectional area of the adsorbent molecule (0.162 nm^2 for nitrogen and 0.195 nm^2 for krypton); M_W molecular weight of the adsorbent gas. The specific surface area (S) per unit mass of the solid can then be obtained by dividing S_T by the mass of powder measured (m), Eq. (3).

Eq. (3): Specific surface area

$$S = \frac{S_T}{m} \quad (3)$$

2.1.4. Applications

Surface area measurement provides vital understanding about performance characteristics of API, excipients and formulations. SAA of candidate compounds is monitored in early and late development owing to its direct correlation with dissolution performance (as per Noyes' Whitney equation). SSA analysis in pre clinical stage is crucial for comparing the results of oral animal pharmacokinetic (PK) studies with future human PK trials [17].

In addition to immediate release dosage forms, the SSA of API can significantly influence the release characteristics of sustained release dosage forms. For example, 'Invega Sustenna®' is extended release parenteral suspension of paliperidone palmitate and single administration this intramuscular injection last for 1 month [18]. As per the innovator's (Janssen Pharmaceuticals) patents and bio pharmaceutical review submitted to US FDA, the desired release profile to achieve the therapeutic concentration (is only achieved when the specific surface area of the submicron particles in the suspension is $> 4 \text{ m}^2/\text{g}$ (corresponding to an effective average particle size of less than 2000 nm) [19].

Role of surface area on dissolution profile of API can be understood by another case study of siramesine hydrochloride. The solubility of siramesine hydrochloride anhydrate is 2 fold higher than that of siramesine hydrochloride monohydrate and therefore the faster dissolution of the anhydrate was expected but both forms exhibited similar dissolution profile in pH 3.4 aqueous medium. The similar dissolution rate, despite different solubility, was attributed to the larger surface area of the monohydrate ($1.00 \text{ m}^2/\text{g}$) compared to anhydrous form ($0.33 \text{ m}^2/\text{g}$) [20].

Material performance properties of APIs such as wettability, adhesion during coatings, and hygroscopicity are influenced by surface area of a material. Higher the surface area, the higher would be water sorption tendency (hygroscopicity) of material. In a study by Puri et al., specific surface areas of amorphous and crystalline celecoxib were compared to study vapor sorption and differential wettability behavior of the two solids forms [21].

Homogeneity of powder blend, compressibility and powder flow are affected due to varying surface area of the samples. Coarser grades of microcrystalline cellulose (MCC), having smaller surface area, have been reported to be more lubricant sensitive than the finer grade. The larger SSA of fine MCC provided greater interparticulate area for compaction and exhibited higher tensile strength. Hence, analysis of SSA along with particle size of

individual excipients/powdered blend/granules guide in excipient selection in product development [22].

Interestingly, Vippagunta et al., utilized SSA measurement for understanding the batch to batch variation in the flow behavior of a drug substance during roller compaction process. Surface amorphization caused the variability in flow behavior and the same was identified from altered SSA values. SSA measurements thus, provide information about the nature of the solid surface including defects, disorder or void space [23].

2.2. Dynamic vapor sorption (DVS)

2.2.1. Introduction

Sorption studies are conventionally performed using vacuum desiccators and the desired relative humidity is maintained using saturated salt solutions. The experiments were limited to moisture uptake and the use of organic vapors/gases was earlier uncommon. This set up is bulky, time consuming, need large sample amount and is less accurate. The data only depicts the sorption curve while desorption and hysteresis cannot be established [24].

In contrast, DVS is a versatile, gravimetric tool to study material properties based on sorption. Commercially, an automated DVS instrument was introduced in 1990s [25,26]. DVS uses a continuous and measured vapor flow (up to 200 ml/min), which significantly reduces the time to reach equilibrium. The sample is automatically weighed throughout the experiment and few milligrams to grams of sample can be studied at different levels of humidity. The continuous flow of vapor complemented with high sensitivity and accuracy of measuring weight change, allow documentation of both rate and extent of sorption/desorption of vapors [24].

2.2.2. Instrument and parameters

DVS is equipped with high thermal conductivity and gas purged aluminium chamber maintained at constant temperature that provides precise control of environment, minimizes thermal gradients and errors due to static charges. A diagrammatic representation of DVS instrument can be referred from already published literature [24,27]. Several DVS models run in vacuum mode that provides numerous additional applications such as *in situ* degassing, multi component dynamic/static isotherms, isobars and study drying kinetics [28]. The flexible design of DVS instrument can further support a high resolution video camera with 200× optical zoom and 2.5× microscope. This allows simultaneous measurement of gravimetric change, visualize moisture induced morphological alterations and chemical interactions [29]. The specifications

of DVS instrument and parameters are given in Table 1. DVS instrument has been recognized in United States and European Pharmacopoeia for studying water solid interactions [30].

The experimental temperature varies with instrument model and is maintained by Peltier elements or resistance cartridge heaters. The sample temperature is accurately measured using a calibrated platinum resistance thermometer. The digital mass flow controllers mix the dry air or nitrogen with saturated moisture or organic vapor and regulate the flow through the sample. Humidity and ultrasonic sensors located adjacent to the sample and reference crucible, constantly record the % RH and vapor pressure respectively [31]. A unique feature of vapor generation and dynamic flow at experimental temperature, in the vicinity of sample crucible, prevents issues of vapor condensation. Hence, the sample and reference crucibles are constructed using chemically inert, anti static and easy to clean materials [27,32].

The vapors used in DVS are primarily moisture, however, in recent times organic vapors such as acetone, have gained importance. Organic vapors have dispersive properties; offer higher resolution with faster and reversible sorption. Organic vapors are primarily used for studying surface area, solvate formation, surface energetics, porosity and solid solvent interactions. However, moisture remains the choice of solvent for evaluating hygroscopicity and material interactions with water. Vapor pressure in case of moisture is depicted by % Relative Humidity (RH), while the ratio of partial and saturation vapor pressure is used in case of volatile organic solvents and gases [24,27].

The vital part of DVS instrument is the thermal microbalance, which has made sorption experiments simple and reliable. The microbalance is isolated from heating units by a water cooled plate that allows measurement of same sample at variable temperature and %RH. The microbalance has sensitive null balance or symmetry of the balance design, which is capable of measuring subtle changes in weight with extreme sensitivity, baseline stability and precision [33].

2.2.3. Working principle

The principle of DVS is documentation of weight change (increase or decrease) of sample on exposure to particular vapor pressure and temperature. The weight change is the manifestation of solid vapor interaction and its uptake or release by mechanisms such as adsorption, absorption, chemical reaction to form hydrate or solvate and condensation. Sorption is a combination of surface adsorption and bulk penetration or absorption of vapor molecules in the sample. The choice of vapor and solid properties govern the

Table 1
Specifications of various DVS instrument parameters [28,31,32,34].

Parameters	Range
Chamber temperature stability	±0.01 °C
Temperature variability	Minimum- 5 20 ± 0.1 °C; Maximum- 40 150 ± 0.1 °C; Organic vapors- 5 85 ± 0.1 °C
Pre-drying or degassing	Upto 400 °C
Vapors	Moisture, organic solvents- Acetone, dichloromethane, dichloroethane, ethanol, chloroform, cyclohexane, ethyl acetate, ethyl benzene, heptane, decane, hexane, isopropanol, methanol, methyl ethyl ketone, octane, nonane, toluene, water, xylene, pinene, hexanol, limonene, butyl amine and gases- carbon dioxide and methane
Experimental Humidity	0-98 ± 0.5% RH
Humidity calibration	Daily, weekly or as needed using deliquescent salts such as Bromide, Sodium Chloride and Potassium Nitrate
Partial pressure of organic vapors and gases	0.03 0.09%
Safety mechanism for organic vapors and gases	Dry nitrogen purging while switching solvents; Gas sensor and alarm
Mass capacity of microbalance	1 1000 mg ± 0.1 µg; 10 5000 mg ± 0.5 µg; Accuracy and Precision of ±0.1% and ±0.01% respectively
Vacuum	10 ⁻⁶ to 760 torr
Number of samples at a time	1 23
Sample crucibles and volume	Hemispherical quartz, metal-coated quartz (180 µl), stainless steel (50 µl), aluminium (20 µl), platinum (50, 100 µl)

sorption behavior of the solid. For example, water adsorbs readily on polar surfaces to a few molecular layers by virtue of hydrogen bonding. The sample properties such as solid state, porosity, surface roughness, polarity, surface energy, hygroscopicity and active surface area drive their sorption behavior. The basic experimental considerations in DVS are listed below [27,35]

- i. Pre treatment of sample by heating and/or purging to displace residual volatile components or moisture and evaluate the initial dry weight of the sample. However, in some cases it is recommended to use the sample in as is receiving state to preserve chemical integrity and solid form during handling and heating.
- ii. After the sample has dried and equilibrated, the sample is subjected to controlled in flow of varying vapor pressures and temperature. The vapor sorption by the sample is continuously measured and recorded in the instrument. The sample should be properly equilibrated at each increment of vapor pressure before proceeding to the next step. An equilibrium criterion of <0.01% weight change in 5 min or a more stringent criterion of <0.005% weight change in 15 min is generally recommended. An indicator of properly equilibrated sample is superimposable sorption and desorption isotherms, while in absence of solid form changes hysteresis may indicate poor equilibration. The duration of the experiment is governed by sorption behavior of material and time taken for equilibration at each step.
- iii. The resulting weight change of the sample, plotted against relative humidity at constant temperature yields the sorption/desorption isotherm. The isotherm should be interpreted carefully to identify the vapor interaction with the solid sample.

The three basic methodologies used in DVS are equilibrium moisture content, water uptake and residual weight method. In *equilibrium moisture content* method, the weight change is measured below the vapor pressure that induces phase transformation in the sample. However, in *water uptake method*, relative mass difference prior to and post *in situ* vapor induced crystallization is measured. Likewise in *residual weight method*, multiple moisture sorption–desorption cycles are conducted for stoichiometric hydrate/solvate formation and the resulting residual weight change is measured. Selection of vapor that can bring required crystallization and solid form change in the sample is paramount. Besides, *water uptake* and *residual weight methods* require standards (pure crystalline/amorphous) for data correlation and quantification purposes. For more details on sensitivity, sample weight, time required for the methods of analysis, readers can refer the publication by Sheokand et al. [27].

2.2.4. Applications

Diverse solids ranging from APIs, excipients such as lactose, trehalose, polymers and amorphous solid dispersion (ASDs) based formulations have been studied using DVS [36,37]. It is extremely useful in understanding heterogeneity of surfaces, physicochemical properties of raw materials, including the susceptibility to undergo physical changes during processing such as wet granulation and drying.

2.2.4.1. Solid form characterization. Yamauchi et al., have reported use of DVS for solid state characterization and contrasting surface and bulk properties of anhydrate and dehydrated hydrates of niclosamide and naproxen sodium. Niclosamide dehydrated hydrate showed a higher rate of moisture sorption than the corresponding anhydrate due to exposure of more hydrophilic groups on the surface. In contrast, the dehydration of the hydrophilic

compound, naproxen sodium, decreased the surface energy resulting in lower water sorption than the original anhydrate form [38].

2.2.4.2. Process induced surface amorphous content. Another area of significant interest is assessment of small amounts of process induced surface amorphous content in APIs and excipients [39]. DVS has been established as a sensitive and rapid tool for quantitative determination of as low as 0.05%w/w amorphous content [27].

Saleki Gerhardt et al., first used the equilibrium moisture uptake approach to study processing induced surface defects and amorphization in crystalline sucrose. Standard calibration curves were prepared with known amounts of %amorphous added into pure crystalline samples to quantify amorphous content in unknown sample. Since the water adsorption by the 100% crystalline sample was almost negligible, the water vapor sorption could be attributed to the amount of amorphous solid present in the mixture. This technique was found to be much more sensitive than the other techniques such as density, DSC and PXRD in detecting the concentration of amorphous solid at very low values, i.e., 1.0% with an accuracy of $\pm 0.5\%$.

Buckton and Darcy used phenomenon of crystallization of amorphous lactose to crystalline lactose monohydrate on exposure to vapor to quantify surface amorphous content. The mass difference before and after the crystallization was used to calculate the amorphous content. This method was sensitive and reproducible with low level of quantification of 0.5%w/w. However, a major drawback of the method was purity of crystalline form of lactose post crystallization. Several reports suggested formation of anhydrous α lactose and anhydrous β lactose along with lactose monohydrate during the crystallization event, which can significantly alter the moisture sorption. Hence, quantification based on hydrate formation can only be possible if the amorphous sample rapidly and consistently converts to the same hydrate form [3,40,41].

Keeping this in view, DVS method has been improved with the use of volatile organic solvents such as acetone. Mackin and Burnett et al., recrystallized the amorphous content without conversion to hydrates or solvates [42]. Instead of using moisture sorption, researchers used acetone vapors for quantification of amorphous content. In contrast to water, acetone has a significantly lower T_g of 93–100 K. This reduces the T_g of amorphous fraction below 25 °C, thus inducing rapid crystallization. Multiple acetone uptake cycles were carried out at 30% and 85% relative pressures to measure the amorphous content. Mackin et al., also concluded that DVS does not require a homogenous mixture of crystalline/amorphous fractions as it analyses the entire sample. This conclusion established DVS as a highly suitable tool for assessment of small amounts of surface amorphous content in predominantly crystalline APIs and excipients [43].

Saleki Gerhardt et al., further extended this concept and used apolar organic probes instead of water vapor [44]. In case of moisture sorption, relative humidity is restricted to low values so as to prevent recrystallization. However, in case of organic apolar probes such as *n* Octane, the entire partial pressure range may be used without observing crystallization phenomena due to relatively large size of probe and lower affinity towards hydrophilic molecules. Since kinetics of *n* Octane sorption is fast, higher experimental throughput and accuracy (0.05%) could be achieved [45].

Thus, a recent trend in DVS based quantification is exploiting volatile organic probes. Muller et al., identified that polar solvents such as moisture have a limited access to surface and pores in hydrophobic APIs. In contrast, organic solvents show absorption into the hydrophobic amorphous samples. The researchers investigated

methanol, ethanol, isopropanol and ethyl acetate as the organic probes for development of a one step DVS method for determination of amorphous content at a lower limit of 0.5% [46]. In another study, Muller et al., highlighted that it necessary to select the parameters like solvent and vapor pressure individually for each API so as to maximize solute solvent interactions for better resolution [47].

2.2.4.3. Flow properties. The different lots of an API can have significant differences in their physical properties such as flow, particle size and surface area. The manufacturing and processing conditions require stringent control for ensuring batch to batch consistency [48]. Vippagunta et al., have presented an interesting example of DVS, where the researchers identified that freshly milled and aged Metformin HCl had significant differences in flow. Based on reduction in peak intensity in PXRD and higher moisture uptake in DVS, the researchers concluded that the freshly milled sample contained surface crystal defects that contributed to flow ability differences. DVS was used to decide the conditioning parameters temperature and %RH to eliminate defects and obtain crystalline order in the freshly milled sample and eliminate the differences in flow [49].

2.2.4.4. Surface properties of ASDs. The key surface properties of amorphous systems measureable by moisture sorption/desorption isotherms are drug polymer interactions, surface chemistry and mechanism of water sorption, moisture induced plasticization and recrystallization [21,50,51]. Bianco et al., have studied surface properties and water uptake mechanism of ASD of sulfathiazole (ST) and its sodium salt (STNa). Increasing the proportion of salt improved the physical stability, however, this was opposed by an increased hygroscopicity. Using DVS, the authors optimized ratio of ST:STNa and demonstrated that salt in the mixtures influenced the RH at which crystallization took place by promoting water uptake and plasticization [52].

2.2.4.5. Surface properties of polymers. Understanding of surface properties of polymers can help in predicting polymer behavior in water, mechanism of water uptake and deciding optimal polymer in designing controlled release products. Sasa et al., correlated surface properties of three cellulose ethers hydroxypropyl (HPC), hydroxyethyl (HEC) and hydroxypropylmethyl (HPMC) with release of water soluble pentoxifylline and vancomycin hydrochloride from their matrices. The drug release was highest from HEC matrices, followed by HPMC and HPC due to better water sorption, polymer swelling and faster release of drug from the matrix [53].

2.2.4.6. Excipient moisture interactions. Excipient water interactions can also influence physical stability of moisture sensitive APIs. Veronica et al., investigated influence of excipients maize starch (MS), microcrystalline cellulose (MCC) and calcium hydrogen phosphate Dihydrate (DCP) having different moisture interaction, on stability of moisture sensitive drug, aspirin. In contrast to the sorption data, % aspirin degradation was maximum in DCP and least in MS. The reasons identified were that DCP increased basicity of the formulation, MCC had lower affinity for water and the moisture scavenging property of MS provided protection against moisture induced degradation [54].

2.3. Hyphenation dynamic vapor sorption near infrared spectroscopy (DVS NIR)

The DVS NIR hyphenation is an approach for real time monitoring of gravimetric behavior and spectroscopy to understand the structural state of pharmaceutical solids. NIR is routinely used as a

process analytical tool for achieving control of unit processes like drying. NIR investigates vibrational phenomena at frequencies greater than 4000 cm^{-1} ; is noninvasive and non destructive technique that requires no sample preparation. Buckton et al. housed NIR probe just 3–4 mm below the sample pan within the DVS. The DVS and the NIR can be synchronized to run together but are operated using independent interface [55].

Hogan and Buckton used this hyphenation to assess low amorphous contents in partially amorphous lactose. NIR allowed relatively faster assessment with complete sample recovery [56].

Li and Buckton reported another interesting example using DVS NIR, to investigate mechanism of water sorption and physical instability in ASDs of griseofulvin and PVP. Griseofulvin increased overall hydrophobicity of the solid dispersion, thus reducing water sorption by PVP. Storage of ASD at elevated humidity of 75%RH, showed no change in mass implying stable ASD with no crystallization. However, NIR produced contrasting results showing disappearance of NIR peaks for amorphous griseofulvin followed by phase separation and generation of crystalline griseofulvin. The NIR spectral region corresponding to water showed reduced peak intensity at 1904 nm (water absorbed into griseofulvin) and increase in peak at 1932 nm (absorbed water in PVP). This suggested that water expelled from crystallization of griseofulvin was sorbed by PVP, thus maintaining a constant net mass for the product [47].

The hyphenation provides complimentary information to deconvolute subtle events, such as crystallization and phase transformation that do not accompany a detectable weight change in DVS. In such cases, NIR can provide insights on solid form of the components leading to a better understanding of the water sorption behavior of solids. In addition, the hyphenation overcomes practical challenges in orthogonal assessment of samples using multiple techniques. In this case, the samples are exposed to identical environmental conditions, allows transfer and correlation of data from two different techniques.

A thorough review of literature indicated that the novel hyphenation of DVS NIR has been used primarily to study crystallization of sugars (lactose, raffinose and trehalose) and monitoring physical transitions in limited number of therapeutic drugs such as salbutamol and theophylline. A significant amount of work is required to establish this technique as a routine tool in surface or bulk characterization of pharmaceutical solids.

2.4. Hyphenation dynamic vapor sorption Raman Spectroscopy (DVS RS)

Another hyphenation of DVS is available with Raman Spectroscopy (RS) to study mechanisms of water solid interactions, phase transformation and chemical changes within the sample. RS is particularly useful due to low Raman activity of water molecules, small sample size (in milligrams) with no preparation, the sample crucibles used in DVS are compatible for Raman analysis, spectra can be captured at each gravimetric measurement or equilibrium and the spectra are well resolved and easy to interpret. The laser irradiates the sample and the vibrations in the molecules scatter this radiation at a different wavelength, called as the Raman effect, which is captured by the detector. Feth et al., have demonstrated DVS RS as a powerful tool to investigate water sorption/desorption induced solid phase transition of APIs. The spectroscopic data provided evidence for origin of changes in moisture content with change in humidity [57]. Similarly, Gift et al., demonstrated usefulness of this hyphenation for investigating water–solid interactions such as stoichiometric and non stoichiometric hydrate formation, deliquescence, amorphous–crystalline transformation and capillary condensation for different APIs [58].

To date, only a limited number of case studies have explored applications of DVS RS in studying bulk properties of the solids. The strengths of DVS RS in investigating moisture induced phase transformation and especially surface characterization of pharmaceutical solids need to be demonstrated further.

2.5. Contact angle measurement

2.5.1. Introduction

Surface energy is a common source of variability in pharmaceutical solids and can be affected by heterogeneous surface chemistry, distribution of facets and anisotropy of crystalline solids, partial or complete amorphization and processing such as size reduction [4,59]. It can be experimentally determined by introducing liquids or gases with known properties in contact with the solid under characterization. One such approach that utilizes liquid probes is, the Contact angle measurement, between a reference liquid and solid surface [4,60]. It is a useful technique for studying and measuring properties of the solid liquid interface. Geometrically, it is the angle formed by a liquid at the three phase boundary where a liquid, gas and solid intersect. Besides surface energy, contact angle measurement is a simple and rapid way to determine wettability, polarity or nature of chemical groups at surface, adsorption/absorption, spreadability and adhesion properties [61,62].

2.5.2. Instrument and parameters

The instrument for contact angle measurement is interchangeably called as optical tensiometer, contact angle goniometer, contact angle meter or drop shape analyzer. It consists of an adjustable sample stage to mount the sample, needle connected to a syringe to allow dispensing of small volume of desired liquid on the sample, an illuminating source and a high speed video camera with the axis parallel to the sample stage to obtain an image of the droplet. The in built image analysis software automatically processes the image to obtain contact angle value [62,63]. The specifications of several instrument models are compiled in Table 2.

Contact angle measurement can be performed directly on powder or either by spreading a uniform layer of powder on a flat surface or compressing it to form compacts [59]. However, the compressed compacts allow drop penetration in case of hydrophilic and porous powders or the droplet might stay on the surface if the powder is hydrophobic. Surface properties of the compact can vary with compression pressure that can lead to miscalculation of contact angle. Roughness on the compact surface should be considered while marking proper baseline or directly measure the roughness corrected contact angle [64]. Ideal probe liquid should be pure,

inert, non volatile and less viscous. Polar probes used in contact angle measurement are ultrapure water, ethanol; the non polar probes primarily used are dichloromethane, hexadecane, diiodo methane and lastly, ethylene glycol possess properties of both polar and non polar probes [59,61].

Contact angle measurements are generally performed at ambient conditions but in some cases, temperature and relative humidity need to be controlled. The instruments are capable of temperature control (both cooling and heating) for sample stage and the droplet. Cooling and heating is typically conducted using circulators that can control the wide range of temperature between -300 and $+400 \pm 0.1$ °C. The optical tensiometer can be equipped with electrically heated chamber to achieve temperatures up to 400 °C [65].

2.5.3. Working principle

A small drop of liquid (of known surface tension) is dispensed onto a solid surface, using a small bore capillary tube positioned above and close to solid surface to minimize kinetic energy. A contact angle is formed at the three phase interface between the solid surface, liquid and vapor surrounding them. The angle of a tangent to the drop originating from the point of contact of drop and surface is measured. The contact angle measurement allows quantification of the wettability of a surface and can further be used to calculate surface energy [61,62]. Contact angle depends on physicochemical properties of the solid surface such as particle size and shape, roughness and porosity. The factors to be considered for probe molecules are purity of the measurement liquid, presence of wetting agents, penetration of liquid into the substrate, drop size and surface swelling [68].

Wetting is the most common and useful parameter calculated from contact angle measurement. It depicts the ability of liquid to spread out or form boundary surface on a solid substrate, and is governed by interaction forces between the three phased interface. The interactions between probe and surface can be categorized into cohesive (interaction between similar molecules such as between the liquid molecules via hydrogen bonds and van der Waals forces) and adhesive forces (forces between dissimilar molecules such as between the liquid and solid molecules e.g. mechanical and electrostatic forces). A balance of these determine the contact angle created at the solid liquid interface. At smaller values of contact angle, the cohesive forces between liquid molecules are weaker than the adhesive forces, thus, liquid interacts with the solid surface leading to its wetting. In contrast, partial wetting represents opposite pattern of interactions. Consequently, a small contact angle ($<90^\circ$) shall be formed if the wetting tendency is more, while a non wetting liquid shall create a contact angle between 90° and 180° with the solid surface. A pictorial representation of various types of contact angle is shown in Fig. 3 [62,68,69].

Table 2

Specifications of contact angle instrument [63,65–67].

Parameters	Values
Angle	$0-180 \pm 0.05$ °C
Needle type and diameter	Stainless steel, PTFE, PP, 0.2, 0.5, 1, 2, 3 mm
Dosing syringes and volume	Glass (450 and 500 μ l), disposable (900 μ l, 1, 3 ml), upto 8, ≥ 10 pL upto 250 μ l
Sample support	Motion in x, y, z direction, 30 mm height adjustment and 360° angular displacement for sample stage
Maximum sample size	$300 \times 300 \times 50$ (Breadth*Thickness*Height), as small as 50 μ m in diameter
Optics	20 and 50X, 3 mm
Camera magnification	20 $\times$
Camera positioning	Field of view (1.17×1.3 to 10.8×8.1 mm) and 7 $\times$ zoom lens, flexible 3D positioning for imaging dosing head and sample
Image production	Monochromatic LED illumination (470 nm) with low thermal radiation using infrared cut filter
Image acquisition rate	25 up to 3400 fps (frames per second)

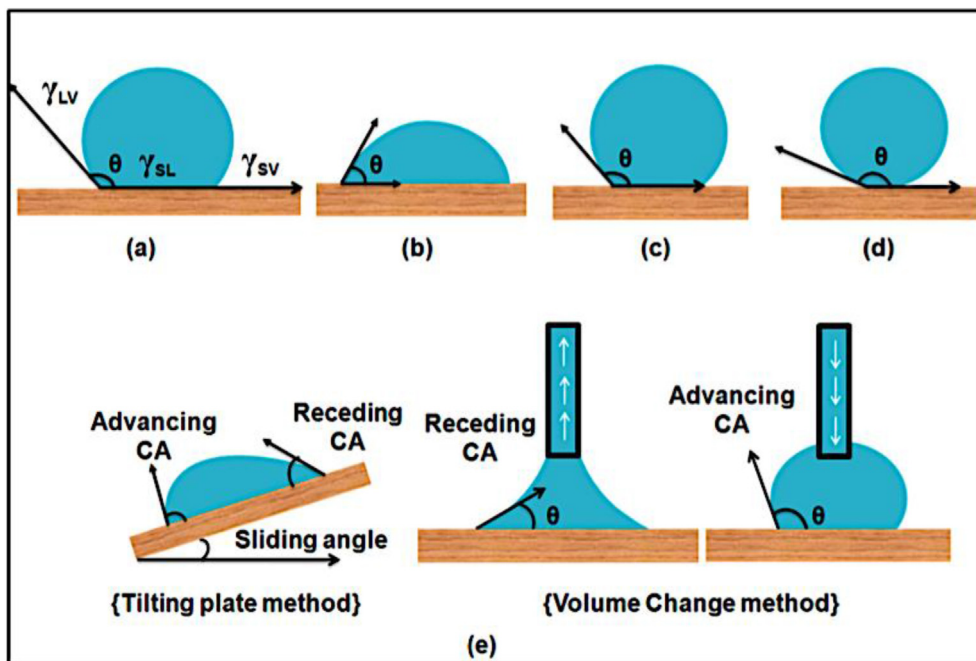


Fig. 3. (a) Interfacial tensions of all three phases that co-exist and static contact angle, (b) Hydrophilic surface contact angle (θ) $< 90^\circ$, (c) Hydrophobic surface contact angle (θ) $> 90^\circ$, (d) Superhydrophobic contact angle (θ) $> 150^\circ$ and (e) Dynamic contact angles for measurement of contact angles for measurement of contact angle hysteresis [78].

Thomas Young proposed an equation to establish relationship between interactions forces at the interface and contact angle. It provides a means of treating contact angle of a liquid with a surface as the mechanical equilibrium of drop resting on the solid surface under the constraints of three forces or surface tensions pulling away from equilibrium point [62]. At equilibrium, the forces are balanced and described using Young's equation as given in Eq. (4), where γ_{lv} , γ_{sl} and γ_{sv} represent surface tension at the interface of the liquid/vapor phases, solid/liquid phases and solid/vapor phases).

Eq. (4): Young's Equation

$$\gamma_{sv} = \gamma_{sl} + \gamma_{lv} \cos \theta \quad (4)$$

The equation can be rearranged to obtain value of liquid vapor surface tension.

$$\cos \theta = \frac{\gamma_{sv} - \gamma_{sl}}{\gamma_{lv}}$$

In case, γ_{sv} is $< \gamma_{sl}$, then $\cos \theta$ will be negative and θ is therefore $< 90^\circ$ and the droplet is likely to wet the surface. This can happen under two situations, either the surface energy of solid is high or liquid has a low surface tension. If γ_{sv} is $> \gamma_{sl}$ then $\cos \theta$ will be positive and θ is $> 90^\circ$. This type of situation arises when solid has low surface energy or liquid has high surface tension (such as water). Young's equation is based on assumption of rigid, flat, nonreactive, inert, homogeneous, insoluble, smooth and nonporous surface. In addition, it is difficult to define surface tension of the solid vapor and solid liquid phases. Therefore, the resulting contact angle value may vary due to different interfacial tensions [62,69].

The interpretation of surface energy from contact angle is based on Young's equation. Several methods describe ways to calculate surface free energy from contact angle values. Good–Girifalco method utilized contact angle measurement of single non polar solvent on the solid surface and its surface energy, to determine the dispersive surface energy (γ_s^{LW}) of the solid as given in Eq. (5) [70].

Eq. (5): Good–Girifalco method:

$$1 + \cos \theta = 2 \sqrt{\frac{\gamma_s^{LW} \gamma_l^{LW}}{\gamma_l^{LW}}} \quad (5)$$

However, two solid forms of a pharmaceutical compound may yield similar values of dispersive surface energies. Thus, alternate methods such as Wu Harmon method can distinguish polar (γ_s^{AB}) and dispersive components (γ_s^{LW}) of total surface energy (γ_s), as given in Eq. (6). The method is based on the principle that free energy of adhesion between two phases is equal to the harmonic mean of two separated phases. The method requires two types of liquid media. In this case, contact angle measurement is performed using a polar (water) and a non polar probe (e.g. diiodomethane) with different surface tension [71,72].

Eq. (6): Wu Harmon method:

$$(1 + \cos \theta) \gamma_{lv} = 4 \left[\frac{\gamma_s^{LW} \gamma_l^{LW}}{\gamma_s^{LW} + \gamma_l^{LW}} + \frac{\gamma_s^{AB} \gamma_l^{AB}}{\gamma_s^{AB} + \gamma_l^{AB}} \right] \quad (6)$$

Surface energy of a solid can also be calculated using van Oss Good method (Eq. (7)) by measuring contact angle using at least three liquids viz. (i) Liquid with only a dispersive component of surface energy, (ii) another liquid with dispersive and an acidic or basic component and (iii) liquid with either a dispersive and a basic or acidic component (whichever the second probe liquid did not have) or a liquid with all three components. The theory subdivides the polar component of surface energy of solids and liquids into two specific components: the surface energy due to acidic interactions (γ_A) and basic interactions (γ_B). The acid component theoretically describes propensity of a surface to have polar interactions with a second surface that has basic characteristics by donating electrons. Conversely, the base component of the surface energy describes the propensity of a surface to have polar interactions with another surface that has acidic properties by accepting electrons [73].

Eq. (7): Van Oss method:

$$(1 + \cos\theta)\gamma_{lv} = 2 \left[\sqrt{\gamma_s^{LW}\gamma_l^{LW}} + \sqrt{\gamma_s^A\gamma_l^B} + \sqrt{\gamma_l^A\gamma_s^B} \right] \quad (7)$$

Another useful parameter calculated from contact angle measurement is the spreading parameter (S). In case the surface energy values (both dispersive and polar) of two materials are known, spreading coefficient (S) of material (1) over the surface of another material (2) can be calculated using Eq. (8). In this manner, in interactions between the two materials, like two powders or a powder and a liquid, can be calculated.

Eq. (8): Spreading coefficient:

$$S_{12} = 4 \left[\frac{\gamma_1^d \gamma_2^d}{\gamma_1^d + \gamma_2^d} + \frac{\gamma_1^p \gamma_2^p}{\gamma_1^p + \gamma_2^p} - \frac{\gamma_1}{2} \right] \quad (8)$$

At positive value of S, complete spreading of material (1) over the surface of (2) is preferred [74].

2.5.4. Methods

The methods for contact angle determination are highly surface sensitive, low cost, readily performed and easy to understand. Besides, the measurements are precise and reproducible when performed carefully. **The evolution of theories and methods for contact angle determination such as captive bubble method, capillary rise method, Washburn method and Wilhelmy plate method have been discussed in detail in number of publications [68,69].** The applications of these methods have been criticized due to significant limitations in accurate measurement of surface energy [4,75,76]. We have compiled three major methods for contact angle measurement, which have been widely, reported in literature and utilized in number of commercial equipment.

Sessile or static drop is the most common method employed for solid surfaces. A drop of liquid is gently dispensed on a surface (outermost 10 Å molecular layers of the solid surface) and the contact angles on each side of the relaxed drop are measured. A prerequisite is that the three phase boundary should be immobile, i.e., thermodynamic equilibrium has been achieved between solid surface, liquid probe and air. Static contact angle offers a quick, easy and quantitative measurement of wettability and surface free energy. However, the application of this method is limited for smooth and homogenous surfaces. In few cases, complexities related to the solid state of API prevent compression of a particulate material into a smooth chemically and physically homogenous powder disc. Errors arise in measurement as either the liquid absorbs into the porous powder or the contact angle is overestimated [59,61,69,77]. The static method produces a single value of contact angle close to global minimum free energy. However, several metastable static contact angles, corresponding to the local free energy minima can exist for a solid surface. Considering the energy barrier between the local free energy minima, the 'static' drop can be in any of the free energy minima within the hysteresis range. Thus, *dynamic* contact angles are preferred for reproducible measurement of contact angle [62].

The dynamic or (quasi) static contact angle can be determined using two main approaches *needle* and *tilting* method [62]. In needle method, also referred to as the volume change method, a small droplet is first placed on the surface and the volume of drop is gradually increased to the maximum without altering the three phase line, while recording the '*advancing*' contact angle at the same time. Likewise, '*receding*' contact angle is measured by gradually decreasing the volume of the droplet, till maximum volume that can be removed without reducing the three phase line is reached. Caution should be practiced in this case to allow delivery

of drop from a point sufficiently close to the solid that the capillary remains in contact with the drop with minimum perturbation of drop geometry. This method is more reliable for determining the advancing angle, while it is difficult to capture the receding angle as the needle disturbs the geometry of the drop. An alternate method for capturing the receding contact angle is the evaporating method. The drop is measured repeatedly as the volume evaporates. The receding contact angle is measured just prior to de wetting and reduction in the three phase line [77].

In tilting method, as the name suggests, the droplet is placed on the surface, which is then gradually tilted typically from 0° to 90°. As the surface is inclined, gravity causes the contact angle on the lower side to increase while the contact angle on the uphill/tilted side to decrease. The *advancing* angle is constantly measured at the front (downhill) as the droplet starts to move. The *receding* angle is measured at the back (uphill) of the droplet at the same time. The tilt angle is strongly dependent on the weight of the drop and the drop retention on the surface. In some cases, the drop will roll off the solid as wetting occurs at the roll off angle. The last valid readings are captured and normally represent the *advancing* and *receding* contact angles. In some cases, the solid can tilt all the way to 90° without releasing the drop [67,79].

Advancing and receding contact angles give the maximum and minimum values the static contact angle can have on the surface. Advancing contact angle is always larger than or equal to the receding contact angle. Besides, the experimental receding angle is usually less reproducible due to roughness, liquid sorption or solid swelling [64]. The difference between receding and advancing angle is called as the contact angle hysteresis and can be as high as 50°. The hysteresis characterizes surface topology and can help quantify contamination, surface chemical heterogeneity and effect of surface treatments and surfactants. The interested readers can refer publication by Huhtamaki et al., for steps, critical considerations and troubleshooting during practical measurement of contact angle [62,69,79].

Roughness corrected contact angles can be measured with combined surface roughness and contact angle measurements. Topography modules equipped in optical tensiometer facilitate roughness measurements at the same spot as the contact angle. The roughness ratio, defined as the ratio between the actual and projected solid surface area (roughness factor is 1 for a smooth surface and >1 for a rough surface), is used to correct the contact angle for roughness [67].

2.5.5. Applications

2.5.5.1. Crystal surface chemistry. The surface properties of crystals are governed by the chemical functional groups exposed on the dominant crystal planes. By virtue of their high total facet area, chemical and physical properties of the dominant planes would ultimately affect the interfacial properties such as wettability, dissolution rate, flowability and tabletability. Kiang et al., established useful correlation between single crystal structure, morphology and role of surface functional groups in wetting of anhydrous (Form A) and monohydrate (Form B) forms of an unknown drug. The combined results from single crystal analysis and attachment energy revealed that the two dominant faces of Form A has carboxylic acid groups while in case of Form B, the single dominant plane has butyl groups. Contact angle measurement was used to experimentally validate this observation and it was ascertained that hydrophilic groups in Form A led to superior wetting than Form B [80]. In similar context, several studies report use of contact angle measurement with other surface sensitive techniques such as Inverse Gas Chromatography (IGC), X Ray Photoelectron Spectroscopy (XPS), Time of Flight Secondary Ion Mass Spectrometry (TOF SIMS) and Atomic Force Microscopy (AFM) to

study face specific structure, chemistry and wettability of pharmaceutical crystals [81–84]. To exemplify, Heng et al., investigated wettability of (001), (100) and (011) facets of aspirin crystals using sessile drop method. It was established that wettability and surface energy of aspirin was anisotropic and facet dependent, due to relative presence of carboxylic acid groups on the surface [76].

Jain et al., also demonstrated practical implications of surface anisotropy on performance of two plate shaped crystals of aspirin Form I. Wettability and surface free energy determined using contact angle established that crystals with (100) plane had higher values for polar components (acidic and basic, due to carbonyl groups) than (001) (that possessed hydrophobic aryl and methyl groups) that provided hydrophilicity to the facets resulting into higher hygroscopicity and degradation on stability [85]. Similarly, Modi et al., explained faster dissolution and high oral bioavailability of plate (hydrophilic surfaces) and acicular shaped (hydrophobic surfaces) crystal habits of celecoxib using wettability and surface energy determination [5].

In addition to surface chemistry, surface heterogeneity contributed by impurities, crystal facets, growth steps, crystal edges, surface pores, local degree of crystallinity and surface functional groups can be evaluated using contact angle measurement. Heng et al., illustrated variations in surface properties of paracetamol Form I with milling. The combined results from contact angle measurement and IGC were used to justify exposure of hydrophobic surface in paracetamol with decrease in particle size [60]. Ho et al., demonstrated and compared sensitivity of IGC and contact angle measurement for monitoring surface heterogeneity in D mannitol samples. The two techniques were scrutinized for detecting surface chemical changes in D mannitol post functionalization with methylene groups, i.e., altered surface chemistry [75].

2.5.5.2. Solid form characterization. The solid forms of an API such as crystalline, amorphous and hydrates possess different physico chemical, mechanical and biopharmaceutical properties. It is necessary to characterize the solid forms to maintain solid state purity during manufacturing, setting specifications/monographs and product development. For example, Shete et al., performed comprehensive solid state characterization of commercial crystalline and amorphous atorvastatin calcium samples. The impact of these properties on wettability and intrinsic dissolution rate (IDR) was evaluated. The authors established that amorphous form showed superior wetting and faster dissolution rate than the crystalline counterparts due to lack of long range order and random surface chemistry [86]. Similarly, Puri et al., have highlighted differential wetting and surface free energy of crystalline and amorphous celecoxib using contact angle measurement. Although the crystalline and amorphous celecoxib surface possessed similar dispersive surface energy, but the amorphous form had a small component of polar energy that enhanced its wettability with polar probes [21].

In addition, the variations in surface properties of similar solid state forms can also affect the biopharmaceutical and pharmacokinetic performance of an API. Laad et al., characterized material properties of four telmisartan samples obtained from different sources, that exhibited same polymorphic form, but different particle shape, particle size distribution, surface energetics and surface chemistry. The role of these properties in telmisartan powder wettability was explored using contact angle and XPS studies. From the parameters adhesion, immersion and spreading the researchers concluded that the four telmisartan samples had different wetting properties, probably due to different surface chemistry [87].

2.5.5.3. Excipient characterization. The excipients play a key role in modulating performance of the dosage form in terms of stability, drug release, bioavailability, taste and texture. The contact angle based wetting, spreading coefficient and surface free energy parameters can provide useful insights on their functional behavior. Oh et al., proved that surface free energy based parameters such as work of adhesion and spreading coefficient, can be used to predict factors affecting polymer adhesion to substrate during film coating process [71]. In a similar manner, Bartkowiak et al., measured surface properties (wetting and surface energy) of mucoadhesive and film forming polymers along with their blends to design novel mucoadhesive buccal and gastro retentive dosage forms [88].

2.5.5.4. Formulation screening and optimization. The surface energy information obtained from contact angle measurement can be used for rationale designing and optimization of the formulation components. For example, Zajic et al., illustrated use of spreading coefficient and surface energy values to select a suitable binder for granulation and predict its performance [89]. Ahfat et al., used surface energy data determined by contact angle studies in predicting mixing behavior of binary and tertiary systems of iron oxide with either microcrystalline cellulose (MCC), magnesium stearate or titanium dioxide [90].

On the other hand, Zhang et al., established that dispersive surface energies calculated from contact angle are as reliable as IGC. The researchers also demonstrated that contact angle approach is contrary for hydrophilic powders due to penetration of drop. The surface energy data could reliably predict the performance of granulating solvent with respect to density, porosity and friability of granules. Thus, based on solvent–drug spreading coefficients, complex processes such as wet granulation can be studied and optimized [91]. Similarly, Baki et al., studied impact of surface properties of pellet cores with differing surface energies on layering efficiency in fluidized bed processor [74].

Several techniques have been explored for characterization of tablet surface in the micro and nanometer range including AFM, TOF SIMS and confocal microscopy (CM). In one such study, Sauri et al., characterized the surface of tablets using contact angle, AFM and CM to establish relationship between surface free energy and texture with dynamic swelling and dissolution rate of drug from matrices [92]. Liu et al., further established that contact angle is a good indicator for prediction as well as exploring the mechanism of increased dissolution. The approach can be used as a performance evaluation tool in excipient and formulation screening [93].

2.6. Inverse gas chromatography (IGC)

2.6.1. Introduction

Following its inception in 1940s, IGC has been established as a sensitive technique for measurement of powder surface properties [94]. It is a dynamic gas sorption technique wherein intermolecular forces between probes (of known properties) and solid are analyzed to quantify the total surface energy of the sample. It offers numerous advantages over other existing methods in its ability to quantify stronger interactions (between substrate and the probe), is unaffected by nanorugosity and surface heterogeneity [95]. These situations are undesirable for contact angle measurement because stronger interactions and roughness alter contact angle values, leading to erroneous measurements (e.g. contact angle values can be close to zero in case of stronger interactions between substrate and the probe). Lastly, IGC is more accurate, versatile and require no sample preparation [4,41,96]. The primary application of IGC is determination of surface energy and surface energy heterogeneity of powders, particulates, fibers, films and semi solids. In addition, bulk properties such as solubility parameter (Hansen and

Hildebrand), Flory Huggins mixing parameter, moisture induced glass transition, BET specific surface area, wettability, adhesion and cohesion can be studied [95,97,98].

2.6.2. Instrument and parameters

The first commercial IGC based surface energy analyzer was launched in 2001. The specifications of the IGC instrument are compiled in Table 3. A unique feature of the compact design of the instrument is stability of temperature, pressure and flow rate in the column oven [99]. IGC does not require powder treatment and assesses surface energy of powders in their genuine true state. The study of impact of humidity on sample surface properties is feasible with the humidity controller. The two columns can be run sequentially, 10 methods can be run in a sequence with up to 250 injections pre programmed in each method. The reproducibility (1% RSD) and precise volume of vapor injection at wide concentration range allows quick and accurate surface energy determination at different sample surface coverage. The automated data analysis software can perform reliable calculations for surface energy heterogeneity, isotherms and thermodynamic parameters [100,101].

2.6.3. Working principle

IGC is reverse of traditional analytical gas chromatography in terms of stationary and mobile phases [94]. In IGC, sample tube is packed with the solid material of interest that acts as the stationary phase. Volatile probes (nonpolar and polar) of known physico chemical properties (polarity, acidity, molecular area, and electron donor/acceptor number) are passed through the sample at a certain dilution via inert carrier gas. The linear movement of probe molecules is hampered by nature and magnitude of interactions with the stationary phase. The resulting change in retention time and volume of probe is recorded [94,95,101].

IGC measures the time taken for the pulse of gas or vapor to travel through the sample to measure wide range of physico chemical properties. Distribution of volatile probes yields information on equilibrium properties (such as phase transitions and thermodynamic properties such as sorption, surface properties of solids, surface area, molecular weight), from retention time or peak area. The net retention time (t_N) is given by difference in retention times of an inert molecule (t_0) and the probe (t_R) [98]. Retention volume, on the other hand, is the measure of quality and quantity of adsorption of probe molecule on the sample. With known sample BET surface area and molecular size of probes, the amount/moles of probe needed to achieve certain coverage can be determined. Retention volumes of a homologous series of alkanes at this coverage can be used to determine dispersive surface energy, while

the specific free energy is calculated from the retention volumes of polar probes [98]. The theory of IGC for calculation of dispersive and specific component of surface free energy and heterogeneity has been extensively reported [94,98,102–104].

2.6.4. Methods

IGC analysis can be performed at (i) finite concentration or (ii) infinite dilution [94,98]. Several experimental considerations during IGC analysis are listed in Table 4. The analysis at infinite dilution (or zero surface coverage) involves injection of very small amounts of probe molecules through the solid sample. The degree of dilution is such that the intermolecular interactions between the adsorbed molecules are negligible with no competition for binding sites and adsorption desorption occurs at monolayer level (obeys Henry's law). The probe molecules are pushed by the carrier gas to explore the whole surface of the solid before exiting the sample tube. The flow of solute molecules leaving the column is detected and recorded. At low surface coverage, the highly energetic sites get occupied that do not actually represent all active sites, but correspond only to a small fraction of it. Hence, the value of surface energy recorded is high. The data can be validated based on observations such as (i) volume of injected vapor in the zero surface coverage region and the absence of probe probe interactions, (ii) a linear isotherm, reproducible, sharp and symmetrical (Gaussian) peaks for both non polar and polar probes and (iii) the retention volume remains constant with change in flow rate.

In finite concentration (finite surface coverage) mode, a known quantity of liquid solute is injected, such that the surface coverage close to a monolayer or even higher could be obtained. The high concentration of probe allows interaction with all active sites on the sample surface. In the finite concentration, retention volume is dependent on injected probe concentration. It provides complementary information to the results of infinite dilution, as it allows construction of adsorption isotherms for full surface characterization. It is a dynamic adsorption method that can provide energy distribution of all surface active sites even the sites adsorbing the probes, possible to study temperature effects, which allows access to desorption isotherm of the probes. The key application is the assessment of the surface heterogeneity through adsorption energy of the probe [106].

2.6.5. Applications

IGC can measure changes in surface energy with utmost accuracy, enabling study of surface crystallization, solid form transformation and process induced disorder. The key benefits of IGC include, non destructiveness towards the surface, well controlled temperature and humidity, measures the outermost layers of a

Table 3
Specifications of IGC instrument [94,99–101].

Parts	Specifications
Carrier gas	Nitrogen, argon, helium
Column oven	2
Insulation material	Teflon
Temperature range	2–180 °C upto 600 °C
Non-polar probes	Hexane, heptane, octane, nonane, decane
Polar probes	Acidic- acetone, ethyl acetate; Basic- chloroform, acetonitrile, dichloromethane
Number of vapor reservoirs	12
Volume of reservoir	50 ml
Injection volume	Wide concentration ratio 1:4000
Humidity control	0–98% RH at 25 °C
Sample tube	Silane-treated glass tube
Dimensions	Length- 30 cm, Outer diameter- 6 mm, Internal diameter- 2, 3, 4 and 10 mm, Surface area- 0.5 m ² ; glass wool to close the ends
Sample size	10–500 mg
Detector	Flame Ionization detector with sensitivity of 10 ⁻⁹ mol

Table 4
Experimental considerations during IGC analysis [4,94,97,105].

Parameter	Implications
Pulse mode	A fixed volume of probe injected into the carrier gas for passage through the sample tube; suitable for rapidly equilibrating substrates
Frontal mode	Probe molecules flow continuously into the carrier gas. Used for slowly equilibrating samples, produce broader peak due to long experiment time
Equilibration time	>12 h
Packing of sample tube	Fill the powder under tapping till there are no visible cracks, hollows or channels in powder; insignificant effect otherwise
Packing length	Inadequate separation at too short length and too long sample packing would lead to long and imprecise retention time due to diffused peaks
Minimum number of probe injections	3 in N ₂ 3 each
Infinite dilution	Measures highest energy sites of the solid surface, which is often <0.1%; surface energy may be overestimated; retention time independent of injection volume
Elution gap in between probes	30 min
Injection of large amount of probe	Reduced retention time as probe interact with lower energy sites as well
Flow rate	Lower the flow rate, more likely the system is in equilibrium, but longer experimental times and broader peaks, reduced accuracy of retention time determination; It is advisable to optimize the flow rate individually

molecule and has a resolution down to very small surface area [106].

2.6.5.1. Crystallization. Interfacial tension of solution plays a critical role in crystallization, as crystal growth rate from solution is higher when the interfacial tension is low. This phenomenon can be regulated by addition of surfactants that preferentially absorb onto the surface of crystals thereby modifying the surface energy of the growing crystals. Kumar et al., demonstrated application of IGC in studying surface free energy change due to the adsorption of surfactant on surface of sucrose crystals. The surfactant increased the sites for specific component while the dispersive free energy decreased due to adsorption. IGC results of the surfactant coated sucrose were used to interpret the thermodynamic effect of surfactants during the crystal growth process [107].

2.6.5.2. Study of solid forms. Yamauchi et al., characterized surface properties of dehydrated hydrate and anhydrous forms of niclosamide and naproxen sodium using IGC. Based on polar probes, the surface chemistry of crystals, i.e., exposure of hydrophilic functional groups and resulting increase in specific energy could be understood. Consequently, the difference in surface energy manifested as differences in the hydration behavior. Hence, surface energy profiles and water sorption isotherms provided thermodynamic and kinetic information on the susceptibility of the materials to undergo physical changes during processing such as wet granulation and drying [38].

Pacheco et al., highlighted differences in surface and bulk properties of anhydrous forms of D mannitol. Surface energy of polymorphs was determined using dynamic contact angle measurements, DVS and IGC. As each technique provides specific information of the solid, the combination of these techniques allowed sound understanding of the polymorphs [108].

IGC is an invaluable tool to study surface properties of amorphous materials. In amorphous systems, determination of T_g can be a challenge due to difficulty in controlling the environment during sample preparation and analysis. Hydrophilic materials can rapidly sorb moisture from the environment and the resulting value of T_g would be lower than the dry sample. However, in case of IGC, the environment in the packed sample tube is highly controlled and *in situ* drying facilitates removal of adsorbed moisture and volatile impurities. Besides, the humidity control provided in the instrument enables study of plasticization and crystallization induced due to moisture [109]. Surana et al., determined T_g and studied plasticizing effect of water by controlling temperature and relative humidity in the column, *in situ* in IGC [110]. Buckton et al., also presented an example to determine T_g of powder surface using IGC.

The work highlighted that there is a concentration gradient of water from surface to bulk, thus, plasticizing the surface to a greater degree than the bulk [111].

IGC can provide information on nature of functional groups exposed on solid surface. Besides, the role of moisture on electron acceptor and donor interaction with the surface and resulting change in physicochemical properties of amorphous materials can be investigated [52]. Ohta and Buckton et al., identified the basis of physical instability of spray dried cefditoren pivoxil powders (SD A and SD B) prepared using spray drying. IGC revealed differences in their surface properties with SD A having a lower dispersive surface energy and high K_D due to stronger interactions with the polar probe. By virtue of different local order of molecules at the surface SD A sorbed more water than SD B at high RH that contributed to differences in their physical stability [112].

IGC is an invaluable method to study transitions in amorphous materials due to controlled environmental conditions. Newell et al., assessed the impact of relative humidity on the surface energy of 100% and partially amorphous lactose prepared by spray drying and milling, respectively. Although the spray dried sample collapsed and crystallized in the column and occupied only a proportion of the original packed volume, surface energy could still be measured reliably. In contrast, for the milled sample, decrease in sample volume was negligible as only a small amount of amorphous content collapsed, while the bulk of the sample was in the form of crystalline lactose [113].

Stability assessment of amorphous pharmaceuticals can be predicted from structural relaxation. The established techniques such as DSC and Isothermal Calorimetry are used primarily to study this phenomenon, however, these techniques measure the properties of the bulk of the sample while crystallization often starts at the surface of amorphous samples. Buckton et al., have studied structural relaxation of ASD upon ageing that was induced *in situ* in IGC. Ageing induced structural relaxation was studied using decane injection. In absence of recrystallization, the decrease in retention volume of decane with aging time implied that the sample relaxed at the surface [114,115].

2.6.5.3. Processing induced disorder. The phenomenon of partial or complete amorphization of crystalline drugs on milling is well known. This is accompanied by changes in surface chemistry and energy of the material [60]. Thielmann et al., used finite concentration method to study energy heterogeneity of untreated, milled and recrystallized lactose of similar particle size. The untreated and recrystallized lactose showed a similar dispersive energy at infinite dilution, but the energy distributions for the two samples was different. In contrast, the milled lactose showed a shift in the

energy distribution of dispersive as well as specific component to higher values. The study was complemented with DVS that suggested formation of amorphous regions in the milled sample contributing to an increase in surface energy [116]. With the help of IGC, Burnett et al., also showed that milled indomethacin possessed relatively higher dispersive surface energy and higher surface basicity than quench cooled and ball milled sample [117]. Luner et al., also established that IGC could successfully predict surface energy differences of molecular crystals subjected to dry and high shear wet milling [118].

Subsequently, the changes in surface energy as a function of RH need to be assessed as sorption of water alters the physicochemical properties at surface and in the bulk [119]. Ohta and Buckton et al., identified the nature of chemical groups exposed on amorphous cefditoren pivoxil generated during milling. Authors reported that the value of K_D/K_A was more suitable than the γ_s^D as the acid/base parameter changed on milling while the dispersive surface contribution remained constant. This was implied in combination with FTIR results, wherein the presence of carbonyl groups (electron donating nature), on surface, caused an increase in the basic nature of cefditoren pivoxil following amorphization. On further exposure to water, the basicity reduced due to shielding of carbonyl groups from acidic polar probes [120].

The annealing or exposure of micronized materials to controlled relative humidity, temperature or organic vapors; induce structural stabilization of process induced amorphous content. Using IGC based surface energy parameter, Puri et al., measured surface specific changes, especially molecular level surface disorder in dry milled drug samples. In addition, the cohesive adhesive forces measured using IGC and AFM, displayed shift from drug drug cohesive forces to drug lactose adhesive forces upon annealing [121].

2.6.5.4. Drug excipient interactions. IGC is a valuable tool in pre formulation studies, as it can yield valuable information on drug excipient interactions. The acid basic contributions at immediate surface of individual drug and excipient can be reliably predicted using IGC. Ohta and Buckton et al., evaluated acid base interactions between cefditoren pivoxil (a basic drug when dissolved) and various Eudragit polymers. The basic to acidic parameter ratios (K_D/K_A) on the powder surface was used to predict incompatibility between carbonyl groups (amphoteric nature) on the surface of cefditoren pivoxil crystal and dimethylaminoethyl groups (basic nature) on Eudragit EPO [122].

Sasa et al., investigated surface properties of cellulose ethers using IGC to find a suitable polymeric matrix to formulate tablets and study drug release. The polymers under study showed large differences in specific component of the enthalpy of adsorption and acid–base properties in the order: HEC > HPMC > HPC, which had direct correlation with water sorption behavior and swelling degree of polymers. The release rate of pentoxifylline and vancomycin from the polymer matrices followed the same order as the polarity of polymers [53].

2.6.5.5. Performance of dry powders for inhalation (DPIs). IGC based interparticulate interaction measurements can serve as a useful means for predicting the aerosol performance of DPIs. Cline et al., used IGC to examine nature and extent of forces present at the surfaces of the drug and carrier for development of DPI. Researchers successfully demonstrated that increasing surface energy interaction between drug and carrier resulted in greater fine particle fraction of the drug [123]. Tong et al., used solubility parameters derived from IGC analysis at infinite dilution to evaluate the influence of solid–solid interactions on *in vitro* aerosol performance of salmeterol xinafoate (SX) samples, with or without the

lactose carrier [124]. Based on surface energy characterization, Jong et al., proved superior aerosolization performance of spray dried colistin over jet milled powders [125]. Maruyama et al., developed a non destructive method, a novel index—the Void Forming Index (VFI)—which is related to the pressure drop, to detect powder agglomeration in IGC [125].

2.6.5.6. Surface modification. In pharmaceutical development, surface modification of excipients is common to improve powder flow, aerosolization, wettability and dissolution. Swaminathan et al., assessed the feasibility of using IGC to measure surface energy changes during lubrication of MCC and lactose with magnesium stearate (MgSt). The researchers witnessed reduction in dispersive energy of MCC–MgSt blends with increase in MgSt concentration. The results were attributed to increasing coverage of the high energy sites on microcrystalline cellulose by magnesium stearate. The measurement of the specific component of energy indicated that MgSt interacted with MCC through non specific forces rather than acid–base interactions. The researchers additionally highlighted the limitations in measuring the surface energies of mixtures that individually have similar surface energies [126]. Similarly, Zhou et al., investigated changes in surface properties of a fine lactose powder processed by mechanical dry coating with MgSt. IGC could probe surface energy and demonstrated that modification of the surface properties correlated with the reduction in cohesion and improved powder flow [127]. In similar context, Das et al., measured surface energy of lactose processed using mechanofusion with MgSt at finite dilution. The surface energy distribution profiles measured at finite dilution were more informative and consistent with the observed flow behavior of mechanofused powders than comparative surface energy determined at infinite dilution [128].

Karde et al., compared surface energy values from sessile drop contact angle and IGC at infinite dilution for excipients (corn starch, lactochem, avicel PH 105) modified using nano silica (Aerosil 200 P, R972). Both the techniques provided comparable decrease in dispersive component of surface energies for all the excipients. The wettability of powders increased with higher values of polar component of surface energy. The study demonstrated that using contact angle and IGC, surface wettability and energetics of powders could be evaluated altogether [129].

3. Microscopic techniques for surface characterization

3.1. Scanning electron microscopy (SEM)

3.1.1. Introduction

The emergence of electron microscopy is an evolution in the field material sciences, as it enables scientists to visualize fine details at a level of submicron, which otherwise would not be possible using a highly sophisticated optical microscope. Outstanding benefits of SEM over optical microscopy (OM) are (1) 250 times higher magnification (250 000 $\times$ in SEM vs. 1000 $\times$ in OM), (2) large depth of field, and (3) around 200 times better lateral spatial resolution (1–3 nm in SEM vs. 200 nm in OM). Interestingly, ultra high resolving power (spatial resolution of 1 nm) is now achievable in SEM instrument equipped with field emission electron source and operated with a beam accelerating voltage > 15 kV [24].

3.1.2. Instrument and parameters

A typical SEM instrument comprises of electron gun to produce electron beam; condenser lens to demagnify the beam; scan coil (scan generator) to control and modify the beam; and specimen holder to mount specimen. All of these are housed in a vertical column attached to a pump, which produces a high vacuum. Finally,

the signal detection system consists of detectors, which collect the signals, and electronic monitor, which produces an image from the signal. A beam of electrons is emitted from a source, known as the cathode. The high vacuum helps to prevent the scattering of the beam and maintain the electron source stable during operation of the SEM. Negatively charged electrons in the form of the beam are accelerated down the column using anode (positive charge electrode) by applying a high voltage (accelerating voltage). The applied voltage is adjustable over 0.1 kV to 30 keV. The source of electrons in SEM can be either heated tungsten wire hairpin filament (having operating life of up to about 100 h) or single crystal of lanthanum hexaboride, LaB₆ (which has an operating life of up to about 1000 h). Schematic representation of SEM instrument is given in a book by Storey and Ymén [24,130].

Sample preparation is an important step in accurate and meaningful analysis using SEM. Normally, the pharmaceutical samples examined in SEM are in the form of 'fine powder'. Though less frequently, analysis of granules, tablets, freeze dried cakes, capsules, single particles (individual crystals) and beads can also be performed. Stainless steel sample holder (known as 'stub') is used for mounting of the specimen using different methods based on the nature of the sample. Sticky pads or a thin smear of thermosetting tapes can be used to fix the fine powder sample [24,130].

Non conductive samples are coated using conductive material to inhibit sample charging by electrons in the beam. Frequently, coatings using gold, carbon, and gold palladium are commonly applied, as they are highly conductive, relatively inert and easy to apply as a coat. Gold and gold palladium are coated using sputter coating and carbon by evaporative coating. Metals coating (gold or gold palladium) are usually not visible in the SEM images when viewed below 15 000 $\times$ magnifications. On the other hand, coating becomes visible at high magnification >15 000 $\times$, which is commonly encountered in high resolution field emission scanning electron microscope (FE SEM). The coating is seen as a fine granular texture on the surface of the specimen and hence could obscure the features of interest or misinterpret as an intrinsic feature of the specimen. This problem can be minimized by using platinum coating material for the specimen to be examined using FE SEM. This is because platinum can coat a much thinner layer as compared to gold. Nowadays, non conductive materials can be analyzed using environmental SEM or variable pressure SEM and overcomes the potential problems of interference arises due to coating of the specimen [131]. Elemental analysis in SEM is carried out on uncoated sample to avoid interferences from the coating materials [132].

In conventional SEM, specimen needs to be examined at a high accelerating voltage (>15 kV) to achieve an ultra high resolution (around 1 nm). However, this can significantly damage the surface of organic molecular solids due to the high energy of the beam, resulting in a loss of fine surface details. A low accelerating voltage (≤ 5 kV) can provide information on the fine surface details but which comes at compromise of resolution. Most of the pharmaceutical specimens are present in micronized form and can be examined at a magnification of 2000 to 10,000 $\times$ and therefore, the higher resolution is not always required [24].

Modern SEM is fitted with a field emission electron source, known as FE SEM. It offers a high lateral spatial resolution of up to 0.8 nm hence it is also called as 'Ultra High resolution SEM'. Ultra high resolution SEM has a special electron source such as 'cold or hot field emission source'. The FE source consists of a fine pointed tungsten wire that is upheld at high vacuum in a high electrostatic field. Thus, the source capability to provide an intensely bright and small diameter electron beam, is advantageous in offering ultra high resolution at a lower accelerating voltage. Moreover, the life expectancy of the FE source is many thousands of hours. In

comparison with conventional SEM (non FE SEM), FE SEM has a unique ability to produce high resolution images at a low accelerating voltage and offers low noise, high quality images. Due to the low voltage operation of FE SEM, it has the advantage of causing less damage to specimens without comprising the resolution [24].

Quantitatively SEM is able to measure size and aspect ratio of the specimen. However, the surface topography was earlier analyzed by visually means (qualitative way). Late recently, Austria based company, Alicona Imaging GmbH, has come up with a 3 D surface reconstruction software, called as MeX, which can quantitatively measure the surface profile of the particle (surface profilometry). The surface profilometry involves measurement of surface morphology, step heights and surface roughness of specimen. Using stereoscopic images, many of images at a single region on specimen surface are captured for greater accuracy using a SEM and then imported into MeX [133].

The environmental SEM (ESEM) has recently been employed for overcoming the problems associated with analysis of wet samples. Conventional SEM has drawback of the requirement of prior sample preparation if sample is present in 'wet' state (e.g. nanosuspension, biodegradable polymeric matrices in hydrated state). For a wet sample needs to be dried in air or using other suitable treatment such as freezing drying. The sample preparation step may cause perturbation in the true surface topography of the sample. ESEM enables direct imaging of wet samples in their native state, without requiring any prior sample treatment [131].

3.1.3. Working principle

SEM is based on the principle of analysis of secondary electrons and backscattered electrons, emitted from specimen when the specimen surface is bombarded with a high energy electron beam. The signals (secondary and backscattered electrons) are collected and analyzed to construct image of the specimen. High energy electron beam penetrates into the surface of specimen to a depth of few micrometers (depending upon accelerating voltage and average atomic number of the specimen) and scattered within a volume, known as the 'interaction volume'. Comparatively, high energy beam (generated by applying a high accelerating voltage) penetrates deeper than a low energy beam. The depth of penetration also depends on the average atomic number of the specimen and the beam penetrates deeper into a specimen containing lighter elements in comparison with the specimen containing heavier elements [24,134].

3.1.4. Applications

Owing to high resolution and a high magnification capability, the images captured using SEM reveals vital information on size, shape and topography, which are frequently monitored as critical material attributes. Topography is surface features of the material, in other words, it is about 'how the surface looks' and its texture and porosity.

Azad et al., prepared nanocomposites in which nanosuspension of the itraconazole (poorly water soluble drug) was spray coated on two types of carriers: large carrier particles (>300 μm) and fine size carrier particles (50 μm). Extensive SEM analysis was carried out to compare particle size, surface morphology and coating thickness of spray coated nanocomposites [135].

Delamination of glass bottles and prefilled syringes need to be critically monitored in the development of parenteral drug products. Due to the transparent nature of the glass flakes, they remain unnoticed during visual inspection test. SEM analysis has been employed for evaluation of delamination tendency of glass vials by visualizing surface changes on inner side of vials or prefilled syringes. SEM EDS is also used to examine the level of metal contaminants present on the surface of the glass material. It can also

reveal changes that occur due to interaction of filled drug solution with the glass surface [136,137].

Ditter et al., established *in vitro* *in vivo* correlation (IVIVCs) for parenteral polymeric microsphere of Naltrexone (Vivitrol®). In this case, the release profile is greatly affected by the particle morphology, size and surface chemistry of the polymer. SEM analysis of three test formulations and Vivitrol® revealed that all four formulations possessed the spherical shape morphology with small pits on the surface but differed in terms of porosity and particle size distribution, that resulted in variable drug release. This study demonstrated the utility of SEM in characterizing formulation with complex release profile and power of SEM in getting in sights on critical quality attributes of the complex formulations [138].

3.2. Hyphenation scanning electron microscopy energy dispersive X Ray spectroscopy (SEM EDS)

SEM EDS is a qualitative tool for rapid analysis of elemental composition of the near surface of sample. The analysis is made easy using modern software that automatically assigns the elements to the peaks and provides information on the relative surface abundance of the elements in the specimen. SEM EDS or EDS can serve as “initial screening tool” to identify elemental impurities in APIs and finished pharmaceutical products [24].

The emitted X rays in SEM are analyzed to get information on elemental composition or chemical characterization of the specimen. The characterization capability of EDS is due to the principle that each element has a unique atomic structure that generates a unique set of peaks in an X ray energy spectrum. The information collected is from 0.5 to 3.0 μ below the actual surface of the sample. In commercially available instruments, the detector for analysis of X rays is attached in SEM instrument; therefore, the technique is known as SEM Energy Dispersive X Ray spectroscopy (EDS or EDX) [139].

The accelerating voltage in SEM EDS analysis must be 1.5–2 times greater than the energy of the highest energy of X ray line to be emitted from the sample. An optimal working distance for EDS measurement is provided by the manufacturer, based on the geometry of detector present in the instrument. The beam spot size and final aperture is required set at ‘high’. It is suggested to begin EDS analysis in low vacuum mode with an accelerating voltage of 15 kV to excite typical metallic contaminants including the lower atomic number elements [139].

The distribution of the metallic excipients such as magnesium stearate (common lubricant) in the physical blends can be analyzed using SEM EDS coupled with X ray photon mapping. Swaminathan et al., at Pfizer Global R&D studied the effect of blending and magnesium stearate (MgSt) concentration on “the changes in surface energy” of the blend of MgSt and microcrystalline cellulose (MCC). Interestingly, SEM EDS along with X ray photon mapping were employed to demonstrate that MgSt was distributed on the surface of MCC particles [126].

3.3. Atomic force microscopy (AFM)

3.3.1. Introduction

High resolution microscopic imaging facilitates characterization of solid surfaces’ down to nanometer scale. In conventional microscopes, the wavelength of the illuminating radiation (visible light or electron beam) limits the depth and resolution of imaging. In contrast, AFM employs a scanning probe to “perceive” the sample and generates three dimensional topographic images from submicron to nanometer scale. AFM is a powerful, non destructive surface analytical tool that does not require extensive sample

preparation, coating or staining, can be performed in air, liquid or vacuum and allow small scale imaging from 10^{-10} m to one tenth of nanometers [140]. Along with atomic resolution imaging (molecular interactions, hydrophobicity, surface roughness, height of the surface up to 10 μ m, morphology and defects), probe and sample interaction can provide information on nano scale chemical, mechanical (modulus, stiffness, viscoelastic, frictional), electrical and magnetic properties [141–143].

3.3.2. Instrument and parameters

The major components of AFM are cantilever/tip assembly, sample stage capable of moving in x (length), y (width), and z (height) directions, scanner, laser and photodiode detector. The most sensitive part of AFM is the cantilever/tip assembly also referred to as the probe. The cantilever ranges from 100 to 200 mm in length, 10–40 μ m in width and 0.3–2 mm in thickness. It is usually soft and loose to be deflected at very small forces, without deforming the sample surface. The dimension, shape (board or V shaped) and material of fabrication govern the functioning of cantilever. For example, the stiffness and resonant frequency are high for shorter and thicker cantilevers. These types of cantilevers find applications in noncontact mode for measurement on slightly harder and rough surfaces. The flexible cantilever further encompasses a pyramid shaped, sharp, micro fabricated tip (diameter <100 Å) for mapping the surface [140]. The rationale for employing a sharp tip is to provide minimal contact area with just a few atoms of the surface, minimize long range forces and obtain atomic resolution data. In addition to traditional silicon and silicon nitride, nano tips constructed from whiskers or carbon fibers have become common due to better resolution. The probe tip can be customized or functionalized using epoxy glue resin or sputter coated with material of interest such as iron oxide, glass or polystyrene beads (1–5 μ m size) for studying material interactions [141–144].

On the other hand, the AFM scanners are constructed from a piezoelectric material such as ceramics that expands or contracts in response to the voltage gradient. The scanners are capable of three dimensional movement with respect to the probe tip. The closed loop feedback system automatically measures and adjusts the scanner movement as per the configuration. The two scanner configurations are: scanned tip or scanned sample. In former, the piezoelectric scanner is attached to tip to move it across the stationary sample. While in latter configuration, the scanner is attached to the sample to monitor its movement while the tip position remains fixed. Lastly, the photodetector consists of a split photo diode that senses changes in intensity of reflected laser beam due to deflections of the cantilever with supreme sensitivity of 0.1 nm.

In AFM, high resolution and artifact free imaging depends on substrate and sample. The substrate should be planar with surface roughness less than the material under investigation. The successful substrates used till date comprise of pyrolytic graphite, mica, evaporated or single crystal gold, silicon wafers and glass slides. The interactions between substrate and sample should be such that there is no displacement of sample during scanning while the morphology and integrity of the sample should be maintained. While studying material interactions (such as adhesion forces), a flat custom grown crystal or surface can be constructed directly on the AFM sample stub by melting or recrystallization. AFM does not require staining or coating of sample surface under study to increase electrical conductivity for measurement. However, a flat surface is ideally required, with height up to 1.5 inches and sample area in the range of 100 nm to 100 μ m square can be examined easily without repositioning of the stage [144].

3.3.3. Working principle

In principle, when the probe tip scans the sample surface (in x, y and z directions), attractive (long range) or repulsive (short range) van der Waals forces between the tip and the sample cause deflections of the cantilever. The deflection is directly proportional to the magnitude of force sensed by the tip. The laser detects the deflections and the beam reflection is focused onto a position sensitive photo diode detector to build three dimensional image of the sample surface. The interaction force between sample and tip is kept constant and described using Hooke's law as $F = 1/4kx$; where F is the force exerted on cantilever during scanning, k is the spring constant or stiffness of cantilever and x is the cantilever deflection [141–143,145,146].

3.3.4. Modes and methods

AFM is capable of resolving subtle details of sample surface in a single scan. Broadly, it can be operated in three modes viz. Static or contact mode, dynamic non contact and tapping mode, that are relevant for surface characterization of pharmaceutical solids [147]. The differences in various modes are compiled in Table 5. In contact mode, the cantilever and sample are constantly in physical contact with separation distance less than 0.5 nm. The tip drags over the sample surface in xy raster pattern. The feedback loop maintains a constant deflection (changes cantilever height to maintain a constant force) of the cantilever with respect to the sample surface by moving the z scanner for each xy coordinate. The cantilever deflects owing to a constant repulsive van der Waals force (with change in z axis), which is directly proportional to topography of the surface. This mode is suitable for flat, smooth and moderately rigid surfaces (crystals and hard polymers), which are not substantially stiffer than the tip. In case the cantilever stiffness is less than the measured surface, the contact may bend and break the cantilever. Surface contamination in form of adsorbed moisture may further require high lateral drag force due to attractive capillary forces contributed by moisture. The continuous contact and high coefficient of friction inevitably damages the sample and the tip, leading to poor quality of images [141,144].

In dynamic mode, the cantilever is vibrated using a piezoelectric crystal oscillating with a frequency close to the resonance frequency of the cantilever. The resonance energy of cantilever is characterized by amplitude, phase and frequency. In non contact mode, the cantilever vibrates just above the surface at a frequency of 100 kHz, with tip sample separation and amplitude of the order 1–10 nm. The feedback system monitors resonance frequency and amplitude of the oscillating cantilever varying due to long range forces at the surface. The motion of the scanner is used to build the image of the sample. The value of spring constant, k is higher than contact mode to overcome attractive forces between tip and the surface. Because of zero physical contact and high separation distance, no sample or tip damage is witnessed, however, it compromises the resolution and scanning speed of the probe [141,146].

Tapping is the most commonly used mode of AFM that combines the benefits of contact and non contact modes. Herein, the tip comes in contact with the sample for only a short duration (in nanosecond range), thus, eliminating the drag force while maintaining the same level of resolution as the contact mode. The tip oscillates (at amplitude of 20–200 nm) and taps on the sample surface under external electrical excitation. The AFM tip briefly touches the sample in each swing while rastering the sample in xy direction, producing a decrease in the oscillation amplitude due to reduction of the vibrating space. As a result, the tip experiences both attractive and repulsive forces during each oscillation. Similar to the constant force in contact mode, the feedback loop keeps this decrease in oscillation amplitude at a constant value by moving the piezoelectric scanner to record the image height. In addition, phase imaging can be recorded in tapping mode. The phase of oscillation is disturbed when the tip touches the sample at the bottom of its swing, inducing a phase difference between the tip and the electrical oscillator. The tapping mode is used on soft surfaces where the constant contact of the probe can damage the sample surface. AFM, with this setup is able to determine the surface topography of a material on atomic scale vertically and nanometer scale horizontally [141,144,146].

In addition to the modes discussed above, AFM offers wide range of applications to study nanomechanical properties (deformation, young's modulus, hardness), cohesive adhesive measurements and electrical properties of crystals that are either beyond the scope of the current discussion or have been covered in more focused manner elsewhere in literature [142].

3.3.5. Applications

AFM is a useful tool for study of surface topography as these properties significantly impact the functional performance and interactions of pharmaceutical APIs, excipients and multi component finished dosage forms.

3.3.5.1. Study of crystal surfaces and crystallization. Differential surface chemistry and solid state properties (packing, density, hygroscopicity), as in case of polymorphs, can pose challenges in numerous unit processes such as mixing, granulation and tableting. Cao et al., investigated three dimensional topography and adhesion forces of single crystals of Form I and II of sulfamerazine. AFM enabled study of crystal dimensions, morphology, surface roughness and even crystal edges [149]. The tip crystal interactions provided information on hydrophilic and hydrophobic nature of the crystal facets.

Price et al., used environmentally controlled AFM mounted with a CCD camera, in combination with DVS, to monitor solid state recrystallization of amorphous lactose. The study illustrated that high resolution capability of AFM, coupled with complementary bulk analytical techniques, provide real time imaging of a typical recrystallization process at nano scale [150]. A similar study by Jones et al., used AFM coupled with bulk techniques such as DVS

Table 5
Comparison of different modes of operation in AFM [141,148].

Parameters	AFM modes of operation		
	Contact mode	Non-contact mode	Tapping
Tip oscillation	Static	Dynamic	Dynamic
Physical contact between sample and probe	Constant	No contact	Intermittent
Interaction force	Repulsive	Attractive	Attractive and repulsive
Feedback parameter	Cantilever deflection	Resonance frequency and amplitude	Amplitude and phase
Scan speed	High	Least	Low
Resolution	High; 0.2–0.3 nm	Low	High
Damage to sample or tip	Yes	No	Minimal

and NIR to study kinetics of dehydration of trehalose dihydrate to α anhydrous trehalose (on exposure to low relative humidity and ambient temperature). Change in surface morphology was used to monitor this phase transformation. Dehydration decreased the surface roughness, which stabilized on complete dehydration and marked the end point of the process [151].

3.3.5.2. Surface properties of excipients. Fahs et al., investigated the surface properties of Hydroxy Propyl Methyl Cellulose (HPMC) films formulated with stearic acid. In this study, AFM was used to understand surface structure, chemistry, roughness, adhesion and frictional properties of polymer at nanoscale [152].

Meeus et al., used AFM for nanoscale surface characterization of a spray dried injectable polymeric matrix consisting of poly (lactic co glycolic acid) and polyvinylpyrrolidone. Information on surface composition and miscibility of the excipients can be obtained from topographical and phase images. AFM data coupled with chemical profiling techniques such as TOF SIMS and XPS, is a useful screening tool in designing novel excipients due to small sample size requirements and robustness of data [153].

3.3.5.3. Drug excipient miscibility and stability assessment. Formulators intend to understand the amorphous drug polymer systems to obtain optimal dissolution performance and avoid phase separation on storage [154]. Lauer et al., carried out AFM based assessment of active ingredient and excipient combinations to identify miscible and stable solid dispersions. Two AFM based parameters (i) phase mapping to directly visualize phase homogeneity of mixtures and (ii) height maps to determine specific molecular surface roughness were investigated. Topographical information can be used for process and formulation optimization in a scientific manner [155].

3.3.5.4. Particle interactions. AFM can be used to investigate inter molecular interactions between “like” and “unlike” surfaces with unprecedented sensitivity. Research has proven that interactions depend on solid form, particle size, surface roughness and surface energy. Shetty et al., used AFM based roughness parameter and topographical imaging to study particle particle interactions in dry powders for inhalation. They concluded that aerosolization of spray dried amorphous ciprofloxacin powders improved on storage at 55% and 75%RH. This happened because recrystallization increased particle roughness that reduced particulate contact area resulting in an increase in % fine particle fraction [156].

AFM can also provide valuable information on surface structure and texture at atomic level, ordered crystalline/amorphous regions, cohesive adhesive and electrostatic forces. The readers can refer more elaborate reviews on basic physics of measurement, models for data interpretation and practical considerations regarding force measurement for characterization of DPIs, already published in literature [142,157–159].

Several analytical tools are available that can guide on characterization, quantification and reversal of amorphization in milled powders. Price et al., used phase and topographical imaging using AFM at elevated humidity conditions to determine surface amorphous content in milled lactose in comparison to the initial recrystallized sample [160].

Tejedor et al., used AFM for characterization of surface amorphization in milled α lactose monohydrate and compared it to unprocessed and spray dried α lactose monohydrate as reference crystalline and amorphous structures, respectively. The combined results obtained from other complementary techniques (Raman, SEM and DSC), validated that amorphization began at the surface

followed by propagation into the bulk by disrupting the crystalline structure [161].

The milling induced surface amorphization can be partially or completely reversed by conditioning (exposure to humidity or organic vapors) that recrystallizes the distorted regions [39]. Jones et al., utilized phase imaging using AFM to visualize recrystallization of surface amorphous content in milled budesonide particles, on exposure to organic vapors [162].

In addition, the AFM based colloid probe technique has been utilized widely to screen novel carriers and container systems for DPI products by measuring drug adhesion to the surfaces of interest [163]. In one such study, Eve et al., determined interaction forces of micronized salbutamol to glass, lactose and polytetrafluoroethylene. Salbutamol functionalized AFM tip was used in tapping mode to determine cantilever deflection as a result of tip sample interaction. The resulting force distance profiles quantified the particle substrate adhesion forces [164]. Several reports demonstrated the strength of AFM for quantification of separation energies between micronized drug drug and drug excipient particles. Hence, AFM is a useful preformulation tool for development of dry powders for inhalation [163,165–168].

Davies et al., demonstrated another useful application of AFM in the field of DPIs. The researchers probed the nature and extent of surface interactions between micronized budesonide drug particles and simulated pulmonary surfactant monolayers. The applicability of AFM for this purpose was illustrated by measuring force of adhesion in tapping mode using budesonide functionalized AFM tips and pulmonary surfactant monolayer [169].

3.3.5.5. Determination of surface energy. AFM, although not a primary technique, is useful in determining surface energy of micronized drug particles. The researchers have established that AFM yields surface energy values close to IGC. In AFM, adhesion force is measured and the range of surface energy value obtained is because of different chemistry exhibited at single particle level. AFM is a good choice, if the effect of environmental or storage conditions need to be assessed. In contrast, IGC provides a better option as a quality control tool for determining batch to batch reproducibility [170].

Similarly, Zhang et al., used AFM to determine surface energy of pharmaceutical powders from adhesion force measurements. The contact adhesion force or pull off force between an AFM tip and lactose was used to determine surface free energy of crystalline and amorphous forms [171].

3.3.5.6. Surface characterization of tablets and tooling. Adhesion of granules to punch faces remains an unpredictable issue during tablet manufacturing. AFM can be used to study intermolecular interactions between drug crystal surface and the punch face [172]. Wang et al., demonstrated the strength of AFM in measuring adhesion forces of three model compounds ketoprofen, ibuprofen and flurbiprofen to a metal surface. The energy of interactions (in contact mode) between iron coated silicon nitride tips and flat surfaces of the three molecules were considered directly proportional to the adhesion force during tablet compression [173].

Bunker et al., used a more realistic experimental set up comprising of ~15 mm in diameter and 4 mm in thickness flat disk of base metal used to make actual punches (two grades of steel HPG S and HPG P). They used AFM to understand the influence of punch face material, surface roughness and % relative humidity (RH) on tableting issues such as sticking [174].

In addition, Seitavuopio et al., demonstrated usefulness of AFM for characterization of microstructure of tablet surfaces. Although, AFM could provide

quantitative values for roughness data, but small measurement area, slow speed and the need for flat samples limits its capabilities for examining entire dosage form such as tablet [175].

4. Spectroscopic techniques for surface characterization

4.1. X ray photoelectron spectroscopy (XPS)

4.1.1. Introduction

XPS, also known as, electron spectroscopy for chemical analysis (ESCA), is a sensitive spectroscopic technique for determination of surface chemistry, including elemental composition, empirical formula, chemical state and electronic state of elements in the material [176]. The technique is non destructive and extremely surface sensitive with capability to perform analysis of 1–12 nm of top surface [177]. Moreover, it provides both qualitative and quantitative (≈ 0.1 atomic%) data of surface under investigation [178].

4.1.2. Instrument and parameters

The major parts of an XPS instrument comprise of a gas cluster ion X ray source, ultra high vacuum stainless steel chamber, electron collection lens and energy analyzer (to sort photoelectrons on basis of energy), sample stage and an electron detector system [179]. The technical details on instrumentation of XPS can be referred in Table 6. Automated, high sample throughput analysis of solid surface is performed under ultra high vacuum conditions to prevent contamination. Solid sample in form of pellets, powders and coated surface can be studied in XPS, provided the material is compatible with ultra high vacuum. The sample can be analyzed in its native state however, treatment such as fracturing, crystal cleaving, cutting or scraping in air or vacuum, ion beam etching to clean surface contamination, exposure to heat to study changes due to heating, reactive gases or solutions, ultraviolet light can be performed for suitable applications [176,179].

4.1.3. Working principle

The principle of XPS is based on photoelectric effect discovered by A. Einstein, wherein the absorbed light ejects electrons from the solid surface. In XPS, the solid surface is irradiated with an X ray beam and the absorption of the X ray photon ejects core electrons (called the photoelectrons) from the surface (Note: thus, cannot detect H and He) [178]. Though, X rays penetrate the sample to several microns, the core electrons from the top 10 nm possess enough energy (≤ 2000 keV) to travel to the detector. XPS is extremely surface sensitive because of short travel distance, zero energy loss and elastic scattering of surface electrons. The hemispherical analyzer detects the kinetic energy of the emitted surface electrons, which helps in determination of binding energy as per Eq. (9) [176,178,179].

Eq. (9): Relationship of binding and kinetic energy

$$BE + h\nu = KE + \phi \quad (9)$$

where, binding energy (BE) is the energy required to emit an electron from the surface, kinetic energy (KE) of emitted electron depends on energy of incident photon ($h\nu$) and ϕ is the work function that depends on the instrument. The signal from emitted electrons can be increased or decreased from surface to bulk by varying the energy of irradiating photon (e.g. use a high energy photon such as dual Al K α /Ag L α 2984.2 eV monochromatic X ray source) [176]. The binding energy is characteristic to the orbital from which the photoelectron emerges and thus is specific to the element and its surrounding chemical environment. Therefore, a change in chemical environment of atom results into different binding and kinetic energies [177,178].

The photoelectron spectrum, (i.e. the emitted electron signal on Y axis is plotted as a spectrum of binding energies on X axis) is generated from counting number of ejected electrons and their kinetic energies. The photoelectrons scattering inelastically and losing kinetic energy within the sample's atomic structure (>15 nm) fail to escape from the surface and contribute to the spectral background [179]. The binding energy gives information on identity of element, chemical state while the quantity of an element is measured from the intensity of photoelectron peak (area under the peak) in an XPS spectrum. The peak shape and position is used to determine chemical state of an element [178].

In XPS, rapid spatial distribution (1–100 μ m) information of individual particle or powder can be obtained using synchrotron based highly focused and bright source of X rays (filtered or monochromatic using a quartz crystal). Likewise, for depth analysis sequential ion sputtering (using Ar $^{+}$ gas cluster ions or high energy monochromatic X ray photons) and measurement is performed to etch the surface and determine elemental composition beneath the topmost surface layer. In mapping mode, relative concentration of an element can be traced in color mode according to its position on the sample surface [179]. The readers can refer publications by Powell et al., Richard T. Haasch, Charles S. Fadley for in depth details on theory, instrumentation and quantification methods of XPS [181–183].

4.1.4. Applications

XPS has been predominantly reported for identification of impurities and determination of surface chemistry of crystal facets, APIs, solid forms, and powders for inhalation.

4.1.4.1. Crystal structure analysis. The surface chemistry and energetics of crystal facets can demonstrate anisotropic behavior due to differential growth rate along a particular axis. Heng et al., studied

Table 6
Specifications of an XPS instrument [178–180].

Parameters	Value
X-ray source	Monochromatic aluminium (Al); Magnesium (Mg); Zirconium (Zr), Dual source (Mg/Al)
Energy range	Mg source- 0–1253.3 eV; Monochromated Al source: 0–1486.6 eV; Zr source: 0–2042.4 eV
X-ray resolution	20.0 μ m
Energy resolution	≤ 0.50 eV
Ultra high vacuum	$\sim 10^{-9}$ Torr
Smallest analytical area	5–10 μ m
Sample heating	<i>In situ</i> upto 800 $^{\circ}$ C
Sample dimension	≤ 25 mm $\times$ 12 mm (diameter $\times$ height); with <i>in situ</i> sample tilting (x, y and z) for near surface depth analysis, sample thickness 1–20 mm
Elements detected	Li–U with chemical bonding information
Imaging/Mapping	Yes
Quantitative Depth profiling	Yes, using Ar $^{+}$, upto depths of 20–200 $\text{\AA}$

crystal facets sliced from a macroscopic paracetamol crystal for surface chemistry. XPS data, along with predicted crystallographic data and contact angle measurements implied that wettability of paracetamol was facet specific and was determined by the number of hydroxyl groups present on the crystal surface [184]. In another study by Heng et al., anisotropic wettability and surface energy of aspirin was demonstrated to be anisotropic and facet dependent due to carboxylic acid group [76]. Likewise, Ho et al., identified differential distribution of hydroxyl groups on crystal facets of D-mannitol that resulted into anisotropic surface energetics and wettability [75].

Modi et al., also demonstrated differential surface chemistry of acicular and plate shaped crystal habits of celecoxib. The scientific evidence gained from XPS and crystallographic studies revealed presence of higher % of hydrophilic groups (O, S, N) on surface of plate shaped crystals. The results correlated well with significantly enhanced dissolution and oral bioavailability of plate shaped crystal habit over acicular crystals [5].

4.1.4.2. Solid form characterization. Puri et al., demonstrated differences in surface chemistry of crystalline and amorphous celecoxib using XPS. A quantitative surface analysis of the two solid forms was performed for specific functional groups in celecoxib such as CF_3 , SO_2 and N of pyrazole rings. As the relative contribution of these functional groups differed, the amorphous form (O groups) had hydrophilic character while the crystalline celecoxib was more hydrophobic (CF_3) at surface. These differences in surface polarity extrapolated into superior wetting of amorphous celecoxib by water, whereas, the crystalline celecoxib displayed good wettability with non polar solvents [21].

In a similar context, Laad et al., elucidated differential surface chemistry in case of four commercially available Telmisartan (TMS) samples. The four samples displayed different values of surface polarity index, expressed as (oxygen + nitrogen) to (carbon) atomic concentration ratio. TMS 2 exhibited higher surface hydrophilicity whereas, TMS 1, 3 and 4 were comparatively more hydrophobic on the surface [87].

4.1.4.3. ASDs. XPS has been reported extensively for studying phase separation in ASDs. XPS enables qualitative and quantitative measurement of surface composition with superior surface sensitivity and spatial resolution [9,185,186]. Chen et al., studied phase separation in ASD of an undisclosed molecule, BMS 817399 and PVP using XPS. The surface composition was determined from ratio of peaks obtained from elements Cl present only in drug molecule and N present in both drug and PVP. The surface composition of initial samples was close to theoretical drug loading of 40% in ASD. In contrast, on exposure to 95%RH, surface drug concentration increased to 69%. The researchers could attribute surface enrichment of ASD to hydrophobic nature of the drug that diffused to solid air interface on phase separation [187].

Song et al., investigated potential drug polymer interactions in ASD of lapatinib and gefitinib prepared with polystyrene sulfonic acid using multitude of techniques such as Ultraviolet Spectroscopy (UV), Fourier Transform Infrared Spectroscopy (FTIR), XPS, ^{13}C and ^{15}N ssNMR. XPS and ssNMR provided conclusive evidence regarding protonation of basic nitrogen atoms in lapatinib and gefitinib. XPS could not differentiate aliphatic secondary amine NH and aniline NH in lapatinib freebase; thus, ssNMR was used as complimentary tool to identify protonation state at atomic level to provide insights on stability of ASDs [188].

4.1.4.4. DPLs. XPS can provide quantitative measure of elemental composition of surface coating of powders. For example, Zhou et al., investigated chemical properties of lactose powder surface coated

with magnesium stearate. XPS detected high surface coverage of magnesium on lactose powders up to a few nanometers depth [127]. Similarly Wang et al., demonstrated surface coating of colistin with rifampicin by detecting C, N, O and S elements [189]. In another study Zhou et al., quantified % coverage of azithromycin on colistin particles to demonstrate reduced moisture sorption due to increase in hydrophobicity at powder surface [190]. In a recent study by Lu et al., XPS was used to determine chemical composition of spray dried simvastatin powders co spray dried with amino acids. As simvastatin does not contain element N, the element N was specific to leucine. Based on atomic concentration of element N, XPS analysis revealed that the surface of the spray dried particles was leucine rich [191].

4.1.4.5. Tablet characterization. Felton et al., quantified interfacial film thickness of coated tablets by coupling ion depth profiling and XPS. The marker elements identified were phosphorous in dicalcium phosphate core tablet and chlorine in Eudragit® RL 30D coating. Interfacial thickness was determined from the region where both phosphorous and chlorine were detected simultaneously [192].

4.2. X ray fluorescence (XRF)

4.2.1. Introduction

XRF is a useful qualitative and quantitative analytical tool for determining elemental composition of solids. Henry Moseley, called as father of XRF technique, discovered a mathematical equation to determine atomic number from emitted X ray frequency of the element. The technique offers rapid analysis with high accuracy and precision. XRF is capable of measuring layer thickness from nm to 100 μm with a spatial resolution of 600–700 nm and can identify and quantify (1–100 ppm) trace elements in complex matrices. In comparison to other methods for elemental analysis, XRF is compact, versatile, non destructive, less expensive and needs no sample preparation for analysis [193].

4.2.2. Instrument and parameters

A commercial XRF was launched in 1950. The typical XRF instrument consists of an X Ray source such as X Ray tube or synchrotron, filter, detector and software package. Based on type of detector, XRF instrument is classified into two types: energy dispersive and wavelength dispersive spectrometers. Former uses semiconductor type detector, which collects entire emitted energy from the sample and decodes it for individual atoms. It has higher signal throughput, which enables analysis of small area and can thus simultaneously identify and quantify all atoms present in the sample. In contrast, wavelength dispersive spectrometer uses crystals to disperse the emitted photons based on wavelength and adjusts the detector to a position to receive X rays of a particular energy. Its advantages include better resolution, high elemental specificity, sensitivity and minimal spectral overlaps [194].

The two instrument types offer several configurations, X ray sources, optics and detectors, which ultimately govern resolution, sensitivity and application of the instrument. The higher the energy of the incident rays, greater is the depth of X ray penetration in the sample. Similarly, in micro XRF, optics systems focus incident X rays to a spot as small as tens of micrometers for enhanced spatial resolution. Similarly in Total Reflection XRF, incident X ray beam is directed at very low angles of incidence and is primarily used to study thin films and quantification of very low levels, from pico to femtograms, of trace elements [194,195]. For greater details and advancements in XRF instrumentation, the interested readers can refer elaborate reviews available in literature [196,197].

4.2.3. Working principle

The principle of XRF primarily involves three steps: excitation, emission and measurement. On irradiation of atoms in the solid using primary X ray radiation, the absorption of incident energy leads to rearrangement of electrons in the atom. Rearrangement of electrons from outer to inner shells, generates secondary fluorescent X rays with energy/wavelength characteristic of the emitting atom. The detector and the data system record and build the X ray spectrum. The quantification of particular atom is done from intensity of the signal detected at its distinctive energy [193].

4.2.4. Applications

In literature, XRF has been used to detect elemental composition. Awad et al. used XRF for studying composition, purity and microstructure of differently prepared Kaolin powders [198]. In another study Shi et al., used fluorescent signal from sulfur atoms in ritonavir, to study phase separation in ASD. The researchers used *in situ* synchrotron X ray fluorescence (XRF) due to its high photon flux and sensitivity. Appearance of heterogeneity in sulfur distribution was used to correlate phase separation and drug dissolution from ASD [199].

In another study by Cahyadi et al., XRF was explored for measurement of coating thickness of tablets. Iron oxide, in the coat was used as the marker for analysis and the amount of iron was correlated to the amount of coating applied on the tablet surface. The general observation of the study was that XRF is useful for surface analysis of materials such as tablet coatings and its associated variability, when the molecular structure of coat contains elemental groups that can be detected by XRF spectroscopy. In this study, iron intensity could not be directly related to coat thickness and was instead used to obtain complementary information on efficiency of the coating process [200]. Junior et al., demonstrated the potential of XRF in deformation of drug products. XRF helped in identification of elemental constituents in surface coating as well as the inner tablet matrix [201]. Till date, the capabilities of XRF remain under explored for characterization of pharmaceutical solids, thus providing unique futuristic opportunities to demonstrate its strength.

5. Mass spectrometry based analytical technique for surface characterization

5.1. Time of flight secondary ion mass spectrometry (TOF SIMS)

5.1.1. Introduction

TOF SIMS is a combination of two sensitive analytical techniques for mapping and qualitative analysis of elemental and molecular composition of solid surfaces to varying depths (<2 nm up to 10 μm). TOF enhances surface sensitivity and detection limits to ppm or ppb range with a mass resolution of 0–2000 amu. The unique strengths of TOF SIMS are that it is extremely surface sensitive (outermost 1–3 monolayers), allows parallel analysis of entire range of elements and molecular species at the surface, ease of charge neutralization and precise spatial resolution (1000 nm) [202,203].

5.1.2. Instrument and parameters

TOF SIMS is operated in ultra high vacuum mode ($>>1 \times 10^{-9}$ Torr) to increase mean free path of the liberated ions. A particle gun particularly comprises of Ga, Au, Bi or Cs liquid metal cluster ion source because of their inert nature. For depth profiling and 3D analysis, dual beam mode is used, wherein the first pulsed beam of energy 2–22 keV (Ga^+ , Au^+ , Au_2^+ , Au_3^+ , C_{60}^+ , O_2^+) raster a crater on the surface (of size $10 \times 10 \mu\text{m}$ to $500 \times 500 \mu\text{m}$) and the second beam of energy 0.25–40 keV (Cs^+ , O_2^+ , Ar^+ , C_{60}^+)

progressively analyzes the crater bottom. Reactive species such as Cs^+ , O_2^+ are employed in depth profiling because of their reactivity and sensitivity for enhancing depth resolution and collection of three dimensional data. The other important parts of the instrument are a sample chamber with a 5 axis manipulator (x, y, z, rotation and tilt) for flexible navigation, charge neutralization system, sample heating and cooling mechanism (130 to $+600 \pm 1^\circ\text{C}$), a linear or circular flight path using electrostatic analyzers or reflecting mirrors to direct the particle beam and time of flight mass analyzer with negative and positive ion extraction modes. In addition, a secondary electron detector is used for parallel imaging (image resolution $<1 \mu\text{m}$) and mass detection. TOF SIMS is equipped with a computer interface for system control and analysis. It allows retrospective analysis of the spectra for chemical mapping of specific regions of interest, after the analysis is finished. The software generates a 4D raw data file (x, y, z, m) for every secondary ion to construct mass spectra, depth profiles and images [203,204].

Sample can be analyzed in form of pressed powder, film, fiber or particles. The foremost criteria for analysis in TOF SIMS is that the sample should remain stable in ultra high vacuum mode and free from even minute amounts of surface contaminants. Practically, sample can be decontaminated by nitrogen purge or light (<1 min) sputtering of the sample surface immediately before analysis. The sample diameter acceptable is 100 mm in any lateral direction with height not exceeding 25 mm. The instrument can handle up to 15 samples at a time due to high speed of analysis. TOF SIMS is primarily non destructive, however, depth profiling in dual beam mode is destructive as particles are removed from the surface [202].

5.1.3. Working principle

In TOF SIMS, focused beam of primary ions strikes the sample leading to sputtering of atoms, molecules or fragments from the few atomic layers of the surface. This is the manifestation of atomic collision and transfer of energy associated with primary ions to the surface atoms. The energy (keV) of the incident beam also governs the depth of penetration of primary ions into the sample surface. The energy of the atomic collision is sufficient to ionize (positive or negative) the surface atoms and overcome the surface binding energy, leading to their emission. The emitted ions are then accelerated into the time of flight mass analyzer [202,204].

A challenge associated with TOF SIMS is the analysis of huge amount of data and interpretation of every molecular ion peak in the mass spectra. In addition, quantification of elements using TOF SIMS is less feasible due to reactivity of organic compounds with primary ions, need for standards/calibration and complexity of spectra due to matrix effects [202,203].

5.1.4. Applications

Nevertheless, TOF SIMS has been extensively reported in literature for studying surface chemical composition.

5.1.4.1. DPIs. In an interesting study by Wang et al., effect of surface composition on aerosolization and dissolution was established for co spray dried colistin and rifampicin powders. TOF SIMS and XPS confirmed that rifampicin non uniformly covered the surface of particles even for the formulation with a mass ratio of colistin/rifampicin to 4:1. The hydrophobicity of rifampicin reduced moisture sorption without retardation of aerosolization or dissolution rate of colistin because of non uniform coating of rifampicin on the particle surface [189]. In another study by the same research group, TOF SIMS was used to study uniformity of azithromycin coating on spray dried colistin particles. TOF SIMS results confirmed that azithromycin occupied 96.5% of particle surface at concentration of 50% w/w in the formulation [190].

5.1.4.2. Characterization of ASDs. Similar to dry powders for inhalation, surface chemistry of ASDs is critical for their stability and performance. Bhujbal et al., demonstrated that a combination of XPS and TOF SIMS is useful for measuring chemical composition and surface heterogeneity with change of process parameters [185]. Bosselmann et al., also used TOF SIMS to probe molecular composition in the uppermost 1–3 monolayers of amorphous ketoprofen coated with hydrophobic plasma polymerized films. TOF SIMS results showed that coating was almost uniform except for few small areas of thin or missing coating. This observation was used to justify partial recrystallization in samples with thin coating layers, on stability [205].

5.1.4.3. Surface modifications. Zhou et al., investigated changes in chemical surface properties of a fine lactose powder processed by a mechanical dry coating with magnesium stearate. The chemical composition measured by TOF SIMS demonstrated homogeneous coverage of magnesium stearate on lactose particles [127].

5.2. Excipient characterization

Further, Bailey et al., performed depth profiling and imaging of multilayer polymer films comprising of alternating polystyrene (PS) and polyvinylpyrrolidone (PVP) layers. The depth profile data illustrated proportionate sample compositions up to depths of $\leq 15 \mu\text{m}$. The study demonstrated usefulness of TOF SIMS in studying imperfections and 3D chemical homogeneity within the polymer layers [206].

6. Miscellaneous technique for surface characterization

6.1. Isothermal calorimetry

6.1.1. Introduction

Calorimeters, is a broad category of instruments that typically measure the heat involved in a process. DSC, an established instrument in this category primarily heats the sample during measurement; is prominently used for identification and characterization of solid forms and study phase transitions. The calorimetric sensitivity of a basic DSC instrument is $\pm 0.5 \text{ mW}$, while the highly sensitive variants possess detection limit of $\pm 0.1 \mu\text{W}$ [207]. The second type of calorimeters is the isothermal calorimeters, implying they operate at a constant temperature and can measure (detection limit) heat change up to a “microwatt” and “nanowatt” range as a function of time. The sample is monitored *in situ*, the technique is non invasive, non destructive and non specific. Isothermal calorimetry is therefore a sensitive tool in thermodynamics and kinetic measurements. It is not *a priori* surface sensitive technique due to its non specific nature, however, it enables study of adsorption experiments using vapors to assess degree of crystallinity and surface amorphization [208,209].

6.1.2. Instrument and parameters

The core part of the instrument is a large oil bath that acts as a heat sink and prevents deflections in bath and calorimeter (temperature) due to thermal gradients in the room. The oil bath encloses the four separate twin type, heat flow calorimetric channels that comprise of a reference and a sample side. The instrument can be configured to accommodate 4 to 48 calorimeters that can be operated simultaneously and independently. The calorimeter and sample ampoule configurations, size, sensitivity and throughput can be read from reference number [210]. The programmable temperature range of the instrument is 4–150 °C, with rapid thermal equilibration. The temperature regulation unit utilizes thermistors that sense and continuously adjust the heating, cooling

and oil flow speed to allow temperature drift of less than $\pm 100 \mu^\circ\text{C}$ over 24 h. This enables performance of short (few hours) and long term (days and weeks) experiments with sensitivity and reliability. In addition, an accessory interface box provides flexibility of installing ancillary parts such as dry gas purge system, humidity, syringe pumps, stirring controls, mass flow controllers and titration modes. Advanced I/O module enables interfacing of up to three independent probes/sources, such as pH probe or light source and computer software [210]. Sample of interest can be in any physical form – solid, liquid or gas or combination, provided that it accommodates within the sample ampoule [208,209].

6.1.3. Working principle

Regardless of the mode of measurement, an isothermal calorimeter measures heat produced or consumed by any process in the sample. The sample is connected to a thermoelectric sensor on one side, while the other side exchanges heat (absorbed or released in the process) with the surrounding heat sink via semiconducting Peltier elements, such that the sample is maintained at a constant temperature. The reference is either kept empty or filled with an identical inert material in same quantity as the sample to reduce baseline noise by eliminating effects of even small thermostat fluctuations on the measured heat flow. The thermoelectric or heat flow sensor generates a potential difference over the sensor in response to a temperature difference between sample and reference side according to the Seebeck effect. The potential is detected as a voltage signal that is proportional to heat flow incurred in the sample. The instrumental output is the rate of heat change (dQ/dt) as a function of time. Heat evolved or absorbed at a specific time can be measured by integrating the heat flow curve [208–212].

6.1.4. Modes and methods

Broadly, the instrument can be run in three modes isothermal, step isothermal and scanning modes. In an isothermal experiment, the thermostat is maintained at a fixed pre programmed temperature while the heat absorbed or released by the sample is measured. In step isothermal mode, multiple isothermal temperature analysis can be performed within an experiment. Data is recorded continuously during step temperature change as well as isothermal dwells. In third mode, scanning rate as low as 2 °C/hr can be used to provide greater resolution of subtle phase transitions and thermal, physical and chemical equilibrium state of sample [210].

Isothermal calorimeter is equipped to perform three major types of experiments viz., (i) *isothermal gas, liquid or relative humidity (RH) perfusion calorimetry*, (ii) *solution calorimetry* and (iii) *isothermal titration calorimetry*. In *perfusion calorimetry*, gas or liquid is passed through or over the sample in a continuous mode. The mass flow controllers, user configurable stirring, injection capabilities and software controls ease experimental set up and flexibility to perform experiments under controlled environmental conditions. Likewise, in RH perfusion mode, saturated and dry vapors are mixed together to achieve a linear ramp or step change in RH over the sample. This is achieved through in built humidity chambers maintained at a particular temperature to ensure 100% saturation of vapor.

The perfusion calorimetry can be used to obtain information on amorphicity, stability, compatibility, adsorption/absorption, hydration and swelling [209,210].

RH perfusion calorimetry is performed for determination of surface amorphous content. The experiment can be performed in two ways (1) use closed ampoules sealed with microhygrostat (glass tube containing solvent saturated by salt) to obtain desired relative humidity or using mass flow controllers where the vapor (moisture or organic vapors) is passed continuously in increments

through the sample [209]. The selected vapor is such that it induces plasticization of the sample. At critical RH or vapor pressure, T_g falls below the experimental temperature, the sample crystallizes and the resulting heat released is quantitatively proportional to the amount of amorphous material. The quantification usually requires pure standards (amorphous and crystalline) to convert the heat data to % amorphous content. It can reliably detect and distinguish several events occurring during the process such as adsorption of water, molecular rearrangement in the sample, recrystallization, expulsion of water from the lattice and polymorphic transitions [208,210,211].

Solution or ampoule breaking calorimetry performs high precision measurements of heat change when solid or liquid is partly or completely dissolved in a solvent. The experimental measurement is in the form of enthalpy of solution ($\Delta H_{\text{solution}}$) that is the sum of enthalpy of crystal lattice dissolution ($\Delta H_{\text{lattice}}$) and enthalpy of solvation ($\Delta H_{\text{solvation}}$). The solid forms (crystalline, amorphous, polymorphs) differ in their $\Delta H_{\text{lattice}}$ and $\Delta H_{\text{solvation}}$ that enhances sensitivity of solution calorimetry for detection of small amounts of amorphous content, polymorph (pseudo polymorphs) screening and dissolution rate or heat of solution [210,213].

In *titration calorimetry*, one sample is added sequentially to another and the subsequent heat change is recorded. The sample can be a solid or powder, suspension, aqueous and organic solvents, biological or non biological sample. The vacuum/pressure ampoules can be employed to simultaneously monitor heat flow and changes in gas pressure (up to 10 Bar) in the ampoule. This is useful in studying volatile organic solutions and detecting small amounts of evolved gas in titration experiments. The applications of titration calorimetry include thermodynamic characterization of solid or liquid molecular interactions, ligand substrate binding profile, mixing enthalpy, dissolution kinetics, absorption and swelling, dose response drug sensitivity assays on viable living cells, characterizing enzymes and chemical kinetics [210,213].

O'Neill et al., have covered details on methods and calibration of instrument in their publication [213]. The calorimetric experiments are prone to errors as the heat change measured is subtle and non specific. The interested readers can refer a publication by Wadso et al., to understand the nature and effects of errors in isothermal calorimetry [214].

6.1.5. Applications

Over the last thirty years, Isothermal calorimetry has been used for characterization of surface properties of powders such as adsorption and detection of low levels of amorphous content.

6.1.5.1. Process induced disorder. Over the years, Isothermal calorimetry has been established as a versatile tool for quantification of low levels of amorphous content. Gaisford et al., also quantified amorphous content using isothermal gas perfusion calorimetry in ball milled salbutamol sulphate. The information was used for optimization of ball milling process to achieve desired particle size while maintaining the crystal lattice [6,40,215,216]. Sebbatu et al., first evaluated microcalorimetry for assessment of crystalline lactose with varying degrees of disorder. The technique captured heat flow from crystallization of amorphous content on exposure to a humidity. It could detect at least 2% disorder in the sample, while comparative analysis using PXRD and DSC had accurate detection over 10% [217]. Similarly, Briggner et al., could quantify amorphous content with resolution of 1%. In addition, the polymorphic change of amorphous lactose to α and β lactose could be monitored [218].

Since, isothermal calorimetry is non specific, every process (desorption, absorption, crystallization etc.) occurring during the experiment is recorded. Darcy et al., further explored the basis of

net heat change (enthalpy of crystallization) observed in calorimetric experiments on exposure of lactose to humidity for the quantitative assessment of the amorphous content [219]. Besides, Hogan et al., highlighted that pre equilibration of the sample to a temperature at which crystallization occurs is essential. Consequently, the researchers evaluated solution calorimetry to detect and quantify small amounts of amorphous content to minimum limit of 0.5%. The methodology could differentiate between samples of varying degrees of residual moisture content and the results illustrated that sample storage impacted the enthalpy of solution. A greater accuracy in quantification of amorphous content can only be achieved if samples are maintained in a controlled and dry environment [220].

Dilworth et al., further improved the methodology and attempted to establish a standard protocol for reproducible measurement of heat change or enthalpy of crystallization. They suggested ways to eliminate discrepancies associated with competitive exothermic and endothermic processes occurring in the sample that are detected in the calorimetric response as artifacts. For accurate and reproducible measurement, the researchers suggested three critical considerations (i) crystallization should be slow to enable better experimental observation and resolution, (ii) integrate all the events, instead of measuring only the crystallization response since the events overlap and are difficult to deconvolute and (iii) a negative deviation from linear response could be the result of polymorphic transitions [111].

Samra et al., used a combination of isothermal calorimetry and DVS to study crystallization of a hydrophobic drug terfenadine in presence of humidity and organic vapors, as the two techniques operate under equilibrium conditions. The crystallization induced by moisture on the surface was self-limiting, preventing further ingress of moisture into the bulk and thus crystallization stopped. A mixture of 1:1 ethanol: propanol induced complete crystallization as indicated by the fact that the crystallized samples attained enthalpy of fusion similar to the original crystalline material [44].

One study by Katainen et al., evaluated the effect of solubility of a sample on solution calorimetry measurements. A correlation was established between heat of solvation of lactose partially dissolved in non aqueous solvents and the amorphous content of the sample. The study clearly demonstrated that partial solubility over total dissolution of a sample in a solvent is sufficient for the determination of amorphicity by solution calorimetry. However, complete insolubility of sample in a solvent would be undesirable for calorimetric measurement [221].

Similarly, in a recent study by Puri et al., RH perfusion micro calorimeter was used to investigate surface disorder on milling and conditioning at different combinations of temperature and %RH. As expected, the freshly micronized sample showed maximum enthalpy of recrystallization in comparison to the unmiconized drug. On annealing, researchers witnessed reduction in enthalpy of recrystallization as a function of storage time. They demonstrated that the surface disorder in predominantly crystalline material reorganized under influence of storage temperature and humidity [121].

6.1.5.2. Sorption studies. The RH perfusion assembly allows measurement of heat of interaction of water vapor sorption on powder samples. The sensitive calorimetric measurement can aid in monitoring the crystallization process, quantify vapor interaction, study batch variations and wetting phenomenon [222,223]. Puri et al., performed comprehensive analysis of crystalline and amorphous celecoxib to differentiate their surface properties using plethora of characterization tools. Isothermal Calorimetry was especially useful in studying vapor sorption processes and distinguish the solid forms based on heat of adsorption. The crystalline

sample showed no measurable response due to minimal surface adsorption while the amorphous form absorbed water into the bulk. Therefore, based on heat change values affinity of moisture towards solid form and surface chemistry could be predicted [21].

Hirschberg et al., studied kinetics of water sorption behavior of excipients upon exposure to humidity. The insights into the nature of water sorption process can be used for deciding optimal processing and storage conditions for excipients. Authors propose to use complimentary techniques to understand the impact of different storage environments on powder flowability for improved handling and processing [224].

6.2. Electron diffraction (ED)

Electron diffraction (ED) employs a beam of electrons to produce diffraction patterns from a sample of interest. Owing to little penetration of the electron beam (in comparison with X rays used in X ray diffraction), ED is a powerful tool in probing atomic details on surface (surface chemistry) of a material. ED data is acquired in a Transmission Electron Microscope (TEM) instrument, wherein the electron beam passes through a thin film of the sample. The sample is tilted to the incident electron beam to obtain diffraction patterns from several orientations of a nano sized crystal. This approach of scanning the sample on available three dimensional reciprocal space can be called as '3D Electron Diffraction' [225]. ED can be collected with a TEM operated with beam energy of 120–300 kV generated using LaB6/W electron gun or Field Emission Gun (FEG) filament. Two most common modes used are 'Selected area electron diffraction' or 'Nanobeam diffraction' mode [225,226].

The interaction of electron beam with material may cause structural damage to beam sensitive samples. Therefore, a low dose, small, semi parallel electron beam is particularly suitable for beam sensitive materials. In selected area electron diffraction mode, a low electron dose rate of $\leq 10\text{e}^-/\text{nm}^2\text{s}$ is used for an initial survey of the region of interest on a sample surface. Once the region of interest is selected, selected area electron diffraction patterns can then be collected using an electron dose rate of $\approx 50\text{e}^-/\text{nm}^2\text{s}$ [227]. Additionally, the sample can be cooled to -180°C using a cryogenic sample holder filled with liquid nitrogen (using Cryogenic TEM), which reduces the rate of electron beam damage [228]. Interested readers can refer a recent publication by Gemmi and coworkers for further details on data collection protocol and structural refinement [225].

Understanding of three dimensional structure can provide information on functional group exposed on the surface of a material (Surface chemistry). Conventional XRD offers structural details

largely in bulk of the material (owing to its higher penetration depth) and the obtained structure averages over a large area. Consequently, local structural variations may not be observed in conventional XRD. On the other hand, ED is a technique that can be used to determine the structure of individual clusters, individual nanoparticle or nanoscale crystals. Interestingly, the structure of a cluster or a grain with the size of 10–100 nm deposited on the surface of a larger crystal can be solved using ED. Moreover, crystal structure of polycrystalline and polyphasic materials can be determined using ED, which cannot be solved using conventional XRD [229].

Despite the promising applications of ED in structural and surface characterization of varieties of materials (eg. minerals, metal organic framework, Zeolites, Proteins), the technique has been underutilized in the surface characterization of pharmaceutical solids. Recently, Ricarte et al., determined a small fraction of crystalline regions in amorphous solid dispersion of griseofulvin and hydroxypropyl methylcellulose acetate succinate, using cryogenic transmission electron microscope. The solid dispersion was thermally annealed *in situ* in TEM grid to assess its sensitivity for crystalline domains in amorphous solid dispersion. The technique was able to detect a nanosize crystal of griseofulvin ($\sim 300\text{ nm}$) that grew in the annealed amorphous dispersion with help of the electron diffraction pattern of griseofulvin [227].

In another study, x ray amorphous extrudate of a 1:1 blend of indomethacin (IDM) and polyvinylpyrrolidone/vinyl acetate copolymer (PVPVA) was prepared using hot melt extrusion (HME). The HME processing conditions were such that there is a significant risk of residual crystallinity if insufficient time or mixing conditions are provided. The prepared the extrudate was found to be amorphous by XRPD and DSC. On the other hand, the electron diffraction pattern (collected using TEM) was successfully employed to detect very low levels of nanometer scale residual crystallinity within a nominally amorphous extrudate [230]. In this way, ED in nanoscale crystallinity analysis served as surface as well as bulk level characterization tool, because the nano domains are typically present within a bulk and on the surface of materials.

6.3. Faraday Pail

Solid surface including pharmaceutical solid can acquire charge in many ways such as corona charging, triboelectrification, spray charging and charging by mechanical fracture of material. Amongst this, tribocharging or triboelectrification is a commonly observed phenomenon in pharmaceutical solids [231]. During common unit operations like micronization, sieving, blending, powder transfer

Table 7

Summary of surface properties and orthogonal analytical techniques used for their characterization.

Surface property	Techniques used
Solid form characterization (polymorphs, hydrates, anhydrides, amorphous forms)	SSA, DVS, isothermal calorimetry, IGC, contact angle measurement, XPS
Drug-Excipient- moisture interactions (hygroscopicity, water sorption tendency, stability, surface reactivity, compatibility, phase transformation)	SSA, DVS, DVS-NIR, DVS-RS, contact angle measurement, IGC, AFM, Isothermal calorimetry
Wettability	SSA, contact angle measurement
Surface defects and amorphous content	SSA, DVS, DVS-NIR, IGC, AFM, Isothermal calorimetry
Surface crystallization	SSA, DVS, DVS-NIR, DVS-RS, AFM, Isothermal calorimetry
Surface energy and heterogeneity	SSA, IGC, contact angle measurement, AFM
Surface topography, roughness	SEM, AFM
Gross surface chemistry and composition (crystal facets, powders)	DVS, Contact angle measurement, IGC, XPS, TOF-SIMS
Elemental composition of surface monolayer	SEM-EDX, XPS, TOF-SIMS, TOF-SIMS, ISS
Particle-particle interactions	SSA, AFM, IGC
Surface modification and coating, adhesion	Contact angle measurement, IGC, AFM, XPS, TOF-SIMS
Surface characterization of tablets and tooling	XPS, XRF, AFM
Mechanism of drug release and dissolution rate	SSA, DVS, DVS-NIR, XRF, XPS
Formulation screening and optimization (selection of binder, granulation, blending/mixing)	DVS, contact angle measurement

Table 8

Summary of analytical techniques for surface characterization of pharmaceutical solids.

Technique	Sample nature	Sample weight	Qualitative/ Quantitative	Non-destructive/ Destructive	Detection limit	Information obtained	Advantages	Limitations
SSA	Powder	50–100 mg	Both	Non-destructive	0.1 m ² /g	m ² /g specific surface area	Wide applications; first step in surface characterization	Conditioning should be carefully performed
DVS	Powder, films	Few mg to g	Both	Non-destructive ^a (Refer section 2.2.3)	1 mg ± 0.1 µg	Sorption/desorption isotherm	Wide applications	Extensive efforts in quantitative method development
Contact angle measurement	Powders, Pellets	100–300 mg	Both	Destructive		Wettability, spreading parameter, dispersive and polar components of surface energy	Ideal for wetting studies	Not an ideal method for powders and determination of surface energy
IGC	Powders	Few mg to g	Both	Non-destructive		Dispersive, polar and acid base parameters of surface energy	Ideal for powders and determination of surface energy	Selection and method development challenging, time consuming
SEM	Powders, pellets, films	Few mg	Qualitative	Non-destructive		Surface morphology	Wide applicability	Small sample spot size
AFM	Powders, pellets, films, intact tablets	Few mg	Both	Non-destructive [†] (refer section 3.3.4)		Surface topography, roughness, cohesive-adhesive forces	Wide applicability	Complicated experimental set up and time consuming
XPS	Powders, pellets, films	Few mg	Both	Non-destructive [#] (refer section 4.1.3)	0.1% atomic weight	Elemental composition, mapping, spatial distribution, depth analysis	Surface sensitive	Bulky, need complementary techniques for assessing chemical environment; X-ray is destructive to organic samples, matrix effects
XRF	Powders, pellets, films, tablets	Few mg	Both	Non-destructive	1–100 ppm	Elemental composition	Surface sensitive	Matrix effects
TOF-SIMS	Powders, pellets, films	Few mg	Qualitative/ Semi-quantitative	Non-destructive [#] (refer section 5.1.2)	ppm–ppb, 0–2000 atomic mass unit	Elemental and molecular composition	Surface sensitive (2 nm–10 µm), detects entire range of elements	Surface contamination deteriorates quality of data, reactivity with organic samples
Isothermal calorimetry	Powders	200 mg	Both	Non-destructive ^a (refer section 6.1.4)	1 µV	Heat change	Sensitive	Non-specific

^a “Few mg” represents sample weight less than 100 mg.

through hoppers, the relative movements of particles and particles collision with surface lead to a generation of charge known as 'Triboelectric charge'. During pharmaceutical unit operation, the relative forces of attraction and repulsion that exist between charged particles can cause adhesion of the material to the wall of processing equipment and agglomerate formation, resulting into failure of the operation and process [232]. Particularly, it is very important to monitor and control the charge of powders in the development of dry powder inhaler products [233,234]. Further, due to the inherent complexity of dry powder inhaler system, it is difficult to assign the influence of a single factor such as charge level/sign on the product performance. Nonetheless, net charge on material is often measured using Faraday Pail (Also known as Faraday Cup). Net charge means the sum of electrostatic charges carried by a powder sample. The net charge on a powder material may be electropositive, electronegative, or neutral.

Faraday pail consists of two cups, outer and inner, which are constructed from a conducting material (e.g. metal). These cups are placed on top of each other separated using an insulating material such as polytetrafluoroethylene (Teflon). Powder of interest is poured into inner cup (measuring cup) while the outer cup serves as a protector by minimizing interference from external factors. The outer cup is grounded to earthing and the inner cup consists of an electrometer with a known capacitor. The charged powder of interest is poured into the inner cup, then the charges present on the powder induces a charge of same magnitude but opposite sign on the inner cup. These induced charges are thus measured in the inner cup using the electrometer [235,236].

Measurement of dynamic charge of material can provide a more realistic picture on charging behavior of a material under the influence of contact material, surface contaminations and airflow rate. The charge of powder in a dynamic state can be measured by modified 'open end Faraday cup'. Working principle of open end Faraday cup is similar to normal faraday except that both ends of cups are open for the purpose sucking the air through [236]. The open end faraday cup is frequently used in evaluation of dry powder inhalation products, as it can measure the charge carried by the aerosolized particles after actuated from an inhaler. Murtomaa et al., used open end faraday cup technique to show that different inhalers and different morphologies of carrier lactose particles have a significant impact on the degree of charge generated by the actuated particles [237]. It is noteworthy that, the electrostatic charging is sensitive to many external factors such as temperature, relative humidity and atmospheric pressure [238]. Therefore, control of environmental conditions during the measurement is necessary for reducing measurement variability.

6.4. Predictive computational tools

In addition to analytical tools, computational methods such as molecular dynamics simulations, attachment energy (AE) calculations, and Monte Carlo simulations have been reported for studying surface characteristics of organic molecular crystals [239,240]. These methodologies commonly utilize single crystal structural data as 'input' for computational calculations. From the reported studies, it is apparent that the predictive computational tools served as an indirect approach towards the characterization of surface properties. Chan et al., reported use of molecular dynamics simulation for investigating molecular interaction at the surface of crystalline materials and found its application in understanding the role of solvent in crystal growth [241].

Similarly, Kale et al., investigated the molecular basis for water sorption tendency of three crystal systems (rivaroxaban, malonic acid and its cocrystal). To understand the role of interaction between water molecules and surface exposed chemical group

present on each crystal facet, it is important to identify the available hydrogen bonding sites in a given crystal structure. Therefore, molecular dynamics simulation calculations were performed to identify the hydrogen bonding sites that are accessible for interaction with H₂O molecules in the crystal structure of the three samples [242].

Predicting crystal morphology to understand the molecular arrangement on the crystal surface (surface molecular environment) using Bravais Friedel Donnay Harker (BFDH) theory is a simplest computational operation, which can be performed using Mercury Software (offered by Cambridge Crystallographic Data Centre, Cambridge, UK). Interestingly, Modi et al., utilized BFDH calculations together with XPS analysis for studying the molecular environment on crystal surfaces of two different crystal habits of celecoxib [243].

7. Summary of surface properties and analytical techniques for their characterization

The review discussed the instrumentation, methods, principle and applications of various analytical techniques for surface characterization of pharmaceutical solids. The analytical capabilities of these techniques are utilized in pre formulation to formulation screening and optimization stages, because surface properties of a solid further govern wettability, dissolution, compatibility and stability of the product. The surface properties of pharmaceutical solids that have been extensively studied using the aforementioned techniques are compiled in Table 7.

Each analytical technique offers unique information (Refer Table 8) and access surface to a varying depth, thus, an orthogonal assessment is generally performed. The qualitative and quantitative composition of a solid surface can be determined from spectroscopic techniques such as SEM EDX, XPS, XRF, TOF SIMS and ISS. Likewise, for determination of surface defects and amorphous content, techniques such as DVS and isothermal calorimetry are preferred. Simultaneously, these techniques facilitate and monitor *in situ* conditioning and recrystallization of surface amorphous content. For determination of surface energy, IGC is the method of choice over AFM and contact angle measurement. In contrast, contact angle measurement is extremely useful for studying surface chemistry, wettability and adhesion properties. AFM, on the other hand, provide details on surface topography, roughness and particle particle interactions. Table 7 shall assist formulation scientists to select appropriate analytical technique to assess desired surface properties of a solid.

8. Conclusions

A multitude of analytical techniques are available for studying surface properties of pharmaceutical solids. The current review captured comprehensive details on instrumentation, methodology and applications of these techniques in surface characterization. Every technique has strengths and limitations and it is imperative to adopt a set of orthogonal tools to understand the surface behavior of a solid. A thorough understanding of surface properties can eliminate risk factors such as batch to batch variation, supplier to supplier variation and ensure uniformity of drug product performance and stability. As time is an invaluable asset for pharmaceutical organizations, the use of screening and optimization tools to determine product performance and stability are extremely useful. The methodology and the instrumentation of these analytical techniques have progressed with time to provide superior sensitivity, specificity and reproducibility. The pharmaceutical organizations continually strive to develop API and material characterization 'tool kits' for better understating of product

performance. The paper provides a strong knowledge base for mechanistic understanding of surface properties and their implications to facilitate robust product development.

Author contributions

Conceptualization, AKB; writing —original draft preparation, Abstract, Introduction, DVS, DVS NIR, DVS RS, contact angle measurement, IGC, AFM, XPS, XRF, TOF SIMS, Isothermal calorimetry, AK; writing —original draft preparation, SSA, SEM, SEM EDX, DPK, writing —review and editing, Abstract, Introduction, DVS, DVS NIR, DVS RS, contact angle measurement, IGC, AFM, XPS, XRF, TOF SIMS, Isothermal calorimetry AK, writing —review and editing SSA, SEM, SEM EDX, ED, Faraday Pail, Predictive computational tools, DPK; review and editing AKB; supervision, AKB; project administration, AKB. All authors have read and agreed to the revised version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- [1] E. Hadjittofis, et al., Influences of crystal anisotropy in pharmaceutical process development, *Pharmaceut. Res.* 35 (5) (2018) 100.
- [2] N. Chieng, T. Rades, J. Aaltonen, An overview of recent studies on the analysis of pharmaceutical polymorphs, *J. Pharmaceut. Biomed. Anal.* 55 (4) (2011) 618–644.
- [3] G. Buckton, Surface characterization: understanding sources of variability in the production and use of pharmaceuticals, *J. Pharm. Pharmacol.* 47 (4) (1995) 265–275.
- [4] G. Buckton, H. Gill, The importance of surface energetics of powders for drug delivery and the establishment of inverse gas chromatography, *Adv. Drug Deliv. Rev.* 59 (14) (2007) 1474–1479.
- [5] S.R. Modi, et al., Impact of crystal habit on biopharmaceutical performance of celecoxib, *Cryst. Growth Des.* 13 (7) (2013) 2824–2832.
- [6] V.-P. Lehto, et al., The comparison of seven different methods to quantify the amorphous content of spray dried lactose, *Powder Technol.* 167 (2) (2006) 85–93.
- [7] J. Feeley, et al., Determination of surface properties and flow characteristics of salbutamol sulphate, before and after micronisation, *Int. J. Pharm.* 172 (1–2) (1998) 89–96.
- [8] S. Sheokand, S.R. Modi, A.K. Bansal, Quantification of low levels of amorphous content in crystalline celecoxib using dynamic vapor sorption (DVS), *Eur. J. Pharm. Biopharm.* 102 (2016) 77–86.
- [9] Z. Chen, et al., Surface enrichment and depletion of the active ingredient in spray dried amorphous solid dispersions, *Pharmaceut. Res.* 35 (2) (2018) 38.
- [10] X. Liu, et al., Characterization of amorphous solid dispersions, *Journal of Pharmaceutical Investigation* 48 (1) (2018) 19–41.
- [11] X.M. Zeng, Particulate analysis – particle size, *Solid State Characterization of Pharmaceuticals* (2011) 387–425.
- [12] M. Thommes, et al., Physisorption of gases, with special reference to the evaluation of surface area and pore size distribution (IUPAC Technical Report), *Pure Appl. Chem.* 87 (9–10) (2015) 1051–1069.
- [13] Pharmacopoeia, U.S. General chapter-846 specific surface area [cited 2020 16 August 2020]; Available from, https://www.usp.org/sites/default/files/usp/document/harmonization/gen-chapter/g11_pf_30_4_2004.pdf.
- [14] Monosorb brochure - Quantachrome instruments [cited 2020 16 August 2020]; Available from, https://www.quantachrome.com/pdf_brochures/07109.pdf.
- [15] Particle surface area [cited 2020 17 August 2020]; Available from, <http://nanocastki.pl/file/2018/07/acorn-area-brochure-en.pdf>.
- [16] S. Brunauer, P.H. Emmett, E. Teller, Adsorption of gases in multimolecular layers, *J. Am. Chem. Soc.* 60 (2) (1938) 309–319.
- [17] S. Balbach, C. Korn, Pharmaceutical evaluation of early development candidates “the 100 mg-approach”, *Int. J. Pharm.* 275 (1–2) (2004) 1–12.
- [18] Patient information leaflet for invega Sustenna [cited 2020 7 September 2020]; Available from, https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/022264s0231bl.pdf.
- [19] M.K.J. François, W.M.A.C. Dries, E.D.G. Basstanie, Aqueous Suspensions of Submicron 9-hydroxyrisperidone Fatty Acid Esters, Google Patents, 2003.
- [20] A. Zimmermann, et al., Influence of the solid form of siramesine hydrochloride on its behavior in aqueous environments, *Pharmaceut. Res.* 26 (4) (2009) 846.
- [21] V. Puri, et al., Wettability and surface chemistry of crystalline and amorphous forms of a poorly water soluble drug, *Eur. J. Pharmaceut. Sci.* 40 (2) (2010) 84–93.
- [22] A. Almaya, A. Aburub, Effect of particle size on compaction of materials with different deformation mechanisms with and without lubricants, *AAPS PharmSciTech* 9 (2) (2008) 414–418.
- [23] R.R. Vippagunta, et al., Application of surface area measurement for identifying the source of batch-to-batch variation in processability, *Pharmaceut. Dev. Technol.* 14 (5) (2009) 492–498.
- [24] R.A. Storey, I. Ymén, *Solid State Characterization of Pharmaceuticals*, John Wiley & Sons, 2011.
- [25] G. Buckton, A. Beezer, J. Newton, A vacuum microbalance technique for studies on the wettability of powders, *J. Pharm. Pharmacol.* 38 (10) (1986) 713–720.
- [26] M.S. Bergren, An automated controlled atmosphere microbalance for the measurement of moisture sorption, *Int. J. Pharm.* 103 (2) (1994) 103–114.
- [27] S. Sheokand, S.R. Modi, A.K. Bansal, Dynamic vapor sorption as a tool for characterization and quantification of amorphous content in predominantly crystalline materials, *J. Pharmaceut. Sci.* 103 (11) (2014) 3364–3376.
- [28] Surface Measurement Systems, Dynamic dual vapor/gas gravimetric sorption analyzer [cited 2020 25 June]; Available from, https://www.surfacemeasurementsystems.com/wp-content/uploads/2017/08/DVS_Vacuum.pdf, 2020.
- [29] Surface Measurement Systems, DVS microscope [cited 2020 25 June 2020]; Available from, <https://sandbox.surfacemeasurementsystems.com/dvs-microscope/>.
- [30] United States Pharmacopoeia <1241>, Water solid interactions in pharmaceutical systems, Available from, https://www.usp.org/sites/default/files/usp/document/harmonization/gen-chapter/g15_pf_36_4_2010.pdf, 2016.
- [31] Surface Measurement Systems, Dual vapor gravimetric sorption analysis [cited 2020 25 June]; Available from, <https://www.surfacemeasurementsystems.com/wp-content/uploads/2014/05/DVS-Resolution-brochure-v1.0.pdf>, 2020.
- [32] Surface Measurement Systems, Dynamic gravimetric vapor sorption analyzer [cited 2020 25 June]; Available from, https://www.surfacemeasurementsystems.com/wp-content/uploads/2014/05/DVS-IntrinsicPlus_E-brochure.pdf, 2020.
- [33] J.Y. Heng, D.R. Williams, *Vapour Sorption and Surface Analysis, Solid State Characterization of Pharmaceuticals*, 2011, pp. 245–285.
- [34] Ta instruments, Vapor sorption analysis [cited 2020 25 June 2020]; Available from, <https://www.tainstruments.com/wp-content/uploads/Q-Sorption-brochure-2019-Web.pdf>.
- [35] A.W. Newman, S.M. Reutzel-Edens, G. Zografi, Characterization of the “hygroscopic” properties of active pharmaceutical ingredients, *J. Pharmaceut. Sci.* 97 (3) (2008) 1047–1059.
- [36] Z. Zhao, et al., Low density, good flowability cyclodextrin- α -D-glucopyranoside binary carrier for dry powder inhaler: anti-hygroscopicity and aerosolization performance enhancement, *Expet Opin. Drug Deliv.* 15 (5) (2018) 443–457.
- [37] V.A. Lovikka, L. Rautkari, T.C. Maloney, Changes in the hygroscopic behavior of cellulose due to variations in relative humidity, *Cellulose* 25 (1) (2018) 87–104.
- [38] M. Yamauchi, et al., Contrasting the surface and bulk properties of anhydrate and dehydrated hydrate materials, *Cryst. Growth Des.* 11 (3) (2011) 692–698.
- [39] J. Shur, et al., Effect of processing history on the surface interfacial properties of budesonide in carrier-based dry-powder inhalers, *Ther. Deliv.* 4 (8) (2013) 925–937.
- [40] G. Buckton, P. Darcy, Assessment of disorder in crystalline powders – a review of analytical techniques and their application, *Int. J. Pharm.* 179 (2) (1999) 141–158.
- [41] G. Buckton, Characterisation of small changes in the physical properties of powders of significance for dry powder inhaler formulations, *Adv. Drug Deliv. Rev.* 26 (1) (1997) 17–27.
- [42] D. Burnett, F. Thielmann, J. Booth, Determining the critical relative humidity for moisture-induced phase transitions, *Int. J. Pharm.* 287 (1–2) (2004) 123–133.
- [43] L. Mackin, et al., Quantification of low levels (< 10%) of amorphous content in micronised active batches using dynamic vapour sorption and isothermal microcalorimetry, *Int. J. Pharm.* 231 (2) (2002) 227–236.
- [44] R.M. Samra, G. Buckton, The crystallisation of a model hydrophobic drug (terfenadine) following exposure to humidity and organic vapours, *Int. J. Pharm.* 284 (1–2) (2004) 53–60.
- [45] P.M. Young, et al., The use of organic vapor sorption to determine low levels of amorphous content in processed pharmaceutical powders, *Drug Dev. Ind. Pharm.* 33 (1) (2007) 91–97.
- [46] T. Müller, et al., Measurement of low amounts of amorphous content in hydrophobic active pharmaceutical ingredients with dynamic organic vapor sorption, *Eur. J. Pharm. Biopharm.* 92 (2015) 102–111.
- [47] T. Müller, et al., Applicability of the one-step DVS method for the determination of amorphous amounts for further different hydrophilic and hydrophobic drugs, *Eur. J. Pharm. Biopharm.* 94 (2015) 333–341.
- [48] S. Zellnitz, et al., Crystallization speed of salbutamol as a function of relative humidity and temperature, *Int. J. Pharm.* 489 (1–2) (2015) 170–176.
- [49] R.R. Vippagunta, et al., Investigation of metformin HCl lot-to-lot variation on flowability differences exhibited during drug product processing, *J. Pharmaceut. Sci.* 99 (12) (2010) 5030–5039.

- [50] A. Paudel, J. Meeus, G. Van den Mooter, Structural characterization of amorphous solid dispersions, in: *Amorphous Solid Dispersions*, Springer, 2014, pp. 421–485.
- [51] Y. Guo, E. Shalae, S. Smith, Physical stability of pharmaceutical formulations: solid-state characterization of amorphous dispersions, *Trac. Trends Anal. Chem.* 49 (2013) 137–144.
- [52] S. Bianco, et al., Bulk, surface properties and water uptake mechanisms of salt/acid amorphous composite systems, *Int. J. Pharm.* 456 (1) (2013) 143–152.
- [53] B. Saša, et al., Analysis of surface properties of cellulose ethers and drug release from their matrix tablets, *Eur. J. Pharmaceut. Sci.* 27 (4) (2006) 375–383.
- [54] N. Veronica, C.V. Liew, P.W.S. Heng, Insights on the role of excipients and tablet matrix porosity on aspirin stability, *Int. J. Pharm.* (2020) 119218.
- [55] R.A. Lane, G. Buckton, The novel combination of dynamic vapour sorption gravimetric analysis and near infra-red spectroscopy as a hyphenated technique, *Int. J. Pharm.* 207 (1–2) (2000) 49–56.
- [56] S.E. Hogan, G. Buckton, The application of near infrared spectroscopy and dynamic vapor sorption to quantify low amorphous contents of crystalline lactose, *Pharmaceut. Res.* 18 (1) (2001) 112–116.
- [57] M.P. Feth, et al., New technology for the investigation of water vapor sorption induced crystallographic form transformations of chemical compounds: a water vapor sorption gravimetry dispersive Raman spectroscopy coupling, *J. Pharmaceut. Sci.* 100 (3) (2011) 1080–1092.
- [58] A.D. Gift, L.S. Taylor, Hyphenation of Raman spectroscopy with gravimetric analysis to interrogate water–solid interactions in pharmaceutical systems, *J. Pharmaceut. Biomed. Anal.* 43 (1) (2007) 14–23.
- [59] G. Buckton, Assessment of the wettability of pharmaceutical powders, *J. Adhes. Sci. Technol.* 7 (3) (1993) 205–219.
- [60] J.Y. Heng, F. Thielmann, D.R. Williams, The effects of milling on the surface properties of form I paracetamol crystals, *Pharmaceut. Res.* 23 (8) (2006) 1918–1927.
- [61] R. Holm, et al., Investigation of surface porosity measurements and compaction pressure as means to ensure consistent contact angle determinations, *Int. J. Pharm.* 498 (1–2) (2016) 355–361.
- [62] T. Huhtamäki, et al., Surface-wetting characterization using contact-angle measurements, *Nat. Protoc.* 13 (7) (2018) 1521–1538.
- [63] First ten angstroms [cited 2020 6 August 2020]; Available from, <https://www.firsttenangstroms.com/pro>.
- [64] G. Zografi, B.A. Johnson, Effects of surface roughness on advancing and receding contact angles, *Int. J. Pharm.* 22 (2–3) (1984) 159–176.
- [65] kruss-scientific, Drop shape analyzer [cited 2020 6 August 2020]; Available from, <https://www.kruss-scientific.com/products/drop-shape/dsa100/drop-shape-analyzer-dsa100/>.
- [66] kruss-scientific, Needle specifications [cited 2020 6 August 2020]; Available from, https://www.kruss-scientific.com/fileadmin/user_upload/website/brochures/kruss-bro-acc-dsa-needle-specifications-en.pdf.
- [67] Biolin Scientific, Optical tensiometer [cited 2020 6 August 2020]; Available from, <https://www.biolinscientific.com/attention/optical-tensiometers>.
- [68] R. Hebbat, A. Isloor, A. Ismail, Contact angle measurements, in: *Membrane Characterization*, Elsevier, 2017, pp. 219–255.
- [69] Y. Yuan, T.R. Lee, Contact angle and wetting properties, in: *Surface Science Techniques*, Springer, 2013, pp. 3–34.
- [70] L. Girifalco, R.J. Good, A theory for the estimation of surface and interfacial energies. I. Derivation and application to interfacial tension, *J. Phys. Chem.* 61 (7) (1957) 904–909.
- [71] E. Oh, P.E. Luner, Surface free energy of ethylcellulose films and the influence of plasticizers, *Int. J. Pharm.* 188 (2) (1999) 203–219.
- [72] S. Wu, Calculation of interfacial tension in polymer systems, in: *Journal of Polymer Science Part C: Polymer Symposia*, Wiley Online Library, 1971.
- [73] C. Van Oss, R. Good, M. Chaudhury, Additive and nonadditive surface tension components and the interpretation of contact angles, *Langmuir* 4 (4) (1988) 884–891.
- [74] G. Baki, et al., Role of surface free energy and spreading coefficient in the formulation of active agent-layered pellets, *Eur. J. Pharm. Biopharm.* 74 (2) (2010) 324–331.
- [75] R. Ho, et al., Determination of surface heterogeneity of d-mannitol by sessile drop contact angle and finite concentration inverse gas chromatography, *Int. J. Pharm.* 387 (1–2) (2010) 79–86.
- [76] J.Y. Heng, et al., Anisotropic surface chemistry of aspirin crystals, *J. Pharmaceut. Sci.* 96 (8) (2007) 2134–2144.
- [77] J.T. Korhonen, et al., Reliable measurement of the receding contact angle, *Langmuir* 29 (12) (2013) 3858–3863.
- [78] I. Ahmad, C.-w. Kan, A review on development and applications of bio-inspired superhydrophobic textiles, *Materials* 9 (11) (2016) 892.
- [79] A. Neumann, R. Good, Techniques of measuring contact angles, in: *Surface and Colloid Science*, Springer, 1979, pp. 31–91.
- [80] Y.-H. Kiang, et al., Crystal structure, crystal morphology, and surface properties of an investigational drug, *Int. J. Pharm.* 368 (1–2) (2009) 76–82.
- [81] T.H. Muster, C.A. Prestidge, Face specific surface properties of pharmaceutical crystals, *J. Pharmaceut. Sci.* 91 (6) (2002) 1432–1444.
- [82] R. Ho, et al., Wetting behavior of ibuprofen racemate surfaces, *J. Adhes.* 84 (6) (2008) 483–501.
- [83] V. Zupancić, et al., Physical characterization of pantoprazole sodium hydrates, *Int. J. Pharm.* 291 (1–2) (2005) 59–68.
- [84] T.H. Muster, C.A. Prestidge, Application of time-dependent sessile drop contact angles on compacts to characterise the surface energetics of sulfathiazole crystals, *Int. J. Pharm.* 234 (1–2) (2002) 43–54.
- [85] T. Jain, et al., Effect of differential surface anisotropy on performance of two plate shaped crystals of aspirin form I, *Eur. J. Pharmaceut. Sci.* 99 (2017) 318–327.
- [86] G. Shete, et al., Solid state characterization of commercial crystalline and amorphous atorvastatin calcium samples, *AAPS PharmSciTech* 11 (2) (2010) 598–609.
- [87] P. Laad, et al., Differential surface properties of commercial crystalline telmisartan samples, *Eur. J. Pharmaceut. Sci.* 49 (2) (2013) 109–116.
- [88] A. Bartkowiak, et al., Surface properties and morphology of selected polymers and their blends designed to mucoadhesive dosage forms, *React. Funct. Polym.* 118 (2017) 10–19.
- [89] L. Zajic, G. Buckton, The use of surface energy values to predict optimum binder selection for granulations, *Int. J. Pharm.* 59 (2) (1990) 155–164.
- [90] N.M. Ahfat, et al., Predicting mixing performance using surface energy measurements, *Int. J. Pharm.* 156 (1) (1997) 89–95.
- [91] D. Zhang, et al., Wettability of pharmaceutical solids: its measurement and influence on wet granulation, *Colloid. Surface. Physicochem. Eng. Aspect.* 206 (1–3) (2002) 547–554.
- [92] J. Saurí, et al., Relationships between surface free energy, surface texture parameters and controlled drug release in hydrophilic matrices, *Int. J. Pharm.* 478 (1) (2015) 328–340.
- [93] T. Liu, et al., Contact angle measurements: an alternative approach towards understanding the mechanism of increased drug dissolution from ethyl-cellulose tablets containing surfactant and exploring the relationship between their contact angles and dissolution behaviors, *AAPS PharmSciTech* 19 (4) (2018) 1582–1591.
- [94] S. Mohammadi-Jam, K. Waters, Inverse gas chromatography applications: a review, *Adv. Colloid Interface Sci.* 212 (2014) 21–44.
- [95] J. Santos, J.T. Guthrie, Analysis of interactions in multicomponent polymeric systems: the key-role of inverse gas chromatography, *Mater. Sci. Eng. R Rep.* 50 (3) (2005) 79–107.
- [96] J. Dove, G. Buckton, C. Doherty, A comparison of two contact angle measurement methods and inverse gas chromatography to assess the surface energies of theophylline and caffeine, *Int. J. Pharm.* 138 (2) (1996) 199–206.
- [97] I.M. Grimsey, J.C. Feeley, P. York, Analysis of the surface energy of pharmaceutical powders by inverse gas chromatography, *J. Pharmaceut. Sci.* 91 (2) (2002) 571–583.
- [98] A. Voelkel, et al., Inverse gas chromatography as a source of physicochemical data, *J. Chromatogr. A* 1216 (10) (2009) 1551–1566.
- [99] Surface measurement systems, Surface characterization [cited 2020 13 August 2020]; Available from, <https://www.surfacemeasurementsystems.com/wp-content/uploads/2014/05/IGC-SEA-brochure-v1.4.pdf>.
- [100] Surface Measurement Systems, Inverse gas chromatography-surface energy analyzer (IGC-SEA) [cited 2020 13 August 2020]; Available from, <https://www.surfacemeasurementsystems.com/products/igc-sea/>.
- [101] Surface measurement systems, Surface Energy [cited 2020 13 August 2020]; Available from, <https://sandbox.surfacemeasurementsystems.com/solutions/inverse-gas-chromatography-sea/>.
- [102] B. Shi, Y. Wang, L. Jia, Comparison of Dorris–Gray and Schultz methods for the calculation of surface dispersive free energy by inverse gas chromatography, *J. Chromatogr. A* 1218 (6) (2011) 860–862.
- [103] L. Lapčák, et al., Surface heterogeneity: information from inverse gas chromatography and application to model pharmaceutical substances, *Curr. Opin. Colloid Interface Sci.* 24 (2016) 64–71.
- [104] M.D. Jones, P. Young, D. Traini, The use of inverse gas chromatography for the study of lactose and pharmaceutical materials used in dry powder inhalers, *Adv. Drug Deliv. Rev.* 64 (3) (2012) 285–293.
- [105] O. Planinšek, G. Buckton, Inverse gas chromatography: considerations about appropriate use for amorphous and crystalline powders, *J. Pharmaceut. Sci.* 92 (6) (2003) 1286–1294.
- [106] H.H. Tong, et al., Surface characterization of salmeterol xinafoate powders by inverse gas chromatography at finite coverage, *J. Pharmaceut. Sci.* 94 (3) (2005) 695–700.
- [107] K.V. Kumar, F. Rocha, On the effect of a non-ionic surfactant on the surface of sucrose crystals and on the crystal growth process by inverse gas chromatography, *J. Chromatogr. A* 1216 (48) (2009) 8528–8534.
- [108] M. Cares-Pacheco, et al., Physicochemical characterization of D-mannitol polymorphs: the challenging surface energy determination by inverse gas chromatography in the infinite dilution region, *Int. J. Pharm.* 475 (1–2) (2014) 69–81.
- [109] A.V. Ambarkhane, K. Pincott, G. Buckton, The use of inverse gas chromatography and gravimetric vapour sorption to study transitions in amorphous lactose, *Int. J. Pharm.* 294 (1–2) (2005) 129–135.
- [110] R. Surana, et al., Determination of glass transition temperature and in situ study of the plasticizing effect of water by inverse gas chromatography, *Pharmaceut. Res.* 20 (10) (2003) 1647–1654.
- [111] S.E. Dilworth, et al., Approaches to determine the enthalpy of crystallisation, and amorphous content, of lactose from isothermal calorimetric data, *Int. J. Pharm.* 284 (1–2) (2004) 83–94.
- [112] M. Ohta, G. Buckton, A study of the differences between two amorphous spray-dried samples of cefditoren pivoxil which exhibited different physical stabilities, *Int. J. Pharm.* 289 (1–2) (2005) 31–38.

- [113] H.E. Newell, et al., The use of inverse phase gas chromatography to study the change of surface energy of amorphous lactose as a function of relative humidity and the processes of collapse and crystallisation, *Int. J. Pharm.* 217 (1–2) (2001) 45–56.
- [114] P. Ke, et al., Investigation of preparation methods on surface/bulk structural relaxation and glass fragility of amorphous solid dispersions, *Int. J. Pharm.* 422 (1–2) (2012) 170–178.
- [115] S. Hasegawa, P. Ke, G. Buckton, Determination of the structural relaxation at the surface of amorphous solid dispersion using inverse gas chromatography, *J. Pharmaceut. Sci.* 98 (6) (2009) 2133–2139.
- [116] F. Thielmann, D.J. Burnett, J.Y. Heng, Determination of the surface energy distributions of different processed lactose, *Drug Dev. Ind. Pharm.* 33 (11) (2007) 1240–1253.
- [117] D. Burnett, et al., Effect of processing route on the surface properties of amorphous indomethacin measured by inverse gas chromatography, *AAPS PharmSciTech* 13 (4) (2012) 1511–1517.
- [118] P.E. Luner, et al., Evaluation of milling method on the surface energetics of molecular crystals using inverse gas chromatography, *Cryst. Growth Des.* 12 (11) (2012) 5271–5282.
- [119] H.E. Newell, et al., The use of inverse phase gas chromatography to measure the surface energy of crystalline, amorphous, and recently milled lactose, *Pharmaceut. Res.* 18 (5) (2001) 662–666.
- [120] M. Ohta, G. Buckton, Determination of the changes in surface energetics of cefditoren pivoxil as a consequence of processing induced disorder and equilibration to different relative humidities, *Int. J. Pharm.* 269 (1) (2004) 81–88.
- [121] V. Puri, J. Shur, A.S. Narang, Elucidating molecular- and particle-level changes during the annealing of a micronized crystalline drug, *Mol. Pharm.* 16 (10) (2019) 4339–4351.
- [122] M. Ohta, G. Buckton, The use of inverse gas chromatography to assess the acid base contributions to surface energies of cefditoren pivoxil and methacrylate copolymers and possible links to instability, *Int. J. Pharm.* 272 (1–2) (2004) 121–128.
- [123] D. Cline, R. Dalby, Predicting the quality of powders for inhalation from surface energy and area, *Pharmaceut. Res.* 19 (9) (2002) 1274–1277.
- [124] H.H. Tong, et al., Predicting the aerosol performance of dry powder inhalation formulations by interparticulate interaction analysis using inverse gas chromatography, *J. Pharmaceut. Sci.* 95 (1) (2006) 228–233.
- [125] T. Jong, et al., Investigation of the changes in aerosolization behavior between the jet-milled and spray-dried colistin powders through surface energy characterization, *J. Pharmaceut. Sci.* 105 (3) (2016) 1156–1163.
- [126] V. Swaminathan, J. Cobb, I. Saracovan, Measurement of the surface energy of lubricated pharmaceutical powders by inverse gas chromatography, *Int. J. Pharm.* 312 (1–2) (2006) 158–165.
- [127] Q.T. Zhou, et al., Characterization of the surface properties of a model pharmaceutical fine powder modified with a pharmaceutical lubricant to improve flow via a mechanical dry coating approach, *J. Pharmaceut. Sci.* 100 (8) (2011) 3421–3430.
- [128] S.C. Das, et al., Use of surface energy distributions by inverse gas chromatography to understand mechanofusion processing and functionality of lactose coated with magnesium stearate, *Eur. J. Pharmaceut. Sci.* 43 (4) (2011) 325–333.
- [129] V. Karde, C. Ghoroi, Influence of surface modification on wettability and surface energy characteristics of pharmaceutical excipient powders, *Int. J. Pharm.* 475 (1–2) (2014) 351–363.
- [130] United States Pharmacopoeia, Monograph: <1181> Scanning Electron Microscopy, I. U.N., Rockville, USA, 2009b.
- [131] A.D. Emanuele, C. Gilpin, Applications of the environmental scanning electron microscope to the analysis of pharmaceutical formulations, *Scanning: The Journal of Scanning Microscopies* 18 (7) (1996) 522–527.
- [132] J. Goldstein, *Scanning electron microscopy and X-ray microanalysis: a text for biologists, materials scientists, and geologists*, Plenum Publishing Corporation, 1992.
- [133] S. Scherer, A. Piffer, The SEM as a profile measurement device, *Imaging & Microscopy* 2 (2002) 18.
- [134] J. Chandler, Methods of analysis in the electron microscope, in: *Proc. R. Microsc. Soc.*, 1980.
- [135] M. Azad, et al., Fast dissolution of poorly water soluble drugs from fluidized bed coated nanocomposites: impact of carrier size, *Int. J. Pharm.* 513 (1–2) (2016) 319–331.
- [136] D. Ditter, et al., Evaluation of glass delamination risk in pharmaceutical 10 mL/10R vials, *J. Pharmaceut. Sci.* 107 (2) (2018) 624–637.
- [137] R.D. Ennis, et al., Glass vials for small volume parenterals: influence of drug and manufacturing processes on glass delamination, *Pharmaceut. Dev. Technol.* 6 (3) (2001) 393–405.
- [138] J.V. Andhariya, et al., Development of in vitro-in vivo correlation of parenteral naltrexone loaded polymeric microspheres, *J. Contr. Release* 255 (2017) 27–35.
- [139] M.L. Henson, T.A. Jergovich, in: *Scanning Electron Microscopy and Energy Dispersive X-Ray Spectrometry (SEM/EDS) for the Forensic Examination of Paints and Coatings*, Taylor & Francis, London, 2001.
- [140] F.M. Etzler, J. Drelich, Atomic force microscopy for characterization of surfaces, particles, and their interactions, in: *Developments in Surface Contamination and Cleaning*, Elsevier, 2012, pp. 307–331.
- [141] R. Jagtap, A. Ambre, Overview Literature on Atomic Force Microscopy (AFM): Basics and its Important Applications for Polymer Characterization, 2006.
- [142] C. Weiss, P. McLoughlin, H. Cathcart, Characterisation of dry powder inhaler formulations using atomic force microscopy, *Int. J. Pharm.* 494 (1) (2015) 393–407.
- [143] D. Rugar, P. Hansma, Atomic force microscopy, *Phys. Today* 43 (10) (1990) 23–30.
- [144] J. Sitterberg, et al., Utilising atomic force microscopy for the characterisation of nanoscale drug delivery systems, *Eur. J. Pharm. Biopharm.* 74 (1) (2010) 2–13.
- [145] H.-J. Butt, B. Cappella, M. Kappl, Force measurements with the atomic force microscope: technique, interpretation and applications, *Surf. Sci. Rep.* 59 (1–6) (2005) 1–152.
- [146] D. Johnson, N. Hilal, Characterisation and quantification of membrane surface properties using atomic force microscopy: a comprehensive review, *Desalination* 356 (2015) 149–164.
- [147] U. Maver, et al., Recent progressive use of atomic force microscopy in biomedical applications, *Trac. Trends Anal. Chem.* 80 (2016) 96–111.
- [148] M. Marrese, V. Guarino, L. Ambrosio, Atomic force microscopy: a powerful tool to address scaffold design in tissue engineering, *J. Funct. Biomater.* 8 (1) (2017) 7.
- [149] X. Cao, C. Sun, T.J. Thamann, A study of sulfamerazine single crystals using atomic force microscopy, transmission light microscopy, and Raman spectroscopy, *J. Pharmaceut. Sci.* 94 (9) (2005) 1881–1892.
- [150] R. Price, P.M. Young, Visualization of the crystallization of lactose from the amorphous state, *J. Pharmaceut. Sci.* 93 (1) (2004) 155–164.
- [151] M.D. Jones, A.E. Beezer, G. Buckton, Determination of outer layer and bulk dehydration kinetics of trehalose dihydrate using atomic force microscopy, gravimetric vapour sorption and near infrared spectroscopy, *J. Pharmaceut. Sci.* 97 (10) (2008) 4404–4415.
- [152] A. Fahs, et al., Hydroxypropyl methylcellulose (HPMC) formulated films: relevance to adhesion and friction surface properties, *Carbohydr. Polym.* 80 (1) (2010) 105–114.
- [153] J. Meeus, et al., Nanoscale surface characterization and miscibility study of a spray-dried injectable polymeric matrix consisting of poly (lactic-co-glycolic acid) and polyvinylpyrrolidone, *J. Pharmaceut. Sci.* 101 (9) (2012) 3473–3485.
- [154] R. Fule, P. Amin, Development and evaluation of lafutidine solid dispersion via hot melt extrusion: investigating drug-polymer miscibility with advanced characterisation, *Asian J. Pharm. Sci.* 9 (2) (2014) 92–106.
- [155] M.E. Lauer, et al., Rapid assessment of homogeneity and stability of amorphous solid dispersions by atomic force microscopy from bench to batch, *Pharmaceut. Res.* 30 (8) (2013) 2010–2022.
- [156] N. Shetty, et al., Effects of moisture-induced crystallization on the aerosol performance of spray dried amorphous ciprofloxacin powder formulations, *Pharmaceut. Res.* 35 (1) (2018) 1–13.
- [157] A. Noy, R.W. Friddle, Practical single molecule force spectroscopy: how to determine fundamental thermodynamic parameters of intermolecular bonds with an atomic force microscope, *Methods* 60 (2) (2013) 142–150.
- [158] M. Tsukada, et al., Adhesion force measurement of a DPI size pharmaceutical particle by colloid probe atomic force microscopy, *Powder Technol.* 141 (3) (2004) 262–269.
- [159] M.D. Jones, et al., An investigation into the dispersion mechanisms of ternary dry powder inhaler formulations by the quantification of interparticulate forces, *Pharmaceut. Res.* 25 (2) (2008) 337–348.
- [160] R. Price, P.M. Young, On the physical transformations of processed pharmaceutical solids, *Micron* 36 (6) (2005) 519–524.
- [161] M.B. Tejedor, et al., Milling induced amorphisation and recrystallization of α -lactose monohydrate, *Int. J. Pharm.* 537 (1–2) (2018) 140–147.
- [162] M.D. Jones, et al., The use of atomic force microscopy to study the conditioning of micronised budesonide, *Int. J. Pharm.* 357 (1–2) (2008) 314–317.
- [163] D. Traini, et al., Comparative study of erythritol and lactose monohydrate as carriers for inhalation: atomic force microscopy and in vitro correlation, *Eur. J. Pharmaceut. Sci.* 27 (2–3) (2006) 243–251.
- [164] J. Eve, et al., A study of single drug particle adhesion interactions using atomic force microscopy, *Int. J. Pharm.* 238 (1–2) (2002) 17–27.
- [165] P.M. Young, et al., Investigation into the effect of humidity on drug drug interactions using the atomic force microscope, *J. Pharmaceut. Sci.* 92 (4) (2003) 815–822.
- [166] J. James, et al., The surface characterisation and comparison of two potential sub-micron, sugar bulking excipients for use in low-dose, suspension formulations in metered dose inhalers, *Int. J. Pharm.* 361 (1–2) (2008) 209–221.
- [167] M.D. Louey, P. Mulvaney, P.J. Stewart, Characterisation of adhesional properties of lactose carriers using atomic force microscopy, *J. Pharmaceut. Biomed. Anal.* 25 (3–4) (2001) 559–567.
- [168] H. Adi, et al., Role of agglomeration in the dispersion of salmeterol xinafoate from mixtures for inhalation with differing drug to fine lactose ratios, *J. Pharmaceut. Sci.* 97 (8) (2008) 3140–3152.
- [169] M.J. Davies, et al., A quantitative assessment of inhaled drug particle pulmonary surfactant interaction by atomic force microscopy, *Colloids Surf. B Biointerfaces* 73 (1) (2009) 97–102.
- [170] M. Davies, et al., Characterization of drug particle surface energetics and Young's modulus by atomic force microscopy and inverse gas chromatography, *Pharmaceut. Res.* 22 (7) (2005) 1158–1166.
- [171] J. Zhang, et al., Determination of the surface free energy of crystalline and amorphous lactose by atomic force microscopy adhesion measurement, *Pharmaceut. Res.* 23 (2) (2006) 401–407.

- [172] V. Wakis, et al., Molecular basis of crystal morphology-dependent adhesion behavior of mefenamic acid during tableting, *Pharmaceut. Res.* 31 (1) (2014) 160–172.
- [173] J.J. Wang, et al., Modeling of adhesion in tablet compression – I. Atomic force microscopy and molecular simulation, *J. Pharmaceut. Sci.* 92 (4) (2003) 798–814.
- [174] M. Bunker, et al., Characterising the surface adhesive behavior of tablet tooling components by atomic force microscopy, *Drug Dev. Ind. Pharm.* 37 (8) (2011) 875–885.
- [175] P. Seitavuopio, J. Rantanen, J. Yliruusi, Tablet surface characterisation by various imaging techniques, *Int. J. Pharm.* 254 (2) (2003) 281–286.
- [176] Kratos analytical. X-ray photoelectron spectroscopy (XPS) [cited 2020 29 September 2020]; Available from, <https://www.kratos.com/applications/techniques/x-ray-photoelectron-spectroscopy>, 2020.
- [177] P.C. Vioglio, M.R. Chierotti, R. Gobetto, Pharmaceutical aspects of salt and cocrystal forms of APIs and characterization challenges, *Adv. Drug Deliv. Rev.* 117 (2017) 86–110.
- [178] J.S. Stevens, S.L. Schroeder, X-Ray photoelectron spectroscopy, *Encyclopedia of Physical Organic Chemistry* (2016) 1–53.
- [179] H.-L. Lee, N.T. Flynn, X-ray photoelectron spectroscopy, in: *Handbook of Applied Solid State Spectroscopy*, Springer, 2006, pp. 485–507.
- [180] T. Scientific, X-ray photoelectron spectroscopy [cited 2020 18 September 2020]; Available from, <https://assets.thermofisher.com/TFS-Assets/MSD/brochures/BR53097-xps-family.pdf>, 2020.
- [181] R.T. Haasch, X-ray photoelectron spectroscopy (XPS) and auger electron spectroscopy (AES). *Practical Materials Characterization*, Springer, 2014, pp. 93–132.
- [182] C.J. Powell, A. Jablonski, Progress in quantitative surface analysis by X-ray photoelectron spectroscopy: current status and perspectives, *J. Electron. Spectrosc. Relat. Phenom.* 178 (2010) 331–346.
- [183] C.S. Fadley, X-ray photoelectron spectroscopy: progress and perspectives, *J. Electron. Spectrosc. Relat. Phenom.* 178 (2010) 2–32.
- [184] J.Y. Heng, et al., Anisotropic surface energetics and wettability of macroscopic form I paracetamol crystals, *Langmuir* 22 (6) (2006) 2760–2769.
- [185] S.V. Bhujbal, et al., Qualitative and quantitative characterization of composition heterogeneity on the surface of spray dried amorphous solid dispersion particles by an advanced surface analysis platform with high surface sensitivity and superior spatial resolution, *Mol. Pharm.* 15 (5) (2018) 2045–2053.
- [186] Y. Chen, et al., A single hydrogen to fluorine substitution reverses the trend of surface composition enrichment of sorafenib amorphous solid dispersion upon moisture exposure, *Pharmaceut. Res.* 36 (7) (2019) 105.
- [187] H. Chen, et al., Moisture-induced amorphous phase separation of amorphous solid dispersions: molecular mechanism, microstructure, and its impact on dissolution performance, *J. Pharmaceut. Sci.* 107 (1) (2018) 317–326.
- [188] Y. Song, et al., Acid–base interactions of polystyrene sulfonic acid in amorphous solid dispersions using a combined UV/FTIR/XPS/ssNMR study, *Mol. Pharm.* 13 (2) (2016) 483–492.
- [189] W. Wang, et al., Effects of surface composition on the aerosolisation and dissolution of inhaled antibiotic combination powders consisting of colistin and rifampicin, *AAPS J.* 18 (2) (2016) 372–384.
- [190] Q.T. Zhou, et al., How much surface coating of hydrophobic azithromycin is sufficient to prevent moisture-induced decrease in aerosolisation of hygroscopic amorphous colistin powder? *AAPS J.* 18 (5) (2016) 1213–1224.
- [191] W. Lu, et al., Amino acids as stabilizers for spray-dried simvastatin powder for inhalation, *Int. J. Pharm.* 572 (2019) 118724.
- [192] L.A. Felton, W.L. Perry, A novel technique to quantify film–tablet interfacial thickness, *Pharmaceut. Dev. Technol.* 7 (1) (2002) 43–47.
- [193] X. Hou, Y. He, B.T. Jones, Recent advances in portable X-ray fluorescence spectrometry, *Appl. Spectrosc. Rev.* 39 (1) (2004) 1–25.
- [194] A. Barhoum, et al., Physicochemical characterization of nanomaterials: polymorph, composition, wettability, and thermal stability, in: *Emerging Applications of Nanoparticles and Architecture Nanostructures*, Elsevier, 2018, pp. 255–278.
- [195] P. Wobrauschek, Total reflection x-ray fluorescence analysis: a review, *X Ray Spectrom.* Int. J. 36 (5) (2007) 289–300.
- [196] C. Vanhoof, et al., Atomic spectrometry update – a review of advances in X-ray fluorescence spectrometry and its special applications, *J. Anal. At. Spectrom.* 33 (9) (2018) 1413–1431, 2018.
- [197] C. Vanhoof, et al., Atomic spectrometry update – a review of advances in X-ray fluorescence spectrometry and its special applications, *J. Anal. At. Spectrom.* 34 (9) (2019) 1750–1767, 2019.
- [198] M.E. Awad, et al., Flow and tableting behaviors of some egyptian kaolin powders as potential pharmaceutical excipients, *Minerals* 10 (1) (2020) 23.
- [199] C. Shi, et al., Direct visualization of drug polymer phase separation in ritonavir–copovidone amorphous solid dispersions using in situ synchrotron X-ray fluorescence imaging of thin films, *Mol. Pharm.* 16 (11) (2019) 4751–4754.
- [200] C. Cahyadi, et al., Comparative study of non-destructive methods to quantify thickness of tablet coatings, *Int. J. Pharm.* 398 (1–2) (2010) 39–49.
- [201] J. Oliveira Junior, et al., Deformation of a Solid Pharmaceutical Form Using Computed Tomography and X-Ray Fluorescence, 2015.
- [202] A.M. Belu, D.J. Graham, D.G. Castner, Time-of-flight secondary ion mass spectrometry: techniques and applications for the characterization of biomaterial surfaces, *Biomaterials* 24 (21) (2003) 3635–3653.
- [203] C.A. Prestidge, T.J. Barnes, W. Skinner, Time-of-flight secondary-ion mass spectrometry for the surface characterization of solid-state pharmaceuticals, *J. Pharm. Pharmacol.* 59 (2) (2007) 251–259.
- [204] T.J. Barnes, I.M. Kempson, C.A. Prestidge, Surface analysis for compositional, chemical and structural imaging in pharmaceuticals with mass spectrometry: a ToF-SIMS perspective, *Int. J. Pharm.* 417 (1–2) (2011) 61–69.
- [205] S. Bosselmann, et al., Plasma deposited stability enhancement coating for amorphous ketoprofen, *Eur. J. Pharm. Biopharm.* 78 (1) (2011) 67–74.
- [206] J. Bailey, et al., 3D ToF-SIMS imaging of polymer multilayer films using argon cluster sputter depth profiling, *ACS Appl. Mater. Interfaces* 7 (4) (2015) 2654–2659.
- [207] Hitachi, Differential scanning calorimetry DSC7000 series [cited 2020 23 August 2020]; Available from, https://www.hitachi-hightech.com/global/product_detail/?pn=ana-dsc7000x.
- [208] S. Gaisford, G. Buckton, Potential applications of microcalorimetry for the study of physical processes in pharmaceuticals, *Thermochim. Acta* 380 (2) (2001) 185–198.
- [209] A. Müllertz, Y. Perrie, T. Rades, *Analytical Techniques in the Pharmaceutical Sciences*, Springer, 2016.
- [210] TA instruments. Microcalorimetry: tam IV & 48 [cited 2020 23 August 2020]; Available from, <https://www.tainstruments.com/wp-content/uploads/BROCH-TAM-IV.pdf>.
- [211] S. Gaisford, Isothermal microcalorimetry for quantifying amorphous content in processed pharmaceuticals, *Adv. Drug Deliv. Rev.* 64 (5) (2012) 431–439.
- [212] G. Buckton, Applications of isothermal microcalorimetry in the pharmaceutical sciences, *Thermochim. Acta* 248 (1995) 117–129.
- [213] M.A. O'Neill, S. Gaisford, Application and use of isothermal calorimetry in pharmaceutical development, *Int. J. Pharm.* 417 (1–2) (2011) 83–93.
- [214] I. Wadso, L. Wadso, Systematic errors in isothermal micro- and nanocalorimetry, *J. Therm. Anal. Calorim.* (2005) 1388–6150. <https://doi.org/10.1007/s10973-005-6864-8>.
- [215] S. Gaisford, et al., Following mechanical activation of salbutamol sulphate during ball-milling with isothermal calorimetry, *Int. J. Pharm.* 393 (1–2) (2010) 75–79.
- [216] C. Gustafsson, et al., Comparison of solid-state NMR and isothermal microcalorimetry in the assessment of the amorphous component of lactose, *Int. J. Pharm.* 174 (1–2) (1998) 243–252.
- [217] T. Sebbatu, M. Angberg, C. Ahlneck, Assessment of the degree of disorder in crystalline solids by isothermal microcalorimetry, *Int. J. Pharm.* 104 (2) (1994) 135–144.
- [218] L.-E. Briggner, et al., The use of isothermal microcalorimetry in the study of changes in crystallinity induced during the processing of powders, *Int. J. Pharm.* 105 (2) (1994) 125–135.
- [219] P. Darcy, G. Buckton, Quantitative assessments of powder crystallinity: estimates of heat and mass transfer to interpret isothermal microcalorimetry data, *Thermochim. Acta* 316 (1) (1998) 29–36.
- [220] S.E. Hogan, G. Buckton, The quantification of small degrees of disorder in lactose using solution calorimetry, *Int. J. Pharm.* 207 (1–2) (2000) 57–64.
- [221] E. Katainen, et al., Evaluation of the amorphous content of lactose by solution calorimetry and Raman spectroscopy, *Talanta* 68 (1) (2005) 1–5.
- [222] V.-P. Lehto, E. Lain, Simultaneous determination of the heat and the quantity of vapor sorption using a novel microcalorimetric method, *Pharmaceut. Res.* 17 (6) (2000) 701–706.
- [223] G. Buckton, J.W. Dove, P. Davies, Isothermal microcalorimetry and inverse phase gas chromatography to study small changes in powder surface properties, *Int. J. Pharm.* 193 (1) (1999) 13–19.
- [224] C. Hirschberg, et al., Effects of water on powder flowability of diverse powders assessed by complimentary techniques, *J. Pharmaceut. Sci.* 108 (8) (2019) 2613–2620.
- [225] M. Gemmi, et al., 3D electron diffraction: the nanocrystallography revolution, *ACS Cent. Sci.* 5 (8) (2019) 1315–1329.
- [226] U. Kolb, et al., Structural characterization of organics using manual and automated electron diffraction, *Polym. Rev.* 50 (3) (2010) 385–409.
- [227] R.G. Ricarte, T.P. Lodge, M.A. Hillmyer, Detection of pharmaceutical drug crystallites in solid dispersions by transmission electron microscopy, *Mol. Pharm.* 12 (3) (2015) 983–990.
- [228] E. Knapke, J. Dubochet, Beam damage to organic material is considerably reduced in cryo-electron microscopy, *J. Mol. Biol.* 141 (2) (1980) 147–161.
- [229] A. Ponce, et al., Advances in the electron diffraction characterization of atomic clusters and nanoparticles, *Nanoscale Advances* (2021) 311–325.
- [230] D.E. Mosesson, et al., Nanometer-scale residual crystals in a hot melt extruded amorphous solid dispersion: characterization by transmission electron microscopy, *Cryst. Growth Des.* 18 (12) (2018) 7633–7640.
- [231] D.A. Engers, et al., Triboelectrification of pharmaceutically relevant powders during low-shear tumble blending, *J. Electrostat.* 64 (12) (2006) 826–835.
- [232] K.C. Pingali, et al., An observed correlation between flow and electrical properties of pharmaceutical blends, *Powder Technol.* 192 (2) (2009) 157–165.
- [233] A. Bailey, The inhalation and deposition of charged particles within the human lung, *J. Electrostat.* 42 (1–2) (1997) 25–32.
- [234] W. Kaialy, A review of factors affecting electrostatic charging of pharmaceuticals and adhesive mixtures for inhalation, *Int. J. Pharm.* 503 (1–2) (2016) 262–276.
- [235] R. Brown, Tutorial review: simultaneous measurement of particle size and particle charge, *J. Aerosol Sci.* 28 (8) (1997) 1373–1391.

- [236] S. Karner, N.A. Urbanetz, The impact of electrostatic charge in pharmaceutical powders with specific focus on inhalation-powders, *J. Aerosol Sci.* 42 (6) (2011) 428–445.
- [237] M. Murtomaa, et al., Effect of particle morphology on the triboelectrification in dry powder inhalers, *Int. J. Pharm.* 282 (1–2) (2004) 107–114.
- [238] A. Bailey, Charging of solids and powders, *J. Electrostat.* 30 (1993) 167–180.
- [239] E.M. Walker, K.J. Roberts, S.J. Maginn, A molecular dynamics study of solvent and impurity interaction on the crystal habit surfaces of ϵ -caprolactam, *Langmuir* 14 (19) (1998) 5620–5630.
- [240] C. Stoica, et al., Understanding the effect of a solvent on the crystal habit, *Cryst. Growth Des.* 4 (4) (2004) 765–768.
- [241] E.J. Chan, R. Tejjwani, L. Derdour, Use of molecular simulation in calculating a characteristic relative growth effect curvature to correlate factors influencing crystalline growth and other properties, *Cryst. Growth Des.* 15 (12) (2015) 5754–5766.
- [242] D.P. Kale, et al., Molecular basis of water sorption behavior of rivaroxaban-malonic acid cocrystal, *Mol. Pharm.* 16 (7) (2019) 2980–2991.
- [243] S.R. Modi, et al., Effect of crystal habit on intrinsic dissolution behavior of celecoxib due to differential wettability, *Cryst. Growth Des.* 14 (10) (2014) 5283–5292.