**ABE557 Literature Review**

During the fall semester, students will have to perform a literature review on their particular process (brewing, food production, pharmaceuticals, etc.) where each member of the group will focus on a thorough review of a major unit operation from their process.

Published review examples………………………………………………………………….pg 3

Student example …………………………………………………………………………….pg 4

* Written example from a former ABE student for another course. This example is a B+-level paper.

**Requirements (see teaching team for more details)**

A literature review will be submitted by each person in the group; each person will pick a major unit operation within their chosen process. Since your senior design project should be novel, or an improvement upon the current state of production, the majority of the works cited should be from the past 5-10 years however some excellent research on many operations may have been conducted up to 50 years ago. Text books can provide an excellent source of information. The length of each review should range between 5 to 10 pages.

Rule of thumb for stand-alone reviews: minimum number of sources = 3.5\*(# of pages).

Example: 10 pg review= 35 reputable sources, minimum

**Literature Review: A Guide**

**Coleen Riley, Undergraduate Teaching Lab Manager, 2018**

*What is a literature review?*

It is a survey of scholarly articles, textbooks, and other sources (for example, dissertations, conference proceedings, published notes, etc.) relevant to a particular field of study, areas of research, novel processes, or theory; it is meant to provide a description, critical evaluation, and a source of recommended future works. The purpose of a literature review is to provide an overview of a topic with relevant pieces of significant literature published on a topic.

*What makes up a literature review?*

A literature review is made up of the following parts:

* Title:
  + Should be concise, descriptive and comprehensible
  + Reader should know what to expect from the paper
  + Include authors’ names and affiliations within academia or industry
  + Order of authors varies by discipline and journal. Typically, the first author listed is the primary author of contents and primary investigator. Names following are followed in order of involvement.
* Abstract:
  + Short summary of a larger body of work.
  + Utilizes specific language to accurately summarize the literature review.
  + Depending on the word restriction of a publication, typically between 200-400 words.
  + Should be the absolute last aspect of the paper written.
  + Includes: intro, theme descriptions, and conclusions.
* Introduction:
  + Describe the overall topic that you are investigating- why it is important, why it is important for people to understand and research.
  + Identify themes and trends in research, methodology, and findings. Give the big picture overview of the topic.
  + Define what you are trying to prove with the review. While scientific papers are meant to be objective, a literature review is actually an expression of a broad opinion.
    - It is important to note, however, that you should still write in an academic manner. No personal pronouns, speak in past tense, etc. While you are expressing an idea, it should be evident through the body of work, not through statements such as “This review proves that…” “this technique should be used because…”.
* Theme 1:
  + Overview of the theme; description of commonalities, differences, nuances; introduce sub-themes
  + Sub-theme A: narrow, but grouped findings
    - Study 1: research questions, methods, related findings, important conclusions
    - Study 2: research questions, methods, related findings, important conclusions
    - Study 3: research questions, methods, related findings, important conclusions
    - Repeat as necessary
  + Sub theme B: (see sub-theme A)
  + Continue with sub-themes that fit within the scope of theme 1. Studies can be repeated if there are multiple findings that fit in multiple sub-themes. If this is the case, it is not required to re-state the methods in detail, simply put enough information to remind the reader what it was.
* Theme 2…
* Theme 3…
* Continue through all themes…
* Conclusion:
  + An evaluation/critique of the existing literature discussed in the themes. This should be ***several paragraphs long***
  + Your literature review is meant to prove something- a literature review is not just meant to show what has been done, but what is yet to be explored. The conclusion if the place to express an opinion about the state of research.
  + Things you should include:
    - What are the contributions of this literature to the field?
    - What are the strengths of the research presented? What are the downfalls?
    - ***What is still not understood?***
    - ***What are next steps for research?*** (This should be an explicit description of how to address unknowns, weaknesses, gaps in knowledge, etc.)

*How do I find reputable sources?*

* Purdue libraries search
* Web of Science database (available through Purdue Libraries page)
* Google scholar
* Science Direct
* PubMed database (available through Purdue Libraries page)

Avoid open-source pages or sources that have not been published in a peer-review journal.

*What are the steps to begin your literature review?*

Step 1: Begin with broad topic of interest

* Begin with broad topic of interest; begin cataloguing pieces of published literature into areas of study.
* Begin defining themes based on catalogued literature
* This step will include reading and understanding publications that may not make it into the review!

Step 2: Outline themes and continue search for relevant publications

* The initial literature review search will most likely not be enough to cover all of the possible themes
* Research questions, methods, and results should all expand upon the theme in different ways.
* While there is no limit to the number of papers that can be included in each section, a good rule of thumb is to start with three, and then continue beyond that as you explore each topic in more detail.

Step 3: Define knowledge gaps, evaluate QUALITY of literature, form opinion, provide future work to address current issues

* While the conclusion of your review will, most likely, be the shortest section of your paper, it will also be the most impactful. This is where you will address gaps in knowledge, areas of work, and suggest future work.
* While searching for valid literature, it is extremely important to assess the quality of the sources. There are several factors that can aid in your assessment:
  + What journal are you obtaining this paper? What impact factor\*\* (more on that later) does this journal have?
  + How many people have cited this paper? The number of citations (while not the \*best\* metric to measure reliability) can indicate where research is heading and what is considered “respectable” in your field. This value is usually shown in searches made in Web of Science and Google Scholar. (I highly recommend utilizing Web of Science. This database can be accessed through the Purdue Libraries page)

\*\*Journal Impact Factor: The impact factor is a measure of the frequency with which the average article in a journal has been cited in a particular year. It is a relative measure which indicates importance or rank of a journal. It should be noted that some areas of research (consumer marketing, food science, etc.) consistently have low impact factors, simply due to the small number of people in that particular area. While you should consider the impact factor while analyzing your sources, it is not the end-all variable that you should consider.

**General Notes:**

* Use consistent formatting. Pick one journal and stick with that formatting.
* No contractions (can’t, won’t, isn’t, etc.)
* No personal pronouns
* All Latin words in italics. (*et al*, *in vivo*, etc.)

**Published literature review examples:**

Psimadas D, Georgoulias P, Valtolassiou V, Loudos G. 2012. Molecular Nanomedicine Towards Cancer: In-Labeled Nanoparticles. J Pharm Sci 101:2271-2280.

Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S. 2012. Host-Gut Microbiota Metabolic Interactions. Science 336:1262-1267.

Gharsallaoui A, Roudaut G, Chambin O, Voilley A, Saurel R. 2007. Applications of spray drying in microencapsulation of food ingredients: An overview. Food Research Int 40:1107-1121.

Hirst MB, Richter CL. 2016. Review of Aroma Formation through Metabolic Pathways of *Saccharomyces cerevisiae* in Beverage Fermentations. Am J Enol Vitic 67:361-370.

**Review Article**

**Just What the Doctor Did Not Order– The Role of Water in Pharmaceutical Hydrates & Amorphous Solids**

Student A

*Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, IN, USA 47907*

**Abstract**

In the pharmaceutical industry, water is involved in many processes throughout the manufacturing of a pharmaceutical drug compound. Each unit operation that is involved in this process is capable of inducing a phase transformation which can lead to the hydration of a drug material which can have a drastic effect on the physical and chemical properties of a drug compound. Changes due to processing operations can occur as well, thus the control of phase transformations due to water throughout a pharmaceutical manufacturing process must be taken under consideration and be better controlled to ensure the quality of the final pharmaceutical drug product. This review article will discuss the role of water throughout the manufacturing process giving special attention to hydrates, amorphous solids, and the phase transformations between the two. This article will also discuss the theory behind these two solid forms along with their effects on final drug product performance, various characterization and screening techniques, and methods on how to better control water throughout the development process to prevent undesired final drug products.

**Keywords:** Hydrates, amorphous solids, water, phase transformation, unit operations, manufacturing

**Introduction**

During the development process of a pharmaceutical drug compound, the pure thermodynamically stable crystal form must be considered1. As the most stable drug form is desired in a pharmaceutical solid, a thorough understanding of the role of water, hydrates, and amorphous solids is essential in finding the optimum solid form for a drug product2. In the various stages involved in the manufacturing process, pharmaceutical solids may come in contact with water where the active pharmaceutical ingredient (API) and excipients in the formulation can have different moisture sorption properties. These differences can lead to alterations in the physical properties of the solid form when contacted by water. Additionally, the drug product should be considered over the specific temperature and relative humidity (RH) range under which that compound will be exposed to during processing1.

Not only are pharmaceutical solids exposed to water during the manufacturing process, but there is also water exposure during storage. The presence of water and moisture can heavily influence intermolecular interactions and the crystalline order of a drug compound, which can lead to extreme changes in physical properties, chemical properties, and stability as these properties are dependent on the presence of moisture. Changes in the crystalline order can also influence the free energy, thermodynamic activity, solubility, dissolution rate, stability, and bioavailability of a pharmaceutical compound3.

Water can interact with the crystalline solid in numerous ways. This can include adsorption of moisture on particle surfaces, crystal hydrate formation, deliquescence, and capillary condensation4. The amount of water that is sorbed is dependent upon the chemical properties, polarity of the compound, RH, temperature, particle-size distribution, specific surface area, deliquescence, structure of the sorbent surface, and porosity of the material4. To ensure that the final drug product consists of the desired properties, special requirements may be necessary for certain steps during the development of a pharmaceutical solid. Therefore, thorough studies must be conducted as the hydration state can alter the final *in vivo* performance of the drug product.

If the water is adsorbed onto the solid surface it may become incorporated into the crystalline lattice of the compound in stoichiometric proportions3 to form crystalline hydrates. Hydrates are formed when water is the solvent of crystallization. In this form of crystalline solid, is included in the structural definition of the crystal3 by forming various hydrogen bonds with the anhydrate drug molecule. With water involved in the manufacturing process, many solid-solid transformations may occur which can lead to undesirable product performance in the final solid dosage form.

For solid dosage forms, moisture adsorption can also provide helpful information in terms of selecting excipients and for controlling humidity during processing stages and storage of the final drug product5. Water that is involved in solid dosage manufacturing can lead to occurrences of hydrate formation, which can greatly alter the properties of an amorphous solid. According to the Cambridge Structural Database (CSD), approximately one third of the pharmaceutical active compounds, which comprise 14.4% of the existing compounds, are capable of forming crystalline hydrates20.Therefore, the role of water in the manufacturing process has gained great importance.

1. **Importance of Hydrates and Amorphous Solids**

A crystalline hydrate can be defined as a crystalline solid containing water as a participant in the crystal lattice7. Molecules with higher molecular weights tend to form hydrates more easily as it is harder to pack the dense lattice. The solvent involved, which in this case is water, helps strengthen intermolecular forces. Hydrates also have the ability to change physically by altering the solubility of the drug compound, which leads to changes in rates of dissolution and bioavailability. In terms of potency, the sensitivity of hydrates compared to the anhydrous form must be considered for processability, flow characteristics, and compressibility. In addition, hydrates have the ability to undergo dehydration and transform into the amorphous state which will be discussed later on. Hydrates serve as the “*most stable, least soluble form of a compound in water8*.” In terms of manufacturing, hydrates uptake various amounts of water, thus water content can change considerably with relative humidity, which can lead to issues with content uniformity8. Several dosage forms use hydrates as the solid form of their API. Hydrates can be selected for a variety of reasons, for they have been proven to be superior over the anhydrous form, but with the selection of this crystalline form, each step of the manufacturing process must be carefully considered9. Some examples of hydrates on the market are: cephalexin, azithromycin, and ampicillin which are marketed as a monohydrate, dihydrate, and trihydrate respectively9.

As many pharmaceutical solids have the ability to alter various physicochemical properties, amorphous solids are given special attention to as well especially because hydrate-amorphous solid transformations are very common. Amorphous solids provide interest in the industry as they tend to be more soluble and provide a dissolution advantage over their crystalline counterparts. An amorphous solid can be defined as a supercooled liquid in which the molecules are arranged in a random manner11. This solid form exhibits short-range order and has physical properties different from those in corresponding states12. Amorphous solids also have the ability to enhance bioavailability thus improving solubility and dissolution rate. However, this state is to be avoided in most scenarios as it can decrease chemical and physical stability, increase variability in product quality, and provide difficulties with analytical characterization13.

Amorphous forms of a drug compound are typically expected to be less chemically stable than that of a crystalline form due to the lack of a three dimensional crystalline lattice, the increase in free volume, and greater molecular mobility14. In the formulation, the choice of excipients play a vital role in minimizing the chemical instability of a drug. Thus, if an amorphous form of a drug is less chemically stable than that of the thermodynamically favored form, the formulation process can be altered in order to maximize the stability14.

Amorphous solids vary from hydrates and other crystalline solids in many ways. In amorphous solids, the interactions are maximized with neighboring molecules and particles. Amorphous solids also go through a phase transition when they go through a glass transition (Tg) temperature. In this transition, these solids go from a glassy state to a supercooled liquid. In addition, amorphous solids tend to deform not slip or break and they are more compressible. Amorphous solids are also more ductile and in terms of their role with water tend to be more hygroscopic than crystalline materials. Therefore, it is more reactive with water and can retain large amounts of water, which may lead to problems with stability. In addition, amorphous solids are more hygroscopic when exposed to a humid environment, and the absorbed moisture acts as a plasticizer, which leads to a large increase in molecular mobility10 which can lead to a less chemically stable product. As amorphous solids have higher energy compared to the crystalline forms, it can recrystallize to a more stable crystalline solid, which may eliminate the benefits it may provide in a formulation15. Thus, considerations regarding the heat and humidity that is required for the amorphous form to crystallize and other factors that may lead to the crystallization of amorphous solids must be considered during processing16. The theory behind crystallization and its relation to Tg is discussed by Yu17 and Hancock *et al*12.

Various unit operations involved in the development may induce hydrates and amorphous character, thus improving control over the manufacturing process where water is involved needs to be optimized to ensure manufacturability and desired physicochemical properties. Unit operations that may induce amorphous and hydrate character include drying, mixing or blending, milling, dry granulation, wet granulation, tablet compression, and tablet coating. Typically, when the water activity is higher than the critical water activity, a hydrate is the more thermodynamically stable form18. Therefore, hydrates are known to have lower Gibbs free energy than anhydrates which leads to its lower solubility. According to the Noyes-Whitney equation, this results in hydrates having lower dissolution rates as well18. As there are differences in the particulate properties between anhydrates and hydrates, hydrate formation can greatly impact mechanical properties of the drug particles18.

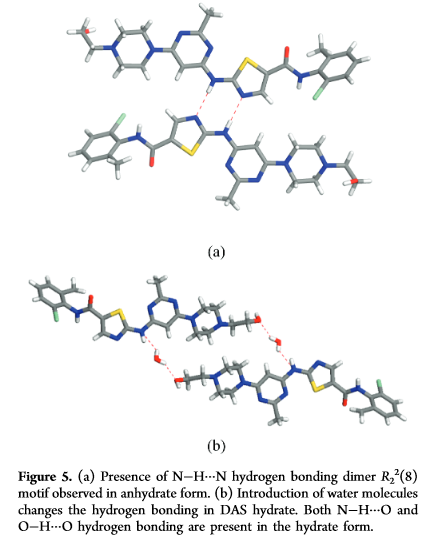
*1.1 Hydrate Classification*

Hydrates are best understood in terms of their solid-state structure19. This crystalline solid can be broken down into three different classes. Class I constitutes the isolated site hydrates. The water molecules in this structure are isolated and are not in contact with one another. A high amount of energy is required in order to remove the water from this class of hydrates. Class II includes the channel hydrates or water forming lattice channels. This class indicates that the water molecules in these hydrates form channel-like chains along a given crystal axis18. Water molecules in Class II hydrates are capable of hydrogen bonding with nearby water molecules18. The water channel helps facilitate water elimination from the hydrated crystal forms. The third class of hydrates or Class III consists of the ion-associated hydrates and they contain ion-coordinated water. This class of hydrates typically requires high dehydration temperatures as the bond strengths are particularly strong18.

* 1. *Hydrogen Bonding and Packing Motifs*

In hydrates, water molecules tend to behave in a tetrahedral distribution nature with two positive and two negative regions of charge3. On the negatively charged region, the water molecules can either accept a hydrogen bond or form a covalent bond3. On the other hand, the positively charged region interacts via a donated hydrogen bond3. In this pharmaceutical crystalline form, water has the ability to interact with other waters. The water molecule may also participate in Van der Waals interactions. The hydrogen bond in this pharmaceutical solid is also strongly directional. Water can bond to other hydrogen bond donors and acceptors. It can also bond to other waters to form chains and networks. Through a CSD analysis, it was found that in many structures that form hydrates, the number of hydrogen bond donor groups was much less than the number of acceptors20. From this analysis, various environments under which water hydrogen bonds to various structures were analyzed. Water most commonly hydrogen bonds to nitrogen and oxygen in environments containing only one hydrogen bond to either a hydrogen or oxygen atom20. The most common interaction for water is in an atmosphere where water forms three hydrogen bonds (two to the hydrogen atoms and one to the oxygen atom)20.

The differences in packing motifs between a hydrated and anhydrate form can be seen through Dasatinib (DAS), an inhibitor of critical oncogenic kinases. DAS anhydrate is a dimer formed through N-H···H interactions21. The dimer in DAS hydrate is different due to the introduction of water. It results in one DAS shifting and two DAS molecules being held together by two water molecules which serve as bridges. Both packing motifs can be seen in Figure 1. As can be seen in Figure 1b the hydrate has both N-H···O and O-H···O hydrogen bonding present21. Another difference between the two forms is that DAS anhydrate dimers tend to pack in a herringbone fashion, whereas DAS hydrate molecules form layers21.

  
**Figure 1:** (a) DAS anhydrate form showing N-H···H hydrogen bonding dimers (b) DAS hydrate showing both N-H···O and O-H···O hydrogen bonds with the introduction of water molecules21.

*1.3 Dehydration processes*

When dealing with pharmaceutical hydrates and amorphous solids, the dehydration process is also of utmost importance as it can also lead to alterations in the physical and chemical properties of a drug product. Dehydration of crystalline hydrates can lead to the conversion to a metastable form or to an amorphous phase which can drastically alter the stability19. The conversion to these forms can lead to other phases being generated with each phase conversion being time dependent22. With dehydration there is also a possibility of impure phases being present in the bulk sample, which can cause a change in the phase conversion rate22.

Through a study done by Lerk *et al,* binding capacities of α-D-glucose that was dehydrated at temperatures ranging from 60 to 135 °C was analyzed23. It was concluded that the crushing strength of the tablets that were compressed from fully dehydrated α-D-glucose monohydrate increased with increasing dehydration temperature. Suggested explanations for these results proposed that the increased binding capacity of the particles was due to the change in the texture of the particles due to the dehydration temperatures3. In another study done by Shefter *et al* the dehydration of ampicillin trihydrate resulted in an amorphous hydrate24. The rates of appearance of the amorphous form and the disappearance of the hydrate were found to be same24. Through this analysis, it was concluded that the dehydration rate is a measure of the physical stability of the hydrate.

1. **Role of water**

When water is involved in a drug product, it can cause significant changes in many properties, thus knowledge of the roles water plays throughout the development process should be required. When a solid form is exposed to water vapor, water molecules may attach to the surface of the solid via Van der Waals, ion-dipole, or specific hydrogen bonding interactions. According to Desiraju, the incorporation of water into crystal structures helps to balance the mismatch between the number of hydrogen bond donors and acceptors in the host molecules. When water is sorbed onto the surface it can be in the form of individual molecules, clusters, monolayers, and multimolecular layers which will eventually lead to condensed water5. When water gets added to a dried product, the molecules tend to be adsorbed onto specific sites until all sites are occupied which constitutes the water monolayer25. The water that makes up the monolayer tends to be very stable25. In addition, water involved in the crystal structure can be present stoichiometrically or nonstoichiometrically26. If the water is present nonstoichiometrically, water has the ability to form hydrogen bonded networks which can be tetrahedrally coordinated26. As water is a small molecule that has the ability to be both a hydrogen bond donor and acceptor, it is found to be the most capable solvent to link to drug molecules to form new crystal structures27.

A definition of hygroscopicity that is used as a standard in the pharmaceutical industry has not been clearly identified, but descriptions of this term always relate to sorption and the retention of moisture5. For the purpose of this paper, hygroscopicity can be defined as “*readily absorbing, becoming coated with, and retaining moisture, but not enough to make a liquid*28.” If a sample is placed in known RH, hygroscopicity has the ability to describe both the amount of moisture in a substance and the rate of moisture uptake5. Hygroscopicity can be influenced due to multiple factors including temperature, partial vapor pressure or RH, chemistry, and the nature of the solid.

Behavior of formulations vary depending on the differences in the water sorption behavior of the excipients as well because excipients play a large role on the phase transitions of the API4. When water is involved with pharmaceutical hydrates, it attempts to maximize its hydrogen bonding interactions within the structure20. As water is the strongest solvate-forming solvent, nearly half of the pre-existing drug substances are capable of forming a stable hydrate29. However, when these hydrates are dried, failure in the drying processes leads to the degradation in the quality of the drug substance due to the liberation of hydrate water molecules which initiate polymorphism29.

An evaluation of hygroscopicity should be performed for drug substances and excipients during the early stages of pharmaceutical development to gain full understanding on the impact that water-solid interactions can have on the various physical and chemical properties5. There are different types of water-solid interactions including adsorption and absorption. Adsorption is more common in crystalline materials. This property is dependent upon surface area and involves the condensation of water on crystal faces and monolayer coverage. Absorption on the other hand is more common for solids with amorphous character. With this property there is migration of water to the interior of the solid and it is independent of surface area. This is also the process in which hydrates form. Sorption is a term used that refers to both absorption and adsorption.

Similar to the crystalline state, molecules in an amorphous solid are capable of absorbing large quantities of water vapor into the solid bulk in addition to surface adsorption30. In amorphous solids, numerous physical changes can occur during water sorption. Water has the ability to dissolve in the solid where it can lower the Tg of the material and behave as a plasticizer and has the ability to enhance crystallization. This is attributed to the ability of an amorphous solid to be very hygroscopic and to the fact that it can contain considerable amounts of water31. Greater mobility and crystallization of the amorphous material can destabilize the amorphous pharmaceutical solid30. The strength of the water-solid interaction within an amorphous compound is dependent on the level of hydrogen bonding that is possible within the lattice of the pharmaceutical drug compound4.

*2.1 Water Activity*

For a pharmaceutical drug compound, the hydration state is also dependent upon the solvent composition1. There have been recent studies conducted on the thermodynamic equilibrium between the anhydrous and hydrated form of theophylline, ampicillin, and quinolones in water-solvent mixtures. From this experiment it was determined that the water activity in mixed solvents was the main factor in determining the hydration state of the drug compounds1.

The amount of water vapor that equilibrates with any material via adsorption can be described by water activity (aw). In pharmaceuticals knowing the aw can help obtain a dosage form with ideal chemical, physical, microbial, and shelf-life properties32. Moisture content does not serve as an accurate measurement, as the composition has to be constant. In this respect, aw enables the evaluation of chemical stability, flow properties, compaction, hardness, and dissolution rates of pharmaceuticals32. It is also temperature dependent. Although a specific aw applies to only one specific temperature, it can decrease with decreasing temperature31. Water activity is also a time dependent property. When an amorphous solid crystallizes, it can cause a significant change in aw31.

Previous studies have been conducted which have given tremendous insight on various properties that water activity has a hand on. In studies conducting slurry experiments it was found that the water-solvent mixture of certain drug compounds demonstrated that the aw in mixed solvents was the leading factor in determining the hydration state of drugs2. Water activity can also be used to “*reduce degradation of API formulations susceptible to hydrolysis*33” and be used to measure the water’s energy level.

This property provides helpful information in regards to the development process. It can be used to determine storage conditions due to its direct correlation with RH33. Through the solvate rule it is known that at higher aw hydrates are more stable than the anhydrous form and that higher hydrates are more stable than lower hydrates33. Additionally, it is possible to preferentially crystallize either the anhydrous or hydrated forms of a compound using aw33. Water activity also has an influence in the dehydration process. When the aw of a products surroundings is lower than the solid itself, a hydrate is exposed to a higher temperature which leads to a form change34.

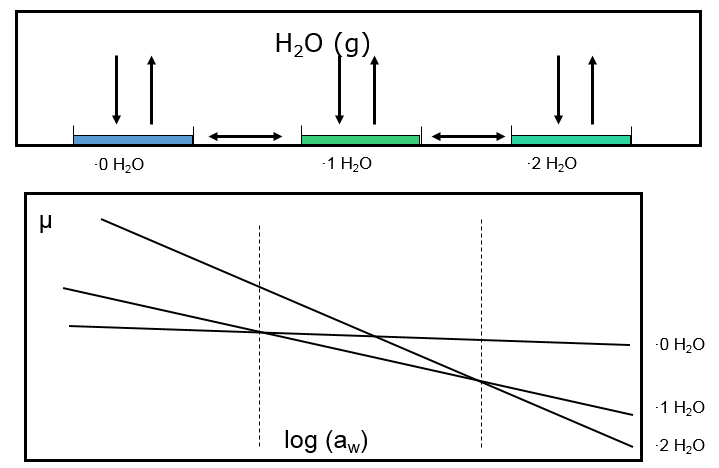
According to the U.S. Food and Drug Administration (FDA), water activity can be defined as the ratio between the vapor pressure of a product “*when in a completely undisturbed balance with the surrounding air media, and the vapor pressure of distilled water under identical conditions*35.” Therefore if the water activity is 0.60, vapor pressure is 60% that of the pure water. Water activity can be represented by equations 1 and 225:

(Eq.1)

(Eq. 2)

where µ represents chemical potential, µ0 represents the standard chemical potential of water, R represents the gas constant, T represents the temperature, aw represents water activity, p represents the vapor pressure of water in the material, p0 represents the vapor pressure of pure water, and ERH represents equilibrium relative humidity. Equation 2 shows that the ratio of the vapor pressures of the solution and solvent are described by aw as mentioned in the definition given previously.

When a surface is exposed to water there are various hydration states that may occur. When an anhydrous solid is exposed to moisture it can form a monohydrate, which results in an increase in aw leading to the dihydrous form. Equation 1 describes how water activity can be related to the chemical potential. Low aw represents low chemical potential which also symbolizes the most thermodynamically stable form. As aw increases there is a transition point that occurs where the chemical potential of the monohydrate is lower than that of the anhydrous. This is the stage where a solid can form a hydrate. The various hydration states are represented by Figure 2. In Figure 2, -0 H2O represents the anhydrate, -1 H2O represents a monohydrate, and -2 H2O represents a dihydrate. The transition point that occurs in the graph of µ vs log (aw) is the dihydrate, which also has the lowest energy. At equilibrium the chemical potential of water within the system must be the same as the chemical potential of water in the surroundings of the compound31.

  
**Figure 2:** Various hydration states represented as a function of water activity to display the relationship between chemical potential and water activity amongst an anhydrate, monohydrate, and dihydrate36

Bradshaw *et al* conducted a study with theophylline where a phase conversion to the monohydrate form was observed during dissolution which correlated to a drop in concentration which lead to a decrease in solubility22. The rate of conversion was dependent on the aw of the solution. With a aw of 1, the conversion was complete in 10 minutes, but with a aw of 0.65 the conversion was chemically inhibited and an insignificant drop in solubility was seen22. This study further supports the conclusion that the solubility of a pharmaceutical drug compound is dependent upon water activity.

Previously when the formation of zinc fumarate was analyzed, aw was varied in a grinding liquid, which served as a useful technique when different hydrated forms were screened37. This idea was implemented by Strobridge *et al* to demonstrate a dissimilar strategy to screen for different forms of a magnesium-based pharmaceutical derivative37. Low amounts of water in the grinding liquid led to the formation of a monohydrate, whereas intermediate amounts led to a tetrahydrate, and the usage of pure water resulted in a hydrated salt37.

* 1. *Unit operations involving water*

As water plays a large role in the pharmaceutical manufacturing process, it affects each unit operation along the process in various ways. During the crystallization phase of the process, water content is defined as the aw, which is a critical parameter for controlling the anhydrate/hydrate form of the final product18. The relative stability of an anhydrate/hydrate system is specified with respect to both temperature and the water activity in the surrounding medium18. It can also be common for new polymorphs to be found in this stage which can further lead to stability issues.

Upon crystallization, the next step in the process is milling. Milling is best known as a dry process, but in preparing suspensions aqueous medium may be required. Additionally, excipients are added to obtain a homogeneous mixture during this stage. Solid-state changes can occur during this processing step due to the energy and heat that is generated, but the presence of water can make a further impact as it can increase molecular mobility.

After milling, the drug compound moves on to wet granulation. In this step, particles are aggregated to larger granules with the addition of a binding agent18. A mixture is exposed to both water and high humidity during this processing step. As temperature increases, there is a higher probability that solid-state transformations may occur. Dehydration may also occur during this step.

Mixing speed in various unit operations can impact the rate that water is distributed, wetting, and the shear forces experienced by particles38. In a study done on mixing speeds through granulation of theophylline, at higher mixing speeds granule temperature increased which resulted in an increase in transformation time between solid states38.

Drying processes also have a large influence on phase transformations that can occur with pharmaceutical hydrates. In a study conducted by Khoo *et al* carbamazepine (CBZ) was dried using multiple methods. Under low pressure (vacuum) dehydration a decrease in intensity was seen in the X-ray powder diffraction (XRPD) pattern as water molecules departed29, thus representing that vacuum dehydration reduced the crystal quality.

During powder compression, water may not be directly involved, but atmospheric water is present and can impact the pharmaceutical solid form. When tableting, the API and excipients are exposed to high pressures which can induce defects or increase the amorphous content. With an increase in amorphous content, the facilitation of water entrapment into the anhydrous part occurs more easily. Dehydration rates increase with the compression force as well18.

Once the tablet has been compressed, a coating can be applied to the drug to control the release or to mask the taste. The coating solution can be aqueous or an organic-solvent based polymer solution. One method of coating to assure a uniform distribution of the coating medium is spray atomization. With this process, there must be a balance between the coating solution addition rate and drying conditions18 to ensure that the distribution occurs evenly. Local water activity on the tablet core-film interface can be high enough to induce hydrate formation and thus undesired changes may be seen in release behavior in the final dosage form18.

1. **Characterization Techniques**

To probe the presence of water in a pharmaceutical solid, numerous methods of characterization can be utilized. Optical microscopy with hot stage where a hydrate is put on a glass slide then heated at a given rate has proven to be successful. The increase in temperature allows the dehydration process to be visible. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) can be used as well. Water loss can be seen as a peak as the solid form is heated. In TGA there is typically a decrease in weight. With TGA it may be difficult to differentiate between different states of binding which may make it more difficult when trying to identify the location of water in a hydrate26. With DSC, the endotherm of dehydration for less stable hydrates may be broad thus it is harder to detect with this method18. However, DSC provides an advantage as it requires a small amount of material for the study1. DSC has also proven to be more useful than TGA in some cases as DSC allows the sample chamber to be precooled which allows analysis of “labile hydrates34”.

Karl Fischer titration and infrared spectroscopy (IR) are also useful characterization techniques. Water is very IR active, thus is easily detectable. Although the Karl Fischer method allows the total water content to be determined, it prevents differentiation between adsorbed moisture and the water of crystallization26. Single crystal and X-ray powder diffraction can be used to see the change in crystal structure as there will be changes in the powder pattern. XRPD is the most commonly used method to characterize pharmaceutical solids as it can distinguish different crystalline structures39. 2D-nuclear magnetic resonance (NMR) can also be used as this provides insight to changes in the chemical environment.

Near-infrared (NIR) spectroscopy can also be useful as it is very sensitive to water. NIR spectroscopy can distinguish bulk water from hydrate water due to different hydrogen bonding environments18. It serves as a powerful tool when dealing with hydrate differentiation as it can distinguish between different hydrates of the same compound and can examine the degree of hydrogen bonding in drugs that are capable of forming multiple hydrates18.

Although many of the other techniques have proven to be useful in characterizing particular solid forms, Raman spectroscopy is becoming the most standard method for samples in aqueous conditions. It is a method that is based upon light scattering and water along with a majority of excipients are weak, poor Raman scatterers. However, this method is beneficial as it requires minimal sample preparation similar to DSC. Low frequency Raman spectroscopy can also be used to identify the different crystalline forms of anhydrous and hydrated compounds via specific lattice vibrational modes39. However, the laser irradiation can induce a phase change in the sample.

In many cases the spectral difference between anhydrous and hydrated compounds are not clear due to the fact that there are only minor spectral differences in crystalline packing39. Additionally, many vibrational and stretching vibrations in the molecules override the lattice vibrational difference39. All of these techniques provide numerous benefits and disadvantages, but each vary with the situation at hand.

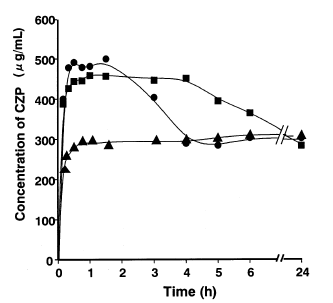
1. **Impact of Water on Performance**

As the solid form of the API can present challenges related to stability, water can mediate drug-excipient interactions through the vapor phase or facilitate interactions at the drug-excipient interface. These mechanisms of water involvement can lead to compromised physicochemical properties. Compared to crystalline solid forms of a drug compound, amorphous solid mechanical properties may be different due to the absence of long range packing14, but water still affects its properties. The effect water has on hydrates and amorphous solids differ in many ways, but both are paid close attention to as final drug performance can be drastically affected.

*4.1 Solubility*

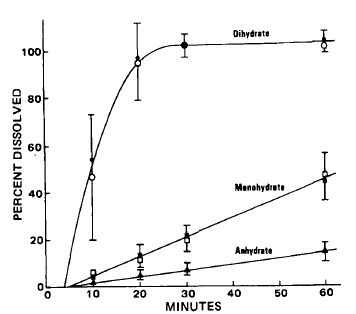
During drug development, issues related to dissolution arise. When solubilizing a crystalline solid the bonds in the lattice are breaking. The breaking of these bonds is heavily dependent upon temperature, pressure, and the nature of the solid form. The anhydrous form of a substance is always more soluble in water than the corresponding hydrate which crystallized from water at the same temperature3. As the hydrate has already intimately interacted with water, the free energy released on crystal dissolution and the further interaction with water is less for the hydrate than for the anhydrate3,36. Therefore the solubility of a hydrate in water will almost always be less than that of an anhydrous form. However, there have also been studies conducted where the hydrate has shown a faster dissolution rate than the anhydrate. Furthermore, the solubility of a hydrate in solvent will almost always be greater than that of the anhydrous form36,40. As the solvent involved in hydrates is water it cannot make as many new hydrogen bonds compared to the anhydrous that does not have water.

In a previously conducted study, the dissolution behaviors of various forms of CBZ were determined by the dispersion method and results obtained from the experiment can be seen in Figure 3. Dissolution experiments were conducted at 37°C in JP13 first fluid. Form III of CBZ showed a rapid increase in concentration with its maximum value being at the initial hours of the experiment41. Concentration decreased as time increased because dihydrate crystals precipitated out. This was due to the phase conversion of the anhydrous form to the dihydrate. This conversion to the dihydrate form resulted in a decrease in bioavailability as the concentration gradually decreased41. From this research it was concluded that the transformation from form III to the dihydrate was faster than that of form I.

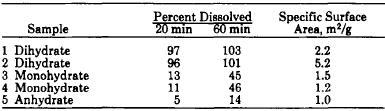
  
**Figure 3:** Dissolution profile of CBZ or CZP polymorphs and dihydrate in JP13 1st fluid with ■ representing form I, ● representing form III, and ▲ representing the dihydrate41.

Contrary to the behavior seen from the studies directed on CBZ, from research conducted on erythromycin dihydrate, the hydrate form of the drug was found to show a more rapid dissolution rate compared to the anhydrous form42. Dissolution rates of five samples of erthyromycin dihydrate, monohydrate, and anhydrate in phosphate buffer were found. From Figure 4 it can be seen that the dihydrate proved to be the fastest dissolving material43. Allen *et al* alluded the dissolution behavior seen to surface area and the net heat of adsorption43. These physical parameters were more closely examined to determine the exact reason, and results from the surface area study can be seen in Table 1.

From the results obtained through this examination, Allen *et al* determined that surface area was not the main factor that affected the dissolution rate of erythromycin. With the two samples of dihydrate, no difference in the dissolution rate was seen even though the surface area was doubled in Sample 2 compared to Sample 1.

  
**Figure 4:** Dissolution profile of erythromycin dihydrate, monohydrate, and anhydrate in phosphate buffer at 37ºC with ● representing Sample 1, ○ representing Sample 2, ■ representing Sample 3, □ representing Sample 4, and ▲ representing Sample 543. It can be concluded that the dihydrate had a faster dissolution rate compared to that of the anhydrate.

**Table 1:** Dissolution rates versus surface area for the anhydrate, monohydrate, and dihydrate forms of erythromycin43.



This physical parameter investigation was further supported when no difference was seen in the values obtained from the net heat of adsorptions.

As the aqueous solubility of the anhydrate is typically greater than the hydrate form, the dissolution rates can show a similar trend. During dissolution of theophylline anhydrate, the solution became supersaturated with respect to the hydrated form, which resulted in precipitation until the concentration reached the solubility limit of the monohydrate crystal form3. In other investigations, it was found that amorphous indomethacin crystallized at room temperature16, thus formulations containing this solid form have a higher chance of crystallizing which makes them less soluble.

When there are differences in the solubility and dissolution rates of a pharmaceutical solid, bioavailability is also altered. A previous study was done on the anhydrous and trihydrate forms of ampicillin. All forms of the ampicillin were given both to dogs and humans and the blood serum concentrations produced by the anhydrate form were found to give higher peaks than the trihydrate3. The differences seen in the concentration levels was attributed to the differences in the aqueous solubility of the anhydrate and trihydrate forms. In a study conducted by Otsuka *et al*, physicochemical properties of an anhydrate and monohydrate were analyzed when placed in low and high humidity3. This study helped conclude that the important factors controlling the bioavailability of a drug product can be caused by the storage period under certain humidities as this can result in phase changes of a pharmaceutical compound3.

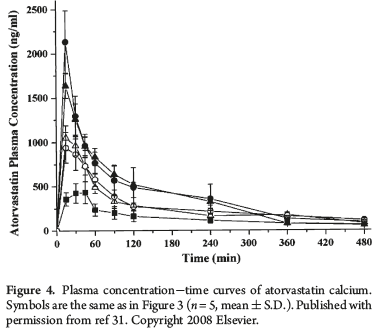
With CBZ, bioavailability tests were carried out in dogs. With a low dose the plasma concentration curves of form I, form III, and dihydrate were all found to be similar. However, with the high dose there were concentration-time curve differences. The plasma concentration was found to be the highest in form I41. The area under the curve (AUC) found for each form gave a ranking of form I > form III > dihydrate41. This relationship determined that the dihydrate did indeed possess the lowest bioavailability compared to the other forms of the pharmaceutical compound41.

Although the solubility of pharmaceutical hydrates tends to be low, amorphous solids have the potential to enhance solubility. The solubility of several amorphous drugs were looked at in previous research studies. The solubility of atorvastatin calcium was 140 µg/mL whereas the amorphous form had a solubility of 460 µg/mL44. However, with the amorphous form, after 24 hours the solubility decreased by more than 50% due to its high Gibbs free energy and lack of long-range order. Although the amorphous form had a decrease in solubility, it still remained above that of the crystalline phase. As seen in Figure 5 the dissolution rate of the amorphous atorvastatin showed a concentration peak at 10-15 minutes which decreased over the course of 5-6 hours due to crystalline formation, while still being above the peaks found from the crystalline forms.

In amorphous solids there is no crystal packing that needs to be disrupted, thus there is no energy barrier to overcome45. This results in an increase in solubility. Solubilities of amorphous indomethacin proved to be consistently greater than those of its crystalline form over a temperature range in two hours46. Similar to the peak seen with atorvastatin, the amorphous indomethacin had a peak solubility within the first 10-15 minutes46. As amorphous solids are the solid form with the highest energy, it serves to be advantageous in terms of solubility and dissolution rate47. With the increase in free energy storage instability comes into play due to relaxation, nucleation, and crystal growth47. As amorphous solids may recrystallize in the GI tract, they are typically not the solid form used in commercial pharmaceutical products as stabilizing them during storage is a big issue47.

* 1. *Stability*

In amorphous solids, temperature and RH can lead to changes in stability. Along with solid-solid phase transformations, water can cause changes in morphology which can lead to agglomeration48. When exposed to high temperature or elevated RH, amorphous drug

  
**Figure 5:** Plasma concentration-time curves of atorvastatin calcium over 480 minutes where ■ is unprocessed atorvastatin calcium particles, ● represents supercritical antisolvent (SAS) processed amorphous atorvastatin calcium precipitated from acetone solution, ▲ represents SAS processed amorphous atorvastatin calcium precipitated from THF solution, ○ represents spray-dried amorphous atorvastatin calcium from acetone solution, and ∆ represents spray-dried amorphous atorvastatin calcium from THF solution44, where there is a concentration peak at 10-15 minutes.

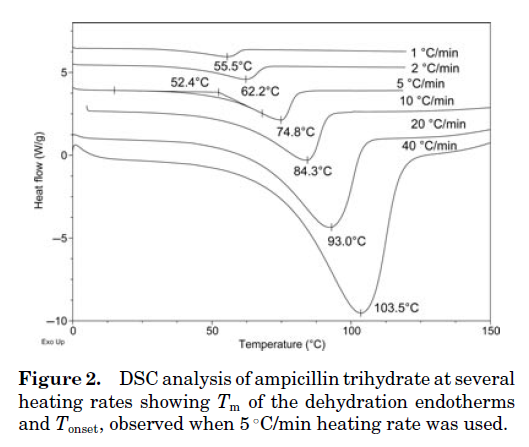
recrystallization may occur as molecular mobility will increase48. Amorphous solids have greater free volume therefore there is more space to move. When water is involved with amorphous solids it increases the free volume which increases the molecular mobility. When the molecular mobility increases, the amorphous solid has a greater change of crystallization. When water is absorbed it reduces the Tg, which increases the molecular mobility49. This increase in molecular mobility leads to a decrease in mechanical strength, decrease in viscosity, an increase in chemical reactivity44, and decrease in physical stability as crystallization is more likely to happen. An amorphous material therefore requires storage at a temperature below its Tg to ensure long term stability.

Methods to improve the stability of amorphous solids have been investigated for many years, and it has been proven that polymeric additives do indeed provide benefits to the stability of this solid form as they inhibit the crystallization of an amorphous form15. In previous studies, it was demonstrated that the inhibition of crystallization had no effect on the Tg70. Wegiel *et al* examined an amorphous formulation of resveratrol, a drug with poor aqueous solubility in hopes of ensuring its physical stability15. This drug compound has a high tendency to crystallize, thus drug-polymer intermolecular interactions were analyzed to stabilize the formulation and the storage for this product15. Several attempts to prepare amorphous resveratrol were made, but it was concluded that a crystallization inhibitor was necessary.

Phase transformations can result in crystal growth which can lead to other complications in the manufacturing process. Metronidazole benzoate can exist as both an anhydrate and as a monohydrate. When the anhydrate transforms to the monohydrate form, there is a large increase in the particle size which leads to physical instability of the suspension3. Forms are tested on accelerated stability and the form changes can be seen using XRPD, IR, TGA, or DSC50.

To determine the stability of a pharmaceutical hydrate, both the anhydrous and hydrated forms are stored in chambers which are maintained at various relative humidities6. Once equilibrium has been achieved, the solid phase is characterized. Through research conducted by Han *et al*, a rapid method of evaluating the physical stability of hydrates was determined6. A humidity controlling device was attached to a sample chamber of a powder X-ray diffractometer. Methods including TGA and variable temperature X-ray powder diffractometry (VTXRD) acquired information regarding the change in weight and the phase during the dehydration process6. From their research, it was concluded that the mechanism of dehydration should be the same at all water vapor pressures that were of interest. The dehydration kinetics of amoxicillin trihydrate was analyzed as a function of temperature and vapor pressure. When the water vapor pressure was above the transition water vapor pressure the hydrate was stable6.

In recent years, studies using DSC-based methods were done to obtain the activation energy of dehydration of hydrates in order to determine the stability of the hydrate. The dehydration activation energy serves as a barrier to chemical transformation as it depends on the crystal lattice energy of a hydrate34. DSC traces of ampicillin trihydrate can be seen in Figure 6. This figure shows an endothermic peak with an onset temperature of 52°C and a peak maximum at 75°C34. The peak maximum shifts to a higher temperature at higher heating rates. From analyzing this compound along with various hydrates, it was determined that lower values of the dehydration activation energy along with the onset temperature indicate that a hydrate may have a low stability threshold34.

  
**Figure 6:** DSC profile of ampicillin trihydrate at various heating rates showing onset temperatures and melting temperatures when a 5°C/minute heating rate was used34.

* 1. *Effects of Water on Other Performance Factors*

Through research done by Sebhatu *et al* it was seen that the amount of sorbed moisture increased with increasing RH51. When the water content increased, there was a decrease in Tg which lead to an increase in material deformation. The tensile strength, or maximum stress a tablet can withstand before breaking, was also analyzed in this study. The increased compactibility of an amorphous solid increased with moisture content which corresponded to an increased compressibility51.The drug substance can also impact compression properties. Sun and Grant conducted a study to show that the incorporation of water into the crystal lattice of *p*-hydroxybenzoic acid (PHBA) facilitated plastic deformation which provided much stronger tablets. Compared to crystalline materials that tend to be highly elastic and brittle upon exposure to external stress, amorphous materials exhibit varying degrees of viscoelasticity5. Moisture sorption into amorphous excipients has different effects on tablet hardness as well5.

In another investigation conducted by Wӧstheinrich and Schmidt, thiamine hydrochloride, a nutritional factor, was prepared by spray granulation. Through their research, the effect of a polymorphic drug substance that dissolved during processing was observed. Spray granulation led to several phase changes. During granulation the monohydrate formed and it was later dehydrated in the final drying stage. Granules that were formed contained the anhydrous form, which were rapidly sorbing moisture during tableting to produce the monohydrate10. Through multiple months of storage, the monohydrate converted to the hemihydrate with an increase in tablet hardness and disintegration time.

When molecular mobility is increased crystallization has a greater chance of occurring as it is kicking the water out. As crystallization occurs water is being pushed out of the nucleus and the water activity of the solid is changing from the solid phase to the crystalline phase. Water also has the ability to increase chemical reactivity in amorphous solids. During hydrolysis water is directly involved in a chemical reaction which results in the cleavage of a covalent bond. In a glassy state, molecules tend to be “frozen” in a certain conformation when water is not present. Thus, the molecular motion is much lower. However when water is introduced there is an increase in the molecular mobility which allows molecules to adopt conformations which are necessary for certain chemical reactions.

Through an experiment conducted with amorphous insulin, water sorption isotherms were obtained which showed the amorphous form absorbing slightly more water at a certain RH52. The level of water plays a significant role in stability because at a certain moisture content reactive groups in the protein and its reactants, which can include water, can acquire enough mobility to facilitate degradation processes52. Thus, it was established that a sharp increase in degradation rate may be observed near the monolayer level of water52.

Macromolecules are known to be inhibitors of crystallization particularly in amorphous drugs53. Aso *et al* witnessed the presence of 10% PVP slowing down the rate of total crystallization of amorphous NIF by a factor of 30053. The use of low-concentration polymers have proven to be effective even though some amorphous formulations contain polymers as its major components. Hancock and Shamblin5 discussed the effect water had on various sugars that ultimately affected drug product performance. The compactibility of β- cyclodextrin was completely lost when its waters of crystallization were removed.

1. **Formation of Hydrates and Amorphous Solids**

There are various methods for creating an amorphous phase. Some of these methods include milling/grinding, lyophilization or freeze-drying, spray drying, rapid cooling of melts or solutions, or removal of solvent or water from a hydrate or solvate. During milling, amorphous behavior can be induced because of the high amount of energy input into the system. In lyophilization, a solvent is removed rapidly leaving the molecules no time to form a crystalline lattice which results in an amorphous solid. This also occurs during spray drying. When removing a solvent or water from a solvate or hydrate, key intermolecular interactions are taken away leaving the crystal structure unstable as the intermolecular interactions are not satisfied. The resulting amorphous structure is stable and it will not return back to its original structure unless it is re-crystallized with its original solvent. Anhydrous crystalline forms may convert to a hydrated form *in-vivo,* but this process results in an impact in dosage form performance as discussed previously.

Hydrates can also be formed in numerous ways. Exposure to water is the most important step in forming this crystalline solid. Hydrates are also formed through solvent-mediated transformations (SMT) where the solvent is water54. With SMT, an anhydrous metastable form of the drug is used where its temperature is below the transition temperature54. This transition temperature represents a temperature in which anhydrous forms are the more stable solid form above it. When a solution is supersaturated with respect to a stable phase, nucleation can occur. Nucleation serves as a criteria for the growth of a stable hydrate form.

1. **Phase Transformations**

As the stability relationship between crystalline solids such as hydrates and amorphous solids changes considerably depending on temperature, pressure, and relative humidity of the environment, product development steps need to be thoroughly regulated to ensure that the final drug product contains the solid form that is desired. Solid phases of the drug compound along with the transitions among them under certain conditions should be meticulously considered. Knowing the mechanism of a phase transformation can be helpful in identifying the potential for these transformations during processing and can prevent unwanted products10. Solid-solid phase transformations are dependent upon internal rearrangements and conformational changes of molecules in the pharmaceutical drug product, thus changes in these properties lead to alterations in the properties of the final drug product.

Hydration is the conversion between crystalline anhydrates and hydrates. At a constant temperature one crystal form is stable over a certain range of water activities. At the critical aw, anhydrate/hydrate or lower hydrate/higher hydrate pairs coexist10. As temperature is increased, the critical aw tends to shift to a lower value. Vitrification refers to the interconversion between an amorphous solid and hydrate. An amorphous solid is susceptible to crystallization at all temperatures via various mechanisms10.

Phase transformations can influence the quality attributes of a pharmaceutical solid as processing characteristics may vary. Common process induced phase transitions include partial or complete formation of metastable polymorphs, an amorphous phase, hydrates, or desolvated forms10. These conversions can occur in each unit operation during the manufacturing process as well. They can occur due to the properties of the API, excipients, and the solvent involved in each processing step.

During storage, the effects of moisture and temperature can cause transformations between hydrated and anhydrous species. In a previous study, anhydrous Form A of cimetidine was converted to the hydrated Form B in water over the course of one year55. Similarly, anhydrous Form A of theophylline was converted to the monohydrate in the presence of water, resulting in a significant decrease in dissolution55. Stress-induced transformations can also occur due to mechanical stress. Hydration was found to decrease significantly with an increase in tableting pressure for theophylline55. Phase transformations caused by mechanical stress are typically known to form metastable and amorphous solids27.

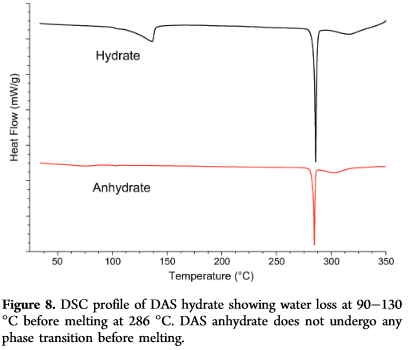
Hydration and dehydration of crystals can occur easily at low temperatures. However, varying the temperature and humidity can result in stable hydrates. Through various RH studies it was found that an amorphous solid dispersion with “*a less hydrophobic API, stronger drug-polymer interactions, and low hygroscopicity was more likely to resist water-induced phase separation*48.” CBZ has four anhydrous polymorphs, a dihydrate, and several solvates2. Commercial tablets of CBZ are formulated with the anhydrous monoclinic form which is the most stable, but when subjected to high humidity this form transforms into the dihydrate2.

Process-induced transformations can be a result of mechanical and thermal stress that is imposed upon a system during processing or after exposure to water. Wet granulation is a processing step during manufacturing that is a leading cause of these transformations. According to Jorgensen *et al,* low drying temperatures after wet granulation can cause a conversion to a metastable polymorph56. During the grinding step, there can be dehydration of the pharmaceutical hydrate. When mixing speeds increase the dissolution rate of anhydrous drugs tends to increase as well. During the granulation process, the rate the granulation liquid is sprayed can greatly affect the possibility for a transformation. When the rate is slower, excipients have the chance to adsorb the water and hinder the formation of hydrates. When mixing speed is increased, more shear force is provided to the granules which leads to nucleation occurring at a faster rate. Thus, it is important to follow process-inducing transformations to increase the quality of pharmaceutical compounds56.

Milling of the pharmaceutical compound can also promote hydrate formation. There is an increase in surface area which can lead to an increase in dissolution and nucleation rate. The quantity of water needed to achieve good granules can be up to 50% (w/w) of the dry powders38. Phase transformations that have occurred during wet granulation include the formation of an amorphous drug57 and the formation of a hydrate form after wet granulation of an anhydrous form58,59. In an investigation conducted by Marsac *et al* it is mentioned that theophylline undergoes an SMT from the anhydrate to the monohydrate form during wet granulation38. Typically when amorphous solids come in contact with water they crystallize via phase transformation. Therefore, although amorphous pharmaceuticals have proved to provide faster dissolution rates than hydrates or other crystalline solids, the use of amorphous solids as marketable dosage forms to augment oral bioavailability has been limited due to the difficulty in preventing recrystallization from the amorphous state during dissolution27.

In a study conducted by Jorgensen *et al*, granulation of wet masses of theophylline and caffeine were prepared by adding different amounts of water to anhydrous material at a low agitation speed56. From NIR spectroscopy, the caffeine hydrate showed a water absorbance maximum at 1960 nm while the anhydrate showed no absorbance in this region56. The absorbance maximum increased with increasing water content56. Through analysis with Raman spectroscopy, bands representing a C=O stretch disappeared and new bands appeared upon hydrate formation of theophylline56.

The hydrate and anhydrate phases of DAS mentioned earlier were analyzed by DSC and the profile can be seen in Figure 721. When the samples are heated there are two endothermic events seen by the DAS hydrate, one representing water loss and the other representing melting. In some cases the anhydrate form can undergo phase transitions before melting as well, but in the case of DAS there is no phase transition observed before melting occurs.

  
**Figure 7:** DSC profile of DAS hydrate and anhydrate where water loss of the hydrate is between 90-130°C and melting is at 286°C21.

Although the hydrate to anhydrate phase transformation can possess adverse effects during product manufacturing, it is an imperative issue in the final dosage form. This was recently observed in thiamine HCl hydrate tablets9. When the API exists as a hydrate, water in the lattice is not available for interaction with the formulation components9. However, it has recently been observed that the water released from the crystal lattice has been used in co-crystal formation9. This further supports the fact that pharmaceutical hydrates and water must be carefully considered throughout the manufacturing process.

1. **Screening Methods**

Hydrates are a unique type of pharmaceutical solid form. As water is a compound that is abundantly present in the air compared to other solvents, the relative stability of forms with different hydration states (including anhydrous) depends on the RH. As form changes can happen during processing, handling, and storage screening for hydrates must always occur. Hydrate screening should be done early on to prevent unwanted surprises during development. Some methods to perform hydrate screening are via solid state method, aqueous solvent systems (‘thermodynamic’) screening, or mechanical grinding. Screening is essential during the development processes as it can help optimize properties of the final formulation.

Pharmaceutical hydrates must be thoroughly characterized to avoid problems with phase transformations which could result in altered physical and/or chemical stability, bioavailability, and processing during product development3. During the initial development of the pharmaceutical dosage form, it should be determined whether the pharmaceutical solid will form a hydrate under certain operating conditions and if so, the temperature and water activity under which the solid form is thermodynamically stable should be determined3.

To determine if hydrates are possible, it is necessary to crystallize the pharmaceutical solid with solvents of varying polarity. In addition, the water activity of the solvent medium may also be varied with mixtures of water and an organic solvent3. If a hydrate is formed, physical properties such as the solubility, dissolution rate, and stability should be determined and compared with those of the anhydrous form or other hydrate form of the same drug compound3. From results obtained from these analyses, if the properties of the hydrate and anhydrate or the properties of two or more hydrates of the same substance differ drastically, the composition of the drug substance and the final product should be thoroughly monitored3.

Due to the benefits offered by amorphous solids, screening should encompass a search for amorphous forms along with hydrates. If there is a possibility of improved solubility for a pharmaceutical compound, screening for an amorphous form should occur16. XRPD and microscopy serve as the two primary methods to determine if an amorphous form has been produced16. If no peaks are seen in XRPD this represents an amorphous solid. With microscopy, an amorphous form can be detected via birefringence. If the solid form lacks this property, an amorphous solid exists. IR and solid-state NMR can also be used to screen for amorphous solids as it shows up as broad lines and alters relaxation times in NMR16.

1. **Controlling Water in Pharmaceutical Manufacturing**

As the best way to avoid issues regarding water in the manufacturing process is to eliminate it, a non-hygroscopic crystalline form of an API is highly desirable. When dealing with a pharmaceutical solid form that has acceptable moisture sorption behavior, a controlled crystallization process should be developed to avoid mixtures of various crystal forms and regions of amorphous solids5. Issues dealing with phase transformations can be minimized by identifying the thermodynamically stable crystal form over the temperature range to which the drug will be subjected. If a metastable form a pharmaceutical drug compound is used, the risk of moisture-induced phase transformations should be evaluated.

Certain processing stages should be given special attention to as well. To maintain constant quality throughout the final drug product, hygroscopicity should be measured during preformulation studies via dynamic vapor sorption (DVS)60. DVS also provides a means of viewing the transformations that can occur between the various solid forms60. During the drying stage, drying materials to the lowest possible water content may induce substantial changes. Other characterization techniques that are helpful include NIR as it can be used to differentiate between surface and bound water, which can be helpful in monitoring drying operations5. Drying processes should also be monitored to see when drying is complete.

When dealing with amorphous solids, thermodynamic and kinetic properties must be considered to ensure stability. The minimum RH at which a crystalline solid has to be exposed to eliminate the amorphous content and the maximum RH at which an amorphous sample must be stored at to stabilize the amorphous state should be identified5.

To anticipate and prevent solid phase transformations during manufacturing each of the crystal forms and amorphous phases of the API and excipients must be thoroughly understood. It is crucial to identify and investigate transformations against hydrates during product development60. Crystallization in the amorphous phase can be prevented with the addition of polymers into the matrix as they help stabilize the amorphous solid27. After the processing steps are clearly defined, monitoring of the incoming raw materials and the physical form present in the final dosage unit is suggested10.

For a manufacturing process, it is necessary to select a crystal form of an API that is least susceptible to phase transformations. When designing manufacturing processes for solid dosage forms, process-induced phase transformations can be anticipated during preformulation studies10. If the drug substance is sensitive to moisture, dry granulation could be utilized rather than wet granulation. Furthermore, during the coating stage the solid-liquid interaction at the surface of moisture sensitive cores can be minimized by applying a seal coat first that uses a solution of low viscosity at a slow spray rate10.

1. **State-of-the-Art Research**

As the hydration process has been an increasing interest in the pharmaceutical manufacturing process, characterization techniques to help identify and better control each process are becoming vital. Various methods to monitor the water activity during the manufacturing process and measure hygroscopicity have been analyzed as early identification and investigation are crucial in ensuring a high quality product.

In recent studies, it has been proved that nuclear quadrupole resonance (NQR) technique is capable of being able to detect various hydrated forms in both the raw material and the final solid dosage form product in means of helping improving quality control61. NQR is a method that is noninvasive and one that excipients do not modify. This method was used on diclofenac sodium as this drug compound exists as many solid forms. Through NQR spectra, it was evident that the active agent was found in the anhydrous form, something that XRPD patterns are not capable of. This technique is also capable of being able to study the hydration and dehydration process to quantify the conditions in which they take place in, as results from this investigation confirmed that the hydration process happened during the manufacturing process. It also serves as a method of helping to better quantify the differences in dissolution rates of final products61.

Recently, studies have been done on γ-Amino butyric acid (GABA) which are used to produce GABAergic drugs for the treatment of neurological disorders62. As a hydrate structure of GABA has never been observed, research was conducted to see which solid form would result from high-pressure crystallization experiments. From *in situ* high-pressure crystallization a GABA monohydrate was obtained62. Crystals were recovered from this crystallization process to seed saturated aqueous solutions of GABA. However only the monohydrate form was obtained with these seeds. Experiments were done at various pressure ranges to see the effect of compression on water molecules. It was concluded that due to the poor compressibility of water within ice, the hydration of GABA was thermodynamically favored at higher pressures. From this study, it can be seen that seeding techniques can promote the realization of unobserved forms of a drug product under certain conditions62.

Polymorphism also continues to be of critical importance in the industry as it is still continuing to be thoroughly screened for to ensure the most stable form of a drug product once manufacturing is complete. Polymorphs of hydrates do exist and they play a role in the transformation pathways. Raijada *et al* studied the transformation pathway of sodium naproxen and the effect its polymorphic dihydrate had on these pathways60. Sodium naxopren exists as an anhydrate (AH), monohydrate (MH), two dihydrate polymorphs (DH-1 and DH-II), and a tetrahydrate (TH). Various hydration pathways were observed: AH was transformed to MH, DH-I, and DH-II. MH was transformed to DH-I which could transform to TH. DH-II was transformed both to DH-1 and TH. It was noted that the behavior of these solid forms in the first sorption cycle varied with those of other cycles. With increasing humidity, DH-II formed from AH and DH-I was formed from MH60. With decreasing humidity DH-I and DH-II transformed to the same MH. From the DVS trace representing the first sorption cycle, DH-II produced a unique shape which was not seen in succeeding cycles60. Furthermore, it was detected that the critical humidity needed for the transformation to the TH varied in the first and second sorption cycles due to the varied tendencies of formation for DH-I and DH-II60. From this analysis, it is evident that the sorption cycles of polymorphic compounds can vary, thus better screening and characterization techniques need to exist to better analyze phase transformations that occur between these crystalline forms to guarantee production of the desired drug product.

Additionally, as the pharmaceutical industry is still facing issues with the poor aqueous solubility of drug candidates, solid dispersions have proven to be a more successful method to improve the dissolution profile63. Solid dispersions are defined as “*a dispersion of one or more API in an inert carrier or matrix at the solid state*63.” The API in the dispersion can exist as separate molecules, amorphous particles, or crystalline particles whereas the carrier can only be in the crystalline or amorphous state63. Although solid dispersions are not popular commercially as there are many issues related to scale-up they have been proven to reduce particle size, enhance wettability, and change the crystalline state in the amorphous solid state63. Amorphous carriers have the ability to increase the wettability and can inhibit the precipitation process of drugs when amorphous solids are dissolved in water, thus improving drug solubility and release rate63. Studies were conducted on amorphous dispersions and results were reported by Wegiel *et al* where polymers were used as crystallization inhibitors with resveratrol15.

1. **Conclusion**

There is a clear need for greater understanding and control of water in the pharmaceutical development of drugs. With the presence of water molecules in the pharmaceutical solid form, there can be a change in the crystal lattice which can affect packing arrangements, various intermolecular interactions, and crystalline disorder. This influences solubility, dissolution rate, stability, and bioavailability of the drug compound. The impact these changes can have in the hydration state is vital for consideration during the drug development process. Water can play a significant role throughout all stages of the development process, thus each step must be carefully considered to prevent undesired drug products. As many physical and chemical properties can be altered with water involved in the lattice or through phase transformations, to ensure the proper dose and final drug product performance better methods of controlling water during manufacturing and more efficient methods of screening should be taken into account. Through various studies done, the effect that water has on physicochemical properties and the effect it has on hydrates and amorphous solids has proven to alter properties, thus more research needs to be done to obtain a better understanding of these pharmaceutical compounds.

In addition, phase transformations of pharmaceutical compounds must be controlled to ensure there is no unexpected phase change during the production and storage of the product to guarantee the intended final drug product. As hydrates have not peaked as much interest as other crystalline forms such as co-crystals and polymorphs, more research should be conducted on this crystalline form to gain a better understanding. As recent studies have shown the improvements made in screening and characterization techniques, phase transformations along with hydrates and amorphous solids will be better understood. With this new found knowledge of these crystalline and solid forms, the role water plays in each system along with their benefits and detriments can be better used to produce the desired, more stable final drug product.

**References**

1. DiFeo, T. J. (2003). Drug Product Development: A Technical Review of Chemistry, Manufacturing, and Controls Information for the Support of Pharmaceutical Compound Licensing Activities. *Drug Development and Industrial Pharmacy*, *29*(9), 939-958.
2. Li, Y., Chow, P. S., Tan, R. B., & Black, S. N. (2008). Effect of Water Activity on the Transformation between Hydrate and Anhydrate of Carbamazepine. *Organic Process Research and Development*, *12*, 264-270.
3. Khankari, R. K., & W. Grant, D. J. (1994). Pharmaceutical Hydrates. *Thermochimica Acta*, *248*, 61-79.
4. Airaksinen, S., Karjalainen, M., Shevchenko, A., Westermarck, S., Leppänen, E., Rantanen, J., & Yliruusi, J. (2005). Role of water in the physical stability of solid dosage formulations. *Journal of Pharmaceutical Sciences*, *94*(10), 2147-2165.
5. Hilfiker, R. (2006). *Polymorphism: in the Pharmaceutical Industry*.
6. Han, J., & Suryanarayanan, R. (1999). A method for the rapid evaluation of the physical stability of pharmaceutical hydrates. *Thermochimica Acta*, *329*, 163-170.
7. [43] "Hydrate". *Encyclopedia Britannica. Encyclopedia Britannica Online.* Encyclopedia Britannica Inc., 2015. Web. 26 Apr. 2015
8. Eddleston, M. D., Madusanka, N., & Jones, W. (2014). Cocrystal Dissociation in the Presence of Water: A General Approach for Identifying Stable Cocrystal Forms. *Journal of Pharmaceutical Sciences*, *103*(9), 2865-2870.
9. Arora, K. K., Thakral, S., & Suryanarayanan, R. (2013). Instability in Theophylline and Carbamazepine Hydrate Tablets: Cocrystal Formation Due to Release of Lattice Water. *Pharmaceutical Research*, *30*, 1779-1789.
10. Zhang, G.Z. G., Law, D., Schmitt, E. A., & Qiu, Y. (2004). Phase transformation considerations during process development and manufacture of solid oral dosage forms. *Advanced Drug Delivery Reviews*, *56*(3), 371-390.
11. Sinko, P. J. (2010). Martin's Physical Pharmacy and Pharmaceutical Sciences (6th ed.).
12. Hancock, B. C., & Zografi, G. (2000). Characteristics and significance of the amorphous state in pharmaceutical systems. *Journal of Pharmaceutical Sciences*, *86*(1), 1-12.
13. Roberts, C. J., & Debenedetti, P. G. (2002). Engineering Pharmaceutical Stability with Amorphous Stability. *American Institute of Chemical Engineers Journal*, *48*(6), 1140-1144.
14. Singhal, D., & Curatolo, W. (2004). Drug polymorphism and dosage form design: a practical perspective. *Advanced Drug Delivery Reviews*, *56*(3), 335-347.
15. Wegiel, L.A., Mauer, L.J., Edgar, K.J., & Taylor, L.S. (2013). Crystallization of amorphous solid dispersions of resveratrol during preparation and storage- Impact of different polymers. *Journal of Pharmaceutical Sciences, 102*(1), 171-184.
16. Byrn, S. R., Pfeiffer, R., Ganey, M., Hoiberg, C., & Poochikian, G. (1995). Pharmaceutical Solid: A Strategic Approach to Regulatory Considerations. *Pharmaceutical Research*, *12*(7), 945-954.
17. Yu, L. (2001). Amorphous pharmaceutical solids: preparation, characterization, and stabilization. *Advanced Drug Delivery Reviews, 48*, 27-42.
18. Giron, D., Goldbronn, C., Mutz, M., Pfeffer, S., Piechon, P., & Schwab, P. (2002). Solid State Characterizations of Pharmaceutical Hydrates. *Journal of Thermal Analysis and Calorimetry*, *68*, 453-465.
19. Vogt, F. G., Brum, J., Katrincic, L. M., Flach, A., Socha, J. M., Goodman, R. M., & Haltiwanger, R. C. (2006). Physical, Crystallographic, and Spectroscopic Characterization of a Crystalline Pharmaceutical Hydrate: Understanding the Role of Water. *Crystal Growth & Design*, *6*(10), 2333-2354.
20. Gillon, A. L., Feeder, N., Davey, R. J., & Storey, R. (2003). Hydration in Molecular Crystals- A Cambridge Structural Database Analysis. *Crystal Growth and Design*, *3*(5), 663-673.
21. Roy, S., Quiñones, R., & Matzger, A. J. (2012). Structural and Physicochemical Aspects of Dasatinib Hydrate and Anhydrate Phases. *Crystal Growth and Design*, *12*, 2122-2126.
22. Seton, L., Khamar, D., Bradshaw, I., & Hutcheon, G. (2012). Process induced transformations: Phase impurities introduced during hydration/dehydration. *Chemical Engineering Science*, *77*, 57-64.
23. Lerk, C.F., Zuurman, K., & Kussendrager, K. (1984). Effect of dehydration on the binding capacity of particulate hydrates. *Journal of Pharmacy and Pharmacology, 36*(6).
24. Shefter, E., Fung, H., & Mok, O. (1973). Dehydration of crystalline theophylline monohydrate and ampicillin trihydrate. *Journal of Pharmaceutical Sciences*, *62*(5), 791-794.
25. Troller, J., & Christian, J. H. B. (1978). *Water Activity and Food*. New York: Academic Press Inc.
26. Khankari, R. K., Law, D., & Grant, D. J. (1992). Determination of water content in pharmaceutical hydrates by differential scanning calorimetry. *International Journal of Pharmaceutics*, *82*, 117-127.
27. Lu, J. (2012). Crystallization and transformation of pharmaceutical solid forms. *African Journal of Pharmacy and Pharmacology*, *6*(9), 581-597.
28. Speight, J. G. (2005). *Lange's Handbook of Chemistry* (16th ed.).
29. Kho, J. Y., Shah, U. V., Schaepertoens, M., Williams, D. R., & Heng, J. Y. (2013). Process-induced phase transformation of carbamazepine dihydrate to its polymorphic anhydrates. *Powder Technology*, *236*, 114-121.
30. Crowley, K. J., & Zografi, G. (2002). Water vapor absorption into amorphous hydrophobic drug/poly (vinylpyrrolidone) dispersions. *Journal of Pharmaceutical Sciences*, *91*(10), 2150-2165.
31. Roos, Y. H. (2008). Water Activity and Glass Transition. In *Water Activity in Foods: Fundamentals and Applications* (pp. 29-45). Oxford, UK: Blackwell Publishing Ltd.
32. Barbosa-Cánovas, G. V., Fontana Jr., A. J., Schmidt, S. J., & Labuza, T. P. (Eds.). (2007). *Water Activity in Foods: Fundamentals and Applications*.
33. Variankaval, N., Lee, C., Xu, J., Calabria, R., Tsou, N., & Ball, R. (2007). Water Activity-Mediated Control of Crystalline Phases of an Active Pharmaceutical Ingredient. *Organic Process Research and Development*, *11*(2), 229-236.
34. Shimanovich, R., Cookie, M., & Peterson, M. L. (2012). A rapid approach to the preliminary assessment of the physical stability of pharmaceutical hydrates. *Journal of Pharmaceutical Sciences*, *101*(10), 4013-4017.
35. U.S. Food and Drug Administration. (1984, April 16). Water Activity (aw) in Foods. Retrieved from <http://www.fda.gov/ICECI/Inspections/InspectionGuides/InspectionTechnicalGuides/ucm072916.htm>
36. Chadwick, K. (2015) Pharmaceutical Hydrates, *class notes*
37. Delori, A., Friscic, T., & Jones, W. (2012). The role of mechanochemistry and supramolecular design in the development of pharmaceutical materials. *CrystEngComm*, *12*(7), 2350-2362.
38. Wikström, H., Marsac, P. J., & Taylor, L. S. (2004). In-line monitoring of hydrate formation during wet granulation using Raman spectroscopy. *Journal of Pharmaceutical Sciences*, *94*(1), 209-219.
39. Liu, H., Chen, Y., & Zhang, X. C. (2006). Characterization of Anhydrous and Hydrated Pharmaceutical Materials with THz Time-Domain Spectroscopy. *Journal of Pharmaceutical Sciences*, *96*(4), 927-934.
40. Grant & Higuchi. *Solubility Behavior of Organic Compounds,* Wiley, 1990.
41. Kobayashi, Y., Ito, S., Itai, S., & Yamamoto, K. (2000). Physicochemical properties and bioavailability of carbamazepine polymorphs and dihydrate. *International Journal of Pharmaceutics*, *193*(2), 137-146.
42. Savjani, K. T., Gajjar, A. K., & Savjani, J. K. (2012). Drug Solubility: Importance and Enhancement Techniques. *International Scholarly Research Network Pharmaceutics*, *2012*.
43. Allen, P. V., Rahn, P. D., Sarapu, A. C., & Vanderwielen, A. J. (1978). Physical characterization of erythromycin: Anhydrate, monohydrate, and dihydrate crystalline solids. *Journal of Pharmaceutical Sciences*, *67*(8), 1087-1093.
44. Jagadeesh Babu, N., & Nangia, A. (2011). Solubility Advantage of Amorphous Drugs and Pharmaceutical Cocrystals. *Crystal Growth and Design*, *11*(7), 2662-2679.
45. Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, *46*(1-3), 3-26.
46. Hancock, B. C., & Parks, M. (1999). What is the True Solubility Advantage for Amorphous Pharmaceuticals? *Pharmaceutical Research*, *17*(4), 397-404.
47. Van den Mooter, G. (2012). The use of amorphous solids dispersions: A formulation study to overcome poor solubility and dissolution rate. *Drug Discovery Today: Technologies, 9*(2), e79-e85.
48. Guo, Y., Shalaev, E., & Smith, S. (2013). Physical stability of pharmaceutical formulations: solid-state characterization of amorphous dispersions. *TrAC Trends in Analytical Chemistry*, *49*, 137-144.
49. Abdul-Fattah, A. M., Truong-Le, V., Yee, L., Nguyen, L., Kalonia, D. S., Cicerone, M. T., & Pikal, M. J. (2007). Drying-induced variations in physico-chemical properties of amorphous pharmaceuticals and their impact on stability (I): Stability of a monoclonal antibody. *Journal of Pharmaceutical Sciences*, *96*(8), 1983-2008.
50. ICH Q1A (R2) Stability Testing of new Drug Substances and Products (2003).
51. Sebhatu, T., Ahlneck, C., & Alderborn, G. (1997). The effect of moisture content on the compression and bond-formation properties of amorphous lactose particles. *International Journal of Pharmaceutics*, *146*(1), 101-114.
52. Pikal, M. J., & Rigsbee, D. R. (1997). The Stability of Insulin in Crystalline and Amorphous Solids: Observation of Greater Stability for the Amorphous Form. *Pharmaceutical Research*, *14*(10), 1379-1387.
53. Sun, Y., Zhu, L., Wu, T., Cai, T., Gunn, E. M., & Yu, L. (2012). Stability of Amorphous Pharmaceutical Solids: Crystal Growth Mechanisms and Effect of Polymer Additives. *The American Association of Pharmaceutical Scientists Journal*, *14*(3), 380-388.
54. Rodríguez-Hornedo, N., Lechuga-Ballesteros, D., & Wu, H. (1992). Phase transition and heterogeneous/epitaxial nucleation of hydrated and anhydrous theophylline crystals. *International Journal of Pharmaceutics*, *85*(1-3), 149-162.
55. Newman, A. W., & Byrn, S. R. (2003). Solid-state analysis of the active pharmaceutical ingredient in drug products. *Drug Discovery Today*, *8*(19), 898-905.
56. Jorgensen, A., Rantanen, J., Karjalainen, M., Khriachtchev, L., Räsänen, E., & Yliruusi, J. (2002). Hydrate Formation during Wet Granulation Studied by Spectroscopic Methods and Multivariate Analysis. *Pharmaceutical Research*, *19*(9), 1285-1291.
57. Morris, K. R., Newman, A. W., Bugay, D. E., Ranadive, S. A., Singh, A. K., Szyper, M., Varia, S. A., Brittain, H.G., & Serajuddin, A.T.M. (1994). Characterization of humidity-dependent changes in crystal properties of a new HMG-CoA reductase inhibitor in support of its dosage form development. *International Journal of Pharmaceutics*, *108*(3), 195-206.
58. Otsuka, M., Hasegawa, H., & Matsuda, Y. (1997). Effect of Polymorphic Transformation During the Extrusion-Granulation Process on the Pharmaceutical Properties of Carbamazepine Granules. *Chemical and Pharmaceutical Bulletin*, *45*(5), 894-898.
59. Räsänen, E., Rantanen, J., Jorgensen, A., Karjalainen, M., Paakkari, T., & Yliruusi, J. (2001). Novel Identification of pseudopolymorphic changes of theophylline during wet granulation using near infrared spectroscopy. *Journal of Pharmaceutical Sciences*, *90*(3), 389-396.
60. Raijada, D., Bond, A. D., Larsen, F. H., Cornett, C., Qu, H., & Rantanen, J. (2013). Exploring the Solid-Form Landscape of Pharmaceutical Hydrates: Transformation Pathways of the Sodium Naproxen Anhydrate-Hydrate System. *Pharmaceutical Research*, *30*(1), 280-289.
61. Limandri, S., Visñovezky, C., Perez, S. C., Schurrer, C. A., Wolfenson, A. E., Ferro, M., & Cuffini, S. L.,Gonçalves de Souza, J., Armani Aguiar, F., Masetto de Gaitani, C. (2011). Nuclear Quadrupole Resonance: A Technique to Control Hydration Processes in the Pharmaceutical Industry. *Analytical Chemistry*, *83*(5), 1773-1776.
62. Fabbiani, F. P., Buth, G., Levendis, D. C., & Cruz-Cabeza, A. J. (2014). Pharmaceutical hydrates under ambient conditions from high-pressure seeds: a case study of GABA monohydrate. *Chemical Communications*, *50*(15), 1817-1819.
63. Vo, C. L., Park, C., & Lee, B. (2013). Current trends and future perspectives of solid dispersions containing poorly water-soluble drugs. *European Journal of Pharmaceutics and Biopharmaceutics*, *85*, 799-813.