POLYMORPHISM
(AS A PART OF PREFORMULATION STUDY)

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DEFINATION:-

Polymorphism: Elements can exist in two or more different forms, known as allotropes of that element .eg.

Carbon: diamond in cubic (tetrahedral lattice arrangement) graphite in sheets of a hexagonal lattice. Similar phenomenon in compounds, scientifically referred to as polymorphism. The term polymorphism was coined by AGUIAR ETAL in 1967.

THUS IT IS DEFINED AS THE ABILITY OF SUBSTANCE TO EXIST AS TWO OR MORE CRYSTALLINE PHASES THAT HAVE DIFFERENT ARRRANGEMENTS OR CONFIRMATIONS OF THE MOLECULES IN THE CRYSTAL LATTICE.

Since 1967 a series of review articles has dealt with polymorphism and its pharmaceutical application but still there is confusion in the terminology used to identify it.

NEED TO STUDY POLYMORPHISM:-

Polymorphs show the same properties in the liquid or gaseous state but they behave differently in the solid state. Different polymorphs of a compound are in general different in structure and properties in the same manner as the crystals of two different compounds. Furthermore polymorphism is remarkably common particularly within certain structural groups. For eg.

CLASS	%OF POLYMORPHISM
Barbiturates	63
Steroids	67
Sulphonamides	40

The effect of polymorphism on bioavailability is the most important consequence for drug substances if the bioavailability is mediated via dissolution. The oldest known example is chloramphenicol palmitate. Others are noviobiocine, griseofulvine, carbamazepine, aspirin and ampicilline. The polymorphism of the excipients may also play an important role in bioavailability. Thus, investigating the polymorphic behavior of drugs and excipients is an important part of the preformulation work.

One latest example is of "HYDOISOINDOLIN", a tachykinine receptor antagonist. Its stable polymorphic form is developed which is having better pharmacokinetic and pharmacodynamic criteria. *

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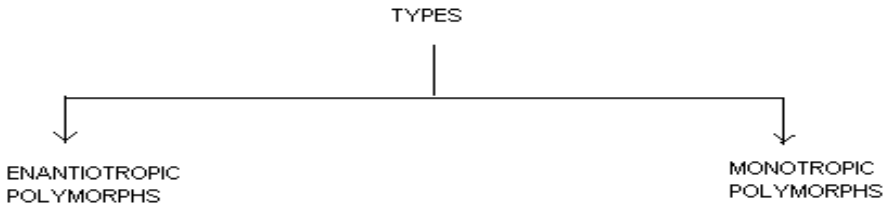
PROPERTIES OF POLYMORPHS:-

Polymorphs show the same properties in liquid or gaseous state but they behave differently in solid state.
Polymorphs differ from each other with respect to physical properties like
Melting and sublimation temperature
Vapour pressure
Solubility and dissolution rate
Stability
Optical and electrical properties
Crystal habit
Hygroscopicity
Heat capacity
Solid –state reactions
Conductivity
Compression characteristics

TYPES OF POLYMORPHISM:-



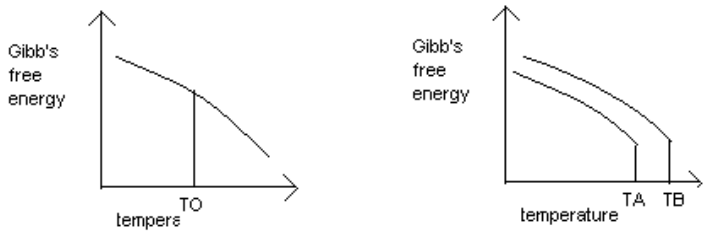
- ❖ Phase transition: the process of transformation of one polymorph into another, which may also occur on storage or during processing, is called phase transition.
- ❖ ENANTIOTROPHS:- If one form stable over certain pressure and temperature range, while the other polymorph is stable over different pressure and temperature range. eg, sulfur

- ❖ MONOTROPHS:- only one polymorph is stable at all temperature below the melting point, with all other polymorph being unstable. glyceryl stearate, chloramphenicol palmitate.

- ❖ Both enantiotropism and monotropism are important properties of polymorphs.

TRANSITION TEMPERATURE:-

"Temperature at which both stable and metastable forms exist in equilibrium with each other."



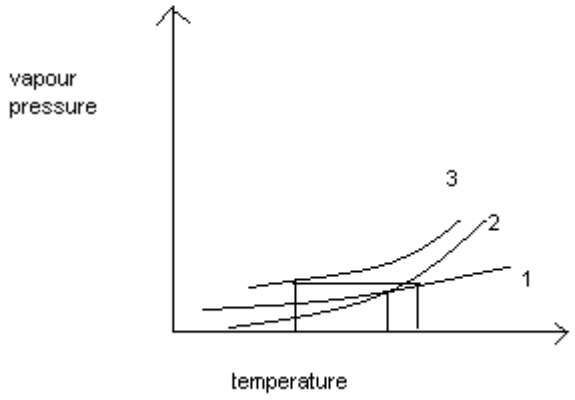
{Relationship between Gibb's free energy and temperature}

- In case of monotropy higher melting form is always thermodynamically stable form.

- In case of enantiotropy lower melting form is thermodynamically stable at the temperature below the transition temperature and higher melting form is stable at the temperature above the transition temperature.

HOW TO DIFFERENTIATE BETWEEN ENANTIOTROPIC AND MONOTROPIC SYSTEM?

Polymorphs can be differentiated by vapor pressure versus temperature curve and solubility versus temp curve.
Here form 1 is stable at tempT1 and if it exist in form 1&2, the phenomena is called enantiotropism. In case of enantiotrophism transition temperature of both the forms are same.
A different situation exists if compound exists as form 1&3. Such phenomenon is referred as monotropism. Here transition temperature of both the forms are different since form 3 is relatively unstable than form 1.



DIFFERENCE BETWEEN ENANTIOTROPY AND MONOTROPY

Enantiotropic pair	Monotopic pair
Reversible phase transition	Irreversible phase transition
metastable ↔ stable	Metastable →stable
lower melting form is thermodynamically stable below the transition temperature and higher melting form is stable above the transition temperature	higher melting form is always thermodynamically stable form

Transition is endothermic (heat of fusion rule)	Transition is exothermic (heat of fusion rule)
Higher m.p. has lower heat of fusion	Higher m.p. has higher heat of fusion

PSEUDOPOLYMORPHISM.

The term pseudo means false. Phenomenon in which solvent molecules get incorporated into crystal lattice of solid are known as solvates. This solvates exist in different crystal form called pseuopolymorphs and the phenomenon is called as pseudopolymorphism. Also known as hydrates when water is solvent. E.g. when the potent synthetic estrogen 'ethynylestradiol' is crystallized from the solvents acetonitrile, methanol, chloroform and saturated with water four different crystalline solvates are formed.

How to differentiate pseudopolymorphs from true polymorphs?
Pseudopolymorphs can be differentiated from true polymorphs by observing melting behavior in silicon oil using hot stage microscopy. Here in this technique psrudopolymorphs evolve gas(steam or solvent vapors)causing bubbling of the oil. While true polymorphs merely melts, forming second globular phase.

METHODS TO IDENTIFY POLYMORPHISM:-

- **Optical crystallography:** it is used in the identification of polymorphs .Crystal exist in isotropic and anisotropic forms . When isotropic crystals are examined the velocity of light is same in all directions while anisotropic crystals have 2 or 3 different light velocities or refractive indices. Video Recording Systems have made it possible to record the events visualized during the heating and cooling stages .The polarizing microscope fitted with a hot and cold stage is very useful for investigating polymorph .It is useful to know ,
    1. The degree of stability of metastable form .
    2. Transition Temperature .
    3. Melting Point
    4. Rates of Transition under various thermal and physical conditions .
    5. Whether to persue polymorphism as a route to an improved dosage form .

- **Hot stage microscopy:** Using this technique fluid phase transformation as a function of temperature is observed. Generally silicon oil hot stage microscopy is used for detection of pseudo polymorphs

- **X-ray diffraction method:** Using Bragg's equation:$n\lambda=2d\sin\theta$ where d=distance for different planes of crystal, λ=wavelengthof x-ray used ,θ=angle of incoming beam, n=order of spectrum
    - X-ray diffraction-a}powder x-ray diffraction→Basically focusing on packing pattern of the atom.b}exact relative location of atom in crystal is determined.

- **NMR technique:** In this technique, powder sample must be rotated at a special angle with respect to magnetic field.

- **FTIR technique:** It has been used to quantify binary mixtures of polymorphs. In identification of polymorphs , only solid samples (as mineral oil mulls & KBr pellets) can be used .
    - In solutions polymorphs of a compound have identical spectra .
    - Advantages: - Rapid.
      Technique is qualitative & quantitative.

- **Dilatometry:** Measure change in volume caused by thermal or chemical effect.Using dilatometry the melting behaviour of Theobroma Oil was studied .Extremely accurate but tedious , time consuming and not widely used .

- **Microcalorimetry:** Used to characterize thermodynamic properties of different molecules.
- **Thermal methods:** a}DSC[Differential scanning calorimetry] b}DTA[Differential thermal analysis]c} TGA [thermal gravimetric analysis]

This method measures heat loss or gain from physical or chemical chages occurring in sample which is recorded as a function of temperature as substance is heated at uniform scale.

Advantage:

I Thermodynamic parameter can be evaluated.

II Heat of Transition from one polymorph to the other.

- Melting point determination

NOTE: DSC and M.P. determination are often useful technique, but only when substance undergoing investigation heated through phase transition without decomposition.

PARAMETERS TO BE CHECKED BY PREFORMULATOR WHILE DOING POLYMORPHISM STUDY:-

1. No of polymorphs
2. Relative degree of stability
3. Presence of glassy state
4. Stabilization of metastable form
5. Temperature stability range
6. Solubility of each polymorph
7. Method of preparation
8. Effect of micronization
9. Excipients incompatibility

Stability characteristics:
If a compound exist polymorphism, one of the form will be more stable (physically)then the other form. Depending upon relative stability there are two forms of polymorphs: 1)stable form having lowest energy state, highest melting point and least aqueous solubility.2)Metastable form having higher energy state, lower melting point and higher aqueous solubility.

Ostwald Rule of Stages (Step Rule):-

Statement:- It is not the most stable state with the lowest amount of free energy that is initially formed but the least stable lying nearest in free energy to the original state.

Relative solubility of polymorphs:-In order to asses the relative increase in solubility of polymorphs with respect to another, a simple solubility ratio can be defined:-

Solubility ratio =solubility of metastable form/solubility of stable form
Examples

Compound	Solubility ratio
glybuzole(37)	**1**
Quinolone dvt	**1**
Tetracycline	**1**
Fluconazole(II/I)	**1.1**
Aspirin	**1.2**
Carbamazepine	**1.2**
Lamivudine(7)	**1.2**

Piroxicam(II/III)	**1.3**
Indomethacine	**1.4**
Methyl prednisolone(41)	**1.7**
Etoposide(28)	**1.9**
Succynil sulfathiazole	**12.7**
Niclosamide(A/H)	**22.9**

From above example, we can say that solubility ratio is higher than one just because of relative higher solubility of metastable form, that leads to increase inapparent solubility. The theoretical estimation of solubility can be done using following equation:-

Log Xic=ΔSm(Tm-T)/2.303Rt

Where Xic=ideal solubility
ΔSm=entropy of melting
Tm=temperature
R=gas constant (1.98cal/k.mol) or (8.28J/k.mol)

But solubility of metastable form may never reach to theoretical solubility due to crystallization of most stable form so data may not be consistent if we use above equation.

Dissolution behavior of the polymorphs:-the absorption rate and bioavailability of drug administered orally is controlled by many factors among which dissolution rate is one of the most important. Therefore physicochemical state such as polymorphism or amorphism of drugs affect bioavailability of pharmaceutical preparation. This is due to the fact that, as the thermodynamic activity of polymorph is lower there is lower apparent solubility and thus absorption is also less.
Order of dissolution rate: Amorphous>metastable> stable

FORMATION OF METASTABLE POLYMORPHS:-

Preparation of metastable polymorphs requires,

1. Supersaturating conditions for the metastable form.
2. Crystallization of the metastable state before the stable polymorph forms.
3. Stable conditions for the metastable polymorph so that conversion to the stable form is prevented.

STABILITY OF METASTABLE FORM :-

- The difference in melting point (Δmp)between polymorphs is measure of the metastable polymorph stability.

- If Δmp<1^0c --neither is significantally more stable.
- If Δmp is 25-50^0c—lower melting species will be difficult to crystallize and will revert rapidly.
- If Δmp is 1-25^0c—unstable form can be obtained easily before solid-solid transition.

***FACTORS AFFECTING POLYMORPHISM*:-**

(A)Temperature and humidity:-

Storage conditions affect physicochemical reactions which are accelerated at higher temperature.(arhenious theory).

Humidity acts as catalyst on the solid surface. Therefore both are the important factors for the prefomulator scientist to consider.eg.

1. Chlortetracycline hydrochloride has two different polymorphs form:α and ß. Alpha form is stable up to 82% RH while beta form is hygroscopic.
2. Zanoterone: The solid-state degradation rate of form IV (hemihydrate A) is found to be greater than that of form III at 40°C/25% RH and 40°C/75% RH. At 40°C/75% RH, the rate of degradation is 4-fold higher for form IV vs. form II.
3. Polymorphic transformation of phenylbutazone and cocoa butter occur after heating.
4. Recent studies done on paracetamol.It consist of form-1 (monoclinic) and form-2 (orthorhombic) At high temp(500k)form-II is stable
5. Leflunomide → Form I and Form II.Here Form I : Stable below transition temperature. (127°c)

(B)Photostability:-

Generally light sensitive drugs are protected from the photolytic degradation by packing them suitably in light resistant container. However the bulk powder of the stable crystalline forms resists photochemical degradation and does not require light resistant system. But still there are fewer reports:

1. For eg:- Photostability of two polymorphs of tamoxifen citrate upon irradiation by visible light and u.v. light investigated using chromatography and spectroscopy. The surface color of pellets prepared with either crystal forms turned from white to brown but the extent of the color change in cross section of form –A pellets was deeper than that of form –B pellets. So form A exhibit higher degree of Photo-instability relative to that of form B.
2. Acetametacin: α, β :- Stable and γ:- Unstable

(C) Effect of solvent:-

Solvent can bring dramatic change in growth mechanism and morphology. Growth kinetic of crystal growing from solution was determined by two important factors:

Degree of molecular roughness
Nature of absorption of the solvent from surface.

(D) **Effect of grinding:-**

Since the physicochemical properties of the same drug polymorphs are affected by mechanical energy .It is very important tool to be taken under consideration by preformulator scientist. Grinding process reduces particle size, so increasing specific surface area and that' why direct effect on dissolution rate and bioavaibility of the preparation. During grinding process solid state polymorphic transformation in to non crystalline or metastable form is caused by mechanical action.

- eg. Prasterone sulfate dihydrate
- Here dihydrate form is more stable than anhydrous form. With increasing grinding time compound become unstable because grinding weakened bonding crystals and water molecules participating in hydrolysis process of the drug.
- The grinding process can easily induce the polymorphic transition of famotidine from form B to form A and this was accelerated by thermal effect.

(e)Effect of tablet compression:-

The mechanical stability and compaction behaviour of the polymorphic form of drug during tableting are very important in practice.
Eg. Polymorphic transformations of phenylbutazone in which form 3 is converted to form2 at > 2000kg/cm^2.

EFFECT OF POLYMORPHISM ON BIOAVAILABILITY:-

If the absorption of active ingredient in drug through G.I.T. is dissolution rate dependent then polymorphism is an important preformulation tool. Here successful utilization of polymorph having significant greater thermodynamic activity (solubility)may provide good therapeutic blood level from otherwise inactive drugs.eg. novobiocin, identified in two different forms : crystalline and amorphous. In tablet or capsule formulation novobiocin is used as sodium salt which is active orally but unstable chemically while insoluble form is stable chemically and orally inactive.(unabsorbable)

HIGH THROUGHPUT CRYSRTALLIZATION:-

A high-throughput (HT) crystallization system uses a combinatorial approach to solid form generation, where large arrays of conditions and compositions are processed in parallel.
Screen 1000's of crystallization conditions.
Small amount of API is required.
Variety of solvents, additives, conditions necessarily generates large set of data.
Solid form discovery in highly polymorphic form.

A fully integrated HT crystallization system consists of experimental design and execution software, robotic dispensing and handling hardware, automated high-speed micro-analytical tools, integrated cheminformatics analysis software.
Systems designed to carry out these experiments generally consist of both hardware and software components that drive and track experimentation, and permit data storage, retrieval and analysis. Such systems should be designed to be flexible and scalable to ensure that a variety of experimental procedures can be carried out either serially or concurrently. Thus, the system can be employed at various stages of drug development, where differences exist in the quality and quantity of compound available. While it is highly desirable to have the ability to mine and model experimental data, and to use the subsequent knowledge to guide further experiments, not all HTcrystallization systems are equipped with these features.

CONCLUSION:-

If it appears that polymorphism is occurring or is likely to occur in the samples supplied for preformulation wok then a cooperative study with the bulk chemists should determine the most stable form chemically and physically.
Differences in the solubility and melting point must also be assessed and then a decision can be made to determine which form to progress through to the next stage of formulation.
Small difference in the stability but higher solubility of a relatively metastable form may lead to a preferential choice of a polymorph other than stable form but this is unlikely and is not encouraged by regulatory authorities.
Risk associated with using the metastable form is that it will convert back to the stable form during the product's life, and give a consequent change in properties.
As polymorphism can have such serious consequences for the bioavailabilities of drugs with low aqueous solubility, it is essential that manufacturers check for the existence of polymorphism and ensure that they use the same appropriate polymorphic form every time they make a product.

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