SOLUBILITY ENHANCEMENT OF CO-CRYSTAL BASED SOLID DOSAGE FORM

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Submitted By

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Under the Guidance of

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TAMIL NADU

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"Dedication is not what others except of you; it is what you can give to others"

DEDICATED TO THE ALMIGHTY AND MY BELOVED FAMILY

Evaluation Certificate

This is to certify that this dissertation entitled **"SOLUBILITY ENHANCEMENT OF CO-CRYSTAL BASED SOLID DOSAGE FORM"** is a bonafide research work Submitted by **Reg. No: 26103001, to "The Tamilnadu Dr. M.G.R. Medical university" Chennai** , in partial fulfillment of the requirement for the award of degree in **Master of Pharmacy (Pharmaceutics)**, was evaluated by us during the examination held on.....

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Declaration by the Candidate

I hereby declare that the work incorporated in the dissertation **"SOLUBILITY ENHANCEMENT OF CO-CRYSATAL BASED SOLID DOSAGE FORM"** is my own work except for the guidance received from my Project Supervisor, Dr. R. SAMBATH KUMAR M.Pharm., Ph.D., Head and professor, Dept. of Pharmaceutics , J.K.K.Nataraja college of pharmacy, Komarapalayam.

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This is to certify that this dissertation entitled **"SOLUBILITY ENHANCEMENT OF CO-CRYSATAL BASED SOLID DOSAGE FORM"** is submitted by **M.Y.ANEEF, Reg. No:26103001,** in partial fulfillment of the requirements for the award of degree in **Master of Pharmacy (Pharmaceutics),** comprises the bonafide work done by him in the Department of pharmaceutics, J.K.K.Nataraja college of pharmacy, Komarapalayam, under my guidance and direct supervision during the academic year **2011-2012.** Certified further that to the best of our knowledge and this report was not copied or does not form part of any other thesis or dissertation.

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M.Y. ANEEF
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INTRODUCTION

Solubility of active pharmaceutical ingredients (API) has always been a concern for formulators, since inadequate aqueous solubility may hamper development of parenteral products and limit bioavailability of oral products. In recent years, the problem has become more acute and more common as pharmaceutical companies cast the drug discovery net ever wider in the anticipation of finding new therapeutic approaches and improving drugs for existing therapeutic areas. Experimental determination of drug solubility is not a single event but is performed multiple times along the drug discovery and development process, the assays and their focus varying with the phase. Among the five key physicochemical screens in early compound screening, P^{Ka}, solubility, permeability, stability and lipophilicity, poor solubility tops of the list of undesirable compound properties. Compounds with insufficient solubility carry a higher risk of failure during discovery and development since insufficient solubility may compromise other property assays, mask additional undesirable properties, influence both pharmacokinetic and pharmacodynamic properties of the compound, and finally may affect the develop ability of the compound. Ideally solubility liabilities should be known prior to any functional evaluations.

Conceptually, solubility is an easy parameter to measure but its meaning and concept of use is often different for discovery and development scientists and this can be a source of misunderstandings and controversy. In a broad sense, solubility may be defined as the amount of a substance that dissolves in a given volume of solvent at a specified temperature. More specifically, compound solubility can be defined as unbuffered, buffered, and intrinsic solubility. Unbuffered solubility, usually in water, means solubility of a saturated solution of the compound at the final pH of the solution (which may be far from pH 7 due to self-buffering)1. Buffered solubility also termed apparent solubility refers to solubility at a given pH, e.g. 2 or 7.5, measured in a defined pH-buffered system and usually neglects the influence of salt formation with counterions of the buffering system on the measured solubility value. Intrinsic solubility means the

solubility of the neutral form of an ionizable compound. For neutral (non-ionizable) compounds all three definitions coincide.

Solubility plays an essential role in drug disposition, since the maximum rate of passive drug transport across a biological membrane, the main pathway for drug absorption, is the product of permeability and solubility. Poor solubility has been identified as the cause of numerous drug development failures. It is one of the components of the Biopharmaceutical Classification Scheme (BCS) and is particularly important for immediate release BCS class II drugs, for which absorption is limited by solubility (thermodynamic barrier) or dissolution rate (kinetic barrier).

TABLE 1: Solubility Classification Scheme

Definition	**Parts of solvent required for one part of solute**
Very soluble	< 1
Freely soluble	1 - 10
Soluble	10 - 30
Sparingly soluble	30 - 100
Slightly soluble	100 - 1000
Very slightly soluble	1000 - 10,000
Insoluble	> 10,000

PROCESS OF SOLUBILISATION:

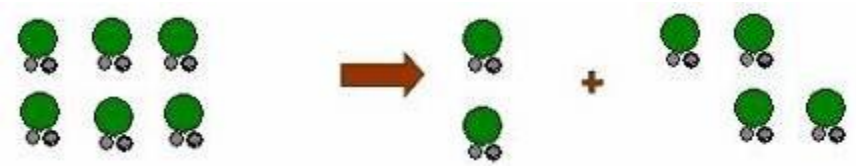
The process of solubilisation involves the breaking of inter-ionic or intermolecular bonds in the solute, the separation of the molecules of the solvent to

provide space in the solvent for the solute, interaction between the solvent and the solute molecule or ion.

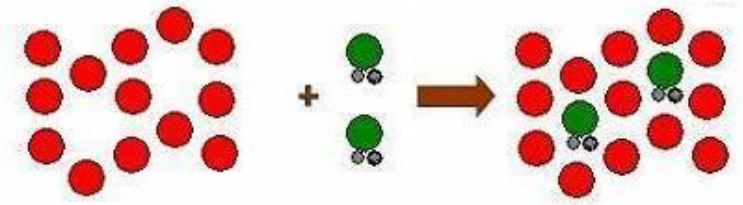
FIGURE 1: Process Of Solubilisation

Step 1: Holes opens in the solvent:

Step2: Molecules of the solid breaks away from the bulk:



Step 3: The freed solid molecule is intergrated into the hole in the solvent



FACTORS AFFECTING SOLUBILITY:

The solubility depends on the physical form of the solid, the nature and composition of solvent medium as well as temperature and pressure of system.[1]

Particle Size

The size of the solid particle influences the solubility because as a particle becomes smaller, the surface area to volume ratio increases. The larger surface area allows a greater interaction with the solvent. The effect of particle size on solubility can be described by[2]

$$\log \frac{S}{S_0} = \frac{2 \gamma V}{2.303 R T r}$$

Where,

S is the solubility of infinitely large particles

S is the solubility of fine particles

V is molar volume

g is the surface tension of the solid

r is the radius of the fine particle

Temperature:

Temperature will affect solubility. If the solution process absorbs energy then the solubility will be increased as the temperature is increased. If the solution process releases energy then the solubility will decrease with increasing temperature[3]. Generally, an increase in the temperature of the solution increases the solubility of a solid solute. A few solid solutes are less soluble in warm solutions. For all gases, solubility decreases as the temperature of the solution increases[4].

Pressure:

For gaseous solutes, an increase in pressure increases solubility and a decrease in pressure decrease the solubility. For solids and liquid solutes, changes in pressure have practically no effect on solubility[4].

Molecular size:

Molecular size will affect the solubility. The larger the molecule or the higher its molecular weight the less soluble the substance. Larger molecules are more difficult to surround with solvent molecules in order to solvate the substance. In the case of organic compounds the amount of carbon branching will increase the solubility since more branching will reduce the size (or volume) of the molecule and make it easier to solvate the molecules with solvent[3].

Polarity:

Polarity of the solute and solvent molecules will affect the solubility. Generally non-polar solute molecules will dissolve in non-polar solvents and polar solute molecules will dissolve in polar solvents. The polar solute molecules have a positive and a negative end to the molecule. If the solvent molecule is also polar, then positive ends of solvent molecules will attract negative ends of solute molecules. This is a type of intermolecular force known as dipole-dipole interaction.

Polymorphs:

A solid has a rigid form and a definite shape. The shape or habit of a crystal of a given substance may vary but the angles between the faces are always constant. A crystal is made up of atoms, ions, or molecules in a regular geometric arrangement or lattice constantly repeated in three dimensions. This repeating pattern is known as the unit cell. The capacity for a substance to crystallize in more than one crystalline form is polymorphism. It is possible that all crystals can crystallize in different forms or polymorphs. If the change from one polymorph to another is reversible, the process is called enantiotropy. If the system is monotropic, there is a transition point above the melting points of both polymorphs. The two polymorphs cannot be converted from one another without undergoing a phase transition. Polymorphs can vary in melting point. Since the melting point of the solid is related to solubility, so polymorphs will have different solubilities[2].

SOLUBILITY ASSAYS IN DISCOVERY AND DEVELOPMENT:

Depending on the experimental set-up, solubility measurements determine either the kinetic or the thermodynamic solubility of compounds. In most cases, kinetic (non-thermodynamic) solubility measurements start from dissolved compound and represent the maximum (kinetic) solubility of the fastest

precipitating species of a compound. The type of precipitating material is not determined and can be amorphous or crystalline, neutral or a salt, exist as a co-crystal or a combination of these possibilities. Kinetic solubility values are strongly time dependent and due to the degree of super-saturation that may occur, values are likely to over predict the thermodynamic solubility and are not expected to be reproducible between different kinetic methods[11].

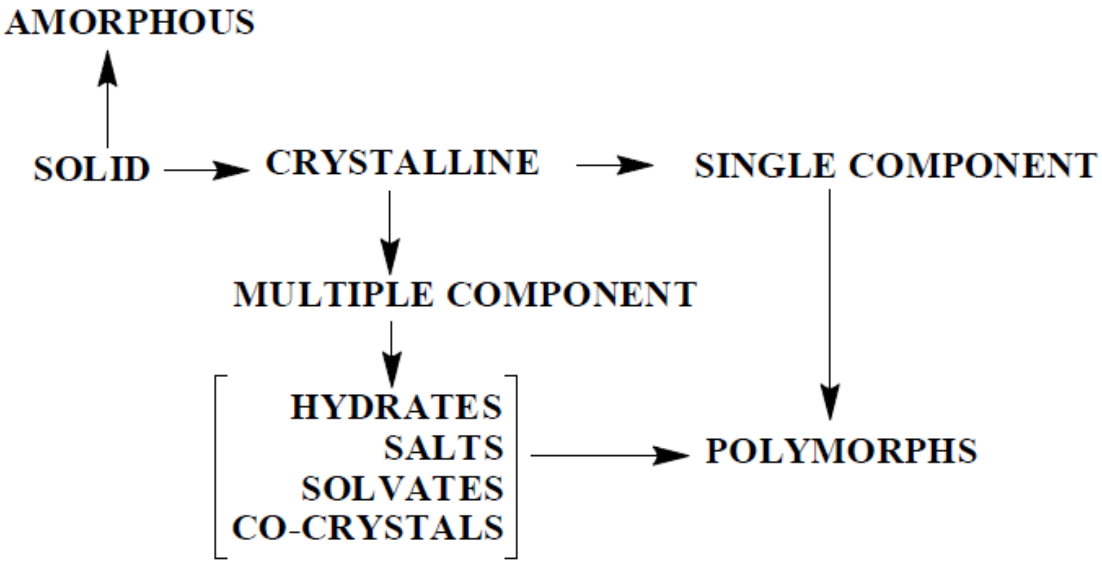
For thermodynamic solubility measurement, the dissolution rate of compounds plays an important role, since the compound's crystal lattice has to be disrupted as part of the solubilization process. Consequently, the amorphous material or poorly crystalline material that is often generated in early discovery almost always exhibits higher solubility in all solvents compared to crystalline drug[12]. Apart from crystallinity, the dissolution rate is affected by a number of additional factors, such as stirring rate, drug solubility, temperature, time, particle size, compound wettability, solvent viscosity (diffusion coefficient), and the polarity of the solvent[13]. In practice, 'equilibrium' solubility is often determined only by single measurements, generally after 24–48 h. However, to really confirm that equilibrium has been achieved, compound solubility has to be constant with time and hence solubility measurements at several time points are necessary.

The key questions addressed by solubility assays in development focus on formulation and solid phase properties of compounds and on the identification of in vitro/in vivo correlations. In early development, thermodynamic assays are performed to confirm earlier kinetic solubility results, to rule out potential artifacts, and to generate quality solubility data with crystalline material to support the discovery scientist in the selection of potential clinical candidates. Re-testing of compound solubility of new batches in this and in subsequent development phases is a must. Frequently, amorphous or partially crystalline material in early batches is gradually replaced in subsequent large scale batches by polymorphs with increasing thermodynamic stability and higher purity which may in turn result in significantly lower compound solubility[14,16].

In early development, solubility screens are also performed in organic solvents. They are required to support initial salt and polymorph screening activities which aim to generate pure, stable, crystalline material in the most desirable physical form of the compound as early as possible for development[17]. Additionally, organic solvent solubility data are very useful for formulators, process chemists, and analytical chemists for formulation, scale-up, and analytical method development, respectively[17]. In much later phases, the solubility profile in organic solvents is necessary to develop efficient cleaning validation protocols for the cleaning of pilot plant and equipment, which comply with regulatory requirements.

CRYSTAL ENGINEERING:

Polymorphism, which is the definite arrangement of molecules within a solid, has been known to influence various physicochemical and biological properties of a crystalline moiety. However, crystal habit has been paid scant attention. Crystallization is commonly employed as the final step for purification of a drug. Use of different solvents and processing conditions may alter the polymorphic state and or habit of the purified drug, leading to variation in raw material characteristics. In addition, crystal habit influences flowability, packing, compaction, syringability, stability and dissolution characteristics of a drug powder.



"Crystal engineering is the understanding of intermolecular Interactions in the context of crystal packing and in the utilization of such Understanding in the design of new solids with desired physical and chemical properties."[102]

Crystal engineering allows for the design of new compositions or multicomponent crystalline phase of matter using existing pharmaceuticals, which allows for a much wider range of possible pharmaceutical compositions than present approaches such as salt formation (ion-pairing).

It has been suggested that pharmaceutical co-crystals could play a major role in the future of pharmaceutical formulations given that they, in principle, will outnumber polymorphs, solvates and pharmaceutical salts combined. The physical properties of interest for specific API's could be systematically optimized by rational design.

New opportunities for producing a larger diversity of solid forms of drug substances exhibiting the proper balance of important properties for development into a viable and effective drug product may be met by co-crystals[18]. Furthermore, exploring the co-crystallization potential around an API increases the intellectual property protection over a particular drug product; thus, reducing the risk of costly litigation and market erosion[19,20].

CRYSTAL ENGINEERING FOR IMPROVING SOLUBILITY AND DISSOLUTION RATES:

Crystal engineering approaches, which can potentially be applied to a wide range of crystalline materials, offer an alternative and potentially fruitful method for improving the solubility, dissolution rate and subsequent bioavailability of poorly soluble drugs. The ability to engineer materials with suitable dissolution characteristics, whilst maintaining suitable physical and chemical stability provides a strong driver for the utilisation of new and existing crystal engineering approaches to drug delivery system design. The challenges of low aqueous solubility provide an ideal situation for the application of crystal engineering techniques for improving bioavailability, whilst also developing stable and robust pharmaceutical products. Therefore potential utility of crystal engineering as an approach for designing efficacious dosage forms for poorly soluble drugs.

Crystal engineering is taken as the design of molecular solids in the broadest sense with the aim of tailoring specific physical or chemical properties. The subject of the approaches is therefore to present those diverse aspects of crystal engineering which may be used to manipulate the solubility and or dissolution rate of the parent molecular components in the crystalline state. At the centre of these available approaches is the need to change surface and molecular assembly in equilibrium with a solution. Consequently, it covers the possible ways; this may be achieved from recent developments in the study of molecular solids and reviews topical issues such as habit modification, polymorphism, solvation, co-crystal formation and surface modification. Particular attention will be paid to the area of co-crystallisation, which is an emerging area of strategic importance to the pharmaceutical sector.

Although numerous strategies exist for enhancing the bioavailability of drugs with low aqueous solubility, the success of these approaches is not yet able to be guaranteed and is greatly dependent on the physical and chemical nature of the molecules being developed. Crystal engineering offers a number of routes to improved solubility and dissolution rate, which can be adopted through an in-depth

knowledge of crystallization processes and the molecular properties of active pharmaceutical ingredients.

CRYSTAL ENGINEERING IN DRUG DEVELOPMENT:

Crystal engineering has been described as the 'exploitation of non-covalent interactions between molecular or ionic components for the rational design of solid-state structures that might exhibit interesting electrical, magnetic, and optical properties'. It is also recognized that it 'is becoming increasingly evident that the specificity, directionality, and predictability of intermolecular hydrogen bonds can be utilized to assemble supramolecular structures of, at the very least, controlled dimensionality'.

Crystal engineering now encompasses many aspects of solid-state intermolecular interactions, structure prediction, control and rationalisation, as well as the synthesis of novel molecular building blocks and crystalline materials, and may be broken down into the components of analysis and synthesis. Within the notion of a crystal as a supramolecular entity lies certain key ideas central to the activity of crystal engineering. These are the nature of the crystallisation process at a molecular level, crystal packing, molecular interaction and directed molecular recognition, which will all be explored to some extent in this review and which should provide some understanding of crystal engineering approaches as a means of addressing the challenges of low aqueous solubility.

It is clear that the crystal and particle engineering strategies have notable potential to strengthen the available methods for addressing problems of low aqueous solubility of drug substances. These methods are applicable not only to molecules of a specific physical and chemical nature, but to a wide range of crystalline materials, although a comprehensive knowledge of drugs at the molecular level is required to determine the appropriate approach to improving solubility and dissolution rate.

SUPRAMOLECULAR TECHNIQUE:

Supramolecular chemistry has grown around Lehn's analogy that 'supermolecules are to molecules and the intermolecular bond, what molecules are to atoms and the covalent bond'. If molecules are built by connecting atoms with covalent bonds, solid-state supermolecules (crystals) are built by connecting molecules with intermolecular interactions. The fundamentals of crystal engineering were described in detail under the term 'molecular engineering'[21]. Modern crystal engineering initially began as a method for understanding the regioselectivity and product distribution in solid-state molecular reactions, termed topochemistry[21]. This field has developed rapidly, particularly with the arrival of modern crystallographic techniques such as four circle diffractometers in the early 1970's followed by the introduction of area detector technology.

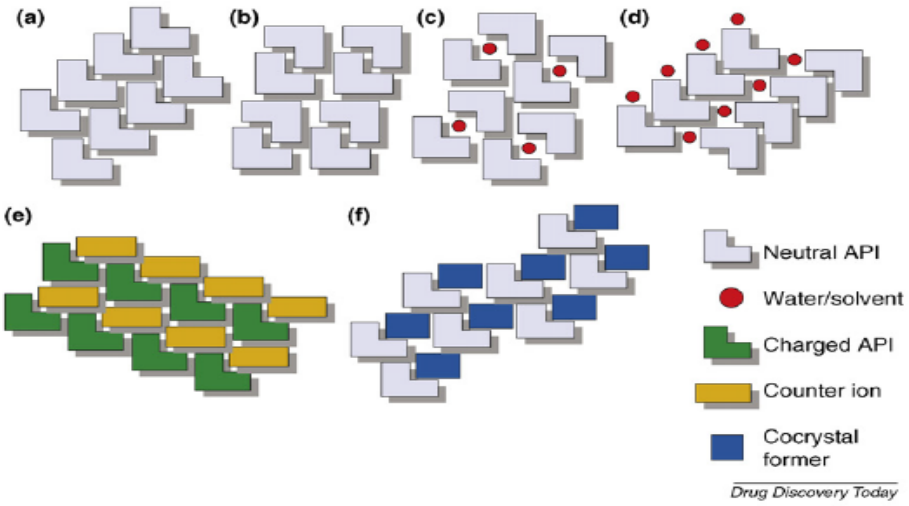
Supramolecular chemistry is an important, interdisciplinary branch of science and compassing ideas of physical and biological processes, defined as 'chemistry beyond the molecule', i.e. the chemistry of molecular aggregates assembled via non-covalent interactions[22,23]. The term 'synthon' was initially established to explain synthetic organic structural features. The term 'supramolecular synthon' established by Desiraju is defined as: "Structural units within super molecules which can be formed and/or assembled by known conceivable synthetic operations involving intermolecular interaction".

TABLE 2: Possible homosynthons and heterosynthons in the supramolecular Chemistry

S.NO	TYPE OF BOND	OCCURRENCE
1	Acid-acid	33%
2	Acid-pyridine	63%
3	Acid-amide	47%
4	Amide-amide	35%
5	Amide-pyridine	4%
6	Amide-pyridine-N-Oxide	78%

In biological processes, supramolecular chemistry is nothing but non-covalent molecular binding recognized by Paul Ehlrich and Emil Fisher's lock-and-key principle through concept of complementarily and selectivity. Progressively non-covalent bonds were understood in more detail and the importance of supramolecular chemistry was well established by 1987. Supramolecular chemistry has broad range of applications in different areas such as catalysis, material technology, green chemistry, data storage and processing. Apart from these supramolecular chemistry has been used in the design and development of new pharmaceutical therapies by understanding the interactions at a drug binding site.

Figure 2: The range of single crystalline forms that is possible for an API: (a) pure API; (b) polymorph of pure API; (c) clathrate hydrate/solvate of API; (d) hydrate/solvate of API; (e) salt of API; (f) pharmaceutical co-crystal. Salts and co-crystals can also form hydrates, solvates, and polymorphs.



COCRYSTALS:

Pharmaceutical materials science being a fundamental branch that continuously provide important insights, theories, and technologies to formulation sciences. During the development of the pharmaceutical industry, crystallization has been engaged more and more extensively for the purification, separation particle formation and co-crystallization of pharmaceutical materials It is estimated that more than 70% of all solid drugs are produced by crystallization. With regards to this, an understanding of the effect of the crystallization process on the final solid state of a drug is vital for several of the activities of the pharmaceutical industry.

The recent advances in this area have brought the possibility to produce pharmaceutical materials by design. In particular, the formation of co-crystals, i.e. crystalline molecular complexes of two or more neutral molecules, represents a potential route to achieve pharmaceutical materials with improved properties of interest, including dissolution rate and stability under conditions of high relative humidity.

Co-crystals consists of API and a stoichiometric amount of a pharmaceutically acceptable co-crystal former. APIs are among the most valuable crystalline substances and crystal engineering has been successfully utilized in the generation of cocrystals of drugs with improved physicochemical properties such as solubility, stability and bioavailability in pharmaceutical development without changing the chemical composition of the API.

These can be constructed through several types of interaction, including hydrogen bonding, pi-stacking, and vander Waals forces. Phase transformations induced during processing or storage affects the mechanisms of conversion of crystalline drugs to co-crystals. Pharmaceutical co-crystals considered better alternatives to optimize drug properties could play a major part in the future of API formulation and can be employed for chiral resolution.

TABLE 3: Type of different bonding interactions

INTERACTION TYPE	EXAMPLE
Very strong H bonds	O...H...O- , F...H...F-
Coordinative bonds	M...N , M...O
Strong hydrogen bonds	O...H...O , N...H...O
Weak hydrogen bonds	C—H...O , O—H..pi
Van der waals interactions	CH3...CH3 , CH3...Ph
Hetero atom interactions	N...Cl ,I...I , Br...Br
pi-stacking	Ph...Ph , nucleobases

Co-crystallization is a result of competing molecular associations between similar molecules, or homomers, and different molecules or heteromers[23]. Hydrogen bonds are the basis of molecular recognition phenomena in pharmaceutical systems and are responsible for the generation of families of molecular networks with the same molecular components (single component crystals and their polymorphs) or with different molecular components (multiple component crystals or co-crystals) in the crystalline state[24] Cocrystals with the same active pharmaceutical ingredient will have strikingly different pharmaceutical

properties (melting point, solubility, dissolution, bioavailability, moisture uptake, chemical stability, etc.), depending on the nature of the second component.

A co-crystal can be described as a crystalline structure formed by two different or more molecular entities where, in contrast to salts, the intermolecular interactions are not ionic, but weak forces like hydrogen bonding and π-π stacking. The big advantage of co-crystals compared to salts is that co-crystallisation is also applicable to non-ionisable molecules where salt formation is not possible.

Co-crystals offer great potential in various fields:

- ***Chiral resolution:*** whether a molecule is ionisable or not, selective diastereomeric co-crystallisation can be attainable using an enantiomerically pure coformer.
- ***Separation and purification:*** co crystals can be a good option, especially with non-ionisable products, to purify some intermediates, consequently avoiding expensive chromatographic techniques.
- ***Crystallisation of non-solid products:*** liquids, pastes and oily products can become a solid form by means of co-crystallisation, leading to more robust and efficient manufacturing processes.
- ***Improvement of solid state properties (of APIs and other organic substances):*** several important characteristics of pharmaceutical substances like solubility, bioavailability, stability, hygroscopicity, morphology, filtration and flowability can be modified by means of co-crystal formation. In the case of APIs, it is remarkable that the number of pharmaceutically acceptable co-crystal formers is larger than the number of counter ions for salt formation.

In the pharmaceutical field, the increase attention of investigation of co-crystal forms of API relates to four main factors:

a) **Design** - many drugs contain functional groups which are complimentary to a range of coformers to form pharmaceutical cocrystals.

b) **Discovery** - numerous processes, including conventional solution crystallisation, liquid assisted grinding, slurry conversion and melts crystallisation, can be used to generate wide ranges of co-crystal forms of drugs.

c) **Diversity**- Pharmaceutical co-crystals demonstrate different physicochemical properties compared with pure crystal forms of API.

d) Development - Co-crystals signify non-obvious forms of drugs and therefore can represent intellectual property for future development. Additionally pharmaceutical co-crystals enhance other essential properties of the APIs such as chemical stability, compressibility, flowability and hygroscopicity[25,26].

Pharmaceutical Co-crystals:

An alternative approach available for the enhancement of drug solubility, dissolution and bioavailability, is through the application of crystal engineering to co-crystals, historically referred to as molecular complexes. The physicochemical properties and the bulk material properties of the API can be modified, at the same time as maintaining the intrinsic activity of the drug molecule.

Pharmaceutical co-crystallization is emerging as an attractive alternative to polymorphs, salts and solvates in the modification of an active pharmaceutical ingredient (API) during dosage form design. The intellectual property implications of creating co-crystals are also highly relevant.

This approach of co-crystal involves the expansion of a supramolecular library of co-crystallizing agents. A hierarchy of guest functional groups is classified within the library according to a specific role to a crystal packing arrangement, which is dependent on the host molecule functionalities. These are obtained from investigation of structure property relationships present in the CSD which contains classes of known crystal structures[27]. Generally in the pharmaceutical industry, Chemists and engineers try to deliver crystalline forms of their active compounds, principally due to the inherent stability of crystalline

materials and the well-established impact of crystallization processes on isolation and purification of chemical substances[28]. Increasing interest is now receiving on the impact of properties of material on drug discovery and development[29], The task of pharmaceutical industry is to quick advance development programs through good confidence with the intention that formulation problems are unlikely to occur and to maximize a compounds potential as a therapeutic[30].

A major tool which is accountable for the majority of directed intermolecular interactions in molecular complex pharmaceuticals is the hydrogen bond. Co-crystals are multi-component crystals depend on hydrogen bonding interactions lacking the transfer of hydrogen ions to form salts. Pharmaceutical co-crystals can be defined as multicomponent crystalline materials comprised of an API and one or more unique co-crystal formers, which are solids under ambient conditions. For nonionizable compounds, co-crystals enhance pharmaceutical properties by modification of solubility, dissolution rate, chemical stability, mechanical behavior, moisture uptake and bioavailability[31].

Recently, Pharmaceutical co-crystallization has only gained widespread attention as a tool of changing the physicochemical properties of drugs, for the reason that co-crystal formation may probably be employed with all drugs, including acidic, basic and non ionizable molecules and a large number of probable 'counter molecules' which possibly considered to be non toxic possibly rising the scope of the pharmaceutical co- crystallization over the salt forms. To salt selection, an correlation can be drawn in which pKa point of view are used to select acid-base pairs that can be converted to salt compounds. Chemistry exhibits that a pKa difference between an acid and a base of at least two units is necessitated to form a salt that is stable in water[32]. In addition, it is significant to remember that salt formation is usually directed at a single acidic and basic functional group. On the contrary co-crystals can concurrently address multiple functional groups in a single API. As well space is not limited to binary combinations that is acid-base pairs as tertiary and quaternary cocrystals are realistic one[33,34].

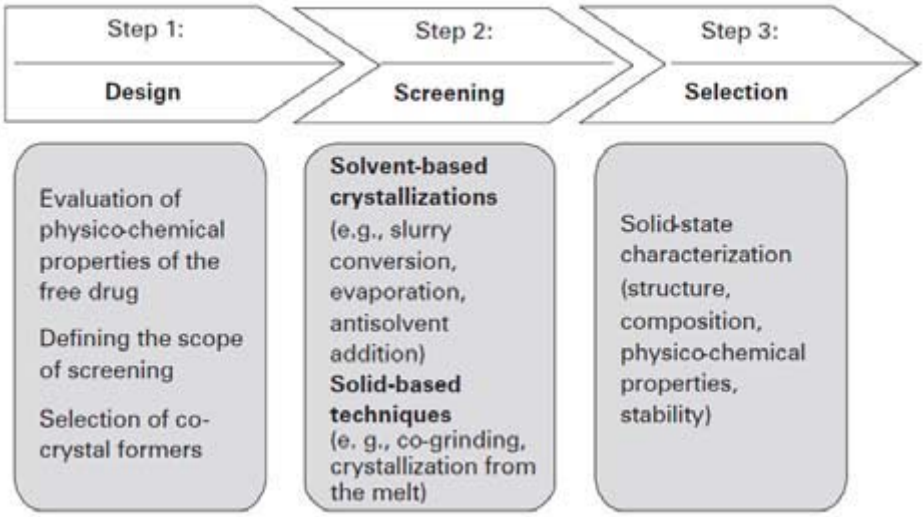
The key difference between solvates and co-crystals is the physical state of the individual components[35]. At room temperature if one component is liquid then the crystals were assigned as solvates, if both components are solids at room temperature then the crystals are called as co-crystals. Though, co-crystals have a propensity to be a product of more rational design and are more stable, predominantly as the co-crystallizing agents are solids at room temperature.

The key remunerations associated with approach of co-crystallization to alter the properties of pharmaceutical solids are the theoretical ability of all types of drug molecules to form co-crystals including weakly ionisable and non-ionizable, and the existence of numerous, potential countermolecules, including preservatives, food additives, pharmaceutical excipients as well as other drugs, for co-crystal synthesis. Major advantage for the pharmaceutical industry is co-crystal synthesis which may offer is an opportunity to address intellectual property (IP) issues by extending the life cycles of old APIs[36].

Co-crystals - Preparation Methods:

Formation of co-crystal described shows the disreputably difficult situation these systems present with regard to preparation it has been recognized to take 6 months to prepare a single co-crystal of appropriate quality for single X-ray diffraction analysis[37]. This is partially as such a heteromeric system will only form if the non covalent forces between two or more molecules are stronger than between the molecules in the corresponding homomeric crystals. Cocrystal design strategies are still being researched and the mechanism of formation is far from being understood. Co-crystals can be prepared by solid and solvent based techniques. The solvent-based techniques involve solvent evaporation, slurry conversion, cooling crystallization and precipitation. The solid based techniques involve net grinding, solvent-assisted grinding and sonication (applied to both to dry or wet solid mixtures) 80^{o} to 85° [38].

Figure 3: A general guideline for co-crystal design and screening.



Co-crystallization from Solution:

The two components must have similar solubility for solution cocrystallization; otherwise the component which has least soluble will precipitate out entirely. On the other hand similar solubility of two components alone will not promise success. It has been recommended that it possibly useful to believe polymorphic compounds, which exist in more than one crystalline form as co-crystallizing components. If a molecular compound exists in numerous polymorphic forms it has showed a structural flexibility and is not locked into a single type of crystalline lattice or packing mode. Therefore, the possibility of conveying such a component into a different packing arrangement in coexistence with another molecule is improved. Obviously polymorphism alone does not promise the functionality of a molecule to act as a co-crystallizing agent, at the same time as the ability of a molecule to contribute in intermolecular interactions clearly plays a critical role[39].

Co-crystal from Small-scale preparation has been described. Scale-up crystallization was carried out in a water-jacketed glass crystallization vessel and temperature was controlled by a circulating water bath. Teflon blade and overhead stirrer with a glass shaft were attached to vessel ports and also a reflux column,

digital thermometer were attached. The API and cocrystal former were added to this vessel and were dissolved in ethanol/methanol mixture and heated to 700 C under reflux for 1 hour. To induce precipitation of co-crystal, temperature was decreased at a rate of 100 C in a stirred, un seeded system. Literate to improve solids recovery decrease the additional temperature[40].

Co-crystallization by Grinding:

The product acquired when preparing co-crystals from grinding is usually consistent with that obtained from solution. This may specify that patterns of hydrogen-bond connectivity are not idiosyncratic or determined by non-specific and uncontrollable effects of solvent or crystallization conditions. Many co-crystal materials can be prepared from both solution co-crystallization and solid-state grinding, some can only be prepared by solid-state grinding where as some can be prepared by solution cocrystallization.

For instance, in the co-crystallization of 2,4,6- trinitrobenzoic acid and indole-3-acetic acid, different crystal forms were prepared from solution when compared with grinding co-crystallization. Disappointment in co-crystals formation by grinding co-crystallization possibly due to an incapability to generate suitable co-crystal arrangements rather than due to the stability of the initial phases. The reason for the successful formation of co-crystal from solution but not from grinding may be of solvent inclusion in stabilizing the supramolecular structure. Even though formation of co-crystal by solidstate grinding has been established for a moment and a late 19th century report is frequently cited as the initial reference to such a procedure, the current technique of liquid assistant grinding has been shown to improve the kinetics and facilitate co-crystal formation and as lead to increased attention of solid-state grinding as a method for co-crystallization[39].

Co-crystallization by Slurry conversion:

Experimentations in slurry conversion were carried out in different organic solvents and water. 100 to 200 ml of Solvent was added and the resulting

suspension was stirred at room temperature for few days. After few days, the solvent was decanted and the solid product was dried under a flow of nitrogen for few minutes. The remaining solids were then characterized using PXRD analysis[40].

Co-crystallization by addition of ant solvent:

This is one of the precipitation methods for co-crystallization of the co-former and drug. In this method, solvents include buffers (pH) and organic solvents. For instance in preparation of aceclofenac-chitosan cocrystals, in which solution of chitosan was prepared by soaking chitosan in glacial acetic acid for few hours. By using high dispersion homogenizer the drug was dispersed in chitosan solution. This dispersion was added to distilled water or sodium citrate solution to precipitate chitosan on drug[40].

Sublimation:

If a compound is sufficiently volatile at accessible vacuum pressures it can be crystallized. This technique is often used in the purification of crude mixtures. Crystals may form from a fusion, or by sublimation; but crystallization almost always takes place from solution[39].

Melting:

Melts have generated an interest in co-crystal formation. By simply melting two co-crystal formers together and cooling, a co-crystal may be formed. If a co-crystal is not formed from a melt, a seed from a melt may be used in a crystallization solution in order to afford a co-crystal[41].

Seeding:

A seed crystal of the same or a similar material is added to a supersaturated solution in order to induce the growth of single crystals of a certain form as the solution evaporates[41].

Pharmaceutical Co-Crystals as Intellectual Property:

Compared to other types of solid forms, co-crystals possessed particular scientific and regulatory advantages, and alongside these advantages were intellectual property issues which give co-crystals with exclusive opportunities and challenges. Researchers accounted the importance about patents on pharmaceutical co-crystals to the pharmaceutical industry[42]. The worth of co-crystals to the pharmaceutical industry should become clearer, mostly with respect to several relevant legal and regulatory issues, as products containing co-crystal technology come out from pharmaceutical development pipelines onto the market.

Characterization of Co-Crystals:

Characterization of co-crystals involves both structures (infrared spectroscopy, single crystal x-ray crystallography and powder x-ray diffraction)[42,44] and physical properties (e.g. melting point apparatus, differential scanning calorimetry, thermo gravimetric analysis)[45,46]. The analytical prospective of NIR spectroscopy for co-crystal screening using Raman spectroscopy as a comparative technique has been reported[47].

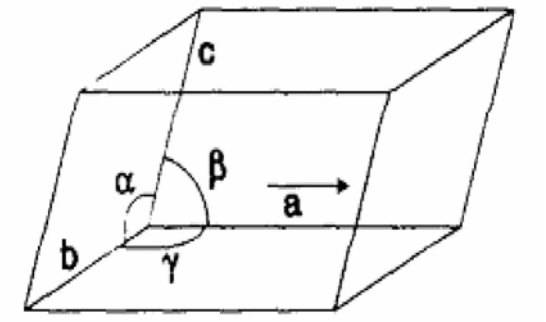
Crystallography:

X-ray crystallography is the study of crystals and their structure by means of the diffraction of X-rays by the frequently spaced atoms of crystalline materials[44,48]. When an X-ray beam is directed at a crystalline sample, the atomic structure of the lattice causes the X-rays to be scattered in a defined manner creating a diffraction pattern. The electron density in the crystal may be deduced from this diffraction pattern, enabling an accurate molecular structure to be determined. To understand this theory further and precisely determine crystal structures it is useful to have a general knowledge of crystal structures and their interaction with X-rays[48,84].

Crystal Structure:

Crystalline solid compounds are highly ordered structures; the molecules are arranged in an exactly regular array that is repeated by translation in three dimensions. Consequently crystal structures consist of structural units that are stacked side by side in all directions, thus forming a lattice in which the molecules in the repeated structural units are thought of as points. The lattice is considered to be infinitely large. The lattice points connect to form unit cells - a unit cell is the smallest group of atoms which has the overall symmetry of a crystal structure. The entire lattice can be built up by repetition of the unit cell in three dimensions. Figure no. 4 depicts a unit cell; it is a parallelepiped defined by sides of length a, b and c, and three angles α, β, γ. The number of molecules in the unit cell is given as Z; the number of molecules in the asymmetric unit is given as Z'[48].

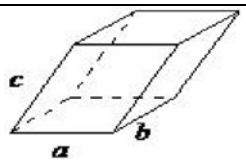
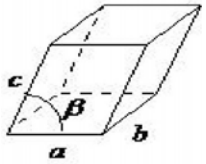
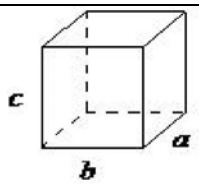
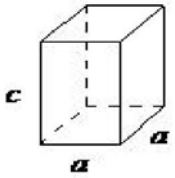
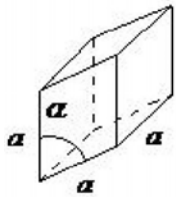
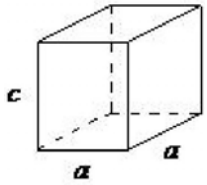
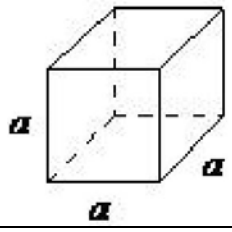
Figure No. 4 The unit cell



Crystal Systems:

Due to restrictions imposed by reflection and rotation symmetry on the unit cell parameters, there are only seven types of crystal systems possible. These are shown in Table no. 3[48].

TABLE 4: TYPES OF CRYSTALS

Crystal System	Restrictions of unit cell	Essential Symmetry	Unit cell geometry
Triclinic	None	None – but can have a centre of inversion	
Monoclinic	$a \neq b \neq c$, $\alpha = \gamma = 90°$, $\beta \neq 90°$	One 2-fold rotation and/or mirror (perpendicular)	
Orthorhombic	$a \neq b \neq c$, $\alpha = \beta = \gamma = 90°$	Three 2-fold rotations and mirrors (mutuallyorthogonal)	
Tetragonal	$a = b \neq c$, $\alpha = \beta = \gamma = 90°$	Three axes are at right angles, one is longer than the other two.	
Rhombohedral	$a = b = c$, $\alpha = \beta = \gamma \neq 90°$	1 threefold axis of rotation.	
Hexagonal	Hexagonal $a = b \neq c$, $\alpha = \beta = 90°$, $\gamma = 120°$	Three equal axes are in one plane to each other, one is at right angles to this plane but not necessarily of the same length as the others. Cross section is hexagonal.	
Cubic	$a = b = c$, $\alpha = \beta = \gamma = 90°$	Four three-fold rotation axes	

Lattice Types:

Within these seven crystal systems there are two possible lattice types: primitive and non-primitive. A primitive lattice (P) only has lattice points at the corners of the unit cell, whereas a non-primitive lattice also has points on the faces or within the unit cell as well as on the corners. Non primitive lattices may be side-centred (A, B or C), face-centred (F) with a lattice point at the centre of each face, body-centred (I) with a lattice point at the centre of the unit cell, or in the special case of trigonal rhombohedra, doubly-centred at (⅓,⅓, ⅔) and (⅔, ⅔, ⅓).

Bravais Lattices:

When the above crystal systems and lattice types are combined, only 14 crystal lattices are possible. These lattices are called Bravais lattices as they were defined by Bravais and Frankenheimer in the19th century. Figure depicts these lattices – they correspond to the seven unit cell shapes of the seven different crystal systems. Each lattice has a centre of inversion and all the points in the lattice are equivalent byinversion[48].

Space Groups:

In a single molecule the various combinations of symmetry operations are called point groups as all the symmetry elements refer to one point[49]. For a crystal, however, the symmetry elements are arranged in space in accordance with the lattice translational symmetry, therefore these sets of symmetry operations are called space groups[50]. There are 230possible space groups; each space group represents a combination of point and space symmetry elements that is compatible with the geometrical requirements of 3-D lattices. The complete crystal structure can be obtained from the asymmetric unit and its appropriate space group symmetry operations.

Each space group is denoted by a symbol comprising of an upper case letter showing the Bravais lattice type followed by lower case numbers and letters to represent the combination of symmetry elements present. Rotation and screw axes

are symbolized by numbers and mirror and glide planes by letters. For example, P21/c is the most common space group consisting of a primitive unit cell with a screw axis parallel to the b-axis and a glide plane perpendicular to b with translation along c. The International Tables for Crystallography list all 230 space groups and their associated systematic absent reflections[50].

CURCUMIN:

Turmeric has been used historically as a component of Indian Ayurvedic medicine since 1900 BC, to treat a wide variety of ailments. Research in the latter half of the 20th century has identified curcumin as responsible for most of the biological activity of turmeric[4,51] Invitro and animal studies have suggested a wide range of potential therapeutic or preventive effects associated with curcumin. At present, these effects have not been confirmed in humans. However, as of 2008, numerous clinical trials in humans were underway, studying the effect of curcumin on various diseases, including multiple myeloma, pancreatic cancer, my elodysplastic syndromes, colon cancer, psoriasis and Alzheimer's disease[5,52].

FIGURE 5: CURCUMIN

TURMERIC COMPOSITION:

CURCUMIN:

The most active component of turmeric is curcumin, which makes up 2 to 5% of the spice. The characteristic yellow colour of turmeric is due to the curcuminoids, first isolated by Vogel in 1842.

Essential oils:

They are about 1.5 and 5.5% of the composition. These essential oils consist of a 60% of sesquiterpene, lactone, turmerone. They also contain zingiberene (25%), α- and γ-atlantone, bisabolene, guaiane, germacrene, 1,8-cineole, borneol, δ-sabinene, caprilic acid, dehydroturmerone, 1-phenyl-HON-pentane, limonene, linalol, eugenol, curcumenol, curcumenone, curlone and phelandrene.

Other active principles:

Turmeric extract is rich in carbohydrates, especially in starch (45-55%). It also contains arabinogalactans (ukonans), potassium salt and resins.

Turmeric is a popular spice frequently used in Indian foods and curry. The biological activities of turmeric are attributed to the active component, curcumin. Curcumin today occupies a unique global niche as *"A Wonder Molecule"*. Curcumin (1, diferuloylmethane) is the active ingredient in the traditional herbal remedy and dietary Indian spice turmeric; it is derived from the rhizome of the herb Curcuma longa.

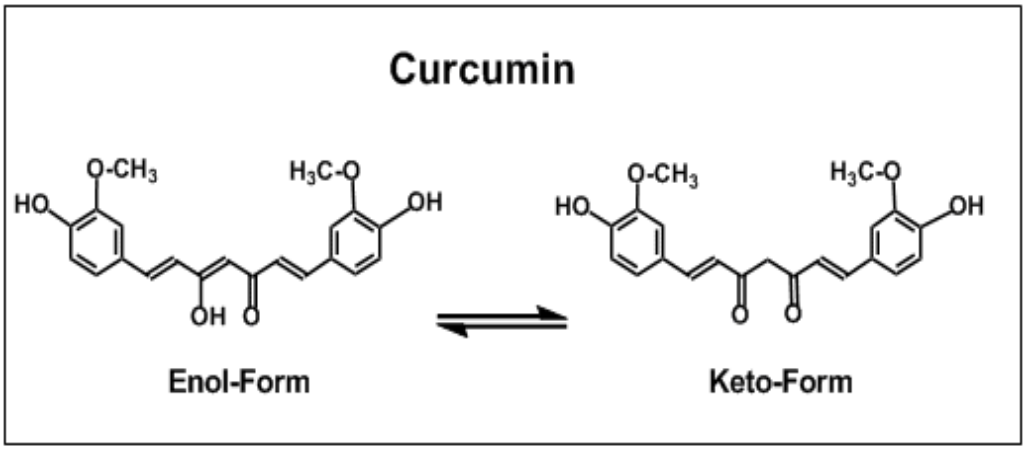
Curcumin is extracted from turmeric by solvent extraction. Powdered turmeric is dissolved in a suitable solvent (such as ethanol, acetone, or methanol) and solvent is partially evaporated. The concentrate is then cooled to allow curcumin to crystallize. After a wash with solvent such as hexane, impurities are removed and residual solvent vaporized to obtain yellow crystals of curcumin.

Curcumin is classified as a hydrophobic polyphenol compound that gives turmeric its bright yellow colour. Besides being a popular dietary supplement, it is used as a food colouring agent. Curcumin holds a high place in ayurvedic medicine as a "cleanser of the body," and today, science has documented several diseased conditions that can be healed by the active ingredients of turmeric[54-7,55-8].

CHEMISTRY:

Curcumin was first chemically characterized in 1910. Chemically, curcumin is bis-α,β-unsaturated β-diketone (diferuloylmethane) which exists in a keto-enol tautomerism where keto is predominant in neutral and acidic pH and stable-enol form is in alkaline pH .

FIGURE 6: TWO FORMS OF CURCUMIN

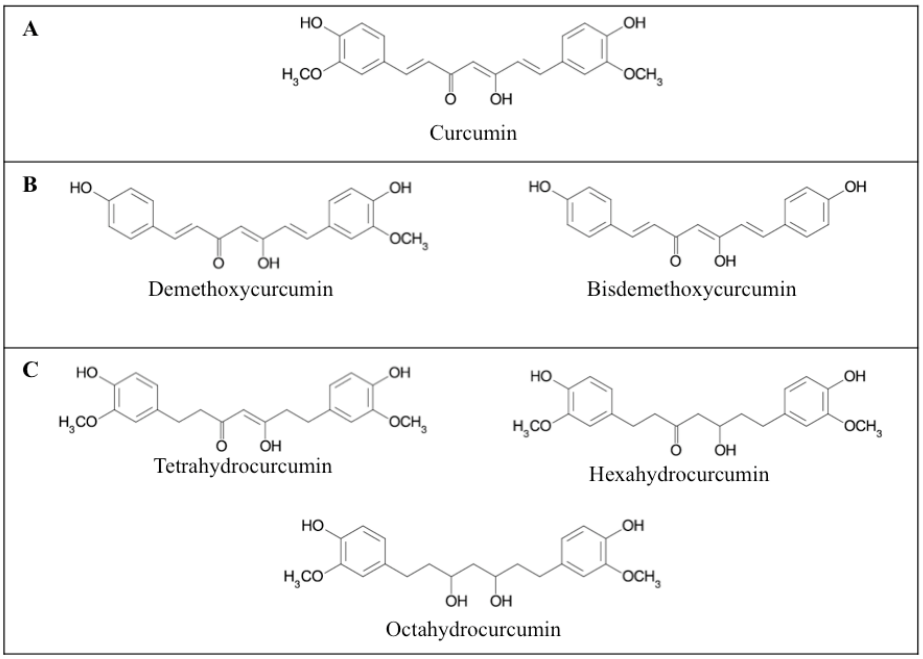


It is a hydrophobic phenol that is insoluble in water, but soluble in acetone, DMSO and alcohol. At pH 3–7, curcumin acts as a potent H-atom donor and at alkaline pH, curcumin acts mainly as an electron donor. Curcumin has a molecular weight of 368.37 and a melting point of 183°C.

Commercial grade curcumin (from Sigma Aldrich) has 70% curcumin, 10-20% of demethoxycurcumin and less than 5% of bis demethoxycurcumin . Commercial grade curcumin is known to have comparable inhibitory effects on carcinogenesis as pure curcumin. This can be attributed to the pro- and anti-oxidant capacities of both the analogue of curcumin [56-11,57-12].

FIGURE 7: DIFFERENT FORMS OF CURCUMIN

Chemical structure of curcuminoids. Curcumin (A), curcumindemethoxy derivatives (demethoxycurcumin and bisdemethoxycurcumin)(B) and hydrogenated curcumin metabolites (tetrahydrocurcumin, hexahydrocurcumin and octahydrocurcumin).



Activities of curcumin:

Curcumin has been shown to exhibit following well established activities:

- Antioxidant[58,9]
- Anti-inflammatory[59,10]
- Antimicrobial[60-11,61-12]
- Anticarcinogenic[62,13]
- Hepato and nephro-protective[63-14,64-15]
- Thrombosis suppressing[65,16]
- Myocardial infarction protective [66-17,67-18]
- Hypoglycemic[68-19,69-20]
- Anti-atherosclerotic [70,21]
- Antirheumatic[71,22]

It also inhibits lipid per oxidation and scavenges super oxide anion, singlet oxygen, nitric oxide and hydroxyl radicals[72,23]. Recent work has also demonstrated that curcumin possesses the ability to prevent protein aggregation in debilitating diseases such as Alzheimer's [73,24]and Parkinson's[74,25] .

Various animal models[75,26] or human studies[76-27,77-28] proved that curcumin is extremely safe even at very high doses. For example, three different phase I clinical trials indicated that curcumin, when taken as high as 12 g per day, is well tolerated [76-27,77-28]. Despite having multiple benefits and extremely superior safety profile, the administration of curcumin to patients has a serious practical problem.

Challenges:

A major challenge in using curcumin for treatment of diseases is the poor aqueous solubility (~20 μg/mL), which significantly limits its availability in biological systems. For the fraction of curcumin that is aqueous soluble, another main challenge to widespread clinical application is the lack of stability. Curcumin undergoes rapid degradation first by hydrolysis, which is then followed by molecular fragmentation [78,29] .

After oral administration, curcumin was poorly absorbed [79,30] and only trace amounts of curcumin compound appeared in blood[76,27], while most orally administered curcumin was excreted in the feces and urine after rapid metabolization in the intestine to form several reduced products [80,31] . One of the causes of reduced bioavailability of curcumin is due to its poor solubility in water at either acidic or physiological pH, which makes curcumin hard to absorb[81,32]**.** Together with membrane permeability, drug solubility is among the key determinants for oral bioavailability.

The reasons for reduced bioavailability of any agent within the body are low intrinsic activity, poor absorption, and high rate of metabolism, inactivity of metabolic products and/or rapid elimination and clearance from the body.

The poor bioavailability of curcumin from biological system[82,33] is due to its:

- Low absorption
- Rapid metabolism
- Fast systemic elimination.

Curcumin decomposes rapidly in neutral and alkaline medium, >90% decomposition occurs within 30 min in pH 7.4 buffer medium[78,29].

The pharmacological safety and efficacy of curcumin makes it a potential compound for treatment and prevention of a wide variety of human diseases. In spite of its efficacy and safety curcumin is still not approved as a therapeutic agent, and the relative bioavailability of curcumin has been highlighted as a major problem for this. Studies to date have suggested a strong intrinsic activity and, hence, efficacy of curcumin as a therapeutic agent for various ailments. However, studies over the past three decades related to absorption, distribution, metabolism and excretion of curcumin have revealed poor absorption and rapid metabolism of curcumin that severely curtails its bioavailability.

The aqueous solubility of curcumin can be improved by increasing the pH of the solution. However, this approach leads to an undesirable outcome: a high rate of degradation by alkaline hydrolysis[83,34]. An attractive alternative approach to addressing the poor aqueous solubility issue is to encapsulate curcumin in surfactant micelles. Several studies have shown that curcumin has a significantly higher solubility (~740 μg/mL) in micellar solutions[84,35].

Biomacromolecules, including gelatin, alginate, and maltodextrin (MD), have been used to improve the water solubility, stability, and bioavailability of curcumin[85-36,86-37] . Physical processing methods, which reduce particle size and generate an amorphous state, have been applied to improve the dispersity of curcumin powder particles.

The problems of poor solubility, instability and poor bioavailability have been overcome by several methods:

- ✓ By adding adjuvants such as piperine to block the metabolic pathways of curcumin.
- ✓ By nano-particle based drug delivery approaches in which curcumin is encapsulated in liposomes [87,38], solid lipid micro particles such as Bovine Serum Albumin [88,39] and chitosan[89,40] or complexed with phospholipids and cyclodextrin[90,41].
- ✓ With concomitant administration of lecithin, quercetin, genistein, eugenol, terpinol etc.

The aqueous solubility of curcumin was enhanced 38-fold in the presence of 1-10% (w/v) rubusoside. The bioavailability of curcumin in water and lipid medium was enhanced by complexation with phosphatidyl choline in equimolar ratio.

Recently synthesis of curcumin-encapsulated polymeric Nanoparticles of N-isopropyl acryl amide with N-vinyl-2-pyrrolidone and poly (ethylene glycol) mono acrylate has been reported[91,42] .

STUDIES CARRIED OUT ON CURCUMIN BIOAVAILABILITY:

Serum Concentration:

One of the major observations related to curcumin studies involves the observation of extremely low serum levels. The first reported study to examine the uptake, distribution, and excretion of curcumin was by Wahlstrom and Blennow in 1978 using Sprague– Dawley rats. Negligible amounts of curcumin in blood

plasma of rats after oral administration of 1 g/kg of curcumin showed that curcumin was poorly absorbed from the gut[92,43] .In 1980, Ravindranath et al. showed that after oral administration of 400 mg of curcumin to rats, no curcumin was found in heart blood, whereas a trace amount (less than 5 μg/mL) was found in the portal blood from 15 min to 24 h after administration of curcumin[79,30].

Pan et al., for example, investigated the pharmacokinetic properties of curcumin administered either orally or intra peritoneal (i.p.) in mice. With oral administration of 1.0 g/kg of curcumin, low plasma levels of 0.13 μg/mL appeared in plasma after 15 min, while a maximum plasma level of 0.22 μg/mL was obtained at 1 h; plasma concentrations then declined below the detection limit by 6 h. Entirely different plasma curcumin levels were found after i.p. administration of 0.1 g/kg. Plasma curcumin levels peaked (2.25 μg/mL) within 15 min of administration and declined rapidly within 1 h[80,31].

Table 5: Serum and Tissue Levels of Curcumin in Rodents and Human after Different Routes of Administration

SPECIES	ROUTE	DOSE	PLASMA/TISSUE	LEVELS
Mice	I.P.	100 mg/kg	Plasma	2.25 µg/ml
Mice	Oral	100 mg/kg	Plasma	0.22 µg/ml
Rat	Oral	1 g/kg	Serum	0.5 µg/ml
Rat	Oral	500 mg/kg	Plasma	0.06±0.01 µg/ml
Rat	I.V.	10 mg/kg	Plasma	0.36±0.05 µg/ml
Human	Oral	10 g	Serum	50.5 ng/ml
Human	Oral	3.6 g	Plasma	11.1±0.6 nmol/ml

Tissue distribution:

Uptake and distribution of curcumin in body tissues is obviously important for its Biological activity, yet only a limited number of studies have addressed this

issue. Ravindranath et al. showed that after oral administration of 400 mg of curcumin to rats only traces of unchanged drug were found in the liver and kidney. At 30 min, 90% of curcumin was found in the stomach and small intestine, but only 1% was present at 24 h[79,30]. In an in vitro study, when everted sacs of rat intestine were incubated with 50–750 μg of curcumin in 10 mL of incubation medium 30–80% of the curcumin disappeared from the mucosal side and no curcumin was found in the serosal fluid. Less than 3% of the curcumin was found in the tissues at the highest curcumin concentration[93,44].

Metabolism:

Various studies have evaluated the metabolism of curcumin in rodents and in humans. Once absorbed, curcumin is subjected to conjugations like sulfation and glucuronidation at various tissue sites. The very first biodistribution study reported the metabolism of major part of curcumin orally administered to rats. Liver was indicated as the major organ responsible for metabolism of curcumin [94-45,95-46]. Holder et al. reported that the major billiary metabolites of curcumin are glucuronides of tetrahydrocurcumin (THC) and hexahydrocurcumin (HHC) in rats. A minor biliary metabolite was dihydroferulic acid together with traces of ferulic acid**.** In addition to glucuronides, sulfate conjugates were found in the urine of curcumin treated rats [30] .Hydrolysis of plasma samples with glucuronidase by Pan et al. showed that 99% of curcumin in plasma was present as glucuronide conjugates. This study also revealed curcumin–glucuronoside, dihydrocurcumin–glucuronoside, tetrahydrocurcumin (THC)–glucuronoside, and THC are major metabolites of curcumin in vivo[80,31].

Elimination:

Systemic elimination or clearance of curcumin from the body is also an important factor, which determines its relative biological activity. An early study by Wahlstrom and Blennow reported that when 1 g/kg curcumin was given orally to rats, 75% of it was excreted in the feces and negligible amounts were found in the urine. Intravenous (i.v.) and i.p. administration of curcumin resulted in biliary excretion of drug from cannulated rats[96,47].

A clinical study with 15 patients and oral curcumin doses between 36 and 180 mg of curcumin daily for up to 4 months found neither curcumin nor its metabolites in urine, but curcumin was recovered from feces[97,48]. The absorption and elimination half-lives of orally administered curcumin (2 g/kg) in rats were reported to be 0.31 ± 0.07 and 1.7 ± 0.5 h, respectively. But in humans, the same dose of curcumin did not allow the calculation of these half-life values because the serum curcumin levels were below the detection limit at most of the time points in most of the experimental subjects.

LITERATURE REVIEW

Ashwini Nangia et al 2011, He worked on fast dissolving curcumin co-crystals and reported novel cocrystals of curcumin with resorcinol and pyrrogallol obtained by liquid assisted grinding. They also characterized by X-ray diffraction, thermal analysis, FT-IR, FT-Raman, and solid-state ^{13}C NMR spectroscopy. The dissolution rates of co-crystals in 40% etoh-Water are faster than that of curcumin[78].

S. Manju, K. Sreenivasan 2011, Studied on hollow microcapsules built by layer by layer assembly for the encapsulation and sustained release of curcumin. Hollow microcapsules fabricated by layer-by-layer assembly (LBL) using oppositely charged polyelectrolytes are an emerging technique for the design of novel drug delivery systems. The possibility of loading a fair amount of active component of poor aqueous solubility is one of the encouraging factors on the wide spread interest of this emerging technology. LBL constructed polyelectrolyte microcapsules based drug delivery systems have the potential for dispersing hydrophobic agent like curcumin in aqueous media. They composed of poly (sodium 4-styrene sulfonic acid) and poly (ethyleneimine). The microcapsules were found to be cytocompatible while the extract of capsules loaded with curcumin showed severe cytotoxicity on the mouse fibroblast cell indicating released curcumin is active[77].

H.Tang et al 2010 Were reported a novel type of polymer-drug conjugates. The high molecular curcumin polymers (polycurcumins) made by condensation polymerization of curcumin. The polycurcumins as backbone-type conjugates have advantages of high drug loading efficiency, fixed drug loading contents, stabilized curcumin in their backbones and tailored water-solubility. The polycurcumins are cytotoxic to cancer cells, but a polyacetal-based polycurcumin (pcurc 8) is highly toxic to SKOV-3, OVCAR-3 ovarian cancers, and MCF-7 breast cancer cell lines. It can be quickly taken up by cancer cells into their lysosomes, where pcurc 8 hydrolyzes and releases active curcumin. In vivo, the polymer showed remarkable antitumor activity in a SKOV-3 i.p. Tumorxenograft animal model[76].

Anindita Mukerjee and Jamboor K. Vishwanatha 2009, Worked on formulation, characterization and evaluation of curcumin- loaded PLGA (polylactic-co-glycolic acid) nanospheres for cancer therapy. PLGA nanospheres were prepared by solid/oil/water emulsion evaporation method and characterized for % yield, encapsulation efficiency, surface morphology, particle size, drug distribution studies, drug-polymer interaction studies and in vitro drug release profiles. The in vitro release study showed that curcumin was released in a sustained manner over a prolonged of time. Intracellular uptake and cell viability assays also demonstrated efficient uptake and action of the curcumin nanospheres in prostate cancer cell lines, thus making it a potential candidate for cancer therapy[75].

R.S. Mulik et al 2010 Investigated the in vitro anticancer activity and photo stability of transferrin mediated solid lipid nanoparticles containing curcumin (Tf-C-SLN). Tf-C-SLN were prepared by homogenization method and characterized by size, zeta potential, entrapment efficiency and stability, transmission electron microscopy (TEM), X-ray diffraction (XRD) and in vitro release study. Microplate analysis and flow cytometry techniques were used for cytotoxicity and apoptosis studies. The cytotoxicity, cell uptake and anticancer activity of curcumin were enhanced with Tf-C-SLN compared to curcumin solubilized surfactant solution and curcumin-loaded SLN. The proposed drug delivery system of curcumin is suitable for the effective delivery of curcumin considering the aspects of sustained release, biocompatible and biodegradable nature, and the targeting effect[74].

Anirban Maitra et al 2007, worked on polymeric nanoparticle encapsulated formulation of curcumin-nanocurcumin. They synthesized nanocurcumin by utilizing the micellar aggregates of cross-linked and random copolymers of N-isopropylacrylamide (NIPAAM), with N-vinyl-2-pyrrolidone (VP) and poly (ethylene glycol) monoacrylate (PEG-A). Physico-chemical characterization of the polymeric nanoparticles by dynamic laser light scattering and transmission electron microscopy confirms a narrow size distribution in the 50

nm range. Unlike free curcumin, nanocurcumin is readily dispersed in aqueous media. They demonstrated that nanocurcumin formulation has comparable efficacy to free curcumin against pancreatic cancer cell lines in vitro, by inhibiting cell viability and colony formation in soft agar[73].

Latheeshjlal. L et al 2011, were studied on formulation and development of buccal drug delivery system containing curcumin. An attempt has been made to improve the bioavailability by using different concentrations of sodium lauryl sulphate as bio enhancer. Buccal bilayer tablets were prepared by direct compression with different ratio of HPMC. K4M. As bioadhesive polymer and ethyl cellulose as backing layer. The formulations were characterized by weight variation, thickness, hardness, friability, mucoadhesive strength, drug content, swelling studies and in vitro diffusion studies. The formulated unidirectional, bilayerd, buccoadhesive tablet for curcumin using HPMC as mucoadhesive agent is superior to oral conventional tablets, as it has the potential to bypass the first pass metabolism and improve the bioavailability of curcumin[72].

Saraswathi R et al 2010, were made an attempt to formulate and evaluate curcumin transdermal drug delivery system. The transdermal patches were made which were of matrix diffusion control system. Solvent casting technique was used to prepare the transdermal patch by using polymers HPMC and ethyl cellulose at various ratios. Interaction of drug and polymer was confirmed by UV-Visible interaction and FTIR studies. In vitro drug diffusion study was also carried out using Franz diffusion cell. In vitro permeation studies show that 1:1 concentration ratio of HPMC and EC bring the satisfactory release of curcumin[71].

R.Patel et al 2009 Investigated the potential of a transfersomes formulation for transdermal delivery of curcumin. Optimization of formulations was done by selecting various processing variables such as effect of lecithin, surfactant ratio, effect of various solvents and effect of surfactants. The transfersomes were formulated by modified hand shaking method using tween 80 and span 80 in various concentrations. Transfersomes formed from lecithin: span 80 in the ratio 85:15 is a promising approach to improve the permeability of curcumin. The

transfersome entrapped curcumin gel gives better permeation as compare to plain drug gel[70].

Guangxi Zhai et al 2009, Developed self-micro emulsifying drug delivery system (SMEDDS) to enhance the oral absorption of curcumin. The formulation of curcumin-loaded SMEDDS was optimized by a simplex lattice experiment design. The in situ absorption property of curcumin loaded SMEDDS was evaluated in rat intestine and the absorption was via passive transfer by diffusion across the lipid membranes. The oral absorption of curcumin in vivo in mice was significantly enhanced by SMEDDS compared with curcumin suspension. These studies show that SMEDDS is a promising formulation for improving oral absorption of drug with poor aqueous solubility[69].

Jithanaukunuru and vidyavathi sankavarapu 2009, Worked on preparation, characterization and evaluation of hepatoprotective activity of NNDMA-(NN dimethylamino) curcumin biodegradable parenteral sustained release microspheres. Microspheres were prepared using solution crystallization method using polycaprolactone. In vivo animal studies for 10 days were carried out for the determination of hepatoprotective activity of the formulation in a ccl_4 model. In this 10-day study they identified that the microsphere formulation offers significant hepato protection than repeated I.V. administration. Prepared NNDMAC microspheres have potential use in liver toxicity/cirrhosis[68].

Mohamed Habiburrahman et al 2010 Investigated the influence of polymers on the release kinetics of curcumin in floating microspheres. The microspheres were prepared by oil in water emulsion or solution crystallization method using HPMC K100 and poloxamer 188 and characterization was done by size analysis, drug incorporation efficiency, % yield, buoyancy percentage and in vitro drug release. Linear regression method was used to evaluate release kinetics. Curcumin absorption and kinetic parameters can be modified by formulating as floating drug delivery system. Formulation variables such as agitation speed and solvent composition doesn't have any significant effect on release profile of

floating microspheres. Compared to HPMC based curcumin microspheres the poloxamer based curcumin microspheres show controlled release kinetics[67].

Andrew VT, et al 2006 observed the theophylline co-crystal prepared possess improved physical stability, specifically in avoidance of hydrate formation. The co-crystals prepared with oxalic acid were found to be more stable. This study demonstrates that co-crystals can be used effectively in physical property improvement[66].

Saranjit Singh, et al 2007 has been investigates the Isoniazid stability by subjecting to different ICH prescribed stress conditions of thermal stress, hydrolysis, oxidation and photolysis. Author reported that drug was stable to dry heat, extensive decomposition under hydrolytic conditions, while it was only moderately sensitive to oxidation stress and the solid drug turned intense yellow on exposure to light under accelerated conditions of temperature (40◦C) and humidity (75% RH). In total, three major degradation products were detected by LC-MS. The product appearing at RRT 4.22 was isolated using preparative LC-MS, and turned out to be a yellow compound that was identified as isonicotinic acid N-(pyridyl-4-carbonyl)-hydrazide based on mass, FTIR and 1H/13C NMR spectral data[109].

P. York, et al 2007 has been described Crystal engineering of active pharmaceutical ingredients to improve solubility and dissolution rates. The concept and theory of crystal engineering and discusses the potential benefits, disadvantages and methods of preparation of co-crystals, metastable polymorphs, high-energy amorphous forms and ultrafine particles[13].

Mutalik S, et al 2008 had prepared co-crystals of aceclofenac by using chitosan. Sodium citrate has been used as a salting out agent to precipitate chitosan on aceclofenac crystals. The dissolution study showed that marked increase in dissolution rate in comparison to pure drug. The dissolution rate of aceclofenac crystals can be attributed to the wetting effect of chitosan, decreased drug crystallinity, altered morphology and micronization. The optimized crystals

showed excellent stability on storage at accelerated conditions. *In vivo* studies it was revealed that the crystals provide a rapid pharmacological response in mice and rat and also it shows improved pharmacokinetic parameters in rats[76].

Sekhon BS 2009 has been described about Pharmaceutical co-crystals which cover Co-crystals construction through several types of interaction, including hydrogen bonding, pi-stacking, and van der Waals forces. Factors affecting co-crystal stability are reported and a co-crystal is only expected toform if it is thermodynamically more stable than the crystals of its components. Phase transformations induced during processing/storage affects the mechanisms of conversion of crystalline drugs to co-crystals[108].

Nate Schultheiss, et al 2009 have been described pharmaceutical cocrystals and Their Physicochemical Properties, advances made over the last 10 years pertaining to physical and chemical property improvements through pharmaceutical cocrystalline materials and, with anticipation, draw closer the fields of crystal engineering and pharmaceutical sciences[2].

Miranda L, et al 2010 Cheney et al described how the supramolecular synthon approach can be used for discovery of novel crystal forms and for enhancing the relevant preclinical properties of a low solubility anti epileptic drug, Lamotrigine[99].

Sudarshanmahapatra, et al 2010 reported structural studies of four new crystal forms, a pure form, a cyclohexane solvate, and co-crystals with 1,4-cyclohexanedione and 4,4-bipyridine. Temperature dependent single-crystalto single-crystal phase transitions are observed for the pure form and for the cyclohexane solvate with an increase in the number of symmetry independent molecules, Ź, upon a lowering of temperature. Other issues related to these solid forms, such as thermal stability, conformational flexibility, and high Ź occurrences, are addressed by using a combined experimental and computational approach [106].

Elbagerma M. A. Et al 2010 employed salicylic acid as a co-crystal formed with the nicotinic acid, DL-phenylalanine, and 6-hydroxynicotinic acid (6HNA) and 3,4-dihydroxybenzoic acid was studied with oxalic acid. The results show that all the co-crystals, prepared in a 1:1 molar ratio, possess unique thermal, spectroscopic, and X-ray diffraction properties. The authors also observed that from the Raman and TRS spectra, vibration modes of the co-crystal were different from those of the starting materials and they concluded that Raman spectroscopy and TRS can be considered as effective tools to evaluate co-crystal formation[104].

Andreas Lemmerer, et al 2010 employed the Kofler hot stage contactmethod to investigate the potential of preparing a co-crystal of 2-chloro-4-nitrobenzoicacid with nicotinamide (nic). The crystal structure determination of 1:1 ratio co-crystal revealed that the two molecules are associated via a carboxylic acid–pyridine hydrogen bond, at the same time nicotinamide forms a centrosymmetric dimer to ultimately form a ribbon architecture which is compared to other known cocrystals of nic. Authors observed that the melting point of the co-crystal is higher than the melting points of either of the pure components, which indicates that the pharmaceutical co-crystal is thermally more stable than the pure compound. Authors supported the relative stability of the co-crystal by molecular modelling calculations[103].

Andreas Lemmerer, et al 2010 reported co-crystals of isoniazid with the dicarboxylic acids malonic, succinic, glutaric, adipic and pimelic acid, andthe mono carboxylic acids 4-hydroxybenzoic acid and 2,4-dihydroxybenzoicacid By surveying the literature through the CSD. Author described that the dominant interaction in all seven co-crystals as the COOH---N hydrogen bond and present an example in which both a covalent and supramolecular synthesis occurs without affecting the vital supramolecular co-crystal forming synthon, the carboxylic acid---pyridine pair functionality, by reacting isoniazid with 2-butanone and acetone while co-crystallizing it with3-hydroxybenzoic acid in a one-pot synthesis[102].

Ashwini Nangia, et al 2010 has been reported the two polymorphs of furosemide i.e Form 2 and 3 of different space group which are characterized by

single crystal X-ray diffraction. Author describes the Known form1 in p1space group, and novel forms 2 and 3 in P21/n and P1 space groups. The stabilization of molecular conformations was analyzed in terms of attractive intramolecular N-H...Cl hydrogen bonds and minimization of repulsives=O...Cl interactions. Phase stability relationships confirm the thermodynamic nature of form 1 in grinding and slurry experiments by Xraypowder diffraction and infrared spectroscopy. The greater stability ofpolymorph 1 is recognized to its more efficient crystal packing, higherdensity, and the presence of sulfonamide N-H...O dimer synthon. Because of the differences in torsion angles and hydrogen bonding in polymorphs 1-3,author classified them as conformational and synthon polymorphs[101].

Dea Herrera-Ruiz, et al 2010 has been reported the acetazolamide (ACZ) co-crystals with 20 co-crystal formers via solvent drop grinding in acetone, acetonitrile, and water which were identified by X-ray powder diffraction(XRPD), IR spectroscopy and differential scanning calorimetry thermogravimetric analysis (DSC-TGA). Single-crystal X-ray diffraction analysis has been done to describe the dominant hydrogen bonding patterns in the co-crystals, 4hbabinds to the thiadiazole acetamide fragment of aczvia C(N)NH---HOOC and O-H---N interactions, while NA is linked through NH---N and N-H---O contacts[100].

Zaworotko*et* al 2010 has been reported the Effect of Crystal Form of lamotrigine (LMT) on Solubility and Pharmacokinetics by crystal engineering. Ten novel crystal forms are reported: LMT methylparaben cocrystal, LMT Nicotinamide cocrystal monohydrate, LMT saccharin salt, LMT adipate salt, LMT malate salt, LMT nicotinate di methanol solvate, LMT Dimethanol solvate, and LMT ethanol monohydrate. LMT saccharin salt and lmt methylparaben cocrystal exhibited the highest concentration in the aqueous media and acidified aqueous media, respectively. Lamotrigine saccharin salt has showing better pharmacokinetic profile compared to lamotrigine alone[99].

AIM AND OBJECTIVE

AIM:

The main aim of present work is to enhance the solubility of co-crystal based solid dosage from.

OBJECTIVE:

1. From physical properties perspective, a key advantage of co-crystals as a solid form of an API is the possibility of achieving the high dissolution rate comparable to that of the amorphous form, while maintaining the long-term chemical and physical stability that crystalline forms provide.
2. A major problem in the use of bioactive herbal ingredient curcumin as a therapeutic agent is its low solubility and bioavailability. Basic goal in the development of co-crystal is to increase the solubility, stability and dissolution rate of pure drug curcumin.
3. In particular substantial advancement methods of crystal engineering supramolecular technique alter the physicochemical properties of curcumin which can relatively undergoes for formulation.

SCOPE OF WORK

Poor dissolution rate, solubility, chemical stability and moisture uptake influence therapeutic efficacy of many pharmaceuticals, and significantly lower the market value of a drug. The increasing prevalence of poorly soluble drugs in development provides notable risk of new products demonstrating low and erratic bioavailabilty with consequences for safety and efficacy, particularly for drugs delivered by the oral route of administration. Although numerous strategies exist for enhancing the bioavailability of drugs with low aqueous solubility, the success of these approaches is not yet able to be guaranteed and is greatly dependent on the physical and chemical nature of the molecules being developed.

The ability to deliver the drug to the patient in a safe, efficient and in cost-effective manner depends largely on the physicochemical properties of the active pharmaceutical ingredient (API) in the solid state. This provides a significant driving force for inventing new approaches to designing pharmaceutical solid materials with specific physicochemical properties. In the last years, crystal engineering of APIs through co-crystallization has gained an increased interest as means of optimizing the physical properties and/or stability of solid dosage forms[101].

A crystal engineering experiment typically involves CSD surveys followed by experimental work to prepare and characterize new compounds that are sustained by supramolecularsynthons. These facilitate understanding of the supramolecular chemistry of the functional groups present in a given molecule and are prerequisites for designing a cocrystal since they facilitate selection of an appropriate cocrystal former(s). However, when multiple functional groups are present in a molecule, the CSD rarely contains enough information to address the hierarchy of the possible supramolecularsynthons. Fortunately, the hierarchy of the supramolecularsynthons that can occur for common functional groups such as carboxylic acids, amides, and alcohols with emphasis upon supramolecular heterosynthons. Furthermore, it is becoming evident that such interactions are key to implementing a design strategy for cocrystals in which a target molecule forms

cocrystals with cocrystal formers that are carefully selected for their ability to form supramolecularheterosynthons with the target molecule.

Drug molecules with limited aqueous solubility are becoming increasingly prevalent in the research and development. Molecules of this type can provide a number of challenges in pharmaceutical development and may potentially lead to slow dissolution in biological fluids, insufficient and inconsistent systemic exposure and consequent sub-optimal efficacy in patients, particularly when delivered via the oral route of administration. Advances in the pharmaceutical sciences have led to establishment of a number of approaches for addressing the issues of low aqueous solubility.

Curcumin derived from the common food spice turmeric has been used for centuries as a remedy for many ailments. Extensive scientific research over the past decade has shown the ability of this compound to modulate multiple cellular targets and hence possesses preventive and therapeutic value against a wide variety of diseases. Curcumin has a diverse range of molecular targets like transcription factors, growth factors and their receptors, cytokines, enzymes, and genes regulating cell proliferation and apoptosis.

Despite its demonstrated efficacy and safety, limited curcumin bioavailability continues to be highlighted as a major concern. As detailed in this work, curtailing curcumin bioavailability and dissolution rate are the main strategies now being explored. Crystal engineering is the attempt to improve the bioavailability and dissolution rate of curcumin in supramolecular technique with Resourcinol as coformer.

However, the limited literature evidence devoted to show improvements in curcumin bioavailability reveals that the curcumin bioavailability enhancement has not gained significant attention. Yet, novel delivery strategies including those of nanoparticles, liposomes, and defined phospholipid complexes offer significant promise and are worthy of further exploration in attempts to enhance the bioavailability, medicinal value, and application of this interesting molecule from Mother Nature.

PLAN OF WORK

- ✓ Procurement of active pharmaceutical ingredient
- ✓ Selection of drug and Co-former.
- ✓ Analytical method optimization.
- ✓ Pre formulation studies.
- ✓ Preparation and Evaluation of co crystals.
- ✓ Characterization of co crystals.
    - Microscopical studies
    - FT-IR Analysis
    - Differential scanning calorimerty (DSC) Studies
    - X-Ray Diffraction Studies
    - Scanning Electron Microscopy (SEM) Analysis
- ✓ Preparation and evaluation of tablets.
- ✓ Solubility Analysis.
- ✓ Micrometric Characterisation.
- ✓ In vitro Studies.
- ✓ In vivo studies.
- ✓ Results and Discussion

DRUG AND EXCIPIENTS PROFILE

DRUG PROFILE

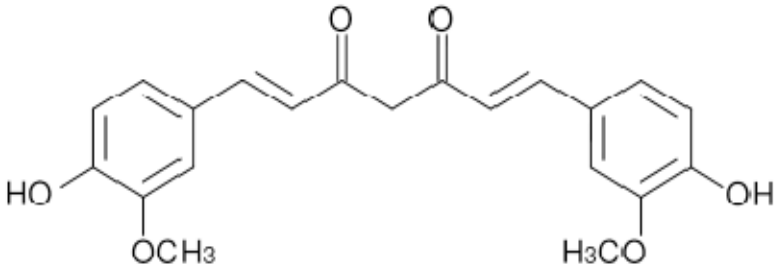
CURCUMIN:

Curcumin is a polyphenol molecule. It is an orange-yellow coloring principle obtained by solvent extraction of turmeric, the ground rhizomes of Curcuma longa L native to South India and Indonesia. In addition to its main application as colouring and flavouring agent in food, it is known it has medicinal activity including antioxidant, anti-inflammatory, anti-digestive trouble. It is also used as a biological stain, pH indicator and indicator for boron. Curcumin is also known as potent anti-tumor agent with inducing apoptosis in cancer cells and inhibiting phorbol ester protein kinase activity.

IUPAC (systematic) Name:

(1E,6E)-1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione.

Chemical Structure:



Appearance: Bright yellow-orange crystalline powder.

IDENTIFIERS:

CAS number: 458-37-7

CHEMICAL DATA:

Molecular formula: $C_{21}H_{20}O_6$

Molecular weight: 368.38

Melting point: 183°C (361°F) (456.15K)

Solubility: Practically insoluble in water.

Freely soluble in acetone (20 mg/ml)

Soluble in ethanol, isopropyl alcohol, DMSO,DMF (1 mg/ml)

Dose: 0.5-3g/day, after the meals.

Odour: Negligible.

Dosage forms: Capsule 400-450 mg

Powder 0.5-5 g (2-3/day)

Tincture

Injectable form 400-600 mg

Pharmacokinetics: Poor absorption.

Oral bioavailability of 60 %.

High first pass and high rate of intestinal metabolism.

Rapid elimination with a mean elimination half-life ($_{t1/2}$)0.3-3 hours.

Side Effects: Diarrhea

Nausea

Skin irritation and allergic reactions

Iron deficiency

Therapeutic uses: curcumin reduces inflammation and oedema

- curcumin accelerates wound-healing
- curcumin's role against cancer
- curcumin's potential to reduce heart disease
- curcumin's therapeutic effects against:
- Arthritis
- Crohn's and inflammatory bowel disease
- Irritable bowel syndrome and ulcerative colitis
- Neurological diseases
- Alzheimer's disease
- Multiple sclerosis

- Diabetes type II
- Cataract formation
- Drug-induced toxicity in the heart, lung and kidney
- Cystic Fibrosis
- Skin diseases: psoriasis, scleroderma and dermatitis
- curcumin may reduce the progression of HIV

COFORMER PROFILE

RESORCINOL:

Although of comparatively low tonnage, the use of resorcinol in oxidative hair dyes and anti-acne creams and peeling agents is relevant for consumer exposure. A total of 150 tonnes of resorcinol was used in oxidative hair dyes by the cosmetics industry in the year 2000 .In oxidative hair dyes, resorcinol is regulated to 5% or below in practice, however, many manufacturers limit the level of free resorcinol in oxidative hair dyes to 1.25%.

Resorcinol is limited to 0.5% in shampoos and hair lotions .Resorcinol is used in pharmaceutical preparations for the topical treatment of skin conditions such as acne, seborrhoeic dermatitis, eczema, psoriasis, corns, and warts. Resorcinol is usually present in anti-acne preparations at a maximum concentration of 2%. The concentration of resorcinol can be much higher in peels, in some cases around 50%.Jessner's solution (resorcinol in ethyl alcohol, 14% w/v; lactic acid, 14%; and salicylic acid, 14%) is commonly used in chemical peeling.1 A specialized medical use of resorcinol is in biological glues (gelatin–resorcinol–formaldehyde glue) for cardiovascular surgery, in particular aortic operations.

IDENTIFIERS:

CAS NO: 108-46-3

FORMULA: $C_6H_4(OH)_2$

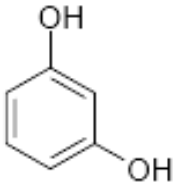
MOL WT: 110.11

H.S. CODE: 2907.11

TOXICITY: Oral rat LD50: 301 mg/kg

SYNONYMS: m-dihydroxybenzene; 1,3-benzenediol;Resorcin; 1,3-Dihydroxybenzene; 3-Hydroxyphenol; C.I. 76505; m-Hydroquinone; m-Benzenediol; 3-Hydroxyphenol; C.I. Developer 4; C.I. Oxidation Base 31; 3-Hydroxycyclohexadien-1-one;

CHEMICAL STRUCTURE:



APPEARANCE: White to off-white needle crystals.

PHYSICAL STATE: White to off-white needle crystals.

ODOUR: weak odour

TASTE: sweetish bitter taste

MELTING POINT: 110 - 113 C.

BOILING POINT: 280 C.

SPECIFIC GRAVITY: 1.272.

SOLUBILITY IN WATER: Completely soluble.

pH: 9.32

FLASH POINT: 127 C.

STABILITY : Stable under ordinary conditions.

APPLICATIONS:

Used externally it is an antiseptic and disinfectant, and is used 5 to 10% in ointments inthetreatment of chronic skin diseases such as psoriasis, hidradenitissuppurativa and eczema of a sub-acute character. It is present in over-the-counter topical acne treatments at 2% or less concentration, and in prescription treatments at higher concentrations.

Weak, watery solutions of resorcinol (25 to 35 g/kg) are useful in allaying the itching in erythematous eczema. A 2% solution used as a spray has been used with marked effect in hay fever and in whooping cough. In the latter disease 0.6 mL of the 2% solution has been given internally. It can be included as an anti-dandruff agent in shampoo or in sunscreencosmetics. It has also been employed in the treatment of gastric ulcers in doses of 125 to 250 mg in pills, and is said to be analgesic and haemostatic in its its action .In large doses it is a poison causing giddiness, deafness, salivation, sweating and convulsions. It is also worked up in certain medicated soaps.

Resorcinol is used in resins as an UV absorber. It is used in manufacturing fluorescent and leather dyes and adhesives (resorcinol formaldehyde resins). It is used as a pharmaceutical to treat acne and other greasy skin conditions in combination with other acne treatments such as sulfur. It is used as an anti-dandruff agent in shampoo and sunscreen cosmetics. It is also used as a chemical intermediate to synthesis pharmaceuticals and other organic compounds. Resorcinol is one of parent material of topical keratolytic agents.

CHEMICAL PROFILE:

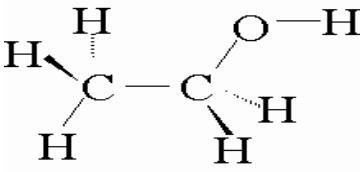
ETHANOL:

Synonyms:

Absolute alcohol, ethyl alcohol, drinking alcohol, ethyl hydrate, ethyl hydroxide, ethylic alcohol, ethylol, grain alcohol, hydroxyethane, methyl carbinol.

Chemical name: Ethanol

Chemical structure:



Molecular formula: C2H6O

Empirical formula: C2H5OH

Molecular weight: 46.07

Functional category: Solvent

Appearance : Colorless liquid

Density: 0.789g/cm3

Melting point: -114°C, 159K, -173°F

Boiling point: 78°C, 351K, 172°F

Vapour pressure: 5.95 kPa (at 20°C)

Log p: -0.18

Acidity (pKa): 15.9

Basicity: (pKb): -1.9

Refractive index: 1.36

Viscosity: 0.0012 Pa s (at 20°C)

Dipole moment: 1.69D

PHARMACOLOGY:

Routes of administration: Intramuscular, Intravenous, Oral, Topical

Metabolism: Hepatic

Toxicity: Pure ethanol will irritate the skin and eyes.

- Long term ingestion can result in serious liver damage.
- Prolonged heavy consumption of alcohol can cause significant permanent damage to the brain and other organs.
- Ethanol within the human body is converted into acetaldehyde by alcohol dehydrogenase and then into acetic acid by acetaldehyde dehydrogenase. Acetaldehyde is more toxic than ethanol. It increases the risk of developing cirrhosis of liver, multiple forms of cancer.
- In pregnant women, large consumption causes fetal alcohol syndrome.

Solvent:

Ethanol is miscible with water and is a good general purpose solvent. It is found in paints, tinctures, markers, perfumes and deodorants. It may also be used as a solvent in cooking, such as in vodka sauce.

Uses: Antiseptic in medical wipes and in antibacterial hand sanitizer gels at a concentration of about 62 % w/w.

Antidote in cases of methanol poisoning.

Fuel in early bipropellant rocket vehicles.

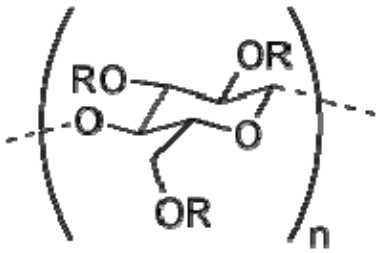
Medicine for depression**,** Anesthetic

EXCIPIENTS

CORBOXYMETHYL CELLULOSE:

Carboxymethyl cellulose or cellulose gum is a cellulose derivative with carboxymethyl groups (-CH_2-COOH) bound to some of the hydroxyl groups of the glucopyranose monomers that make up the cellulose backbone.

Chemical structure:



R = H or CH_2CO_2H

Synonyms: Carboxymethylcellulose, carmellose.

IUPAC name: 2,3,4,5,6-pentahydroxyhexanal

Molecular formula: $C_8H_{16}NaO_8$

Molecular weight: 263.19761

IDENTIFICATION NUMBERS:

Cas Number: 9000-11-7

Pubchem: 6328154

Chemspider: 8677

Chembel: 23393

H-Bond Donar: 6

H-Bond Accecptor: 8

Density: 1.396

Melting Point: 257-263^{0}C

Appearance: Free Flowing White To Off-White Powder

Odour: Slight Or Odourless

Solubility: Soluble In Water

P^H: 7-10

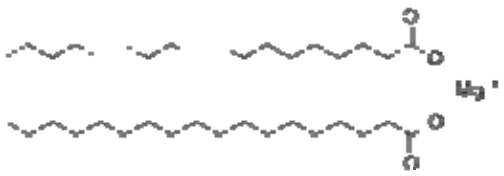
Stability: Stable Under Normal Conditions

Catogories: Pharmaceutical Excipient**.**

MAGNESIUM STEARATE:

Magnesium stearate is a white substance which is solid at room temperature .it is a salt containing two equivalents of stearate the anion of stearic acid and one magnesium cation. It is generally considered safe for human consumption at levels below 2500 mg/kg per day.

Chemical structure:



IUPAC name: Magnesium octadecanoate.

Molecular formula: $C_{12}H_{25}NaO_4S$

Molecular weight: 288.379

Identification numbers

Cas Number: 557-04-0

Pubchem: 11177

Chemspider: 10704

Chembel: 9254

H-Bond Donar: 0

H-Bond Accecptor: 4

Density: 1.028

Melting Point: 150^0C

Appearance: Light White Powder

Odour: Slight

Solubility: Negligible In Water, Insoluble In Ether, Slightly Soluble In Benzene.

P^H: 3-7.5

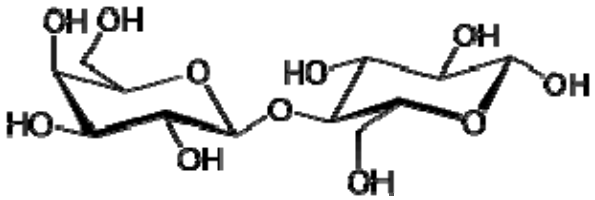
Stability: Stable Under Normal Conditions

Catogories: Diluent.

LACTOSE:

Lactose is adisaccharide sugar that is found notably in milk and is formed from galactose and glucose.It makes up around 2to8 % of milk,although the amount varies among species and individuals.it is exctracted from sweet or sour whey.

Chemical structure:



Synonyms: Milk sugar, 4-O-β-D-galactopyranosyl-D-glucose, D-Lactose, Lactose, anhydrous, Aletobiose, Galactinum, Tablettose, Lactin, Saccharumlactin, Fast-flo,beta-D-Lactose

IUPAC name: (2R,3R,4S,5R,6S)-2-(hydroxymethyl)-6-[(2R,3S,4R,5R,6R)-4,5, 6-trihydroxy-2-(hydroxymethyl)oxan-3-yl]oxyoxane-3,4,5-triol

Molecular formula: $C_{12}H_{22}O_{11}$

Molecular weight: 342.296480g/mol

IDENTIFICATION NUMBERS:

Cas Number: 63-42-3

Pubchem: 6134

Chemspider: 5904

Chembel: 1159651

H-Bond Donar: 8

H-Bond Acceptor: 11

Density: 1.525 G/Cm^3

Melting Point: 202.8 °C

Boiling Point: 668.9 °C

Appearance: White Solid

Odour: Slight

Solubility: 21.6 G/100 Ml

P^H: 6

Stability: stable under normal condition

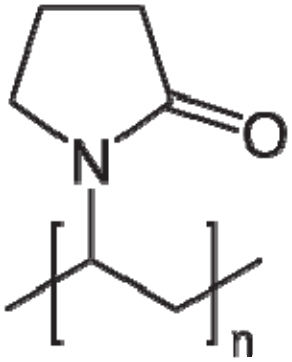
Pharmacological action: Substances that sweeten food, beverages, medications, etc., such as sugar, saccharine or other low-calorie synthetic products**.**

Category: As filler in pharamaceutical industry and in dairy products**.**

POLYVINYL PYROLLIDINE:

Polyvinylpyrrolidone (**PVP**), also commonly called Polyvidone or Povidone, is a water-soluble polymer made from the monomer N – vinylpyrrolidone.

Chemical structure:



Synonyms: PVP, Povidone, Polyvidone, Poly[1-(2-oxo-1-pyrrolidinyl)ethylen] 1-Ethenyl-2-pyrrolidon homopolymer 1-Vinyl-2-pyrrolidinon-Polymere Copovidone

IUPAC name: Polyvinylpyrrolidone

Molecular formula: $(C_6H_9NO)_n$

Molecular weight: 111.14176

IDENTIFICATION NUMBERS:

Cas Number: 900-33-98

Pubchem: 6917

Chemspider: 6651

H-Bond Donar: 0

H-Bond Accecptor: 1

Density: 1.2 G/Cm³

Melting Point: 150 - 180 °C (Glass Temperature)

Appearance: White To Light Yellow, Hygroscopic, Amorphous Powder

Odour: Odourless

Solubility: Soluble In Water And Other Polar Solvents

P^H: 3&7

Stability: Stable Under Normal Conditions

USES: Pharmaceutical Aid, For All Pathological Conditions Where There Is Decr In Mass Of Liquid Blood.

Catogories: Polymer.

TALC:

It is a mineral composed of hydrated magnesium silicate , in loose form it is widely used as talcum powder.it occours as foliated to fiberousmasses,it is the softest known mineral and it can be easily scractched by fingernail.

Synonyms:

talcum powder, hydroxyl magnesium silicate, English chalk.

IUPAC name: dioxosilane; oxomagnesium; hydrate

Molecular formula: $Mg_3Si_4O_{10}(OH)_2$

Molecular weight: 379.26568

Identification numbers

Cas Number: 16211421

Pubchem: 24866335

Chemspider: 25073

H-Bond Donar: 0

H-Bond Acceptor: 12

Density: 2.58 – 3.83

Melting Point:

Appearance: Light Dark Green,White Or Brown Powder

Odour: Odourless

Solubility: Practically Insoluble In Water

P^H: 3&7

Stability: Stable Under Normal Condition**.**

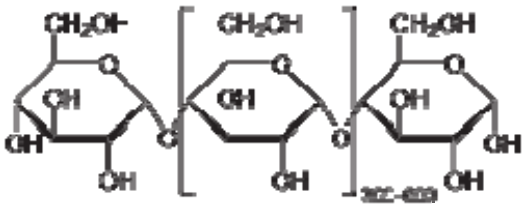
Catogories: Silicate Material.

STARCH:

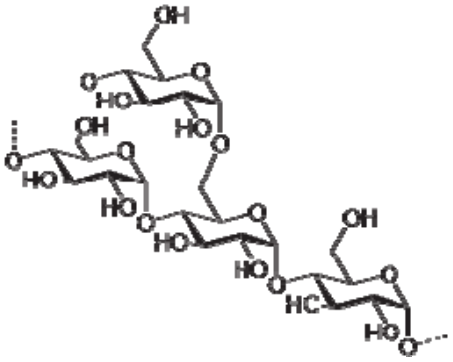
Starch or amylum is a carbohydrate consisting a large number of glucose units joined together by glycosidic bonds. This polysaccharide is produced bt all green plants as an energy store.it is the most common carbohydrate in human diet and is contained in large amounts in such staple foods as potatoes, wheat, corn etc.

Chemical structure:

Amylose



Amylopectin



Synonyms: amylum

IUPAC name: 4-O-α-D-Glucopyranosyl-α-D-glucopyranose

Molecular formula: $(C_6H_{10}O_5)_n$

Molecular weight: 342.116211 Da

Identification Numbers:

Cas Number: 9005-25-8

Pubchem: 47208163

Chemspider: 388469

Density: 1.5 G/Cm^3

Melting Point: 256-258 Dec

Appearance: White Powder

Odour: Odourless

Solubility: Insoluble In Cold Water And Alcohol**.**

P^H: 3&7

Stability: Stable Under Normal Conditions

Catogories: Dietery Supplement And Pharmaceutical Bulk Agent**.**

MATERIALS AND EQUIPMENTS

The best possibly supplied pharma grade materials available were used, for this research, which supplied by the manufacturer and all the other reagents and chemicals used are of analytical grade.

TABLE 6: Materials Used For Study

S.NO	MATERIALS	MANUFACTURER
1	Curcumin	ITC,Guntur
2	Resorcinol	Himedia Laboratories pvt Ltd, Mumbai
3	Ethanol	S.D. Fine Chemicals Ltd
4	Polyvinyl pyrollidine K30	Research lab fine., Mumbai
5	Carboxy methyl cellulose	Loba chemi, Mumbai
6	Lactose	HI-Pure, Chennai
7	Magnesium stearate	Himedia Laboratories pvt Ltd, Mumbai
8	Talc	Research lab fine., Mumbai

Table 7: Equipments Used for the Study

S.NO	Equipments	Manufacturer
1	Electronic Analytical Balance	Shimadzu, Japan
2	UV-Visible Spectrophotometer UV-1700	Shimadzu, Japan
3	FT-IR Spectrophotometer IR 200	Thermo electron corporation
4	Differential Scanning Calorimetry	NETZSCH DSC 204
5	Scanning Electron Microscope	ZEISS Electron Microscope, EVO MA 15
6	X-Ray Diffractometer	EnrafNonius CAD4- MV31
7	Dissolution test apparatus	LABINDIA Dissso 2000

ANALYTICAL METHOD OPTIMIZATION

Number of analytical methods was available for estimation of Curcumin such as ultra-violet spectroscopy, reverse phase HPLC with UV detection, gas chromatography, mass spectrometry and spectro fluorimetric method. The following method was used for further studies.

UV-VISIBLE SPECTROSCOPY:

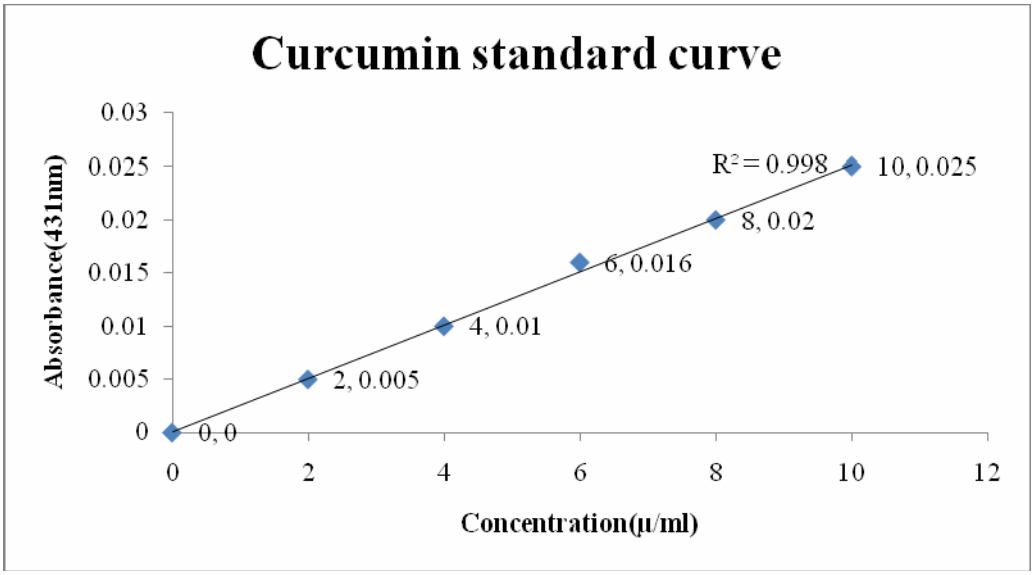
- **Standard Curve of Curcumin in pH 1.2 buffer :**

1mg of Curcumin was dissolved in 100 ml of pH-1.2 buffer by proper ultrasonication for 5-10 mins and further dilutions were made by using pH1.2 buffer to obtain concentrations ranging 2, 4, 6, 8 and 10 µg/ml. The absorbance of solution was measured at 431 nm using UV Visible Spectrophotometer. The readings obtained are tabulated in Table5 and the graph was given in Graph1.

TABLE 8: Standard Curve of Curcumin In Ph 1.2 Buffer

Concentration(µg/ml)	Absorbance(431nm)
0	0
2	0.005
4	0.01
6	0.016
8	0.02
10	0.025

GRAPH 1: Standard Curve of Curcumin In Ph 1.2 Buffer



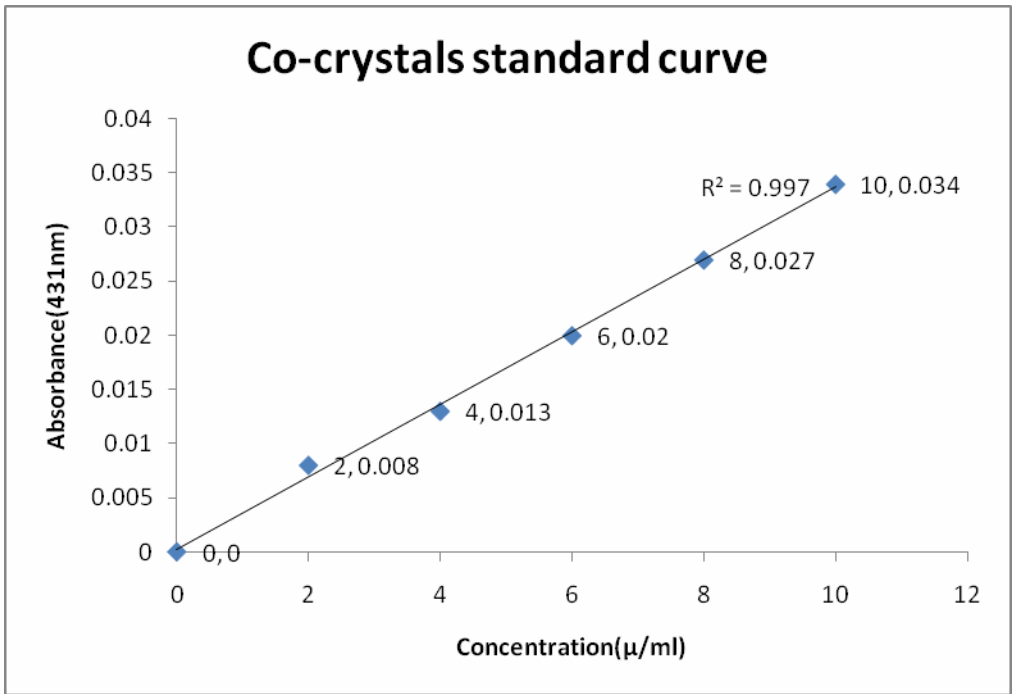
❖ **Standard Curve of Co-crystal in pH 1.2 buffer :**

1mg of Crystals was dissolved in 100 ml of pH-1.2 buffer by proper ultrasonication for 5-10 mints and further dilutions were made by using pH1.2 buffer to obtain concentrations ranging 2, 4, 6, 8 and 10 µg/ml. The absorbance of solution was measured at 431 nm using UV Visible Spectrophotometer. The readings obtained are tabulated in Table and the graph was given in Graph.

TABLE 9: Standard Curve of Co-crystal in pH 1.2 buffer

Concentration(µg/ml)	Absorbance
0	0
2	0.008
4	0.0013
6	0.002
8	0.0027
10	0.0034

GRAPH 2: Standard Curve of Co-crystal Crystals



PREFORMULATION STUDIES

SELECTION OF THE DRUG:

Turmeric is a popular spice frequently used in Indian foods and curry. The biological activities of turmeric are attributed to the active component, curcumin. Curcumin today occupies a unique global niche as "A Wonder Molecule". Curcumin (1, diferuloylmethane) is the active ingredient in the traditional herbal remedy and dietary Indian spice turmeric; it is derived from the rhizome of the herb Curcuma longa[5, 6].

Curcumin is classified as a hydrophobic polyphenol compound that gives turmeric its bright yellow color. Besides being a popular dietary supplement, it is used as a food coloring agent. Curcumin holds a high place in ayurvedic medicine as a "cleanser of the body," and today, science has documented several diseased conditions that can be healed by the active ingredients of turmeric [7, 8].

SELECTION OF THE CO-FORMER:

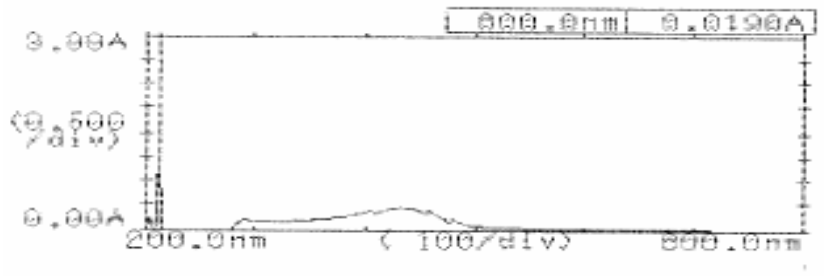
Resorcinol considered generally recognized as safe (GRAS) for dye`s and cosmetic preparation and for some topical pharmaceutical formulations. Potential co-crystal co-formers that satisfy the complementarities rules for the functional groups present in the co-crystallizing system are selected for co-crytallisation process.

CHARACTERISATION OF DRUG:

UV-VIS spectrum of Curcumin:

UV-VIS spectrum of Curcumin in 40% EtOH-Water was determined and spectrum was shown in graph no.3. It gave a peak at 431 nm, the λ max which is similar to the obtained reference (429 nm).

FIGURE 8: UV-VIS spectrum of Curcumin in 40% EtOH-Water



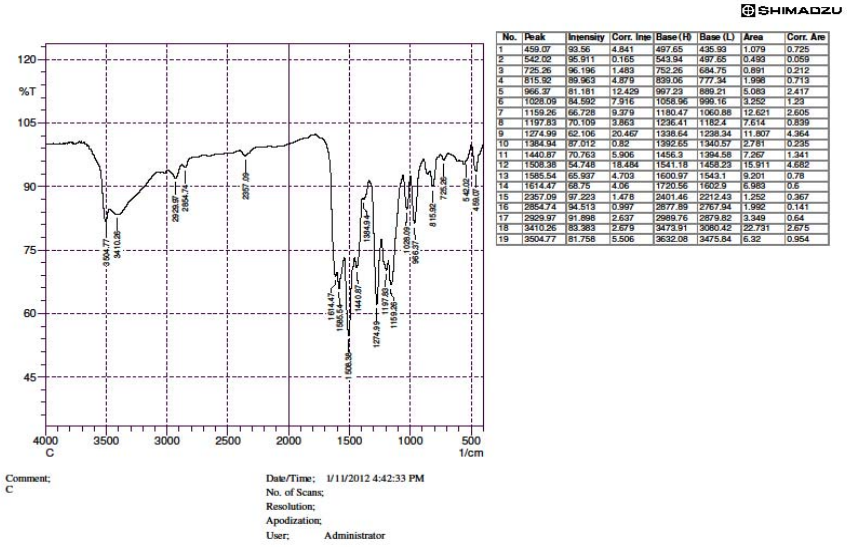
FTIR (Fourier Transform Infra-red Spectroscopy) Studies of pure curcumin:

IR spectroscopy was conducted using a FTIR Spectrophotometer (shimadzu) and Potassium bromide pellet method was employed and background spectrum was collected under identical conditions. The spectrum of pure curcumin, was recorded in the wavelength region of 4000–400 cm^{-1}.

TABLE 10: FTIR Studies of pure curcumin

GROUP	WAVE NUMBER (CM^{-1})
O-H	3504.77
C=O	1614.47
Aromatic C=C	1585.54
Phenol C-O	1440.87
Enol C-O	1197.83

FIGURE 9: FTIR SPECTRUM OF PURE DRUG



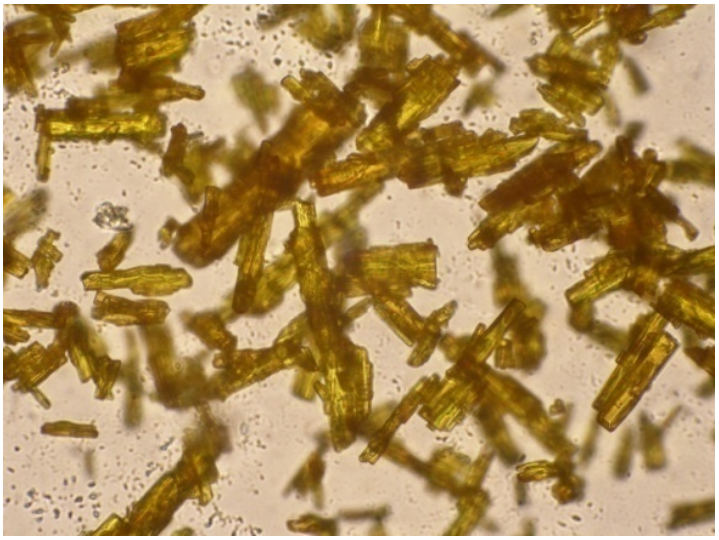
No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	459.07	93.56	4.841	497.65	435.93	1.079	0.725
2	542.02	95.911	0.165	543.94	497.65	0.493	0.059
3	725.26	96.196	1.483	752.26	684.75	0.891	0.212
4	815.92	89.363	4.879	899.06	777.34	1.998	0.712
5	966.37	81.181	12.429	997.23	899.21	5.083	2.417
6	1028.09	84.592	7.916	1058.96	999.16	3.252	1.23
7	1159.26	66.728	9.379	1180.47	1060.88	12.621	2.605
8	1197.83	70.109	3.863	1236.41	1182.4	7.614	0.939
9	1274.99	62.106	20.467	1338.64	1236.34	11.807	4.364
10	1384.94	87.012	0.82	1392.65	1340.57	2.781	0.235
11	1440.87	70.763	5.906	1456.3	1394.58	7.267	1.341
12	1508.38	54.748	18.484	1541.18	1458.23	15.911	4.682
13	1585.54	65.937	4.709	1600.97	1543.1	9.201	0.78
14	1614.47	68.75	4.06	1720.56	1602.9	6.983	0.6
15	2357.09	97.223	1.478	2401.46	2212.43	1.252	0.367
16	2854.74	94.513	0.997	2877.89	2767.94	1.992	0.141
17	2929.97	91.898	2.637	2989.76	2879.82	3.349	0.64
18	3410.26	83.383	2.679	3473.91	3080.42	22.731	2.675
19	3504.77	81.758	5.506	3632.08	3475.84	6.32	0.954

In FT-IR analysis , the spectrum of pure curcumin showed an intense and well-defined bands characteristic to curcumin at 3504.77cm^{-1} (OH-stretching vibration), 1614.47 cm^{-1} (C=O stretching), 1585.54 cm^{-1} (Aromatic C=C), 1440.87 cm^{-1} (Phenol C-O) and 1197.83cm^{-1} (Enol C-O). This FTIR analysis shows that curcumin is pure. Interpretation of IR spectra of curcumin has shown in Table and spectrum shown in Graph.

Microscopic studies:

Morphology of Curcumin was studied by microscopy. Curcumin shows small rod like crystals. The microscopic photograph shown in Figure.

FIGURE 10: Photography of Curcumin

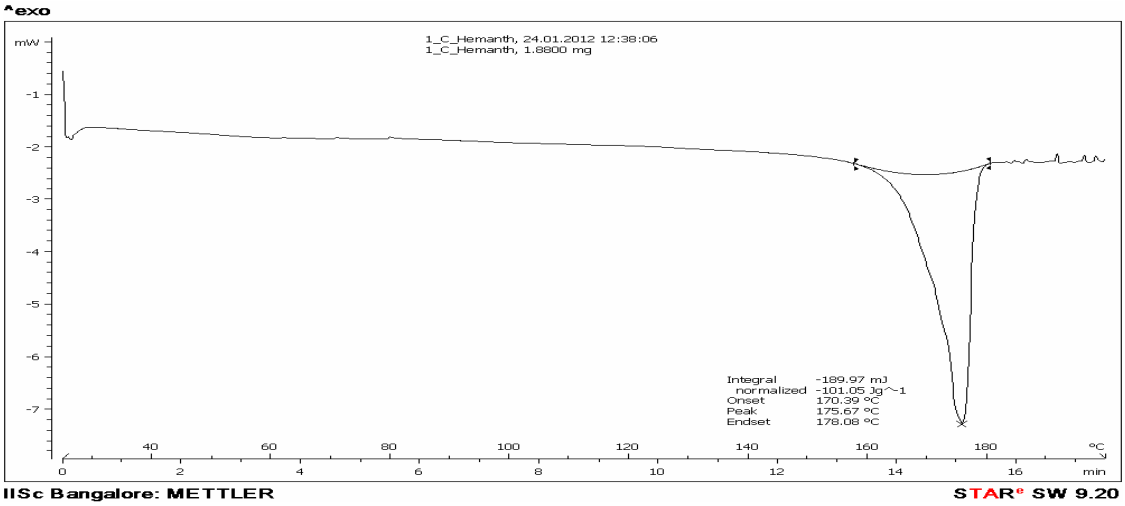


Differential scanning calorimetry (DSC) of pure curcumin:

Thermal analysis of pure curcumin, were recorded on a DSC (NETZSCH DSC 204). The temperature axis and cell constant of DSC were previously calibrated with indium. A heating rate of 10^0 C/min was employed with nitrogen pursing. Powder samples (5- 8mg) was weighed into an aluminum pan and analyzed as sealed with pin holes and an empty aluminium pan was used as reference.

DSC thermo grams of curcumin shows sharp endothermic peak at 175.67 C. This indicates pure crystal form. A DSC thermo gram of curcumin was shown in figure10.

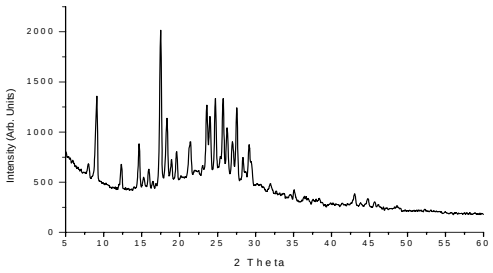
FIGURE 11: DSC thermo gram of pure curcumin



Powder X-Ray Diffraction (P-XRD):

X-ray powder diffractometry (XRPD) is a powerful technique for the identification of the crystalline solid phases. Every crystalline solid phase has a unique XRPD pattern, which can form the basis for its identification. The study was carried out using X-Ray Diffractometer using Cu kα radiation. The tube operated at 45 kV, 9mA and data was collected over an angular range from 0 to 60 2θ of the diffraction angle in continuous scan mode using a step size of 0.050 2θ and a time of 0.1 s.

FIGURE 12: Powder XRD of pure curcumin

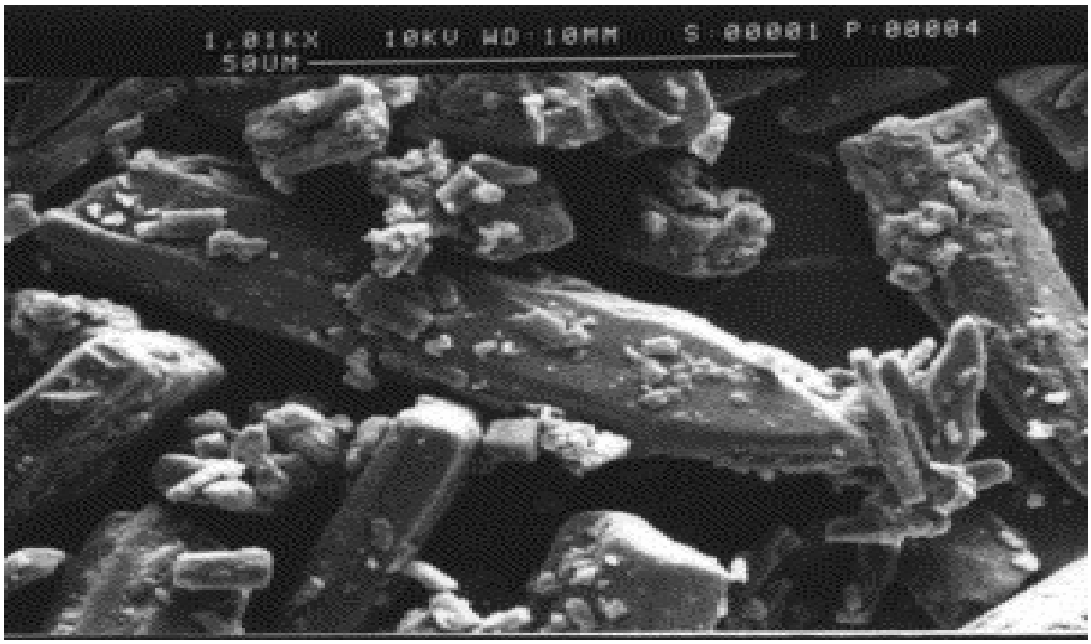


The X-ray powder diffraction (XRD) spectra of curcumin in figure11 shows characteristic peak of pure curcumin having 100% relative index at 17^0 of 2θ range indicates pure curcumin.

Scanning Electron Microscopy (SEM) Studies of pure curcumin:

The surface characteristics of pure curcumin were studied by SEM (ZEISS Electron Microscope, EVO MA 15). The specimens were scanned with an electron beam of acceleration potential of 20 kV and the images were collected as secondary electron mode. It shows small rod like crystals.

Figure 13: SEM Photograph of pure curcumin



CHARACTERISATION OF COFORMER

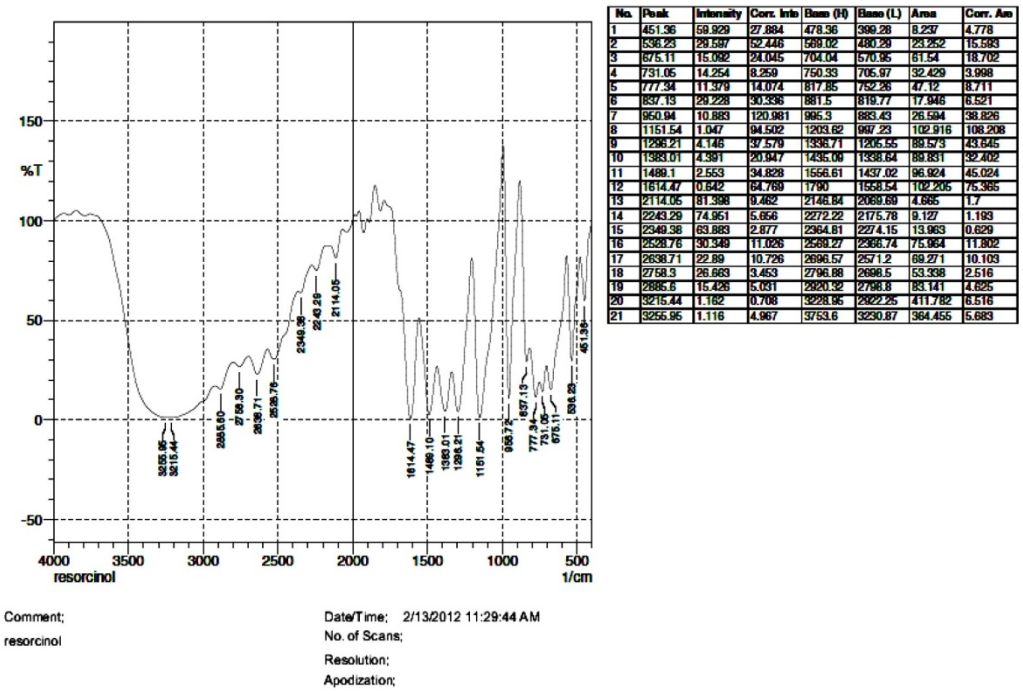
RESORCINOL:

IR spectroscopy was conducted using a FTIR Spectrophotometer (shimadzu) and Potassium bromide pellet method was employed and background spectrum was collected under identical conditions. The spectrum of pure curcumin, was recorded in the wavelength region of 4000–400 cm^{-1}.

TABLE 11: FTIR Studies of Resorcinol

GROUP	WAVE NUMBER (CM^{-1})
O-H	3255.95
Aromatic C=C	1614.47
Phenol C-O	1296.21

FIGURE 14: FTIR studies of resorcinol

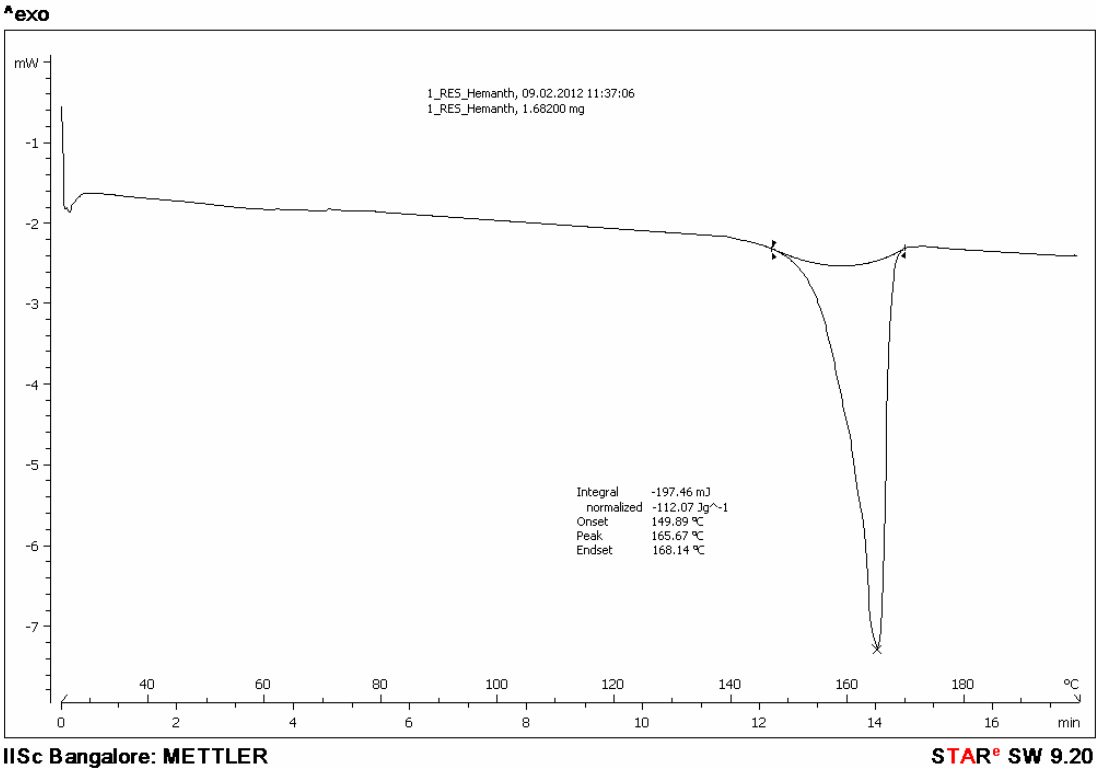


No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	451.36	59.929	27.884	478.36	399.28	8.237	4.778
2	536.23	29.597	52.446	589.02	480.29	23.252	15.593
3	675.11	15.092	24.045	704.04	570.95	61.54	18.702
4	731.05	14.254	8.259	750.33	705.97	32.429	3.998
5	777.34	11.379	14.074	817.85	752.26	47.12	8.711
6	837.13	29.228	30.336	881.5	819.77	17.946	6.521
7	950.94	10.883	120.981	995.3	889.43	26.594	38.826
8	1151.54	1.047	94.502	1203.62	997.23	102.916	108.208
9	1296.21	4.146	37.579	1336.71	1205.55	89.573	43.645
10	1383.01	4.391	20.947	1435.09	1338.64	89.831	32.402
11	1489.1	2.553	34.828	1556.61	1437.02	96.924	45.024
12	1614.47	0.642	64.769	1790	1558.54	102.205	76.365
13	2114.05	81.398	9.462	2146.84	2069.69	4.685	1.7
14	2243.29	74.951	5.656	2272.22	2175.78	9.127	1.193
15	2349.38	69.883	2.877	2364.81	2274.15	13.963	0.829
16	2528.76	30.349	11.026	2589.27	2368.74	75.964	11.802
17	2638.71	22.89	10.726	2696.57	2571.2	69.271	10.103
18	2758.3	26.663	3.453	2796.88	2698.5	53.398	2.516
19	2885.6	15.426	5.031	2920.32	2798.8	83.141	4.825
20	3215.44	1.162	0.708	3228.96	2922.25	411.782	6.516
21	3255.95	1.116	4.967	3753.6	3230.87	364.455	5.683

DSC STUDIES:

Thermal analysis of Resorcinol, were recorded on a DSC (NETZSCH DSC 204). The temperature axis and cell constant of DSC were previously calibrated with indium. A heating rate of 10^0 C/min was employed with nitrogen pursing. Powder samples (5- 8mg) was weighed into an aluminum pan and analyzed as sealed with pin holes and an empty aluminium pan was used as reference.

FIGURE 15: DSC thermo gram of pure resorcinol

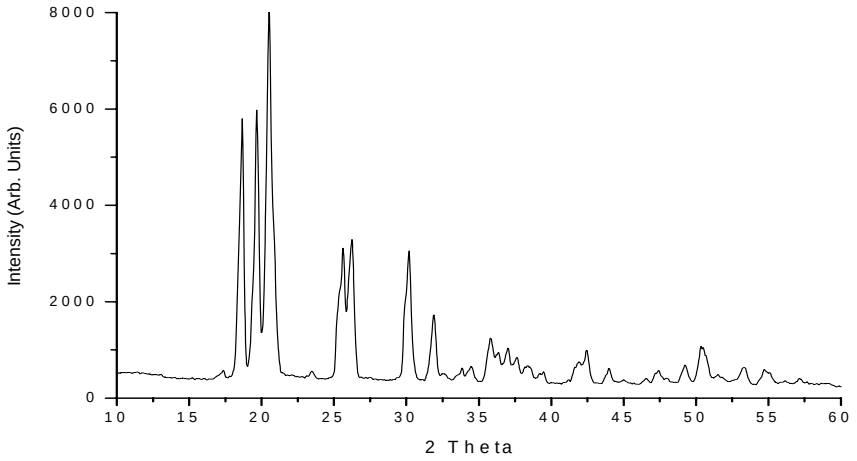


DSC thermo grams of resorcinol shows sharp endothermic peak at 165.37^{0}C. This indicates pure crystal form. A DSC thermo gram of resorcinol was shown in figure13.

XRD STUDIES:

X-ray powder diffractometry (XRPD) is a powerful technique for the identification of the crystalline solid phases. Every crystalline solid phase has a unique XRPD pattern, which can form the basis for its identification. The study was carried out using X-Ray Diffractometer using Cu kα radiation. The tube operated at 45 kV, 9mA and data was collected over an angular range from 0 to 60 2θ of the diffraction angle in continuous scan mode using a step size of 0.050 2θ and a time of 0.1 s.

FIGURE 16: XRD spectra of pure resorcinol

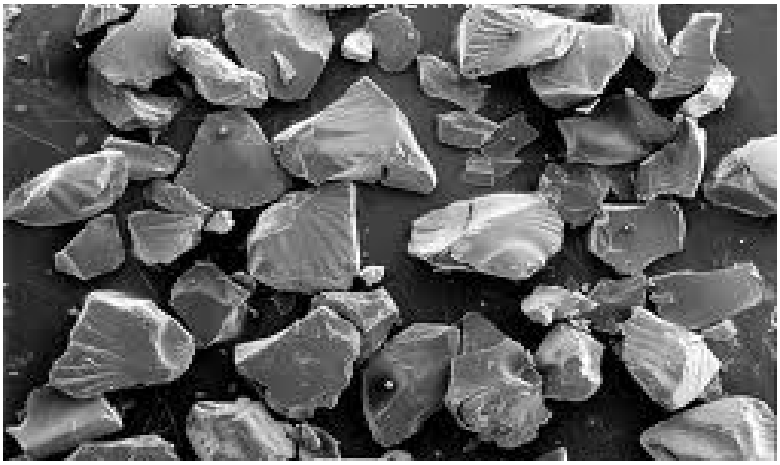


The X-ray powder diffraction (XRD) spectrum of Resorcinol in figure14 shows characteristic peak at 20^0 of the 2θ range providing 100% relative intensity and indicates pure resorcinol.

Scanning Electron Microscopy (SEM) Studies of Resorcinol:

The surface characteristics of pure Resorcinol studied by SEM (ZEISS Electron Microscope,EVO MA 15). The specimens were scanned with an electron beam of acceleration potential of 20 kV and the images were collected as secondary electron mode. It shows spherical crystals.

Figure 17: SEM Photograph of pure resorcinol



SUPRAMOLECULAR SYNTHESIS

PREPARATION OF CRYSTALS:

Liquid Assisted Grinding (L.A.G.) Method:

Curcumin and resorcinol weighed according to molar ratio (1:1) bases were grinded in a mortar and pestle using small quantity of ethanol (4 to 5 drops) by liquid assisted grinding method. The crystals formed were collected separately and preserved.

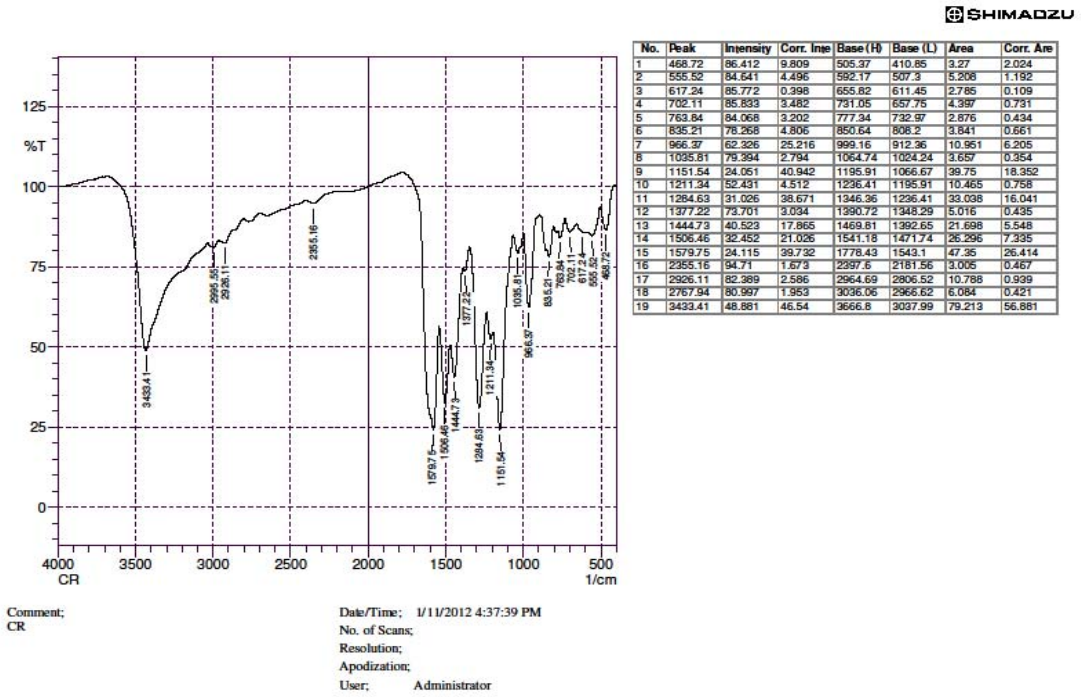
In FT-IR analysis, the spectrum showed an intense and well-defined bands characteristic to curcumin at 3433.41 cm^{-1} (OH-stretching vibration), 1579.75 cm^{-1} (C=O stretching), 1506.46 cm^{-1} (Aromatic C=C), 1284.63 cm^{-1} (Phenol C-O) and 1035.81 cm^{-1} (Enol C-O). Interpretation of IR spectra of crystals prepared by liquid assisted grinding method has showed in table.

TABLE 12: FTIR Studies of prepared crystals

GROUP	WAVE NUMBER (CM^{-1})
O-H	3433.41
C=O	1579.75
Aromatic C=C	1506.46

Phenol C-O	1284.63
Enol C-O	1035.81

FIGURE 18: FTIR spectra of crystals by L.A.G. method

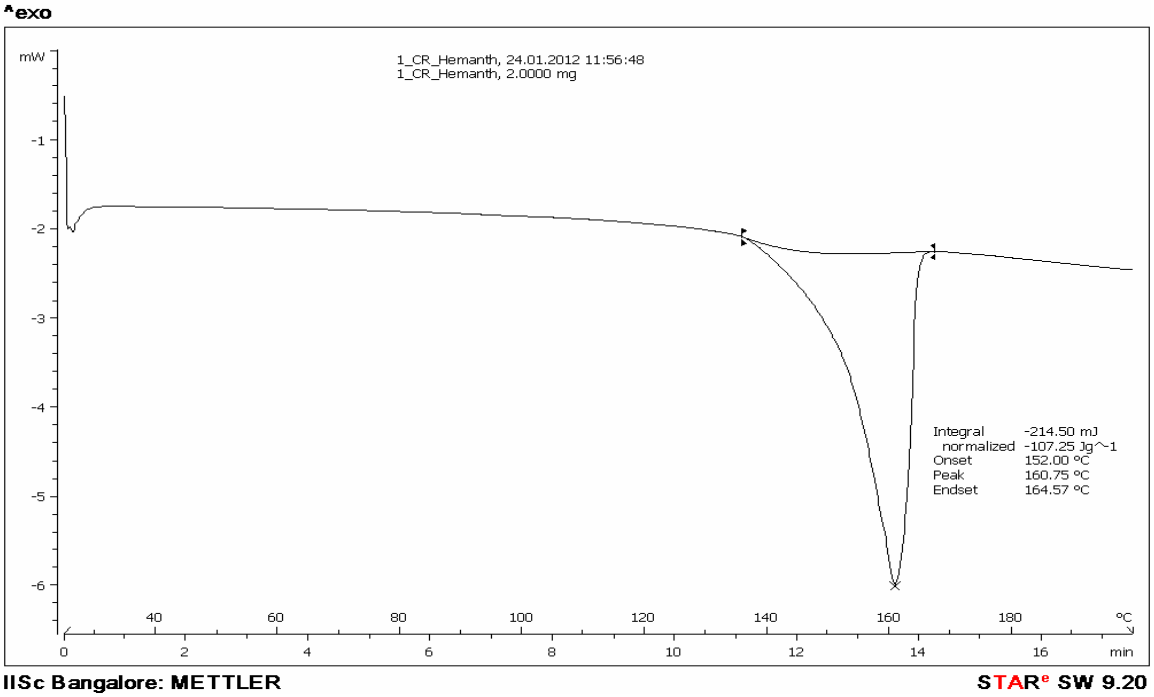


No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	468.72	86.412	9.809	505.37	410.85	3.27	2.024
2	555.52	84.641	4.496	592.17	507.3	5.208	1.182
3	617.24	85.772	0.398	655.82	611.45	2.785	0.109
4	702.11	85.833	3.482	751.05	657.75	4.397	0.731
5	763.84	84.068	3.202	777.34	732.97	2.876	0.434
6	835.21	76.268	4.806	850.64	808.2	3.841	0.661
7	966.37	62.326	25.216	999.16	912.36	10.951	6.205
8	1035.81	79.394	2.794	1064.74	1024.24	3.657	0.354
9	1151.54	24.051	40.942	1195.91	1066.67	39.75	18.352
10	1211.34	52.431	4.512	1236.41	1195.91	10.465	0.758
11	1284.63	31.026	38.671	1346.36	1236.41	33.038	16.041
12	1377.22	73.701	3.034	1390.72	1348.29	5.016	0.435
13	1444.73	40.523	17.865	1469.81	1392.65	21.698	5.548
14	1506.46	32.452	21.026	1541.18	1471.74	26.296	7.335
15	1579.75	24.115	39.732	1778.43	1543.1	47.35	26.414
16	2355.16	94.71	1.673	2397.6	2181.56	3.005	0.467
17	2926.11	82.389	2.586	2964.69	2806.52	10.788	0.939
18	2767.94	80.997	1.953	3036.06	2966.62	6.084	0.421
19	3433.41	48.881	46.54	3666.8	3037.99	79.213	56.881

Differential scanning calorimetry (DSC) of curcumin-resrcecinol crystals by L.A.G. Method:

DSC experiments were carried out to study the thermal behaviour of the crystal form in relation to the individual components. DSC thermal data are shown in figure. DSC study of curcumin and resorcinol shows endothermic peak at 175.67^{0}C and 112.02^{0}C C while DSC study of prepared cocrystal shows sharp endothermic value at 165.75^{0}C, the sharp endothermic values of crystal form and the individual components agreed with the measured melting range in the melting point determination. The thermal profile of crystal form was distinct, with a different melting transition from that seen with either of the individual components. This indicates the formation of novel crystal phase: crystal form of Curcumin with resorcinol (1:1 molar ratio). This single endothermic transition indicates the absence of any unbound or absorbed solvent or water and also demonstrates the stability of the phase until the melting point.

FIGURE 19: DSC thermo gram of prepared crystals

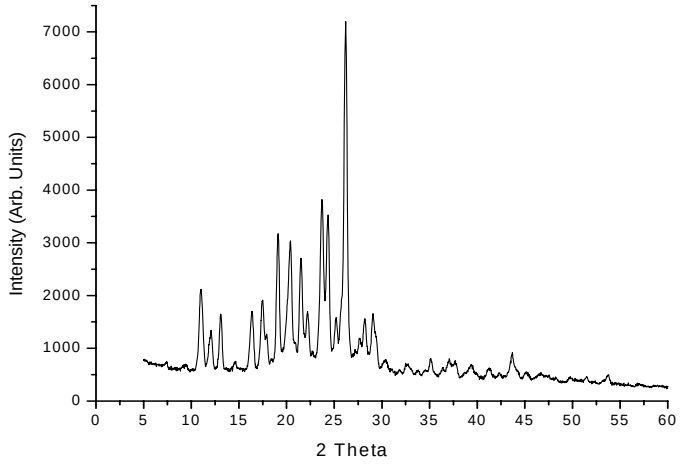


Crystal PXRD Crystallography:

X-ray powder diffractometry (XRPD) is a powerful technique for the identification of the crystalline solid phases. Every crystalline solid phase has a unique XRPD pattern, which can form the basis for its identification. The study was carried out using X-Ray Diffractometer using Cu kα radiation. The tube operated at 45 kV, 9mA and data was collected over an angular range from 0 to 60 2θ of the diffraction angle in continuous scan mode using a step size of 0.050 2θ and a time of 0.1 s.

FIGURE 20: PXRD of prepared crystals

Prepared crystal

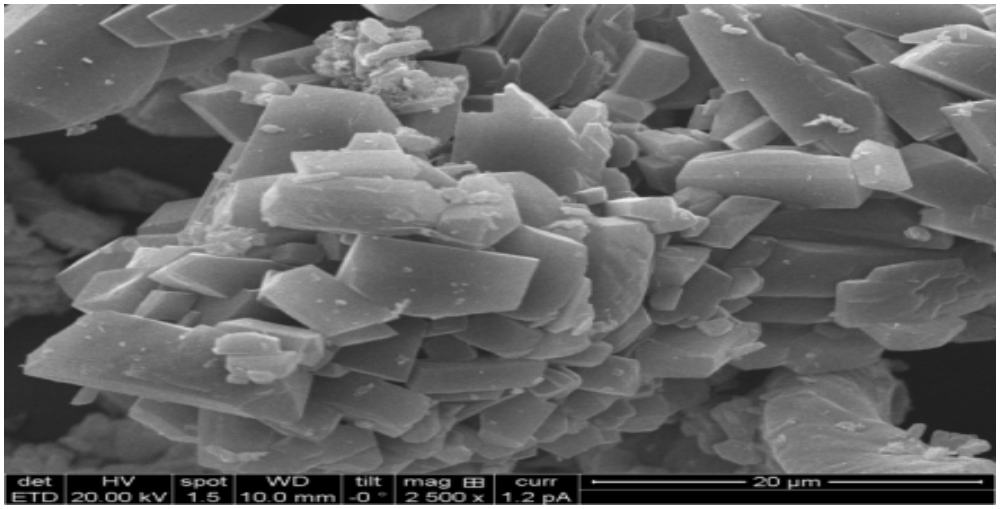


Predicted PXRD pattern of crystal form shown in figure19, which was different from its pure drug powder XRD pattern, this indicates the formation of new multi component crystalline phase.

Scanning Electron Microscopy (SEM) Studies of Prepared crystals:

The surface characteristics of pure Resorcinol studied by SEM (ZEISS Electron Microscope,EVO MA 15). The specimens were scanned with an electron beam of acceleration potential of 20 kV and the images were collected as secondary electron mode. It shows spherical crystals.

Figure 21: SEM Photograph of Prepared crystals



SOLUBILITY STUDIES:

The solubility studies have been performed for the pure drug and prepared crystals. In pH 1.2 buffer the pure drug showed a solubility of 0.071 mg/ml, liquid assisted grinding method showed a solubility of 0.157 mg/ml respectively. The crystals prepared by liquid assisted grinding method have shown the greater solubility than the pure drug.

TABLE 13: Solubility Data in P^H 1.2 Buffer

SAMPLE		SOLUBILITY (mg/ml)
Pure drug in water		0.003±0.01
Crystals	In water	0.017±0.01
	In P^H 1.2 buffer	0.157±0.01
	In P^H 7.2 buffer	0.124±0.01

ASSAY OF CRYSTALS:

1mg of curcumin crystals prepared by liquid-assisted grinding method were taken and dissolved in 40% EtOH-Water. From that 1ml was taken and diluted to 10 ml. The absorbance of solution was measured at 431 nm using ELICO UV –Visible Spectrophotometer and drug content was calculated.

TABLE 14: Determination of drug content

SNO	CRYSTALS	DRUG CONTENT
1	Crystal by L.A.G. method	87.24%

ANGLE OF REPOSE

A funnel was kept vertically in a stand at a specified height above a paper which placed on horizontal surface. The funnel bottom was closed and 5g (or) 10 g (or) any weighed quantity of sample was filled in a funnel. The bottom of funnel was opened and allowed the powder to flow freely to form smooth conical heap. The radius of the heap (r) and the height of the heap (h) were measured. The value of angle of repose was calculated by using the following formula.

$$\theta = \tan^{-1}\frac{h}{r}$$

Table 15: Angle of repose

ANGLE OF REPOSE	FLOW
<25	Excellent
25-30	Good
30-40	Passable
>40	Very poor

BULK DENISITY AND TAPPED DENSITY

A quantity of 5 gm of powder weighed and transferred to a measuring cylinder and observed the volume occupied by the sample. The initial volume was noted. Bulk density was calculated using the formula.

$$\text{Density} = \frac{\text{Mass}}{\text{Bulk Volume}}$$

The powder in the measuring cylinder was tapped for specific times at a height of 2.5cm at an interval of 2 seconds. The powder in the graduated cylinder was tapped for specific times at a height of 2.5cm at an interval of 2 seconds. The final volume occupied by the sample was noted and tapped density was found out using the formula.

$$\text{Density} = \frac{\text{Mass}}{\text{Tapped Volume}}$$

POWDER COMPRESSIBILITY:

Based on the apparent bulk and the tapped density, the percentage compressibility of bulk was determined by the following formula.

$$\text{\% Compressibility} = \frac{\text{Tapped density - Initial bulk density}}{\text{Tapped density}} \times 100$$

100 (Carr's index)

Table 16: Compressibility index

Flow character	Compressibility index
Excellent	≤10
Good	≤ 11-15
Fair	16-20
Passable	21-25
Poor	26-31

Very poor	32-37
Very very poor	≥ 38

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Table 17: Hausner's ratio limits

Flow character	Hausner's ratio limits
Excellent	1.00 – 1.11
Good	1.12 – 1.18
Fair	1.19 – 1.25
Passable	1.26 – 1.34
Poor	1.34 – 1.45
Very poor	1.46 – 1.59
Very very poor	≥ 1.60

PHARMACEUTICAL CHARACTERISATION OF COCRYSTALS:

FORMULATION OF TABLETS:

A tablet is a pharmaceutical dosage form. It comprises a mixture of active substances and excipients, usually in powder form, pressed or compacted from a powder into a solid dose. The excipients can include diluents, binders or granulating agents, glidants (flow aids) and lubricants to ensure efficient tabletting; disintegrants to promote tablet break-up in the digestive tract; sweeteners or flavours to enhance taste; and pigments to make the tablets visually attractive.

The compressed tablet is the most popular dosage form in use today. About two-thirds of all prescriptions are dispensed as solid dosage forms, and half of these are compressed tablets. A tablet can be formulated to deliver an accurate dosage to a specific site; it is usually taken orally, but can be administered sublingually, buccally, etc.

FORMULATION OF CO-CRYSTAL TABLETS

The general method of direct compression is used here for the formulation of tablets. At first all the suitable ingredients were weighed according to their molar ratios for the accuracy of weighing, then all are mixed finely in mortor and pestle for 30minutes the passed through the 60 no. sieve then the magnesium stearate was added and again passed through the same sieve. After completing the process go for direct compression.

TABLE 18: Quantity of Raw materials per Tablet (in mg)

S. No	Ingredients	F1	F2	F3	F4	F5	F6
1	Co-Crystals	54	54	54	54	54	200
2	*Lactose*	35	35	55	15	35	0
3	Starch	35	35	15	55	35	0
4	Polyvinylpyrollidine K30	15	35	35	35	55	0
5	Carboxymethyl cellulose	55	35	35	35	15	0
6	Magnesium stearate	4	4	4	4	4	0
7	Talc	2	2	2	2	2	0
Total weight		**200**	**200**	**200**	**200**	**200**	**200**

TABLE 19: PRE COMPRESSION PARAMETERS

Formulation	Angle of repose ±S.D	Bulk density (gm/ml) ± S.D	Tapped density (gm/ml) ± S.D	Compressibiliy index (%) ± S.D	Hausner's ratio ± S.D
F1	$16^0 57' \pm 0.19$	0.336 ± 0.01	0.36 ± 0.02	14.29± 0.36	1.01 ± 0.04
F2	$16^0 48' \pm 0.17$	0.323 ± 0.03	0.33± 0.02	13.23± 0.16	1.13 ± 0.03
F3	$16^0 36' \pm 0.12$	0.326 ± 0.02	0.34± 0.02	12.75± 0.18	1.11 ± 0.02
F4	$17^0 39' \pm 0.15$	0.345 ± 0.01	0.35± 0.02	11.94± 0.23	1.10 ± 0.04
F5	$17^0 28' \pm 0.14$	0.328 ± 0.02	0.32± 0.02	13.87± 0.15	1.12 ± 0.03
F6	$16^0 25' \pm 0.18$	0.349 ± 0.03	0.31± 0.02	15.62± 0.13	1.13 ± 0.02

The angle of repose values were ranged from $16.27^o \pm 0.19$ to $20.28.^o \pm 0.14$. The results were found to be below 17; hence they have excellent flow ability. The Hausner's ratio value ranged from 0.139 ± 0.04 to 0.142± 0.03, It has shown that crystals have good flow property and can be compressed in to tablets.

EVALUATION OF FORMULATED TABLETS

The formulated tablets were evaluated for the following parameters

PHYSICAL APPEARANCE

Prepared Tablets were evaluated for the smoothness and absorbance of cracks, chips and other undesirable characteristics.

WEIGHT VARIATION

10 tablets were selected, weighed collectively and individually. From the collective weight, average weight was calculated. Each tablet weight was then compared with average weight to ascertain the weight of the tablets within the permissible limits. Not more than two of the individual weights deviated from the

average weight by more than 7.5% for >300 mg tablets and none by more than double that percentage.

FRIABILITY

Friability of the tablet was checked by Roche friabilator. In this device **10** previously weighed tablets were placed in the apparatus, rotated at 20 rpm for 5 minutes to give the result of 100 revolutions and the tablets were deducted and weighed after completion of the test. The percentage friability was calculated by using the following formula**.**

W_O = initial weight, W = final weight.

THICKNESS

The thickness was measured by using verniercallipers and values were tabulated. Three tablets of each batch were measured. Average and standard deviation was calculated.

HARDNESS

Hardness of the tablets was determined by using the Monsanto hardness tester. The lower plunger was placed in contact with the tablet and a zero reading was taken. The plunger was then forced against a spring by turning a threaded bolt until the tablet fractured. As the spring was compressed a pointer rides along a gauge in the barrel to indicate the force.

DRUG CONTENT

10 tablets of each formulation were weighed and powdered. The quantity of powder equivalent to 10 mg of curcumin was transferred into a 100 ml standard flask and volume made up with 0.1 N hydrochloric acid and absorbance of the resulting solution was observed at 431 nm.

DISINTEGRATION TIME

Many reports suggest that conventional DT apparatus may not give correct value of DT. Here measured volume of phosphate buffer of p^H 1.2 was taken in 25ml measuring cylinder. Temperature was maintained at 37 ± 2^0C formulated tablet was put into it and time required for complete disintegration of the tablet was noted**.**

UNIFORMITY OF DISPERSION TEST

Two tablets from each batch were separately kept in 100 ml water and gently stirred for 2 minutes. The dispersion was passed through 22 mesh. The tablets were considered to pass the test if no residue remained on the screen.

Table 20: Evaluation Chart of Tablet

Formul-ations	Weight variation in mg ± S.D	Thickness in mm ± S.D	Diameter in mm ± S.D	Hardness In Kg/cm^2 ± S.D	Friability (%)	Drug content (%) ± S.D
F1	200.1± 0.05	3.0±0.01	1.01± 0.014	4.0 ± 0.16	0.25	99.36 ± 0.04
F2	200.8 ±0.01	3.1±0.03	1.01± 0.027	3.9 ± 0.13	0.29	96.41± 0.03
F3	200.5± 0.02	3.1±0.02	1.00± 0.015	3.8 ± 0.12	0.36	97.64± 0.01
F4	200.3±0.04	2.9±0.02	1.01± 0.015	3.9 ± 0.12	0.35	97.85± 0.03
F5	200.2±0.01	3.1±0.02	1.02± 0.018	3.9 ± 0.15	0.36	96.63± 0.02
F6	200.7±0.04	3.0±0.03	1.02± 0.016	3.8 ± 0.10	0.35	98.24± 0.02

The weight variation test were done for F1 to F6 formulations and found to be 200.1 ± 0.05 to 200.8 ± 0.01. The % deviation is coming within 3% to 5% range for this test accepted % deviation should be 5 % for 200-250 mg tablet. F1 to F6 formulations come within limit and passed the test.

The friability test done for the F1 – F6 formulations was ranged from 0.25 to 0.36 which exactly falls within the limit of standard (0.1 to 0.9 %).

The thickness was carried out according to the procedure. The thickness of the tablets ranges from 2.9 ± 0.02 to 3.1 ± 0.03.

The diameter of the tablet ranges from 1.00 ± 0.015 to 1.02 ± 0.018.

The hardness for F1 to F5 formulations ranged from 3.8 ± 0.10 to 4.0 ± 0.13, which showing that those are within the limits.

The drug content was determined and the results were given. The drug content was determined by measuring absorbance. The drug content F1 to F6 showed in the range of 96.63± 0.02 to 99.36± 0.03 and F1 showed maximum drug content 99.36± 0.03.

Table 21: Disintegration time

Formulations	Disintegration time(min) ± S.D
F1	4.5 ± 1.23
F2	5.0± 1.13
F3	4.9 ± 0.92
F4	5.2± 0.95
F5	5.1± 1.19
F6	5.2± 1.32

Disintegration was determined and the results were given. The disintegration time for F1 – F6 were ranging 4.5± 095 to 5.2± 1.36 min.

Table 22: Uniformity of dispersion

Uniformity of dispersion was carried out. The residue remaining on the screen was found to be nil hence all the formulations from F1 to F6 passed the test.

Formulations	Uniformity of dispersions	
	Residue remaining on screen	Result
F1	Nil	Pass
F2	Nil	Pass
F3	Nil	Pass
F4	Nil	Pass
F5	Nil	Pass
F6	Nil	Pass

DISSOLUTION STUDIES

USP 2 paddle apparatus is most suitable and common choice for dissolution test of crystal tablets as compared to USP 1(basket) apparatus due to specific physical properties of tablets. In paddle apparatus the paddle speed of 25-75 rpm is commonly used. Since the dissolution of crystal tablet is very fast when using USP monograph conditions hence slower paddle speed may be utilized to obtain a comparative profile.

Dissolution studies were carried out by USP type II (paddle apparatus) at 50 rpm in 1.2 P^H phosphate buffer. Temperature was maintained at 37 ±0.5 ℃. Aliquots of dissolution media were withdrawn at 0,5,10,15,30,45,60,75,90,105 minutes of time intervals and the sample was filtered. Same quantity of fresh media was replaced. The filtered solution was used to determine the drug content. Absorbance was we as used at 431 nm by UV/Visible spectrophotometer.

KINETIC STUDIES

To analyze the *in vitro* release data various kinetic models were used to describe the release kinetics. The zero order describes the systems where the drug release rate is independent of its concentration given in Eq(1). The first order describes the release from system where release rate is concentration given in Eq (2). Higuchi (1963) described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion given in Eq(3).

Where, K_0 is zero-order rate constant expressed in units of concentration/time and t is the time.

Where, C_0 is the initial concentration of drug and K is first order constant.

Where, K is the constant reflecting the design variables of the system.

The plots were made by taking cumulative % drug release vs time for zero order kinetic models, log cumulative of %drug remaining vs time for first order kinetic models, cumulative % drug release vs square root of time for higuchi models and log cumulative % drug release vs. log time for korsmeyer models remai

ACCI .ITY STUDIE

Sele studies as per I.C.H guidelines. analyzed for weight variation, hardness, friability, drug content and In vitro dissolutions study for every month for a period of three months.

IN-VIVO PHARMACOLOGICAL ACTIVITY:

MATERIALS AND METHODS

Animals used

Healthy adult wistar rats (180-200gm) were maintained in a well ventilated room with 12:12 hour light or dark cycle in polypropylene cages. The animals were fed with standard pellet fed and water was given ad libitum. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Antiulcer activity

Ethanol induced gastric ulcer

Animals were randomly divided into four groups each of 6 rats. Group I treated with 1% w/v CMC (10 ml/kg p.o), Group II treated with Pure Curcumin (100mg/kg p.o) Group III treated with prepared crystals (100mg/kg p.o) respectively for 5 days and Group IV treated with Omeprazole (20 mg/kg p.o) were administered 30min prior to induction of gastric ulcer. On the 5th day, Gastric ulcers were induced with ethanol at a dose of 8ml/kg[103]administered to all groups by orally. The animals were anaesthetized 6 h with ether and stomachs were incised along the greater curvature and the ulcer index for each rat was taken as the mean ulcer score.

Measurement of ulcer index

The stomachs were excised and were examined for hemorrhagic lesions in glandular mucosa. Immediately after the animals were sacrificed, their stomachs were dissected out, cut along the greater curvature and the mucosa were rinsed with cold normal saline to remove blood contaminant, if any. The sum of the length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the percentage of inhibition (%I) was calculated as described by[104]using the following formula:

$$\%I = \frac{(USc - USt)}{USc} \times 100$$

Where USc = ulcer surface area in control and USt = ulcer surface area in treated animals.

Histopathological studies

The freshly excised stomachs were washed with saline and preserved in 10% formaldehyde solution for histopathological studies. The sections of stomachs

stained with hematoxylin and eosin, were assessed for histopathological changes such as congestion, edema, hemorrhage and necrosis[105]. The microscopic slides were photographed.

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnett's test p values less than 0.05 were considered as significance.

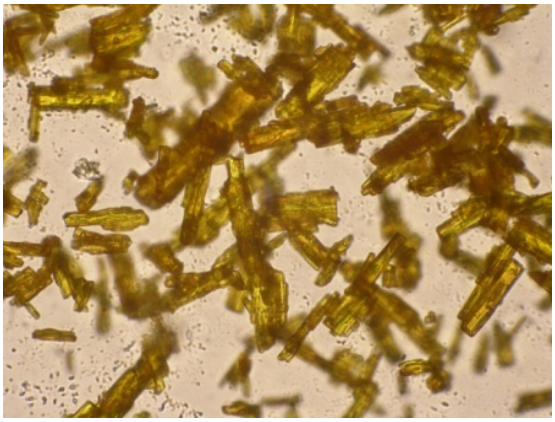
RESULTS AND DISCUSSION

MICROSCOPIC STUDIES:

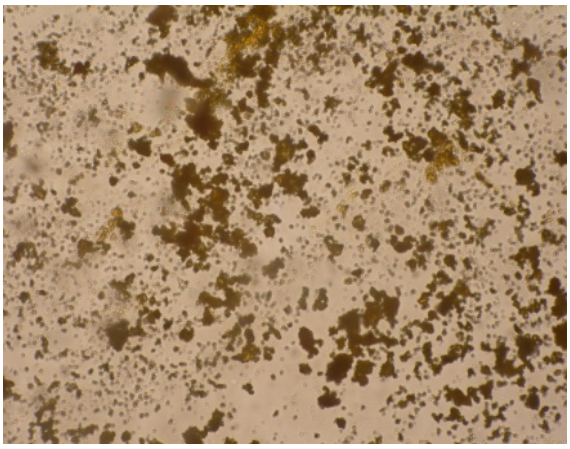
A pinch of prepared curcumin crystals were taken on separate glass slides and cover slip is placed on the slides. Observed in microscope at 45X magnifications. Photography of samples was taken and shown in Figures.

FIGURE 22: Photography of curcumin and prepared crystals

Pure drug



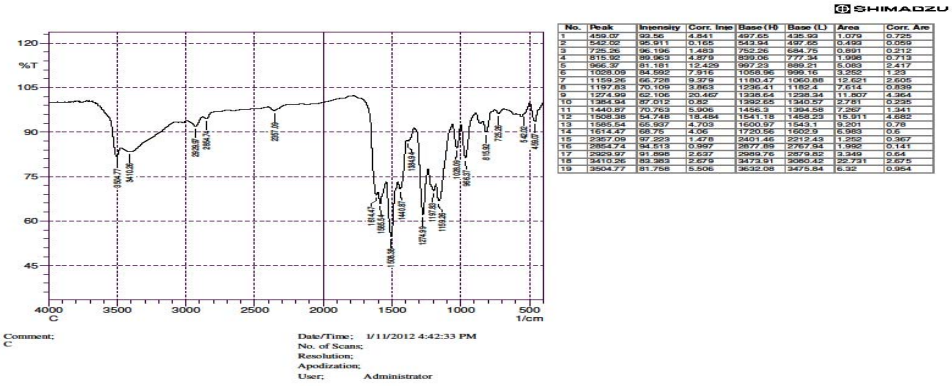
L.A.G. Crystals



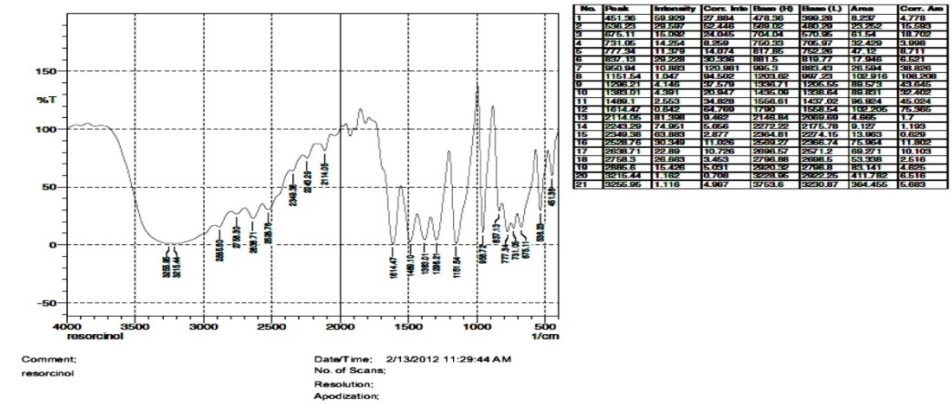
Results from microscopic studies of the liquid assisted grinding crystals showing different form of powder like crystals when compared with that of the broad size crystals of the pure curcumin.

Comparative studies of FT-IR Spectra

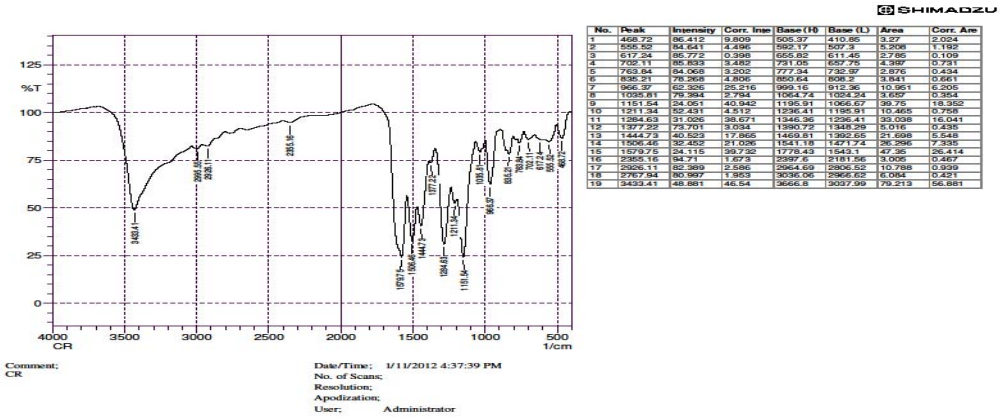
FIGURE 23: Comparative FTIR spectra of curcumin, resorcinol and prepared crystals



No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	459.07	93.56	4.641	497.65	435.93	1.079	0.725
2	542.02	95.911	0.165	543.94	497.65	0.493	0.059
3	735.36	96.196	1.483	752.26	684.75	0.891	0.212
4	815.92	89.963	4.879	839.06	777.34	1.998	0.713
5	966.37	81.181	12.499	997.23	889.21	5.083	2.417
6	1028.09	84.592	7.916	1058.96	999.16	3.252	1.23
7	1159.26	66.728	9.379	1180.47	1060.68	12.621	2.605
8	1197.83	70.109	3.863	1236.41	1182.4	7.614	0.839
9	1274.99	62.106	20.467	1338.54	1238.34	11.807	4.364
10	1384.94	87.012	0.82	1392.65	1340.57	2.781	0.235
11	1440.87	70.763	5.906	1456.3	1394.58	7.267	1.341
12	1508.38	54.748	18.484	1541.18	1458.23	15.911	4.682
13	1585.54	68.997	4.703	1600.97	1543.1	8.201	0.78
14	1614.47	68.75	4.06	1730.56	1602.9	6.983	0.6
15	2357.09	97.223	1.478	2401.46	2312.43	1.252	0.367
16	2854.74	94.513	0.997	2877.89	2767.54	1.992	0.141
17	2929.97	91.895	2.637	2989.76	2879.82	3.349	0.64
18	3410.26	83.383	2.679	3473.91	3080.42	22.731	2.675
19	3504.77	81.758	5.506	3632.08	3475.84	6.32	0.954



No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	451.36	59.909	27.884	478.36	366.28	8.237	4.778
2	536.21	29.597	52.448	589.02	480.29	23.252	15.583
3	675.11	15.069	64.045	704.04	570.35	61.54	18.709
4	731.05	14.254	8.259	750.33	705.97	32.429	3.998
5	777.34	11.379	14.974	817.85	752.26	47.12	8.711
6	837.13	29.228	90.236	881.5	819.77	17.946	61.521
7	960.94	10.883	120.881	995.3	885.43	26.584	38.826
8	1151.54	1.047	94.502	1203.82	1062.23	102.916	106.208
9	1256.21	4.146	37.579	1338.71	1206.55	89.573	43.645
10	1383.01	4.901	30.947	1426.08	1358.64	86.867	27.403
11	1488.1	2.553	34.829	1556.61	1437.02	96.464	46.054
12	1614.47	0.642	64.769	1730	1558.54	102.205	75.305
13	2114.06	81.398	91.482	2146.84	2068.89	4.895	1.7
14	2243.29	74.951	5.456	2272.22	2175.78	9.127	1.193
15	2540.38	63.883	2.877	2564.81	2527.4.15	13.983	0.629
16	2628.76	30.349	11.026	2689.27	2586.74	75.964	11.902
17	2838.71	22.89	10.726	2896.57	2571.2	68.271	10.103
18	2918.3	26.663	3.453	2786.88	2698.5	53.398	2.516
19	2885.6	15.426	5.031	2920.32	2786.8	85.141	4.825
20	3215.44	1.162	10.708	3228.96	2922.25	411.782	16.516
21	3256.95	1.116	4.967	3753.6	3230.87	364.455	5.683



No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	468.72	86.412	9.809	505.37	410.86	3.27	2.024
2	555.52	84.641	4.496	592.17	507.3	5.206	1.192
3	617.24	85.772	0.398	655.82	611.45	2.785	0.109
4	702.11	85.833	3.462	731.05	657.75	4.397	0.791
5	763.84	84.068	3.202	777.34	732.97	2.876	0.434
6	838.21	76.268	4.806	860.64	808.2	3.641	0.661
7	966.37	62.326	25.216	999.16	912.36	10.951	6.205
8	1035.81	79.394	2.784	1064.74	1024.24	2.657	0.354
9	1151.54	24.051	40.942	1195.91	1066.67	39.75	18.352
10	1211.34	52.431	4.512	1236.41	1195.91	10.465	0.755
11	1264.63	31.026	38.671	1346.36	1236.41	33.036	16.041
12	1377.22	73.701	3.034	1390.72	1348.29	5.016	0.435
13	1444.73	40.523	17.865	1469.81	1392.65	21.598	5.548
14	1506.46	32.452	21.026	1541.18	1471.74	26.296	7.335
15	1579.75	34.115	39.732	1778.43	1543.1	47.35	26.414
16	2355.16	94.71	1.673	2397.6	2181.56	3.005	0.467
17	2926.11	82.989	2.586	2964.69	2806.52	10.788	0.939
18	2767.94	80.997	1.953	3036.06	2966.62	6.064	0.421
19	3433.41	48.881	46.54	3666.8	3037.99	79.213	56.881

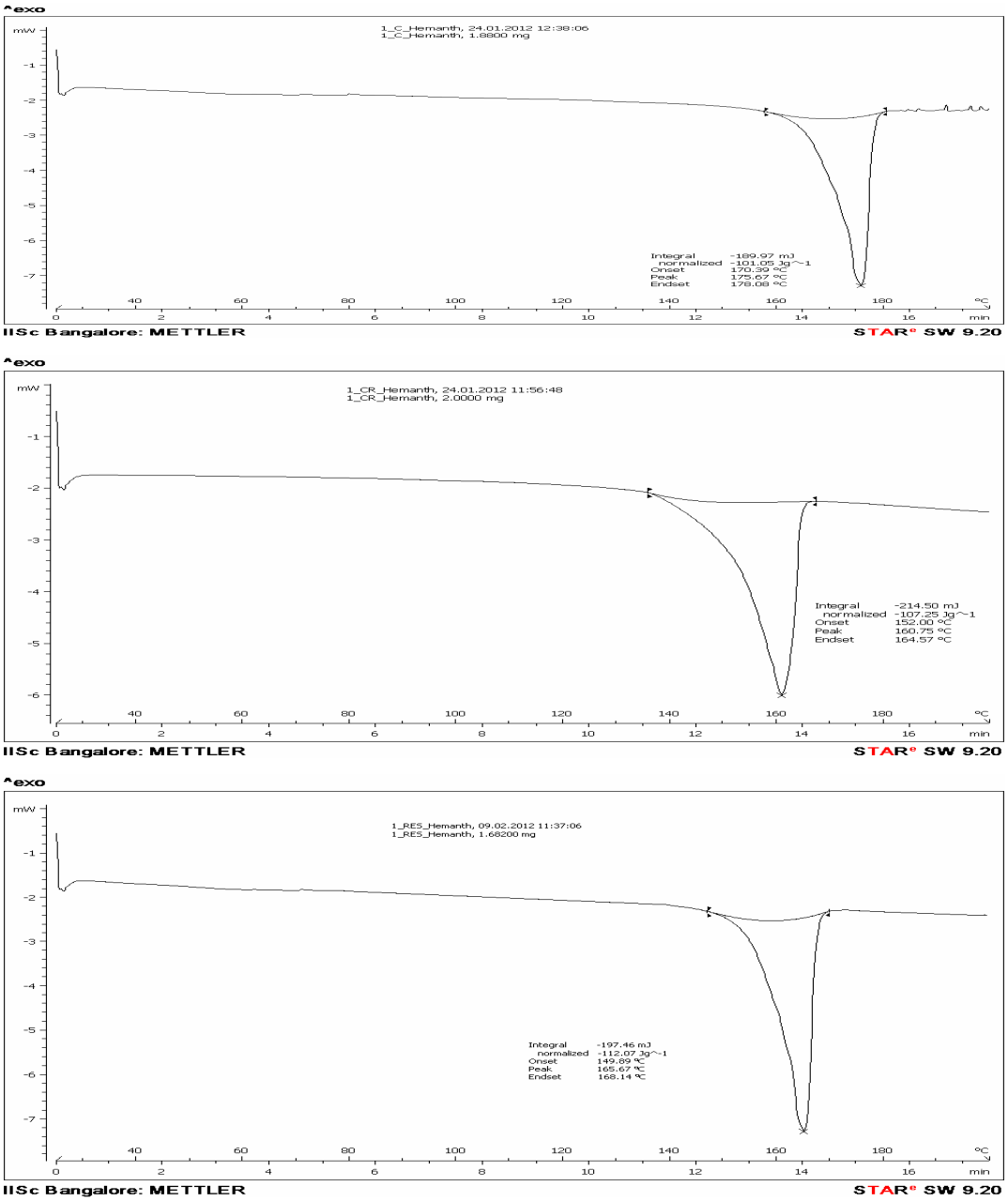
Comparative FTIR stretching vibrations (cm-1) of pure drug, resorcinol and prepared crystals

Sample/Peaks	Pure drug	Resorcinol	Crystals by L.A.G.Method
O-H	3504.77	3255.95	3433.41
C=O	1614.47	---	1506.46
Aromatic C=C	1585.54	1614.47	1444.73
Phenol C-O	1440.87	1296.21	1211.34
Enol C-O	1274.99/1197.83	---	1151.54

FTIR results show that the crystals prepared from liquid assisted grinding method has change in their peak intensity when compared to pure drug and others. But only a slight change in peak wavelength was identified for pure drug, and crystals prepared from liquid assisted grinding method.

Comparative studies DSC thermo grams:

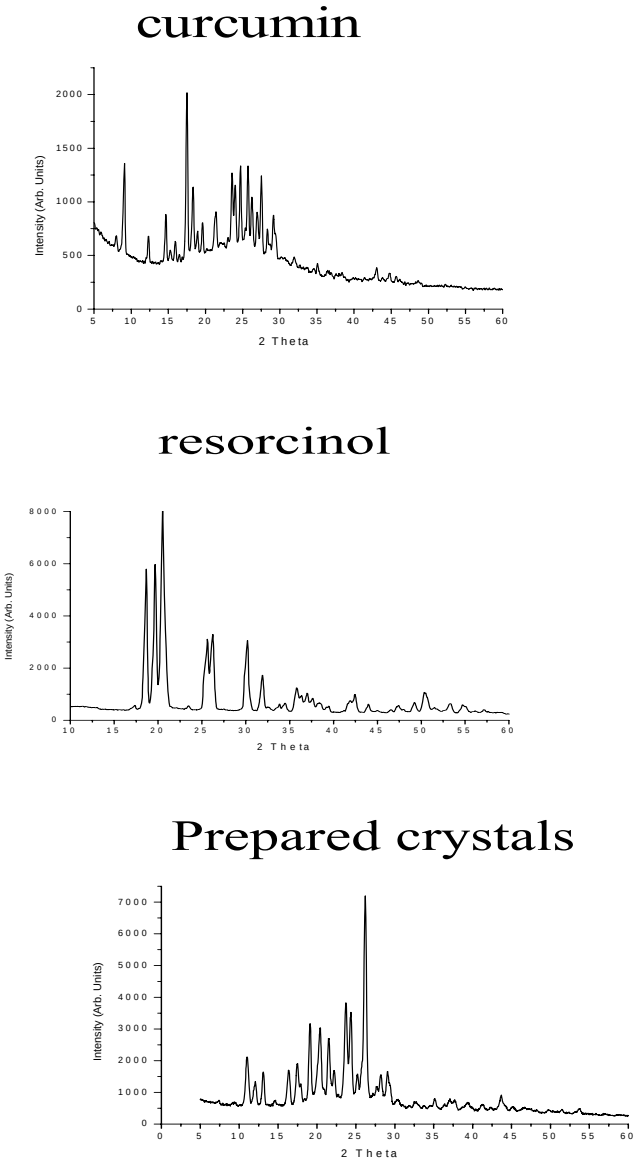
FIGURE 24: Comparative DSC thermo grams of curcumin, resorcinol and prepared crystals



The above DSC thermo grams showing different thermal peaks at various positions which clearly showing the formation of co-crystal.

Comparative Studies of Xrd Graphs of Pure Curcumin Resorcinol And Prepared Crystals Crystals

FIGURE 25: Comparative Pxrd Of Curcumin Resorcinol And Prepared Crystals

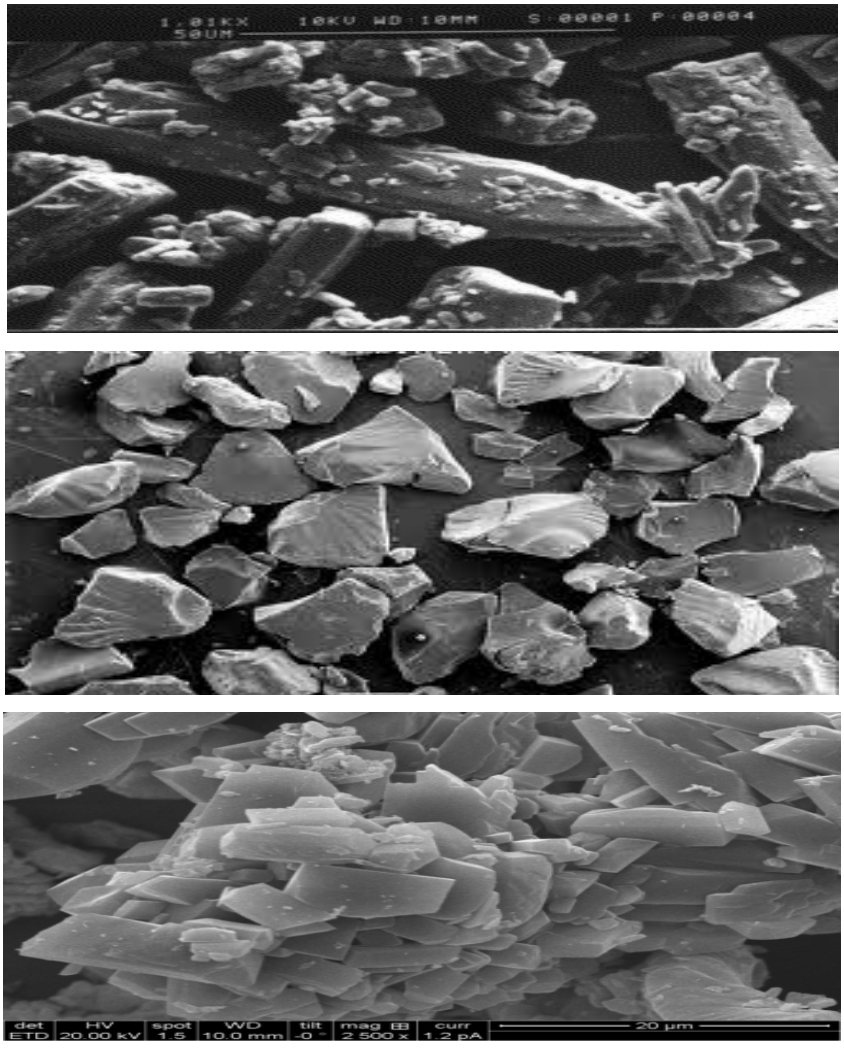


By comparing the above graphs, each graph showing 100% relative intensity at different 2θ ranges, which showing clearly that the formulated crystals having crystal property.

Comparative Scanning electron microscopy (SEM) studies:

SEM photography of prepared cocrystal shows uniform block or rod like crystals while curcumin showing different type of crystals and resorcinol showing pellet like crystals when comparing. This indicates the formation of Crystal form. SEM photographs of Curcumin, Resorcinol and prepared crystals are shown in figure 20.

FIGURE 26: Comparative SEM of Curcumin, Resorcinol and Prepared crystals



CRYSTAL STRUCTURE ANALYSIS:

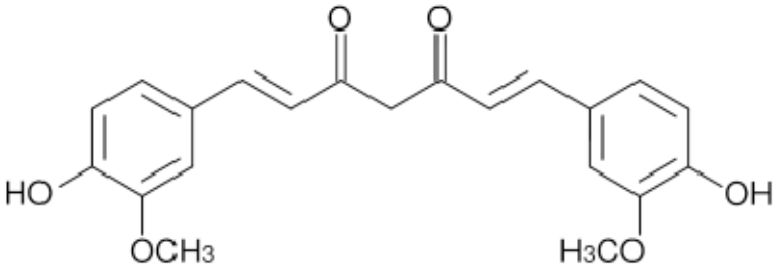
Crystal engineering of organic materials relies on the supramolecular building of crystalline solids based on the interaction of functional groups point of view. For the study of these crystalline materials, it is important to have a knowledge and understanding of intermolecular interactions between various functional groups.

A distinguishing feature of co-crystals, as compared to other crystalline forms of APIs, is that these multi-component systems can be manipulated using crystal engineering. By definition, crystal engineering involves modification of the crystal packing of a solid material by changing the internal arrangement of the molecules that regulate the breaking and forming of non-covalent bonds (e.g., hydrogen bonding, van der Waals forces, π-stacking, electrostatic interactions). In the case of multi-component systems, the co-crystallizing agent brings additional multiplicity into a crystallizing system, thus increasing its diversity in terms of resulting solid-state forms of APIs.

Given that the properties of materials are dependent on their solid-state structure, it is clear that specific characteristics of the APIs can be tailored systematically by varying the co-crystal former. An important initial step in co-crystal design is, therefore, the selection of an appropriate co-crystallizing agent.

The 1:1 co-crystal stoichiometry is sustained by O-H...O hydrogen bonds between the phenolic OH groups of the co-formers to the carbonyl group of curcumin.

Curcumin



Resorcinol

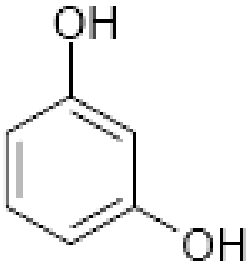
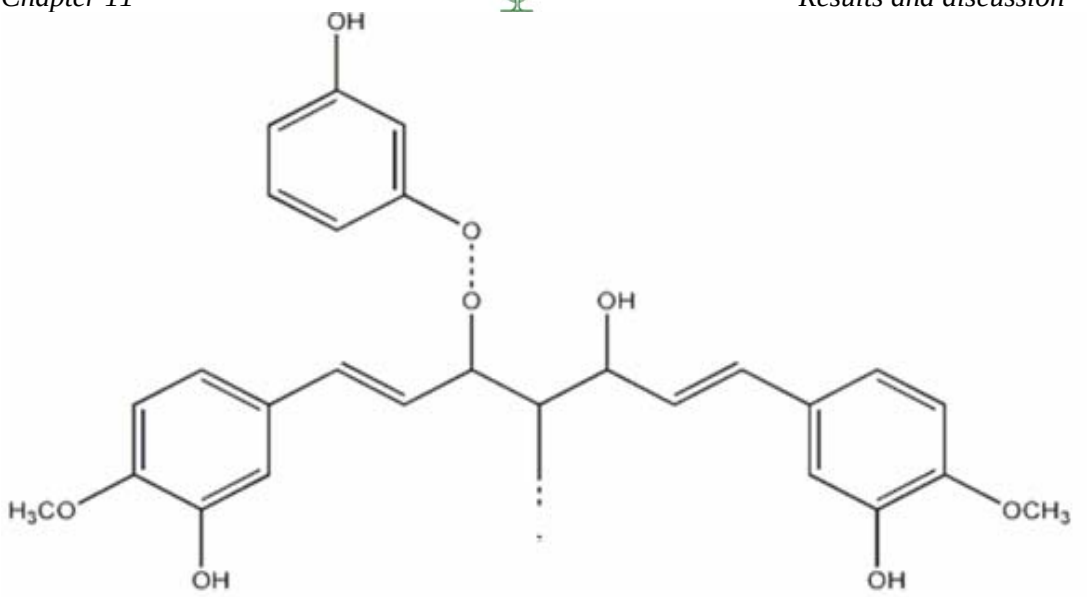


Figure 22: Expected structure of prepared crystals crystal



INVITRO DISSOLUTION STUDIES

TABLE 23: COMPARITIVE INVITRO DISSOLUTION STUDY OF PURE DRUG AND PREPARED CRYSTALS

S. No	Time interval	PURE DRUG	CRYSTALS
1	0	0.00	0.00
2	5	0.18±0.03	2.12±0.02
3	15	0.38±0.04	9.54±0.01
4	30	0.69±0.03	14.85±0.02
5	45	1.58±0.03	22.25±0.01
6	60	5.56±0.01	29.15±0.02
7	75	10.24±0.02	33.31±0.01
8	90	13.33±0.01	37.23±0.02
9	105	16.62±0.002	39.45±0.02

Figure 28: Percentage Drug Release Of Pure Drug And Crystals

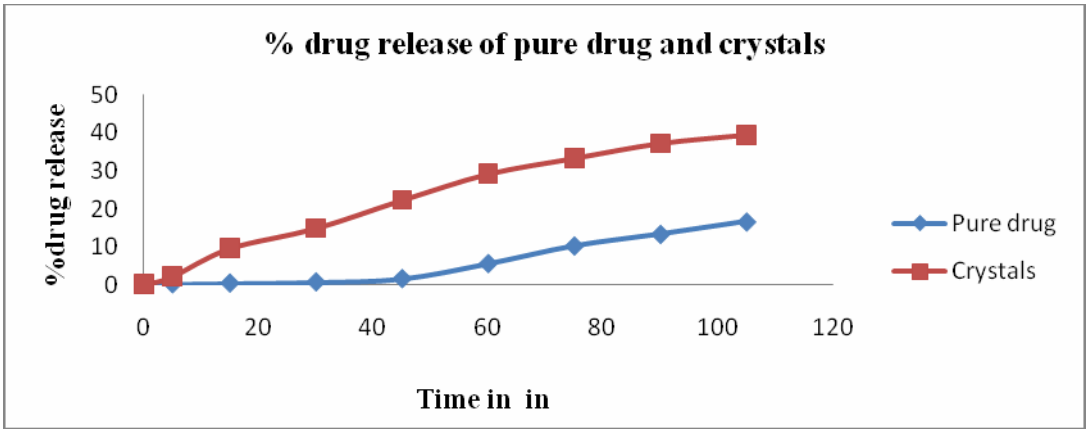
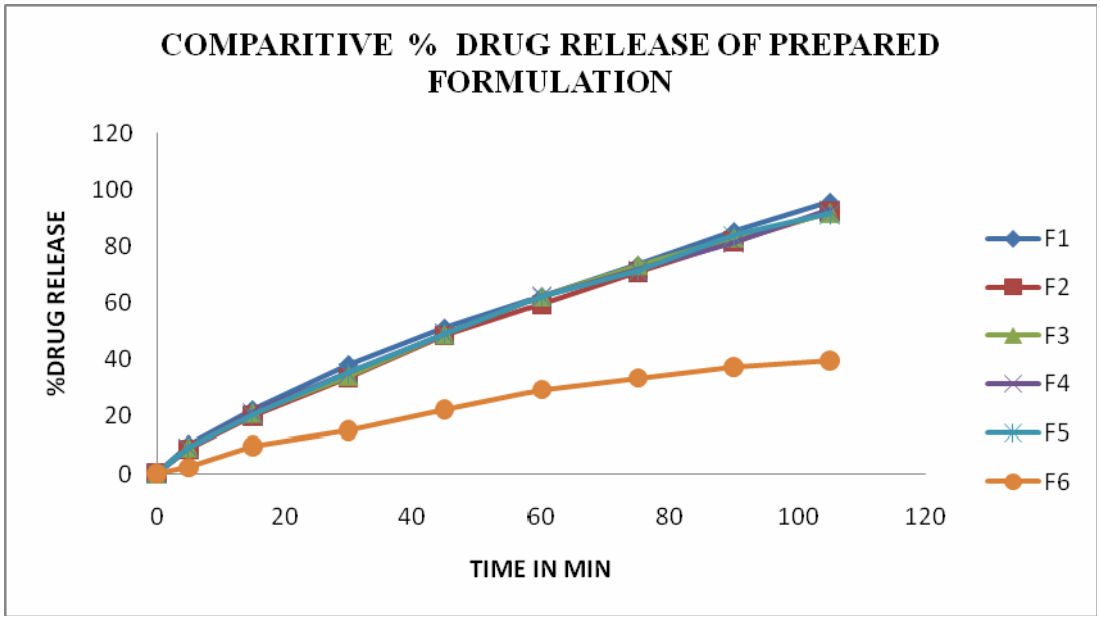


TABLE 24: COMPARITIVE INVITRO DISSOLUTION STUDY OF PREPARED FORMULATION (Tablets)

S. No	Time interval	F1	F2	F3	F4	F5	F6
1	0	0.00	0.00	0.00	0.00	0.00	0.00
2	5	9.98±0.01	8.55±0.02	9.19±0.03	9.01±0.02	8.99±0.01	2.12±0.01
3	15	22.12±0.02	20.12±0.02	21.11±0.03	21.18±0.02	20.22±0.02	9.54±0.02
4	30	38.12±0.02	33.44±0.01	34.17±0.01	35.15±0.01	35.12±0.03	14.85±0.02
5	45	50.98±0.01	48.44±0.01	48.88±0.02	49.11±0.03	49.12±0.02	22.25±0.02
6	60	62.12±0.01	59.32±0.05	62.22±0.02	62.24±0.02	62.12±0.01	29.15±0.01
7	75	73.14±0.01	70.71±0.01	73.30±0.02	71.41±0.01	71.12±0.02	33.31±0.03
8	90	85.15±0.02	81.81±0.02	83.11±0.01	81.21±0.02	83.75±0.02	37.23±0.02
9	105	95.54±0.02	92.24±0.01	91.84±0.01	92.82±0.03	91.51±0.01	39.45±0.01

GRAPH 3: Comparative invitro dissolution



RELEASE KINETICS ANALYSIS:

The data obtained from *in vitro* release studies were subjected to Zero order, first order, Higuchi's model, Korsmeyer's model and Hixson-Crowell model. Release parameters were given in Tables. Correlation coefficient (R) values obtained from PCP Disso software was used to test the applicability of release models. Graphs for various kinetic models for drug release of Curcumin and the drug release from prepared crystals in pH 1.2 buffer were given below.

TABLE 25: RELEASE KINETICS OF PURE DRUG

Time in min	Log Time	$\sqrt{Time}$	Cumulative % drug Release	Cumulative % drug remain	Log cumulative % drug release	Log cumulative % drug remained
0	0	0	0	100	0	2
5	0.698	2.24	2.12	97.88	0.326	1.990
15	1.176	3.87	9.54	90.46	0.979	1.956

30	1.477	5.48	14.85	85.15	1.171	1.930
45	1.653	6.70	22.25	77.75	1.347	1.890
60	1.778	7.745	29.15	70.85	1.464	1.850
75	1.875	8.66	33.31	66.69	1.522	1.824
90	1.954	9.48	37.23	62.77	1.570	1.797
105	2.021	10.25	39.45	60.55	1.596	1.782

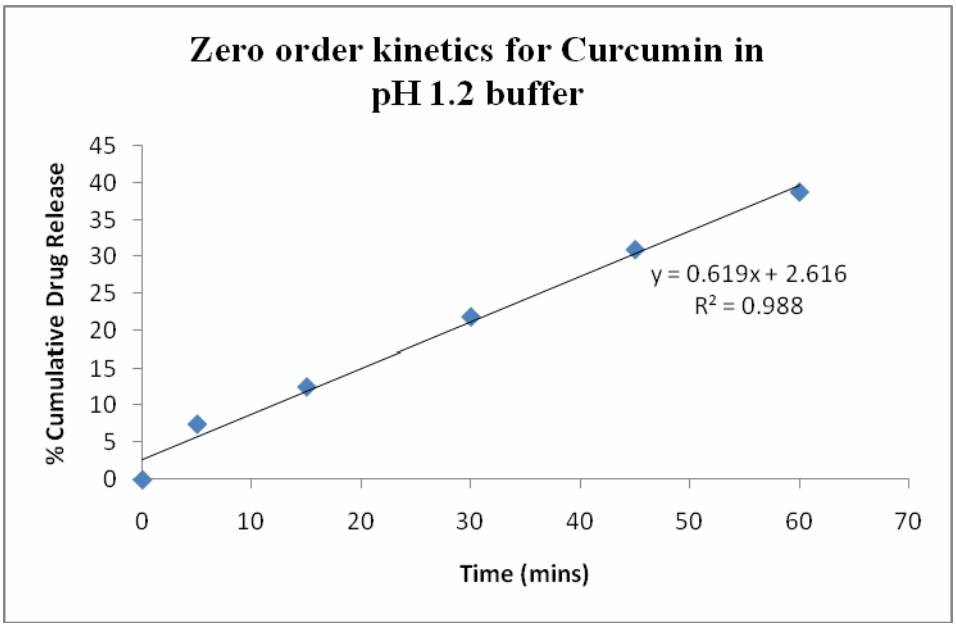
TABLE 26: RELEASE KINETICS OF CRYSTALS BY LAG METHOD

Time in min	**Log Time**	**$\sqrt{Time}$**	**Cumulative % drug Release**	**Cumulative % drug remain**	**Log cumulative % drug release**	**Log cumulative % drug remained**
0	0	0	0	100	0	2
5	0.698	2.24	17.1	82.9	1.232	1.918
15	1.176	3.87	38.63	61.37	1.586	1.787
30	1.477	5.48	48.51	51.49	1.685	1.711
45	1.653	6.70	57.62	42.38	1.760	1.627
60	1.778	7.745	69.78	30.22	1.844	1.480
75	1.875	8.66	79.79	20.21	1.901	1.305
90	1.954	9.48	85.45	14.55	1.931	1.162
105	2.021	10.25	97.24	2.76	1.987	0.440

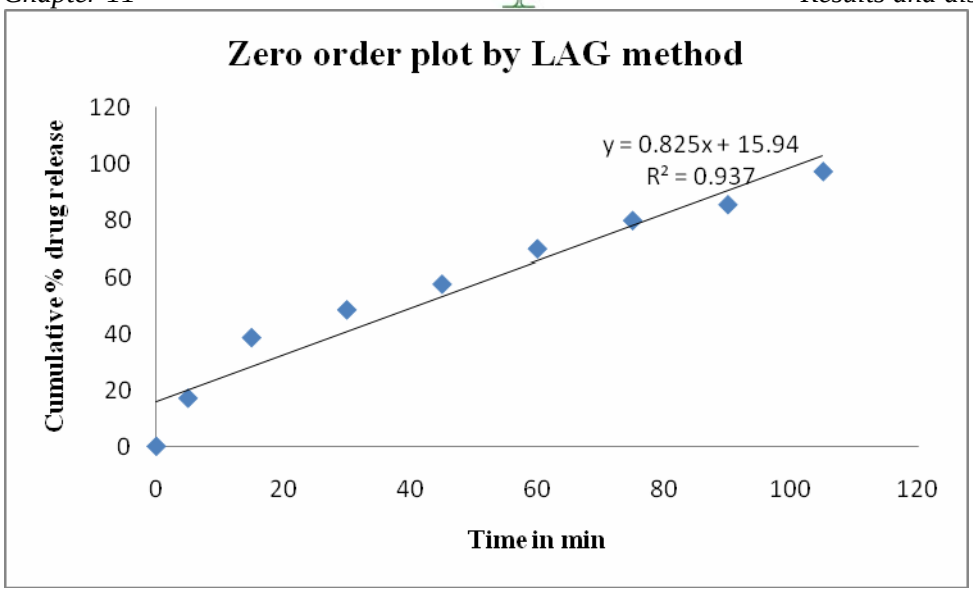
Table 27: Release Parameters of Pure Drug and Crystals

SAMPLES		Zero order model	First order model	Higuchi model	Krosmeyer model
Pure drug	R^2	0.988	0.215	0.963	0.974
Crystal by L.A.G. method	R^2	0.937	0.873	0.994	0.910

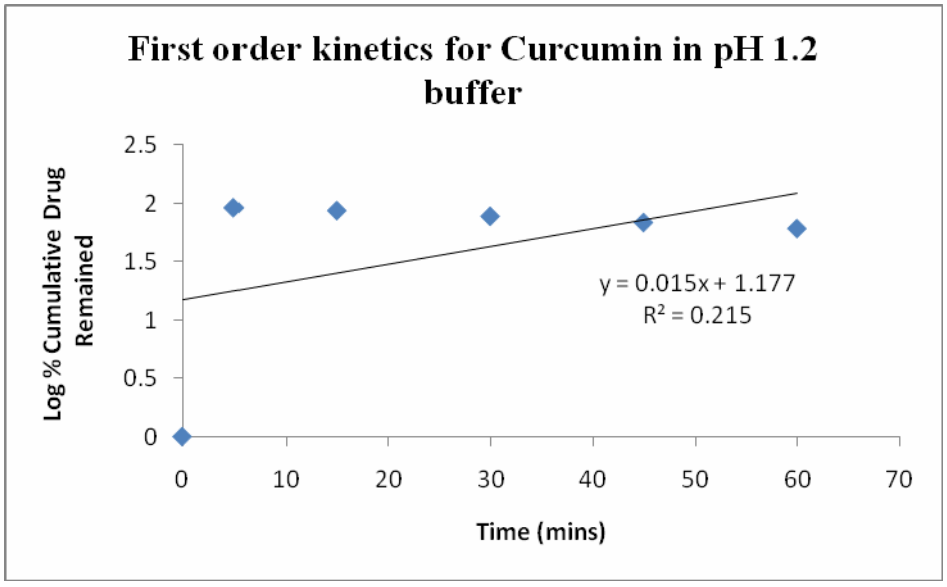
GRAPH 4: Zero Order Release Model for Curcumin in pH 1.2 buffer



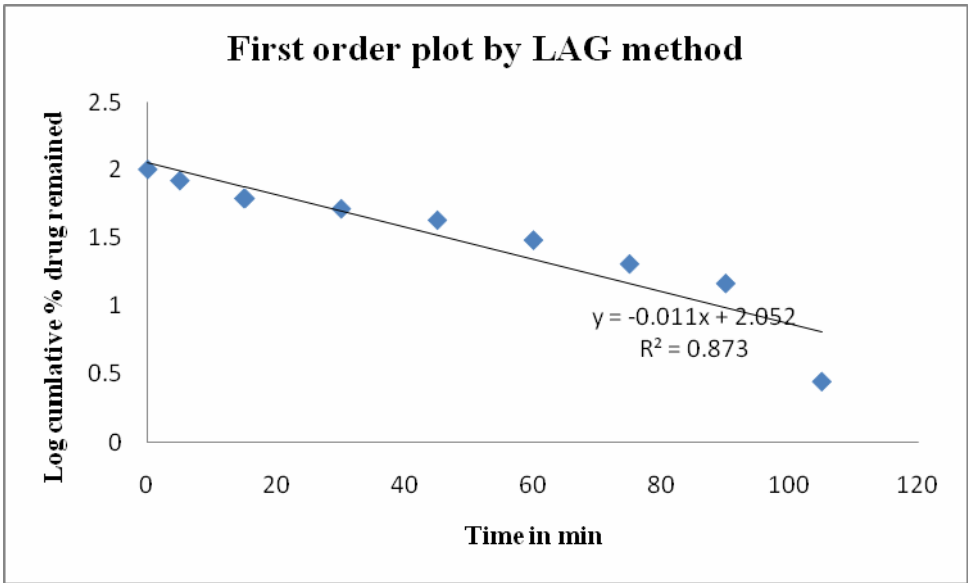
GRAPH 5: Zero Order Release Model for Curcumin crystals by liquid assisted grinding method in pH 1.2 buffer



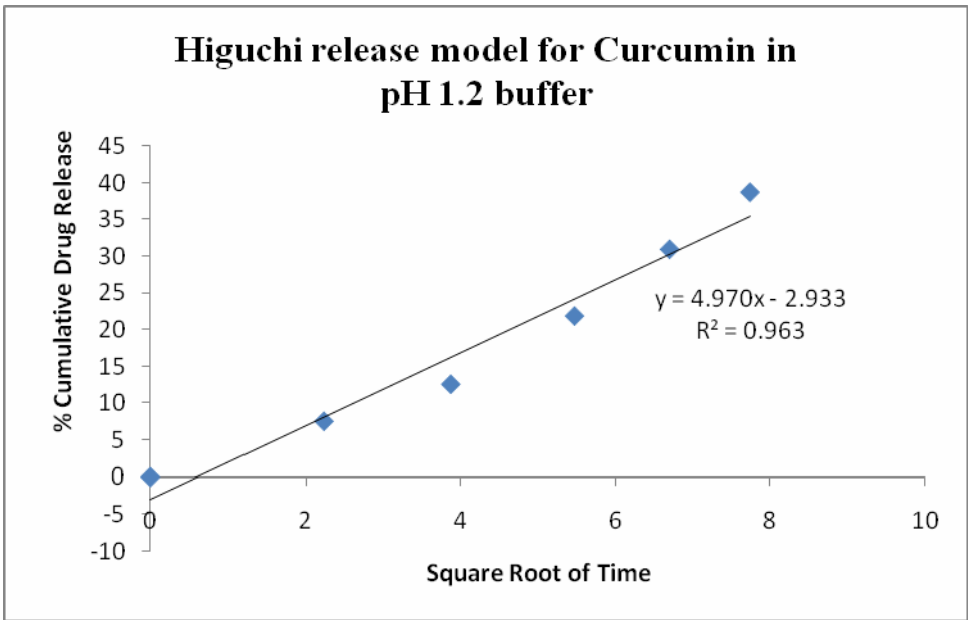
GRAPH 6: First Order Release Model for Curcumin in pH 1.2 buffer



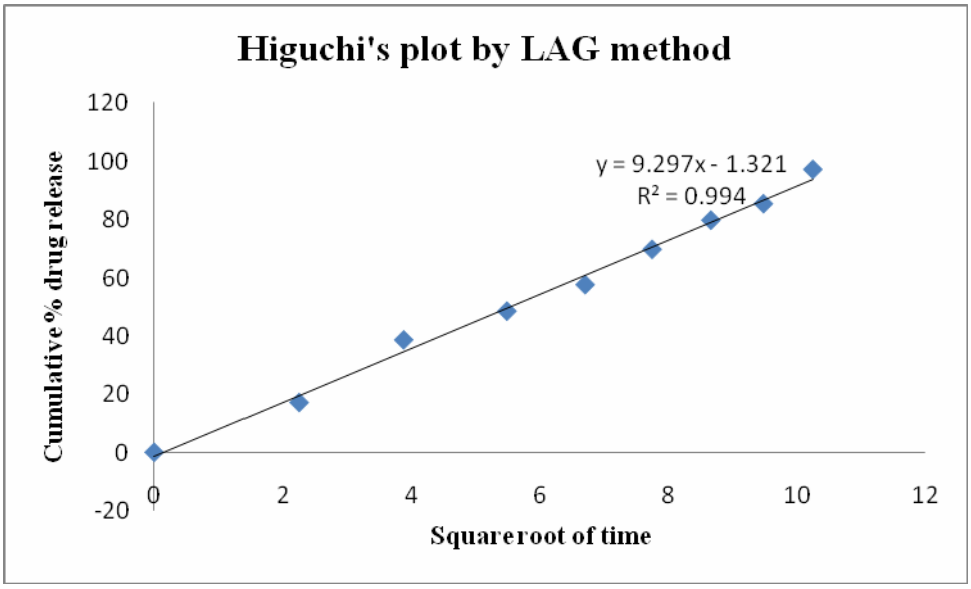
GRAPH 7: First Order Release Model for Curcumin crystals by Liquid assisted grinding method in pH 1.2 buffer



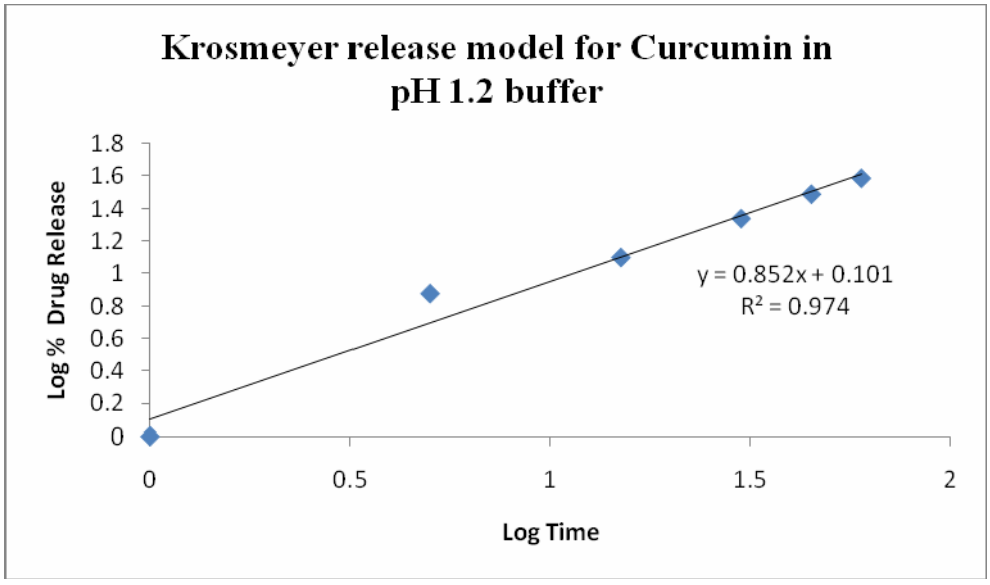
GRAPH 8: Higuchi Release Model for Curcumin in pH 1.2 buffer



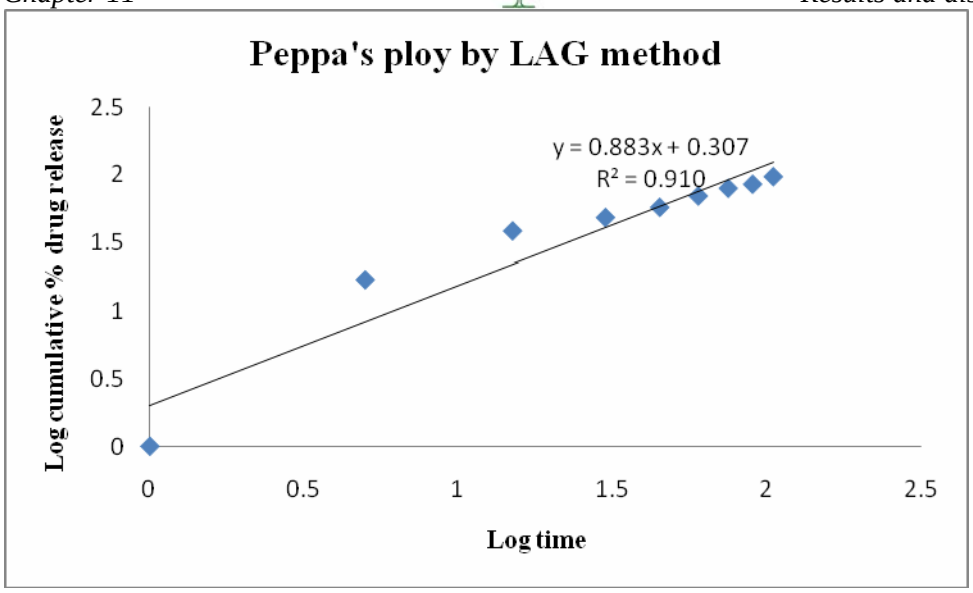
GRAPH 9: Higuchi Release Model for Curcumin crystals by Liquid assisted grinding method in pH 1.2 buffers



GRAPH 10: Krosmeyer Release Model for Curcumin in pH 1.2 buffer



GRAPH 11: Krosmeyer Release Model for Curcumin crystals by Liquid assisted grinding method in pH 1.2 buffer


Peppa's ploy by LAG method
y = 0.883x + 0.307
R² = 0.910
Log cumulative % drug release
Log time
2.5
2
1.5
1
0.5
0
0
0.5
1
1.5
2
2.5

Correlation Coefficient (R) values obtained from PCP Disso V3 software was used to test the applicability of release models. The release of pure drug and crystals follows zero order kinetics. The m value is 0.619 (>0.5) in ethanol- water. Whereas the drug release from crystals by L.A.G. method follow Higuchi release.

Best fit models for drug and crystal forms in various systems were given in Table 27. From the dissolution results, the crystals prepared by liquid-assisted grinding method shows increased drug release when compared to pure drug.

ACCELERATED STABILITY STUDIES

Selected formulation F1 was subjected to stability studies as per I.C.H guidelines. Samples were kept at 40 °C / 75% RH and analyzed for weight variation, hardness, friability, drug content and In vitro dissolutions study for every month for a period of three months.

TABLE 28: Accelerated Stability Studies

S. No.	Parameters	Initial	30days	60days	90 days
1	Weight variation	200.3±0.03	200.3±0.03	200.3±0.02	200.3±0.01
2	Drug content	95.45±0.03	95.43±0.02	95.43±0.01	95.42±0.12

IN-VIVO STUDIES

Effect of Co-crystals on gastric ulcer induced by Ethanol

The prepared crystals showed significant anti-ulcer effect against ulcers induced by Ethanol compare than Curcumin treated groups. In ethanol induced ulcer model, Curcumin-Resorcinol crystals at a dose of 100 mg/kg body weight showed protective effect of 76.63%, where as Omeprazole showed protection index of 81.47% at a dose of 20 mg/kg body weight (**Table -29**).

Histopathological studies suggest that the ethanol damage to the gastrointestinal mucosa starts with microvascular injury, namely disruption of the vascular endothelium resulting in increased vascular permeability, edema formation and epithelial lifting. Hence histopathological studies conformed, Curcumin-Resorcinol crystals showed significant inhibition in ethanol induced gastric lesions **(Figure 28 and 29).**

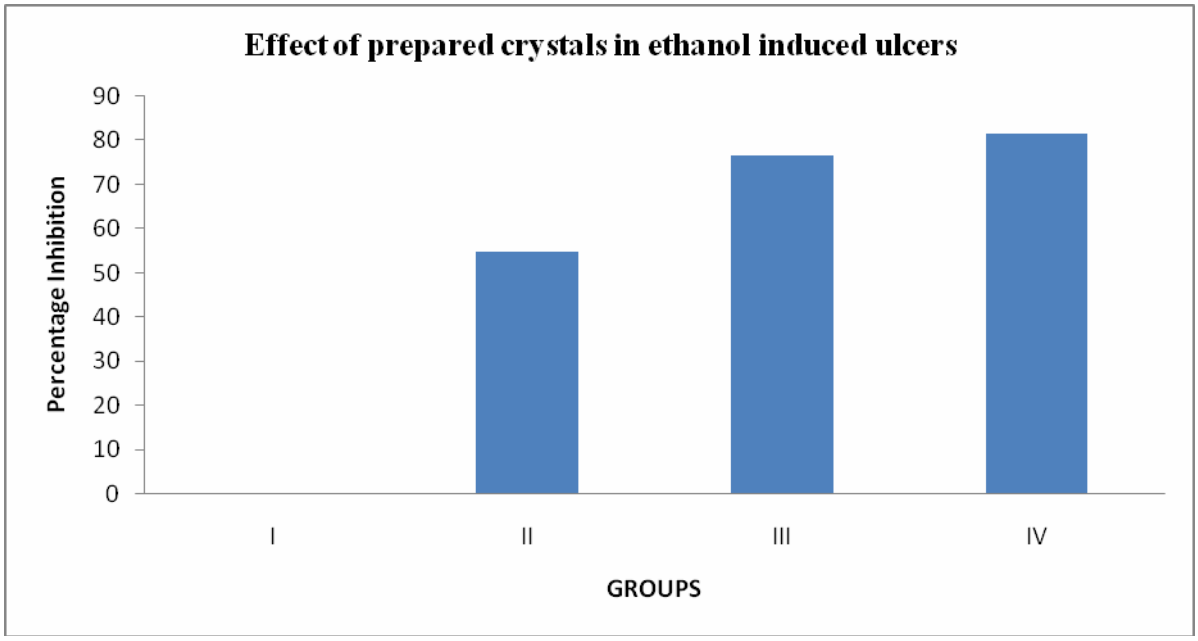
Table 29: Effect of Curcumin-Resorcinol crystals *in* ethanol (8 ml/kg) induced gastric ulcer in rats

Group	Design of Treatment	Ulcer Index	Percentage Inhibition (% I)
I	Control (1% w/v CMC, 10 ml/kg b.w) p.o	20.67 ± 0.71	---
II	CURCUMIN (100mg/kg b.w) p.o	9.33 ± 0.33*	15.86
III	Curcumin-Resorcinol crystals (100mg/kg b.w) p.o	4.83 ± 0.17**	76.63
IV	Omeprazole (20mg/kg b.w) p.o	3.83 ±0.31**	81.47

Data are represented as mean ± S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison test. $^{*}P < 0.01$ and $^{**}P < 0.001$ as compared to control (n = 6 in each group).

b.w= Body weight.

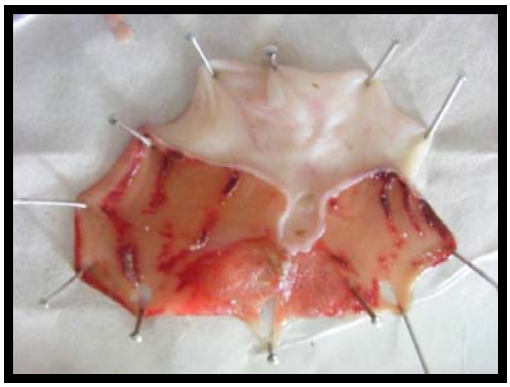
GRAPH 12: Effect of prepared crystals in ethanol (8ml/kg) induced gastric ulcer in rats



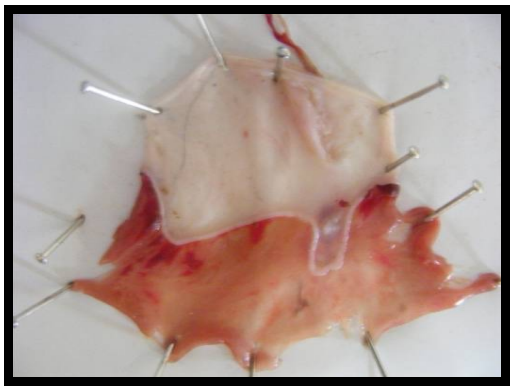
GROSS PATHOLOGY OF STOMACH

AFTER EXPOSURE TO ETHANOL

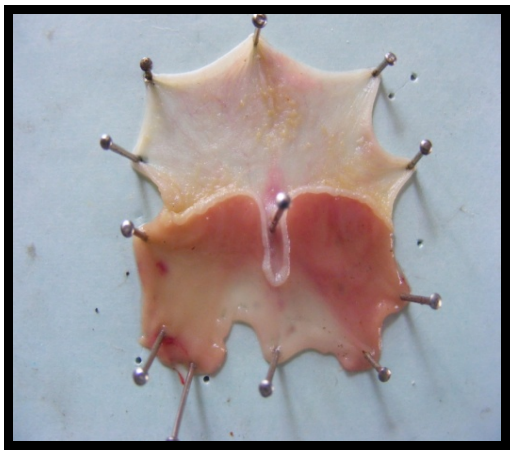
Figure 29: Effect of Curcumin-Resorcinol crystals pretreatment on ethanol-induced gastric ulcer in rats.



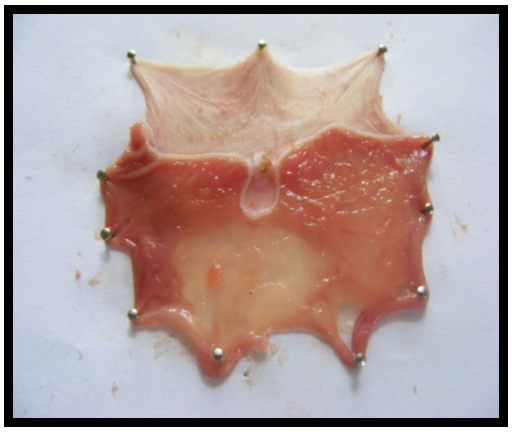
A



B



C



D

(A) Stomach after ethanol treatment,

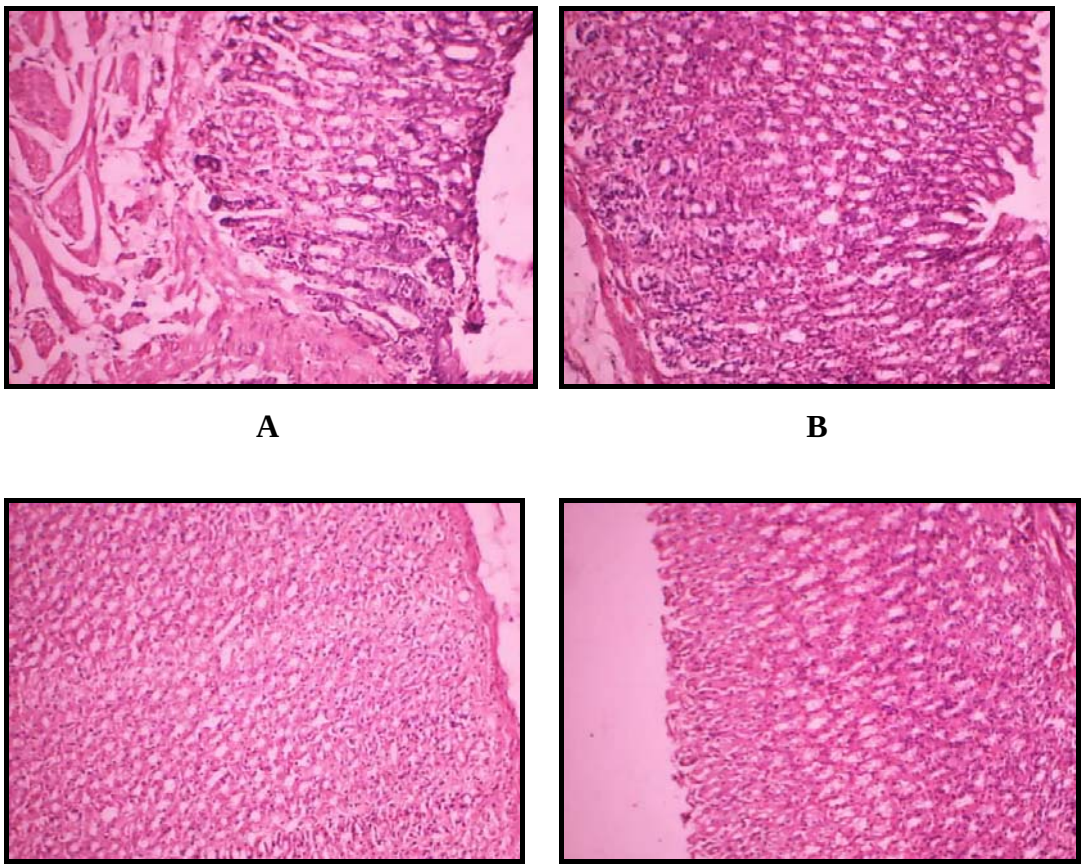
(B) Stomach treated with CURCUMIN 100 mg/kg plus ethanol,

(C) Stomach treated Curcumin-Resorcinol crystals -100 mg/kg plus ethanol,

(D) Stomach treated with omeprazole-20 mg/kg plus ethanol.

HISTOPATHOLOGY OF ETHANOL INDUCED GASTRIC ULCER

Figure 30: An effect of Curcumin-Resorcinol crystals pretreatment on ethanol - induced gastric ulcer in rats.
Stomach tissue was stained with hematoxylin and eosin (100 x).



A **B**

C **D**

(A) Stomach after ethanol treatment,

(B) Stomach treated with CURCUMIN 100 mg/kg plus ethanol,

(C) Stomach treated Curcumin-Resorcinol crystals -100 mg/kg plus ethanol,

(D) Stomach treated with omeprazole-20 mg/kg plus ethanol.

SUMMARY

The crystal and particle engineering strategies have notable potential to strengthen the available methods for addressing problems of low aqueous solubility of drug substances. These methods are applicable not only to molecules of a specific physical and chemical nature, but to a wide range of crystalline materials, although a comprehensive knowledge of drugs at the molecular level is required to determine the appropriate approach to improving solubility and dissolution rate. The formation of molecular complexes and co-crystals is becoming increasingly important as an alternative to salt formation, particularly for neutral compounds or those having weakly ionizable groups.

The curcumin resorcinol cocrystals of 1:1 molar ratio supramolecular complexs was formulated. It characterized in terms of SEM, FT-IR, DSC, XRPD, and subjected to solubility, micromeritic, In-vitro dissolution studies, In-vivo studies.

Slight variation in the wave lengths of the functional group in the molecular complexs of FT-IR spectrum. The **melting points** obtained from the thermographs of molecular complex were different from that of the pure drug and the excipient which confirms the formation of the new crystal phases. New peaks were observed in the crystalline pattern.

From micromeritics studies of crystal form shows, the angles of repose values were ranged from **16.27^{o} ± 0.19 to 20.28.^{o} ± 0.14**. The results were found to be below **17**; hence they have excellent flow ability. The Hausner's ratio value ranged from **0.139 ± 0.04 to 0.142± 0.03,** it has shown that crystals have good flow property and can be compressed in to tablets.

The weight variation test were done for F1 to F6 formulations and found to be **200.1 ± 0.05 to 200.8 ± 0.01.** The % deviation is coming within 3% to 5% range for this test accepted % deviation should be 5 % for **200-250** mg tablet. F1 to F6

formulations come within limit and passed the test. The friability test done for the F1 – F6 formulations was ranged from **0.25 to 0.36** which exactly falls within the limit of standard (0.1 to 0.9 %). The thickness of the tablets ranges from **2.9 ± 0.02 to 3.1 ± 0.03**. The hardness for F1 to F5 formulations ranged from **3.8 ± 0.10 to 4.0 ± 0.13,** which showing that those are within the limits. The drug content F1 to F6 showed in the range of **96.63± 0.02 to 99.36± 0.03** and F1 showed maximum drug content **99.36± 0.03.** Disintegration was determined and the results were given. The disintegration time for F1 – F6 were ranging **4.5± 095 to 5.2± 1.36 min.**

From In-vitro dissolution studies it shows similar dissolution profile, With the percentage release of pure drug shows **39.45 %** for 105 min, while the crystals of tablet show **97.24%**

Curcumin and Omeprazole are potentially preventing gastric lesions development in the gastric wall during the acute phase of gastric ulcer diseases, but curcumin was more potent in its effect. From the in-vivo studies the antiulcer activity of curcumin was evaluated gastric ulcer disease at dose of 100 mg/kg (dissolved in saline solution).Therefore, the comparison between such recommended dose of curcumin and one of the proton pump inhibitors (PPIs) Omeprazole 20mg/kg is worth-while. Since, the pharmacological control of gastric acid secretion is the main desired goal for gastro-cytoprotection, particularly, the H+/K+-ATP ase (acid proton pump) inhibitors. Form the formulated co-crystals showed significant anti-ulcer effect against ulcers induced by Ethanol compare than Curcumin treated groups. In ethanol induced ulcer model, co-crystal at a dose of 100 mg/kg body weight showed protective effect of **76.63%,** where as Omeprazole showed protection index of **81.47%** at a dose of 20 mg/kg body weight.

CONCLUSION

Despite lack of precedence in marketed products and concerns about the safety and toxicity of co-crystal forming agents, there is growing interest and activity in this area, which aims to increase the understanding of co-crystal formation and methods of preparation. Although, some recent developments in crystal and particle engineering have been included nowadays, consideration of established approaches such as the use of high-energy amorphous and metastable crystalline forms is still widespread. In particular substantial advancement methods of crystal engineering, supramolecular technique alters the physicochemical properties of Curcumin which can relatively undergoes for formulation.

The instability of curcumin at physiological pH may be ascribed to the β-diketone linker in the seven carbon chain of curcumin. A crystal engineering approach was utilized to prepare 1:1 cocrystals of curcumin with resorcinol by liquid-assisted grinding. We reasoned that the reactivity of the keto-enol group could be modified through hydrogen bonding with phenolic compounds in co-crystals, which in turn might provide more soluble and stable curcumin solid-state forms. A solid form screen of curcumin with phenolic coformers afforded novel cocrystals with resorcinol .These curcumin cocrystals were characterized by FT-IR, X-ray powder diffraction, and thermal techniques. The present results on more soluble cocrystals of curcumin could provide faster dissolving solid forms of curcumin that are relatively stable for drug development.

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