INCLUSION COMPLEXATION OF GEFITINIB WITH CYCLODEXTRINS

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Yann-Huei Phillip Lee

Certificate of Approval:

_____	_____
Daniel L. Parsons Professor Pharmacal Sciences	Jayachandra B. Ramapuram, Chair Assistant Professor Pharmacal Sciences
_____	_____
William R. Ravis Professor Pharmacal Sciences	George T. Flowers Interim Dean Graduate School

INCLUSION COMPLEXATION OF GEFITINIB WITH CYCLODEXTRINS

Yann-Huei Phillip Lee

A Thesis

Submitted to

the Graduate Faculty of

Auburn University

in Partial Fulfillment of the

Requirements of the

Degree of

Master of Science

Auburn, Alabama
August 9, 2008

INCLUSION COMPLEXATION OF GEFITINIB WITH CYCLODEXTRINS

Yann-Huei Phillip Lee

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Signature of Author

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Date of Graduation

THESIS ABSTRACT

INCLUSION COMPLEXATION OF GEFITINIB WITH CYCLODEXTRINS

Yann-Huei Phillip Lee

Master of Science, August 9, 2008
(B.S., Auburn University, 2004)

70 Typed Pages

Directed by Jayachandra Babu Ramapuram

Gefitinib, a very slightly soluble new drug, is used in the treatment of locally advanced or metastatic non-small cell lung cancer (NSCLC) in patients who have previously received chemotherapy. This study examined the complexation of gefitinib with three selected cyclodextrins, β-cyclodextrin (βCD), hydroxypropyl-β-cyclodextrin (HPβCD), and randomly methylated β-cyclodextrin (RMβCD), with the objective of improving the solubility and dissolution of the drug.

Phase solubility studies were performed with the different cyclodextrins to characterize the complexation in the liquid state. The complexation in the solid state was characterized by differential scanning calorimetry and x-ray diffraction analyses. The dissolution studies were performed using USP dissolution testing equipment and the dissolution samples were analyzed by an UV spectrophotometric assay. The solubility of gefitinib was significantly improved by all cyclodextrins (CDs) in the study. The

association rate constants (K_s) calculated from the phase solubility diagrams indicate that gefitinib can form a stable inclusion complex with all three CDs. The freeze-dried formulations showed substantial increases in the dissolution of gefitinib with all three CDs compared to gefitinib alone, while the kneaded and physical mixtures showed no improvement in the dissolution. Furthermore, addition of the hydrophilic polymers polyvinyl pyrrolidone (PVP) and hydroxypropyl methyl cellulose (HPMC) markedly enhanced the dissolution of gefitinib from CD complexes. The gefitinib-HPβCD (1:1) complex yielded 50% dissolution in 1 hr whereas PVP or HPMC in association with the complex increased the dissolution up to 95% within 1hr.

In conclusion, gefitinib can form a stable inclusion complex with all three cyclodextrins as demonstrated by the liquid and solid state complexation studies. HPβCD showed the greatest improvement in the dissolution of gefitinib followed by RMβCD and βCD.

ACKNOWLEDGEMENTS

I’d like to thank my advisor, Dr. Ramapuram for his guidance and support, the professors in the School of Pharmacy for their input, my fellow graduate students for their help, and my Mother, Father, and Brother for their love.

Style manual: The AAPS Journal

Computer software: Microsoft Word 2000

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INTRODUCTION

Gefitinib is an anilinoquinazoline with the chemical name 4-Quinazolinamine, N-(3-chloro-4- fluorophenyl) -7-methoxy-6- [3-4-morpholin) propoxy] and the following structural formula:

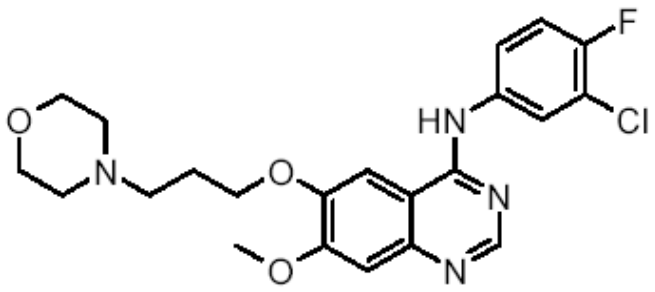

Figure 1-1: The molecular structure of gefitinib.

It has the molecular formula $C_{22}H_{24}ClFN_4O_3$, a relative molecular mass of 446.9 and is a brown-colored powder. Gefitinib is a free base with has pKa's of 5.4 and 7.2 and therefore ionizes progressively in solution as the pH falls. Gefitinib is soluble in aqueous solvents at pH 1, but is practically insoluble above pH 7 and the solubility dropping sharply between pH 4 and pH 6. In non-aqueous solvents, gefitinib is freely soluble in glacial acetic acid and dimethylsulphoxide, soluble in pyridine, and sparingly soluble in tetrahydrofuran, and slightly soluble in methanol, ethanol (99.5%), ethyl acetate, propan-2-ol, and acetonitrile.

Gefitinib is a new drug used in the treatment of locally advanced non-small cell lung cancer. There is no absolute data on the bioavailability of gefitinib in humans; while in animal studies, a bioavailability of about 50% has been reported. Gefitinib is 90% protein bound in vitro to serum albumin and α-1-acid glycoprotein, and has a mean steady state volume of distribution of 1400 liters. Gefitinib is metabolized in the kidneys, but renal elimination accounts for only 4% of the administered dose. The predominant mode of excretion is via feces (approximately 86%). The elimination half-life ranges from 6 to 49 hours, with no clear evidence of dose-dependency, and values being similar after single doses or repeat administration. The bioavailability can be very well increased by improving the solubility and dissolution of the drug. If the drug absorption is limited by its solubility and dissolution, various approaches can be followed to improve these. Some examples are: 1) solvent dispersion on an inert carrier, 2) micronization of drug particles, 3) nanoparticle formation, 4) hot-melt extrusion, and 5) cyclodextrin complexation. This research proposes to study the effect of cyclodextrin complexations on gefitinib solubility and dissolution.

Cyclodextrins (CDs) are crystalline, cyclic oligosaccharides with a bucket-like structure having a hydrophobic internal cavity and a hydrophilic exterior cavity. The interior of the toroid is hydrophobic as a result of the electron rich environment provided in large part by the glycosidic oxygen atoms. This structure allows the formation of inclusion complexes in which lipophilic compounds are noncovalently bound within the cavity. It is the interplay of atomic (Van der Waals), thermodynamic (hydrogen bonding), and solvent (hydrophobic) forces that accounts for the stable complexes that may be formed with chemical substances while in the apolar environment of the

cyclodextrin cavity. The complex exists in an equilibrium dependent upon the concentrations of the cyclodextrin, the guest chemical and water.

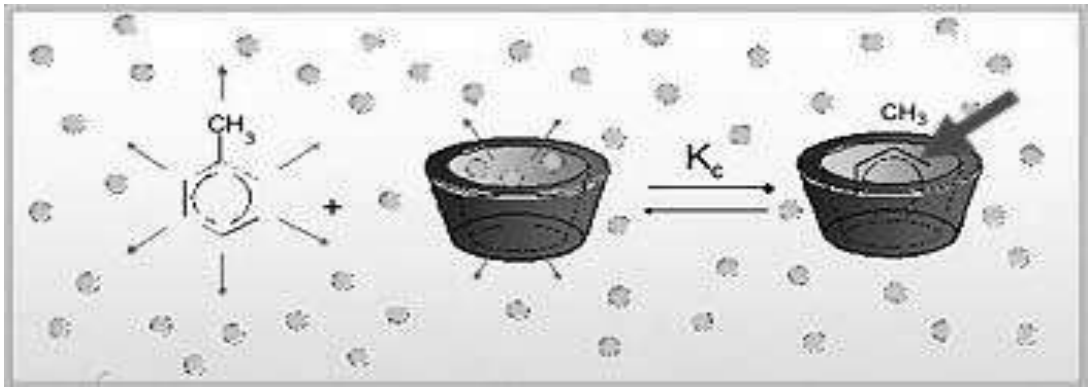

Figure 1-2: The cyclodextrin complexation equilibrium in water.

Cyclodextrins have been employed in the pharmaceutical industry to increase the aqueous solubility and stability of drugs and have been used in both parenteral and oral drug delivery systems. There are three types of naturally occurring cyclodextrins—alpha, beta, and gamma, seen in Figure 1-3.

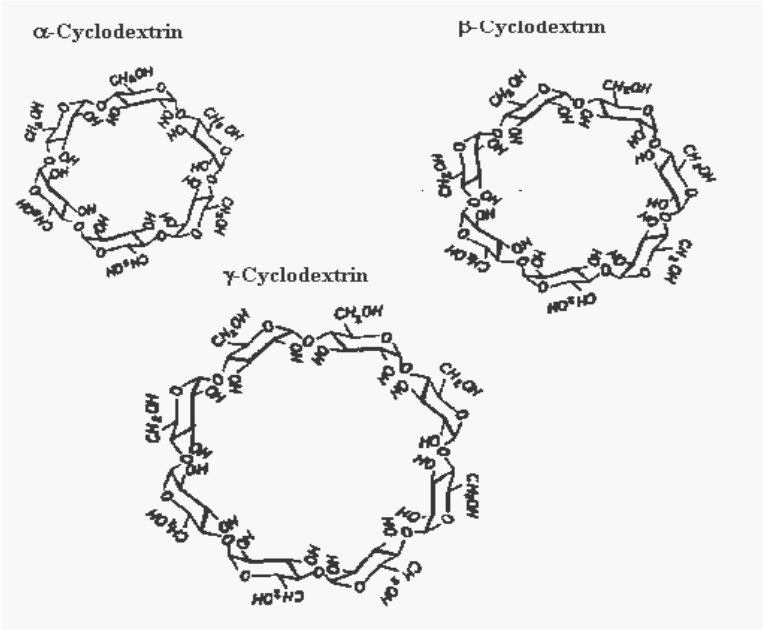

Figure 1-3: The three naturally occurring cyclodextrins.

β-cyclodextrin (βCD) in particular is useful for complexation of average size molecules, such as most drugs. The promising advantages of β-cyclodextrin as a drug carrier are limited by its low aqueous solubility (1.8 g/100 ml). Therefore chemical modification of βCD is used to produce products of very high water solubility (>50 g/100 ml) and minimal toxicity. Furthermore, the inclusion ability of βCD is amply magnified due to chemical modification and presently about 50 cyclodextrin derivatives are commercially available.

Since the usefulness of cyclodextrins vary according to molecular weight, cavity size, intrinsic solubility in water and other solvents, safety, and cost, the study must match the intended use of the drug with the appropriate cyclodextrin or cyclodextrin derivative. Many different chemicals may be introduced into the cyclodextrin molecule by reaction with the hydroxyl groups lining the upper and lower ridges of the toroid, for example, methyl, hydroxypropyl, carboxymethyl, and acetyl. The randomness of position and type of substitution causes the resultant modified cyclodextrins to be amorphous, which contributes to its greatly enhanced aqueous solubility compared to the crystalline natural form. One such modification, hydroxypropyl-β-cyclodextrin (HPβCD), has the best balance of enhanced aqueous solubility, and the range of drugs which can form stable complexes. It is very soluble in water, greater than 500 mg/ml at room temperature compared to 18 mg/ml for βCD. Hydroxypropyl-β-cyclodextrin is also moderately soluble in methanol and ethanol, which allows for greater drug incorporation into powdered formulations using co-solvent techniques.

Inclusion complexes are entities comprising two or more molecules, where the cyclodextrins are usually the host molecules, and include a guest molecule held only by

physical forces without covalent bonding. Cyclodextrins are capable of forming complexes with compounds with a size compatible with the cyclodextrin's inner cavity. Geometric factors, rather than chemical, are key to determining which guest molecules can be complexed. The complexed molecules are oriented inside the host to achieve the maximum contact between the hydrophobic parts of the guest molecule to the cavity.

The driving force of complexation includes various effects, one of which is the substitution of the energetically polar and apolar interaction between the included water and the cyclodextrin cavity, and between water and the guest molecules, by the more favorable interaction between the guest and the cavity. Another is the strain release on complexation, and also the Van der Waals and hydrogen bonding interactions.

Stability and solubility of the complexes are entirely independent properties. It is not the most insoluble inclusion complex which is the most stable one in solution. However, a direct correlation exists between the complex stability and the enhancement of the guest molecule's poor solubility. With increasing temperatures, the complexes disassociate, with association constant values to decrease rapidly.

We believe that gefitinib can form stable inclusion complexes with cyclodextrins to improve the solubility and dissolution for this drug for enhanced absorption and oral bioavailability.

The objectives of the present study are:

1) To characterize the inclusion complexation of gefitinib in the liquid state: Phase solubility studies of gefitinib with three different cyclodextrins (β-cyclodextrin,

hydroxypropyl-β-cyclodextrin, and randomly methylated-β-cyclodextrin) were conducted and stability rate constants of the complexes were calculated.

2) To characterize the inclusion complexation of gefitinib in the solid state: The inclusion complexes of gefitinib with various cyclodextrins were prepared by freeze drying and the formation of complexes in the solid state was confirmed by differential scanning calorimetry (DSC) and x-ray diffraction analysis.
3) To study the dissolution improvement of gefitinib by complexation with cyclodextrins: The dissolution studies were conducted according to USP guidelines.

LITERATURE REVIEW

Gefitinib

Gefitinib is a tyrosine kinase inhibitor used for the treatment of non-small cell lung cancer (NSCLC). It is taken in 250 mg oral doses once daily, higher doses showing no improvement but increased toxicity. It has been approved for continued monotherapy in patients with locally advanced/metastatic NSCLC who have failed both platinum and docetaxel-based chemotherapies, or in patients who are or have benefited from gefitinib. It has a molecular mass of 446.9 and is a brown-colored powder. Gefitinib has pKa's of 5.4 and 7.2 and therefore ionizes progressively in solution as the pH falls. Gefitinib is sparingly soluble at pH 1, but is practically insoluble above pH 7 and the solubility drops sharply between pH 4 and pH 6. In non-aqueous solvents, gefitinib is freely soluble in glacial acetic acid and dimethylsulphoxide, soluble in pyridine, and sparingly soluble in tetrahydrofuran, and slightly soluble in methanol, ethanol (99.5%), ethyl acetate, propan-2-ol, and acetonitrile.[1]

There is no absolute data on the bioavailability of gefitinib in humans; while in animal studies, a bioavailability of about 50% has been reported. Gefitinib is 90% protein bound in vitro to serum albumin and α-1-acid glycoprotein, and has a mean steady state volume of distribution of 1400 liters. Gefitinib is metabolized in the kidneys, but renal elimination accounts for only 4% of the administered dose. The predominant mode of excretion is via feces (approximately 86%). The elimination half-life ranges

from 6 to 49 hours, with no clear evidence of dose-dependency and values being similar after single doses or repeat administration.[1] Furthermore, upon oral administration, gefitinib induces gastro-intestinal adverse effects such as nausea, vomiting and diarrhea, which are dose dependent. Clearly, there is a need to improve the dissolution of this drug in order to improve the bioavailability.

Cyclodextrins

Cyclodextrins have well-known effects on drug solubility and dissolution, bioavailability, safety, and stability. There are various factors influencing inclusion complex formation and an understanding of these factors is necessary for proper handling of these versatile materials. Some important considerations in selecting CDs in drug formulation are their commercial availability, regulatory status, and patent status. CDs, are expected to solve many problems associated with the delivery of different novel drugs through different delivery routes.[2]

Cyclodextrins were called cellulosine when first described in 1891. Soon after, Schardinger identified the three naturally occurring cyclodextrins: alpha, beta, and gamma. These new compounds were referred to as Schardinger sugars. For 25 years between 1911 and 1935, Pringsheim in Germany was the leading researcher in this area, demonstrating that these sugars formed stable aqueous complexes with many other chemicals. By the mid 1970's, each of the natural cyclodextrins had been structurally and chemically characterized and many more complexes had been studied. The natural cyclodextrins are produced from starch by the action of cyclodextrin glycosyltransferase, an enzyme produced by several organisms, Bacillus macerans being the earliest source.[3]

Structurally, cyclodextrins consist of 6, 7, or 8 (α, β, and γ respectively) D-glucopyranosyl units connected by alpha-(1,4) glycosidic linkages. The most stable three dimensional molecular configuration for these non-reducing cyclic oligosaccharides takes the form of a toroid with the larger and smaller opening of the toroid presenting secondary and primary hydroxyl groups, respectively, to the solvent environment. The interior of the toroid is hydrophobic as a result of the electron rich environment provided in large part by the glycosidic oxygen atoms. It is the interplay of atomic (Van der Waals), thermodynamic (hydrogen bonding), and solvent (hydrophobic) forces that accounts for the stable complexes that may be formed with chemical substances while in the apolar environment of the cyclodextrin cavity. The complex exists in an equilibrium dependent upon the concentrations of the cyclodextrin, the guest chemical and water. The rate at which the associated complex is formed is determined in large part by the accessibility of the guest molecule to the cyclodextrin cavity and the magnitude of the net thermodynamic driving force.[3]

Cyclodextrins are able to form water-soluble inclusion complexes with many lipophilic water-insoluble drugs. In aqueous solutions drug molecules located in the central cavity are in a dynamic equilibrium with free drug molecules. Furthermore, lipophilic molecules in the aqueous complexation media will compete with each other for a space in the cavity. Due to their size and hydrophilicity only insignificant amounts of cyclodextrins and drug-cyclodextrin complexes are able to penetrate into lipophilic biological barriers, such as intact skin.[4]

Cyclodextrin Toxicity

Natural cyclodextrins are practically not absorbed under normal physiological conditions, and the metabolites produced are not unusual, and they are therefore considered safe for oral use. In the U.S. since 1997, β-cyclodextrins have been considered GRAS for certain food uses. However, β-cyclodextrin is not safe for parenteral use, since it forms insoluble complexes in the kidneys, causing nephrotoxicity.[5]

Chemically modified cyclodextrins were introduced partially in order to improve the parenteral safety profile of β-cyclodextrin. Randomly methylated β-cyclodextrin has been shown to have very good complexing ability, in addition to being very water soluble. However, its lipophilicity can create irritation and hemolysis. Hydroxypropyl β-cyclodextrin has been shown to be safe at very high oral doses, and observed to have no irreversible adverse effects in i.v. doses up to 400 mg/Kg.[5]

Solubility and Dissolution Improvement

A study compared the physicochemical characteristics of the solid complexes prepared by traditional methods (kneading, freeze-drying and spray-drying) and using a supercritical fluid technology to enhance the solubility of Naproxen, a poorly soluble anti-inflammatory drug, with β-cyclodextrin. In physical mixtures and kneaded systems, the drug's endothermic properties are present, suggesting incomplete encapsulation, while freeze dried and spray dried systems show complexation.[6]

The effect of β-cyclodextrin on the aqueous solubility and dissolution rate of celecoxib was investigated. Celecoxib, a specific inhibitor of cycloxygenase-2 (COX-2),

is a poorly water-soluble nonsteroidal anti-inflammatory drug with relatively low bioavailability. The possibility of molecular arrangement of inclusion complexes of celecoxib and β-cyclodextrin were studied using molecular modeling and structural designing. A phase-solubility profile indicated that the solubility of celecoxib was significantly increased in the presence of β-cyclodextrin. Solid complexes were prepared by freeze drying, evaporation, and kneading methods and characterized using differential scanning calorimetry, powder x-ray diffractometry, and scanning electron microscopy. Freeze-dried complexes showed a higher dissolution rate than the other complexes.[7]

Solid dispersions of Celecoxib have also been prepared with hydroxypropyl-β-cyclodextrin by various methods such as physical mixture, cogrinding, kneading, and coevaporation. The dispersions were characterized by differential scanning calorimetry, X-ray diffraction patterns, infrared spectroscopy, and nuclear magnetic resonance studies. The DSC thermograms of the dispersions indicated the potential of a heat-induced interaction between Celecoxib and cyclodextrin that could influence in vitro drug dissolution. The dispersions exhibited faster rates of dissolution compared to that of Celecoxib alone.[8]

The method of co-grinding with CDs was applied to a poorly water soluble drug, ONO-8713 (solubility; 0.92 l g/ml in H_2O at 25°C), as a method to prepare nanoparticles. ONO-8713 was co-ground with various CDs in a vibration mill. When ONO-8713 was co-ground with βCD, 85% of the drug was recovered as nanoparticles with a mean particle size of 120 nm. Nanoparticle yield achieved 90% when hydroxypropyl-β-cyclodextrin was used as a co-grinding additive. It was found that the amount of drug nanoparticles depended on the characteristics of the CDs. This phenomenon was

probably due to the difference in the cavity size of CDs along with the variation of substitution groups that affected the interaction between CDs and drug, and the affinity between CD and drug. Zeta potential analysis suggested that the CD would form a layer covering on the particle surface and alter the charge of the particles, improving the stability and total yield of the nanoparticle.[9]

The inclusion behavior of sulfobutyl ether-7 derivative of β-cyclodextrin (SBE7βCD) in solution and solid state was compared with that of natural β-cyclodextrin toward a poorly water-soluble anti-inflammatory agent, rofecoxib (ROFX). A phase solubility method was used to evaluate the stoichiometries and stability constants of ROFXβCD and ROFX-SBE7βCD complexes. Solubility enhancement was much greater for the rofecoxib-SBE7βCDcomplex compared to drug-βCD complex. The stability constant obtained for the SBE7βCD inclusion complex of rofecoxib was higher. Finally, dissolution profiles obtained suggest that SBE-βCD is more effective than β-cyclodextrin in improving the pharmaceutical properties of rofecoxib.[10]

In another study, the influence of natural β-cyclodextrin and its hydrophilic derivatives (HPβCD and SBE7βCD) on the in vitro dissolution rate, in vivo absorption, and oral bioavailability of a poorly water soluble anti-inflammatory agent, valdecoxib (VALD) was studied. Equimolar drug-cyclodextrin solid complexes were prepared by kneading and coevaporation methods and characterized by infrared spectroscopy, differential scanning calorimetry, and X-ray diffraction. In the liquid state, the cyclodextrin complexes were studied using phase solubility analysis. Drug solubility and dissolution rate in distilled water were notably improved by employing the CDs. It was

found that the cyclodextrin complexes of drug showed significant improvement in anti-inflammatory activity.[11]

Parent β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin can form 1:1 solid complexes with an orally active angiotensin-converting enzyme inhibitor, captopril, while hydrophobic perbutanoyl-β-cyclodextrin (TBβCD) forms a solid dispersion or solid solution with the drug. The binary system of captopril-HPβCD or captopril-TBβCD and the ternary system of captopril-TBβCD-HPβCD in different molar ratios were prepared by the kneading method, and the release behavior of the drug was investigated. The release rate of captopril from the binary HPβCD system was rather fast, whereas that from the binary TBβCD system was comparatively slower, the retarding effect being dependent on the amounts of TBβCD. It was difficult to prolong plasma levels of captopril by administering orally either the binary HPβCD or TBβCD system in dogs. On the other hand, the ternary captopril-TBβCD-HPβCD system gave a plasma profile comparable to that of a commercially available sustained release preparation.[12]

Complexes between members of a homologous series of alkylparabens and β-cyclodextrin have been prepared by both kneading and co-precipitation methods and their behavior studied by DSC, thermogravimetric, infrared and powder X-ray diffraction techniques. Results showed that complexation did occur by both the kneading and co-precipitation methods. DSC and IR techniques confirmed these results and thermogravimetric indicated the presence and number of water molecules in each complex.[13]

Salbutamol laurate is a novel salt form of the well-known bronchodilator, salbutamol (albuterol). Its polymorphism and inclusion in 2-hydroxypropyl-β-cyclodextrin were

investigated by thermogravimetry, differential scanning calorimetry, infrared and powder X-ray diffraction techniques. Two polymorphic forms of the salt were identified. Conditions for inclusion complex formation between the salt and (2-hydroxypropyl)-β-cyclodextrin, namely prolonged co-grinding and kneading, were established by a combination of the above methods.[14]

Chemical analysis, DSC and solubility determinations have been applied to the study of solid inclusion complexes of a pesticide, 2, 4-dichlorofenoxyacetic acid as guest, and β-cyclodextrin as host, in order to produce experimental evidence of the inclusion process and of the stoichiometry of the inclusion compound. Three processing methods were studied and compared: physical (mechanical) mixing; kneading; and spray-drying. The phase-solubility diagram of complex formation in solution has been also established. The stoichiometric ratio of the complexes was found to be 1:1 by solubility and DSC, being confirmed by chemical analysis. Spray-drying was found to be most suitable method for preparing the complexes.[15]

A study aimed at developing a tablet formulation was based on an effective flurbiprofen-cyclodextrin system, able to allow a rapid and complete dissolution of this practically insoluble drug. Three different cyclodextrins were evaluated: the parent β-cyclodextrin, and two amorphous, highly soluble β-cyclodextrin derivatives, i.e., methyl-β-cyclodextrin and hydroxyethyl-β-cyclodextrin. Equimolar drug-cyclodextrin binary systems prepared according to five different techniques (physical mixing, kneading, sealed-heating, coevaporation, and colyophilization) were characterized by differential scanning calorimetry, x-ray powder diffractometry, infrared, and optical microscopy. The drug solubility improvement obtained by the different binary systems varied from a

minimum of 2.5 times up to a maximum of 120 times, depending on both the cyclodextrin type and the system preparation method. All formulations containing drug-cyclodextrin systems gave a higher drug dissolved amount than the corresponding ones with drug alone. It can be reasonably expected that the obtained drug dissolution rate improvement will result in an increase in its bioavailability, with the possibility of reducing drug dosage and side effects.[16]

Microencapsulation of lemon oil was undertaken by kneading with α-cyclodextrin, at an α-cyclodextrin to lemon oil ratio of 88:12 (w/w). The resulting paste samples of the complex were vacuum- or spray-dried. Ten selected lemon oil flavor volatiles in the complex were analyzed periodically after kneading. The results indicated that the levels of these volatiles were not significantly different irrespective of mixing time or type of drying (vacuum- or spray-drying) used. An optimum mixing time was found to be 15 min, at which time the maximum encapsulation of lemon oil was obtained in the complex powder.[17]

For another study, the possibility of improving dehydroepiandrosterone (DHEA) solubility and bioavailability by high-energy cogrinding with α-cyclodextrin (αCD) in the presence or absence of different auxiliary substances was investigated. In all cases, ternary products exhibited higher drug solubilizing properties than the binary DHEA-α-cyclodextrin coground system. Glycine was the most effective component. The best combinations were characterized by differential scanning calorimetry and X-ray powder diffractometry and evaluated for dissolution rate. The presence of glycine favored destruction of DHEA crystalline structure during cogrinding, as evidenced by the strong reduction in both time and vibration frequency of milling necessary to obtain total drug

amorphization. Both ternary products showed better dissolution properties than the drug alone, affording, respectively, a 40 and 60% increase of dissolution efficiency.[18]

The aim of another study was to enhance the low solubility of eflucimibe, a new chemical entity to treat dyslipidemiae, by complexation with γ-cyclodextrin. The complex was prepared using the kneading method. The interaction evolution was studied during process by comparison of the semi-solid and physico-chemical states of the product. The evolution of the semi-solid state was followed by torque measurement while the evolution of physico-chemical state was studied by differential scanning calorimetry, infrared spectroscopy and by determination of the drug solubilization profile. The interaction, which occurs during the process, is characterized by a modification of the product consistency and by a disappearance of the drug endothermic peaks, a disappearance of a drug spectral band and an improvement in the drug solubilization profile. Indeed, after complete interaction, the drug quantity solubilized in specific conditions increased about 44-fold compared to those of untreated drug. Moreover, the comparison of the physico-chemical and semi-solid states during the kneading process clearly shows that when the interaction takes place, a solidification of the paste occurs. The results suggested that the formation of new solid phase allows an enhancement of the solubility of eflucimibe.[19]

The purpose of another study was to investigate the effect of moisture condition during the cogrinding process on fine drug particle formation. Cogrinding of CDs and pranlukast (PRK) hemihydrate was performed in various moisture conditions and the formation of PRK submicron particles was investigated. The moisture content in the cogrinding process significantly affected the fine particle formation. More than 90% of

PRK loaded transformed to submicron particles when coground with αCD, βCD or γCD containing the specific amount of water for each CD system. Fine particle formation of PRK was considered as a particular phenomenon to cyclodextrins, since the submicron particles could not be formed when d-mannitol, lactose or microcrystalline cellulose (MCC) was used as a cogrinding additive. Moreover, the appearance and disappearance of fine particle formation was found to be reversible depending on the existence of water during the grinding process.[20]

Glimepiride is one of the third generation sulfonylureas used for treatment of type 2 diabetes. Poor aqueous solubility and slow dissolution rate of the drug lead to irreproducible clinical response or therapeutic failure in some cases due to subtherapeutic plasma drug levels. Consequently, the rationale of the study was to improve the biological performance of this drug through enhancing its solubility and dissolution rate. Inclusion complexes of glimepiride in βCD, HPβCD and SBEβCD, with or without water soluble polymers were prepared by the kneading method. Binary systems were characterized by thermogravimetric analysis, IR spectroscopy and X-ray diffractometry. Phase solubility diagrams revealed an increase in solubility of the drug upon cyclodextrin addition. All the ternary systems containing βCD or HPβCD showed higher dissolution efficiency compared to the corresponding binary systems. In conclusion, the association of water soluble polymers with glimepiride-CD systems leads to great enhancement in dissolution rate, increased duration of action and improvement of therapeutic efficacy of the drug.[21]

Inclusion complexes of clofibrate with β-cyclodextrin were prepared by coprecipitation, kneading and sealed heating methods and characterized by UV

spectrophotometry, differential scanning calorimetry, X-ray diffractometry and infrared spectroscopy. All results were in keeping with the formation of a 1:1 inclusion complex. Dissolution studies showed that clofibrate entrapped in inclusion complexes dissolved much faster than uncomplexed liquid clofibrate. The complex prepared by the sealed heating method showed the greatest improvement in dissolution rate. These results show that a more easily manipulated, solid form of clofibrate may be obtained through formation of its inclusion complex with βCD, and that complex formation simultaneously enhances the solubility and dissolution rate of this drug.[22]

Inclusion complexes of tolbutamide with β-cyclodextrin and hydroxypropyl-β-cyclodextrin were prepared using different methods: kneading, coprecipitation and freeze-drying. Inclusion complexation in aqueous solution and in solid phase was studied by the solubility method, X-ray diffractometry, thermal analysis and Raman spectroscopy. The solubility of tolbutamide increased as a function of cyclodextrin concentration, showing B_S and A_L type diagrams for β-cyclodextrin and hydroxypropyl-β-cyclodextrin, respectively. The dissolution rate of tolbutamide from the inclusion complexes was much more rapid than tolbutamide alone.[23]

The methods of obtaining and physicochemical properties of inclusion complexes of amlodipine (AM) and felodipine (FL) with methyl-β-cyclodextrin (MβCD) clathrates were studied. Solid complexes were obtained by two methods: kneading and lyophilization with the drug and MβCD at the molar ratio of 1:1. The identity of the obtained clathrates was confirmed by IR, 13C-NMR spectra and DSC measurements. The process of AM and FL complexation with MβCD involve the aromatic ring, the carbonyl groups in the ester bonds and the carbon atoms of the ring linked via the ester

bonds. One of the aims of complexation was to improve the drug solubility, so the dissolution rate of the obtained clathrates was tested. As a result of the inclusion complexes formation obtained both by kneading and lyophilization, the solubility of AM increased 3 times. The inclusion complex formation with FL and MβCD brought the most dramatic increase in FL solubility, which increased 16 times.[24]

The effects of hydroxypropyl-β-cyclodextrin and methyl-β-cyclodextrin on the solubility of ketoconazole were examined. Products were prepared by physical mixing, kneading and spray-drying in four molecular ratios. Kneaded products in a ratio of drug: cyclodextrin (1:2) and spray-dried products showed the highest dissolution rate. Phase solubility diagrams of ketoconazole with these cyclodextrins at 25 $^{\circ}$C in water and simulated intestinal medium were constructed. A solubility diagram of the A_L type was obtained with hydroxypropyl-β-cyclodextrin, and of the A_P type with methyl-β-cyclodextrin. The complexes were characterized by thermal methods. Multicomponent systems were prepared with tartaric acid. The effects of water-soluble polymers, e.g., polyvinyl pyrrolidone, on the aqueous solubility of ketoconazole were investigated. The particle size of ketoconazole (70 μm) is reduced to 12 μm by the preparation of spray-dried products. As the solubility in water increased, the partition coefficient, surface tension and wetting angle values decreased.[25]

The possible competitive displacement of a drug from its cyclodextrin-based inclusion complex by a third substance was investigated by studying the dissolution behavior of tolbutamide-β-cyclodextrin inclusion complex in demineralized water and in aqueous solution of different surfactants. Physical mixtures and kneaded systems were prepared in 1:1 and 1:2 drug-β-cyclodextrin mol/mol ratios and they were characterized

by hot-stage microscopy, differential scanning calorimetry, and X-ray powder diffractometry. The release behavior of tolbutamide from its inclusion complex was studied by dissolution of the binary systems in water and in aqueous solutions of three surfactants: polysorbate 20, poloxyl 23-lauryl ether, and sodium lauryl sulfate. When demineralized water was used as the dissolution medium, the fastest dissolution of tolbutamide was obtained from the 1:2 kneaded system followed by the 1:1 kneaded system. The presence of poloxyl 23-lauryl ether and sodium lauryl sulfate in the media caused a decrement in the rate and extent of dissolution of the drug from both kneaded systems in comparison with that obtained from the same systems in water. However, the release of tolbutamide from the kneaded systems remains unaffected when polysorbate 20 was present in the dissolution medium. Results of this study suggest that the simultaneous presence of β-cyclodextrin and surfactants of proper molecular structure in a pharmaceutical formulation can yield an unexpected dissolution of the drug.[26]

Griseofulvin-cyclodextrin interactions were investigated in an aqueous environment and in solid state. Two cyclodextrin derivatives (α-cyclodextrin and 2-hydroxypropyl α-cyclodextrin) were used to prepare different physical mixtures and kneaded systems, and the drug/cyclodextrin ratios were 1:1 and 1:2 mol/mol. Scanning electron microscopy (SEM), hot-stage microscopy (HSM), differential scanning calorimetry (DSC), thermogravimetry (TG), and X-ray powder diffractometry were employed to characterize pure substances and their kneaded counterparts and all of the binary systems. The solubility of griseofulvin was increased in accord with the quantity of cyclodextrin added. HSM examination revealed that 2-hydroxypropyl-α-cyclodextrin was dissolved in the droplets of melted griseofulvin, but did not show any interactions between melted

griseofulvin and α-cyclodextrin particles. The presence of the griseofulvin endothermic peak in the DSC curves suggested the absence of any griseofulvin/cyclodextrin inclusion compound in the solid state. In TG, data of weight loss owing to the dehydration of cyclodextrins was similar for both kneaded systems and physical mixtures. X-ray diffraction patterns exhibited the amorphous nature of 2-hydroxypropyl-α-cyclodextrin and the crystalline nature of griseofulvin and binary systems. Griseofulvin dissolution profiles from all binary systems showed an improvement in drug dissolution, which indicates that a drug/cyclodextrin inclusion compound was formed in the aqueous dissolution medium.[27]

Equimolar combinations of econazole, a very poorly water soluble antifungal agent, with β-cyclodextrin and statistically substituted methyl-β-cyclodextrin were investigated for both solid state characterization (differential scanning calorimetry, hot-stage microscopy, infrared spectroscopy, scanning electron microscopy) and dissolution properties (dispersed amount method). The influence of the preparation method (physical mixing, ball-milling, kneading, and sealed-heating) on the physicochemical properties of the products was evaluated. Kneading and sealed heating techniques led to amorphous products in the case of systems with methyl-β-cyclodextrin, whereas crystalline drug was still clearly detectable in all products with β-cyclodextrin. Independently of the preparation technique, all combinations with methyl-β-cyclodextrin yielded better performance than the corresponding ones with β-cyclodextrin. However, the influence of the preparation method was clearly more marked for products with methyl-β-cyclodextrin. In fact, the sealed-heated with the β-derivative showed an increase of drug dissolution efficiency of 130% with respect to the corresponding physical mixture, in

comparison to the 70% increase obtained from that with β-cyclodextrin. Moreover, whereas the difference in dissolution efficiency values between coground products was only about 8% in favor of the β-derivative, it reached 80 and 90% between sealed-heated and kneaded products, respectively.[28]

Binary systems of ketoprofen with native crystalline β-cyclodextrin and amorphous statistically substituted methyl-β-cyclodextrin were investigated for both solid phase characterization (differential scanning calorimetry, powder X-ray diffraction, infrared spectroscopy, scanning electron microscopy) and dissolution properties (dispersed amount and rotating disc methods). Grinding, kneading, sealed-heating and colyophilization of equimolar combinations of ketoprofen with methyl-β-cyclodextrin, as well as colyophilization of analogous combinations with β-cyclodextrin, led to amorphous products. Crystalline drug, instead, was still clearly detectable in coground, kneaded and sealed-heated products with β-cyclodextrin. Both the preparation method, and even more the nature of the carrier, played an important role in the performance of the system. Colyophilized and sealed-heated products showed the best dissolution properties. However, independently of the preparation technique, all combinations with methyl-β-cyclodextrin yield better performances than the corresponding ones with the β-cyclodextrin. Moreover, the intrinsic dissolution rate of ketoprofen from a simple physical mixture with the β-cyclodextrin derivative was five-fold higher than that from the best product with the parent β-cyclodextrin.[29]

The complexing, solubilizing and amorphizing abilities toward ibuproxam (a poorly water-soluble anti-inflammatory agent) of some randomly substituted amorphous β-cyclodextrin derivatives were investigated and compared with those of the parent β-

cyclodextrin. Equimolar drug-cyclodextrin solid systems were prepared by blending, cogrinding, coevaporation, and colyophilization. All the derivatives showed greater solubilizing efficacies toward ibuproxam than the parent one, due to their higher water solubility. Colyophilized products were in all cases the most effective, followed by coground and coevaporated systems, whose dissolution efficiencies were over four times higher than the corresponding physical mixtures and about 15 times higher than the pure drug.[30]

The effect of ternary complexation of naproxen, a poorly water soluble anti-inflammatory drug, with hydroxypropyl-β-cyclodextrin and the basic amino acid L-arginine on drug dissolution properties has been investigated. Equimolar binary (drug-cyclodextrin or drug-arginine) and ternary (drug-cyclodextrin-arginine) systems were prepared by blending, cogrinding, coevaporation, and characterized by differential scanning calorimetry, thermogravimetric analysis, FT-IR spectroscopy, X-ray diffractometry. The dissolution behavior of naproxen from the different products was evaluated by means of a continuous flow through method. The results of solid state studies indicated the presence of strong interactions between the components in ternary coevaporated and coground systems, which were both of totally amorphous nature. In contrast, the presence of either free drug or free arginine was detected when the third component (cyclodextrin or amino acid) was physically mixed, respectively, to the drug-arginine binary system (as physical mixture, coevaporate, or co-ground product) or to the drug-cyclodextrin binary system (as physical mixture, coevaporate, or coground product). All ternary combinations were significantly more effective than the corresponding binary drug-cyclodextrin and drug-arginine systems in improving the naproxen dissolution rate.

The best performance in this respect was given by the ternary coevaporate, with about a 15 times increase in terms of both drug relative dissolution rate and dissolution efficiency. The synergistic effect of the simultaneous use of arginine and cyclodextrin on the dissolution rate of naproxen was attributed to the combined effects of inclusion in cyclodextrin and salt formation, as well as to a specific role played by arginine in this interaction.[31]

The inclusion complex between sulfaproxyline (SP) and βCD was prepared by the freeze-drying and the kneading method. Complex formation was confirmed in the solid state by X-ray diffractometry and by infrared spectroscopy. The interaction between SP and β-cyclodextrin in solution was studied by the solubility method and 13C-NMR spectroscopy. Phase solubility studies in water revealed an A_N type diagram. Complexation was found to improve the dissolution rate of SP.[32]

The interactions of nabumetone (NAB) with α-cyclodextrin and γ-cyclodextrin were studied in aqueous solution by means of phase-solubility analysis. Solid dispersions of NAB with αCD, βCD, MβCD, and HPβCD were prepared by coevaporation and kneading and also by coprecipitation in the case of γCD. X-ray diffractometry, thermal analysis and infrared spectroscopy (FTIR) were used to study the possible complexation of the drug with the different cyclodextrins. Solid dispersions of NAB with γCD showed a remarkable improvement in the dissolution rate of NAB.[33]

The application of molecular modeling to the inclusion complexation of natural and modified CDs with carboxylic acid derivatives as guest molecules was examined. Information was available on the thermal behavior in the solid-state of benzoic acid (BA), salicylic acid (SA), and various substituted aminosalicylic acids (3-aminosalicylic acid

(3-ASA), 4-aminosalicylic acid (4-ASA), and 5-aminosalicylic acid (5-ASA), as well as on the thermal behavior of 1:1 molar ratio physical and kneaded mixtures of these acids with each of three different cyclodextrins, βCD, HPβCD, and γCD. The thermal behavior of the binary mixtures was modeled using stepwise multiple regression (SMR). Two models for the prediction of the percentage mass loss and enthalpy of dehydration of the physical mixtures were established with correlation coefficients of 0.79 and 0.92, respectively. Decreased correlation in the thermal behavior of kneaded mixtures indicated significant interaction and possible formation of inclusion complexes.[34]

An inclusion complex between warfarin and β-cyclodextrin was obtained to improve the in vitro bioavailability of the drug in acidic media. Inclusion complexation in solution was studied by the phase solubility technique. The apparent stability constant was influenced by the pH of the medium ranging from 633.26 M^{-1} (at pH 1.2, where the drug was in the unionized form) to 99.81M^{-1} (at pH 7.4, where the drug was in the ionized form). Phase solubility study showed an AL-type diagram indicating the formation of an inclusion complex in 1:1 molar ratio. Solid binary mixtures of the drug with β-cyclodextrin were prepared by several methods (physical mixing, kneading, co-evaporation, freeze-drying). Physicochemical characterizations were performed using differential scanning calorimetry, powder X-ray diffractometry and dissolution studies. Preparation method influenced the physicochemical properties of the binary mixtures. An inclusion complex was obtained by freeze-drying, with a high solubility and drug dissolution rate. The physical stability of the complex was also studied. After one year storage in a glass container at room temperature no significant changes were detected in the diffractogram, thermogram and dissolution profile of the freeze-dried product.[35]

The solubility of miconazole in water increased in the presence of CDs. The apparent K values calculated from the phase solubility diagrams of βCD, hydroxypropyl-α-cyclodextrin, hydroxyethyl-α-cyclodextrin, HPβCD, and αCD were 695 ± 39.6, 363 ± 34.1, 333 ± 18.5, 312 ± 31.0, 305 ± 27.6, and 293 ± 17.6 M-1, respectively. Solid 1:1 molar complexes were prepared by freeze-drying and kneading and characterized by UV spectroscopy, differential scanning calorimetry, and electron microscopy. The dissolution rate increased 28-255 fold and the solubility 9-55 fold. Oral bioavailability in rats increased 2.3 fold by complexation with hydroxypropyl-α-cyclodextrin. Human cadaver skin retained 2.6 fold more drug from the miconazole-cyclodextrrin complex and hairless mice skin retained 8.4 fold more drug from the HPαCD complex than from miconazole solution alone in 24 h.[34]

Solid complexes between gliclazide and βCD were prepared by kneading, coprecipitation, neutralization, co-grinding and spray-drying. Characterization of gliclazide-cyclodextrin inclusion complexes was performed using X-ray diffractometry and cross polarizing/magic angle spinning ~3C-nuclear magnetic resonance spectroscopy. These techniques clearly demonstrated the existence of solid-state inclusion compound formation. The complexes obtained by neutralization and spray-drying showed enhanced dissolution rates of gliclazide.[37]

Natural crystalline (α-, β-, γ-) and amorphous derivative (hydroxypropyl-β- and methyl-β-) cyclodextrins were selected as potential carriers for obtaining, through a co-grinding technique, a stable activated amorphous form of glyburide with improved dissolution properties. Differential scanning calorimetry was used to investigate solid-state modifications of the drug induced by cogrinding with the selected carriers in a high

energy vibrational micro-mill. X-ray powder diffraction and FTIR spectroscopy were employed as additional techniques to support DSC data. Equimolar drug: cyclodextrin physical mixtures were co-ground for different times (up to 60 min) at constant vibration frequency (24 Hz). A progressive drug amorphization with increasing grinding time was observed in all binary systems, but, interestingly, different degrees of sensitivity to the mechanical-chemical activation were evident. In fact, blends with natural cyclodextrins, despite the initial higher crystallinity than those with the amorphous derivatives, required the same or shorter cogrinding times (60 min) to achieve complete drug amorphization. Stability studies indicated no appreciable drug recrystallization in co-ground products after 4 months storage in sealed containers at 25°C or 1 month at 25°C and 75% RH. No stability differences were detected between products with natural or derivative cyclodextrins. The results accounted for the suitability of cyclodextrin co-grinding technique to obtain and stabilize glyburide in the activated amorphous form.[38]

An optimized kneading method for the preparation of lycopene-cyclodextrin binary systems was developed leading to solubilization of lycopene in water and 5% (w/v) dextrose solution. Storage stability characteristics of the binary systems were studied in the lyophilized products. At 4°C, storage stability of lycopene-cyclodextrin binary systems in water or 5% (w/v) aqueous dextrose solutions was limited Addition of the antioxidant sodium metabisulfite increased the stability of lycopene-HPβCD binary system in water at -20°C, the lyophilized lycopene-cyclodextrin binary systems were stable for at least 2 weeks.[39]

The possibility of obtaining inclusion complexes between omeprazole (OME) and γCD by kneading, spray-drying, coprecipitation, and freeze-drying was evaluated. All

methods led to the isolation of a true inclusion compound, as evidenced by DSC, infrared, and X-ray diffractometry on powder (PXRD). Moreover, PXRD and scanning electron microscopy (SEM) afforded data concerning crystallinity and surface characteristics of the solid phases obtained. In all cases, a significant increase in the release rate with respect to the drug alone was found, and it was attributed to the formation of an inclusion compound. Among the solid phases obtained, the coprecipitated product presented the highest dissolution rate.[40]

The inclusion complexes of β-cyclodextrin-isoproturon were prepared using techniques such as kneading, coevaporation, and co-precipitation. These complexes were characterized by UV, FTIR, and NMR spectroscopy. To investigate the stoichiometry of the complexes, the phase solubility method was adopted. Most of the complexes showed an increase in the dissolution rate of the herbicide. The best results were obtained when inclusion complexes were prepared by the co-precipitation and co-evaporation methods.[41]

The complexation of gliclazide (GL) with a partially methylated β-cyclodextrin was studied. Phase-solubility and 1H NMR spectroscopy were employed to investigate the complexation behavior in solution and to demonstrate the complexation in liquid medium with the participation of both azabicyclooctyl and tolyl moieties of GL in the inclusion process. Solid systems prepared by kneading, co-grinding and spray drying were checked using DSC and HSM to assess the formation of the inclusion compound. Evidence of complexation was found for the co-ground and spray-dried systems.[42]

γ-Cyclodextrin and dimethyl-β-cyclodextrin were used as solubilizing agents for the very poorly water-soluble drug clotrimazole, an imidazole derivative antifungal agent. Solid products were prepared by physical mixing, kneading, precipitation and spray-

drying in 1:1 and 1:2 drug:cyclodextrin molar ratios. Drug interactions were studied by thermoanalytical methods such as DSC, X-ray diffractometry, and FTIR. The results demonstrated the formation of inclusion complexes in some products.[43]

A physical mixture and a kneaded product containing sulfadimidine and β-cyclodextrin were prepared. The morphologies of these products and of the sulfadimidine bulk substance were studied by scanning electron microscopy. The thermoanalytical behavior of the samples was studied. The existence of an inclusion complex in the products could not be proved. However, an increase in dissolution rate was observed. The reason in the case of the physical is the regular distribution of the active agent crystals and the β-cyclodextrin crystals, and in the case of the kneaded product in the formation of new recrystallized particles.[44]

MATERIALS AND METHODS

Materials

Gefitinib was from Sequoia Research Products Ltd, United Kingdom. The glacial acetic acid came from Fisher Scientific, PA. The β-cyclodextrin was from ISP Technologies, Inc., NJ, while the hydroxypropyl-β-cyclodextrin and the randomly methylated-β cyclodextrin were from Cyclodextrin Technologies Development, Inc., FL.

Gefitinib Standard Curve

A standard curve was prepared to calculate the concentrations of gefitinib in the solubility as well as the dissolution study samples. Gefitinib is freely soluble in glacial acetic acid, so a series of samples with known concentrations were made. First, a 5 mg/mL stock solution of gefitinib in glacial acetic acid was prepared by adding 50 mg of gefitinib to 10 mL of acetic acid. This stock solution was diluted into a series of 10 mL solutions in increasing concentration, at 2, 5, 10, 25, 40, 50, 60, 75, 90, and 100 μg/mL. Samples from each solution were UV spectrophotometrically analyzed (DU460; Beckman, CA) at 340 nm. A plot was then made showing concentration of gefitinib vs. UV absorbance.

The Effect of Cyclodextrins on Gefitinib's Solubility: Phase Solubility Studies

Phase solubility studies were conducted according to the method of Higuchi and Connors.[45] Solubility studies were performed by preparing samples of 10 mL distilled water with gefitinib and cyclodextrin. Approximately 5 mg of gefitinib were added to each 10 ml of distilled water. Increasing amounts of each cyclodextrin were then placed in each sample so any improvement in gefitinib's solubility from the addition of cyclodextrin can be determined. To form complexes in solutions, the suspensions were sonicated to ensure molecular dispersion. The suspensions were sonicated for 30 minutes, repeated 5 times, with 1 hour in between for the suspension to settle. The samples were then filtered through a 0.45 μm membrane filter (Millipore, MA). The filtered samples were analyzed by UV spectrophotometry. Solubility diagrams were constructed by plotting the molar concentration of gefitinib dissolved (solubility) versus the molar concentration of complexing agents. From these diagrams, the stability constants for the complexation of gefitinib with cyclodextrins were calculated.

Formulation of Solid Gefitinib-Cyclodextrin Complexes

The physical mixtures and kneaded formulations were in 1:1 gefitinib:cyclodextrin ratio, while the freeze dried formulations had 1:1 as well as 1:2 ratio for each cyclodextrin.

The physical mixtures were prepared first by calculating the amounts needed to produce 250 mg of each formulation in 1:1 molar ratio. Gefitinib , 70.6 mg, 57.0 mg, and 63.5 mg was added to sample vials in addition to βCD, HPβCD, and RMβCD,

respectively, to reach 250 mg of the formulations. The samples were then shaken rigorously in order to achieve even mixing.

The kneaded formulations were also prepared in 1:1 molar ratio. The same amounts were used as in the physical mixtures to make 250 mg of each formulation. The cyclodextrin and gefitinib were put in a mortar and wetted with a few drops of distilled water and then kneaded. The kneading continued for 30 minutes, and the product was then at 37ºC for 24 hours (Thermolyne 5000 Compact CO_2 Series; Barnstead, MA). The formulations were then removed from the mortar and kept in sample vials.

The freeze-dried formulations were prepared in both 1:1 as well as 1:2 gefitinib-cyclodextrin ratios. The 250 mg of 1:1 ratio formulations were made with the same amounts of gefitinib and each cyclodextrin as with the kneaded and physical mixtures. The 1:2 formulations were prepared by measuring 49.3 mg, 38.6 mg, and 43.7 mg of gefitinib and βCD, HPβCD, and RMβCD, respectively, to reach 300 mg for the formulations. In order to ensure that both the gefitinib and cyclodextrin for freeze drying, 30 mL of approximately 0.5 M acetic acid solution was added to each formulation. This solution was prepared by adding 3 mL of acetic acid to 97 mL of distilled water. Each formulation was then frozen at -70ºC. After freezing, the formulations were freeze dried for approximately 48 hours (FreeZone 4.5; Labconco, KS). The freeze dried samples were then stored in sample vials.

Dissolution Studies

Dissolution experiments were performed at 37°C on a SRII 6-Flask Dissolution Test Station (Hanson Research, CA) according to the dispersed amount method.

Approximately 0.025 moles of gefitinib were added to 1L of water in a 1L beaker and paddle stirred at 50rpm. At fixed time intervals (5, 10, 15, 20, 30, 60, 90, 120, 180, and 240 minutes), 10 mL samples were drawn with a syringe and spectrophotometrically assayed for gefitinib content as in the solubility studies. A correction was applied for the cumulative dilution caused by replacement of the sample with an equal volume of fresh medium. This procedure was performed again for the physical mixtures, the kneaded formulations, and the freeze-dried formulations of gefitinib and cyclodextrin. After preliminary results, additional formulations were made including 10% and 25% w/w of polyvinyl pyrrolidone (PVP) and hydroxypropyl methyl cellulose (HPMC) to the HPβCD inclusion complex (Table 3-1). Each test was repeated three times, using samples containing an equivalent amount of gefitinib in each formulation.

Cyclodextrin	Formulation
Effect of βCD	Pure drug Physical mixture Kneaded mixture Inclusion complex (1:1) Inclusion complex (1:2)
Effect of HPβCD	Physical mixture Kneaded mixture Inclusion complex (1:1) Inclusion complex (1:2)
Effect of RMβCD	Physical mixture Kneaded mixture Inclusion complex (1:1) Inclusion complex (1:2)
Effect of polymers on dissolution of HPβCD formulations	1. Inclusion complex (1:1) A) PVP 10% w/w B) PVP 25% w/w 2. Inclusion complex (1:1) C) HPMC 10% w/w D) HPMC 25% w/w

Table 3-1: Various CD formulation compositions in the dissolution studies of gefitinib.

Differential Scanning Calorimetry

The differential scanning calorimetry DSC of samples to determine the formation of an inclusion complex in the solid state was conducted on a modulated differential scanning calorimeter (Q200 DSC; TA Instruments, DE) at Auburn University Department of Bioengineering. A 5 mg mass of gefitinib, β-cyclodextrin, hydroxypropyl-β-cyclodextrin, randomly methylated-β-cyclodextrin and each of the formulations was measured into aluminum pans. Changes in the melting temperatures, determined as the onset temperatures, were used as an indication of complex formation.

X-Ray Diffraction

The powder X-ray diffraction patterns of gefitinib, the cyclodextrins, and the different formulations were recorded by using an automated Philips X'Pert X-ray diffractometer (Almelo, The Netherlands). Samples were irradiated with monochromatised Cu-Kα radiation and analyzed between 2 angles of 5 and 408. The voltage, current, and time per step used were 40 kV, 55 mA, and 1 s, respectively.

RESULTS AND DISCUSSION

Standard Curve

The graph in Figure 4-1 shows the standard curve constructed to equate absorbance to the concentration of gefitinib in the solution from the samples in the solubility and dissolution tests.

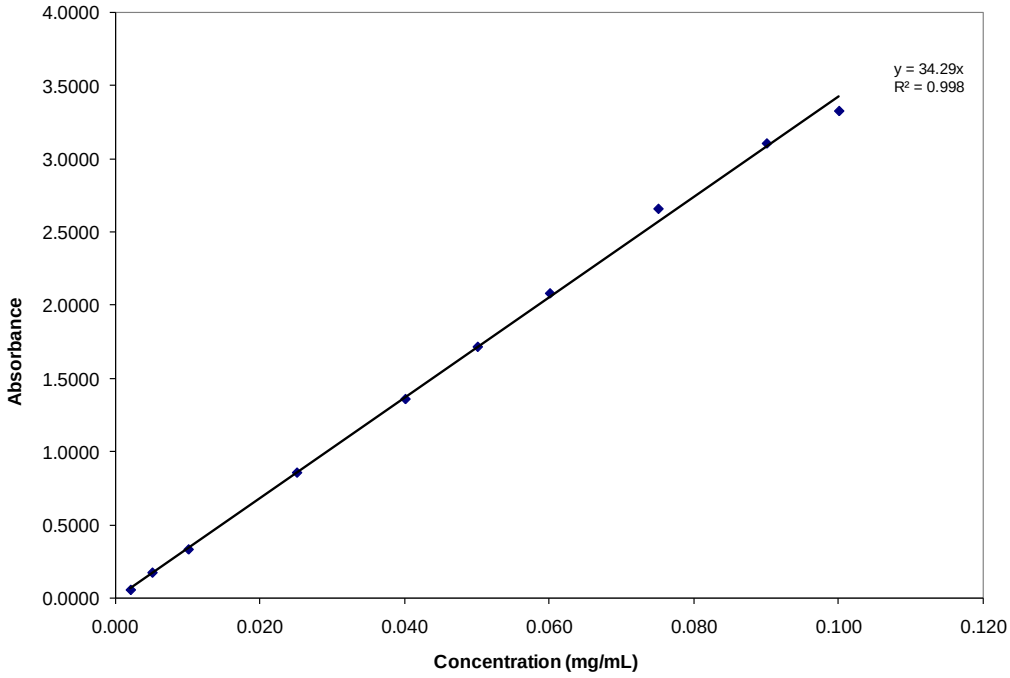

Figure 4-1: The standard curve for gefitinib absorbance.

Phase Solubility Studies

The phase solubility diagram of gefitinib as a function of concentration of various CDs at room temperature is shown in Figures 4-2, 4-3, and 4-4. The solubility of

gefitinib with an increase in concentration of CDs indicates an A_L type of phase solubility diagram. An apparent 1:1 stability constant (K_s) of the complex was calculated from the slope (R) and intercept (S_o) of the phase solubility diagram according to the equation[45]:

$$K_S = \frac{R}{S_0(1-R)}$$

The K_s of gefitinib-βCD, gefitinib-HPβCD and gefitinib-RMβCD complexes were calculated to be 735.9, 446.6 and 1021.9 M^{-1}, respectively. The K_s values of all three gefitinib-cyclodextrin complexes make them suitable for practical applications in terms of improving the solubility related oral bioavailability. If the complex is too weak, there is little improvement in the solubility of the drug. On the other hand, if the complex is too strong, as indicated by an association rate constant greater than 10,000 M^{-1}, the complex can not dissociate easily and oral absorption of the complex is not possible. Only the drug that is dissociated from the cyclodextrin complex is absorbed. Thus the stability constant values (association rate constants) of the present study indicate that they are suitable complexes for improving oral bioavailability.[46]

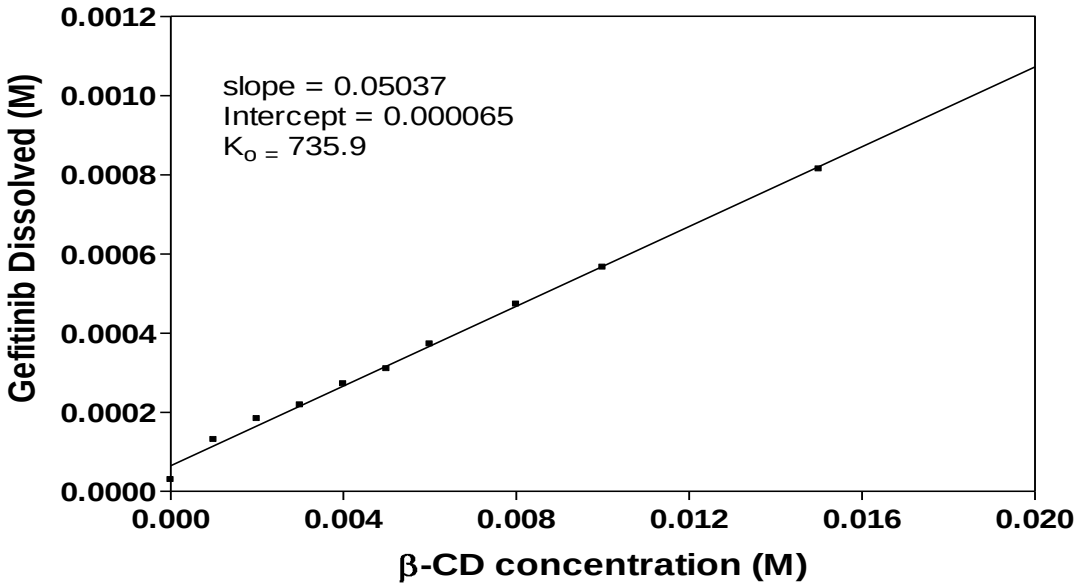

Figure 4-2: Phase solubility diagram of gefitinib with βCD at room temperature.

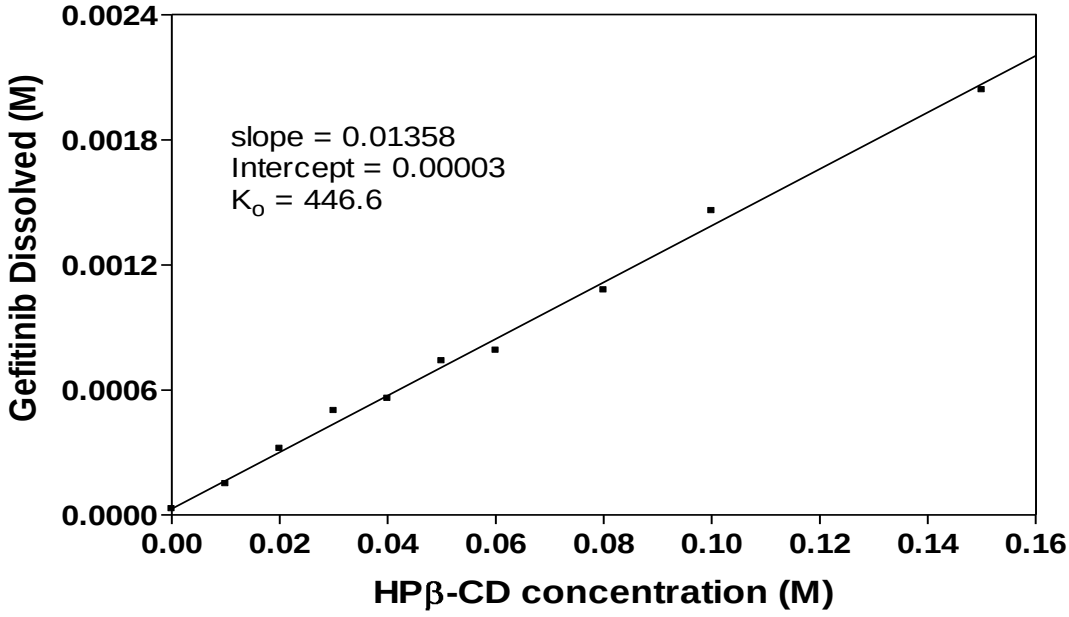

Figure 4-3: Phase solubility diagram of gefitinib with HPβCD at room temperature.

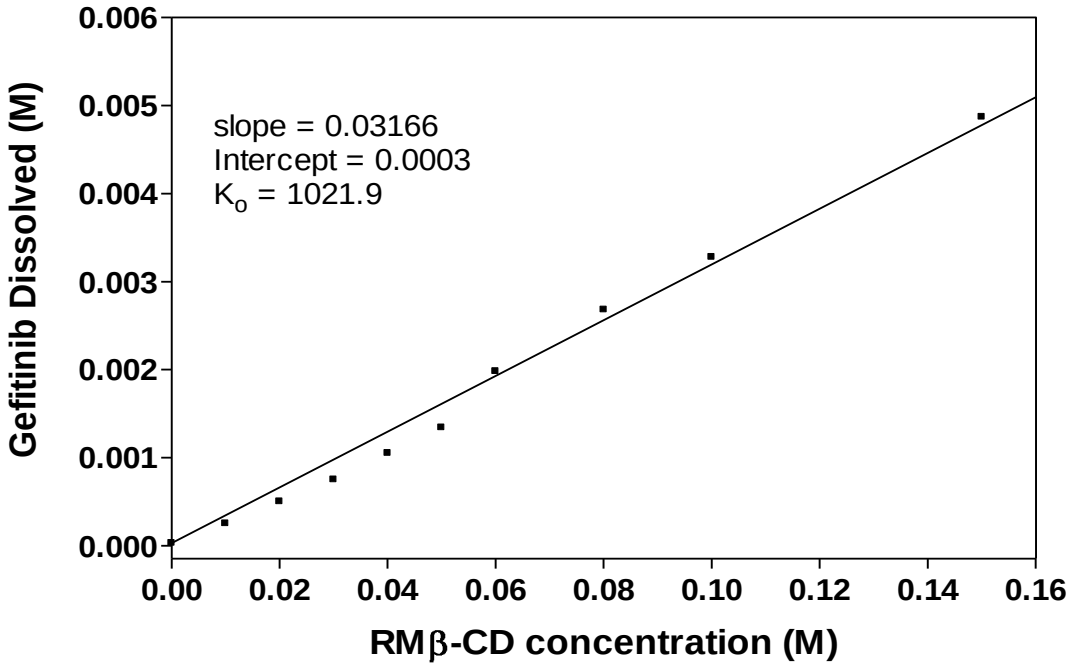

Figure 4-4: Phase solubility diagram of gefitinib with RMβCD at room temperature.

Gefitinib Solubility Curves	**Calculated equation**
Gefitinib Standard Curve	Absorbance = 34.298 * Concentration
Gefitinib with βCD	Moles (gefitinib) = 0.05037 * Moles (βCD)
Gefitinib with HPβCD	Moles (gefitinib) = 0.01358 * Moles (HPβCD)
Gefitinib with RMβCD	Moles (gefitinib) = 0.03166 * Moles (RMβCD)

Table 4-1: Gefitinib solubility curves with their calculated equations.

Inclusion Complexation in the Solid State

The inclusion complexes of gefitinib with CDs were prepared and characterized in the solid state. The existence of gefitinib-CD complex in the solid state was confirmed by x-ray diffractometry and differential scanning calorimetry. The cyclodextrin freeze dried inclusion complexes and their physical mixtures and kneaded mixtures were analyzed to determine if the inclusion complex has been formed by the method used to prepare the cyclodextrin-gefitinib complex. The x-ray diffraction patterns of powder samples made with βCD, HPβCD and RMβCD are shown in Figures 4-5, 4-6, and 4-7, respectively. The strong diffraction peaks of gefitinib indicate the crystalline nature of the drug, whereas the βCD is much less crystalline, while CD derivatives (HPβCD and RMβCD) are amorphous as evidenced from the absence of diffraction peaks in Figures 4-6 and 4-7, respectively. The characteristic diffraction peaks of gefitinib are significantly decreased for the inclusion complex of βCD. The characteristic gefitinib peaks are completely absent in the inclusion complexes of gefitinib with HPβCD and RMβCD, whereas some of these peaks are evident in the kneaded mixtures of gefitinib. This indicates that the gefitinib-cyclodextrin inclusion complexes constitute a new solid state. There was an amorphous structure in both HPβCD and RMβCD complexes. The x-ray diffraction patterns of the physical mixtures still showed the characteristics peaks of gefitinib, indicating that little to no complexation occurred.

More direct evidence of complex formation was obtained from DSC thermograms for HPβCD and RMβCD complexes are shown in Figures 4-8 and 4-9, respectively. Gefitinib shows an endothermic peak corresponding to its melting point (~ 196^{o}C). The kneaded mixtures of gefitinib with HPβCD and RMβCD also show the endothermic peak

that is characteristic of gefitinib. This indicates that there was not complete interaction of gefitinib with HPβCD and RMβCD on kneading. For inclusion complexes the characteristic melting point peak of gefitinib almost completely disappeared, showing the interaction of gefitinib with HPβCD and RMβCD. These results indicate that the inclusion complexes prepared by freeze drying exist in the new solid state.

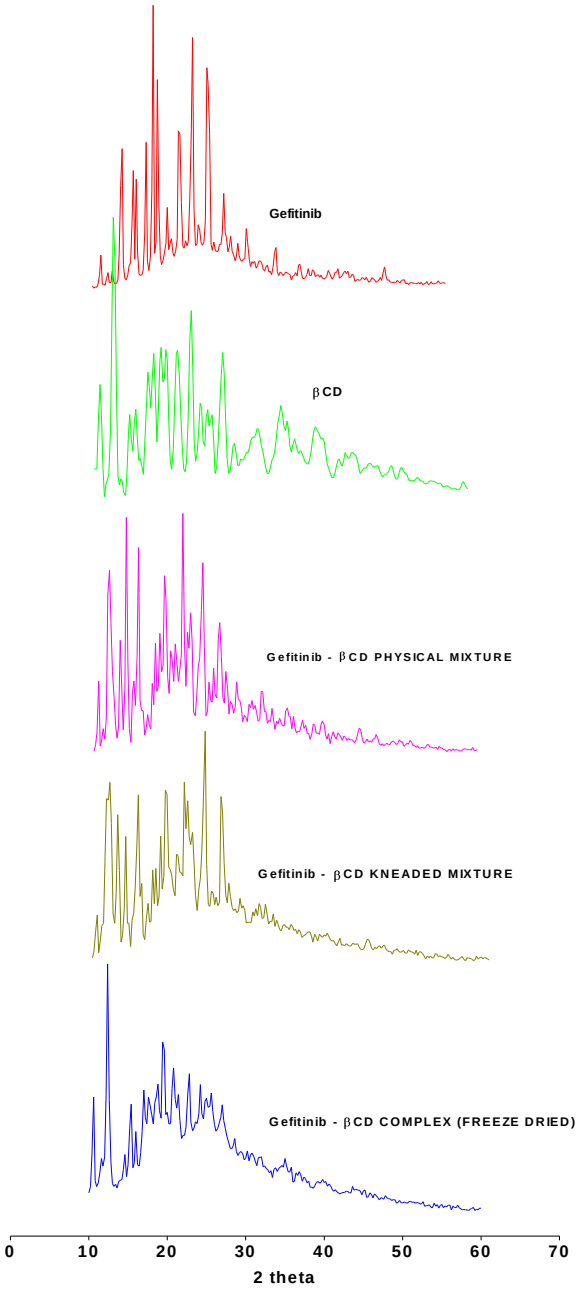

Figure 4-5: X-ray diffraction pattern of gefitinib, βCD, their physical mixture (1:1), kneaded mixture (1:1), and inclusion complex (freeze dried, 1:1).

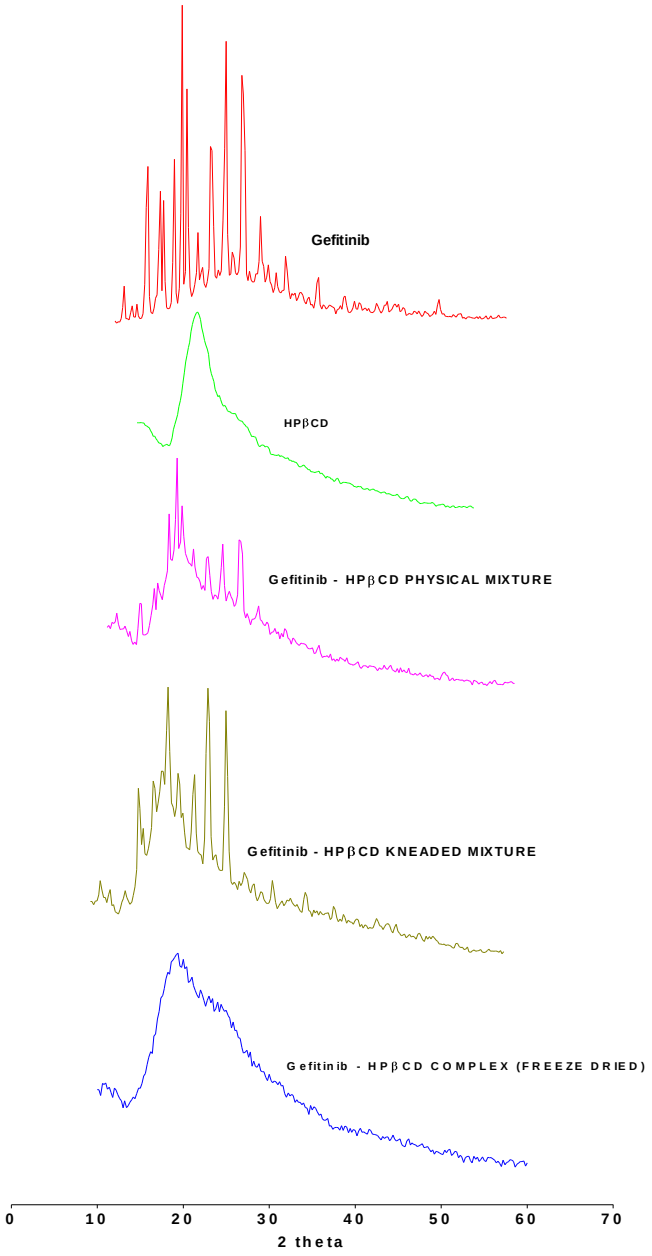

Figure 4-6: X-ray diffraction analysis of gefitinib, HPβCD, their physical mixture (1:1), kneaded mixture (1:1), and inclusion complex (freeze dried, 1:1).

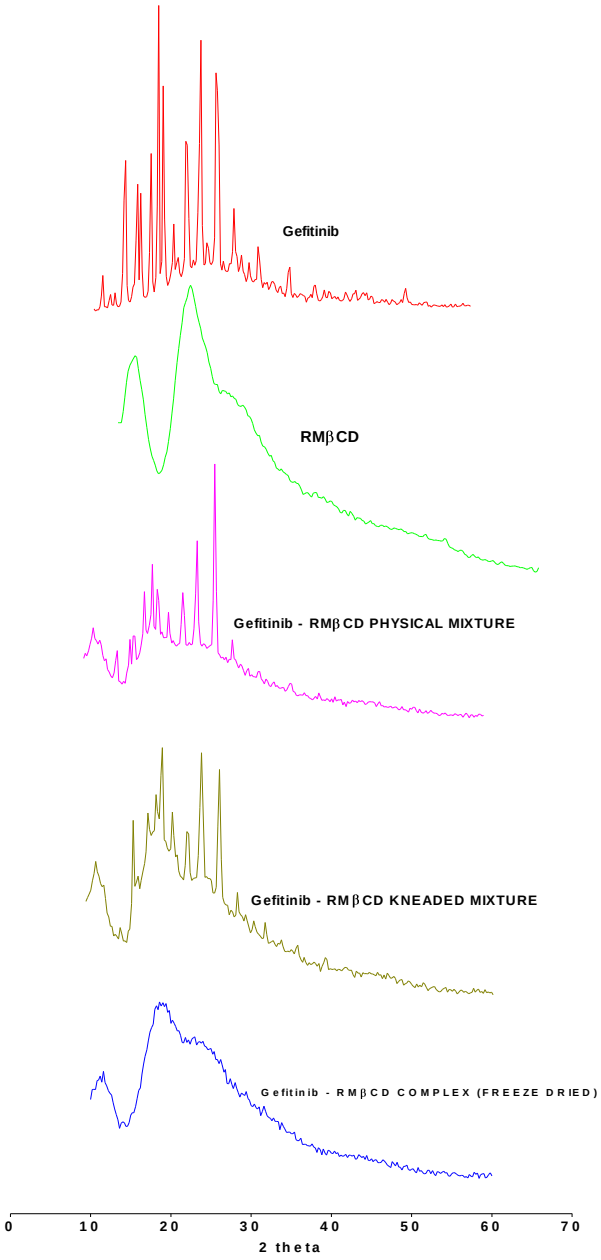

Figure 4-7: X-ray diffraction analysis of gefitinib, RMβCD, their physical mixture (1:1), kneaded mixture (1:1), and inclusion complex (freeze dried, 1:1).

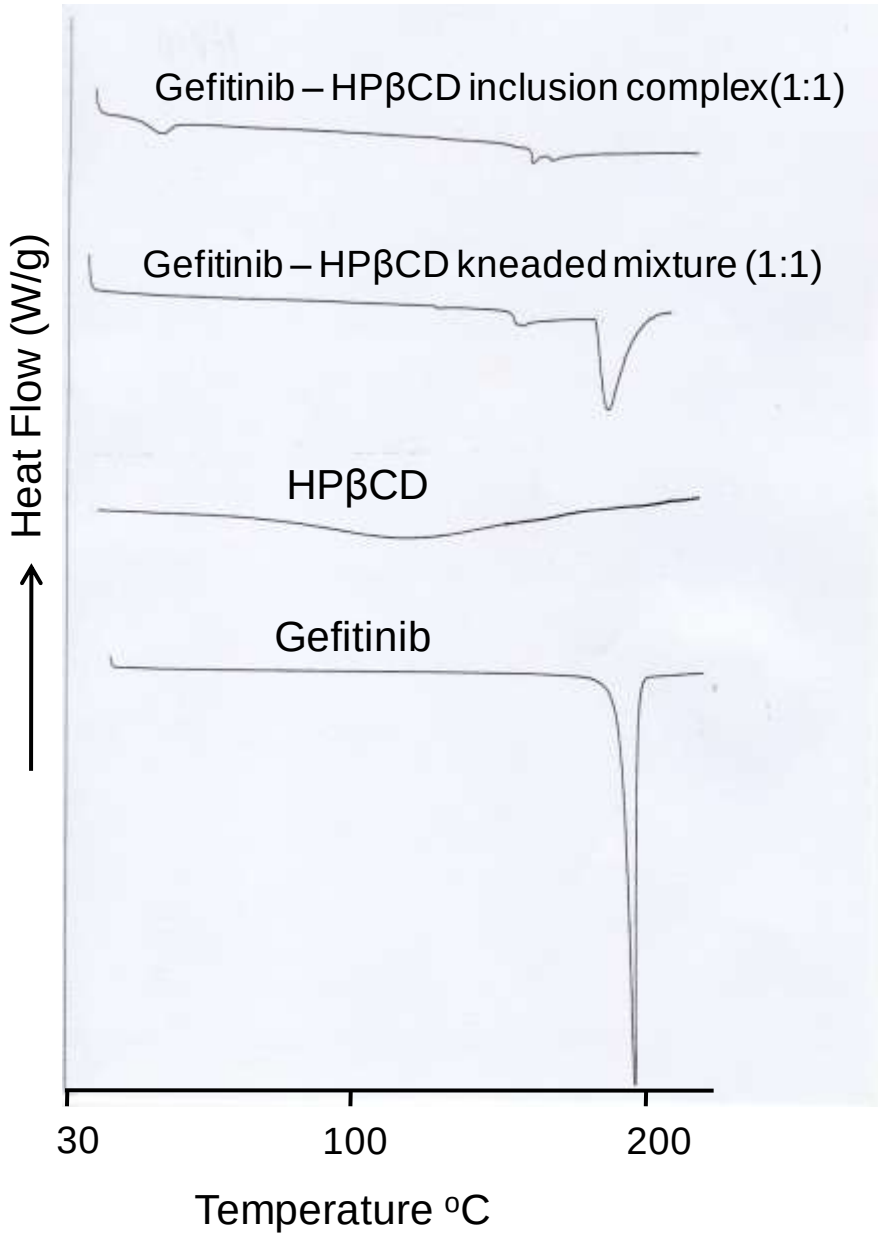

Figure 4-8: Differential scanning calorimetry analysis of gefitinib, HPβCD, their kneaded mixture (1:1), and inclusion complex (freeze dried, 1:1).

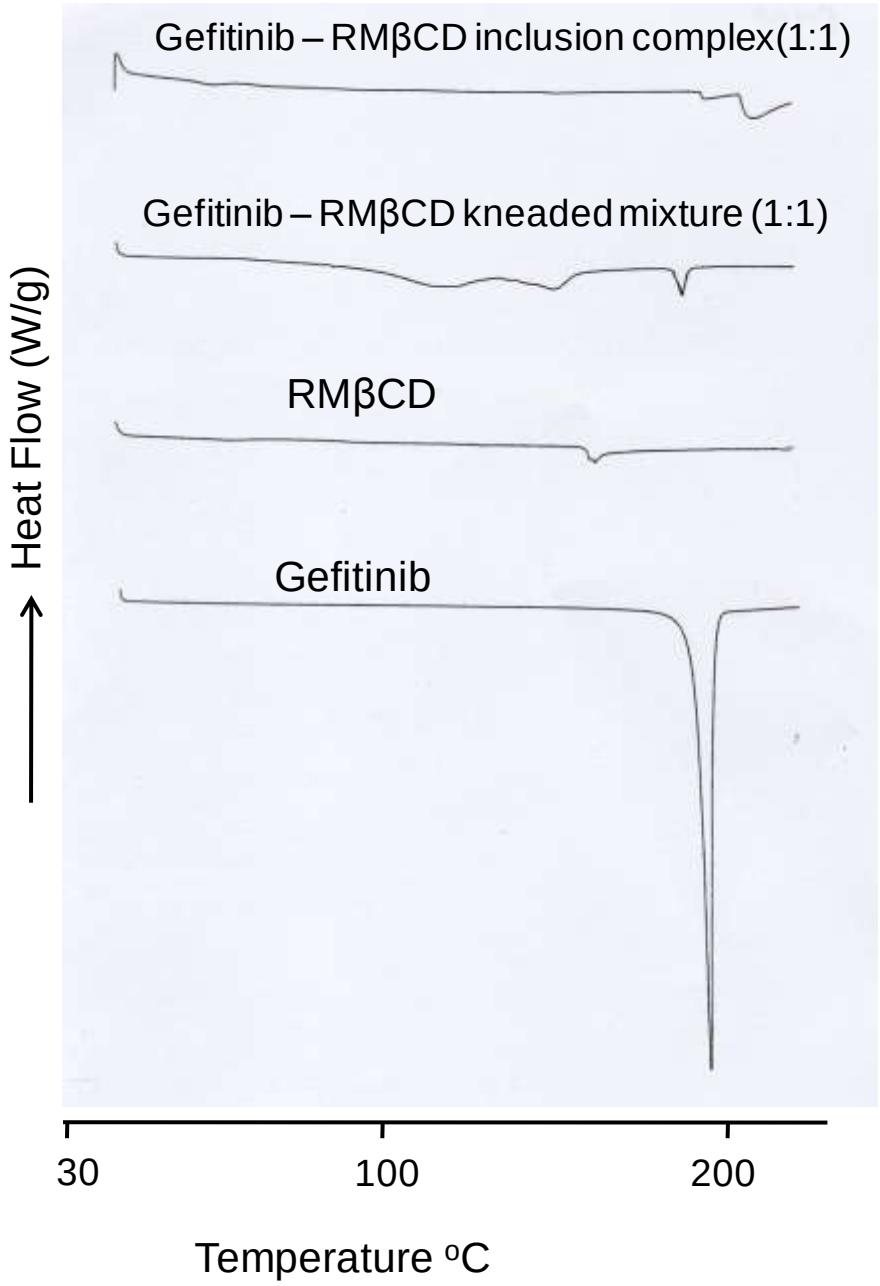

Figure 4-9: Differential scanning calorimetry analysis of gefitinib, RMβCD, their kneaded mixture (1:1), and inclusion complex (freeze dried, 1:1).

Dissolution Studies

Dissolution studies on various gefitinib-cyclodextrin systems were conducted to demonstrate the influence of the type of cyclodextrin, and the complexation method on dissolution kinetics and the total amount of drug in solution. It is generally assumed that the complexes show higher dissolution as compared with the pure drug.[47] But the objective is to achieve higher solubility that is characteristic of inclusion complexes. The dissolution profiles of gefitinib and various binary systems of β-cyclodextrin are presented in Figure 4-10. It is evident that the physical mixture prepared with β-cyclodextrin did not show any improvement in the dissolution of gefitinib. The kneaded mixture showed improvement in the dissolution of the drug. The concentration dissolved from pure drug and kneaded mixtures at 1 hr were 10% and 30%, respectively. The βCD inclusion complex (1:1) showed rapid dissolution; about 35% drug dissolved in 10 min, but the total amount of drug dissolution was similar to kneaded mixture. The inclusion complex (1:2) showed substantially higher dissolution (60% dissolution in 240 min) compared to control (22% dissolution in 240 min).

The dissolution profiles of gefitinib and various binary systems of hydroxypropyl β-cyclodextrin are presented in Figure 4-11. The physical mixtures prepared with hydroxypropyl β-cyclodextrin did not show any improvement in the dissolution of gefitinib. The kneaded mixture also showed little improvement in the dissolution of the drug. The concentration dissolved from pure drug and kneaded mixture at 1 hr was 10% and 20%, respectively. The HPβCD inclusion complex (1:1) showed rapid dissolution; about 50% drug dissolved in 10 min. The total amount of drug dissolution in 240 min was 50% which is two fold higher than the kneaded mixture. The HPβCD inclusion

complex (1:2) showed much higher dissolution (65% dissolution in 240 min) compared to gefitinib alone (22% dissolution in 240 min).

The dissolution profiles of gefitinib and various binary systems of randomly methylated β-cyclodextrin are presented in Figure 4-12. The physical mixtures prepared with randomly methylated β-cyclodextrin did not show any improvement in the dissolution of gefitinib. The kneaded mixture showed slight improvement in the dissolution of the drug. The concentration dissolved from pure drug and kneaded mixtures were 10% and 20%, respectively. The RMβCD inclusion complex (1:1) showed rapid dissolution; about 40% drug dissolved in 10 min, and the total amount of drug dissolution in 240 min was at ~40% which is two fold higher than the kneaded mixture. The inclusion complex (1:2) showed substantially higher dissolution (70% dissolution in 240 min) compared to gefitinib alone (22% dissolution in 240 min).

Two hydrophilic polymers, polyvinyl pyrrolidone and hydroxypropyl methyl cellulose were individually blended at 10% and 25% w/w concentrations with gefitinib-HPβCD inclusion complex (1:1). The dissolution profiles of these blends containing PVP and HPMC as co-solubilizers are presented in Figures 4-13 and 4-14, respectively. The addition of hydrophilic polymers markedly enhanced the dissolution of gefitinib. The gefitinib-HPβCD (1:1) complex yielded 50% dissolution in 1 hr whereas 10% w/w and 25% w/w PVP in association with the complex increased the dissolution to 80% and 90%, respectively. Similarly, addition of HPMC at 10% w/w and 25% w/w to the complex increased the dissolution to 85% and 95%, respectively, in 1 hr.

The freeze dried complexes showed improved dissolution relative to the physical and kneading complexes with all the CDs. This suggests formation of an inclusion complex

by freeze drying with a reduction of the crystallinity of the products as confirmed by x-ray diffraction studies.

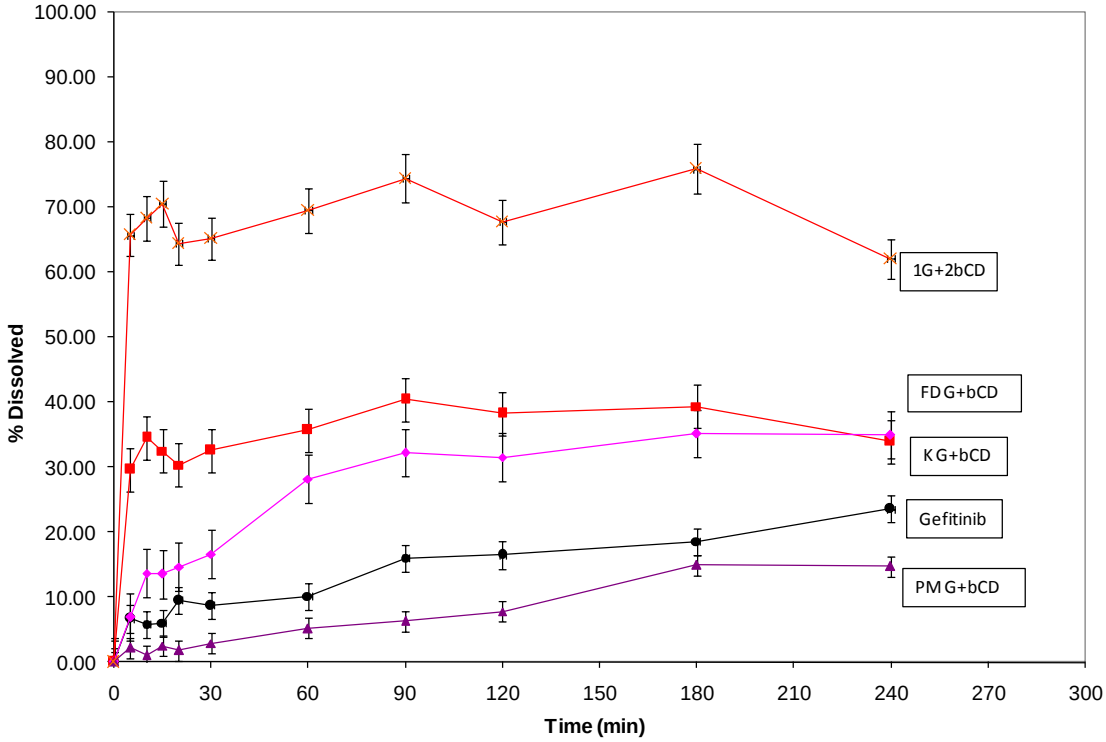

Figure 4-10: Dissolution of gefitinib-βCD formulations.

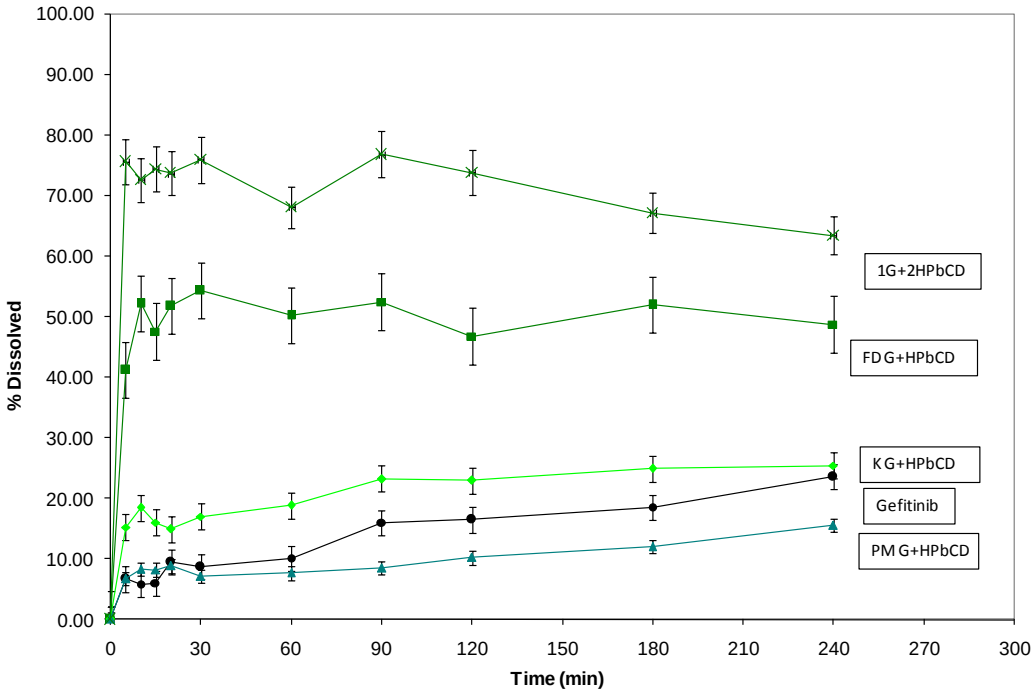

Figure 4-11: Dissolution of gefitinib-HPβCD formulations.

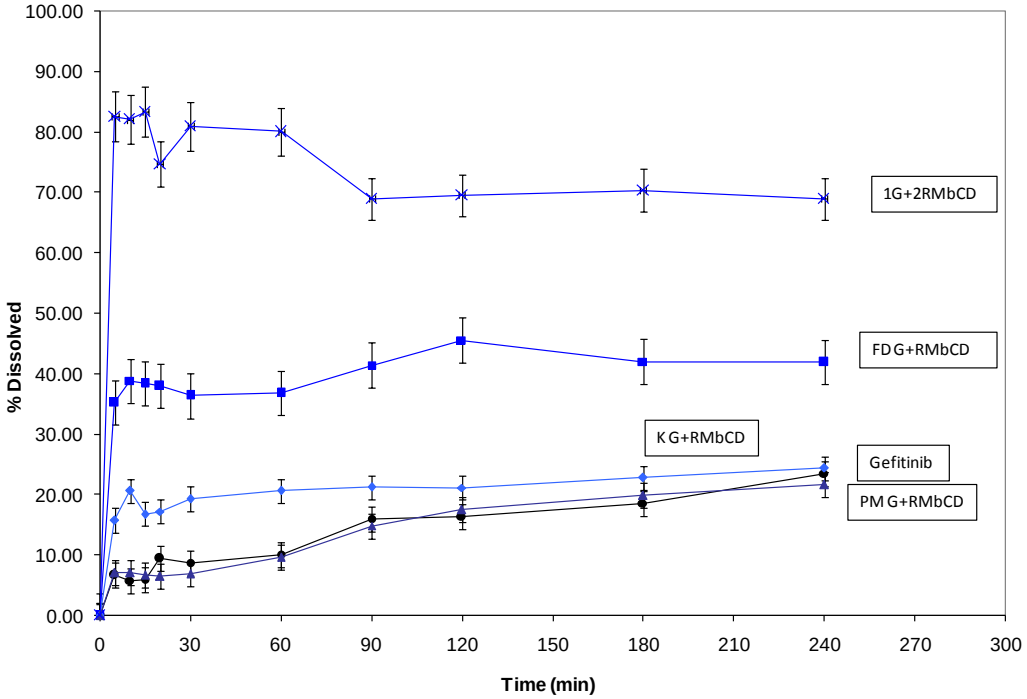

Figure 4-12: Dissolution of gefitinib-RMβCD formulations.

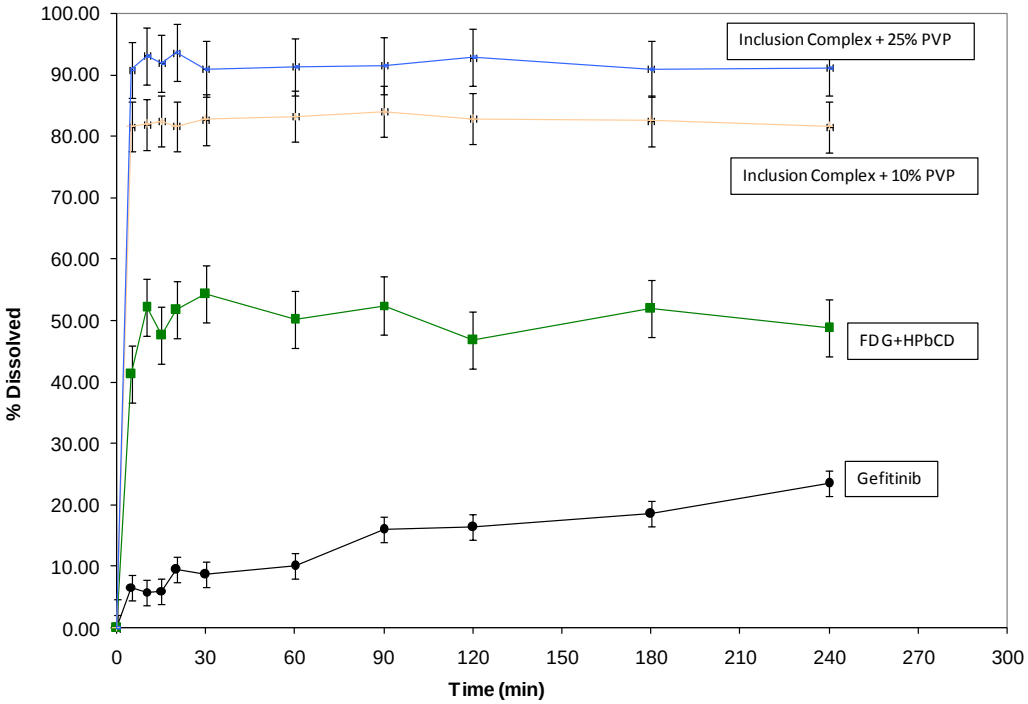

Figure 4-13: Effect of PVP on the dissolution of gefitinib-HPβCD complex.

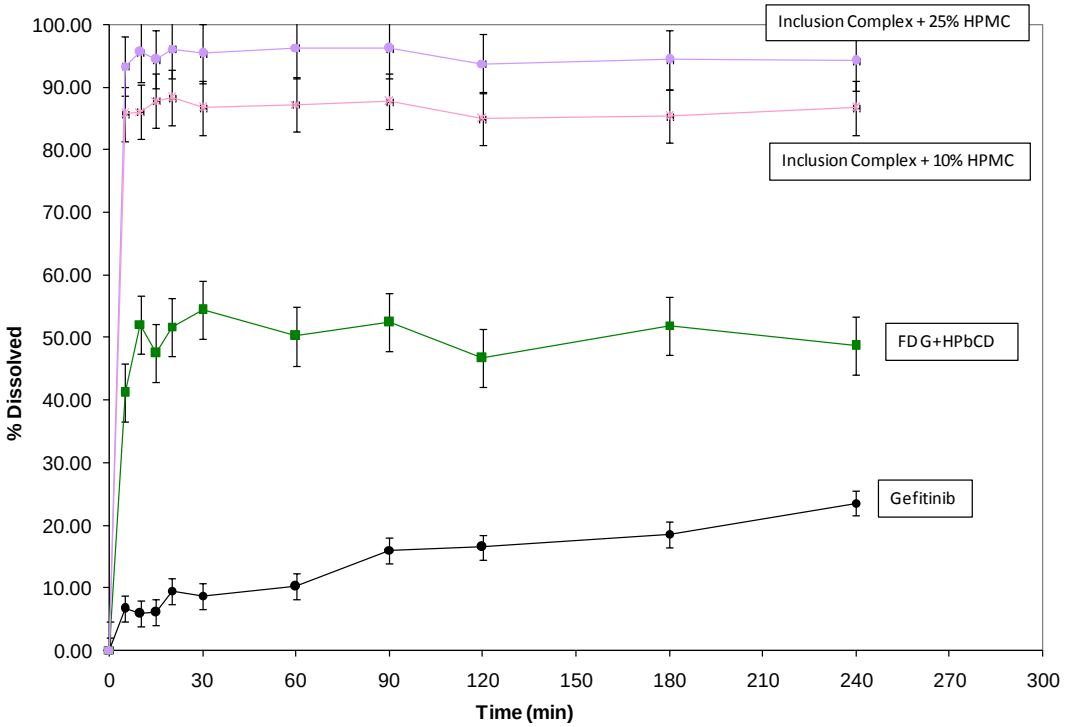

Figure 4-14: Effect of HPMC on the dissolution of gefitinib-HPβCD complex.

Time (min)	Gefitinib and βCD				Gefitinib and HPβCD			
	Physical Mix	Kneaded	Freeze Dried	1:2 ratio	Physical Mix	Kneaded	Freeze Dried	1:2 ratio
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	2.21	6.97	29.59	65.68	6.77	15.22	41.25	75.62
10	1.04	13.66	34.53	68.27	8.29	18.42	52.16	72.58
15	2.53	13.53	32.49	70.43	8.20	16.03	47.55	74.37
20	1.84	14.67	30.34	64.37	8.84	14.89	51.76	73.70
30	2.89	16.63	32.51	65.15	7.12	16.98	54.37	75.94
60	5.20	28.19	35.65	69.47	7.70	18.84	50.20	68.06
90	6.27	32.19	40.42	74.37	8.53	23.23	52.45	76.82
120	7.82	31.49	38.22	67.67	10.21	22.96	46.76	73.77
180	14.95	35.15	39.33	75.87	12.07	24.94	51.94	67.13
240	14.74	34.99	33.91	61.98	15.54	25.41	48.74	63.44

Time (min)	Gefitinib and RMβCD				Gefitinib with polymer formulations				
	Physical Mix	Kneaded	Freeze Dried	1:2 ratio	10% PVP	25% PVP	10% HPMC	25% HPMC	Gefitinib
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	3.99	15.78	35.29	82.59	81.55	90.79	85.73	93.40	6.66
10	4.25	20.62	38.85	82.12	81.92	93.06	86.07	95.67	5.83
15	4.79	16.78	38.43	83.35	82.41	91.89	87.87	94.50	6.02
20	4.87	17.24	38.02	74.69	81.63	93.56	88.36	96.17	9.50
30	5.10	19.33	36.39	80.95	82.70	90.95	86.74	95.41	8.73
60	6.58	20.65	36.81	80.06	83.20	91.26	87.24	96.32	10.14
90	7.90	21.23	41.46	68.96	84.03	91.44	87.75	96.24	16.02
120	8.80	21.18	45.57	69.53	82.78	92.77	85.02	93.79	16.45
180	10.46	22.83	42.01	70.36	82.52	90.92	85.39	94.42	18.57
240	11.93	24.36	41.95	68.95	81.50	91.13	86.77	94.23	23.55

Table 4-2: Average percent dissolved for various gefitinib-cyclodextrin systems.

CONCLUSION

The association rate constants calculated from the phase solubility diagrams (735.9, 446.6 and 1021.9 M^{-1}) for βCD, HPβCD, and RMβCD, respectively indicate that gefitinib can form a stable complex with all three CDs. The freeze dried formulations showed substantial increases in the dissolution of gefitinib with all three of the cyclodextrins compared to gefitinib alone; the most with HPβCD, followed by RMβCD, then βCD. The kneaded mixtures showed some improvement, while the physical mixtures showed no improvement in the dissolution of gefitinib over the drug itself. X-ray diffraction analysis and differential scanning calorimetry (DSC) studies indicated the formation of inclusion complexes in the solid state by various cyclodextrins. The cyclodextrin derivatives HPβCD, and RMβCD formed amorphous complexes with gefitinib and provided greater improvement in the solubility and dissolution of the drug.

The addition of hydrophilic polymers markedly enhanced the dissolution of gefitinib from the CD complexes. The gefitinib-HPβCD (1:1) complex yielded 50% dissolution in 1 hr whereas the addition of 10% and 25% w/w PVP increased the dissolution to 80% and 90%, respectively. Similarly, addition of HPMC at 10% w/w and 25% w/w to the complex increased the dissolution of the complex to 85% and 95%, respectively in 1 hr.

In conclusion, gefitinib can form a stable inclusion complex with all three cyclodextrins as demonstrated by the liquid and solid state complexation studies.

HPβCD showed the greatest improvement in the dissolution of gefitinib followed by RMβCD and βCD, with further improvement upon the addition of PVP or HPMC.

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