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**DRUG- CYCLODEXTRIN INCLUSION COMPOUNDS. STUDIES OF THE FORMULATION OF PHARMACEUTICAL PRODUCTS CONTAINING CYCLODEXTRINS.**

Ramachandra P. Hegde  
*University of Rhode Island*

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DRUG-CYCLODEXTRIN INCLUSION COMPOUNDS.

STUDIES OF THE FORMULATION OF PHARMACEUTICAL PRODUCTS  
CONTAINING CYCLODEXTRINS

BY

RAMACHANDRA P. HEGDE

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY  
IN  
PHARMACEUTICAL SCIENCES

UNIVERSITY OF RHODE ISLAND

1985

DOCTOR OF PHILOSOPHY DISSERTATION

OF

RAMACHANDRA P. HEGDE

Approved

Dissertation Committee

Major Professor

CT. R. G. S.  
[Signature]  
[Signature]  
[Signature]

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UNIVERSITY OF RHODE ISLAND

1985

## ABSTRACT

Interaction of beta-cyclodextrin with ampicillin, phenobarbitone, and phenytoin was studied and complexation was observed with all three drugs. Analytical methods for the quantification of these drugs were validated. In order to obtain an inclusion compound in the solid powdered form, a variety of methods (freeze drying, spray drying, kneading, and solvent evaporation) was evaluated. Among these methods freeze drying was the most feasible and reproducible method from a laboratory scale to a pilot scale and to the manufacturing level. Methods using an industrial model freeze drier were developed which resulted in an increase in yield from 53% to more than 90%. However, because of ampicillin instability in freezing conditions, it was not possible to obtain an inclusion compound in the solid powdered form.

Physico-chemical properties of the inclusion complexes were evaluated by a variety of methods such as stability, solubility, X-ray diffractometry, differential scanning calorimetry, infrared and proton magnetic resonance spectroscopies, photomicrographs, etc. The stability of ampicillin in an acidic medium (pH 2.0) was considerably improved by complex formation with beta-cyclodextrin. The observed apparent pseudo-first order rate constants ( $\text{hr}^{-1}$ ) of ampicillin were  $1.8 \times 10^{-2}$ ,  $1.2 \times 10^{-2}$ , and  $1.1 \times 10^{-2}$  for ratios of 1:0.0 and 1:1.0, 1:0.0 and 1:1.5 and 1:0.0 and 2.0 of ampicillin to beta-cyclodextrin, respectively. Furthermore, it was estimated that the complex degrades nine times slower than the drug itself, and the apparent stability constant was found to be  $458.46\text{M}^{-1}$ . Aqueous solubility of phenobarbitone and phenytoin beta-cyclodextrin complexes were five and eleven fold larger than that of phenobarbitone and phenytoin alone, respectively. A phase solubility

diagram was constructed and the apparent stability constants were calculated for phenobarbitone and phenytoin beta-cyclodextrin complexes. The calculated stability constants were  $4.54 \times 10^3 M^{-1}$  and  $766 M^{-1}$  for phenobarbitone and phenytoin complexes respectively. Thus, the formation and presence of the inclusion complex were confirmed by stability and solubility analysis. Furthermore, the proton magnetic resonance spectra of phenobarbitone beta-cyclodextrin complex strongly indicated the presence of the inclusion complex in the liquid phase. The mode of interaction and elucidation of the presence of the inclusion complex in the solid phase were studied by X-ray diffraction, differential scanning calorimetry, infrared spectra photomicrographs, etc. The X-ray diffraction spectra and differential scanning calorimetry thermograms clearly suggested the presence of the inclusion complex in the freeze dried material but not in the physical mixture. The infrared spectra and photomicrographs evaluations were such that a definitive, unambiguous interpretation could not be made; however, they were useful to some extent to show the interaction with beta-cyclodextrin.

A solution was prepared from the phenobarbitone beta-cyclodextrin complex. Unlike an official USP preparation, the solution prepared from the complex did not require the addition of alcohol to keep the phenobarbitone in solution. Four months' physical and one month's chemical stability data indicated a considerable potential especially for pediatric use. It was possible to prepare a pharmaceutically elegant suspension from the phenytoin beta-cyclodextrin complex. The suspension possessed desirable qualities of a pharmaceutically elegant suspension, such as ease of dispersion, high sedimentation volume, etc.; in addition, the suspension had a low viscosity. Physical stability was good even in

freezing conditions and a syringeability study suggested a potential for intramuscular use.

A flow study was designed using a recording flowmeter with small quantities of material in an attempt to predict the flow rate during large scale operations. The data indicated that the flowmeter was sensitive enough that a small quantity of material could be used to predict the flow rate, effect of formulation and processing variables on production scale quantities. The flow study, and the bulk properties of beta-cyclodextrin suggested that the material was fairly free flowing and compressible. The freeze dried phenytoin beta-cyclodextrin complex lacked flowability and compressibility; nevertheless, it was possible to compress tablets of a suitable size and weight using the wet granulation method. All the properties of tablets were measured and found to be within the USP standards. Dissolution studies in different test media strongly suggested that the dissolution rate for phenytoin beta-cyclodextrin complex tablets was superior to all other phenytoin formulations or preparations. Furthermore, due to in situ complexation in the dissolution medium, the dissolution rate of the physical mixture of the freeze dried phenytoin and beta-cyclodextrin was found to be similar to the dissolution rate of the freeze dried complex.

DEDICATED

TO

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## PLAN OF THE THESIS

This manuscript is divided into seven sections, numbered in Roman numerals. The sections are: I. Introduction, II. Experimental, III. Results and Discussion, IV. Conclusions and Suggestions for future work, V. References, VI. Bibliography, VII. Appendix. The tables and figures are numbered in Roman and Arabic numerals respectively.

In this thesis the term " Inclusion complex, or Inclusion compound ", is often used. The meaning of the term is defined on page # 10. The Results and Discussion are organized into the following main sections: A. Physico-chemical properties of complexes, B. Preparation of the complexes, C. Preparation and evaluation of dosage forms.

In some cases, the figures in this manuscript depict a horizontal dotted line. It represents the calculated percent drug dissolved based on the equilibrium solubility. When experimental points are joined by lines on figures, no mathematical relationships are implied. All the error bars represent standard deviation unless otherwise marked differently in the key to the figure.

## PUBLICATIONS AND PRESENTATIONS

In addition to based on the work presented in this manuscript, the following papers were published or presented.

### PUBLICATIONS:

1. Studies of powder flow using a recording powder flowmeter and measurement of the dynamic angle of repose.  
J. Pharm. Sci., 74, 1, 11-15 (1985).  
Ramachandra P. Hegde, J. L. Rheingold, S. Welch and C. T. Rhodes.
2. Preparation of a phenytoin beta-cyclodextrin complex and evaluation of a suspension and a tablet dosage form prepared from the complex.  
Ramachandra P. Hegde and C. T. Rhodes.  
Pharm. Acta. Helv., 60, 2, 53-57 (1985).
3. Studies of the interaction of beta-cyclodextrin with ampicillin, methicillin, and phenytoin.  
P. Hsyu, Ramachandra P. Hegde, B. K. Birmingham, and C. T. Rhodes.  
Drug Development and Ind. Pharm., 10 (4), 601-611 (1984).

### PRESENTATIONS:

1. Preparation of a phenytoin beta-cyclodextrin complex and evaluation of a suspension and a tablet dosage form prepared from the complex, presented at the A. Ph. A. annual meeting, Montreal, Canada, May, 1984.  
Ramachandra P. Hegde and C. T. Rhodes.

2. Studies of the interaction of beta-cyclodextrin with ampicillin, methicillin, and phenytoin, presented at the A. Ph. A. annual meeting, Montreal, Canada, May 1984.  
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Ramachandra P. Hegde, J. L. Rheingold, S. Welch and C. T. Rhodes.
4. Utility of dynamic angle of repose measurement for pharmaceutical systems, presented at the NERPA meeting, CT., June 1983.  
Ramachandra P. Hegde, J. L. Rheingold, S. Welch and C. T. Rhodes.

MANUSCRIPT ACCEPTED FOR PRESENTATION

1. Inclusion complexation of phenytoin and phenobarbitone with beta-cyclodextrin in solution and solid phases, to be presented at the A. Ph. A. Academy of Pharmaceutical Sciences national meeting, to be held on October 20-24, 1985, in Minneapolis, Minnesota.  
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## I INTRODUCTION

### A- Historical background

The first reference to cyclodextrin was made in a publication of Villers in 1891 (1), who isolated a small amount of a crystalline substance from a culture of Bacillus amylobacter, grown on a medium containing starch. Villers named his crystalline product " Cellulosine " because of its similarity to cellulose. Then Schardinger (2-4) observed that Villers cellulose often formed on starch-based culture media as a product of putrefying microorganisms. Schardinger succeeded in isolating a bacillus which he named Bacillus macerans. This is the most frequently used source of the enzyme glycosyl-transferase by which cyclodextrin is now produced.

After Schardinger, it was Pringsheim (5) who played the major role in the cyclodextrin research, and he discovered the complexing power of the cyclodextrins. From the mid-thirties on, pure cyclodextrins were prepared, first by Freudenberg and his co-workers, who also elucidated the chemical structure of cyclodextrins and discovered gamma-cyclodextrin (6). The cyclodextrins, also called cycloamyloses, Schardinger dextrins, or Cycloglycopyranoses, are cyclic oligosaccharides in which glucose units are linked by alpha 1-4 glycosidic bonds (7,8). French (7) in the mid-fifties demonstrated the existence of delta and epsilon cyclodextrins. Thoma and Stewart (9) described further homologues containing 11 and 12 glycopyranose units.

## B-Properties of cyclodextrins

### 1. Physico-chemical properties

Since the discovery of the Schardinger dextrans, these cyclic compounds have been of special interest as they relate to starch (7). However, with respect to various chemical reactions, especially their biochemical behavior, the Schardinger dextrans vary from starch in important aspects (7,10). One of the rather remarkable properties of the Schardinger dextrans is their resistance to hydrolysis by the common starch-splitting enzymes; for example, it has been repeatedly reported that the Schardinger dextrans are resistant to beta amylase action. Since the Schardinger dextrans, are cyclic, there are no reducing end groups; hence they are resistant to beta amylase attack. The Schardinger dextrans have been reported (11) to be stable to alpha type amylases. Three types of cyclodextrins are most common: (a) Alpha-cyclodextrin containing six glucose units arranged in a ring and called alpha-Schardinger dextrin or cyclohexaamylose. (b) Beta-cyclodextrin containing seven glucose units and named beta-Schardinger dextrin or cycloheptaamylose. (c) Gamma-cyclodextrin containing eight glucose units and named gamma-Schardinger dextrin or cyclooctaamylose. The structure and molecular dimensions of these cyclodextrins are shown in Figs. 1 and 2., and some of their more important physico-chemical properties are given in Table I (8,12).

French and co-workers (13) found that the alpha-dextrin is essentially completely resistant to beta amylase, the beta-dextrin is attacked very slowly indeed and gamma-dextrin is attacked about 1 % as rapidly as is starch. So it is clear that the ring size exerts an effect; possibly the smaller rings have greater rigidity and hence can

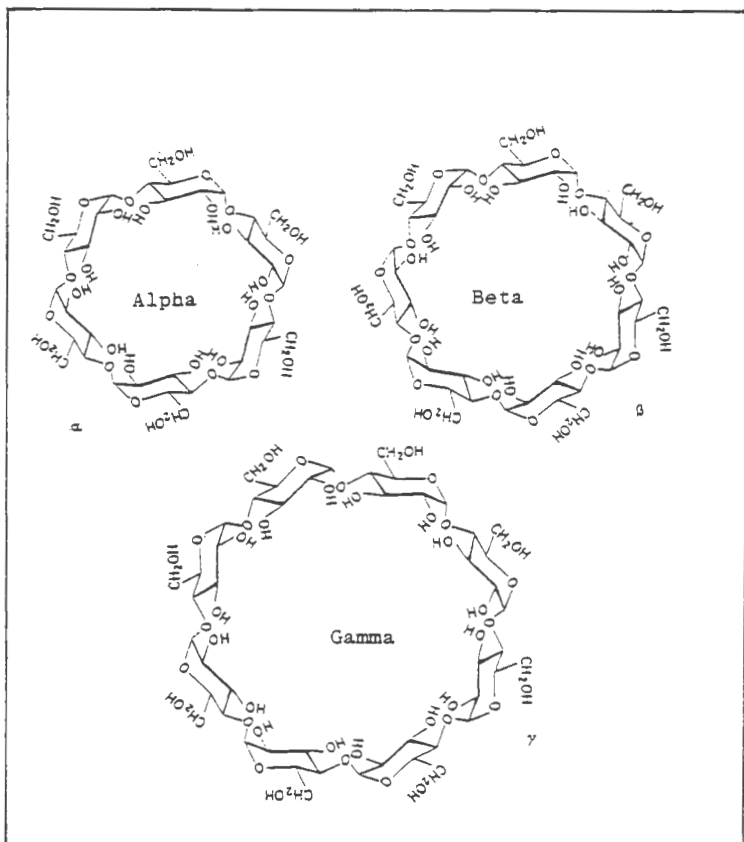


Fig. 1 Structures of Alpha, Beta, and Gamma Cyclodextrins.  
(Taken from Ref. 8).

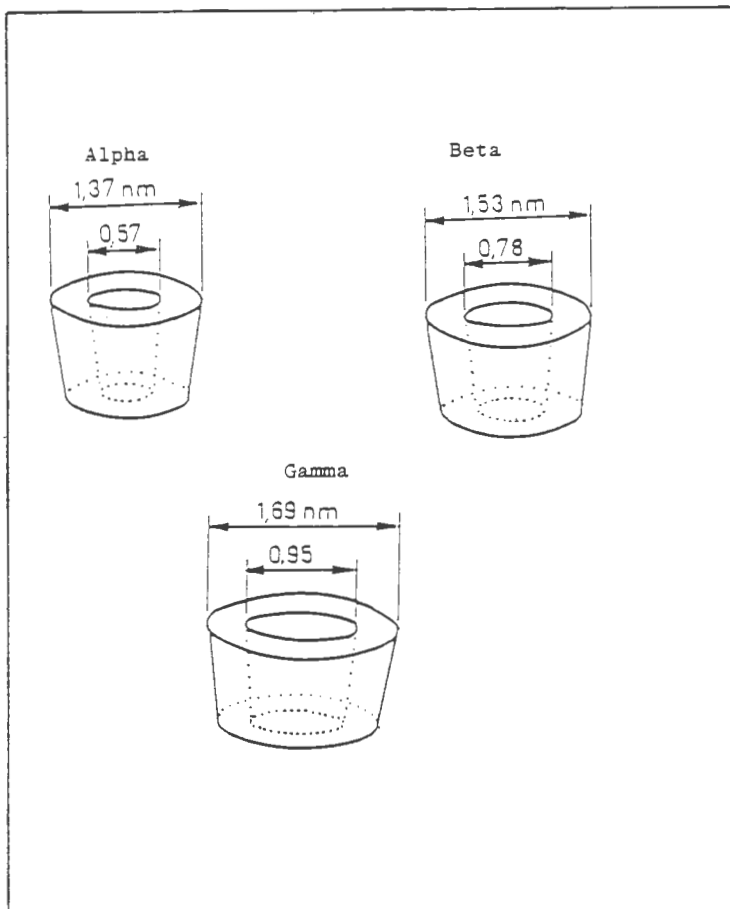


Fig. 2 Dimensions of Alpha, Beta, and Gamma Cyclodextrins.  
(Taken from Ref. 23).



Table I  
Some physico-chemical properties of cyclodextrins

	Alpha	Beta	Gamma
Molecular weight	972	1135	1297
Diameter of cavity	4.5-6.0A <sup>0</sup>	8.0A <sup>0</sup>	10A <sup>0</sup>
Volume of cavity	176A <sup>03</sup>	346A <sup>03</sup>	510A <sup>03</sup>
Number of water molecules taken up by cavity	6	11	17
Molecules per unit cell	4	2	6
Solubility in water gm./100ml. at 25 <sup>0</sup> C.	14.5	1.85	23.2

not adapt their shape to that required by the enzyme. Researchers are still left with the enigma as to why ring closure exerts such a profound effect on the starch chain. In particular, interest has been focused on the question as to which part of the starch structure gives rise to the cyclic molecules. The cyclodextrins are not sensitive to alkalis, and they have no well defined melting points: from 300°C on they start to decompose. The density of cyclodextrins is 1.42-1.45g/cm<sup>-3</sup>, solubility data, molecular weight, cavity diameter, etc. are given in Table I. The cyclodextrins are white crystalline powders, very stable, slightly sweet in taste, and they are more resistant to hydrolysis than the linear dextrins (8). There are no reported data available in the literature with respect to flow and compression characteristics of cyclodextrins (Some of these properties are the subject of this study).

In solution, the cyclodextrin's cavity cannot be regarded as an empty space. For example, the diameter of the cavity in alpha-cyclodextrin is 4.5Å and its height is 6.7Å and therefore the total volume is 64ml/mole. The energy required to maintain an empty space of this dimension would be approximately 40kcal/mole. This value is so high that it is hardly conceivable that the cavity could remain empty (8). The other two cyclodextrins (beta and gamma), having a larger diameter, this energy requirement is even higher. The viscosity of aqueous cyclodextrin solutions differs only insignificantly from that of water (14). One of the most striking properties of cyclodextrins is their ability to form inclusion complexes (8,15). This property will be discussed in detail separately.

## 2. Cyclodextrin metabolism and toxicity

Detailed studies of metabolism and toxicity are necessary for any compound intended for use in pharmaceuticals or in food. Therefore, utilization of the cyclodextrins in the pharmaceutical or food industries depends to a large extent on knowledge of the effects they have on living organisms. In 1963 it was discovered that, in rats, 14C-labeled alpha and beta cyclodextrins are distributed into tissues and organs similarly to 14C-labeled starch (10). It is remarkable that, on feeding 14C-starch, the 14C-level of the  $^{14}\text{CO}_2$  expired reached a maximum after one hour; whereas, in the case of 14C-beta cyclodextrin, the maximum appeared after approximately nine-hours. The total quantity of expired  $^{14}\text{CO}_2$  when related to the carbohydrate consumed, was about equal in both experiments. The cyclodextrins are not metabolized as rapidly as starch; this is because they are cleaved more slowly than linear dextrans (10), and are not hydrolyzed by enzymes which attack terminal groups.

Szejtli et al. (16), reported a study in which they fed rats with beta-cyclodextrin and glucose, both uniformly labeled with 14C, and measured the radioactivity level of blood and exhaled air. With glucose, about 2 % of the initial radioactivity was found to be present in blood within 10-min. With labeled beta-cyclodextrin, only 0.5 % of the initial radioactivity could be recovered from blood and this value was reached only between the 4th and 10th hour following administration. The amount of exhaled radioactivity was practically identical with rats treated orally with 14C-glucose or 14C-beta cyclodextrin and followed for a 24-hr period. No significant difference was found in the tissue distribution of radioactivity (17). Further studies (15,16) indicated that beta cyclodextrin is metabolized and that the intestinal flora are probably

responsible for the first step in the degradation of cyclodextrin. Attempts to detect the intact beta-cyclodextrin ( by high performance liquid chromatography ) in blood following oral administration of <sup>14</sup>C-labeled substance gave negative results; no significant radioactivity was found at retention times that correspond to the beta-cyclodextrin.

Chronic six months oral toxicity of the beta-cyclodextrin was studied with doses up to 1.6gm./body weight kg./day in rats, and up to 0.6gm./body weight kg./day in dogs (18). No toxic effects were observed with respect to weight gain, food consumption, or biochemical values of clinical significance. Pathologic and histopathologic investigations following the six-month treatment did not reveal any sign of toxicity in the digestive organs, central nervous system, cardiovascular system, or in any other organ tested. In addition, the beta-cyclodextrin was also devoid of embryotoxic effect. Chromosomal tests performed in rats treated for six months did not reveal increased incidence of spontaneous aberrations or gene mutation (18). The cyclodextrins administered in high doses (above 50mg./body weight kg.) subcutaneously, intraperitoneally, or intravenously, can induce renal damage in the rat, but at lower doses the compound can be administered without ill effects (20,21).

All toxicity tests have shown that the orally administered cyclodextrin is innocuous. According to the report (22) of the Food and Agriculture Organization (FAO), enzymatically modified starch-- this includes cyclodextrins-- is also toxicologically harmless. The FAO report concludes that there is no need to conduct a toxicological examination in the case of enzymatically modified starch products. However, considering the reduced activity of starch-degrading enzymes

towards cyclodextrins, and also their retarded resorption and slow metabolism when administered orally, it is the author's opinion that additional toxicological studies of cyclodextrins seem nevertheless to be justified, especially if the parenteral route of administration were to be used.

### 3. Cyclodextrin derivatives

Among the natural cyclodextrins, beta-cyclodextrin is widely used in many fields because of its cavity size ( $8.0 \text{ \AA}^0$ ), and the ease with which it can be obtained at a relatively low cost on a large scale (8,15,23). The present cost of this material is about \$ 500 per kilogram. Numerous derivatives of cyclodextrins have already been reported (8). However, for biomedical applications only a small number of cyclodextrin derivatives have been tested (24).

Recently, the chemically modified cyclodextrins have received considerable attention because their physico-chemical properties are different from those of the natural cyclodextrins (25). Among the properties that can be modified are solubility, membrane permeability, chemical reactivity, and dissociation constant (26). These properties will provide a rational basis for the design of formulations, and a means of improving the efficiency of drug activity; however, it should also be noted that modified cyclodextrins cause more hemolysis and local tissue damage than do natural cyclodextrins (27). Hence, the practical application of modified cyclodextrins must await the results of exhaustive toxicological studies.

#### C-Inclusion complexes

For a half century chemists speculated that certain molecular structures might enclose other structures of suitable size and geometry.

It was not until late 1940's that this form of molecular architecture was actually shown to exist. Then, as so often happens, when the time is ripe, the discovery was made almost simultaneously by several research groups (15,28-33). The term "Einschlussverbindung" (inclusion compound) was introduced by Schlenk (33). Schlenk defines inclusion compounds as addition compounds in which one entity fits into and is surrounded by the crystal lattice of the other. These inclusion compounds may be called "no bond" complexes and are characterized by the lack of adhesive forces between the components of the complex. It is not a chemical interaction which is the primary force which causes an inclusion compound to form, although this may be a factor in the net complexation observed. There are some other names for inclusion compounds used in the literature, such as, abduct, clathrate, molecular compound, complex, etc. Inclusion complexes are known in six different forms (34), depending on the architecture of the "host" structures and the shapes of the cavities they enclose (Fig. 3). The structural arrangements that allow this snug "hand in glove" or "lock and key" fitting of molecular shapes differ from one family of inclusion compounds to another (34). In any event, according to John F. Brown Jr. (34), the discovery and detailed understanding of inclusion compounds have provided a powerful support for the hypothesis that such "hand in glove" or "lock and key" structures can indeed account for biochemical specificity, and hence that inclusion structures play a central role in the functioning of living organisms.

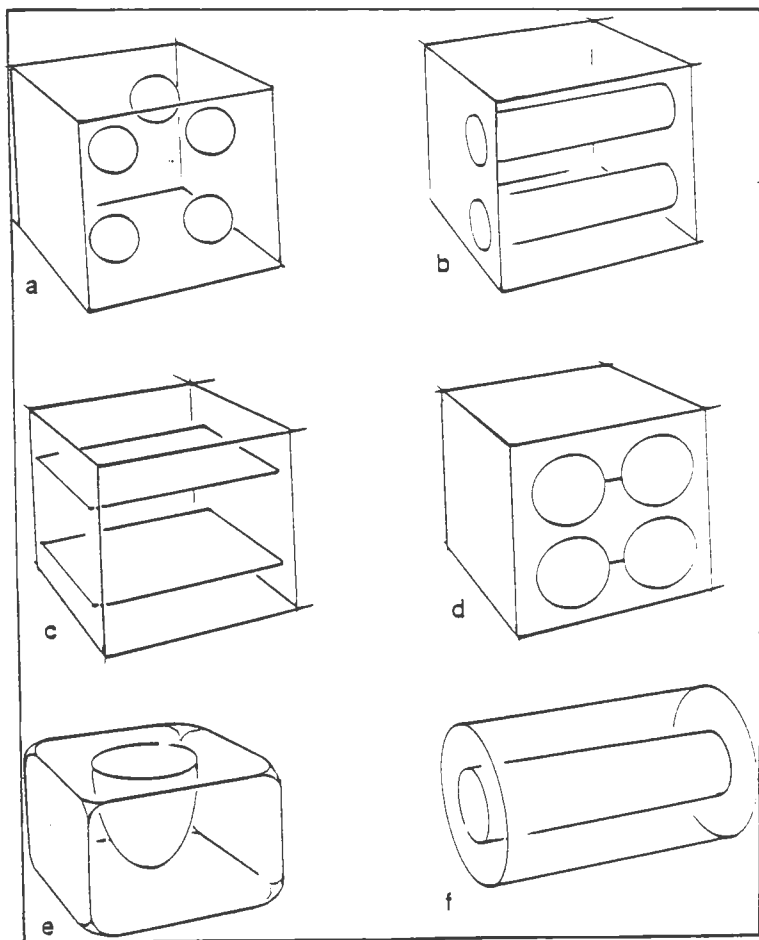


Fig. 3: Six different forms of inclusion complexes.

Key: a- Spherical complex; b- Canal complex; c- Layer complex  
d- Molecular sieve complex; e- Hollow space complex; and  
f- Cylindrical complex.(Taken from Ref. 34).

### 1. Steric requirements

The steric configurations of the molecules are such that the " host " molecule can spatially enclose the " guest " molecule, leaving unaffected the bonding systems of the components. This type of formation is typified by the Schardinger dextrans or cyclodextrins (35). According to Cohen and Lach (36), geometrical rather than chemical factors are decisive in determining the kind of guest molecules which can penetrate into the cyclodextrin cavity to form an inclusion complex. The extent of the complex formation also depends on the polarity of the guest molecule. Because of the cyclodextrins' cyclic structures and relatively large open space within each molecule (  $6.0 \text{ \AA}^0$  for alpha,  $8.0 \text{ \AA}^0$  for beta, and  $10.0\text{-}11.0 \text{ \AA}^0$  for gamma ), they have been reported to form complexes with different sized molecules (36). For example, naphthalene is too bulky for alpha-cyclodextrin, and anthracene fits only into gamma-cyclodextrin. On the other hand, propionic acid fits well with alpha-cyclodextrin, but it is too small to fit properly in larger cavities. Similarly the role of molecular dimensions is well demonstrated by the complex formation with halogenated benzenes (28). Reaction of the cyclodextrins with halobenzenes show that 1:1 complexes may be prepared from chloro, bromo, and iodobenzenes. Chlorobenzene reacts only with alpha-cyclodextrin, bromobenzene reacts with alpha and beta-cyclodextrins, and iodobenzene reacts with beta and gamma-cyclodextrins. According to Van Hoodonk and Breebaart-Hansen (37), the diameter of the cavity in beta-cyclodextrin is about  $8.0 \text{ \AA}^0$  and the size of the benzene ring is about  $6.8 \text{ \AA}^0$ ; therefore a substituted benzene ring can penetrate into the ring. Certain chemical groups or substituents may promote complex formation or stability. However, the stability of the complex is proportional to the hydrophobic



character of the substituents. Thus, a methyl, ethyl, or phenyl substituent will increase the complex formation or stability. A methyl group in the ortho position to a carbonyl group has a shielding effect on the hydrophilic carbonyl group, thereby increasing the hydrophobicity of the whole molecule. A similar substituent in the para position has a relatively weak effect. Hydroxyl groups hinder complex formation, but their hydrophilic effects decrease in the order ortho, meta, and para (38).

## 2. Complexation by cyclodextrins

Many drugs are amenable to inclusion complex formation (36,39-43) with cyclodextrins. As shown in Fig. 1, the lining of the cyclodextrin cavity is formed by hydrogen and glucosidic oxygen atoms. Therefore, this surface is rather apolar (8). In aqueous solution the apolar cyclodextrin cavity is occupied by water molecules that are in an energetically unfavored state ( polar-apolar repulsion ) and are, therefore readily replaced by an appropriate " guest molecule " that is less polar than water. Schematic illustration of the complexation process is as shown in the Fig. 4 (8). The small circles represent water molecules, the large ones represent cyclodextrin rings. The outer surface of the cyclodextrin ring is hydrated, but the water molecules in the ring cavity are in an energetically unfavorable condition because of the nonpolar surface of the cavity. The hydrophobic part of the potential guest molecule is highly hydrated, while the nonpolar aromatic ring repulses the water molecules. The result of the complex formation is that the nonpolar part of the guest molecule penetrates into the nonpolar cavity, thereby establishing an energetically favorable nonpolar-nonpolar interaction, while the protruding hydrophilic part of

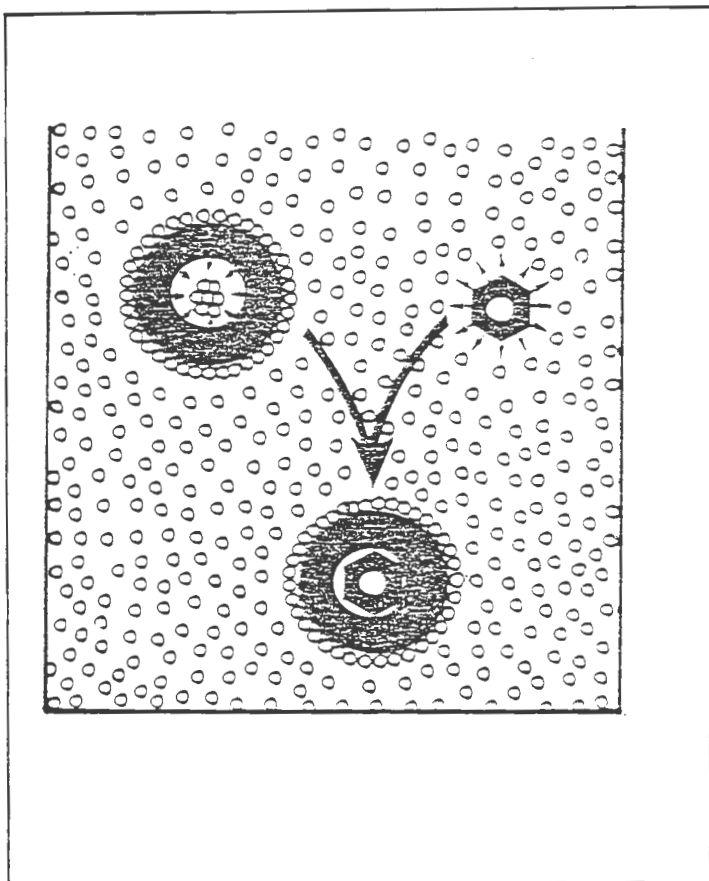


Fig. 4: Schematic representation of the formation of cyclodextrin inclusion complexes. (Taken from Ref. 23).

the guest molecule outside retains its hydrated shell. Among the various changes (44) that occur upon complexation with cyclodextrins the following may be of considerable advantage:

- a. Organoleptic properties
- b. Conversion of a liquid drug to a solid form
- c. Physico-chemical properties
- d. Content uniformity
- e. Dissolution
- f. Bioavailability
- g. Target organ oriented dosage forms

#### D-Preparation of the inclusion complexes

The method of preparation of inclusion complexes depends on the properties of the guest components (8,15). The most common procedure is to stir or shake an aqueous solution of cyclodextrin (cold or warm) with the guest molecule or its solution. Equilibrium is reached with intense stirring and slow cooling in a few hours. The guest molecule content cannot be increased by repeating the heating and cooling steps. After having attained the equilibrium, water can be removed by any one of the following methods.

##### 1. Solvent evaporation

Equimolar quantities or up to a ten-fold excess of water soluble substances are dissolved in concentrated warm or cold aqueous solutions of the cyclodextrins. The inclusion compounds crystallize out on slow cooling or evaporation (45).

## 2. Kneading

In this case the cyclodextrin is not dissolved, it is kneaded with a small amount of water to make a slurry and then guest components are added to the slurry of the cyclodextrin. On stirring in a mixer, the viscosity of the mixture increases, giving a paste which can be dried and powdered.

## 3. Freeze drying and Spray drying

These two methods are suitable when fine particles of the complexes are desired. Equimolar quantities of host and guest molecules are dissolved in a saturated solution of the cyclodextrins. Substances which are not water-soluble can sometimes be dissolved by adding an appropriate amount of 28% ammonium hydroxide solution. The solution are then either freeze dried or spray dried (8).

### E-Methods of investigating the complexes

#### 1. Solubility

There are many examples which demonstrate the effect of cyclodextrins on the solubility of substances that are sparingly soluble in water (39-43). The solubility of the guest component increases linearly with the amount of cyclodextrin added. According to the method of Higuchi and his co-workers (46,47) the stability constant and stoichiometry can be determined from the phase solubility diagram. The phase solubility diagram can be constructed by plotting the total molar concentration of substrate as a function of the total molar concentration of ligand. The ascending linear part of a solubility diagram (Fig.5,A) is generally ascribed to the formation of a 1:1 complex and the stability constant can be calculated as follows.

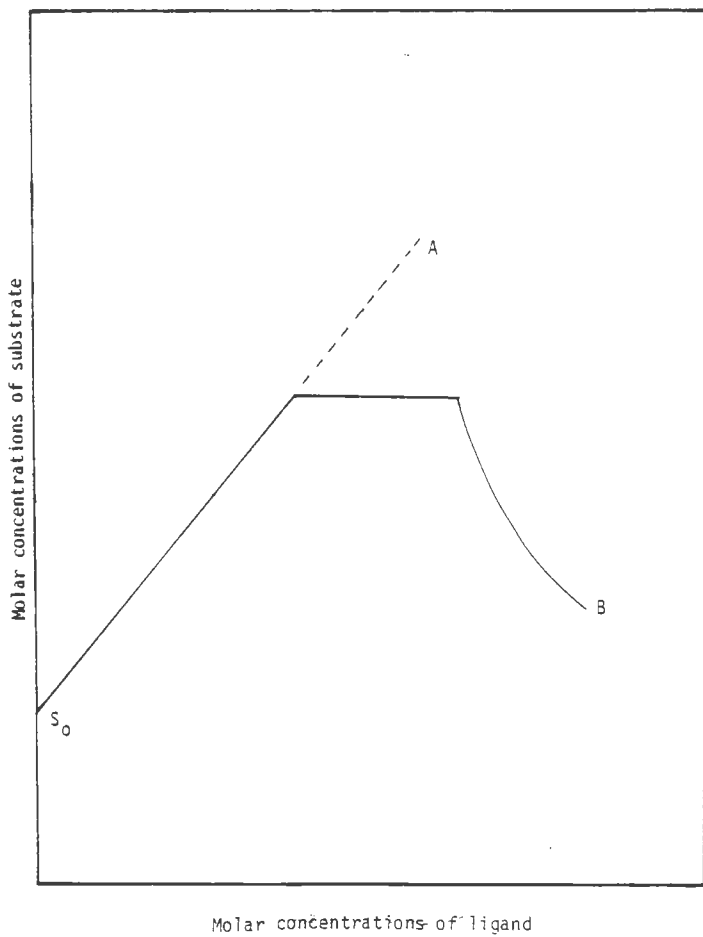


Fig. 5: Phase solubility diagrams

$$K = \text{Slope} / S_0 (1 - \text{Slope}) \quad \text{Eq. (1)}$$

Where  $S_0$  is the equilibrium constant of the substrate  $S$ , in the absence of the ligand  $L$ , and thus  $S_0$  is equal to the intercept of the plot in Fig. 5,A. In some cases, the phase solubility diagram shows a plateau region before the descending part of the curve (Fig.5,B), making it possible, on the basis of the length of the plateau, to estimate the stoichiometry. In such plots, the formation of the 1:2 complex is assumed and the stability constant can be calculated by using Eq. 1 and 2 according to (45,46) as follows:

$$(S_t - S_0) / L_t - (S_t - S_0) = K_{1:1} S_0 + K_{1:1} K_{1:2} S_0 (L_t - (S_t - S_0)) \quad \text{Eq. (2)}$$

Where  $L_t$  is the total ligand concentration and  $S_t$  is the equilibrium solubility of the substrate in the presence of ligand.

## 2. Diffusion

Gas diffusion provides one of the most direct demonstrations of random molecular motion. Without this phenomenon, the perfume industry would not exist, and skunks would be much less feared. The diffusion properties of cyclodextrin complexes as reported in the literature (8,48) have been used to investigate the complex formation by cyclodextrins. For example, when indomethacin solution was separated by a semipermeable membrane from a buffer solution, a 1:1 equilibrium was observed (47). Whereas, when the indomethacin solution was separated by a semipermeable membrane from a buffer solution saturated with beta-cyclodextrin a higher concentration of indomethacin was observed on the side containing cyclodextrin, because, the diffusion rate of the complex was slower due to the increase in molecular weight and extensive hydration of beta-cyclodextrin. Thus, the concentration of indomethacin was higher on

the side containing cyclodextrin, indicating that the majority of the indomethacin molecules were formed in a complex.

### 3. X-ray spectroscopy

Takeo and Kuge (49,50) published X-ray diffraction diagrams of several alpha, beta, and gamma cyclodextrins complexes. According to their studies, inclusion complexes can be detected quickly and directly by X-ray diffraction. If the diffraction pattern does not correspond to those of pure components, a true inclusion complex may exist. In the case of liquid guest molecules X-ray powder diffraction is the most useful method for the detection of inclusion complex formation. Since the liquid guest molecules produce no diffraction pattern at all, if the diffractogram differs significantly from that of uncomplexed cyclodextrin, formation of a crystal lattice of a new type and complex formation can be established. Single X-ray structure analysis is the best method for detecting complex formation (15).

### 4. Infrared (IR) spectroscopy

The complex formation may be proved by IR spectroscopy, the characteristic bands of cyclodextrin, which represent the overwhelming part of the complex, are influenced by complex formation. Bands due to the included part of the guest molecule are generally shifted or their intensities are altered (51).

### 5. Differential scanning calorimetry (DSC)

In certain cases cyclodextrins complex formation can be proved by DSC. Kurozumi et al. (51) have found that freeze dried mefenamic acid gives at 232<sup>0</sup>c an endothermic peak; its mechanical mixture with freeze dried beta cyclodextrin gives the same peak at the same temperature. But the beta cyclodextrin complex prepared by freeze drying or

crystallization from water does not give any peak. This behavior is usually characteristic of the inclusion complexes.

#### 6. Proton magnetic resonance (PMR) spectroscopy

Demarco and Thakkar (52) investigated cyclodextrin inclusion complex formation by PMR spectrometry. In their study, they observed that the beta-cyclodextrin spectrum was shifted upfield in the presence of guest molecules. According to Demarco and Thakkar, if the guest molecule is accommodated in the cyclodextrin cavity, then the hydrogen atoms located in the interior of the cavity (C-3-H and C-5-H) will be considerably shielded by the guest and hence the signal will be shifted upfield. Whereas, the hydrogen atoms on the outer surface (C-2-H, C-4-H, and C-6-H) will not be affected by the guest molecule, hence the signals of those protons will remain unchanged. The cyclodextrins contain six, seven, and eight units of glucose, and due to the multiplicity of (C-3-H and C-5-H) protons located in the interior of the cavity, an exact evaluation was impossible.

#### 7. Other methods

Inclusion of a guest molecule can also be investigated by monitoring changes in its conductivity (53-55), apparent changes in its basicity (56,57), changes in the optical spectra, thermodynamic parameters, etc. (8,58).

#### F-Present and possible future potential of cyclodextrins in pharmaceutical products.

##### 1. Organoleptic properties

It is possible to improve the organoleptic properties of drug molecules by inclusion complexation with cyclodextrins (8,15,59,60). For instance, femoxetine is a selective serotonin uptake inhibitor with



antidepressant properties (59). This compound is used as a water soluble salt, but it has a very bitter taste which hinders the development of oral liquid formulations. A study was undertaken to obtain a liquid formulation of fexofenadine with acceptable organoleptic properties through inclusion complexation with the beta-cyclodextrin (59), and the results were favorable.

## 2. Conversion of a liquid drug to a solid form

Liquid compounds can be converted into the solid form by inclusion complexation with cyclodextrins (61). Organic nitrates have been used for a long time for the treatment of angina pectoris, and they are still widely used. For example, one of the best known drugs is nitroglycerin, which is a highly explosive liquid drug and therefore cannot be tableted. However, by complexation with cyclodextrin, it is possible to convert the drug into solid form and tablets can be manufactured (61). In addition, complexes of unsaturated fatty acids, ascaridol, clofibrate, methyl pentynol, etc., have been converted into a solid form by complexation with cyclodextrins (62,63). These reports suggest that the product obtained in this way can be used to manufacture tablets.

## 3. Physico-chemical stability

Inclusion complexation can be regarded as micro-encapsulation, because each guest molecule is surrounded by cyclodextrin molecules and is thus, from the microscopical point of view, encapsulated. This phenomenon can be exploited by industries which manufacture drugs, foodstuffs, plant protective agents, etc. The use of cyclodextrin complexes in industries, particularly in the pharmaceutical field, can result in the following improvements in stability.

a. Stabilization of light or oxygen sensitive substances

The extremely labile vitamin D3 (cholecalciferol) should be mentioned here; heat, light, and oxygen all increase the degradation of vitamin D3 by oxidation, but these effects can be inhibited by the inclusion complexation (64,65). Similarly, inclusion of vitamin A in alpha-cyclodextrin increased its stability against heat (66). The sensitivity to light of clofibrate (67) and guaiazulene (68) are reduced by the inclusion complexation with the cyclodextrins.

b. Modification of the chemical activity or stability of guest molecules

Lach and Chin (41) have reported that the alkaline hydrolysis of benzocaine becomes considerably slower in the presence of beta-cyclodextrin. In 1 % beta-cyclodextrin solution the half-life of benzocaine is increased five-fold. Chin, Chung, and Lach (69) studied in detail the alkaline hydrolysis of esters of various aminobenzoic acids and acetylsalicylic acid.

It was found that the rate of hydrolysis decreased when inclusion was complete. If, however, incorporation of the guest molecule was incomplete then the hydrolysis rate increased. Since the inclusion was complete, the rate of hydrolysis of para, meta, and ortho aminobenzoates was decreased by the beta-cyclodextrin. Beta-cyclodextrin has also been shown to have a stabilizing influence on procaine, atropine, aspirin, and phenylbutazone (70). Indomethacin is stabilized by beta-cyclodextrin but not by alpha-cyclodextrin (71). In addition, there are many reports on the modification of the chemical activity or stability of guest molecules by inclusion complexation with the cyclodextrins (8,15,72,73).

c. Fixation of very volatile substances

A reduction in the volatility can be demonstrated by an increase in the boiling point of both liquids and solids that sublime. Uekama et al. (67) demonstrated the reduction in volatility of drugs forming inclusion complexes by differential thermal analysis and thermogravimetry. They used this technique to show the reduced volatility of the clofibrate beta-cyclodextrin complex. The differential thermal analysis of clofibrate gave an endothermic peak around 150<sup>0</sup>C corresponding to the boiling point. This endothermic peak disappeared with the formation of an inclusion complex. Furthermore, the thermogravimetry curves showed significant reduction in the volatility of clofibrate as a result of complex formation. Uekama et al. (74) obtained similar results with the complexes of derivatives of cinnamic acid and beta-cyclodextrin, and the complexes of benzaldehyde and alpha, beta, and gamma-cyclodextrins (75). Szejtli et al. (76) produced beta-cyclodextrin complexes of 25 different aromatic substances, and the solid complexes obtained enabled better handling of the products. They contained all the constituents of the original substances. Nakai et al. (77) used the thermogravimetry method to study the volatility of the physical mixtures, the ground mixtures, and the inclusion complexes of parahydroxybenzoic acid with the alpha and beta-cyclodextrins. The sublimation of parahydroxy benzoic acid was considerably reduced by the inclusion complex. The inclusion complex with alpha-cyclodextrin showed the greatest reduction of sublimation, most probably due to a more tight fit within the cavity of alpha-cyclodextrin molecule.

#### 4. Content uniformity

This United States Pharmacopeia (USP) content uniformity test is designed to establish the dose equivalency of solid dosage forms. The

content uniformity of a solid dosage form cannot be better than that of the granulation of those dosage forms. There are three possible factors inherent in the tablet manufacturing process which may cause difficulties in achieving a good content uniformity namely: (a) nonuniform distribution of drug throughout the powder mixture or granulation, (b) segregation of the powder mixture or granulation during the manufacturing process, and (c) tablet weight variation. These three factors may cause more difficulties in achieving a good content uniformity with potent low-dose drugs (oral contraceptives, etc.). It is not difficult to comprehend why a perfect physical mixture never occurs or why the segregation occurs with powder mixtures intended for direct compression or why with wet granulations drug migration is likely. Some or all of these problems may be alleviated by complexation with cyclodextrins. If a drug gives a stable complex, then the crucial question is the required dose. In addition to the required dose (active ingredient), tablets contain a number of inert materials. These include diluents, binders, disintegrants, and lubricants. If the required dose is 50mg or more it may not be difficult to distribute the active ingredient uniformly in the inert materials to compress a tablet with suitable weight. However, if the required dose is a few milligrams or less, it is very difficult, if not impossible, to achieve a good content uniformity, since more than 90% of the tablet weight is filled with the inert materials.

By the inclusion complexation technique, a drug can be dispersed at a molecular level, and usually the drug content in the inclusion complex is in the range of 15-25% (8). If the required dose is a few milligrams or less then it may not be difficult to disperse uniformly 100mg of the complex instead of 15 or 25mg of drug into the inert materials. By this

type of molecular dispersion it is conceivable that some or part of the previously mentioned factors may be reduced in achieving a good content uniformity of potent low dose drugs.

### 5. Dissolution

The number of papers and patents describing drug-cyclodextrin complexes, their stability, dissolution rate, bioavailability, and pharmacological effects has been growing rapidly in pharmaceutical research (8,15,23). Solubility changes have been observed as a function of complexation and substantial increases in drug stability have also been reported (78,84). These reports also showed that the improved aqueous solubility by complexation resulted in an enhancement of the dissolution rate of various drugs (78,84). The dissolution-absorption process of an orally administered drug can be approximated by the following simple kinetic model.

$$D \xrightarrow{K_d} GI \xrightarrow{K_r} B \quad \text{Eq. (3)}$$

Where D is the oral dose of the drug, GI is the concentration of drug dissolved in the gastrointestinal tract, B is the concentration of drug in blood;  $K_d$  represents the rate constant of dissolution, and  $K_r$  represents the rate constant of absorption.

When  $K_d < K_r$ , then the dissolution is the rate determining step in the drug absorption. In such cases  $K_d$  may be increased by complexing with cyclodextrins. For example, the dissolution rate of proscillaridin complexes was much greater than that of proscillaridin itself (85). Interaction of digitalis glycosides with cyclodextrins has been reported (86). It has been clearly shown that the dissolution rate (in acidic medium) of the complexed form of digoxin is about 100-fold more than that of digoxin itself. In addition, during the dissolution experiments, the

simultaneous conversions of digoxin to hydrolysis products were also followed by HPLC. The rapid dissolving form of the complex showed the reduced decomposition compared to that of digoxin alone. The dissolution of menadion (vitamin  $k_3$ ), and its beta-cyclodextrin complex in water was found to be increased by 10 to 12-fold (23). Improved dissolution characteristics of acetoexamide has been reported by complexation with cyclodextrins (87). Similarly, there are many examples which clearly demonstrate that complexation with cyclodextrins has improved aqueous solubility ( 78-84 ) and dissolution rate of various drugs (8,71,74,88,89).

Dissolution tests have two possible uses. They may, as indicated in the above discussion, be predictive of the rate and extent of drug entering a patient's blood stream. However, a quantitative relationship between dissolution and bioavailability can not be assumed for any given drug; such a relationship is not necessarily simple. Secondly, dissolution tests have value as a method of controlling batch to batch variation. USP XX requires dissolution tests for virtually all conventional solid oral dosage forms. Thus to some extent dissolution has become an end itself.

## 6. Bioavailability

Bioavailability of a drug product is defined as the rate and extent of absorption. Biological availability is determined by comparing blood levels produced by a test product with those produced by an intravenous injection, an aqueous solution, and a commercial dosage form of the drug. When making comparisons of the biological availability of products, normally the following three parameters are determined.

- a. Maximum plasma concentration
- b. Time to reach maximum plasma concentration
- c. Area under the curve

Whenever a drug is administered by the oral route, there is a possibility that part of the dose may not reach the blood due to incomplete absorption (90,91). This result may arise for a variety of reasons, such as poor dissolution of the drug in the gastrointestinal fluids (92-95), first-pass liver metabolism (90), etc. In instances where bioavailability is incomplete, the ratio of oral to intravenous blood level curve areas is less than unity (96). Hence, a great deal of investigation is required to appreciate fully the role of the gastrointestinal tract in drug absorption. For example, a thorough investigation is required with respect to interactions of drug with dietary factors, physiological and physico-chemical factors (97), etc. In this section, enhancement of bioavailability by cyclodextrin inclusion complexation is discussed. Improving the absorption of drugs may be one of the important practical applications of cyclodextrin complexes. Following the oral administration of the drug, practically no cyclodextrin is absorbed (23). Cyclodextrin is only a carrier agent; it transports the lipophilic guest molecule through an aqueous milieu to the lipophilic membrane of cells in the gastrointestinal tract. There the guest molecule is absorbed, since the membrane has a higher affinity for a lipophilic guest molecule than the cyclodextrin itself (23). The relationship of complexation to the absorption and distribution of a drug in the body is well documented in the literature (98).

The dissociation and association reactions of cyclodextrin complexes in solution are very rapid, and the equilibrium of free and complexed

drug is established instantaneously (23,99). This may perhaps be used to improve medication through the use of tablets containing both the hydrophobic drug complexed to cyclodextrin and the same drug in the free form. The former would enter the circulation very rapidly, while dissolution of the latter would occur slowly and form a drug depot (100).

Andersen, F.M., et al. (59), reported the absorption of femoxetine (a selective serotonin uptake inhibitor with antidepressant properties) in five human volunteers. In this study, five subjects were given six sugar-coated tablets each containing 100mg of femoxetine salt and femoxetine beta-cyclodextrin complex formulated as a suspension. The dose of the suspension was equivalent to 600mg femoxetine salt. The study was single dose and cross-over design. Each volunteer was given the doses with an interval of at least two weeks between each administration. The blood level was determined by gas chromatography. The area under the blood level curve (AUC) was calculated using the trapezoidal rule. The AUC for the cyclodextrin complex varied from 23 to 96  $\text{ng}\cdot\text{h}\cdot\text{kg}\cdot\text{ml}^{-1}\cdot\text{mg}^{-1}$ , while the corresponding values were 30 to 120  $\text{ng}\cdot\text{h}\cdot\text{kg}\cdot\text{ml}^{-1}\cdot\text{mg}^{-1}$  for the sugar-coated tablet. There was statistically no difference between the AUCs for the two formulations. The maximum plasma concentration was reached within one to four hours. The variations were similar to those observed in AUCs. In conclusion, the bioavailability of femoxetine beta-cyclodextrin complex, formulated as a suspension, was found to be similar to that observed from a sugar-coated tablet of femoxetine salt. However, this study would have been more meaningful if the results of the above study had been compared with (a) femoxetine tablet, preferably the uncoated tablet, or (b) with the same dosage form.



In vivo absorption studies were undertaken to find if the in vitro dissolution enhancement of digoxin from its cyclodextrin complex increases in the absorption of the drug (86). In this study, digoxin tablets containing 100/ug digoxin and its gamma-cyclodextrin complex containing 100/ug digoxin were administered orally to six dogs. The concentration of digoxin in the plasma sample was determined by enzyme immunoassay. The maximum plasma concentration of  $0.90 \pm 0.14\text{mg/l}$  at 45 minutes was obtained. This concentration level was three times higher than that of digoxin alone. The area under the plasma concentration time curve of the complex up to 24 hours was found to be 5.4 times as much as that of digoxin alone. In addition, the area under the curve of the complex containing 50/ug of digoxin was found to be superior to that of 100/ug digoxin alone. Thus, the improved bioavailability of digoxin by gamma-cyclodextrin complexation suggests a decrease in doses and fewer side effects in oral digitalis glycoside therapy.

Ukema, K., et al. (87), studied the hypoglycemic action of acetohexamide-beta-cyclodextrin complex with that of acetohexamide by oral administration in five male rabbits. The complex equivalent to 30mg/kg of acetohexamide was administered as a suspension in 80ml of water. In each case at least seven days were allowed between each blood glucose estimation. The reduction in the blood glucose level was observed in the system containing the complex. When results were compared using paired Student's t-test, the difference was found to be statistically significant. However, it is the opinion of the author of this thesis that, a detailed study should be made to elucidate the absorption mechanism of cyclodextrin complex.

Seo, H., et al. (88), studied in vivo absorption of spironolactone (SP) to determine whether or not the enhanced in vitro dissolution of SP from its beta or gamma-cyclodextrin complex increased the GI absorption of the drug. SP is a steroidal aldosterone antagonist that is widely used in the treatment of hypertension, edematous states, etc. Because of its low solubility in water, the bioavailability of the SP preparation is known to vary significantly among brands and batches (authors reported with proper references). In the above study, four male beagle dogs were administered orally a capsule containing (5mg/kg of body weight as SP), the drug, and its complex. The administration sequence was based on a crossover design with an interval of more than one week. The plasma concentration was determined by high pressure liquid chromatography. The standard curve range was from 20 to 200ng/ml. The beta and gamma-cyclodextrin complexes produced a maximum plasma concentration of  $103 \pm 28.3$  ng/ml and  $131 \pm 14.7$  ng/ml. at 90-minutes, respectively. These concentrations were about two to three times higher than that of SP alone. Similarly, the areas under the curves of complexes up to 24 hours were found to be more than two times greater than SP alone. The authors did not report any significant difference in time to reach the maximum concentration ( $T_{max}$ ). It is the authors' opinion of the above study that the enhanced bioavailability of SP produced by beta or gamma-cyclodextrin complexation makes possible the use of a lower dose with fewer side effects in (oral) SP therapy.

Tsuruoka, M., et al. (95), studied the absorption of freeze dried phenytoin, phenytoin and its beta-cyclodextrin complex in a group of four female beagle dogs. Dogs were given orally 300mg of phenytoin and its complex equivalent to 300mg phenytoin. The concentration of phenytoin

was determined by gas chromatography. There was a twofold increase in the area under the curve of the blood level. Further, it was reported in this study that increased bioavailability of phenytoin by means of beta-cyclodextrin complexation suggested the possibility of smaller doses and fewer side effects in phenytoin therapy. The details of the studies such as, statistical evaluations, etc., were not clearly reported.

Indomethacin and its beta-cyclodextrin complex were administered orally to rats (100). The rats were treated orally with 30mg/kg indomethacin and its complex equivalent to 30mg of indomethacin. Indomethacin level in the blood was measured by high pressure liquid chromatography. The maximum blood level was found between 1 and 4 hours after treatment. It was approximately 25% higher in the case of the complex. The details of the experimental design, such as number of animals, statistical evaluations, sensitivity of the assay method, etc., were not reported; however, based on the reported data it appears that the beta-cyclodextrin complex may be used to increase the bioavailability of indomethacin.

Tokumara, T., et al. (101), studied the bioavailability of cinnarizine with beta-cyclodextrin in three male beagle dogs. Two tablets of cinnarizine and its complex containing 25mg of cinnarizine in each tablet were administered orally to dogs. The concentration of the drug in the plasma was determined by high pressure liquid chromatography. In less than an hour the complex gave the maximum plasma level of  $166.9 \pm 22.4$ ng/ml, which was 8.6 times as high as that of cinnarizine alone. This initial increase in drug absorption might be due (according to the authors) to the 30 times higher dissolution rate of the complex than that of intact cinnarizine alone; however, there was no significant

difference between the areas under the plasma concentration-time curves (AUC). The AUC of cinnarizine and its complex up to eight hours was  $267.2 \pm 102.9$  and  $374.2 \pm 97.2$  ng·h/ml, respectively. This might be due (authors' opinion) to the large stability constant of the complex, estimated to be approximately  $6.2 \times 10^3 \text{M}^{-1}$  in water at 20°C.

Fromming and Weyermann (102), investigated the absorption of an orally administered salicylic acid and its beta-cyclodextrin complex in ten human volunteers (7 male and 3 female). The percentage of absorbed salicylic acid at a given time was reported as follows. From salicylic acid in one hour 24.8%, whereas from the complex 43.0% appeared in the blood; in two hours the values were 56.8% and 82.5%, respectively. Hence, a significant difference in blood level was reported.

Nambu, N., et al. (103), have reported the bioavailability of the powdered inclusion compounds with beta-cyclodextrin of four kinds of nonsteroidal anti-inflammatory drugs and the freeze dried drug alone. These drugs were flufenamic acid (FFA), ibuprofen (IPE), ketoprofen (KPF) and indomethacin (IMC). This study was done on three healthy albino rabbits and six healthy male beagle dogs. The drugs were administered to the stomach of each rabbit through a sonde, using fresh suspensions containing 100mg of FFA, 200mg of IPF, 100mg of KPF, and 50mg of IMC. In the case of the beagle dogs, the cross-over method was used with an interval of one week. The dogs were administered 100mg of KPF orally as a powder. The bioavailability results of only this drug in dogs were reported. The plasma concentration of FFA was determined by fluorescence spectrophotometer, the concentrations of IPF, KPF, and IMC were determined by gas chromatography. The details of the analytical methods and conditions such as sensitivity, reproducibility, accuracy, were not

reported. The FFA inclusion compound gave a high blood level compared with the simply freeze dried drug. The actual blood level was not reported. It was observed or estimated from the blood level curve that the maximum plasma concentrations of FFA freeze dried inclusion compound and freeze dried FFA were approximately 6ug/ml and 2.5ug/ml, respectively. The time to reach maximum plasma concentration was approximately one hour. However, there was a double maxima, and it is in the authors' opinion that further investigation is required to elucidate the double maxima on the blood level curve. A similar effect was observed in the case of IMC. In the case of IPF the freeze dried complex gave a high blood level compared to the simply freeze dried drug. The exact plasma concentration was not reported. Among the four drugs, KPF gave the highest blood level, and no double maxima phenomenon was observed. The KPF recovery in the urine was 60.5% for the freeze dried inclusion compound and 40.0% for the simply freeze-dried drug. In order to confirm the results obtained in the rabbits, a bioavailability study was carried out using beagle dogs. In the area under the curve up to four hours after administration the ratio was found to be 1.52 to 1.00 for the inclusion compound KPF against the simply freeze dried drug. There was a significant difference in the blood levels up to four hours, as determined by Student's-t-test.

Cyclodextrin complexes are not necessarily limited to oral use. Cyclodextrin complexes in suppositories have improved dissolution and bioavailability. For instance, Iwaoku, R. et al. (104), studied the absorption of phenobarbital from suppositories containing beta-cyclodextrin. In this study, five male albino rabbits were used, and a test suppository containing approximately 50mg phenobarbital was

inserted into the rectum. An interval of more than two days was allowed prior to the next experiment. Assay of phenobarbital in blood samples withdrawn from the ear vein was performed by gas chromatography; however, details of assay sensitivity, reproducibility were not reported. Blood levels of the drug containing the complex were much higher during the initial three hour period. There was a statistically significant difference in the extent of absorption compared to the rate of absorption.

The literature is replete with other reports on application of cyclodextrin complexes for the bioavailability of various drugs (105-117).

Summarizing the biological availability data, the author of this thesis feels as follows: Some of the published data demonstrate unambiguously that the cyclodextrin inclusion compounds are shown to improve bioavailability (in animals as well as in humans) of certain drugs. However, some of the reported studies are not well designed or may lack certain essential details; hence, reported results should be accepted with some reservations. However, there is a substantial number of papers that show that orally administered cyclodextrins are safe and useful in the enhancement of bioavailability. There are some cases in which it has been shown that cyclodextrins are not only useful in the enhancement of bioavailability but also in the reduction of side effects. It has been reported that for digoxin there was a correlation between in vitro dissolution enhancement by complexation and in vivo absorption in animals.

## 7. Target organ oriented dosage forms

It is understood by the author of this thesis that certain research groups in the U.S. pharmaceutical industry are exploring the possibility of developing drug delivery systems containing cyclodextrins for target organ oriented dosage forms. In particular, they are focussing their attention on systems containing drugs administered by the parenteral route for the treatment of cancer.

### G-The development process for a new pharmaceutical dosage form

Today the discovery, development, and marketing of a new drug is a time consuming and costly procedure. The development of a new drug usually begins with a decision made by the scientific advisory board of a pharmaceutical company. When the decision is made to create a drug for use in a particular state, virtually all departments within the company or institute become involved in its development, such as chemical and biological research, pharmaceutical development, regulatory department, etc. This multifaceted approach is frequently so complex that for this reason at first only regulatory and technical aspects are discussed.

#### 1. Regulatory aspects

When the Federal Food, Drug, and Cosmetic Act of 1938 was passed, a new era of drug development began. Prior to the institution of clinical testing in humans, a Notice of Claimed Investigational Exemption for a new drug must be filed with the Food and Drug Administration (FDA) by the sponsor. This form is also referred to as an IND or investigational new drug. These regulations appear in section 505 of the act (118-120). A "new drug" is one not presently recognized by experts in the field of clinical pharmacology to be safe and effective based on currently available clinical evidence. All the definitions of a new drug may be

found in the FDA consumer (121). The Supreme Court held in FDA: Prem that " new drug " could include a new dosage form, not just a new chemical entity. Originally a sponsor could begin clinical trials immediately after filing his IND; today, however, the regulations provide for a 30-day waiting period after which, if the FDA has not responded negatively, the clinical trials may commence. The sponsor, upon completion of a sufficient amount of clinical work to demonstrate the safety and effectiveness of the new drug for the use or uses for which it is intended, may then submit a new drug application (NDA) to the FDA. This application must include: (a) detailed reports of the preclinical studies, (b) reports of all clinical studies, (c) information on the composition and manufacture of the drug and on the controls and facilities used in its manufacture according to current good manufacturing practices (CGMPs), good clinical practices (GCPs), etc., (d) samples of the drug and its labeling. Once a new drug application (NDA) is approved, any change in the manufacturing, packaging, or other physical properties of the drug that may have an effect on safety and effectiveness must be covered by a supplemental new drug application. Because of today's myriad regulations, the NDA submission has become a compilation of information that could be compared in size to any one of the well known encyclopedias. Currently, the period from the time of synthesis of a compound to its release for marketing is generally some ten-year or longer (122-124). The FDA does try to minimize duplication of effort in preparing application for drugs about which some of the needed information is already available, by allowing use of that information with assurance that the new product will be equivalent to established marketed products. In this regard, the detailed description of the



concept of an abbreviated new drug application (ANDA) was published in 1975 (125). Unlike the NDA, which requires submission of well controlled clinical studies to demonstrate effectiveness, data to show safety, and detailed description of the manufacturing and packaging of a drug as well as stability data, the ANDA requires the following: a description of the components and composition of the dosage form to be marketed; brief statements that identify the place where the drug is to be manufactured; the name of the supplier of the active ingredients; an outline of the methods and facilities used in the manufacture and package, etc. (126). The purpose of the ANDA procedure is to eliminate unnecessary and costly animal and human experimentation and to make all drug substances not covered by patents readily available to the consumer in a competitive market. As discussed in the sections B and C of this introduction, detailed studies of the metabolism and toxicity have shown that the orally administered beta-cyclodextrin is innocuous. The cyclodextrins administered in high doses subcutaneously, intraperitoneally, or intravenously can induce renal damages in the rat, but at lower doses the compound can be administered without any ill effects (20,21). Discussion with people from the pharmaceutical industry suggests that several companies are working on the formulation and evaluation of pharmaceutical products containing cyclodextrins, while other companies are waiting to see a cost reduction and improved availability of cyclodextrins before proceeding with their studies. For instance, Squibb is evaluating parenteral administration of a pharmaceutical product containing cyclodextrin, while in Japan prostaglandin E1 is now commercially available in the form of its cyclodextrin complex (15,23).

## 2. Technical aspects

The development of a pharmaceutical formulation involves a number of considerations, such as, physico-chemical stability, availability of materials, cost factors, equipment, proper facilities, etc. As previously mentioned in section F of this introduction, cyclodextrins can be used for the preparation of inclusion compounds which demonstrate improved physico-chemical stability, dissolution, bioavailability, etc., of pharmaceuticals. At present, even though the cyclodextrins are somewhat expensive for large scale use because of their monumental potential in various fields, in the future the cost of the cyclodextrins may well become more reasonable for pharmaceutical purposes. When a formulator is called on to develop a suitable dosage form for a new drug, he or she would first consider solid dosage forms. Formulation and stability problems arise less frequently with solid dosage forms than with liquid pharmaceutical preparations (127). However, the formulations of solid dosage forms are not an easy task. The development of a solid dosage form can be traced through three stages; formulation design, process development, and process validation, prior to actual production. The process development and validation are often considered as a separate stage in dosage form development. The technical aspects of solid dosage forms are as follows.

- a. The determination of chemical and physical characteristics of the active ingredient and its compatibility with potential formulation ingredients.
- b. The determination of formulation tolerances, flow characteristics, compressibility, selectivity of methods of manufacturing, etc.

c. The evaluation of the final product to meet requirements for a variety of properties such as, appearance, hardness, friability, weight variation, disintegration, dissolution, consumer acceptability, etc.

Some or all of these evaluations ought to be done in order to develop a solid dosage form. When a formulator faces the problem of getting sufficient quantity of active ingredients, as is quite common, an innovative modification of techniques to a semi-micro scale may well be needed. In this thesis some of these problems are considered and possible solutions are developed.

#### H- Objectives of this study

As has been noted in previous section of this introduction, the literature has a wealth of publications which report on the ability of cyclodextrins ( especially beta-cyclodextrin ) to form inclusion compounds with a wide variety of drugs and other guest molecules. Cyclodextrin complexes have attracted a number of different types of scientific study ( for example, some biochemists have used cyclodextrin complexes as a model for evaluating enzyme activity ). A number of possible pharmaceutical applications of cyclodextrins have been suggested. In particular, there are abundant data which clearly demonstrate that, for some drugs, chemical or physical stability, organoleptic properties, and dissolution can be radically improved by use of cyclodextrins.

The literature is replete with papers which report laboratory studies of the interaction of a wide variety of drugs with cyclodextrins. The number of interaction studies described in the literature is almost equal to the number of researchers in the pharmaceutical field. However, there is a distinct paucity of papers which describe how such inclusion

compounds can be conveniently incorporated into pharmaceutical dosage forms. From the pharmaceutical point of view, if an inclusion compound with cyclodextrins is provided in the solid powdered form, it may be more widely used, since it is especially convenient for oral administration. However, there have been very few reports published concerning the inclusion compounds obtained in the solid powdered form. Additionally, although a number of methods are described in the literature for the production of the drug-cyclodextrin inclusion compounds at the laboratory level, little attention appears to have been given to producing these products at the pilot scale or manufacturing level. The success of a new drug or drug-complex depends on the development of practical formulations which can be readily processed by an economically viable method for general use.

The objectives of the present study are:

1. To prepare some drug-betacyclodextrin inclusion compounds in the solid powdered form by various techniques, with a view to providing information concerning which methods are most likely to be of use commercially.
2. To investigate properties of the complexes in aqueous solution and in solid state.
3. To evaluate the preformulation properties of inclusion compounds, such as, bulk properties, flow characteristics, compressibility, dissolution, etc.
4. To accomplish preliminary laboratory scale-up studies.
5. To prepare and evaluate potentially commercially acceptable pharmaceutical dosage forms of selected drugs.

The drugs selected for this study are ampicillin, phenobarbitone, and phenytoin. These compounds have a wide range of pharmacologic, and

physico-chemical properties and therefore allow us to explore a variety of different formulation and processing problems.

Data published by other authors ( post Jan.1982 ) are discussed, together with the data obtained in this study in the Result and Discussion section of this thesis. It is noteworthy that despite the wide interest in the pharmaceutical exploitation of cyclodextrins there is at the time of writing ( June 1985 ) still no pharmaceutical product containing cyclodextrins approved for marketing in the U. S.

## II EXPERIMENTAL

A-Materials

## 1. Drugs

Ampicillin anhydrous (43F-0746)

(Sigma Chemical Company, St. Louis, MO)

Phenobarbitone (G19204F04)

(Amend Drug and Chemical Co., Irvington, NJ)

Phenytoin (63F-3449)

(Sigma Chemical Company, Co., Irvington, NJ)

## 2. Formulation components and chemicals

Acetonitrile (724629)

(Fisher Scientific Company, Fair Lawn, NJ)

Ammonium hydroxide (E701005)

(Allied Chemical Division, Morrison, NJ)

Boric acid (710413)

(Amend Drug and Chemical Co., Irvington, NJ)

Citric acid (z6698M31)

(Amend Drug and Chemical Co., Irvington, NJ)

Colloidal magnesium aluminum silicate (82470)

(R.T. Vanderbilt Company Inc., New York, NY)

Dicalcium phosphate dihydrate (9187)

(Encompress, Edward Mendell Co., Carmel, NY)

Dimethylacetamide (33F-0831)  
(Sigma Chemical Company, St. Louis, MO)

Ethanol  
(U.S. Industrial Chemicals, Inc., Newark, NJ)

Glycerol (J26123K29)  
(Ruger Chemical Co., Irvington, NJ)

Hydrochloric acid (026490)  
(E.I. Dupont De Nemours & Co., Inc., Wilmington, DE)

Magnesium carbonate (27061M31)  
(Ruger Chemical Co., Irvington, NJ)

Magnesium stearate (772716)  
(Fisher Scientific Company, Fair Lawn, NJ)

Methanol (743275)  
(Fisher Scientific Company, Fair Lawn, NJ)

Methyl cellulose 1500cps (MM121926c)  
(Ruger Chemical Co., Irvington, NJ)

Microcrystalline cellulose (14261)  
(Avicel PH 101, FMC corporation, Philadelphia, PA)

Monobasic potassium phosphate (722964)  
(Fisher Scientific Company, Fair Lawn, NJ)

Peppermint oil (J26477M16)  
(Amend Drug and Chemical Co., Irvington, NJ)

Phosphoric acid (y-115)  
(Allied Chemical Division, Morrison, NJ)

Polyethylene glycol 200 (B3459A26)  
(Ruger Chemical Co., Irvington, NJ)

Polyethylene glycol 300 (J19526B10)  
(Ruger Chemical Co., Irvington, NJ)

Polyethylene glycol 400 (D14508k06)  
(Ruger Chemical Co., Irvington, NJ)

Potassium chloride (950373)  
(J.T. Baker Chemical Co., Phillipsburg, NJ)

Propylene glycol (025395)  
(J.T. Baker Chemical Co., Phillipsburg, NJ)

Sodium hydroxide (720859)  
(Fisher Scientific Company, Fair Lawn, NJ)

Sodium Starch glycolate (2166)  
(Explotab, Edward Mendell Co., Carmel, NY)

Sorbitol (H22101P20)  
(Ruger Chemical Co., Irvington, NJ)

Spearment oil (H18261110)  
(Amend Drug and Chemical Co., Irvington, NJ)

Sugar  
(Sweet Life Products Corp., Suffield, CT)

### 3. Equipment

Apple Computer and Printer  
(Apple Computer, Cupertino, CA)

Brinkmann Mini Spray Drier  
(Brinkmann Instruments, Inc., Westbury, NY)

Carver Laboratory Press  
(Fred S. Carver Inc., Menobone1 Falls, WI)



Chart Strip Recorder

(Cole-Parmer Instrument Company, Chicago, IL)

Constant Temperature Refrigerated Bath

(Blue M Electric Company, Blue Island, IL)

Differential Calorimeter

(Perkin Elmer, Norwalk, CT)

Erweka Hardness Tester

(Erweka Apparatabau, W. Germany)

Fisher-Wheel Sieve Shaker

(Central Scientific Company, Chicago, IL)

Fixed Wavelength Detector

(Water Associates, Milford, MA)

Hull Freeze Drier

(Hull Corp. Hatboro, PA)

Infrared Spectrophotometer

(Perkin Elmer, 457, Grating Infrared Spectrophotometer, NJ)

Kitchen Aid Mixer

(The Hobart Mfg. Co., Troy, OH)

Markson Digital pH meter

(Markson, Science Inc., Delmar, CA)

Mettler Balances

(Mettler Instrument Corporation, Princeton, NJ)

Mettler Digital/Analog converter

(Mettler Instrument Corporation, Princeton, NJ)

Microscope

(Leitz, Wetzlar, Germany)

Millipore Filter Holder  
(Millipore Corporation, Bedford, MA)

Millipore Vacuum Pump  
(Millipore Corporation, Bedford, MA)

uBondapak C<sub>18</sub>  
(Water Associates, Milford, MA)

Ohaus Moisture Balance  
(Ohaus Scale Corporation, Florham Park, NJ)

Proton Magnetic Resonance  
(Varian EM390, Palo Alto, CA)

Roche Friabilator  
(Erweka Abrasion Tester, Erweka GmbH, Frankfurt, Germany)

Solvent Delivery System  
(Water Associates, Milford, MA)

Stokes Hot Air Oven  
(Stokes-Penwalt Co., East Stroudsburg, PA)

Stokes Model F Tablet Press  
(Stokes-Penwalt Co., East Stroudsburg, PA)

Stokes Model B<sub>2</sub> Rotary Tablet Press  
(Stokes-Penwalt Co., East Stroudsburg, PA)

Thickness Gauge  
(Vanderkamp, Vankel Industries, Chatham, CA)

Turbula Rapid Blender  
(Wiky A. Bachoven Co., Troy, OH)

USP Disintegration Apparatus  
(Vanderkamp, Vankel Industries, Chatham, CA)

USP Dissolution Apparatus

(Vanderkamp, Vankel Industrues, Chatham, CA)

UV Spectrophotometer

(Hitachi 200, Perking Elmer Corporation, Norwalk, CT)

Virtis Freeze Drier

(Virtis Company, Gardiner, NY)

WISP 710B

(Water Associates, Milford, MA)

X-ray Diffractometer

(Philips Electronin Division, Mount Vernon, NY)

Glassware and common laboratory equipment as available in  
the College of Pharmacy

## B- Methods

1. Studies of the interaction of beta-cyclodextrin with  
ampicillin, phenobarbitone and phenytoin.

### a. Ampicillin

Interaction of beta-cyclodextrin with ampicillin was assessed by studying the influence of the beta-cyclodextrin on the rate of hydrolysis of the ampicillin at 37<sup>o</sup>C in hydrochloric acid buffer of pH 2.0. The hydrochloric acid buffer was prepared in accordance with USP XX (128). A known amount of ampicillin was added to each of a series of different molar concentrations of the beta-cyclodextrin solution. The molar concentrations of ampicillin to beta-cyclodextrin were 1:1.0, 1:1.5, and 1:2.0. The solutions were transferred into a 100 ml volumetric flask, stoppered and then kept at 37<sup>o</sup>C in constant temperature bath. Samples

were taken at 0,3,6,9, and 12-hour time intervals. After appropriate dilution all samples were assayed by High Pressure Liquid Chromatography (HPLC). This assay procedure was basically that described by Das Gupta, V., et al.(129). This method has been validated and is currently in routine use in a pharmaceutical company. Hence there was no need to fully validate the method again. However, the reproducibility, accuracy and precision were determined, these values are reported in Appendix A, 1.

The HPLC system consisted of a solvent delivery system, the WISP (Waters Intelligence Sample Processor), and a variable wavelength detector. The separation was performed on a 3.9mm (id) x 30cm uBondapack C<sub>18</sub> (reversed phase) column. The chromatographic peaks were recorded using a chart recorder. The mobile phase employed in the analysis of ampicillin consisted of 1.4gm./l potassium phosphate in acetonitrile/water (7:93 v/v). The pH was adjusted to 4.1 with phosphoric acid. The following chromatographic conditions were employed (129): flow rate = 2.0ml/min.; detector wavelength=240nm; 0.05 AFUs (Absorptions Full Scale Units); and injection volume 20  $\mu$ l.

A series of dilutions from a standard solution ( $3.15 \times 10^{-3}$  M) was made and the peak height was determined. A calibration curve was constructed by plotting the peak height of each standard dilution against its known concentration (Appendix A, 1).

Beer's law states:

$$A = abc \qquad \text{Eq. (4)}$$

Where A = absorbance or peak height

a = absorptivity

b = sample path length

c = concentration

Applying Beer's law, the slope of the straight line obtained by the absorbance (peak height in this case) as a function of concentration is equivalent to the term  $abc$  in Eq. (4).

By rearranging Eq. (4) to

$$c = A/\text{slope} \quad \text{Eq. (5)}$$

The concentration of an unknown sample may be calculated.

A graph of log concentration of the drug as a function of time was then plotted. The hydrolytic degradation constant ( $k_d$ ) and half-life ( $t_{1/2}$ ) with and without beta-cyclodextrin were calculated using Eqs. (6) and (7), respectively.

$$k_d = -\text{Slope} \times 2.303 \quad \text{Eq. (6)}$$

$$t_{1/2} = 0.693/k_d \quad \text{Eq. (7)}$$

The stability constant was calculated using Eq. (8) according to Griffiths and Bender (130).

$$k_{\text{obs}} - k_o = - (k_{\text{obs}} - k_o)/K (\text{Beta-CD}) + k_c - k_o \quad \text{Eq. (8)}$$

Where  $k_o$  and  $k_c$  are the pseudo-first order rate constants for the degradation of uncomplexed and complexed ampicillin, respectively.  $k_{\text{obs}}$  is the observed pseudo-first order rate constant, and  $K$  is the apparent stability constant for the complex. A plot of  $(k_{\text{obs}} - k_o)$  as a function of  $(k_{\text{obs}} - k_o) / \text{beta-cyclodextrin concentration}$  was then plotted. From the slope and intercept of the plot  $k_o$ ,  $k_c$ , and  $K$  were calculated.

## b. Phenobarbitone

Interaction of beta-cyclodextrin with phenobarbitone was studied by solubility analysis. The procedure used in this study was the solubility method of Higuchi and Lach (131). Quantities in excess of the normal solubility of phenobarbitone were weighed into 20-ml vials and added to 10-ml of aqueous solution containing various concentrations of beta-cyclodextrin. The vials were capped, sealed with a tape, and then agitated on a mechanical shaker in a constant temperature bath at 25°C until the system reached equilibrium. Two replicates were studied to determine the equilibrium point by assaying the samples at 17, 24, and 40 hours as follows. At the end of each time period, contents of the vials were filtered through (0.45u) filter paper. Aliquot portions of the filtrate were properly diluted and assayed for drug content. It was ascertained that a 24-hour time point was sufficient to reach the equilibrium point.

Based on the principles described above in section B, 1; a calibration curve was generated (Appendix A, 2). A series of dilutions from a standard solution ( $1.72 \times 10^{-3}$  M) was made and injected using the following chromatographic conditions which are a minor modification of the published method (132): flow rate = 1.5ml/min.; detector wavelength-254nm; injection volume - 10ul. The same HPLC system was used as described in section B, 1. The mobile phase employed in the analysis of phenobarbitone consisted of acetonitrile, methanol, and water (20:20:60 v/v). A phase solubility diagram was constructed according to the method described by Higuchi and Connors (47). The stability constant was calculated from the phase solubility diagram as described in the section E, 1 (Eq. 1) of the introduction.

### c. Phenytoin

Interaction of beta-cyclodextrin with phenytoin was studied by solubility analysis. The solubility was determined in a pH 7.0 using the Therell and Stenhagen buffer system (133). The method was as described in section 1, b, except that the drug was assayed by ultra-violet absorption at the wavelength of 225nm. The USP (128) value for this drug is at the wavelength of 258nm in water. However, at this wavelength there was no maximum absorption observed even with the USP standard of this drug. Hence,  $E_{1\text{cm}}^{1\%}$  was determined as in the Isolation and Identification of Drugs edited by Clarke (134). The value was found to be 27.8 which is within 5% of the value reported by Clarke. Hence the reported wavelength (225nm) at which a maximum absorption was observed and taken as  $\lambda_{\text{max}}$  in a given medium. Based on the principles described in the method section B, 1, a calibration curve was generated (Appendix A, 3) from a series of dilutions of a standard solution ( $7.93 \times 10^{-5}\text{M}$ ). It was determined that the cyclodextrin did not interfere with the spectrophotometric measurements at the concentration employed (Appendix A, 4). The stability constant was calculated from the phase solubility diagram as described in the section E, 1 (Eq. 1) of the introduction to this thesis.

### 2. Preparation of Drug-cyclodextrin complexes

a. Solvent evaporation : Equimolar quantities of drugs and beta-cyclodextrin were added to a 50:50 mixture of water and ethanol. All the solids were dissolved by warming (up to  $40-45^{\circ}\text{C}$ ) and stirring the mixture. The solution was kept at room temperature for approximately five days to allow complete evaporation of solvents. Moisture content was checked using a moisture balance.

b. Kneading : Equimolar quantities of drugs and beta-cyclodextrin were dry mixed thoroughly in a Kitchen-Aid Mixer for about 30-min. This mixture was kneaded with a small amount of water to make a slurry and the slurry was stirred well. The slurry was allowed to stand for about an hour and then dried at 35<sup>o</sup>C for approximately two-hours and screened through 20 mesh screen.

c. Freeze drying : Equimolar quantities of drugs and beta-cyclodextrin were dissolved in water. It was necessary to add in some cases less than 2 % of 28 % ammonium hydroxide solution to dissolve the drug completely. The solution was filtered through a 100 mesh screen and then freeze dried using a laboratory model and as well as an industrial model freeze drier. The details of the freeze drying operating conditions are given in Table II. This freeze drying procedure was basically that described by Kurozumi, et al.(51).

d. Spray drying : As described in the freeze drying procedure, solutions were prepared and spray dried using a laboratory model spray drier. The details of the operating conditions are in Table III.

### 3. Evaluation of the complexes

a. Stability and solubility analysis : As described in the section B, 1 of the introduction, stability and solubility studies were carried out.

b. Infrared (IR) spectroscopy : Approximately one to two mg of drug was ground thoroughly with 80-90mg of potassium bromide (KBr) in order to give a thin transparent disk. Then IR spectra were measured using Perkin Elmer, 457, grating infrared spectrophotometer. Dr. Sandeep Gupta (Department of Pharmacognosy, University of Rhode Island) supervised this work.



Table II

## -Freeze drying procedure-

- \*1. Prefreeze the solution to be freeze dried.
2. Turn on refrigeration before turning on vacuum pump.
3. Do not proceed unless the temperature is between  $-40$  to  $-50^{\circ}\text{C}$  or lower.
- \*4. When the temperature is  $-40^{\circ}\text{C}$  or lower, load samples.
5. Turn vacuum pump on
6. Operate vacuum pump ballast closed.
7. When the desired vacuum or pressure is reached (approximately 200u), turn the heater on.
- \*8. Dry the product between 40 to 50, till a marked decrease in chamber pressure or vacuum occurs.
9. When the drying cycle is complete, turn off heater and vacuum.
10. Slowly break the vacuum.
11. Turn off refrigeration.
12. Unload the product.

---

\*- Necessary only for laboratory freeze drier.

\*\* - Difficult to monitor the product temperature and chamber pressure using a laboratory freeze drier.

\*\* - These two parameters were monitored using an industrial model freeze drier (Appendix B).

Table III  
Spray drying procedure

1. Heat the unit to the desired temperature.
2. As soon as the inlet temperature has stabilized, adjust and stabilize the outlet temperature.
3. Turn on compressed air pressure to the desired value.
4. When the desired values have been achieved, the unit is ready for the spraying operation.
5. Adjust the aspirator to regulate the spraying.

---

\*- Following conditions were used for the present study.

Inlet temperature- 120 to 140<sup>o</sup>C

Outlet temperature - 80 to 90<sup>o</sup>C

Air pressure - 70 to 80 PSI

Pump scale - 5

Aspirator scale - 10

Heating scale - 10

Flow indicator scale - 50

c. X-ray diffractometry : Specimens were placed in a shallow squareshaped glass holder. The holder was flat on one side, approximately measuring 1-5/16" x 1-3/16" x 1/8". The  $(2\theta)$  10-50<sup>0</sup> diffraction angles were measured from the CrKalpha (2.29 A<sup>0</sup>) at a scanning speed of 2<sup>0</sup>/min. and 500 cycles per second. Dr. Thomas Rockett (Department of Chemical Engineering, University of Rhode Island) supervised and advised in the interpretation of spectra.

d. Measurement of lattice parameters : A single crystal of the complex was obtained from the solvent evaporation method, and lattice parameters were measured using Weissenburg and Precession Photographs. Dr. Clair Cheer (Department of Chemistry, University of Rhode Island) supervised and assisted in this work.

e. Proton magnetic resonance (PMR) spectroscopy : Approximately 10-20mg of samples were dissolved in deuterium oxide solvent and filtered (a cotton plug was used as a filter on the pipet tip). The PMR spectra were taken using a Varian EM 390, spectrometer. Due to deuterium oxide fixed position, this peak was used as an internal standard in the comparison of the chemical shifts of the host and the guest. Dr. Nandu Bongale and Mr. Amarendra Mikkeleni (Department of Medicinal Chemistry, University of Rhode Island) supervised and advised in the interpretation of spectra.

f. Differential scanning calorimetry (DSC) : Samples of approximately 5-10mg were placed in aluminum pans and the 120-400<sup>0</sup>C temperature range was measured using a Perkin Elmer Calorimeter. The heating rate was 5<sup>0</sup>/min. and the range was 5mcal/sec. at a chart speed of 2cm/min. Dr. Mahendra Dedhiya (Miles Pharmaceuticals, West Heaven, CT.) supervised this work.

g. Photomicrographs : Samples were placed on a mounting slide with a drop or two drops of low refraction liquid. Microphotographs were taken on a Leitz microscope. All samples were photographed using the same liquid at 125x magnification.

#### 4. Properties of drug-cyclodextrin complexes

##### a. Drug content in the freeze dried complex

###### (i) Phenobarbitone beta-cyclodextrin complex

Approximately 100mg of a weighed sample of the complex was dissolved in 100ml water and 1ml was diluted to 10ml. Three injections were made in triplicate, and concentration was determined by HPLC as described in the experimental section B, 1b.

###### (ii) Phenytoin beta-cyclodextrin complex

Approximately 100mg of a weighed sample of the complex was dissolved in 100ml of a pH 9.0 buffer (Therell and Stenhagen buffer system). Samples were appropriately diluted for spectrophotometric analysis at 225nm. The concentration of the drug was determined as described in the experimental section B, 1c.

##### b. Scale-up and Yield improvement

The freeze drying method for the preparation of the complexes was scaled-up from a laboratory freeze drier to an industrial model freeze drier. The yield was improved from 57% to more than 95%. This significant improvement in the yield was achieved from an open tray drying system to a closed tray drying system. These systems are depicted in Figures given the Results and Discussion of this thesis.

##### c. Particle size distribution

A nest of sieves, sizes (U. S. Standard) 20,40,60,80,100,140, and 200, was used to measure the particle size distribution. A known amount

of sample was placed on the top screen and the sieve stack was shaken for 30-min. on a Fisher-Wheel Sieve Shaker. The fractions in each sieve were collected, weighed and percent underweight values were calculated.

d. Bulk and Tap densities

A sample of 50 cm<sup>3</sup> of powder was carefully introduced into a tared 100 cm<sup>3</sup> graduated cylinder, weighed, tapped 100 times and measured the resulted volume. If V is the tapped volume in cm<sup>3</sup> of W grams of material in the cylinder, then the tapped density in gm/cm<sup>3</sup> is given by

$$Td = W/V \quad \text{Eq. (9)}$$

and bulk density

$$Bd = W/50 \quad \text{Eq. (10)}$$

Where Td and Bd represent the tapped and bulk densities, respectively.

Compressibility was then determined as follows.

$$\% \text{ Compressibility} = (Td - Bd)/Td \times 100 \quad \text{Eq. (11)}$$

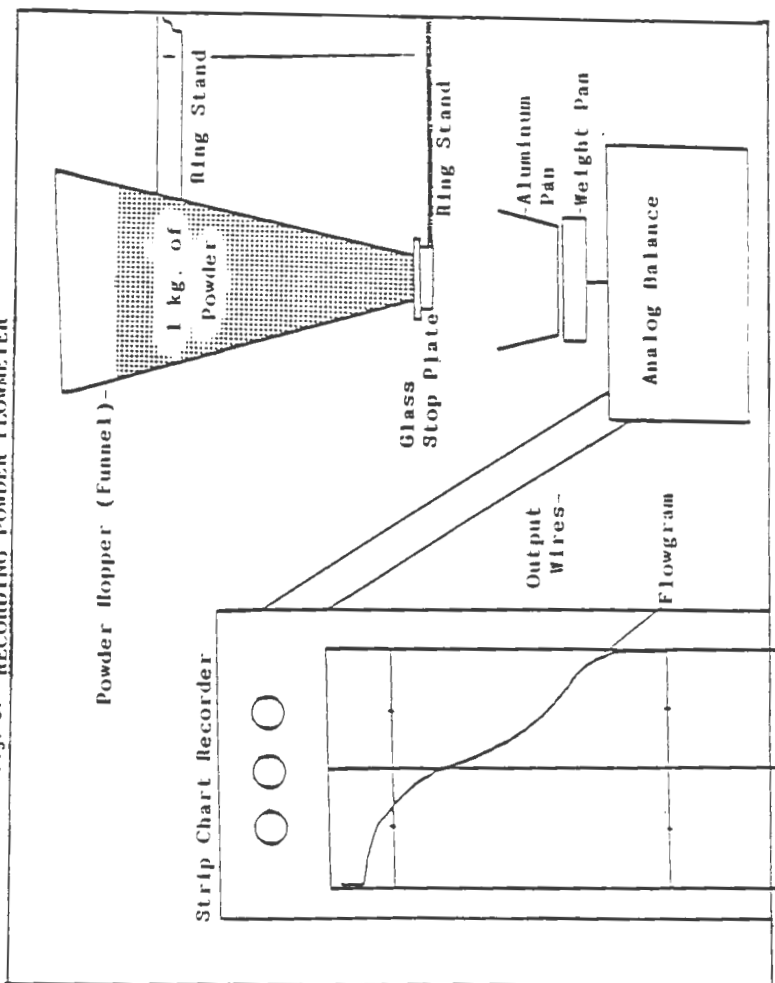
e. Moisture content

The moisture content of each material was determined using an Ohaus Moisture Balance, operating at a temperature of 80°C (4 watt light) for a period of 30-min. The percent loss was directly read from the balance.

f. Flowability studies

Powder flow was determined using a recording flow meter (Fig. 6). The powder flow meter design, procedure, calculations of linearity index, etc., were developed and described by Jordan and Rhodes (135), and Rudnic, et al.(136). The same procedure was used in this study. However, the use of the flow meter was extended to study the effects of small mass and geometry on flow rates. This miniturization of flow studies was undertaken so that flow data could be obtained under

Fig. 6: RECORDING POWDER FLOWMETER



conditions when only small amounts of material are available. This makes recording powder flowmeter data useful in numerous quality control and in-process powder flow testing situations. Hence, a stainless steel hopper, a conical glass funnel, measuring 12cm (top diameter) by 38cm (length) and 1.9cm (orifice diameter), and a cylindrical glass funnel, measuring 5cm (top diameter) by 26.5cm (length) and 1.5cm (orifice diameter) were used to determine mass and geometry effects on flow rates. The delivery of a specified weight was timed, then mass flow rates were determined for three runs of each system. The value is only a mean flow rate, and the flow rate change during the flow process. A measure of this change is the linearity value, which was calculated as follows.

$$f_l = (r^2 - 0.8) \times 100 \quad \text{Eq. (12)}$$

Where  $f_l$  is the powder flow index and  $r^2$  is the least squares correlation coefficient.

#### g. Compressibility studies

Compression measurements were made using an instrumented Stokes B rotary press, located in the Department of Pharmaceutics of the University of Rhode Island. This tablet press was instrumented with four piezo-electric transducers and interfaced with an Apple IIe computer. The software to the Apple computer (developed by Mr. J. Hoblitzell) enabled the calculation of compression and ejection forces. In addition, it also calculates mean area under the compression, ejection curves, mean area to height ratio for compression and standard deviation for all the above parameters.

C- Formulation of liquid dosage forms.

1. Solution preparation and evaluation

The compositions of two potential formulations are shown in Tables IV and V. Once the dosage form was prepared, its properties were evaluated on daily basis to weekly, and then one month. These evaluations were as follows:

- a. Visual examination for clarity, such as, precipitation, crystal growth, change in color, odor, etc.
- b. Assay of drug content (HPLC) after samples had been stored at room temperature for one month.

2. Suspension preparation and evaluation

A final suspension formula is given in Table VI. The suspension was evaluated as follows.

- a. Visual examination : The supernatants of the stored suspensions were examined for evidence of opalescence caused by very small particles, which are slow to settle.
- b. Sedimentation study : The sedimentation volumes were recorded in terms of the ultimate settled height (Hu), to the original height Ho (137).

$$\% \text{ Sediment} = Hu/Ho \quad \text{Eq. (13)}$$

- c. Measurement of redispersibility study : To standardize the evaluation of redispersibility a blender was modified (Fig. 7), such that the cylinder would turn through  $360^\circ$  at a speed of 20-22 rpm. The number of revolutions necessary to restore the suspension to homogeneity was recorded. Similar methods of evaluation have been reported in the literature (137).

- d. Photomicrographs : As described in the experimental section B, 3g.



Table IV  
Phenobarbitone solution formulation # 1

	% Ingredients
Phenobarbitone beta-cyclodextrin complex *	2.0
Glycerol	40.0
Sorbitol	20.0
Simple Syrup **	20.0
Distilled water to make	100.00

---

Dose = 20mg/5ml

\* - Contains 20% drug

\*\* - Prepared according to the USP XX

Preparation - Dissolved the complex in the mixture of glycerol, sorbitol, and simple syrup. Sufficient water was added to the mixture to desired volume. The final product was mixed well by means of a magnetic stirrer and filtered through 0.45u filter paper. Upon filtration the product was flavored ( Orange oil approximately three drops ) and mixed well.

Table V  
Phenobarbitone solution formulation # 2

	% Ingredients
Phenobarbitone beta-cyclodextrin complex	2.0
Glycerol	10.0
Sorbitol	40.0
Simple Syrup	20.0
Distilled water to make	100.0

Preparation : As described in Table IV.

Table VI  
Phenytoin suspension formulation

	% Ingredients
Phenytoin beta-cyclodextrin complex *	10.0
50 : 50 mixture of water and glycerol quantity sufficient to make	100.0

---

Dose = 100mg/5ml

\* Complex contains 20% drug.

Preparation : The complex was dispersed in the 50 : 50 mixture of water and glycerol and then made up to desired volume. The suspension was homogenized using an hand homogenizer, flavored with Banana flavor, and mixed well by means of a Motor Generator Monomatic stirrer.

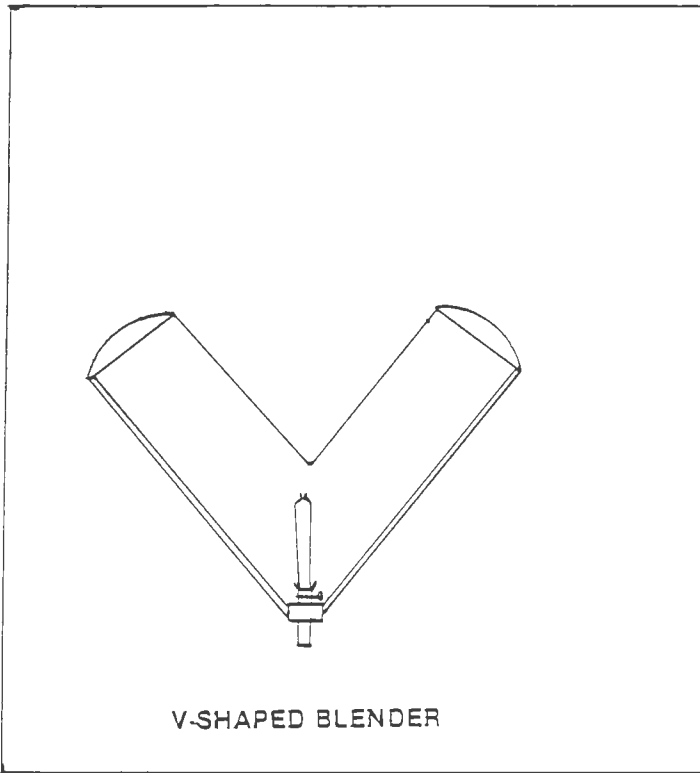


Fig. 7: A device for redispersibility measurement of the suspension.

#### D- Formulation and evaluation of solid dosage form.

Tablet formulation and preparation are given in Table VII. Tablets were evaluated as follows:

##### a. Appearance

Tablets were examined using a 10X magnifying glass for chipping, cracking, picking or mottling of the surface as an in-process check.

##### b. Weight

The weight of each individual tablet was determined after dedusting. This procedure was repeated for twenty tablets. The data from the tablets were analyzed for sample mean and standard deviation.

##### c. Thickness

The thickness of ten tablets was determined by first dedusting, and then placing each of them in the jaws of a micrometer. The measurements were recorded and analyzed for mean value and standard deviation.

##### d. Hardness

The hardness of ten tablets was determined by placing each tablet in the hardness tester (Erweka), which recorded the breaking strength of the tablet in kilograms. This procedure was repeated and the data were analyzed for sample mean and standard deviation.

##### e. Friability

This test is a measure of the abrasion resistance which was determined by first weighing twenty tablets after dedusting, then placing them in a tumbling chamber for four minutes or 100 revolutions. The tablets were again dedusted and weighed after tumbling, and the percent friability was determined as follows:

$$\% \text{ Friability} = ( I_w - F_w ) / I_w \times 100 \quad \text{Eq. (14)}$$

Where  $I_w$  and  $F_w$  represent the initial and final weights, respectively.

Table VII  
Phenytoin tablet formulation

	% Ingredients
Phenytoin beta-cyclodextrin complex *	80.00
Sodium starch glycolate	1.50
Magnesium stearate	0.75
Microcrystalline cellulose to make	100.00

---

Dose = 50mg/tablet

\* - Contains 20% drug

Preparation : Prewighed complex and sodium starch glycolate (0.75%) were thoroughly mixed for fifteen minutes in a Kitchen Aid Mixer, and wetted using water as a granulating agent. The wet mass was sieved (10 #) and dried at 40<sup>o</sup>C to approximately a moisture level of 5% as determined by a moisture balance. Dried mass was re-sieved (20 #) to produce uniform granules, then mixed for ten minutes with the remaining (0.75%) sodium starch glycolate and microcrystalline cellulose (app. 18%). The magnesium stearate was mixed in a Turbula Rapid Blender for three minutes with the tablet matrices and tablets were compressed. Punch diameter was approximately 0.95cm and target weight was 312.50mg.

f. Ease of manufacturing

The noise and vibration from the tablet press were carefully monitored subjectively to identify any problems in manufacturing tablets.

g. Disintegration

Tablet disintegration was tested using the USP apparatus, as described in the U. S. Pharmacopeia (128). The time needed for all the palpable fragments to pass through the screen at the bottom of the cage was detected visually and was recorded. Six tablets were used in each test, and mean and standard deviations were calculated.

h. Dissolution

When a drug was to be measured for rate and extent of dissolution, a sample of the lot of drug to be used was placed in various concentrations in a spectrophotometer to measure the  $\lambda_{max}$  for that particular drug. Once this value was determined, the spectrophotometer was set at that wavelength, and each sample was analyzed for absorbance. The result was recorded on a Beer's plot (as described in the method section B, 1) and correlation between absorbance and concentration was recorded. The monograph, as it appeared in the USP (128) was used as a reference for determining the dissolution medium for the test. In addition, dissolution was determined in acidic (pH 2.2) and basic media (pH 7.4). The required acidic and basic media were prepared in accordance with the USP (128). A sample of three and six tablets were used for each system. The samples at specified time intervals were withdrawn and diluted with dissolution medium to read the absorbance between 0.1 and 0.7. Because of the rather limited aqueous solubility of the phenytoin, samples of the tablets were carefully cut in to two halves and based on weighed portions

of the tablets, dissolved drug concentrations were determined. The concentrations were determined from the Beer's plot (Appendix A, 5).



### III Results and Discussion

The results and discussion are organized into the following main sections.

#### A. Physico-chemical properties of complexes

- a. Stability
- b. Solubility
- c. X-ray diffractometry
- d. Measurement of lattice parameters
- e. Differential scanning calorimetry
- f. Infrared spectroscopy
- g. Proton magnetic resonance spectroscopy
- h. Photomicrographs

#### B. Preparation of the complexes

- a. Solvent evaporation
- b. Kneading
- c. Spray drying
- d. Freeze drying

#### C. Preparation and evaluation of dosage forms

## A. Physico-chemical properties

### a. Stability

Complex formation of ampicillin with beta-cyclodextrin was studied using different molar concentrations of the beta-cyclodextrin with an ampicillin solution. Figure 8, depicts the results of this study. It is readily evident that the beta-cyclodextrin has a stabilizing effect on the ampicillin hydrolysis rate. The disappearance of ampicillin displayed a pseudo-first order kinetic behavior in the solutions with or without beta-cyclodextrin. From this figure and using equations six and seven the observed pseudo-first order rate constants and half-lives were calculated. The results are reported in Table (VIII). An analysis of variance (ANOVA) was used to evaluate any statistical significance among the treatments. There was a significant difference ( $\alpha=0.05$ ) between the treatments with 1:0.0 and 1:1.0 molar concentrations of ampicillin to beta-cyclodextrin. Furthermore, due to the single replicate nature of the data and the constant time period between observations it was deemed more appropriate to evaluate the colinearity of the degradation curves presented in Fig. 8. This analysis detected a significant difference ( $\alpha = 0.05$ ) between the slopes associated with the treatments using ratios of 1:0.0 and 1:1.0 of ampicillin to betacyclodextrin. In order to determine the optimal ratio of beta-cyclodextrin to ampicillin, a graph was constructed by plotting the observed degradation rate constants as a function of the beta-cyclodextrin concentration. As seen in Fig. 9, the observed degradation rate constant asymptotically approaches a minimal value as the beta-cyclodextrin concentration is increased. This saturation behavior is a characteristic of reactions which proceed

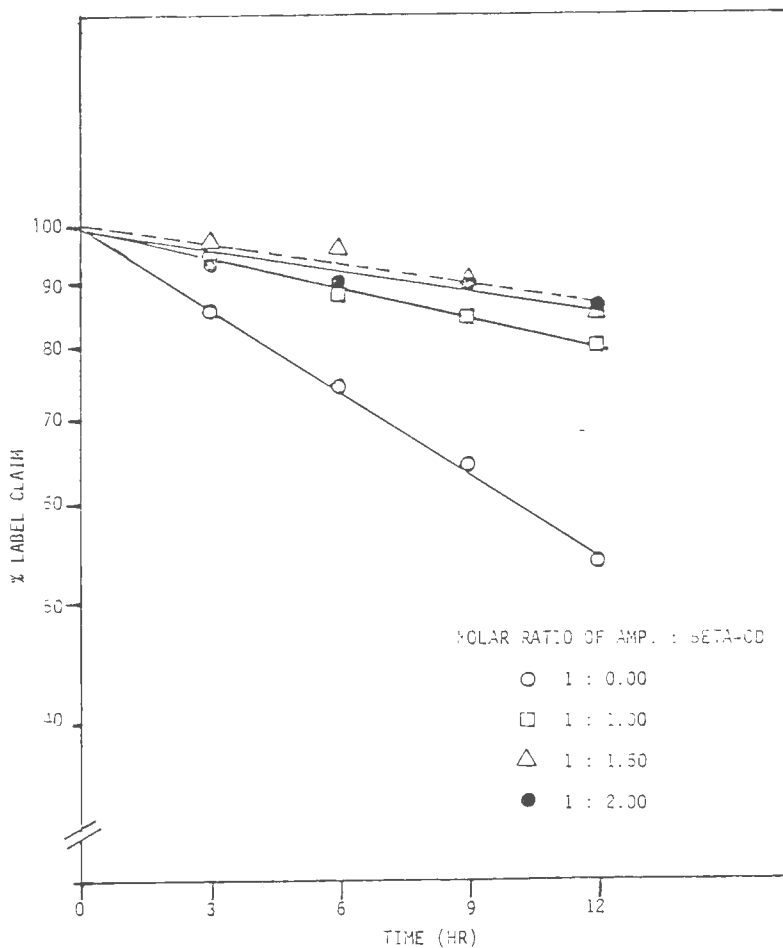


Fig. 8: Hydrolysis of ampicillin with different molar concentrations of beta-cyclodextrin at pH 2.0 and 37°C

\* Each point represents an average of three determinations

Table VIII

Degradation constants and Half-lives of ampicillin with different molar concentrations of beta-cyclodextrin at pH 2.0 and 37<sup>o</sup>C

Molar ratio of Amp. and Beta-CD	$k_d(\text{hr})^{-1}$	$k_d'(\text{hr})^{-1}$	$t_{1/2}(\text{hr})$	$t_{1/2}'(\text{hr})$
1.0:0.0	0.052	-	13.30	-
1.0:1.0	-	0.018	-	38.50
1.0:1.5	-	0.012	-	57.75
1.0:2.0	-	0.011	-	63.00

$k_d$  : The degradation constant of ampicillin without beta-cyclodextrin  
 $k_d'$  : The degradation constant of ampicillin with beta-cyclodextrin  
 $t_{1/2}$  : The half-life of ampicillin without beta-cyclodextrin  
 $t_{1/2}'$  : The half-life of ampicillin with beta-cyclodextrin

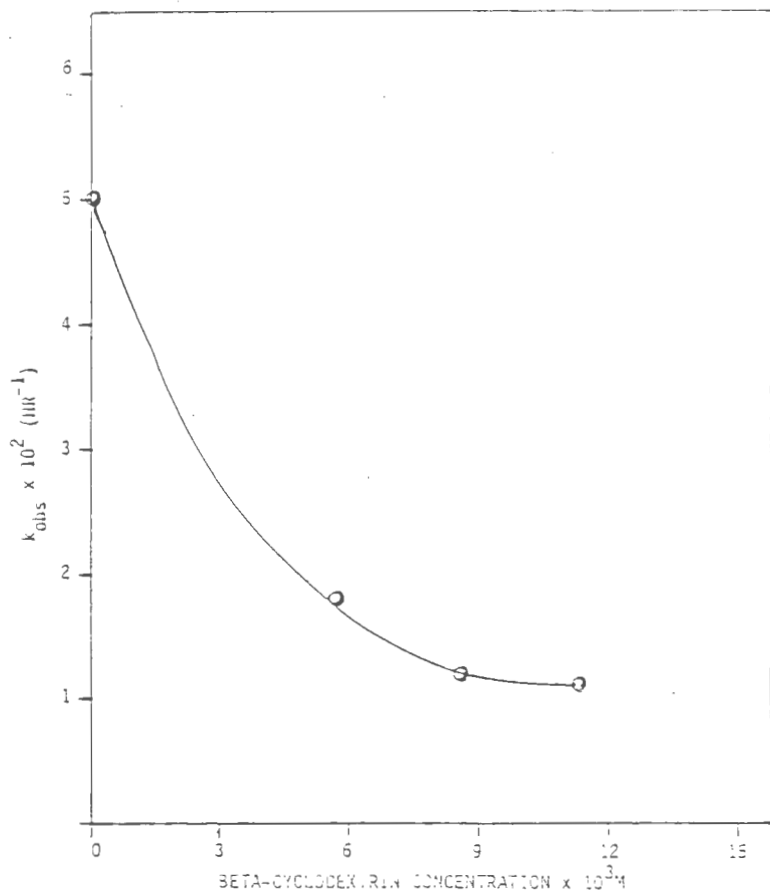
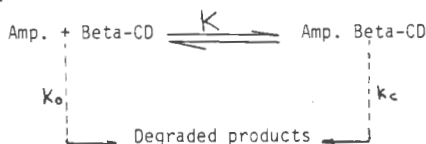


Fig. 9 Effect of beta-cyclodextrin concentration on the pseudo-first order rate constant for the hydrolysis of ampicillin at pH 2.0 and 37°C.

through a complex prior to the rate-determining step and may be accommodated by the mechanism illustrated in the following scheme (130).



Where Amp., Beta-CD, and Amp. Beta-CD, represent ampicillin, beta-cyclo-dextrin and the inclusion complex of ampicillin with the beta-cyclodextrin, respectively. The pseudo-first order rate constants for the degradation of uncomplexed and complexed ampicillin are  $k_0$  and  $k_c$  and  $K$  is the apparent stability constant for the complex. In Fig. 10 the rate constants of Fig. 9 are plotted according to Eq. (8).

For example:

$$k_{\text{obs}} - k_0 = - (k_{\text{obs}} - k_0)/K(\text{Beta-CD}) + k_c - k_0$$

A plot of  $k_{\text{obs}} - k_0$  as a function of  $(k_{\text{obs}} - k_0)/\text{beta-cyclodextrin}$  is shown in Fig. 8. From the slope and intercept of the plot  $k_0$ ,  $k_c$ , and  $K$  were obtained and they are reported in Table (IX). It is clear from the data that the complex degrades nine times slower than the drug itself, which is an indicative of a fairly stable complex. In addition the calculated stability constant ( $458.46\text{M}^{-1}$ ) is also characteristic of the extent of complex formation and may be suitable for practical application, since too labile complexes result in premature release of the drug and too stable complexes result in a retarded or incomplete release of the drug. It has been reported (23) that only those complexes with stability constant between 200 - 5000 seem to be suitable for practical applications. The author of this thesis found no literature data available on the ampicillin beta-cyclodextrin complex with which to compare the results from this study.

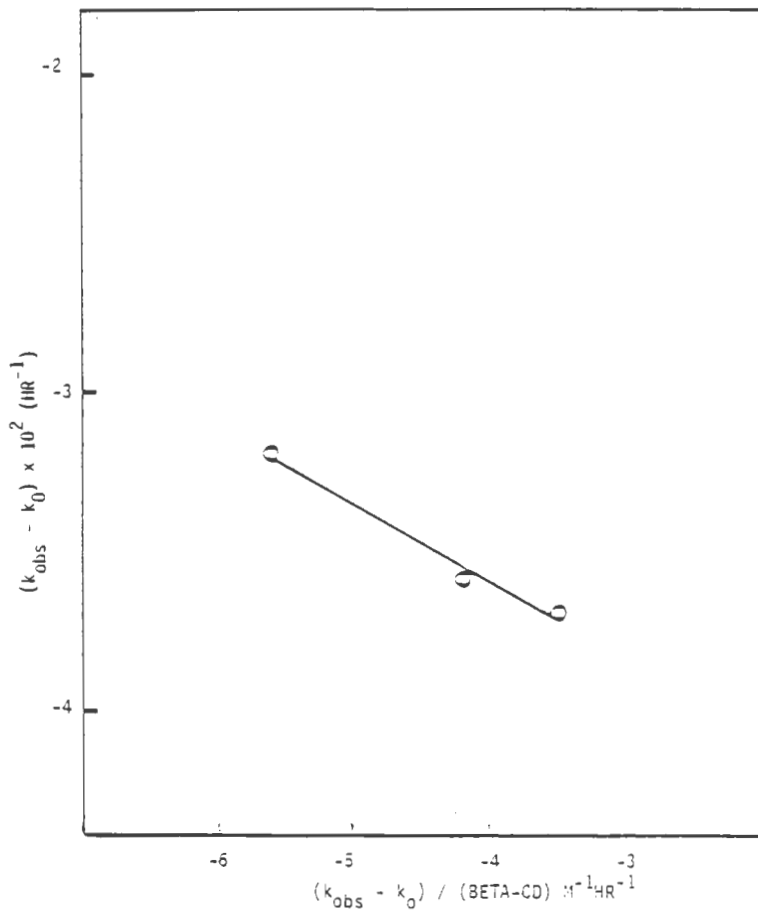


Fig. 10: Plot of the rate data according to Eq. 8

Table IX

Degradation constants and stability constant of the inclusion complex of ampicillin-beta-cyclodextrin

Degradation constant ( $k_0$ ) of the drug.	$5.00 \times 10^{-2} \text{hr}^{-1}$
Degradation constant ( $k_c$ ) of the complex	$0.55 \times 10^{-2} \text{hr}^{-1}$
Stability constant (K) of the complex	$458.46 \text{M}^{-1}$



## b. Solubility

The solubility method of Higuchi and Lach (131) was used to study the complex formation of phenobarbitone and phenytoin. Interactions of these two drugs were studied by solubility analysis. A phase solubility diagram of phenobarbitone was constructed according to the method described by Higuchi and Connors (47) and is depicted in Fig. 11. There was a five-fold increase in the solubility of phenobarbitone due to complex formation. According to the authors of the phase solubility techniques, if a plot of the total molar concentration of substrate as a function of the total molar concentration of ligand and the complex is of a 1:1 type then a straight line with a positive slope will result. Thus, from such plots the stability constant (K) can be calculated using Eq. 1 as follows:

$$K = \text{Slope}/S_0(1-\text{Slope})$$

Where  $S_0$  is the solubility of the substrate (phenobarbitone) in the absence of the ligand (beta-cyclodextrin). In other words,  $S_0$  is the intercept as shown in Fig. 9, and using the slope from this figure, the stability constant can be obtained. However, if the slope is greater than unity, as in this case (Fig. 9), it becomes impossible to calculate the stability constant using the above equation. Hence, the derivation of the above equation was reexamined, and it was modified to calculate an approximate apparent stability constant as follows.

Higuchi and Connors derived the above Eq. 1 from the following equation.

$$S_t = (mKS_0^m L_t) / (1 + KS_0^m) + S_0 \quad \text{Eq. (15)}$$

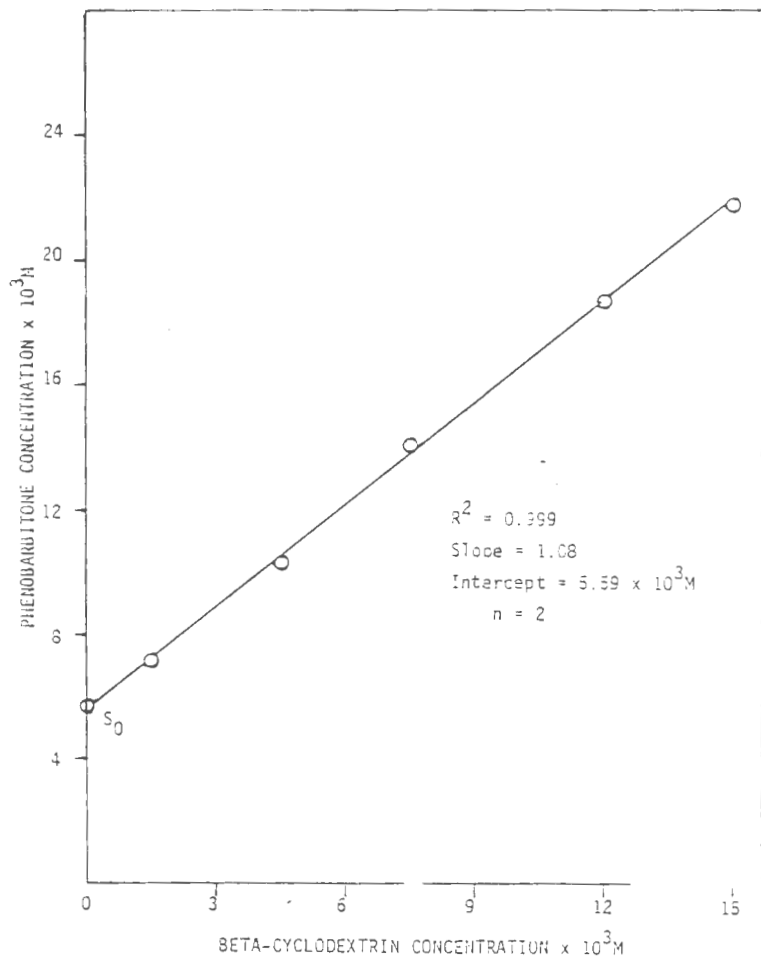


Fig. 11: Solubility of phenobarbitone as a function of beta-cyclodextrin in water at 25<sup>0</sup>C.

Where  $S_t$  and  $L_t$  represent the total molar concentration of substrate and ligand, respectively.  $S_0$  is as defined before and  $m$  is the stoichiometric coefficient.

A plot of  $S_t$  as a function of  $L_t$  for the formation of a soluble complex should therefore yield a straight line. The intercept is equal to  $S_0$  and the slope is given by:

$$\text{Slope} = mK S_0^m / (1 + K S_0^m) \quad \text{Eq. (16)}$$

If  $m$  is known or for simplicity is equal to 1, then equation 16 becomes:

$$K = \text{Slope} / S_0(1 - \text{Slope})$$

However, as pointed out earlier, if the slope is greater than unity, the assumption of a 1:1 complex alone is manifesting untenable, and the calculation of the stability constant according to the above equation is impossible. In such cases, according to the authors of the more general expression (Eq. 15), it may be used by assigning a value to  $m$ .

In order to determine the value of  $m$ , the complex was isolated (preparation is discussed in Section IIB, 2) and assayed. The results are reported in Table (X). Based on the percent drug content in the complex, the value of  $m$  is calculated as follows.

The molecular weight of phenobarbitone is 232gm/mole and that of the beta-cyclodextrin is 1135gm/mole. Hence, the number of moles in 20% of the drug is equal to  $8.62 \times 10^{-2}$  and that of beta-cyclodextrin is equal to  $7.05 \times 10^{-2}$ . The ratio of substrate to ligand gives 1.22, indicating that the complex formula is  $S_{1.2}L$ . Based on these calculations a value for  $m$  was obtained and substituted into the equation(16).

$$\text{Slope} = mK S_0^m / (1 + K S_0^m) \quad \text{Eq. (16)}$$

By rearranging Eq. 16 to

Table X

Drug content in the freeze dried phenobarbitone beta-cyclodextrin complex.

Batch No	Batch size (in liters)	% Drug content
1	10	20.00
2	10	19.68

$$K = \text{Slope} / S_0^m (m - \text{Slope}) \quad \text{Eq. (17)}$$

When  $m = 1.2$  Eq. (17) results to

$$K = \text{Slope} / S_0^{1.2} (1.2 - \text{Slope}) \quad \text{Eq. (18)}$$

The calculated apparent stability constant is  $4.54 \times 10^3 \text{M}^{-1}$  by using the slope and intercept values from Fig. 9. This value is close to the value ( $3.60 \times 10^3 \text{M}^{-1}$ ) obtained by Thoma and Stewart (9). Although the stability constant value obtained by Thoma and Stewart is close to the value obtained in the present study, there is a discrepancy. This discrepancy can be explained as follows. Firstly, the reported stability constant value by Thoma and Stewart was from the interaction study carried out at  $30^\circ\text{C}$ , whereas, the present study was carried out at  $25^\circ\text{C}$ . Hence there is a temperature effect on the stability constant. Secondly, unless a solid complex is isolated and its stoichiometry analyzed, calculation of the exact stability constant may lead to an approximation. It is the opinion of the author of this thesis that Thoma and Stewart did not isolate the complex to determine the stoichiometry; hence there is a discrepancy in the stability constant values. Further, Higuchi and Connors (47) also pointed out in their phase solubility techniques that the stoichiometry and the equilibrium constants may be ambiguous quantities. Hence (according to them), whenever there is an ambiguity or conflicting results, a solid complex should be isolated and its stoichiometry analyzed and compared with a graphical estimate of the stoichiometric ratio. Thus, the phenobarbitone beta-cyclodextrin complex may be a molecular ratio of 1.2:1.0, rather 1:1.

The results of the interaction of phenytoin with beta-cyclodextrin are depicted in Fig. 12. There was an eleven-fold increase in the solubility of phenytoin due to the complex formation.

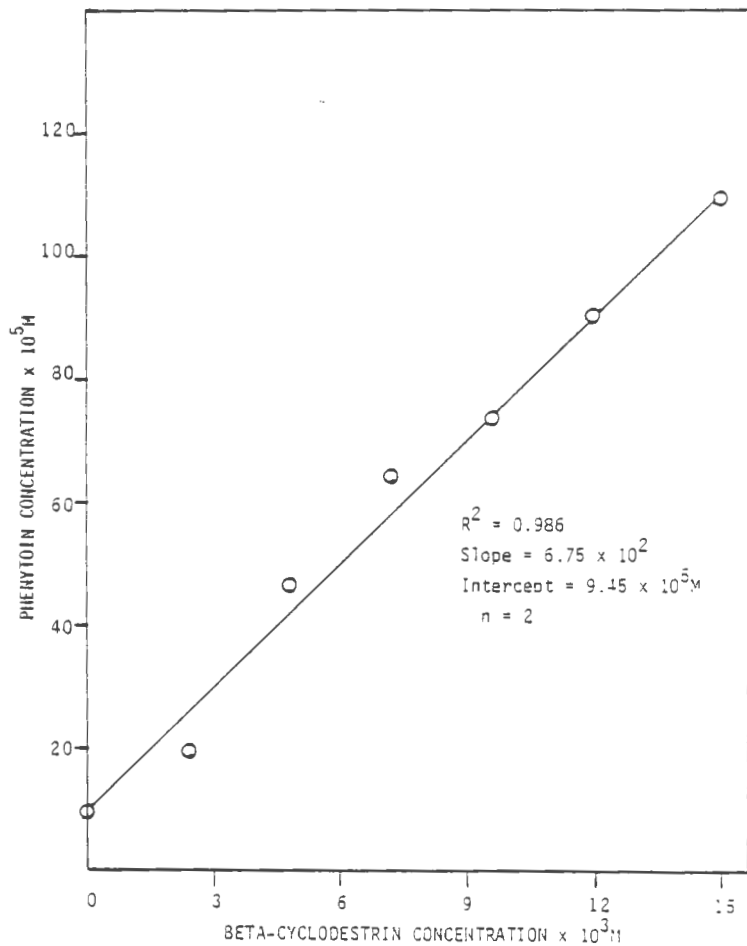


Fig. 12: Solubility of phenytoin as a function of beta-cyclodextrin in pH 7.0 buffer at 25°C.

Uekama (117) reported the solubilities of several drugs in water both in the presence and in the absence of beta-cyclodextrin. Among the reported drugs is phenytoin. These results are reported in Table (XI) along with the results of the present study. Although the results are very close to each other there is a slight discrepancy. This discrepancy may be due to the concentration of cyclodextrin in the solubility study. Since Uekama did not use a saturated solution of beta-cyclodextrin (Table XI), there is a slight decrease in the total solubility of phenytoin. However, he obtained a higher solubility in the absence of cyclodextrin; hence, it may be said that both results are comparable. Uekama reported neither the range of the cyclodextrin concentration used nor the stability constant of the complex.

A phase solubility diagram was constructed from the solubility data of phenytoin and is shown in Fig. 12. From this phase diagram, and using Eq. 1, the stability constant ( $756M^{-1}$ ) of the complex was obtained. As shown in Fig. 12, in this interaction study, the slope is less than unity and hence there was no need to determine a value for  $m$  (Eq. 16) to calculate the stability constant. However, a solid complex was isolated (preparation is described in Section IIB, 2) and the drug content in the complex was analyzed by a spectrophotometric method. The results of the drug content in the complex are reported in Table XII. Based on the drug content in the complex, the ratio of the substrate concentration to the ligand concentration (obtained as described earlier) gave a value of 1.05. This value is an indication of the complex with a molecular ratio of 1:1.

Lach and Cohen (40) studied the interaction of nineteen compounds with alpha and beta-cyclodextrin. The data in their study indicate that

Table XI

Comparison of phenytoin solubility data with the reported literature data.

Solubility at 25 <sup>o</sup> C in water (ug/ml), $S_0$	Solubility in beta-cd solution (ug/ml), $S_c$	$S_c/S_0$
*28	282	10.00
**25	277	11.00
-----		
* Reported values (117)		
** Determined vlues by the author of this thesis in pH 7.00 buffer.		
Concentration of the bet-cyclodextrin used:		
* $13 \times 10^{-3}M$		
** $15 \times 10^{-3}M$		



Table XII

Drug content in the freeze dried phenytoin beta-cyclodextrin complex

Batch No	Batch size (in liters)	% Drug content
1	* 1	18.88
2	8	18.30
3	10	18.90

\* - Prepared by using a laboratory freeze drier.

those drugs which are least soluble in water show the greatest increase in solubility as a function of quantity of cyclodextrin present. In addition, in their study, the drugs which are low in molecular weight and more soluble show the highest slopes. Similar results are shown in Table XIII, phenobarbitone shows the highest slope compared to phenytoin, since phenobarbitone is more soluble than phenytoin. However, increase in solubility of phenytoin relative to its initial solubility is eleven-fold compared to only a five-fold increase in phenobarbitone solubility. This increase in solubility, especially for phenytoin, has a considerable potential in the development of suitable dosage forms. The extent of complex formation (as evidenced by the stability constant) of phenobarbitone is greater than that of phenytoin. This stronger interaction of phenobarbitone may be due to the size of the molecule. Since the size of the phenobarbitone molecule is smaller than the phenytoin molecule (Table XIII), smaller molecules may fit well into the beta-cyclodextrin cavity to form a more stable complex. Cohen and Lach (36) also felt that geometrical rather than chemical factors are decisive in determining the extent of complex formation.

#### c. X-ray diffractometry

The X-ray diffractometer allows us to examine the atomic arrangement of a material, giving information on crystal geometry and structure. It has been reported (49, 50) that an inclusion complex can be detected quickly and directly by the X-ray methods. As per these reported studies, a true inclusion complex may exist if the diffraction pattern does not correspond to those of pure components.

Figures 13 and 14 show the X-ray diffraction patterns of phenytoin and the beta-cyclodextrin. As shown in the Fig. 13c, the diffraction

Table XIII

Slopes of interaction isotherms, solubility data and stability constants.

Drug	Stability constant $K(M^{-1})$	Slope	Increase in solubility	M. wt.
* Phenobarbitone	$4.53 \times 10^3$	1.08	5-fold	232
* * Phenytoin	766	0.0675	11-fold	252

\* Solubility = 1.0mg/ml in water at 25<sup>o</sup>C (134).

\*\* Solubility = 7.0ug/ml in water at 24<sup>o</sup>C (138).

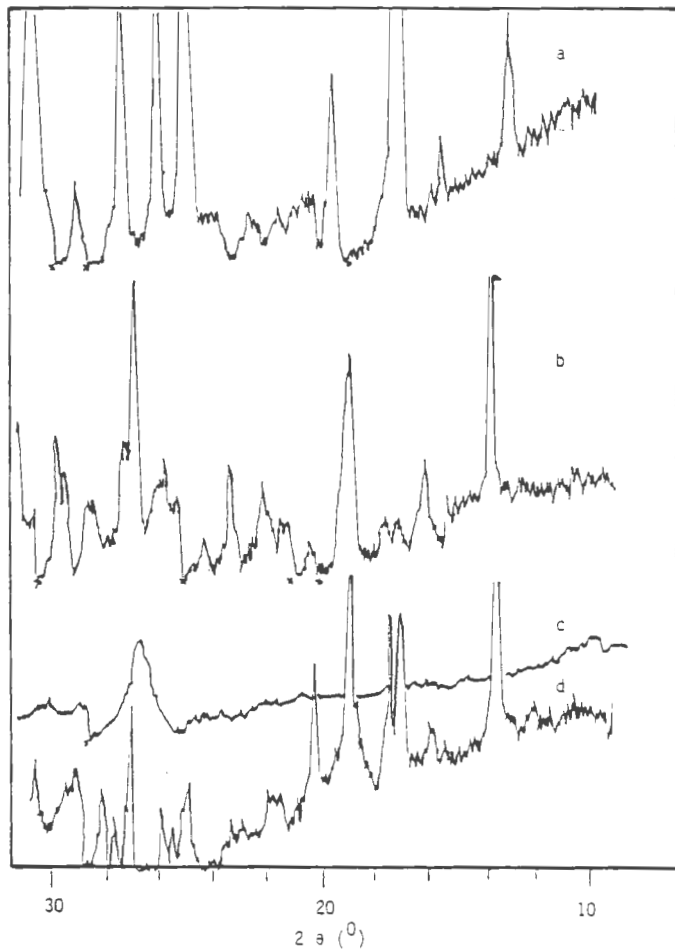


Fig. 13: Powder X-ray diffraction patterns

Key: a- Phenytoin, b- Beta-cyclodextrin, c-Freeze dried complex, d- Physical mixture of a and b.

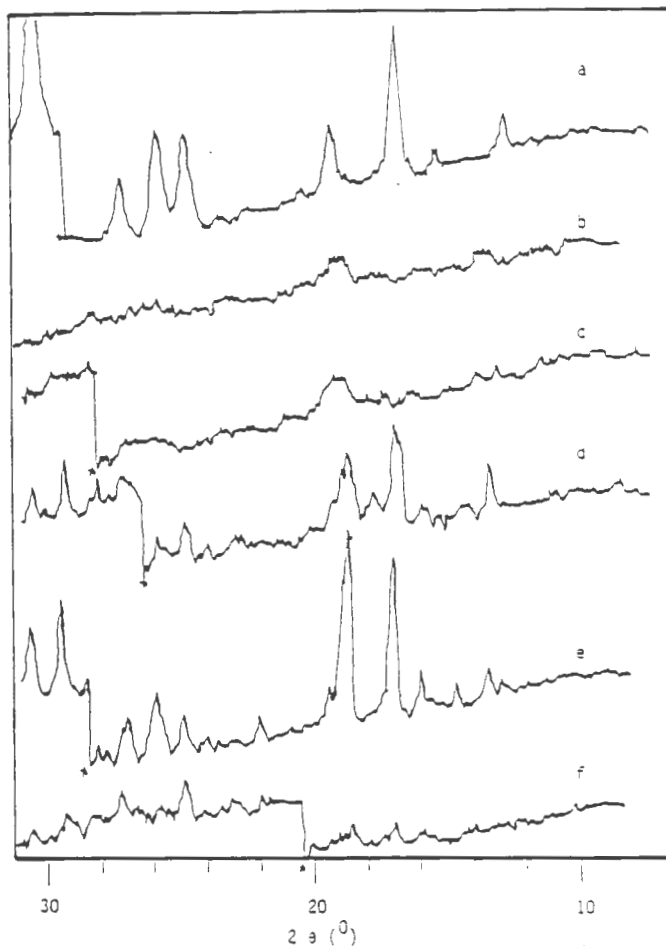


Fig. 14: Powder X-ray diffraction patterns

Key: a- Freeze dried pentytoin, b- freeze dried beta-cyclodextrin, c- Physical mixture of a and b, d- Kneaded complex, e- Solvent evaporated complex, f- Spray dried complex.

pattern of the freeze dried material is different from that of the pure components and their physical mixture. The diffraction pattern of the physical mixture was found to be a simple superimposition of those of the components. The X-ray diffraction pattern shown in the Fig. 14b, indicates that the beta-cyclodextrin, which was originally in a crystalline form (Fig. 13b), is transformed to an amorphous state after freeze drying. Similarly, the phenytoin was originally in a crystalline form; which did not transform to an amorphous state after freeze drying of phenytoin alone (Fig. 14a); however, the complex prepared by the freeze drying method was transformed to an amorphous state and indicated the formation of a new solid phase. This new solid phase is clearly shown in Fig. 13c, around  $28^{\circ}$ . The complex prepared by the other methods, such as kneading, solvent evaporation, etc., did not transform into an amorphous state as evidenced by the diffraction patterns (Fig. 14d and 14e). However, unlike the physical mixture, the diffraction patterns are not a simple superimposition of the pure components. The diffraction patterns of the complexes differed from those of the physical mixture (diffused diffraction patterns), thus indicating the existence of the complex as a separate molecular species.

Seo, et al.(88), studied the X-ray diffraction patterns of spirinolactone beta-cyclodextrin systems. In their studies they also observed that, the diffraction patterns of the physical mixtures were simply the superimposition of each component pattern, while those of complexes were apparently different. Similarly, Uekama, et al. (87), also interpreted the powder X-ray diffraction patterns of acetohexamide beta-cyclodextrin complex and their physical mixture. In addition, Tokumura, et al. (166), also reported the X-ray diffraction patterns of

cinnarizine beta-cyclodextrin complex and their physical mixture. In their results also, the diffraction pattern of the physical mixture was found to be a simple superimposition of those of the components, and that of the complex was apparently different.

Figures 15 and 16 show the diffraction patterns of phenobarbitone the complex. The X-ray diffraction patterns shown in Fig. 15b indicate that, like phenytoin, phenobarbitone also retains its crystalline form even after freeze drying. However, the phenobarbitone beta-cyclodextrin complex prepared by the freeze drying method is completely transformed to an amorphous state (Fig. 15c). The physical mixture pattern is a simple superimposition of the pure components (Fig.16c). Hence, it is clear that the diffraction patterns of the complex differed from that of the physical mixtures, indicating the existence of the complex as a separate molecular species.

#### d. Measurement of lattice parameters

It was possible to obtain a single crystal of the phenytoin beta-cyclodextrin complex by the solvent evaporation method. The results are reported in Table (XIV). As shown in the Table, one of the axial lengths (A) has almost doubled, indicating a different molecular order. This new molecular order may be due to inclusion complexation of phenytoin with the beta-cyclodextrin. Based on the X-ray results and solubility characteristics, it is possible by inclusion complexation that the axial length (A) may have changed. However, in order to reach a definitive conclusion, this question is deferred for further study. Since, the author of this thesis could find no data reported in the literature on the measurement of the lattice parameters of inclusion complexes.

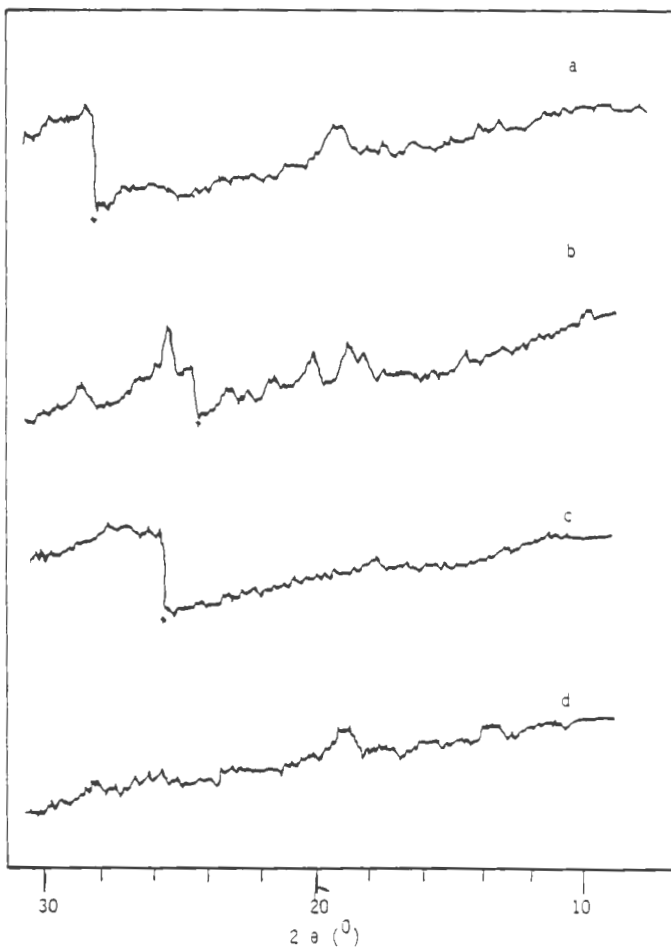


Fig. 15: Powder X-ray diffraction patterns  
Key: a- Freeze dried beta-cyclodextrin, b- Freeze dried phenobarbitone, c- Freeze dried complex, d- Spray dried complex.



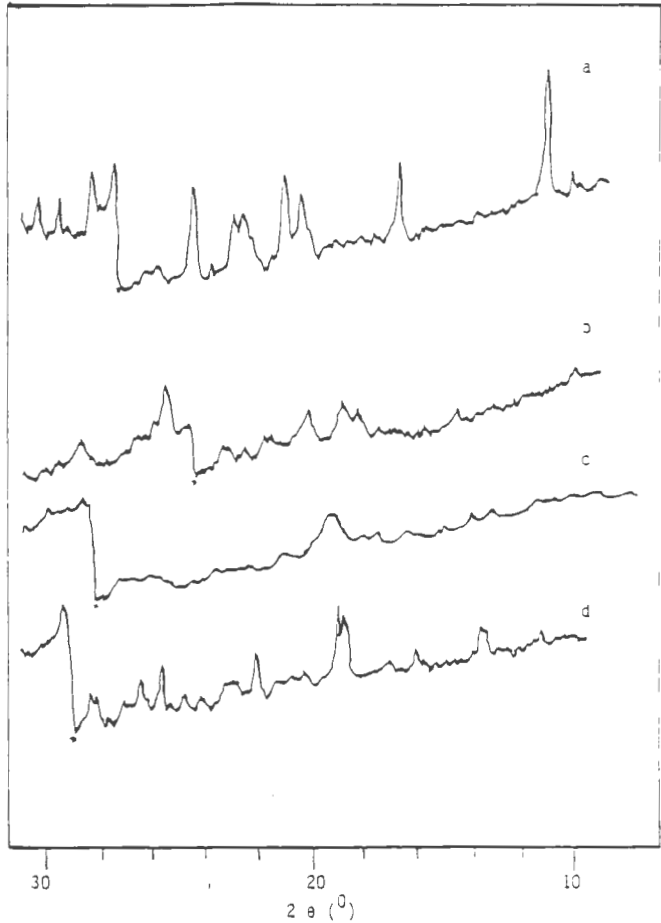


Fig. 16: Powder X-ray diffraction patterns  
Key: a- Phenobarbitone, b- Freeze dried phenobarbitone,  
c- Freeze dried beta-cyclodextrin,  
d- Physical mixture of b and c.

Table XIV

Lattice parameters data

Axial length	A	B	C
* 1	21.29	10.33	15.10
** 2	42.18	11.04	15.05

\*- Reported values of beta-cyclodextrin unit cell dimensions.  
Carbohydrate Research, 99, 103 (1982)

\*\* - Measured values of phenytoin beta-cyclodextrin complex prepared by  
the solvent evaporation method.

## e. Differential scanning calorimetry (DSC)

Cyclodextrins complex formation may be proved by DSC. Figure 17 shows the DSC curves of freeze dried phenytoin and the freeze dried complex. In Figs. 17 and 18 the freeze dried phenytoin and beta-cyclodextrin gave an endothermic peak around  $295^{\circ}\text{C}$  ( $568^{\circ}\text{K}$ ), which corresponds to the melting points of the phenytoin and beta-cyclodextrin. The freeze dried complex and the physical mixture (Figs. 17 and 18) also gave an endothermic peak around the same temperature (Fig. 18). However, the pattern of the freeze dried complex thermogram is clearly different from that of the physical mixture. These thermograms suggest that there is some interaction in the freeze dried complex but not in the physical mixture. Because of the very narrow range of the melting points of the phenytoin and beta-cyclodextrin (Table XV), it was not possible to elucidate clearly the presence of the inclusion complex. In order to demonstrate the absence of the inclusion complex in the physical mixture, DSC curves of phenobarbitone and the beta-cyclodextrin were depicted as shown in Figs. 19 and 20. Since there is clearly a wide range of difference in melting points (Table XV), it was possible to distinguish the presence and absence of the true inclusion complex by observing the endothermic peaks of the two different molecules. This phenomenon is elucidated in the Fig. 20. These thermograms strongly suggest the presence of the inclusion complex in the freeze dried material but not in the physical mixture.

Similarly, Kurozumi, et al. (51) have found that freeze dried mefenamic acid gives at  $232^{\circ}\text{C}$  an endothermic peak; its mechanical mixture also gives the same peak at the same temperature; but the complex

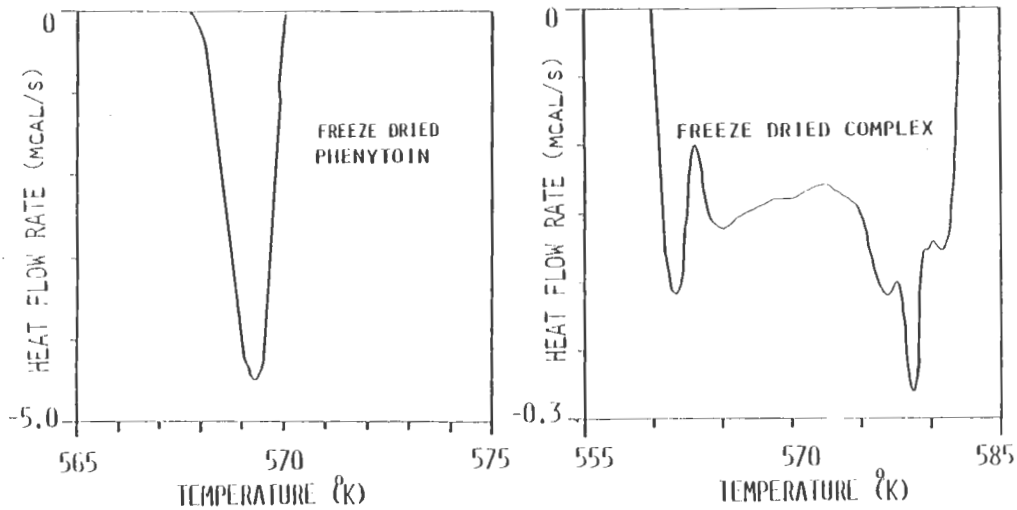


Fig. 17: DIFFERENTIAL SCANNING CALORIMETRY (DSC) THERMOGRAMS

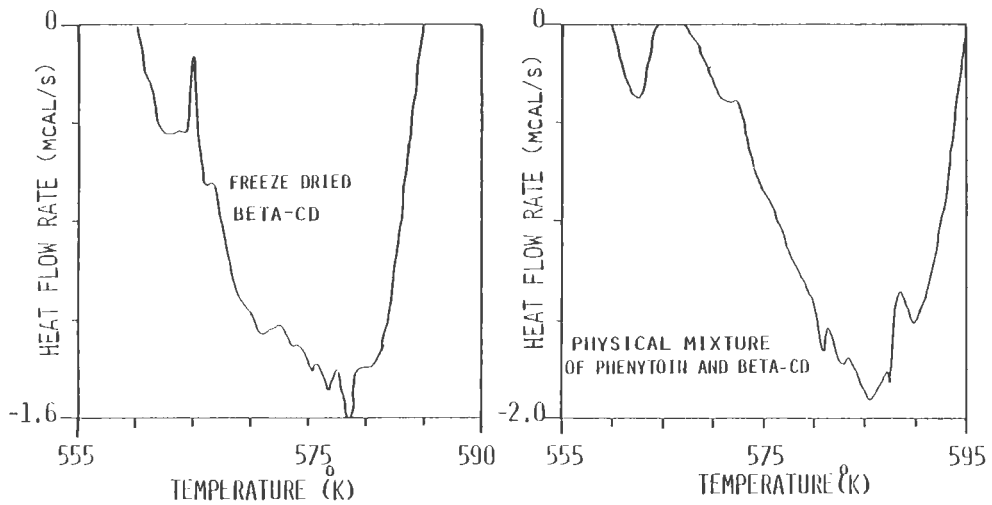


Fig. 18: DIFFERENTIAL SCANNING CALORIMETRY (DSC) THERMOGRAMS.

Table XV

Melting points data on phenytoin, phenobarbitone and beta-cyclodextrin.

Drug	Melting Point ( $^{\circ}\text{C}$ )
Phenytoin	295 - 298
Phenobarbitone	174 - 177
Beta-cyclodextrin	300 - 310

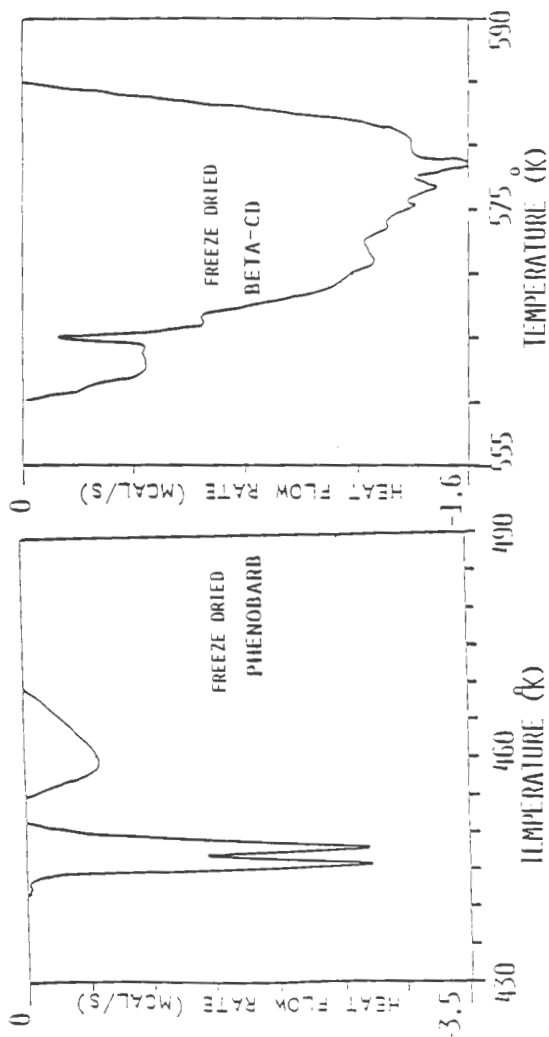


Fig. 19: DIFFERENTIAL SCANNING CALORIMETRY (DSC) THERMOGRAMS.

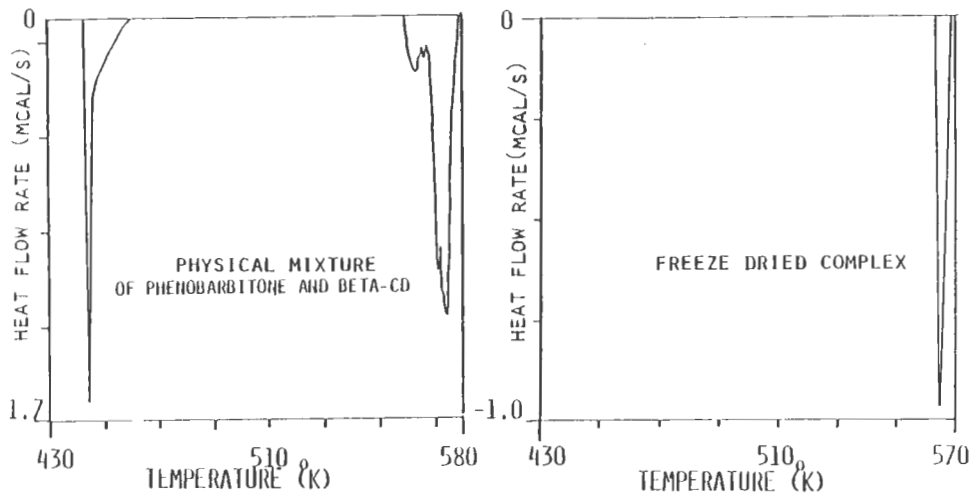


Fig. 20: DIFFERENTIAL SCANNING CALORIMETRY (DSC) THERMOGRAMS.



prepared by the freeze drying method does not. Hence, this behavior is usually a characteristic of inclusion complexes.

#### f. Infrared (IR) spectroscopy

Figures 21 and 22 show the IR spectra of the phenytoin and beta-cyclodextrin. The complex formation may be proved by IR because bands due to the included part of the guest molecule are generally shifted or their intensities are altered. However, the results shown in Figs. 21 and 22 can not be readily interpreted in an unambiguous manner since the physical mixture and the freeze dried complex show by and large similar patterns. This similarity in the patterns of freeze dried complex and the physical mixture can be explained as follows.

- (i) In general, the mass of the guest molecule in a complex does not exceed more than 25% (8) of the mass of the complex.
- (ii) This phenytoin beta-cyclodextrin complex contains around 20% drug in the complex (Table XII).

For IR spectra, one or two mg of sample is used with 80-90 mg of potassium bromide. Hence, in the one or two mg complex, the active drug content is only a few micrograms. Therefore, if any bands are altered due to the included part of the guest molecules, these alterations may possibly obscure the spectrum of the host. Similar explanations are reported in the literature (8) in the study of pyrethrin-cyclodextrin complexes.

#### g. Proton magnetic resonance (PMR) spectroscopy

Recently, nuclear magnetic resonance (NMR) methods have contributed greatly to the understanding of the cyclodextrin interaction with different guest molecules. The PMR investigation of the complexes of cyclodextrins was technically difficult due to the low solubility of the

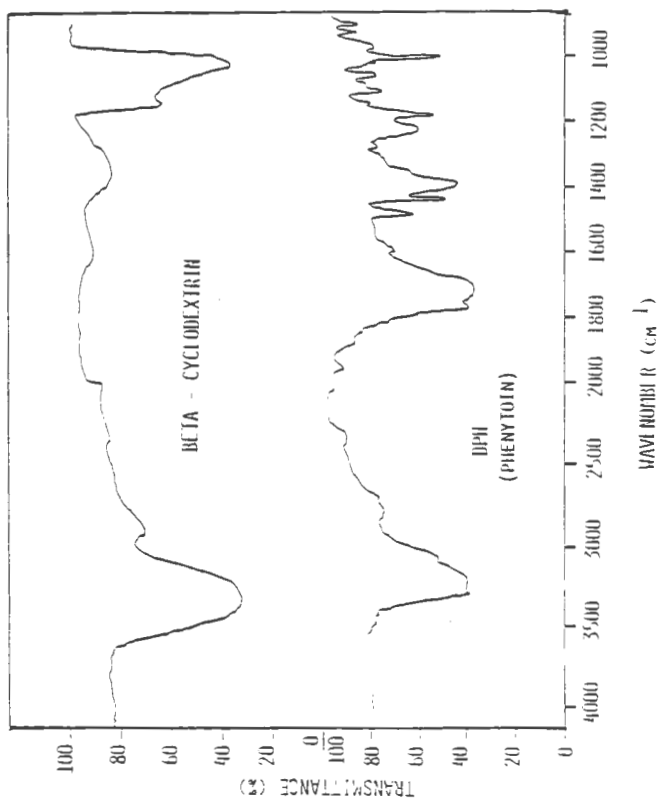


FIG. 21: INFRARED (IR) SPECTRA.

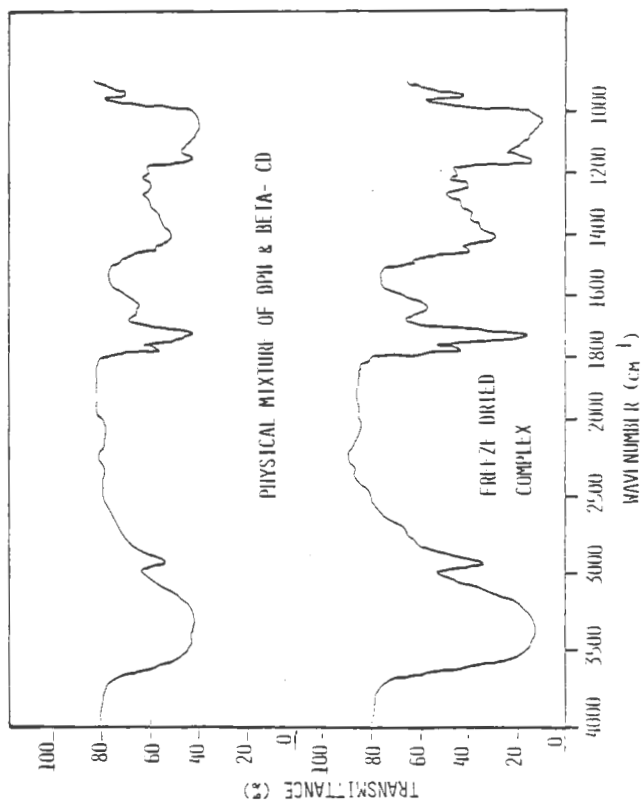


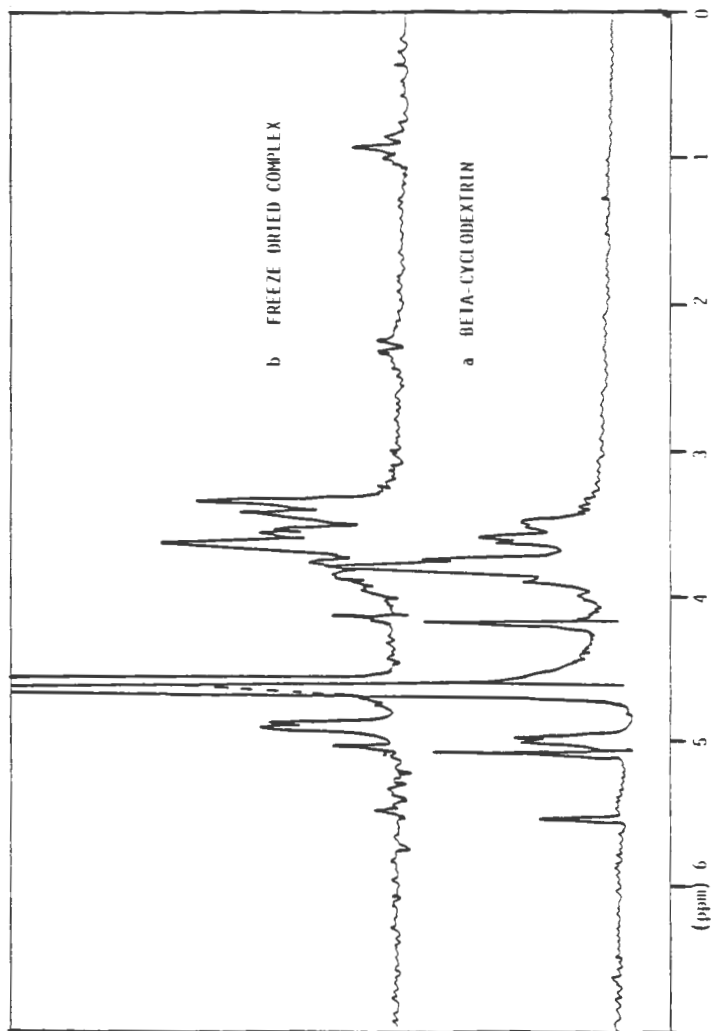
Fig. 22: INFRARED (IR) SPECTRA.

complexes in deuterium oxide. In organic solvents, such as dimethylsulfoxide, chloroform, etc., complex can be dissolved; however it has been reported (8) that the complex decomposes, and the cyclodextrin complex of the solvent is formed. This is possible since in the complex formation the nonpolar part of the guest molecule penetrates into the nonpolar cyclodextrin cavity, thereby establishing an energetically favorable nonpolar-nonpolar interaction. When an organic solvent is used to dissolve the complex, if the solvent is more polar than the included guest molecule, then a complex with the organic solvent will be formed.

In this study, since the phenytoin beta-cyclodextrin complex did not dissolve in the deuterium oxide, it was very difficult to show the complex formation in the liquid phase. However, the phenobarbitone complex readily dissolved in the deuterium oxide, and the results are shown in Fig. 23. The large peak at approximately 4.6ppm is common to both spectra due to small amounts of deuterium hydroxide and water as impurities. All the peaks observed in the Fig. 23a, are those of the beta-cyclodextrin. The deuterium oxide peak is fixed in both spectra; this peak was used as an internal standard for the comparison of the chemical shift observations. From the spectra of both the beta-cyclodextrin and the complex, it is clear that all of the protons of the complex located in the internal cavity of the beta-cyclodextrin are experiencing a shielding effect; hence signals are shifted upfield (Fig. 23b). These spectra strongly suggest the presence of the complex in the liquid phase.

Demarco and Thakkar (52) reported that the protons located in the interior cavity of the beta-cyclodextrin (C-3-H, and C-5-H) will be considerably shielded by the guest molecule; hence the signals will be

Fig. 23: NUCLEAR MAGNETIC RESONANCE (NMR) SPECTRA OF PHENOBARBITONE BETA-CYCLODEXTRIN COMPLEX



shifted upfield. Whereas, the protons on the outer surface (C-2-H, C-4-H, and C-6-H) will not be affected by the guest molecule; hence the signals of those protons will remain unchanged. In addition, Frank and Cho (32) also observed similar results in the study of the complexing behavior of dinoprostone with beta-cyclodextrin in water.

#### h. Photomicrographs

Figures 24, 25 and 26 show the morphology of recrystallized beta-cyclodextrin, phenytoin, and phenobarbitone before and after freeze drying. All the photomicrographs were taken at the same magnification (125x). As shown in the Fig. 24a, the beta-cyclodextrin is a crystalline material, however after freeze drying it has completely transformed into an amorphous state. This amorphous state is also confirmed in the X-ray analysis (Fig. 14b). Whereas phenytoin and phenobarbitone (Figs. 25b and 26b) as seen in the X-ray analysis (Figs. 14a, and 15b) did not transform into an amorphous state, the complex prepared by the freeze drying method of these two drugs as shown in Figs. 27b, and 28b, are completely transformed into an amorphous state. This was also previously observed in the X-ray analysis (Figs. 13c, and 15c). The complex prepared by various methods are shown in Figs. 27a, 28a, and 29. It is very difficult to prove in an unambiguous manner the formation of an inclusion complex in these photomicrographs; it can be readily shown in Fig. 29b that, some kind of interaction has taken place in the complex prepared by the solvent evaporation method. As previously discussed, a single crystal isolated from this preparation was taken for lattice parameters measurement and showed an increase in one of the axial length. The results are reported in the Table (XIV). Based on this photomicrograph, the lattice parameters data, and also from the X-ray

Fig. 24a: RECRYSTALLIZED BETA-CYCLODEXTRIN AT 125x.

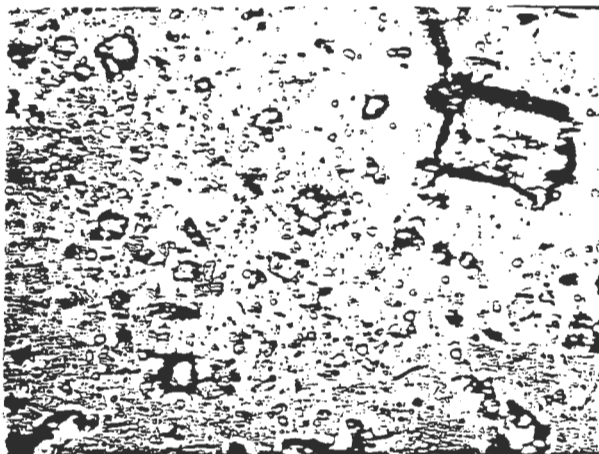


Fig. 24b: FREEZE DRIED BETA-CYCLODEXTRIN AT 125x.

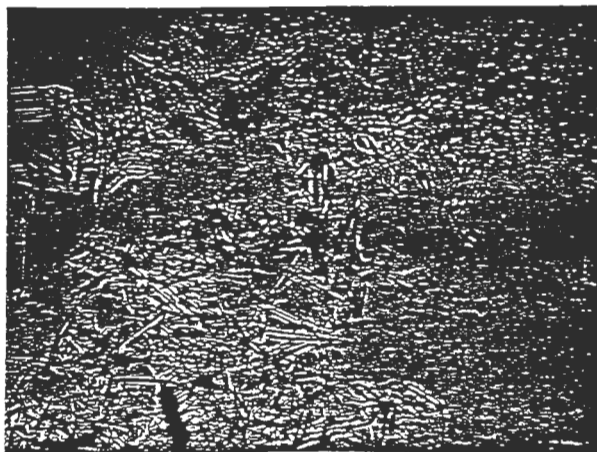


Fig. 25a: PHENYTOIN AT 125x.

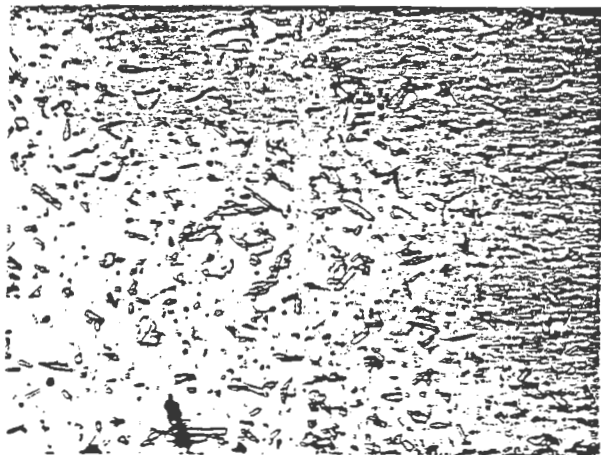


Fig. 25b: FREEZE ORIED PHENYTOIN AT 125x.

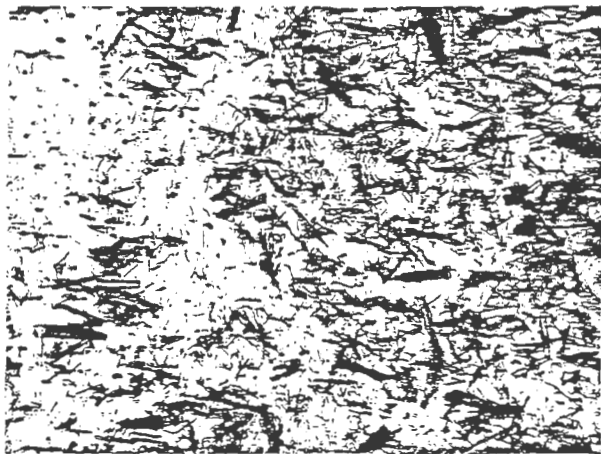




Fig. 26a: PHENOBARBITONE AT 125x.

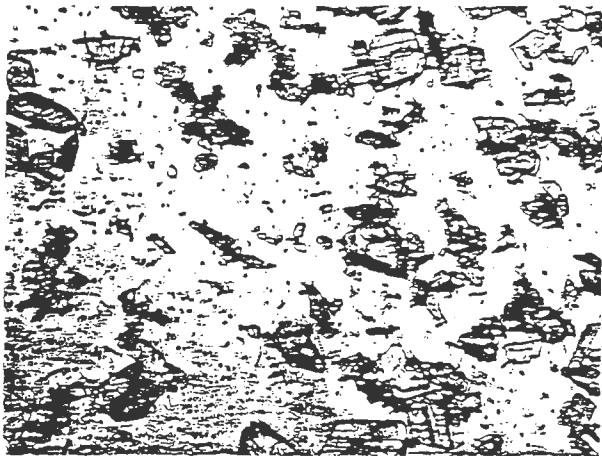


Fig. 26b: FREEZE DRIED PHENOBARBITONE AT 125x.

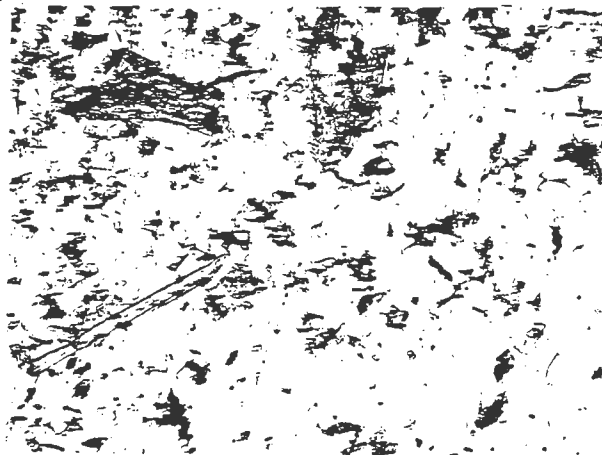


Fig. 27a: SPRAY DRIED PHENYTOIN COMPLEX AT 125x.

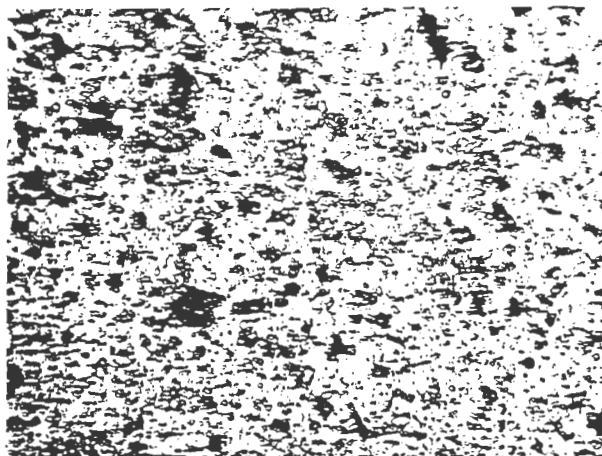


Fig. 27b: FREEZE DRIED PHENYTOIN COMPLEX AT 125x.

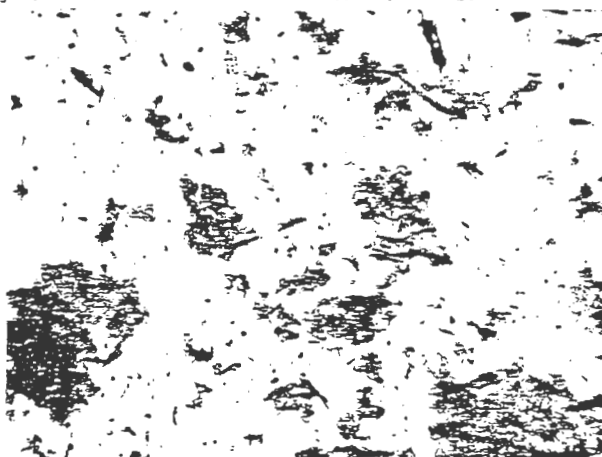


Fig. 28a: SPRAY DRIED PHENOBARBITONE COMPLEX AT 125x.

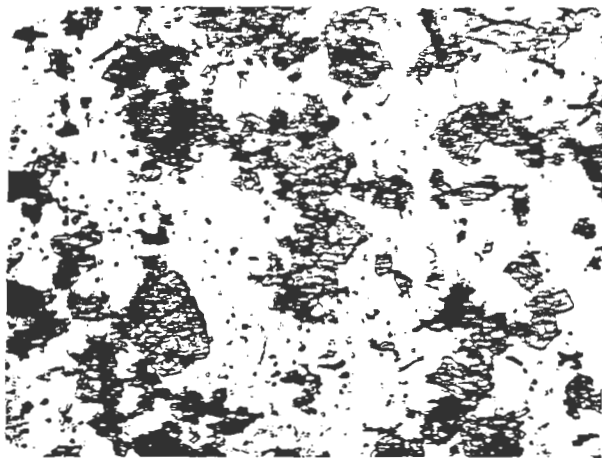


Fig. 28b: FREEZE DRIED PHENOBARBITONE COMPLEX AT 125x.

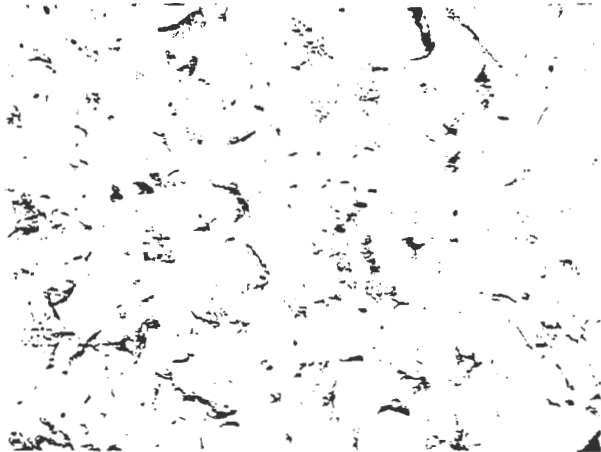
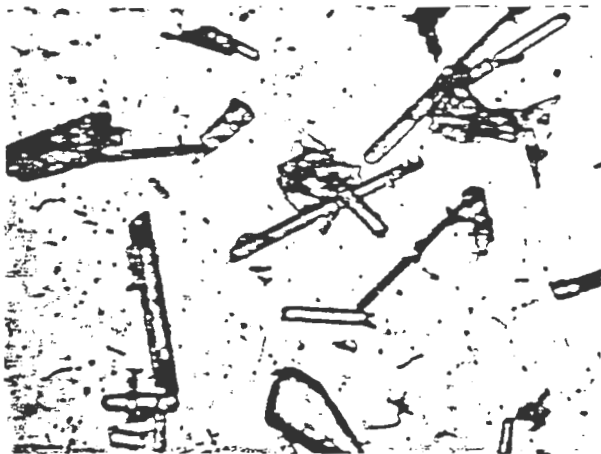


Fig. 29a: KNEADED PHENYTOIN COMPLEX AT 125x.



Fig. 29b: SOLVENT EVAPORATED PHENYTOIN COMPLEX AT 125x.



diffraction, it is conceivable that inclusion complex may be present in the solvent evaporated material.

## 8. Preparation of the complexes

### a. Solvent evaporation

Solvent evaporation is a simple method however, it is a costly and time consuming procedure because:

(i). The solvent of a small batch size of 200ml requires at least 10-12 days to evaporate at room temperature.

(ii). The solvent is 50% ethanol, which is expensive and without a solvent recovery system this process may not be economically feasible.

A phenytoin beta-cyclodextrin complex was prepared by this procedure with a satisfactory yield of 95%. The final end product was in a crystalline form. As discussed in Section IIIA, d., the measurement of lattice parameters showed some interaction with the beta-cyclodextrin. However, in order to collect a reasonable quantity of material to begin formulation work, it would take several months' time to collect a workable amount of material. Further, even in the literature this method of preparation of inclusion compounds has not been explored.

### b. Kneading

In the preparation of inclusion compounds the most common technique is to stir or shake an aqueous solution of cyclodextrin with a guest molecule or its solution. In aqueous solution the apolar cyclodextrin cavity is occupied by water molecules that are in an energetically unfavorable state (polar - apolar repulsion) and are, therefore readily replaced by an appropriate guest molecule that is less polar than water (Fig. 4). In this kneading process the cyclodextrin is not dissolved, it is kneaded with a small amount of water to make a slurry, and then guest

components are added to the slurry of cyclodextrin. Since neither the guest nor host molecules are dispersed at a molecular level, it is difficult to conceive the formation of inclusion compounds in this process. However, this method of preparation has been reported in the literature (8).

A phenytoin-beta-cyclodextrin complex was prepared by this method with a satisfactory yield of 92%. Only the X-ray-analysis (Fig. 14), showed ambiguously the presence of the inclusion complex as a separate molecular species. Thus, it is interesting to see the effect of kneaded complex on formulations.

#### c. Spray drying

Figs. 27a and 28a show the morphology of spray dried phenytoin and phenobarbitone complex with beta-cyclodextrin. It appears from the photomicrographs that the complex may be a physical mixture of the pure components (Figs. 24a, 25a and 26a). These types of results may be conceivable from the spray drying process as follows.

During the spray drying process the surface liquid is quickly evaporated and a tough shell of solids may form in its place. As drying proceeds, the liquid in the interior of the droplet must diffuse through this shell; however, the diffusion of the liquid occurs at a much slower rate than does the transfer of heat through the shell to the interior of the droplet. The resultant build-up of heat causes the liquid below the shell to evaporate at a far greater rate than it can diffuse to the surface. The internal pressure causes the droplet to swell; and if the shell is nonelastic, it ruptures, producing either fragments or ruptured hollow spheres and perhaps some intact spheres. Therefore, when the cyclodextrin solution with the drug is sprayed, if the shell is

nonelastic, it may rupture producing fragments of drug and cyclodextrin or there may be fragments of both species together. Hence, if the shells of cyclodextrin solids are nonelastic it may not be possible to produce an intact cyclodextrin species containing drug molecules.

As shown in Figs. 14 and 15, only the X-ray analysis showed some kind of interaction with beta-cyclodextrin rather than a physical mixture of the pure components. The photomicrographic evaluations are highly subjective; therefore based on the X-ray analysis, it is felt that the inclusion complex exists as a separate molecular species.

However, there was only a 10% yield of the complex prepared by this method. The low yield is due to the poor design of the equipment rather than technique, because of the following reasons:

- (i). The solution was sprayed as fine droplets into a moving stream of hot air, where they did not evaporate rapidly before reaching the wall of the drying chamber.
- (ii). The resultant build-up of liquid droplets on the wall of the drying chamber did not dry into a fine powder to give a good yield.
- (iii). The temperature of the system was fluctuating constantly during the spraying process.
- (iv) The peristaltic feed pump was not functioning properly.

Therefore, because of the unsatisfactory yield,, this method was not explored further.

#### d. Freeze-drying

Among the various methods of preparing the inclusion complexes, this method was satisfactory at a laboratory level. However, the ampicillin inclusion compound could not be prepared by this method since it has been

(114) reported that the rate of decomposition of the ampicillin increases upon freezing. Savello and Shangraw (139) showed that for a 1% sodium ampicillin solution in 5% dextrose, the percentage of degradation at four hours is approximately 14% at  $-20^{\circ}\text{C}$ , compared to 10% at  $5^{\circ}\text{C}$ . Therefore, only phenytoin and phenobarbitone beta-cyclodextrin complexes were prepared firstly by using a laboratory freeze drier.

As shown in Fig. 13c, the phenytoin beta-cyclodextrin complex prepared by the freeze drying method strongly suggested the presence of inclusion complex as a separate molecular species. In addition the DSC thermograms (Fig. 17) also suggested that the freeze dried complex is not a physical mixture. Similarly, the DSC thermograms of the phenobarbitone complex (Figs. 19 and 20) and the NMR spectra (Fig. 23) strongly indicated the presence of the inclusion complex in solid phase as well as in liquid phase.

Although there are different methods of preparing the inclusion complexes, they are not suitable at the pilot scale or manufacturing level. Kurozumi *et al* (51) prepared the inclusion compounds of non-steroidal antiinflammatory and other slightly soluble drugs with alpha and beta-cyclodextrins. They reported that the freeze-drying method was successful in obtaining the inclusion compounds of all the test drugs. However, they did not describe how such inclusion compounds can be produced at the pilot scale or manufacturing level. Similarly Lach and Cohen (40) studied the interaction of nineteen compounds with cyclodextrins, but they did not report how to harvest these inclusion compounds.

In the present study, preparation of the inclusion compounds using the freeze drying method is expanded from a laboratory freeze drier to an



industrial level freeze-drier. Since the laboratory freeze drier was not capable of handling more than a liter per batch, the batch size was limited to one liter. The total time required to complete the batch was 4-5 days. Although theoretically the yield per batch (with 2% solids) was 20 grams, the experimental yield was only 18 grams. Therefore, in order to begin formulation work by using the laboratory freeze dryer it would take months to collect a reasonable quantity of material. Hence, the success of a new drug or complex for dosage forms formulation work is dependent upon scale-up evaluation from laboratory procedure to routine production operations.

With this in mind two phenytoin beta-cyclodextrin complex batches were made using an industrial level freeze dryer at Miles Pharmaceuticals in West Haven, Connecticut. The first batch yield (Table XVI) was much poorer than expected (43% less). This process was carried out in an open tray freeze-drying system under a vacuum, thus, directly exposing the drying material to the applied vacuum. A large portion of the dried fluffy end product may have been inadvertently vacuumed away during the drying cycle. In order to increase the yield and decrease the percent loss, another batch was made with a minor modification to the open tray system (Fig. 30a). As shown in the Fig. 30b, a cover with an opening in the center was placed onto the open metal tray. In addition, a stainless steel screen (30 mesh) was used to cover the center opening (Fig. 30c). This equipment resulted in a reduction in direct exposure and prevented the loss of the drying material to the applied vacuum. The yield was significantly improved, limiting the loss to 27% (Table XVI).

The results of preliminary scale-up batches indicated that the preparation of the complex by freeze drying may be scaled-up to a

Table XVI

Preliminary scale-up results of the phenyton beta-cyclodextrin complex.

Batch No.	Batch size (liters)	Yield obtained (gms.)	Yield expected (gms)	% Loss
1	10	113	198	43
2	8	116	158	27

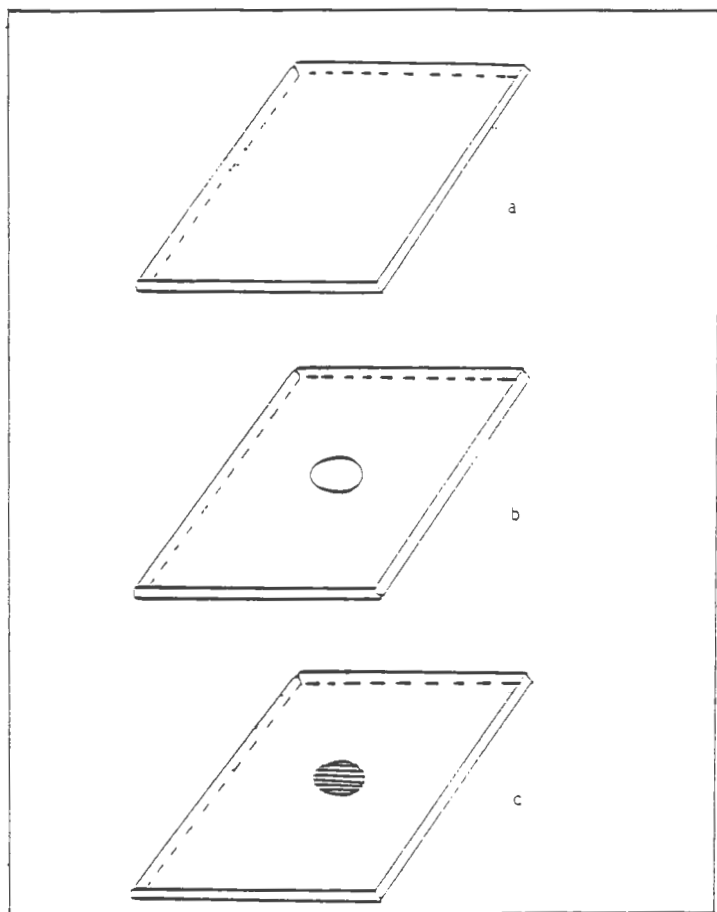


Fig. 30: STAINLESS STEEL TRAYS USED FOR FREEZE DRYING.

Key: a- Open tray, b- Open tray with cover having an opening in the center, c- Tray with center opening covered by a stainless steel screen.

manufacturing level. In addition moisture content determinations of the complex and the drug content analysis (Table XVII) suggested that the method is also reproducible. It was felt that it may be possible to improve the yield by using finer screens to cover the center opening in Fig. 30b. A 60 mesh stainless steel screen over the center opening improved the yield from 73% to 90% (Table XVIII). Thus, it was clearly shown that the preparation of the phenytoin inclusion compound can be scaled-up to manufacturing level. The total length of time required to complete the ten liters batch was little less than two days (Appendix B, 1), compared to four to five days to complete the liter batch by using the laboratory freeze drier. In addition, in the laboratory freeze drier, it was not possible to determine the final end product temperature without breaking the vacuum system. However, as shown in Appendix B, 1, it was possible to determine quantitatively the final end product temperature.

A phenobarbitone beta-cyclodextrin complex was also prepared using the same technique to improve the yield. It was possible to get a yield of more than 90%. The total length of time required to complete the ten liter batch was less than two days (Appendix B, 2).

### C. Preparation and evaluation of dosage forms

#### 1. Liquid dosage forms

The compositions of two potential liquid formulations are given in Tables IV and V. These preparations were visually examined for clarity, ( precipitation, crystal growth) change in color, odor, etc. After two weeks the formulation containing 10% glycerol, 40% sorbitol, and 20% syrup (Table V) showed slight precipitation and microbial growth. However, the formulation (Table IV) containing 40% glycerol, 20% sorbitol

Table XVII

Some properties of the phenytion beta-cyclodextrin complex.

Batch No	Batch size	Moisture content	% Drug content
1	10	6.50	18.90
2	8	5.70	18.30

Table XVIII

Scale-up results of the phenytoin beta-cyclodextrin complex.

Batch no.	Batch size (liters)	Yield obtained (gms.)	% Yield
1	10	113	57
2	8	116	73
3	10	160	80
4	10	160	80
5	10	180	90

and 20% syrup did not show any precipitation or visual evidence of microbial growth after samples had been stored at ambient temperature up to four months. The probable reason for an increase in stability of the formulation is the higher concentration of glycerol, since glycerol itself acts as a preservative when it is present in higher concentrations (130). This formulation was compared with an official elixir (128) with respect to physical properties as well as one month's chemical stability data. The results are in Table XIX. The official formula contains 45% glycerol, 15% syrup and 15% alcohol. The alcohol content of the official preparation is required to keep the phenobarbitone in solution; however, the alcohol content in the official elixir may not be desirable, especially for pediatric use. For the phenobarbitone beta-cyclodextrin complex the addition of alcohol was not required. It has been reported (140,141) that in the case of elixir of phenobarbitone, propylene glycol, and a combination of poly-alcohols can be used as substitutes, and formulations using these substitutes have been proposed. Peterson and Hepponen (142), developed a formula containing 35% of propylene glycol, 20% syrup, and 0.4% of phenobarbitone with 0.1% flavoring oil. They did not report any chemical stability data, however, after three months physical stability was satisfactory. In order to aid in masking the bitter taste of phenobarbitone, and since propylene glycol does not add any sweetness as does the glycerol of the official formula, they increased the syrup content from the 15% of the official formula to 20%. However, their taste tests showed that the propylene glycol preparations were less acceptable than the official elixir.

The formulation (Table IV) of the present study does not contain any propylene glycol, and the concentration of the glycerol is similar to

Table XIX

One month chemical stability of solution prepared from the phenobarbitone beta-cyclodextrin complex, stored at room temperature.

Product	% Label claim *
Formulation #1 (Table IV)	94.80
Official elixir **	97.60

\* An average of three injections.

\*\* Contains 45% glycerol, 15% syrup, 15% alcohol, and 0.4% phenobarbitone.



that of the official preparation. The taste was better than or equal to the official formulation. Furthermore, it is the opinion of the author of this thesis that the complex could readily be used as a powder for reconstitution were any chemical stability problems to arise in the preparation.

A phenytoin suspension was prepared (Table VI) from the complex and its physical stability up to two years did not show changes in appearance, color, odor, etc. The results of the sedimentation study (Fig. 31), indicated that the sedimentation volume was slowly decreased during the first period of the week, then decreased gradually. However, even after six months, the sedimentation volume was approximately 60% of its original value. The redispersibility study showed that ten revolutions were required to restore the suspension to homogeneity. The blender rotation was 20 to 22 rpm. Hence, the suspension was easily redispersed within 30 seconds. In order to compare the ease of redispersability using the technique described in Experimental Section IIc, 2c, a light magnesium oxide suspension was introduced as a control. The percentage of solid content was the same as in the phenytoin suspension (Table VI) in the same suspending medium. However, due to formation of a concrete cake, it was not possible to redisperse the light magnesium oxide suspension, even after five minutes. In contrast, the phenytoin beta-cyclodextrin complex suspension, which contained amorphous freeze dried material, formed a flocculated suspension. A floc, or floccule, by definition (143), is a loose aggregation of individual particles that are held together by comparatively weak particle-to-particle bonding forces; the sediment is loosely packed and has a scaffold-like structure. Particles do not bond tightly to each

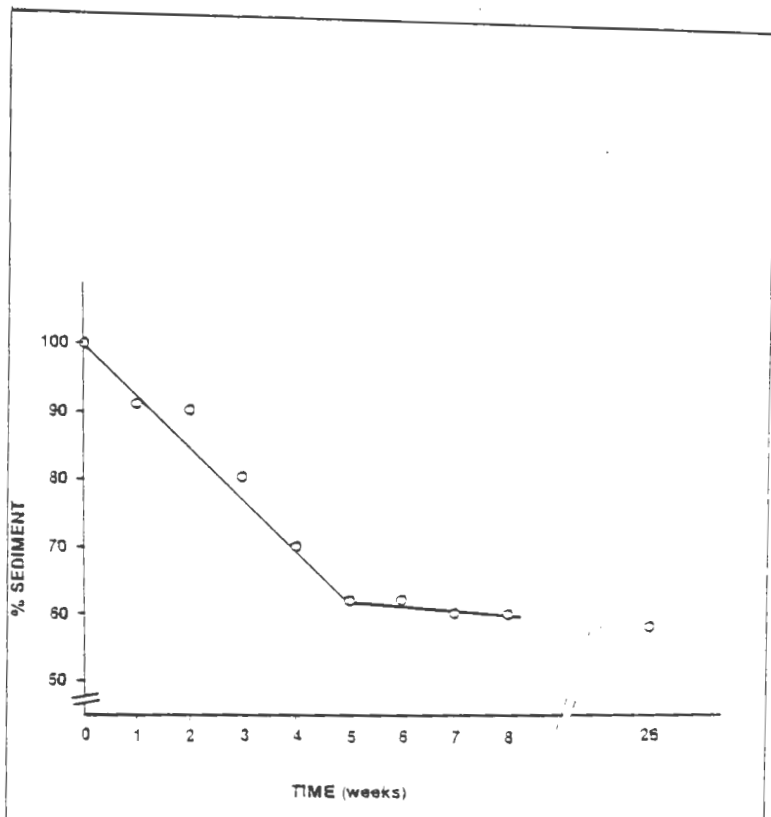


Fig. 31: Sedimentation study of the suspension prepared from the phenytoin beta-cyclodextrin complex.

other, and a hard, concrete cake does not form. The sediment of our suspension was easy to redisperse, so as to form the original suspension.

Generally, a typical suspension formula contains drug, suspending agent, suspending medium, flocculating agent, electrolytes, preservatives, etc. (144,145). But the formula selected in this study (Table VI) contains only drug or suspensoid and suspending medium. It may also be noteworthy that a 50:50 mixture of water and glycerol as the suspending medium was chosen because of the poor solubility of phenytoin (138,146) plus the low solubility of beta-cyclodextrin in the suspending medium (147). The drug complex exhibits a minimum degree of solubility and thereby it is possible to achieve a maximum chemical stability. Hence, despite the high concentration of the solid content (10%) without any extra additives, the suspension possessed desirable qualities (144, 145 and 148) such as ease of dispersion, high sediment volume, etc. These properties are indicative of a pharmaceutically elegant suspension. Furthermore, the suspension was stored in a freezer (app.  $-15^{\circ}\text{C}$ ) for a week. Since the freezing point of a 50:50 mixture of water and glycerol is  $-22.0^{\circ}\text{C}$  (149), the suspension did not freeze at all. This property is another of the desirable qualities of a pharmaceutically elegant suspension. Figures 32, 33 and 34 depict the morphology of the suspension stored at freezing station, refrigerator and at ambient conditions. As expected, it is evident from Fig. 32 that the suspension stored at freezing temperatures showed a slight increase in particle size, whereas Figs. 33 and 34 showed little or no change in the particle size. It is the author's opinion that one week storage at freezing conditions is too severe, hence some change in particle size is to be expected. The photomicroscopic technique is one of the oldest and most

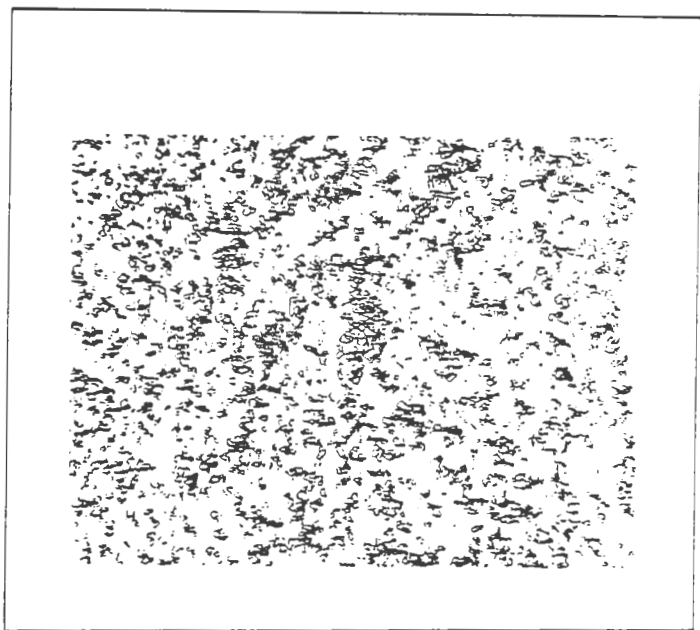


Fig. 32: Photomicrograph of the suspension stored at  $-15^{\circ}\text{C}$  (125x)

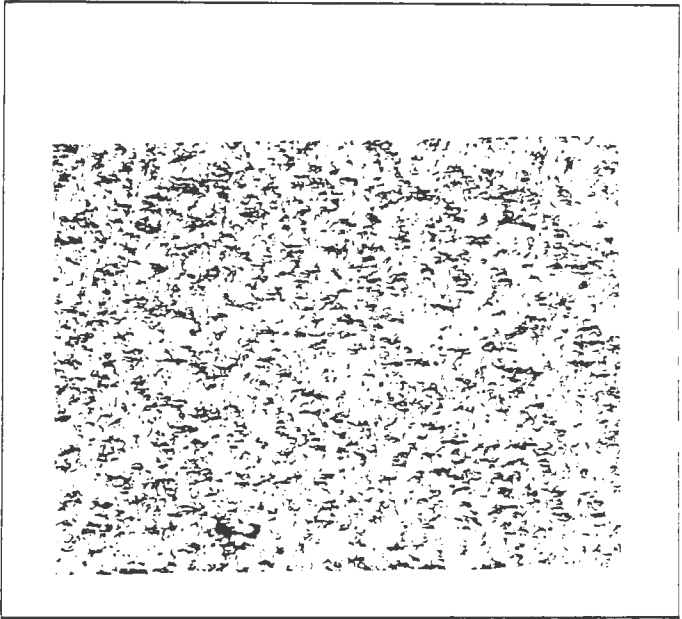


Fig. 33: Photomicrograph of the suspension stored at 5-8°C (125x).

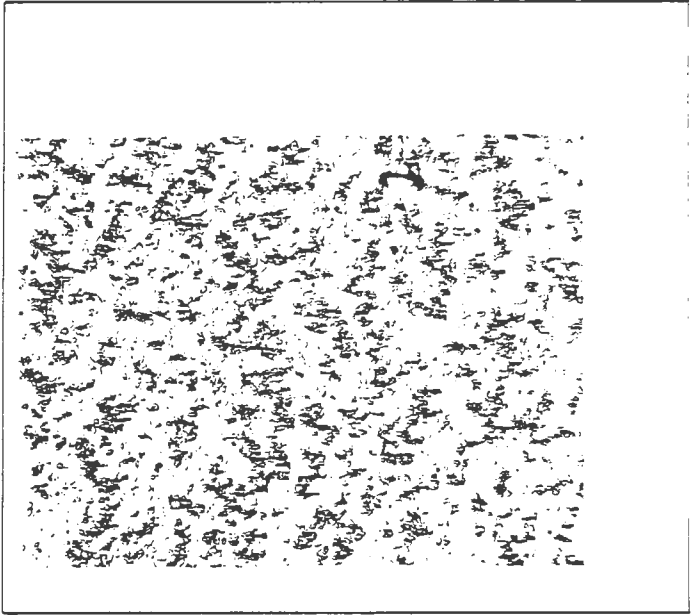


Fig. 34: Photomicrograph of the suspension stored at room temperature.

useful for detecting changes in particle size and crystal form. Nash (145) also used a similar technique in the evaluation of physical stability of a suspension and also particle size measurement. However, he reported that particle size measurement using a photomicrograph gives an approximation but not an accurate value. Therefore Nash (145) suggested the use of a Coulter Counter, an electronic particle counter that measures the resistance caused by the presence of a particle in an electrolyte. Since it was not possible to use such an electronic particle counter, an estimation of the particle size was determined from Fig. 32. An average of length of 25 particles was less than 25 $\mu$ .

Another, distinct advantage is that, since this suspension contains freeze dried material, it is presented to the body in fine particles just like micronized particles ready for dissolution process immediately upon administration (150). In addition, the suspension is less viscous and it was possible to pass 5ml of the suspension through a 22-gauge needle without a great difficulty. Hence, it may be possible to use the suspension for intramuscular administration.

Anderson, F.M. et al (59), reported that the bioavailability of femoxetine beta-cyclodextrin complex, formulated as a suspension, was found to be similar to that observed from a sugar-coated tablet of femoxetine salt. Their study would have been more meaningful if the bioavailability results had been compared with femoxetine suspension instead of its salt form. However, it was shown in their study that the salt form of femoxetine is equivalent to the inclusion complex of femoxetine.

## 2. Solid dosage forms

As discussed in Section G (Technical aspects) of the Introduction portion of this thesis when a formulator is called on to develop a suitable dosage form for a new drug, he or she would likely first consider solid dosage forms, due to several reasons, mainly:

- a. Formulation and stability problems arise less frequently with solid dosage forms than with liquid pharmaceutical preparations (130).
- b. Solid dosage forms are preferred because of their ease of administration, dose uniformity, etc.

When a formulator faces the problem of getting a sufficient quantity of active ingredients, an innovative modification of techniques to a semi-micro scale may well be needed. One of the most important considerations in solid dosage forms manufacture is a uniform flow rate of solid mixtures. Modern high speed tableting or capsule-filling machines are capable of producing thousands of units per minute. Thus, an acceptable flow of solid mixtures is essential. Among the various methods used (151) to predict powder flow, various flowmeters have received considerable attention (135,136,152-155). These methods are useful if sufficient quantities of active ingredients are available. However, when a formulator faces the problem of getting a sufficient quantity of active ingredient, then the question becomes one of predicting the effect of formulation and processing variables on production scale quantities using a small quantity of material. In order to answer this question, miniturization of flow studies was undertaken and results are given in Table XX.

It is evident in Table XX that good flow rate and a high linearity index were recorded through hoppers I and II as the mass was increased up



Table XX - Comparisons of mass flow rates<sup>a</sup> with linearity values<sup>b</sup>

	Hopper I <sup>c</sup>		Hopper II <sup>d</sup>		Hopper III <sup>d</sup>	
	Flow rate (Range)	Linearity	Flow rate (Range)	Linearity	Flow rate (Range)	linearity
Dicalcium phosphate dihydrate, g.						
100	-	-	-	-	49.7 (5.0)	19.0
200	162.9 (27.6)	17.7	83.1 (6.80)	17.5	43.7 (1.9)	18.1
300	177.8 (11.0)	18.2	85.2 (4.60)	19.5	40.4 (3.3)	16.7
400	202.8 (10.0)	19.1	89.7 (5.50)	19.6	-	-
600	206.7 (13.8)	19.5	95.7 (6.40)	19.7	-	-
800	201.5 (5.30)	19.6	95.5 (4.60)	19.6	-	-
1000	203.9 (8.00)	19.5	91.9 (1.70)	19.2	-	-

<sup>a</sup>g/s (An average of three trials). <sup>b</sup>  $(r^2 - 0.8) \times 100$ ; I- Stainless steel hopper from Stokes single punch tablet press. II- Conical glass funnel. III- Cylindrical glass funnel.

to 60% of the hopper capacity. However, the flow rate and linearity index were decreased through hopper III as the mass was increased from 30 to 100% of the hopper capacity. Furthermore, effects of different levels of lubricant on flow rates were determined with a constant mass (200gm). The results are plotted in Fig. 35. It is evident that lower concentrations of magnesium stearate (0.3 and 0.6%) resulted in increasing flow rates from all three hoppers whereas, higher concentrations of lubricant levels (1.0 to 5.0%) resulted in a linear and faster decrease in flow rates with larger orifice diameter (hopper I) compared with the smaller orifice diameters (hoppers II and III). Since the three hoppers are different in geometry and in size, variation in flow rate was to be expected. However, the results indicate that although different hoppers affect the quantitative nature of the results, the same general trends are apparent. Hence, this study indicates that it may be possible to use a recording powder flowmeter with small quantities of material in an attempt to predict the flow rate, effect of formulation and processing variables on production scale quantities. In addition, for routine quality control or in-process powder flow testing situations, using a small quantity of material is cost effective. This makes the recording powder flowmeter data useful in numerous quality control and in-process powder flow testing situations. The reproducibility and sensitivity of recording powder flowmeter data for a number of systems are well documented in the the literature (152-155).

As also mentioned in Section G (Technical aspects) of the Introduction to this thesis, cyclodextrins are expensive for large scale use. Table XXI shows some physical properties of beta-cyclodextrin. It has reasonably good flow rate and compressibility. However, as shown in

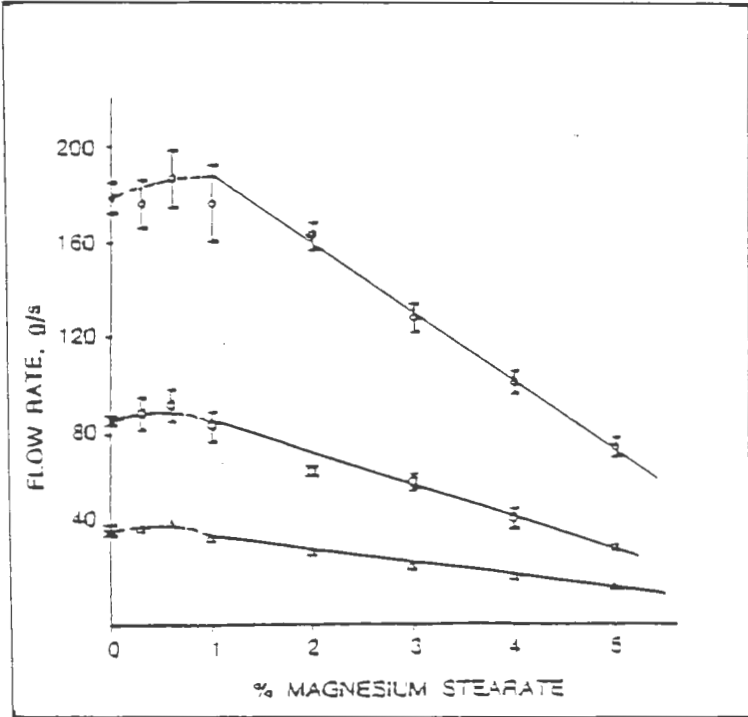


Fig. 35: Effect of different levels of lubricant on flow rates with constant mass (200gm.) of dicalcium phosphate dihydrate (Emcompress).

Key: - Stainless steel hopper type I; - Conical glass funnel type II; - Cylindrical glass funnel type III

Table XXI

Some physical properties of the beta-cyclodextrin

Particle size distribution (in microns)		%	
0 - 74		11.80	
74 - 125		25.70	
125 - 149		32.00	
149 - 177		15.00	
177 - 250		12.20	
250 - 420		3.10	
Flow rate gm/sec	Bulk density gm/cc	Tap density gm/cc	Compressibility %
3.2	0.54	0.68	20.58
All the above values are an average of three trials.			

Table XXII, the phenytoin beta-cyclodextrin complex prepared by various methods does not have either good flow or compressibility properties. Without a good flow it is very difficult if not impossible, to develop solid dosage forms. The usual dose of phenytoin is 100mg three times a day. Thus, based on the concentration of phenytoin (20%) in the complex, it is estimated that the total amount of the complex required would be 500mg (equivalent to 100mg phenytoin). Therefore, the complex would contribute a major portion of the total tablet weight.

In order to enhance the flow properties especially of the freeze dried complex (poor flow, Table XXII), different concentrations of the complex were mixed with a direct compression vehicle (Emcompress) and flow rates evaluated. As the concentration of the complex was increased (Fig. 36), flow rate decreased drastically. An acceptable flow rate was found only when the concentration of complex was less than 5%. Thus, direct compression was not found to be feasible, since the final tablet weight would be more than a few grams. An attempt was made to prepare slugs, however, due to lack of compressibility and flowability of the complex, slugs could not be prepared. Thus wet granulation was the only method available for tableting. Since freeze dried materials are generally highly soluble, it was thought that after addition of a granulating agent the material might agglomerate rapidly; however, no agglomeration problem was observed during the wet granulation process. It was then possible to develop a formula (Table VII) with an acceptable weight and size. It has been reported by Szejtli, J. (8), who is one of the authorities in the field of cyclodextrin inclusion complexes that, "if the required dose amounts to several hundred milligrams, then especially with low molecular weight drugs, it would be necessary to

Table XXII

Some physical properties of the phenytoin beta-cyclodextrin complex prepared by the various methods.

Complex prepared by	Flow Rate mg/s	Bulk mg/cc	Tap mg/cc	Compressibility %
Freeze drying	33	65	167	61.08
Spray drying	302	402	744	45.97
Kneading	1836	480	727	33.98
Solvent evaporation	3270	512	717	28.60
Emcompress*	4666	733	916	19.98

\* Used as a reference standard.

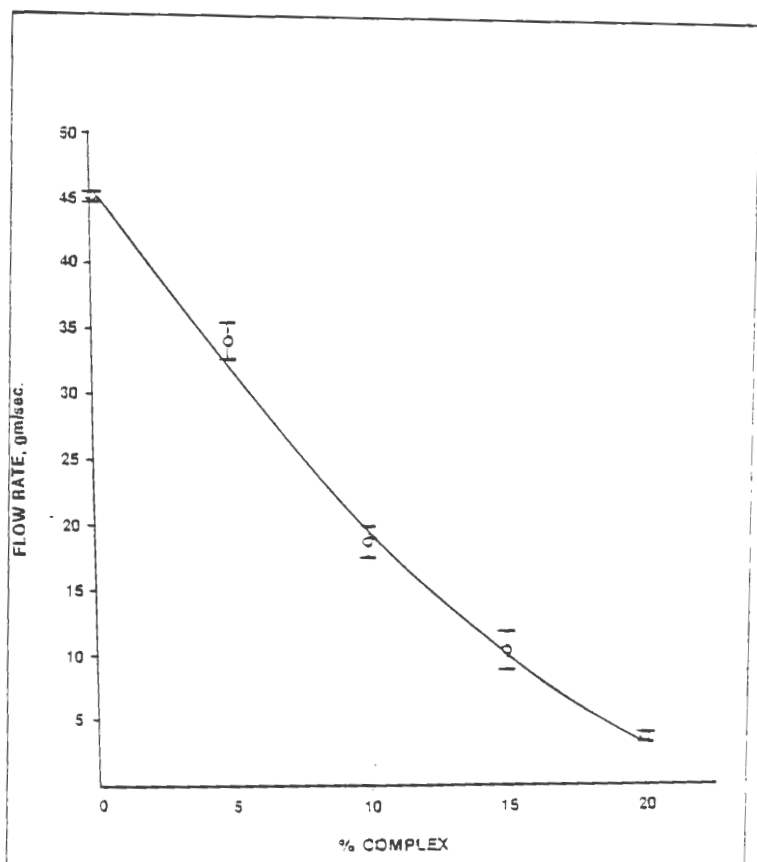


Fig. 36: Flow study of the freeze dried phenytoin beta-cyclodextrin complex.

disperse several grams of cyclodextrin complex tablets. Thus no practical application of the complex seems to be likely." However, the phenytoin dose is 100mg, ( by complexation with beta-cyclodextrin) and using the wet granulation method it was possible to make a tablet of 625mg containing 100mg phenytoin. It is in the opinion of the author of this thesis that if the drug content in the complex is less than 10%, and if the molecular weight of the drug is very low, practical application of cyclodextrin complexes for tableting is highly unlikely.

Table XXIII shows the particle size distribution, bulk properties, flow characteristic and compressibility of formulation prepared from the phenytoin beta-cyclodextrin complex. The complex was prepared by the freeze drying method and then wet granulated. By wet granulation it was possible to achieve good flow rate and compressibility. Furthermore, tablets were compressed using an instrumented tablet press and Fig. 37 depicts the results of compressibility. It is evident from Fig. 37 that there is almost a linear relationship between hardness and compression force, thus there was no problem during the compression. Tablets were examined for a variety of properties including weight variation, hardness, disintegration time, etc. Table XXIV lists all of these properties. All the examined properties meet the USP standards. Fig. 38 shows the dissolution test data performed on weighed portions of the tablets; the figure shows that at 60 minutes more than 90% of the drug had dissolved. Fig. 39 depicts the dissolution test data performed in accordance with the USP monograph dissolution medium. In this plot (Fig. 39) the dotted line represents the percent drug dissolved based on the equilibrium solubility of phenytoin (138). The dissolution results of three different products are shown in this plot. The three products are:



Table XXIII

Some physical properties of granulation prepared from the phenytoin beta-cyclodextrin complex.

Particle size distribution (in microns)		%	
0 - 74		24.25	
74 - 125		8.75	
125 - 149		6.24	
149 - 177		3.54	
177 - 250		7.75	
250 - 420		48.75	
Flow rate	Bulk density	Tap density	Compressibility
gm/s	gm/cc	gm/cc	%
7.0	0.51	0.68	25
All the above values are an average of three trials.			

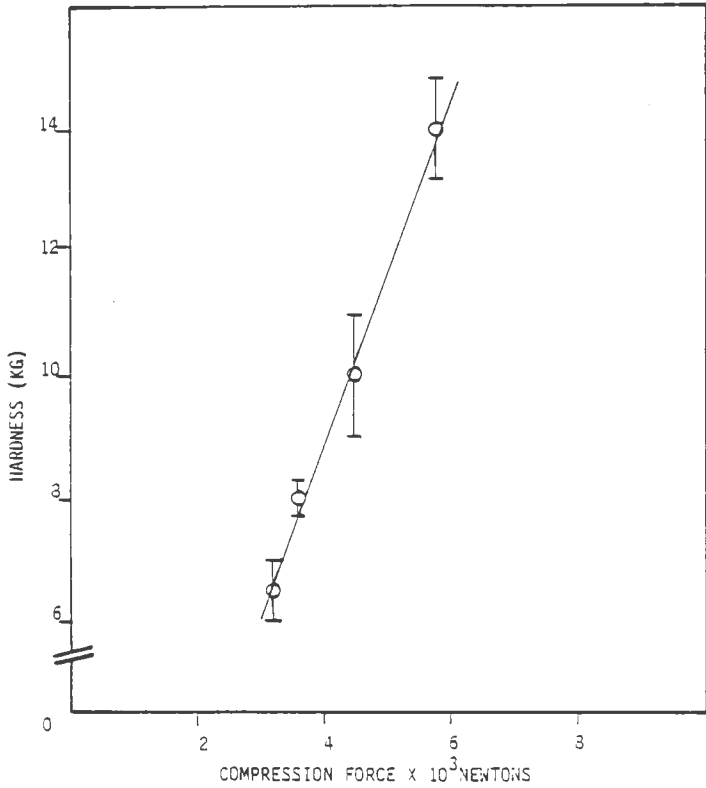


Fig. 37: Compression study of the formulation prepared from the freeze dried phenytoin beta-cyclodextrin complex.

Some physical properties of the tablets prepared from the phenytoin beta-cyclodextrin complex.

Weight variation (mg)	Hardness (kg)	Thickness (inches)	Friability %	Disintegration time (minutes)
335	7.5	0.187		7.0
333	6.5	0.188		6.5
322	9.0	0.188		5.0
335	8.5	0.187		5.5
321	7.0	0.190		4.0
334	7.5	0.187		6.0
334	8.0	0.187		
324	7.5	0.187		
324	6.5	0.187		
323	6.5	0.187		
325	7.0	0.188	0.18	
327	7.5	0.186		
325	8.0	0.188		
325	7.5	0.188		
323	7.0	0.187		
320	7.0	0.191		
322	6.5	0.188		
326	7.0	0.188		
325	7.0	0.180		
320	7.5	0.190		
Mean 326 * (5.0)	7.33 (0.67)	0.188 (0.001)		5.7 (1.1)
*-Standard deviation				

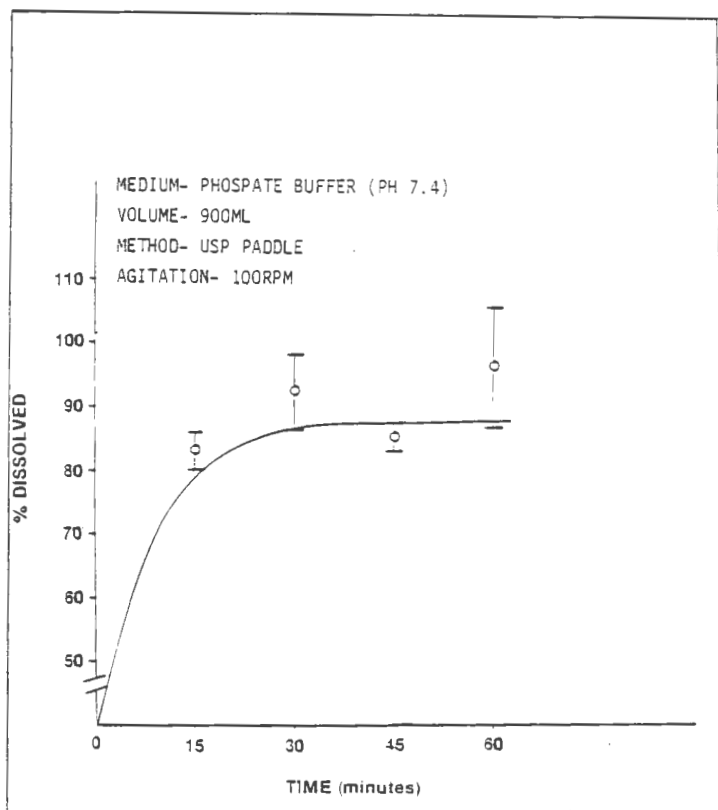


Fig. 38: Dissolution study of the tablets prepared from the freeze dried phenytoin beta-cyclodextrin complex.

\* Each point is an average of six determinations.

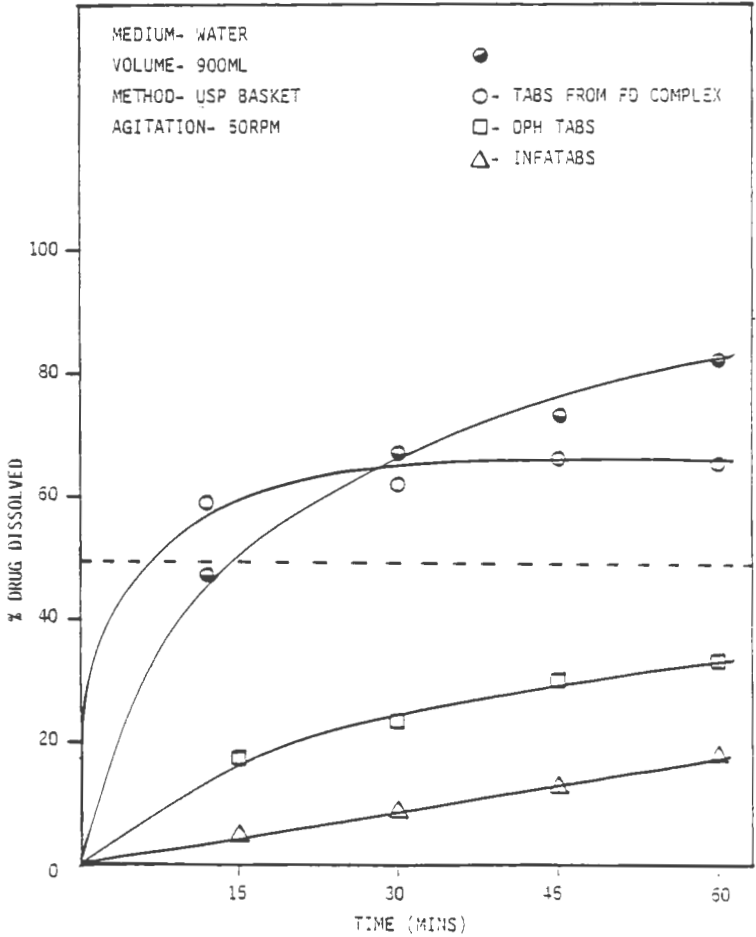


Fig. 39: Dissolution studies of the phenytoin beta-cyclodextrin systems.

\* Each point is an average of three determinations.

tablets prepared from the freeze dried beta-cyclodextrin complex; Infatabs from Parke-Davis; and phenytoin tablets prepared by Mr. S.R. Ghanta (graduate student). It is evident that the phenytoin beta-cyclodextrin complex showed highest percent dissolved (more than 60% at 30 minutes). Because of the low aqueous solubility of phenytoin (138), dissolution was conducted with half tablets. However, as shown in the same plot (Fig. 39), there was no significant improvement in the dissolution rate in water. The tablets prepared from the freeze dried complex reached the equilibrium solubility within fifteen minutes; whereas, the other two phenytoin products did not reach the equilibrium solubility even after 60 minutes. Further, it was thought that, since the Infatabs are used as chewable tablets, they may erode very slowly and for this reason showed slowest dissolution rate. However, the instructions on the Infatabs bottle indicate that the tablets can be either chewed thoroughly before being swallowed or swallowed whole. Therefore these tablets were taken for comparison. Generally, chewable tablets are hard, they erode very slowly in the mouth, so that a slow dissolution rate is expected. However, when these tablets were crushed, passed through 60 mesh sieve and filled into capsules, then dissolution was compared (Fig. 40) with the similar preparation of the tablets prepared from the freeze dried complex. As shown in Fig. 40, the freeze dried complex showed significant improvement in the percent drug dissolved. Figure 41 shows the dissolution of phenytoin beta-cyclodextrin complex tablets after two years' storage at ambient temperatures, compared with a sodium phenytoin capsule (Zenith Labs). Tablets from the freeze dried complex reached the equilibrium solubility within 30 minutes. As shown in Fig. 41, the half tablets showed

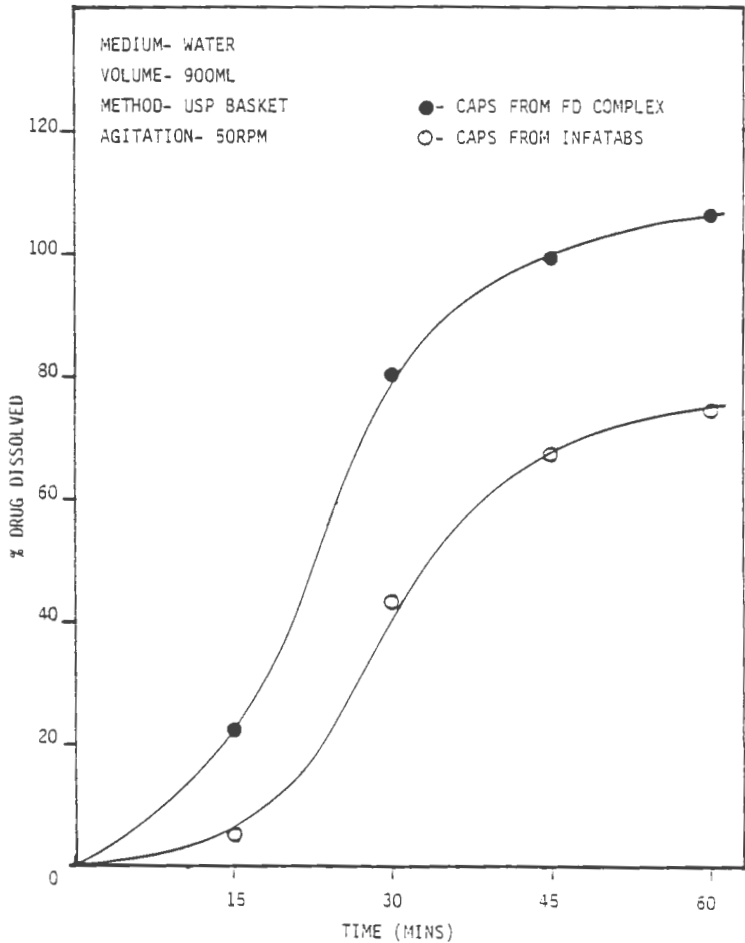


Fig. 40: Dissolution studies of the phenytoin beta-cyclodextrin systems.

\* Each point is an average of three determinations.

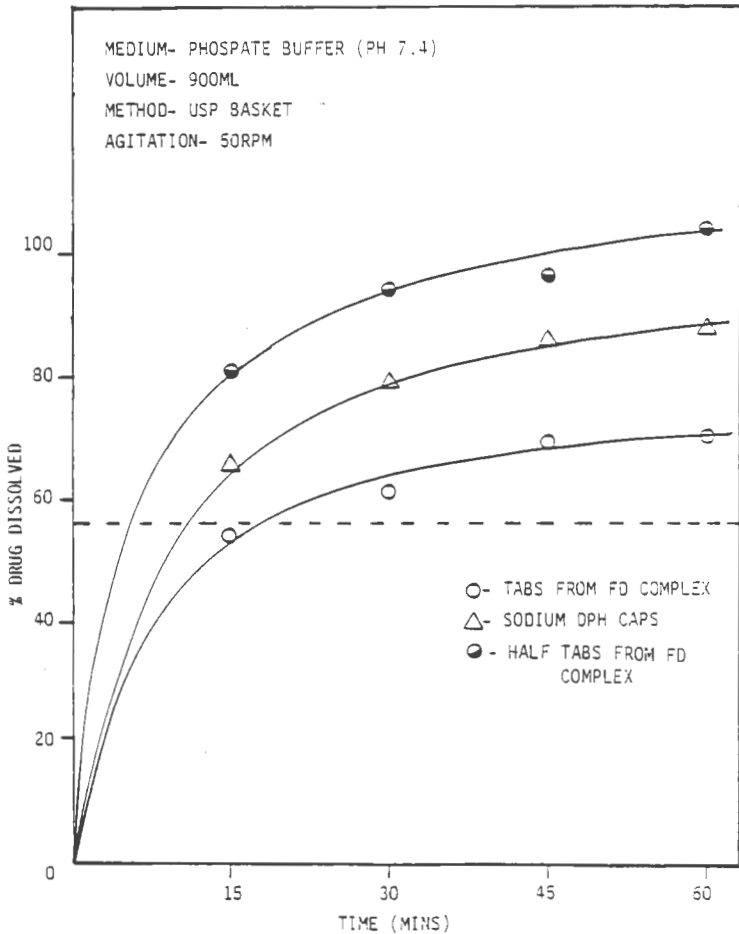


Fig. 41: Dissolution studies of the phenytoin beta-cyclodextrin systems.

\* Each point is an average of three determinations.



significant improvement in the dissolution rate compared to sodium phenytoin capsules.

Dissolution was also investigated in an acidic medium. The results are depicted in Fig. 42. In this plot, dissolution results of sodium phenytoin, freeze dried complex, physical mixture of phenytoin beta--cyclodextrin, and phenytoin powder alone are shown. As expected, the physical mixture and phenytoin did not improve dissolution. However, the dissolution rate of the complex and sodium phenytoin powder was by and large equal after a 30 minutes time period. Figure 43 shows the dissolution results of the freeze dried complex tablet (full and half) and sodium phenytoin capsule. As observed in Fig. 42, approximately 60% of the drug was dissolved from the complex within 60 minutes. The sodium phenytoin capsule showed a higher percent dissolution (apprx. 70%) within 60 minutes. This increase in percent dissolved may be due to the effect of formulation and processing. However, the percent drug dissolved from the half tablets was higher at the 30 minute time period than sodium phenytoin capsule. These results indicated that even in the acidic medium the dissolution of the phenytoin beta-cyclodextrin complex was better or equal to the sodium phenytoin capsules.

Since there was no significant difference between total percent drug dissolved in water and in an acidic medium, further dissolution work was carried out in water according to the USP (128). Figure 44 shows the dissolution results of phenytoin beta-cyclodextrin complex prepared by the various methods. As indicated earlier the dotted line represents the equilibrium solubility of phenytoin in water at 37°C. In this plot the complex prepared by spray drying, kneading, etc, showed less than 40%

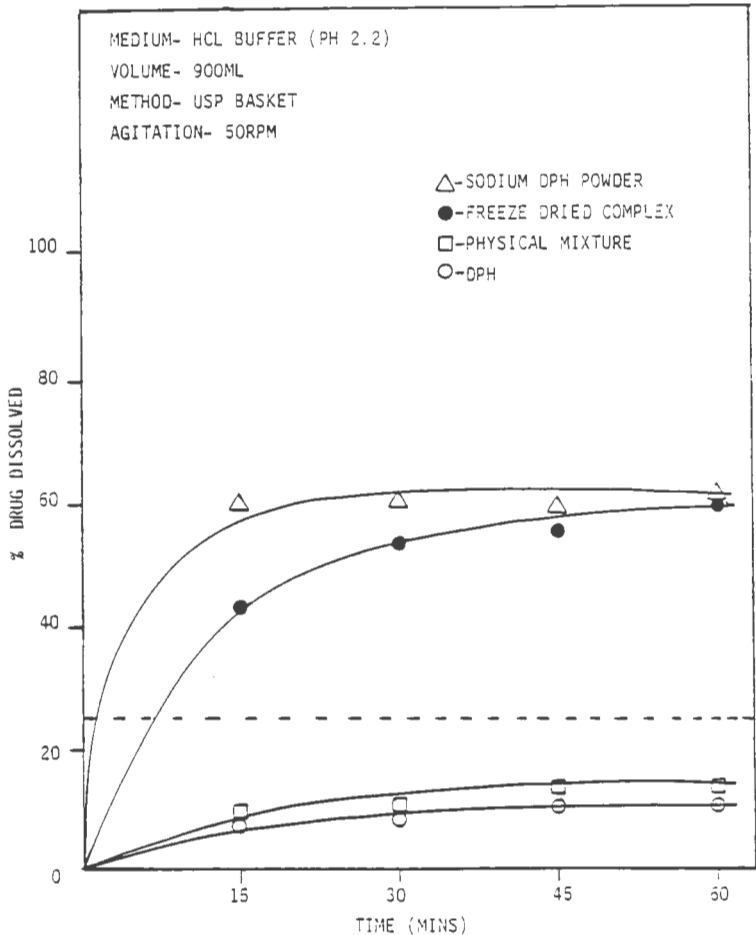


Fig. 42: Dissolution studies of the phenytoin beta-cyclodextrin systems.

\* Each point is an average of three determinations.

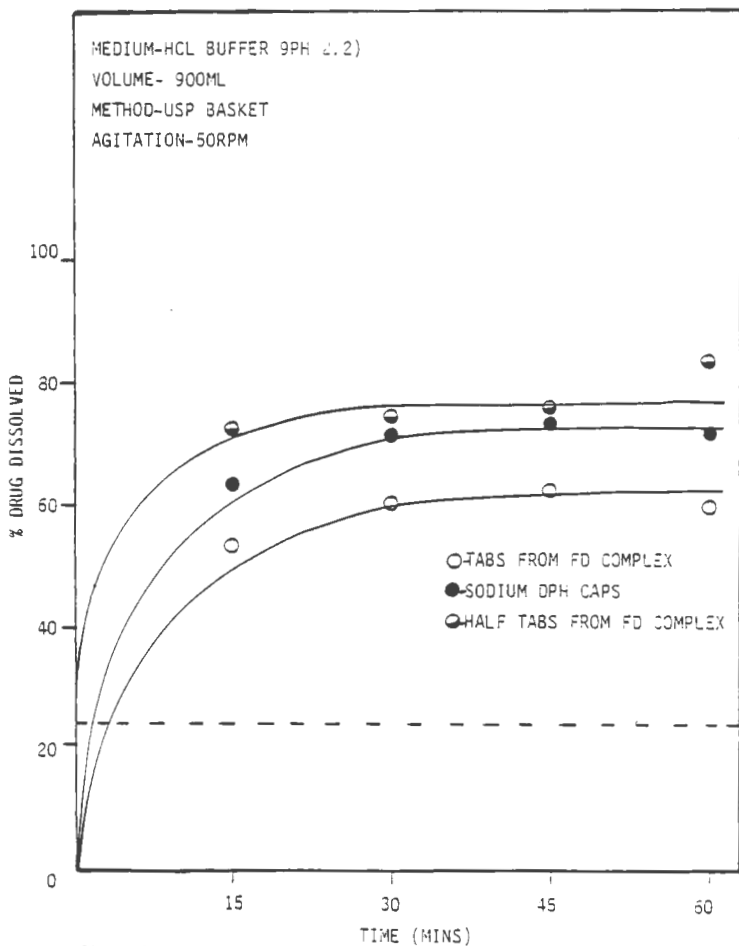


Fig. 43: Dissolution studies of the phenytoin beta-cyclodextrin systems.

\* Each point is an average of three determinations.

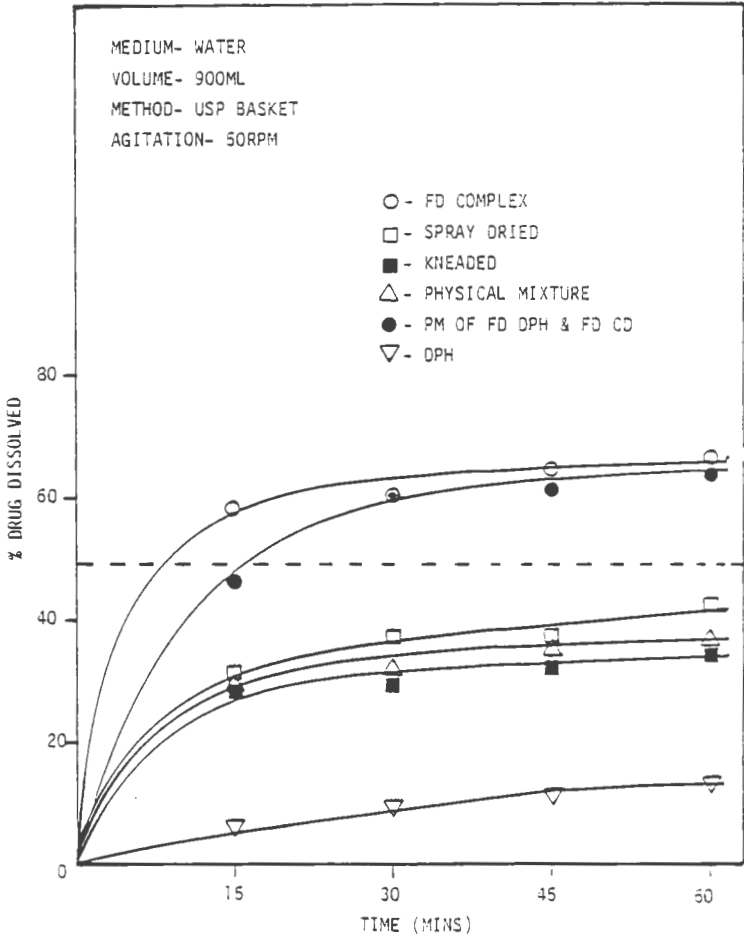


Fig. 44: Dissolution studies of the phenytoin beta-cyclodextrin systems.

drug dissolved even at 60 minutes. Whereas, the complex prepared by the freeze drying method showed more than 60% dissolved at 60 minutes.

This increase in percent dissolved is due to the amorphous nature of the freeze dried complex as seen earlier in X-ray analysis (Fig. 13c) and photomicrographs (27b). However as shown in Table XXV, there was no significant difference in disintegration time of phenytoin beta-cyclodextrin complex tablets prepared by the various methods. Furthermore, it was thought that a decrease in particle size of phenytoin may increase the dissolution rate similar to the freeze dried complex. Hence, phenytoin was freeze dried alone, compressed into tablets and dissolution was carried out. The results are shown in Fig. 44. As shown in Fig. 44, the freeze dried phenytoin did not improve the dissolution rate. However, when the freeze dried phenytoin was mixed with freeze dried beta-cyclodextrin, the tablets prepared from that mixture showed (Fig. 44) the similar results as obtained from the freeze dried complex. As shown in Fig. 44, there is a slight difference at 15 minutes time period; however, after that time period, the percent drug dissolved was by and large equal to the freeze dried complex. This unique phenomenon, may be due to the in situ complex formation in the dissolution medium. It is the opinion of the author of this thesis that as far as can be ascertained, this unique phenomenon (in situ complexation) is the first report of the interaction of beta-cyclodextrin with phenytoin.

Phenytoin is a high melting ( $293^{\circ}\text{C}$ ), weakly acidic (138,146) and poorly water soluble drug (138). Because of these physicochemical properties, phenytoin is subject to erratic and incomplete bioavailability (156-159). Phenytoin has been classified as a drug with "high risk potential" with respect to bioavailability problems (160,161).

Table XXV

Disintegration time of the phenytoin beta-cyclodextrin complex tablets  
Prepared by the various methods.

Method of preparation	Disintegration time in minutes (Standard deviation)
Freeze drying	6.5 (1.2)
Spray drying	6.0 (2.3)
Kneading	7.3 (3.0)
Solvent evaporation	7.8 (2.5)

It has been reported that the rate of dissolution of phenytoin is influenced by particle size (162), characteristics of excipients (163), manufacturing procedures and dosage form (164).

Chakrabarti et al. (162) reported that significant differences in bioavailability were observed when phenytoin crystals of different particle size were administered. In their investigation 3 dogs were administered  $390 \pm 10$ mg capsules in a cross-over design with an interval of one week between two administrations. The radioimmunoassay method was used in the analysis of drug content. The largest area under the curve (AUC) and maximum concentration ( $C_{max}$ ) were observed with smallest particle size (0-32 $\mu$ ), followed by 75 and 100 $\mu$  particle size respectively. Chakrabarti et al (162) did not report the time required to reach maximum ( $T_{max}$ ) plasma level. The paired t-test was used to show the significant difference between the  $C_{max}$  and the AUC among the treatments. They concluded that the AUC is inversely related to the particle size. In addition, their results were correlated with the faster dissolution of phenytoin of smaller particle size. It is the opinion of the author of this thesis that since the phenytoin beta-cyclodextrin complex prepared by the freeze drying method produced amorphous particles (less than 25 $\mu$ ), and therefore a smaller dose would be enough to achieve the therapeutic range of plasma level (10-20  $\mu$ g/ml).

Yakou et al (163), reported the effect of manufacturing procedures on the dissolution and human bioavailability of phenytoin. The dissolution characteristics of commercial phenytoin, freeze dried phenytoin, 20% simple blend of phenytoin with lactose and starch, and 20% solvent deposition of phenytoin powder were determined in pH 1.2 and 7.5 test media. The results are summarized as follows:

- a. The solvent deposition of phenytoin with PVP, showed the fastest dissolution rate (app. 15% in ten minutes). This increase in dissolution rate is probably due to improvement in the wettability of the phenytoin crystals as well as decrease in particle size.
- b. Based on the results of the in vitro studies, 20% phenytoin solvent deposition powder and the phenytoin crystals were selected for a bioavailability study in humans. A cross-over study was carried out in six healthy male volunteers, and 300mg of phenytoin was administered orally. The plasma samples were assayed for phenytoin by the method of enzymeimmunoassay. The urine samples were assayed for phenytoin by gas-liquid chromatography.
- c. The commercial crystals gave a very low plasma level and showed a significant difference was found at each time period. The coprecipitated powder gave 7.63-11.6 times larger AUC than the phenytoin crystals in individual volunteers and the mean was 8.97 times larger. The urinary excretion study also revealed a lower excretion rate of phenytoin from the crystals.
- d. It was concluded from the above study that the phenytoin crystals (between 177-350 $\mu$ ) gave significantly lower bioavailability than the coprecipitated powder due to poor dissolution characteristics of the formulation. However, the authors of this bioavailability study did not report the particle size range of the coprecipitated phenytoin powder. They simply reported that when the same batch of phenytoin crystals was processed by solvent deposition, the particle size might have reduced in the course of the manufacture to below the critical range (less than 50 $\mu$ ) of crystal size distribution. Further, they concluded that a



manufacturing method affects the bioavailability of phenytoin preparations substantially.

Sekikawa et al (164) reported the dissolution behavior and absorption of phenytoin and phenytoin polyvinylpyrrolidone (PVP) coprecipitate in five male subjects. The dissolution characteristics of phenytoin and coprecipitated phenytoin with PVP were determined in pH 1.2 and 7.5 test media. The results are summarized as follows:

- a. The dissolution rate of phenytoin coprecipitate was markedly increased (app. 31.50% in fifteen minutes) in the dissolution media. The concentrations of phenytoin following the dissolution of 1:3 or 1:5 coprecipitates were 2.3 times as much as phenytoin solubility (25ug/ml) in the test media. The dissolution rate of coprecipitate with PVP of lower molecular weight was greater (K-15 K-30 K-90).
- b. Based on the results of the in vitro studies, a bioavailability study was conducted in five male subjects, and 250 mg of phenytoin was administered orally. A cross-over design was used. Urine was collected at hourly intervals for the first nine hours and at a convenient time intervals up to 120 hours after administration. The urine samples were assayed for the main metabolite (5-p-hydroxyphenyl-5-phenylhydantoin) by a gas-liquid chromatography. Blood samples were not taken .
- c. Following the administration of the coprecipitate, the maximum value of mean urinary excretion rate of the metabolite appeared in the 8-12 hour period. Excretion rate of the coprecipitate was almost twice that of the phenytoin alone in the 8-12 hour time period. The intersubject variation of excretion rates were considerably smaller when the coprecipitate was administered. The recoveries of the metabolites excreted in urine for 120 hours following the administration of the

coprecipitate and phenytoin alone were 84.3% and 54.7% respectively. Extent of bioavailability of phenytoin in phenytoin PVP-coprecipitate was 1.54 times greater than that of phenytoin alone. According to the authors of the above study, higher excretion patterns may be considered to be a reflection of the in vitro dissolution behavior of each preparation. High dissolution rate and supersaturation of phenytoin from the coprecipitate resulted in the large improvement of the rate and extent of the bioavailability of poorly water-soluble phenytoin. Furthermore, Yakou et al (165) also reported the particle size dependency on dissolution rate and human bioavailability of phenytoin and phenytoin polyethylene glycol solid dispersions. The dissolution characteristics of phenytoin and phenytoin solid dispersion prepared at various ratios of polyethylene glycol 4000 were determined in pH 1.2. The results are summarized as follows:

- a. At lower ratios of phenytoin to polyethylene glycol (0.5:10, and 1:10) the bulk concentration reached a plateau of 43ug/ml (10.75%) in less than five minutes, while higher ratios (2:10, 3:10 and 4:10) gave rapid rises to 32ug/ml (8.0%) in five minutes with subsequent small increases.
- b. These results suggested that a ratio of less than 1 to 10 is required to disperse phenytoin completely in polyethylene glycol.
- c. Further, the dissolution rate of different particle size suggested a critical particle size between 74-149u for higher dissolution rate.
- d. Based on the results of the in vitro studies, a bioavailability study was conducted in five male subjects, and 300mg of phenytoin crystals (size 44-53um), 200mg of physical mixture with polyethylene glycol, and 200mg of the solid dispersion powders were administered orally. A

three-way cross-over study was used. Blood samples were taken at 2, 4, 6, 8, 12, 24, 32, 36, and 48 hour, and urine was also collected at the same intervals. The plasma samples were assayed for phenytoin by enzyme immunoassay and the levels of the intact phenytoin in urine were assayed by gas-liquid chromatography. The details of the assay methods are not reported. Phenytoin solid dispersion gave the highest plasma level (3.74ug/ml) in 5.6 hours, followed by the physical mixture and phenytoin alone. The physical mixture gave a plasma level of (1.92ug/ml) in 6.8 hours, and phenytoin gave a lowest plasma level of 0.75ug/ml in 5.7 hours.

e. The areas under the plasma level curve were: phenytoin solid dispersion 73.86h.ug/ml, phenytoin physical mixture 50.26h.ug/ml, and phenytoin crystals 18.45h.ug/ml. There was a significant difference among the treatments as shown by Student's-t-test.

f. The urinary excretion rate from the solid dispersion of phenytoin in polyethylene glycol exceeded that from the physical mixture or the phenytoin crystal at any time.

g. It was concluded from the above study that the phenytoin solid dispersion showed superior dissolution and bioavailability. These characteristics of the phenytoin solid dispersion should offer the clinical advantages of quick drug release and excellent bioavailability in phenytoin therapy.

As previously mentioned in Section F, 6 (Bioavailability) of the Introduction to this thesis Tsurika et al (95) studied the absorption of freeze dried phenytoin, phenytoin, and its beta-cyclodextrin complex in a group of four female beagle dogs. They reported that there was a two-fold increase in the area under the curve of the blood level. The

Student's-t-test showed a significant difference in the blood level curves. In addition, it was reported that an increase in bioavailability of phenytoin by means of beta-cyclodextrin complexation suggested the possibility of smaller doses and fewer side effects in phenytoin therapy.

The results of the in vitro dissolution studies of phenytoin obtained by Yakou et al (163), Sekikawa et al (164), and Yakou et al (165) can be compared with the results obtained by the author of this thesis. These results are summarized in Table XXVI. As shown in Table XXVI, it is clearly evident that phenytoin beta-cyclodextrin showed superior in vitro dissolution results in all media. However, direct comparison may be questionable since dissolution results are a function of dissolution methodology, dissolution volume, agitation speed, etc. Despite these differences, it is in the opinion of the author of this thesis that the phenytoin beta-cyclodextrin complex dissolution rate is superior among all the preparations. Hence, it may be possible to use a smaller dose in in vivo studies with fewer side effects. Furthermore, the reported phenytoin preparations such as solid dispersion, coprecipitation, etc. are prepared at a laboratory scale. The feasibility of formulation and incorporation into a suitable dosage form are not explored. Physico-chemical properties are not fully tested, and hence direct practical application is skeptical. However, the scale of production of the phenytoin beta-cyclodextrin complex was increased from a laboratory scale to an industrial scale. Physico-chemical properties, feasibility of formulation, etc. are studied in this thesis.

Anderson, et al. (59), reported a preparation of an inclusion complex of femoxetine with beta-cyclodextrin by a precipitation method. The method of preparation was time consuming and laborious (one liter

Table XXVI

Comparisons of the *in vitro* dissolution data of the tablets prepared from the phenytoin beta-cyclodextrin complex with the literature data of other phenytoin tablets preparations.

Phenytoin tablets preparation	% Drug dissolved in different media Time (minutes)		
	pH 1.2	pH 7.5	Water
* 1. Solvent deposition	7.50 (10)	7.50 (10)	NR
** 2. Coprecipitation with PVP	31.50 (15)	25.23 (15)	NR
*** 3. Solid dispersion in Polyethylene glycol (0.5 : 10.0)	10.75 (5)	NR	NR
	pH 2.2	pH 7.4	Water
4. Beta-cyclodextrin complex	53.50 (15)	56.00 (15)	58.30 (15)

\*- According to reference (163).

\*\* - According to reference (164).

\*\*\* - According to reference (165).

NR - Not Reported

batch took one week to collect the precipitate and one day to dry in vacuum). The yield was only 87%. Scale-up evaluations, reproducibility, etc. were not reported. However, they had developed a suspension dosage form only on a small scale (100ml) from the complex. Physical properties of the suspension such as ease of dispersion, sedimentation volume, freeze thaw study, etc., were not studied. Hence, the formulation feasibility and physical stability are to be investigated.

Sea, et al. (88), studied the inclusion complex formations of spironolactone with three cyclodextrins (alpha, beta, and gamma). They obtained the solid complex by a precipitation method, it took seven days to precipitate the complex and then 48-hr to dry the complex at 60°C. The percent yield, scale-up, dosage form development, etc., were not reported. It is the opinion of the author of this thesis that the method is time consuming and also that the preparation of the solid complex of aqueous labile drugs can be a problem. However, the above authors did not report any stability problems.

Iwaoka, et al. (104), studied the absorption of phenobarbitone from suppositories containing beta-cyclodextrin. They dissolved the drug and beta-cyclodextrin in a molar ratio of 1:1 in hot water (temperature not reported), the solution was then filtered and left to crystallize (length of time not reported). In this study also details of the preparation of the complex, scale-up evaluation, etc., were not reported. However, after rectal administration of the suppository containing the beta-cyclodextrin complex to rabbits, the blood concentration of the drug was higher than that following administration of the phenobarbitone suppository. In the present thesis, the complex was prepared on a large scale; a batch of ten liters took less than two days, and the yield was

more than 90%. It is the opinion of the author of this thesis that the complex obtained by the freeze drying method was amorphous; hence it may be more easily uniformly dispersed in a melted suppository base than the crystalline complex obtained by Iwaoka et al. (104).

Tokumura, et al. (166), studied an inclusion complex of cinnarizine with beta-cyclodextrin in aqueous solution and in the solid state. They confirmed the inclusion formation by the solubility, powder X-ray diffractometry, differential scanning calorimetry and proton magnetic resonance spectroscopy methods. In order to prepare the complex, coprecipitation and neutralization methods were used. Scale-up evaluation, batch size, drug content in the complex, percent yield, etc., were not reported. Even though they reported that the dissolution rate of cinnarizine in the inclusion complex was 30 times larger than that of cinnarizine alone, they did not report on how one can conveniently incorporate the complex into a suitable dosage form.

The literature is replete (87, 95, 101-103) with papers which report laboratory techniques of the preparation of inclusion complexes. It has been shown that dissolution and bioavailability of drugs improved by complexation with beta-cyclodextrin. However, how such inclusion complexes can be scaled-up from a laboratory scale to a pilot scale, formulation development, etc., have not been reported in detail.

#### IV Conclusions and suggestions for future work

The following are believed to be the salient conclusions of this work as reported and discussed in the previous section.

The data obtained in this thesis indicate that the beta-cyclodextrin interacts with ampicillin, phenobarbitone, and phenytoin. The mode of interaction was studied by a variety of methods, which include stability, solubility, X-ray diffractometry, differential scanning calorimetry, infrared and proton magnetic resonance spectroscopies, photomicrographs, etc. It was found that in the preparation of the complexes, the freeze drying method was the most feasible and reproducible from a laboratory scale to a pilot scale production. However, because of ampicillin instability under freezing conditions, it was not possible to obtain the inclusion compound in the solid powdered form. Phenobarbitone and phenytoin beta-cyclodextrin complexes were prepared using both a laboratory model freeze drier and an industrial model freeze drier. Modifications to the techniques in the freeze drying process allowed the yield to be improved to almost 90%.

Properties of the complexes in solutions were evaluated by stability, solubility and proton magnetic resonance spectroscopy methods. In addition, to confirm the presence of the inclusion complex in the solid phase, X-ray diffractometry and differential scanning calorimetry techniques were found to be useful. Infrared spectra and photomicrographs were difficult to interpret in an unambiguous manner. However, these two methods are deemed to be useful to some extent; hence, they can not be ruled out in the evaluation of physico-chemical properties of the complexes. Liquid dosage forms were prepared from the



phenobarbitone and phenytoin beta-cyclodextrin complexes. A solution dosage form was prepared from the phenobarbitone beta-cyclodextrin complex. Unlike an official preparation there was no need to use ethanol to keep the phenobarbitone in solution. Hence, this dosage form may have a considerable potential, mainly for pediatric use. Evaluation of the physical stability of the suspension prepared from the phenytoin beta-cyclodextrin complex were indicative of a pharmaceutically elegant suspension. In addition, another distinct advantage is that this suspension contains freeze dried material so it is presented to the body in fine particles and therefore ready for the dissolution process immediately upon administration.

Despite the lack of flowability and compressibility of the phenytoin beta-cyclodextrin complex, it was possible to manufacture tablets of suitable size and weight. All the examined physical properties of the tablets were found to be within the USP standards. Dissolution studies of the tablets in different dissolution media indicated that the phenytoin beta-cyclodextrin had greatly improved dissolution compared to the uncomplexed drug. Due to in situ complexation of the physical mixture of the freeze dried phenytoin and beta-cyclodextrin, tablets prepared from the mixture showed a similar dissolution profile to that obtained from tablets prepared from the complex. However, a similar dissolution profile was not observed from the tablets prepared from the nonfreeze dried phenytoin and beta-cyclodextrin. Hence, it may be concluded that, when the substrate disperses at a molecular level, it comes in direct contact with the molecularly dispersed ligand and in situ complexation takes place in the dissolution medium. It appears that the rate limiting step to form in situ complexation is the solubility of a

substrate in the vicinity of the solution of a ligand. In order to increase the dissolution rate by in situ complexation, the following techniques may be useful:

- a. A physical mixture of a weakly acidic drug and cyclodextrins can be mixed with a GRAS (Generally Recognized As Safe) buffering agent to compress a tablet along with other tableting ingredients.
- b. When such tablets disintegrate in the dissolution medium, the buffering agent will cause a rise in pH in the close proximity of disintegrated tablet. When there is an increase in pH, a weakly acidic drug will dissolve and may form an in situ complex with the molecularly dispersed cyclodextrins. This technique makes it possible to increase the dissolution rate of weakly acidic drugs. However, there are some assumptions to be made to make this technique work. Firstly, weakly acidic drugs are stable in the presence of a buffering agent in the liquid as well as solid phase. Secondly, the amount of a buffering agent incorporated is sufficient to increase the pH in the dissolution medium so as to increase the solubility of weakly acidic drugs. Furthermore, due to an increase in the pH, acidic drugs will ionize (depending on  $pK_a$ ) and the ionized species may not form a complex of suitable stability constant unlike unionized species. A similar technique may also be useful for basic drugs with appropriate buffering agents.

In addition, the following suggestions can be given for future work.

1. It would be interesting to investigate the interaction of an ampicillin with gamma-cyclodextrin, provided the cost is reasonable.
2. Under controlled humidity and temperature facilities, micronization or co-grinding of ampicillin with cyclodextrins might be explored to

substrate in the vicinity of the solution of a ligand. In order to increase the dissolution rate by in situ complexation, the following techniques may be useful:

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1. It would be interesting to investigate the interaction of an ampicillin with gamma-cyclodextrin, provided the cost is reasonable.
2. Under controlled humidity and temperature facilities, micronization or co-grinding of ampicillin with cyclodextrins might be explored to

obtain the inclusion compound in solid powdered form, with subsequent evaluations of the physico-chemical properties of the complex.

3. It is planned to conduct bioavailability studies of dosage forms prepared from the phenytoin beta-cyclodextrin complex in humans. However, because of today's myriad regulations, the dosage forms manufactured at URI (University of Rhode Island) in the Department of Pharmaceutics facility cannot be used for testing in humans. The FDA (Food and Drug Administration) requires brief statements that identify the place where the drug is manufactured and tested, an outline of the methods and facilities used in the manufacture, etc., and these regulations requirements can not be met at URI. Hence, it is decided to manufacture and conduct bioavailability studies of the dosage forms in appropriate (FDA approved) facilities, firstly in healthy humans (at least six) then in epileptic patients.

4. At present, even though cyclodextrins are somewhat expensive for large scale use, because of their monumental potential in various fields, in the future cost of the cyclodextrins may well become more reasonable for pharmaceutical purposes. Hence, it would be interesting to study the interaction of alpha and gamma-cyclodextrins with other pharmaceuticals to improve their physico-chemical properties such as, stability, solubility, dissolution, etc.

5. Furthermore, since the aqueous solubility of alpha and gamma-cyclodextrins is higher than for the beta-cyclodextrin, the complexes prepared from them may be useful for parenteral dosage forms.

6. Although the literature reports the existence of delta, epsilon, and further homologues of cyclodextrins containing 9-12 glycopyranose units, they are not available commercially in the pure form. However, even if

they become available in the future, complex formation will tend to diminish as the higher homologues of cyclodextrins increase in internal diameter. The probable reasons are as follows: The number of water molecules taken up by alpha, beta, and gamma cyclodextrins are 6, 11, and 17, respectively. As the cyclodextrins' cavity is enlarged (with higher homologues), the properties of the water filling it will approach those of bulk water. Hence, the lowering of the energy of the entrapped water may account for the failure of the inclusion; alternatively, the inclusion "fit" may be too "loose".

7. Recently, the chemically modified cyclodextrins have received considerable attention because their physico-chemical properties are different from the natural cyclodextrins. However, the practical application of modified cyclodextrins will have to await the results of exhaustive toxicological studies.

Although a number of drug-cyclodextrin inclusion compounds are reported in the literature at the laboratory level, little attention appears to have been given to producing these products at the pilot scale or manufacturing level. The results reported in this thesis clearly demonstrate that inclusion compounds not only can be produced at the pilot scale but also can be conveniently incorporated at a large scale into pharmaceutically acceptable dosage forms.

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APPENDIX

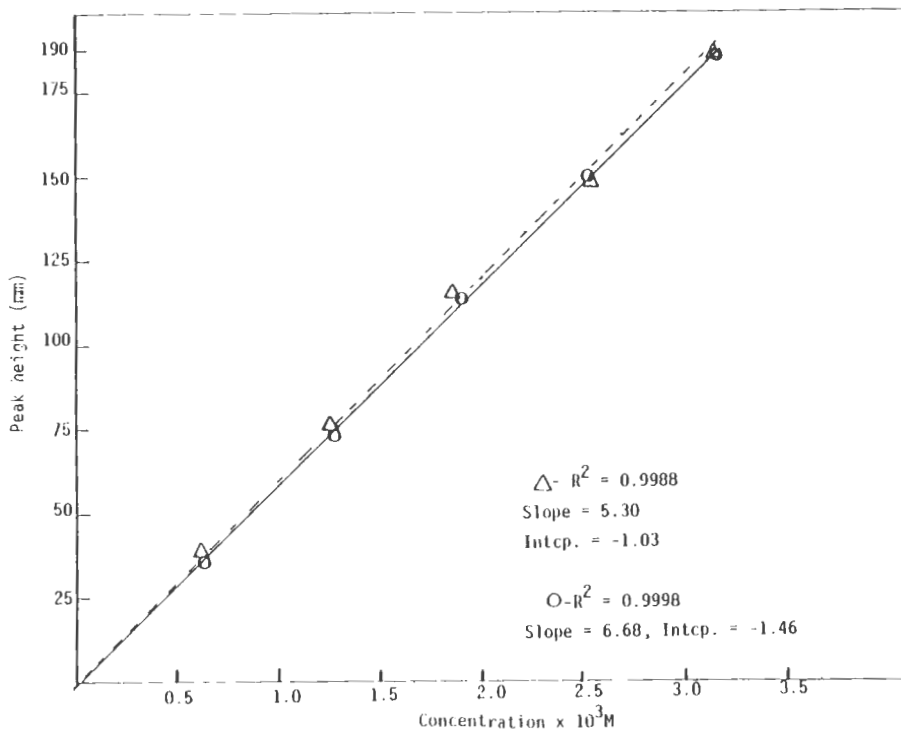


Fig. 45 Beer's plot for ampicillin using HPLC .

## Appendix A, 1

Precision of HPLC assay method for ampicillin

Drug concentration $\times 10^3 M$	No of injections	Peak height	RSD(%)*
2.55	3	25200	2.15
		25153	
		24250	
2.52	3	19645	1.86
		20330	
		20248	
2.58	6	24786	0.74
		24858	
		24564	
		24773	
		24839	
		24396	

\* Relative Standard Deviation =  $\frac{S. D.}{\text{Mean}} \times 100$

\*\* A guideline for methods validation in New Drug Applications (NDA) was followed in this analytical work.

## Appendix A, 1 (contd).

Accuracy of HPLC assay method for ampicillin

Amount added (mg.)	Amount found (mg.)	Recovery (%)
1.003	1.012	100.90
0.802	0.772	96.17
0.602	0.591	98.12
0.401	0.410	102.30
0.201	0.203	101.20
	Mean	= 99.74
	Standard deviation	= 2.52

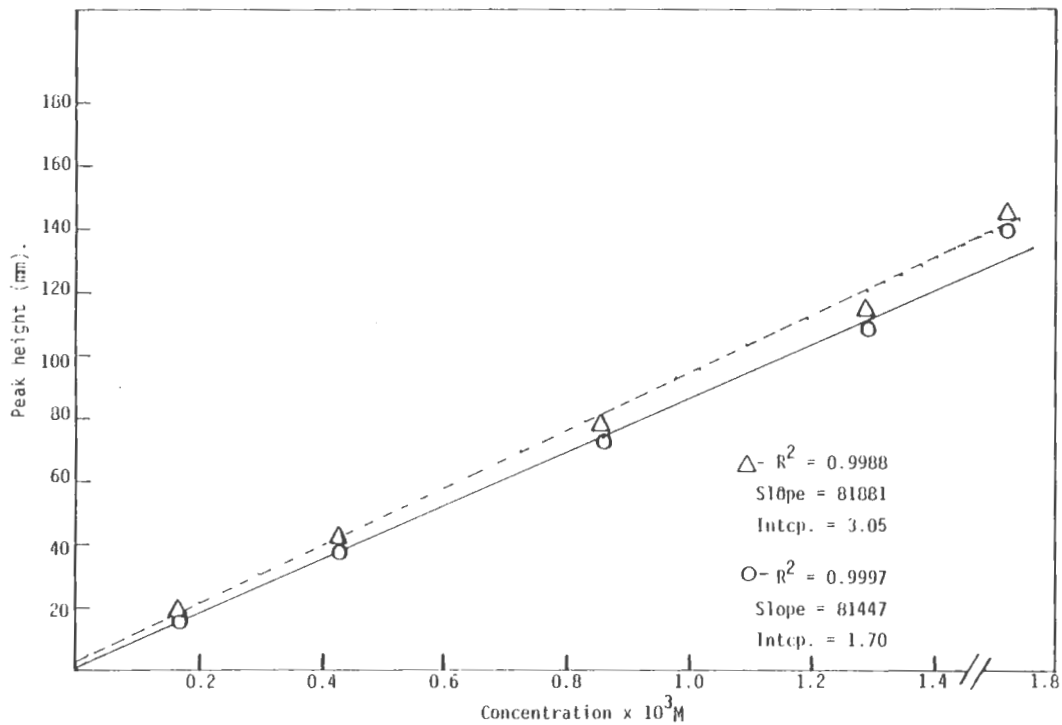


Fig. 46 Beer's plot for phenobarbitone using HPLC.

## Appendix A, 2

## Precision of HPLC assay method for phenobarbitone

Drug concentration $\times 10^{-1}M$	No of injections	Peak height	RSD (%)
1.72	6	20860	1.69
		20796	
		21388	
		20842	
		21426	
		21600	
0.43	6	5620	1.60
		5386	
		5410	
		5557	
		5494	
		5532	

## Appendix A, 2 (contd).

## Accuracy of HPLC assay method for phenobarbitone

Amount added (ucg)	Amount found (ucg)	Recovery (%)
300	300.56	100.19
300	300.20	100.06
300	299.65	99.88
100	98.70	98.70
100	99.00	99.00
100	98.30	98.30
40	38.88	97.20
40	38.10	95.25
40	38.40	96.00

Mean = 98.29

Standard deviation = 1.77



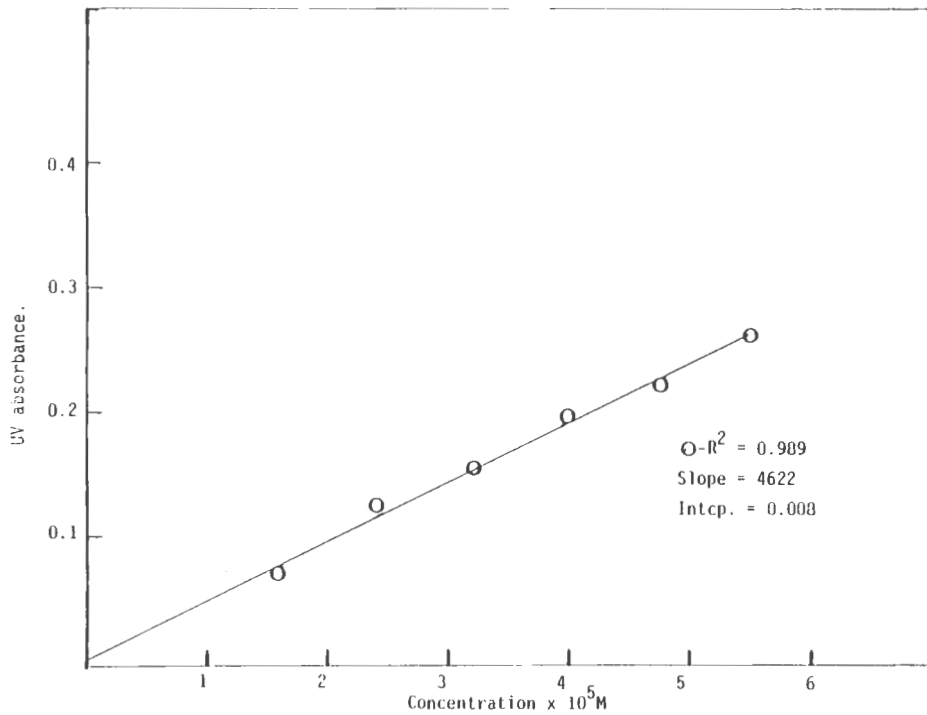


Fig. 47 Beer's plot for phenytoin using UV in an aqueous medium.

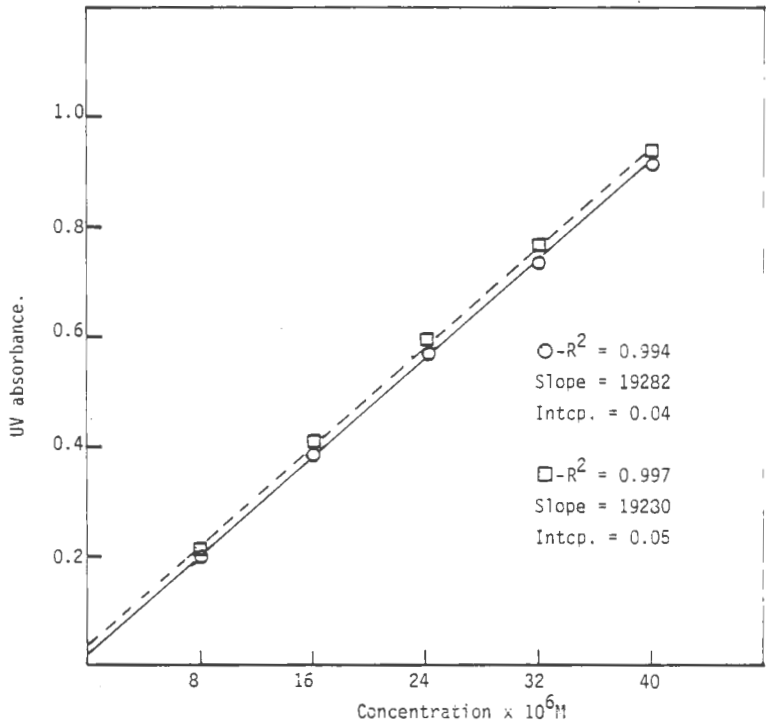


Fig. 48 Beer's plot for phenytoin using UV in pH 2.2

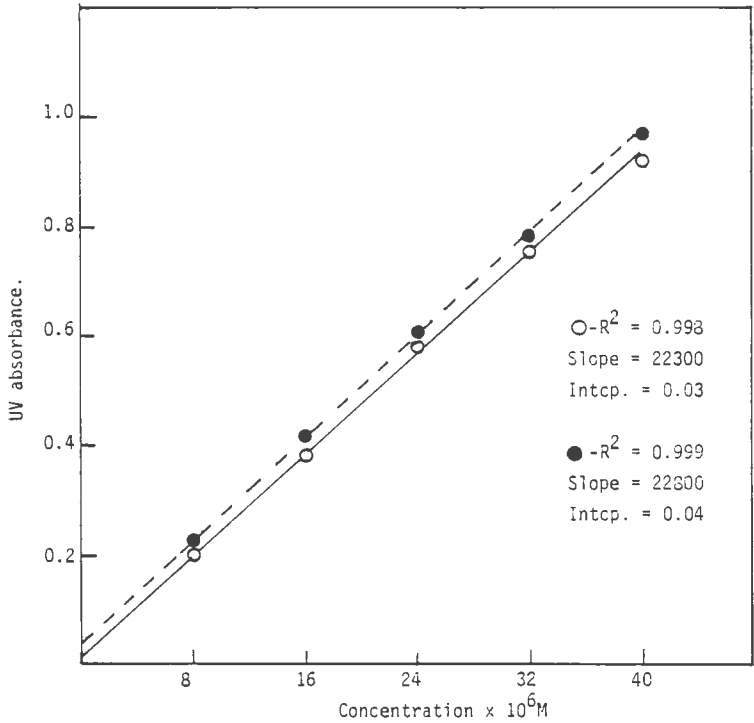


Fig. 49 Beer's plot for phenytoin using UV in pH 7.4

## Appendix A, 3

Precision of spectrophotometric assay method for phenytoin

Drug concentration $\times 10^5 M$	UV Absorbance	RSD (%)
1.60	0.206	3.93
	0.224	
	0.218	
	0.210	
	0.229	
	0.216	
3.17	0.763	1.47
	0.738	
	0.747	
	0.768	
	0.750	
	0.749	

## Appendix A, 3 (contd).

## Accuracy of spectrophotometric assay method for phenytoin

Amount added (ucg)	Amount found (ucg)	Recovery (%)
4	3.78	94.50
4	3.85	96.25
4	3.90	97.50
8	7.94	99.25
8	7.80	97.50
8	7.91	98.88

Mean = 97.31

Standard deviation = 1.75

## Appendix A, 4

Molar absorptivity ( ) of beta-cyclodextrin in pH 7.0 buffer

Concentration of beta-cyclodextrin $\times 10^3 \text{M}$	at 225nm
1.25	
2.50	
5.00	7.15
10.00	
15.00	

\* Since the molar absorptivity of phenytoin (10191) was more than 1000-fold compare to beta-cyclodextrin, hence it was felt that interference of beta-cyclodextrin may be insignificant.

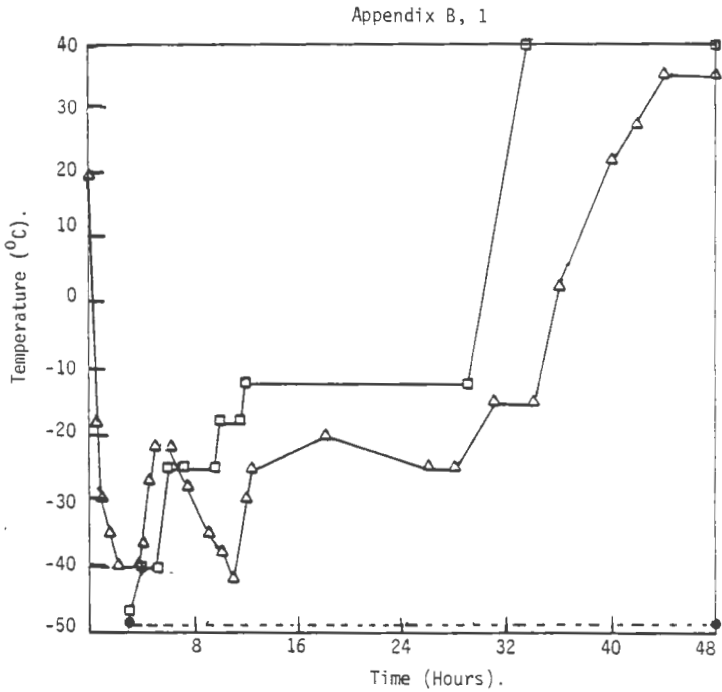


Fig. 50 Freeze drying cycle of phenytoin beta-cyclodextrin complex.

Key :  $\Delta$  - Product temperature,  $\square$  - Heat applied,  
 $\bullet$  - Condenser temperature.

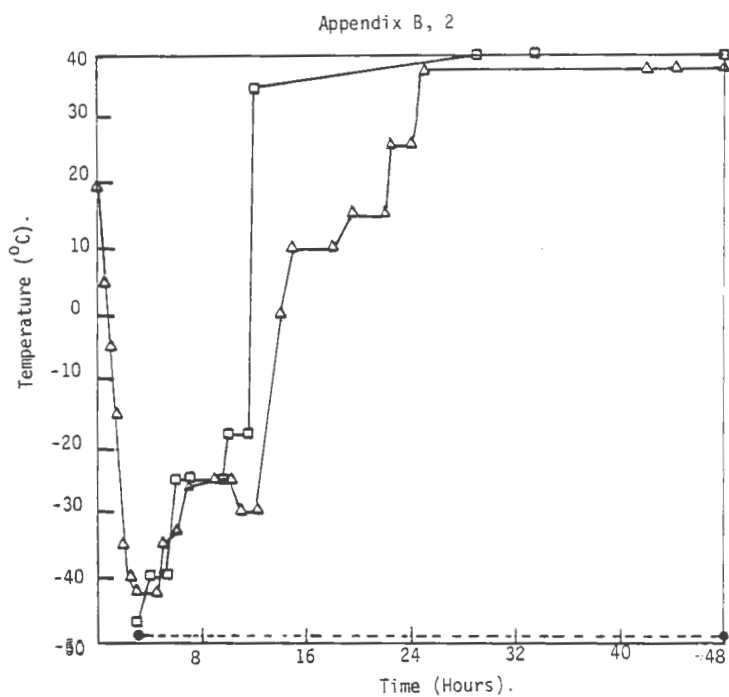


Fig. 51 Freeze drying cycle of phenobarbitone beta-cyclodextrin complex.

Key : Δ - Product temperature, □ - Heat applied,  
● - Condenser temperature.