DISSOLUTION RATE AND BIOAVAILABILITY STUDY ON NEW NITROFURANTOIN FORMULATIONS

BY

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A P P R O V A L

This thesis submitted by Michael Aidelomo Akade meets the regulations governing the award of the degree of Master of Science (Pharmaceutics) of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria; and it is approved for its contribution to science and literary presentation.

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D E C L A R A T I O N

I hereby declare that this thesis has been written by me and that it is record of my research work done under the supervision of Dr. D.K. Agrawal and Dr. J.A.K. Lauwo. It has not been presented in any previous application for a higher degree. All quotations are indicated and sources of information specifically acknowledged by means of references.

A Akademi

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30. 3. 85

Date

D E D I C A T I O N

To my wife, Taiwo, for her love and understanding;
my daughter, Omoyeme; and my eldest brother
for his exemplary and dedicated life, and his
fatherly love;

and

our Lord, Jesus Christ, for His abundant grace.

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ABSTRACT

Drug biological availability has been broadly reviewed and an attempt made to link dissolution of solid dosage forms with bioavailability. A brief review of solid dispersion technique and its application in the resolution of pressing pharmaceutical problems has been presented.

Nitrofurantoin, an antibacterial drug for urinary tract infection and as research drug material has been reviewed. In-vitro studies were done to find a suitable carrier for the drug, and the effect on dissolution properties of the drug, of solid dispersion technique has been studied. Carriers investigated were lactose, mannitol, PEG 6000, PVP 44,000, and succinic acid. PEG 6000, and mannitol were used in preparing 10% w/w drug-carrier fusions which were tested in-vitro using dissolution rate tests. The 10% drug - PEG 6000 fusion exhibited superior dissolution and as such PEG 6000 was selected for further studies. In the study that followed, 5, 10, 20, 30, 40, and 50% drug - PEG 6000 fusions were prepared and comparative study was made of dissolution characteristics. From this study it was deduced that the 30% drug - carrier fusion was optimum for tablet formulation.

Formulation studies involving the formulation and production of tablet formula A (30% drug in Avicel) as physical mixture; formula B (30% drug in PEG 6000)

as a physical mixture; and formula C (30% drug in PEG 6000) as a solid dispersion were carried out. The tablets were subjected to official quality control tests and were found satisfactory. They were later employed for in-vivo absorption studies that covered a three-week period. Three human volunteers, using a cross-over design, took part in the study. Plasma level determinations were used for estimating bioavailability of the drug in the different formulations.

In-vitro dissolution studies indicated higher dissolution for the drug-carrier solid dispersion as compared to the physical mixtures of the same carrier and of Avicel PH 101 with the drug. Physical mixture of Avicel with the drug exhibited slightly lower dissolution (4%) against the PEG 6000 physical mixture with the drug.

In-vivo absorption studies indicated that drug - PEG 6000 solid dispersion's extent of absorption if taken as 100% the drug-Avicel mixture's amount absorbed was 19% lower, while the drug-PEG 6000 physical mixture was 31.3% lower. The three formulations were not bioequivalent because they were absorbed to different extents even though the time taken to attain peak blood levels was one hour in all three formulations. For the three formulations absorption rates were grossly different. For the solid dispersion the rate was 50% higher than the drug-Avicel physical mixture, 33% higher than the drug-PEG 6000 physical mixture in the first 30 minutes.

In the second 30 minutes the rate for the solid dispersion compared to the drug - Avicel physical mixture had fallen to 25%, and remained at 33% for the drug - PEG 6000 physical mixture.

By the end of the second hour the absorption rate differences narrowed considerably to a mere 1% in the drug - Avicel physical mixture though remaining as high as 21% between thesolid dispersion of drug - PEG 6000 and its physical mixture. No further blood levels were observed in all three formulations in all three volunteers at the end of four hours.

There appeared to be no direct correlation between in-vitro dissolution rates and in-vivo absorption for the drug-Avicel mixture and the drug - PEG 6000 mixture. But the significantly higher in-vitro dissolution rate of the solid dispersion compared to the mixtures correlated with in-vivo absorption of these formulations.

Results of this study has indicated the possibility of reducing the quantity of drug substance required in any given formulation following the solid dispersion of the drug in a suitable carrier, to give the same desired pharmacologic effect in most patients thereby minimizing the toxicity of the drug.

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Abbreviations and Symbols

UV	Ultra violet radiation
TLC	Thin layer chromatography
IR	Infra red radiation
HPLC	High performance liquid chromatography
PEG	Polyethylene glycol
PVP	Polyvinyl pyrrolidone
pH	Negative logarithm of hydrogen ion concentration
pKa	Negative logarithm of dissociation constant
GI	Gastro - intestinal
et al.	and others
µm	micrometre (10^{-6} metre)
CNS	Central nervous system
ad lib	As much as required
G6PD	Glucose-6-phosphate dehydrogenase
rpm	revolutions per minute
S.D	standard deviation

Part One: General Literature Review

CHAPTER ONE

INTRODUCTION

Chapters one and two are devoted to a review of the literature in respect of the general and basic principles involved in the project.
Bioavailability, formulation and dosage form - related factors influencing bioavailability, dissolution rate, and the solid dispersion technique have been considered. A review of the therapeutic significance, and basic pharmacokinetics of nitrofurantoin were also done. This general literature review has also considered the work done on the drug by previous research workers. The more specific literature reviews have been presented in the appropriate chapters. The objective and scope of the project have been presented at the end of the general review of the literature.

1.1 DRUG BIOAVAILABILITY

A dosage form is designed to have the ability to deliver its active constituent to its site of action in an amount sufficient to elicit the desired pharmacologic response. This property of a dosage form is referred to as physiologic availability, bioavailability or biologic availability.

Bioavailability has been defined as the rate and extent of absorption of a drug from its dosage form into the systemic circulation (Blanchard and Sawchuk,

1979; Ritschel, 1976; Roger et al., 1981c).
The venous and arterial blood is considered as systemic circulation during the absorptive phase following oral administration. The blood in the portal vein is excluded (Ritschel, 1976b).

According to this definition, while the absorption of an intravenously administered drug is rapid and complete, the rate and extent of absorption in an individual from tablets or capsules are usually not precisely known. However, for reasons of convenience, stability and other factors, tablets and capsules would continue to be used for administering drugs orally.

Pharmacokinetics concepts in bioavailability of drug products

The most readily available site for measuring the level of drugs in the body is the blood or plasma. It is also the most logical site, since it is the blood that picks up a drug from the site of absorption, distributes it to the tissues and then delivers it to the organs of elimination . Usually, a peripheral vein is used as the sampling site. The concentration of a drug in the blood at the sampling site is a net result of the physiological processes that govern drug absorption, distribution, metabolism and excretion (Tozer, 1979).

It is possible to determine the concentration of a drug in a selected body fluid over a period of

time following administration of its preparation, thereby obtaining an indication of the extent and rate of absorption of the drug from the preparation being assayed. Measurements such as these form the basis for carrying out comparative bioavailability estimations of alternative formulations of a drug. Though the body fluid frequently used for these measurement is blood, other fluids like urine and saliva may be used (The Pharmaceutical Codex, 1979; Ritschel, 1976b; Tozer, 1979).

For estimations based on blood sampling, the most commonly used method involves the construction of plasma concentration - time curves.

Plasma concentration - Time curves

Serial samples of plasma are withdrawn from the subject and examined for drug content at specified periods following administration of the preparation. If the drug concentrations are plotted against time, a curve (figure 1.1) is obtained which usually shows a fairly steep rise to a peak concentration followed by a slow reduction in concentration over a longer period of time.

The shape of the curve depends on a number of factors. Elimination of the drug begins as soon as it appears in the blood. Absorption, distribution, metabolism and excretion are all capable of modifying the shape of the curve.

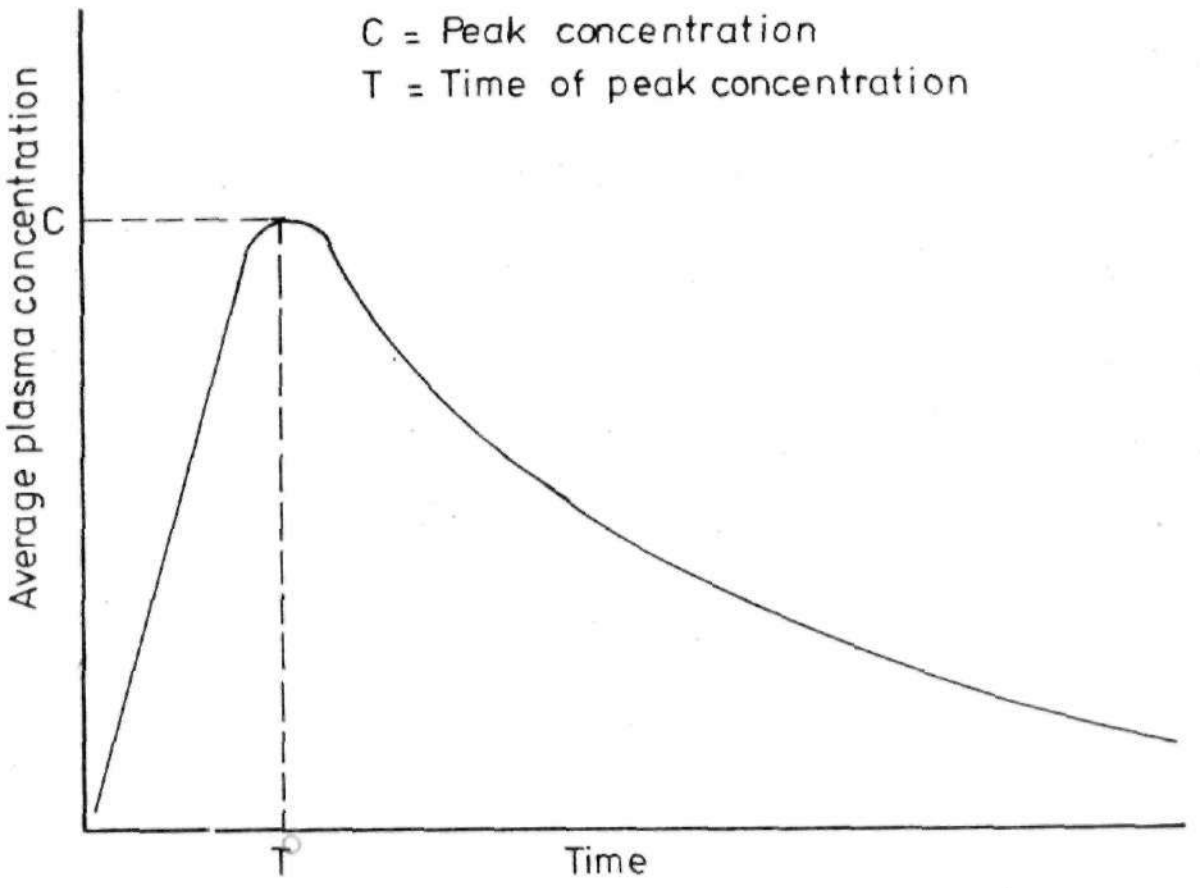


Fig. 1.1 A typical plasma concentration - time curve

There are three important parameters which can be derived from the plasma concentration - time curve. These are the peak concentration, C; the time of peak concentration, T; and the area under the curve, AUC.

Peak concentration represents the highest concentration of the drug achieved in the blood. It is attained when the rate of appearance of the drug in the blood no longer exceeds its rate of removal by distribution, metabolism and excretion (Ritschel, 1976b).

Time of peak concentration is related to the rate of absorption and can be used in determining the rate of absorption of the drug being assayed.

Area under the curve is related to the total amount of drug absorbed from an administered dose. It is one approach in the determination of extent of absorption. The other approach is by determination of the total amount of unchanged drug excreted in urine. The values obtained from these parameters may or may not be related to the therapeutic activity, and if there is a relationship, it may not be linear. Nevertheless, a plasma concentration - time curve can be used as basis for comparing alternative formulations (Ritschel, 1976b).

1.2 FORMULATION AND DOSAGE FORM RELATED FACTORS INFLUENCING DRUG BIOAVAILABILITY

Variation in bioavailability may be due to patient related, or dosage form related factors. A review of the best known patient related factors has been made by Meyerschn, (1979). In the review, the role of GI fluids, gastric emptying, intestinal transit, blood flow, GI drug metabolism, drug-food and drug-drug interactions, disease states and physiologic disorders, age, first-pass metabolism, as well as factors influencing dose-dependent availability were discussed.

Dosage form related factors which can produce profound differences in bioavailability include formulation and manufacturing variables such as particle size; the chemical form and solubility of the drug; the type and quantity of excipients used; the compaction pressure and other factors (Blanchard and Sawchuk, 1979).

Dosage form properties often account for some modifications in the pharmacological activity of therapeutic agents by altering their intrinsic absorption pattern. The schematic diagram in figure 1.2 shows drug concentration in the blood as a function of time after oral administration of a fixed amount of drug in three different dosage forms designated I, II and III. The properties of the dosage form in this hypothetical example produce a

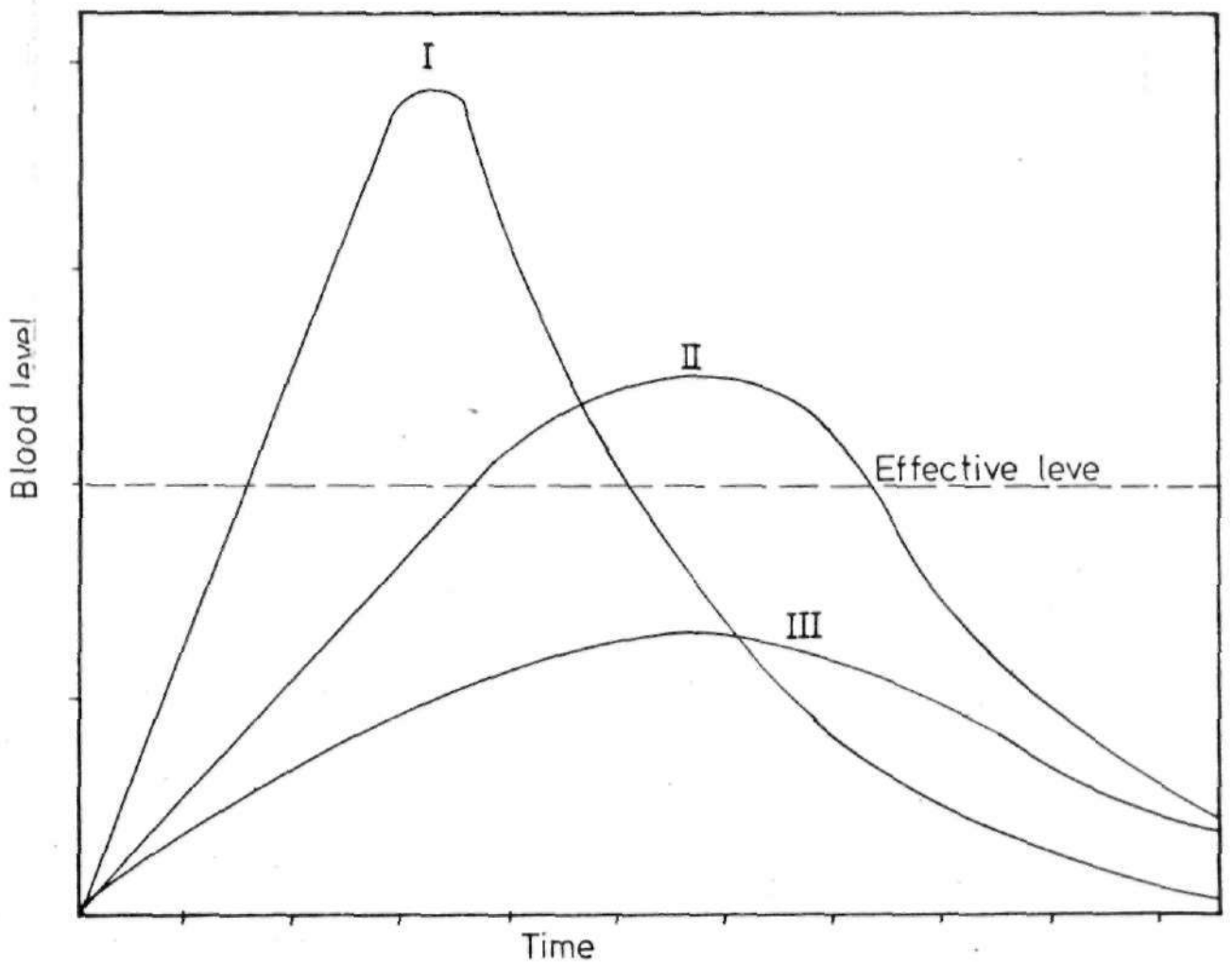


Fig 1.2 Blood level - time curves showing possible effects of dosage form on blood levels after oral administration of equal amounts of active agent

(From Poole, J.W., (1979) Chapter three of Principles and Perspectives in Drug Bioavailability edited by Blanchard, J., Sawchuk, R.J., and Brodie, B.B. S. Karger, Boehm, AG 4011 Basle, P 62)

change in the overall absorption rate which is reflected in the blood level - time curves shown. Activity occurs when the blood concentration reaches the 'effective' drug level (figure 1.3) the onset of pharmacologic action is significantly earlier with formulation I than with formulation II, and is never attained with formulation III.

The duration of the effect is often reflected by the length of time that the blood level of drug is equal to or higher than the minimum effective level. Again it can be seen from the example in figure 1.2 that the duration of effect obtained from formulation I is significantly longer than that obtained after equal dose of formulation II. From this general and over-simplified example, it can be seen readily that dosage form factors can have a significant effect on the onset, duration and intensity of pharmacologic action of a particular active component.

Figure 1.2 also illustrates the effect of formulation on overall biological availability. This availability is frequently expressed as a ratio of the amount of test formulation absorbed to the amount of standard formulation absorbed.

$$\text{Bioavailability} = \frac{\text{area under curve or cumulative amount excreted for test formulation}}{\text{area under curve or cumulative amount excreted for standard formulation.}}$$

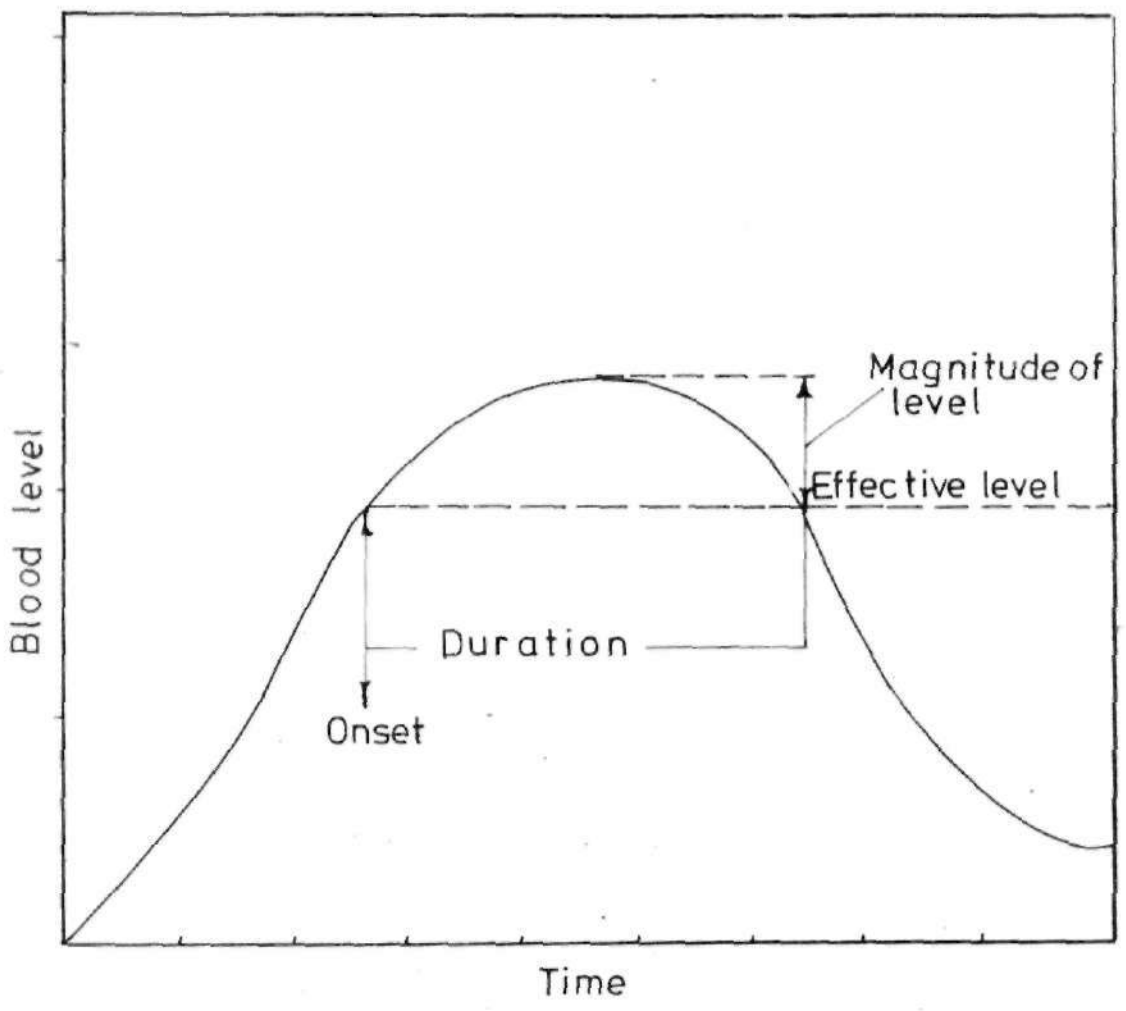


Fig 1.3 Relationship of blood level-time curve and pharmacologic effect

(From Poole, J.W., (1979) Chapter three of Principles and perspectives in Drug Bioavailability edited by Blanchard, J., Sawchuk, R.J., and Brodie, B.B.; S. Karger, Boehm, AG 4011 Basle, p 62)

A preparation that exhibits 100% availability may be compared with the test solid dosage form. The ratio obtained for the areas under the two blood level-time curves indicates the availability of the drug from a dosage form. In the example, the areas under the curves for formulations I and II are essentially the same. This means that although the drug is more rapidly absorbed when administered as formulation I, the total absorbed when administered from both forms is approximately the same. However for formulation III, the area is significantly less (about 50%) than that of the other two. If in the case of the first two formulations the drug were completely absorbed, the bioavailability of the drug when administered as the dosage form designated III is only about 50%.

The various formulation and dosage form related factors which have been extensively discussed by Poole, (1979), Ritschel, (1976b), Wade (1976) and many writers in this field include: particle size, crystal form, solvate, salt, complex, pH and solubility characteristics. Other factors are excipients and adjuvants as well as manufacturing methods.

Poole, (1979) in his discussion showed that bioavailability of some drugs have been improved by particle size reduction, and how particle size

reduction in recent years has been accomplished in several instances by the preparation of microcrystalline molecular dispersions of poorly soluble drugs in solid matrices of water-soluble carriers. Various adaptations of the technique of solid dispersion has been put forward by different research workers.

Fincher, (1968) published a review article on the effect of particle size on drug absorption and activity.

The crystal form in which a drug is presented to the patient is an important factor influencing the bioavailability of drugs. The crystal form of a drug substance may change because the substance exhibits polymorphism. Polymorphism has been reported to be relatively common, being exhibited by at least one-third of all organic compounds. Apart from the polymorphic forms in which substances may exist, they can also occur in non-crystalline or amorphous forms. The energy required for a molecule of drug to escape from the crystal in which it is located is very much greater than that required to escape from an amorphous powder. Therefore, the amorphous form of a compound is always more soluble than a corresponding crystal form and may exhibit correspondingly different therapeutic properties (Poole, 1979). Examples of drugs in which this has

been observed in-vitro are novobiocin and chloramphenicol.

The property of many organic medicinal compounds to form solvent addition compounds or solvates may also affect their bioavailability. When water is the solvent associated with the drug moiety, the solvate form is called a hydrate.

The solubility and rate of solution of a solvate may significantly differ from that of the non-solvated form of the drug. Drugs for which significant differences in dissolution rates have been observed include caffeine, theophylline and glutethimide. In each of these the anhydrous form dissolved more rapidly. But for fludrocortisone acetate and succinylsulfathiazole, the solvated forms dissolved more rapidly, than the non-solvated forms (Shefter and Higuchi, 1963).

Differences in bioavailability between anhydrous and hydrated form of the amphoteric compounds, penicilline and ampicillin has been reported by Poole and Bahal, (1968).

Complex formation is a mechanism that can influence the biopharmaceutical properties of a drug. Drugs may interact reversibly to form complexes with substances occuring in the body, with other drug or with pharmacologically inactive components of the pharmaceutical dosage form. Such a drug complex may

differ in its physico-chemical properties, from the pure drug. For instance its ability to permeate biological membranes may become altered. Such complexes are thus pharmacologically inactive since dissociation prior to drug absorption usually fails to occur (Poole, 1979). The effect of complexation on drug absorption depends on the magnitude of the stability constant of the complex. Frequently there may be little or no effect on the rate of absorption and on the overall bioavailability, however some drugs which are intrinsically poorly absorbed can be converted to non-absorbable complexes under certain circumstances and their bioavailability is further decreased. Complex formation according to Poole (1979) can be used in formulation of dosage forms in order to increase solubility or stability of drug substances or drug products. The drug complex in such systems is diluted in the biological fluids after administration which results in the dissociation of the complex with little or no effect on the overall absorption properties of the active compound.

The components in a dosage form other than the active drug substance may influence the absorption and subsequently the performance of a drug product. These additives or excipients may either facilitate or inhibit the absorptive process. The importance of excipients in the determination of the final drug effect was clearly demonstrated by an outbreak of

phenytoin toxicity in Australia. The toxicity occured when patients previously maintained on a stable dose of 'Dilantin' capsules in which calcium sulphate had been used as inert excipient, were given capsules (containing same quantity of active compound) but with the calcium sulphate substituted for with lactose as 'inert' excipient. Presumably the inorganic ion had complexed with phenytoin reducing the absorption (Wade, 1976).

The role of surface active agents is more complex and not always easy to predict. These substances are frequently used to increase solubility or act as emulsificants, but they may either increase or decrease absorption depending on the agent, the quantity, the route of administration, the drug in question, and the nature of the formulation. It was reported that these surface active agents may change the absorptive surface iteself in the small intestine, and that in other cases, the agents promote micelle formation, thus enabling lipid soluble substances to disperse in an aqueous medium (Wade, 1976).

The factors associated with formulation and processing which may affect dispersion, rate of dissolution and subsequently the bioavailability of drugs vary according to the type of preparation. Because tablets are compressed, quick dispersion and dissolution of the drug are more difficult to achieve.
It is generally accepted that efficient

disintegration is an important requirement for a tablet but it does not necessarily ensure good dispersion and dissolution of the drug because the size and nature of the particles which are produced are also controlling factors. The most important factor is the rate of dissolution of the drug from the disintegrating particles (The Pharmaceutical Codex, 1979). Dissolution rate may itself be affected by the granulation, or other mixing procedures; the degree of compression and the presence of fillers, binders, or surface active agents.

1.3 RELATIONSHIP BETWEEN BIOAVAILABILITY AND DISSOLUTION RATE

A dosage form must provide a stable environment during storage, so must it deliver the active ingredient in a form available for absorption upon administration. In as much as oral solid dosage forms must dissolve before absorption can occur; dissolution testing provides the means of evaluating this absorption phenomenon. The results of dissolution testing are useful in various ways depending upon the purpose for which the test was performed.

i) In research and development, dissolution testing is performed for assessing factors governing the release of the active ingredient, and more specifically for evaluating the influence of excipients or other processing variables on dissolution rate and hence on drug bioavailability.

ii) During the early stages of product design dissolution rate tests done in conjunction with in-vivo absorption studies can indicate the extent to which the release characteristics of the dosage form would influence blood levels of the drug.

iii) Where evidence for dissolution-rate limited absorption exists, monitoring changes in the formulation, (e.g particle size or salt form) by means of dissolution rate test provides a rational basis for optimizing a formulation.

As previously mentioned, the most widely used dosage form owing to convenience and economic factors involved in manufacturing, is the compressed tablet. However, tablet formulations also pose some of the most difficult pharmaceutical problems which may influence the bioavailability of the active ingredient. One of the major problems encountered with tablets is the decreased effective surface area of the active ingredients as a result of granulation and compression of the drug particles. Unless a compact tablet readily disintegrates and deaggregates when it comes in contact with the body fluids, the bioavailability of drugs from such dosage forms usually become reduced. Figure 1.4 is a schematic diagram showing the steps involved in the disintegration, deaggregation and dissolution of compressed tablets or powders in capsules.

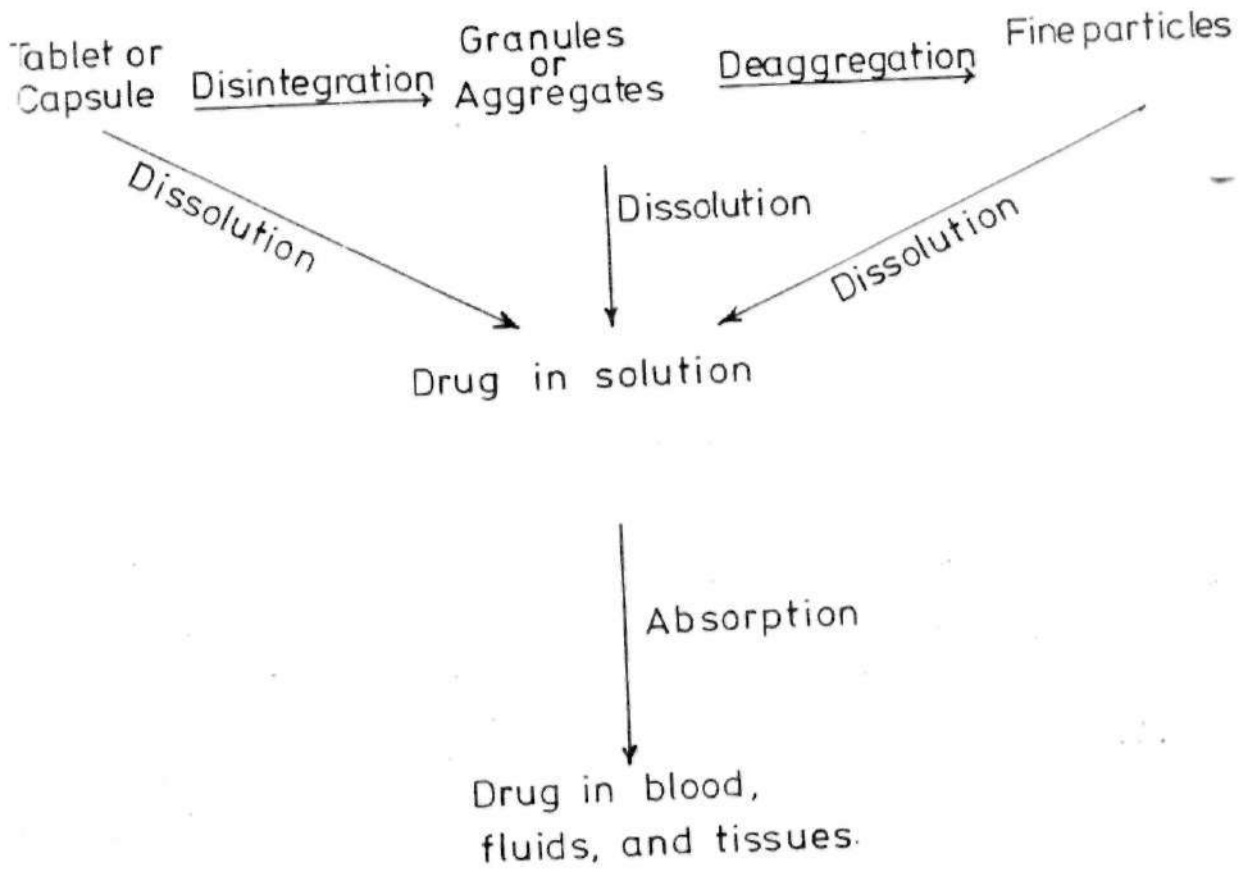


Fig.1.4 Schematic representation of disintegration, deaggregation and dissolution of a drug from solid dosage forms

(From Poole, J.W., (1979) Chapter Three of Principles and Perspectives in Drug Bioavailability edited by Blanchard, J., Sawchuk, R.J., and Brodie, B. S. Karger, Boehm, AG 4011 Basle, P 76)

A tablet may first disintegrate into granules, which are then reduced to fine particles and eventually after dissolution, to drug in solution. However, dissolution occurs at all levels, from the intact dosage form, the granules and from the particles. Since the surface area of the drug in the intact tablet is limited, the rate of dissolution from the intact compact is negligible except for very soluble drug (Poole, 1979).

In certain instances, dissolution rate or the time taken for the drug to dissolve in the fluids at the absorption sites becomes the rate limiting step in the absorption process. Whenever this occurs, anything which affects it will also affect absorption. Under these conditions, dissolution rate can affect the onset, intensity and duration of response by controlling the overall bioavailability of the drug from the dosage form.

Noyes and Whitney (1897) propounded a scientific law describing the dissolution of a substance in a non-reacting solvent. The law may be represented by the equation:

$$\frac{dc}{dt} = K.S.\ (C_s - C_t)$$

Where, dc/dt, is the dissolution rate;
S, is the surface area of the dissolving solid;
K, is the dissolution rate constant which incorporates factors such as agitating intensity, and the diffusion coefficient of the dissolving substance; C_t, is the concentration of the drug in the dissolution medium at some finite time, t;

and C_s is the concentration of the drug in the diffusion layer surrounding the solid material. Since the diffusion layer is a thin film saturated with drug, C_s is essentially equivalent to the concentration of a saturated solution of the drug in the solvent.

The rate of dissolution is governed by the rate of diffusion of solute molecules through the diffusion layer into the body of solution. On the basis of this law, the dissolution rate of a drug may be modified by manipulating the related factors. For instance, since dissolution rate is directly proportional to surface area, a reduction in particle size would result in increased surface area and consequently in more rapid dissolution.

In instances when the intrinsic dissolution rate is so low that a drug is not completely absorbed, more rapid dissolution can be attained by increasing the specific surface area available to the gastric fluids.

This has frequently produced increases in both rate and total amount of drug absorbed, i.e greater bioavailability.

I.4 SOLID DISPERSIONS

Dissolution has been reported to be the rate limiting step in the gastrointestinal absorption of some drugs from solid dosage forms, with the relationship between solution rate and absorption being particularly remarkable for drugs with poor water solubility (Fincher, 1968; Chiou and Riegelman 1971a).

Numerous attempts have been made to modify the dissolution characteristics of some drugs to ensure more rapid and more complete absorption (Chiou and Riegelman, 1971b, Goldberg et al, 1965).

It has been demonstrated that dissolution rate is a function of the surface area exposed to the dissolution medium. That a drug would dissolve more rapidly when its specific particle size is decreased has been long establised (Goldberg et al, 1965a). One of the first observations of this phenomenon was made with work on sulfadiazine, in which blood level determinations showed that the drug in suspension containing particles 1 to 3μm in size was absorbed more rapidly and more efficiently than from a suspension containing particles 7 times larger.

Maximum blood levels were about 40% higher and occurred 2 hours earlier.

Increased bioavailability following particle size reduction has been observed with griseofulvin. The extent of absorption of an oral dose increased two and half times, when the surface area was increased approximately six-fold. Micronized griseofulvin permitted a 50% decrease in dosage, where as a satisfactory clinical response was attained (Rovin, 1980). Some of the more commonly applied methods of particle size reduction are:-

(a) conventional trituration and grinding
(b) fluid energy micronization
(c) controlled precipitation by change of solvents or temperature, application of ultrasonic waves and spray-drying
(d) ball milling
(e) administration of liquid solution from which upon dilution with gastric fluids, the dissolved drug may precipitate in very fine particles.
(f) administration of water soluble salts of the poorly soluble compounds from which the parent neutral forms may precipitate in ultra fine form in GI fluids.

Although the reduction of particle size can be easily and directly accomplished by methods (a) - (d), the resultant fine particles may not produce the expected faster rate of dissolution and absorption. This primarily results from the possible aggregation and

agglomeration of the fine particles due to their increased surface energy and consequently stronger vander Waals attraction between the non-polar molecules. This and the fact that pure fine powders of poorly soluble drugs have poor wettability in water (wetting of powders is the first step for them to dissolve and sometimes disperse in fluids) are serious disadvantages. While on the other hand, drugs with plastic properties are difficult to subdivide by methods (a) - (c) since they have the tendency to stick together even if fine powders can be produced by controlled precipation (Chiou and Riegelman, 1971a).

However a unique technique known as solid dispersion first demonstrated by Sekiguchi and Obi in 1961 which does not have the above disadvantages has been the subject of much study in recent times by various research workers.

The solid dispersion technique involves the formation of a eutectic mixture (solid at room temperature) of a drug and a pharmacologically inert, readily soluble carrier. To achieve this, the drug and the carrier are melted and mixed and the resulting homogeneous liquid is cooled until it solidifies and then the solid mass is finally powdered by some simple communition technique and sieved.
Crystals in a eutectic mixture are usually quite small and fine grained and when placed in water, the soluble

carrier substance dissolves rapidly, and extremely fine particles of the drug are released.

A study of solid solutions and eutectic mixtures in solid dispersions by Goldberg et al, (1965) revealed that encouraging results could be obtained from such studies, but they observed that the dissolution rates were below theoretical expectations. This they attributed to possible solid state interactions resulting in stronger crystal lattice. They also reported significant degree of solid-solid solubility rather than the simple eutexia earlier proposed and that the increased dissolution rates previously attributed to eutectic mixtures are more properly explained by the existence of solid solutions. They concluded that though factors such as thermal degradation, sublimation, and polymorphic transitions are limitations to the application of the technique, solid solutions and perhaps eutectic mixtures provide a unique approach for increasing dissolution rates.

In three other elaborately planned studies Goldberg and co-workers in 1966 examined solid solutions vis-a-vis eutectic mixtures employing separate binary systems.
From a urea - acetaminophen system they found out that there was a simple eutectic mixture with negligible formation of solid solution.
Dissolution rate studies showed that the presence of urea enhanced the

dissolution rate of acetaminophen by virtue of a microenvironmental solubilization, with a fused mixture at the eutectic composition giving similar, but prolonged enhancement of dissolution rate as compared to a physically mixed sample. They reported that their results indicated that particle size reduction of acetaminophen by eutectic formation with urea did not increase the dissolution rate of the drug. They observed that decreasing particle size 50% by sieving selection yields a 20% increase in the dissolution of acetaminophen. With this study, Goldberg et al,(1965) showed that the value of eutectic formation as a means of enhancing dissolution rate to be somewhat questionable since an enhancement of dissolution rate by simple eutectic formation alone was not demonstrated.

A succinic acid-griseofulvin system was found to be a eutectic mixture showing considerable solid solubility of griseofulvin in succinic acid. From its study, Goldberg et al, (1965) were able to establish that when two compounds exhibit solid state interactions, they frequently demonstrate interaction in aqueous solutions; and that griseofulvin and succinic acid display a solution phase interaction manifested by a linear increase in the solubility of griseofulvin with increasing concentration of the carrier. Evaluation of the dissolution rates of

samples indicated that the solid solution used in their experiment dissolved 7 times faster than the pure material. And that the presence of solid solution in eutectic mixtures in proportions as high as 60% more reasonably explains increased dissolution rates as compared to effects produced by reduction in particle size. Thus this study demonstrated a method of physical modification which could prove more important than micronization in enhancing the absorption and therapeutic effect of many water insoluble drugs.

Goldberg and co-workers, (1966c) studied a chloramphenicol urea system. A solid solution of chloramphenicol in urea was found to dissolve twice as rapidly as a physical mixture of the same composition and almost 4 times as rapidly as the pure drug. In this study they reported observing that the chloramphenicol-urea eutectic dissolved somewhat faster than the pure drug of comparable particle size, where Sekiguchi and his co-workers (1961) had previously been unable to detect any differences in dissolution rates of the eutectic mixture and the pure drug. The experimental discrepancies has been claimed to be ascribable to differences in method of determining dissolution rates. The method adopted by Sekiguchi and co-workers involved a higher degree of agitation.

The use of high shear in in-vitro dissolution investigations is capable of obviating differences arising from microenvironmental factors which would be significant in-vivo. Goldberg et al, (1966c) then suggested that three individual (or possibly connected) factors are involved in the dissolution of chloramphenicol. The factors suggested were:-

(i) local solubilization
(ii) particle size reduction
(iii) presence of significant amount of the rapidly soluble alpha solid solution.

They claimed that results of dissolution studies with the beta solid solutions indicated that despite the presence of significant amount of urea in the sample the solution rate was approximately equal to that of the pure drug. They ascribed this to the mere presence of a material as soluble as urea which in the crystal would tend to increase the wetting of the particle and in this manner alone increase effective surface area, thereby increasing dissolution rate. The inability to demonstrate this effect may be attributable to the formation of a crystal lattice in which the chloramphenicol is bound at least as tightly as in the pure crystal.

In a series of studies, Chiou and Riegelman, (1969) made their own contribution to the application of solid dispersions. They employed different grades

of PEG, and other carriers. They reported that there was increased dissolution from solid dispersions of griseofulvin over pure drug and better dissolution rate from wetted pure griseofulvin than from non-wetted pure drug. The result of this work suggested that one of the ways in which water soluble carriers increase dissolution rates of poorly water soluble drugs dispersed in them was by increased wettability. The superior dissolution rate of the dispersions made by the melt method over those by the solvent method, was contained in their report.

Chiou and Riegelman, (1971b) studied the dissolution rates of a water insoluble cardiac glycoside, (digitoxin) and steroids (17-methyltestosterone, hydrocortisone acetate and prednisolone acetate) in solid dispersions using PEG 6000 as carrier. They observed increased dissolution rates from the dispersions, noting that super-saturation of each drug could be attained when an excess amount of the drug was used. The increased dissolution rates were attributed to molecular and/or colloidal dispersion of the drugs in the matrix of PEG 6000.

Solid dispersions may be defined as the dispersion of one or more active ingredient(s) in an inert carrier or matrix at solid state prepared by the melting, (fusion) solvent, or melting-solvent methods. This definition indicates that solid

dispersions are homogeneous one phase solid systems. The term " coprecipitates" is frequently used to refer to preparations obtained by the solvent method.

Methods of Preparation

Three methods currently used to prepare solid dispersions are:-

(i) melting method

(ii) solvent method

(iii) melting-solvent method.

The melting or fusion method is a technique for preparing fast-release solid dispersion for dosage forms. Basically, in this method, a physical mixture of a drug and a water-soluble carrier is gradually warmed in a suitable oil bath until it melts. The melted mixture is then cooled and rapidly solidified under vigorous stirring. The final solid mass is crushed and pulverised prior to sieving. Goldberg et al, (1966a -c) as well as Chiou and Riegelman, (1969) employed this method with some modifications. To facilitate faster cooling and solidification, the homogeneous melt was poured in a thin layer upon ferrite plate or steel plate and cooled by flowing air or water on the opposite side of the plate.

The advantages of the fusion method include simplicity and economical, while the main disadvantages is that many substances (drug or carrier) may

decompose or evaporate during the fusion process at high temperature. For example, succinic acid, a carrier once used for dispersing griseofulvin (Goldberg et al, 1966a) is quite volatile and may also partially decompose by dehydration near its melting point. However, the evaporation problem can be avoided if the physical mixture is heated in a sealed container. Melting under vacuum or a blanket of an inert gas such as nitrogen may be employed to prevent oxidation of the drug or carrier.

The melting point of a binary system depends on its composition. By proper control, the melting point of a binary system may be much lower than the melting points of its two components. Under such a condition, this simple melting method can still be used to prepare solid dispersions, even if the pure drug may undergo decomposition at or near its melting point. This principle was used to prepare solid dispersions of steroids and a cardiac glycoside in PEG 6000 (Chiou and Riegelman, 1971b).

In the solvent method, solid solution or mixed crystals of organic or inorganic compounds are made by dissolving a physical mixture of the solid components in a common solvent followed by evaporation of the solvent. The main advantage of the method is that

thermal decomposition of the drug or carrier is avoided because of the low temperature required for evaporation of organic solvents.

Some disadvantage of solvent method are higher cost of preparation; difficulty of removing liquid solvent completely; possible adverse effect of the supposedly negligible amount of the solvent on the chemical stability of the drug; problem in selecting a common, volatile solvent; and difficulty in reproducing crystal forms.

The melting-solvent method is based on the finding that about 5 - 10% W/W of liquid compounds could be incorporated into PEG 6000 without significant loss of its solid property. Solid dispersions could be prepared by first dissolving a drug in a suitable solvent and then incorporating the solution directly into the melt of PEG obtainable below 70° without removing the liquid solvent (Chiou and Riegelman, 1971a).

This unique method has the advantages of both melting and solvent methods. From a practical stand point, its application is limited to drugs with a low therapeutic dose say 50mg. The feasibility of this method was demonstrated on spironolactone - PEG 6000 and griseofulvin - PEG 6000 systems (Chiou and Riegelman, 1971a).

The disadvantages include the possibility of the

selected solvent or dissolved drug being immiscible with the melt of the solid carrier. Another disadvantage is that the polymorphic form of the drug precipitated in the solid dispersion may be affected by the liquid solvent used.

In reviewing the classification of solid dispersions along lines of major fast-release mechanism, Chiou and Riegelman, (1971a) considered them under six groups, viz -

Simple eutectic mixtures. They are usually prepared from the rapid solidification of the fused liquid of two liquids which show complete liquid miscibility and negligible solid-solid solubility. These properties are illustrated in the phase diagram presented in figure 1.5. Thermodynamically, such a system is regarded as an intimately blended physical mixture of its two crystalline components. When a eutectic (composition E in figure 1.5) composed of a poorly soluble drug is exposed into aqueous medium in fine crystalline form.

A number of factors may contribute to the faster dissolution rate of a drug dispersed in a eutectic mixture.

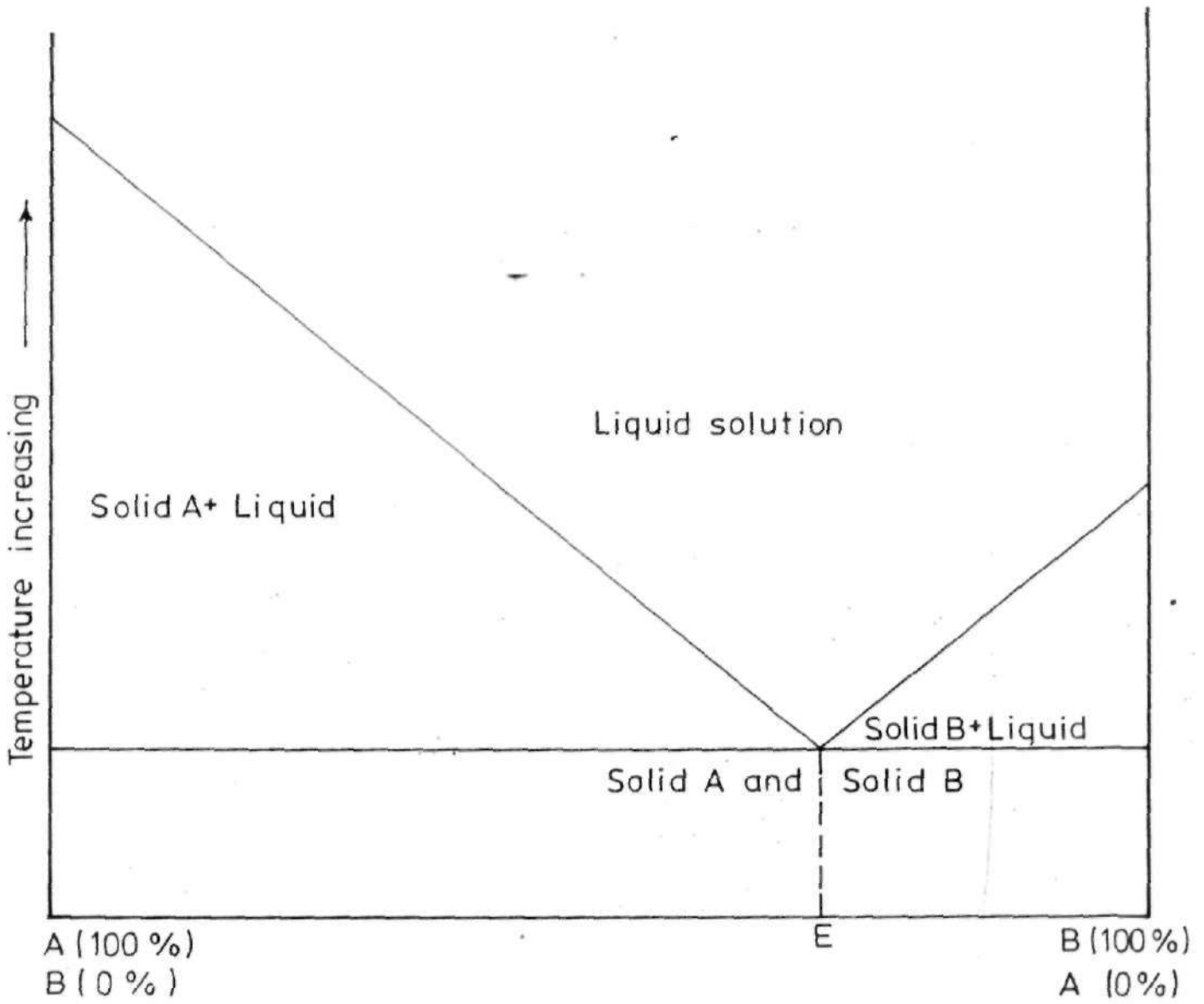


Fig.1.5 Phase diagram of a simple eutectic mixture with negligible solid solubility

From Chiou, W.L; and Riegelman, S. (1971)
Journal of Pharmaceutical Sciences,
Vol. 60, No. 9, p 1284.

They include the absence of aggregation* and agglomeration** between the fine crystallites of the pure hydrophobic drug.

Solid solutions. They are made up of solid solutes dissolved in solid solvents. It is often called a mixed crystal because the two components crystallize together in a homogeneous one-phase system.

Solid solutions are generally classified to reflect the extend of miscibility between the two components of the crystalline structure of the solid solution.

Based on the extent of miscibility between the two components there are:

(i) continuous or isomorphous, unlimited, complete solid solutions, and

(ii) discontinuous or limited, restricted, partial, incomplete solid solutions.

* An aggregate is a particle or an assembly of particles held together by strong inter or intramolecular atomic cohesive forces. Usually the aggregate is stable to high speed mixing or ultrasonic forces.

** An agglomerate is a gathering of two or more particles and/or aggregates held together by relatively weak cohesive forces.

Based on the crystalline structure, there are:

(i) substitutional solid solutions, and
(ii) interstitial solid solutions.

Glass solution and glass suspension

A glass solution is a homogeneous glassy system in which a solute dissolves in a glassy solvent. The glassy or vitreous state is usually a result of an abrupt quenching of a melt. It is characterized by transparency and brittleness below the glass forming temperature. On heating, it softens progressively and continuously without a sharp melting point.

A glass suspension refers to a mixture in which precipitated particles are suspended in a glassy dispersing medium.

A number of compounds have been shown to be capable of forming glasses rapidly upon cooling from the liquid state. These compounds include sucrose, glucose, ethanol and 3-methyl hexane.
Glass formation is common in many polyhydroxyl molecules, presumably due to their strong H-honding which may prevent their crystallization (Chiou and Riegelman, 1971a). Citric acid, a normal constituent of animals has been found to be capable of glass formation. Pure polyvinyl pyrrolidone (PVP) and other polymers dissolved in suitable organic solvents may become glassy after evaporation of the solvents.

An amorphous precipitation of a drug in a crystalline carrier. In this type the drug is presumed to be precipitated out as an amorphous form in the crystalline carrier. Since the amorphous form is the highest energy form of a pure drug, it should under almost all conditions, produce faster dissolution rate and better absorption than the crystalline form whether the crystals are, or are not dispersed in a carrier. Amorphous novobiocin actually has a 10-fold higher solubility than its crystalline form. A much faster dissolution and higher blood levels were also found in the amorphous form of the drug.

It has been postulated that a drug with a high super cooling property has more tendency to solidify as an amorphous form in the presence of a carrier (Chiou and Riegelman, 1971a).

Compounds or complex formation. Though strictly speaking these should not be classified as sold dispersions, but owing to their frequent occurence during the preparation of solid dispersions by the standard methods, they have attracted the attention of workers in this field. Although the water soluble polymers have been considered as ideal carriers for the solid dispersion of poorly soluble drugs, the implication of possible complexation should not be overlooked, as some of them actually retard the pharmacological response to drugs incorporated therein.

Examples where complexation has been implicated for retarding drug effects include: PVP's effect on penicillines, novobiocin, prostigmine, hexobarbital, cuinine and hexylresorcinol and the effect of PEG 6000 on phenobarbital (Chiou and Riegelman, 1971a).

Combination and miscellaneous mechanisms

Quite often a solid dispersion does not entirely belong to any of the major groups, but is made up of a combination of different groups. Under this circumstance, the observed increase in dissolution and absorption rates may be due to contributions made by different mechanisms.

The review by Chiou and Riegelman, (1971a) also touched upon the many methods used singly or in combination for acquiring information regarding the physical nature of any given solid dispersion, including their advantages and disadvantages.

Chiou and Niazi, (1971) worked on the dispersion of sulphathiazole in urea employing two polymorphic forms. They found that solid solubilities depended on the polymorphic form of the drug, and that the increased dissolution rate of the dispersion changed with aging.

Corrigan and Timoney, (1976) being aware of the lack of quantitative data on the release of drugs from PEG systems of constant surface area, carried out a systematic investigation of dissolution from hydroflumethiazide - PEG systems. The effect of

polyethylene glycol on the discs of hydroflumethiazide from discs of constant surface area prepared by three methods showed that incorporation of PEG increased the dissolution of hydroflumethiazide. The increase was found to be a function of the molecular weight of the carrier and of the ratio of drug to carrier. They reached the conclusion that the observed enhancement of the drug's dissolution rate at low and intermediate polymer weight fractions was due to the formation of soluble complexes in the diffusion layer during dissolution while the formation of solid solutions may be considered to explain the observed differences at high weight fractions.

Allen et al, (1977) in studies on the dissolution rates of corticosteroids using sugar glass dispersions, reached the conclusion that the observed increase dissolution rate was attributable to the presence of the drug in a very fine state of sub-division and to increased wettability.

The possibility of overcoming bioavailability problem of a nabilone-a cannabinoid derivative has been studied. Solvent method was used and an effective way of preventing the conversion of nabilone into the non-bioavailable polymorphic forms was found by keeping the drug dispersed in povidone.

This was the first reported use of the solid dispersion technique for the prevention of undesirable crystalline tranformation. The povidone dispersion maintained the drug in the bioavailable form for at least two years at room temperature. Nabilone is capable of existing in at least four distinct polymorphic forms depending on the crystallization conditions and solvent. All the forms appeared to be equally hydrophobic and insoluble. (Thakkar et al, 1977).

Geneidi et al, (1980a) studied glibenclamide solid dispersed by fusion method in mannitol, sorbitol, a 50:50 sorbitol: mannitol mixture, and polythene glycols 4000 and 6000. Results showed that the drug was stable and did not decompose during fusion, with the solid dispersion exhibiting marked increase in the level of drug dissolved compared to that from the plain drug. The degree of dissolution enhancement achieved by PEG 4000, was reported to be similar to that by PEG 6000.

The magnitude of release of the drug from fusion was found to be much greater than that obtained from marketed glibenclamide products.

Geneidi et al, (1980b) in another study employed other carriers for dispersing glibenclamide and investigated the dissolution rate characterisitcs.

They reported marked increase in the dissolution rate of the drug. Differential thermal analysis (DTA) and IR spectral studies on the drug and its coprecipitate showed that no polymorphic forms were formed during the preparation. But evidence of complex formation between glibenclamide and PVP was obtained.

The coprecipitates were found to have high dissolution rates compared to two marketed preparations.

Bloch et al, (1982) studied varying composition of chlorthalidone and urea. Dissolution rate studies of the physical, molten and compressed molten mixture gave results showing enormous increase in dissolution of molten discs from 10 to 35% W/W ratio of drug in urea, compared to the physical mixtures having corresponding composition. No significant differences were found in the dissolution rates between molten discs and compressed molten discs. They reported that crushing and compressing seemed to have had influence on dissolution rates. Aging studies showed no significant change in dissolution rate characteristics after 4 and 8 weeks. It was the view of these workers that dissolution enhancement by urea was due to the fact that each single crystallite of the poorly wettable drug was very intimately encircled by the soluble carrier which readily dissolves and causes the water to contact and wet the drug particles.

Their study indicated that a physical mixture produces about 10 times dissolution enhancement while melting with the carrier produces about 130 times increase in the dissolution of the drug compared to its intrinsic value.

Deshpande and Agrawal, (1982a) have reported observing increase in dissolution rates of chlorothiazide, hydrochlorothiazide, flumethiazide and cyclopenthiazide solid dispersions using polyethylene glycol as carrier.

In a separate presentation, Deshpande and Agrawal, (1982b) studied the influence of various dispersing agents on the dissolution rate of hydrochlorothiazide and observed that the drug decomposes in the presence of dextrose. They reported the superior drug release from urea as compared to the other carriers which were sorbitol and tartaric acid.

Meshali et al, (1983) reported the results of a study done on the solid dispersion of trimethoprim with different classes of sugar (reducing and non-reducing) prepared by the fusion method.

A marked increase in the dissolution rate as well as the bioavailability in humans of the solid dispersions as compared to the drug alone were observed. The study therefore clearly established that solid dispersion of trimethoprim with sugars can remarkably enhance the rate and extent of its absorption in humans.

CHAPTER TWO

NITROFURANTOIN

2.1 A REVIEW OF THE THERAPEUTIC SIGNIFICANCE OF THE DRUG

Nitrofurantoin is solely a urinary antimicrobial agent (Martindale, 1982; Conklin and Hollifield, 1965; Bron et al, 1970; Roger et al, 1981b), frequently used in the prophylaxis and treatment of acute and chronic urinary tract infections. It is bactericidal in-vitro to most gram-positive and gram-negative urinary-tract pathogens and minimum inhibitory concentrations have been reported to range from 4 to 100 µg per ml, however pseudomonas aeruginosa is usually resistant and most strains of proteus spp. are moderately resistant. Nitrofurantoin is most active in acid urine and if the pH exceeds 8, most of the antimicrobial activity is lost. Resistance rarely develops during treatment with the drug. (Martindale, 1982).

Mechanism of action

It is thought that nitrofurantoin acts by inhibiting acetyl-coenzyme A synthesis thereby blocking bacterial carbohydrate metabolism. This inhibition may extend to human tissues, causing polyneuritis (Roger et al., 1981b).

2.2 BASIC PHARMACOKINETICS OF NITROFURANTOIN

On absorption, 25 to 60% is bound to plasma proteins while about 40% of a dose is excreted rapidly in the urine as unchanged nitrofurantoin. The plasma half-life is about 20 minutes. (Martindale,1982).

Physico-chemically, nitrofurantoin is a weak acid (pKa 7.2) with relatively low aqueous solubility characteristics at pH values normally recorded for the various segments of the alimentary canal of man. As a result of this, the drug shows a particle size dependence in its dissoulution properties (Stoll et al., 1973), and in its bioavailability in man (Paul et al., 1967; Stoll et al., 1973; Bates et al., 1973).

Usually, large amounts of the drug are recovered from the excretory fluids (bile and urine) after either oral or parenteral drug administration. However, detectable faecal drug levels are only encountered occasionally (Conklin, 1971).

The drug is excreted in the kidney by glomerular filtration and is both excreted and reabsorbed in the tubules. Nitrofurantoin clearance in the kidney is handled by a weak acid transport system which is influenced by pH. Under acid conditions, tubular reabsorption is increased resulting in possible higher kidney tissue drug levels. Alkaline conditions reduce this effect, evidently yielding greater drug concentration in urine. During renal impairment, nitrofurantoin urinary

excretion is significantly reduced (Conklin, 1971).

Conklin, (1971) has reported that studies conducted with intravenously administered nitrofurantoin established that the drug levels in both blood and urine were proportional to the dose. The result has indicated that urinary excretion of the drug may be used as a reliable indicator for the in-vivo absorption of nitrofurantoin, while results obtained from numerous studies showed that similarities or differences between nitrofurantoin formulations are readily discernible using this approach (Conklin, 1971).

Bates et al., (1974) have studied the effect of food on the absorption of the drug from commercial dosage forms. Results showed considerably increased absorption in non fasting as compared to fasting subjects.

Toxicity of nitrofurantoin

Administration of nitrofurantoin has been reported to cause nausea, vomitting and diarrhoea in an appreciable number of patients. Gastrointestinal effects have also been observed in some patients receiving the drug intravenously (Harvey, 1980). Polyneuritis, usually related to high blood concentration has been reported in patients with impaired renal function or following prolonged administration of large doses. Allergic reactions such as skin rashes, fever, and more rarely, serious acute pulmonary sensitivity reactions

including symptoms of asthma and oedema have been reported. Chronic pulmonary symptoms may develop insidiously in patients on long-term therapy and these are not always reversible. Nystagmus and alopecia have also been reported to occur during treatment with the drug (Harvey, 1980).

Other adverse effects of the drug include megaloblastic anaemia, agranulocytosis, and haemolytic anaemia in persons with genetic deficiency of glucose - 6 - phosphate dehydrogenase and in the presence of liver damage (Harvey, 1980; Martindale, 1982).

2.3 A GENERAL REVIEW OF WORK DONE ON NITROFURANTOIN BY PREVIOUS WORKERS

Studies have been conducted relating the effect of varying the crystal size of orally administered nitrofurantoin on absorption and urinary excretion in the rat and man and emesis in dogs.

Urinary excretion in the rat and man demonstrated a slower rate of absorption from larger crystal size than from fine crystals with comparable total urinary excretion from a suitably selected size or size range (average crystal size of about 100 μm or its equivalent surface area falling between 187.5 to 75 μm limits). Studies with dogs revealed that the larger crystals were much less provocative of emesis than the smaller crystal size. (Paul et al., 1967).

A study has been carried out to develop a turbidimetric method for the estimation of nitrofuran compounds in biological fluids. A microbiological assay procedure for nitrofurantoin and its active congeners was developed using S. faecalis. The results of the assay subjected to statistical analysis showed that the extent of inhibition and the form of regression of responses were affected by the concentration of the indicator organism, time of incubation, and quantity of serum or uring. Optimal conditions for the estimation were established using nitrofurantoin and furazolidone in serum and urine. The method was found to be rapid and reliable for the estimations of smalll amounts of the drug in biological fluids (Gang and Shaikh, 1972).

A study of in-vitro dissolution and in-vivo absorption of nitrofurantoin from deoxycholic acid has been carried out. Results indicated that the coprecipitates showed significant increases in both the initial urinary excretion and the total cumulative amount of unchanged nitrofurantoin when compared to either the physical mixture or the pure drug (Stoll et al., 1973).

Single lots of 14 commercially available 50 and 100mg nitrofurantoin products have been evaluated in-vitro and in-vivo. All products tested met USP XVIII specifications for drug content, disintegration time, and dissolution rate. Statistically significant differences were observed in bioavailability, as

determined from cross-over urinary excretion studies in 14 volunteers. No useful correlation was observed between the extent of urinary excretion and either the disintegration or dissolution characteristics of the dosage forms tested (Meyer et al., 1974).

The pH - dependent dissolution rate of nitrofurantoin from commercial suspensions, tablets and capsules has been investigated. Results provided evidence that the dissolution rate of nitrofurantoin from different pharmaceutical dosage forms can be markedly affected by pH. The dissolution medium (PH 7.2 Phosphate buffer) recommended by the USP is unable to discern inherent particle-size dependent differences in the rate of solution and incidence of side effects of nitrofurantoin from commercial, microcrystalline drug tablet and macrocrystalline drug capsule dosage forms. The dissolution rate characteristics of the drug from these two dosage forms in simulated gastric fluids (PH I.12) and the existence of a relationship between these in-vitro results and reported toxicity data suggest that the USP XVIII dissolution rate specification for nitrofurantoin products should be modified (Bates et al., 1974).

The influence of two linear polymers, PEG 6000 and PVP 25,000, on the dissolution rate of three poorly soluble drugs, nitrofurantoin, ethotoin and coumarin has

been studied. Povidone 25,000 produced better drug solubilization that PEG 6000 with the drug - polymer ratios studied, and an increase in the weight fraction of either polymer gave more rapid dissolution. TLC and IR studies ruled out any interaction between the drugs and PEG 6000. IR spectrophotometry provided evidence that there was complex formation between nitrofurantoin and PVP 25,000 (Geneidi et al., 1976).

In a series of in-vitro and in-vivo experiments, the dissolution, bioavailability and pharmacokinetics of three commercial nitrofurantoin preparations have been studied and compared. Plasma and urine levels were determined using a HPLC method. From the in-vivo results, one of the formulations was significantly less absorbed and excreted than the other two preparations. The other two formulations, A and Ceduran were bioequivalent, but in view of the reduction of side-effects Ceduran seemed to be the drug of choice. An in-vitro dissolution test for nitrofurantoin using the USP XIX disintegration apparatus provided profiles comparable to the in-vivo results (Bron et al., 1979).

During an investigation, the feasibility of formulating controlled-release microspheres of a reservoir type using egg albumin as the core matrix and nitrofurantoin has been studied. The albumin microspheres and albumin microspheres coated with ethylcellulose were tested. In-vitro release rates of nitrofurantoin from

the egg albumin beads was significantly reduced as compared to the nitrofurantoin powder. Ninety percent of the drug dissolved from the nitrofurantoin powder in about one hour while approximately two hours were needed for half of the drug to be released from the albumin beads. The release of nitrofurantoin from ethylcellulose coated microcapsules followed an apparent zero order rate, although the drug was not completely released from the beads during the eight hours dissolution time (Jun and Lai, 1983).

A method for the determination of furadantin (nitrofurantoin) in urine has been described. Furadantin was separated from interfering urinary pigments by a chromatographic absorption technique. Furadantin concentrations were then determined spectrophotometrically from the absorbance (375nm) of the eluate, or from the eluate further treated to form the phenylhydrazone of nitrofuraldehyde from furadantin and the determination carried out colorimetrically. Furadantin concentrations as low as 1 or 2ug per 100ml. can be determined by this chromatographic method (Bender et al., 1956).

Watari, et al., (1983) investigated the influence of route administration in the absolute bioavailability and GI tract absorption of nitrofurantoin in rabbits and reported that the disposition of nitrofurantoin was described by a one-compartment model with simultaneous

first-order and Michaelis-Menten type elimination kinetics and bioavailability estimated by non linear assessement. The plasma levels following oral administration were significantly lower than those after intravenous administration. From the different absolute F values, they concluded that the reduction of bioavailability following oral administration could not be attributed to metabolism by intestinal microflora or to the hepatic first-pass effect, but may be probably due to gastric degradation of the drug. The investigation of factors influencing bioavailability such as water volume taken with the drug, change of gastric emptying and effect of particle size were also carried out. Increase of volume of water administered tended to improve bioavailability and a particle size dependency was observed.

2.4 OBJECTIVE AND SCOPE

The primary objective of this project has been to work towards the optimal use of nitrofurantoin, particularly the enhancing of the bioavailability of the drug at its site of action and consequently greater therapeutic efficacy and possible reduced incidence of toxicity in greater number of patients.

The possibility of increasing the dissolution and absorption rates of nitrofurantoin preparations by using the solid dispersion technique was investigated.

Six carriers were screened but only two were capable of producing comparable solid dispersions. Solid dispersions of the drug in the two inert, water-soluble carriers were prepared by the melting method. The stability of the solid dispersions were assessed using TLC and UV spectrophotometry.

Preformulation dissolution rate studies were carried out using powder samples. Drug: carrier ratio of 1:10 was used in preparing solid dispersions and physical mixtures with the two selected carriers. Drug: carrier ratios of 5, 10, 20, 30, 40, and 50% solid dispersions of the better of the two carriers, were then tested for dissolution rates. The 30% drug carrier solid dispersion was selected for formulation studies.

The 30% drug in carrier solid dispersion was formulated into a tablet and compared with tablets made from the drug/PEG 6000 physical mixture and Avicel physical mixture of corresponding composition, formulated and produced under identical manufacturing procedures. Tablet evaluation and quality control tests were carried out according to USP XVIII.

A study aimed at establishing an *in-vitro* dissolution rate and *in-vivo* absorption correlation was then carried out on the prepared and tested tablets. An *in-vivo* absorption studies was done on 3 fasted human volunteers, using a balanced cross-over design.

PART TWO - EXPERIMENTAL

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

The active principle:

GENERIC NAME: Nitrofurantoin (analytical grade) from the Cedona, Haarlemmer Pharmaceutische Fabriek B.V., Haarlem (The Netherlands).

APPEARANCE: Lemon-yellow, fine powder

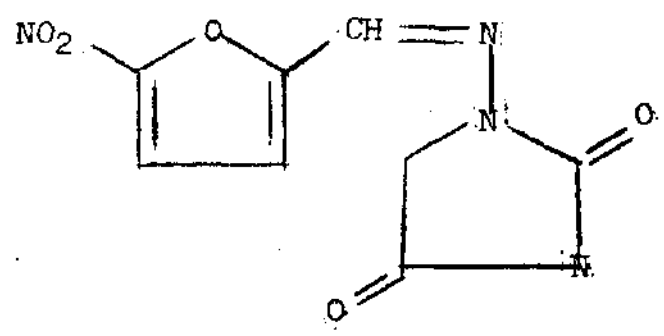
CHEMICAL NAMES:

(i) I - [(5-nitrofurfurylidene) amino] hydantoin

(ii) I - [(5-nitrofurfurylidene) amino] imidazolidene 2, 4 - dione.

EMPIRICAL FORMULA: $C_8 H_6 N_4 O_5$

STRUCTURAL FORMULA:



MOLECULAR WEIGHT: 238.16

MELTING RANGE: 270 - 272 °

MOISTURE CONTENT: Not more than 1% determined by drying at 105°.

SOLUBILITY: I in 5000 parts of water, (Martindale, 1982); 79.5mg per litre (24°).

113.4mg per litre (30°); 174.1mg per litre (37°) and 251.2mg per litre (45°) (Cadwallader and Jun, 1976).

DOSES:

Therapeutic, 50 - 150mg four times daily by mouth.

Prophylactic, 50 - 100mg at bedtime.

USES: Broad spectrum antimicrobial agent used clinically for treating specific urinary tract infections.

Other Materials

1 Polyethylene glycol (reagent grade) BDH chemical Ltd., Poole BH 124 NN, England.

PHYSICAL STATE: Hard wax-like, non-hygroscopic flakes.

MELTING RANGE: 58 - 62°

STRUCTURAL FORMULA: $HOCH_2 (CH_2 OCH_2)_n CH_2 OH$.

MOLECULAR WEIGHT: 6000

COLOUR: Creamy-white

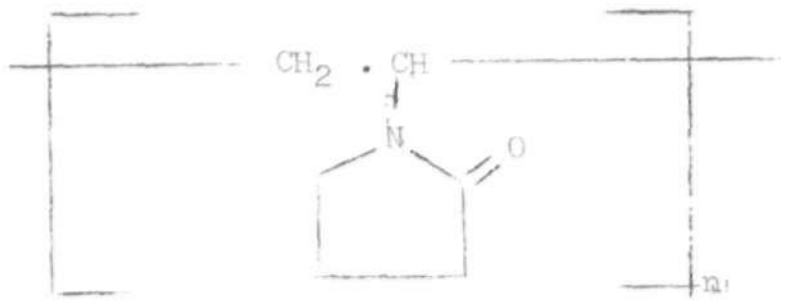
ODOUR: Almost odourless

SOLUBILITY: I in 2 parts of water, I in 2 part of chloroform, freely soluble in alcohol, but practically insoluble in ether. (Martindale, 1982).

OTHER PROPERTIES: Polythylene glycol 6000, is nono-volatile. It is chemically and physiclogically inactive. It has been used as inert matrix in several solid dispersions by Chiou and Riegelman, (1971b);Corrigan and Timoney (1976): Deshpande and Agraval, 1982a), Geneidi et al. (1976); Geneidi et al., (1980a).

2 Polyvinylpyrrolidone (PVP). povidone (reagent grade), BDH Chemicals Ltd.,

STRUCTURAL FORMULA:



EMPIRICAL FORMULA: $(C_6 H_9 NO)_n$

MOLECULAR WEIGHT: 44,000

COLOUR: White

ODOUR: Odourless

NATURE: Hygroscopic powder

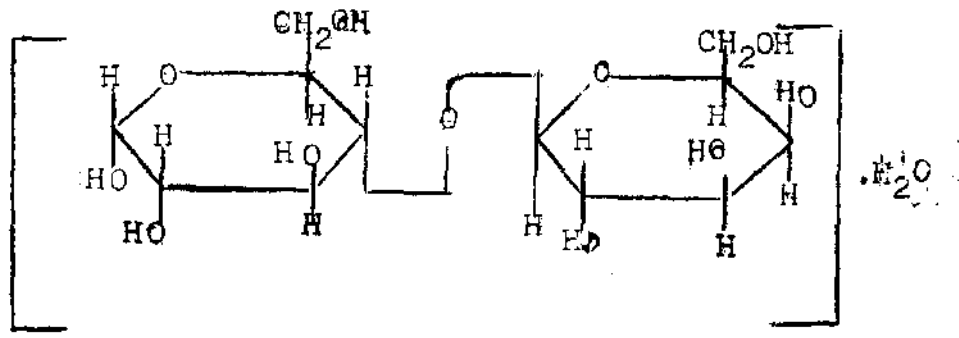
SOLUBILITY: Soluble in water, alcohol, chloroform and isopropyl alcohol; practically insoluble in acetone and ether. (Martindale, 1982).

USES: Povidone is used as suspending agent and dispersing agent usually in concentrations of up to 10 percent. It is also used as tablet binding and granulating agent usually in concentration of 0.5 to 5 percent. It is particularly useful in the preparation of effervescent tablets which are highly sensitive to water, since it can be incorporated as a solution in an organic solvent such as alcohol. It is also useful in the preparation of heat sensitive tablets since granules prepared with a solution of povidone in an organic solvent of low boiling point can be dried in air at ordinary temperature.

3 Lactose (reagent grade), BDH Chemicals Ltd.

CHEMICAL NAME: 4 - O - β - D - Galactopyranosyl - D - Glucopyranose monohydrate

STRUCTURAL FORMULA:



EMPIRICAL FORMULA: $C_{12}H_{22}O_{11}\cdot H_2O$

MOLECULAR WEIGHT: 360.3

COLOUR: Creamy white

TASTE: Faintly sweet

ODOUR: Odourless

SOLUBILITY: I in 5ml of water, and 2.6 ml of hot water, very slightly soluble in alcohol, insoluble in chloroform and ether (Martindale, 1982).

USES: Lactose is used as diluent to give bulk to powders, and in compressed tablets. It is used in the production of penicillin and as sterilized lactose as diluent for antibiotic powders.

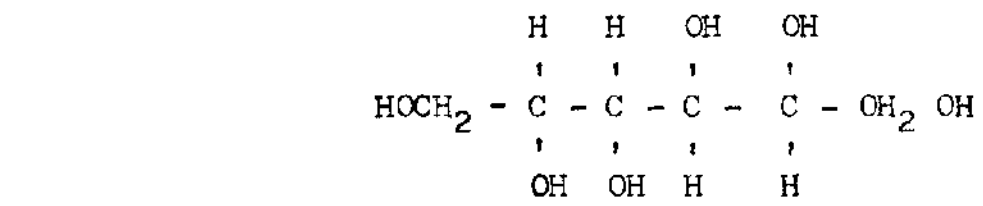
Lactose is widely used as an addition to milk for infant feeding.

4 Succinic acid (reagent grade) BDH Chemicals Ltd.

NATURE: White powder, which undergoes sublimation

5 Mannitol (reagent grade) BDH Chemicals Ltd.

STRUCTURAL FORMULA:



EMPIRICAL FORMULA: $C_6 H_{14} O_6$

MOLECULAR WEIGHT: 182.17

MELTING RANGE: 165 - 169°

SOLUBILITY: I in 5.5 of water, slightly soluble in Pyridine, very slightly in alcohol soluble in alkaline solutions, practically insoluble in ether. (Martindale, 1982).

COLOUR: White

ODOUR: Odourless

TASTE: Sweet

PHYSICAL STATE: Free flowing granules

USES: This hexahydric alcohol has been used as diluent and excipient in pharmaceutical preparations. It is also use as diagnostic agent for kidney function. It is an osmotic diuretic in high doses (50 to 200g daily) by intraveous infusion as 5 to 20% solution.

Reagents:

Purified stearic acid (general purpose) Hopkin & Williams Ltd., Chadwell Health, Essex, England

Benzene (reagent grade) BDH Chemical Ltd.,

Phosphoric Oxide SLR, Fisons Scientific Apparatus, Loughborough, Leics, England

N,N-Dimethylformamide (general purpose) Hopkin & Williams Ltd. Chadwell Health Essex, England).

Acetone BDH Chemicals Ltd., Ethyl alcohol, (Absolute)
James Burrough Ltd., Fine Chemicals Division 60
Montford Place, London SE II.
Microcrystalline cellulose, Avicel, PH 101
Starch (Laboratory Chemicals) May and Baker Ltd.,
Dagenham, England.
Toluene (Chemically pure) Shermond, Sussex, England
Talc, Zinc powder BDH Chemical Ltd.

THE EQUIPMENTS

SP 30 UV-VISIBLE SPECTROPHOTOMETER (PYE-UNICAM LTD.,
York Street, Cambridge, England CB1 2PX.).
Silica cuvettes PS 3875 IA 10mm PO
ERWEKA dissolution apparatus and accessories Erweka
Appatebau - G.M. B.H. 6056 Heusenstamm Kr. Offenbach -
Main. CENTRIFUGE Remi instruments, 14 Shah Industrial
Estate, Veera Desai Rd., Andheri (W), Bombay - 400 058,
India.
ERWEKA Granulator, Type AR 400 Erweka Apnatebau
SINGLE PUNCH, tabletting machine (KORSCH)
Friabilator, Type TA 3R (ERWEKA)
Oscillating granulator, Type AR 400 (ERWEKA)
Analytical balance, Type H35 AR Mettler, Gallenkamp

3.2 PRELIMINARY STUDIES

3.2.1 ABSORPTION SPECTRUM FOR NITROFURANTOIN

To determine the wavelength of peak absorbance for nitrofurantoin, a 10µg per ml aqueous solution was run on spectrophotometer from 200nm to 405nm. The absorbances observed are presented in table 3.1 while the graphical representation is made in figure 3.1.

Table 3.1 Absorption - spectrum of nitrofurantoin in water (10 µg per ml)

Wavelength, (nm)	Absorbance	Wavelength, (nm)	Absorbance
200	0.508	305	0.210
205	0.444	310	0.194
210	0.389	315	0.196
215	0.333	320	0.214
220	0.293	325	0.247
225	0.281	330	0.291
230	0.292	335	0.345
235	0.313	340	0.399
240	0.332	345	0.455
245	0.349	350	0.512
250	0.368	355	0.562
255	0.396	360	0.605
260	0.428	365	0.637
265	0.467	370	0.655
270	0.493	375	0.656
275	0.488	380	0.645
280	0.452	385	0.614
285	0.400	390	0.567
290	0.346	395	0.511
295	0.290	400	0.446
300	0.244	405	0.378
410	0.313	455	0.022
415	0.246	450	0.023
420	0.192	465	0.022
425	0.145	470	0.020
430	0.104	475	0.030
435	0.076	480	0.033
445	0.042	490	0.018
450	0.030	495	0.021

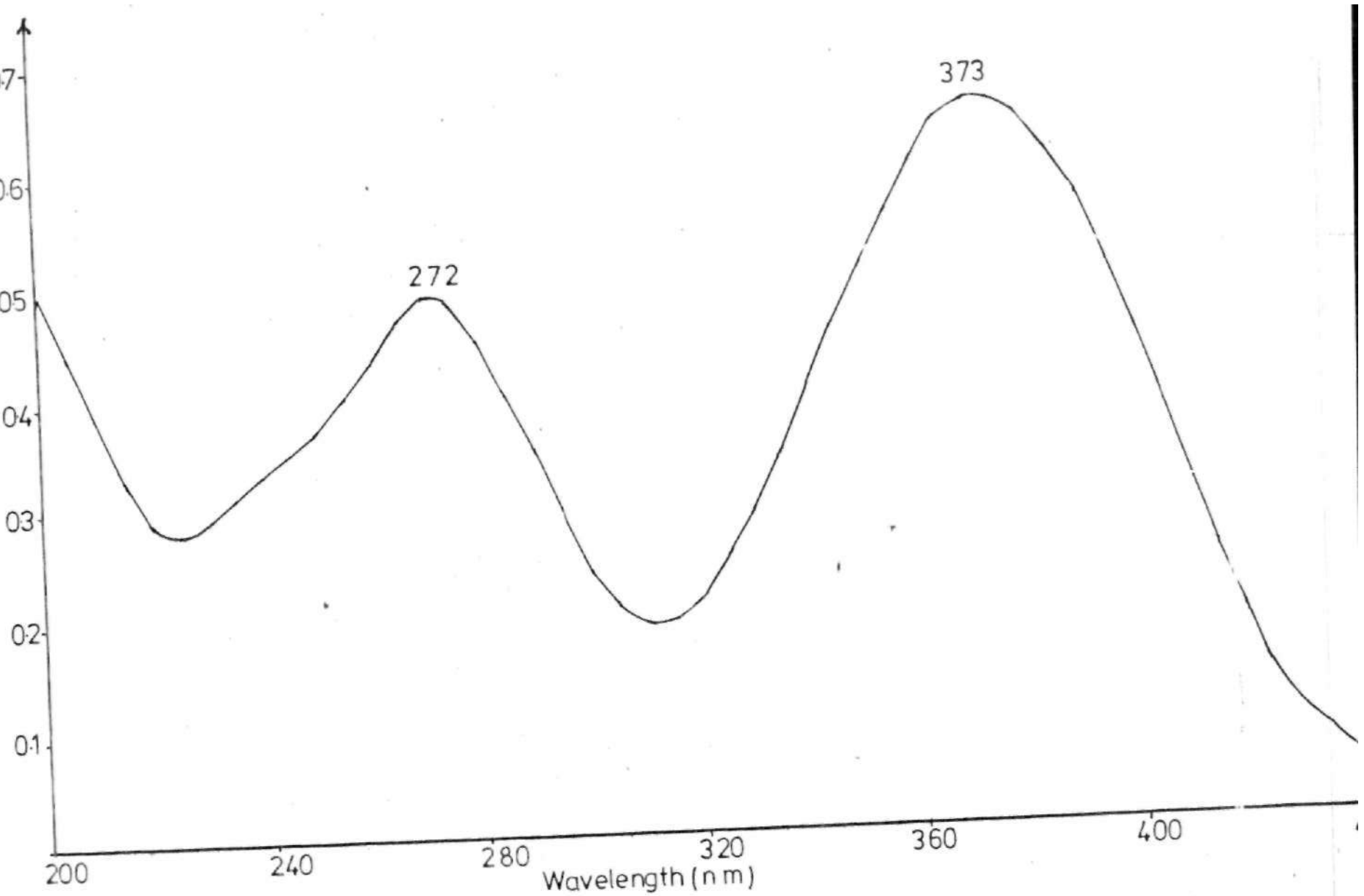


Fig. 3.1 Absorption spectrum for nitrofurantoin solution in water (10 µg/ml)

3.2.2 LAMBERT-BEER'S PLOT

To establish the applicability of UV spectrometry for the quantitative determination of nitrofurantoin in aqueous solutions, compliance with Beer-Lambert's linearity plot was investigated at the wavelength of maximum absorption (373nm).

Procedure

A 20 µg per ml stock solution of the drug, was serially diluted and absorbances of resulting dilute solutions recorded. The observations are presented in a tabular form in table 3.2 and graphically in figure 3.2.

Table 3.2 Concentrations and absorbances of solutions of nitrofurantoin in water used for the Lambert-Beer's plot.

Concentrations of dilute solutions - µg per ml.	Absorbances* at 373nm
1	0.071
2	0.140
3	0.211
4	0.283
5	0.351
8	0.566
10	0.696
15	1.042
20	1.207

* Mean of three observations.

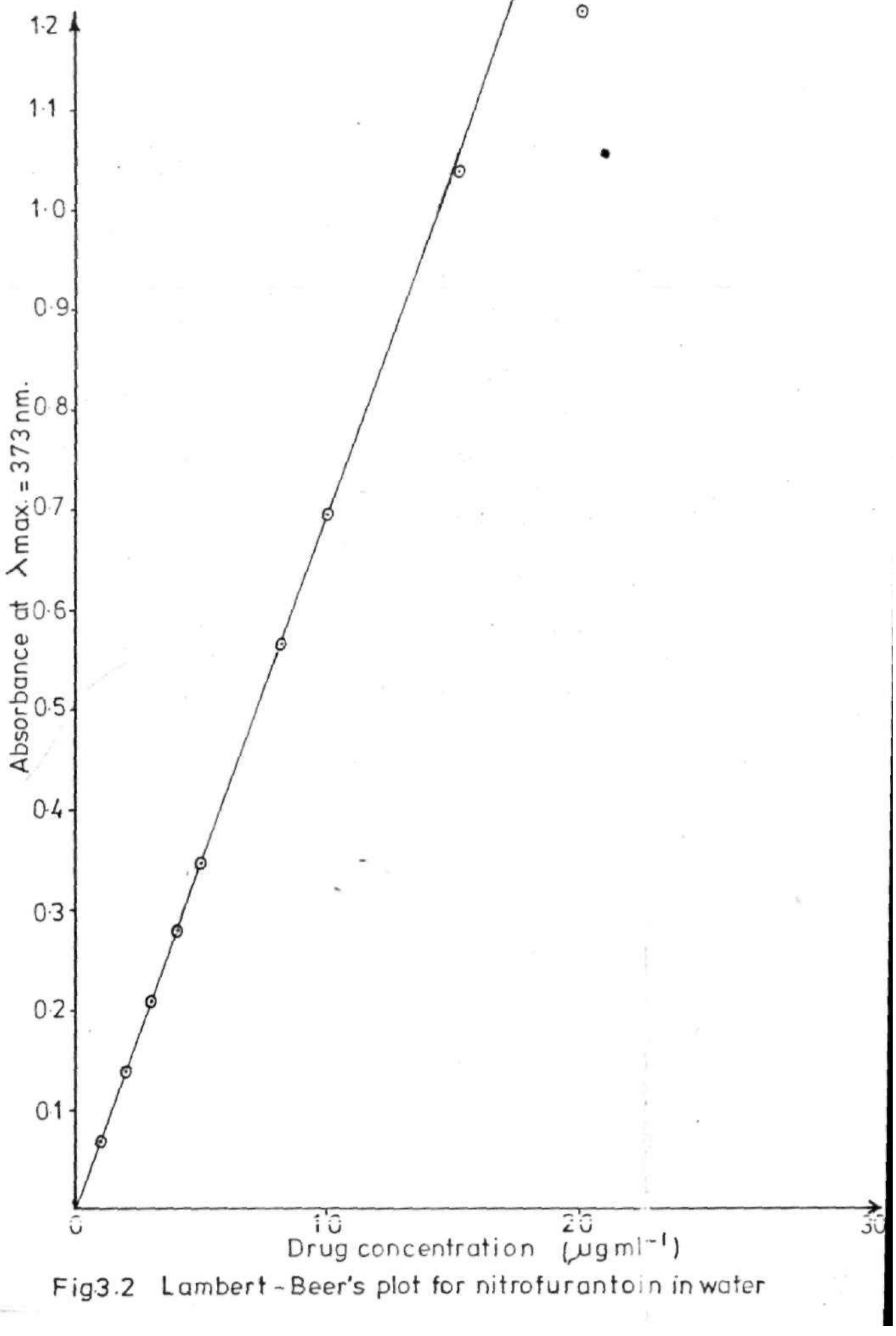


Fig3.2 Lambert - Beer's plot for nitrofurantoin in water

Calculation

The slope of the plot in fig. 3.2 was calculated by linear regression analysis and its value was 0.0696. The intercept was zero.

3.2.3 INTERACTIVE EFFECT OF LIGHT ON NITROFURANTOIN SOLUTIONS IN WATER

This investigation was carried out to study the possible effect of normal laboratory lighting on the stability of aqueous solution of the drug.

Procedure

A 5 µg per ml solution of the drug was prepared in water and divided into two parts. One part was exposed to a constant light illumination* in a well-stoppered flask. The other part was kept in another flask protected from light by wrapping with aluminium foil. These containers and their contents were kept this way for 72 hours. Samples were withdrawn periodically for analysis to monitor drug levels in them. The result of this investigation is presented in table 3.3 and graphically in figure 3.3.

* Constant illumination was facilitated by 4 fluorescent light tubes of 40w, 5x120cm each in a $10m^2$ room with white plastered walls.

Table 3.3 Photolytic degradation of solutions of pure nitrofurantoin in water.

Time (hr.)	Percentage drug Content	
	Exposed Container	Proctected Container
0	100.00	100
0.5	95.43	100
1.0	89.86	100
1.5	86.08	100
2.0	80.52	100
2.5	77.93	100
5.0	75.94	100
11.0	62.82	99.60
14.0	60.24	99.60
72.0	10.54	99.60

3.2.4 EFFECT OF TEMPERATURE ON THE STABILITY OF NITROFURANTOIN IN WATER

As much of the drug would be subjected to tests of various kinds, some of which would be at the simulated temperature of the human body, this study was carried out to estimate the effect of this temperature on the drug content of aqueous solutions.

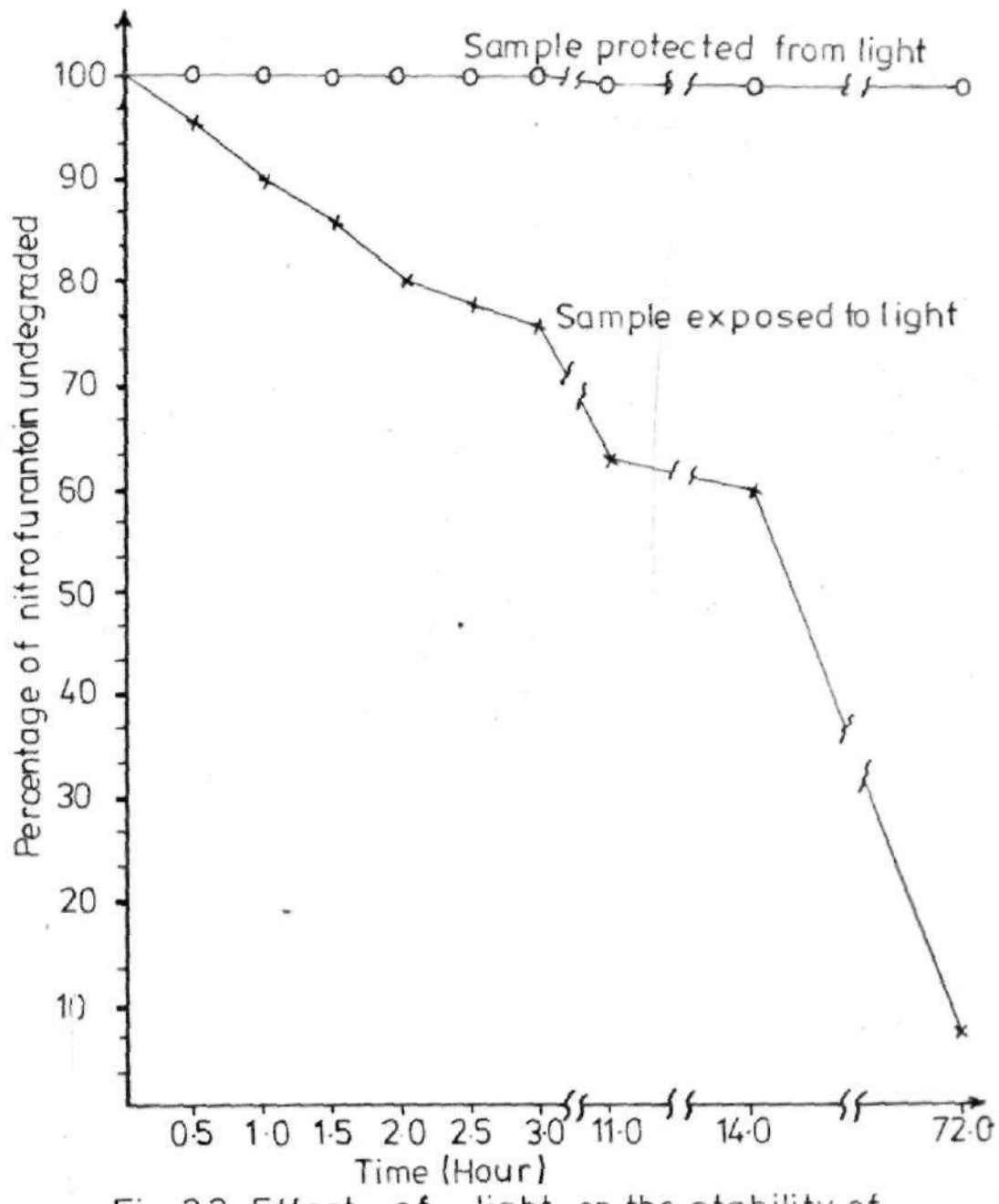


Fig. 3.3. Effect of light on the stability of nitrofurantoin solution in water

Procedure

A 5 µg per ml aqueous solution of the drug was placed in a well-stoppered volumetric flask wrapped with aluminium foil (to protect the drug from light) and kept in a constant temperature water bath maintained at 37° ± 0.5 for 4 hours. Samples were taken and assayed periodically. Concentrations corresponding to absorbances observed were obtained from the standard curve by calculation. The results are shown in table 3.4 and figure 3.4.

Table 3.4 Drug content variation with time at 37°.

Time (minutes)	Drug content (% w/v)
0	100
30	100
60	100
90	100
120	100
180	100
240	100

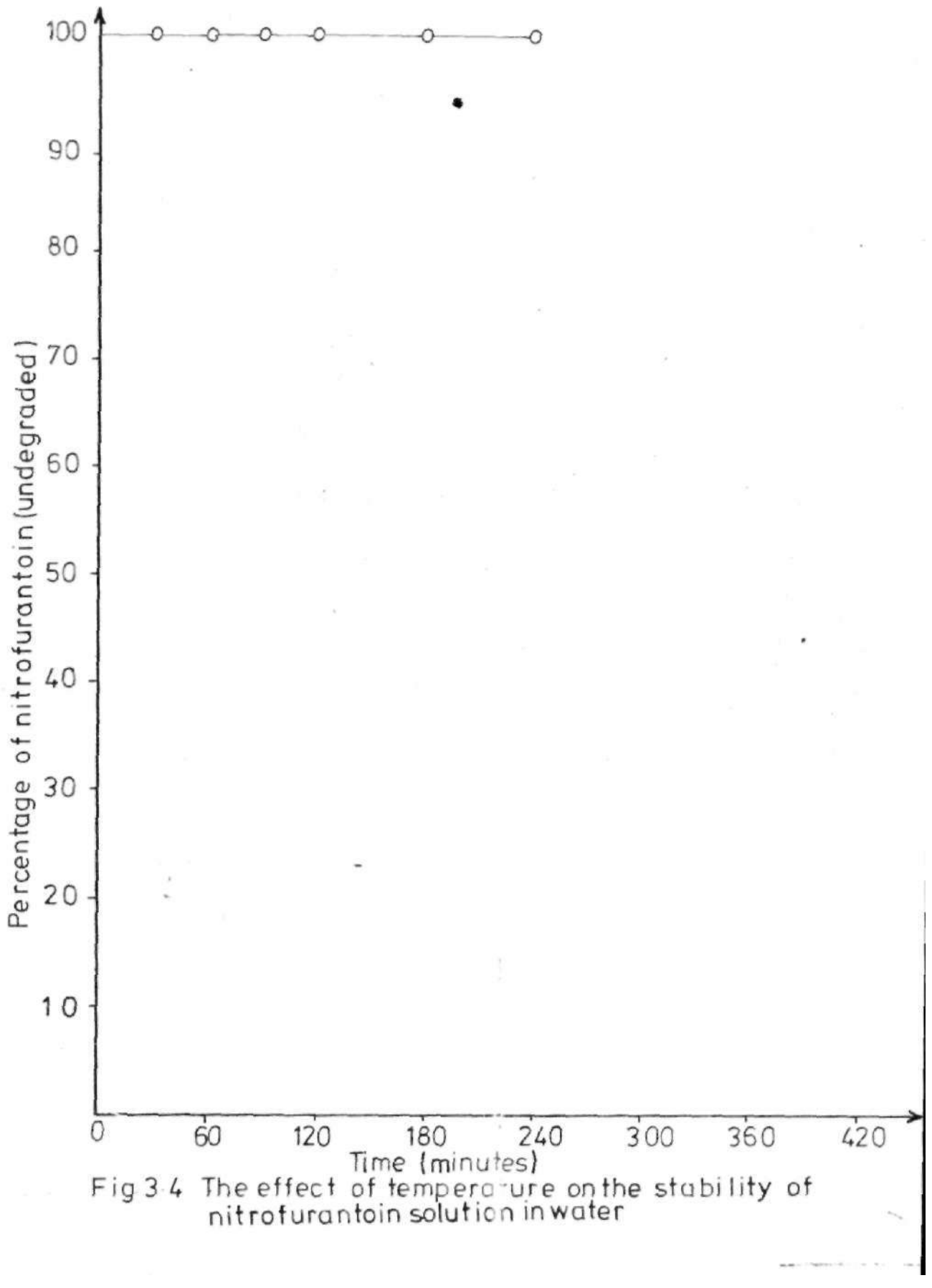


Fig 3.4 The effect of temperature on the stability of nitrofurantoin solution in water

3.2.5 SCREENING OF THE PROPOSED CARRIERS

Introduction

A preliminary survey was planned for six proposed carriers, namely, lactose, mannitol, polyethylene glycol (PEG) 6000, polyvinylpyrrolidone (PVP) 25,000 and 44,000 and succinic acid. Attempts to produce solid dispersions by the met (fusion) method or coprecipitates by the solvent method gave the following results:-

Lactose: A satisfactory melt or fusion was not formed. The solvent method approach to produce a coprecipitate with solvents like chloroform, ethanol and methanol was unsuccessful.

Mannitol: This carrier formed a satisfactory melt with the drug, subject to stability studies good performance. Mannitol did not produce a coprecipitate with the drug in the solvents (chloroform, ethanol and methanol) tried.

Polyethylene glycol 6000: This carrier readily formed a satisfactory melt. With the solvents (chloroform, ethylalcohol, and methanol) tried, no coprecipitate was formed.

PVP 25,000: This molecular weight grade of Povidone was not available for trial at the time this survey was being done.

PVP 44,000: This carrier did not form a satisfactory melt with the drug. The solvent method gave a glassy and sticky coprecipitate with chloroform as solvent.

The mass remained sticky until the solvent was completely evaporated, and this took several weeks, in a vacuum desiccator. The dry coprecipitate was glassy and powdering by means of mortar and pestle was difficult and a little unpleasant.

Succinic Acid: This carrier was found to be subliming and since a sealed container system (adequate for maintaining the drug and carrier composition and reproducible) could not be provided, the melt method could not be investigated further. A satisfactory coprecipitate did not form between the drug and this carrier in the solvents tried.

Conclusion

Melts of the drug in mannitol and in PEG 6000 were selected for comparative in-vitro dissolution studies.

3.2.6 [illegible] OF CARRIER INTERFERENCES

Introduction::

During the drug content evaluation stage the drug would be in aqueous solutions of the selected carriers. The aim of this survey was to find out what absorbances would be due to the presence of different carriers in the various solutions. If these absorbances were found to be significant, the appropriate zeroing of the spectrophotometer with corresponding concentrations of the carriers would be carried out.

Procedure

0.5% aqueous solutions of both mannitol and PEG 6000 were made separately. For each carrier, the 0.5% solution was diluted to make the final carrier concentration 0.03% (being the highest concentration of any of the carrier to be encountered during the in-vitro dissolution test) and their corresponding absorbances recorded at the wavelength of peak absorbances for the drug (Table 3.5)

Results

The carriers have shown minimal absorbance at the working wavelength. The carriers are not expected to adversely affect the readings obtained.

Table 3.5 Absorbances for 0.03% aqueous solutions of PEG 6000 and mannitol at the analytical wavelength (373nm)

Carrier	Absorbance
PEG 6000	0.003
Mannitol	0.001

3.2.7 DETERMINATION OF THE SOLUBILITIES OF NITROFURANTOIN

Introduction

This preliminary investigation of the solubility of nitrofurantoin (as pure drug) was carried out at room temperature (which was 28°

at the time of these determinations) and at 37° in water. Solubility studies were also carried out at 37° using varying concentrations, (0.1 - 5%) of aqueous solutions of PEG 6000 and mannitol.

Procedure

Excess powder of the drug were placed in the various solvent systems in triplicates inside amber coloured clinbritic bottles. The bottles and their contents were placed in a shaker bath with thermostat set at 37° ± 0.5. Small samples were withdrawn at intervals for analysis until there was no further increase in drug content. Each smaple upon withdrawal was filtered through membrane filters (HA 5.0 μ), and Iml of filtrate diluted to 25ml. The absorbances were recorded and the corresponding drug content in the dilute solutions calculated. The drug content of the concentration filtrates were thereby derived.

The solubility of the drug at room temperature with water as solvent was determined using the same procedure given above, but with the thermostat bath of the shaker bath set at 28°. The results of study are presented in table 3.6.

Table 3.6 The solubility of nitrofurantoin in aqueous solutions of PEG 6000 and mannitol.

Solvent System	Temperature (°C)	Solubility (mg per litre)
Water	28	111.71
Water	37	196.81
0.1% Mannitol	37	191.42
0.5% Mannitol	37	195.01
1.0% Mannitol	37	202.55
5.0% Mannitol	37	210.10
0.1% PEG 6000	37	222.67
0.5% PEG 6000	37	244.57
1.0% PEG 6000	37	251.40
5.0% PEG 6000	37	325.74

3.2.8 DISCUSSION

The results from this set of preliminary studies has shown that:

i) There are two peaks recorded in the absorption spectrum. There was one peak at 272nm, and another was located at 373nm. For its greater sensitivity, 373nm was selected for use in measurements involving concentration of the drug in aqueous solutions.

ii) One effective way of protecting aqueous solutions of nitrofurantoin is by wrapping the containers with aluminium foil. This study has demonstrated the photolytic degradation of nitrofurantoin and the need to protect samples of the drug from direct sunlight or fluorescent light (Bender et al., 1956; Buzard et al., 1956; Conklin and Hollifield, 1965; Patton et al., 1970) The study on photolytic effect on solutions of nitrofurantoin has helped to solve any problem that might arise when samples for quantitative determinations have been prepared and there is a major electrical power failure for a prolong period. Provided the solutions are adequately protected from light in well stoppered containers, loss of appreciable quantities of the drug in solution would be greatly minimized, thereby ensuring that valid, reproducible readings would still be obtained when normal power supply is restored, and the determination made.

iii) A Lambert-Beer's plot for aqueous solutions of nitrofurantoin has been constructed. The graph had a gradient of 0.0696 and a zero intercept. Reliable absorbances may be expected only for aqueous drug concentrations in the 2 to 10 ug per ml (2 to 10mg per litre) range. This is the range of sensitivity of the graph.

iv) Working at a temperature of 37° does not result in thermal decomposition of aqueous solutions of nitrofurantoin. The total time taken for this study was 240 minutes. This time was far in excess of the 120 minutes needed for any in-vitro dissolution rate study. It may also be inferred from this study that purely thermal degradation is not likely to reduce drug levels within the human body, although other metabolic processes that may require this range of temperature may exist. This study has clearly indicated that photolytic degradation of aqueous solutions of nitrofurantoin, may be a major source of instability. Thermal stability above 37° was not the subject of this study, and comments on this aspect of the drug's stability in aqueous solution cannot therefore be made here.

v) Aqueous solubility of the drug, and its solubility in various solvent systems and at different temperatures have been determined.
The results from this study, have established that the physical presence of the carriers actually enhance aqueous solubility. The greater the concentration of the carriers tried the greater the observed solubility. This study has again demonstrated that it is important to keep temperature constant during solubility determinations. Also during dissolution rate studies, temperature should be kept

constant, just as agitation intensity and diffusion coefficient of the drug in any given dissolution medium according to Noyes-Whitney equation of

$$\frac{dc}{dt} = K.S.\ (C_s - C_t)$$

This temperature effect is incorporated into the constant, K. This solubility estimation determined the size of powder used for each in-vitro dissolution rate test.

vi) The proposed carriers have been screened. An insight on how they may be used to promote greater product stability and bioavailability, through the solid dispersions technique has been gained.
Mannitol and PEG 6000 have been selected for comparative studies, to find out which is better for product formulation. The basis of further study between mannitol and PEG 6000, would be in-vitro dissolution rate tests.

vii) The selected carriers in their highest possible concentrations in aqueous solutions to be encountered during the various stages of the project would not contribute significantly to absorbance readings that would be recorded at 373nm. However, appropriate zeroing of the spectrophotometer with correct solutions of the carrier involved in any given measurement process would remove every doubt.

With these findings and strict adherence to their provisions as necessary precautions, it is expected that sources of error in all experimental work in this project would be reduced considerably.

CHAPTER FOUR

IN-VITRO DISSOLUTION RATE STUDIES ON POWDERS

4.1 PREPARATION OF SOLID DISPERSIONS, PHYSICAL MIXTURES, AND PURE DRUG POWDERS FOR DISSOLUTION STUDIES

Introduction

Powders were prepared and used for in-vitro dissolution rate studies, to compare the pure drug, drug-carrier solid dispersions, and physical mixtures dissolution characteristics utilizing mannitol and polyethylene glycol 6000 as carriers. Drug: carrier ratios of 1:10 were prepared both as physical mixtures and as solid dispersions. The solid dispersions were prepared by the melting method.

Procedure

A 1:10 nitrofurantoin in mannitol melt was prepared by placing 9 g of mannitol in a clean, dry porcelain dish and gradually heated on a paraffin bath until it started to melt at 141°. At this point, 1 g of pure drug was added a little at a time with continuous stirring. The final temperature of the molten mixture was 144°, and had a creamy, lustrous appearance. The colour was lemon-yellow. The molten mass was poured on a stainless steel plate and rapidly cooled by running cold tap water under the plate. The cooled solid mass had a lemon-yellow colour and retained its glassy, lustrous apparance. The cooled solid was left to dry in a desiccator under phospheric oxide (phosphorus pentoxide)

at room temperature for one week, after which it was powdered using mortar and pestle. The powder was sieved through a set of standard sieves on a sieve shaker operated for 5 minutes, and 150/90 μm particles collected. For the 1:10 drug-PEG 6000 fusion, the same procedure as described above was followed. The carrier started to melt at 56° and the final temperature of the molten mixture was 61°. The melt had a lemon-yellow colour, lustrous in appearance, but this lustre disappeared on cooling while the lemon-yellow colour was retained.

The 1:10 nitrofurantoin-mannitol physical mixture was obtained by blending 9 g of mannitol (powdered and sieved to collect 150/90 μm particles) with 1 g of pure nitrofurantoin powder similarly treated. The colour of the final mixture was lemon-yellow.

The 1:10 nitrofurantoin - polythylene glycol 6000 physical mixture was prepared following the procedure used in preparing the nitrofurantoin-mannitol physical mixture given above.

The pure drug powder was sieved and particles in the 150/90 μm size range collected.

4.2 STABILITY STUDIES ON THE SOLID DISPERSIONS USING TLC AND UV SPECTROPHOTOMETRY

Introduction

This investigation aimed at assessing the stability of the prepared solid dispersions was carried out using TLC and UV spectrophotometry.

Some research workers have applied TLC, UV, and/or IR spectra in evaluating the stability of fused mixtures.

Chiou and Riegelman, (1971b) used UV spectra and TLC to test whether a cardiac glycoside (digitoxin) and some steriods solid dispersed in PEG 6000 had decomposed following the fusion process. Results indicated that UV spectra and molar extinction coefficients of the pure and processed drugs were identical within the limits of experimental error. A single spot with the same Rf value was found for both the pure and processed compounds on the TLC plates. However, the sample of the processed digitoxin showed two additional spots, one slightly darker and one lighter than the spots of pure digitoxin. The result probably indicated some decomposition of digitoxin due to the fusion process, although it could not be detected by the UV spectra.

Goldi et al. (1976) have applied TLC in assessing nitrofurantoin, ethotoin and coumarin solid dispersed in PEG 6000 and coprecipitated with PVP 25,000. From results obtained, TLC showed that the drug/PEG 6000

dispersion was resolved into two separate spots of R_f (x 100) values of 52, 73, 3, and 78 for PEG 6000, ethotoin, nitrofurantoin and coumarin respectively which were identical to the pure components. The nitrofurantoin/PVP 25,000 coprecipitate was not resolved into two separate spots as were coumarin/PVP 25,000 and ethotoin/PVP 25,000 coprecipitates. This may be attributed to the existence of complexation between nitrofurantoin and PVP 25,000.

Allen et al, (1977) have used both TLC and UV for assessing the stability of some corticosteroids which had been used in preparation of dispersion systems. Their results indicated that none of the corticosteroids demonstrated any decomposition product after being subjected to the preparation of the dispersion systems. TLC revealed no additional spots and there was good correspondence with the reference samples. UV spectra of the dispersion systems in deionized water revealed no decomposition products, and the absorbance values were unchanged. A light amber discolouration of the melt and of the cooled glass was observed during the dextrose and sucrose glass formation process, indicating a possible slight decomposition of the sugars. The colour change appeared not to have interferred with the fast-release characters of the carriers.

Meshali et al, (1983) have also reported the use of UV spectra and TLC characteristics in evaluating the

stability of trimethoprim in sugar glass dispersions. Their results showed that all preparations of reducing and non-reducing sugars gave the same spectra as pure trimethoprim, and with the same wavelength of maximum absorption.

Procedure

Thin-layer Chromatography (TLC)

A slurry was formed by mixing 25g of silica gel, 5g of calcium sulphate and 60 ml of distilled water in a round bottom flask, and then spread evenly on 10x20mm glass sheets, giving slurry thickness of 250 µm. The plates were left in a level position to dry and the drying time was one hour. The plates were activated by drying in an oven at 100° for one hour, and then stored in a desiccator until required for use. The solvent system of Acetone: Benzene: Glacial Acetic acid mixture
80 20 1% v/v
was placed to cover the bottom of the trough to a depth of about 1cm. The interior of the tank was lined with Whatman filter paper (chromatography grade) to ensure that the solvent completely saturated the internal atmosphere of the trough. Equilibration time of one hour was allowed, for each run. To the plates, spots in aliquots of about 10 µl of 0.1% alcoholic solutions of the pure drug, drug/PEG 6000 and drug/mannitol melts obtained by dilution were applied, 2cm from the lower edge of the plate, (origin). The plates were developed

at room temperature and each run was stopped when the solvent front had moved about 15cm. from the origin. The plates were removed from the trough and allowed to dry.

Detection of spots and Results

Plates containing spots of the drug and its preparations were visualized by a screened UV light at a 366nm. Rf values of pure nitrofurantoin and that in the melts were found to be approximately 0.95. No additional spots were located. These results are shown in figure 4.1.

UV-Visible Spectrophotometric Scanning

A 10µg per ml. aqueous solutions of the pure drug and melts were prepared and samples scanned from 200nm to 450nm on the spectrophotometer.

Results

There were no extra peaks, nor were there significant shifts in the spectra as compared to spectrum of the pure drug. The result is depicted in figure 4.2.

4.3 IN-VITRO DISSOLUTION RATE STUDIES

Introduction

Dissolution rate tests are intended to measure the time required for a given drug in an oral solid dosage form to go into solution under a specified set of conditions. Any such test affords an objective means towards the evaluation of the physiologic availability

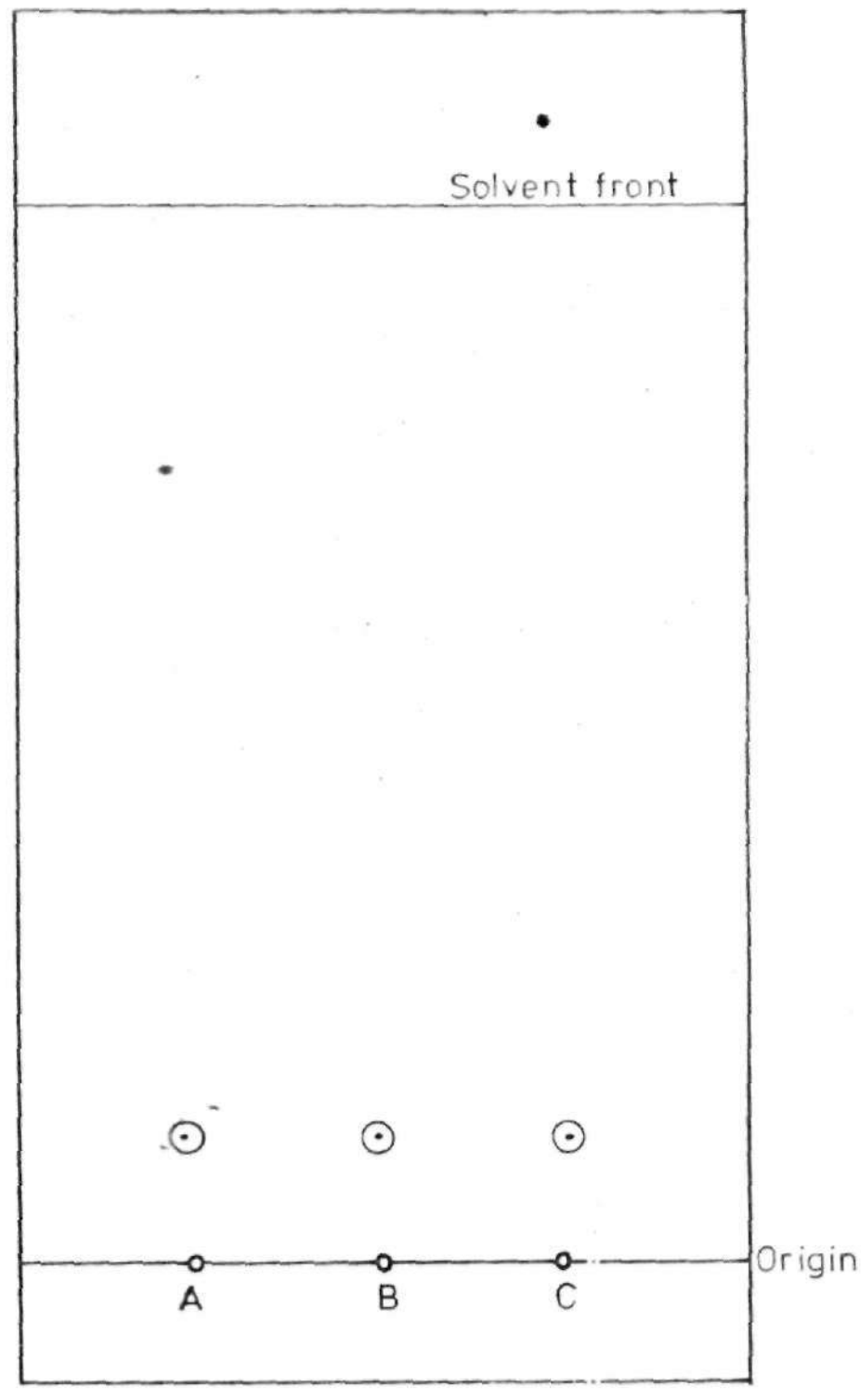


Fig. 4.1 TL Chromatograms

A: Pure drug;
B: drug / PEG 6000 solid dispersion;
C: drug / mannitol solid dispersion.

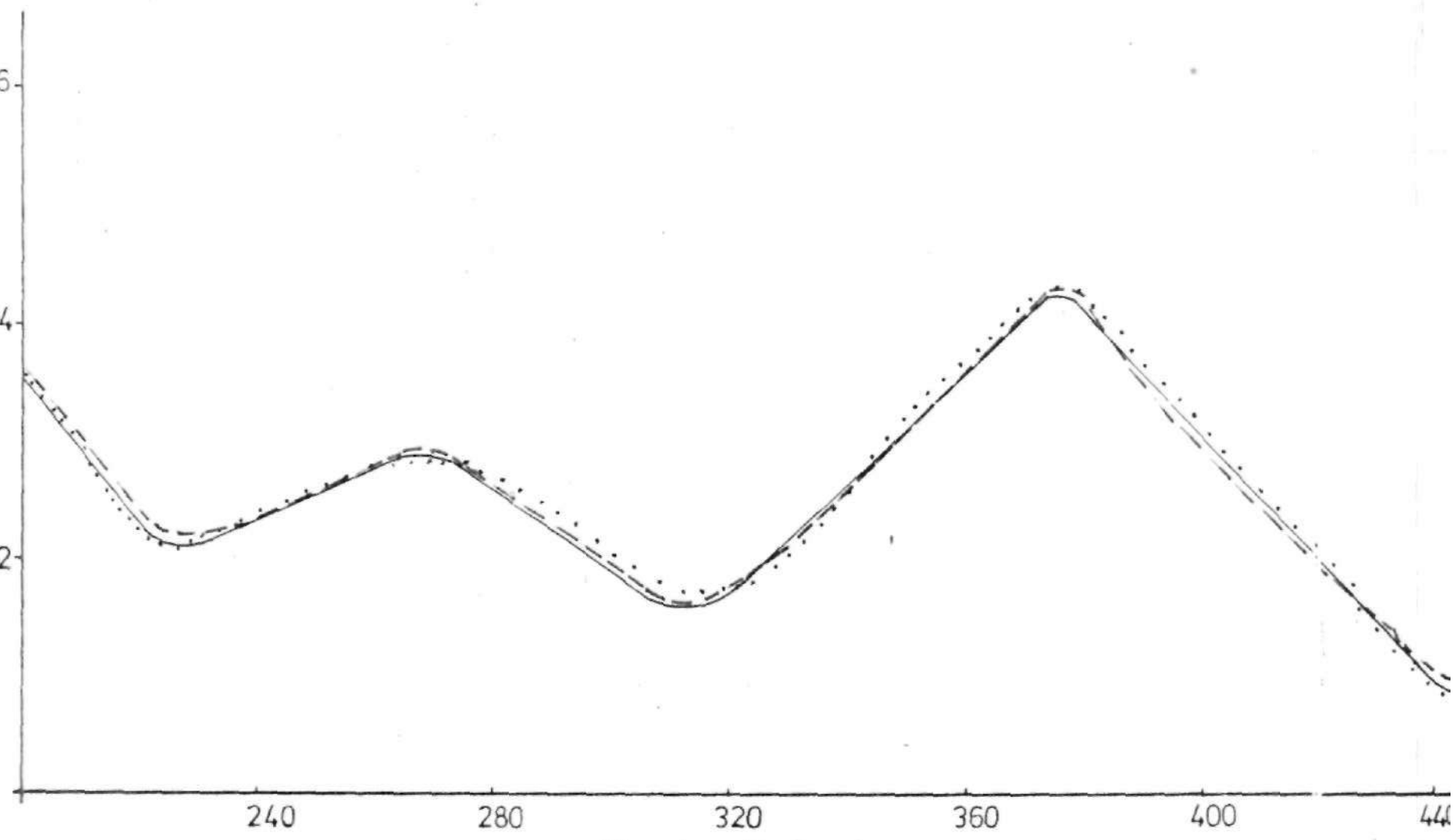


Fig. 4.2 Absorption spectra for pure drug, —— (A); 10% drug - mannitol melt (B); – – – and 10% drug - PEG 6000 melt (C) .....

of the drug under survey since drug absorption and physiologic availability are largely dependent upon having the drug in the dissolved state. Suitable dissolution rates are an important property of a good tablet product. However, it has been emphasized that dissolution rate is not necessarily a measure of safety or therapeutic efficacy, which must be initially established through appropriate in-vivo studies and clinical evaluation though in some cases it has been possible to correleate dissolution rates with physiologic availability of the drug. Dissolution rate testing is done not only on finished dosage forms but can also be used to assess the characteristics of the pure drug or the effects of excipients, formulation, and processing or manufacturing variables. Another application of dissolution rate tests is for in-process quality control to ensure that a given pharmaceutical product is uniform within the same batch and from one batch to another. For some time to come, in-vitro dissolution rate tests are likely to remain the principal means of evaluating the rate of release of an active constituent from a tablet dosage form.

There is at this time a proliferation of dissolution methods and apparatus, so much so that almost every worker in the field may be ascribed one.
The crucial test of a given in-vitro model is its ability to correlate in-vivo conditions. In the design and use of a reliable in-vitro dissolution model, it is essential

that, that model is capable of simulating in-vivo dissolution process.

The USP XX and NF XV dissolution apparatus is described here. Modifications which were made for experimental purposes would be outlined.

The apparatus essentially, consists of a litre glass or other inert material transparent vessel with a slightly concave bottom. It is fitted with a variable speed motor and a cylindrical stainless steel basket. The whole assembly is immersed in a water bath at 37 ± 0.5°. The motor shaft is placed in the centre of the vessel to which the top part of the basket is attached.

The detachable part of the basket is made of welded seam, 375 um stainless steel cloth formed into a cylinder 3.66 cm high and 2.55cm in diameter. The motor is regulated between 25 and 200 revolutions per minute and is maintained at the rate specified in the individual drug monograph within ± 5%. The vessel is immersed in the constant temperature bath and 900 ml of the dissolution medium specified in the individual monograph placed in it.

Exrerimental Modification Adopted

1. The 1 litre glass vessel was used as a holder for an amber-coloured, 600ml bottle (used here to screen the drug from light during the runs), and 400ml of distilled water (dissolution medium) used

2. In place of the cylindrical stainless steel basket (unsuitable for holding powder samples having particle size 150/90 μm) a cellulose cylindrical bag of about the same dimensions (made from Whatman No. 105 lens cleaner) was used, held to the motor shaft by means of a rubber band. The sampling position was determined using the recommended guide by the British Pharmacopoeia 1980, Volume Two (figure 4.3).

Procedure

For each in-vitro dissolution rate test, the quantity of powder containing about 150mg of nitrofurantoin were accurately weighed directly into the cylindrical cellulose bags.

The speed of the motor shaft controlling the stirrer was maintained at 50 rpm, and 400ml of distilled water at $37 \pm 0.5^{\circ}$ used during each run. At 10 minutes intervals, 1ml quantities of dissolution medium were withdrawn and replaced with 1ml of pure distilled water warmed to $37 \pm 0.5^{\circ}$. Each withdrawn 1ml sample was diluted to 25ml with distilled water and the absobance of each sample recorded.

Calculations

$$\text{Concentration of drug in dilute solution} = \frac{\text{absorbance}}{\text{slope of the standard curve}}$$

The concentration of nitrofurantoin in each withdrawn sample is equal to the dilution factor multiplied by the concentration of nitrofurantoin in the dilute solution.

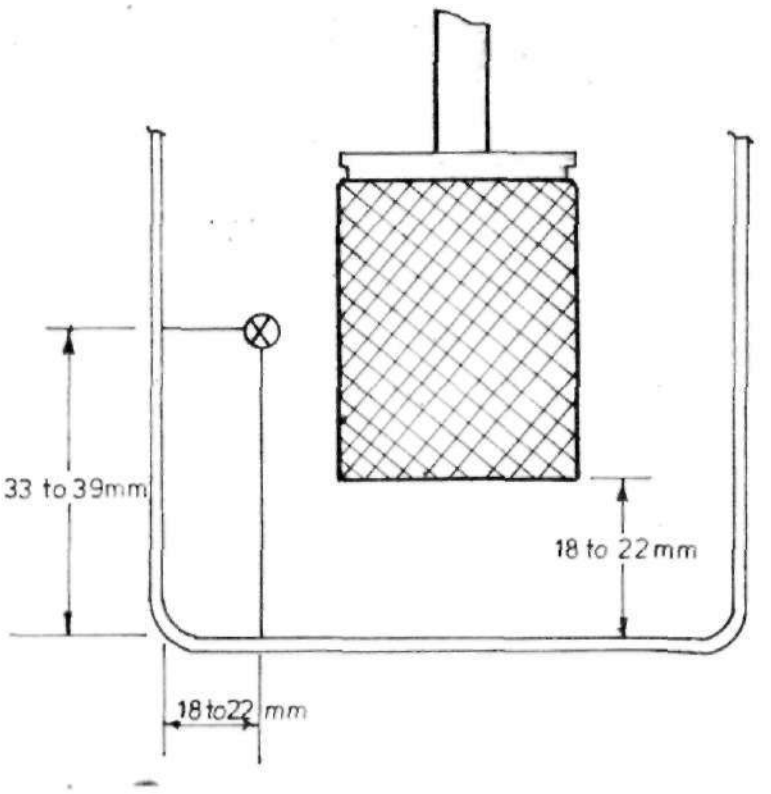


Fig. 4.3 Assembled apparatus for determination of dissolution rate

⊗ = Sampling position

(From British Pharmacopoiea (1980) Volume II, Her Majesty's Stationery Office at the University Press, Cambridge, p A115)

Drug content

Percentage cumulative dissolved drug was calculated from:

$$\% \text{ cumulative drug dissolved} = \frac{\text{cumulative drug dissolved in } \mu g}{\text{drug content in } \mu g} \times \frac{100}{1}$$

The drug content of the various physical mixtures and solid dispersions were determined (table 4.1).

Table 4.1 - Percentage drug content in various drug-carrier combinations.

Drug/carrier type	% Drug content
Drug-mannitol physical mixture,	100.58%
Drug-mannitol melt,	99.86%
Drug/PEG 6000 physical mixture	99.86%
Drug/PEG 6000 melt	99.86%

Table 4.2 - Percentage nitrofurantoin dissolved in water at 37 ± 0.5°

Time (min.)	PERCENTAGE DRUG DISSOLVED				
	A	B	C	D	E
10	12.8	6.7	12.8	6.2	14.4
20	15.3	9.8	15.6	9.3	20.2
30	15.9	12.1	18.0	12.5	26.5
40	17.1	13.6	19.7	15.4	29.3
50	17.4	15.2	20.7	19.4	32.0
60	17.6	16.4	22.8	22.2	37.4
70	18.3	18.2	24.9	24.8	46.3
80	18.5	19.0	26.1	26.2	53.5
90	18.3	20.4	27.6	27.1	59.0

A: Pure drug

B: 10% drug-mannitol physical mixture

C: 10% drug-mannitol solid dispersion

D: 10% drug-PEG 6000 physical mixture

E: 10% drug-PEG 6000 solid dispersion

4.4 Results and discussion

Table 4.2 shows the percentage of nitrofurantoin released from the respective powder samples. Fig. 4.4 graphically represents the time dissolution profiles for the pure drug, physical mixture, and dispersions of the drug with mannitol and PEG 6000. The final results have shown that while melts of the drug with PEG 6000 have dissolved more than 3 times as the pure drug, those of mannitol was only, one and half times better released than the pure drug. The release of the drug from the physical mixture with PEG 6000, was only about half the total released from the fusion with the same carrier. On the basis of better release of drug from the PEG 6000 melt compared to the mannitol melt, it was decided that various drug: PEG 6000 ratios be prepared and compared to determine the optimum drug-carrier combination.

5, 10, 20, 30, 40, and 50% nitrofurantoin solid dispersions were prepared by the melt method as described earlier. During the preparation, the following observations were made (Tables 4.3 and 4.4).

Table 4.3 - Physical characteristics of the freshly molten drug- PEG 6000 solid dispersions

Amount of drug (%)	Temperature (°C)	Colour	Appearance
5	61	lemon yellow	lustrous and glassy
10	61	" "	" "
20	76	" "	" "
30	76	" "	Viscous, lustrous and glassy
40	84	Deep lemon yellow	Highly viscous, slightly glassy
50	84	"	semisolid, slightly glassy.

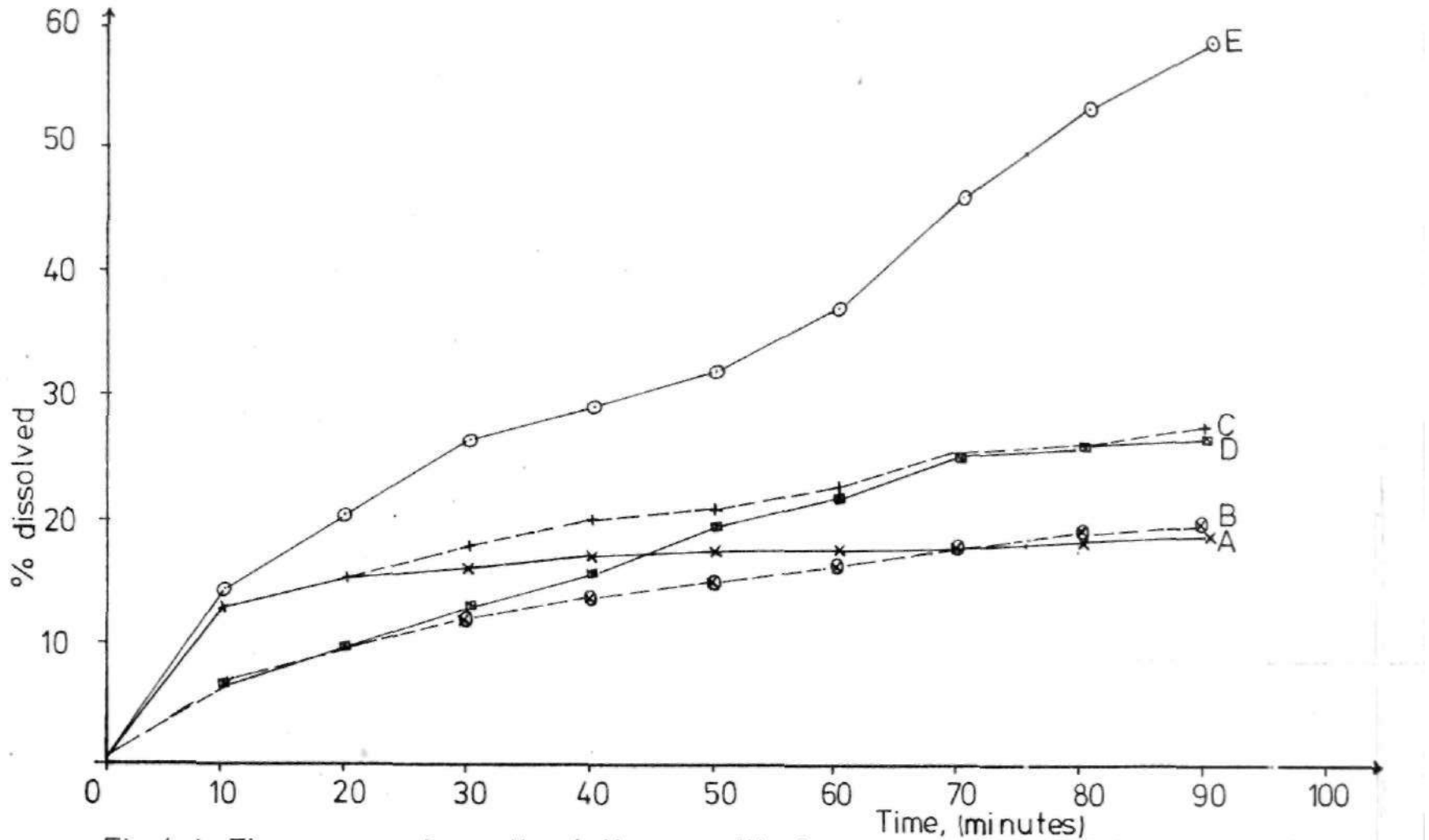


Fig. 4.4 Time - percentage dissolution profile for pure drug; 10 % drug-mannitol mixture; 10% drug-mannitol melt; 10 % drug - PEG 6000 mixture and 10 % drug - PEG 6000 melt. A —x—x—x pure drug B ---o---o---o drug - mannitol mixture; C ---+---+---+ drug - PEG 6000 mixture; D ■——■——■ drug-mannitol melt; E —o—o— drug - PEG 6000 melt.

Table 4.4 - Physical characteristics of drug - PEG 6000 cooled solid dispersions.

Amount of drug (%)	Colour	Appearance
5	Lemon yellow	Non-glassy and pale
10	"	" " " "
20	"	" " " "
30	"	" " " "
40	Deep	" " " " more pale
50	Deep	" " " " " "

Drug content determinations done on each of the solid dispersions showed that nitrofurantoin content were as shown in table 4.5.

Dissolution rate studies readings obtained from the various runs for the 5, 10, 20, 30, 40 and 50% drug/PEG 6000 solid dispersions are presented in tables 4.6 and 4.7; while figure 4.5 is a graphical representation.

The drug in the solid dispersions appears not to have undergone any chemical change. Evidence of thermal degradation has not been found. Results of both TLC and UV spectrophotometric evaluations have confirmed the stability of the drug in the fusion process.

Table 4.5 - Drug content of various drug/PEG 6000 solid dispersions.

Amount of drug (% w/w)	% drug content
5	99.86
10	99.86
20	99.14
30	99.00
40	99.80
50	100.58

The validity of these evaluations is attested to by various reports. (Allen et al., 1977; Meshali et al., 1983; Geneidi et al., 1976; Chiou and Riegelman, 1971b; Corrigan and Timoney, 1976). Results of TLC and UV spectrophotometric assay were further confirmed by results of the drug content determination in the drug-carrier physical mixtures and solid dispersions.

Results of the in-vitro dissolution tests between mannitol and PEG 6000 revealed that PEG 6000 is a better carrier than mannitol. Hence the selection of PEG 6000 for use as carrier in varied drug-carrier proportions of 5, 10, 20, 30, 40 and 50%.

Table 4.6 Dissolution of drug in water from various solid dispersions at 37° (1)

Time (min.)	Cumulative percentage dissolved		
	A	B	C
10	20.2	14.4	18.8
20	25.5	20.2	23.2
30	29.5	26.5	27.7
40	33.0	29.3	31.6
50	36.5	32.0	36.6
60	38.8	37.4	40.3
70	47.2	46.3	43.0
80	54.5	53.5	48.2
90	59.5	59.0	53.2

A: 5% drug/PEG 6000, B: 10% drug/PEG 6000,
C: 20% drug/PEG 6000

The result of the initial dissolution rate, indicate that there was merit in the preparation of drug/carrier solid dispersion as against simple physical mixing. This has confirmed similar indications observed by various workers of the effect of solid dispersion on drug solubility, dissolution and absorption characteristics (Goldberg et al., 1965; Chiou and Riegelman, 1969; Chiou and Niazi, 1971; Corrigan and Timoney, 1976; Geneidi et al., 1980;

Table 4.7 Dissolution of drug in water from various solid dispersions at 37° (II)

Time (min.)	Cumulative percentage dissolved		
	A	B	C
10	18.9	15.7	11.7
20	22.5	16.6	14.7
30	25.6	24.9	20.1
40	29.2	24.9	20.1
50	33.4	29.2	23.1
60	37.9	33.0	26.0
70	42.3	35.7	29.6
80	47.5	39.4	33.1
90	50.4	42.5	36.8

A: 30% drug/PEG 6000, B: 40% drug/PEG 6000, C: 50% drug/PEG 6000

Bloch et al., 1982, Deshpande and Agrawal, 1982; Mesholi et al., 1983;) to mention a few. The fact that the physical mixtures of the drug with various carriers have displayed better dissolution than the pure drug is suggestive of the effect of local solubilization on the solubility of a drug substance (Goldberg et al., 1966c)

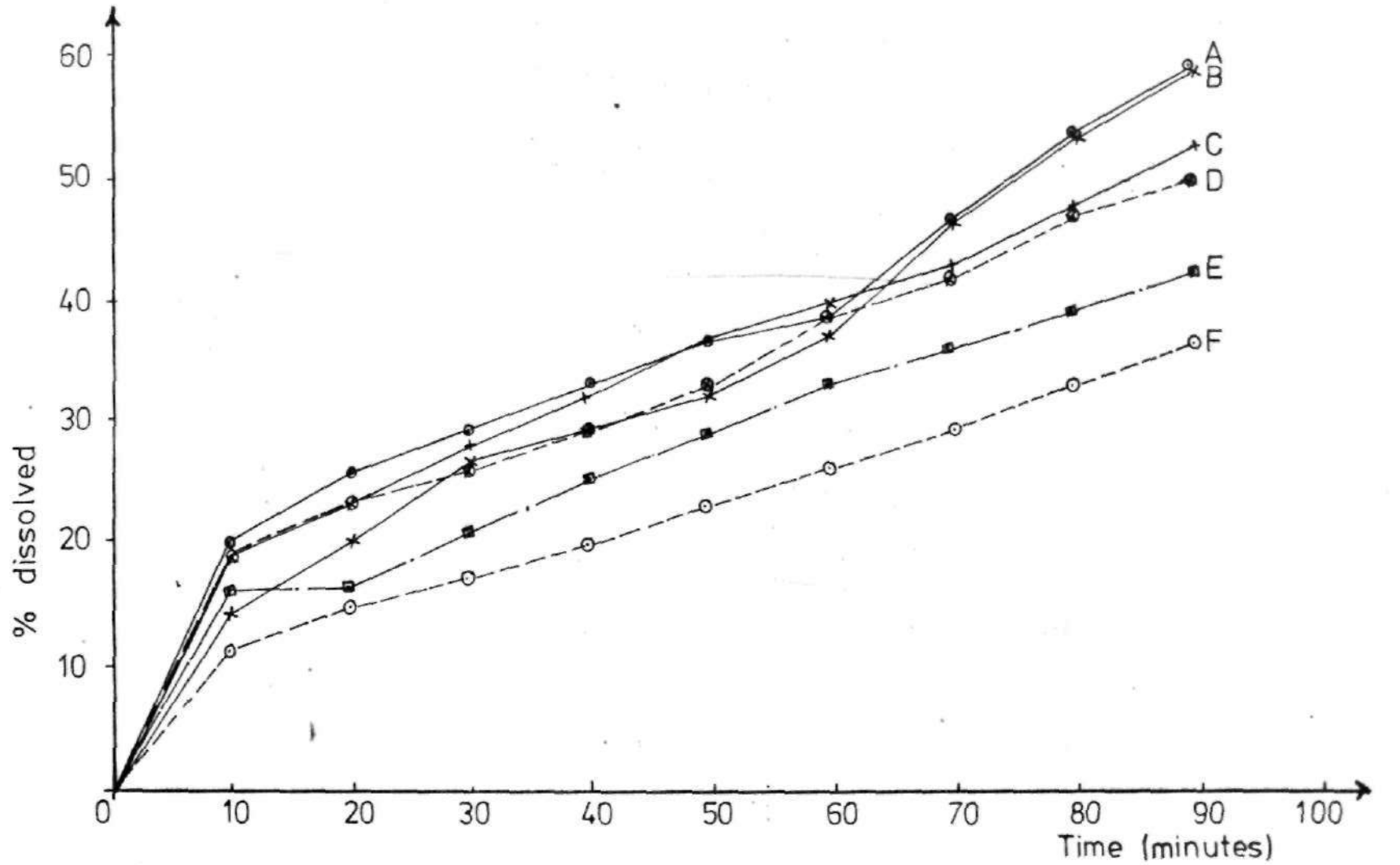


Fig. 4.5 Time - percentage dissolution profile for 5, 10, 20, 30, 40, and 50 % nitrofurantoin solid dispersion in PEG 6000.

A ⊙—⊙—⊙— 5 % drug in carrier; B —×—×— 10 % drug in carrier;
C —|—|— 20 % drug in carrier; D - - -⊗- - -⊗- - -⊗- - - 30 % drug in carrier;
E —·—▫—·—▫ 40 % drug in carrier; F -⊙----⊙----⊙---- 50 % drug in carrier

It is possible that this local solubilization plays a role in enhancing drug dissolution from the solid dispersions along with the mechanism of particle size reduction and other factors (such as the formation of rapidly soluble alpha solid solution (Goldberg et al., 1966c).

The comparative study on solid dispersion with varying drug-carrier proportions has indicated that the drug: carrier molecular weight ratio of approximately 240:6000 (i.e 4% drug in carrier) would probably produce the greatest dissolution enhancement for nitrofurantoin/PEG 6000 solid dispersion. In fact, the tendency has been towards this, with the 5% drug-carrier solid dispersion having a release percentage of 59.5 and the 50% proportion having only a release percentage of 36.8. In view of the bulk of the resultant tablet, if a 5% drug in PEG 6000 combination were used, the 30% drug/PEG 6000 solid dispersion was selected for formulation studies. This was in a bid to reach a compromise and still be able to produce an optimal tablet formula. It is expected that with this formula, an excessively bulky tablet would not be produced, and the tablet would have an optimum total release as well as rate of release of drug content.

For these sets of comparative in-vitro dissolution studies, the dissolution medium has been glass distilled water. Though, this is not the dissolution

medium mentioned in the monograph for the drug, it was selected for (i) its simplicity; (ii) to study the effect of not simulating any one given gastric fluid; and (iii) in the light of allegation of inconsistencies in formulae underlying official USP dissolution rate specifications for nitrofurantoin (Bates et al., 1973).

CHAPTER FIVE

FORMULATION STUDIES AND EVALUATION

5.1 FORMULATION AND PRODUCTION OF NITROFURANTOIN TABLETS

Introduction

This part of the study was undertaken to investigate the effect of (i) molecular drug dispersion and (ii) change in excipient on overall drug product quality. Tablets were formulated and produced based on the working formula in table 5.1.

Table 5.1 Composition of prepared tablets of 50mg nitrofurantoin.

Constituents	%	Quantities Used per tablet (mg)	per 200 tablets (g)
Drug	20.0	50.0	10.0
Diluent (carrier)	46.6	116.5	23.3
Disintegrant	33.0	82.5	16.5
Lubricant	0.2	0.5	0.1
Glidant	0.2	0.5	0.1
	100.0	250.0	50.0

Three different tablet formulae were produced by using different diluents in the same proportion thus: In formulations A, B, and C, the diluents are Avicel PH 101, PEG 6000 (as a simple mixture), and PEG 6000 (in which the drug had been dispersed as a 30% solid dispersion) respectively.

Though no particular conventional or commercial production formula was used, the objective was to produce high quality tablets in conformity with current quality control standards and suitable for comparative bioavailability studies, with no intention however of making absolute comparison with existing commercial nitrofurantoin tablets.

Procedure

The disintegrant, starch granules, (selected after a preliminary study) was prepared from maize starch powder and 10% starch mucilage.
kneading of the starch powder with the mucilage was done and the wet mass was granulated through 1.0μ sieve then dried overnight at room temperature.
The dry granulation was done using a 0.8μ sieve, and the granules packed in a clean, dry bottle from which required amounts were taken from time to time.
The exact constituents and quantities used in formulations A, B, and C are given in table 5.2.

Table 5.2 Constituents and quantities used in different nitrofurantoin tablet formulations

	QUANTITIES USED IN FORMULATION		
CONSTITUENT	A/mg	B/mg	C/mg
Nitrofurantoin	50.0	50.0	50.0
Polyethylene glycol 6000	-	116.5	116.5
Avicel PH 101	116.5	-	-
Starch granules	82.5	82.5	82.5
Stearic Acid	0.5	0.5	0.5
Talc	0.5	0.5	0.5
	250.0	250.0	250.0

A: 30% drug/Avicel (physical mixture)
B: 30% drug/PEG 6000 (physical mixture)
C: 30% drug/PEG 6000 (solid dispersion).

The technical differences in the manufacturing processing of the different formulations now follow:

Formulation A.

A homogeneous blend of the required quantity of the drug and Avicel was obtained by mixing in a cube mixer for 10 minutes, at the end of which, the starch granules stearic acid and talc were added and mixed for 10 minutes.

The resulting granules were compressed using 10mm dies and flat punches, producing flat tablets with bevelled edges weighing 250mg each prepared using a

single punch tabletting machine and a constant force of about 7kg wt.

Formulation B.

The PEG 6000 powder was blended with the drug powder in the cube mixer run for 10 minutes. The required amounts of starch granules, stearic acid, and tale were added to the mixture and further blended bv running the mixer for another 10 minutes. The compression process was same as for formula A.

Formulation C.

The required quantity of the drug-PEG 6000 solid dispersion powder was weighed and blended with starch granules, tale and stearic acid in the cube mixer for 10 minutes. The granules were compressed into tablets using 10 mm. dies and flat punches to produce flat tablets with bevelled edges.

5.2 TABLET EVALUATION AND TESTING

(a) ORGANOLEPTIC PROPERTIES

The result of the organoleptic examination of tablet diameter, thickness, colour, shape and presence or absence of coating is presented in table 5.3.

(b) WEIGHT VARIATION

Twenty tablets were individually weighed and the mean weight also recorded. Table 5.4 shows the individual tablet weights.

Table 5.3 Organoleptic properties of nitrofurantoin tablets.

	FORMULATION		
Properties evaluated	A	B	C
Tablet diameter (mm)	10.5	10.5	10.5
" thickness	2.70	2.71	2.71
" colour	lemon yellow	lemon yellow	lemon yellow
" shape	flat, bevelled edge	flat bevelled edge	flat bevelled edge
" coating	None	None	None

A: 30% drug/Avicel(physical mixture) B; 30% drug/PEG 6000 (physical mixture)
C: 30% drug/PEG 6000 (solid dispersion).

Conclusion

According to USP XVIII, for tablets having average weight between 130mg and 324mg, the official deviation in percentage about the average is ± 7.5 using 20 tablets of which not more than 2 tablets may deviate for the batch to be satisfactory.

Since no batch contained tablets which deviated by more than 4.8% (formulation B), the three batches were satisfactory in respect of uniformity of weight.

(c) HARDNESS

The hardness of the tablets in the three batches were assessed using the Monsanto tablet hardness tester. For each batch, 3 different sets of five

Table 5.4 Tablet weight variation

FORMULATION	A		B		C	
Tab. No.	Tablet weight (mg)	% Variation from mean	Tablet weight (mg)	% Variation from mean	Tablet weight (mg)	% Variation from mean
1	256	+1.6	256	+1.6	256	+1.3
2	252	-	250	-0.8	256	+1.3
3	257	+2.0	252	-0.42	250	-1.1
4	248	-1.6	240	-4.8	250	-1.1
5	251	-0.3	254	+0.77	252	-0.3
6	249	-1.2	252	-0.02	255	+0.9
7	257	+2.0	250	-0.8	248	-1.9
8	252	-	255	+1.2	252	-0.3
9	253	+0.4	255	+1.2	253	+0.1
10	251	-0.4	257	+2.0	256	+1.3
11	253	+0.4	253	+0.4	257	+1.7
12	246	-2.4	257	+2.0	249	-1.5
13	249	-1.2	251	-0.42	248	-1.9
14	252	-	255	+1.2	255	+0.9
15	257	+2.0	255	+1.2	249	-1.5
16	251	-0.4	249	-1.2	254	+0.5
17	248	-1.6	245	-2.8	257	+1.7
18	253	+0.4	260	+3.2	249	-1.5
19	255	+1.2	240	-4.8	250	-1.1
20	250	-0.8	255	+1.2	260	+2.9
Mean Weight (mg)	252.00		252.05		252.80	

A: 50% drug/Avicel (physical mixture),
B: 30% drug /Avicel (physical mixture),
C: 30% drug/PEG 6000 (solid dispersion).

Tablets per set were examined. The result of the test is shown in table 5.5.

(d) FRIABILITY

The tests were carried out using ERWEKA friabilator. 20 tablets per batch were brushed lightly to remove surface powder and weighed accurately. The tablets were allowed to drop from a distance of 6 inches in a drum rotating at speed of 25 r.p.m, operated for 4 minutes, after which the tablets were dusted and again weighed.
Tablet friability in percentage is given by:

$$\frac{\text{Change in weight of 20 tablets}}{\text{Original weight of 20 tablets}} \times \frac{100}{1}$$

By official standards, a test is deemed satisfactory if weight loss is less than 0.8% (Gunsel and Kanig, 1976). A summary of the results obtained is presented in table 5.6 and it shows that the three tablet batches satisfied the official requirements in respect of friability.

(e) UNIFORMITY OF CONTENT

Procedure

10 tablets per batch were individually tested. Each tablet was powdered and 25mg portions weighed accurately and dissolved in water and the volume made up to 250ml.
5ml of the aqueous solution was diluted to 50ml and the absorbance measured at 373nm.
The result of assay is shown in table 5.7.

Table 5.5 Hardness test result for tablets

Tablet Number	HARDNESS RECORDED (kg. wt.)		
Formulation	A	B	C
1	4.6	5.4	5.6
2	4.0	4.8	4.6
3	5.4	4.8	4.8
4	5.0	5.2	5.0
5	5.4	4.4	4.6
MEAN	4.88	4.92	4.92

The tablets contain

A: 30% drug /Avicel (physical mixture)

B: 30% drug/PEG 6000 (physical mixture)

C: 30% drug/PEG 6000 (solid dispersion)

Table 5.6 Friability test results of formulated tablets.

Formulation	A	B	C
Original Weight (g)	5.03	5.07	5.09
Change in Weight (g)	0.01	0.01	0.01
Friability (%)	0.10	0.21	0.21

A: 50% drug/Avicel (physical mixture)

B: 50% drug/PEG 6000 (physical mixture)

C: 50% drug/PEG 6000 (solid dispersion).

Table 5.7 Content uniformity for nitrofurantoin tablets.

	Formulation					
	A		B		C	
Tablet	Drug Content (mg)	% diff	Drug Content (mg)	% diff	Drug Content (mg)	% diff.
1	50.30	+0.6	50.30	+0.6	49.58	-0.8
2	50.30	+0.6	50.30	+0.6	49.58	-0.8
3	50.30	+0.6	49.93	-0.1	49.93	-0.1
4	49.58	-0.8	49.58	-0.8	49.58	-0.8
5	49.93	-0.1	49.93	-0.1	4958	-0.8
6	49.93	-0.1	50.30	+0.6	49.92	-0.1
7	49.58	-0.8	50.30	+0.6	49.58	-0.8
8	50.30	+0.6	49.93	-0.1	49.93	-0.1
9	50.30	+0.6	49.58	-0.8	50.30	+0.6
10	49.58	-0.8	49.93	-0.1	50.30	+0.6

A: 30% drug/Avicel (physical mixture);

B: 30% drug/PEG 6000 (physical mixture),

C: 30% drug/PEG 6000 (solid dispersion).

Conclusion

The official position on this tablet property is that if the individual monograph required testing for content uniformity, 10 tablets should be assayed and of the total, 9 must assay within ± 15% of the declared potency and none may exceed ± 25%. That if not more than 2 exceed the 15% limit, 20 tablets of the 30 representative tablets originally selected are also assayed. None of these may fall outside the limits. This is according to the USP XVIII, while according to the NF XIII only one of the 30 tablets is permitted to fail the test.

The tablets in the 3 batches passed the test for content uniformity, since none of the tablets assayed was outside the ± 15% limit.

(f) DISINTEGRATION TIME

The time taken for 6 tablets per batch to disintegrate in water which had a constant temperature of 37 ± 0.5° was recorded as shown in table 5.8.

(g) DISSOLUTION RATE

One tablet per run was used in this test. The basket method was used, with each tablet contained in the basket, and the motor shaft rotating at a speed of 100 r.p.m.

The volume of dissolution medium (distilled water) was 400 ml maintained at 37 ± 0.5°
At every run, 5ml of solution was withdrawn, filtered using millipore filter HA 0.45µ, white grid, 25mm, and 2ml of the filtrate diluted to 25ml and the absorbance measured, 373nm, The recorded absorbances are the mean of 3 runs.
The absorbances and calculated cumulative drug concentrations percentages are presented in a tabular from in table 5.9 and graphically in figure 5.1.

Table 5.8 Tablet disintegration times.

FORMULATION	DISINTEGRATION TIME /Minutes/
A	0.5
B	10.0
C	10.0

A: 30% drug/Avicel (physical mixture),
B: 30% drug/PEG 6000 (physical mixture)
C: 30% drug/PEG 6000 (solid dispersion).

Table 5.9 Dissolution of nitrofurantoin in water from various tablet formulations at 37°

Formulation	A	B	C
Time (min.)	% Dissol.	% Dissol.	% Dissol.
10	55.8	36.5	33.3
20	65.4	58.6	62.6
30	68.2	66.7	81.4
40	71.6	68.4	93.2
50	74.2	77.0	93.2
60	76.7	81.0	96.2
70	78.4	84.8	98.2
80	79.6	86.3	100.0
90	81.6	81.7	100.0
100	83.2	89.0	99.7
110	84.7	89.3	99.7
120	85.8	90.2	99.1

A: 30% drug/Avicel (Physical mixture)

B: 30% drug/PEG 6000 (Physical mixture)

C: 30% drug/PEG 6000 (solid dispersion).

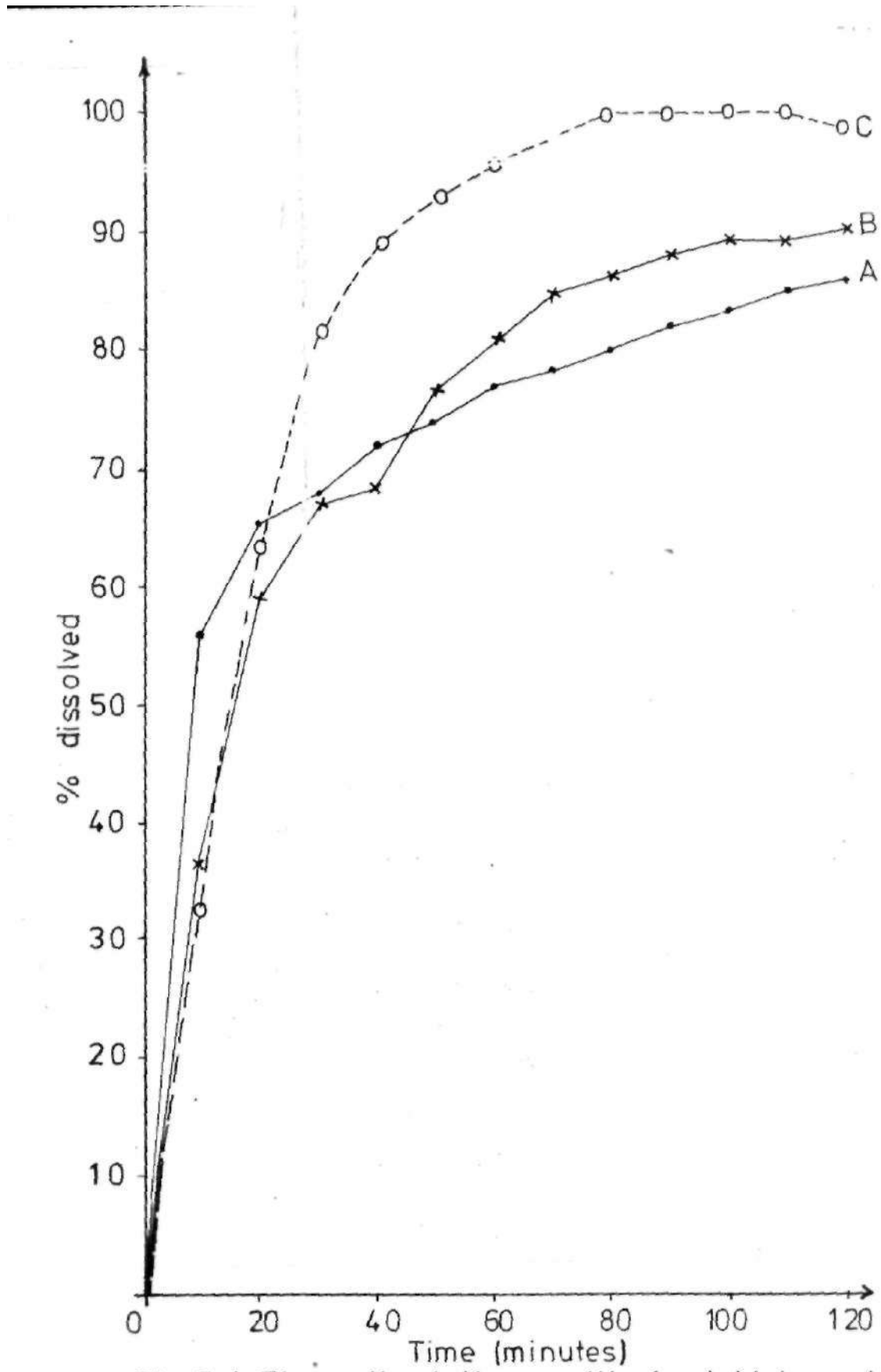


Fig. 5.1 Time-dissolution profile for tablets made from drug-Avicel physical mixture (A); drug-PEG 6000 physical mixture (B); and drug-PEG 6000 solid dispersion (C).

5.3 RESULTS AND DISCUSSION

For the three tablet formulations produced, the results of the quality control tests have attested to their compliance with official standards. The tablets were produced by direct compression. Of the three formulations, that produced using Avicel consistantly exhibited minor deviation in respect of mean weight, hardness, friability, but considerable deviations in respect of time disintegration and dissolution. This is indicative of one fact that any in-built tablet formula deviation tends to be transmitted to affect the general characteristics of that product. This supports the current thinking of industrial pharmacists that the quality of a given formulation has to be built into it (hence the call for in-process quality control tests) as against emphasis on the quality control testing of the finished product. With the results of the quality assurance tests, the three tablet formulations would now be subjected to further in-vivo absorption comparative studies. Meanwhile results of the in-vitro dissolution study established the superiority of formulation C compared to the formulation made from drug/carrier physical mixtures. This greater drug dissolution from formulation C may have been due to any one or a combination of these mechanisms.

(1) local solubilization

(2) increased drug wettability

(3) particle size reduction via the formation of either rapidly soluble solid solution or eutectic mixture.

It is hoped that a definite mechanism or pathway by which this observed increased dissolution rate was brought about would soon be investigated and resolved.

CHAPTER SIX

BIOAVAILABILITY STUDIES

6.1 INTRODUCTION

In chapter one certain basic and general principles pertaining to drug bioavailability were discussed. In this project, in-vitro dissolution and in-vivo absorption correlation in nitrofurantoin tablets have been investigated. An in-vivo study has been carried out to evaluate the effects of (i) solid dispersion (formulation C versus formulationB) and (ii) change in formulation constituents by replacing the inert excipient, PEG 6000 with another one, Avicel PH 101 (formulation A versus formulation B) on the dissolution and absorption characteristics of nitrofurantoin.

Nitrofurantoin is not a new drug, but since the formulations in the project are new ones, the need arose to fulfil compendial regulation that bioavailability must be considered. (The Pharmaceutical Codex, 1979). Previous workers have utilized different methods for the quantitative determination of the drug in biological samples. Some of these workers and the result of their work have been discussed in chapter two. Others who have done some work in this area would now be briefly introduced.

Buzard et al., (1956) developed a simple colorimetric method for the determination of nitrofurantoin and two

other nitrofurans in plasma.

Conklin and Hollified, (1965) introduced the nitromethane-hyamine procedure for the determination of nitrofurantoin in urine. The procedure was modified for assay of the drug in whole blood or plasma by the same workers in 1966. The modified procedure has a sensitivity of 2 µg per millilitre and has been reported to be specific for nitrofurantoin, but inadequate for quantitating nitrofurantoin blood levels in subjects who received normal therapeutic doses.

Mattok et al, (1970) introduced the improved nitromethane-hyamine method for use with blood samples. In this method, smaller blood volumes (0.8ml) were required and the sensitivity was 0.2µg per millilitre.

Conklin and Hollifield, (1970) proposed a modified nitromethane-hyamine method for the estimation of nitrofurantoin in urine, in the presence of phenazopyridine and its metabolities.

In most of these methods, studies in humans were carried out on normal human subjects.

After a survey of the literature, a search for reagents, chemicals and equipments was carried out. On the basis of available reagents, chemicals, and

equipments, the Buzard et al., (1956) colorimetric determination was standardized. After establishing its reproducibility, it was adopted for use. The original authors had recorded that the reaction involved, was specific for nitrofurans and had a recovery of 100% (nitrofurazone), 92% (nitrofurantoin), and 97% (furazolidone) respectively.

The method is based on the formation and colorimetric estimation of 5-nitro-2-furfuraldehyde phenylhydrazone.

6.2 DESIGN AND PROCEDURE

6.2.1 Absorption spectrum for 5-nitro-2-furfuraldehyde phenylhydrazone

Procedure

To determine the wavelength of maximum absorbance, of 5-nitro-2-furfuraldehyde phenylhydrazone in toluene, a 6µg per millilitre aqueous solution of nitrofurantoin (obtained by dissolving 50mg of pure nitrofurantoin in 50ml of N, N-dimethylformamide and serially diluting to give a 20µg per ml solution of which 1.5 ml was made up to 3ml with water). It was derivatised by adding 1ml each of 1.5% phenylhydrazine hydrochloride and 5N hydrochloric acid, in centrifuge tubes. The tubes were mixed by shaking and heated for 25 minutes at 70°. After cooling the tubes for 5 minutes in cold tap water, extraction was done by adding 5ml of toluene to each tube, and shaken vigorously for 25 to 30 times and then centrifuged. The absorbance of the clear toluene layer

was scanned on the spectrophotometer from 370nm to 470nm, with pure toluene as blank.

Results

The absorbances obtained are presented in tabular form (table 6.1) and graphically as absorption spectra (fig. 6.1). A λ max of 426nm was recorded.

Table 6.1 Absorption spectrum for 5-nitro-2-furfuraldehyde phenylhydrazone in toluene.

Wavelength (nm)	Absorbance	Wavelength (nm)	Absorbance
370	0.080	425	0.360
375	0.097	430	0.354
380	0.120	435	0.334
385	0.140	440	0.230
390	0.168	445	0.267
395	0.198	450	0.233
400	0.213	455	0.194
405	0.243	460	0.160
410	0.270	465	0.140
415	0.307	470	0.124
420	0.340		

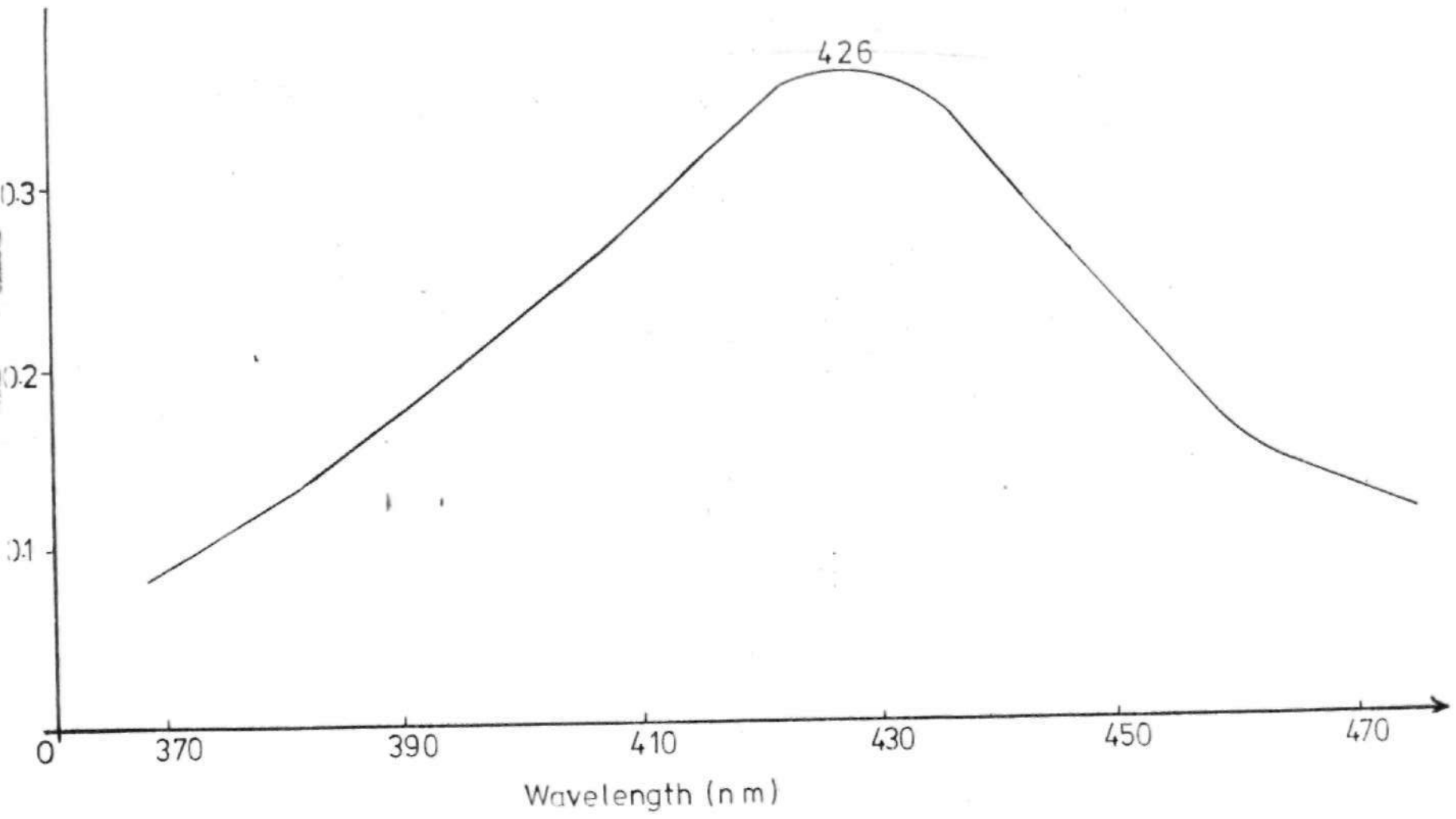


Fig. 6.1. Absorption spectrum for 5-nitro-2-furfuraldehyde phenylhydrazone in toluene

6.2.2 LAMBERT-BEER'S PLOT FOR 5-NITRO-2-FURFURALDEHYDE PHENYLHYDRAZONE IN TOLUENE

Introduction

A Lambert-Beer's Plot was constructed on pure nitrofurantoin, at the wavelength of maximum absorption (λ max., 426nm),

Procedure

50mg of pure nitrofurantoin was dissolved in 50ml of N, N-dimethylformamide the solution was serially diluted with water to obtain a 20 µg per millilitre solution. 0.25, 0.5, 1.0, 1.5, and 2.0ml of the 20 µg/ml solution were placed in 5 separate centrifuge tubes and each tube made up to 3ml with water. The derivatisation and extraction procedure outlined in 6.2.1 was followed. The clear toluene layer was analysed at 426nm, using toluene as blank.

Results

The results of the analysis are presented in table 6.2 and figure 6.2. The method had a sensitivity of 0.4 µg per ml. and linearity up to 1.2µg per ml in the Lambert-Beer's plot. The slope of the graph was 0.31.

Table 6.2 Absorbances of 5-nitro-2-furfuraldehyde phenylhydrazone solutions in toluene at 426nm.

Concentration of toluene extract (µg per ml)	Absorbances* (426nm)
0.2	0.058
0.4	0.126
0.8	0.254
1.2	0.374
1.6	0.480

6.2.3 DRUG RECOVERY FROM WHOLE BLOOD

Introduction

To determine the level of drug recovery from blood specimen, the same volumes of the 20µg per ml standard solution of nitrofurantoin in water as those used for plotting the Lambert-Beer's plot, were again extracted after derivatization in the presence of blood.

Procedure

0.25, 0.5, 1.0, 1.5, and 2.0 ml of the 20µg per ml aqueous solution of nitrofurantoin were diluted to 3 ml each using human whole blood. The derivatization, extraction and estimation at 426 nm were same as described in 6.2.1.

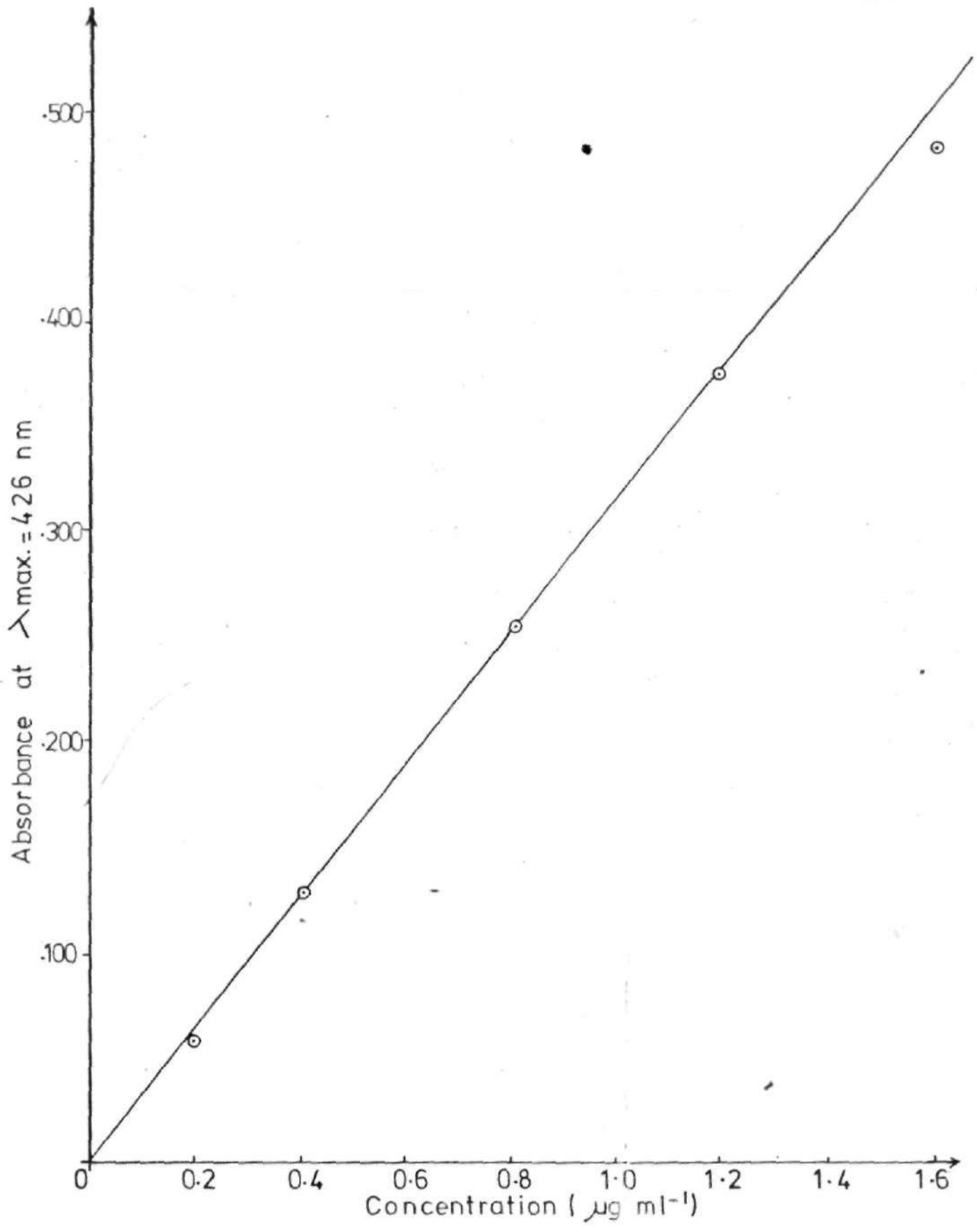


Fig. 6 . 2. Lambert - Beer's plot for 5 - nitro - 2 - furfuraldehyde phenylhydrazone in toluene

Table 6.3 Recovery of nitrofurantoin (as 5-nitro-2-furfuraldehyde phenylhydrazone) from whole blood.

Absorbance (426nm)	Drug quantity added (µg/ml)	Drug quantity found (µg/ml)	Recovery (%)
0.053	0.2	0.17	85.0
0.105	0.4	0.34	85.0
0.217	0.8	0.70	87.5
0.322	1.2	1.04	86.7
0.431	1.6	1.39	86.9

Results and discussion

An extraction efficiency of approximately 86% from whole blood of 5-nitro-2-furfuraldehyde phenylhydrazone has been recorded in this study. This implies that for realistic estimation of plasma drug concentration, a correction factor of 1.163 (i.e whatever plasma concentration is recorded, each determination must be multiplied by the correction factor) must be applied. This is necessary because this set of estimations, the Lambert-Beer's plot was constructed with values obtained from extraction of nitrofurantoin from water and extraction from aqueous solutions in the presence of blood have not been exactly equivalent.

6.2.4 IN-VIVO ABSORPTION STUDIES OF NITROFURANTOIN IN HUMAN VOLUNTEERS

Introduction

A total of three healthy human volunteers (adult; males; ages 21 - 30; average height, 1.72 metres; average weight, 64 kg) took part in the study. The volunteers were to be screened to exclude the following defects:

i) renal impairment

ii) liver damage

iii) glucose-6-phosphate dehydrogenase (G6PD) deficiency

iv) history of allergy to nitrofurantoin or structurally related compounds. But for reasons of non availability of materials and facilities, the screening for liver damage and G6PD deficiency were not carried out. However, medical advice was sought and assurances were given that if any adverse reaction is detected, in any volunteer, dosing would be stopped immediately.

Each volunteer was orally given a single dose of 100mg nitrofurantoin, on the day of the test, with 250ml of drinking water. Only one day was devoted to the study every week. A washout period of about 7 days was allowed between the weekly drug administration. Food intake was restricted during the entire test period of about 4 hours. Caffeine, alcohol and smoking were prohibited in all subjects during the study. The day preceding the day of

test, each volunteer had supper followed by at least eight hours of overnight fast (i.e no snacks or breakfast were allowed)

Procedure

On the day of each test, prior to oral ingestion of the 2x50mg tablets, approximately 5ml of blood specimen were collected by venipuncture. The specimen were placed in sample bottles containing sequestrene (anticoagulant). Further blood collections were made from each volunteer at 0.5, 1, 2, and 4 hours after the administration of the drug. 250ml of water was administered to each volunteer every hour during the study. The volunteers were allowed food ad. lib. after the last specimen of blood (approximately 5ml each sampling) had been collected. The latin-square cross-over design (table 6.4) was used for the study.

Table 6.4 Latin square balanced cross over design used for the in-vivo absorption study,

Volunteer	1	2	3
Product taken (week one)	A	B	C
(week two)	C	A	B
(week three)	B	C	A

Analysis

The blood specimens collected were immediately labelled on collection. The samples were not pooled but assayed separately.

The blood samples were initially centrifuged to separate the components from plasma. For each estimation, 3ml of plasma was placed in 15 ml centrifuge tubes. Derivatisation was done byadding 1ml each of 1.5% phenylhydrazine hydrochloride(freshly prepared) and 5N hydrochloric acid. The tubes were mixed by shaking and heated at 70^{o} for 25 minutes. After cooling the tubes for 5 minutes in cold tap water, 5ml of toluene was shaken vigorously about 25 to 30 times and then centrifuged. The absorbence of the toluene layers were determined spectrophotometrically at 426nm, with pure toluene as blank. The absorbances of the control plasma (that obtained prior to administration of the drug) were subtracted from the absorbance of the plasma specimens obtained after the ingestion of the drug. The amount of nitrofurantoin present in the tube was given by dividing the corrected absorbance by the slope of the Lambert-BeerIs plot and multiplying by the correction factor, i.e.

$$\text{nitrofurantoin concentration } (\mu g \text{ per ml}) = \frac{\text{absorbance} \times 1.163}{0.31}$$

Results

The results of these estimations are presented in tables 6.5 and 6.6 and the graphical representation is figure 6.3.

Table 6.5 Plasma drug concentration versus time

Formulation	A		B		C	
	corrected plasma drug concentration (µg per ml)					
Time (hr.)		S.D ±		S.D ±		S.D ±
(Week one)						
0.5	0.47	0.09	0.64	0.00	1.17	0.05
1.0	1.00	0.05	0.91	0.01	1.35	0.06
2.0	0.34	0.05	0.12	0.05	0.49	0.03
4.0	0.00	0.00	0.00	0.00	0.00	0.00
(Week two)						
0.5	0.56	0.04	0.65	0.02	1.12	0.02
1.0	0.86	0.05	0.90	0.01	1.29	0.02
2.0	0.47	0.03	0.24	0.36	0.36	0.05
4.0	0.00	0.00	0.00	0.00	0.00	0.00
(Week three)						
0.5	0.48	0.01	0.59	0.03	0.98	0.06
1.0	0.96	0.01	0.86	0.02	1.12	0.08
2.0	0.44	0.01	0.27	0.04	0.46	0.01
4.0	0.00	0.00	0.00	0.00	0.00	0.00

A; 30% drug/Avicel (physical mixture);

B; 30% drug/PEG 6000 (physical mixture);

C; 30% drug/PEG 6000 (solid dispersion).

Table 6.6 Mean plasma concentration versus time

Time (hr.)	Mean corrected plasma drug concentration (µg per ml.) A	B	C
0.5	0.50	0.64	1.09
1.0	0.94	0.89	0.25
2.0	0.42	0.21	0.44
4.0	0.00	0.00	0.00

6.2.5 RESULTS AND DISCUSSION

Apart from the data obtained from the in-vivo absorption study, (tables 6.5, 6.6 and figure 6.3), the following affects were reported by all three volunteers:

In the 20th minute, there was tenseness in the head which persisted until the 35th minute, when nausea set in. Dryness in the mouth, drowsiness and dizziness were effects which became pronounced during the 50th minute. The mechanism by which these effects manifested were not investigated in this study. They may have been centrally mediated and could have been initiated by local gastric irritation.

Some measure of in-vivo absorption and in-vitro dissolution correlation seem to have been shown by results obtained. At the end of one hour dissolution rate studies, the observed trend was: formulation C gave better dissolution

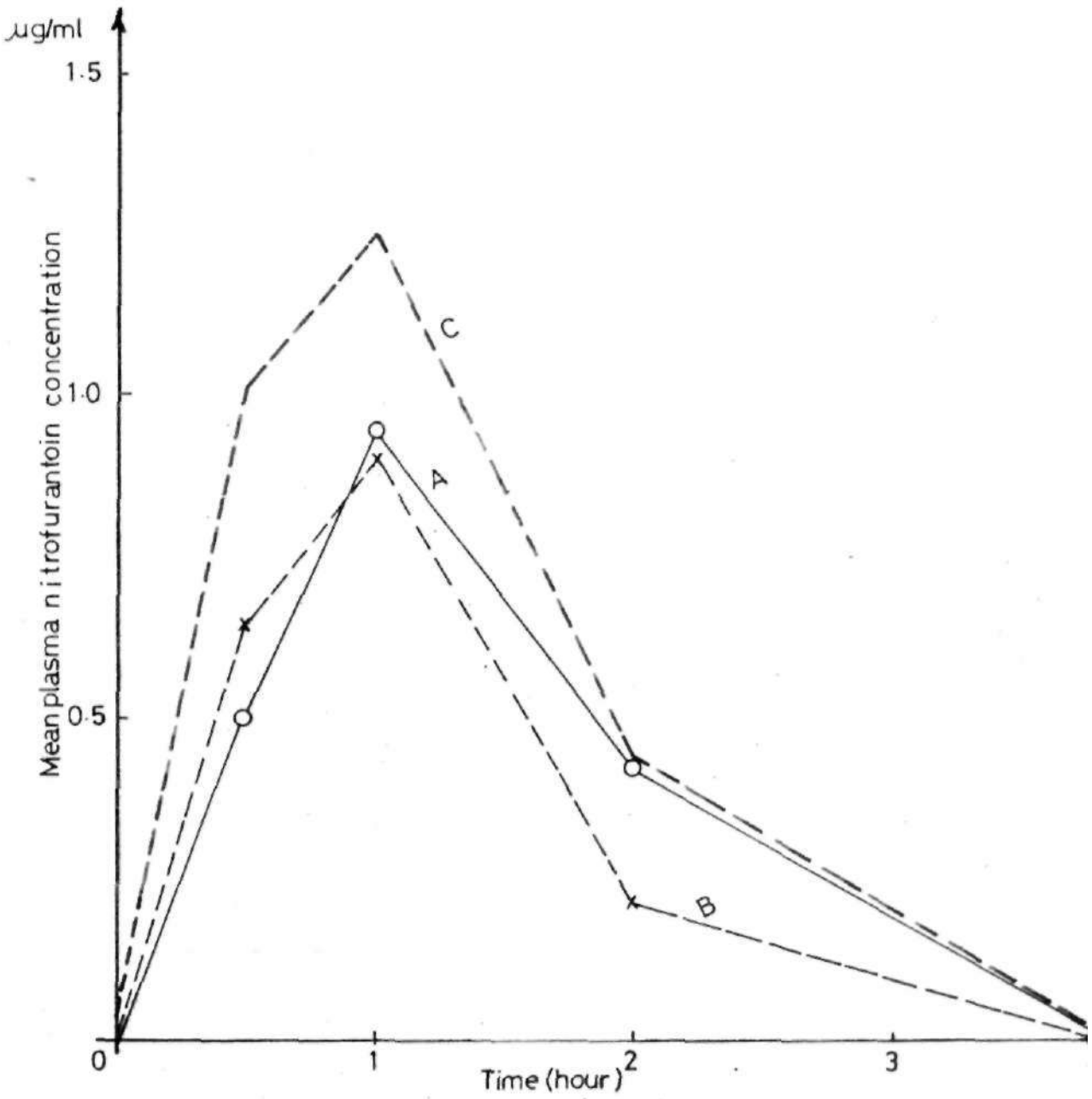


Fig. 6:3. Plasma concentration-time curves for three formulations of nitrofurantoin following absorption A – 30% drug / Avicel physical mixture; o——o——o; B – 30% drug / PEG 6000 physical mixture; x– – –x– – –x; C – 30% drug/ PEG 6000 solid dispersion; ———

compared to both formulations B and A. This trend was observed during the in-vivo absorption study. The correlation has not been completely linear, as observed in-vitro better dissolution of formulation B than formulation A. had been slightly reversed in favour of formulation A in the in-vivo absorption study. In terms of extent of absorption (AUC) formulation C was absorbed to the largest extent followed by formulation A, which was followed very closely by formulation B.

This study has confirmed that solid dispersion of nitrofurantoin enhances both its in-vitro dissolution rate and its in-vivo absorption as against simply physically mixing drug and carriers. This is indicative of the involvement of more than just local solubilization brought about by the presence of the carriers (Goldberg et al., 1966c). The effect of change of inert excipient has not been as dramatic as the effect produced by solid dispersion.

Since all the three formulations have produced similar physical symptoms, it has become clear that solid dispersion does not produce any immunity against toxic tendencies inherent in drugs. But when improved formulations are produced via solid dispersion, it is expected that the reduction of quantity of drug required in tablet formulae for administration to patients to produce the same if not better desired pharmacologic effects, would more than compensate for slightly higher production costs as well as ensure greater safety to the

larger number of patients.

Absolute bioavailabilities of the three formulations have not been reported because they have not been compared against any known standard tablet formulation.

The minimum inhibitory concentration of at least 4µg per ml (Martindale, 1982) was not attained in any of the blood samples. But since about 20 to 60% of the total absorbed drug is bound to plasma proteins (Martindale, 1982) the quantities detected in this study are probably only representative. It has not been established if the protein bound drugs are available for the derivatisation process.

Since large amounts are removed via the excretory fluids of bile and urine (Conklin, 1971) after oral or parenteral administration, the urine is considered best suited for the estimation of this drug. This is more so, when it is realised that nitrofurantoin is solely a urinary tract antibacterial agent (Martindale, 1982; Conklin and Hollifield, 1965; Bron et al., 1970; Roger et al., 1981b) and urinary concentration at the optimum pH is critical (Martindale, 1982).

In this study, the subjects were fasted so that the observable effects could be most appropriately ascribed to the product formulation alone since it has been established that food has a very considerable effect on the absorption of nitrofurantoin (Bates et al., 1974) as compared to effects in fasting subjects.

This study has provided further evidence that dissolution rate-limited absorption exists with nitrofurantoin. This rate limited absorption is probably due to particle size dependence (Watari et al., 1983; Fincher, 1968). It follows therefore, that if a product optimization is the goal, solid dispersion is a potentially beneficial approach.

SUMMARY

The photolytic degradation of aqueous solutions of nitrofurantoin has been studied. The degradative process is considerably minimized by protecting all solutions of the drug from light at all times. An extension of this finding has been to suggest that all forms of the drug should be packaged in airtight, light proof containers.

A sensitivity of 2 to 10 mg per litre has been established for the spectrophotometric estimation of aqueous solutions of nitrofurantoin.

Purely thermal degradation of the drug in persons having normal body temperature are unlikely judging from results of the in-vitro study on the effect of a temperature of 37° on the stability of the drug.

The determination of aqueous solubilities of the drug and its solubilities in aqueous solutions of mannitol and PEG 6000 at emperatures of 28° and 37° has indicated that the physical presence of these carriers actually improves aqueous solubilities. Results have shown that the higher the concentration of the carriers, the greater the improvement on drug solubility.

Lactose, mannitol, PEG 6000, PVP 44,000 and succinic acid were screened for use as carriers. Mannitol and PEG 6000 separately formed suitable comparable solid dispersions with the drug. PEG 6000 exhibited better dissolution enhancement in the drug, than did mannitol.

The drug naturally forms an approximately 4% combination with the carrier PEG 6000 on a molecular basis, and a study of 5, 10, 20, 30, 40, and 50% drug/PEG 6000 solid dispersions revealed greater solubility in the 5% drug-carrier solid dispersion. For purposes of optimizing the formulations (for the provision of a tablet that is not excessively bulky) a compromise was reached by selecting the 30% drug/carrier solid dispersion.

Stability studies carried out on the solid dispersions using TLC and UV spectrophotometry revealed no degradation products.

A formulation study has been done to investigate the effects of (i) molecular drug dispersion and (ii) changes in inert excipients on the overall drug product quality, dissolution and absorption characteristics. Three different tablet formulations produced using different diluents (Avicel physical mixture, PEG 6000 physical mixture, and PEG 6000 solid dispersion for formulations A, B and C respectively), complied with official quality control specifications and were then subjected to in-vivo absorption evaluation in three human volunteers.

An in-vitro dissolution and in-vivo absorption correlation has been exhibited by way of better dissolution of tablets containing drug-carrier solid dispersion compared to the two tablet formulations containing drug-carrier physical mixtures and also showed

improved absorption. The correlation was not completely linear as formulation A was absorbed to a greater extent than formulation B. (contrary to in-vitro dissolution rate observed).

Absolute bioavailabilities of any of the three tablet formulations with a standard or marketed product has not been considered. But comparative bioavailabilities of the three formulation, if formulation C more bioavailable than formulation A, while formulation B is the least bioavailable.

Results have confirmed that solid dispersion enhances both the in-vitro dissolution and in-vivo absorption characteristics of nitrofurantoin. This suggests that simple local solubilization alone cannot explain the effects of the carriers observed. The in-vivo absorption study is indicative of the immense beneficial effects of solid dispersion which would be derived when the quantity of drug incorporated in table formulations are reduced and the tablets still give comparable desirable pharmacologic responses. It is expected that the patients' discomfort due to inherent drug toxicity would be reduced. It is hoped that, in attempts to be made to put to practical uses, the unique technique of solid dispersion, emphasis would be laid on adequate performulation studies aimed at among other things, the determination of the optimum drug; carrier ratio.

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