# STABILITY STUDIES AND INFLUENCE OF A LOW-FAT MEAL ON THE PHARMACOKINETIC AND BIOAVAILABILITY OF NIFEDIPINE IN HEALTHY VOLUNTEERS

BY

SUNDAY OLAWALE OKENIYI

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN PHARMACEUTICAL CHEMISTRY.

DEPARTMENT OF PHARMACEUTICAL AND MEDICINAL CHEMISTRY,
FACULTY OF PHARMACEUTICAL SCIENCES,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA.

SEPTEMBER, 1991.

## DECLARATION

I hereby declare that this thesis has been written by me and it is a record of my research work, carried out under the joint supervision of Dr. Abdullahi Mustapha and Dr. Ibrahim Abdu-Aguye. It has not been presented in any previous application for higher degree. The work of other investigators are acknowledged and referred to accordingly.



Sunday Olawale Okeniyi
Department of Pharmaceutical and Medicinal Chemistry,
Faculty of Pharmaceutical Sciences,
Ahmadu Bello University,
Zaria, Nigeria.

## CERTIFICATION

This thesis entitled "STABILITY STUDIES AND INFLUENCE OF A LOW-FAT MEAL ON THE PHARMACOKINETIC AND BIOAVAILABILITY OF NIFEDIPINE IN HEALTHY VOLUNTEERS" by SUNDAY OLAWALE OKENIYI meets the regulations governing the award of the degree of MASTER OF SCIENCE (PHARMACEUTICAL CHEMISTRY) of AHMADU BELLO UNIVERSITY, and is approved for its contribution to science, knowledge and literary presentation.

---------- Date: 2-1-92

INTERNAL EXAMINER
Dr. A. Mustapha, B.Sc.(Pharm),Ph.D., MPSN.
Head, Dept. of Pharmaceutical and Medicinal Chemistry,
Faculty of Pharmaceutical Sciences,
Ahmadu Bello University,
Zaria.

---------- Date: 2-1-92

INTERNAL EXAMINER
Dr. I. Abdu-Aguye, M.B.B.S., M.Sc., F.M.C.P.
Head, Dept. of Pharmacology and Clinical Pharmacy,
Faculty of Pharmaceutical Sciences,
Ahmadu Bello University,
Zaria.

---------- Date: 02/01/92

EXTERNAL EXAMINER
Dr. H.A.B. Coker, B.Sc.(Pharm), Ph.D.(Glasgow), MPSN.
Ag. Head, Department of Pharmaceutical Chemistry,
School of Pharmacy, College of Medicine,
University of Lagos,
Lagos.

---------- Date: 20/3/92

Professor E.G. Kolawole, B.Sc., Ph.D., F.P.R.I.
Professor of Chemistry, and
Dean of Postgraduate School,
Ahmadu Bello University, Zaria.

DEDICATION

Dedicated

to

My wife, Kikelomo

and

My son, Olawale Jnr

for their steadfast love, constant

companionship, support and

continual inspiration.

ACKNOWLEDGEMENT

I wish to acknowledge with due respect and gratitude, the erudite supervision, understanding guidance, patience, constant encouragement and valuable recommendations given by Dr. Abdullahi Mustapha and Dr. Ibrahim Abdu-Aguye.

I also wish to express my sincere gratitude and appreciation to Dr. M.K. Choudhury, Dr. T.T. Iyaniwura, Dr. Isa Hussaini, Mr. D.S. Ogunleye, Mr. Francis Obeta, Mal. Iliyasu and host of others, all of whom have tremendously helped me in one way or the other.

I cannot forget Dr. Ifedapo Raji and Miss M.T. Bakare both whom have in no small way contributed to the beginning and successful completion of this course and for making my stay in Zaria particularly interesting. The same goes for a host of others friends and associates like SamBic, the Adeshinas, the Akandes, the Oladipos, Aston Ajiboye, the Odetokuns, the Oshuduns, RMR, Ayo Lesotho, the Agboolas, the Adeyemos, Balami special and Abba Jay and a host of others whose names cannot be mentioned. I remain indebted.

My sincere gratitude to my parents, my sisters and my loving inlaws for their prayers and kind moral support and to all the volunteers who participated in this study, I say thank you.

My appreciation to Mr. Chris Eneogwe for secretarial assistance and to the members of staff, academic and technical of the department of Pharmaceutical and Medicinal Chemistry for their assistance.

I am most grateful to my wife, Kikelomo and my son, Olawale Jnr for their love, patience and understanding about my absence throughout the period of the study.

Finally, I thank God for everything.

## ABSTRACT

Identification tests for nifedipine based on the thin-layer chromatography and melting point were carried out on the three brands of nifedipine employed in this study as specified in the B.P. (1988) addendum (1990). The results obtained were in conformity with the B.P. 1988 specifications under identification tests for nifedipine. The presence of nifedipine in the tablets was examined by running the ultra-violet absorption spectrum of the reference nifedipine and comparing it with the U.V. spectra of the three brands of nifedipine samples. The spectra exhibited maxima which were at the same wavelength.

HPLC and UV methods of chemical assay were also employed to determine the total drug content for the three brands. The results complied favourably with the stated amount in the B.P. 1988 for nifedipine content (98.00 - 102.0C%).

The influence of day-light, fluorescent light, red-light, temperature and ultraviolet irradiation on the stability of nifedipine tablets and solution was evaluated. HPLC method for estimating drug content in tablet dosage forms was employed. Stability test of nifedipine solution in methanol showed loss of drug content and decomposition when exposed to day-light, fluorescent and ultraviolet irradiation while there was no signifi-cant decomposition nor loss of drug content under

red-light and elevated temperature up to 50 C. Stability test carried out on nifedipine tablets showed no loss of drug content, decomposition nor colour change, indicating the stability of nifedipine tablets under all the conditions of irradiation.

Dissolution rate profiles of three different brands of nifedipine tablets in 0.1M methanolic-hydrochloric acid was ingestigated by means of B.P. 1988 rotating basket method. The drugs showed similarities in their respective dissolution rate profiles. Total release of the active ingredient for the three brands was obtained in less than 30 minutes.

Disintegration time test was carried out on the three brands of nifedipine tablets (S, 0, and K). There was no considerable variation in their disintegration times. The times taken for the three brands to disintegrate was less than 8 minutes. They all passed the official requirement of 15 minutes for tablet disintegration.

Invivo bioavailability studies was carried out in healthy human subjects in fasting and non-fasting states. A simple, reliable and rapid HPLC method of nifedipine estimation preceeded by an extraction technique was employed for the determination of plasma and saliva nifedipine levels, pharmacokinetic parameters for plasma and saliva nifedipine samples were generated by computer application.

Statistically significant higher levels of nifedipine were detected in saliva compared to plasma in the absorption phase in both fasting and non-fasting states following oral ingestion of 20mg of the drug. There were very good linear correlations between concentrations of nifedipine in plasma and saliva in both fasting and non-fasting states. The overall absorption-elimination profile for plasma did not differ significantly compared to that for the saliva in both fasting and non-fasting states as indicated by the Area under the Curves.

The low fat meal ingested appeared to reduce the rate of absorption and absorption rate constant of nifedipine, and increase the time lag in both plasma and saliva. The total availability of nifedipine was however not significantly affected.

TABLE OF CONTENTS


| TITLES | PAGES |
|---|---|
| Title page | i |
| Declaration | ii |
| Certification | iii |
| Dedication | iv |
| Acknowledgement | v |
| Abstract | vi |
| Table of contents | ix |
| List of Tables | xi |
| List of Figures | xiii |
| List of Schemes | xv |
| List of Appendices | xvi |
| Abbreviations | xvii |
| **CHAPTER ONE: INTRODUCTION** | |
| 1.1 Introduction | 1 |
| 1.2 Pharmacokinetic studies | 2 |
| 1.3 Pharmacokinetic parameters | 9 |
| 1.4 Bioavailability | 12 |
| 1.5 Factors affecting bioavailability of drugs | 16 |
| 1.6 Dissolution rate studies (Invitro availability) | 23 |
| 1.7 Factors influencing dissolution rate | 26 |
| 1.8 Relationship between bioavailability and dissolution rate | 27 |
| 1.9 Salivary excretion of drugs | 29 |
| **CHAPTER TWO: GENERAL LITERATURE REVIEW** | |
| 2.1 History and Chemistry | 32 |
| 2.2 Absorption and distribution | 35 |
| 2.3 Metabolism and excretion | 36 |
| 2.4 Side effects | 38 |
| 2.5 Drug interactions, dosage and administration | 39 |
| 2.6 Place of Nifedipine in therapy | 40 |
| 2.7 Objective and Scope of study | 42 |

PAGE


CHAPTER THREE: MATERIALS AND METHODS

3.1 Drugs.....44
3.2 Equipment.....44
3.3 Identification tests.....46
3.3.1 U.V. Absorption spectra.....46
3.3.2 Thin-layer chromatography.....46
3.3.3 Melting point.....48
3.4 Solvent system.....48
3.5 Chemical assay.....49
3.5.1 HPLC method.....49
3.5.2 U.V. spectrophotometric method.....51
3.6 Stability studies.....52
3.6.1 Effect of light.....52
3.6.2 Ultra-violet irradiation.....53
3.6.3 Effect of temperature.....54
3.7 Dissolution rate tests.....55
3.7.1 Disintegration tests.....59
3.8 Invivo absorption studies.....59
(a) Fasting state.....59
(b) Non-fasting state.....60
3.9 Analysis.....61
3.9.1 Extraction procedure.....61
3.9.2 Preparation of samples for HPLC analysis.....62
3.9.3 Preparation of standard solutions and standard curve.....62

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Identification tests.....64
4.2 Solvent system.....68
4.3 Chemical assay.....72
4.4. Stability studies.....76
4.5 Dissolution rate tests.....88
4.6 Disintegration tests.....88
4.7 Extraction procedure.....93
4.8 Invivo absorption studies.....95
CONCLUSION.....110
REFERENCES.....114
APPENDICES.....120

## LIST OF TABLES


| TABLE | | PAGE |
|---|---|---|
| 1 | Chromatographic parameter for nifedipine and 4-dimethylaminobenzaldehyde under different conditions using μ-Bonda-Pak $C_{18}$ radial Pak catridge (Waters Associates) | 69 |
| 2 | Effect of pH on the capacity factor (R) and resolution (d) for nifedipine and 4-dimethyl-aminobenzaldehyde using solvent system 0.1M sodium acetate (pH 4): methanol (45:55) (pH 5.2) and μ-Bonda-Pak $C_{18}$ radial Pak catridge (Waters Associates) | 70 |
| 3 | Quantitative determination of nifedipine tablets using HPLC and U.V. spectrophotometric methods of analysis | 75 |
| 4 | Effect of day-light, fluorescent light and red-light on the stability of nifedipine tablets and solution in methanol using HPLC method of analysis | 79 |
| 5 | Kinetic parameters of nifedipine solution (100 μg/ml) after exposure to normal day-light, fluorescent light and red-light | 83 |
| 6 | Effect of ultra-violet radiation on the stability of nifedipine tablets and solution in methanol | 85 |
| 7 | Effect of elevated temperature on the stability of nifedipine tablets and solution in methanol | 87 |
| 8 | In-vitro dissolution release rates of three brands of nifedipine tablets in 0.1M HCl maintained at 37 ± 0.5°C using B.P.(1988) Rotating Basket method with a stirring rate of 100 r.p.m. | 90 |
| 9 | Disintegration times for nifedipine tablets in water maintained at 37 ± 0.5°C (B.P. 1988) | 92 |
| 10 | Solvent/solvent combination and percentage extraction recoveries of nifedipine from biological matrix | 94 |
| 11 | Mean plasma and saliva nifedipine concentration in healthy volunteers following oral administration of 20mg nifedipine in fasting and non-fasting state | 101 |

TABLE | PAGE

12 Pharmacokinetic parameters of nifedipine derived from the mean plasma and saliva concentration data after a single 20mg oral dose in fasting and non-fasting state..... 104

## LIST OF FIGURES


| FIGURE | | PAGE |
|---|---|---|
| 1 | Steps involved in the distribution and elimination of drugs in the body | 2 |
| 2 | A single compartment model | 5 |
| 3 | Two compartment model | 6 |
| 4 | Semi-logarithmic plasma concentration time plot of a two compartment model | 7 |
| 5 | A three compartment model | 8 |
| 6 | A typical logarithmic concentration-time plot after a single oral dose | 9 |
| 7 | A typical plasma-concentration-time curve | 14 |
| 8 | Apparatus for the dissolution of tablets and capsules | 56 |
| 9 | UV absorption spectra for three brands of nifedipine in 0.1M hydrochloric acid (200 - 400nm) | 65 |
| 10 | UV absorption spectra for three brands of nifedipine in methanol (200 - 400nm) | 66 |
| 11 | Effect of pH on the capacity factor (R) for nifedipine and 4-dimethylaminobenzaldehyde using solvent system 0.1M sodium acetate (pH 4): Methanol (45:55) and μ-Bonda-Pak $C_{18}$ radial-Pak Catridge (Waters Associates | 71 |
| 12 | Calibration curve for nifedipine (20.0 - 100.0 μg/ml) using the High Performance Liquid Chromatographic (HPLC) method of analysis | 73 |
| 13 | Calibration curve for nifedipine (5.0 - 40.0 μg/ml) in 0.1M hydrochloric acid using UV spectrophotometric method of analysis ($\lambda$max = 349nm) | 74 |
| 14 | Chromatogram showing 4-dimethylaminobenzaldehyde (I) and the unexposed nifedipine (II) | 77 |
| 15 | Chromatogram showing 4-dimethylaminobenzaldehyde (I), nifedipine (II) and the day-light decomposed product (III) after exposure to normal day-light (6 hours) | 78 |

FIGURE PAGE


16 Effect of day-light (A), fluorescent light (B) and red-light (C) on the stability of nifedipine (100 µg/ml) solution in methanol.....80

17 Graphical plot of the logarithmic transformed data on the effect of day-light (A), fluorescent light (B) and red-light (C) on the stability of nifedipine solution in methanol.....81

18 High Performance Liquid Chromatogram of nifedipine solution (II), UV-decomposed product (III) and internal standard (I) after exposure to 48 hours under UV-light (366nm).....84

19 Effect of ultraviolet irradiation (366nm) on the stability of nifedipine solution in methanol.....86

20 Calibration curve for nifedipine (5.00 - 30.00 ug/ml) in 0.1M HCl solution using UV spectrophotometer. $\lambda$max = 349nm.....89

21 Cumulative concentration profile of dissolution rate studies of three brands of nifedipine tablet in 0.1M HCl solution.....91

22 Calibration curve for nifedipine in plasma sample (0 - 50.0 ng/ml).....96

23 Calibration curve for nifedipine in saliva sample (0 - 200ng/ml).....97

24 HPLC chromatogram of plasma nifedipine sample (II) at time of maximum concentration (Cmax) and internal standard (500ng/ml) (I).....98

25 HPLC chromatogram of saliva nifedipine sample (II) at time of maximum concentration (Cmax) and internal standard (1000ng/ml) (I).....99

26 The mean plasma concentration time curve after oral ingestion of 20mg nifedipine tablets in fasting (A) and non-fasting (B) states.....102

27 The mean saliva concentration time curve after oral ingestion of 20mg nifedipine tablets in fasting (A) and non-fasting (B) states.....103

## LIST OF SCHEMES


| SCHEME | | PAGE |
|---|---|---|
| 1 | Schematic representation of disintegration, deaggregation and dissolution of drug from solid dosage forms | 25 |
| 2 | General chemical structure of dihydropyridines | 32 |
| 3 | Schematic representation of 4-(2'-nitrosophenyl)-pyridine | 34 |
| 4 | Schematic representation of 4-(2'-nitrophenyl)-pyridine | 34 |
| 5 | Pathways of metabolism of nifedipine | 37 |

## LIST OF APPENDICES


| APPENDIX | | PAGE |
|---|---|---|
| 1 | Dissolution rate studies of nifedipine tablets in 0.1M HCl maintained at 37 ± 0.5°C using B.P. (1988) Rotating Basket method | 120 |
| 2 | Preparation of acetate and phosphate buffer | 121 |
| 3 | Apparatus for the disintegration of tablets | 122 |
| 4 | Comparison of different light sources | 124 |
| 5 | Questionnaire for volunteers participating in the invivo bioavailability studies | 125 |
| 6 | Plasma and saliva concentration of unchanged nifedipine (ng/ml) in 6 healthy volunteers following oral administration of the tablets (20mg) in fasting and non-fasting states | 126 |
| 7 | Pharmacokinetic parameters of nifedipine in plasma and saliva of individual volunteers after a single 20mg oral dose in fasting and non-fasting state | 128 |

## ABBREVIATIONS

| | | |
|---|---|---|
| B.P. | = | British Pharmacopoeia |
| U.S.P. | = | United States Pharmacopoeia |
| T.L.C. | = | Thin-Layer Chromatography |
| H.P.L.C. | = | High-Performance Liquid Chromatography |
| HCl | = | Hydrochloric acid |
| pH | = | Hydrogen-ion concentration |
| U.V. | = | Ultraviolet |
| hr. | = | Hour |
| Min. | = | Minute |
| ml | = | Millilitre |
| Mg | = | Milligramme |
| µg | = | Microgramme |
| ng | = | Nanogramme |
| g | = | Gramme |
| $^{o}C$ | = | Degree centigrade (Celsius) |
| r.p.m. | = | revolutions per minute |
| Log. | = | Logarithm |
| Ln | = | natural logarithm |
| Mw | = | Molecular weight |
| mm | = | Millimetre |
| Rf | = | Retardation factor |
| THF | = | Tetrahydrofuran |
| TEA | = | Triethylamine |

# CHAPTER ONE

## 1.1 INTRODUCTION

Food has been used to describe a unique formation of what one takes into the body system, which being digested, nourishes the body to maintain life and sustains or promotes growth. While drug is a chemical compound of synthetic, semisynthetic, natural or biological origin which interacts with human or animal cells. The interaction may be quantified, whereby these resulting actions are intended to prevent, to cure or to reduce ill effects in the human or animal body, or to detect disease-causing manifestations. Drugs are also defined as substances that are body foreign by nature or amount, often initiate a sequence of biochemical and physiological processes. Body foreign substances, semantically distinguished as food, luxury, or drug are absorbed, distributed in and eliminated from tissues and organs in either intact or modified form by a multitude of mechanisms. The rates at which these processes occur simultaneously are characteristic for every drug with the restrictions of a number of variables. These rates determine the onset, the time course, and the intensity of beneficial or toxic effect to the body.

## 1.2 PHARMACOKINETIC STUDIES

Pharmacokinetics deals with the changes of drug concentration in the drug product and changes of concentration of a drug and/or its metabolite(s) in the human or animal body following administration, i.e, the changes of drug concentration in the different body fluids and tissues in the dynamic system of liberation, absorption, distribution, body storage, binding, metabolism, and excretion. Applied to therapy, clinical pharmacokinetics are used for selecting and adjusting drug dosage schedules and to facilitate the interpretation of measured serum concentration of drugs.

The transfer of a drug from its absorption site to the blood, and the various steps involved in the distribution, and elimination of the drug in the body are as follows:

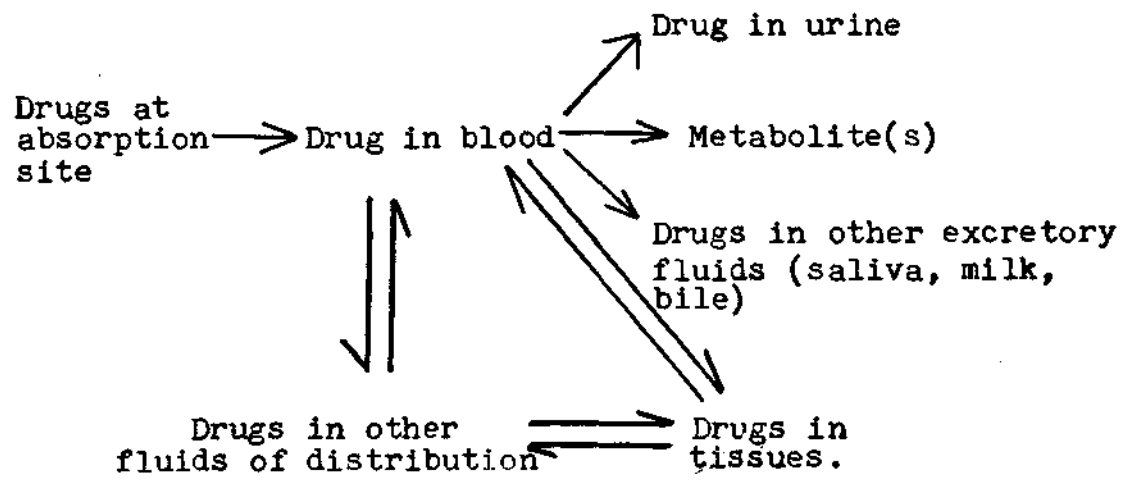



Fig. 1: Steps involved in the distribution and elimination of drugs in the body

The primary standard of a pharmacokinetic study is based on the data obtained following intravenous administration of drug in which the entire dose administered is placed directly into the blood stream, the pharmacokinetic parameters obtained are unaffected by all the potential rate-limiting and metabolic factors associated with drug absorption following other routes.

It has been established that the intensity of the pharmacologic effects of many drugs is related to their concentration and/or to the concentration of their metabolite(s) in plasma (Levy, 1986).

Attempts are made in pharmacokinetics to describe biological event(s) in mathematical terms, by developing mathematical models to represent the body as a system of compartments with no apparent physiologic or anatomic reality. A model in pharmacokinetics is a hypothetical structure which can be used to characterize with reproducibility the behaviour and the fate of a drug in biological systems when given by a certain route of administration and in a particular dosage form. A compartment is an entity which can be described by a definite volume and a concentration of drug contained in the volume.

Compartment models are used to describe the behaviour of a drug in biological systems and could be classified into one, two or multicompartment models. Before any

equations may be derived from these models, some basic assumptions have to be made. Firstly, drug enters the system only via the central compartment. Secondly, reversible transfer occurs between central and peripheral compartments, and thirdly, the exit of drugs from all compartments in the system is described by first order kinetics (i.e, the rate at which a drug is removed from a compartment is directly proportional to the drug concentration in it at that time). The proportionality constant relating the rate and amount is the first order elimination rate constant which has units of reciprocal time for example, $min^{-1}$, $hr^{-1}$ (Gibaldi and Perrier, 1975b). All three assumptions are relatively valid for most drugs. The majority of drugs enter the body via the blood stream, and are eliminated from the blood stream by the kidney. There are a few exceptions which are primarily eliminated by biotransformation, and if given in high enough doses, result in saturation of the metabolic enzyme system. The elimination kinetics then become zero-order, i.e. constant and independent of drug concentration.

The single or one compartment model is the simplest model which depicts the body as a single homogenous unit in which the drug entering the body distributes instantly between the blood and other body fluid or tissues (i.e, the exchange of drug between

the plasma and tissue proceeds rapidly compared with the rate of elimination. The whole body is then considered mathematically as a single compartment. This model is useful for the pharmacokinetic analysis of blood, plasma or serum concentration. It is also useful for analysis of salivary and urinary excretion data for drugs which rapidly distribute between plasma and other body fluids and tissues upon entry into the systemic circulation.

Assumptions that the body behaves as a one-compartment model does not mean that the drug concentrations in all body tissues at any given time are the same. However, a one compartment model does assume that any changes that occur in the plasma quantitatively reflects changes occurring in tissue drug-levels. Assuming instantenous distribution after an intravenous injection into this model (Fig. 2), the concentration (Co) in the plasma immediately after injection is equal to the dose (D) divided by the volume of this compartment (Vdc).

$$Co = \frac{D}{Vdc} \quad \text{.....(Equation 1).}$$

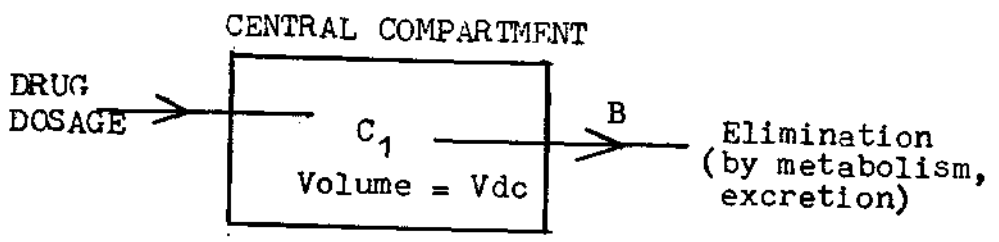



FIG. 2: A single compartment model. Rapid intravenous injection of a drug into a single compartment.

This apparent volume of distribution (Vdc) of the drug is not a true volume, but that volume into which all the drug in the body would appear to be distributed to achieve a concentration the same as that in the plasma. Elimination follows a first order kinetics after instantenous distribution which means that a constant fraction of drug is eliminated per unit time.

The two compartment model contains a central compartment and at least one other peripheral compartment (Fig. 3) and this is usually considered when the distribution of the drug is so slow that it cannot be disregarded. Although, these compartments lack physical

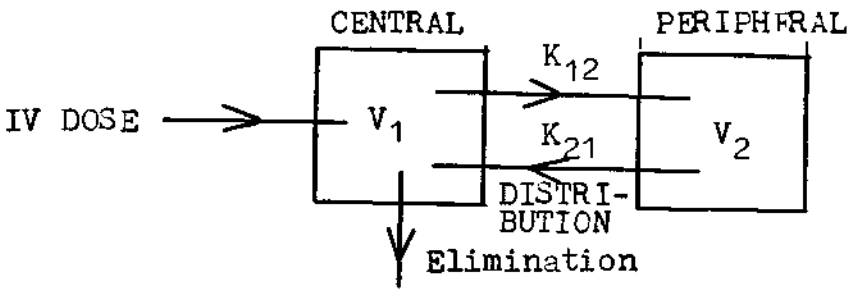



FIG. 3: Two Compartment Model

or anatomical reality, for many drugs the central compartment may correspond to the plasma or blood volume together with the extracellular fluid of highly perfused tissues such as muscle, skin or adipose tissue into which drugs may enter more slowly. The combined effect of two compartments gives rise to a biphasic curve on IV injection with two distinct linear portions

when drawn on a semilog ordinate scale (Fig. 4). Although drug distribution to the peripheral compartment is slow, it is usually much faster than elimination. Thus the initial rapid fall in concentration, known as the $\alpha$ or distribution phase, mainly represents the relatively rapid process of drug distribution from central to peripheral compartment. Once distribution is complete, the curve enters the relatively slow $\beta$ or elimination phase during which drug disappearance is determined mainly by irreversible elimination from the central compartment.

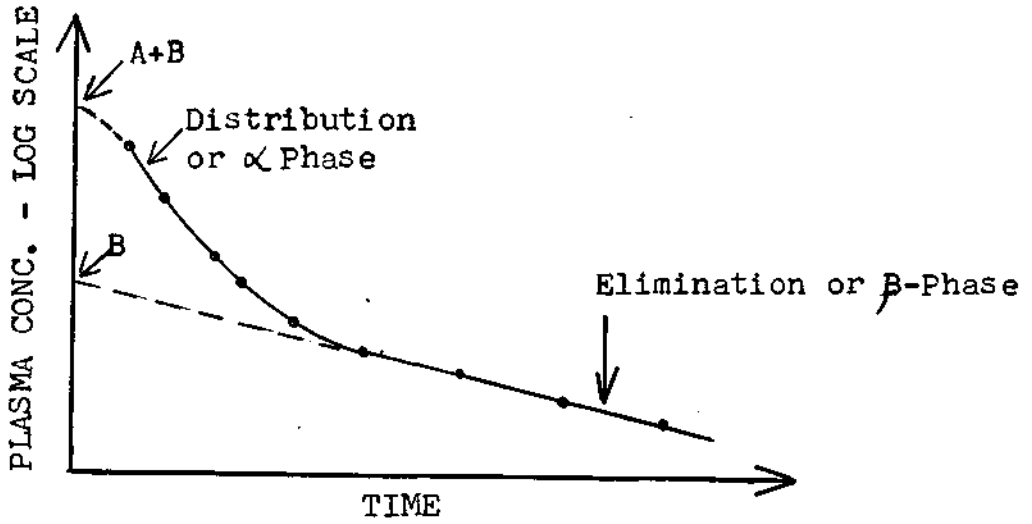



FIG. 4: Semi logarithmic plasma concentration time plot of a two compartment model.

The two compartment may be expanded to contain additional compartment which can be described mathematically as the sum of as many individual exponential

functions as there are relevant compartments (Multi-compartment Models). In reality, a maximum of three compartments is allowed in assay techniques (Paxton, 1981).

The three compartment model is a design or a modified model for oral dosing in which an additional compartment is incorporated to represent the volume (Va) from which absorption occurs at a first-order rate (Fig. 5). In this model, it is assumed that the entire dose is rapidly introduced into the site of absorption, from which it is absorbed into the central compartment.

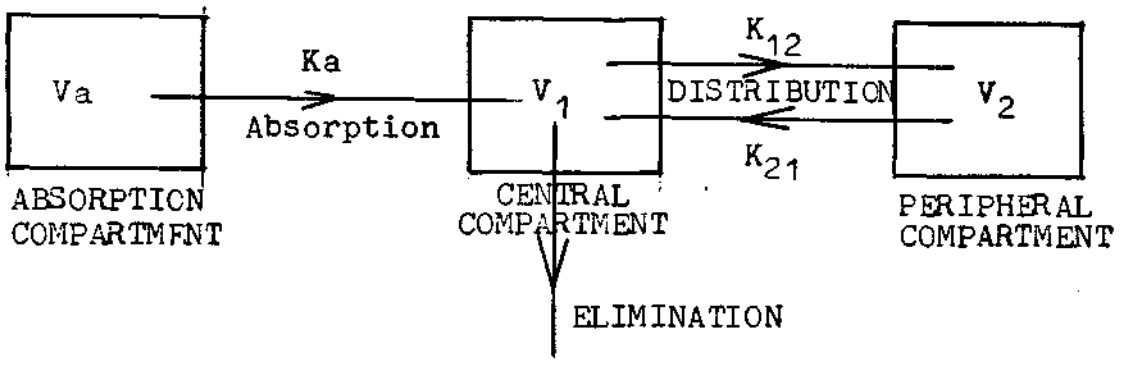



FIG. 5: A Three Compartment Model.

A typical concentration-time curve for a single oral dose is shown in Figure 6. Similar plots are obtained for other administration routes, such as intramuscular or subcutaneous, which inolve a preliminary absorptive phase.

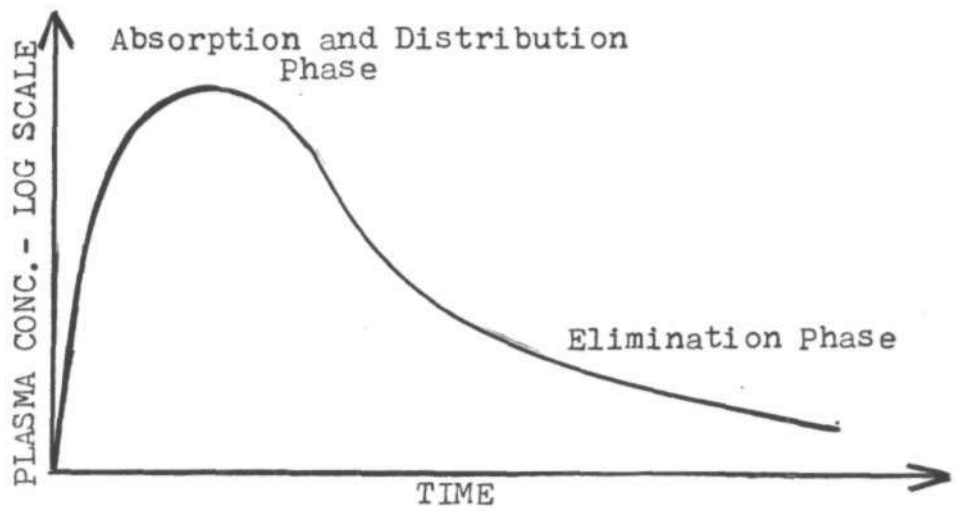



FIG. 6: A Typical log concentration-time plot after a single oral dose.

## 1.3 PHARMACOKINETIC PARAMETERS

Some of the most useful pharmacokinetic parameters obtained following drug administration are half-life, area under the curve of blood level versus time, systemic and total clearance, apparent volume of distribution and rate constants.

Half-life is the time taken for the post-absorption plasma concentration of a drug to be reduced by fifty percent of its original value (Paxton, 1981; Wagner, 1980). It can be determined for both one and two-compartment models from the terminal data of the log concentration-time plot. It is calculated by dividing 0.693 (ln 2) by the calculated slope of each exponential phase of multi-phasic blood level versus time curve.

$$t_{\frac{1}{2}} = \frac{\ln 2}{K} \quad \text{..... (Equation 2)}$$

Although $t_{\frac{1}{2}}$ is a poor index of drug elimination processes and dosage requirements in individual patients, it is a useful parameter in the determination of suitable dosage intervals and the time required to attain the steady-state during constant intravenous or chronic oral therapy.

Area under the curve is the integral of drug blood level over time from zero to infinity and is a measure of quantity of drug absorbed in the body. The methods of calculating area under the curve are discussed by various authors (Wagner, 1967; Gibaldi and Perrier, 1975c). These methods include planimetery, cut and weigh method, trapezoidal rule and computer application. Many authors have also used the area analysis to develop a pharmacokinetic pathway and to obtain the rate constants associated with the proposed pharmacokinetic model.

Systemic or plasma clearance is the volume of plasma from which a drug is totally and irreversibly removed per unit time. It is a direct index of drug elimination. Total plasma clearance of a drug, which results from all processes of elimination (such as hepatic metabolism, renal and faecal excretion, exhalation by the lungs), is the sum total of individual clearances of the drug by the various organs and tissues and can be expressed by two types of relationship. Firstly, one can divide the absorbed dose by the AUC, as the latter value basically represents the total bioavailable drug subsequently elimi-
nated:

$$Cls = \frac{F{\cdot}D}{AUC} \quad \text{.....(Equation 3)}$$

Secondly, the product of the elimination constant and apparent volume of distribution equals the clearance.

$$Cls = \beta{\cdot}Vd \quad \text{.....(Equation 4)}$$

The clearance has dimensions of volume per unit time, and expresses the efficiency with which a drug is eliminated, and is made up of the sum of all the clearances due to individual organs. If only one organ is involved, for example the kidney, the plasma clearance is equal to renal clearance.

The apparent volume of distribution is defined as that volume in which the amount of drug in the body would need to be uniformly distributed to produce the observed plasma concentration. The volume of distribution (Vd) is inversely proportional to blood level, i.e. the larger the volume of distribution, the more extensively the drug distributes from the blood stream into the various tissues, organs, and binding sites of the body. Various ways of calculating volume of distribution are known, but these depend on the pharmacokinetic profile of the drug (Gibaldi and Perrier, 1975b). The simplest method after IV injection in a one compartment model is by back extrapolation to find Co at zero time. More widely used, especially after oral doses or in multi-compartmental models, is the volume term derived from

the AUC which accounts for the observed plasma concentrations on the basis of the total amount of drug in the body at all times after distribution equilibrium has been attained:

$$Vd_{area} = \frac{F.D}{AUC} \quad \text{.....(Equation 4)}$$

## 1.4 BIOAVAILABILITY

The term bioavailability is defined as the rate and extent to which active drug ingredient or therapeutic moiety from a drug product becomes available at the site of action which is normally estimated by its concentration in fluids, rate of excretion or acute pharmacological effect (Skelly, 1976).

According to this definition, the absorption of an intravenously administered drug is rapid and complete. However, for reasons of convenience and stability, most drugs are administered orally which occur mainly from the gastrointestinal tract. Therefore, their rate and extent of absorption in an individual are usually not precisely known (Blanchard and Sawchuk, 1979).

The therapeutic action of a drug is dependent on an adequate concentration of the drug reaching the site of action and this concentration is dependent on the passage of the drug across biological membranes. The

rate and extent of absorption of a drug by the body is influenced by a number of biological and physiological factors but it may also be affected by the rate at which the drug dissolves in the body fluid at or before the site of absorption. Frequently this dissolution is the rate-limiting factor in the absorption process.

Effective dispersal of the drug particles in the body fluid and its solubility may both affect the rate of dissolution. These factors may be dependent on the physicochemical characteristics of the drug itself and on other materials and manufacturing processes used in the preparation of the dosage form.

Bioavailability or bioequivalence studies are usually based on measurement of the active drug moiety or/and its metabolite(s) in biological fluids as a function of time. In-vivo bioavailability testing in humans usually dictates that the biological fluid sampled be limited to blood, urine or saliva. The dosage form is administered to healthy subjects who are usually in the fasted state (approximately 10 hours) and who are not allowed to take food for some hours (usually 4 hours) following administration. Blood samples are then collected before and after drug administration at a frequency sufficient to permit an estimate of peak plasma or serum concentration of the active ingredient and the total area under the plasma level versus time curve. In most studies where oral dosage forms are

compared, the sampling times after each drug administration will be identical. Typical plasma concentration versus time curve that may be obtained after oral administration of a dosage form is shown in Figure 7.

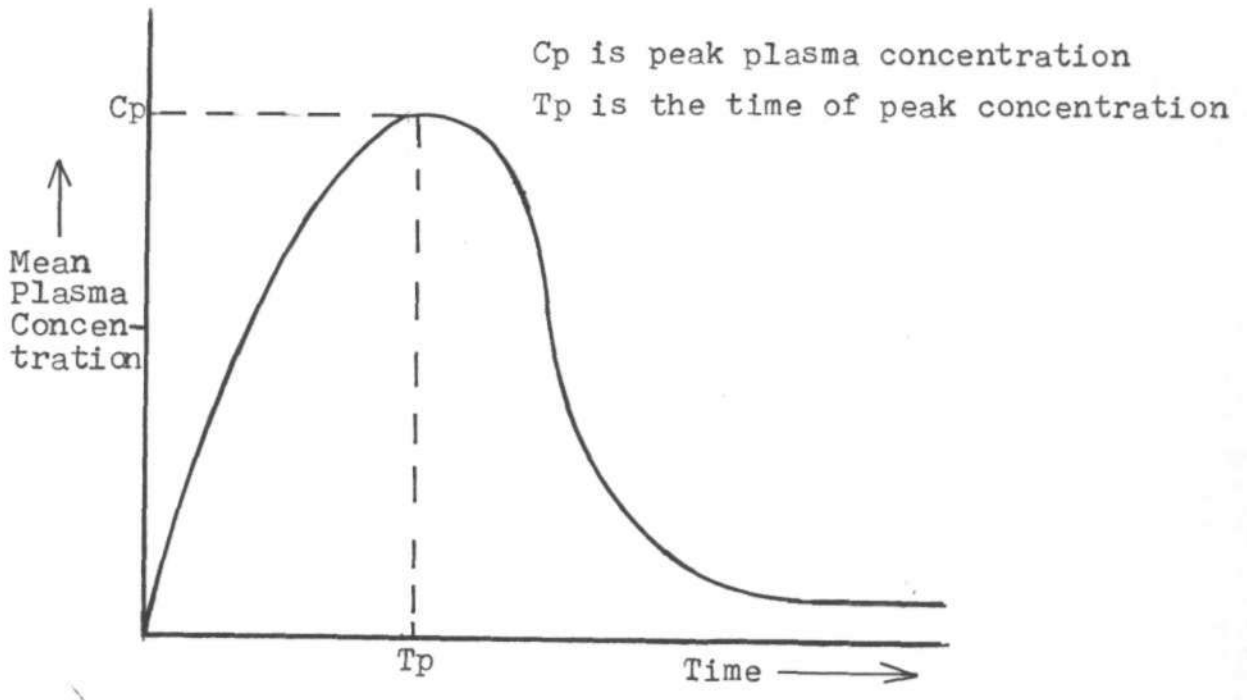



FIG. 7: A Typical Plasma-Concentration-Time Curve.

The shape of the curve is dependent on a number of factors. Elimination of the drug begins as soon as it appears in the blood and absorption, distribution and elimination (metabolism and excretion) will all modify the shape of the curve.

There are three important parameters that can be derived from this curve, namely the peak-plasma concentration (Cmax), the time of the peak-plasma

concentration ($t_{max}$) and the area under the plasma level versus time curve (AUC).

The first two parameters, Cmax and $t_{max}$ are related to and used as a measure of the rate of availability from the dosage form. The extent of absorption from the dosage form is related to the third parameter, AUC. In a bioequivalence study, the objective is to determine whether the tested dosage form is equivalent to the reference form. Alternatively, one might demonstrate that no statistically significant differences exist between the plasma levels derived from each drug product at each sampling time.

The determination of "absolute" bioavailability of a drug involve a comparison of area under the plasma-concentration-time curve (AUC) obtained following oral and intravenous administration of the drug. For practical reasons, intravenous administration is often not possible, and in such cases "relative" bioavailability is determined by comparing the AUC's of the test drug and a secondary reference standard such as an orally administered solution of the drug or a dosage form which has been accepted as a standard (Gibaldi and Perrier, 1975a; Blanchard and Sawchuk, 1979).

A number of circumstances usually arise thereby making the determination of the availability of a drug following administration of a single dose difficult.

The plasma level of some drugs may be very low such that an assay method sensitive enough to measure these levels may not be available. Other drugs have very long half-lives, and the determination of the total area under a plasma concentration-time curve requires plasma samples to be obtained for excessively long periods of time. Thus, it is sometimes desirable to determine availability based on steady-state plasma drug levels following multiple dosing (Gibaldi and Perrier, 1975a).

For the estimation of availability, the area under the plasma concentration time curve (AUC) determination is required. The most commonly employed methods for calculating AUC's are the 'cut and weigh' method, planimetery, trapezoidal rule (Gibaldi and Perrier, 1975c) and computer application.

## 1.5 FACTORS AFFECTING BIOAVAILABILITY OF DRUGS

The compressed tablet is the most widely used oral dosage form, and is also commonly involved in drug bioavailability and bioequivalence problems. This is not surprising owing to the reduction in drug surface area in the compressed formulation and the wide variety of drug forms, tabletting methodologies, excipients and coating that are used.

Absorption may be affected not only by the different dosage forms, but also by similar dosage forms from different manufacturers and different batches of a dosage form from a single source. The most common conventional oral dosage forms, in decreasing order of relative dissolution and absorption rates are solutions, suspensions, capsules, tablets, coated tablets and sustained-release preparations.

Dosage form related factors which can produce profound differences in bioavailability include formulation and manufacturing variables such as particle sizes, the chemical form and solubility of the drug; the type and quantity of excipients used and the compaction pressure (Blanchard and Sawchuk, 1979). Other factors include crystal form, salt, complex and pH of the active ingredients.

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 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 non-solvated form of the drug. Drugs for which significant differences in dissolution rates have been observed include caffeine, theophylline and glutethimide.

Complex formation is a mechanism that can influence the biopharmaceutical properties of a drug. Drugs may interact reversibly to form complexes with substances occurring in the body, with other drugs 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 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 drug effect was clearly demonstrated by an outbreak of phenytoin toxicity in Australia. The toxicity occurred when patients previously maintained on a stable dose of "Dilantin" capsules in which calcium sulphate was used as inert excipient, were given a different brands of capsules in which the calcium sulphate was substituted for 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 itself 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 drug 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 disintegration particles. Dissolution rate may itself be affected by the granulation, or other mixing procedure; the degree of compression and the presence of fillers, binders or surface active agents.

Interaction between drugs may influence the rate and extent of drug absorption. Such interactions may be direct, as in chelation of tetracycline by polyvalent metal ions, or indirect as with the increased rate of acetaminophen (paracetamol) absorption due to the stimulating effect of metoclopromide on the stomach emptying rate. However, propantheline reduced the rate of acetaminophen absorption by delaying the stomach emptying rate (Welling, 1980).

The volume of co-administered fluid may have a considerable effect on drug absorption. Although from a physical view-point, drug absorption might be expected to occur more readily from concentrated solutions than from dilute solutions, the reverse appears to be the case. The pharmacological activity of sodium phenobarbitone and also the circulating levels of salicylate, were greater from dilute than from concentrated solutions (Borowitz and Moore, 1969). Increased bioavailability of drug from dilute solution may be related to the tendency for a compound to follow net mucosal to senosal fluid flux when hypotonic solutions are introduced into the gastrointestinal tract. However, it may be related also to more rapid stomach emptying of large fluid volumes.

The most commonly used route of administration of drug is the oral route which involve absorption occurring mainly from the gastrointestinal tract. Through this route of administration, apart from the physico-chemical properties of the drug, the extent and rate of absorption are determined by other factors which include disintegration in the gastrointestinal tract, dissolution in intestinal fluids, degradation by gastric enzymes and acids, changes of stomach acidity, presence of food or other drugs, inadequate blood supply and changes in the rate of stomach emptying or intestinal motility.

Other factors affecting drug disposition include age, pregnancy, diet, stress, disease state, presence of other medications, cigarrette smoking and exposure to chemicals such as insecticides and various organic solvents. Gender is also a significant variable in some animals but appears to be less so in humans (Paxton, 1981).

The systemic availability of drugs may also be reduced by being metabolized in the gastrointestinal epithelium during absorption, or by the liver before reaching systemic circulation (first pass effect).

Influence of food and specific dietary components on drug absprption has been extensively reviewed by Welling (1977), Melander (1978). The reviews reflect the complex and unpredictable nature of drug-food interactions, and the difficulty of establishing patterns which may be used as guidelines for optimum drug therapy. The presence of food has been shown to decrease, delay, increase and often not to affect drug absorption. Observed effects have been influenced by the type of drug and dosage form, the nature and size of meal, fluid intake, the conditions of the subject and the time sequence between eating and drug administration (Wellings, 1977).

Although, drug bioavailability studies are routinely carried out in healthy human volunteers,

drugs are administered in practice to patients who may be suffering from a variety of illnesses. Diseases concerning the cardiovascular system, the liver and the gastrointestinal tract may alter circulating drug or metabolite level after oral dosing. Surgical removal of part of the gastrointestinal tract reduces the absorption of most compounds, suggesting that patients who have undergone such procedures may need to be monitored carefully when taking drugs whose absorption efficiency is critical.

## 1.6 DISSOLUTION RATE STUDIES (INVITRO AVAILABILITY)

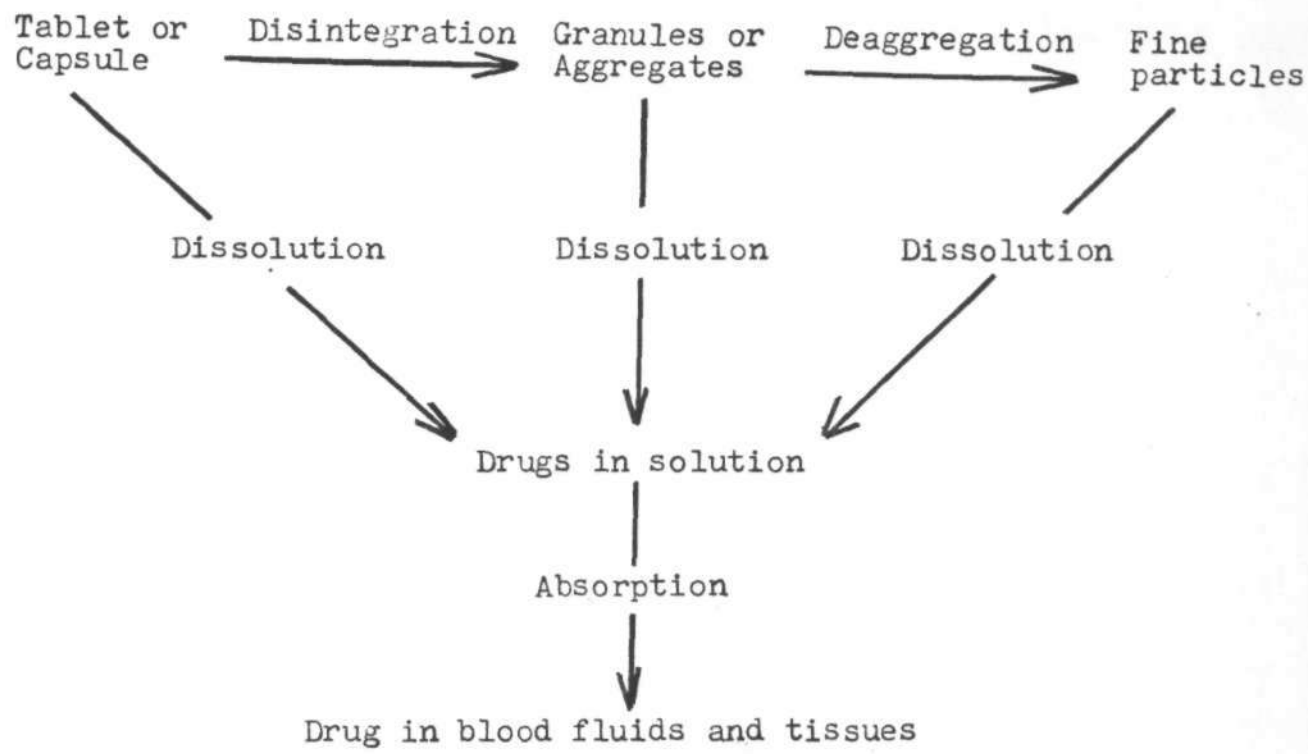
The study of the kinetics of drug release from a solid dosage form is of great importance in the formulation of drugs to ensure that a given dose of an active ingredient is released to the patient, in suitable amounts after administration.

The dissolution rate studies is used in measuring the time required for a given percentage of a drug substance in a solid dosage form to go into solution under specified conditions. It is an invitro test and is intended to provide a step towards the evaluation of the physiological availability of the drug substance, but as currently described, it is not designed to measure the safety or the effectiveness of the solid dosage form. Both parameters must be demonstrated

initially by means of an appropriate invivo studies and clinical evaluation. Since drug absorption and physiological availability depend on having the drug substance in the dissolved state, suitable dissolution characteristics are an important property of a satisfactory solid dosage formulation.

The physical characteristics and the composition of the drug can have effect on the rate of disintegration, deaggregation and dissolution of the drug. These can, as such, affect the rate of absorption and resultant blood levels of the drug. When drugs are administered in solid dosage forms, the rate at which they are released into solution may often be slower than other processes involved in absorption, so that dissolution becomes the rate-limiting factor in absorption. In such cases, anything that affects dissolution will also affect absorption, so that dissolution becomes the rate-liming factor in absorption. Dissolution can therefore, affect the onset, intensity and duration of a response by controlling the overall bioavailability of the drug from the dosage form.

The steps involved in the disintegration, deaggregation and dissolution of compressed tablets or powders in capsules are shown in Scheme I.




SCHEME I: Schematic representation of disintegration, deaggregation and dissolution of a drug from solid dosage forms (from Poole, 1979).

Dissolution occurs at all levels from the intact dosage form, the granules and the particles. Since the surface area of the drug in the intact dosage is limited, the rate of dissolution from the intact compact is negligible except for very soluble drugs (Poole, 1979). It has been reported that the rate of absorption of a drug is the function of its dissolution rate (Levy and Hayes, 1960). Wagner (1971b) demonstrated that the process involved when a solid dosage form is exposed to fluid is dependent on dissolution.

Dissolution rate test is used as a means of quality assurance of a good pharmaceutical dosage form. It is used

to ensure that a given product is uniform within the same batch and from batch to batch during an in-process quality control. The characteristics of the pure drug or the effects of excipients, the processing and manufacturing variables can be assessed using dissolution rate test. Also the stability of a drug after an extended shelf-life can be estimated.

The rate of dissolution depends on the surface area of the solid, which in turn, depends on how finely the drug is comminuted or pulverized. It also depends on the energy and energy states within crystals of the drug.

## 1.7 FACTORS INFLUENCING DISSOLUTION RATE

The rate of dissolution of a drug can be affected by solubility, particle size, disintegration rate, chemical form and surface area of the solid dosage form (Ravens, 1981).

In the process of an in-vitro dissolution rate test, it is essential to control some factors which are known to cause variations within and between control laboratories in order to ensure reproducibility of results.

In relation to the dissolution rate apparatus, it is important to use a standardized equipment designed in such a way as to simulate or feign invivo dissolution processes. The official dissolution apparatus as

specified in the British Pharmacopoeia (BP 1988) and the United States Pharmacopoeia (USP 1980) requires the stimer shaft eccentricity to be an imperceptible wobble, the sampling position to be half way between the basket and the wall and half way up the basket; the speed of the drive motor to be constant (100 r.p.m).

The British Pharmacopoeia (BP 1988) and the United States Pharmacopoeia (USP 1980) specified the use of deaerated dissolution medium. Aerated medium contains air bubbles which can form on the surface of the basket mesh altering the flow of the medium from the basket. Samples are to be filtered to remove solid particles which will give false spectrophotometric readings.

The temperature of the dissolution medium and the rate of agitation seems to be the only two invivo parameters which can be reproduced with ease in the laboratory. The British Pharmacopoeia (BP 1988) and the United States Pharmacopoeia (USP 1980) specify a temperature of 37 $\pm$ 0.5°C and agitation rate of 100 r.p.m. These are important since the dissolution of drugs depends on the temperature of the medium and the rate of agitation.

## 1.7 RELATIONSHIP BETWEEN BIOAVAILABILITY AND DISSOLUTION RATE

Any drug upon administration must be able to deliver the active ingredient in the most suitable form for absorption i.e. able to deliver the incorporated medicament into the blood stream. The bioavailability of oral

dosage forms is mostly evaluated by the rate of dissolution of the drug. Dissolution rate is useful in various ways and the results obtained in this test depends on the purpose for which it was performed.

(i) Dissolution rate tests are performed in conjunction with invivo absorption studies during the early stages of product design, and they indicate the extent to which the released characteristics of the dosage form would influence blood levels of the drug.

(ii) Dissolution rate test is also performed in research and development for assessing factors which govern the release of active ingredient and for evaluating the influence of processing variables such as excipients on dissolution rate and subsequently on drug bioavailability.

(iii) Dissolution rate test provides a rational basis for improving and optimizing a formulation where evidence of limited absorption exists by monitoring changes in the formulation (e.g. particle size or salt form).

The compressed tablet which is the most widely used dosage formulation also poses some or most of the difficult pharmaceutical problems which may influence its bioavailability. One of such problem is the

decreased effective surface area of the active ingredients as a result of granulation and compression of the drug particles. Bioavailability of drug from a tablet usually becomes reduced unless it readily disintegrates and deaggregates when it comes in contact with the body fluids. Bioavailability problems may now be anticipated from drug products that have slow rate of dissolution. This slow dissolution may be a reflection of:-

(1) The low solubility of the active drug moiety.
(2) The importance of particle size or surface area on dissolution of the active drug.
(3) Presence of different crystal forms of the drug, and
(4) The presence of inactive excipients in the drug product which may interfere with the dissolution of the active drug.

## 1.9 SALIVARY EXCRETION OF DRUGS

Saliva is defined as a liquid secreted in the mouth to soften food in readiness for digestion and body use. The pH of saliva range from 5.8 - 7.1 (mean 6.4). About 500-1500ml of saliva is secreted per day at approximately 0.6ml per minute (Wolff et al., 1979).

Saliva performs a number of important functions. It facilitates swallowing, keeps the mouth moist, serves as solvent for the molecules that stimulates the taste buds, aids speech by facilitating movements of the lips and tongue and keep the mouth and teeth clean.

The observation that drug concentration in saliva are often proportional to the concentrations in plasma has led to the suggestion that in therapeutic drug monitoring, or in pharmacokinetic studies in general, saliva might be substituted for plasma. Saliva can be obtained by non-invasive techniques, generally following the stimulation of salivary flow by chewing on semi-solid material or by spraying citric acid on the tongue.

There is evidence that many organic compounds enter saliva by passive diffusion process, where lipid solubility and the degree of ionization dependent on plasma and salivary pH, are important factors. In addition it has been shown for some drugs that their concentration in saliva equals the free or protein-unbound concentration in plasma, which is an advantage because drug plasma concentration generally represent both bound and unbound drug. If saliva is to be used in therapeutic monitoring then the saliva-to-plasma concentration ratio should be constant over a wide plasma concentration range. For a number of anticonvulsant drugs and digoxin a consistent correlation between their concentrations in saliva and plasma has been established under steady-state conditions (Reynolds et al., 1976). Many drugs have not been sufficiently studied during continuous treatment, because single dose studies showed great discrepancies in saliva/plasma ratio (Dahnof and Breimer, 1978).

The use of saliva concentrations is an ideal method for drug therapy monitoring because it is non-invasive, a large number of samples can be obtained and is particularly useful in ambulatory, pediatric and geriatic patients.

In principle, the analytical procedures which have been developed for the assay of drugs in plasma are equally suitable for drug monitoring in saliva. However, salivary concentrations of some high protein-bound drugs are far lower than plasma concentrations which may require modification of existing procedures or development of more sensitive methods (Wolff et al., 1979).

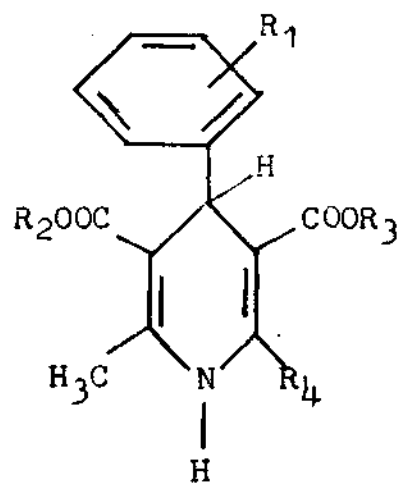
# CHAPTER TWO

# GENERAL LITERATURE REVIEW

## 2.1 HISTORY AND CHEMISTRY

Nifedipine is an active substance belonging to a new class of compounds (1,4-dihydropyridines) which are of great importance within the cardiovascular field as calcium channel blockers. It is reported to be the most superior pharmacologically in its class. Other compounds in this class include nitrendipine, nimodipine, nisoldipine, nilvadipine, nicardipine, bendipine, manidipine, MPC-1304, felodipine, NB-818, amlodipine, isradipine and flordipine (Anhoff and Persson, 1990).

The general chemical structures of dihydropyridines are given below (Scheme 2).




SCHEME 2: General Chemical Structure of Dihydropyridines.

| Compound | MW | $R_1$ | $R_2$ | $R_3$ | $R_4$ | Other substituents |
|---|---|---|---|---|---|---|
| Nifedipine | 346 | 2-$NO_2$ | $CH_3$ | $CH_3$ | $CH_3$ | |
| MPC-1304 | 388 | 2-$NO_2$ | $CH_3$ | $CH_2COCH_3$ | $CH_3$ | |
| Nitrendipine | 360 | 3-$NO_2$ | $CH_3$ | $CH_2CH_3$ | $CH_3$ | |
| Nimodipine | 418 | 3-$NO_2$ | $CH(CH_3)_2$ | $(CH_2)_2OCH_3$ | $CH_3$ | |
| Nisoldipine | 388 | 2-$NO_2$ | $CH_3$ | $CH_2CH(CH_3)_2$ | $CH_3$ | |
| Nivaldipine | 397 | 3-$NO_2$ | $CH(CH_3)_2$ | $CH_3$ | CN | |
| Nicardipine | 479 | 3-$NO_2$ | $CH_3$ | $CH_2N(CH_3)CH_2C_6H_5$ | | |
| Benidipine | 505 | 3-$NO_2$ | $CH_3$ | (R)-Benzyl-3-piperidinyl | $CH_3$ | |
| Manidipine | 610 | 3-$NO_2$ | $CH_3$ | 2-(4-Diphenylmethyl)-1-piperazinyl)ethyl | $CH_3$ | |
| Felodipine | 384 | 2,3-Cl | $CH_3$ | $CH_2CH_3$ | $CH_3$ | |
| NB-818 | 457 | 2,3-Cl | $CH_3$ | $CH(CH_3)_2$ | $CH_2OCONH_2$ | |
| Amlodipine | 419 | 2-Cl | $CH_3$ | $CH_2CH_3$ | $CH_2O(CH_2)_2NH_2$ | |
| Isradipine | 371 | =N, =N, O | $CH_3$ | $CH(CH_3)_2$ | $CH_3$ | |
| Flordipine | 500 | 2-$CF_3$ | $CH_2CH_3$ | $CH_2CH_3$ | $CH_3$ | 1-(2-(4-morpholinyl)-ethyl) |

The preparation containing nifedipine (4-2'-nitrophenyl)-2,6-dimethyl-3,5-dicarbomethoxy-1,4-dihydropyridine) as active ingredient is called adalat.

It is available in three formulations:

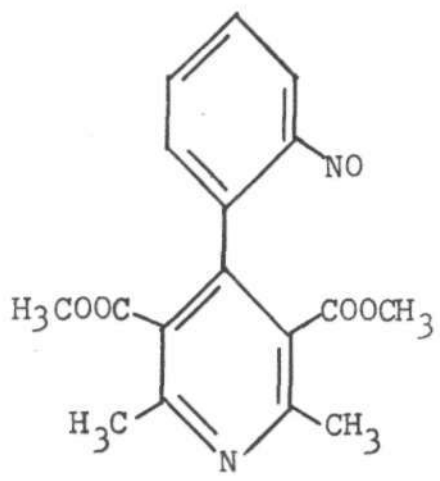
(1) Adalat (capsules of 10mg nifedipine)

(2) Adalat 5 (capsules of 5mg nifedipine)

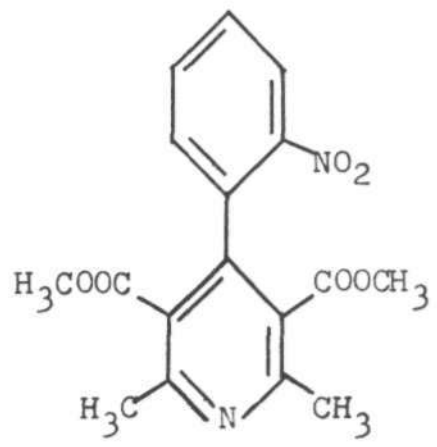
(3) Adalat retard (tablets of 20mg nifedipine).

The nitrogroup is firmly bound to the benzene ring. As a result, the chemical reactivity of this compound differs fundamentally from that of the nitrates in therapeutic use.

Nifedipine is a sensitive substance which decomposes in day light to give the 4-(2'-nitrosophenyl)-pyridine homologue (Scheme 3) and under ultraviolet light to give the oxidation product, 4-(2'-nitrophenyl)-pyridine homologue (Scheme 4) (Piergiorgio et al., 1981).




4-(2'-nitrosophenyl)-pyridine
Scheme 3




4-(2'-nitrophenyl)-pyridine
Scheme 4

Nifedipine is an odourless and flavourless crystals. The substance is highly photosensitive, particularly when dissolved, but is protected from light and consequently stable in the capsule form provided.

Nifedipine is readily soluble in acetone and chloroform, more sparingly soluble in ethanol and practically insoluble in water and carbon tetrachloride.

## 2.2 ABSORPTION AND DISTRIBUTION

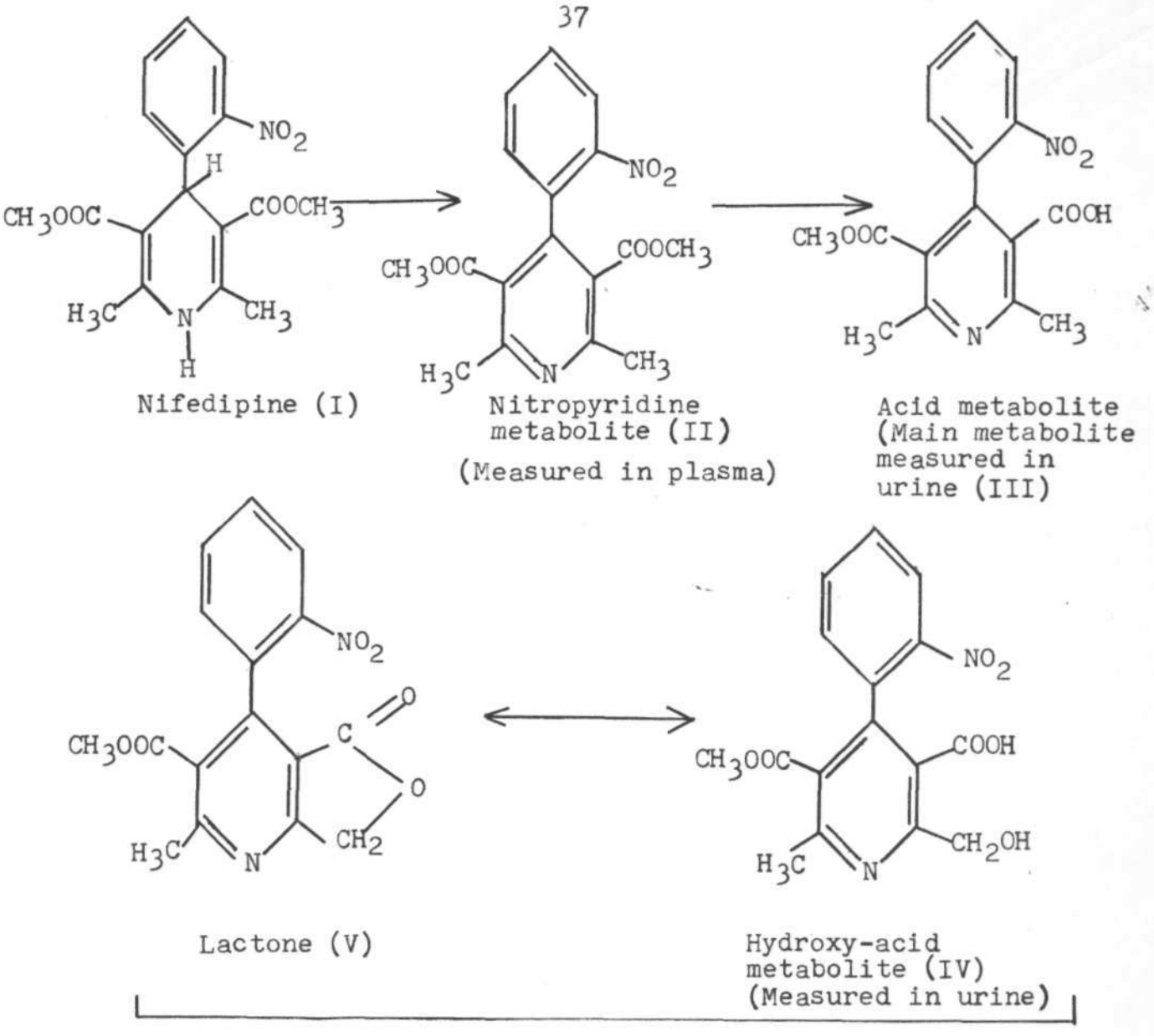
The active ingredient of Adalat, nifedipine is 90% or more absorbed in normal subjects as well as in patients (Horster et al., 1972). Absorption begins quickly. With the use of a $^{14}C$-labelled compound, activity is detectable in the serum 3 minutes after sublingual and at most 20 minutes after oral administration. With both routes of administration, maximum serum concentrations are attained in 1-2 hours after administration (Patzchke et al., 1975).

In view of the short half-life of approximately 1-3 hours, there is no reason to assume that the unchanged active ingredient would accumulate during prolonged treatment. The major portions of both the active ingredient and its metabolites are bound to serum proteins. Portions that are not bound to serum proteins rapidly diffuse into all organs and tissues. Animal studies showed that after administration of a

$^{14}$C-labelled-nifedipine, the concentrations in the (exsanguinated) myocardium were markedly higher than those in skeletal muscle. Within two days, more than 97% of the intravenously or orally administered activity was eliminated from the body. At this time the concentration remaining in the liver was less than 0.4%. There is no evidence of selective storage of the substance or its metabolites in any of the tissues (Duhm et al., 1972).

## 2.3 METABOLISM AND EXCRETION

Nifedipine is a dihydropyridine calcium channel blocker which undergoes extensive first-pass metabolism in man following oral administration (Forster et al., 1983; Waller et al., 1984). Metabolism is apparently via a single pathway involving initial oxidation to give the pyridine analogue, followed by conversion to two metabolites (acid and hydroxyacid) which are excreted in urine (Horster et al., 1972; Scheme 5).




SCHEME 5: Pathways of metabolism of nifedipine (based on Horster et al., 1972).

Both metabolites, in contrast to the dihydropyridine derivative are pyridine derivatives. In the main acid metabolite (III), one of the two ester groups is hydrolysed to free acid. The nitro-group on the benzene ring is unchanged. In the other hydroxy-acid metabolite (IV), the

methyl group adjacent to the carboxyl group has been oxidised to a hydromethyl group (Raemsch, 1980).

The structures of the two main metabolites have been confirmed by thin-layer chromatography, high pressure liquid chromatography, high voltage electrophoresis, mass spectroscopy, infra-red and nuclear magnetic resonance spectra as well as by synthesis (Medenwald et al., 1972; Kondo et al., 1980). These metabolites which were detected in animal studies (rat, dog) were also demonstrated by chromatography in human serum and urine (Schlossmann et al., 1975; Kondo et al., 1980). They are pharmacologically inactive (Schlossmann et al., 1975).

Following sublingual or oral administration of 10mg of Adalat per subject, 70-80% of the dose is renally excreted in the form of metabolites. More than 90% of this quantity is contained in the 24-hour urine. The half-life of excretion is about 4-5 hours. The portion that is not excreted via the kidney is excreted with faeces via liver and bile (Horster et al., 1972).

## 2.4 SIDE EFFECTS

Side effects from nifedipine are dose related and occur in approximately 20% of patients (Lewis, 1983). The most common side effects occur secondary to the vasodilating properties of nifedipine and include

headache (7%), flushing (5-7%) and dizziness (3-12%). The character, severity and incidence of side effects with nifedipine use is no greater during long term (>6 months) treatment with the drug than during shorter courses or therapy (Terry, 1982). The side effects may be mitigated by reducing the nifedipine dose or by combining the drug with a β-blocker. This combination of nifedipine and β-blockers has not been found to place patients at greater risk than sole nifedipine therapy and thus may be advantageous in the treatment of angina pectoris or hypertension in patients with compromised cardiovascular function. Discontinuance of the drug due to side effects has been necessary in 2 to 6% of patients (Gerstenblith et al., 1982; Terry, 1982), while the occurrence of a true "withdrawal syndrome" has not been noted.

## 5 DRUG INTERACTIONS, DOSAGE AND ADMINISTRATION

Most studies on nifedipine suggest that it has no effect on plasma digoxin concentrations, although, some studies have reported increased concentrations and clinical effects of digoxin. Nifedipine does not alter the pharmacokinetics of metoprolol, atenolol, nadolol or propanolol, but a few case reported have described severe hypotension or cardiac failure associated with nifedipine/β-blocker use in high risk patients with severe angina pectoris, triple-vessel disease, impaired left ventricular function and a prior history or myocardial infarction.

For the clinical management of patients with angina pectoris, hypertension or Raynaud's phenomenon, nifedipine dosage should be individualised. The current recommendation is for a starting dosage of 10mg capsule 3 times daily, during or after meals and this can be carefully titrated up to 20mg 3 times daily in patients with hypertension, Raynaud's phenomenon and up to 90-120mg per day (in divided doses) in patients with angina. If an immediate effect is required, the capsules should be bitten open and the liquid contents allowed to remain in the mouth for faster sublingual absorption. A sustained release tablet formulation of nifedipine is now available for the treatment of hypertension. The recommended dosage is 20mg twice daily, after food, and can be increased if necessary to 40mg twice daily.

## 2.6 PLACE OF NIFEDIPINE IN THERAPY

Clinical trials to date support the view that nifedipine should be considered a first line choice in patients with a predominant vasospastic component to their angina, i.e variant or unstable disease, and in those patients whose angina is complicated by hypertension or congestive heart failure. There is also increasing evidence that nifedipine, used alone, may be a useful alternative in the clinical management of stable exertional angina. However, further well

designed comparative trials are necessary to fully define its position in relation to other drugs such as β-blocker and nitrates. Combination of nifedipine and a β-blocker have always produced more favourable results than monotherapy. However, if this combination is used in patients with impaired left ventricular function, care should be exercised, since there have been isolated reports of excessive negative inotropic or hypotensive effects.

Nifedipine has also been used successfully in patients with various grades of hypertension. Sublingual nifedipine is possibly the drug of choice in situations requiring acute blood pressure reduction because of its convenient route of administration, and its lowering of elevated blood pressure without over-correction or excessive adverse effects. Nifedipine is also an extremely useful adjunct for controlling blood pressure in patients refractory to conventional treatment regimens with combinations of β-blockers, diuretics and various vasodilators. Conflict still surrounds the suitability of nifedipine, used alone, in the long term management of patients with mild to moderate hypertension. It may be considered an effective and reasonably safe alternative to β-blockers and diuretics, but comfirmation in well designed long term trials is still needed. A sustained release formulation of nifedipine, allowing twice daily administration has been marketed, and this preparation

appears to maintain a 24-hours control of blood pressure as well as the conventional capsule formulation of nifedipine.

Nifedipine has been used in the management of Raynaud's phenomenon and more effectively reduced the symptoms than did prazosin. It would seem to be a rational first-line choice in this clinical setting.

Thus, nifedipine has shown itself to be a useful addition to the drugs available to the clinician for the management of angina and/or hypertension. It may also be of particular benefit to patients with Raynaud's phenomenon and preliminary data suggest it may have a role in treating pulmonary hypertension, especially if some predictor for identifying those likely to respond can be found.

## 2.7 OBJECTIVE AND SCOPE OF STUDY

Nifedipine, a dihydropyridine calcium channel blocker is strongly lipophilic, highly non-polar, practically insoluble in water and only slightly soluble in intestinal fluids (Kozjek et al., 1987). Food constituents may interact directly or indirectly with drugs in a number of ways. Results of various studies on the influence of food and diet on the gastrointestinal absorption of a number of drugs have shown that the observed effect of food on drug absorption is a function of the type and size of meal, the chemical and physical form of the drug, the

dosage form and the time relationship between eating and drug administration (Wellings, 1977).

Although, Nigerian foods contain essentially the same major constituents comparable to similar types of foods in other countries, it is clear that very little work has been reported using Nigerian foods and Nigerian subjects specifically. Bearing in mind that most clinical tests have been performed using individuals who take balanced diet, it is probable that different results might be obtained using Nigerian individuals and Nigerian food. The objective of this work therefore is to assess the influence of a low-fat meal which cuts across most breakfast table in Nigeria, consisting of corn gruel (pap) and fried bean cake (akara) on the pharmacokinetic and bioavailability of nifedipine tablets using healthy subjects.

The study also aim at quality control assessment of different brands of nifedipine tablets. The effect of temperature, ultraviolet radiation and different light conditions on the stability of nifedipine tablets and solutions will be investigated.

The study will employ a sensitive, rapid and selective High Performance Liquid Chromatographic (HPLC) method for estimating nifedipine in tablets, plasma and saliva samples.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 DRUGS

1. Adalat retard tablets containing 20mg nifedipine, Production Series No. BL 711, Manufacturer Bayer, Lever Kusen, West Germany, referred to in the text as "formulation S".

2. Nifedin retard tablets containing 20mg nifedipine, Production Series No. 8200, Manufacturer Medicalex SRL-Italy, referred to in the text as "formulation O".

3. Adalat retard tablet containing 20mg nifedipine, Production Series No. 782E, Manufacturer MS-0429-0001, Farmacêutica Responsável Shidue Ishitani CRF, referred to in the text as "formulation K".

## 3.2 EQUIPMENT

- Waters 204 HPLC Model equipped with 441 Model U.V. detector fitted with 254nm filter, Model U6K septum-less injector and SE 120 model recorder was used. The column used for the chromatographic separation was a μ-Bondapak radial Pak catridge, Polyethylene 15cm x 8.0mm I.D. packed with 10 μm reversed phase $C_{18}$-support, Waters Part No. 85721, (Waters Associates, Inc., Milford).

- Gallenkamp melting point apparatus.
- SP8-100 Ultraviolet Spectrophotometer (Pye-Unicam Ltd, York Street, Cambridge, England CB1 2PX).
- Camag. U.V. lamp type 2900.
- Erweka dissolution apparatus and accessories, Erweka Appatebau-G.M.B.H. 6056. Heusenstamm k.r., Offenbach-Main.
- Erweka disintegration apparatus and accessories, Erweka Appatebau-G.M.B.H. 6056 Heusenstamm Kr, Offenbach-Main.
- Autovortex-Whirlmixer (Gallenkamp, England).
- Mechanical shaker (Gallenkamp, England).
- Centrifuge (Gallenkamp, England).
- pH Meter (PW 2418, Philips, England).
- Vacuum pump and compressor, RBS, Edwards, Britain.
- Analytical balance, Type H35 AR Mettler Gallenkamp.
- Quickfit Conical centrifuge tubes 15ml.
- 100 µl Syringe (Hamilton Reno, Nevada).

REAGENTS

- Methanol - Analar grade (BDH Chemicals, Poole, England)
- Triethylamine - Analar grade (BDH Chemicals, Poole, England).
- Hydrochloric acid - Analar grade (BDH Chemicals, Poole, England)
- Tetrahydrofuran - Analar grade (BDH Chemicals, Poole, England).
- Sodium Acetate (Anhydrous) - BDH Chemicals, Poole, England).

- Disodium hydrogen phosphate - (BDH Chemicals, Poole, England).
- Acetic acid - Analar grade (BDH Chemicals, Poole, England).
- Dichloromethane - (G.P.R., Hopkin & William, Chadwell Heath, Essex, England).
- 4-Dimethylaminobenzaldehyde - (BDH Chemicals, Poole, England).

METHODS

## 3.3 IDENTIFICATION TESTS

### 3.3.1 U.V. Absorption Spectra

A solution (5 µg/ml) of the three brands of nifedipine and that of the standard powder was prepared in methanol and 0.01M hydrochloric acid respectively. The absorbances and the absorption spectra corresponding to the three brands and that of the standard authentic sample was then recorded using methanol and 0.01M hydrochloric acid as the reference respectively.

### 3.3.2 Thin Layer Chromatography

The B.P. 1988 addendum 1990 thin layer chromatography method is descried for the identification test. The apparatus consists of a coated glass plate (20cm x 10cm) which was prepared by spreading a homogenous suspension of the coating substance (silica gel) to form a uniform layer 0.1 to 0.3mm thick on the carefully cleaned plate by using a spreading device designed for the purpose.

The coated plate was allowed to air dry and then heated in an oven at 105° for 1 hour and allowed to cool protected from moisture.

Method

A TLC tank was prepared by lining the walls with sheets of filter paper; the mobile phase made up of a mixture of 60 volumes of cyclohexane and 40 volumes of ethylacetate was poured into the tank, saturating the filter paper in the process, to a depth of 5 to 10mm. The tank was closed and allowed to stand for 1hr at 25°C.

A narrow strip of the silica gel was removed from the vertical sides of the chromatoplate and a 5 µl of 50 µg/ml methanolic solutions of standard nifedipine and brands S.O.K. were each applied in the form of circular spots about 2 to 6mm in diameter on a line parallel with one end of the plate. The spots were at least 10mm apart. The side of the plate was marked at a distance of 15cm from the line of application and the solvent was allowed to evaporate. The plate was then placed in the tank ensuring that it is as nearly vertical as possible with the spots above the level of the mobile phase. The tank was then closed and allowed to stand at 25°C until the mobile phase has ascended to the marked line. The plate was then removed, dried and visualised under ultraviolet light (254 nm).

### 3.3.3 Melting Point

The melting point was determined as described in B.P 1988 addendum 1990 under identification tests.

Method

A quantity of the powdered tablets (brands S, O and K) equivalent to 0.2g of nifedipine was extracted with four quantities each of 5ml of acetone, filtered and evaporated carefully to dryness on a water bath. A sufficient portion of the dried residue and the standard powder were transferred to a series of dry capillary tubes and packed well by tapping on a hard surface so as to form a tightly packed column about 3mm in height. The capillary tubes were then introduced into the melting point apparatus. The temperature at which the last particle disappeared was then taken and this was regarded as the melting point of the substance.

## 3.4 SOLVENT SYSTEM

In developing a suitable mobile phase for the analysis of nifedipine using the high-performance liquid chromatography with 4-dimethylaminobenzaldehyde as the internal standard, some solvent combinations were tried. The constituents of the mobile phase were premixed, filtered under vacuum and ultrasonicated

before use. The pH of the mobile phases was also noted.

The mobile phases tried consisted of either 0.01M disodium hydrogen phosphate (pH 6.1) or 0.01M sodium acetate buffer (pH 4) mixed with methanol or acetonitrile, tetrahydrofuran and triethylamine in different ratios (see results).

The effect of pH on the capacity factor of the mobile phases was also investigated to obtain the best separation of the drug and its internal standard (see results).

## 3.5 CHEMICAL ASSAY

### 3.5.1 HPLC Method

Twenty nifedipine tablets were accurately weighed and the average weight determined. These were then crushed with pestle and mortar into powder form. A quantity of the powder equivalent to 0.02g of nifedipine was weighed and transferred into a 50ml volumetric flask (wrapped with aluminium foil to prevent photodegradation) with 25ml methanol and shaken on a Gallenkamp mechanical shaker for twenty minutes. Sufficient methanol was added to produce 50ml. The solution was filtered and 1ml of the filtrate was diluted to 10ml with methanol.

0.5ml of the resulting solution was added to 0.5ml of 4-dimethylaminobenzaldehyde, 50 µg/ml (internal standard)

and then whirlmixed on a vortex automixer. 10 µl of the mixture was then injected into the HPLC. The peak height ratios were determined and the concentration determined from the calibration curve.

The percent content of nifedipine was calculated by using the formula:

$$\frac{\text{Concentration observed from calibration curve}}{\text{Expected Concentration}} \times 100$$

Calibration Curve

Standard nifedipine solution of 1mg/ml was prepared by weighing 50mg of nifedipine powder dissolved in a 50ml volumetric flask with methanol. The flask was wrapped with aluminium foil. The mixture was then shaken to obtain a homogenous solution. 1.0, 2.0, 3.0, 4.0 and 5.0ml of the standard solution were transferred into series of 50ml volumetric flasks, enough methanol was added to volume and solutions of various concentrations (20 - 100 µg/ml) were obtained and treated as below:

0.5ml each of the resulting solution (20 - 100 µg/ml) were pipetted into a 10ml centrifuge tube, each of which contained 0.5ml of 50 µg/ml of 4-dimethylaminobenzaldehyde (internal standard) and whirlmixed on a vortex automixer for 15 seconds. 10 µl each of the whirlmixed solution were then injected into the HPLC. The mobile phase used was a mixture of 55 volumes of methanol and 45 volumes

0.01M acetate buffer (pH 4.0). The pH of the mobile phase was adjusted to 5.2 with 0.2M sodium acetate solution. The flow rate was 2.0ml/min at room temperature, at approximate pressure of 2 x 1000 p.s.i. The chart speed was 0.25cm/min. The peak height ratios were determined and plotted against the respective concentrations.

### 3.5.2 U.V. Spectrophotometric Method

0.02g equivalent of the powdered tablets was dissolved in 25ml of methanol in a 50ml volumetric flask; sufficient methanol was added to produce 50ml. The flask was wrapped with aluminium foil to prevent photodegradation of drug and shaken for 20 minutes on a Gallenkamp mechanical shaker. The solution was filtered and 0.5ml of the filtrate was added to enough 0.1M hydrochloric acid in a 10ml volumetric flask and shaken for 5 minutes. The absorbance of the resulting solution was measured at the maximum 349nm using 0.1M hydrochloric acid as blank. The percent content of nifedipine was calculated using the relationship:-

$$\frac{\text{Absorbance of Tablet}}{\text{Absorbance of Standard Powder}} \times 100$$

Calibration Curve

A standard solution of nifedipine containing 0.5mg/ml was prepared from the 1mg/ml stock solution

of nifedipine by transferring 5ml of the stock solution to a 10ml volumetric flask and diluting with methanol. 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0ml of the standard solution were then transferred into series of 100ml volumetric flasks, sufficient 0.1M hydrochloric acid was added to volume and shaken for 5 minutes. Solutions of various concentrations (5.0 - 30.0 µg) were obtained. The absorbance was determined in 1cm cell at the wavelength of maximum absorbance at 349nm using 0.1M hydrochloric acid as blank. The graph of absorbance against concentration was plotted.

## 3.6 STABILITY STUDIES

### 3.6.1 Effect of Light

#### Procedure

(a) Nifedipine solution

0.5ml of 100 µg/ml of standard methanolic nifedipine solution was placed in series of 10ml centrifuge tubes labelled 0, 30, 60, 90, 120, 180, 240, 300 and 360 mins respectively and exposed to three sources of light: Direct sunlight, Fluorescent light and Red light. Each of the centrifuge tubes was withdrawn from the sources of light at the expiration of the particular time and then wrapped with aluminium foil and kept in a light proof box until prior to analysis.

To each of the centrifuge tubes, 0.5ml of 50 µg/ml of 4-dimethylaminobenzaldehyde as internal standard was added and then whirlmixed on a vortex automixer for 15 seconds. 10 µl of each of the whirlmixed solution were injected into the HPLC.

(b) Nifedipine tablets

Nifedipine tablets (20mg) were placed in series of clean watch glass labelled and exposed to the three sources of light as described under nifedipine solution above. Samples (20 tablets) were removed from the sources of light at the expiration of the particular time and assayed as described under 3.5.1.

3.6.2 ULTRA-VIOLET IRRADIATION

(a) Nifedipine solution

Nifedipine powder 50mg was dissolved in 50ml of methanol to give a solution of 1 mg/ml. The solution was wrapped with aluminium foil to prevent photodegradation. 10ml of the solution was diluted to 100ml to give a solution of 100 µg/ml. 60ml of the solution was placed in a beaker and irradiated with U.V. lamp with a fixed wavelength at 366nm. The time of irradiation extended from 0 - 72 hrs. 10ml samples were removed at intervals of 0, 6, 12, 24, 48, and 72h respectively and analysed as described under 3.5.1. Non-irradiated solution at room temperate was used as control.

(b) Nifedipine tablets

Nifedipine tablets (20mg) were placed in a clean watch glass and irradiated with a 366nm U.V. lamp. The time of irradiation extended from 0 - 72 hrs. Samples (20 tablets) each were removed at intervals of 0, 6, 12, 24, 48 and 72h respectively and assayed as described under 3.5.1. Non-irradiated tablets at room temperature were used as control.

3.6.3 EFFECT OF TEMPERATURE

(a) Nifedipine solution

5ml each of 100 µg/ml of standard nifedipine solution in methanol was placed in series of sample bottles labelled 25, 30, 35, 40, 45 and 50°C respectively and each placed on a water-bath which had been calibrated at the particular temperature on the tubes. The labelled tubes were then withdrawn from the water bath after 60 minutes at the respective temperature on the tubes and then wrapped with aluminium foil to prevent further effect of light on the solution until assayed.

(b) Nifedipine tablets

Nifedipine tablets were placed in a covered sample bottles and left on a calibrated water bath for 60 minutes each at 25, 30, 35, 40, 45, and 50°C respectively. The tablets were then withdrawn after the expiration of

the time at the respective temperature from the water bath and then assayed as described under 3.5.1.

## 3.7 DISSOLUTION RATE TESTS

The B.P. 1988 basket method is described for the dissolution rate tests. The apparatus consists of a cylindrical, flat-bottom glass vessel with an internal diameter of 100mm, a flanged upper rim, and a capacity of 1000ml; a variable speed motor; a cylindrical stainless steel basket assembly made of woven wire cloth, with a nominal aperture size of 425 micrometer; joined down one side with a narrow welded seam and with a flanged rim at each end. A speed of 100 revolutions per minute is used. The motor drives the basket by means of a shaft which is approximately 6mm in diameter and about 300mm long in such a way that the basket revolves smoothly and without perceptible wobble.

The shaft passes through the vessel and opening in the lid of the vessel and is positioned so that its axis is not more than 2mm from the vertical axis of the vessel. It carries at its lower end a stainless steel disc of 25mm in diameter in which there is a 2mm vent.

The vessel is securely clamped in a water bath such that displacement vibration from other equipment including water circulating device is minimised. The water bath is set to maintain the temperature of the dissolution medium in the glass vessel at $37 \pm 0.5^{\circ}C$. The apparatus for the dissolution of tablets and capsules is shown in Figure 8.

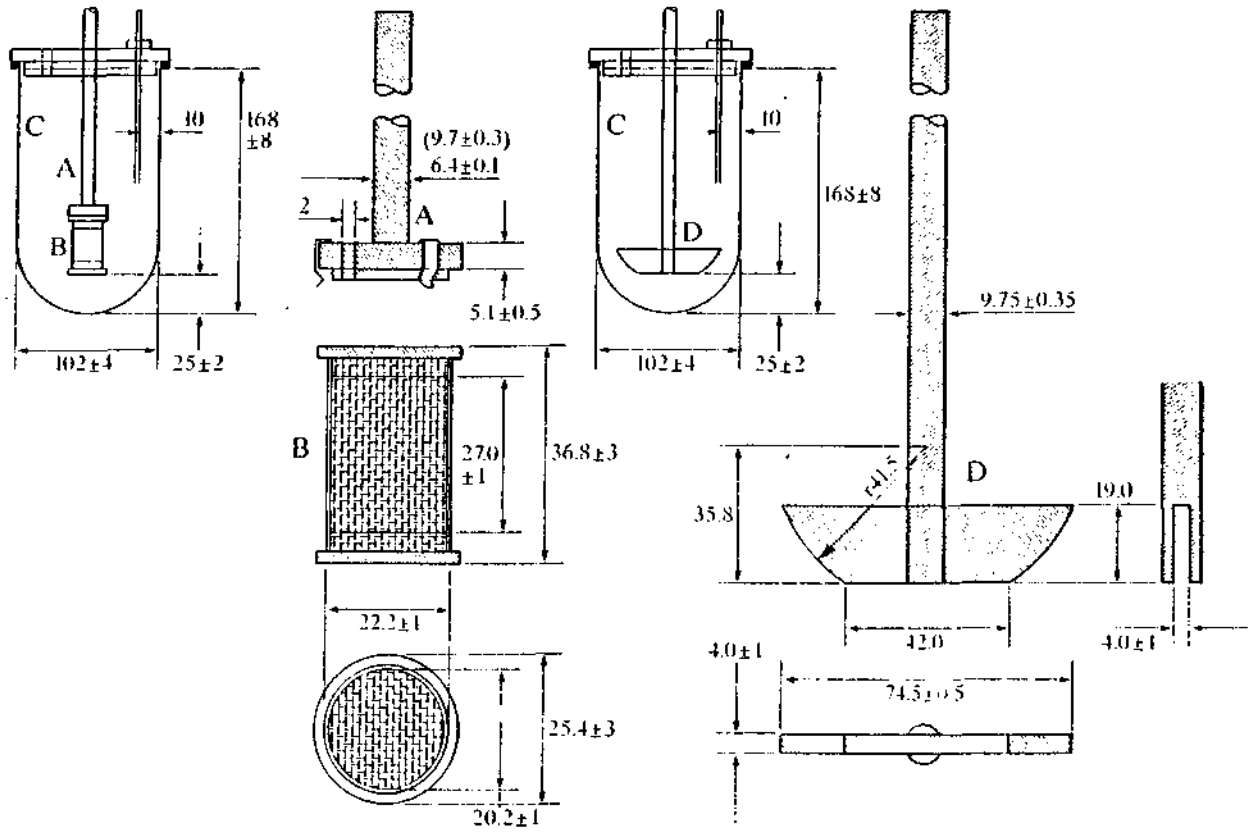



Fig. 8: Apparatus for the dissolution of tablets and capsules.

Procedure

One litre of 0.1M methanolic hydrochloric acid, as the dissolution medium, was introduced into the vessel and immersed in a constant temperature water bath, maintained at 37 ± 0.5°C. For each test, one tablet was placed in the basket. When the required temperature was attained, the apparatus was assembled and the distance between the bottom of the basket and the internal surface of the vessel adjusted to between 18 and 22mm. The motor was started at a rotational speed of 100 revolutions per minute. A sample of 10ml of the dissolution medium was withdrawn at 5, 15, 30, 45 and 60 minutes. Samples were withdrawn from a point half-way between the basket wall and the wall of the vessel, level with the midpoint of the side of the basket, to ensure that the normal pattern of flow of the dissolution medium was not disturbed. An equivalent volume (10ml) of the dissolution medium previously maintained at 37 ± 0.5°C was replaced into the dissolution medium. The dissolution run was performed five times. Samples of the dissolved drug were filtered, and assayed by reference to a standard calibration curve. The absorbance of each sample was measured at a maximum of 349nm.

Cummulative concentration at a given time was calculated using the formula of Wurster and Taylor (1965).

$$Cn = Cn\ measured + \frac{X}{1000} \sum_{s=1}^{n=1} (Cn\ measure)$$

Cn measured denotes the spectrophotometrically measured concentration.

Cn denotes the concentration of nth sampling expected in the medium if previous sample had not been removed.

X is the volume of the sample removed at each time withdrawal was made.

1000 is the volume of the dissolution medium in millilitres.

Calibration Curve

50mg of nifedipine powder was accurately weighed, and dissolved in 100ml of methanol in a 100ml volumetric flask. Thus, the standard solution contains 500 µg/ml. 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0ml of the standard solution were transferred into series of 100ml volumetric flasks; enough hydrochloric acid was added to volume. Solutions of various concentrations (5 - 30µg) were obtained. Absorbances were measured at 349nm in 1cm cell using 0.1M hydrochloric acid as blank. Graph of absorbance versus concentration was plotted.

3.7.1 Disintegration Tests

This was carried out as specified by B.P. (1988) using an Erweka disintegration apparatus.(see Appendix 3).

Procedure

One tablet each was placed in the tubes of the basket, a disc was added to each tube. The assembly was suspended in the beaker containing distilled water maintained at 37 ± 0.5°C and the apparatus was operated for fifteen minutes.

The mean of five replica determination was calculated for each brand of tablets.

## 3.8 INVIVO ABSORPTION STUDIES

(a) Fasting State

Six healthy male volunteers aged 21-30 years (Mean 24 ± 3) and body weights 54 - 70kg (mean, 60 ± 3) took part in the study. Prior to the commencement of the study, the volunteers had to submit a written informed consent in a form of questionnaire (Appendix 5). None of the subjects had received any medication for a period of 2 weeks prior to the study. Beverages containing alcohol and caffeine were not permitted during the study.

On the day of each study, an indwelling cannula with a heparin lock was inserted into the antecubical vein of the volunteer. Basal measurements were performed after a minimal period of stabilization of 15 mins on a couch. Each subject was given a single tablet containing 20mg of nifedipine (Adalat) with 100ml of water after overnight fasting. They remained on the couch and continued to fast for a period of 4h. Blood samples (5ml) were withdrawn prior to the dose and at 0.5, 1, 2, 3, 4, 6, 8 and 12h after the dose. The samples were collected in heparinized tubes, centrifuged for 10 mins, and the plasma was harvested into glass tubes wrapped with aluminium foil, and kept at -20°C until analysis. Salivary samples (5ml) were also collected after stimulation with a rubber band at the same times as the plasma was collected. The saliva samples were collected in wrapped plain plastic containers, centrifuged for 10 mins and harvested into plastic tubes wrapped with aluminium foil and stored at -20°C until assayed.

(b) Non-Fasting State

The above procedure was followed after a 7-day washout period. The same dose (20mg tablet) was taken by each of the volunteers after a low fat meal consisting of 300ml corn gruel (pap) with loballs each of fried bean cake (akara) before dosing. All volunteers received equal and identical meal portions.

## 3.9 ANALYSIS

### 3.9.1 Extraction procedure

Different solvents and solvent combinations were tried in developing the extraction procedure. These are as follows:-
(a) dichloromethane (b) ethylacetate (c) chloroform (d) chloroform : dichloromethane (2:1) (e) dichloromethane : ethylacetate (5:3) (f) Ethylacetate : dichloromethane (1:2) (g) Ethylacetate : dichloromethane : chloroform (2:2:1).

Plasma (0.5ml) spiked with 50ng nifedipine was placed in a series of 15ml centrifuge tubes to which 500ng/ml of 4-dimethylaminobenzaldehyde had been added. Samples were extracted with 5ml each of the solvent/solvent combination, whirlmixed on an autovortex mixer for 15 seconds and centrifuged for 15 minutes. The aqueous layer was removed using an eppendof pipette and the organic extracts were transferred to a clean 15ml centrifuge tubes and evaporated to dryness under a stream of nitrogen at 40°C. The dry residue was dissolved in 100µl of methanol and 15 ul aliquot was injected into the HPLC.

The above extraction procedure was repeated with 50ng/ml of nifedipine without plasma and percentage extraction recoveries of nifedipine was calculated using the relationship:-

$$\frac{\text{Peak height ratio of plasma nifedipine extraction}}{\text{Peak height ratio of nifedipine extraction}} \times 100$$

3.9.2 Preparation of samples for HPLC analysis

Plasma samples (0.5ml) for HPLC analysis was deproteinized with 0.5ml acetic acid and extracted with 5ml dichloromethane and treated as described under the above extraction procedure.

Saliva samples (0.5ml) to which 1000ng 4-dimethylaminobenzaldehyde had been added was also extracted with dichloromethane and treated as in the above extraction procedure.

3.9.3 Preparation of standard solutions and standard curve

Working solutions of appropriate concentrations were prepared every week by dilutions of the stock solutions with methanol.

Plasma calibration curve was prepared by spiking blank plasma sample (0.5ml) with 10, 20, 30, 40, 50ng nifedipine and with 500ng 4-dimethylaminobenzaldehyde as internal standard. The saliva calibration curve was also prepared by spiking blank saliva samples (0.5ml) with 25, 50, 75, 100, 150 and 200ng nifedipine and with 1000ng 4-dimethylaminobenzaldehyde as internal standard. The samples were processed as described above in the preparation of samples for HPLC analysis.

Standard curve was constructed by plotting the peak height ratio of nifedipine to the internal standard against the drug concentration in each standard. The level of drug in an unknown samples (plasma or saliva) was derived from this curve.

# CHAPTER FOUR

## RESULTS AND DISCUSSION

Identification, chemical assay, dissolution rate and disintegration time tests on three brands of nifedipine have been studied. The effect of ultra-violet radiation, temperature and different light conditions on the stability of nifedipine tablets and solutions was also investigated. Also stated in this Chapter are the results of the influence of a low-fat meal on the pharmacokinetic and bioavailability of nifedipine tablet.

The results of the various studies were carefully recorded. Tables were drawn where appropriate as were graphical representations. The data were statistically analysed where appropriate. Students paired t-test was used for all the data except for those from dissolution studies where one-way analysis of variance was found to be more suitable. P values greater than 0.05 was considered to be statistically significant.

### 4.1 IDENTIFICATION OF NIFEDIPINE TABLETS

The ultraviolet absorption spectra of nifedipine in 0.1M hydrochloric acid and methanol is shown in Figures 9 and 10 respectively with three absorption peaks each at 206, 234 and 349nm in 0.1M HCl and 204, 220 and 363nm in methanol which demonstrates the effect

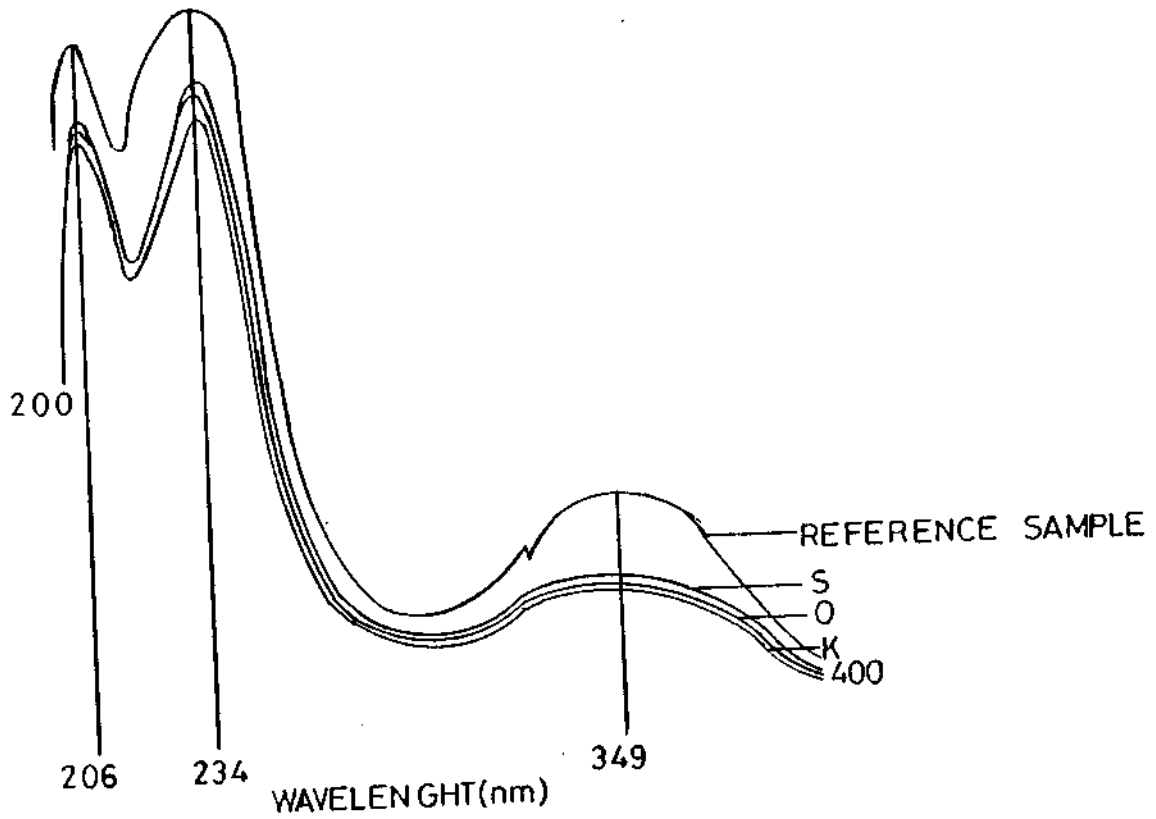



FIG.9 UV ABSORPTION SPECTRA FOR THREE BRANDS OF NIFEDIPINE IN 0.1M HYDROCHLORIC ACID (200 - 400nm)

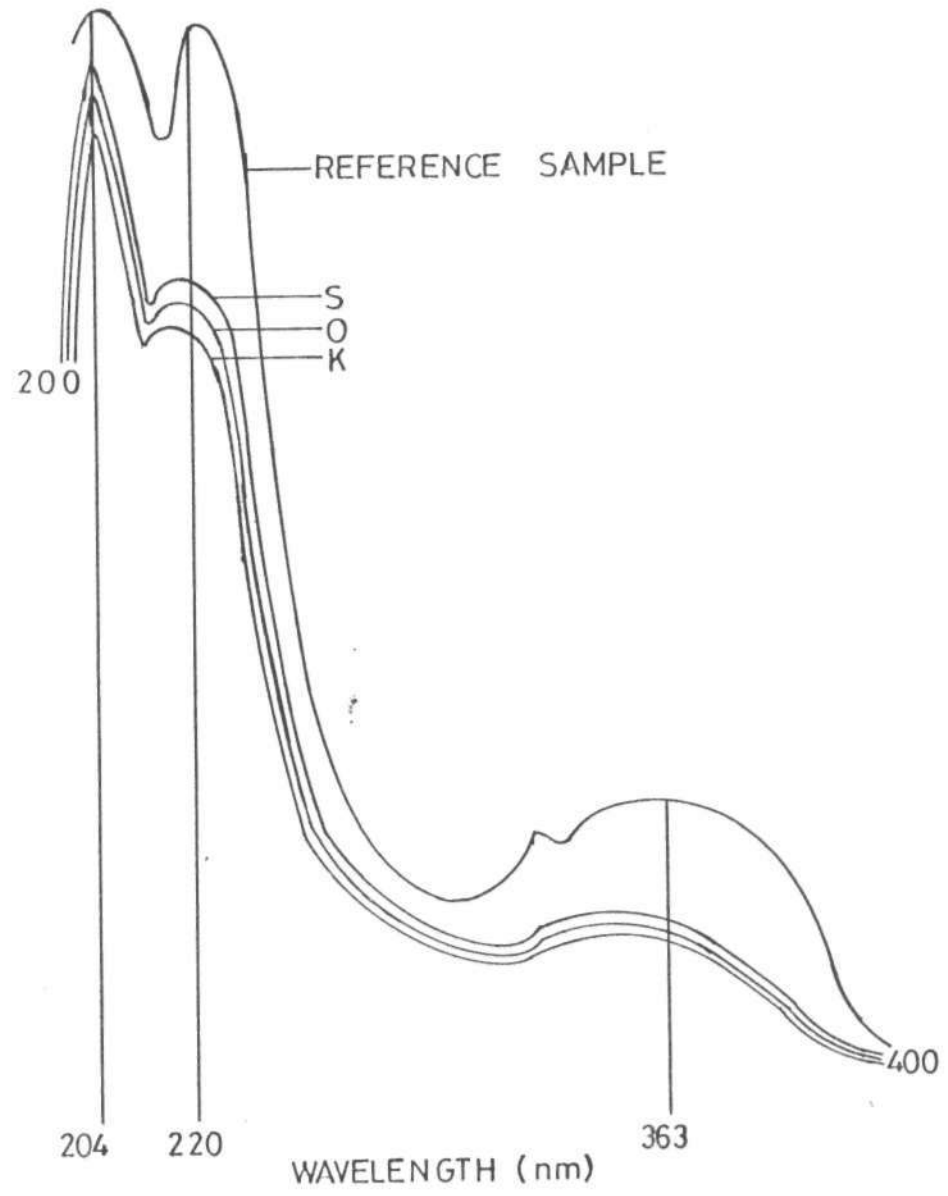



FIG.10 UV ABSORPTION SPECTRA FOR THREE BRANDS OF NIFEDIPINE IN METHANOL (200 - 400 nm)

of various substituents in the structure of nifedipine upon the position and intensity of absorption bands. These peaks corresponds to the aromatic nucleus, the pyridine nucleus and the large substituent groups attached to the structure of nifedipine. Substituents like chromophoric groups enhance and influence the absorption of a compound in the UV region, the larger the number of substituent present in a compound, the greater the absorbance. The effect of solvents upon the position and intensity of absorption bands is also indicated by the little difference observed in the absorbances of the samples in the two solvents used (0.01M HCl and methanol).

The result obtained with the thin-layer chromatography on examination of the spotted coated plate under UV light (254nm) gave four principal spots of the same size and in similar position with Rf value of 0.73. Each spot corresponds to the three brands investigated (S, O and K) and the reference sample.

The melting points of the extracted nifedipine residue obtained were 174, 173, 173 and 174°C for brands S, O, K and the reference sample respectively. The B.P. 1988 value for the melting point of nifedipine is 171-175°C. The values obtained for the samples are within acceptable limits of the official melting point range. These results indicate that the products in circulation were pure and unadulterated.

## 4.2 SOLVENT SYSTEM

The solvent combination tried in the development of the mobile phase used for this work are given in Table 1. The solvent pH, capacity or resolution factors and retention times of the sample for each of the solvent combination are also given in Table 1. From the table, it can be seen that the solvent systems C and G gave the highest resolution or capacity factor which is a measure of chromatographic separation. The time of analysis for the two solvent systems was less than 10 mins. Solvent system C was suggested for the work since it has the highest value of R, better peaks symmetry and no baseline depression as observed in the chromagram using other solvent combinations. Solvent system C has the added advantage of cost saving in that only two solvents were involved compared to solvent G where four different solvents were used. Table 2 and Figure 11 shows the effect of pH on the value of R (capacity factor). The result indicated that pH 5.2 was found to be most suitable for the solvent system. This is also within tolerable range for the life span of the column used (pH 3.4 - 6.5).

Another added advantage is that solvent system C allowed separation of the drug (nifedipine) and its internal standard from endogenous plasma and saliva samples or from the components of the pharmaceutical

Table 1: Chromatographic parameter for Nifedipine and 4-dimethylaminobenzaldehyde under different conditions using μ-Bonda-Pak $C_{18}$ radial-Pak catridge (Waters Associates).

| CODE | SOLVENT COMBINATION AND RATIO | pH | FLOW RATE | RETENTION TIME FOR NIFEDIPINE | RETENTION TIME FOR 4 DAB. | CAPACITY FACTOR R. |
|---|---|---|---|---|---|---|
| A | 0.01M disodium hydrogen phosphate: Methanol (45 : 55) | 7.1 | 2.0 | 9.2 min. | 5.6 min. | 1.80 |
| B | 0.01M disodium hydrogen phosphate: Acetonitrile (45 : 55) | 6.3 | 1.80 | 9.9 min. | 4.4 min. | 1.35 |
| C | 0.01M sodium acetate (pH 4): Methanol (45 : 55) | 6.8 | 2.0 | 9.4 min. | 5.8 min. | 2.50 |
| D | 0.01M Sodium Acetate : Acetonitrile: THF (5:6:1) | 5.2 | 1.5 | 4.5 min. | 3.0 min. | 1.33 |
| E | 0.01M Sodium Acetate (pH 4) : Acetonitrile (5:7) | 6.3 | 1.0 | 10.4 min. | 7.4 min. | 1.00 |
| F | 0.0.1M disodium hydrogen phosphate: MeOH : THF (45:50:5) | 5.5 | 1.5 | 11.8 min. | 8.00 min. | 1.50 |
| G | 0.01M $Na_2HPO_4$:MeOH:THF:TEA (45:55:10:5) | 7.2 | 1.5 | 9.8 min. | 5.4 min. | 2.00 |
| H | 0.01M Sodium Acetate : MeOH : THF : TEA (4:6:1:0.5) | 7.4 | 1.0 | 5.0 min. | 4.4 min. | 1.60 |
| I | 0.01M Sodium Acetate : MeOH : TEA (4:6:2) | 7.1 | 2.0 | 13.6 min. | 8.4 min. | 1.80 |
| J | 0.01M Sodium Acetate : MeOH : THF (45:50:5) | 6.6 | 2.0 | 11.6 min. | 7.80 min. | 1.25 |

Table 2: Effect of pH on the capacity factor (R) and resolution (d) for Nifedipine and 4-dimethyl-aminobenzaldehyde using solvent system 0.01M Sodium Acetate (pH 4) : Methanol (45:55). (pH 5.2) and μ-Bonda Pak $C_{18}$ radial Pak catridge (Waters Associates).

| pH of MOBILE PHASE | CAPACITY FACTOR (R) | RESOLUTION (d) |
|---|---|---|
| 4.4 | 2.40 | 1.65 |
| 4.8 | 2.70 | 1.70 |
| 5.2 | 3.80 | 2.40 |
| 5.6 | 3.20 | 1.80 |
| 6.1 | 3.05 | 1.90 |
| 6.5 | 2.80 | 1.20 |
| 6.8 | 2.50 | 1.40 |
| 7.2 | 3.0 | 1.40 |

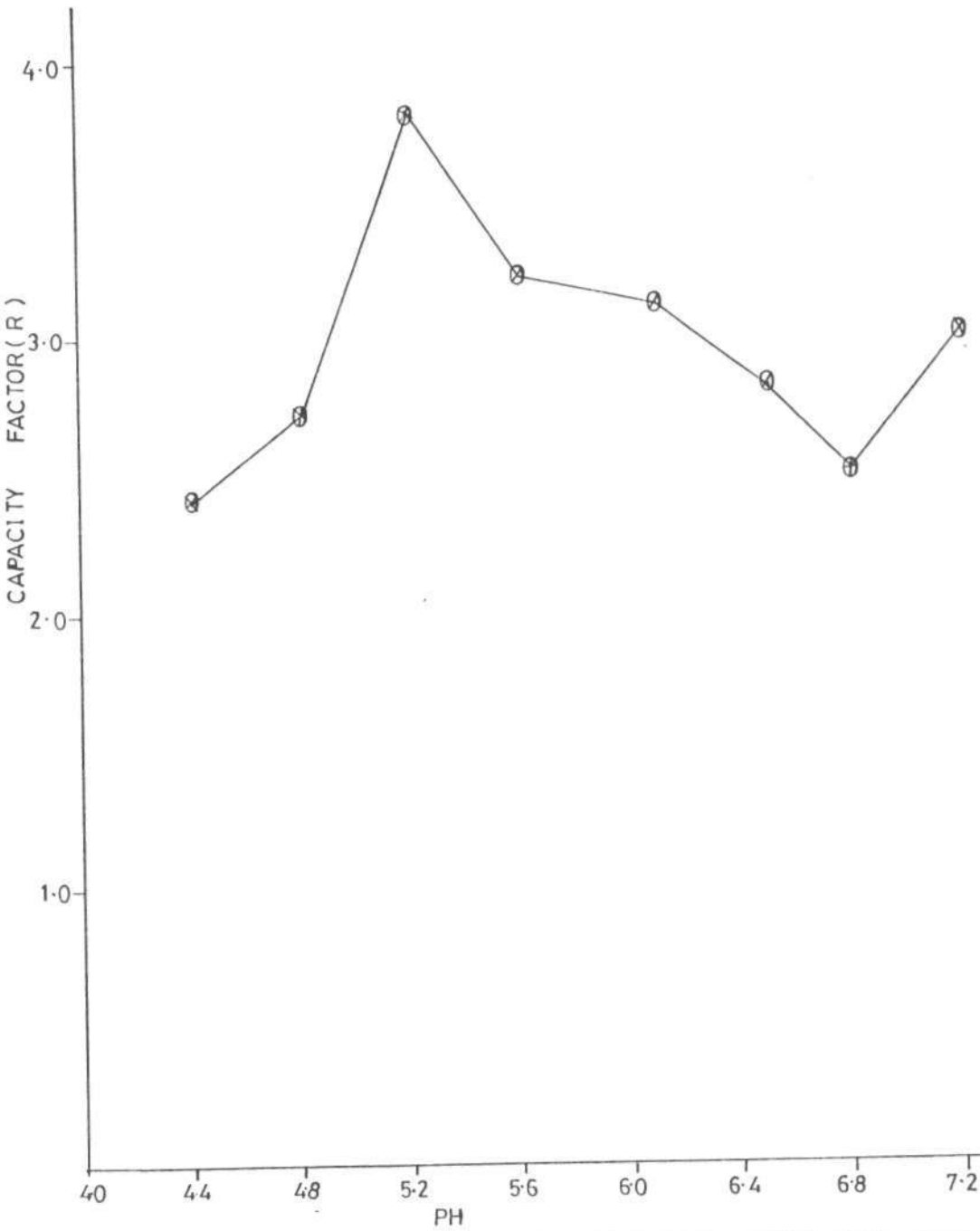



FIG 11: EFFECT OF PH ON THE CAPACITY FACTOR(R) FOR NIFEDIPINE AND 4-DIMETHYL AMINO BENZALDEHYDE USING SOLVENT SYSTEM 0.01M SODIUM ACETATE PH4 : METHANOL (45:55 AND μ-BONDA PAK C18 RADIAL COLUMN(WATERS ASSOCIATES)

preparations without peak overlapping either with the internal standard, endogenous material from the plasma and saliva samples or solvent effect. It also allows the detection and quantification of each compound at low levels.

## 4.3 CHEMICAL ASSAY OF NIFEDIPINE

Linear calibration curves with good correlation coefficients (r = 0.9995 and 0.993) was obtained for nifedipine as shown in Figures 12 and 13 for HPLC and UV spectrophotometric methods of assay respectively. All the three brands analysed contained between 100.01 and 101.25 percent of the active ingredient (see Table 3) thereby indicating that the three brands have complied with the B.P. 1988 addendum 1990 specifications for drug content (98.0 - 102.00%). The result showed that the two methods of analysis employed for the purpose of comparison are very similar. This also showed that chemical equivalence between the different brands of nifedipine analysed conformed with the general manufacturing practice by the different manufacturers.

Chemical equivalence may not necessarily infer physiological equivalence. However, taking into consideration other factors, one can say that the different generic products of nifedipine used in this analysis may infer similar bioavailability data upon administration.

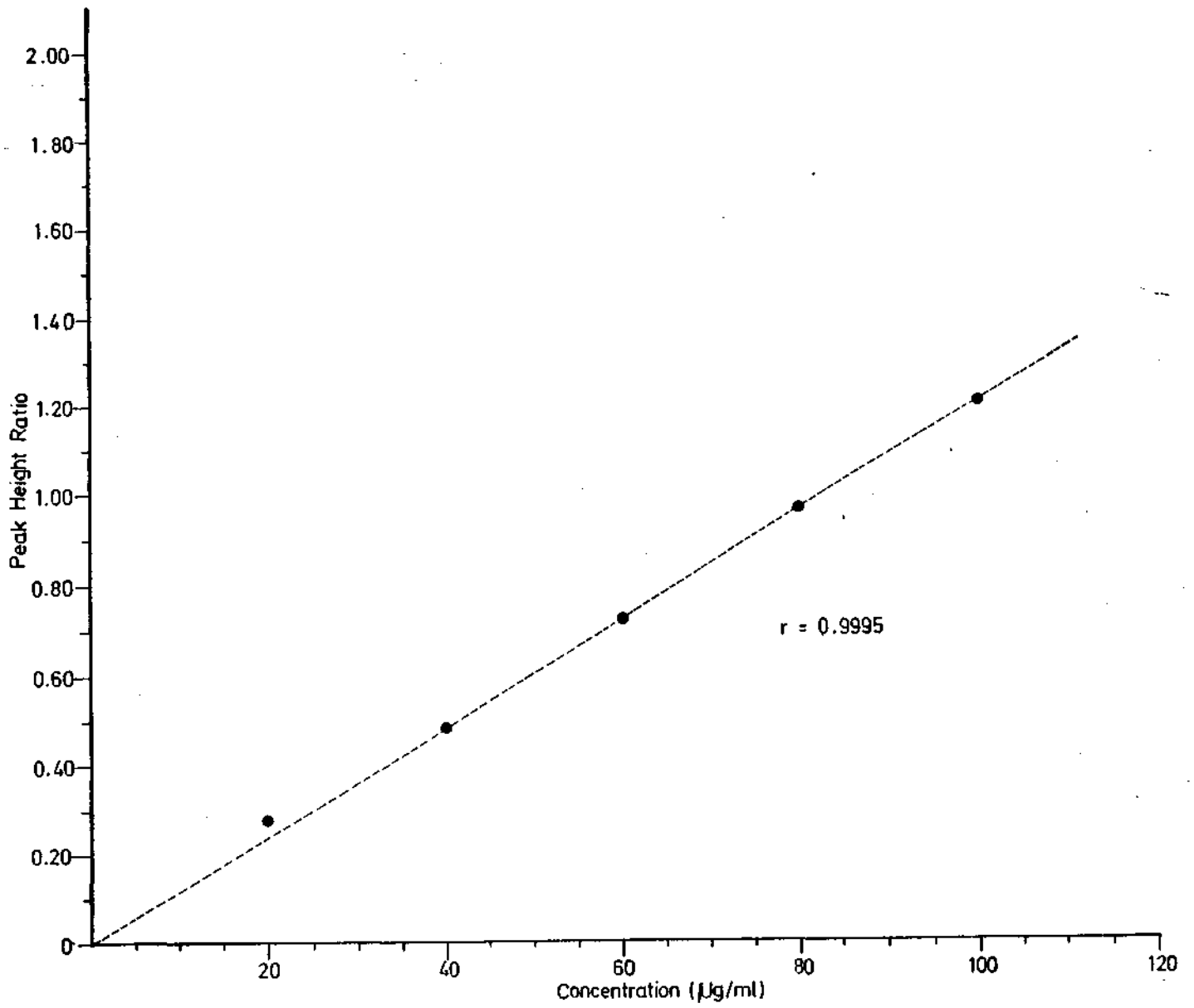



Fig. 12: Calibration curve for nifedipine (20.0 - 100.0 µg/ml) using the High Performance Liquid Chromatographic (HPLC) method of analysis.

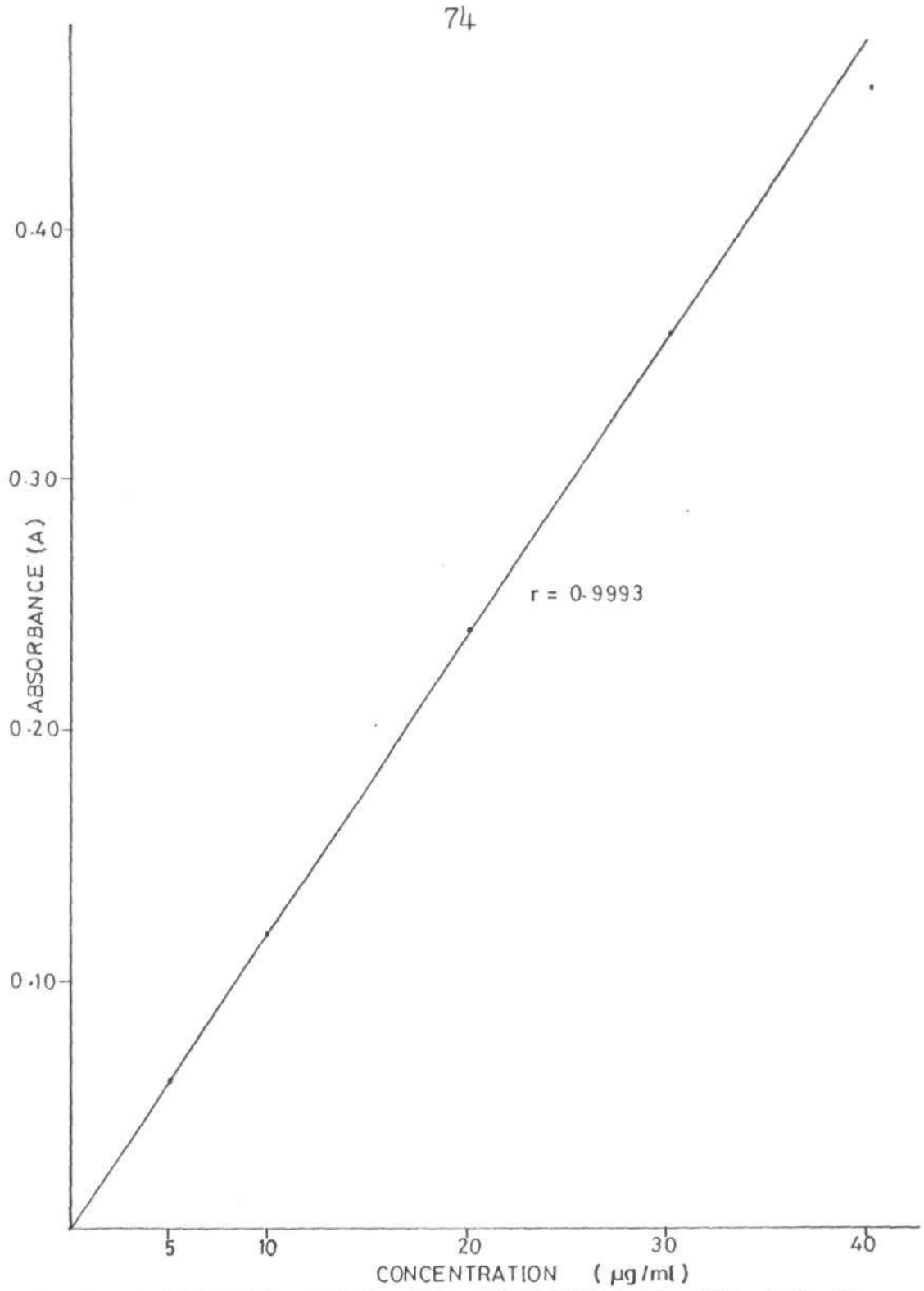



FIG. 13 CALIBRATION CURVE FOR NIFEDIPINE ( 5.0 – 40.0 µg/ml ) IN 0.1M HYDROCHLORIC ACID USING UV SPECTROPHOTOMETRIC METHOD OF ANALYSIS. ( $\lambda$MAX = 349nm)

Table 3: Quantitative Determination of Nifedipine tablets using HPLC and U.V Spectrophotometric methods of analysis.

| COMPOUND | DRUG CONTENT (mg) | | PERCENTAGE ± S.D. | |
|---|---|---|---|---|
| | HPLC METHOD | UV SPECTROPHO-TOMETRIC METHOD | HPLC METHOD | UV SPECTRO-PHOTOMETRIC METHOD |
| S | 20.012 | 20.002 | 101.06 ± 0.35 | 100.01±0.34 |
| O | 20.186 | 20.166 | 100.93 ± 0.17 | 100.83±0.14 |
| K | 20.216 | 20.208 | 101.08 ± 0.29 | 101.04±0.28 |

S.D. = Standard Deviation.

## 4.4 STABILITY STUDIES

Figure 14 shows HPLC chromatogram of the internal standard, 4-dimethylaminobenzaldehyde (I), and nifedipine (II) with retention times of 5.8 and 9.4 minutes respectively. The chromatogram of nifedipine solution after daylight exposure for 6 hours is shown in Figure 15. The shoulder III may possibly be a photodecomposition product of nifedipine. Table 4 and Figure 16 shows the concentration of nifedipine remaining on exposure to day, fluorescent and red lights for 6 hours. Table 5 shows the kinetic parameters obtained from the logarithmic transformations of the data while Figure 17 shows the graphical plot of the logarithmic transformed data which revealed a first order kinetic.

Nifedipine tablets showed neither a decrease in drug content nor colour change under the three different light conditions (day light, fluorescent light and red light). B.P. 1988 specified drug content for nifedipine to be between 98.00 - 102.00%. Exposed nifedipine tablets complied favourably with this specification when compared with the control.

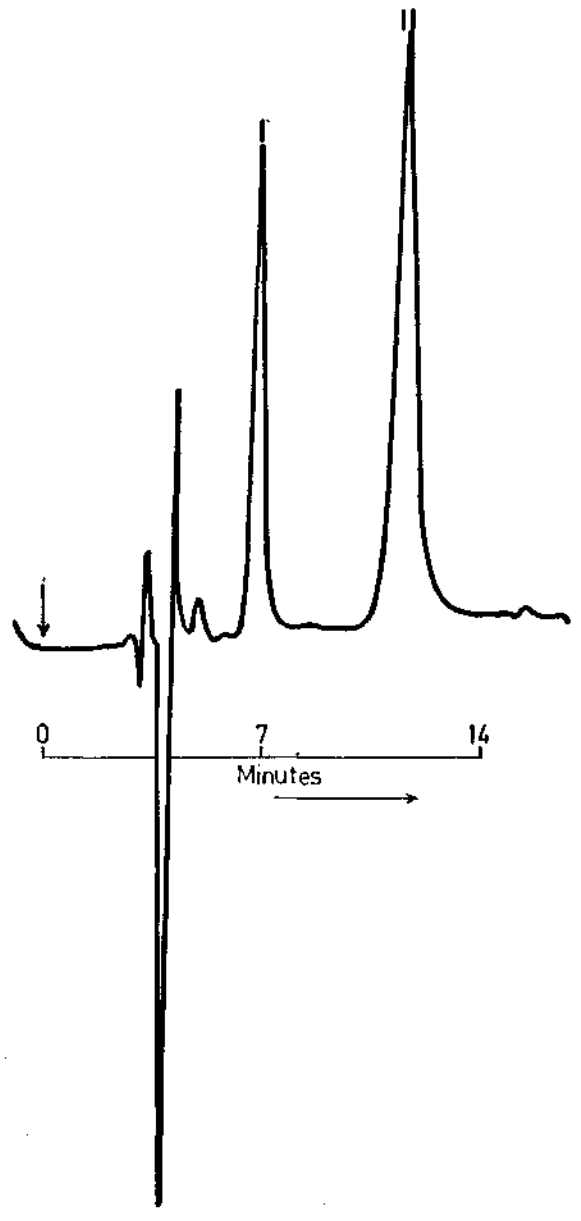



Fig. 14: Chromatogram showing 4-dimethylaminobenzaldehyde (I) and the unexposed nifedipine (II).




Fig. 15: Chromatogram showing 4-dimethylamino-benzaldehyde (I), nifedipine (II) and the day-light decomposed product (III) after exposure to normal day-light (6 hours).

Table 4: Effect of daylight, fluorescent light and red light on the stability of nifedipine tablets and solution in Methanol using HPLC method of analysis.

| Time (Mins) | Concentration (μg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | DAY LIGHT | | FLUORESCENT LIGHT | | RED LIGHT | |
| | Tablets | Solution | Tablets | Solution | Tablets | Solution |
| 0.00 | 100.25 ± 1.60 | 100.00 ± 2.19 | 100.25 ± 1.60 | 100.00 ± 2.19 | 100.25 ± 1.60 | 100.00 ± 2.19 |
| 30.00 | 100.10 ± 1.35 | 60.96 ± 3.39 | 100.20 ± 0.80 | 75.32 ± 0.90 | 100.25 ± 1.30 | 99.86 ± 2.38 |
| 60.00 | 100.20 ± 0.55 | 58.84 ± 1.47 | 100.19 ± 1.10 | 74.57 ± 0.99 | 100.20 ± 0.93 | 99.45 ± 2.04 |
| 90.00 | 100.15 ± 1.85 | 57.69 ± 0.57 | 100.06 ± 2.00 | 72.84 ± 0.85 | 100.10 ± 1.10 | 99.23 ± 2.28 |
| 120.00 | 100.10 ± 0.90 | 53.22 ± 2.11 | 100.10 ± 1.90 | 69.72 ± 1.70 | 100.19 ± 0.52 | 99.23 ± 2.20 |
| 180.00 | 100.18 ± 0.75 | 52.87 ± 2.70 | 100.14 ± 0.22 | 64.38 ± 0.09 | 100.20 ± 0.92 | 99.17 ± 2.15 |
| 240.00 | 100.12 ± 0.65 | 52.47 ± 0.54 | 100.20 ± 0.85 | 64.10 ± 0.54 | 100.05 ± 1.22 | 99.12 ± 2.17 |
| 300.00 | 100.05 ± 1.90 | 52.07 ± 0.42 | 100.17 ± 1.20 | 63.72 ± 0.52 | 100.10 ± 0.35 | 98.84 ± 1.73 |
| 360.00 | 100.10 ± 1.10 | 47.22 ± 0.33 | 100.02 ± 2.20 | 61.87 ± 0.25 | 100.30 ± 0.80 | 98.84 ± 1.73 |

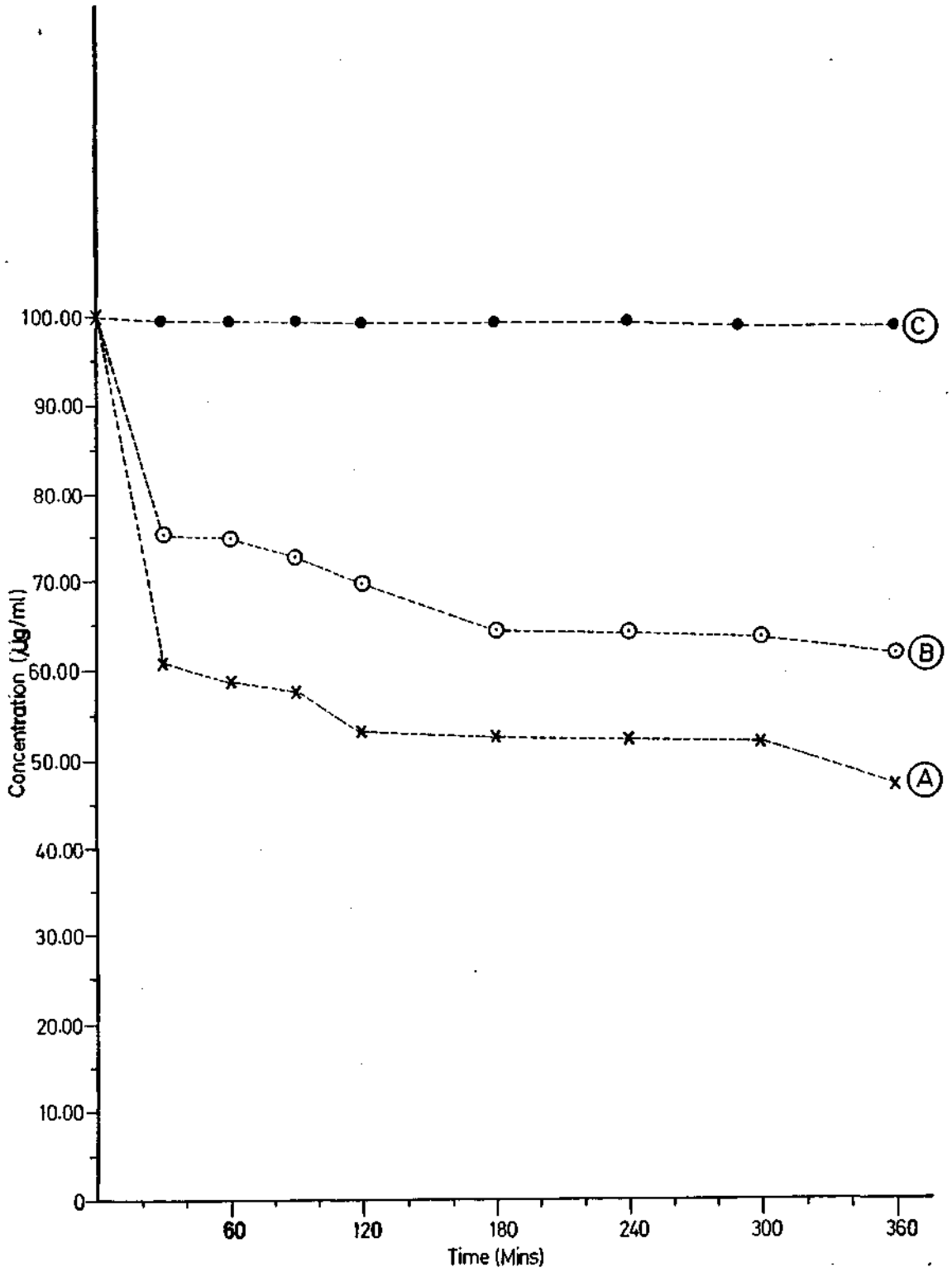



Fig. 16: Effect of day-light (A), fluorescent light (B) and red-light (C) on the stability of nifedipine (100 µg/ml) solution in methanol.

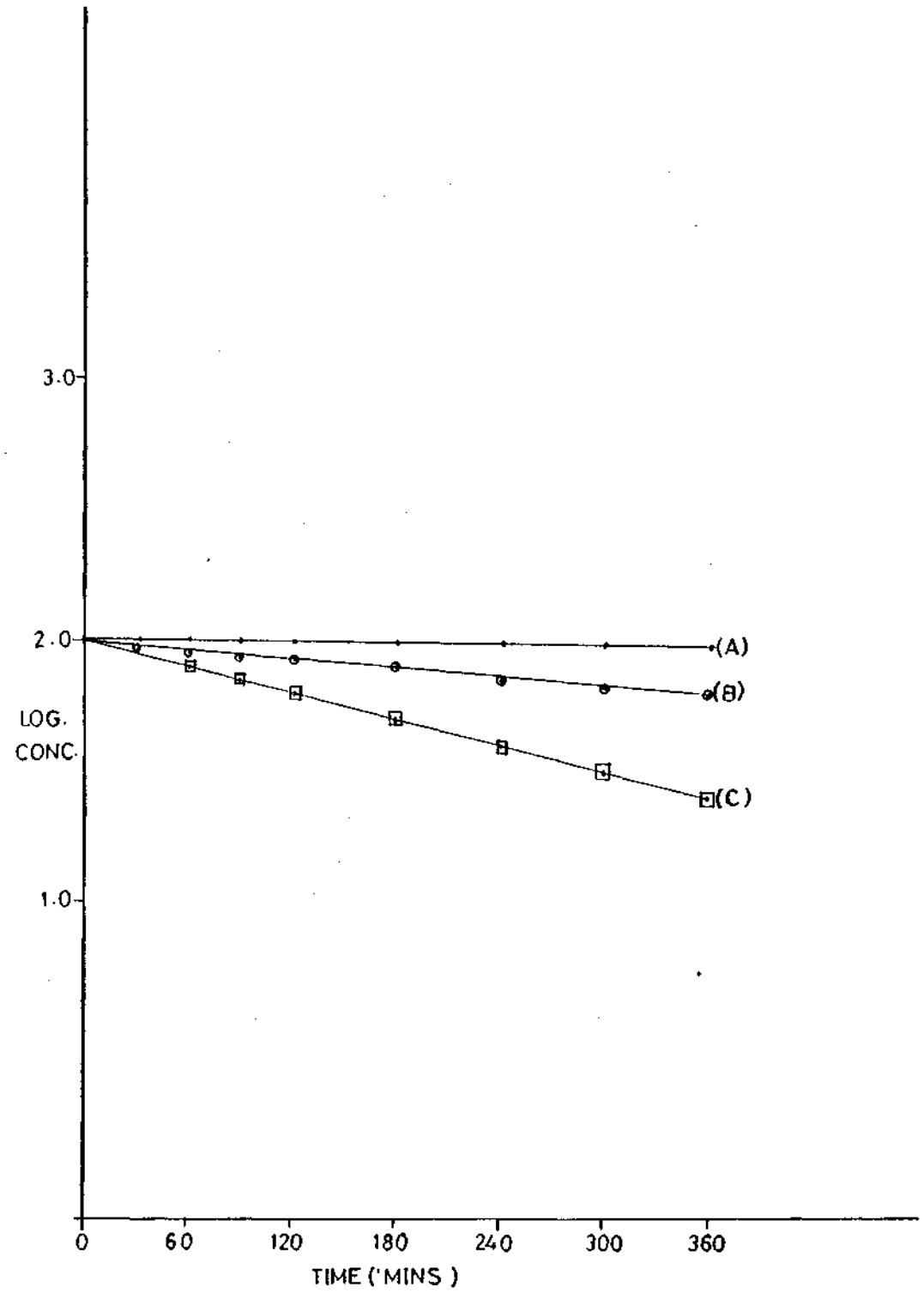



FIG 17 GRAPHICAL PLOT OF THE LOGARITHMIC TRANSFORMED DATA ON THE EFFECT OF DAYLIGHT(A) FLUORESCENT LIGHT (B) AND REDLIGHT(C) ON THE STABILITY OF NIFEDIPINE SOLUTION IN METHANOL

Figure 18 shows HPLC chromatogram of the internal standard, 4-dimethylaminobenzaldehyde I and nifedipine solution (II) after UV irradiation at 366nm for 48 hours. The third peak (III) with retention time 9.0 minutes may possibly be the UV decomposed product of nifedipine. Nifedipine tablets exposed to UV irradiation at 366nm for 72 hours showed no colour change nor decrease in drug content when compared with the percent content of the control (unexposed nifedipine). Table 6 and Figure 19 shows the concentration of nifedipine tablets and solution remaining after the UV exposure at the particular times.

The result of the effect of elevated temperature on nifedipine solution and tablets showed no significant decrease in drug content (see Table 7).

Our result on exposure of nifedipine solution in methanol to different sources of light showed that it decomposes under day light and fluorescent light and ultra-violet irradiation as evident from the chromatograms (Figures 15 and 18). The finding is in agreement with the result of Testa et al.,(1979).

The result also demonstrated the stability of nifedipine solution in methanol under red-light and to an elevated temperature of $50 \pm 0.5^{o}C$. The stability of nifedipine tablets under all the conditions of light and temperature is also demonstrated from our result and this could be attributed to the tablet coating employed by different manufacturers.

It can be concluded that nifedipine solution in methanol is unstable under day light, fluorescent light and UV light and therefore should not be exposed to such lights.

Table 5: Kinetic parameters of nifedipine solution (100 µg/ml) after exposure to normal daylight, fluorescent light and red light.

| Source of light | Rate constant $min^{-1}$ | Half-life ($t_{\frac{1}{2}}$) $hr^{-1}$ |
|---|---|---|
| Normal day light | $2.11 \times 10^{-3}$ | 5h. 28 min. |
| Fluorescent light | $1.34 \times 10^{-3}$ | 8h. 35 min. |
| Red light | $3.26 \times 10^{-5}$ | 354h. 14 min. |

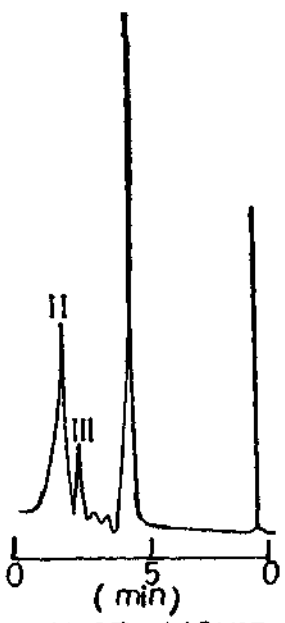



FIG 18 HIGH PERFORMANCE LIQUID CHROMATOGRAM OF NIFEDIPINE SOLUTION(II), UV DECOMPOSED PRODUCT(III) AND INTERNAL STANDARD(I) AFTER EXPOSURE TO 48 HOURS UNDER UV LIGHT 366(nm)

Table 6: Effect of ultra-violet radiation on the stability of nifedipine tablets and solution in methanol.

| Hours of Exposure | Temperature of UV lamp compartment (°C) | Concentration (µg/ml) Tablets | Solution |
|---|---|---|---|
| 0.00 | 33.00 ± 1.00 | 100.12 ± 0.56 | 100.00 ± 2.19 |
| 6.00 | 33.00 ± 1.00 | 100.10 ± 2.72 | 63.10 ± 0.95 |
| 12.00 | 34.00 ± 0.50 | 99.85 ± 1.02 | 50.12 ± 0.72 |
| 24.00 | 34.00 ± 0.50 | 100.04 ± 0.90 | 35.48 ± 1.83 |
| 48.00 | 36.00 ± 0.50 | 99.98 ± 1.50 | 19.95 ± 2.62 |
| 72.00 | 38.00 ± 1.00 | 99.90 ± 1.43 | 12.58 ± 0.92 |

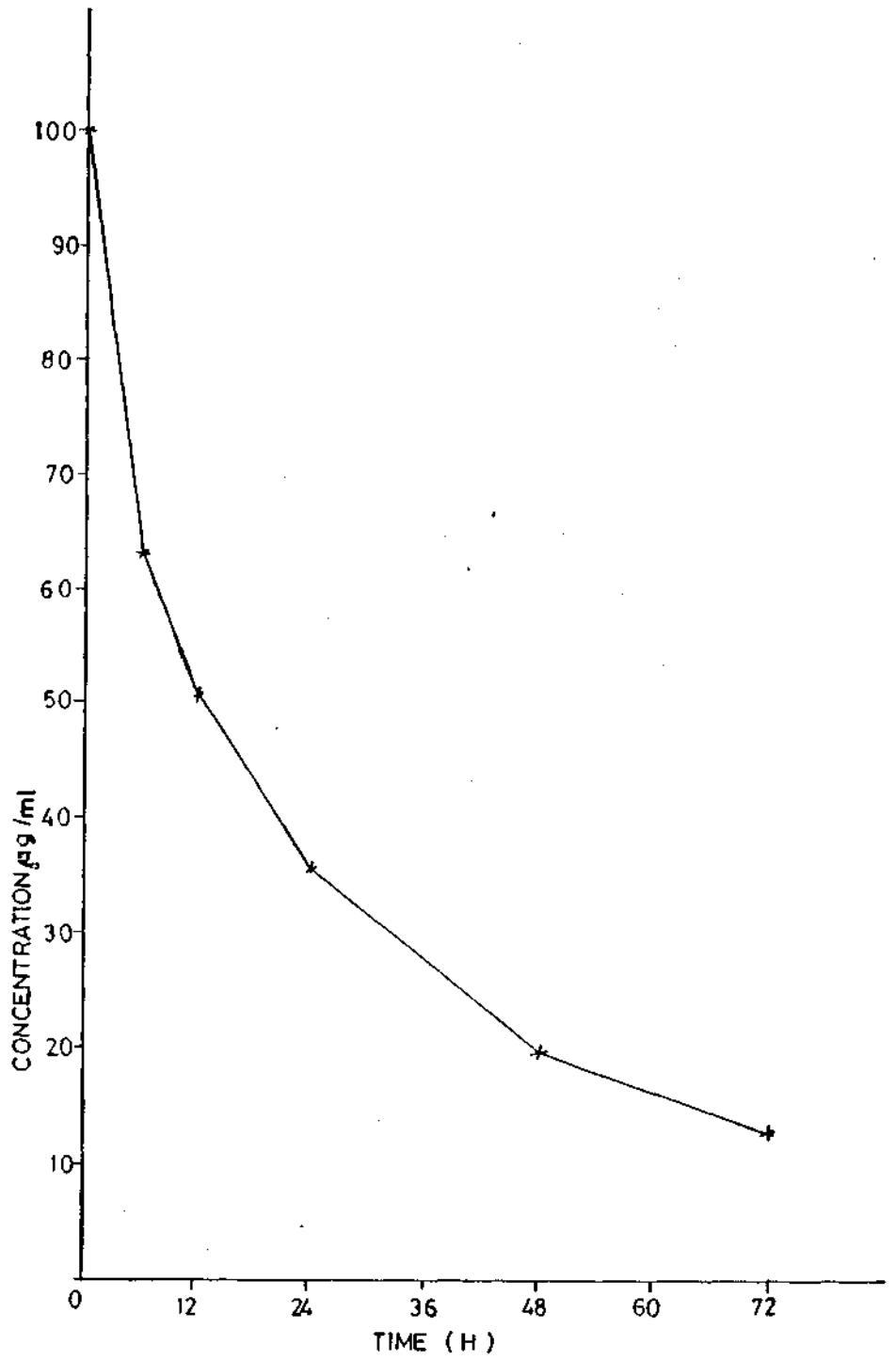



FIG 19 EFFECT OF ULTRAVIOLET IRRADIATION ( 366 nm ) ON THE STABILITY OF NIFEDIPINE SOLUTION IN METHANOL

Table 7: Effect of elevated temperature on the stability of nifedipine tablets and solution in methanol.

| Temperature (°C) | Concentration (µg/ml) Tablets | Solution |
|---|---|---|
| 25.00 ± 0.5 | 100.12 ± 0.56 | 100.00 ± 2.19 |
| 30.00 ± 0.5 | 100.20 ± 1.11 | 100.12 ± 2.60 |
| 35.00 ± 0.5 | 100.10 ± 0.20 | 99.84 ± 0.90 |
| 40.00 ± 0.5 | 100.05 ± 0.84 | 99.79 ± 1.80 |
| 45.00 ± 0.5 | 100.00 ± 1.00 | 99.93 ± 1.35 |
| 50.00 ± 0.5 | 100.11 ± 0.25 | 99.89 ± 1.65 |

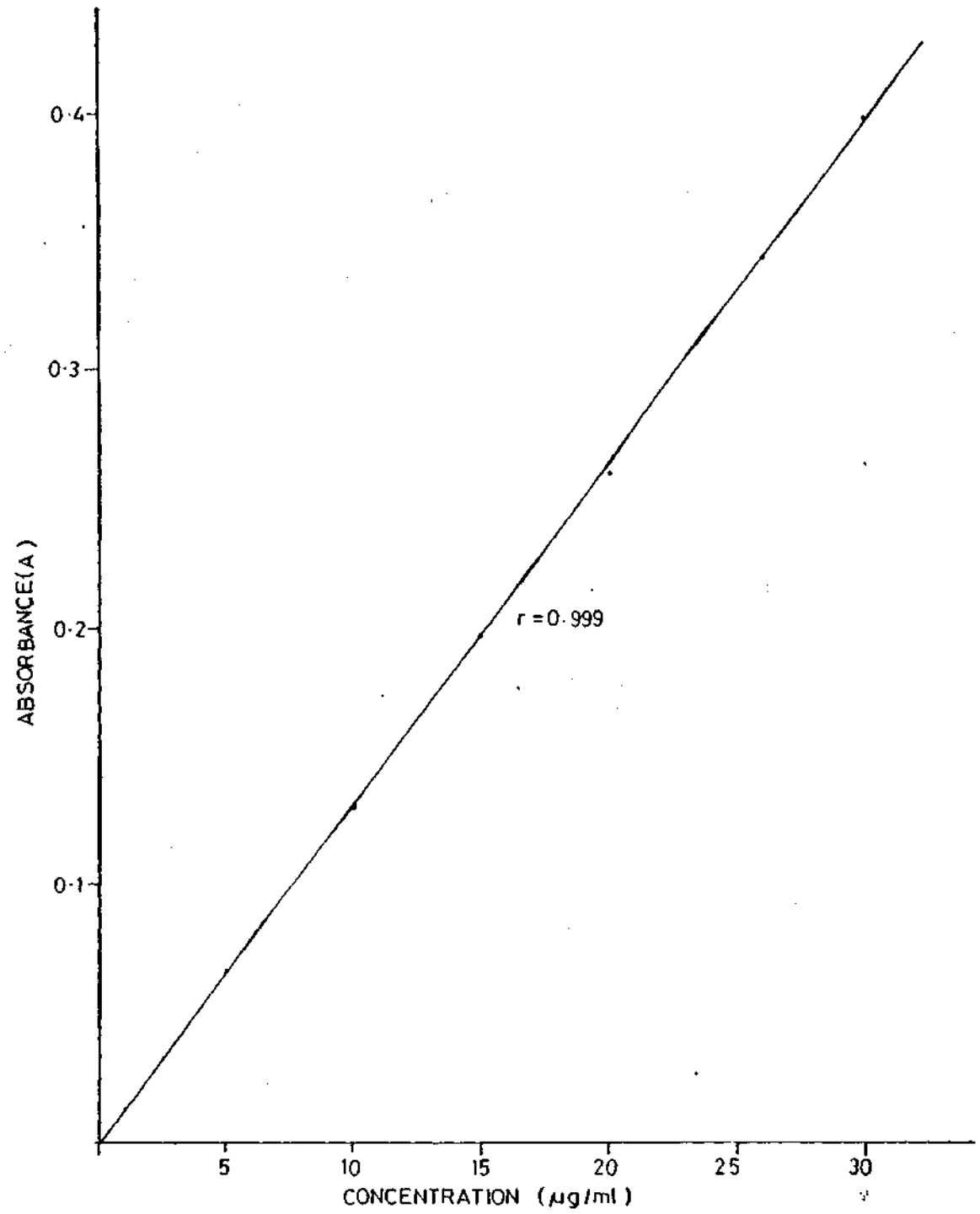
## 4.5 DISSOLUTION RATE TESTS

Linear calibration curve with good correlation coefficient (r = 0.999) of nifedipine in 0.1M hydrochloric acid using U.V. Spectrophotometer is shown in Figure 20. The dissolution rate profiles of nifedipine tablets designated S, O, and K in 0.1M hydrochloric acid are shown in Table 8. Figure 21 shows the graphical representation of the dissolution rate profiles for the three brands of nifedipine tablets in 0.1M HCl solution. The plotted points represent average of five dissolution tests.

Total release of the active ingredient for all the brands was obtained in less than 30 minutes. The BP 1988 official requirement for dissolution rate test stated that for each tested dosage form, the amount of active ingredient in solution is not less than 70% of the stated amount at the end of 45 minutes unless otherwise stated in the monograph. The result obtained in this test complied favourably with this specification for all the brands tested.

## 4.6 DISINTEGRATION TIME TEST

The results obtained for the disintegration of the three brands of nifedipine is shown in Table 9. This shows that the times taken for the three brands of nifedipine tablets to disintegrate was less than 8 minutes. The official B.P. (1988) time-limit for tablet disintegration is 15 minutes. All the brands passed the official requirement for tablet disintegration.




FIG·20 CALIBRATION CURVE FOR NIFEDIPINE ( 5.00 - 30.00 µg/ml ) IN 0.1M HCL SOLUTION USING UV SPECTROPHOTOMETER

$\lambda_{MAX}$ = 349nm

Table 8: Invitro dissolution release rates of three brands of nifedipine tablets in 0.1M methanolic hydrochloric acid maintained at 37 ± 0.5°C using B.P. 1988 rotating basket method with a stirring rate of 100 r.p.m.

| Compound/ Time (min) | Cumulative Percentage Released ± S.D. S | O | K |
|---|---|---|---|
| 5 minutes | 10.06 ± 0.87 | 9.55 ± 0.70 | 6.06 ± 1.03 |
| 15 minutes | 56.50 ± 2.50 | 55.24 ± 0.97 | 43.40 ± 2.05 |
| 30 minutes | 100.008 ± 0.64 | 100.04 ± 0.73 | 100.09 ± 0.38 |
| 45 minutes | 99.98 ± 1.32 | 99.20 ± 0.82 | 99.95 + 1.32 |
| 60 minutes | 99.06 ± 0.59 | 98.19 ± 0.32 | 98.62 ± 0.36 |

S.D. = Standard Deviation

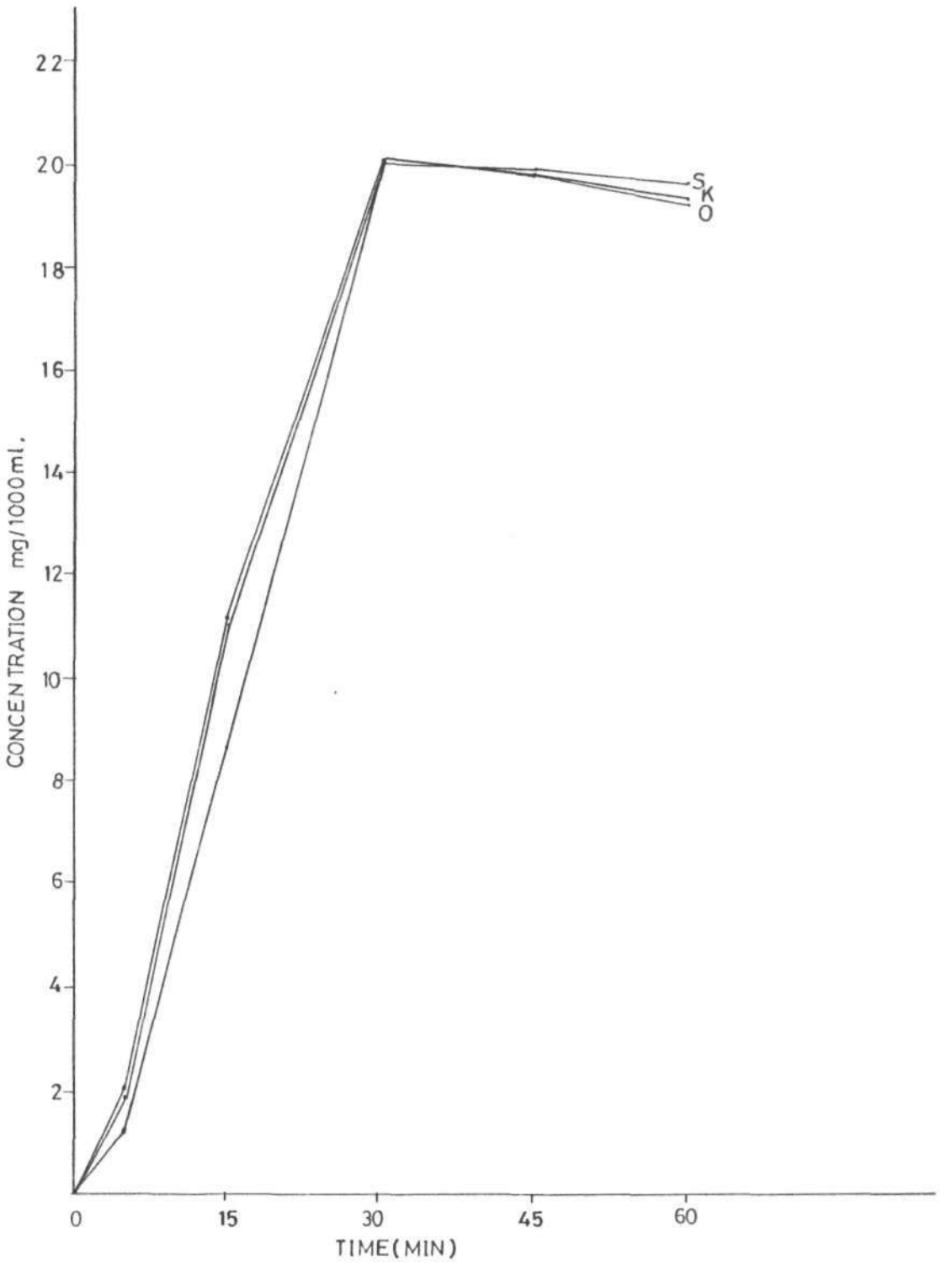



FIG 21 CUMULATIVE CONCENTRATION PROFILE OF DISSOLUTION RATE STUDIES OF THREE BRANDS OF NIFEDIPINE TABLET IN 0.1M HCL SOLUTION

Table 9: Disintegration times for nifedipine tablets in water maintained at 37 ± 0.5°C (BP. 1988)

| Tablet No. | Disintegration Time (Min) | | |
|---|---|---|---|
| | S | O | K |
| 1 | 6.5 | 5.0 | 7.5 |
| 2 | 7.0 | 6.0 | 7.5 |
| 3 | 7.0 | 6.0 | 8.0 |
| 4 | 7.5 | 5.5 | 8.2 |
| 5 | 6.0 | 6.5 | 7.0 |
| Mean ± S.D. | 6.80 ± 0.63 | 5.80 ± 0.31 | 7.44 ± 0.24 |

S.D. = Standard Deviation

## 4.7 EXTRACTION PROCEDURE

The solvent/solvent combinations tried in developing an extraction procedure suitable for the isolation of nifedipine from the biological matrix are given in Table 10. The percentage extraction recoveries of nifedipine in each of the solvent are also given in the table. From the table, it can be seen that the highest extraction recovery was obtained with dichloromethane while the lowest recovery was got using chloroform. Dichloromethane was then chosen as the organic solvent for the extraction.

Solvent extraction is the usual way of isolating a drug from the biological matrix. Nifedipine is highly lipophilic and have sufficiently high distribution constants for a quantitative extraction (> 99%) into the organic solvents used. In the liquid chromatography methods for nifedipine extraction, more volatile solvents such as ethylacetate, chloroform and n-pentane-dichloromethane have been used to facilitate solvent extraction (Anhoff and Persson, 1990).

Table 10: Solvent/solvent combination and percentage extraction recoveries of nifedipine from Biological Matrix

| Solvent/solvent combination | % Extraction Recoveries ± S.D. |
|---|---|
| Ethylacetate | 72.0 ± 1.80 |
| Chloroform | 45.60 ± 2.20 |
| Dichloromethane | 98.10 ± 1.40 |
| Chloroform : Dichloromethane (2:1) | 56.05 ± 1.90 |
| Ethylacetate:Dichloromethane (3:5) | 88.00 ± 0.50 |
| Ethylacetate:Dichloromethane: Chloroform (2:2:1) | 90.10 ± 1.70 |
| Dichloromethane:Ethylacetate (2:1) | 93.45 ± 1.10 |

## 4.8 INVIVO ABSORPTION STUDIES

Linear calibration curves with good correlation coefficients (r = 0.9996 and 0.999) of nifedipine are shown in Figures 22 and 23 for plasma and saliva samples respectively. Nifedipine was detected in both plasma and saliva samples of all the six subjects within the 12 hours of sampling in both fasting and non-fasting state. Figures 24 and 25 shows HPLC chromatogram of the extracted plasma and saliva samples at the time of maximum concentration which gave peaks corresponding to retention time of reference nifedipine sample of 9.4 minutes. (Retention time of internal standard, 4-dimethyl-aminobenzaldehyde 5.8 minutes). This peak was absent from the blank plasma and saliva extract which was collected before the ingestion of nifedipine tablet.

Following oral administration of 20mg nifedipine tablets, peak plasma concentrations (Cmax) ranged from 35.95 to 39.40ng in fasting volunteers and from 34.55 to 39.45ng in non fasting volunteers. This finding is in agreement with the results of Kleinbloesem et al. (1984), Kozjek et al. (1987). The peak salivary concentration (Cmax) ranged from 59.51 to 63.90ng in the fasting state and from 54.60 to 60.85ng in non-fasting state. Time to reach peak concentration ($t_{max}$) ranged from 2 to 3 hours in the fasting state and 3 to 4 hours in the non-fasting state.

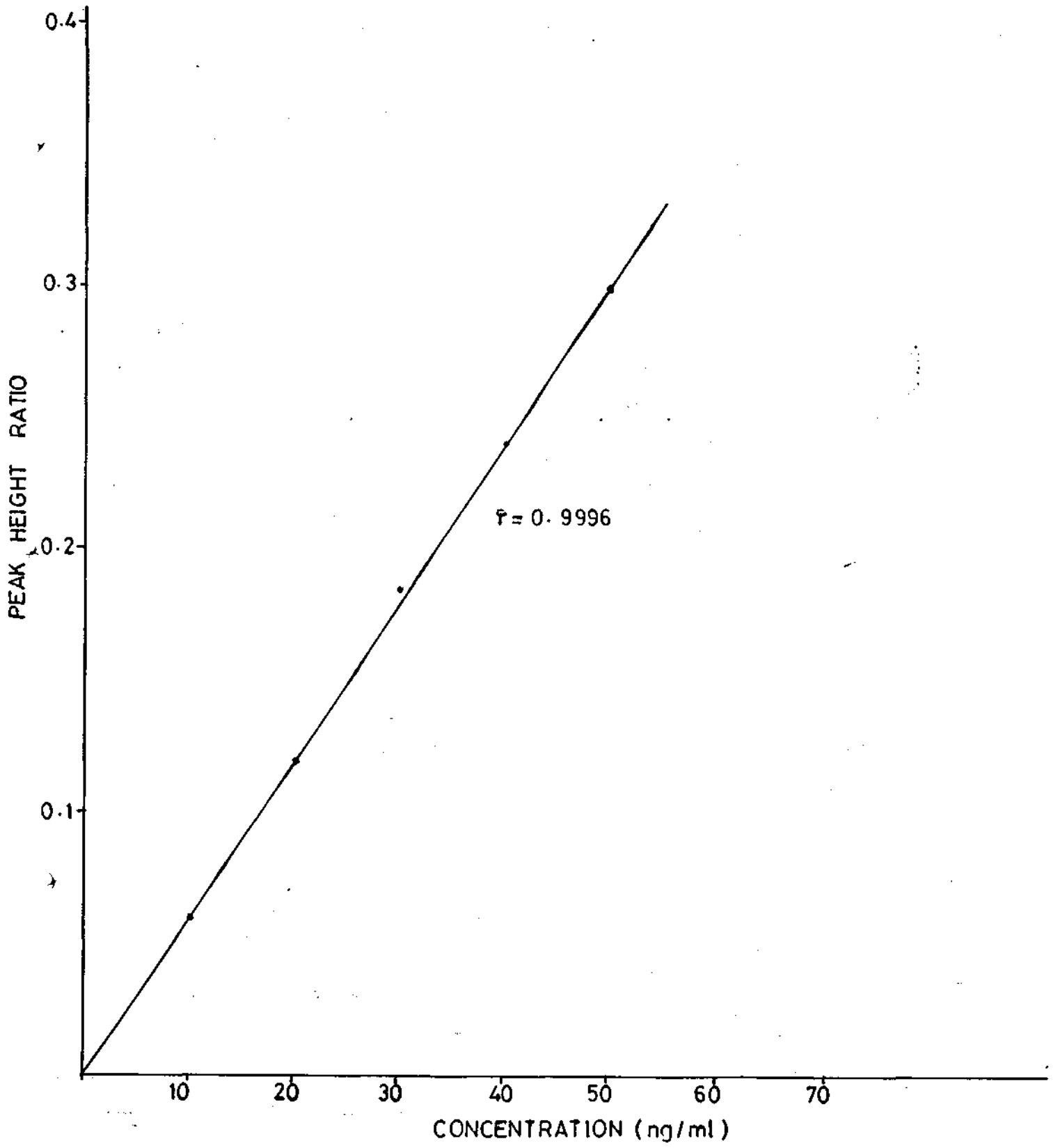



FIG. 22: CALIBRATION CURVE FOR NIFEDIPINE IN PLASMA SAMPLE (0 - 50.0ng/ml)

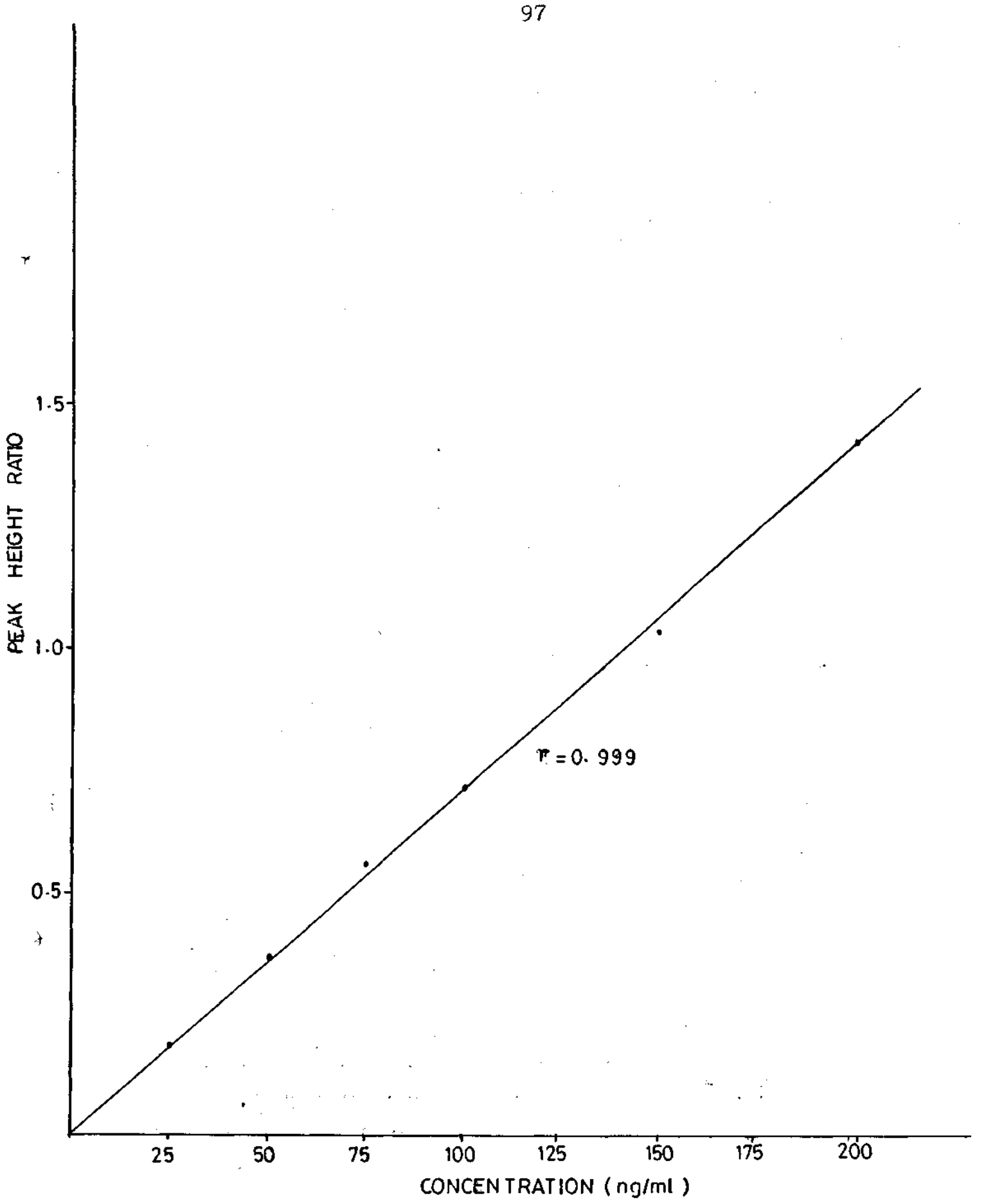



FIG 23 CALIBRATION CURVE FOR NIFEDIPINE IN SALIVA SAMPLE
( 0– 200ng/ml )

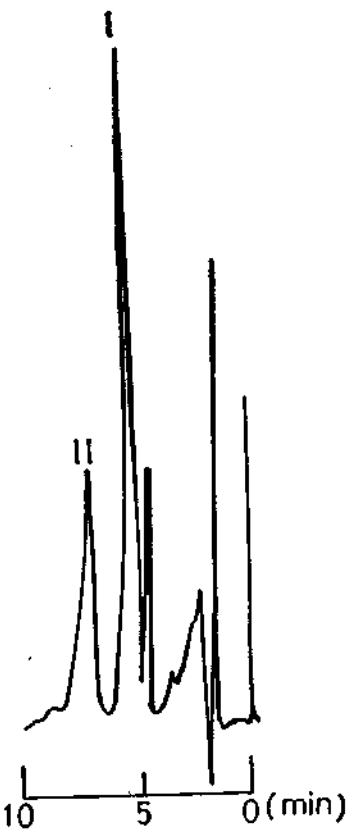



FIG. 24 HPLC CHROMATOGRAM OF PLASMA NIFEDIPINE SAMPLE(II) AT TIME OF MAXIMUM CONCENTRATION(C MAX) AND INTERNAL STANDARD (500 ng/ml)(II)




FIG25 HPLC CHROMATOGRAM OF SALIVA NIFEDIPINE SAMPLE(II)AT TIME OF MAXIMUM CONCENTRATION (C MAX) AND INTERNAL STANDARD (1000ng/ml)(I)

There were differences as well as similarities in the plasma and saliva concentration levels for the fasting and non-fasting volunteers (Appendix 6).

The mean nifedipine plasma and saliva concentration levels for the fasting and non fasting volunteers are shown in Table 11. Figures 26 and 27 shows the mean plasma and saliva concentration time curves for the fasting and non-fasting states respectively.

The pharmacokinetic parameters obtained from the mean concentration data for nifedipine tablets in plasma and saliva of fasting and non-fasting volunteers are shown in Table 12. There were differences as well as similarities in the calculated individual pharmacokinetic. Parameter (see Appendix 7). For example, the time to reach peak concentration ($t_{max}$), elimination half-lives and other parameters calculated for each person were slightly different within subjects, in agreement with the findings of Levy et al. (1965). The differences observed are due to individual variability or varying degree of the first pass effect. An orally administered drug may be degraded or metabolised within the gut lumen by enzymes in the mucosa. Drug metabolism prior to the drug entering into the systemic circuit at any of these sites after oral administration is referred to as presystemic or first-pass elimination.

**Table 11:** Mean plasma and saliva nifedipine concentration in healthy volunteers following oral administration of 20mg nifedipine in fasting and non-fasting state.

| TIME (h) | CONCENTRATION (ng/ml) ± S.D. | | | |
|---|---|---|---|---|
| | PLASMA | | SALIVA | |
| | FASTING STATE | NON-FASTING STATE | FASTING STATE | NON-FASTING STATE |
| 0.5 | 16.54 ± 1.09 | 6.33 ± 1.08 | 22.29 ± 1.97 | 10.20 ± 1.28 |
| 1.0 | 27.73 ± 1.36 | 14.80 ± 4.64 | 37.05 ± 6.61 | 20.34 ± 0.66 |
| 2.0 | 37.28 ± 1.82 | 27.50 ± 4.57 | 59.50 ± 2.45 | 32.63 ± 3.09 |
| 3.0 | 36.53 ± 0.40 | 34.63 ± 3.16 | 55.66 ± 10.33 | 58.08 ± 2.09 |
| 4.0 | 28.50 ± 1.21 | 31.47 ± 6.22 | 49.13 ± 7.41 | 45.94 ± 5.51 |
| 6.0 | 20.04 ± 0.72 | 20.02 ± 4.80 | 37.50 ± 4.52 | 37.12 ± 3.91 |
| 8.0 | 13.80 ± 1.18 | 13.47 ± 2.45 | 25.82 ± 1.85 | 22.07 ± 3.39 |
| 12.0 | 6.34 ± 1.38 | 8.55 ± 3.30 | 13.62 ± 2.37 | 10.25 ± 1.68 |

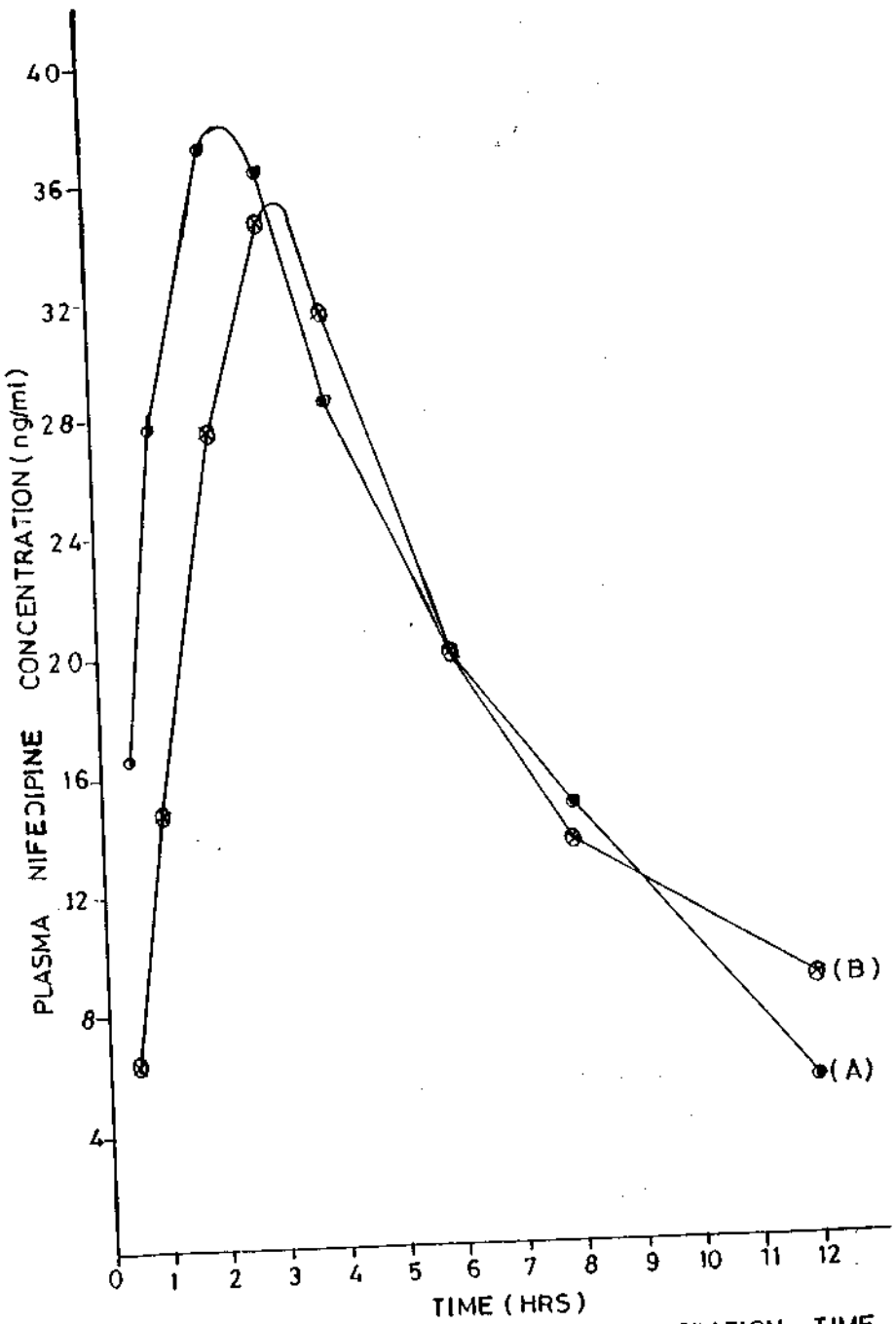



FIG 26 THE MEAN PLASMA CONCENTRATION TIME CURVE AFTER ORAL INGESTION OF 20 mg NIFEDIPINE TABLETS IN FASTING (A) AND NON FASTING (B) STATES

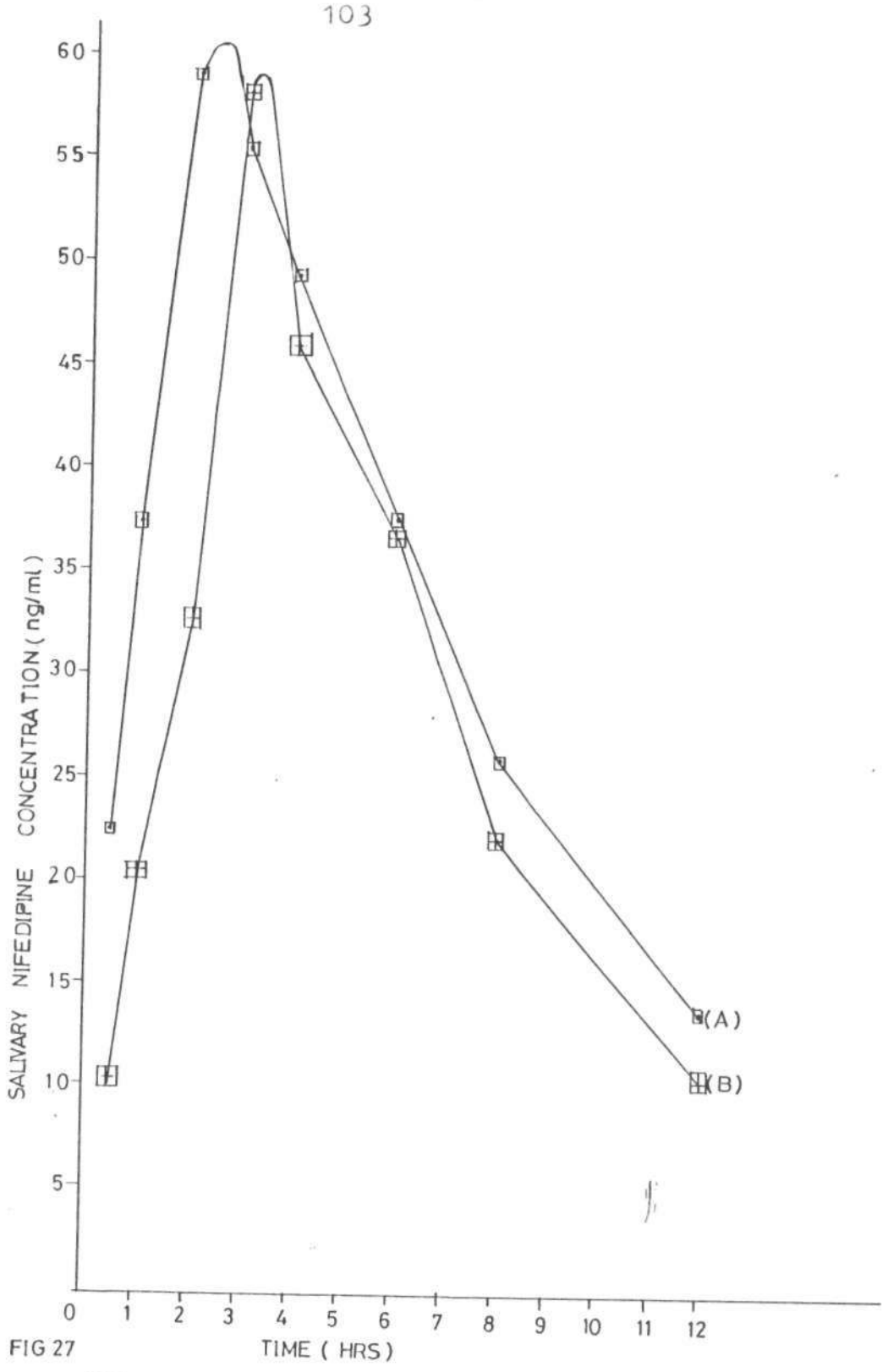



FIG 27 THE MEAN SALIVA CONCENTRATION TIME CURVE AFTER ORAL INGESTION OF 20mg NIFEDIPINE TABLETS IN FASTING(A) AND NON FASTING(B) STATES

**Table 12:** Pharmacokinetic parameters of nifedipine derived from the Mean Plasma and Saliva Concentration data after a single 20mg oral dose in fasting and non-fasting state.

| PARAMETER | PLASMA | | SALIVA | |
|---|---|---|---|---|
| | FASTING STATE | NON-FASTING STATE | FASTING STATE | NON-FASTING STATE |
| Lag time (h) | 0.13 ± 0.037 | 0.36 ± 0.058 | 0.17 ± 0.08 | 0.34 ± 0.018 |
| Absorption half-life $t_{\frac{1}{2}}$ (h) | 0.71 ± 0.077 | 1.30 ± 0.34 | 1.05 ± 0.58 | 1.72 ± 0.12 |
| Absorption rate constant Ka ($h^{-1}$) | 0.96 ± 0.104 | 0.57 ± 0.168 | 0.86 ± 0.38 | 0,41 ± 0.03 |
| Maximum concentration $C_{max}$ (ng/ml) | 37.78 ± 1.21 | 36.90 ± 1.67 | 61.69 ± 1.34 | 58.08 ± 2.09 |
| Time to Peak $t_{max}$ (h) | 2.33 ± 0.47 | 3.50 ± 0.5 | 2.33 ± 0.47 | 3.50 ± 0.5 |
| Elimination half-life $t_{\frac{1}{2}}\beta$ (h) | 3.23 ± 0.33 | 2.85 ± 0.68 | 3.44 ± 0.75 | 2.47 ± 0.13 |
| Elimination rate constant Ke ($h^{-1}$) | 0.22 ± 0.02 | 0.26 ± 0.07 | 0.21 ± 0.045 | 0.27 ± 0.036 |
| Area Under the Curve | | | | |
| AUC(0-12h) ng/ml/h | 235.73 ± 6.65 | 219.57 ± 29.16 | 401.29 ±25.37 | 338.47 ± 28.87 |
| AUC(0-∞) ng/ml/h | 265.93 ± 13.67 | 257.44 ± 48.26 | 469.35 ±41.98 | 375.26 ± 35.68 |
| Volume of distribution (L) | 349.56 ± 20.64 | 317.65 ± 46.38 | 210.64 ±37.48 | 190.81 ± 9.91 |
| Clearance (L/h) | 75.40 ± 3.69 | 80.40 ± 14.60 | 43.42 ± 3.58 | 53.79 ± 5.24 |

S.D. = Standard Deviation

Although many factors influence the rate of drug absorption, pharmaceutical formulation and the characteristics of the subjects are major sources of individual variations. Subjects characteristics can produce striking differences in the efficacy of a given drug product. One characteristic that may influence drug absorption is the rate of gastric emptying. It may affect both the rate and extent of absorption of the drug (Abdel-Monem and Henkel, 1978). Gastric emptying is an important rate-limiting step in the absorption of nifedipine (Waller et al., 1984). Intra-subject variability in drug disposition is well documented (Koup and Gibaldi, 1980). The degree of intra-subject variability changes from drug to drug and may differ from one panel of subjects to another depending on age and other factors (Koup and Gibaldi, 1980).

The plasma and salivary levels of the non-fasting volunteers (at 0.5h) was significantly lower than that of the fasting volunteers ($P < 0.001$). The $t_{lag}$ which is the time it takes a drug to appear in the biological fluid was longer in the non-fasting volunteers than in the fasting volunteers ($P < 0.01$). However, the fasting volunteers had a higher nifedipine concentration level at 2 hours, (time to reach Cmax). This was significantly different from that of the non-fasting volunteers ($P < 0.01$). The mean maximal plasma and saliva levels (Cmax) for the fasting state was attained at $2.33 \pm 0.47$h

compared with 3.5 $\pm$ 0.5h for the non-fasting state. This is in agreement with the results of Challenor et al. (1985 and 1986), Renwick et al. (1988) in which food caused a delay in the time to reach peak plasma levels. Food constituents have been shown to interact directly or indirectly with drugs in a number of ways. It can act as a mechanical barrier preventing drug access to the mucosal surface of the intestine as reported by Kaumeiere et al. (1979) that food delayed the absorption of sulphamethoxydrazine and most sulphonamides. Peak drug levels in plasma of the former were not reached until 10 hours after dosing compared to 7 hours in fasting individuals.

Consistently higher concentrations of nifedipine in saliva relative to those in plasma for both fasting and non-fasting state were observed during the absorption phase following oral ingestion. The magnitude of Cmax for both plasma and saliva in fasting and non-fasting states were statistically insignificant ($P > 0.05$). The level of significance of Cmax between plasma and saliva for both fasting and non-fasting states were however statistically significant ($P < 0.001$).

There were good correlations between plasma and saliva nifedipine levels in fasting and non-fasting states respectively ($r = 0.965$ and $0.956$). A good correlation between plasma concentration in fasting and non-fasting volunteers ($r = 0.835$) and also between saliva concentrations in fasting and non-fasting ($r=0.875$)

were also observed. Similarly, identical saliva and plasma nifedipine time to peak drug levels ($t_{max}$) was established in this study. Ogunbona et al. (1986) established similar result in the study of salivary excretion of chloroquine in man. They suggested that equilibrium is reached rapidly between plasma and saliva in line with Posti's hypothesis that saliva should be regarded as an integral part of the central compartment (Posti, 1982) rather than a deep pharmacokinetic compartment as suggested by Galeazzi et al. (1976).

Posti (1982) developed an anatomical-physiological model to explain the finding of higher salivary drug levels relative to serum drug levels during the early stages following oral ingestion of a drug. In this model it is hypothesized that (1) during absorption the concentration of drug in arterial blood is higher than that in venous blood. This concentration difference being at any moment directly proportional to the rate of absorption, and (2) the concentration of drug in saliva is in equilibrium with that in arterial blood. The duration of the elevated saliva to plasma concentration ratio for nifedipine is consistent with the suggestion that the elevated ratio will occur during absorption and the patterns of saliva/plasma nifedipine concentration-time curves are in agreement with the general patterns predicted by Posti (1982).

One conclusion derived by Posti (1982) is that the difference in AUC between the saliva and plasma drug concentration-time curves within appropriate time limits during absorption is proportional to the amount of drug present in the system at time t.

Comparing the bioavailability of nifedipine in fasting and non-fasting states, it is obvious from results obtained that, the major cause of differences in rates of absorption of nifedipine in the two states is their differing dissolution rates. This is reflected in the longer lag time and absorption half-life found in the non-fasting state. However, measured area under the plasma concentration curve to 12h ($AUC_{(0-12h)}$ for fasting and non-fasting volunteers did not differ significantly ($P < 0.05$) while the area under the nifedipine saliva concentration curve for fasting volunteers was significantly higher than those of the non-fasting volunteers ($P < 0.001$). The level of significance of $AUC_{(0-12h)}$ between saliva and plasma for both fasting and non-fasting states were also statistically significant ($P < 0.001$).

This increased AUC can be explained by considering the fact that, the time required to reach peak concentration is representative of the rate of drug absorption while AUC is proportional to the amount or extent of drug absorption. The greater the total amount of drug absorbed the greater the AUC and the greater will be the bioavailability (Abdel-Monem and Henkel, 1978).

This study suggested that higher values of nifedipine is obtained in saliva corresponding to higher bioavailability and that fasting volunteers maintained higher nifedipine plasma and salivary level than the non-fasting volunteers. In addition, fasting volunteers attained their peak nifedipine plasma and saliva levels faster than the non-fasting volunteers. Accordingly, it could be noted that the overall absorption-elimination profile for plasma did not differ significantly as indicated by the Areas Under the Curve.

The low fat meal appeared to reduce the rate of nifedipine absorption while the time it takes the drug to appear in the biological matrix and rate of nifedipine elimination were increased as indicated from the terminal half-life ($t_{\frac{1}{2}}\beta$) and the lag time ($t_{lag}$). Thus the presence of the low fat meal appears to influence the rate of dissolution of nifedipine. This may be related to longer retention in the stomach as suggested by the delayed time to peak. The total biological availability of nifedipine in the fasting and non-fasting states were however not statistically affected by the ingestion of the low-fat meal.

## CONCLUSION

The brands of nifedipine tablets (S, O and K) employed in this study satisfied the B.P. 1988 addendum 1990 official requirement for nifedipine content (98.00 - 102.00%). Similarly, they all passed the B.P. 1988 official identification tests employed. These results indicate the purity and unadulteration of the products in circulation.

A solvent system which has the added advantage of cost saving, better peaks symmetry, lesser analysis time and high value of resolution factor was developed for the analysis of nifedipine in both pharmaceutical preparations and biological fluids.

It has been demonstrated in the present study that nifedipine tablets are stable to different light and temperature conditions investigated and that nifedipine solution in methanol is only stable under red-light and elevated temperature up to 50$^{o}$C while it is unstable to other sources of light used.

The results of dissolution rate test and disintegration time test conducted for the three brands of nifedipine showed similarities in their respective dissolution rate profiles and disintegration times. All the brands investigated passed the official B.P. 1988 test for dissolution rate and disintegration times.

A simple and easily reproducible extraction technique for isolation of nifedipine from biological matrix has also been developed with a very high extraction recovery (>98.00%) in this study.

Higher concentration of nifedipine were detected in saliva compared with plasma in the absorption phase of nifedipine following oral ingestion, the levels thereafter being comparable. Plasma/saliva nifedipine elimination half-lives calculated were similar.

Measured area under the concentration-time curve (AUC) differed in the plasma and saliva in both fasting and non-fasting states, the difference being proportional, during absorption, to the amount of drug present in the system at time t (Kamali et al., 1987). There were marked differences in the rate of absorption by each individual in both fasting and non-fasting state. This could be due to a varying degree of hepatic first-pass effect or individual variability.

The low fat meal employed in this study appeared to reduce the rate of nifedipine absorption as indicated from the delayed time to peak in both plasma and saliva. A higher but statistically insignificant bioavailability of nifedipine was obtained in the fasting state.

The higher saliva concentrations of nifedipine observed in this study could have two uses: its specific identification and estimation in the saliva of patients

suffering from nifedipine overdose may assist the clinician to decide further treatment; and the measurement of the biological or systemic availability of nifedipine formulations could be achieved without the discomfort, possible hazard and necessary attendance of medical staff required for repeated venepunctures. Salivary level determination proved as useful as the plasma levels in the determination of some pharmacokinetic parameters of the drug. Due to the higher concentration of drug in the saliva, it is suggested that for some lipid soluble drugs, saliva could be used to determine their pharmacokinetic parameters.

The result of this study has shown that total biological availability of nifedipine is unaffected on ingestion of a low fat meal but the rate of absorption is reduced while $t_{lag}$ which is a measure of the appearance of drug in the plasma is increased.

Conclusively, it is suggested that further research work be conducted to investigate influence of other food components such as high fat meal, low and high carbohydrate meal, low and high protein meal to confirm the present observation on the bioavailability and pharmacokinetic of nifedipine.

Further work is also suggested on the stability test of nifedipine in carrying out dissolution rate studies on the exposed tablets.

There is need also for further studies involving the interactions of nifedipine with other commonly used drugs, such as paracetamol, chloroquine, ascorbic acid, vitamin B complex, cimetidine, etc.

## REFERENCES

ABDEL-MONEM, M.M. and Henkel, J.G. (1978): Drug product selection based on bioequivalence. Essentials of Drug Product Quality. The C.V. Mosby Company pp. 23-32.

ANHOFF, M. and Persson, B.A. (1990): Chromatography of calcium channel blockers: Review. Journal of Chromatography, 531: 181-213.

BLANCHARD, J. and Sawchuk, R.J. (1979). Drug Bioavailability: An Overview. Principles and Perspectives in Drug Bioavailability by Blanchard, J.; Sawchuk, R.J. and Brodie, B.B. Boehm S. Karger, A.G. 4011, Basel, Switzerland pp. 1-16.

BOROWITZ, J.L. and Moore, P.F. (1969): Pharmacological activity of sodium phenobarbitone. Journal of American Medical Association, 208: 1189.

BRITISH PHARMACOPOEIA (1988) ADDENDUM 1990, University Press, Cambridge. Identification Tests and Chemical Assay of Nifedipine, pp. 1245-1246.

BRITISH PHARMACOPOEIA (1988), Vol. II. University Press, Cambridge. Dissolution and Disintegration Test for Tablets and Capsules, APPENDIX XIID, A143-A144.

CHALLENER, V.F., Renwick, A.G., Gruchy, B.S., Waller, D.G. and George, C.F. (1985): The effect of food and posture on the pharmacokinetics of a novel formulation of nifedipine. British Journal of Clinical Pharmacology, 20(5): 550-551.

CHALLENOR, V.F., Waller, D.G., Gruchy, B.S., Renwick, A.G. and George, C.F. (1986). The effect of food and posture on the pharmacokinetics of a biphasic release preparation of nifedipine. British Journal of Clinical Pharmacology, 22: 565-570.

DAHNOF, M. and Breimer, D.D. (1978): Therapeutic drug monitoring in saliva. Clinical Pharmacokinetics. 3: 39-57.

DUHM, B., Maul, W., Medenwald, H., Patzschke, K., and Wegner, L.A. (1972) cited by Sorkin E.M., Clossold, S.P., and Brogden, R.N. (1985): Nifedipine: A review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy, in Ischaemic Heart Disease, Hypertension and Related Cardiovascular Disorders: Drugs 30: 182-274.

FOSTER, T.S., Hamann, S.R., Richards, V.R., Bryant, P.J., Graves, D.A. and McAllister Jr., R.G. (1983); Nifedipine kinetics and bioavailability after single intravenous and oral doses in normal subjects. Journal of Clinical Pharmacology, 23: 161-170.

GALEAZZI, R.L., Benet, L.Z. and Sheiner, L.B. (1976): Saliva: A deep pharmacokinetic compartment. Clinical Pharmacology and Therapeutics, 20: 278-289.

GERSTENBLITH, G., Ouyang, P., Achuff, S.C., Bulkley, B.H. and Becker, L.C. (1982): Nifedipine in unstable angina, a double blind, randomized trial. New England Journal of Medicine, 306: 885-889.

GIBALDI, M. and Perrier, D. (1975a): Bioavailability. Pharmacokinetics, Volume I. Ed. Swarbrick, J. pp. 129-173.

GIBALDI, M. and Perrier, D. (1975b): One-compartment model. Pharmacokinetics, Volume I Ed. Swarbrick, J. pp. 1-43.

GIBALDI, M. and Perrier, D. (1975c): Trapezoidal Rule. (Appendix 4). Pharmacokinetics, Volume I Ed. Swarbrick, J. pp. 293-296.

HORSTER, F.A., Duhm, B., Maul, W., Medenwald, H., Patzschke, K. and Wegner, L.A. (1972): Clinical investigations on the pharmacokinetics of radio actively marked 4-(2'-nitrophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethylester. Cited by Renwick, A.G., Robertson, R.C., Macklin, B., Challenor, V., Waller, D.G. and George, C.F. (1988): The Pharmacokinetics of oral nifedipine - a population study: British Journal of Clinical Pharmacology, 25: 701-708.

KAMALI, F., Fry, J.R. and Bell, G.D. (1987). Salivary excretion of paracetamol in man. Journal of Pharmacy and Pharmacology, 39: 150-152.

KAUMEIERE, S., Kissinger, E. and Neiss, A. (1979): The effect of gastro-intestinal filling on the absorption of sulfamethoxydiazine. International Journal of Clinical Pharmacology and Biopharmacy; 17: 412-415.

KLEINBLOESEM, C.H., Brummelen, P.V., Vandelinde, J.A., Voogd, P.F. and Breimer, D.D. (1984): Nifedipine: Kinetics and dynamics in healthy subjects. Clinical Pharmacology and Therapeutics, 35(6): 742-749.

KONDO, S., Kuchiki, A., Yamamoto, K., Akimoto, K., and Takahashi, K. (1980): Identification of nifedipine metabolites and their determination by gas chromatography. Chemical and Pharmaceutical Bulletin 28: 1-7.

KOUP, J.R. and Gibaldi, M. (1980): Some comments on the evaluation of bioavailability data. Drug Intelligence and Clinical Pharmacy, 14: 327-330.

KOZJEK, F., Primozic, S., Mahar, A., Karba, R. and Raemsch, K.D. (1987): The bioavailability of oral nifedipine formulations: A statistical and simulation approach. Biopharmacy Drugs Disposition, 8: 23-35.

LEVY, G. and Hayes, B.A. (1960): Physicochemical basis of the buffered acetylsalicylic acid controversy. New England Journal of Medicine, 262: 1053-1058.

LEVY, G., Leonards, J.R. and Procknol, J.A. (1965). Development of invitro dissolution test which correlate quantitatively with dissolution rate-limited drug absorption in man. Journal of Pharmaceutical Sciences, 54(12): 1719-1722.

LEVY, G. (1986): Applied Pharmacokinetics - A Prospectus. In: Applied Pharmacokinetics Principles of Therapeutic Drug Monitoring, 2nd Edition (Applied Therapeutics Inc.) p. XXI.

LEWIS, J.G. (1983): Adverse reactions to calcium antagonists. Drugs, 25: 196-222.

MEDENWALD, H., Schlossmann, K. and Wunsche, C. (1972) cited by Sorkin, E.M., Clissold, S.P. and Brogden, R.N. (1985): Nifedipine: A review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy, in Ischaemic Heart Disease, Hypertension and Related Cardiovascular Disorders: Drugs 30: 182-274.

MELANDER, A. (1978): Influence of food and specific dietary components on drug absorption. Clinical Pharmacokinetics, 3: 337.

OGUNBONA, F.A. and Oluwatudimu, O.O. (1986): Effect of a non-European (Nigerian) meal on the bioavailability of nitrofurantoin in man. International Journal of Pharmaceutics, 29: 191-193.

PATZECHKE, K., Schlossmann, K., Horster, F.A. (1975) cited by Sorkin, E.M., Clissold, S.P. and Brogden, R.N. (1985): Nifedipine: A review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy, in Ischaemic Heart Disease, Hypertension and Related Cardiovascular Disorders. Drugs 30: 182-274.

PAXTON, J.W. (1981): Elementary pharmacokinetics in Clinical Practice 2: basic concepts of drug pharmacokinetics. New Zealand Medical Journal, 94: 342-345.

PIERGIORGIO, P., Angelo, R., Pierantonio, B. (1981): High Performance Liquid Chromatography of nifedipine, its metabolites and photochemical degradation products. Journal of Chromatography, 210: 516-521.

POOLE, J.W. (1979): Effects of formulation and dosage form on drug bioavailability In: Principles and Perspectives in Drug Bioavailability by Blanchard, J., Sawchuk, R.J. and Brodie, B.B. Karger, A.G. 4011 Basle (Switzerland) pp. 59-89.

POSTI, J. (1982): Saliva: An integral part of the central compartment. Pharm. Acta Helv. 57: 83-92.

RAEMSCH, K. (1980): Pharmacokinetics of Nifedipine. II Mediterranean Symposium on New Aspects of Therapy in Angina pectoris and Hypertension, Madrid, September 19-20.

RAVENS, K.G. (1981): Effects of nifedipine on glucose tolerance in man. 8th International Congress of Pharmacology, Tokyo 19-24 July, 1981. Abstract No.1843 pp. 776.

RENWICK, A.G., Robertson , D.R.C., Macklin, B., Challenor, V., Waller, D.G. and George, C.F. (1988). The pharmacokinetics of oral nifedipine - a population study. British Journal of Clinical Pharmacology 25: 701-708.

REYNOLDS, F., Ziroyanis, P.N., Jones, N.F. and Smith, S.E. (1976): Salivary phenytoin concentrations in epilepsy and in chronic renal failure. Lancet ii 384-386.

SCHLOSSMANN, K., Medenwald, H. and Rosenkranz, H. (1975): Investigations on the metabolism and protein binding of nifedipine. 2nd International Adalat Symposium, pp.33-39.

SKELLY, J.P. (1976): Estimation of concentration of drugs in biological fluids. Journal of Clinical Pharmacology, 16: 539.

TERRY, R.W. (1982): Nifedipine therapy in angina pectoris: Evaluation of safety and side effects. American Heart Journal, 104: 681-689.

TESTA, R., Dolfini, E., Reschiotto, C., Secchi, C. and Biondi, P.A. (1979), cited by Pietta, P., Rava, A. and Biondi, P. High Performance Liquid Chromatography of nifedipine, its metabolites and photochemical degradation products (1981). Journal of Chromatography, 210: 516-521.

UNITED STATES PHARMACOPOEIA XX (1980) Edition Fifteenth. Mack Publishing, Easton, pp. 11-13.

WADE, D.N. (1976): The therapeutic performance of drug products. Drug Treatment, Ed. Avery, S.; Avis Press, Sydney pp. 143-160.

WAGNER, J.G. (1967): Method of estimating relative absorption of a drug in series of clinical studies in which blood levels are measured after single and/or multiple doses. Journal of Pharmaceutical Sciences, 56: 652.

WAGNER, J.G. (1971b): "Theory of tablet disintegration and factors affecting disintegration time of tablets and capsules." Biopharmaceutics and Relevant Pharmacokinetics by Wagner, J.G. and Pernarowski, M. First Edition. Drug Intelligence Publications, Hamilton, Illinois pp. 89-97.

WAGNER, J.G. (1980): Bioavailability trials: A modern perspective. Pharmacy International, p. 184.

WALLER, D.G., Renwick, A.G., Gruchy, B.S. and George, C.F. (1984): The first-pass metabolism of nifedipine in man. British Journal of Clinical Pharmacology, 18: 951-954.

WELLING, P.G. (1977): Influence of food and diets on absorption of drugs. Pharmacokinetics and Biopharmacy. 5: 291.

WELLING, P.G. (1980): Drug bioavailability and its clinical significance. Progress in Drug Metabolism, Vol. 4 by Bridges, J.W. and Chasseaud, I.F.A. Willey-Interscience Publication, pp. 161-163.

WEBER, L.J. and Mclean D.L. (1975): Comparison of different light sources. Electrical Measurement Systems for Biological and Physical Scientists, Addison-Wesley Publishing Company, Reading, Massachusetts Menlo Park, California, pp. 522.

WOLFF, F.A., Mattie, H. and Breimer, D.D. (1979): Salivary excretion and drug monitoring. Therapeutics Relevance of Drug Assays; Vol. 14, pp. 17-19.

WURSTER, D. and Taylor, P. (Jr) (1965): Dissolution kinetics of certain crystalline form of prednisolone. Journal of Pharmaceutical Science 54(5): 671-676.

## APPENDIX 1

Dissolution rate studies of Nifedipine Tablets in 0.1M HCl maintained at 37 ± 0.5°C using B.P. (1988) Rotating Basket Method.

(a) Mean Absorbances measured at 349nm.

| Time (Mins) | Absorbance (A) (nm) | | |
|---|---|---|---|
| | S | O | K |
| 5.0 | 0.026 | 0.025 | 0.016 |
| 15.0 | 0.033 | 0.033 | 0.031 |
| 30.0 | 0.037 | 0.036 | 0.039 |
| 45.0 | 0.028 | 0.028 | 0.029 |
| 60.0 | 0.023 | 0.022 | 0.024 |

(b) Cumulative concentration (Cn)

| Time (Mins) | Concentration (Cn) Mg/1000ml | | |
|---|---|---|---|
| | S | O | K |
| 5.0 | 2.01 | 1.911 | 1.210 |
| 15.0 | 11.30 | 11.05 | 8.680 |
| 30.0 | 20.01 | 20.07 | 20.08 |
| 45.0 | 19.96 | 19.85 | 29.91 |
| 60.0 | 19.67 | 19.24 | 19.32 |

## APPENDIX 2

### PREPARATION OF ACETATE AND PHOSPHATE BUFFER

(a) ACETATE BUFFER (pH 4.0): (a) Dissolve 11.55ml of 0.2M solution of acetic acid in 1000ml of distilled water; (b) dissolve 16.4g of anhydrous sodium acetate or 27.2g of sodium acetate trihydrate in 1000ml of distilled water. Mix 41.0ml of (a) with 9.0ml of (b) and dilute to a volume of 100ml with distilled water to obtain a solution of pH 4.0.

(b) PHOSPHATE BUFFER (pH 7.4): (a) Dissolve 9.465g of anhydrous disodium hydrogen phosphate in distilled water and dilute to 1000ml, (b) dissolve 9.073g of potassium dihydrogen phosphate in distilled water and dilute to 1000ml. Mix 80ml of (a) with 20ml of (b) to obtain a solution of pH 7.4.

(c) PHOSPHATE BUFFER (pH 7.0): Mix 50ml of 0.2M potassium dihydrogen phosphate with 30ml of 0.2M sodium hydroxide and dilute with distilled water to 200ml to obtain a solution of pH 7.0.

(d) PHOSPHATE BUFFER (pH 6.95): Dissolve 0.624g of disodium hydrogen phosphate dihydrate and 0.363g of potassium dihydrogen phosphate in sufficient distilled water to produce 100ml to obtain a solution of pH 6.95.

## APPENDIX 3

### APPARATUS FOR THE DISINTEGRATION OF TABLETS

The apparatus consists of

(a) a rigid basket-rack assembly supporting 6 cylindrical glass tubes 77.5mm long, 21.5mm in internal diameter, and with a wall thickness of about 2mm.

(b) a cylindrical disc for each tube, each 20.7mm in diameter and 9.5mm thick, made up of transparent plastic with a relative density of 1.18 to 1.20, and pierced with five holes, each 2mm in diameter, one in the centre and the other four spaced equally on a circle of radius 6mm from the centre of the disc. Four equally-spaced grooves are cut in the lateral surface of the disc in such a way that at the upper surface of the disc they are 9.5mm wide and 2.55mm deep and at the lower surface 1.6mm square.

(c) The tubes are held vertically by 2 super-imposed transparent plastic plates 90mm in diameter and 6mm thick, perforated by 6 holes having the same diameter as the tubes. The holes are equidistant from the centre of the plate and are equally spaced from one another. Attached to the under-side of the lower plate is a piece of wooven guaze made from stainless steel wire 635 μm in

diameter and having nominal mesh apertures of 2.00mm. The upper plate is covered with a stainless steel disc perforated by six holes each about 22mm in diameter, which fits over the tubes and holds them between the plastic plates. The holes coincide with those of the upper plastic plate and the upper ends of the glass tube.

(d) The plates are held rigidly in position and 77.5mm apart by vertical metal rods at the periphery and a metal rod is also fixed to the centre of the upper plate to enable the assembly to be attached to a mechanical device capable of raising and lowering it smoothly at a constant frequency of between 28 and 32 cycles per minute, through a distance of 50 to 60mm.

(e) The assembly is suspended in the liquid medium in a suitable vessel, preferrably a 1000ml beaker. The volume of liquid is such that the wire mesh at its highest point is at least 25mm below the surface of the liquid, and at its lowest point is at least 25mm above the bottom of the beaker.

(f) A suitable device maintains the temperature of the liquid at $37 \pm 0.5^{\circ}C$.

## APPENDIX 4

### COMPARISON OF DIFFERENT LIGHT SOURCES

| LIGHT SOURCES/ PROPERTIES | SOLAR ENERGY | SODIUM LIGHT | FLUORESCENT LIGHT | RED LIGHT |
|---|---|---|---|---|
| BRIGHTNESS | Has the greatest brightness | Brighter than flourescent light | Brighter than red light | Has the least brightness |
| INTENSITY/ RADIATION $\lambda$ = WAVE-LENGTH | Radiates $8.33 \times 10^{25}$ $kwh^3$ of energy into space every-day. $(0.29<\lambda<3)\mu m$ | Radiates yellow colour $\lambda \approx 0.6\mu m$ | Since white light is always taken as yellow light, the intensity will be assumed to be the same as that of sodium light $\lambda \approx 0.6\mu m$ | Radiates red colour $\lambda = 0.7\mu m$ |
| REFLECTION | Greatest reflection | Reflection is less than that of fluorescent light. | High reflec-tion. | Has the least reflection |
| RECOMMENDA-TION OR USE | Natural sun-light. Solar energy may be tapped for some technologi-cal purposes like: (1) Electri-city gene-ration. (2) Pumping system (3) Irriga-tion,etc | Street lightening | General purpose, industrial lightening. | Used in a situation where sub-dued light is required |

Culled from Weber, L.J. and Mclean, D.L. (1975).

## APPENDIX 5

## QUESTIONAIRE FOR VOLUNTEERS PARTICIPATING IN THE INVIVO BIOAVAILABILITY STUDIES

SERIAL NUMBER: .....

NAME: .....

AGE: ..... SEX: ..... (M) ..... (F)

HEIGHT: ..... WEIGHT: .....

CONTACT ADDRESS: .....
.....
.....

BRIEF MEDICAL HISTORY

.....

.....

Have you ever had any of the following diseases?
(Tick that which is applicable).

Malaria .....
Measles .....
Hypertension.....
Angina pectoris.....
Anaemia .....
Eye problem .....

If yes, when did you complete your medication?

.....

Time last meal was taken (Tick that which is applicable)

12 hours ago .....
10 hours ago .....
8 hours ago .....
2 hours ago .....

Components of last meal taken:.....

.....

APPENDIX 6: Plasma and saliva concentration of unchanged nifedipine (ng/ml) in 6 healthy volunteers following oral administration of the tablets (20mg) in fasting and non-fasting states.

PLASMA

| TIME (hr) | CONCENTRATION (ng/ml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | | II | | III | | IV | | V | | VI | |
| | F | NF | F | NF | F | NF | F | NF | F | NF | F | NF |
| 0.5 | 16.20 | 5.50 | 18.10 | 8.10 | 17.50 | 6.95 | 16.90 | 44.80 | 14.85 | 6.85 | 15.67 | 5.80 |
| 1.0 | 27.00 | 8.95 | 29.30 | 23.60 | 28.70 | 10.70 | 29.20 | 15.60 | 25.30 | 15.30 | 26.85 | 14.70 |
| 2.0 | 35.00 | 26.50 | 35.10 | 32.80 | 36.90 | 23.60 | 39.40 | 30.10 | 38.70 | 31.90 | 38.60 | 20.10 |
| 3.0 | 37.10 | 29.20 | 35.95 | 35.00 | 36.20 | 32.55 | 37.00 | 39.45 | 36.10 | 35.60 | 36.90 | 36.00 |
| 4.0 | 27.20 | 34.55 | 27.30 | 37.60 | 28.30 | 38.20 | 30.35 | 32.20 | 29.15 | 23.40 | 28.86 | 22.85 |
| 6.0 | 20.20 | 23.35 | 18.66 | 26.40 | 20.60 | 24.10 | 21.40 | 13.60 | 19.95 | 15.55 | 19.60 | 17.10 |
| 8.0 | 14.00 | 14.22 | 13.20 | 15.30 | 14.66 | 16.40 | 14.48 | 9.80 | 13.68 | 14.60 | 12.60 | 10.50 |
| 12.0 | 4.90 | 8.35 | 6.90 | 10.60 | 8.77 | 14.35 | 5.55 | 3.90 | 6.20 | 7.85 | 5.90 | 6.25 |

F = FASTING STATE

NF = NON-FASTING STATE

SALIVA

| TIME (hr) | CONCENTRATION (ng/ml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | | II | | III | | IV | | V | | VI | |
| | F | NF | F | NF | F | NF | F | NF | F | NF | F | NF |
| 0.5 | 23.00 | 10.10 | 19.97 | 9.70 | 25.85 | 12.41 | 20.30 | 9.10 | 22.90 | 8.65 | 21.70 | 11.25 |
| 1.0 | 41.50 | 20.50 | 27.30 | 19.80 | 44.80 | 20.65 | 39.05 | 21.40 | 28.76 | 19.30 | 40.90 | 20.40 |
| 2.0 | 65.24 | 35.90 | 40.47 | 31.70 | 63.90 | 29.40 | 60.95 | 33.30 | 41.94 | 36.90 | 61.45 | 28.55 |
| 3.0 | 61.12 | 58.80 | 59.51 | 54.60 | 58.05 | 60.85 | 55.12 | 59.90 | 62.44 | 56.50 | 57.10 | 57.80 |
| 4.0 | 53.50 | 48.70 | 56.58 | 43.50 | 49.60 | 55.75 | 40.60 | 47.60 | 56.59 | 39.60 | 37.90 | 40.50 |
| 6.0 | 37.95 | 40.75 | 41.54 | 37.20 | 41.95 | 41.30 | 36.08 | 39.80 | 39.01 | 31.20 | 28.45 | 32.80 |
| 8.0 | 27.20 | 27.60 | 25.83 | 22.40 | 28.76 | 24.15 | 22.90 | 21.75 | 25.63 | 19.80 | 24.60 | 16.70 |
| 12.0 | 10.50 | 11.50 | 16.20 | 8.90 | 17.10 | 10.80 | 12.40 | 12.85 | 13.80 | 9.70 | 11.70 | 7.75 |

F = FASTING STATE
NF = NON-FASTING STATE

**APPENDIX 7:** Pharmacokinetic parameters of nifedipine in plasma and saliva of individual volunteers after a single 20mg oral dose in fasting and non-fasting state.

PLASMA

| VOLUNTEERS | I | | II | | III | | IV | | V | | VI | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PARAMETERS | F | NF | F | NF | F | NF | F | NF | F | NF | F | NF |
| Lag time (h) | 0.09 | 0.26 | 0.08 | 0.34 | 0.13 | 0.32 | 0.14 | 0.38 | 0.18 | 0.40 | 0.17 | 0.44 |
| $t_{\frac{1}{2}}$ (h) | 0.84 | 0.04 | 1.66 | 0.56 | 1.61 | 0.34 | 0.73 | 0.77 | 0.77 | 1.81 | 0.78 | 1.30 |
| Ka ($h^{-1}$) | 0.83 | 0.67 | 1.05 | 0.44 | 1.14 | 1.52 | 0.95 | 0.89 | 0.90 | 0.38 | 0.90 | 0.53 |
| $C_{max}$ (ng/ml) | 37.10 | 37.60 | 35.95 | 38.20 | 36.90 | 36.00 | 39.40 | 35.60 | 38.70 | 34.55 | 38.60 | 39.45 |
| $t_{max}$ (h) | 3.0 | 4.0 | 3.0 | 4.0 | 2.0 | 3.00 | 2.0 | 3.0 | 2.0 | 4.0 | 2.0 | 3.0 |
| $t_{\frac{1}{2}}\beta$ (h) | 3.01 | 3.52 | 3.40 | 3.60 | 3.88 | 2.42 | 3.16 | 3.33 | 3.05 | 2.45 | 2.87 | 1.75 |
| Ke ($h^{-1}$) | 0.23 | 0.19 | 0.204 | 0.19 | 0.18 | 0.29 | 0.22 | 0.21 | 0.23 | 0.28 | 0.24 | 0.40 |
| $AUC_{(0-12h)}$ | 229.40 | 263.83 | 229.22 | 249.31 | 244.17 | 181.05 | 245.39 | 206.39 | 234.55 | 221.67 | 231.65 | 195.15 |
| $AUC_{(0-\infty)}$ | 250.71 | 317.63 | 263.01 | 323.82 | 293.21 | 202.92 | 270.70 | 244.04 | 261.81 | 251.23 | 256.11 | 204.97 |
| Vd (L) | 346.93 | 319.63 | 372.48 | 320.68 | 381.41 | 344.89 | 336.88 | 393.13 | 335.84 | 281.79 | 323.84 | 245.77 |
| Cl (L/h) | 79.77 | 62.97 | 76.04 | 61.76 | 68.21 | 98.58 | 73.88 | 81.95 | 76.39 | 79.61 | 78.10 | 97.57 |

F = FASTING STATE
NF = NON-FASTING STATE

SALIVA

| VOLUNTEERS | I | | II | | III | | IV | | V | | VI | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PARAMETERS | F | NF | F | NF | F | NF | F | NF | F | NF | F | NF |
| Lag time (h) | 0.19 | 0.34 | 0.089 | 0.34 | 0.18 | 0.35 | 0.26 | 0.35 | 0.05 | 0.37 | 0.27 | 0.31 |
| $t_{\frac{1}{2}}$ (h) | 0.87 | 1.73 | 1.88 | 1.86 | 0.59 | 1.85 | 0.59 | 1.70 | 1.84 | 1.52 | 0.54 | 1.63 |
| Ka ($h^{-1}$) | 0.79 | 0.40 | 0.37 | 0.37 | 1.17 | 0.37 | 1.18 | 0.41 | 0.38 | 0.46 | 1.28 | 0.42 |
| Cmax (ng/ml) | 61.87 | 58.80 | 59.51 | 54.60 | 63.90 | 60.85 | 60.95 | 59.90 | 62.44 | 56.50 | 61.45 | 57.80 |
| $t_{max}$ (h) | 2.0 | 3.0 | 3.0 | 3.0 | 2.0 | 3.0 | 2.0 | 3.0 | 3.0 | 3.0 | 2.0 | 3.0 |
| $t_{\frac{1}{2}}$ B (h) | 3.20 | 2.70 | 2.67 | 2.37 | 4.73 | 2.52 | 3.89 | 2.56 | 2.54 | 2.34 | 3.60 | 2.34 |
| Ke ($h^{-1}$) | 0.22 | 0.26 | 0.26 | 0.30 | 0.14 | 0.27 | 0.18 | 0.27 | 0.27 | 0.30 | 0.19 | 0.30 |
| $AUC_{(0-12h)}$ | 421.99 | 372.95 | 403.29 | 328.23 | 440.79 | 369.12 | 376.99 | 353.48 | 399.07 | 310.64 | 365.60 | 296.41 |
| $AUC_{(0-\infty)}$ | 470.37 | 417.81 | 465.75 | 358.62 | 557.61 | 408.47 | 446.59 | 400.77 | 449.58 | 343.35 | 426.21 | 322.55 |
| Vd (L) | 195.95 | 186.75 | 165.57 | 190.48 | 245.03 | 178.40 | 251.37 | 183.67 | 162.84 | 196.43 | 243.10 | 209.13 |
| Cl (L/h) | 42.52 | 47.87 | 42.94 | 55.77 | 35.87 | 48.96 | 44.78 | 49.90 | 44.49 | 58.25 | 46.92 | 62.01 |

F = FASTING STATE
NF = NON-FASTING STATE