# THE EVALUATION OF INDOMETHACIN AND THEOPHYLLINE ORAL CONTROLLED/MODIFIED-RELEASE DOSAGE FORMS: *IN VITRO - IN VIVO* CORRELATIONS

by

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# ABSTRACT

Over the past few decades many researchers have investigated the utility of *in vitro - in vivo* correlations for the assessment of dosage forms. These investigations are, however, dependent on reproducible dissolution data and well conducted biostudies in order to establish meaningful and robust correlations. Despite the fact that the establishment of such correlations is perhaps idealistic, considerable interest has still been shown in this area of research.

Various Controlled/Modified Release Dosage Forms (CMRD's) of theophylline, a weakly basic drug, and indomethacin, a weakly acidic drug, were assessed in order to establish *in vitro - in vivo* correlations. Dissolution rate studies were carried out using either the USP basket or paddle apparatus. The dissolution rate studies were conducted in a range of dissolution media of varying pH. Bioavailability studies were conducted on the dosage forms used by the Biopharmaceutics Research Institute at Rhodes University. The results of these biostudies were kindly made available for use in this research project.

Type A correlations were established using a mathematical simulation process whereby expected *in vivo* responses are simulated and compared to actual profiles obtained for the dosage forms. In order to perform the simulations the dissolution rate profiles were stripped and using linear regression and the methods of residuals the dissolution rate order and the relevant dissolution rates were obtained. The results of the simulations indicated that the *in vivo* serum concentration-time curves could be accurately predicted for the theophylline dosage forms but to a lesser extent, for the indomethacin formulations.

The dissolution rate studies indicated that the paddle method is a suitable method for dissolution rate studies of theophylline CMRD's, although it appeared that the optimum pH of the dissolution medium was formulation dependent. Dissolution rate

studies conducted on indomethacin formulations indicated that the USP specified basket method for extended-release indomethacin formulations was not able to distinguish between two formulations which exhibited different *in vivo* profiles. The conversion to the paddle method was, however, able to highlight the differences between these formulations.

The use of three dimensional topographs to depict dissolution rate profiles was demonstrated for formulations of both theophylline and indomethacin. The topographs enabled the successful differentiation between bioinequivalent formulations. The dissolution rate profiles were also fitted to the Weibull equation and the parameters obtained from this were compared to the Weibull parameters obtained from the *in vivo* absorption plots obtained using the Wagner-Nelson method. The results indicated that the Weibull function was suitable to describe both the *in vivo* and *in vitro* data.

The following recommendations for the preformulation dissolution studies of weakly acidic and weakly basic drugs are proposed. The dissolution rate studies of weakly acid drugs, such as indomethacin, should be carried out over a range of pH utilising the paddle apparatus. Three dimensional topographs based on the dissolution data should be constructed and used as a comparative tool for different formulations. Based on these comparisons the appropriate formulation can then be selected for a pilot scale *in vivo* bioavailability study.

The dissolution rate studies of weakly basic drugs, such as theophylline, should be carried out over a range of pH utilising the paddle apparatus. The dissolution data should then be used to simulate the expected *in vivo* profile and on this basis the appropriate formulation selected for a pilot scale bioavailability study. The above approach to the preformulation studies of new CMRD's would allow for the more careful selection of new dosage forms and could thus eliminate costly and unnecessary bioavailability studies performed on inferior formulations.

# CHAPTER 1

## Introduction to *in vitro* - *in vivo* correlations

### 1.1 Overview

The development of complex oral controlled/modified release dosage forms, (CMRD's), has brought with it many advantages including increased patient acceptance and compliance.. These developments have, however, also brought about their unique biopharmaceutical problems. Historical claims that all the drug present in a solid oral dosage form is available for absorption clearly no longer applies as the understanding of all the complex processes involved in drug absorption become more clearly understood.

These developments have also brought about the need for *in vitro* test procedures during the early stages of research and development which could limit the number of costly and time consuming *in vivo* tests. One method of achieving this is with the use of *in vitro* - *in vivo* correlations. Langenbucher has suggested that it is not possible to immediately correlate an *in vitro* parameter with an *in vivo* plasma concentration-time curve as the two entities have nothing in common (1). Some link must be established between the *in vitro* parameters and the *in vivo* response obtained after administration of a solid oral dosage form. The most common approach today is the use of *in vitro* dissolution rate testing. Parameters obtained from the dissolution tests are then, by mathematical processes, equated with the parameters obtained *in vivo*. The premise is that dissolution of a dosage form must occur *in vivo* before any drug can be available for absorption.

In order to establish a correlation between the two elements, some third factor is required to link them. Generally two approaches can be employed. Firstly, the *in vivo* dissolution input function can be derived by a method of deconvolution from the

*in vivo* response and directly compared with the *in vitro* dissolution. Secondly, the opposite process can also be employed whereby using a process of convolution of the *in vitro* dissolution, an expected *in vivo* response can be predicted (1).

*In vitro - in vivo* correlations therefore serve as a link between *in vitro* data and *in vivo* data. In theory, correlating dissolution to clinical responses would appear to be a worthwhile undertaking for the assessment of new dosage forms, especially during the developmental stages. It is, however, ironic that most correlations have been established after bioavailability problems have been identified. *In vitro - in vivo* correlations have therefore been used as methods of explaining product failures rather than predicting product performance *in vivo*. Hence, there exists a need for correlations to be established during the research and development stage of product design, as opposed to their use as a retrospective tool.

Many different attempts have been made to establish *in vitro - in vivo* correlations, both for immediate release solid oral dosage forms as well as sustained release preparations. Despite reservations as to whether meaningful *in vitro - in vivo* correlations can be obtained, the literature is replete with reports of attempts at correlating these two aspects. Needless to say, these attempts have produced both positive and negative results. The literature is abundant with reports showing the variability in the clinical response between administered drug products that contain chemically equivalent amounts of a drug (2). Early retrospective *in vitro - in vivo* correlations led to research projects which centred on intentionally varying a particular parameter of either the drug; i.e. by changing the polymorph, or the dosage form in order to ascertain whether such variations would correlate with changes in bioavailabilty. These changes were expected to be reflected by either changes in the absorption rate, total drug absorbed or some other relevant *in vivo* parameter.

Hűttenrauch and Speiser (3) have, however, indicated that due to the many sources

of variability and their possible additive effect, the establishment of an *in vitro - in vivo* correlation should be seen as an unrealistic problem. From the biopharmaceutical point of view the necessity arises to consider and evaluate both the *in vitro* and *in vivo* processes. In drug research, both must be considered as indispensable, but more importantly the one cannot be substituted for the other. The *in vitro* methods are important in the development of pharmaceutical procedures and optimisation of the dosage forms, while *in vivo* tests are invaluable in obtaining quantitative estimates of the behaviour of a dosage form in a living organism.

## 1.2 Historical Development of *In vitro - In vivo* correlations

In the early years of tablet assessment the physical appearance of the dosage form represented the only control procedure of the manufacturing process. However, the recognition that tablets could pass intact through the gastrointestinal tract brought about the development of the disintegration test for the assessment of solid dosage forms. These tests assumed that if the tablet disintegrated, its active component or drug content would automatically be available. There was, however, also an awareness that the rate of dissolution of drug particles played a fundamental role in the determination of drug availability. Coinciding with these developments researchers began to develop *in vivo* assessment procedures which included the studies of plasma and urinary concentrations of a drug as a measure of the physiological availability. Dissolution tests can only be regarded as valuable tools if they provide results which have been correlated with quantitative measures of physiological availability (4, 5).

Initially, the disintegration test was considered to be the sole criterion of drug availability. This presupposed that the disintegration of a dosage form must be followed by dissolution under essentially uniform conditions. For many dosage forms this has been shown, to some extent, to be the case (4). However, pioneering studies conducted by Levy (6) found that there was no direct relationship between

disintegration of salicylate tablets and the amount of salicylate excreted in the urine. Furthermore, the disintegration test was not able to distinguish between rapidly and slowly dissolving small granules or primary particles. The test therefore gave no handle on any changes in the dissolution characteristics due to changes in the physical characteristics of the drug compound (5). Campagna *et al* (7) suggested that while the disintegration test may be a useful test as an industrial control procedure, it has no value as an index of the bioavailability of compressed tablets. The replacement of the disintegration test with a dissolution test was suggested.

It is now a well established fact that the therapeutic efficacy of any dosage form is dependent on factors related to both the *in vitro* dissolution characteristics of the drug and its *in vivo* bioavailability. For the drug to be available it must be released from the dosage form (8). In the late 1960's the dissolution rate test became acceptable as a test procedure for tablet evaluation. Many dissolution test procedures are in use today which vary from extremely gentle procedures to relatively active turbulent mixing. Wood (4) noted that each procedure must have its *in vivo* correlation established before it may be validly used as a criterion of release properties. The dissolution rate of a specific dosage form is, in essence, a parameter which is arbitrary and very dependent on the methodology utilised in obtaining it. Any changes in the type of apparatus used, the dissolution medium, agitation speed etc, can dramatically modify the dissolution pattern. It is therefore important that the *in vitro* dissolution test be shown to have some bearing on the *in vivo* situation in order for a meaningful correlation to be established (8).

The establishment of *in vitro - in vivo* correlations can be attempted in various ways, including (a) pharmacological correlations based on clinical observations, (b) semiquantitative correlations based on the drug's blood concentrations or urinary excretion data and (c) quantitative correlations arising from absorption kinetics and calculation of *in vivo* dissolution rate and absorption rate constants. In theory, the

correlation of *in vitro* dissolution rates with clinical responses would be ideal, however, a clinical response is a very poor tool for accurate measurement of bioavailability (2, 8). This is particularly true where the clinical response is an all or nothing response, which is often the situation. In cases where *in vivo* differences are observed, the *in vitro* parameter can be altered to optimise the correlation. This is achieved by varying the test conditions of the *in vitro* test such as the method itself, the media used, rate of agitation etc (8). Shah (9) noted that an *in vitro - in vivo* correlation can generally be achieved with the use of any reproducible method. However, proper selection of the medium and the degree of agitation are necessary so as to permit discrimination amongst products. Measurement of blood levels at the site of action would also be a useful approach but the current state of technology does not allow this (8).

Numerous variables have been used to establish correlations between *in vitro* and *in vivo* data. Variables derived from *in vivo* data which have been correlated with *in vitro* variables have been summarised by Wagner (10) and include the following:

i) Plasma or serum concentration-time plots or the corresponding numerical values,

ii) Peak plasma or serum concentrations, $C_{max}$,

iii) AUC during some time interval, $AUC_{0 \rightarrow t}$,

iv) $AUC_{0 \rightarrow T}$ - i.e. area under the blood curve to the last blood sample at time T,

v) $AUC_{0 \rightarrow \infty}$ - i.e. area under the entire blood concentration time curve,

vi) Rate constant for absorption, $k_a$, or the half-absorption time derived by applying a pharmacokinetic model to urinary excretion data or blood data,

vii) Amount of drug excreted per a given time interval,

viii) Plots of cumulative amount of drug excreted versus time,

ix) Percent absorbed time plots derived by pharmacokinetic

analysis of blood or urinary excretion data,

x) Pharmacological responses such as lowering of blood sugar levels, blood pressure, pain relief etc.

Many variables derived from *in vitro* data, using various methods, have been correlated with *in vivo* variables. These include:

i) Disintegration time (4, 11, 12),

ii) Time for a certain percentage of the drug to dissolve *in vitro* eg. $t_{20\%}$, $t_{50\%}$ etc (13, 14, 15, 16, 17),

iii) Concentration or amount in solution at a given time (9, 11, 12, 18, 19),

iv) Percent dissolved time plots (9, 20, 21, 22),

v) Rate of dissolution versus time plots (6),

vi) First order plot of percent to be dissolved on logarithmic scale versus time (13),

vii) Plots of percent dissolved on probability scale versus time on logarithmic scale,

viii) Rate constants or dissolution half times derived by kinetic analysis,

ix) Intrinsic dissolution.

Wagner (10) noted that the best variable to correlate *in vivo* data is the time taken for 50% of the drug to dissolve *in vitro*, i.e. $t_{50\%}$. The reason given for this choice is that: (1) its value indicates the central tendency of the dissolution data and (2) its use does not commit the use of any formal kinetic interpretation of the data. If absorption is dissolution rate limited then the best *in vivo* parameter is the time for 50% of the drug to be absorbed.

Differences in bioavailability observed *in vivo* between two different formulations, products or lots should be reflected by similar differences in the *in vitro* dissolution

test. Correlations can be judged as poor when:

i) significant differences in the dissolution are not reflected in the plasma or urine bioavailability data,

ii) differences in the *in vivo* data are not shown by the *in vitro* data,

iii) the rate orders are inverse between the two data sets and

iv) dissolution is faster or slower than is consistent with the *in vivo* data (23).

The failure to establish meaningful correlations can possibly be due to the choice of incorrect *in vitro* testing conditions, such as excessive agitation rates, thereby masking differences between products (23). Within reason, the closer the *in vitro* test conditions are to those prevailing in the physiological environment , the greater is the chance the results obtained will be reflective of those occurring *in vivo*. The testing of dosage forms with inappropriate conditions such as excessive agitation, unphysiological pH, high concentrations of organic solvents etc, is unlikely to have any real assessment value (24).

## 1.3 Early Case Studies

It was probably the work of Levy and co-workers (6) that led to and maintained the interest in the subject of dissolution and bioavailability and the subsequent proposal of the first *in vitro - in vivo* correlation. Through carefully designed studies on various commercial brands of aspirin tablets the authors found that the absorption rate of the salicylate was dependent on the dissolution rate of the drug. A quantitative relationship was obtained between the amount of aspirin excreted and the amount of aspirin dissolved *in vitro*. The authors noted that such relationships would be of extreme importance for the dissolution test to qualify as a predictive tool for bioavailability.

Levy *et al* (20) suggested that the development of more generalised *in vitro* dissolution rate testing could proceed via two pathways, namely: (i) the development of a single test which would correlate the *in vivo* absorption of a number of different drugs administered in a single type of dosage form or (ii) the development of a single *in vitro* test which would correlate with the absorption of a single drug administered in several different dosage forms. A correlation of the type as described in (ii) above was established for three different formulations of aspirin. The authors concluded that the successful quantitative correlation of drug absorption with the results of a single *in vitro* dissolution test was an encouraging indication that the development of more generalised dissolution rate tests was feasible (20).

Numerous other researchers have endeavoured to establish meaningful correlations. Many have done so successfully using a multitude of different dissolution procedures (9, 13, 14, 15, 16, 18, 19, 21, 22, 25). On the other hand, various researchers have found that no correlation was possible for some specific dosage forms tested (11, 12, 26, 27, 28).

Weintraub and Gibaldi (21), also working with various dosage forms of aspirin , found that they could correlate the percent dissolved *in vitro* to time T with the percent absorbed to time T. Good correlations were found using various *in vitro* test conditions employing the rotating flask method of dissolution (21). Aoyagi and co-workers (13) established correlations for griseofulvin tablets using dissolution conditions which provided for both sink and non-sink environments. They correlated the amount dissolved at $t_{5\%}$ and $t_{30\%}$ with the amount of drug absorbed at 1 hour using normal-reciprocal and log-log regressions.

In a study conducted by Shah *et al* (25) using chlorothiazide tablets employing the paddle method of dissolution a correlation for bioequivalent products was established. The authors concluded that the correlation established, would

reasonably assure that a product meeting the dissolution limits would be bioequivalent with the existing products. Studies conducted on phenytoin dosage forms, also by Shah *et al* (9) found positive correlations between dissolution data obtained at 30 and 60 minutes and the $C_{max}$ and $t_{max}$ values. The best correlation was obtained using the basket apparatus, followed by the spin filter method and then the paddle method.

McNamara and co-workers (14) established *in vitro - in vivo* correlations for furosemide tablets. It was found that the amount of furosemide excreted in the urine could be associated with the percent drug dissolved *in vitro* at 30 min. It was further established that dissolution carried out in media of pH 5.6, as opposed to previous studies carried out in media of pH 4.6, allowed for the assessment of batch uniformity and bioavailability of furosemide tablets. Attempts were also made to establish correlations between the $MDT_{in\ vivo}$ and the $MDT_{in\ vitro}$ in these studies, but these proved to be statistically insignificant.

In a bioavailability and dissolution study on digoxin tablets Fraser *et al* (18) found that good correlations existed between the area under the serum concentration-time curve from 0 to 6 hours and the amount of digoxin dissolved in one hour. Good correlations were also established using the reciprocal of $t_{60\%}$ and the AUC. These studies were able to distinguish between products of differing bioavailabilities and it was concluded that the *in vitro* test could be employed to control this variable.

Wagner *et al* (19), in a study on the *in vivo* and *in vitro* availability of commercial warfarin tablets, found that the results obtained *in vivo* correlated very well with the results of the *in vitro* rate of dissolution. No correlations were, however, found between the *in vivo* data and the disintegration tests conducted on the same tablets. The authors concluded that the establishment of the correlation did not necessarily imply that other commercial warfarin tablets, irrespective of the manufacturer, would provide satisfactory results *in vivo*.

Using the USP paddle and basket apparatus and a commercial dissolution simulator (Sartorius Dissolution Simulator, Sartorius Filters Inc., Calif.), an apparatus designed to simulate *in vivo* dissolution, Yau and Meyer (15) carried out dissolution studies on a number of different dosage forms of methamine, nitrofurantoin and chlorothiazide. Using the data obtained from the dissolution simulator more meaningful correlations were obtained for the tablets, except the chlorothiazide, when compared to those obtained using the data from the USP dissolution methods. *In vivo* measurements, such as the cumulative percent of drug excreted at specific times and the maximum urinary excretion rates, were correlated with the *in vitro* data including the cumulative percent of drug dissolved and $t_{15\%}$ and $t_{50\%}$. The authors concluded that the general applicability of the dissolution simulator was limited to a few drugs because only two dosage forms could be tested simultaneously and its small volume restricted it to testing highly water soluble drugs.

A good correlation between the $t_{max}$ value and the percent of drug dissolved at 60 minutes was found by Meyer *et al* (17) in a study on phenobarbital tablets. No correlations were, however, found between the blood concentration-time curve and the *in vitro* dissolution rates.

The failure to establish positive or meaningful and potentially predictive *in vitro - in vivo* correlations is often due to the dissolution method which is employed (26, 27). DiSanto *et al* (26), in a study on prednisone tablets, found that the dissolution procedure which they employed was not sufficiently predictive of the commonly utilised *in vivo* bioavailability parameters of $C_{max}$ and AUC.

Smolen (29) has indicated that it should be possible to establish a single *in vitro* test which could adequately reflect the bioavailability of at least a number of drugs, but that the *in vitro* conditions would have to optimised. Prior to the development of any *in vitro - in vivo* correlation careful selection of sensitive variables and

parameters to characterise both the *in vitro* and *in vivo* bioavailability would have to be made. Using the established correlations the assessment of similar formulations could be made, providing that the untested products do not differ drastically in their formulation from the products from which the correlation was established (30).

## 1.4 Limitations of *In vitro - In vivo* correlations

In certain situations poor correlations are obtained for reasons related to the drug itself. Such factors may include the fact that the *in vivo* dissolution rate is not the rate limiting step for the availability such as is observed for very polar drugs. For certain drugs, differences in the dissolution rate do not necessarily reflect differences in the total amount of drug absorbed (8). Smolen (29) has further reported that an awareness of the limitations of *in vitro* dissolution tests must be exercised. This is especially true if the tests are conducted under a set of arbitrarily chosen conditions. Skelly (31) noted that a single method cannot be extrapolated across different types of formulations. This was found to be specifically the case for slow release dosage forms. Kaplan (32) reported that the *in vitro* release or dissolution rates do not necessarily equate to *in vivo* absorption rates. Furthermore, the dissolution rate of a drug is often an arbitrary parameter and appropriate studies must be performed with individual drugs in order to establish meaningful relationships between *in vitro* and *in vivo* data.

In order for there to be any value in the development of *in vitro - in vivo* correlations there must be a direct relationship between the two aspects of the attempted correlation. It must be demonstrated experimentally that the *in vitro* dissolution reflects the *in vivo* performance. If this is not the case then the data can be of no real value in predicting or assessing the clinical effectiveness of a dosage form (8). This was summarised by Kaplan (32) when he said, "the bioavailability implications of dissolution should never be accepted on faith, rather it has to be proved through

carefully designed *in vitro - in vivo* correlation studies".

Smolen (33) warned that the improper choice of process variables, such as excessively high rate of agitation, can mask significant bioavailability differences between formulations. On the other hand, however, the dissolution test can be overly sensitive in detecting differences that are of little or no clinical significance. This could lead to discarding drug products that may be satisfactory in terms of their *in vivo* performance.

Before any correlation can be established some mathematical manipulation of either the *in vitro* or *in vivo* data, or both, must be carried out. This is necessarily accompanied by a number of assumptions which include the following:

i) the system must behave linearly over the entire concentration range studied or if this is not the case then the kinetics must be well established,

ii) the dissolution rate is the rate limiting step in the absorption process,

iii) the drug is only absorbed from solution,

iv) the drug does not show a narrow "Absorption Window" and

v) the *in vitro* dissolution rate which is calculated using specific *in vitro* conditions are relevant to the drug's *in vivo* dissolution mechanism (8).

It however remains unlikely that any one *in vitro* dissolution test can actually reproduce the *in vivo* dissolution of the drug in the gastrointestinal tract. Many of the physiological factors such as gastric secretions, affect of food, stomach emptying time, first pass metabolism etc all greatly affect the bioavailability of a drug, but are all difficult to reproduce *in vitro* (8, 34, 35).

## 1.5 Advancements in *In vitro* - *In vivo* correlations

The development of more complex dosage forms, such as CMRD's, has brought about the realisation that the standard methods of correlating *in vitro* and *in vivo* performance are not adequate. The fact that slow release dosage forms are designed to release their drug content over extended periods of time, implies that they will necessarily encounter a milieu of varying pH as they move through the gastrointestinal tract. These pH changes will therefore play a significant role in the dissolution of the drug from its dosage form and therefore pH becomes an important variable that must be considered and evaluated in the design process. The situation is therefore much more complex for slow release products than for conventional dosage forms (8, 31, 36, 37).

This problem was exemplified by two quinidine gluconate formulations. The two formulations were found to be bio-inequivalent despite their similar dissolution profiles at pH 1.0 which indicated the products to be almost identical regarding their *in vitro* release characteristics. By repeating the dissolution studies under slightly altered conditions of pH the poorly available product was able to be discriminated from the fully bioavailable formulation (37). At this time the FDA concluded "that because controlled-release products which had virtually the same rate of dissolution over time in the same media were not equivalent *in vivo*, conventional dissolution testing might not be a reliable predictor for controlled-release products" (36). A comprehensive analysis of the problem indicated that if the dissolution had been determined as a function of pH, the possible lack of bioequivalence of the formulations may have been recognised (31). This together with the complexity of the many factors which are involved in *in vitro* dissolution rate testing led to the development of a multidimensional topographical procedure as a tool for decision making by Skelly and his associates (36).

The multidimensional graphs using a topographical plotting technique were found to be quite effective in showing the effects of pH and buffer composition on the dissolution of test products as well as the *in vitro* condition that best correlates with *in vivo* data (37). The authors concluded that whilst relying on a dissolution test carried out at a single pH is better than not conducting any test at all, *in vitro* topographical characterisation would provide much greater assurance of lot to lot uniformity and possibly bioavailability of controlled release formulations. As indicated before, this approach can however not entirely replace the assurance provided by a single dose or a steady state bioavailability study (36).

Some researchers have pointed out that the use of single-point correlations, such as those already described, may lead to misinterpretation of the results since the methods are often based on arbitrarily chosen data points (33). It is preferable to attempt to correlate the entire *in vivo* response time profile with the entire dissolution rate profile. Such correlations can result in developing dissolution test procedures that can reliably predict the time course of the *in vivo* response (8, 33). Two approaches have been attempted to predict the average blood concentration profiles that would be observed for a drug product *in vivo* from *in vitro* dissolution data. The first of these is purely mathematical where conversion methods are used to optimise the amount of information which can be obtained from conventional dissolution data. The second approach is one in which special *in vitro* dissolution-simulator models are designed specifically to predict bioavailability through feedback mechanisms (8).

An example of a mathematical approach to the problem of *in vitro - in vivo* correlations is the application of statistical moment theory to evaluate the *in vivo* dissolution and absorption time. This method was introduced by Riegelman and Collier (38). The use of statistical moments in pharmacokinetics follows from the fact that drug transit through the body has been recognised as a stochastic process (38, 39). If a single molecule is introduced to the body at time zero it is clearly quite

unpredictable as to how long the molecule resides in the body. However, if we consider a large group of molecules, their behaviour is more regular. The mean residence time (MRT) is a characteristic of this collective behaviour and is the mean of the residence times of the individual molecules (39, 40). The main advantage of this method is that it describes the overall characteristics of the concentration versus time curve and is independent of a description of the pharmacokinetic model for drug elimination (41). Riegelman and Collier (38) suggested that the difference in the MRT after the administration of a test dosage form and the MRT after the administration of an aqueous solution is eventually equivalent to the mean *in vivo* dissolution time MDT. The generally accepted pharmacokinetic definition of mean residence time is based on plasma concentration data. Cutler (39) however proposed that urinary excretion data can be used in place of plasma concentration data, especially when a significant proportion of the drug is excreted in the urine unchanged.

A second mathematical approach to the establishment of *in vitro - in vivo* correlations is the use of numerical convolution/deconvolution. Convolution and deconvolution algorithms are best described by Linear Systems Analysis. According to this the input may be the kinetics of dissolution, absorption or the combined dissolution - absorption process. The response of the system may be the amounts or concentration of the drug, or one of its metabolites, or any other suitable pharmacological effect. Langenbucher (42) concluded that the technique requires a minimum of prior assumptions, the only prerequisite is that the body system behaves linearly and time-invariantly.

A further approach to the development of correlations between *in vitro* and *in vivo* data is the use of so-called dissolution simulators. These were introduced in an attempt to enhance the capability of *in vitro* dissolution as a predictor of the *in vivo* behaviour of dosage forms. Many of these systems, however, required highly complex and costly apparatus which were not necessarily advantageous over the

traditional systems (8). One such system which enjoyed some popularity is the Sartorius Dissolution Simulator which was designed to be used together with the Sartorius Absorption Simulator. The absorption simulator was designed to simulate the passive drug transport processes across a lipoidal membrane which occur *in vivo* between the gastrointestinal tract and the plasma. The function of the dissolution simulator is to simulate drug dissolution and its subsequent absorption into the plasma (33). This system has been used by Smolen (33) and Yau *et al* (15) with mixed success. Smolen advanced the system by incorporating a feedback control which was triggered by the *in vivo* data generated from a reference tablet. This method allows for the automatic determination of the time-varying process variables necessary to predict *in vivo* profiles. In addition it simultaneously generates the predicted blood concentration-time curves for one or more drug products undergoing *in vitro* dissolution testing (33). These systems, however, have the disadvantage that they are too expensive, complex and require highly qualified personnel to operate and interpret the data. It should also be noted that in order to prove any systems reliability it is necessary to show that the test can identify between acceptable and unacceptable batches of formulations (8).

It is now generally accepted among pharmaceutical scientists that the present state of science and technology does not always allow for the establishment of meaningful *in vitro - in vivo* correlations for slow release dosage forms (43). It is further accepted that a single *in vitro - in vivo* correlation for different products of the same drug cannot be accomplished at this time. It appears that a separate correlation will have to be developed for each manufacturer's product (44). *In vitro* tests are desirable for the purposes of (i) providing the necessary process and stability determinations of the release characteristics, and (ii) facilitating in the approval process when minor formulation changes or site of manufacture changes are made(43). The *in vitro* dissolution procedure used for the quality control should be validated by appropriate *in vivo* bioavailability studies (44).

In 1988 the US Pharmacopoeial Subcommittee defined *in vitro - in vivo* correlations as "the establishment of a relationship between a biological property, or a parameter derived from a biological property produced by a dosage form, and a physicochemical characteristic of the same dosage form" (45). The subcommittee also classified the correlation methods into four levels, namely A, B, C and D, in descending order of priority (44, 45, 46).

In level A correlations, the *in vitro* dissolution curve of a product is compared with the *in vivo* dissolution curve, i.e. the curve which is produced by deconvolution of the plasma concentration data by model-dependent or model independent methods. The *in vitro* and *in vivo* profiles should be superimposable. Currently, this is the preferred type of correlation for slow release formulations. There should be a 1:1 relationship between the *in vitro* and *in vivo* profiles (45, 46).

Level B correlations compare the mean *in vitro* dissolution time with either the mean residence time of the product *in vivo* or the mean *in vivo* dissolution time. This type of correlation utilises the principles of statistical moment analysis. These correlations are not considered to be 1:1 correlations as they do not reflect the actual *in vivo* plasma level curves (45, 46).

In level C correlations one dissolution parameter, such as $t_{50\%}$, $t_{90\%}$ etc, is related to one pharmacokinetic parameter such as AUC, $C_{max}$ or $T_{max}$. They represent a single point correlation and do not reflect the complete blood concentration curve and are useful for the manufacturing of the product in that they can porvide information on the lot to lot uniformity. They should not be relied upon to justify changes such as formulation modifications or manufacturing site changes (46).

Level D correlations are qualitative relationships of *in vitro* and *in vivo* parameters. These include relating disintegration to *in vivo* performance. The USP considers these type of correlations of little value (45, 46). In the testing of any slow release

dosage form attempts should be made to demonstrate level A correlations. If this is not possible then attempts at Level B or C correlations should be made (44).

The current thinking is that dissolution should be carried out over a full range of physiological pH values. It is also recommended that dissolution tests be carried out over a range of different agitation rates and that the media be confined to aqueous systems only. Finally, the dissolution profile should be characterised over the entire release profile with a minimum of at least three time points (44). These concepts were first proposed by Levy in 1967 (47).

It has been reported that standard pharmacokinetic methods can be used to simulate plasma drug concentrations when a drug is given according to a specific input (48). An example of this method was first introduced by Leeson and coworkers (49). The proposed method employs a formulation's dissolution data and the drug's pharmacokinetic parameters. From this, anticipated plasma concentrations for various formulations are predicted. The potentially most desirable formulation is then selected for a pilot bioavailability study (49). This process has been termed as "biorelevant dissolution". The use of the *in vitro/in vivo* procedures offer a valuable approach to developing new controlled/modified release dosage forms whilst minimising the need for clinical testing, saving time and unnecessary expense. The application of these methods allow for the development of new products with less trial and error (48).

# CHAPTER 2

## Experimental Procedures

### 2.1 Introduction

#### 2.1.1 Dissolution Studies

In biopharmaceutics, rate of dissolution usually refers to the rate at which a solid drug dissolves from an intact dosage form or from fragments of the dosage form. Dissolution of a drug usually occurs not only from the particles into which a dosage form eventually breaks up, but also to some small extent from the intact dosage form before disintegration and deaggregation occur (10). The process of drug dissolution involves the transfer of individual drug molecules from the solid state into an aqueous environment. Since dissolution may be the rate-limiting step in drug absorption it is important to investigate the dissolution properties of all new dosage forms.

The gastrointestinal tract acts as a natural sink since it has been shown that the drug is absorbed instantaneously following dissolution. Accordingly, *in vitro* dissolution rate studies should be carried out under *in vitro* sink conditions. Under sink conditions the solute concentration is much lower than the solubility limit of the solute. Conversely, under non-sink conditions the concentration of the solute increases in the medium until the solubility limit of the solute is reached. Therefore, dissolution rate limited absorption implies that there is very little or no build up of dissolved drug at the absorption site. Hence in dissolution rate studies it is thought that sink conditions lead to a better chance of achieving good *in vitro - in vivo* correlations. Sink conditions are usually achieved by using a large volume of dissolution medium or by replacing the solution constantly with fresh medium at a specified rate (8, 51, 52).

The most common theory of dissolution is the film theory or the diffusion layer model which was proposed by Nernst (53). Essentially the process involves two steps: (i) solution of the solid at the interface of the solid and the dissolution medium and (ii) diffusion from the stagnant layer into the bulk of the medium. The drug is thus found at a uniform concentration throughout the bulk of the dissolution medium. The first step is very rapid and results in the formation of a stagnant layer around the dosage form. The second process is much slower and is therefore considered as the dissolution rate limiting step (8, 31).

The traditional mathematical expression which is used to describe the dissolution process is the Noyes-Whitney equation which was first proposed in 1897 and modified by Underwood and Cadwallader (55):

$$\frac{dW}{dt} = kS(C_{sat} - C_{sol}) \qquad \text{Eqn 2.1}$$

where dw/dt = dissolution rate

k = dissolution rate constant

S = surface area

$C_{sat}$ = concentration of the saturated solution

$C_{sol}$ = the concentration at any time

In order for the dissolution rate constant to be defined, the surface area must be kept constant and the dissolution medium must be maintained at sink conditions. This will provide the intrinsic dissolution rate which is described as the mass of drug dissolved in a system with a constant surface area. The intrinsic dissolution rate is of use in studying the solubility characteristics of a substance but has limited value

in biopharmaceutical applications because the surface of a dosage form changes with time. Of more importance is the apparent dissolution rate which may be described as the total mass of drug dissolved per unit time. Under sink conditions equation 2.1 can be rewritten as:

$$\frac{dW}{dt} = kS \qquad \text{Eqn 2.2}$$

A dosage form must not only serve as a vehicle for drug delivery but must also provide a stable environment during storage. During research and development, dissolution rate studies should be carried out to ascertain the effects of excipients and other process variables on the release of the drug from the dosage form . The test conditions need, however, to be optimised in order to yield accurate, reliable and reproducible results which can be correlated with *in vivo* phenomena (56).

Over the past decades numerous methods have been described for dissolution rate studies of solid oral dosage forms (8, 32, 50, 51, 57, 58, 59, 60, 61, 62, 63, 64). An ideal method should meet six basic criteria, namely: reproducibility, sensitivity, correlation with *in vivo* results, flexibility, simplicity and the potential for automation. The methods should also be economically practical (32). In the last two decades there has been a proliferation of literature concerning the problems and deficiencies of dissolution testing. The more flexible a standard method is, the more easily it can be adapted to accommodate new findings. A good method should also yield data which can be quantitatively related to the theoretical dissolution rate equations. Lastly, the method should also be sensitive enough to distinguish between products which differ only slightly (51).

The methods available for dissolution rate testing can be grouped according to various characteristics, such as; the geometry of the vessel, the method of

agitation, the degree of agitation, the way by which solvent enters and leaves the system and the achievement of sink or non-sink conditions. Methods available for dissolution rate studies include those in which the dissolution vessel is agitated, systems in which the dosage form moves through the medium, apparatus in which the dissolution medium flows through the dissolution vessel and methods which have some form of mechanical agitation of the dissolution medium.

#### 2.1.1.1 Vessel agitation

These methods include the Wruble method (65, 66), the rotating flask apparatus (67, 68) and the Souder and Ellenbogen method (58, 69) (See figure 2.1 a, b, c). The method of agitation of the latter is similar to that described in the British Pharmacopoeia 1948 and 1953. The rotating flask method became the official method of the National Formulary in 1967 (58) for CMRD's and was based on the method of Souder and Ellenbogen (58, 69). The apparatus consists of a flask in which the dosage form is sealed with the dissolution medium in cylindrical bottles which are rotated through 360° end-over-end in a water bath at 37°C. Variations of this method have been used by Nessel *et al* (70) and Chaudhry *et al* (71). Adaptations include the use of different volumes of media, agitation speeds and vessel size and shape. This method has however fallen into disuse as the CMRD's are now being tested by the official compendial methods (8). A major drawback of this method is the difficulty of withdrawing samples without interfering with the process. It also does not lend itself to automation which is of particular use in the dissolution assessment of CMRD preparations for which sampling times may continue for up to twenty four hours.

#### 2.1.1.2 Dosage form movement

A second group of apparatus are those in which the dosage form moves through the dissolution medium. Modified versions of the USP/NF disintegration apparatus (72)

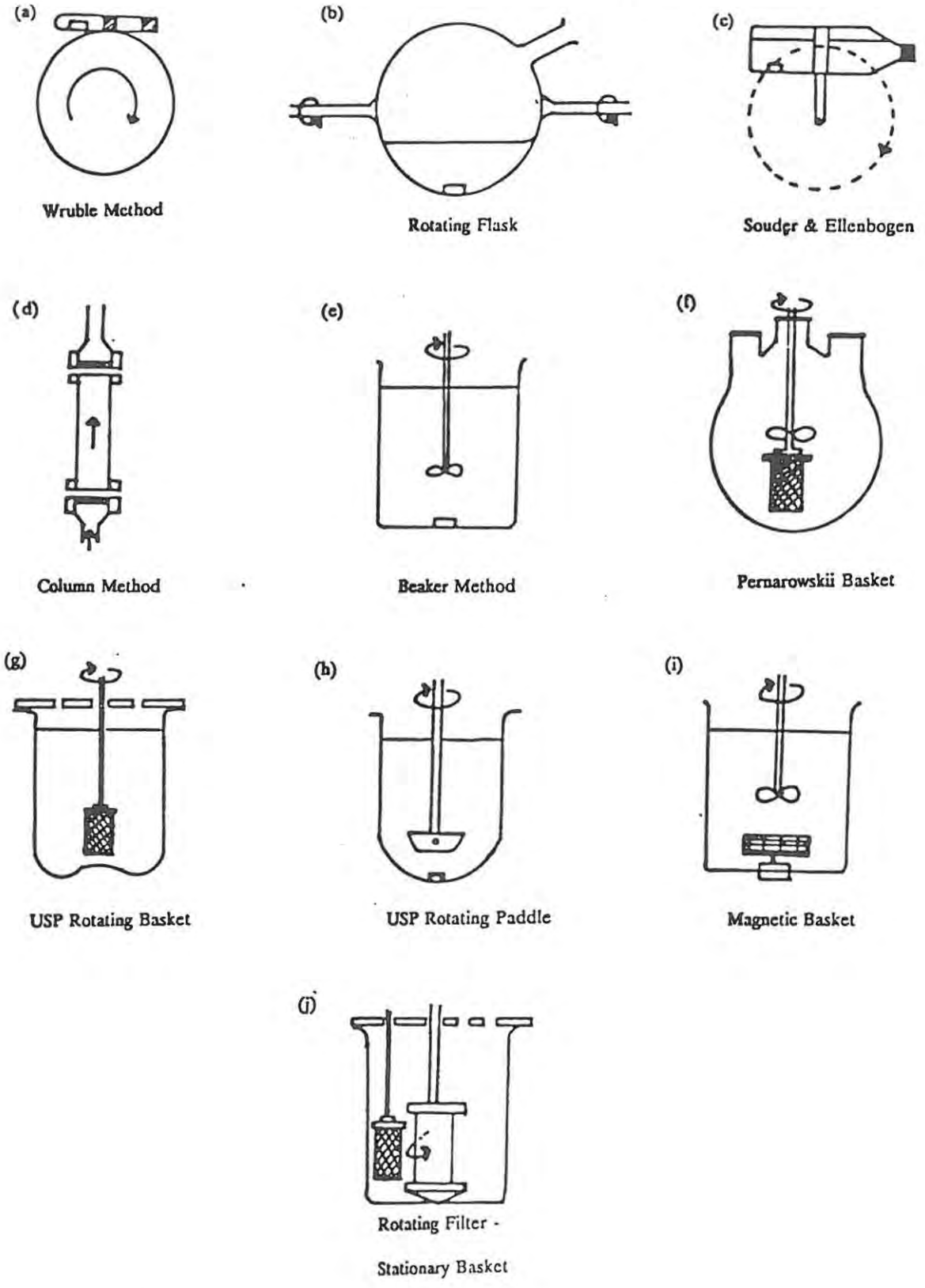



Figure 2.1 Dissolution apparatus

have been widely used. Major modifications of the disintegration apparatus cited in the literature include the elimination of the plastic disc and the replacement of the 10-mesh screen with either a 30- or 40-mesh screen (73, 74, 75, 76, 77, 78, 79). Advantages of this method include the fact that it can be automated and that information about tablet disintegration times are also available.

Drawbacks of these methods include the fact that there is only a single high level of agitation which is difficult to assess. With the use of varying sizes of dissolution beaker the agitation intensity also varies. As a result of the excessive agitation, general correlation with *in vivo* results have not been successful (8).

2.1.1.3 Flow-through methods

A third group of dissolution apparatus are those in which the dissolution medium flows through the system. An example of such an apparatus is the flow-through cell or the column-type flow-through apparatus (fig 2.1d) (32). This method has been proposed in response to several disadvantages in the previously discussed methods - such as the variability in the rate of shear over the particles which leads to large variations in the individual rates of dissolution. In this method the dosage form is held in a cylindrical cell, made of glass or another suitable material and is immersed into a waterbath at 37°C. At the bottom of the cell there is either a porous glass plate i.e. scintered glass, or a bed of glass beads which should be capable of dispersing the solvent to provide laminar flow. A filter is also incorporated into the top of the cell. A modification of this apparatus is the cascade barrier bed which was used by Rippie *et al* (80). The system can be run either as a closed system (62, 81) or as an open system (51, 82, 83, 84, 85). An advantage of the open system is the ability to maintain sink conditions. This is especially important for drug substances which form saturated solutions in volumes of 10-20% of the 900ml normally used in the official compendial methods. The open flow-through method

allows for the maintenance of near perfect sink conditions since fresh dissolution media is continually presented to the dosage form. Conversely, in the closed system fresh media is only presented to the dosage form once, which implies that the drug concentration of the medium gradually increases as the drug dissolves.

Further advantages of the flow through method include such features as built-in filtration, the convenience of changing the pH during testing, and fewer external variables resulting in more reproducible results. Another advantage is that the dosage form may be placed in a fixed position which makes it easier to maintain a constant and repeatable fluid-flow pattern around the dosage form than is the case with the paddle or rotating basket methods (8). Furthermore, problems such as wobbling, shaft eccentricity, verticity, vibration, stirrer position etc, which occur in the official USP methods, do not exist (32).

The open flow through systems also have their inherent disadvantages, the most important of which is the tendency of the filter to clog due to the unidirectional flow. Dissolution characteristics may change if particles adhere to the filter causing turbulence at the solid/liquid interface. The parameters of flow rate and the influence of ripple effects must also be investigated further and understood (8, 32, 50).

2.1.1.4 Beaker methods

The fourth group of dissolution apparatus can be classified as those having a large dissolution vessel with a mechanical agitator of some description. This group encompasses a large number of methods including the Levy and Hayes beaker method (86, 87, 88, 89, 90, 91), the Pernarowski basket stirrer apparatus (92) and the USP rotating paddle method (72). Other methods include the stationary basket method used by Withey (60), the magnetic basket dissolution apparatus used by Needham (91) and the rotating filter - stationary basket (63, 93) (fig. 2.1e - j).

Goodhart *et al* (64) developed and evaluated an apparatus for both testing disintegration and dissolution rates of tablets and capsules in 1973. These methods of dissolution have gained widespread use for many tablet and capsule formulations. They are relatively simple to use and are easily adapted to automated use and allow for a number of determinations to be run simultaneously (93, 94, 95, 96).

(i) The USP Rotating Basket Apparatus

In 1970 the USP dissolution apparatus, described as the rotating basket apparatus first appeared (figure 2.2). This is based on the Pernarowski basket stirrer apparatus described in 1968 (92) and which consisted of a simple stirrer flask with a round bottom and three necks. It also had a tablet/capsule container built into it. The basket method has, because of its official status, been widely applied for both conventional dosage forms, such as tablets and capsules, as well as for CMRD's (89, 97). The official apparatus (See figure 2.2) consists of a covered cylindrical vessel with a hemispherical bottom made of glass or any other suitable transparent material. The vessel has a capacity of 1000ml and must be flanged at the top. A fitted cover with openings for a thermometer, the drive shaft and the sample withdrawal apparatus may be used to limit evaporation. The vessel is partially immersed in a suitable waterbath so that the temperature of the medium in the vessel is kept constant at 37° ± 0.5°C. No part of the assembly, including the environment in which the apparatus is placed should cause any significant agitation or vibration. The stainless steel basket assembly consists of two parts, viz. the basket which is constructed of 40-mesh stainless steel and a shaft to which the basket is attached. The shaft is placed so that its axis of rotation does not deviate by more than 2mm from the vertical axis. It must also rotate smoothly and without significant wobble. A speed regulating device must also be included so that the

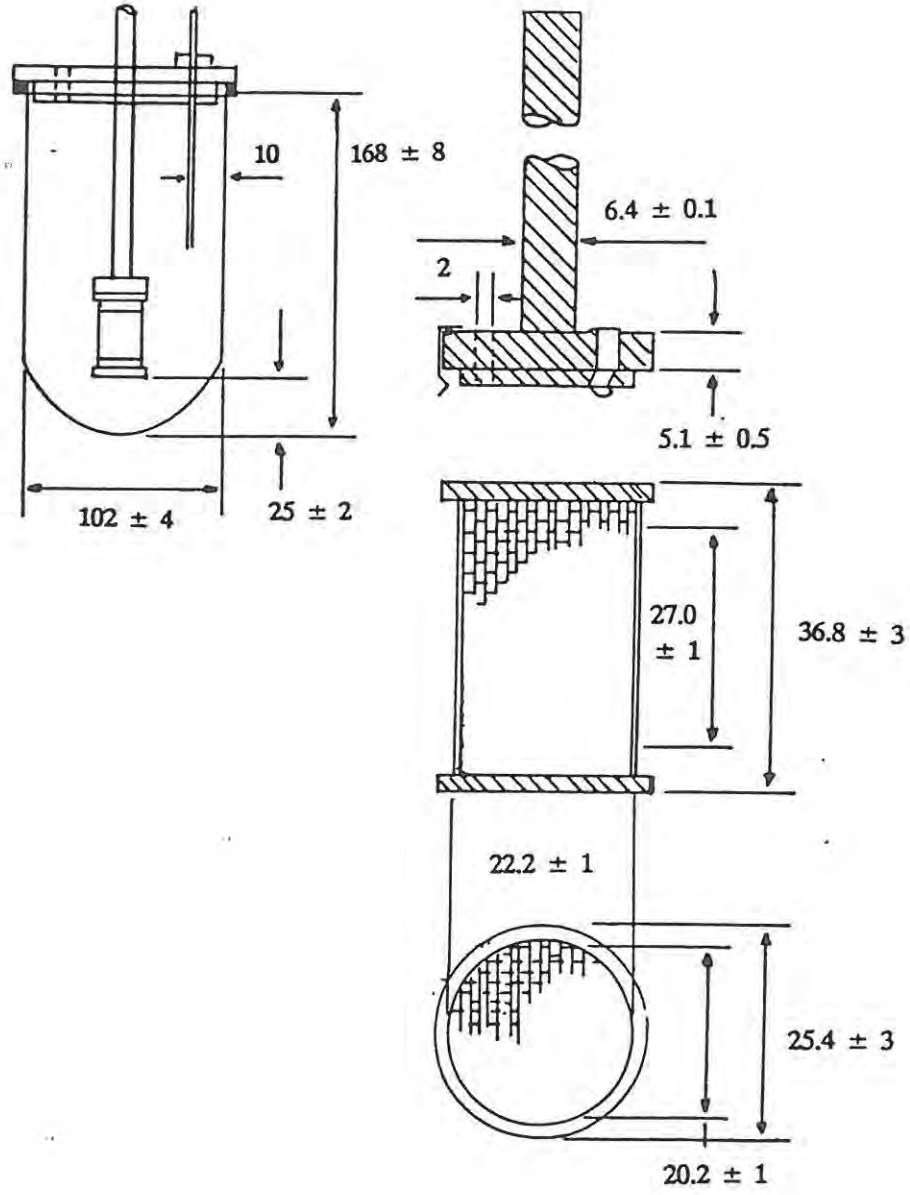



Figure 2.2 The USP Basket Apparatus (All dimensions given in mm)

rotational speed of the shaft can be selected and controlled to within ±4%. The distance between the inside bottom of the dissolution vessel and the basket must be maintained at 25 ± 2mm (8, 72).

Various modifications to the basket apparatus have been described. Mesh sizes of 10-, 20- and 30-mesh have been evaluated. Each type of mesh presented their own problems such as clogging or releasing of particles. Baskets with 80-mesh screens have been used for the testing of microencapsulated particles. This particular mesh size was selected since it retained the particles whilst still allowing the dissolution medium to penetrate the basket without clogging (8).

A variety of dissolution media are used and are outlined in the individual drug monographs in the USP (72). If the dissolution medium is a buffered solution, the pH should be adjusted to within ±0.05 pH units of the specified pH (32). Dissolution media used in the testing of CMRD's include USP simulated gastric fluid for one or two hours followed by USP simulated intestinal fluid (24).

Even though today many *in vitro* dissolution studies are performed using the basket apparatus, the method has nevertheless attracted much criticism over the years. This method has also shown to be adversely affected by a number of variables which led to the development of an alternative method, namely the rotating paddle apparatus.

(ii) The USP Rotating Paddle Apparatus

The second official compendial method noted in the USP XXII/NF XVI (1990) (72) is the rotating paddle dissolution apparatus (fig. 2.3). This method of dissolution rate testing is becoming increasingly popular (98, 99). The construction of the apparatus is very similar to that of the rotating basket with a 1000ml vessel made of glass or other suitable transparent material and a variable speed drive.

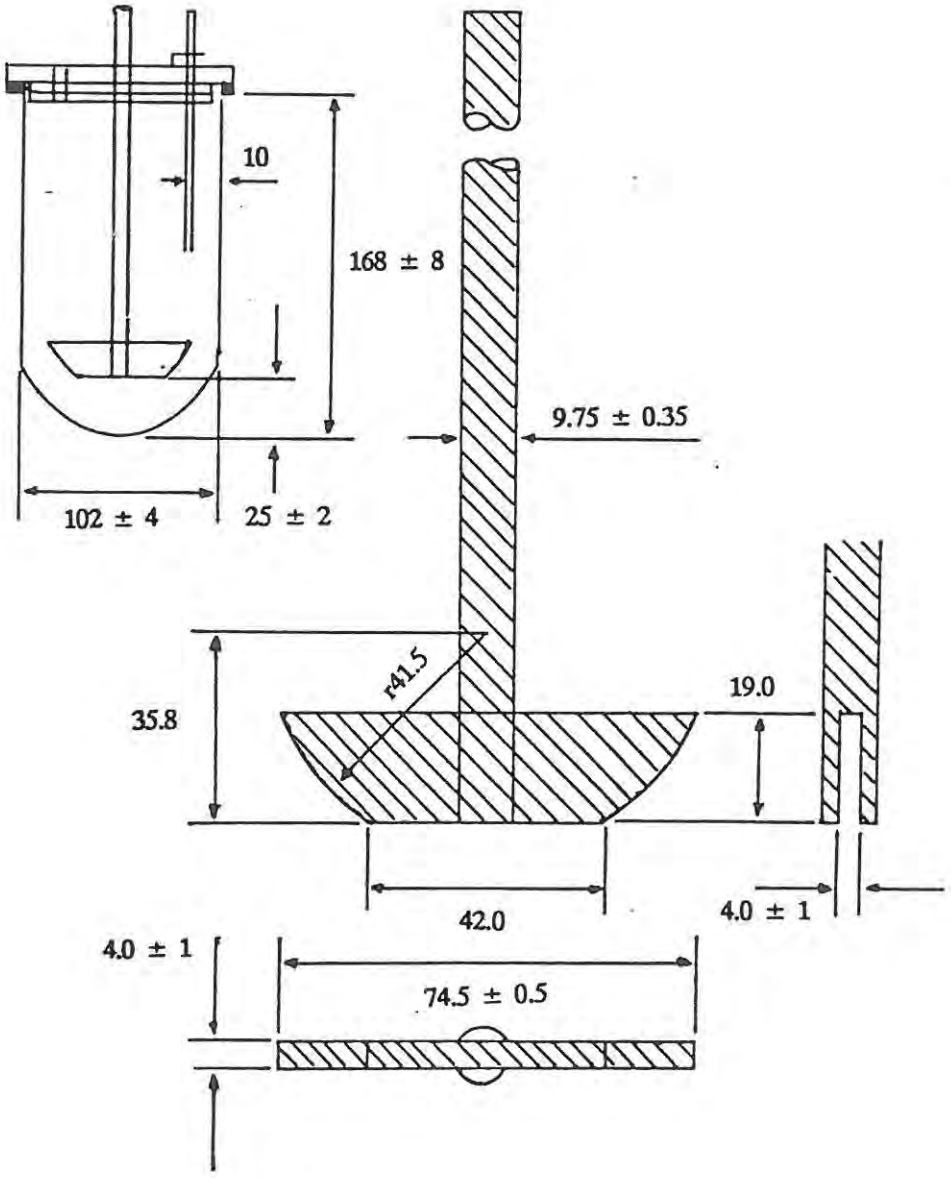



Figure 2.3 The USP Paddle Apparatus (All dimensions given in mm)

The difference is that a paddle, formed from a blade and shaft, is used as the stirring element in place of the basket. The blade passes through the diameter of the shaft so that the bottom of the blade is flush with the bottom of the shaft. During the course of the test, the blade must remain 25 ± 2mm from the inside bottom of the vessel. The blade and shaft consist of a single metallic entity and may be coated with a suitable non-reactive fluorocarbon polymer. The dosage unit is allowed to sink to the bottom of the vessel before rotation of the blade is started.

For dosage forms which float on the dissolution medium a small loose piece of non-reactive material, such as a few turns of wire or a glass helix, may be used to sink the dosage form (8, 72, 100). This has however been criticised as being impractical. The helix may also become clogged with sticky material form the capsule. This may influence the release of particles from the dosage form and hence influence dissolution. The wire may also react with the dosage form or the dissolution media especially if acidic media are used. There are also no strict definitions for the size of the helix which may lead to many interpretations of its exact description. Some researchers have modified the paddle method to include a basket, similar to the USP basket, to hold the dosage form (101).

Discrepancies in dissolution rates determined with the use of the paddle apparatus have been traced to minor variations in the vertical alignment of the paddle shafts. To minimise errors the base of the apparatus must be horizontal, the shafts of the paddles must be vertical, each shaft must be positioned along the vertical axis of the dissolution vessel and the paddles must be positioned at a standard depth in the vessel (102). It has also been found that the curvature of the base of the dissolution vessel may influence the results. Vessels with a curvature which is less than that of a sphere cause a higher bias. These differences can be ascribed to differences in flow patterns brought about by the vessel shape (103).

(iii) Limitations of the official methods

The methods described have a number of shortcomings which may affect the results. At relatively low agitation intensities, together with the shape of the dissolution vessel, mound formation of the granules or particles at the bottom of the beaker may occur. This phenomenon may lead to a decreased surface area being presented to the medium (68). Researchers have also reported that the location of the mound may influence the dissolution results, i.e. whether the mound of particles forms in the centre of the beaker or at the periphery, as would be the case with beaker type vessels (104, 105).

Lerk and Laga (86) have shown that the positioning of the dosage form can have an effect on the dissolution rate. The highest rates were found when the dosage form was placed in the centre of the vessel. Dissolution rates were found to decrease as the dosage form was moved to the periphery of the vessel. Furthermore, it was found that higher values were determined for tablets that were placed against the wall of the basket than when they were placed centrally.

Two types of agitators have been used in the beaker method, viz. the marine propeller and straight-bladed turbine impeller. Both of these produce mainly radial and rotational flow patterns. The marine propeller produced longer mixing times than the straight-bladed impeller. Mixing times also appeared to be dependent on the direction of rotation of the propeller and the angle of the propeller blades from the horizontal - a decrease in the angle producing an increased mixing time (86, 106). In order to centre the dosage form, the authors recommended that the beaker method be modified to use round bottomed beakers and straight bladed impellers (86).

Flow patterns in the dissolution vessel must be consistent in order to obtain reproducible results. Dissolution rate data are also affected by the geometry of the

stirring device, shape of the dissolution vessel, alignment of the stirring device and the presence of sampling probes and a thermometer in the dissolution medium. Flow patterns have been found to affect the dissolution rates through slight differences in the shape of the bottom of the dissolution vessels (107). It was shown that the differences in shape altered the hydrodynamics within the vessel and subsequently affected the dissolution patterns. It was also noted that particles tended to aggregate at the periphery of the vessel where they tend to remain undisturbed.

Withey and Bowker (105), in an investigation into the flow patterns in various dissolution methods, found undesirable mixing and flow characteristics in all the methods that they investigated. It has also been found that the mixing times and hydrodynamics of the dissolution medium are dependent on or affected by the density and viscosity of fast dissolving drugs and excipients (106). The effect of the return of large volumes of dissolution medium to the vessel and the effects of the agitator shaft length have been reported by Carstensen *et al* (108). They found that the longer shaft length resulted in increased dissolution rates.

Sampling positions in the dissolution vessel also influence the results of the dissolution test. Variations in the sampling point should be avoided as this causes poor reproducibility, especially at low rotational speeds of the basket (105, 106). This phenomenon seems to occur as a result of inadequate mixing in the bulk of the dissolution medium (109). The USP XXII/NF XVI (72) recommends that samples be removed at a point midway between the top of the basket or paddle and the surface of the medium, not closer than 1cm form the sides of the flask. The BP 1980 (110) varies slightly - it recommends a sampling point halfway between the basket wall and the wall of the vessel, level with the midpoint of the side of the basket. This has since changed in the BP 1988 (111) to the same specifications as those in the USP XXII.

Vibration can also be a problem in a dissolution system and may have many causes.

This problem was particularly evident in early systems where the drive motor and water pump were not separated from the waterbath and the dissolution vessels. This has the effect of changing the flow patterns of the dissolution medium and introduces unwanted energy into the dynamic system (8, 112). In a study on the effects of vibration on the dissolution process, Beyer and Smith (113) stated that excessive vibration of the apparatus can lead to significant differences in the dissolution times for different formulations. Newer designs of dissolution apparatus do not have this problem since the drive motor and water recirculation pump are separated from the waterbath.

Corrosion of the dissolution vessel is also a problem which occurs with the USP rotating basket apparatus. This was shown by Mattock *et al* (114) who exposed the basket to USP simulated gastric dissolution fluid for 42 hours - this led to the basket being unsuitable for further investigation. This problem can be minimised by using more dilute HCl solutions or replacing them with buffered solutions. Alternatively the basket can be coated with a very thin layer of gold to make it more acid resistant, but this is costly (8). Corrosion of the basket may also lead to excessive abrasion of the dosage form resulting in increased dissolution rates (114). A further problem of the 40-mesh screen of the basket apparatus is the clogging of the basket by particulate matter (115). This leads to an impairment of visual observation of the capsule or tablet in the basket.

Dissolved gases may also affect the dissolution process. If this is the case, then the dissolution medium must be degassed prior to commencement of the procedure. The effect of dissolved gases include a change in the pH of the dissolution media, although this is less likely to be the case in buffered media. Dissolved gases may also influence the flow patterns within the medium as the bubbles rise to the surface. Air bubbles may also collect on the basket surface thereby altering the mesh porosity. In addition, air bubbles may attach to the dosage form before disintegration thereby altering the disintegration and deaggregation processes via

an alteration of the effective surface area exposed to the dissolution medium (8).

A further problem with air in the system is the entrapment of air within the basket itself. Two types of these bubbles seem to occur with the basket method, viz., entrapment of air in the basket device causing floating of the dosage form at the top of the basket, and the entrapment of an air bubble at the base of the basket. There, however, appears to be no significant effect due to these (8). A possible method of avoiding the first situation was proposed by Sarapu and Clark (116) who suggested that the surface of the basket drive plug be made conical in shape.

The variability that occurs in the results from different laboratories and to an extent within laboratories have raised questions about the reproducibility of dissolution testing (117). One way to improve this situation was seen in the use of specially prepared test samples which can act as calibrators for dissolution testing apparatus. Three tablets were selected for a collaborative study - these included a nondisintegrating 300mg salicylic acid tablet, a disintegrating 50mg prednisone tablet and a 100mg nitrofurantoin tablet. For official compendial use the latter has fallen away. Operating standards, apparatus and limits were also decided upon. The study concluded that appropriate standards had been identified as dissolution calibrators and that acceptance limits for these had been established.

In a study conducted by Prasad *et al* (118) to evaluate different performance standards it was concluded that no single standard can predict the suitability of the basket or the paddle dissolution apparatus. This was found after tests were conducted using the USP prednisone calibrator which proved to be sensitive to perturbations by the basket method and not the paddle method. The converse was found for a prednisone standard from the National Centre for Drug Analysis (NCDA).

Storage conditions may affect the dissolution rate of the calibrator, the acceptance range may be too broad and validation procedures may be run too infrequently. In

order to continually monitor the dissolution apparatus, calibrators should be run at the same time as samples. The use of *in vitro* dissolution calibrators was demonstrated by Mazeul *et al* (119). The standard was a preselected lot of the same formulation as that which was being tested. This standard was run simultaneously with the product being tested and adequately compensates for small changes in test conditions, such as slight pH and temperature changes, which may influence dissolution rates. This, therefore, allows a continuous control of the dissolution apparatus.

It has also been shown that large sampling probes may cause significant disturbances in flow patterns in the dissolution medium. This was particularly evident in automated systems where the sampling probe remains *in situ* for extended periods (120). The use of small capillary type probes are recommended as this reduces interference of the hydrodynamic conditions.

2.1.1.5 The Bio-Dis$^{R}$ Apparatus

The Bio-Dis$^{R}$ dissolution apparatus was specifically designed to assess the dissolution characteristics of extended release tablets and multiparticulate systems. Impetus for its design were derived from the fact that:

(a) the need for a robust, simple and relatively inexpensive method for dissolution of controlled-release formulations;

(b) the need to carry out dissolution testing employing sequential changes of pH values without the need for manual transfer of the media as in the compendial methods;

(c) the need to include automation of the procedure and allow the use of various programme sequences, including those to 24 hours (121).

The apparatus (fig 2.4) consists of six rows of six glass containers of 225 ml

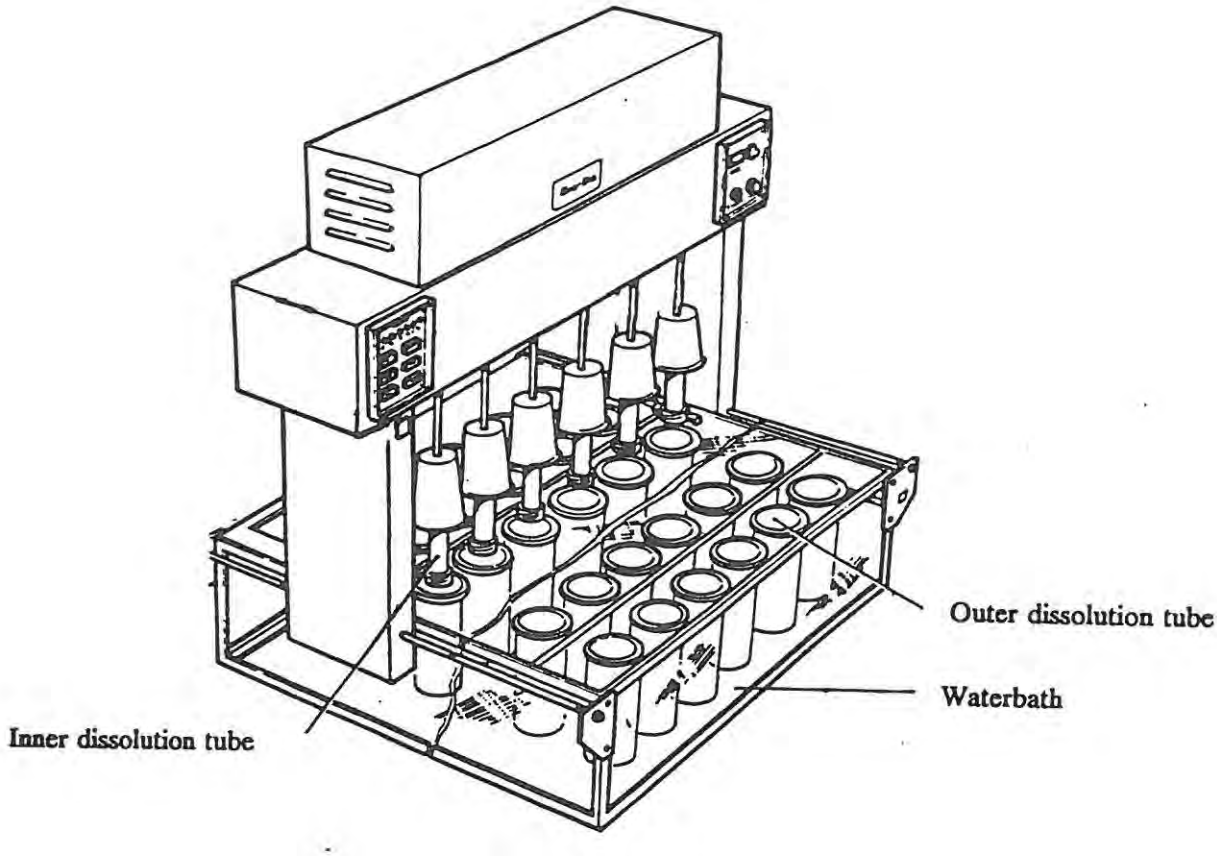



Figure 2.4 Bio-Dis dissolution apparatus.

capacity called the outer dissolution tubes. The inner dissolution tubes consist of a glass tube of approximately 10 cm in length closed off at each end with a plastic mesh filter holder. The top of the tube is screwed into the shafts of the shaft driver. The shaft driver moves the inner tubes into the outer dissolution tubes through a gentle up and down motion. The degree of agitation can be precisely controlled via a variable speed adjuster. The top and bottom ends of the inner tubes are fitted with either 74, 250, 420 or 840 micron mesh screen in order to prevent the particles from falling into the outer dissolution tubes. All the outer tubes are suspended in a suitable waterbath maintained at 37°C.

Weintraub and Gibaldi (68) have reported that dosage forms, including CMRD's, may produce a cone or mound of particles or pellets at the base of the vessel. Furthermore, a semi-stationary film of drug concentration may form in the cone when the paddle or basket apparatus are used, thereby retarding the drug release.

Increasing the agitation rate may lead to a faster dissolution rate but may simultaneously lead to an increased abrasion of the pellet membranes. These problems are adequately overcome by the Bio-Dis apparatus (121).

The Bio-Dis dissolution apparatus is designed so that pellets, solid particles and tablets move through the dissolution manner analogous to their movement in the rotating bottle apparatus. The pellets are kept moving and only come into contact with the liquid and the soft mesh i.e. they undergo similar conditions to those that they would undergo *in vivo*. The rate of release of drug form the pellets is thus not hindered by a semi-stationary film of drug concentration around the pellets. It is therefore expected that the Bio-Dis apparatus will give excellent correlation of drug release with the *in vivo* situation with a wide number of sustained release preparations (121).

This apparatus has recently been employed by Esbelin *et al* (122), in a study to investigate the dissolution properties of various extended release formulations of theophylline.

### 2.1.2 Analytical methods

Many techniques are available for the analysis of dissolution samples. Two such methods which have gained widespread use are UV spectrophotometric and High Performance Liquid Chromatographic (HPLC) methods. These methods each have their own advantages and disadvantages.

The major advantage of UV spectrophotometric over chromatographic methods is that the samples for analysis do not have to be manipulated prior to introduction into the spectrophotometer. A second factor which makes this method particularly suited to routine analysis is that it is a very rapid technique, requiring only a few

seconds to analyse a single sample using the highly specialised equipment that is available today. A disadvantage of these methods is that they lack specificity i.e. if two compounds in a sample absorb UV radiation at the same wavelength the absorption value obtained will be due to both compounds. For these types of situations it is necessary to separate each component by HPLC methods.

The major advantage of HPLC methods is its specificity. The specificity is obtained from the fact that compounds are injected onto a column from which they are eluted at different times. Thus if a sample contains more than one compound they may be separated on the column and detected as two separate entities by a suitable detection system. Drawbacks of HPLC methods include the fact that some manipulation of the sample is often necessary prior to analysis and that the analysis time per sample usually takes several minutes. Recently, however, a fully automated HPLC apparatus for dissolution rate studies has been developed (123).

### 2.1.3 Bioavailability

Bioavailability is an absolute term that indicates the measurement of both the true rate and total amount or extent of a drug that reaches the systemic circulation following the administration of a dosage form. Absolute bioavailability can only be determined following the administration of an intravenous dose, whereas comparative bioavailability studies allow the assessment of relative bioavailability. Bioavailability is usually assessed relative to a standard and is often also referred to as "biological availability" or "systemic availability" (124). The activity of a drug at a site in the body is usually related, in a quantitative relationship, to the concentration of the drug in the blood and to a lesser extent to its concentration in other fluids. There is usually also a relationship between the availability of the drug and the rate at which the body excretes or metabolises the drug (56). The absorption rate constant, $k_a$, is the net effect of the rate of dissolution of the drug in the biological fluids at the site of absorption and the net transfer of the compound from the site of absorption to the circulation (10). In order to provide a general evaluation of the overall rate and extent of the absorption of a drug, several parameters are used.

The serum concentration time curve is the focal point of bioavailability assessment and is obtained when serial bloods sample are taken after the administration of a dosage form (124).

Pharmacokinetics is mainly associated with the quantitative characterisation and treatment of the time course of the drug through the different compartments of the body. Pharmacokinetics can be defined as the application of kinetics to *Pharmakon* which is the Greek word for drugs and poisons. The problem of establishing adequate methods to interpret *in vivo* data frequently leads to the use of compartment methods. The pharmacokinetic compartment models are an approximation of the biological system. The models are mathematical equations which are used to describe and interpret the data which are obtained by *in vivo* experimentation. Correctly selected models allow for the simulation and prediction of pharmacokinetic data. Most drug behaviour in a biological system can be described by either a one or two compartment model (8, 10, 125).

A number of model independent parameters can be derived or calculated. These include such parameters as $t_{max}$, $C_{max}$ and AUC. The maximum concentration, $C_{max}$, and the time at which the maximum occurs, $t_{max}$, are a crude indication of the absorption rate and can be directly estimated from the serum concentration versus time curve or such data. A disadvantage of these parameters is that they do not only refer to the absorption process but also to the disposition of the drug. Although $t_{max}$ is influenced by the absorption rate, any absorption processes which occur after $t_{max}$ have no influence on that parameter (125).

Area under the plasma/serum concentration curve (AUC) is defined as the area under the serum concentration versus time curve from zero time to the last sample time point or to infinity ($AUC_{0\rightarrow\infty}$). The area under the curve provides an estimate of the extent of the absorption.

In statistical moment theory, as already mentioned, the absorption process is regarded as a stochastic process. Statistical moments are characteristic of the shape of the statistical distribution curve, such as the time course of a serum concentration time curve following a single dose and are therefore only dependent on the observed time course data and hence also independent of the pharmacokinetic compartmental model. The use of this approach allows the separation of the absorption and disposition processes. From the serum concentration versus time curve, the area under the moment curve (AUMC) can be calculated. The ratio of the $AUMC_\infty$ to the $AUC_\infty$ yields the *in vivo* mean residence time (MRT). The MRT method of pharmacokinetic analysis has the advantage over other methods in that it is a non-compartmental method (38).

The zero moment represents the $AUC_\infty$. The $AUMC_\infty$ is defined as the area under the curve of the product of time, t, and the serum concentration, $C_p$, from zero time to infinity. The MRT can thus be calculated as follows:

$$MRT = \frac{\int_0^\infty t\, C_p\, dt}{\int_0^\infty C_p\, dt} = \frac{AUMC_\infty}{AUC_\infty} \qquad \text{Eqn 2.3}$$

The MRT is defined as the mean time for intact drug molecules to move through the body and it provides information about all the kinetic processes including the *in vivo* release from the dosage form, drug absorption and the disposition processes (38). The MRT for non-instantaneous input (n.i.v) involves a mean absorption time (MAT) and is defined as:

$$MRT = MAT_{n.i.v} + MRT_{i.v} \qquad \text{Eqn 2.4}$$

The MAT refers to the mean time involved in the *in vivo* release and absorption processes as they occur in the input compartment. In order to evaluate the MAT, the $\bar{M}RT_{i.v}$ should be subtracted from the $MRT_{n.i.v}$. The MAT can however be approximated in cases where the concentration time curve yields a terminal log-linear slope from which the terminal rate constant, $\lambda_z$, can be derived. The reciprocal of $\lambda_z$ is deducted from the MRT to give $MAT_{uncorr}$, the uncorrected mean absorption time.

$$MAT_{uncorr} = MRT_{n.i.v} - \frac{1}{\lambda_z} \qquad \text{Eqn 2.5}$$

When using multi-compartment kinetics this would result in an error, however, if a solution and solid dosage form were evaluated in the same person, the error term would be constant. For an extravascular dose equation 2.5 takes on the following form, where $MAT_{true}$ refers to the exact value of MAT:

$$MAT_{true} - MAT_{uncorr} = \frac{1}{\lambda_1} - \frac{1}{\lambda_{21}} \qquad \text{Eqn 2.6}$$

When no intravenous data are available MAT offers significant advantages over the use of $t_{max}$ as a means of comparing absorption rates since the latter depends on the disposition and absorption of the drug whereas MAT is independent of the disposition (38, 126).

A further parameter which can be estimated from bioavailability data is the absorption rate. This constant can be determined in a number of ways. One method is by the use of the Wagner-Nelson method (8, 125, 127) which assumes a one compartment open model and linear kinetics. The method has, however, also been successfully applied to a two compartment model in certain instances by Wagner (128).

Assuming that the drug elimination is by a first order process, then:

Amount absorbed = Amount in body + amount eliminated

$$A_{abs}(t) = V\,Cp + CL\;AUC_{0-t} \qquad \text{Eqn 2.7}$$

The fraction absorbed, $F_a$, to time t is given by:

$$F_a = \frac{C_p + k\,AUC_{0-t}}{k\,AUC_{0-\infty}} \qquad \text{Eqn 2.8}$$

The value of the absorption rate constant, k, can be estimated from the slope of the terminal concentration-time data if the absorption is sufficiently slow.

The Wagner-Nelson method has the following advantages: (i) it does not require intravenous data, (ii) no prior estimate of the volume of distribution is required and (iii) no limitations are placed on the order of the absorption process. Whilst the method is convenient, a number of shortcomings exist in determining the absorption profile without intravenous data.

### 2.1.4 Correlative methods

Dissolution data can be presented in a number of different ways. The simplest format is to plot dissolution profiles graphically representing the percent or amount dissolved versus time on cartesian axes. More recently, dissolution data have been presented in the form of multi-dimensional plots. These are three dimensional representations of the percent or amount of drug dissolved versus time against some other parameter of the dissolution test conditions, such as pH.

In order to obtain more meaningful information from *in vitro* dissolution data some mathematical manipulation must be performed. *In vitro* drug release involves a number of complex kinetic processes and it is therefore illogical to assume that *in vitro* dissolution will fit a specific zero or first order equation. The problem is greatly facilitated by the application of a general mathematical function in which the entire dissolution curve is described in terms of meaningful parameters. The Weibull function is an example of such a mathematical distribution.

#### 2.1.4.1 The Weibull Function

The Weibull function, which is very versatile, was first proposed by Rosin in 1933 and reinvestigated by Weibull in 1951 (129). The distribution is now often referred to as the Rosin-Rammler-Sperling-Weibull, RRSW, distribution. The function has been applied to a number of distributions such as yield strength of steel fibres, size of insects and the failure rate of electronic components (129, 130). The function has been applied to linearise dissolution data by Langenbucher (131, 132) and has also been applied by many other researchers to describe dissolution data and dissolution curves (133, 134, 135).

The Weibull function parameters were interpreted by Christensen *et al* (134) for drug

release from CMRD's. Riegelman and Upton (136) described the application of the function to *in vitro* dissolution rate data and to the *in vivo* absorption rate data obtained after the administration of the same dosage form to a group of volunteers. When the Weibull function was compared to a complex function proposed by Pederson, both equations were found to fit the data equally well (133).

The Weibull function is represented in the following equation:

$$F = F^{\infty} \left[1 - e^{-\left(\frac{t - T_0}{t_d}\right)^{\beta}}\right] \qquad \text{Eqn 2.9}$$

In the above equation F is the dependent variable and is representative of the fraction of the administered dose which is dissolved at time t. The fundamental form of the equation is not defined for data points within the time period 0 to $t_0$ and an ordinate value of F = 0 is usually assigned in this range.

$F^{\infty}$ gives an indication of the amount of active ingredient which is released from the dosage form at infinite time under the given experimental conditions. It characterises the actual drug content of the dosage form. Analytical or method errors such as incorrect calibration, dilution, volume, flow rate and interactions or degradation in solution will affect the $F^{\infty}$ value.

The lag time, $t_0$, describes any processes which occur before the onset of dissolution, e.g. removal of a tablet layer, disintegration of a tablet or the rupturing of a capsule. In most cases this will be equal to zero. The time parameter, $t_d$, represents a scaling factor of the time axis with two individual curves differing in $t_d$ appearing only as stretched or shortened along the *x*-axis. When $t\text{-}t_0 = t_d$, then $F = 1\text{-}e^{-1} = 1\text{-}0.368 = 0.632$ i.e. $t_d$ is representative of the time required for 63.2%

of the drug dose to be dissolved.

The shape parameter, $\beta$, defines the shape of the curve and is a non-dimensional number ranging from zero to values greater than one. When $\beta = 0$ the system describes zero order dissolution with $\beta = 1$ describing a system in which simple first order exponential characteristics are displayed. Decreasing values of $\beta$ correspond with a steeper initial part of the curve followed by a flattened tail in the final part. For values of $\beta$ greater than 1 and $\beta \rightarrow \infty$ the curve takes on a sigmoidal shape, increasing in sigmoidicity as the value of $\beta$ increases (131, 136).

Langenbucher has applied the Weibull distribution function to linearise dissolution data. Equation 2.9 takes on the following form:

$$\log [-\ln(1-m)] = b\log(t-T_i) - \log a \qquad \text{Eqn 2.10}$$

A linear relationship is obtained for a log-log plot of -ln(1-m) versus t (131). The Weibull distribution has also been applied to describe *in vivo* absorption kinetics by Piotrovskii (137). Using theophylline data he concluded that the Weibull function adequately described absorption data and may be considered as one of the possible approximations of the complex process of absorption.

### 2.1.4.2 Computational Predictive Methods

Data from dissolution studies have also been employed in the prediction of *in vivo* responses. These methods are often referred to as computational conversion predictive methods. Leeson and co-workers (49) applied such an approach to develop controlled release products in lieu of the trial and error approach of

conducting multiple bioavailability studies. The use of *in vitro* dissolution data as a basis for the design and development of any product with specific *in vivo* performance necessarily implies an *in vitro-in vivo* correlation. As discussed in chapter 1 the use of univariate correlation methods are of little utility for CMRD's. It would appear that relating the *in vitro* dissolution behaviour of a CMRD to the whole plasma concentration time curve could be of great value during dosage form development.

Although this technique is based upon theoretical considerations, it represents a tool for the development of controlled release dosage forms. The ability to predict a dosage form's *in vivo* performance based on *in vitro* measurements in combination with a drug's pharmacokinetic parameters clearly offers distinct advantages.

The success of the above approach for the prediction of plasma level curves is based on four assumptions:

i) The products *in vitro* dissolution must mimic the *in vivo* dissolution;

ii) The main pharmacokinetic parameter of the drug in the controlled release dosage form which should not differ from those found in the conventional dosage form is clearance;

iii) There is no absorption window for the drug under study; and

iv) Plasma concentrations are directly related to the drugs efficacy. Although a correlation between *in vitro* dissolution rate and bioavailability might still be achieved even if plasma drug concentrations do not relate to pharmacological effect, the value of such a correlation would be of no practical value.

The procedure used to predict the *in vivo* profiles is summarised in the following four steps:

i) Determination of the products *in vitro* dissolution rate and the subsequent development of an appropriate dissolution equation;

ii) The development of a pharmacokinetic model equation for the drug. Ideally this information should be obtained from data derived from the administration of a solution of the drug or if this is not possible, from a rapidly dissolving dosage form;

iii) Redefinition of the pharmacokinetic model by substituting the *in vitro* dissolution equation for the dosage form into the differential form of the pharmacokinetic model equation; and

iv) The generation of the expected plasma concentration-time curve using the parameters obtained in (i) and (ii) and the equation derived in (iii).

An important aspect of this method is the careful determination of the *in vitro* dissolution rate, since the method is almost totally dependent on this information. In general a three component model is used. The general equation which assumes that the CMRD has an immediate release fraction, a fast first order releasing fraction and a slow first order releasing fraction is as follows:

$$A_d = F_i D + F_f D[A] + F_s D[B]$$

*where*:

$$A = 1 - \exp(-K_f t)$$

$$B = 1 - \exp(-K_s t)$$

Eqn 2.11

where $A_d$ is the amount dissolved, D is the dose, $F_i$ the immediate release fraction, $F_f$ is the fraction in the faster exponential portion, $F_s$ is the fraction in the slower release portion, $K_f$ is the dissolution rate constant for the fast fraction, $K_s$ is the dissolution rate constant for the slower fraction and t is the time (49). Models for a three component release, a model for a zero order release followed by first order release and a model which employs two compartment kinetics have been developed and are shown below.

and are shown below.

Model A : Equations for a first order release with three fractions.

$$C_{im} = \frac{k_a F_{im} D}{(K_a - K_{el})\, V_d} \left[ \exp(-K_{el}\, t) - \exp(-K_a\, t) \right]$$

$$C_f = \frac{k_a K_f F_f D}{V_d} [A + B + C]$$

*where*:

$$A = \frac{\exp(-K_f\, t)}{(K_a - K_f)\,(K_{el} - K_f)}$$

$$B = \frac{\exp(-K_a\, t)}{(K_f - K_a)\,(K_{el} - K_s)}$$

$$C = \frac{\exp(-K_{el}\, t)}{(K_f - K_{el})\,(K_a - K_{el})}$$

$$C_s = \frac{K_a K_s F_s D}{V_d} [D + E + F]$$

*where*:

$$D = \frac{\exp(-K_s\, t)}{(K_a - K_s)\,(K_{el} - K_s)}$$

$$E = \frac{\exp(-K_a\, t)}{(K_s - K_a)\,(K_{el} - K_a)}$$

$$F = \frac{\exp(-K_{el}\, t)}{(K_s - K_{el})\,(K_a - K_{el})}$$

The plasma concentration time curve is generated from the following equation:

$$C_p = C_{im} + C_f + C_s$$

Model B: Equations for zero order release prior to first order release.

$$C_z = \frac{K_0}{V_d K_e} [1 - \exp(-K_e t)] -$$

$$\frac{K_0}{V_d (K_e - K_a)} [\exp(-K_a t) - \exp(-K_e t)]$$

Model C: Equations for first order release following zero order release.

$$C = \frac{K_a K_d (D - t_0 K_0)}{(K_a - K_d)(K_e - K_d) V_d} \exp(-K_d t)$$

$$+ \frac{K_a}{V_d (K_e - K_a)} \{\frac{K_0}{K_a} [1 - \exp(-K_a t_0)] - \frac{K_d (D - t_0 K_0)}{K_a - K_d} \exp(-K_a t)\}$$

$$+ \frac{K_a}{V_d (K_e - K_a)} \{\frac{K_d (D - t_0 K_0)}{K_e - K_d} - \frac{K_0}{K_a} [1 - \exp(-K_a t_0)]\} \exp(-K_e t)$$

$$+ \frac{K_0}{V_d} \{\frac{1}{K_e} [1 - \exp(-K_e t_0)]$$

$$- \frac{1}{K_e - K_a} [\exp(-K_a t_0) - \exp(-K_e t_0)]\} \exp(-K_e t)$$

Model D: Equations for first order release, assuming a three component dosage form, using a two compartment model.

$$C_{im} = \frac{K_a F_{im} D}{V_d} [A+B+C]$$

*where:*

$$A = \frac{(K_a - K_{21}) \exp(-K_a t)}{(\beta - K_a)(K_a - \alpha)}$$

$$B = \frac{(\alpha - K_{21}) (\exp(-\alpha t)}{(\alpha - \beta)(K_a - \alpha)}$$

$$C = \frac{(\beta - K_{21}) \exp(-\beta t)}{(\alpha - \beta)(\beta - K_a)}$$

$$C_f = \frac{K_a K_f F_f D}{V_d (K_a - K_f)} [D+E+F+G]$$

*where:*

$$D = \frac{(K_f - K_{21}) \exp(-K_f t)}{(\beta - K_f)(K_f - \alpha)}$$

$$E = \frac{(K_{21} - K_a) \exp(-K_a t)}{(\beta - K_a)(K_a - \alpha)}$$

$$F = \frac{(\alpha - K_{21})(K_a - K_f) \exp(-\alpha t)}{(\alpha - \beta)(K_f - \alpha)(K_a - \alpha)}$$

$$G = \frac{(\beta - K_{21})(K_f - K_a) \exp(-\beta t)}{(\alpha - \beta)(\beta - K_f)(\beta - K_a)}$$

$$C_s = \frac{K_a K_s F_s D}{V_d (K_a - K_s)} [H + I + J + K]$$

*where:*

$$H = \frac{(K_s - K_{21}) \exp(-K_s t)}{(\beta - K_s)(K_s - \alpha)}$$

$$I = \frac{(K_{21} - K_a) \exp(-K_a t)}{(\beta - K_a)(K_a - \alpha)}$$

$$J = \frac{(\alpha - K_{21})(K_a - K_s) \exp(-\alpha t)}{(\alpha - \beta)(K_s - \alpha)(K_a - \alpha)}$$

$$K = \frac{(\beta - K_{21})(K_s - K_a) \exp(-\beta t)}{(\alpha - \beta)(\beta - K_s)(\beta - K_a)}$$

The plasma concentration time curve is generated from the following equation:

$$C_p = C_{im} + C_f + C_s$$

Definition of variables:

$C_{im}$ concentration due to immediate release fraction

$C_f$ concentration due to fast release fraction

$C_s$ concentration due to slow release fraction

$K_a$ absorption rate constant

$K_e$ elimination rate constant

| | |
|---|---|
| $t$ | time |
| $V_d$ | apparent volume of distribution |
| $D$ | dose |
| $F_{im}$ | immediate release fraction of dose |
| $F_f$ | fast release fraction |
| $F_s$ | slow release fraction |
| $K_f$ | rate constant of fast fraction |
| $K_s$ | rate constant of slow fraction |
| $K_0$ | zero order release rate constant |
| $K_d$ | first order release rate constant |
| $t_0$ | time of zero order release |
| $\alpha$ | elimination rate constant for alpha phase |
| ß | elimination rate constant for beta phase |
| $K_{21}$ | distribution rate constant |

The foregoing equations were used (*vide infra*: chapters 3 and 4) to simulate drug concentration versus time profiles using the appropriate pharmacokinetic constants obtained from the *in vitro* and *in vivo* studies reported.

This technique can be modified so that it is no longer necessary to design a number of different formulations. The desired plasma concentration-time curve is generated using the drugs pharmacokinetic parameters and then the *in vitro* characteristics of the dosage form are computed in order to obtain the dosage form which will provide that particular response.

## 2.2 Methods

### 2.2.1 Chemicals, Drugs and Reagents

i) Ortho-Phosphoric acid, 85%, Holpro Analytics, RSA

ii) Sodium dihydrogen phosphate, SAARChem, RSA

iii) Sodium hydroxide, BDH Chemicals, Analar, RSA

iv) Hydrochloric acid, 34%, Holpro Analytics, RSA

v) HPLC grade Methanol, Burdick and Jackson, USA

vi) HPLC grade Acetonitrile, Burdick and Jackson, USA

vii) Theophylline powder, batch L085295, Lennon Limited, RSA

viii) Indomethacin powder, batch L066133, Lennon Limited, RSA

ix) Theodur$^{R}$ 300mg tablets, batch A800413, Adcock Ingram, RSA

x) Theophylline Test Product 1, batch 6081, Lennon Limited, RSA

xi) Retafyllin$^{R}$ 300mg tablets, batch 01-IS, Orion Pharmaceutica, Finland

xii) Indocid R$^{R}$ 75mg capsules, batch N4048, Logos Pharmaceuticals, RSA

xiii) Indomethacin Test Product 2, batch 5791, Lennon Limited, RSA

xiv) Caffeine powder, batch L046158, Lennon Limited, RSA

xv) Naproxen powder, batch 23092, Lennon Limited, RSA

xvi) Decon, Atomic Research, RSA

### 2.2.2 Equipment

i) Pharmatest PTW-S Dissolution Apparatus, Pharmatest, Germany

ii) Beckman DU 68 Spectrophotometer, Beckman Instruments, USA

iii) Model M510 constant flow pump, Waters Associates, USA

iv) Model 710B WISP Automatic Sample Injector, Waters Associates, USA

v) Model 481 Lambda Max LC Spectrophotometer, Waters Associates, USA

vi) $C_{18}$ 150mm x 4.6mm i.d. Hypersil 5$\mu$m column, Phenomenex, USA

vii) Model 561 Strip Chart Recorder, Perkin Elmer, USA

### 2.2.3 Additional Equipment

i) Model 601 Digital Ionanalyser, Orion Research, USA

ii) Model 2004MP Analytical Balance, Sartorious, USA

iii) Adjustable volume pipettes, Pipetteman, SA

iv) Rheodyne 7125 Injector, Rheodyne Inc., USA

v) Model 1040A Diode Array Ultraviolet Detector, Hewlett Packard, USA

vi) Model 85A Data Processor, Hewlett Packard, USA

vii) Microlab-P Programmable Automatic Pipette, Hamilton-Bonaduz-Ag,Switzerland

viii) Vortex-Genie, Scientific Industries, USA

ix) 10ml plastic syringes, Promex, SA

x) Micro sample filters, 45$\mu$m, Millipore, SA

### 2.2.4 Dissolution Studies

#### 2.2.4.1 Buffer Preparation

Phosphate buffer was utilised in all dissolution rate studies. A quantity of 18.5ml of ortho phosphoric acid was measured into a 5.0L volumetric flask and made up to volume with distilled water. The resultant 0.05M acid solution was adjusted to the required pH using sodium hydroxide. The pH of all buffers was adjusted to within 0.05 pH units. The buffers were allowed to equilibrate to 37°C before the dissolution studies were initiated.

2.2.4.2 pH meter calibration

The pH meter was calibrated daily using pH 4.01 and 7.00 calibrators. The calibration of the pH meter was checked against a pH 6.00 reference buffer to determine the precision of the meter on a day to day basis.

After calibration of the instrument the pH of the pH 6.0 buffer was measured and the value recorded on a daily basis over a four week period. The pH was determined to be pH 6.03 ± 0.02 (mean ± S.D). The mean pH determined was well within the tolerance limit set by the USP of ±0.05 pH units.

2.2.4.3 The Basket Apparatus

The dissolution apparatus used consisted of two main components, the waterbath and the mechanicals which included the drive mechanisms and water pump. The waterbath holds six pyrex glass vessels of one litre capacity. Each vessel was covered with a perspex cover with three openings, a central one for the shaft and two others for sample withdrawl and fluid replacement. The temperature of the waterbath was maintained at 37 ±0.5 °C. The basket assembly consisted of a stainless steel shaft and a 40-mesh screen basket. A single tablet or capsule was placed in the basket and immersed into 900ml of equilibrated dissolution medium. Rotation of the baskets was initiated immediately and the time noted as time t = 0. The baskets were rotated at 75 or 100 r.p.m. Three dosage forms were tested in each study. Samples, of either 3ml or 5ml, were withdrawn from the dissolution medium at specified time intervals using a 10ml disposable syringe fitted with a 15cm luer lock needle, to which a sample filter was attached. Samples were taken from a point midway between the basket and the vessel wall at half the height of the basket (see figure 2.5). Sample volumes were immediately replaced with an equal volume of equilibrated fresh dissolution medium.

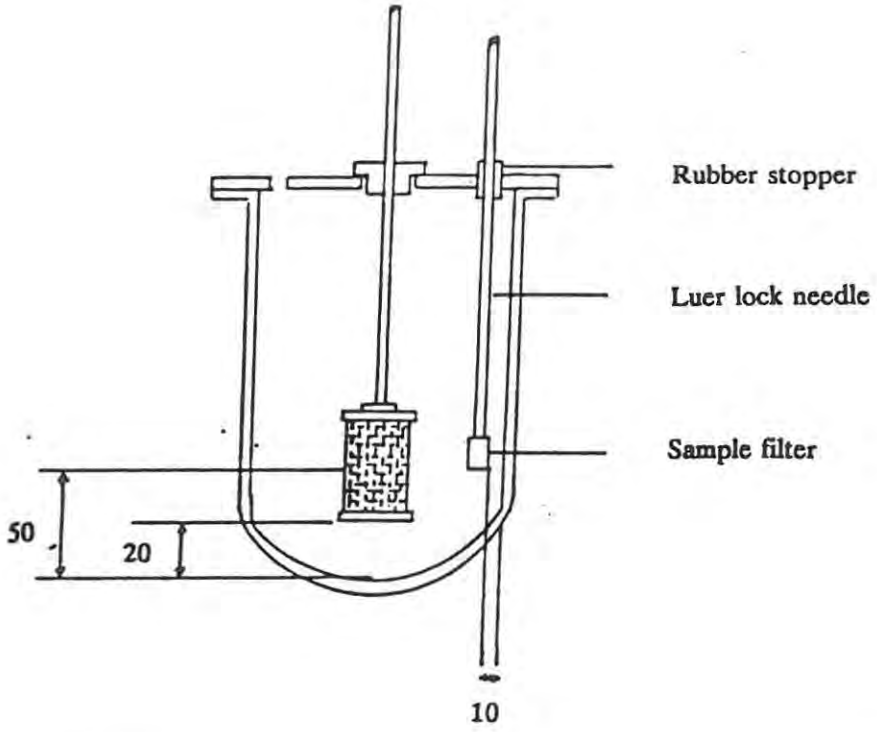



Figure 2.5 The Basket Apparatus showing sample removal specifications.

2.2.4.4 The Paddle Apparatus

The apparatus used was as described in section 2.2.4.3 with the following modification. The basket assembly was replaced with paddles consisting of a stainless steel shaft ending in a paddle coated with teflon. Rotation of the paddle was initiated immediately after introduction of the dosage form to the dissolution medium. Capsules were weighted with a single twist of stainless steel wire around the centre of the dosage form. The paddle rate was set at 50 or 75 rpm. Samples were withdrawn at a position in accordance with the USP XXII specifications. Three dosage forms were tested in each study.

2.2.4.5 Acid exposure dissolution method

This method involved the exposure of the relevant dosage form for one hour to a

0.1N hydrochloric acid medium. A 0.1N HCl medium was prepared by measuring 64.2ml HCl solution and diluting this to 6.0 L with distilled water. The resultant pH was 1.2. After the 1hr exposure, a sample was withdrawn from the dissolution vessel and the medium was discarded by gently pouring it off, taking care not to disturb the dosage form. The acid medium was then replaced with the required phosphate buffer medium and the test was continued. Three dosage forms were tested in each study.

### 2.2.5 Sample analysis

#### 2.2.5.1 UV Analysis

Analysis of all samples was performed using a UV spectrophotometer fitted with a 1cm micro flow-through cell and a sample aspirator. The instrument was calibrated for each dissolution study. Calibration curves were programmed into the instrument in order to obtain the results as amount dissolved per millilitre.

(i) Calibration standards

Stock solutions of indomethacin (103 $\mu$g/ml) and theophylline (404 $\mu$g/ml) were prepared by weighing the appropriate mass of the respective raw material and dissolving it in 10ml methanol and diluting to 100ml with phosphate buffer. Stock solutions were stored at 4°C. Appropriate dilutions were prepared in 10 or 20ml volumetric flasks with A grade glass pipettes to obtain calibrators in the range of 5.40 - 97.75$\mu$g/ml and 20.20 - 404.00$\mu$g/ml for indomethacin and theophylline respectively. All calibrators were prepared in the appropriate buffer. Calibration lines were obtained by analysing the calibrator solutions. Data obtained from this were then utilised to program the spectrophotometer.

(ii) Sample preparation

No manipulation of the indomethacin samples was necessary prior to UV analysis. A 1 in 10 dilution of the theophylline samples was achieved by diluting 400$\mu$l of sample to 5ml with the appropriate buffer. Each sample was vortex mixed prior to analysis.

2.2.5.2 HPLC Analysis

Analyses of certain batches of dissolution samples were carried out using HPLC.

(i) Chromatographic system

The modular chromatographic system used in all the HPLC analyses consisted of a solvent delivery system, autosampler, strip chart recorder and a UV LC spectrophotometer. Separation was achieved on a $C_{18}$ column.

(ii) Mobile phase preparation

The optimum mobile phase for indomethacin consisted of 55% v/v acetonitrile and 45% v/v 0.05M phosphate buffer of pH6.0. The pH of the resultant solution was readjusted to pH3.5 using sodium hydroxide. In order to select the mobile phase conditions for indomethacin separation, the following aspects were considered in terms of their influence on the retention time of indomethacin:

a) effect of pH of the mobile phase

b) effect of percentage organic phase content

c) effect of buffer molarity

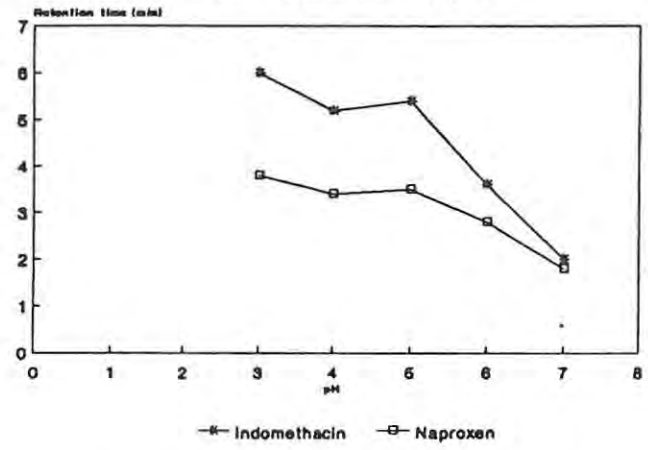
d) effect of organic solvent type

The effect of pH, percent organic phase, buffer molarity and type of organic solvent are summarised in figures 2.6a - d. From these it is evident that the optimum conditions are as follows:

- 55% v/v Acetonitrile
- 45% v/v 0.05M phosphate buffer
- pH adjusted to 3.5.

Methanol was found to be an unsuitable organic solvent because very large volumes were necessary to obtain elution of the indomethacin peak. Methanol also caused poor peak resolution.

Figure 2.6 (a) Effect of pH




(b) Effect of Buffer molarity

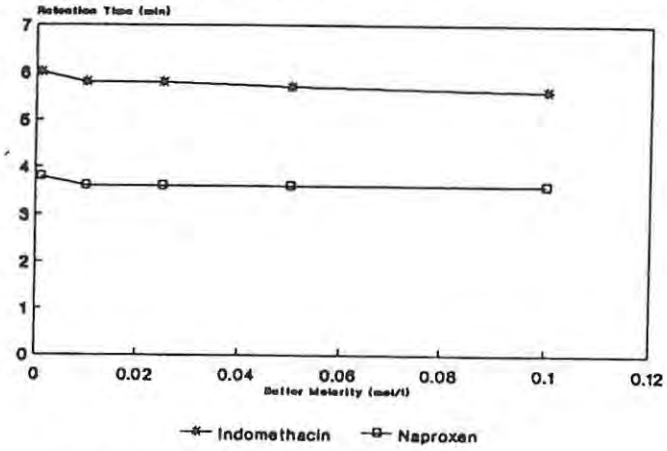
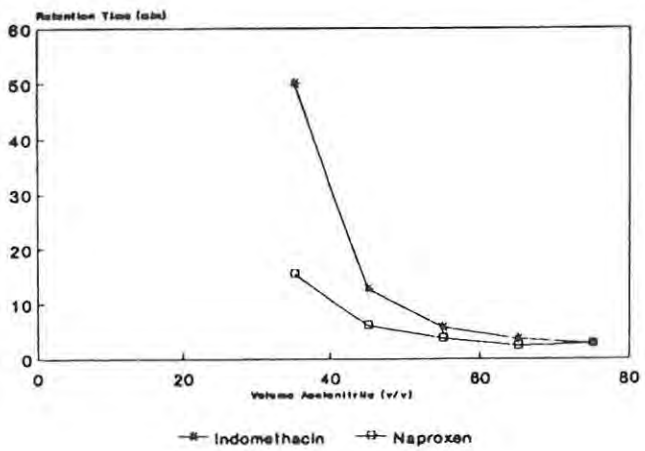
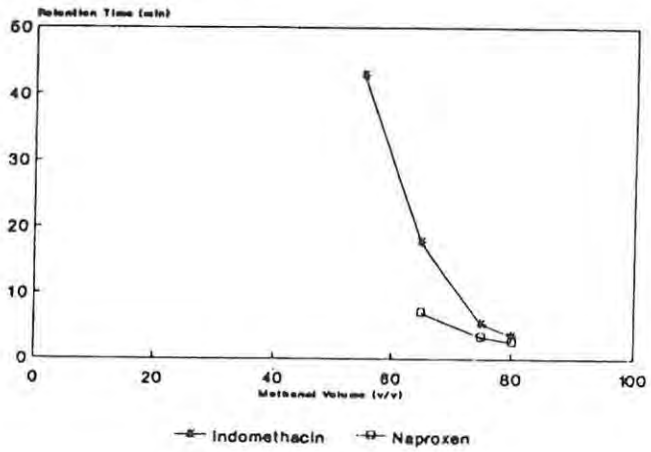



Figure 2.6(c) Effect of Acetonitrile




(d) Effect of Methanol



For the theophylline studies the chromatographic method used was based on that described in the USP XXII (72). The mobile phase was prepared by mixing 35% v/v methanol and 65% v/v 0.05M phosphate buffer of pH 6.0. The resultant solution was then readjusted to pH6.0 with sodium hydroxide.

The mobile phases were filtered and degassed through a 45$\mu$m filter (Millipore).

(iii) Calibration standards

a) Indomethacin

A stock solution of indomethacin (100 $\mu$g/ml) was prepared by dissolving 10mg indomethacin in 10ml methanol and diluting to 100ml using 0.05M pH 6.2 phosphate buffer. Appropriate dilutions were made in order to obtain a set of calibrators over the range 1.00 - 80.00$\mu$g/ml. Three additional standards were prepared to serve as *in vitro* standards. Naproxen was selected as the internal standard and was prepared by dissolving an appropriate mass of naproxen in phosphate buffer in order to obtain a 2.04$\mu$g/ml working solution.

b) Theophylline

A stock solution of theophylline (399 ug/ml) was prepared in 0.05 M phosphate buffer. Appropriate dilutions were prepared to yield calibrators in the range 19.95 - 399.00 $\mu$g/ml. Calibrators were prepared in buffer of the same pH as the dissolution medium used in the particular dissolution study. Caffeine was selected as the internal standard. A stock solution was prepared by dissolving 41.8mg caffeine in 200ml phosphate buffer. A dilution was prepared to yield a working solution of 6.27 $\mu$g/ml.

The calibration lines for both indomethacin and theophylline were found to be linear

over the concentration ranges studied. This was found to be the case for both the UV analysis and the HPLC analysis. Correlation coefficients were determined to be 0.99 or better for all determinations. Within run precision was assessed by running three spiked samples. The coefficients of variation were found to be 4.07%, 3.64% and 3.3% for the upper, middle and lower end of the concentration range used. Figures 2.7 and 2.8 are examples of calibration lines for indomethacin and theophylline respectively.

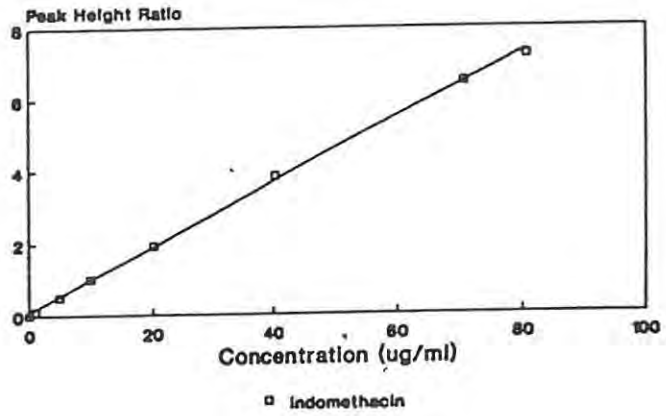



Figure 2.7 Calibration Line for indomethacin

x Coefficient = 0.090

Constant = 0.087

r-squared = 0.998.

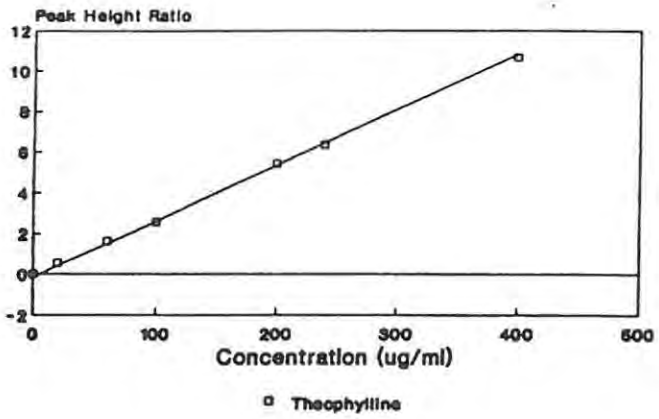



Figure 2.8 Calibration Line for theophylline

x Coefficient = 0.028

Constant = -0.142

r-squared = 0.992

(iv) Chromatographic conditions

(a) Indomethacin

| | | |
|---|---|---|
| Column | : | reverse phase $C_{18}$ |
| HPLC system | : | see section 2.2.5.2(i) |
| Detection wavelength | : | 254nm |
| Sensitivity | : | 0.01 Aufs |
| Flow rate | : | 1ml/min |
| Pressure | : | 800 psi |
| Temperature | : | ambient |
| Recorder input | : | 10mV |
| Mobile phase | : | see section 2.2.5.2(ii) |

(b) Theophylline

| | | |
|---|---|---|
| Column | : | reverse phase $C_{18}$ |
| HPLC system | : | see section 2.2.5.2(i) |
| Detection wavelength | : | 280nm |
| Sensitivity | : | 0.1 Aufs |
| Flow rate | : | 1ml/min |
| Pressure | : | 1400 psi |
| Temperature | : | ambient |
| Recorder input | : | 10mV |
| Mobile phase | : | see section 2.2.5.2(ii) |

(v) HPLC UV 3-D chromatograms

For both theophylline and indomethacin products 3-D chromatograms were obtained for the 24h dissolution samples. These were carried out using a diode-array detector connected in line to the chromatographic system described above.

2.2.6 Bioavailability Studies

All *in vivo* data used in this research project was obtained from bioavailability studies conducted by the Biopharmaceutics Research Institute at Rhodes University, Grahamstown. To compare the rate and extent of indomethacin and theophylline absorption from different dosage forms, single-blind, randomised crossover studies were conducted. The trial periods were separated by a 7 day washout period. The subjects were screened according to the normal procedures for a clinical trial. A complete medical history was taken and haematological examination, clinical chemistry and urine analysis was conducted. All studies were approved by the Rhodes University Ethical Standards Committee and informed consent was obtained

from each volunteer.

(i) Study BRI 14/89

Data for the indomethacin studies were obtained from BRI study number 14/89 (138) For the trial, eight (8) healthy, non-smoking male subjects were used. Each was given one capsule of Indocid R and one capsule of Test Product 2 during the two phases of the trial, which were conducted according to a balanced design. The trial was conducted according to the following protocol:

| Time: | Comment: |
|---|---|
| 0hrs | The subjects reported to the clinic after a ten hour overnight fast. An indwelling needle was inserted into an arm vein and a zero time sample was collected. The respective dosage form was administered with 200ml water after a standard light breakfast. Subjects remained sedentary in bed for the first 8hrs. |
| 0.5 | Blood sample |
| 1.0 | Blood sample |
| 1.5 | Blood sample |
| 2.0 | Blood sample, 200ml water administered |
| 2.5 | Blood sample |
| 3.0 | Blood sample, standard light snack provided |
| 3.5 | Blood sample |
| 4.0 | Blood sample, 200ml water administered |
| 5.0 | Blood sample |
| 6.0 | Blood sample, standard low fat meal given to volunteer |

| | |
|---|---|
| 8.0 | Blood sample, standard light snack given to volunteer |
| 10.0 | Blood sample, standard low fat meal given to volunteer |
| 12.0 | Blood sample |
| 24.0 | Blood sample |

After 6 hours the subjects were allowed to take water *ad libitum*. Serum samples were centrifuged and separated into two portions and frozen at -20°C until the time of analysis. The serum concentrations of indomethacin were determined by HPLC with ultraviolet detection following a solid phase extraction procedure. Results are shown in Appendix B.

(ii) Study BRI 1/89 and 15/90

For study BRI 1/89 (139) six (6) and for BRI 15/90 (140) ten (10) healthy male subjects were used. The protocol for the theophylline studies differed slightly and a summary is given below. The subjects were each given a single Theodur 300mg tablet, a Retafyllin tablet or a single tablet of Test Product 1 during the different periods of the trials.

| Time: | Comment: |
|---|---|
| 0.0hrs | As per above , but no breakfast was given. |
| 0.5 | Blood sample |
| 1.0 | Blood sample |
| 2.0 | Blood sample, 200ml water administered |
| 3.0 | Blood sample |
| 4.0 | Blood sample and standard light breakfast given to volunteer |

| | |
|---|---|
| 5.0 | Blood sample |
| 6.0 | Blood sample, standard low fat meal given |
| 7.0 | Blood sample |
| 8.0 | Blood sample, standard light snack given to volunteer |
| 10.0 | Blood sample |
| 12.0 | Blood sample |
| 24.0 | Blood sample |
| 36.0 | Blood sample |
| 48.0 | Blood sample |

Serum samples were centrifuged, separated and stored at -20°C until analysis. Serum concentrations of theophylline were determined by means of a fluorescence immunoassay technique (Abbott-TDX). Results are shown in Appendix A.

## 2.3 Data Manipulation

### 2.3.1 *In vitro* data

#### 2.3.1.1 3-D plots

Three dimensional plots or topographs were constructed of the dissolution profiles of indomethacin and theophylline dissolution studies. The plots were constructed with the aid of a computer software package designed at the School of Pharmaceutical Sciences. Plots were obtained by entering the sets of data in the following form : time points on the *x*-axis, range of pH on the *y*-axis and percent dissolved on the *z*-axis.

2.3.1.2 % Dissolved versus Time plots

Before any plots of the data could be constructed the data obtained from the UV analysis first had to be transformed into % dissolved of the labelled amount from the concentration data generated by the spectrophotometer. This was achieved by employing a custom designed PlanPerfect (WordPerfect Corporation, USA) computer worksheet. The % dissolved data obtained were then plotted using a graph plotting package, plotting time on the *x*-axis and % dissolved on the *y*-axis.

2.3.1.3 Dissolution rates

Percent remaining to be dissolved data were necessary in order to plot the log (ln) % remaining to be dissolved versus time plots. These plots were used to calculate the dissolution rates and to determine the order of the dissolution processes. The dissolution rates of the pseudo first order processes were determined by linear regression analysis of the log-normal plots using a computer software package (SPS, Rhodes Pharmacy). The zero order dissolution rate constants were determined by linear regression of amount dissolved versus time plots. This provided an estimate of the dissolution rate constant and an estimate of the correlation coefficient.

2.3.1.4 Weibull distribution analysis

Weibull distribution analysis of the dissolution data was performed using the computer software package MINSQ (Micromath, USA). The user function was entered into MINSQ and the iterative program was supplied with initial estimates of the parameters $F^{\infty}$, $t_d$, $t_0$, and $\beta$.

### 2.3.2 *In vivo* data

#### 2.3.2.1 Plots of serum concentration versus time

Plots were generated with a computer graphics package using the data obtained from the bioavailability trials as in Appendix A and B.

#### 2.3.2.2 Parameter estimations

In order to obtain the relevant parameters from the serum concentration data, the data sets were entered into a BIOPAK data analysis package (Statistical Consultants Inc.). The following parameters were calculated using BIOPAK's Pharmacokinetic module:

i) Area under the curve (AUC);

ii) Area under the moment curve (AUMC);

iii) The time for the maximum concentration to occur ($t_{max}$); and

iv) The maximum serum concentration ($C_{max}$)

These parameters were determined for the individual plots and the mean parameters calculated from these. Parameters were also estimated for the mean data set.

#### 2.3.2.3 Wagner-Nelson analysis

Percent drug absorbed, and hence the percent drug remaining to be absorbed, was obtained using the Wagner-Nelson method for the individual data and the mean data. The calculations were achieved with the assistance of a computer software package (SPS, Rhodes Pharmacy). The data were entered as sets of concentration values with their corresponding time points. The point at which log-linearity was assumed to commence was also entered in order to obtain an estimate of $k_e$.

2.3.2.4 Weibull distribution analysis

The data obtained from the Wagner-Nelson analysis were also fitted to the Weibull function. This was performed in the same manner as described in section 2.3.1.4.

2.3.3 *In vitro* - *In vivo* Correlations

2.3.3.1 Type A correlations

To achieve a level A correlation between *in vitro* and *in vivo* data, the *in vitro* dissolution curve of a dosage form was compared with the *in vivo* response curve. An example of a level A correlation is the Leeson predictive model (148).

2.3.3.1 (i) Leeson predictive model

The Leeson method depends on both the *in vitro* and *in vivo* parameters of the drug in its dosage form. The calculations to obtain data for the profile were generated with the aid of MathCAD (MathSoft, USA), a computational software package. The relevant formulae were entered into the database as depicted in section 2.1.4.2. Model worksheets were designed to simulate first order processes (Model A) and zero order processes followed by first order (Model B & C). For the simulation of the indomethacin profiles model D was employed. In cases where the simulated profile was obtained with the aid of more than one model, the cumulative profile was obtained by a summation of the data generated by each model.

In order to generate the required data the following general population parameters, obtained from the literature, were entered into the relevant model: $k_a$, $k_e$, Dose, Vd, $k_d$ and a time range. Plots were constructed with the aid of a graphics package.

2.3.3.1 (ii) Wagner-Nelson plots versus % dissolved plots

Overlay plots of percent absorbed *in vivo* and percent dissolved *in vitro* were constructed to determine whether any correlation existed between these two aspects.

2.3.3.2 Type B correlations

These correlations are not considered as 1:1 correlations since they do not reflect the actual *in vivo* plasma level curves.

2.3.3.2 (i) MRT versus $T_d$

Mean residence times (MRT) *in vivo* were calculated from the parameters generated by BIOPARAMS (SPS, Rhodes Pharmacy) and compared to the $t_d$ values obtained for the *in vitro* dissolution data by the Weibull analysis.

2.3.3.2 (ii) $T_d$ *in vitro* versus $t_d$ *in vivo*

The Weibull parameter, $t_d$, was compared for the *in vitro* dissolution and *in vivo* Wagner-Nelson data. $T_d$ values for the dissolution studies carried out at the differing pH's were compared directly to the *in vivo* parameters.

2.3.3.3 Type C correlations

To establish a level C correlation a specific *in vivo* parameter is compared to an *in vitro* parameter. The *in vivo* parameters $C_{max}$, $t_{max}$, and AUC were compared with the $t_{50\%}$ and $t_{90\%}$ *in vitro*, as well as the Weibull parameter $F^{\infty}$.

# CHAPTER 3

# THEOPHYLLINE STUDIES

## 3.1 Introduction

### 3.1.1 Oral Controlled/Modified Release Dosage Forms (CMRD's)

Over the past four decades, pharmaceutical manufacturers have introduced an increasing number of products which are reported to provide extended therapeutic responses. Controlled-release dosage forms cover a wide range of "prolonged activity" preparations that provide continual release of their active ingredients at a predetermined rate and for a predetermined period. The majority of these products have been designed for oral administration, however, recently there have been significant developments in the design of delivery systems for intramuscular and subcutaneous drug administration and for use as ocular inserts, intra-uterine devices and transdermal patches.

Enteric coating was the first attempt at delaying the release of an active ingredient from a dosage form as well as to prevent gastric irritation during transit along the gastro-intestinal tract. It was only in 1952 that the first practical sustained-release type of dosage form was marketed. This has since led to a great deal of research activity in this area of pharmaceutics (8).

The prime goals of these types of formulations are to provide an extended duration of action, to minimise toxicological effects, to improve therapeutic efficacy and to ensure patient compliance or simply to improve the convenience of the dosing regimen.

3.1.1.1 Definitions

Over the years a number of different terms have been used to describe these formulations; it is therefore necessary to classify these (124, 141, 72).

(i) *Delayed-release*: These systems utilise repetitive, intermittent dosing of a drug from one or more immediate release units which are incorporated into a single dosage form. Examples of these dosage forms include repeat action tablets and capsules and enteric-coated tablets where timed-release is achieved by a barrier coating. These dosage forms do, however, not produce or maintain uniform blood concentrations within the therapeutic range but are nevertheless more effective than conventional dosage forms. The USP defines these dosage forms as "dosage forms which release a drug at a time other than promptly after administration".

(ii) *Sustained-release*: These include any drug delivery system that achieves slow release of drug over an extended period of time. If these systems are capable of maintaining constant blood concentrations they are considered as *controlled-release* systems. If this is not achieved but the dosage form still extends the duration of action over that achieved by a conventional delivery system, they are considered as *prolonged-release* systems. The USP defines these dosage forms as those "that allow at least a twofold reduction in dosing frequency as compared to that drug presented as a conventional dosage form".

(iii) *Site-specific* and *receptor release*: These systems refer to the targeting of a drug to a specific biological location. In the case of site-specific release, the target is a particular organ or tissue; for receptor release, the site is a receptor of the drug in a specific organ or tissue.

3.1.1.2 Advantages and disadvantages of CMRD's

Patient compliance has for many years been recognised as a necessary and important component for the success of self administered drug therapy. The general advantage of these dosage forms therefore lies in the fact that the frequency of

dosing is reduced. Economic advantages are seen in the hospital situation since nursing time for drug distribution and administration is minimised. Adequate overnight therapy is provided without the need for the patient to be awakened during the night. The nature of the kinetics of these dosage forms implies that less drug should be utilised than for conventional dosage forms to achieve successful therapy.

Therapeutic advantages include control of the level of sleep throughout the sleep period, control of nocturnal seizures associated with epilepsy, control of enuresis, control of migraine headache on awakening and the control of appetite over extended periods of time (124). Variations in drug concentrations with high peak concentrations and sub-therapeutic concentrations commonly associated with conventional therapy is reduced with controlled-release products. With the reduction of toxic effects due to high blood concentrations, a better control of the disease state can often be achieved at lower concentrations. The severity and frequency of side effects may also be reduced. In addition, the method by which the extended release is achieved can improve the bioavailability of some drugs (124, 142).

Disadvantages include the lack of precision of the dose between patients depending on the stomach emptying time, thus affecting the absorption rate. Poor dosage form design may result in incomplete dosage release or dose dumping which may lead to toxic drug concentrations. Too frequent administration of these dosage forms may also lead to toxic concentrations. Accidental or intentional poisonings also pose special problems since the slow release of the drug and its extended absorption often results in a slowed clearance, making it more difficult to terminate the effects of the drug if severe toxic side effects develop (10, 124, 142).

3.1.1.3 Desirable properties of drugs for use in CMRD's

The drug should be effective when administered orally and resistant to decomposition in the gastrointestinal tract. The drug should also have a short duration of action, have an elimination rate constant of less than 12 hours and be effectively absorbed throughout the gastrointestinal tract. The drug dose should also be small since these formulations usually contain three or four times the amount of drug used in conventional dosage forms. The final product size should also be of a suitable size for ingestion (142).

Rowland and Beckett (143) suggested that drugs with a slow onset of action should be formulated with only part of the dose in the slow release form. With drugs which have an inherent long duration of action due to their particularly long half-lives, there is very little need to formulate these in slow release products. Drugs which are poorly absorbed and those which have a narrow "therapeutic window" should not be used in CMRD's. Other properties of the drug which must be assessed include the aqueous solubility, $pK_a$, partition coefficient, protein binding and molecular size (124, 142).

3.1.1.4 Design of CMRD's

Before a CMRD can be developed, the drugs which are to be incorporated into the dosage form must first be evaluated in terms of their pharmacological properties, chemistry, therapeutic indications and toxicity. From the pharmaceutical approach the only variables which can be modified are the strength, disintegration and dissolution properties of the dosage form (144).

A practical problem in the design of a CMRD is the amount of drug which must be incorporated into the dosage form. Factors which influence the dose size include the biological half life of the drug, intrinsic activity, intended duration of

prolongation of the therapy and the parallel loss of drug occurring prior to systemic availability. Further considerations include a knowledge of the initial dose and the frequency of administration (142). An important parameter in the design of these systems is a knowledge of the input rate constant, $k_a$, which is a combination of the *in vivo* dissolution and absorption. If, however, the elimination half life is very long then the elimination rate constant, $k_e$, will determine the overall kinetics, and any changes in $k_a$ will have little effect. The purpose of CMRD's is to improve therapy by maintaining a constant and uniform plasma concentration at steady state. This can be achieved by minimising the ratio of the maximum and minimum concentrations ($C_{max}/C_{min}$) at steady state and can be made possible if the release of the drug from the product results in zero order or sufficiently slow first order absorption of the drug from the gastrointestinal tract. This will result in the absorption of the drug into the body being much slower that the rate of elimination (flip-flop kinetics) (145).

If $k_e$, $k_a$, and the conventional dose size, $D_c$, required to produce a satisfactory blood concentration are known, the maintenance dose, D, can be determined (142). The method is based on the argument that in order to maintain a blood concentration at steady state the rate in must equal the rate out, so that the total dose is given by:

$$D = D_c + \frac{0.693 D_c f h}{t_{\frac{1}{2}}} \qquad \text{Eqn 3.1}$$

or

$$D = C_{ss}\, V + k_e\, f\, h \qquad \text{Eqn 3.2}$$

where:
- $D_c$ = the dose from a conventional dosage form which gives a desired clinical response,
- f = a fraction relating the peak drug concentration after $D_c$ to the optimum therapeutic concentration of the drug in the body,
- h = the number of hours required for a sustained level of the drug,
- $t_{½}$ = the biological half life of the drug.
- $C_{ss}$ = concentration at steady state
- V = volume of distribution
- $k_e$ = elimination rate constant

Rowland and Beckett (143) have indicated that this equation can lead to a higher drug quantity than is required if no account is taken of the effect of the maintenance dose of the drug that is released from zero time to $t_{max}$. The consequence of this is that the initial dose will be too large.

### 3.1.1.5 Evaluation of CMRD's

Lazarus and Cooper (141) reported that the assessment of CMRD's should ideally be performed by properly designed *in vivo* trials in which actual measurements of blood concentration are compared to the concentrations resulting from the administration of a solution of the drug or a rapidly disintegrating tablet. The pharmacokinetics of the drug in these dosage forms should also be well established. The most common method for the determination of bioavailability is the establishment of plasma concentration-time curves as discussed in section 2.3.2.1. From this, the $C_{max}$, $t_{max}$, and AUC can be determined. Consequently the onset and duration of action can be estimated provided that the therapeutically effective minimum and maximum concentrations have been previously determined (141, 142, 144) and is illustrated in Figure 3.1.

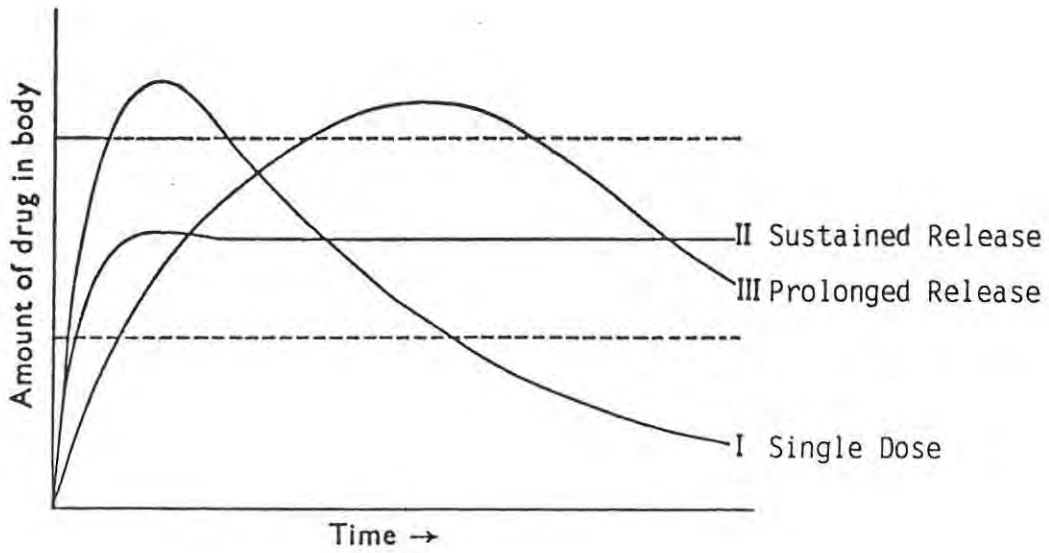



Figure 3.1 Relationship between drug activity and time.

Urinary excretion of a drug or of a drug and its metabolites have also been used to measure the bioavailability of a dosage form. This method has several advantages which include such factors as the fact that samples are more readily obtainable and that the method is non-invasive. Concentrations of drug in urine are usually higher than in the blood and thus less sensitive analytical methods can be used (144). This method is, however, only of use when the drug is excreted predominantly in the unchanged form. It must also be shown that the urinary drug concentrations are proportional to the blood concentrations. Important parameters which can be derived from excretion data include the cumulative amount excreted and the rate at which the excretion occurs.

3.1.1.6 Controlled/Modified Release Formulations (124, 146, 147, 148)

There are several types of CMRD's based on one or more different mechanisms (Table 3.1).

| Table 3.1 Controlled release mechanisms | |
|---|---|
| 1) | Reduced solubility of the drug |
| 2) | Ion exchange resin complexes |
| 3) | Slow eroding matrix tablets |
| 4) | Strongly swelling hydrophillic gel tablets or capsules |
| 5) | Porous inert matrix tablets |
| 6) | Polymer controlled delivery systems, including: |
| | (a) Disintegrating or dispersable coating |
| | (b) Diffusion-controlling membrane |
| | (c) Microencapsulation |
| 7) | Osmotic pumps |

Systems employing some of the above mechanisms include:

(i) *Coated Pellets or Granules*

The manufacture of these types of dosage forms involves the manufacture of beads, pellets or granules. Groups of these are then coated with a coating of varying thickness to retard the release of the drug. The drug is coated onto the nuclei prior to the coating procedure. Some of the nuclei are left uncoated to provide the drug in an immediate release portion which is intended to establish the initial therapeutic concentration. Materials used for the coating of the pellets include beeswax, carnauba wax and bayberry wax with glyceryl monostearate or similar fatty acid esters. The fact that the drug dose is divided into many smaller portions greatly increases the probability that the whole dose will be available for absorption. The uncoated and coated pellets are then blended and placed into hard gelatin capsules or compressed into tablets.

(ii) *Tablets with slow release cores*

These formulations consist of a drug core which is evenly mixed with a substance of low solubility i.e. a hydrophobic material, which permits the drug to dissolve slowly or to be leached from the material. The core is coated with a film which also contains the drug, excipients and binders.

(iii) *Tablet mixed-release granules*

These systems contain granules prepared in the standard manner as well as granules which are coated with a material which will retard the drug release. Materials used to achieve this include beeswax, glyceryl monostearate, glyceryl monopalmitate, stearic acid and cetyl alcohol. The two types of granules are lubricated and compressed into tablets.

(iv) *Multiple layer tablets*

The development of modern compressing machines has facilitated the production of tablets with multiple layers of granules. One layer provides the initial dose, the second a dose at an intermediate release rate and a third layer provides an additional portion at a slow rate of release.

(v) *Ion-exchange resins*

The development of these systems stems from the fact that the ionic concentration of the gastrointestinal fluids varies only within narrow limits. The rate of interchange of ions and hence the release of a drug can thus be fairly well controlled. An ionic drug is allowed to diffuse through an ion-exchange resin where it replaces the inorganic ions on the resin. A slow displacement reaction occurs when the drug-resin complex comes into contact with intestinal fluids. A decreased rate of solution is thus obtained and hence a prolonged action is possible.

(vi) *Osmotic systems*

These dosage forms are designed as mini-osmotic pumps. The system was first described by Theeuwes (149) in 1975. The drug substance is contained in a solid core which is surrounded by a semipermeable membrane that has a single micro-opening. In operation, the system imbibes water at a rate driven by the osmotic pressure difference which occurs across the membrane. The rate is also controlled by the membrane. The system delivers drug at a constant zero order rate as long as it contains excess osmotic driving agent.

### 3.1.2 Introduction to Theophylline

#### 3.1.2.1 Properties of theophylline

Theophylline, designated as 1, 3-dimethylxanthine, is closely related chemically to caffeine and theobromine. It is also occasionally referred to as theocin. The structure of theophylline is shown in Figure 3.2.

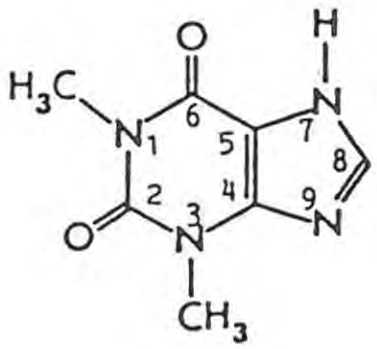



Figure 3.2 Structure of theophylline.

Theophylline occurs as a white, odourless, crystalline powder with a bitter taste and has been reported to exist in both anhydrous and monohydrate forms. The compound is sparingly soluble in water (8.3mg/ml). It is a weakly basic compound with a pKa of 8.6 due to the free proton on the nitrogen in position 7 which is dissociable. A full analytical profile is well documented (150).

Theophylline is a bronchodilator that is effective in the treatment of acute and chronic asthma. The precise mechanism of action is not fully understood but it is known that theophylline inhibits phosphodiesterase. This results in a delayed degradation of 3'5'-cyclic adenosine monophosphate. Theophylline therefore exerts its effect by a different mechanism to that of other bronchodilators. As with the other xanthine compounds, theophylline can potentially induce a transient diuresis, stimulate the central nervous system, produce cerebral vasoconstriction, increase gastric secretion and inhibit uterine contractions (151).

The bronchodilator effect of theophylline increases in proportion to the logarithm of the serum concentration in the range of 5 - 20 $\mu$g/ml. The ability of the compound to prevent exercise-induced bronchospasm also relates directly to the serum concentration, with the greatest effect occurring at concentrations of 15$\mu$g/ml. This effect is maintained with continuous dosing and maintenance of the serum concentration in the range of 10 - 20$\mu$g/ml.

Minor caffeine-like side effects, including slight nausea, insomnia, nervousness and headache are common after the rapid attainment of concentrations in excess of 10$\mu$g/ml but less than 20$\mu$g/ml. Most users, however, develop a tolerance for these effects. More severe and persistent adverse effects may, however, occur at concentrations above 20$\mu$g/ml and include nausea, vomiting, severe headache, diarrhoea, irritability and in cases of severe toxicity, hyperglycaemia, hypotension, cardiac arrhythmias, seizures, brain damage and even death may occur (See Figure 3.3) (151, 152, 153).

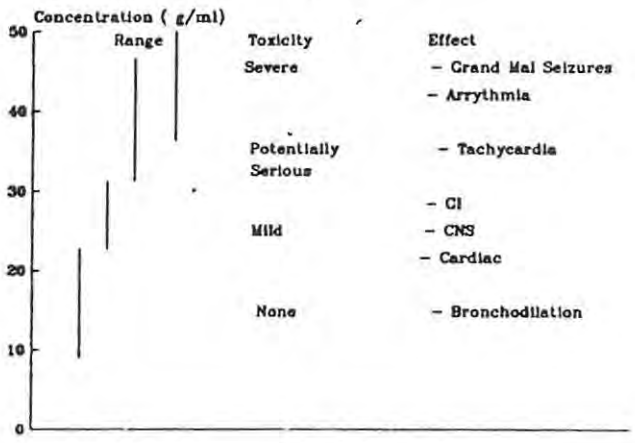



Figure 3.3 Rank order of theophylline effects. (Adapted from Hendeles,L. *et al*, *Drug Intell. Clin. Pharm* 11, 12, 1977).

Theophylline is readily absorbed after oral administration and is highly bioavailable ($F > 0.9$). Rectal suppositories have however been shown to produce a delayed and erratic absorption of theophylline. Following administration, peak concentrations are reached within 0.5 to 2.0h. The plasma concentration-time course after intravenous administration fits a two compartment open model (154). The $\alpha$ or early distribution phase is, however, very rapid and is usually complete within 30 to 45 minutes. Consequently many researchers have applied a 1 compartment open model to perform pharmacokinetic analysis of theophylline data. The volume of distribution averages approximately 0.5 l/kg with a range of 0.3 - 0.7 l/kg. The $V_d$ is apparently not affected by age, sex, history of cigarette smoking, asthma or acute pulmonary oedema. The elimination of theophylline is highly variable with an average elimination half life of 8hrs and an elimination rate constant of $0.087hr^{-1}$. The variability of the elimination appears to be due primarily to differences in the rate of hepatic metabolism, which changes with age, physiological abnormalities, smoking habits, aberrations in diet and concurrent drug therapy (151, 152, 153, 155).

The fact that theophylline has a half-life of 4 - 9 hours and a narrow therapeutic range makes it an ideal candidate for controlled release. Standard dosing schedules of every six hours may result in low trough levels in the early hours of the morning resulting in breakthrough symptoms. The use of properly designed CMRD's may eliminate this early "morning dip" and as a result many CMRD preparations of theophylline have been developed over the past decades (156).

3.1.2.2 Overview of some dissolution studies conducted on theophylline

To-date there is no official dissolution method described in either the USP XXII (72) or the BP 1988 (111) for the dissolution rate testing of theophylline extended release tablet preparations. Due to this fact various methods have been employed to characterise the dissolution properties of theophylline CMRD's. Jonkman *et al*

(156), in a study to develop a simple method, used a modification of the disintegration apparatus of the European Pharmacopœia employing an initial 2 hour acid exposure followed by a phosphate buffer of pH6.8. The results indicated that the dissolution of Theodur, which is a tablet of coated theophylline pellets embedded in a matrix, under these conditions correlated favourably with the *in vivo* data. Furthermore, it was shown that the dissolution of theophylline from Theodur was not dependent on the pH of the system, but that the release from Neulin was pH-dependent.

In an investigation into the effect of pH on the dissolution of Theo-24 which is a capsule containing theophylline coated pellets designed for once a day dosing, Vashi and Meyer (157) found that the release of theophylline from the dosage form was highly pH-dependent. These investigators found that the drug release was more rapid in simulated intestinal fluid than in simulated gastric fluid. These results correlated well with the *in vivo* results from a study conducted using the product in dogs under normal conditions and under conditions of achlorhydria.

In a further dissolution study of Theodur, Jonkman *et al* (158) exposed the dosage form to an acidic medium followed by a phosphate buffer using the compendial paddle apparatus at 75 rpm. The researchers found that a slight dip occurred in the dissolution profile at about 30% release. This they ascribed to the fact that the tablet consists of two compartments, which contain one third and two thirds of the dose respectively. It was also found that the dissolution rate profile correlated well with the *in vivo* results in that the dissolution profile indicated a delayed release of theophylline corresponding to the delayed absorption.

Buckton *et al* (159) conducted dissolution studies on various formulations of theophylline including Theodur. Dissolution studies were carried out under varying conditions of pH. It was found that drug release from the Theodur tablet, in contradiction with previous studies, was pH-dependent with the product showing

a more rapid release in intestinal fluid than in simulated gastric fluid. These authors also simulated the expected *in vivo* response using dissolution data obtained at the various pH's. An *in vivo* study, however, was not carried out using these dosage forms to confirm their predictions.

Certain formulations of theophylline have been shown to be influenced by the presence or absence of food. Maturu *et al* (160) developed a modified dissolution method to simulate *in vivo* fed conditions. The method involves the prior exposure of the dosage form to peanut oil. The results indicated that the release rates were increased, decreased or unchanged, depending on the particular formulation, by the peanut oil pretreatment and that the changes correlated with the *in vivo* absorption rates found in an *in vivo* study. These authors also determined that the dissolution rate from Theodur tablets was pH-independent and would therefore, according to the criteria set out by Skelly *et al* (31), not likely show a food-drug interaction. However, this was not found to be the case with Theodur Sprinkle (a capsule containing theophylline granules intended for paediatric use) which, from dissolution studies, indicated that no food-drug interaction would be expected although this was exhibited *in vivo* (31). Further studies conducted by Aiache *et al* (161) concluded that the peanut oil pretreatment dissolution method provided good correlations with the *in vivo* data for various other formulations of theophylline CMRD's.

The above findings are not in agreement with the results found in studies conducted by El-Arini *et al* (162). Dissolution studies were conducted on Theodur tablets and Theodur Sprinkle capsules to determine the effect of pH, peanut oil in the dissolution medium and the presence of bile salts. The results indicated that the release rate of theophylline from the Theodur tablets was affected by different conditions of pH. The dissolution profiles indicated that the release rate was significantly higher in alkaline media than in acidic media. The addition of the peanut oil and bile salts did not have an effect on the overall dissolution profile.

Theodur Sprinkle showed a lag time upon which dissolution continued at a rate independent of the pH of the dissolution media. The addition of 10% peanut oil to the media ,however, produced a decreased dissolution in media of varying pH. This suggests that in the presence of high fat meals there should be a delayed absorption and a decreased bioavailability of theophylline from Theodur Sprinkle.

In a study to investigate the effect of food on the absorption of theophylline from CMRD's, Macheras *et al* (163) conducted dissolution rate studies on 4 controlled release theophylline dosage forms in milk. It was found that the dissolution of theophylline from Theodur was little affected by the milk media and that overall, the dissolution profiles were the same when compared to dissolution studies carried out in normal dissolution media.

Despite the availability of many different types of CMRD's of theophylline many researchers (164, 165, 166, 167, 168, 169) are still investigating the design of improved types of release mechanisms to retard the release of theophylline from dosage forms. Together with these developments researchers have also attempted to correlate theophylline *in vitro* data with *in vivo* data. To this effect, a comprehensive study was conducted by Dietrich *et al* (164). In their investigations, they found that several successful correlations could be obtained provided that analogous data were correlated, i.e. in order to obtain a good correlation an *in vivo* parameter with a corresponding *in vitro* parameter must be used. They also found that for every comparison that agreed, another was usually found that did not.

## 3.2 Experimental

### 3.2.1 Test Product 1 versus Theodur

#### 3.2.1.1 Statement of the problem

Following a bioavailability study (Study BRI 15/90) (140) conducted on Test Product 1 (a theophylline controlled release formulation) versus Theodur, it was found that the rate of absorption of theophylline from the Test Product was significantly more rapid than the rate calculated for Theodur. These results were in contrast to data obtained from dissolution studies carried out on the test product during the R & D phase. The dissolution studies revealed that the release of theophylline from the Test Product would be very similar to that of the reference formulation.

#### 3.2.1.2 Objectives

In order to find possible explanations for these discrepancies, it was decided to conduct a series of dissolution rate studies on the two formulations in order to establish wether the *in vivo* findings could have been predicted more accurately.

### 3.2.2 Methods

#### 3.2.2.1 Bioavailability Study

The studies were conducted as described in section 2.2.6(ii). Results of the serum concentrations are depicted in Table A3.4 for Theodur and in Table A3.5 (see Appendix A) for the Test Product.

3.2.2.2 Dissolution Studies

Three replicate dissolution rate determinations on each dosage form were carried out.

(i) Dissolution media

Dissolution media were prepared as set out in section 2.2.4.1. Media of pH 3.0, 4.0, 5.0, 6.0, 6.8 and 7.5 were utilised in the various dissolution studies.

(ii) Sampling times

Samples were taken according to the following time schedule: 0.0 (blank), 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0, 12.0 and 24.0 hours after initiation of the dissolution test.

(iii) Sample analysis

All samples were assayed for drug content according to the method set out in section 2.2.5. Analysis of certain studies was also carried out by HPLC as set out in section 2.2.5.2.

3.2.2.3 Data manipulation

Data obtained from the bioavailability studies and dissolution studies were analysed according to the procedures set out in section 2.2.3.

### 3.2.3 Results and Discussion

#### 3.2.3.1 Bioavailability Studies

The serum concentrations of theophylline are presented in Tables A3.4 and A3.5 (Appendix A) for Theodur and the Test Product. The statistical results from these studies are shown in table A3.19 (Appendix A). The mean serum concentration versus time curve is depicted in Figure 3.4.

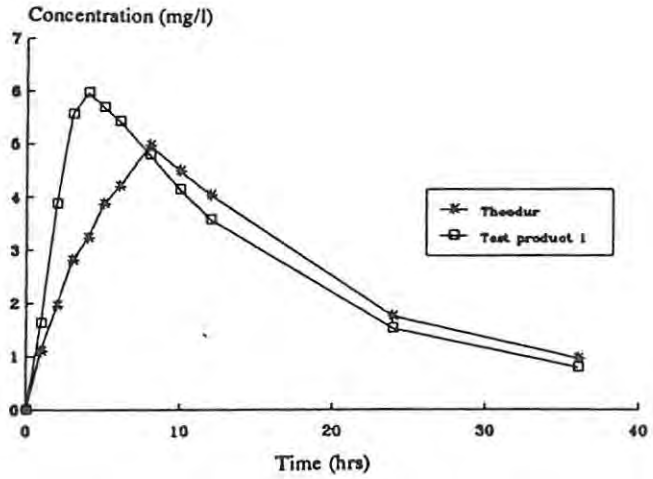



Figure 3.4 Mean serum concentration versus time curve of 10 subjects.

The results indicate that the absorption of theophylline from the Test Product was significantly more rapid than that from Theodur. The mean $t_{max}$ was found to be 4.8h ± 2.3 and 7.5h ± 2.2 (mean ± S.D.) for the Test Product and Theodur respectively. The $C_{max}$ values were determined as 6.29 ± 1.53 mg/l and 5.27 ± 0.84 mg/l for the Test Product and Theodur respectively. The two formulations were, however, found to be equivalent in terms of their extent of absorption. This is clear when the $AUC_{last}$ for the two products are compared (see Table 3.2).

Table 3.2 Mean Bioavailability Parameters.

| Parameter | Test Product 1 | Theodur |
|---|---|---|
| | Mean ± S.D. | Mean ± S.D. |
| $AUC_{last}$ (mg.h/l) | 96.60 ± 20.28 | 93.40 ± 20.69 |
| $AUC_{inf}$ (mg.h/l) | 109.25 ± 25.12 | 109.90 ± 30.15 |
| $C_{max}$ (mg/l) | 6.29 ± 2.30 | 5.27 ± 0.84 |
| $T_{max}$ (hr) | 4.80 ± 2.30 | 7.50 ± 2.20 |

3.2.3.2 Analysis of samples and chromatography

Analysis of dissolution samples by UV spectrophotometric analysis was rapid, accurate and precise. The results of these determinations are given in Tables A3.1 and A3.2 of Appendix A. Sampling with the syringe fitted with the luer lock needle and sample filter enabled rapid, accurate removal of the medium from precisely the same point within the vessel. Since samples of 3ml were removed, this volume was immediately replaced with an equivalent volume of equilibrated fresh medium in order to maintain sink conditions.

Since the results of the UV analysis indicated that dissolution was occurring beyond 100% of label claim, it was decided to analyse the dissolution samples using HPLC. Analysis of samples using this method was found to be rapid and precise. Standard deviations of less than 4.0% were determined and each analysis was completed in under 6 minutes. A guard column was placed in line before the HPLC column to remove any particulate material which may have been injected into the system. Figure 3.5 is representative of the chromatography achieved for theophylline.

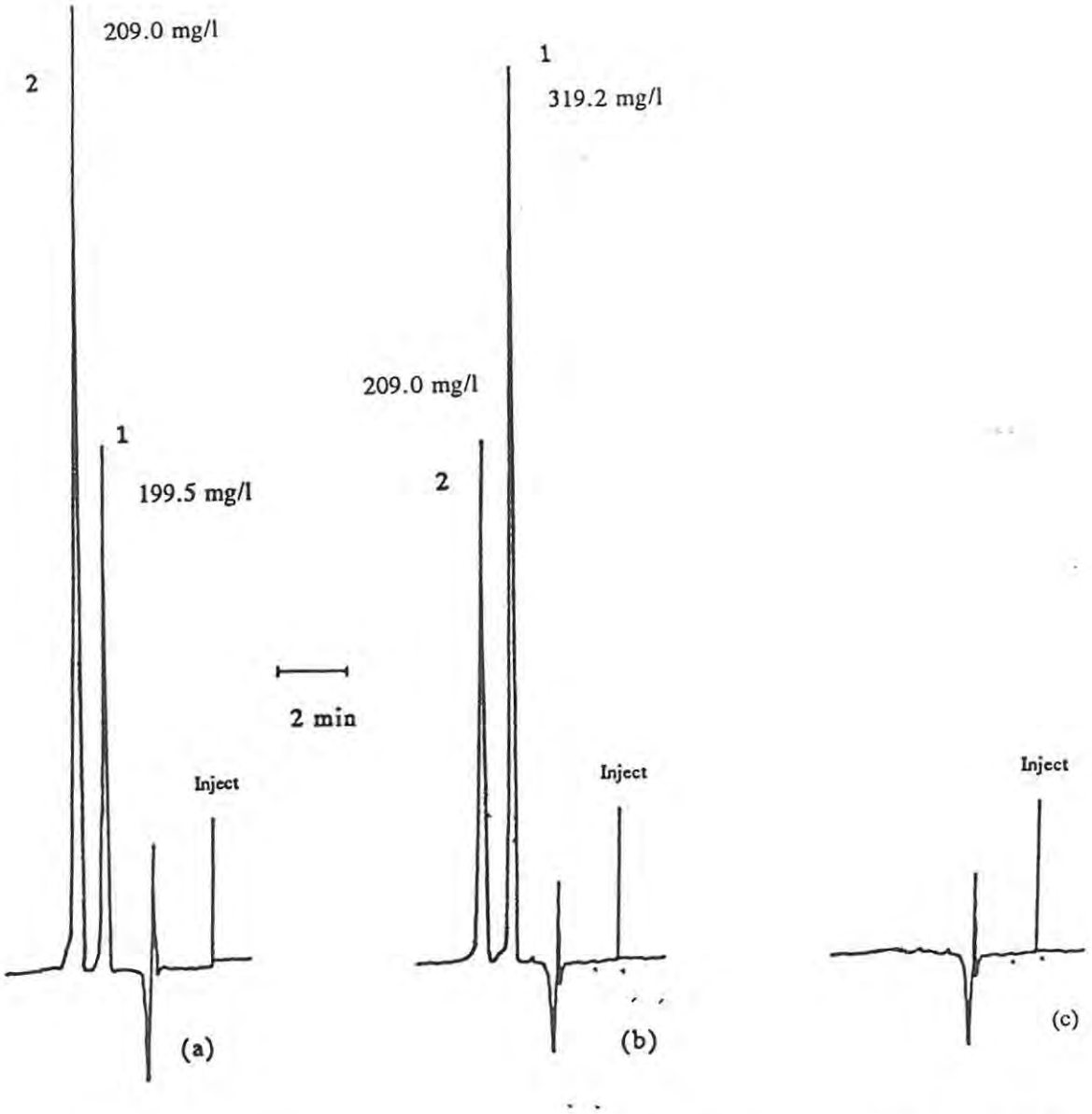



Figure 3.5a, b and c . . Chromatogram of theophylline (1) and caffeine (2) in a calibrator (a) a representative dissolution sample (b) and a blank (c).

Results of the concentration of drug determined by both methods were found to compare very favourably. Differences between the UV and HPLC data differed by no more that 3% for any single sample. Inspection of some of the 24h dissolution samples indicated more than 100% release. In order to determine whether this was

due to any contaminating/interfering species, possibly the excipients of the dosage forms, a series of three dimensional chromatograms were constructed. These are depicted in Figures 3.6 and 3.7. Inspection of these indicate that the three dimensional chromatograms are identical for a sample of pure theophylline powder and a 24hr dissolution sample. The occurrence of release percentages in excess of 100% was therefore not due to any contaminating species in the samples. Furthermore, quality control data obtained from the suppliers indicated that the content of the dosage forms were within the limits of specifications of the label claim.

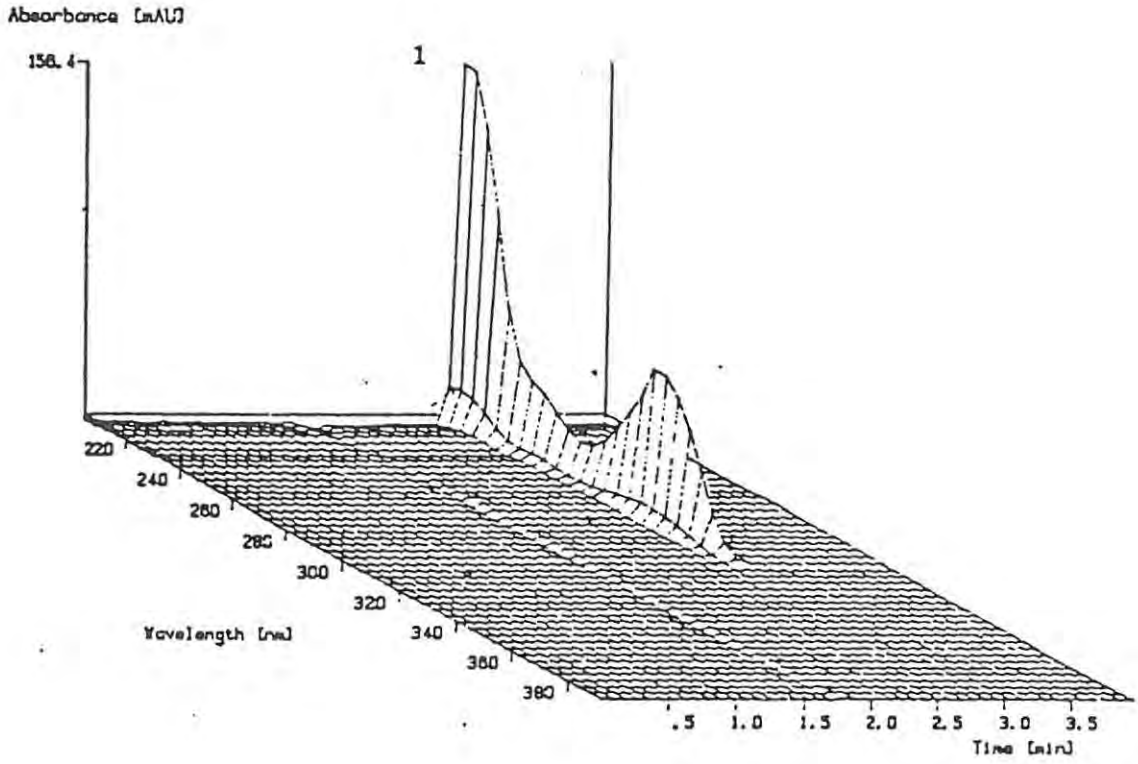



Figure 3.6 Three dimensional chromatogram of theophylline (1) powder.

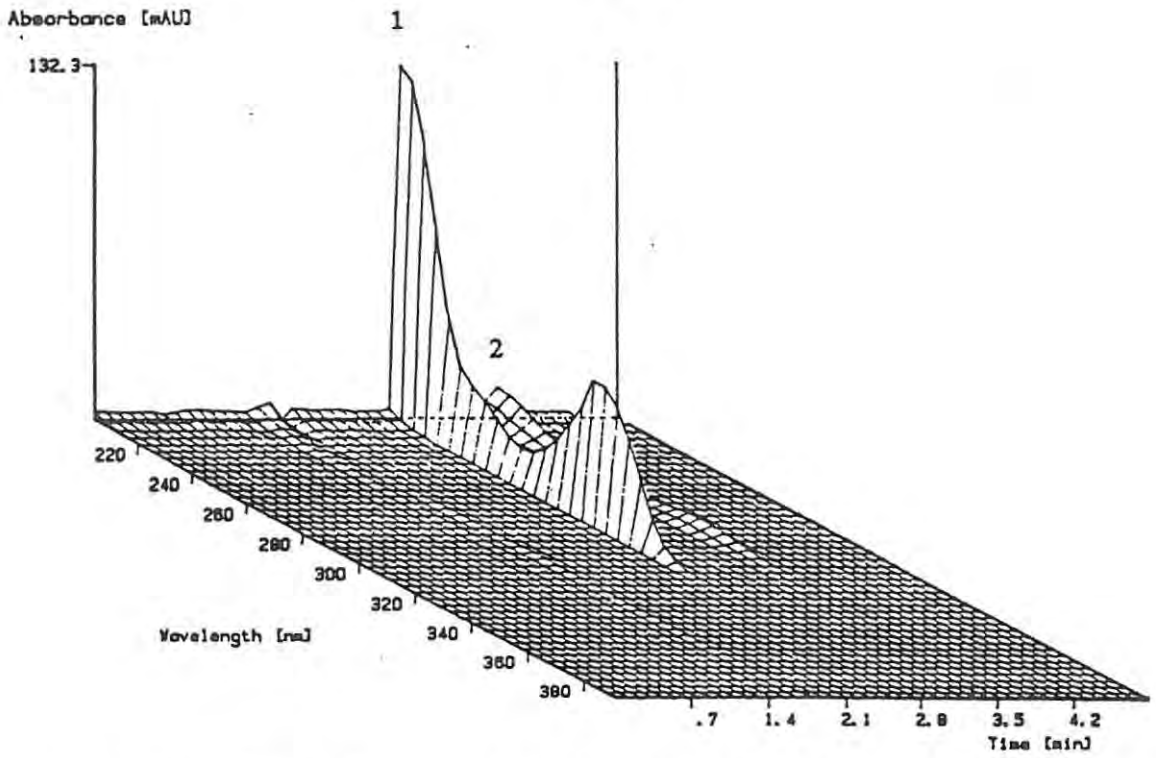



Figure 3.7 Three dimensional chromatogram of a 24 hour dissolution sample containing caffeine (2) as internal standard.

3.2.3.3 Dissolution Rate Studies

Visual inspection of the dissolution process revealed that the Theodur tablets showed a swelling of the tablet matrix after about one hour into the test. The swelling was more pronounced in media above pH 5.0. After 2 to 6 hours the tablets showed varying degrees of disintegration depending on the pH of the medium, with this event occurring earlier at the higher pH's. At the 24 hour sample, the tablet matrix had broken up completely and only beads were still visible in the dissolution medium. The Test Product also exhibited early signs of swelling, with fines breaking free from the matrix. During the course of the tests, the Test Product showed considerable surface erosion but did not disintegrate.

The dissolution of theophylline from Test Product 1 and Theodur are depicted in Figure 3.8a - f.

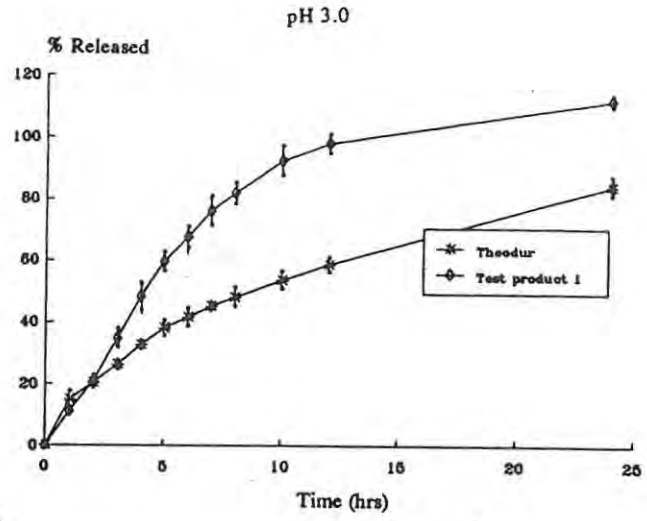



Figure 3.8 (a) Mean dissolution rate profile of Test Product 1 and Theodur.

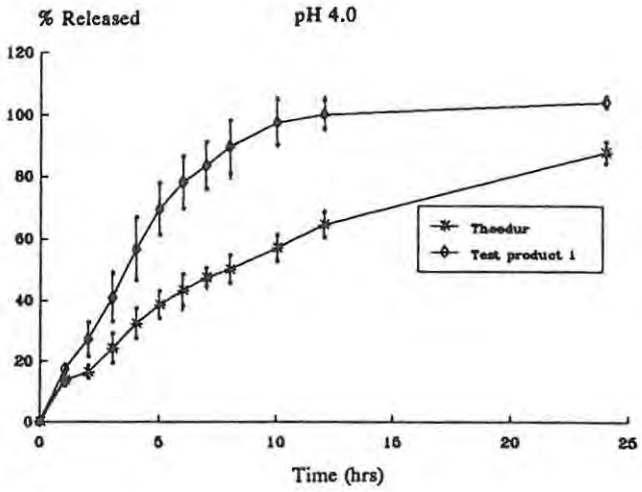



Figure 3.8 (b) Mean dissolution rate profile of Test Product 1 and Theodur.

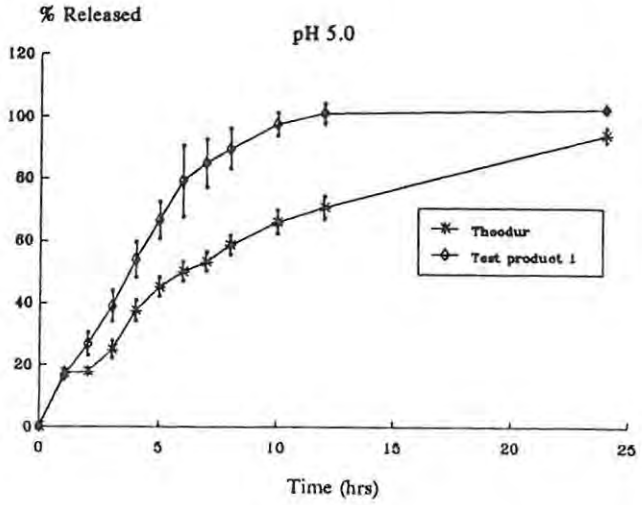



Figure 3.8 (c) Mean dissolution rate profile of Test Product 1 and Theodur.

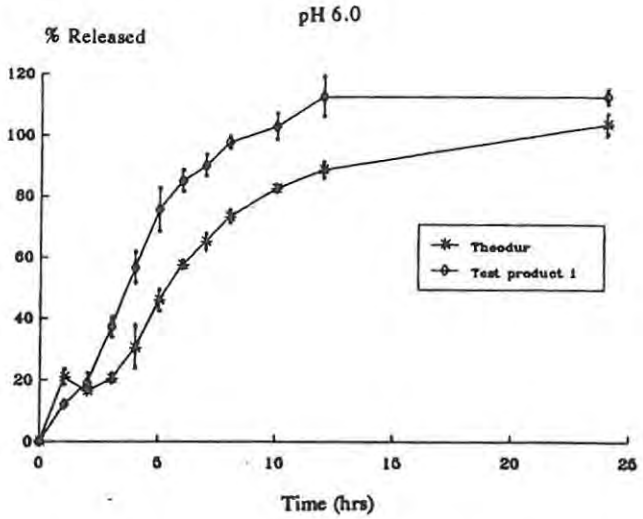



Figure 3.8 (d) Mean dissolution rate profile of Test Product 1 and Theodur.

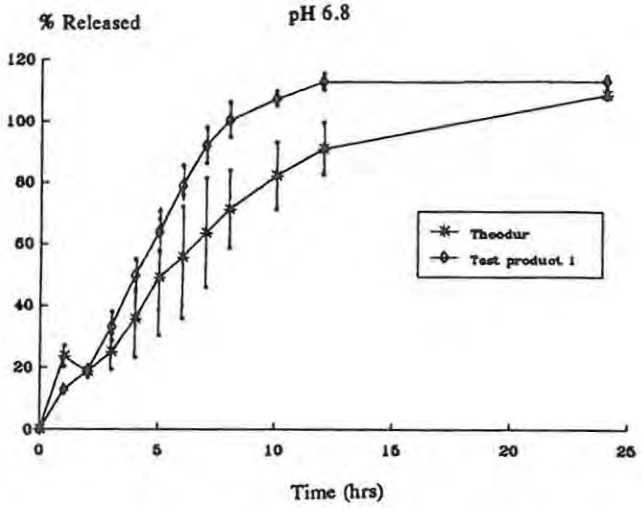



Figure 3.8 (e) Mean dissolution rate profile of Test Product 1 and Theodur.

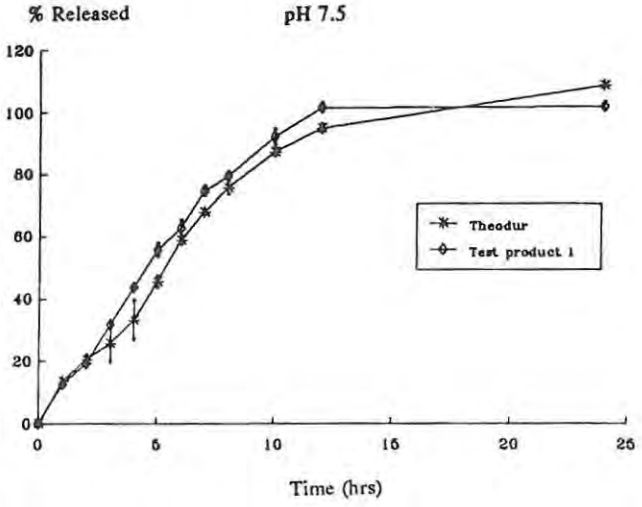



Figure 3.8 (f) Mean dissolution rate profile of Test Product 1 and Theodur.

Inspection of these plots indicate that the rate of release of theophylline from the Test Product is more rapid than that from Theodur at all pH's except pH7.5. It is also evident that the release of theophylline from the Test Product is not affected by the pH of the dissolution medium. This is, however, not the case for Theodur which appears to show some dependency on the pH of the medium and is in agreement with the results found by Buckton *et al* (170). D'Arcy (171) reported that the dose dumping of theophylline from Theo-24, following food intake, is consistent with the increased dissolution which occurs following exposure of the dosage form to alkaline bile salts and/or pancreatic juices. Hendeles and co-workers (172) have reported that Theo-24 showed a dose dumping effect when administered with a meal.

From these plots and plots of log % remaining to be released, the orders of dissolution rate were determined. The rates of the relevant processes were determined by linear regression analysis. The results of these determinations are depicted in Tables 3.3 and 3.4. The goodness-of-fit data are depicted in Table A3.20 (Appendix A).

Table 3.3 Results of dissolution rate order determinations.

| pH | Test Product 1 | Theodur |
|---|---|---|
| | Rate Order | Rate Order |
| 3.0 | Zero order for 6 hours followed by first order or two first order fractions | 20% instant release fraction with a single first order fraction equivalent to 80% of the dose |
| 4.0 | Zero order for 5 hours followed by a first order fraction | 15% instant fraction with a single first order fraction |
| 5.0 | Zero order for 6 hours followed by a first order fraction | 18% instant release fraction with a single first order fraction |
| 6.0 | Zero order for 5 hours followed by a first order fraction | 15% instant release fraction with a single first order fraction |
| 6.8 | Zero order release only | 20% instant release with zero order release for 8 hours followed by a first order fraction |
| 7.5 | Zero order for 6 hours followed by a first order fraction | 10% instant release fraction with zero order release for 8 hours followed by first order fraction |

Table 3.4 depicts the various calculated values for the relevant rate constants determined at each pH.

Table 3.4 Summary of dissolution rates for the Test Product 1 and Theodur.

| Dissolution pH | Product | |
|---|---|---|
| | Test Product 1 | Theodur |
| 3.0 | $K_0 = 35.43mg/h$<br>$K_d = 0.56h^{-1}$<br>or<br>$K_f = 0.38h^{-1}$<br>$K_s = 0.24h^{-1}$ | $K_s = 0.07h^{-1}$ |
| 4.0 | $K_0 = 42.96mg/h$<br>$K_d = 0.32h^{-1}$ | $K_s = 0.09h^{-1}$ |
| 5.0 | $K_0 = 39.69mg/h$<br>$K_d = 0.35h^{-1}$ | $K_s = 0.12h^{-1}$ |
| 6.0 | $K_0 = 56.10mg/h$<br>$K_d = 0.45h^{-1}$ | $K_s = 0.22h^{-1}$ |
| 6.8 | $K_0 = 41.79mg/h$ | $K_0 = 31.58mg/h$<br>$K_d = 0.38h^{-1}$ |
| 7.5 | $K_0 = 33.15mg/h$<br>$K_d = 0.36h^{-1}$ | $K_0 = 29.46mg/h$<br>$K_d = 0.39h^{-1}$ |

where: $K_0$ = zero order release rate constant

$K_d$ = first order release rate constant after zero order release

$K_s$ = rate constant of slow fraction

$K_f$ = rate constant of fast fraction

As is apparent from the above two tables, the dissolution rate order is pH dependent. This is of significance as a CMRD is exposed to various pH's as it traverses the gastrointestinal tract. Since the solubility of theophylline is independent of pH, the rate limiting step of theophylline release from the matrix will be the interaction of the tablet matrix with the environment in which it is found. This seems to be the case for the Theodur matrix more so than for the Test Product matrix. It was found that the dissolution rate profile for Test Product 1 suggested

the existence of two possible release mechanisms, namely a zero order fraction followed by a first order fraction or two first order fractions.

### 3.2.3.4 3-D Dissolution profiles

As previously mentioned, the concept of presenting dissolution data in the form of three dimensional plots was first introduced by Skelly and coworkers (36, 37). By definition, CMRD's are designed to release their drug content over an extended period of time and will therefore be exposed to a varying milieu of pH's as they move through the GI tract. The pH range that such a dosage form will encounter varies from pH 1.0 in the fasting stomach, through pH 4 - 5 in the duodenum to about pH 7 - 8 in the intestinal tract. The basic assumption for all *in vitro - in vivo* correlations is that the rate limiting step in drug absorption is the availability of the drug at the absorption site. Dissolution studies carried out at a single pH will therefore be representative of the *in vivo* dissolution only at a particular point in the GI tract. Drugs may show regional differences in absorption which is dependent on pH and the environment, particularly those compounds which show marked changes in solubility in the pH range experienced in the gut (136). Dissolution rate studies carried out over a range of pH should therefore provide more information about the dissolution characteristics of a dosage form under varying conditions. Three dimensional dissolution rate profiles of the Test Product and Theodur are depicted in Figures 3.9 and 3.10.

Inspection of Figure 3.9 indicates that the dissolution contour of the Test Product is fairly uniform throughout the pH range tested. The dissolution rate is slightly higher at the higher pH's. Particular note must be made of the individual profiles at the lower pH's. In this region it appears that the release of theophylline is not influenced by the pH of the medium. It is therefore expected that *in vivo*, the release of theophylline from the Test Product will not be adversely affected by the

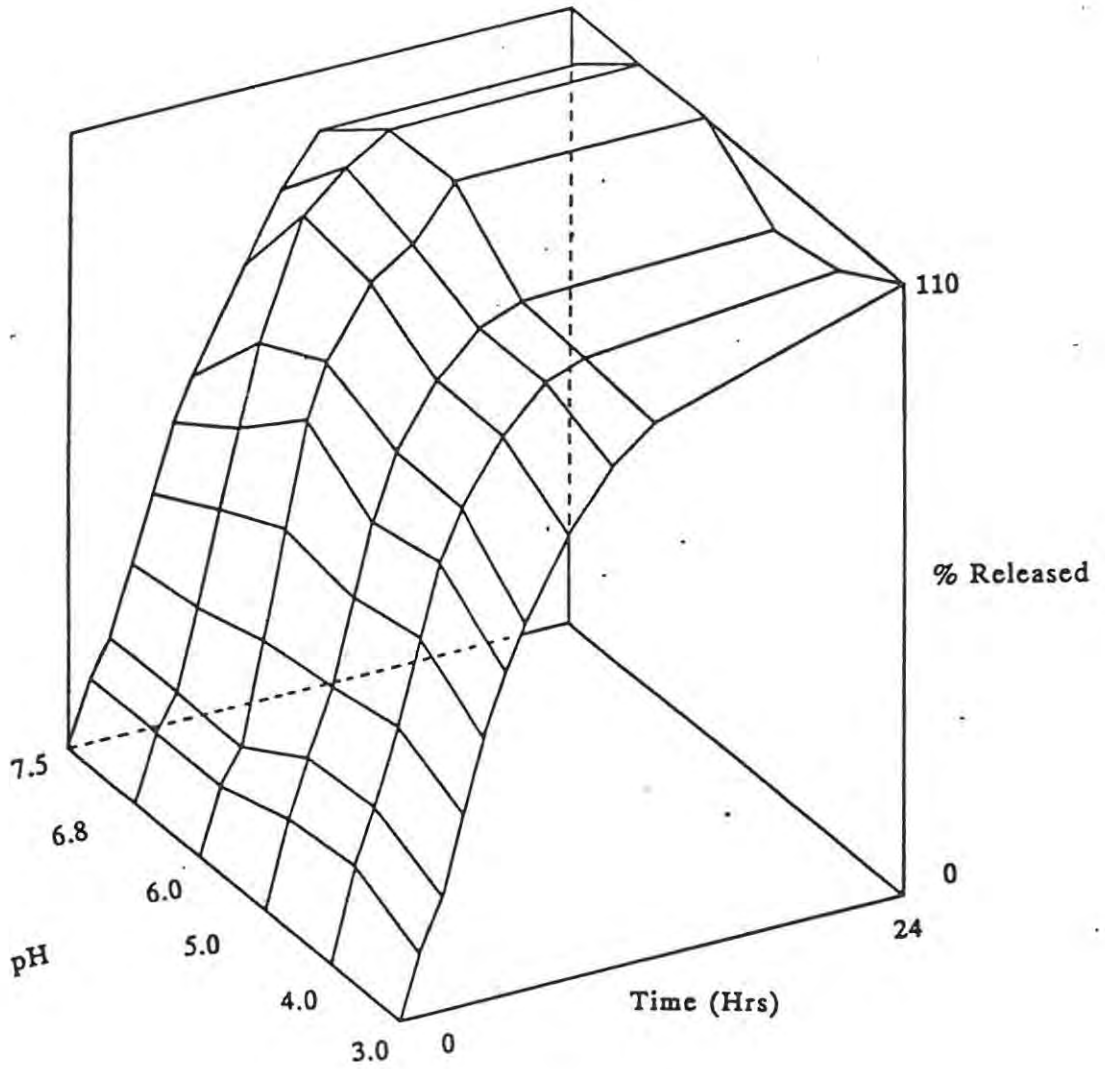



Figure 3.9 3-D dissolution profile of Test Product 1.

low pH's of the stomach and duodenum. Inspection of Figure 3.10, however indicates that the release of theophylline from Theodur is retarded at pH's below 6.0. It can therefore, be expected that the *in vivo* release of theophylline may be delayed in the regions of the GI tract where the pH is below 6.0.

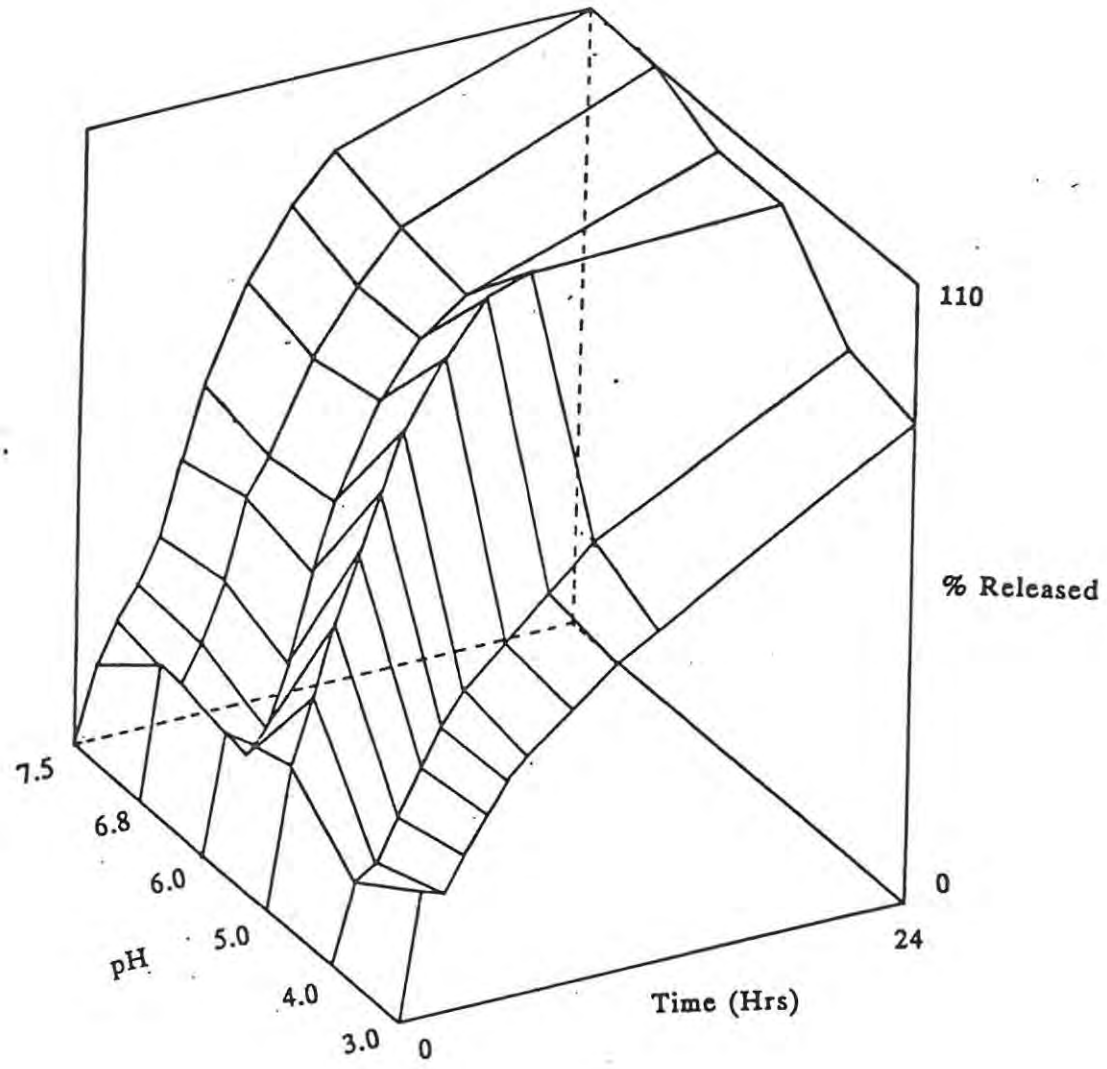



Figure 3.10 3-D dissolution profile of Theodur.

These observations are significant when the *in vivo* serum concentration profiles are considered, from which it is apparent that the absorption of theophylline from the Test Product is more rapid than from Theodur. This can possibly be explained by the above, i.e. more theophylline is available for absorption from the Test Product at the absorption sites and therefore a more rapid $C_{max}$ is attained.

above, i.e. more theophylline is available for absorption from the Test Product at the absorption sites and therefore a more rapid $C_{max}$ is attained.

The results obtained using the 3D data presentation technique are , however, in contrast to those found by Skelly (31). In a dissolution study of Theodur it was found that the dissolution of theophylline from Theodur was not affected by the pH of the dissolution medium. It should, however, be noted that the authors do not report the test conditions employed in their studies. Also, using this technique of three dimensional visualisation of the dissolution profiles, Skelly was able to identify a product which had exhibited dose dumping when administered under fed conditions.

3.2.3.5 Simulation of Serum Profiles (Type A Correlation)

This type of correlation can be regarded as a Type A correlation since the entire serum profile is generated using *in vitro* and *in vivo* data. Simulations for the Test Product and Theodur were obtained using the parameters as set out in Tables 3.3 and 3.4.

(i) Test Product 1

Predicted serum concentration data are given in Table A3.8 (Appendix A). The predicted profiles are depicted in Figure 3.11a - g. The plot of Test Product 1 describes the actual *in vivo* profile obtained following the administration of a single 300mg tablet of the formulation. Table 3.5 is a summary of the pharmacokinetic parameters used for the simulations and are based on the population parameters for theophylline (153). Literature values were used since an intravenous study was not performed to obtain the volume of distribition ($V_d$) nor was a study performed following the administration of an immediate release product (eg. a solution) to obtain the necessary $k_a$.The equations as set out in Models A, B and C (section 2.1.4.2) were used to perform the simulations.

Table 3.5 Pharmacokinetic parameters of theophylline employed in simulations.

| Parameter | Value |
|---|---|
| $k_e$ | 0.087 $h^{-1}$ |
| $k_a$ | 1.20 $h^{-1}$ |
| $V_d$ | 0.50 l/kg |

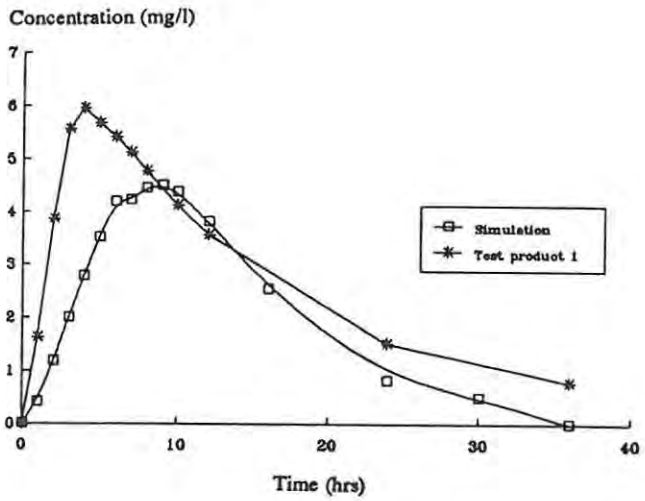



Figure 3.11 (a) Simulated profile at pH 3.0 using zero order followed by first order release fraction.

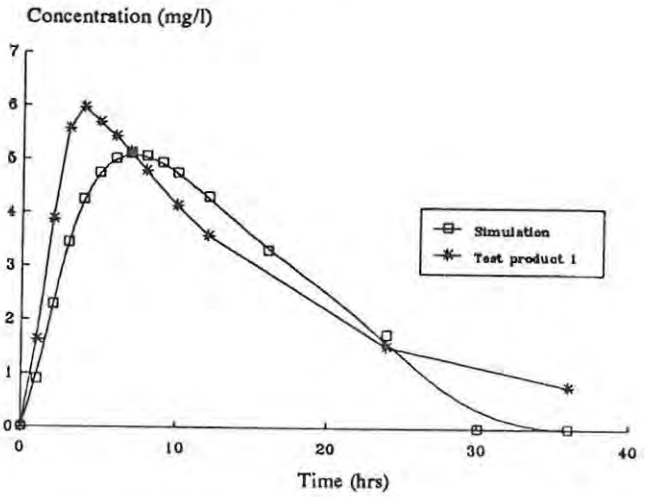



Figure 3.11 (b) Simulated profile at pH 3.0 using two first order release fractions.

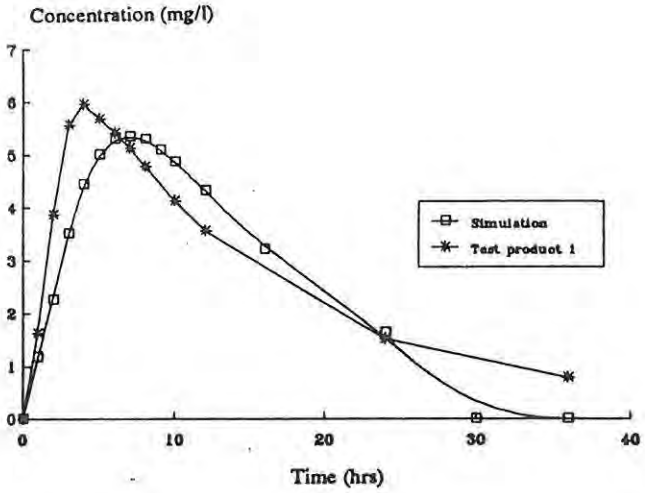



Figure 3.11 (c) Simulated profile at pH 4.0.

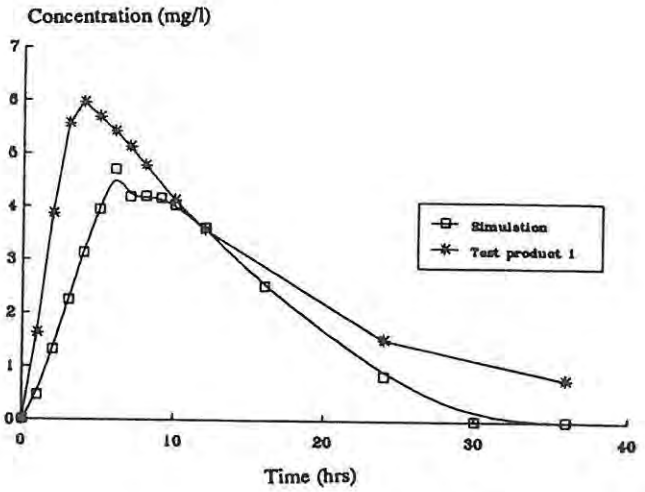



Figure 3.11 (d) Simulated profile at pH 5.0.

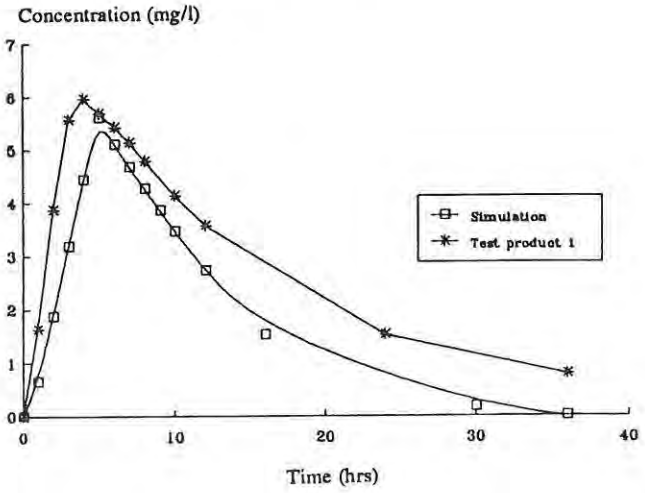



Figure 3.11 (e) Simulated profile at pH 6.0.

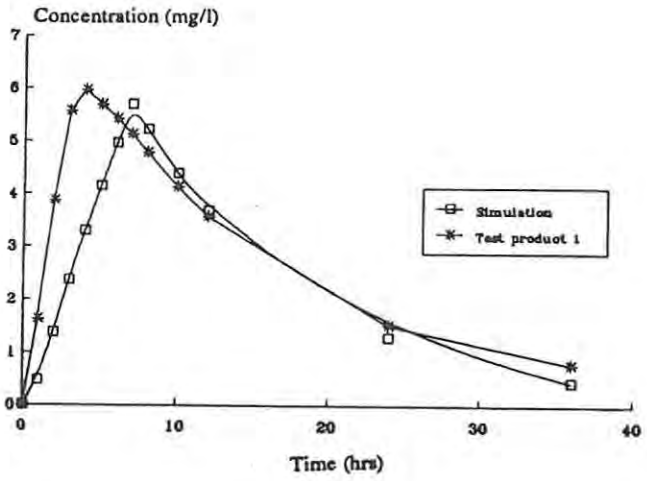



Figure 3.11 (f) Simulated profile at pH 6.8.

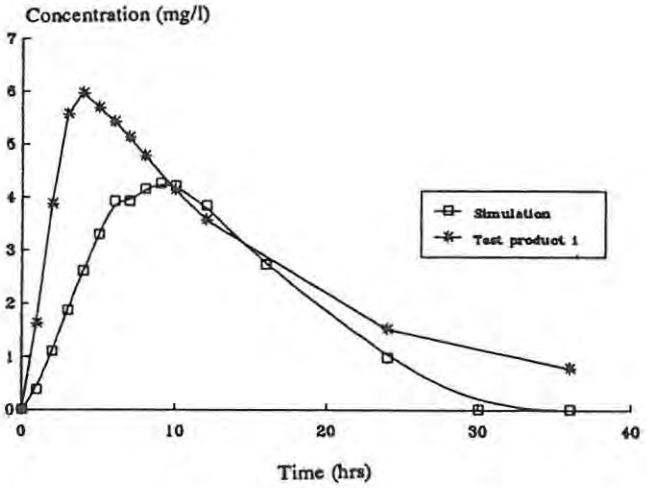



Figure 3.11 (g) Simulated profile at pH 7.5.

For pH 3.0, two possible profiles are depicted which are based on the possibility that the dissolution profile could be interpreted in two ways, i.e. zero order followed by first order or two first order release fractions. Inspection of the profiles reveal that the predictions from dissolution data obtained at pH 4.0, 6.0 and 6.8 approximate the actual *in vivo* profiles obtained for the Test Product. The simulation obtained at pH 6.0 seems to be particularly similar to the actual experimental profile, with both showing similar $t_{max}$ values of 5 and 4 hours for the observed and predicted profiles respectively. It appears, therefore that a simulation based on dissolution data obtained at pH 6.0 adequately predicts the expected

serum concentration-time course.

(ii) Theodur

Similar simulations were performed to determine the expected serum concentration-time profiles following the administration of a single 300mg Theodur tablet. The results of the simulations are reported in Table A3.7 (Appendix A) and depicted in Figures 3.12 a - f.

The simulations obtained from dissolution data at the lower pH's i.e. pH 3.0, 4.0 and 5.0, did not provide accurate predictions. In each of these cases the predicted profiles showed noticeably lower values for $C_{max}$ compared to the values obtained for the experimental data. However, the predicted values for $t_{max}$ compared very favourably with the actual value. Simulations obtained for pH 6.0, 6.8 and 7.5 closely followed the actual profiles obtained. The profile of particular interest, is that obtained at pH 6.0. It is clear that the simulated profile is almost entirely superimposable onto the actual profile. The $C_{max}$ values are 4.96 and 4.77 mg/l for the actual and simulated profile respectively with the $t_{max}$ value for both being 8 hours.

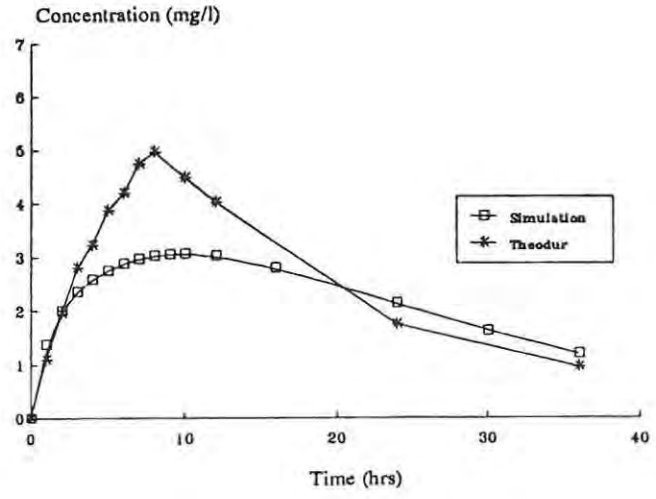



Figure 3.12 (a) Simulated profile at pH 3.0.

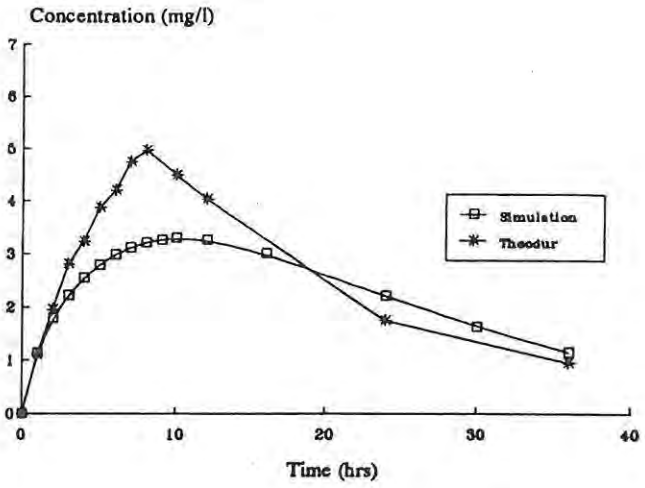



Figure 3.12 (b) Simulated profile at pH 4.0.

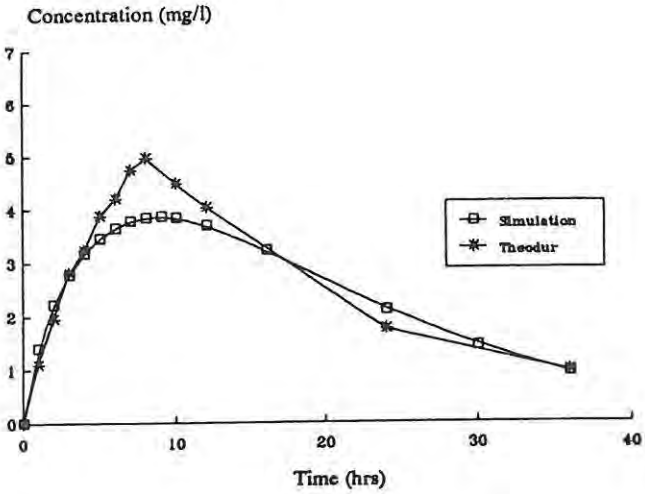



Figure 3.12 (c) Simulated profile at pH 5.0.

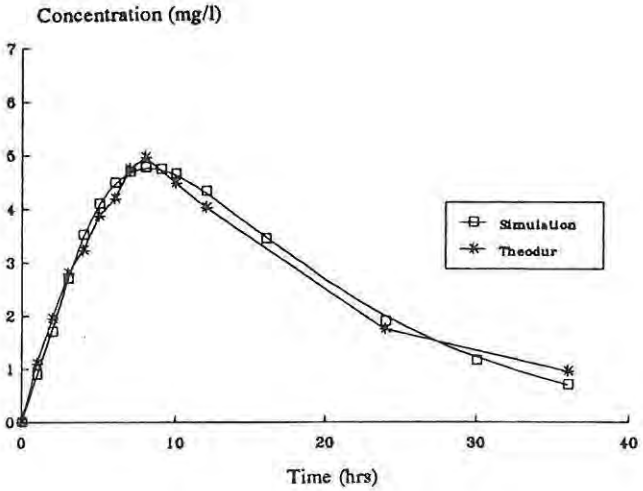



Figure 3.12 (d) Simulated profile at pH 6.0.

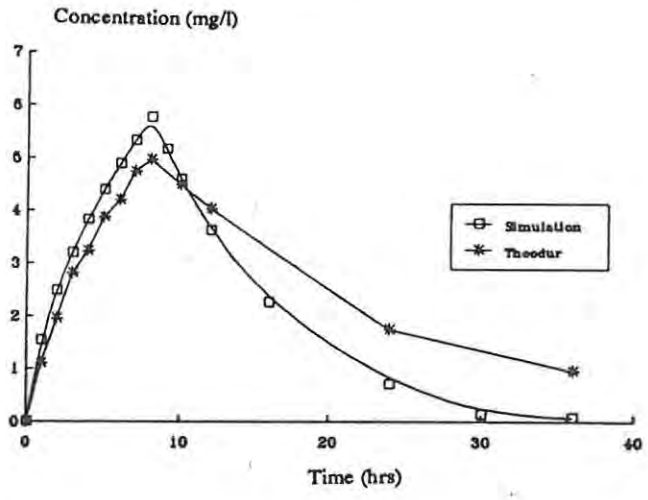



Figure 3.12 (e) Simulated profile at pH 6.8.

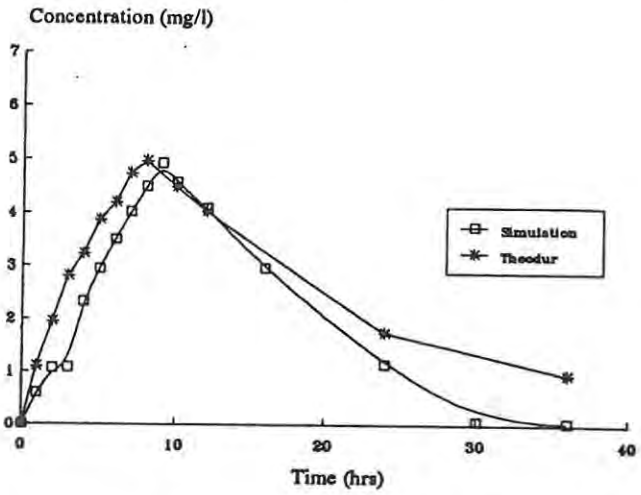



Figure 3.12 (f) Simulated profile at pH 7.5.

3.2.3.6 Weibull Distribution Analysis

(i) Analysis of dissolution data

The observed dissolution rate data and the predicted values calculated according to the Weibull equation are depicted in Figures 3.13 and 3.14 for the Test Product and Theodur respectively. The Weibull function proved to be a robust and versatile

function as it enabled the description of all the dissolution profiles studied. In all cases the correlation coefficients were greater that 0.99.

The detailed parameter values from the Weibull function analysis of the two products over the range of pH are summarised in Table 3.6 and 3.7.

Table 3.6 Weibull function analysis of dissolution data for Test Product 1.

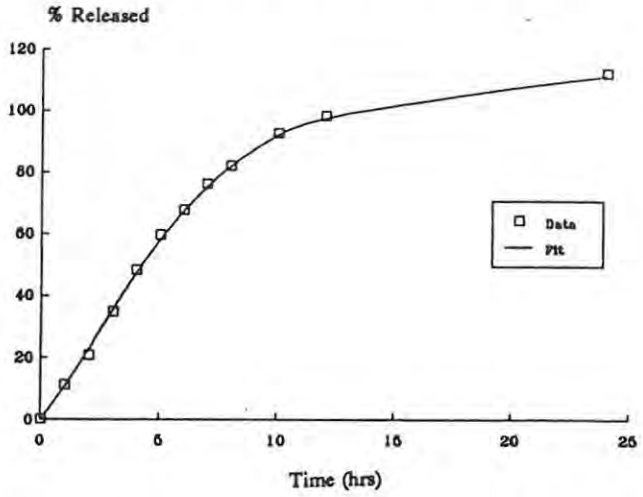
| Parameter | Dissolution pH | | | | | |
|---|---|---|---|---|---|---|
| | 3.0 | 4.0 | 5.0 | 6.0 | 6.8 | 7.5 |
| $t_0$ (h) | 0.07 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $t_d$ (h) | 6.30 | 4.81 | 4.85 | 4.96 | 5.40 | 6.09 |
| $\beta$ | 1.25 | 1.30 | 1.38 | 1.63 | 1.75 | 1.44 |
| $F^{\infty}$ (%) | 111.50 | 104.36 | 103.69 | 111.35 | 113.50 | 104.78 |

Table 3.7 Weibull function analysis of dissolution data for Theodur.

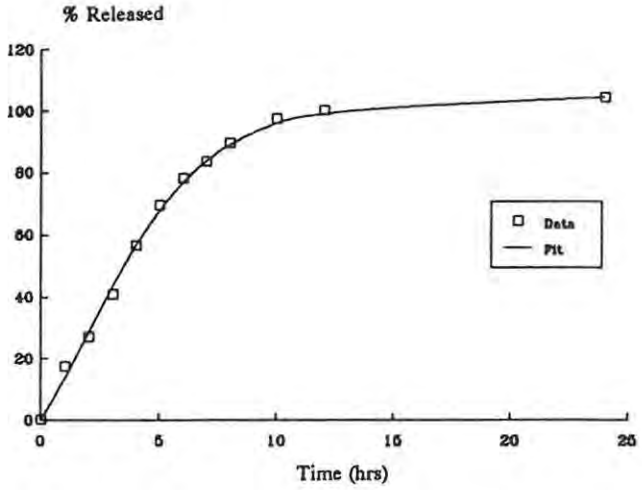
| Parameter | Dissolution pH | | | | | |
|---|---|---|---|---|---|---|
| | 3.0 | 4.0 | 5.0 | 6.0 | 6.8 | 7.5 |
| $t_0$ (h) | 0.00 | 0.56 | 0.88 | 0.34 | 0.00 | 0.00 |
| $t_d$ (h) | 15.49 | 13.64 | 9.62 | 6.78 | 8.31 | 7.31 |
| $\beta$ | 0.80 | 0.80 | 0.80 | 1.44 | 1.19 | 1.46 |
| $F^{\infty}$ (%) | 109.37 | 111.10 | 107.62 | 102.56 | 118.10 | 109.30 |

The lag time, $t_0$, only occurred for the Theodur formulation at pH 4.0, 5.0 and 6.0. No lag time was, however, evident at pH 3.0. All other dissolution profiles did not exhibit a lag time. The mean dissolution time, $t_d$, was slower for the Theodur formulation at all pH's studied when compared to the values obtained for the Test Product. These findings are in agreement with the *in vivo* findings of the Test Product exhibiting a faster absorption rate.

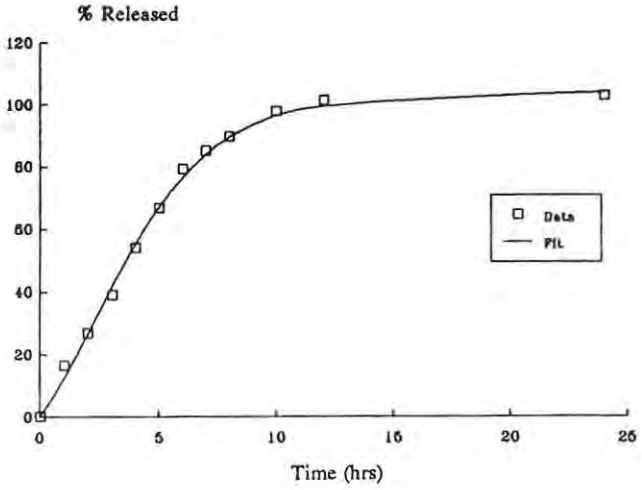
Figure 3.13 Dissolution rate profiles and Weibull fits for Test Product 1.



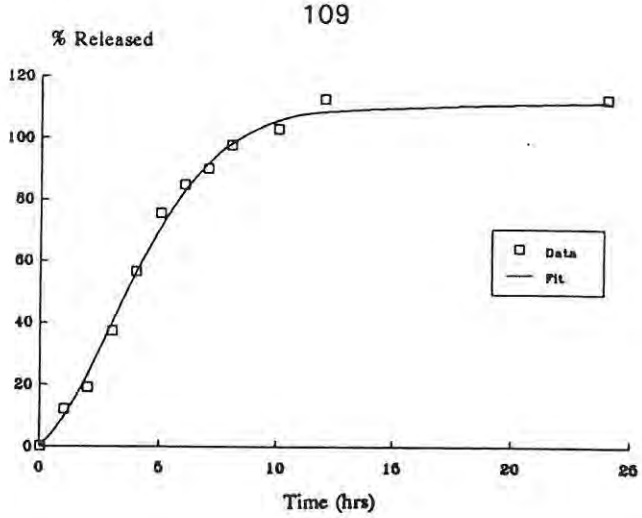

(a) pH 3.0



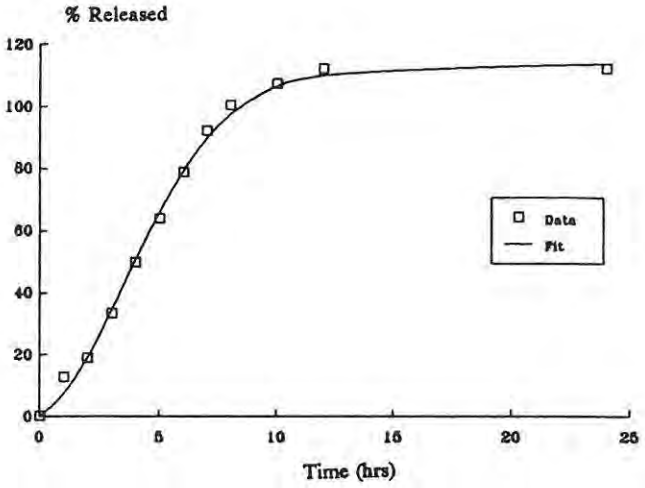

(b) pH 4.0



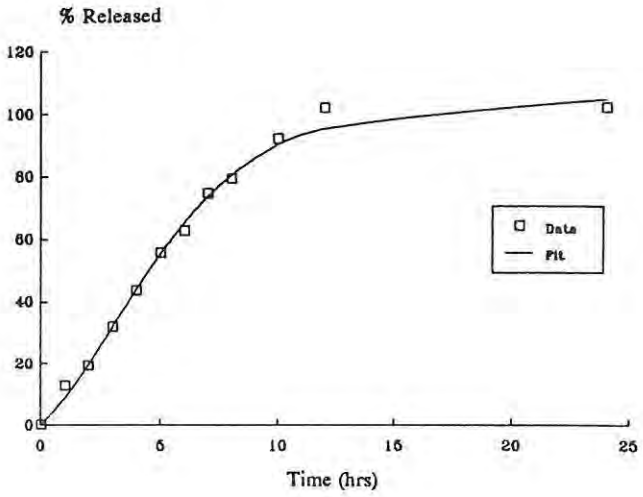

(c) pH 5.0




(d) pH 6.0

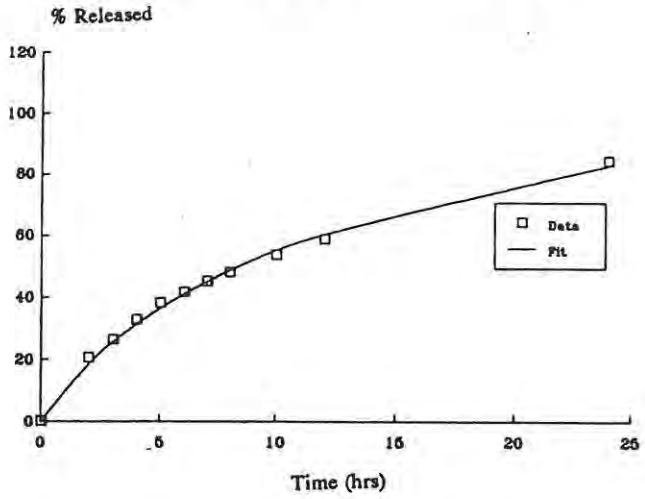



(e) pH 6.8

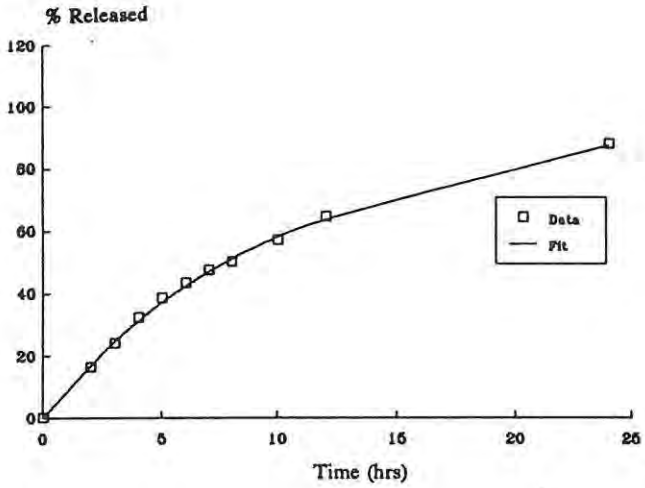



(f) pH 7.5

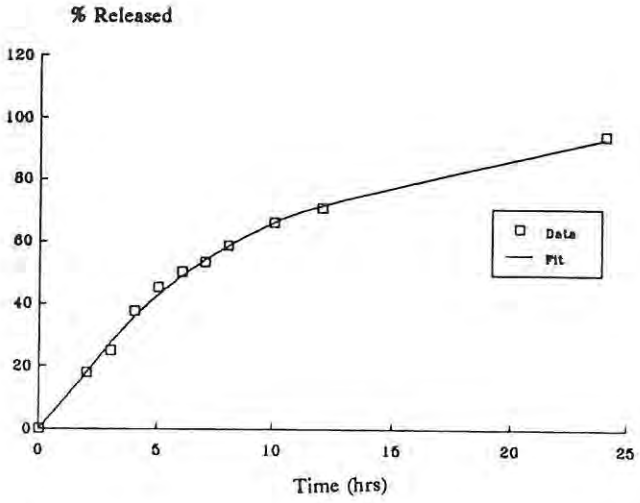
Figure 3.14 Dissolution rate profiles and Weibull fits for Theodur.



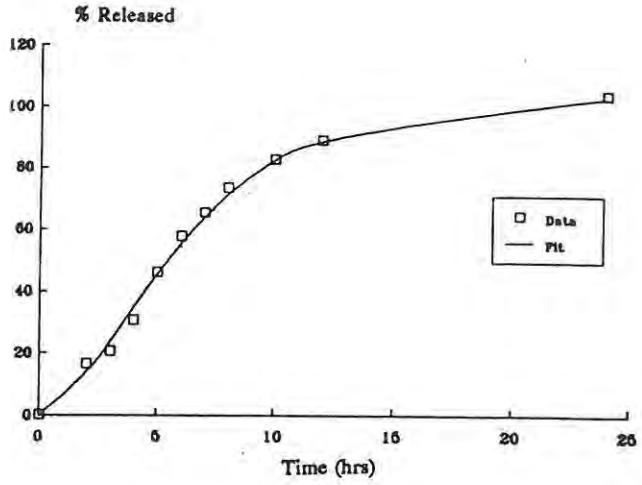

(a) pH 3.0



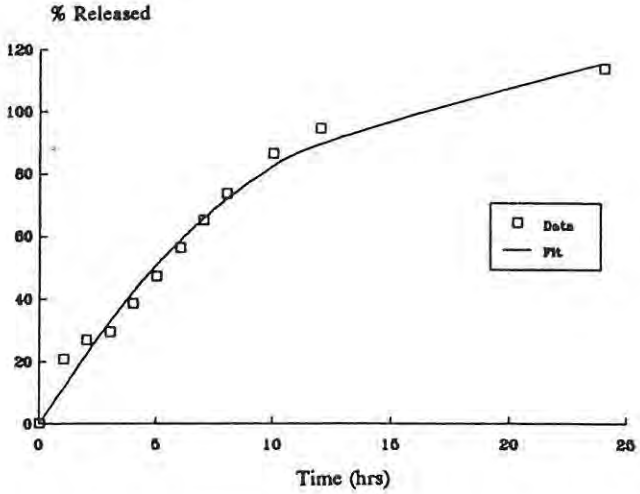

(b) pH 4.0



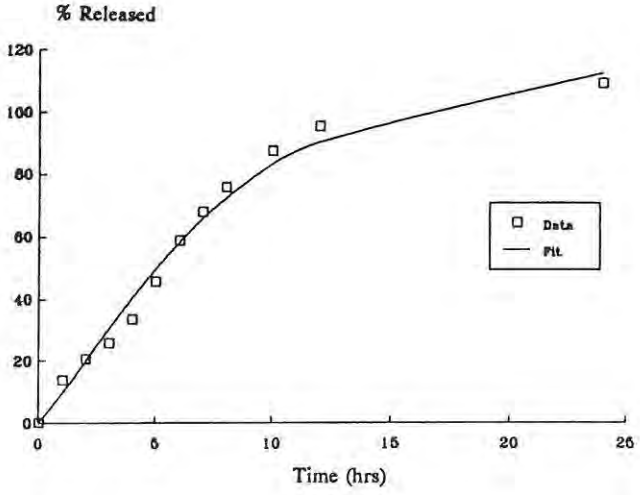

(c) pH 5.0




(d) pH 6.0




(e) pH 6.8




(f) pH 7.5

The $F^{\infty}$ values give some indication of the total amount of drug dissolved. In all cases this was above 100%. The values for the $\beta$ exponent are concordant with the slopes and shapes of the curves. All the curves, except those of Theodur at pH 3.0 - 5.0, take on a sigmoidal shape corresponding to $\beta$ values greater than 1.

(ii) Weibull Fits to Wagner-Nelson Absorption plots

The serum concentration-time data from the Test Product and Theodur were transformed using the Wagner-Nelson method assuming a one compartment model and linear kinetics to obtain the absorption rate plots. These were subsequently fitted to the Weibull equation. The plots for the mean profiles (n = 10) can be seen in Figures 3.15 a - b.

The Weibull function appeared to be robust enough to enable a good description of the absorption rate data, albeit with larger values for the least squares summation of the estimates than for the *in vitro* dissolution rate data. The Weibull parameters obtained for the analysis of the data are shown in Tables A3.13 and A3.14 (Appendix A) and a summary is given in Table 3.8.

The mean profile data were obtained from a plot representing the mean profile obtained from ten subjects. This profile was then fitted to the Weibull equation and the mean parameters depicted accordingly. The mean of the individual parameters represents the mean obtained from each individual value relating to individual profiles fitted to the Weibull equation.

Table 3.8 Weibull function analysis of absorption data for Test Product 1 and Theodur.

| Parameter | Test Product 1 | | Theodur | |
|---|---|---|---|---|
| | Mean Profile Data | Mean of Individual Parameter | Mean Profile Data | Mean of Individual Parameter |
| $t_0$ (h) | 0.40 | 0.25 | 0.00 | 0.15 |
| $t_d$ (h) | 1.63 | 2.19 | 4.48 | 4.48 |
| $\beta$ | 1.35 | 1.75 | 2.32 | 1.32 |
| $F^\infty$ (%) | 101.80 | 103.00 | 103.07 | 104.32 |

The time for 63.2% of the drug to be absorbed *in vivo*, $t_d$, was markedly less for the Test Product. This is in accordance with the $t_d$ values for the *in vitro* dissolution. All the curves had a $\beta$ value greater than 1 and accordingly all showed apparent sigmoidicity. The infinity value, $F^\infty$, obtained from the analysis of absorption rate plots, is not a true estimate of the bioavailability since it is expressed relative to the AUC for that preparation. The values for both products, however, were found to be close to 100%.

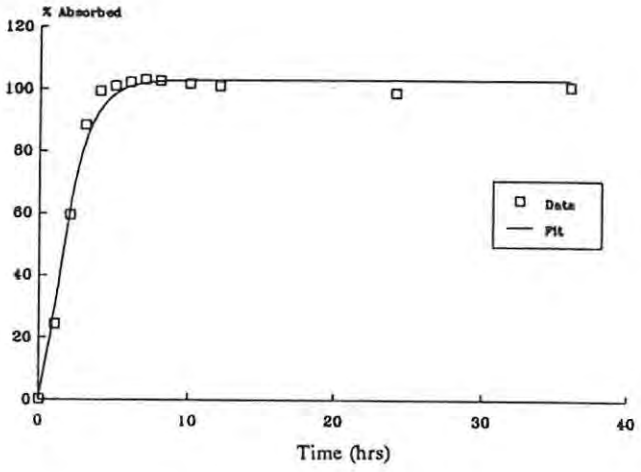



Figure 3.15 (a) Mean Wagner-Nelson plot of serum concentration-time curve after administration of Test Product 1 to ten volunteers. The solid line represents the Weibull fit to the data.

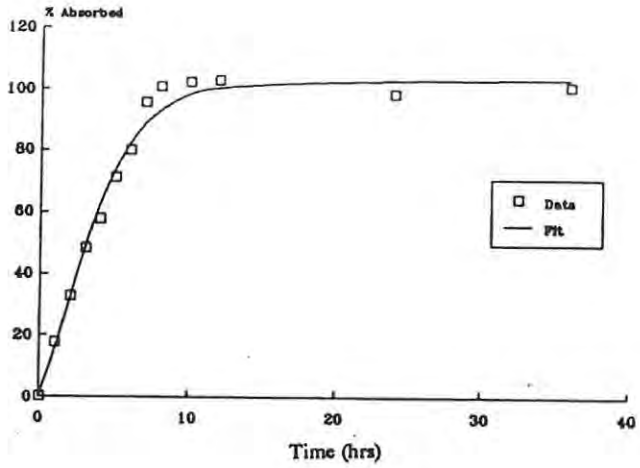



Figure 3.15 (b) Mean Wagner-Nelson plot of serum concentration-time curve after administration of Theodur to ten volunteers. The solid line represents the Weibull fit to the data.

Riegelman and Upton (136) compared the plots of the Weibull fit to the mean absorption data of a theophylline preparation, with the curve generated from the mean parameters obtained from 5 individual curves. The latter curve was found to deviate above 60% absorption and displayed a more rapid apparent absorption than is seen in the curve derived from the mean of individual absorption data. The mean absorption or dissolution time for processes which may be described by the function occurs when 63,2% of the process is complete. However, distribution describing complex CMRD's may exhibit a mean time intermediate between 50 and 63.2%, with a zero order process having a mean of 50%. The above authors evaluated correlations between *in vivo* and *in vitro* lag times and the mean absorption or dissolution times. It was found that correlations from parameters of individually fitted data were superior to those correlations obtained using fits to mean data. This is a phenomena common to all averaged *in vivo* and *in vitro* data and therefore caution must be exercised in interpreting mean data, i.e. individual data should preferably be used.

Erni and Eckert (173) correlated the Weibull function of the *in vivo* and *in vitro* data over the whole time period. It was shown that for a particular dosage form of bufuralol, the time required to dissolve or absorb 63.2% of the drug was the most accurate figure for establishing a correlation. Following linear regression of the data it was established that the regression line intercepted the data points at a point corresponding to 63.2% *in vitro* and *in vivo* (Figure 3.16). Similar curves were plotted using the Weibull fits to the *in vivo* and *in vitro* data from Test Product 1 and Theodur obtained at pH 6.0 (Figures 3.17 a and b).

Inspection of the plots (Figures 3.17 a and b) indicate that the current data were not in agreement with the above findings of Erni and Eckert (173) as the curves from the correlated data did not cross the straight line at 63% for either the *in vivo* or *in vitro* data. The $t_d$ values do not give a good indication of the correlation of the data obtained in the present study.

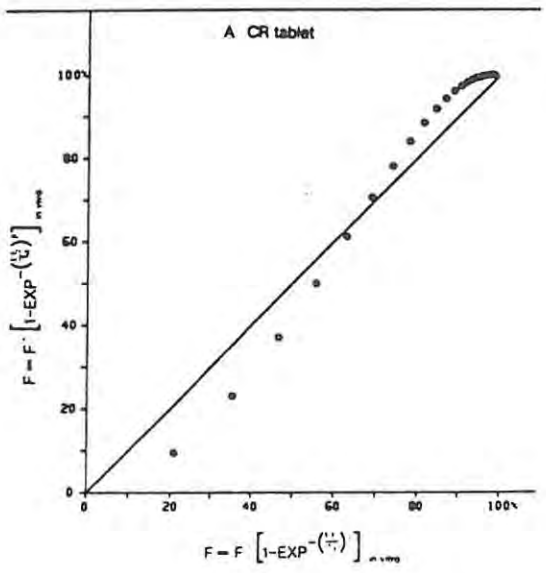



Figure 3.16 Correlation of Weibull function for *in vitro* and *in vivo* data. Reproduced from Erni and Eckert (173).

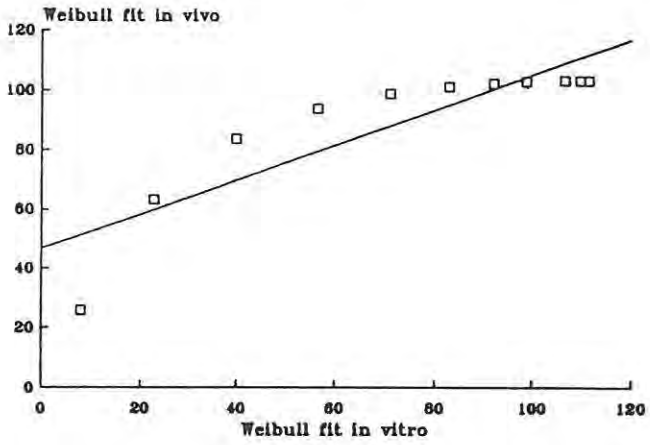



Figure 3.17 (a) Correlation of Weibull function for *in vitro* and *in vivo* data for Test Product 1.

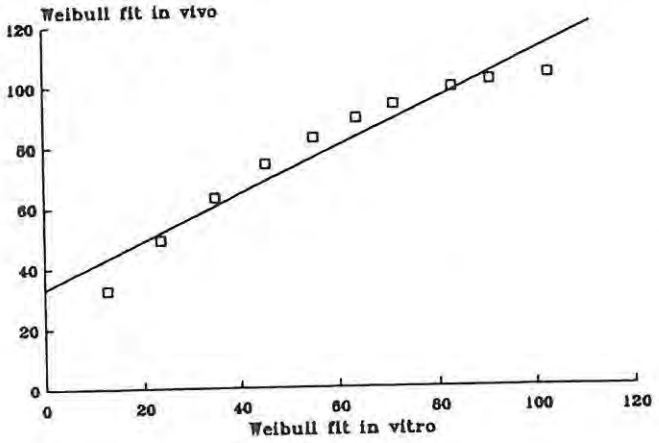



Figure 3.17 (b) Correlation of Weibull function for *in vitro* and *in vivo* data for Theodur.

3.2.3.7 Single point correlations

(i) Moment Analysis

Results of the moment analysis of the data for Test Product 1 and Theodur are summarised in Tables A3.16 and A3.17 (Appendix A). A summary of the parameters

is given in Table 3.9 below.

An indication of the efficiency of the sustained release preparations is usually seen when values for MRT are long. In almost all cases the MRT was longer for Theodur than for the Test Product. The MAT is considerably shorter for the Test Product than for Theodur, and is in agreement with the shorter $t_{max}$ value for the Test Product . Since no data were available for an administered solution of theophylline, the MDT could not be calculated. Where dissolution is the rate limiting step, which can be ascertained if solution data are available, the evaluation of MDT gives some indication of the *in vivo* equivalent to the time required *in vitro* for 63.2% of the drug to dissolve.

Table 3.9 Summary of Pharmacokinetic and Moment Analysis parameters for Test Product 1 and Theodur.

| Parameter | Test Product 1 | | Theodur | |
|---|---|---|---|---|
| | Mean Profile Data | Mean of Individual Parameter | Mean Profile Data | Mean of Individual Parameter |
| $k_e$ ($h^{-1}$) | 0.07 | 0.07 | 0.06 | 0.07 |
| $AUC_\infty$ (mg/l.h) | 105.86 | 106.47 | 106.31 | 106.24 |
| $AUMC_\infty$ (mg/l.$h^2$) | 1804.75 | 1854.71 | 2132.93 | 2152.42 |
| MRT (h) | 17.05 | 17.13 | 20.06 | 19.49 |
| MAT (h) | 1.79 | 1.98 | 3.46 | 3.67 |

### 3.2.4 Retafyllin versus Theodur

#### 3.2.4.1 Statement of the problem

Dissolution studies conducted on Retafyllin tablets following a 22 month storage period indicated that the dissolution profile had shifted over that period, i.e. the dissolution rate had decreased. A repeat bioavailabilty study [BRI 1/89 (initial) (139) and 22/90 (repeat) (174)] conducted on the product indicated that the product was still fully available *in vivo*. It therefore appeared that the dissolution study being used as a batch monitor was not able to accurately reflect the dissolution properties of the product. The initial dissolution studies revealed that a "jelly-like" layer formed around the Retafyllin tablets after initial exposure to acidic dissolution media.

#### 3.2.4.2 Objectives

In order to establish a method of dissolution that would be predictive of the *in vivo* findings it was decided to conduct similar studies as outlined in section 3.2.1. An investigation into the possible causes of the above mentioned "jelly-like" layer was also conducted.

### 3.2.5 Methods

#### 3.2.5.1 Bioavailability Study

The bioavailability study was conducted as outlined in section 2.2.6 (i). The initial study was conducted in 1989 and repeated 22 months later using the same batch of tablets. The determined serum theophylline concentrations are summarised in Table A3.6 (Appendix A).

3.2.5.2 Dissolution Studies

For the general methods employed, refer to section 3.2.2.2.

(i) Acid exposure dissolution method

The method used has been described in section 2.2.4.5 with sample times set out as in section 3.2.2.2. Three replicates of each dissolution rate test for each dosage form were carried out.

3.2.5.3 Data Manipulation

Data from the bioavailability study and the dissolution studies were processed according to the methods described in section 2.2.3.

### 3.2.6 Results and Discussion

#### 3.2.6.1 Bioavailability Study

The mean serum concentration-time curve (n = 10) for the repeat study is depicted in Figure 3.18. The $T_{max}$ was found to be 10.0 hours with a $C_{max}$ of 4.30 ± 0.32 mg/l (mean ± S.D.).

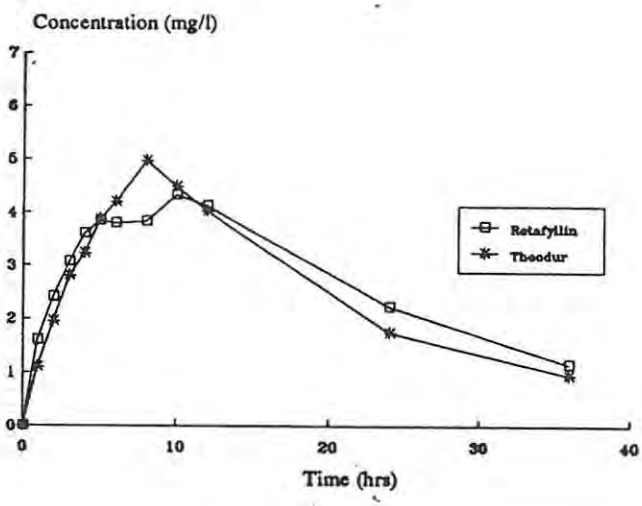



Figure 3.18 Serum concentration-time curve of Retafyllin following administration of a single 300mg tablet to ten volunteers.

#### 3.2.6.2 Acid Exposure Dissolution Method

Original dissolution studies conducted on Retafyllin employed an initial exposure of the dosage form to a 1 hour acid medium. This was then followed by further dissolution testing in media of pH 6.8. Inspection of the dosage form during these test showed that the tablet was surrounded by a "jelly-like" layer when transferred to the buffered medium. These phenomena led to several repeats of this particular dissolution test procedure in order to establish whether this occurrence was a single

event or whether it was due to the test method. A comparison of the results obtained on three different occasions (see Figure 3.19) indicated that the dissolution profiles were not in agreement with each other, with very large interday variations being observed. In order to find a possible explanation for these phenomena dissolution studies were undertaken exposing the dosage form only to the buffered medium at pH 6.8. Inspection of the dosage form under these conditions showed the absence of any "jelly-like" film surrounding the tablet. A plot of dissolution profiles obtained from three individual tests (see Figure 3.20) indicated that the test procedure, excluding the initial acid exposure, resulted in highly reproducible results with very small interday variation.

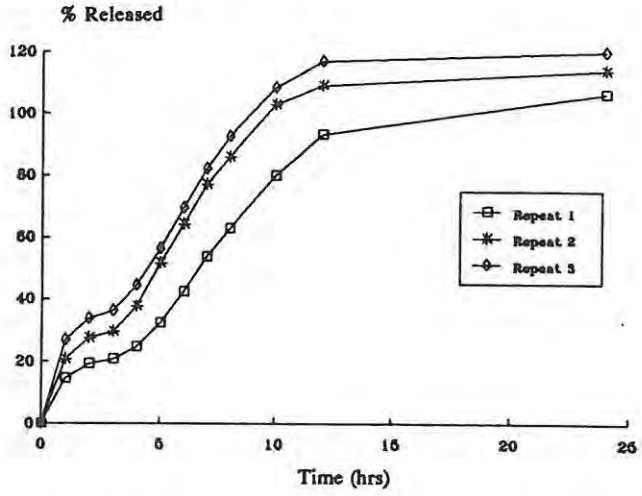



Figure 3.19 Dissolution profiles of Retafyllin following dissolution in acid and buffered medium of pH 6.8 (Acid pretreatment).

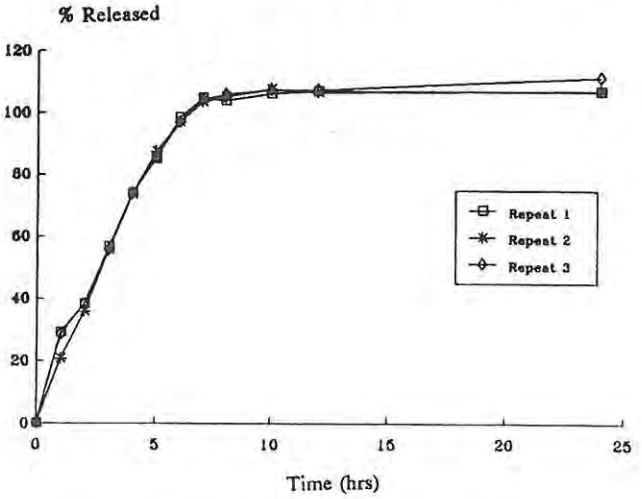



Figure 3.20 Dissolution profiles of Retafyllin following dissolution in buffered medium of pH 6.8 only (no acid pretreatment).

In similar studies conducted on Theodur, it was found that the acid exposure did not have any effect on the dissolution of theophylline from the tablet.

### 3.2.6.3 Standard Dissolution Studies

Visual inspection of the dissolution medium indicated that fine material was suspended in the bulk of the medium after 1 hour. After about 2 hours, the tablet showed signs of swelling and disintegration with complete disintegration after about 7 hours. These trends were evident throughout the pH range tested. The dissolution rate profiles are depicted in Figures 3.21 a - f.

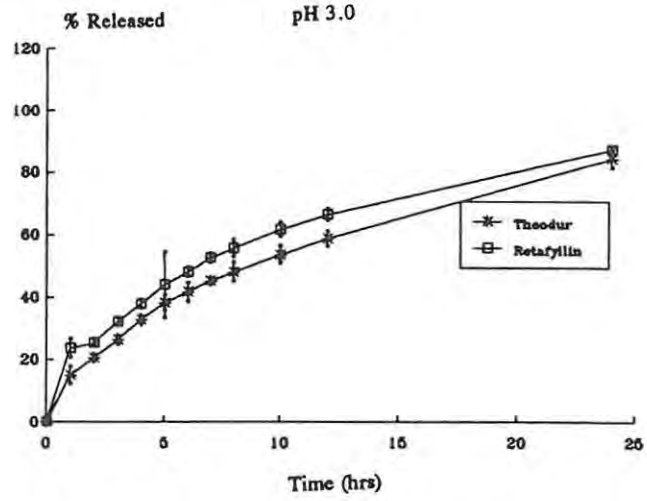



Figure 3.21 (a) Mean Dissolution Rate Profile of Retafyllin and Theodur.

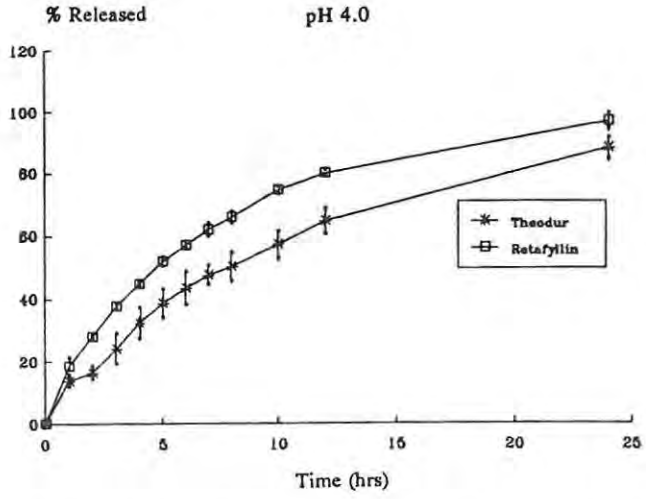



Figure 3.21 (b) Mean Dissolution Rate Profile of Retafyllin and Theodur.

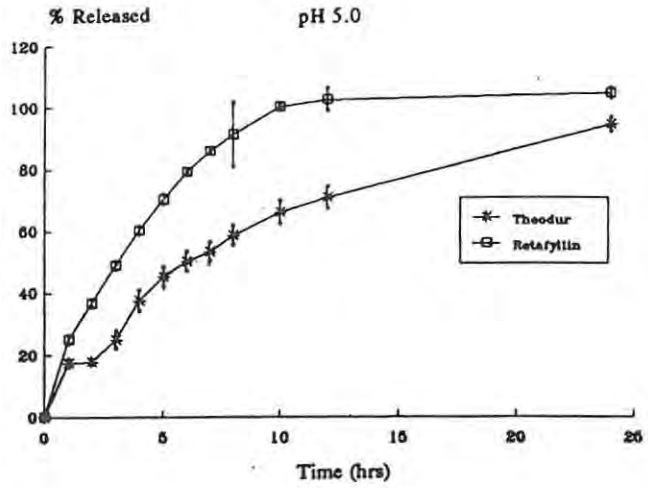



Figure 3.21 (c) Mean Dissolution Rate Profile of Retafyllin and Theodur.

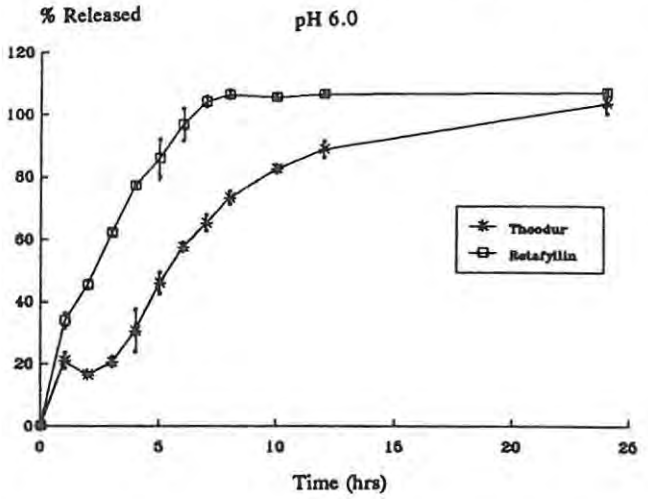



Figure 3.21 (d) Mean Dissolution Rate Profile of Retafyllin and Theodur.

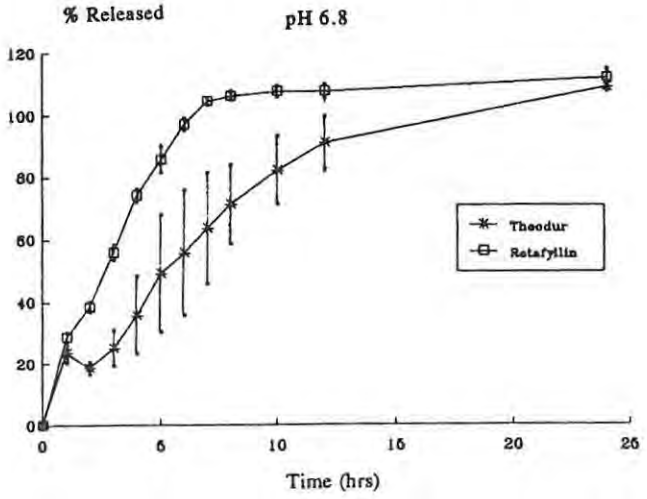



Figure 3.21 (e) Mean Dissolution Rate Profile of Retafyllin and Theodur.

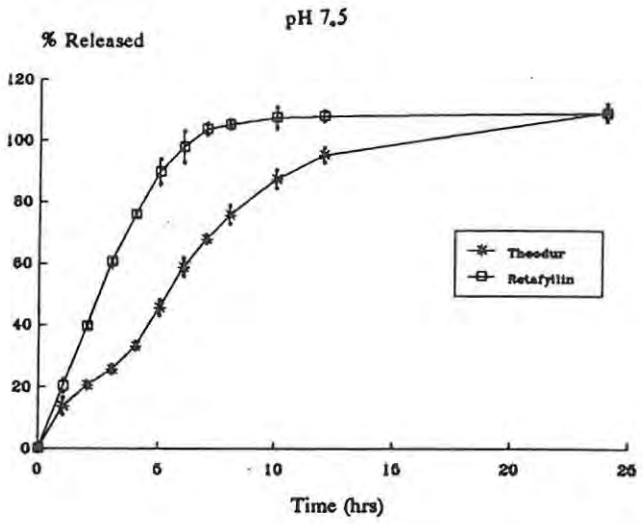



Figure 3.21 (f) Mean Dissolution Rate Profile of Retafyllin and Theodur.

The results of the dissolution studies are summarised in Table A3.3 (Appendix A). Inspection of the above plots show that the dissolution rate of Retafyllin and Theodur are similar at pH 3.0. Beyond this, the dissolution rate of theophylline from the Retafyllin matrix seems to be more rapid than that from Theodur. Furthermore, it also appears that the dissolution rate for Retafyllin increases with an increase in pH. It therefore appears that the dissolution of theophylline from Retafyllin is affected by low pH environments suggesting that the absorption profile would closely approximate that of Theodur, since, Theodur was also found to be pH dependent.

From these plots and plots of log % remaining to be released, the orders of dissolution were determined. The rates of the relevant processes were determined by linear regression analysis. The results of these determinations are depicted in Table 3.10. A comparison of these results with those obtained for Theodur (Table 3.3) indicate that the rates for Retafyllin are, on the whole, more rapid but that the orders of dissolution are very similar over the pH range studied.

Table 3.10 Summary of dissolution rate order and rate constants for Retafyllin.

| pH | Dissolution rate order | Dissolution rate constant |
|---|---|---|
| 3.0 | 20% instant release fraction with a single first order fraction | $K_s = 0.08\ h^{-1}$ |
| 4.0 | A single first order fraction | $K_s = 0.14\ h^{-1}$ |
| 5.0 | Two first order fractions, a slow fraction of 70% and a fast fraction of 30 % | $K_f = 0.39\ h^{-1}$<br>$K_s = 0.25\ h^{-1}$ |
| 6.0 | A single first order fraction | $K_s = 0.45\ h^{-1}$ |
| 6.8 | Two first order fractions, a fast fraction of 45% and a slow fraction of 55% | $K_f = 1.07\ h^{-1}$<br>$K_s = 0.25\ h^{-1}$ |
| 7.5 | Two first order fractions, a fast fraction of 25% and a slow fraction of 75% or zero order for 4 hours followed by first order | $K_f = 1.15\ h^{-1}$<br>$K_s = 0.46\ h^{-1}$<br>or<br>$K_0 = 54.60$ mg/hr<br>$K_d = 1.15\ h^{-1}$ |

### 3.2.6.4 3-D Dissolution Profile

A three dimensional topograph of the dissolution profiles obtained for Retafyllin is depicted in Figure 3.22. From this it is evident that the dissolution is pH dependent at the lower pH's. Comparing this to the profile of Theodur (see Figure 3.10) it is apparent that the two profiles are similar in shape at the low pH values. It is also evident that the dissolution rates are faster for Retafyllin than Theodur since the individual profiles show a steeper slope for Retafyllin.

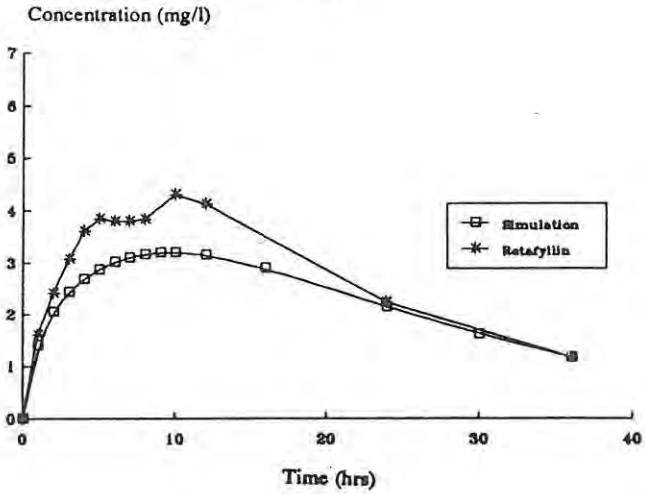



Figure 3.23 (a) Simulated profile at pH 3.0.

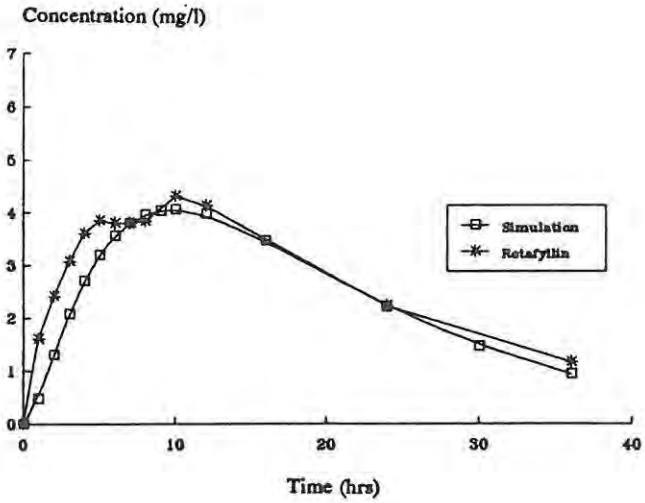



Figure 3.23 (b) Simulated profile at pH 4.0.

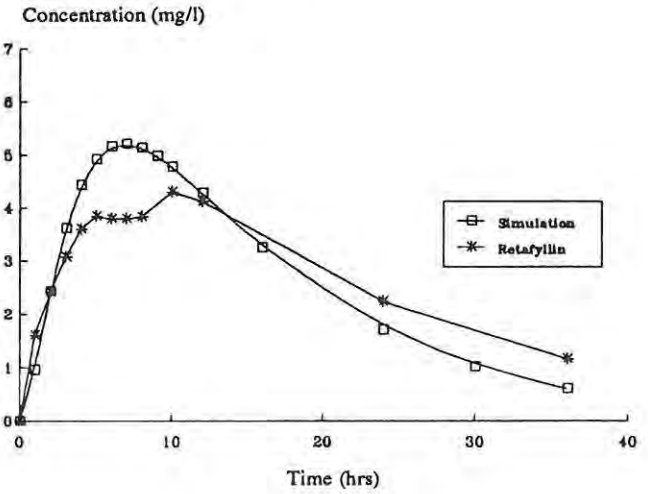



Figure 3.23 (c) Simulated profile at pH 5.0.

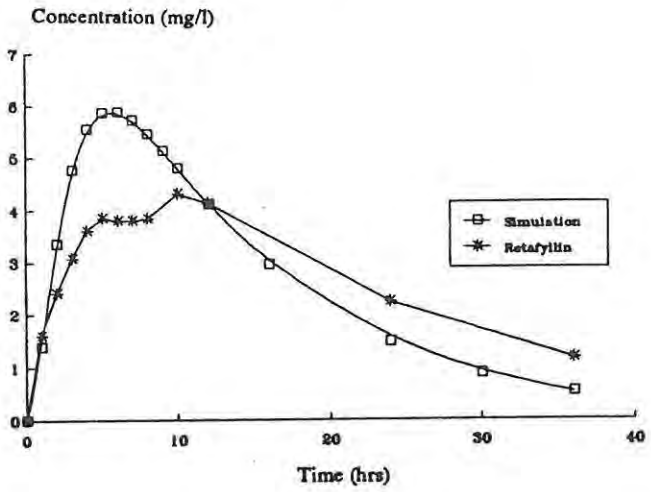



Figure 3.23 (d) Simulated profile at pH 6.0.

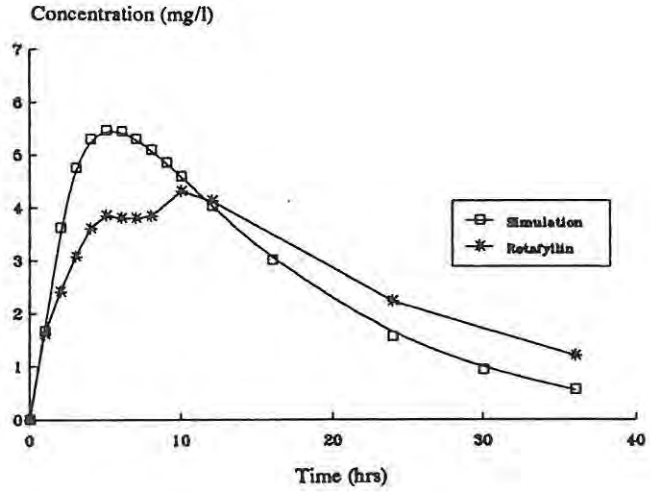



Figure 3.23 (e) Simulated profile at pH 6.8.

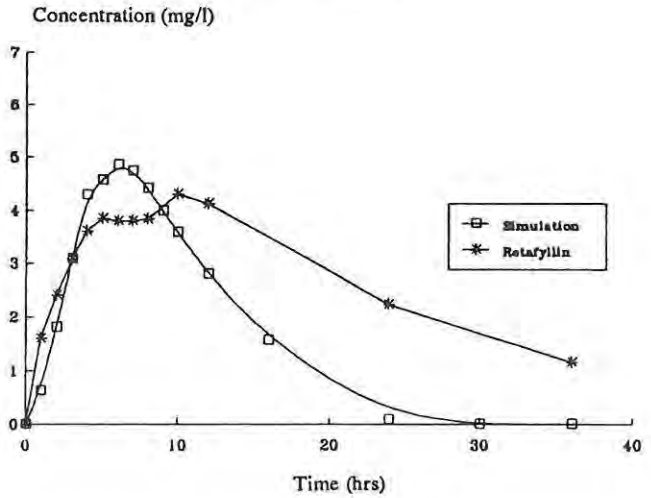



Figure 3.23 (f) Simulated profile at pH 7.5 using two first order fractions.

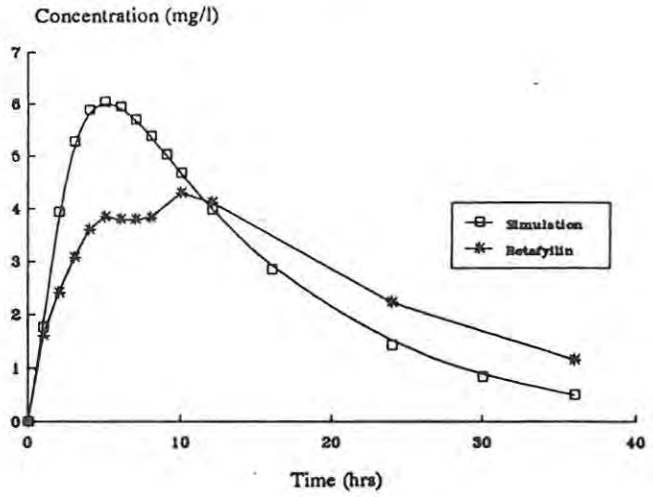



Figure 3.23 (g) Simulated profile at pH 7.5 using a zero order fraction followed by a first order fraction.

The pH at which the best simulation was obtained was found to be pH 4.0 as opposed to pH 6.0 for the other formulations. Since it was found that the dissolution profile for Retafyllin determined at pH 7.5 could be interpreted in two ways, i.e. a zero order fraction followed by a first order fraction or two first order fractions, two simulations were obtained.

The $T_{max}$ value of 10 hours compares favourably with the *in vivo* value of 10 hours. The predicted $C_{max}$ was determined as 4.05mg/l which is in close agreement with the *in vivo* value of 4.30mg/l. The simulated profile at pH 6.0 exhibited a much shorter $T_{max}$ and a correspondingly higher $C_{max}$ value, which were not in agreement with the *in vivo* determined values.

3.2.6.6 Weibull Distribution Analysis

(i) Analysis of Dissolution Data

The observed dissolution rate data and the predicted values calculated according to the Weibull equation are depicted in Figures 3.24 a - f. The Weibull function again proved to be a robust and versatile function as it enabled the description of all the dissolution profiles. In all cases the correlation coefficients were greater that 0.99. The results of the Weibull function analysis are summarised in Table 3.11.

Table 3.11 Weibull function analysis of dissolution data for Retafyllin.

| Parameter | pH | | | | | |
|---|---|---|---|---|---|---|
| | 3.0 | 4.0 | 5.0 | 6.0 | 6.8 | 7.5 |
| $t_0$ (h) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $t_d$ (h) | 11.53 | 8.27 | 4.55 | 3.24 | 5.03 | 3.44 |
| $\beta$ | 0.73 | 0.82 | 1.06 | 1.10 | 1.73 | 1.40 |
| $F^{\infty}$ (%) | 104.79 | 106.54 | 107.38 | 109.55 | 115.26 | 108.95 |

The values calculated for the parameters are in close agreement with those determined for Theodur (Table 3.7) except for the values of $t_d$. These were found to be higher for Theodur when compared to the Retafyllin formulation. This is in agreement with the fact that the dissolution rates for Retafyllin are higher since the lag time is shorter. The high values of $\beta$ observed at pH 3.0 - 5.0 are in accordance with the shapes of the dissolution profiles, i.e. the curves are initially relatively flat and gradually build up to a plateau.

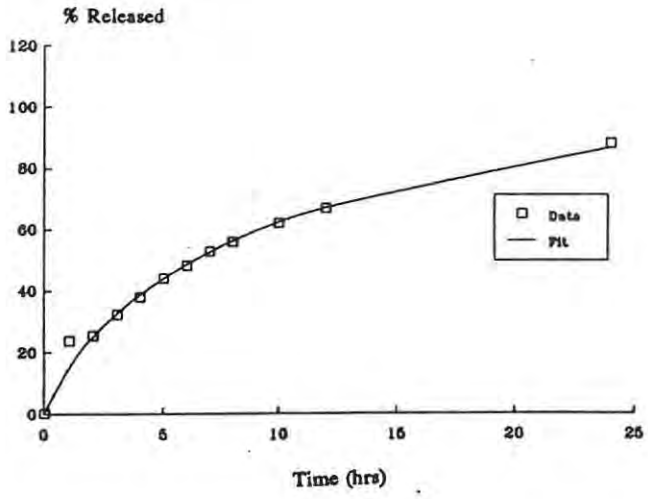



Figure 3.24 (a) Dissolution Rate Profile and Weibull function fit for Retafyllin at pH 3.0.

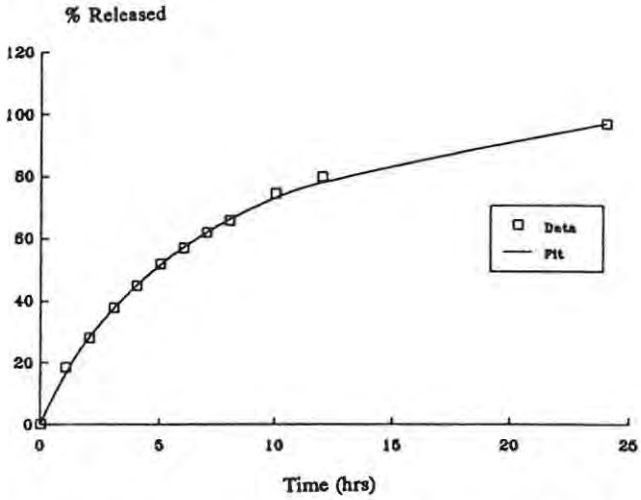



Figure 3.24 (b) Dissolution Rate Profile and Weibull function fit for Retafyllin at pH 4.0.

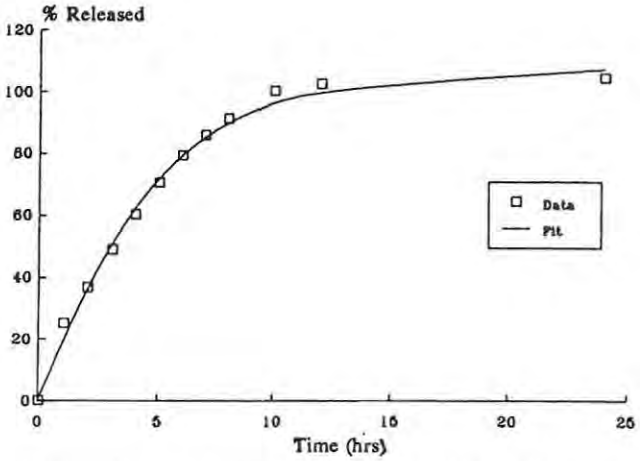



Figure 3.24 (c) Dissolution Rate Profile and Weibull function fit for Retafyllin at pH 5.0.

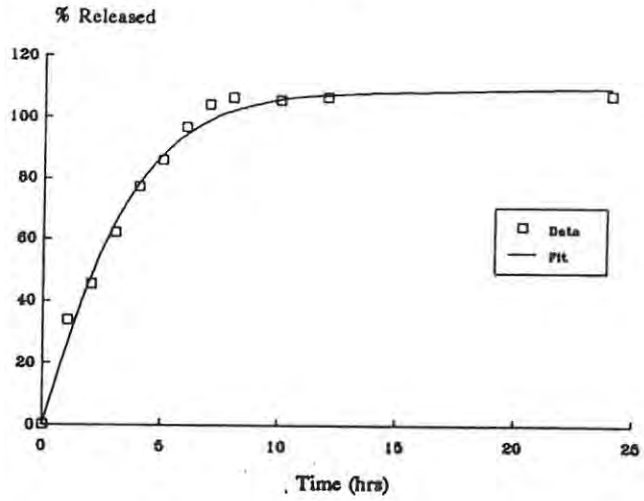



Figure 3.24 (d) Dissolution Rate Profile and Weibull function fit for Retafyllin at pH 6.0.

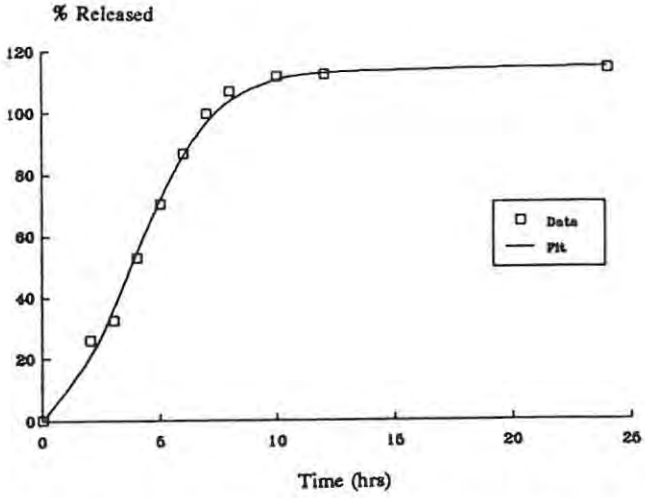



Figure 3.24 (e) Dissolution Rate Profile and Weibull function fit for Retafyllin at pH 6.8.

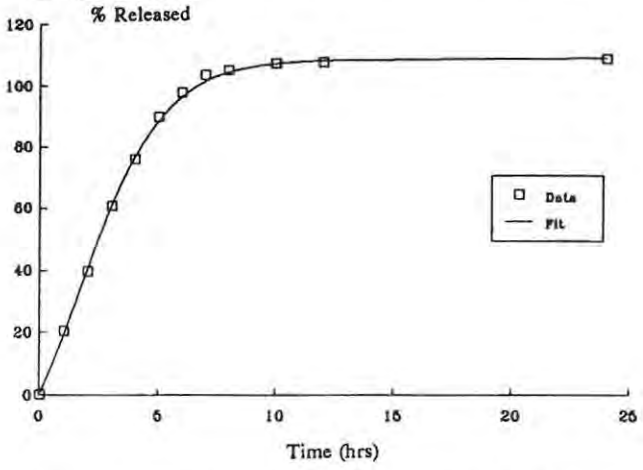



Figure 3.24 (f) Dissolution Rate Profile and Weibull function fit for Retafyllin at pH 7.5.

(ii) Weibull Fits to Wagner-Nelson Absorption plots

The serum concentration-time data of Retafyllin were transformed using the Wagner-Nelson method, assuming a one compartment model and linear kinetics. These were subsequently fitted to the Weibull equation. The plots for the mean profiles can be seen in Figure 3.25.

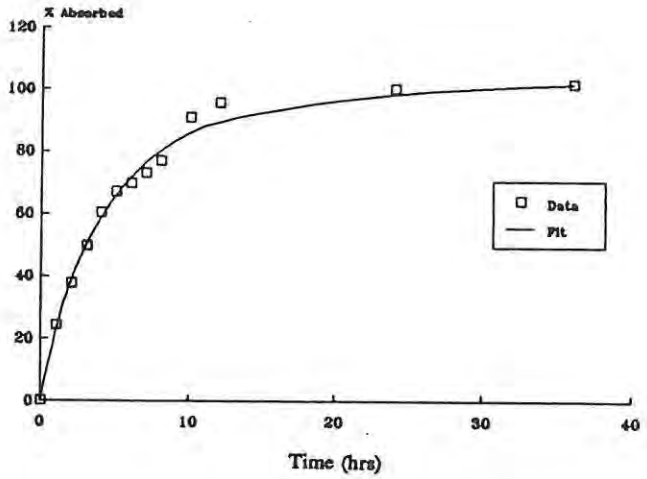



Figure 3.25 Mean Wagner-Nelson plot of serum concentration-time curve after administration of Retafyllin to ten volunteers. The solid line represents the Weibull fit to the data.

The Weibull function again appeared to be robust enough to enable a good description of the absorption rate data, albeit with larger values for the least squares summation of the estimates, than for the *in vitro* dissolution rate data. The Weibull parameters obtained for the analysis of the data are shown in Table A3.15 (Appendix A) and a summary is given in Table 3.12.

Table 3.12 Weibull function analysis of absorption data for Retafyllin.

| Parameter | Mean Data | Mean of Parameter |
|---|---|---|
| $t_0$ (h) | 0.00 | 0.17 |
| $t_d$ (h) | 4.73 | 4.12 |
| $F^\infty$ (%) | 101.78 | 101.58 |
| $\beta$ | 0.84 | 0.95 |

The time for 63.2% absorption *in vivo*, $t_d$, is in close agreement with that found for Theodur and it would therefore be expected that the two products would behave similarly *in vivo*, which is indeed the case.

3.2.6.7 Single point correlations

(i) Moment Analysis

Results of the moment analysis of the data for Retafyllin are summarised in Table A3.18 (Appendix A). A summary of the parameters is given in Table 3.13 below.

Table 3.13 Summary of Pharmacokinetic and Moment Analysis parameters for Retafyllin.

| Parameter | Mean Data | Mean of Parameter |
|---|---|---|
| $k_e$ ($h^{-1}$) | 0.06 | 0.06 |
| $AUC_\infty$ (mg/l.h) | 115.60 | 120.31 |
| $AUMC_\infty$ (mg/l.$hr^2$) | 2466.70 | 2819.68 |
| MRT (h) | 21.34 | 22.78 |
| MAT (h) | 3.97 | 3.72 |

Values for MRT and MAT of Retafyllin are in close agreement with those found for Theodur. The high value of MRT indicates that the formulation is effective as a CMRD.

(ii) Other correlations

Attempts were made to establish correlations of $t_{max}$ with $t_{50\%}$ or $t_{90\%}$ and $C_{max}$. Except for a possibly meaningful correlation of $t_{max}$ with $t_{50\%}$ obtained in medium of pH 3.0 (see Figure 3.26), these correlations were not successful and meaningless. These types of correlation attempts are more suited to instant release dosage forms and not to CMRD's.

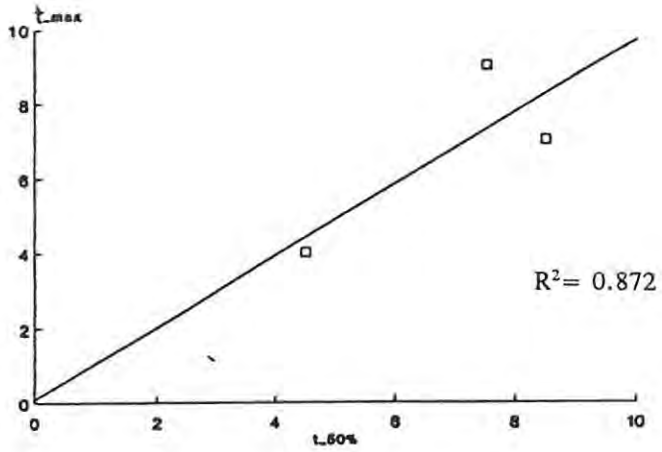



Figure 3.26 Correlation of $t_{max}$ and $t_{50\%}$ for Theodur, Test Product 1 and Retafyllin using data obtained at pH 3.0.

3.2.7 Conclusions

The results obtained from both UV and HPLC analysis correlated well and it was therefore decided to perform all routine analysis of the dissolution samples using UV spectrophotometric methods. The occurrence of % theophylline released in excess of 100% of labelled claim may possibly be due to the tablet content having been in excess of 300mg. No limits are however stated in the current compendia for

extended release tablet formulations of theophylline. It is, however, unlikely that the tolerance limits would be in excess of 110% of labelled claim.

From the dissolution rate studies conducted on Theodur it appears that the dissolution rate is dependent on the pH of the dissolution medium. From the results it is apparent that the dissolution rate increases with an increase in the pH of the dissolution medium. The dissolution rates were, however, found to be very similar in media of pH 6.8 and 7.5. The dissolution rate orders for Theodur were found to be first order or a combination of zero and first order processes. Test Product 1, however, exhibited zero order release over the whole pH range tested. The dissolution rate studies of Retafyllin indicated that the dissolution process occurred via first order rate processes. No pH dependency was exhibited by the Test Product but that of Retafyllin was shown to be dependent on the pH of the dissolution medium.

According to the manufacturers information on Theodur, the formulation contains a fraction of theophylline available as an immediate release fraction. The present dissolution studies were able to demonstrate this at all pH's studied.

The utility of three dimensional plotting of dissolution data clearly has merit in the characterisation of CMRD's over a range of dissolution pH's. The topographs constructed for the products discussed allowed the accurate explanation of the observed *in vivo* behaviour of the three products tested. Contrasting results for Theodur were found when compared to the results found by Skelly (31). This may however be due to the fact that Skelly employed different test conditions in his research.

Classical pharmacokinetics advocates that pharmacokinetic models and parameters must be obtained from individual subjects's plasma concentration data and then the averages of these are calculated. It has, however, been correctly suggested that the

alternate procedure of determining this information from average plasma concentrations may lead to pharmacokinetic constants that differ from the theoretically correct ones.

In order to employ the simulation models, Leeson (49) suggested that the simulations can be carried out using either individual data or average plasma data with equal assurance of meaningful results. This is so since the system predicts the plasma concentration-time curve only relative to the behaviour of a solution or an immediate release formulation. Hence, even if the pharmacokinetic parameters are obtained from the plasma concentration of an individual who received a solution, and using these parameters to predict the plasma concentration-time curve in that same individual when a CMRD is administered, the projections and interpretations are no more accurate than using the corresponding constants derived from average plasma data.

Using pharmacokinetic parameters obtained from mean data, accurate predictions were, however, possible for both the Test Product and Theodur, albeit only at one dissolution pH, namely pH 6.0. This may be of significance in the future lot assessment of Theodur and the possible future development of the Test Product. This approach would almost certainly allow for more accurate assessment of the products following extended storage conditions, change of location of manufacture or minor formulation changes. This implies that fewer expensive *in vivo* studies need to be conducted. It should be noted that these correlations are of utility during pre-formulation studies conducted on CMRD's. It appears from the above that the simulation obtained for Retafyllin, using dissolution data obtained at pH 4.0 provided the best estimate of the *in vivo* response. Dissolution studies conducted in media of pH 4.0 therefore appear to be suited as a lot to lot monitor for Retafyllin, as opposed to the initial acid exposure dissolution test.

The Weibull function was found to be a versatile tool and was able to describe both

the dissolution data and the absorption data. The correlation of the *in vitro* and *in vivo* Weibull parameters did not allow for meaningful correlations. Hence, even though the Weibull function adequately describes the dissolution rate profiles and the absorption profiles, it is of little utility in the prediction of the expected *in vivo* performance of a dosage form.

### 3.2.8 Summary

The utility of presenting dissolution data in the form of three dimensional topographs was illustrated with success. It is therefore evident that the dissolution of dosage forms should perhaps be undertaken over a range of pH in order to successfully characterise the dissolution characteristics of individual formulations.

The simulation of plasma concentration time profiles employing pharmacokinetic data and dissolution parameters has enabled reasonable predictions of the *in vivo* situation. However, it must be noted that it does not appear that a single dissolution test at a single specified pH can be used in the assessment of all formulations. It appears from the data presented that each formulation has its own characteristic dissolution test requirement in order to establish a correlation between the *in vivo* and *in vitro* situation.

Due to the nature of CMRD's it is perhaps not surprising that the Level C correlations have little value in the assessment of such products. Attempts at establishing these correlations did not yield any meaningful results.

APPENDIX A

Table A3.1 Dissolution Results from Theodur using Paddle Apparatus

| Time (Hrs) | pH | | | | | |
|---|---|---|---|---|---|---|
| | 3.00 | 4.00 | 5.00 | 6.00 | 6.80 | 7.50 |
| 0.00 | 0.00[1] ± 0.00[2] | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 1.00 | 22.27 ± 2.70 | 13.77 ± 1.97 | 17.44 ± 1.28 | 20.87 ± 2.51 | 23.58 ± 3.33 | 13.71 ± 0.09 |
| 2.00 | 20.51 ± 0.97 | 16.40 ± 2.05 | 17.82 ± 0.84 | 16.37 ± 0.31 | 18.56 ± 1.92 | 20.47 ± 1.53 |
| 3.00 | 26.23 ± 1.41 | 24.10 ± 4.79 | 24.87 ± 2.84 | 20.63 ± 1.58 | 25.10 ± 5.77 | 25.67 ± 3.70 |
| 4.00 | 32.65 ± 1.24 | 32.22 ± 4.99 | 37.48 ± 3.32 | 30.48 ± 6.73 | 33.63 ± 12.50 | 45.49 ± 6.30 |
| 5.00 | 37.95 ± 2.53 | 38.18 ± 4.37 | 45.10 ± 3.06 | 45.83 ± 3.34 | 48.94 ± 18.83 | 58.68 ± 1.80 |
| 6.00 | 41.50 ± 2.87 | 42.72 ± 5.06 | 50.06 ± 3.10 | 57.37 ± 1.24 | 55.50 ± 20.03 | 67.81 ± 1.20 |
| 7.00 | 43.91 ± 1.19 | 47.44 ± 3.04 | 53.17 ± 3.02 | 65.03 ± 2.50 | 63.38 ± 17.71 | 75.60 ± 0.60 |
| 8.00 | 48.03 ± 2.96 | 50.03 ± 4.46 | 58.47 ± 3.03 | 73.14 ± 2.13 | 71.07 ± 12.36 | 87.12 ± 2.00 |
| 10.00 | 53.63 ± 2.86 | 56.98 ± 4.24 | 65.93 ± 3.70 | 82.29 ± 1.23 | 82.05 ± 10.90 | 94.90 ± 1.20 |
| 12.00 | 58.59 ± 2.39 | 63.40 ± 3.97 | 70.55 ± 3.46 | 88.51 ± 2.49 | 90.79 ± 8.46 | 100.80 ± 1.40 |
| 24.00 | 84.30 ± 2.97 | 87.67 ± 3.35 | 94.14 ± 2.24 | 103.45 ± 3.34 | 108.55 ± 1.46 | |

[1] Mean of three determinations
[2] ±S.D.

Table A3.2 Dissolution Results from Test product 1 using Paddle Apparatus

| Time (Hrs) | pH | | | | | |
|---|---|---|---|---|---|---|
| | 3.00 | 4.00 | 5.00 | 6.00 | 6.80 | 7.50 |
| 0.00 | 0.00[1] ± 0.00[2] | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 1.00 | 11.16 ± 0.93 | 17.36 ± 1.22 | 16.32 ± 1.07 | 12.11 ± 0.89 | 12.86 ± 0.35 | 12.63 ± 0.38 |
| 2.00 | 20.81 ± 1.96 | 27.01 ± 5.61 | 26.67 ± 3.71 | 19.09 ± 3.06 | 18.82 ± 1.94 | 19.18 ± 0.79 |
| 3.00 | 34.80 ± 2.99 | 40.79 ± 7.89 | 38.83 ± 4.95 | 37.19 ± 3.21 | 33.15 ± 4.64 | 31.75 ± 0.57 |
| 4.00 | 48.11 ± 4.39 | 56.52 ± 10.06 | 53.90 ± 5.70 | 56.49 ± 5.03 | 49.70 ± 5.30 | 43.69 ± 0.37 |
| 5.00 | 59.49 ± 3.11 | 69.45 ± 8.32 | 66.45 ± 5.94 | 75.31 ± 7.02 | 63.91 ± 6.51 | 55.54 ± 2.03 |
| 6.00 | 67.52 ± 3.43 | 78.00 ± 8.42 | 79.01 ± 11.36 | 84.70 ± 3.35 | 78.68 ± 6.70 | 62.37 ± 2.48 |
| 7.00 | 76.05 ± 4.64 | 83.37 ± 7.58 | 84.82 ± 7.69 | 89.92 ± 3.33 | 91.88 ± 5.70 | 74.64 ± 1.60 |
| 8.00 | 81.75 ± 3.44 | 89.45 ± 8.42 | 89.47 ± 6.50 | 97.45 ± 1.80 | 100.18 ± 3.66 | 79.33 ± 1.36 |
| 10.00 | 92.38 ± 4.76 | 97.19 ± 7.24 | 97.37 ± 3.69 | 102.60 ± 4.18 | 107.23 ± 2.39 | 92.18 ± 2.30 |
| 12.00 | 97.78 ± 3.01 | 99.98 ± 4.76 | 100.88 ± 3.04 | 112.33 ± 6.48 | 112.64 ± 2.64 | 101.66 ± 1.51 |
| 24.00 | 111.60 ± 1.74 | 104.10 ± 1.80 | 102.30 ± 0.63 | 112.33 ± 2.50 | 112.00 ± 1.50 | 102.00 ± 1.30 |

[1] Mean of three determinations
[2] ±S.D.

Table A3.3 Dissolution Results from Retafyllin using Paddle Apparatus

| Time (Hrs) | pH | | | | | |
|---|---|---|---|---|---|---|
| | 3.00 | 4.00 | 5.00 | 6.00 | 6.80 | 7.50 |
| 0.00 | 0.00[1] ± 0.00[2] | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 1.00 | 23.63 ± 2.84 | 18.48 ± 2.88 | 25.03 ± 1.39 | 33.66 ± 2.35 | 28.60 ± 1.43 | 20.27 ± 1.98 |
| 2.00 | 25.26 ± 1.12 | 29.97 ± 0.55 | 36.65 ± 1.24 | 45.28 ± 1.42 | 38.33 ± 1.51 | 39.50 ± 0.80 |
| 3.00 | 31.96 ± 1.24 | 37.63 ± 0.81 | 48.71 ± 1.40 | 61.90 ± 0.89 | 55.79 ± 2.48 | 60.65 ± 0.92 |
| 4.00 | 37.70 ± 1.38 | 44.72 ± 0.57 | 60.13 ± 1.21 | 77.07 ± 0.55 | 73.86 ± 2.05 | 75.85 ± 0.87 |
| 5.00 | 43.91 ± 1.05 | 51.69 ± 1.43 | 70.23 ± 1.42 | 85.74 ± 5.95 | 85.51 ± 4.20 | 89.67 ± 3.95 |
| 6.00 | 47.86 ± 1.43 | 56.77 ± 1.26 | 79.24 ± 0.74 | 96.43 ± 5.13 | 96.77 ± 2.04 | 97.35 ± 5.12 |
| 7.00 | 52.45 ± 1.46 | 61.76 ± 2.02 | 85.59 ± 0.72 | 103.78 ± 1.61 | 104.20 ± 1.12 | 103.40 ± 1.72 |
| 8.00 | 55.50 ± 2.69 | 65.72 ± 1.89 | 91.06 ± 1.02 | 106.19 ± 1.30 | 105.96 ± 1.49 | 104.94 ± 1.44 |
| 10.00 | 61.62 ± 2.24 | 74.34 ± 1.21 | 100.05 ± 1.09 | 105.45 ± 0.86 | 107.30 ± 1.79 | 107.22 ± 3.26 |
| 12.00 | 66.36 ± 1.76 | 79.80 ± 1.08 | 102.33 ± 3.64 | 106.32 ± 0.62 | 107.30 ± 2.12 | 107.62 ± 1.72 |
| 24.00 | 86.75 ± 1.07 | 96.46 ± 2.61 | 104.18 ± 1.63 | 106.93 ± 1.15 | 111.48 ± 2.80 | 108.72 ± 2.00 |

[1] Mean of three determinations
[2] ±S.D.

Table A3.4 Theophylline Serum Concentrations following the administration of a single 300mg Theodur Tablet. (BRI 15/90).

| Time (Hrs) | Concentration of Theophylline (mg/l) | | | | | | | | | | Mean ± S.D. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 ± 0.00 |
| 0.50 | 0.49 | 1.32 | 0.63 | 0.81 | 0.71 | 0.33 | 1.17 | 1.07 | 1.46 | 1.07 | 0.91 ± 0.37 |
| 1.00 | 0.56 | 1.22 | 0.92 | 1.03 | 1.22 | 0.48 | 1.39 | 1.27 | 1.27 | 1.64 | 1.10 ± 0.36 |
| 2.00 | 1.03 | 1.36 | 1.57 | 1.31 | 1.64 | 4.27 | 2.22 | 1.88 | 1.82 | 2.18 | 1.95 ± 0.89 |
| 3.00 | 1.39 | 1.93 | 2.16 | 1.82 | 2.21 | 6.77 | 3.45 | 3.37 | 2.54 | 2.45 | 2.81 ± 1.53 |
| 4.00 | 1.75 | 2.37 | 2.59 | 1.89 | 4.38 | 6.26 | 3.48 | 3.65 | 2.92 | 3.02 | 3.23 ± 1.33 |
| 5.00 | 3.04 | 2.42 | 3.68 | 2.49 | 5.04 | 6.07 | 4.17 | 4.91 | 3.39 | 3.46 | 3.87 ± 1.18 |
| 6.00 | 3.59 | 2.83 | 3.99 | 2.82 | 5.34 | 5.34 | 4.61 | 4.64 | 4.15 | 4.38 | 4.19 ± 0.90 |
| 7.00 | 3.95 | 4.22 | 5.49 | 3.28 | 5.31 | 4.74 | 5.36 | 5.43 | 5.10 | 4.55 | 4.74 ± 0.74 |
| 8.00 | 3.80 | 5.02 | 5.64 | 4.04 | 5.33 | 4.77 | 5.56 | 5.83 | 4.65 | 4.97 | 4.96 ± 0.67 |
| 10.00 | 3.59 | 5.22 | 4.72 | 3.97 | 4.00 | 3.51 | 5.83 | 5.45 | 3.61 | 4.88 | 4.48 ± 0.85 |
| 12.00 | 3.27 | 5.22 | 4.24 | 3.43 | 3.34 | 2.96 | 5.46 | 4.88 | 2.46 | 4.93 | 4.02 ± 1.06 |
| 24.00 | 0.91 | 2.79 | 1.95 | 1.84 | 1.17 | 1.26 | 3.14 | 1.88 | 0.99 | 1.59 | 1.75 ± 0.74 |
| 36.00 | 0.56 | 1.30 | 1.17 | 0.69 | 0.68 | 0.99 | 1.87 | 0.99 | 0.57 | 0.81 | 0.96 ± 0.40 |

Table A3.5 Theophylline Serum concentration following the administration of a Single 300mg Tablet of Test product 1 (BRI 15/90).

| Time (Hrs) | Concentration of Theophylline (mg/l) | | | | | | | | | | Mean ± S.D. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 ± 0.00 |
| 0.50 | 0.54 | 0.74 | 0.36 | 0.73 | 0.53 | 0.58 | 0.73 | 0.81 | 1.01 | 1.42 | 0.75 ± 0.30 |
| 1.00 | 0.79 | 1.48 | 0.64 | 1.81 | 0.81 | 0.54 | 2.73 | 2.34 | 3.05 | 3.12 | 1.63 ± 1.06 |
| 2.00 | 0.84 | 4.54 | 1.45 | 1.31 | 5.44 | 1.89 | 6.91 | 4.35 | 5.77 | 6.20 | 3.87 ± 2.28 |
| 3.00 | 1.88 | 6.36 | 5.05 | 2.30 | 7.00 | 5.59 | 7.49 | 5.44 | 6.55 | 8.03 | 5.57 ± 2.05 |
| 4.00 | 3.03 | 6.86 | 6.16 | 2.45 | 7.76 | 6.58 | 7.77 | 5.57 | 5.82 | 7.61 | 5.96 ± 1.87 |
| 5.00 | 3.22 | 6.27 | 6.02 | 2.81 | 7.66 | 6.28 | 6.94 | 5.21 | 5.73 | 6.75 | 5.69 ± 1.56 |
| 6.00 | 3.51 | 5.81 | 5.67 | 3.01 | 7.03 | 5.90 | 6.84 | 5.36 | 4.88 | 6.21 | 5.42 ± 1.31 |
| 7.00 | 3.51 | 5.35 | 5.19 | 3.43 | 6.66 | 5.18 | 6.86 | 4.97 | 4.61 | 5.52 | 5.13 ± 1.12 |
| 8.00 | 3.59 | 5.20 | 4.91 | 3.56 | 6.05 | 4.51 | 6.10 | 4.79 | 4.41 | 4.72 | 4.78 ± 0.86 |
| 10.00 | 3.05 | 4.38 | 4.19 | 3.99 | 4.89 | 3.83 | 5.75 | 3.90 | 3.51 | 3.80 | 4.13 ± 0.75 |
| 12.00 | 3.04 | 3.82 | 3.60 | 3.82 | 4.21 | 3.12 | 4.86 | 3.45 | 2.72 | 3.05 | 3.57 ± 0.64 |
| 24.00 | 1.09 | 1.53 | 1.50 | 2.12 | 1.62 | 1.03 | 2.74 | 1.63 | 0.91 | 0.99 | 1.52 ± 0.37 |
| 36.00 | 0.62 | 0.86 | 0.84 | 1.13 | 0.90 | 0.54 | 1.32 | 0.81 | 0.44 | 0.46 | 0.79 ± 0.29 |

Table A3.6 Theophylline Serum concentrations following the administration of a Single 300mg Retafyllin Tablet (BRI 1/89)

| Time (Hrs) | Concentration of Theophylline (mg/l) | | | | | | Mean ±S.D. |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 ± 0.00 |
| 0.50 | 1.14 | 1.76 | 1.14 | 1.92 | 0.92 | 0.59 | 1.25 ± 0.21 |
| 1.00 | 1.18 | 1.98 | 1.70 | 2.38 | 1.46 | 0.95 | 1.61 ± 0.21 |
| 2.00 | 1.72 | 2.96 | 2.44 | 3.18 | 2.34 | 1.84 | 2.41 ± 0.24 |
| 3.00 | 2.17 | 3.79 | 3.22 | 4.10 | 2.90 | 2.24 | 3.07 ± 0.32 |
| 4.00 | 2.43 | 3.42 | 3.70 | 4.43 | 4.43 | 3.16 | 3.60 ± 0.32 |
| 5.00 | 2.38 | 4.22 | 3.69 | 4.44 | 4.66 | 3.64 | 3.84 ± 0.34 |
| 6.00 | 2.48 | 4.20 | 4.00 | 4.11 | 4.51 | 3.52 | 3.80 ± 0.30 |
| 7.00 | 2.56 | 4.16 | 3.60 | 3.80 | 4.81 | 3.81 | 3.79 ± 0.30 |
| 8.00 | 2.48 | 3.73 | 3.87 | 3.67 | 4.73 | 4.49 | 3.83 ± 0.32 |
| 10.00 | 4.24 | 3.85 | 4.67 | 3.13 | 4.44 | 5.44 | 4.30 ± 0.32 |
| 12.00 | 4.47 | 3.59 | 5.11 | 2.75 | 4.04 | 4.77 | 4.12 ± 0.35 |
| 24.00 | 2.11 | 2.09 | 2.41 | 1.67 | 1.73 | 3.35 | 2.23 ± 0.25 |
| 36.00 | 0.91 | 1.49 | 1.05 | 1.14 | 0.66 | 1.70 | 1.16 ± 0.16 |

Table A3.7 Model predicted Theophylline Serum Concentrations (Using models A,B and C [see section 2.1.4.2) based on dissolution parameters obtained from Theodur.

| Time (Hrs) | Dissolution pH | | | | | |
|---|---|---|---|---|---|---|
| | 3.00 | 4.00 | 5.00 | 6.00 | 6.80 | 7.50 |
| 0.00 | 0.00[1] | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.00 | 1.38 | 1.14 | 1.40 | 0.89 | 1.55 | 0.59 |
| 2.00 | 1.99 | 1.78 | 2.22 | 1.70 | 2.49 | 1.05 |
| 3.00 | 2.35 | 2.22 | 2.77 | 2.70 | 3.20 | 1.69 |
| 4.00 | 2.58 | 2.55 | 3.17 | 3.53 | 3.82 | 2.33 |
| 5.00 | 2.75 | 2.79 | 3.45 | 4.11 | 4.38 | 2.94 |
| 6.00 | 2.88 | 2.98 | 3.64 | 4.48 | 4.88 | 3.50 |
| 7.00 | 2.96 | 3.11 | 3.77 | 4.69 | 5.33 | 4.01 |
| 8.00 | 3.02 | 3.21 | 3.83 | 4.77 | 5.75 | 4.48 |
| 10.00 | 3.06 | 3.29 | 3.83 | 4.65 | 4.58 | 4.57 |
| 12.00 | 3.02 | 3.26 | 3.69 | 4.32 | 3.62 | 4.09 |
| 24.00 | 2.13 | 2.21 | 2.10 | 1.90 | 0.70 | 1.15 |
| 36.00 | 1.20 | 1.16 | 0.93 | 0.70 | 0.08 | 0.04 |

[1] Predicted Concentration of Theophylline (mg/l)

Table A3.8 Model predicted Theophylline Serum Concentrations (using Models A, B & C, [see section 2.1.4.2) based on dissolution parameters obtained from Test Product 1.

| Time (Hrs) | Dissolution pH | | | | | |
|---|---|---|---|---|---|---|
| | 3.00 | 4.00 | 5.00 | 6.00 | 6.80 | 7.50 |
| 0.00 | 0.00[1] | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.00 | 0.89 | 1.18 | 0.46 | 0.65 | 0.48 | 0.38 |
| 2.00 | 2.28 | 2.27 | 1.32 | 1.87 | 1.39 | 1.10 |
| 3.00 | 3.44 | 3.52 | 2.24 | 3.18 | 2.36 | 1.87 |
| 4.00 | 4.24 | 4.44 | 3.12 | 4.43 | 3.29 | 2.61 |
| 5.00 | 4.74 | 5.01 | 3.94 | 5.59 | 4.15 | 3.29 |
| 6.00 | 4.99 | 5.29 | 4.70 | 5.09 | 4.95 | 3.92 |
| 7.00 | 5.09 | 5.35 | 4.18 | 4.67 | 5.68 | 3.92 |
| 8.00 | 5.05 | 5.28 | 4.20 | 4.26 | 5.21 | 4.14 |
| 10.00 | 4.74 | 4.87 | 4.03 | 3.46 | 4.38 | 4.21 |
| 12.00 | 4.29 | 4.32 | 3.60 | 2.72 | 3.67 | 3.83 |
| 24.00 | 1.74 | 1.64 | 0.84 | 0.17 | 1.29 | 0.97 |
| 36.00 | 0.50 | 0.50 | 0.00 | 0.00 | 0.45 | 0.30 |

[1] Predicted concentration of Theophylline (mg/l)

Table A3.9 Model predicted Theophylline Serum concentrations using (Models A,B & C, (see section 2.1.4.2) based on dissolution parameters obtained from Retafyllin.

| Time (Hrs) | Dissolution pH | | | | | |
|---|---|---|---|---|---|---|
| | 3.00 | 4.00 | 5.00 | 6.00 | 6.80 | 7.50 |
| 0.00 | 0.00[1] | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.00 | 1.40 | 0.48 | 0.95 | 1.39 | 1.66 | 1.76 |
| 2.00 | 2.05 | 1.30 | 2.42 | 3.35 | 3.61 | 3.93 |
| 3.00 | 2.43 | 2.07 | 3.62 | 4.76 | 4.76 | 5.27 |
| 4.00 | 2.69 | 2.70 | 4.43 | 5.54 | 5.29 | 5.88 |
| 5.00 | 2.87 | 3.19 | 4.91 | 5.86 | 5.46 | 6.04 |
| 6.00 | 3.01 | 3.55 | 5.15 | 5.88 | 5.43 | 5.94 |
| 7.00 | 3.10 | 3.79 | 5.20 | 5.71 | 5.29 | 5.69 |
| 8.00 | 3.16 | 3.95 | 5.13 | 5.44 | 5.08 | 5.37 |
| 10.00 | 3.20 | 4.05 | 4.77 | 4.78 | 4.58 | 4.66 |
| 12.00 | 3.15 | 3.96 | 4.28 | 4.10 | 4.03 | 3.98 |
| 24.00 | 2.14 | 2.21 | 1.70 | 1.47 | 1.56 | 1.42 |
| 36.00 | 1.15 | 0.93 | 0.61 | 0.52 | 0.56 | 0.50 |

[1] Predicted concentrations of Theophylline (mg/l).

Table A3.10 Wagner-Nelson Analysis -% Absorbed : Theodur

| Time | Subject | | | | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 0.05 | 1.00 | 17.45 | 1.00 | 14.22 | 11.17 | 5.46 | 15.53 | 13.69 | 27.31 | 14.03 | 14.42 |
| 1.0 | 11.35 | 16.63 | 14.56 | 18.54 | 19.39 | 8.03 | 18.79 | 16.71 | 24.92 | 22.03 | 17.86 |
| 2.0 | 21.56 | 22.11 | 25.60 | 24.71 | 27.70 | 71.52 | 30.74 | 25.73 | 37.21 | 30.83 | 32.57 |
| 3.0 | 30.26 | 28.26 | 36.25 | 35.29 | 38.69 | 117.31 | 48.34 | 46.71 | 53.49 | 36.54 | 48.24 |
| 4.0 | 39.48 | 35.62 | 44.89 | 38.61 | 75.96 | 113.48 | 50.94 | 53.20 | 64.24 | 46.53 | 57.64 |
| 5.0 | 67.82 | 38.67 | 64.31 | 51.46 | 91.48 | 118.47 | 62.22 | 73.68 | 77.24 | 55.34 | 70.96 |
| 6.0 | 83.25 | 45.41 | 72.36 | 60.17 | 102.00 | 115.63 | 70.55 | 73.33 | 96.43 | 70.98 | 79.76 |
| 7.0 | 95.66 | 66.19 | 99.45 | 71.58 | 107.57 | 107.86 | 83.29 | 87.54 | 120.31 | 77.49 | 92.55 |
| 8.0 | 98.41 | 80.11 | 106.55 | 88.86 | 113.91 | 112.92 | 89.09 | 97.33 | 118.62 | 87.52 | 100.54 |
| 10.0 | 105.10 | 90.45 | 101.37 | 96.74 | 103.85 | 100.66 | 99.25 | 102.09 | 110.56 | 95.89 | 101.92 |
| 12.0 | 108.90 | 98.34 | 101.74 | 95.80 | 101.95 | 98.14 | 100.94 | 103.64 | 97.45 | 106.04 | 102.73 |
| 24.0 | 95.59 | 101.06 | 97.19 | 103.09 | 96.54 | 98.19 | 99.69 | 97.79 | 96.65 | 97.36 | 98.09 |
| 36.0 | 101.43 | 99.69 | 101.05 | 99.19 | 101.25 | 103.04 | 100.08 | 100.67 | 101.31 | 100.77 | 100.61 |

TableA3.11 Wagner-Nelson Analysis - % absorbed : Test Product

| Time | Subject | | | | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | P | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.5 | 11.37 | 10.12 | 6.00 | 3.62 | 6.30 | 8.32 | 8.24 | 12.57 | 14.71 | 17.31 | 11.00 |
| 1.0 | 17.01 | 20.58 | 10.00 | 15.45 | 9.84 | 8.08 | 31.05 | 35.15 | 45.03 | 38.78 | 24.24 |
| 2.0 | 19.17 | 64.45 | 23.25 | 25.63 | 66.50 | 28.46 | 80.56 | 70.48 | 89.05 | 80.38 | 59.12 |
| 3.0 | 42.58 | 93.78 | 80.56 | 45.52 | 89.72 | 84.79 | 91.44 | 91.69 | 107.44 | 109.62 | 88.08 |
| 4.0 | 69.78 | 106.35 | 102.94 | 50.44 | 104.52 | 105.69 | 99.24 | 98.78 | 104.31 | 112.76 | 99.14 |
| 5.0 | 78.00 | 104.21 | 106.63 | 59.46 | 109.52 | 108.85 | 94.51 | 98.28 | 109.83 | 110.00 | 100.75 |
| 6.0 | 88.63 | 103.36 | 106.92 | 65.79 | 108.02 | 110.49 | 97.62 | 103.47 | 104.00 | 110.29 | 102.11 |
| 7.0 | 93.45 | 102.09 | 104.84 | 76.45 | 111.60 | 106.71 | 102.05 | 104.30 | 105.69 | 108.17 | 102.91 |
| 8.0 | 99.98 | 104.74 | 105.43 | 82.02 | 107.29 | 102.84 | 97.57 | 106.07 | 108.15 | 103.94 | 102.54 |
| 10.0 | 97.91 | 102.14 | 103.21 | 96.79 | 102.45 | 102.82 | 100.94 | 100.51 | 104.60 | 101.81 | 101.58 |
| 12.0 | 106.05 | 101.84 | 101.71 | 100.74 | 101.76 | 100.94 | 97.53 | 100.44 | 100.62 | 99.97 | 100.79 |
| 24.0 | 96.97 | 97.56 | 97.42 | 100.82 | 97.52 | 97.41 | 101.17 | 99.61 | 98.16 | 98.29 | 98.44 |
| 36.0 | 100.95 | 100.87 | 100.92 | 99.69 | 100.87 | 100.97 | 99.67 | 100.12 | 100.60 | 100.61 | 100.56 |

Table A3.12 Wagner-Nelson analysis - % absorbed : Retafyllin

| Time | Subject | | | | | | Mean |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| 0.05 | 18.86 | 35.27 | 16.56 | 34.24 | 12.02 | 8.49 | 18.45 |
| 1.0 | 20.07 | 34.73 | 25.13 | 43.43 | 19.34 | 13.86 | 24.31 |
| 2.0 | 30.26 | 61.47 | 37.34 | 60.36 | 32.73 | 27.41 | 37.79 |
| 3.0 | 39.47 | 80.46 | 50.72 | 80.28 | 42.54 | 34.39 | 49.68 |
| 4.0 | 45.93 | 75.79 | 60.28 | 90.46 | 65.84 | 49.19 | 60.28 |
| 5.0 | 47.42 | 94.52 | 63.02 | 95.21 | 73.38 | 57.88 | 67.01 |
| 6.0 | 51.38 | 97.24 | 70.45 | 95.81 | 76.09 | 58.73 | 69.74 |
| 7.0 | 55.11 | 99.55 | 67.67 | 92.43 | 84.63 | 65.17 | 72.89 |
| 8.0 | 56.23 | 93.94 | 74.43 | 93.99 | 88.42 | 77.48 | 76.78 |
| 10.0 | 91.38 | 101.95 | 92.54 | 91.49 | 93.96 | 97.28 | 90.68 |
| 12.0 | 103.51 | 102.31 | 106.44 | 90.86 | 97.40 | 94.21 | 95.38 |
| 24.0 | 101.29 | 97.21 | 101.26 | 98.64 | 100.80 | 104.51 | 99.98 |
| 36.0 | 98.16 | 101.09 | 97.04 | 106.48 | 100.54 | 99.50 | 101.48 |
| 48.0 | 100.77 | | 101.44 | 98.09 | 99.76 | 99.74 | 99.41 |

Table A3.13 Weibull function analysis of absorption data for Theodur

| Parameter | Subject | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| To | 0.50 | 0.00 | 0.00 | 0.00 | 0.50 | 0.50 | 0.00 | 0.00 | 0.00 | 0.00 |
| Td | 4.38 | 6.92 | 4.79 | 5.96 | 3.16 | 1.46 | 4.80 | 4.48 | 3.52 | 5.31 |
| $\gamma^*$ | 103.71 | 103.29 | 103.02 | 103.56 | 104.82 | 108.36 | 103.03 | 102.79 | 107.18 | 103.42 |
| $\beta$ | 1.93 | 1.49 | 1.87 | 1.33 | 1.96 | 3.13 | 1.15 | 1.38 | 1.40 | 1.23 |

Table A3.14 Weibull function analysis of absorption data for Test product 1

| Parameter | Subject | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| To | 0.0 | 0.05 | 0.50 | 0.00 | 0.50 | 0.50 | 0.35 | 0.16 | 0.30 | 0.01 |
| Td | 3.96 | 1.93 | 2.23 | 5.30 | 1.57 | 2.12 | 1.22 | 1.60 | 1.02 | 1.52 |
| $\gamma^*$ | 101.04 | 102.81 | 104.11 | 102.86 | 104.78 | 104.14 | 98.97 | 102.20 | 104.51 | 105.65 |
| $\beta$ | 1.76 | 1.93 | 2.20 | 1.18 | 1.77 | 3.18 | 1.38 | 1.34 | 1.3 | 1.77 |

Table A3.15 Weibull function analysis of absorption data for Retafyllin

| Parameter | Subject | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| To | 0.50 | .00 | 0.00 | 0.00 | 0.50 | 0.00 |
| Td | 6.51 | 2.04 | 4.84 | 1.70 | 3.67 | 5.95 |
| $\gamma^*$ | 103.75 | 101.66 | 102.50 | 98.11 | 108.96 | 102.46 |
| $\beta$ | 0.91 | 0.86 | 0.88 | 0.84 | 1.00 | 1.20 |

Table A3.16 Pharmacokinetic parameters for Test product 1

| Parameter | Subject | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| $K_e$[1] | 0.07 | 0.07 | 0.06 | 0.05 | 0.07 | 0.08 | 0.06 | 0.06 | 0.08 | 0.09 |
| $AUC_\infty$[2] | 72.85 | 113.11 | 104.77 | 110.29 | 124.94 | 87.16 | 162.92 | 107.84 | 84.86 | 95.97 |
| $AUMC_\infty$[3] | 1347.20 | 1901.62 | 1886.52 | 2715.89 | 2011.94 | 1248.82 | 3222.75 | 1929.89 | 1098.45 | 1185.06 |
| MRT[4] | 18.49 | 16.18 | 18.01 | 24.63 | 16.10 | 14.33 | 19.78 | 17.89 | 12.94 | 12.35 |
| MAT[4] | 3.40 | 1.58 | 2.16 | 4.30 | 1.49 | 2.07 | 1.64 | 1.39 | 0.83 | 0.90 |

[1] $hr^{-1}$ [2] mg/lhr [3] $mg/l.hr^2$ [4] hr

Table A3.17 Pharmacokinetic parameters for Theodur

| Parameter | Subject | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| $K_e$[1] | 0.07 | 0.06 | 0.06 | 0.07 | 0.07 | 0.06 | 0.04 | 0.07 | 0.07 | 0.08 |
| $AUC_\infty$[2] | 69.03 | 132.42 | 115.83 | 88.13 | 88.27 | 101.69 | 172.24 | 118.21 | 73.43 | 103.22 |
| $AUMC_\infty$[3] | 1192.07 | 3005.27 | 2515.60 | 1753.78 | 1453.56 | 1813.41 | 4580.45 | 2199.91 | 1196.07 | 1814.06 |
| MRT[4] | 17.23 | 22.70 | 21.72 | 19.90 | 16.47 | 17.83 | 26.59 | 18.61 | 16.29 | 17.57 |
| MAT[4] | 3.69 | 5.43 | 4.00 | 4.68 | 2.83 | 1.42 | 3.98 | 3.72 | 2.70 | 4.28 |

[1] $hr^{-1}$ [2] mg/lhr [3] $mg/l.Hr^2$ [4] hr

Table A3.18 Pharmacokinetic analysis of Retafyllin

| Parameter | Subject | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| $K_e$[1] | 0.06 | 0.04 | 0.05 | 0.06 | 0.08 | 0.04 |
| $AUC_\infty$[2] | 104.06 | 134.94 | 128.67 | 97.10 | 99.06 | 158.01 |
| $AUMC_\infty$[3] | 2308.27 | 3895.12 | 2888.04 | 1884.29 | 1639.17 | 4306.16 |
| MRT[4] | 22.18 | 28.26 | 22.44 | 19.41 | 16.55 | 27.25 |
| MAT[4] | 5.22 | 2.11 | 4.01 | 2.34 | 3.86 | 4.76 |

Table A3.19 Inferential statistics for Theodur and Test Product 1.

| Parameter | Ratio of means | Confidence limit | Classical | Westlake |
|---|---|---|---|---|
| $AUC_{last}$ | 1.05 | 95% | 93 - 114 | 88 - 112 |
| ln $AUC_{last}$ | 1.06 | 95% | 94 - 115 | 87 - 113 |
| $AUC_{inf}$ | 1.07 | 95% | 87 - 112 | 87 - 113 |
| ln $AUC_{inf}$ | 1.07 | 95% | 88 - 115 | 87 - 113 |
| $C_{max}$ | 1.04 | 95% | 102 - 136 | 67 - 133 |
| ln $C_{max}$ | 1.05 | 95% | 101 - 135 | 69 - 131 |

Table A3.20 Goodness-of-fit data ($R^2$ values) for Dissolution rate determinations.

| Dissolution pH | Product | | |
|---|---|---|---|
| | Theodur | Test Product 1 | Retafyllin |
| 3.0 | $K_s$: 0.9976 | $K_0$: 0.9947<br>$K_d$: 0.9468<br>or<br>$K_f$: 0.9880<br>$K_s$: 0.9947 | $K_s$: 0.9994 |
| 4.0 | $K_s$: 0.9996 | $K_0$: 0.9988<br>$K_d$: 0.9932 | $K_s$: 0.9989 |
| 5.0 | $K_s$: 0.9946 | $K_0$: 0.9994<br>$K_d$: 0.9991 | $K_f$: 0.9975<br>$K_s$: 0.9969 |
| 6.0 | $K_s$: 0.9995 | $K_0$: 0.9998<br>$K_d$: 0.9990 | $K_s$: 0.9976 |
| 6.8 | $K_0$: 0.9958<br>$K_d$: 0.9897 | $K_0$: 0.9968 | $K_f$: 0.9577<br>$K_s$: 0.9845 |
| 7.5 | $K_0$: 0.9936<br>$K_d$: 0.9944 | $K_0$: 0.9956<br>$K_d$: 0.9898 | $K_f$: 0.9993<br>$K_s$: 0.9888<br>or<br>$K_0$: 0.9958<br>$K_d$: 0.9888 |

CHAPTER 4

# INDOMETHACIN STUDIES

## 4.1 Introduction to Indomethacin

Indomethacin is a non-steroidal, anti-inflammatory drug with anti-pyretic and analgesic properties. The compound was discovered in the Merck Sharpe and Dohme Research Laboratories in 1961 and is still in wide use today in a number of dosage forms. Indomethacin is designated as 1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid. The structure of indomethacin is shown in Figure 4.1.

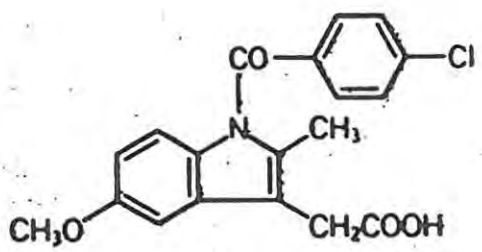



Figure 4.1 Structure of indomethacin.

Indomethacin is defined as the Form 1 crystalline, non-solvated free acid moiety of the compound. It appears as a yellow to yellow-tan crystalline powder which is odourless and almost tasteless. The compound is a weak acid with a pKa of 4.5. It is practically insoluble in water and has varying solubility, which is pH dependent, in phosphate buffer. A summary of the solubility of indomethacin in phosphate buffer is shown in Table 4.1. A full analytical profile has been well documented (175).

Table 4.1 Solubility of indomethacin in phosphate buffer.

| pH of buffer | Solubility (mg/100ml) |
|---|---|
| 5.6 | 3.00 |
| 6.2 | 11.00 |
| 7.0 | 54.00 |

Indomethacin has been used effectively in the treatment of moderate to severe rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, bursitis and acute gouty arthritis. It has also been found effective in the treatment of neonates who suffer from patent ductus arteriosus (175, 176). Although the compound is widely used and effective, toxicity often limits its use. Indomethacin is rapidly and completely absorbed from the gastrointestinal tract following oral absorption. The plasma concentrations which are necessary to produce the anti-inflammatory effects have not been accurately determined and there appears to be no dose-effect relationship. A very high proportion of patients receiving indomethacin treatment suffer from side effects. Complaints usually involve gastrointestinal complications such as anorexia, nausea, abdominal pain and in severe cases single or multiple ulcerations of the GI tract. The most common CNS side effect is severe frontal headache. Other CNS effects include dizziness, vertigo, light-headedness and mental confusion.

Indomethacin is available in 25 and 50mg capsules, 75mg sustained release formulations and as 100mg suppositories. The initial dose is 25mg twice daily to a total daily dose of 100 - 200mg per day. The drug should be taken in divided doses with food or immediately following a meal (176). In 1982 a sustained release formulation of indomethacin, Indocid R, was released (177) and has the advantage of once or twice daily dosing. Indocid R is reported to be formulated with 25mg indomethacin available as an instant dose and 50mg available as a slow release fraction (177, 178, 179).

A number of studies have been conducted over the past few years to establish the efficacy and pharmacokinetics of indomethacin (177, 178, 179, 180 - 185). Few studies, however, report complete sets of pharmacokinetic data (180, 181) for the various formulations of indomethacin. Values for the pharmacokinetic parameters vary greatly from study to study. Indomethacin is usually described by a two compartment pharmacokinetic model. The apparent volume of distribution usually falls within the range of 0.34 - 1.57 l/kg (182) although values as low as 0.07 - 0.14 l/kg have also been reported (180). The elimination half-life, $\beta$, is usually reported to be within the range of 2.9 - 8.5 hours (178, 181, 182). It has been suggested that indomethacin may undergo enterohepatic recycling, which would explain the slow terminal phase (177, 180). This is often very unpredictable and sporadic and may be an indication of continued reabsorption 4 - 48 h after dosing (180). This leads to the fact that no researcher has been able to calculate consistent pharmacokinetic data of indomethacin to date.

Indomethacin is readily absorbed following oral administration of the conventional dosage form. However, indomethacin capsules of differing formulations may show considerable differences in the serum concentration-time curve. Causes for such differences are not yet understood. Despite the large inter-subject variation, peak plasma concentrations observed are approximately dose-proportional and average between 0.79 - 2.3 $\mu$g/ml, 1.60 - 3.68 $\mu$g/ml and 3.04 - 6.80 $\mu$g/ml following administration of 25, 50 and 75 mg doses respectively (178).

To-date, no clinical studies have demonstrated a correlation between serum concentrations and clinical effects (186). Tannenbaum (187) has suggested that although no such correlations have yet been demonstrated, it does not exclude the possibility, but that more carefully designed trials may be necessary. Green (188) has suggested that there is no convincing evidence for the use of controlled release formulations of indomethacin. Green's suggestions are based on the fact that there is no evidence that elevated serum concentrations of indomethacin will result in

greater relief of symptoms and that no studies have shown an improved efficacy of sustained release over conventional indomethacin formulations. However, a study conducted by Yeh *et al* (184) reported that Indocid R did indeed show sustained release qualities when compared to three times daily dosing of a 25mg of conventional indomethacin formulation. It must, however, be borne in mind that CMRD's are designed not only to provide for sustained serum concentrations, but also for the convenience of the patient. This was demonstrated for indomethacin in a study conducted by Jalava *et al* (189) in which it was shown that indomethacin administered as a CMRD improved patient compliance when compared to immediate release and suppository formulations.

In a study on the chronopharmacokinetics of indomethacin, Guissou *et al* (190) showed that indomethacin was influenced by circadian changes. It was found that the pharmacokinetics of oral Chrono-I, a prolonged release form of indomethacin, were markedly modified as a function of its administration time. The most striking variation occurred when the dose was administered at 20h after administration of the initial dose, when the plasma indomethacin concentration did not show a sharp peak. The authors suggested that the circadian changes may be partly due to changes in the rate of hepatic demethylation of the drug.

The development of the Oros$^{R}$ system, by Theeuwes (149), has been discussed previously. A formulation of indomethacin, Osmosin, based on this technology was developed in 1983 (191, 192). The formulation was designed to release a dose of 85mg of indomethacin sodium trihydrate at a predetermined zero order rate of 7 mg/h. Problems were, however, encountered with the dosage form. Reports of intestinal bleeding and perforation following the administration of Osmosin have been cited (193). This led to the withdrawal of the product in 1983. One suggestion for these side effects was reported as having been due to the dosage form adhering to the mucosa and thus releasing indomethacin in intimate contact with the gut wall. This was however disputed by Wilson and Hardy (194), who

conducted a study on the transit of the osmotic tablet through the GIT. The results indicated that the tablet moved through the GIT at an equivalent rate to other indomethacin dosage forms and that it was therefore unlikely that the reported adverse effects were due to tablet adhesion.

Gueurten and Dubois (195) conducted dissolution studies on indomethacin to establish the effect of pH on the dissolution rate. Dissolution studies were conducted in media of pH 5.10, 5.70, 6.00, 6.30, 6.60 and 7.20. The authors found that the pH of the dissolution medium had a marked effect on the dissolution rate constant, with the rate at pH 7.20 being 200 times greater than that determined at pH 5.10. It was further demonstrated that the influence of slight changes in pH were not significant beyond pH 6.20.

In dissolution studies conducted by Herzfeldt (196), it was established that optimal results were obtained using either the USP rotating basket or paddle methods for 25 and 50 mg capsules of indomethacin. It was, however, found that dissolution rates for indomethacin release from a sustained release formulation were faster when the basket apparatus was employed when compared to the paddle method. The USP monograph for indomethacin extended-release capsules (72) requires the dissolution test to be carried out using the basket apparatus at 75 rpm in a phosphate buffer medium of pH 6.2 and the test must be carried out over a 24h period.

Komuro (197) conducted dissolution studies on various extended-release formulations of indomethacin employing the paddle apparatus and a flow-through apparatus. These results indicated that the flow-through apparatus was more advantageous over the paddle method for *in vitro* tests on indomethacin extended-release formulations. The reasons cited for this include the fact that the flow-through apparatus is comparatively mild and mechanical disintegration of the granules do not occur.

Dissolution studies undertaken by Rowe and Carless (198) on various extended-release dosage forms of indomethacin, including Indocid R, indicated that problems were associated with the extrapolation of the *in vitro* data to the *in vivo* situation. The authors found that dissolution studies undertaken according to the beaker method of Levy and Hayes did not adequately show the extended release properties of the dosage forms tested. In was also demonstrated that Indocid R exhibited a smoother *in vivo* profile than the microencapsulated formulations which showed very rapid and high peak serum concentrations. These findings were consistent with the dissolution results for the microcapsules which showed an initial burst phase, i.e. an initial rapid dissolution rate, followed by zero order release. The dissolution of Indocid R was found to be square root time dependent, i.e. the release indicated a diffusion controlled process from a matrix.

In a study conducted by Soininen and Langenskiöld (198) on the influence of polysorbate 80 on the dissolution rate of indomethacin from five different formulations, it was found that the dissolution rate was not affected by the inclusion of polysorbate 80. The dissolution tests were carried out according to the USP XX with and without 0.01% polysorbate 80.

Ramtoola and Corrigan (200) conducted dissolution rate tests on various compressed discs of indomethacin and indomethacin-citric acid. The dissolution studies conducted were not intended to determine the intrinsic dissolution rate. It was established that the dissolution rate increased with an increase in the dissolution agitation intensity. The ratio of the rate at a stirring speed of 180 rpm to the dissolution rate at 60 rpm was found to be 1:24.

Despite the availability of various extended-release formulations of indomethacin, many researchers (201 - 206) have attempted to develop new types of release mechanisms. Bechgaard *et al* (204) conducted studies on two multiple unit formulations of indomethacin. The experimental formulations consisted of capsules

containing enteric coated pellets of different sensitivity to an alkaline environment. These authors found that the experimental formulations were equivalent to Indocid R.

Aiache *et al* (207) conducted studies on indomethacin suppositories in an attempt to establish *in vitro - in vivo* correlations. A promising correlation was established between *in vivo* and *in vitro* data of indomethacin suppositories. The *in vitro* dissolution rates were determined using a specially modified flow-through apparatus. Two types of suppositories were assessed, namely a fatty based product and a PEG based product. A bioavailability study was conducted on the two dosage forms from which it was concluded that the two products were equivalent. The *in vitro - in vivo* correlations were based on the simulation of the plasma concentration-time curves using the drug's pharmacokinetic parameters obtained from a single subject and the release parameters obtained from the dissolution rate test. The absorption of the drug was assumed to be zero order. It was demonstrated by the authors that the simulated profiles were almost identical to the actual *in vivo* profiles obtained in the bioavailability study.

It must, however, be borne in mind that rectal dosage forms are exposed to an environment where the pH is fairly consistent. This is of significance for indomethacin since its dissolution is highly pH dependent. The establishment of good *in vitro - in vivo* correlations, based on data obtained from dissolution studies carried out at a high pH, are more likely than those based on dissolution data obtained from studies conducted under conditions similar to those found in the upper GIT (pH 2 - 5), where the solubility of indomethacin is highly dependent on the pH.

## 4.2 Experimental

### 4.2.1 Test Product 2 versus Indocid R

#### 4.2.1.1 Statement of the problem

Dissolution rate studies carried out on Test Product 2 (a capsule formulation containing 75mg indomethacin as pellets) during research and development indicated that the dissolution profile for Test Product 2 closely followed that of Indocid R (Figure 4.2). The dissolution studies were conducted according to the specification as detailed in the USP monograph for Extended-Release indomethacin formulations. Based on the results of this dissolution study a bioavailability study (Study BRI 14/89) (138) was conducted on Test Product 2, using Indocid R as the reference product. The results of the biostudy indicated that Test Product 2 exhibited a delayed absorption when compared to Indocid R. These findings were in contrast to the results of the dissolution rate studies.

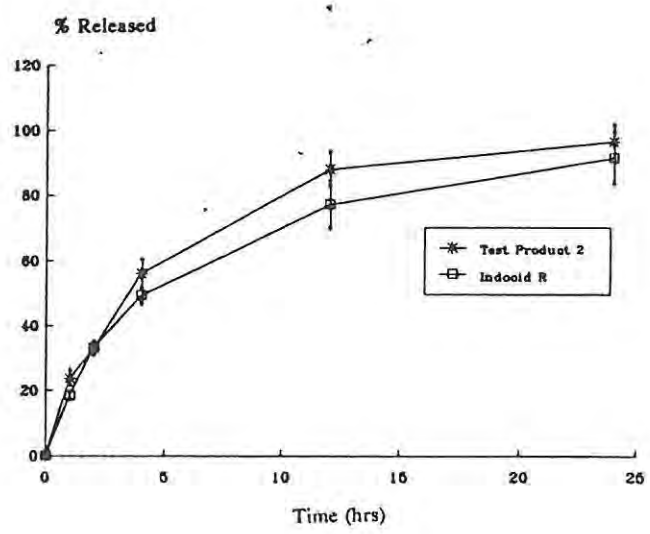



Figure 4.2 R & D Mean (n = 6) dissolution rate profiles obtained at pH 6.2 of Test Product 2 and Indocid R.

4.2.1.2 Objectives

From the above findings it is evident that the official dissolution rate test for the products under study, was not able to accurately predict the *in vivo* situation. In order to find a possible explanation for the results, it was decided to conduct a series of dissolution rate studies on the two products with the aim of finding dissolution conditions which would be more predictive of the *in vivo* situation.

## 4.2.2 Methods

### 4.2.2.1 Bioavailability Study

The study was conducted as described in section 2.2.6(i). Results of the serum concentrations are depicted in Table B4.5 for Indocid R and in Table B4.6 (Appendix B) for Test Product 2.

### 4.2.2.2 Dissolution Studies

Three replicate dissolution rate determinations on each dosage form were carried out.

(i) Dissolution media

Dissolution media were prepared as set out in section 2.2.4.1. Media of pH 4.5, 5.0, 5.5, 6.0, 6.2 and 7.0 were utilised in the various dissolution studies.

(ii) Sampling times

Samples were taken according to the following time schedule: 0.0 (blank), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0 and 24.0 hours after initiation of the dissolution test.

(iii) Sample analysis

All samples were assayed for indomethacin content according to the method set out in section 2.2.5. Analysis of certain studies was also carried out by HPLC as described in section 2.2.5.2.

### 4.2.2.3 Data manipulation

Data obtained from the bioavailability study and dissolution studies were analysed according to the procedures detailed in section 2.2.3.

## 4.2.3 Results and Discussion

### 4.2.3.1 Bioavailability study

The indomethacin serum concentrations are presented in Tables B4.5 and B4.6 (Appendix B) for Indocid R and Test Product 2, respectively. The statistical results from these studies are shown in Table B4.13 (Appendix B). The mean serum concentration versus time curve is depicted in Figure 4.3.

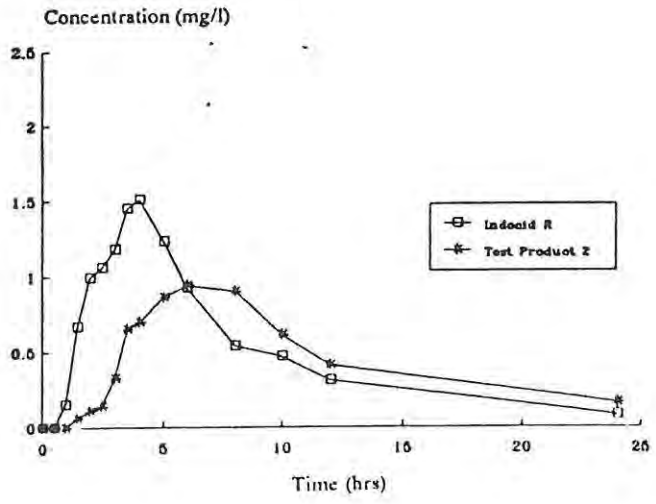



Figure 4.3 Mean serum concentration versus time time curve from eight subjects.

The results indicate that the absorption of indomethacin from Test Product 2 was notably slower than that from Indocid R. The mean $t_{max}$ was found to be 6.5h ± 2.8 and 3.6h ± 1.4 (mean ± S.D.) for Test Product 2 and the reference product, respectively. Only in two subjects were the $t_{max}$ values for the Test Product the same as for the reference product. The mean $C_{max}$ value for the Test Product was 1.5 μg/ml ± 0.4 and 2.1 μg/ml ± 0.6 for Indocid R. In five out of eight cases, $C_{max}$ values for the Test Product were lower than for the reference. When the $AUC_{last}$ (Table 4.2) for the two products are compared, it appears that they are similar with respect to the extent of indomethacin absorption, but they exhibit entirely different release rates resulting in large differences in $C_{max}$ and $t_{max}$ values. Due to the large differences in the $t_{max}$ and $C_{max}$, based on statistical analysis data (138), it was concluded that the two products were not bioequivalent.

Table 4.2 Mean Bioavailability Parameters.

| Parameter | Test Product 2 | Indocid R |
|---|---|---|
| | Mean ± S.D. | Mean ± S.D. |
| $AUC_{last}$ (mg.h/l) | 10.29 ± 4.07 | 11.11 ± 3.03 |
| $AUC_{inf}$ (mg.h/l) | 17.42 ± 4.52 | 13.58 ± 2.83 |
| $C_{max}$ (mg/l) | 1.5 ± 0.4 | 2.1 ± 0.6 |
| $T_{max}$ (h) | 6.5 ± 2.8 | 3.6 ± 1.4 |

4.2.3.2 Analysis of samples and Chromatography

Analysis of dissolution samples by UV spectrophotometric analysis was rapid, accurate and precise. The results of these determinations are given in Tables B4.1 - B4.4 of Appendix B. Since samples of 5ml were removed, this volume was immediately replaced with an equivalent volume of equilibrated fresh medium in order to maintain sink conditions.

As for the theophylline studies, some of the results of the UV analysis indicated that dissolution was occurring beyond 100% of label claim. It was therefore to decided to analyse the dissolution samples using HPLC as set out in section 2.2.5.2. Analysis of the samples was again found to be rapid, accurate and precise. Figures 4.4 is representative of the chromatography achieved for indomethacin.

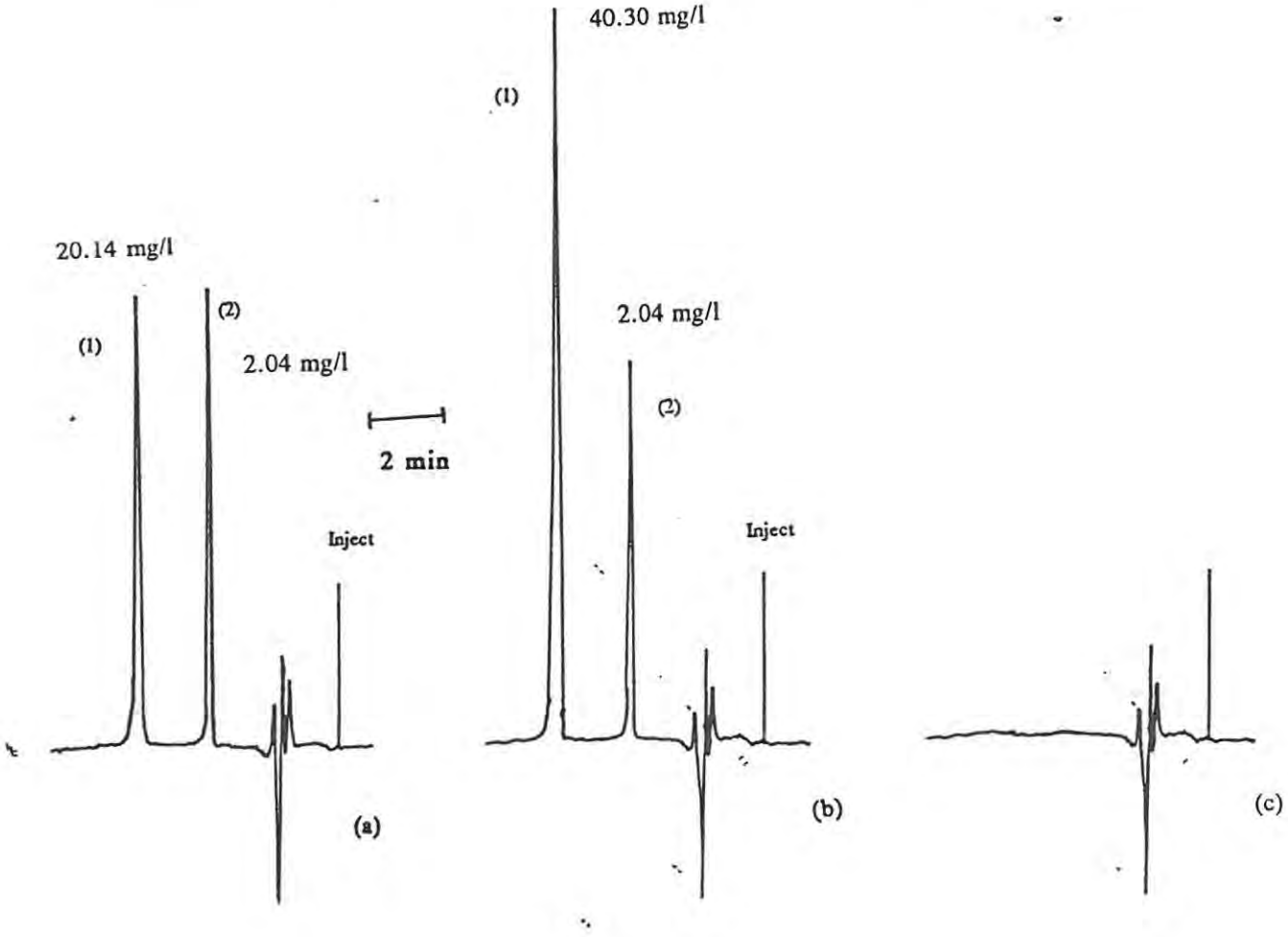



Figure 4.4a and b Chromatogram of indomethacin (1) and naproxen (2) (internal standard) in a calibrator (a) a representative dissolution sample (b) and a blank sample (c).

Results of the concentration of drug determined by both methods were found to compare very favourably. Inspection of some of the 24h dissolution samples indicated more than 100% release. In order to determine whether this was due to contamination, a series of three dimensional chromatograms were constructed. These are depicted in Figures 4.5 and 4.6. Inspection of these indicated that the

three dimensional chromatograms are identical for a sample of pure indomethacin powder and a 24h dissolution sample. The occurrence of release percentages in excess of the label claim was therefore not due to any contaminating species in the samples. Content uniformity assays conducted on both formulations studied, revealed that the indomethacin content of the capsules was within the USP limit of 90 - 110% of labelled claim. The release of indomethacin beyond 110% can therefore not be ascribed to excessive indomethacin content.

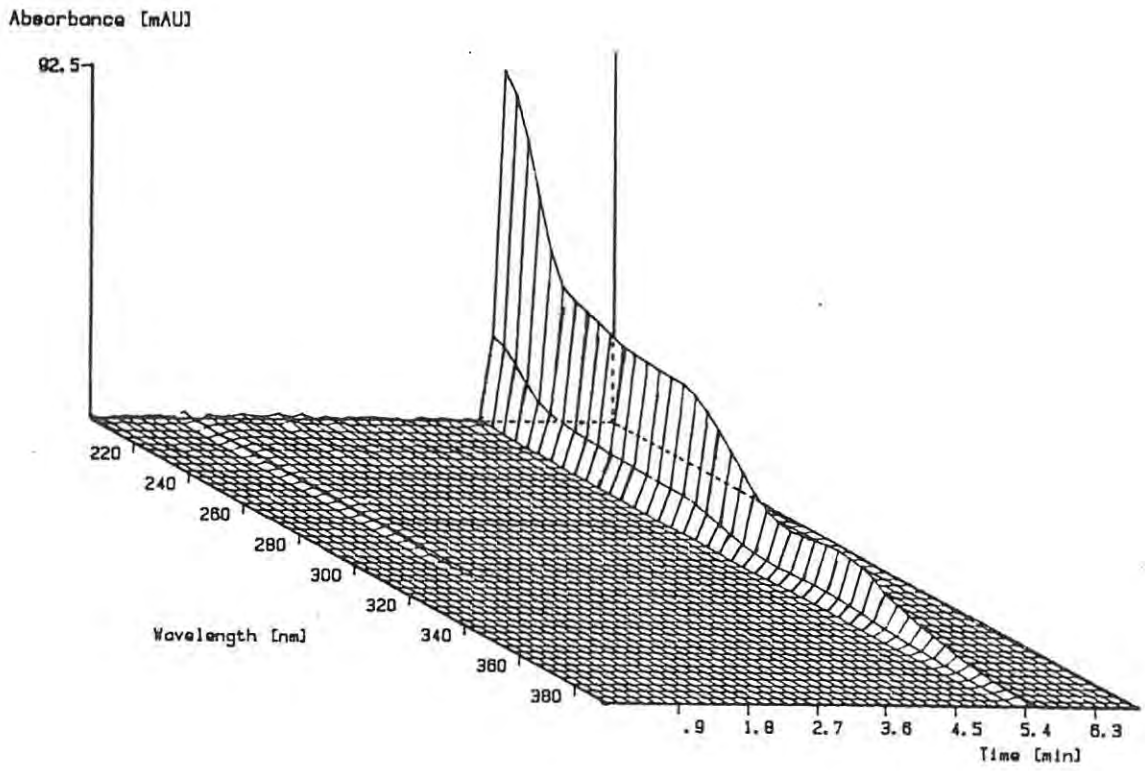



Figure 4.5 Three dimensional chromatogram of indomethacin powder.

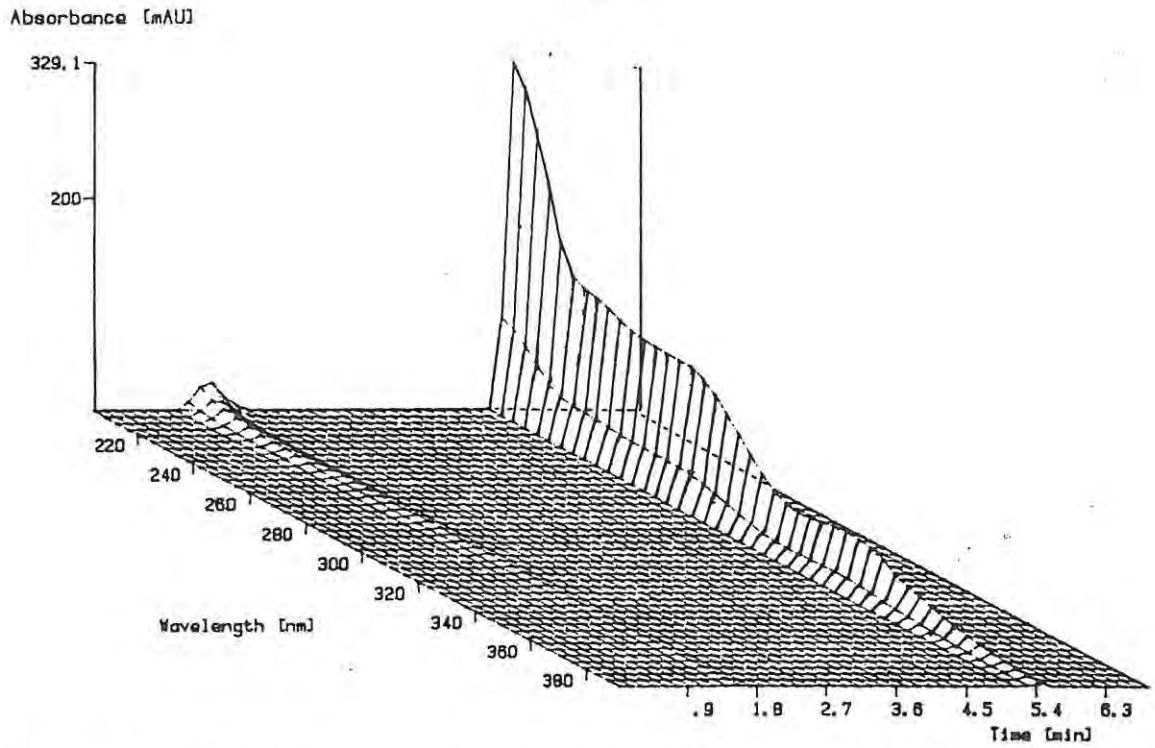



Figure 4.6 Three dimensional chromatogram of a 24h dissolution sample.

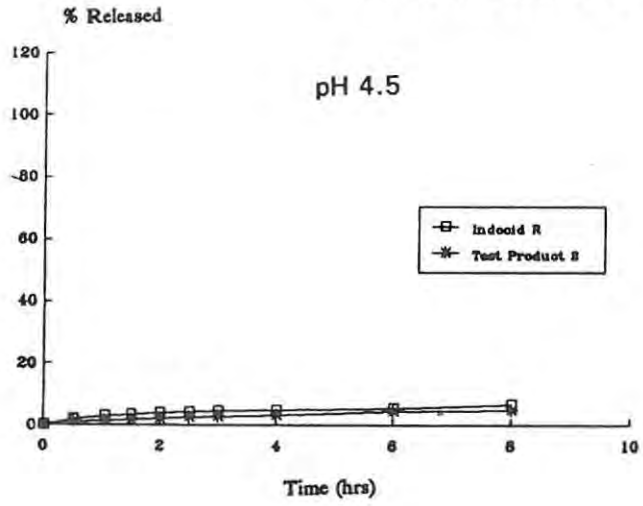
4.2.3.3 Dissolution Rate Studies

(i) Basket Apparatus

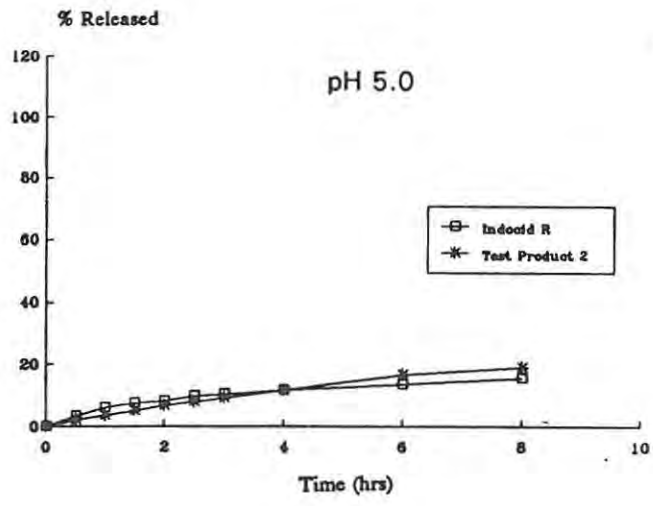
Visual inspection of the dissolution process revealed that the Test Product and reference capsules burst after 2 - 5min of exposure to the dissolution medium. The baskets retained the pellets of the Test Product throughout the test. However, the granules of the reference formulation were only retained during the initial portion of the test upon which fine material sifted into the bulk of the dissolution medium, forming a small undisturbed mound at the base of the dissolution vessel. Upon completion of the dissolution test the baskets were inspected and this revealed that the Test Product left no material after 24h. However, the baskets which contained Indocid R had a white waxy material still present after 24h.

The mean dissolution profiles for Test Product 2 and Indocid R are depicted in Figures 4.7 a - f.

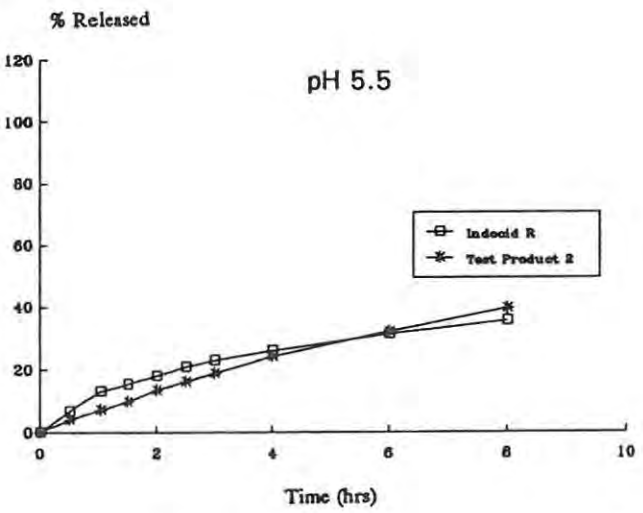
Figure 4.7 Mean Dissolution Rate Profiles of Test Product 2 and Indocid R using the basket apparatus.



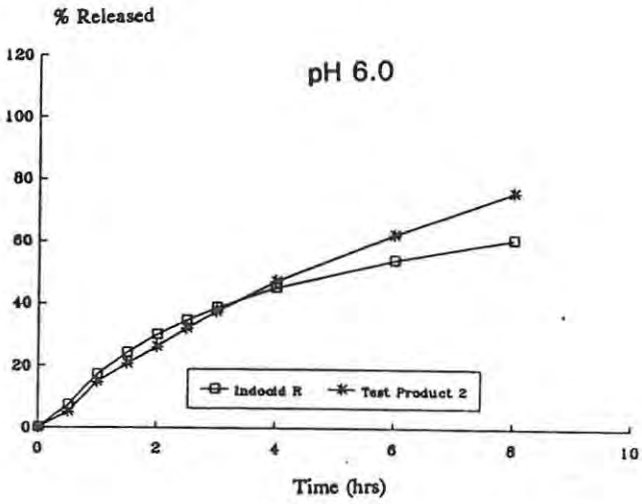

(a)



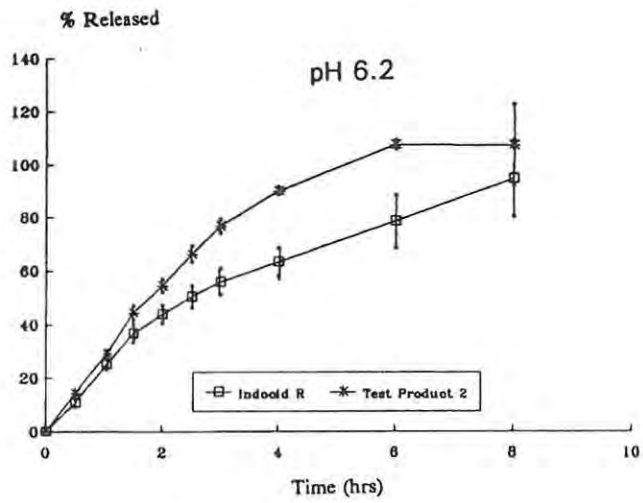

(b)



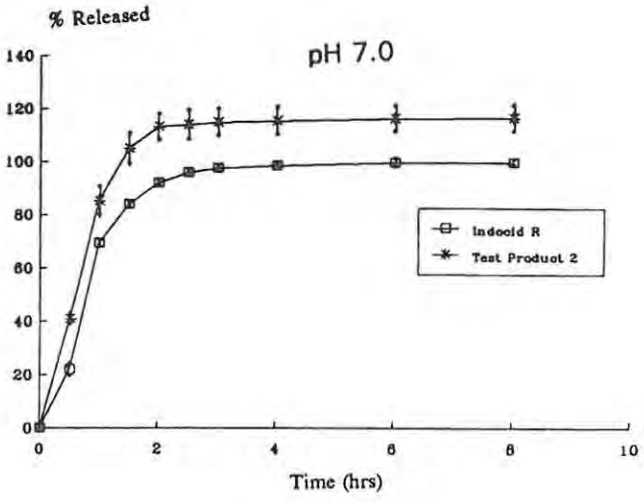

(c)




(d)




(e)




(f)

Inspection of the dissolution profiles of the two products indicates that the rate of release of indomethacin from the Test Product is similar to that of Indocid R at all the pH's studied except pH 6.2 and 7.0. The findings at pH 6.2 are in agreement with those reported during the R & D phase of the Test Product, i.e. the Test Product showing a faster release rate than the reference product.

Inspection of the various profiles obtained using the basket apparatus, as prescribed in the USP monograph for indomethacin, suggests that the products could be expected to behave similarly or that in fact the Test Product could show a faster rate of absorption. These findings are in contrast to those found in the biostudy. It is also evident that the dissolution of indomethacin is pH dependent. This is due to the fact that indomethacin is only sparingly soluble in media with a pH less than 6.0. A further fact to consider is the effect of pH on the formulation and it's possible retarding effect on the release of indomethacin at the various pH.

From plots of log % remaining to be released versus time, the orders of dissolution rate were determined by linear regression and by the method of residuals. The results of these determinations are depicted in Tables 4.3 and 4.4. The goodness-of-fit data are depicted in Table B4.14 (Appendix B).

Table 4.3 Results of dissolution rate order using the basket apparatus.

| pH | Test Product 2 | Indocid R |
|---|---|---|
| | Rate Order | Rate order |
| 4.5 | First order | First order |
| 5.0 | First order | First order |
| 5.5 | First order | 15 % instant release fraction with a single first order fraction equivalent to 85 % of the dose |
| 6.0 | First order | 25 % instant release fraction with a 75 % first order fraction |
| 6.2 | Two first order fractions representing 45% and 55% of the total dose | 10 % instant release fraction with a first order fraction of 90 % |
| 7.0 | --- | First order |

Table 4.4 Summary of dissolution rates using the basket apparatus.

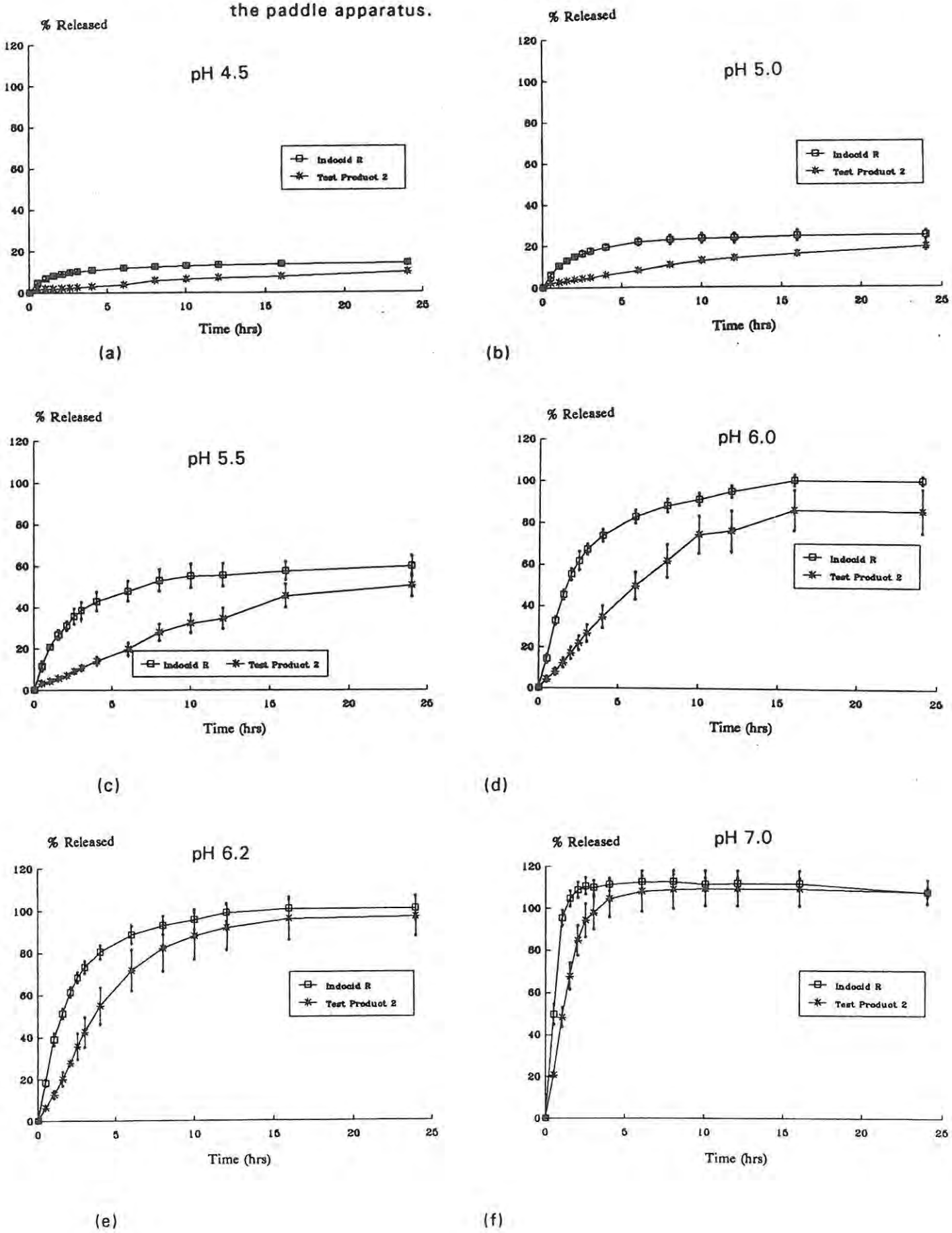
| Dissolution pH | Product | |
|---|---|---|
| | Test Product 2 | Indocid R |
| 4.5 | $K_s = 0.0052h^{-1}$ | $K_s = 0.0052h^{-1}$ |
| 5.0 | $K_s = 0.0258h^{-1}$ | $K_s = 0.0168h^{-1}$ |
| 5.5 | $K_s = 0.0615h^{-1}$ | $K_s = 0.0405h^{1-}$ |
| 6.0 | $K_s = 0.1790h^{-1}$ | $K_s = 0.0995h^{-1}$ |
| 6.2 | $K_s = 0.4260h^{-1}$<br>$K_f = 0.7680h^{-1}$ | $K_s = 0.2360h^{-1}$ |
| 7.0 | --- | $K_s = 1.248h^{-1}$ |

No dissolution rate was determined for the Test Product at pH 7.0 since insufficient data points were available to perform an accurate determination. It is apparent from the above results that the dissolution rate of indomethacin is highly dependent on the pH of the dissolution medium. The rate at pH 6.2 is approximately 45 times greater than the rate determined at pH 4.5 for both products. The differences in rates between the two formulations can possible be ascribed to differences in the interaction of the matrices with the dissolution medium.

(ii) Paddle apparatus

In an attempt to more accurately predict the *in vivo* phenomena, dissolution rate studies employing the paddle apparatus were embarked upon. The dissolution rate studies were conducted under identical conditions as those employed with the basket apparatus using a paddle rate of 50 r.p.m. Visual inspection of the dissolution test revealed that the capsule contents were dumped within 2 - 3min after introduction to the dissolution medium. The Indocid R capsules, however, remained intact for longer periods in media of lower pH. The mass of granules from the Indocid formulation showed a high degree of dispersion in the bulk of the medium, whereas movement of the pellets within the dissolution medium, of the Test Product, was less pronounced. After 24h the reference product left a flaky

Figure 4.8 Mean Dissolution Rate Profiles of Test Product 2 and Indocid R using the paddle apparatus.



material in the medium. No visible material from the Test Product was left in the dissolution medium at the time of the 24h sample.

The mean dissolution profiles obtained, using the paddle apparatus, are depicted in Figure 4.8 a - f. From these plots it is again evident that the dissolution process is pH dependent. Inspection of the profiles also indicates that the release of indomethacin from the Test Product is slower than from the reference product. This is evident throughout the pH range tested. These findings are therefore in closer agreement with the *in vivo* findings. The results of the rate order determinations are depicted in Tables 4.5 and 4.6. The goodness-of-fit data are depicted in Table B4.15 (Appendix B).

Table 4.5 Results of dissolution rate order determinations.

| pH | Test Product 2 | Indocid R |
|---|---|---|
| | Rate Order | Rate order |
| 4.5 | First order | First order |
| 5.0 | First order | First order |
| 5.5 | Two first order fractions representing a fast fraction of 80% and a 20% slow fraction | Two first order fractions, a fast fraction of 50% and a slower fraction of 50% |
| 6.0 | First order | Two first order fractions, a fast fraction of 26% and a slow fraction of 74% |
| 6.2 | First order | Two first order fractions, a fast and slow fraction each equivalent to 50% |
| 7.0 | First order | First order |

Table 4.6 Summary of dissolution rates using the paddle apparatus.

| Dissolution pH | Product | |
|---|---|---|
| | Test Product 2 | Indocid R |
| 4.5 | $K_s = 0.0043h^{-1}$ | $K_s = 0.0037h^{-1}$ |
| 5.0 | $K_s = 0.0089h^{-1}$ | $K_s = 0.0063h^{-1}$ |
| 5.5 | $K_s = 0.0034h^{-1}$<br>$K_f = 0.0245h^{-1}$ | $K_s = 0.0092h^{1-}$<br>$K_f = 0.0650h^{-1}$ |
| 6.0 | $K_s = 0.1247h^{-1}$ | $K_s = 0.3580h^{-1}$<br>$K_f = 0.4870h^{-1}$ |
| 6.2 | $K_s = 0.2090h^{-1}$ | $K_s = 0.2420h^{-1}$<br>$K_f = 1.21h^{-1}$ |
| 7.0 | $K_s = 1.59h^{-1}$ | $K_s = 3.04h^{-1}$ |

where: $K_s$ = Fast first order rate constant

$K_f$ = Slow first order rate constant.

A comparison of the dissolution rate orders obtained from the two procedures used, indicates that the orders determined in both cases are very similar. In all cases the dissolution rate orders were found to be first order. However, an important difference to note is the inability of the paddle method to detect the presence of the 33% instant release fraction present in the Indocid R formulation. Although the analysis of the data obtained from the basket apparatus did not indicate a 33% instant release fraction it did indicate the presence of an immediate release fraction in the order of 10 - 25%, at pH 5.5, 6.0 and 6.2. Inspection of the rates of dissolution indicates that in almost all cases the rates determined by the basket apparatus were faster than those determined by the paddle method. These differences may, however, be due to the choice of paddle rate and not due to the change in procedure.

4.2.3.4 3-D Dissolution profiles

As previously mentioned, CMRD's are exposed to a range of pH as it traverses the GI tract. Dissolution studies conducted in dissolution media of varying pH would therefore be more predictive or indicative of the overall *in vivo* conditions. Three dimensional dissolution profiles of Test Product 2 and Indocid R obtained using the basket apparatus are depicted in Figures 4.9 and 4.10.

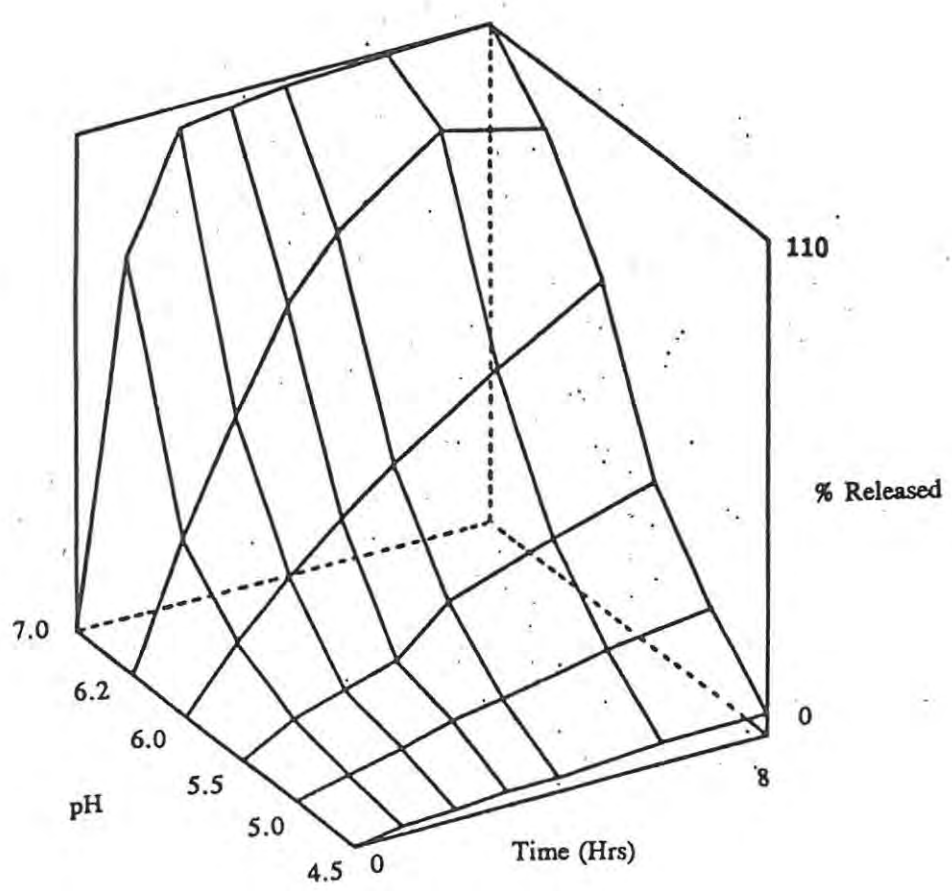



Figure 4.9 3-D Dissolution Rate Profile of Test Product 2 using the basket apparatus.

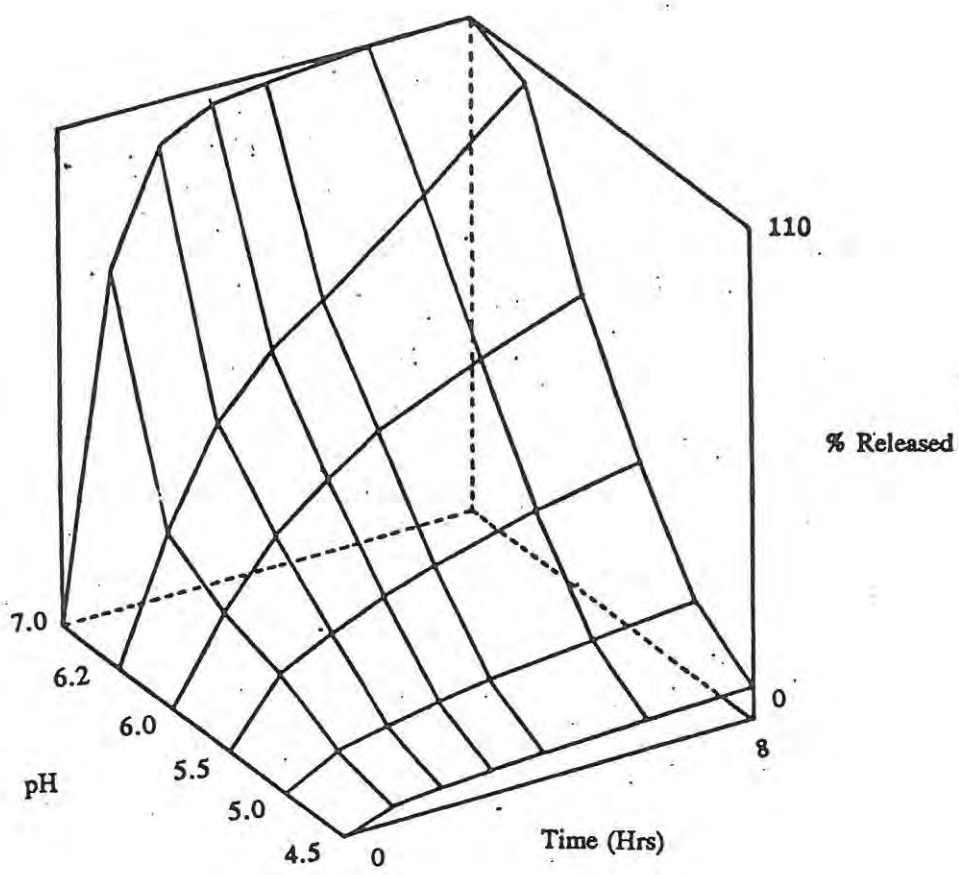



Figure 4.10 3-D Dissolution Rate Profile of Indocid R using the basket apparatus.

Inspection of the profiles indicates that the topographs for the two products are very similar over the entire pH range tested. The region of particular interest is the area of the lower pH's, namely pH 4.5, 5.0 and 5.5. The profiles are very similar in this region which would indicate that the products can be expected to behave similarly in the upper portions of the GIT.

Similar topographs were constructed using the data obtained from the dissolution studies employing the paddle apparatus. These are depicted in Figures 4.11 and 4.12 for Test Product 2 and Indocid R respectively. From an inspection of the topographs it appears that they differ in shape.

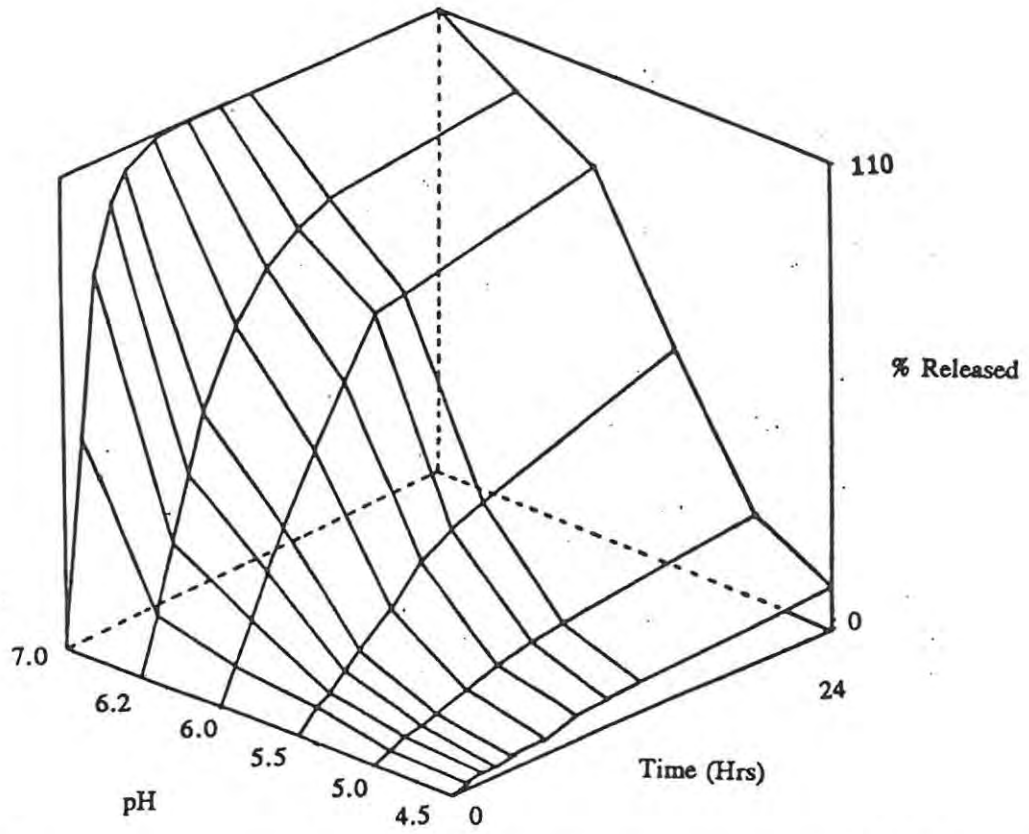



Figure 4.11 3-D Dissolution Rate Profile of Test Product 2 using the paddle apparatus.

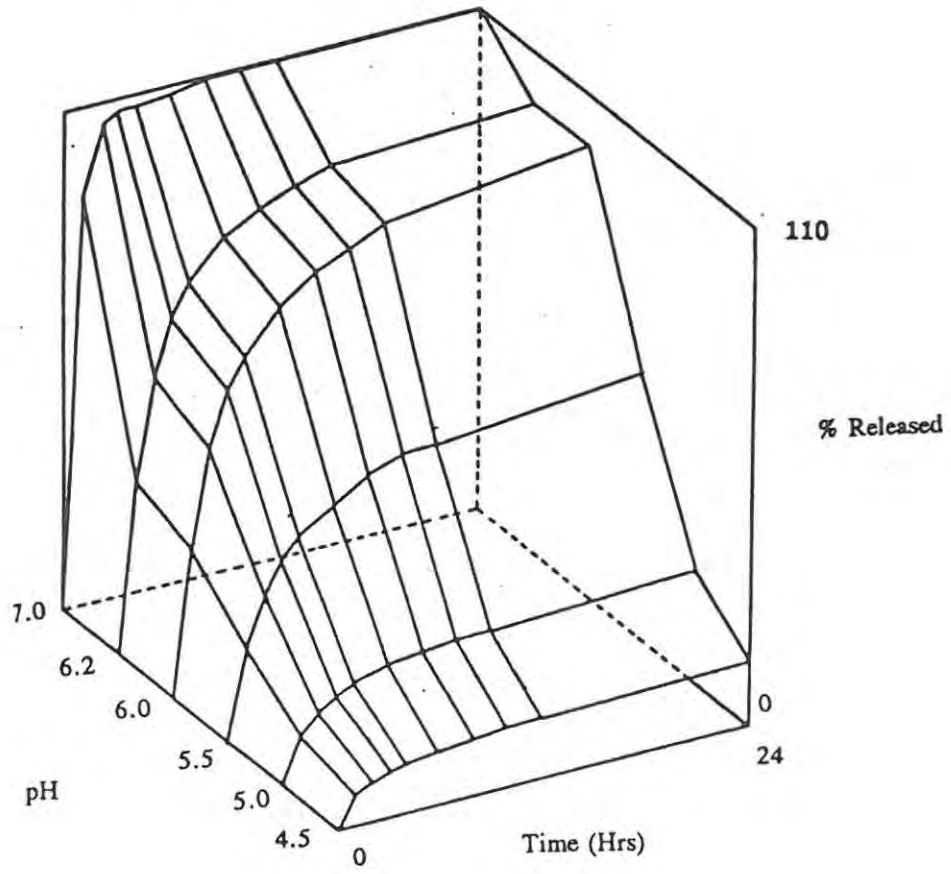



Figure 4.12 3-D Dissolution Rate Profile of Indocid R using the paddle apparatus.

The slope of the Test Product appears to be shallower than that of the reference product. If the region of pH 4.5 to 5.5 is considered, it is clear that the release of indomethacin from Indocid R is more rapid than from the Test Product. These findings are concordant with the *in vivo* results which indicated that indomethacin was more rapidly absorbed from the reference product than from the test formulation.

#### 4.2.3.5 Simulated Serum Profiles (Type A Correlation)

Simulations for Test Product 2 and Indocid R were obtained using the dissolution rate data as set out in Tables 4.3 - 4.6. Due to the lack of comprehensively reported pharmacokinetic parameters for indomethacin in any single research paper, the relevant parameters were obtained from studies conducted by Chaudhari *et al* (181), Alvan *et al* (182) and Yeh *et al* (184). These are summarised in Table 4.7.

Table 4.7 Pharmacokinetic parameters for indomethacin.

| Parameter | Value |
|---|---|
| $\alpha$ (181) | $0.737h^{-1}$ |
| ß (181) | $0.170h^{-1}$ |
| $k_a$ (181) | $1.359h^{-1}$ |
| $V_d$ (182) | 0.831/kg |
| $k_{21}$ (184) | $2.00h^{-1}$ |

(i) Test Product 2

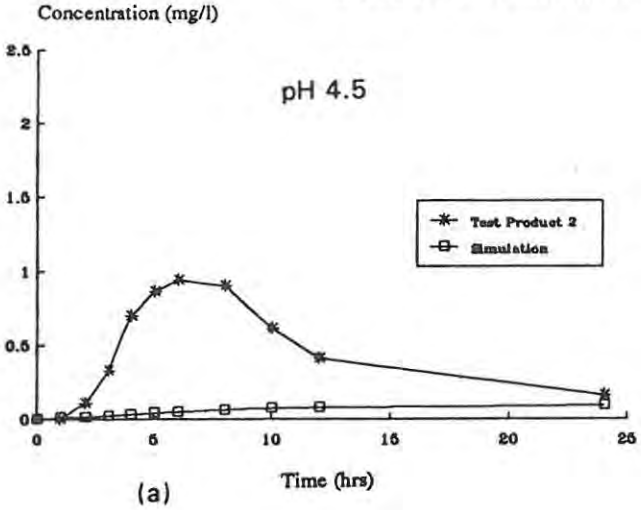
Predicted serum concentration data are given in Tables B4.8 (basket apparatus data) and B4.10 (paddle apparatus data) (Appendix B). The predicted profiles, simulated using dissolution data obtained with the basket apparatus, are depicted in Figures

4.13 a - e. The plot of Test Product 2 represents the *in vivo* profile obtained following the administration of a single 75mg capsule of the formulation. The equations, as set out in model D of section 2.1.4.2, were used to perform the simulations.

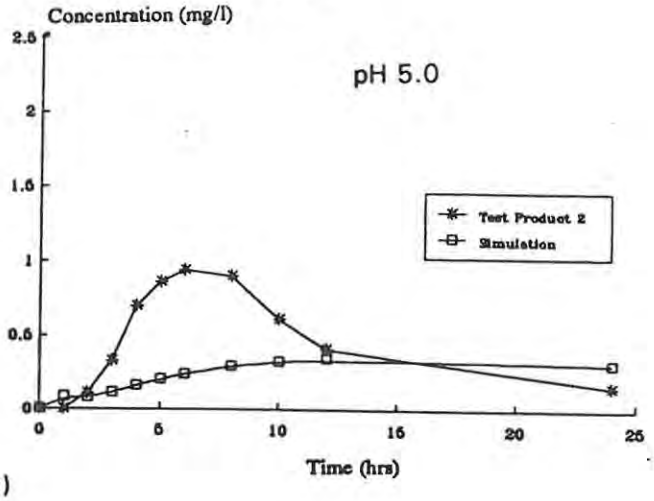
Inspection of the simulated profiles indicates that the simulations obtained from dissolution data obtained at the low pH's, i.e. pH 4.5, 5.0 and 5.5, do not provide accurate predictions of the *in vivo* situation. The predicted serum profiles from the dissolution data obtained at the other pH's appear to be slightly better, however, they also appear not to be accurate reflections of the actual profiles. The profile obtained at pH 6.0 does show some similarities where the predicted profile shows a lag time similar to that found in the *in vivo* situation, although the time to reach the maximum concentration is longer and the predicted plasma concentrations are somewhat elevated for a longer period of time.

Similar predictions were made using the dissolution data obtained following the dissolution studies conducted by the paddle method. These predicted profiles are depicted in Figures 4.14 a - f. Inspection of these again indicated that the predictions obtained are not truly reflective of the *in vivo* situation. The possible exception is the profile obtained at pH 6.2. The two curves again show similar lag times and $t_{max}$ values. The predicted maximum concentration is, however, again higher than the determined $C_{max}$.

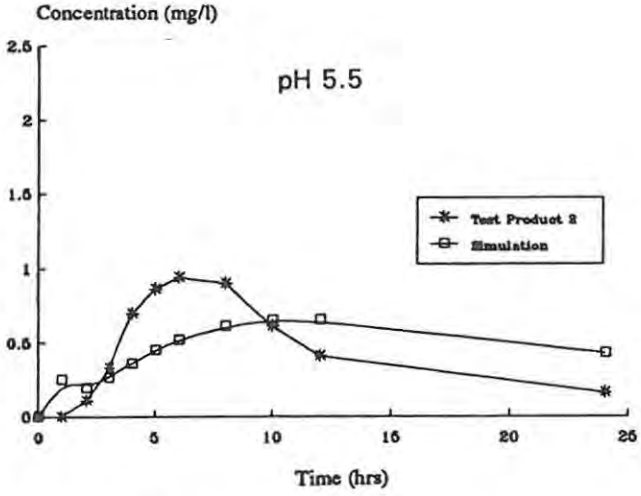
Figure 4.13 Simulated serum concentration profiles for Test Product 2 utilising dissolution data obtained from the basket apparatus.



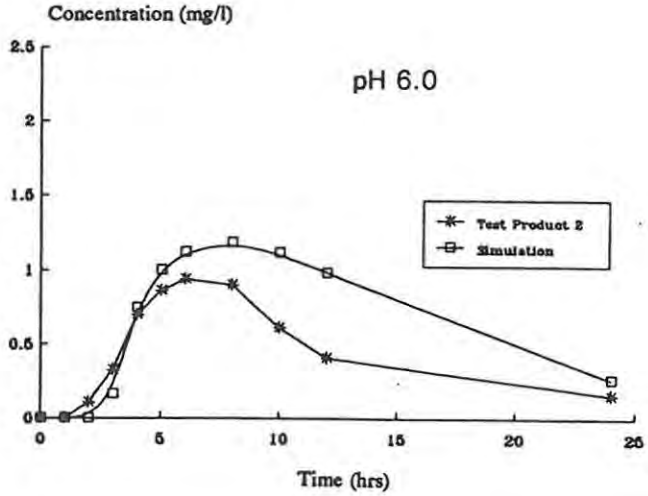

(a)



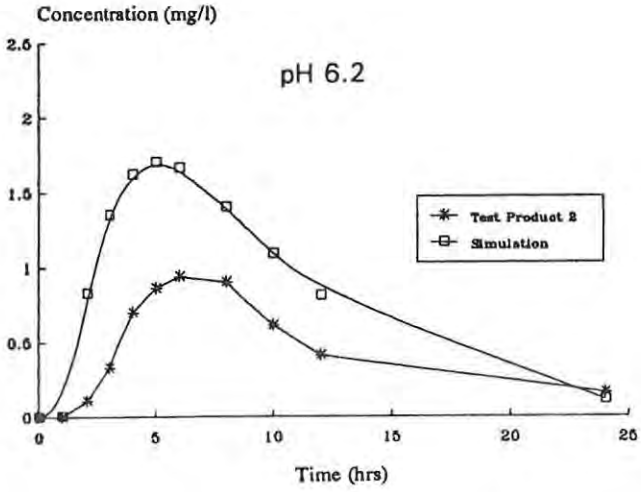

(b)




(c)

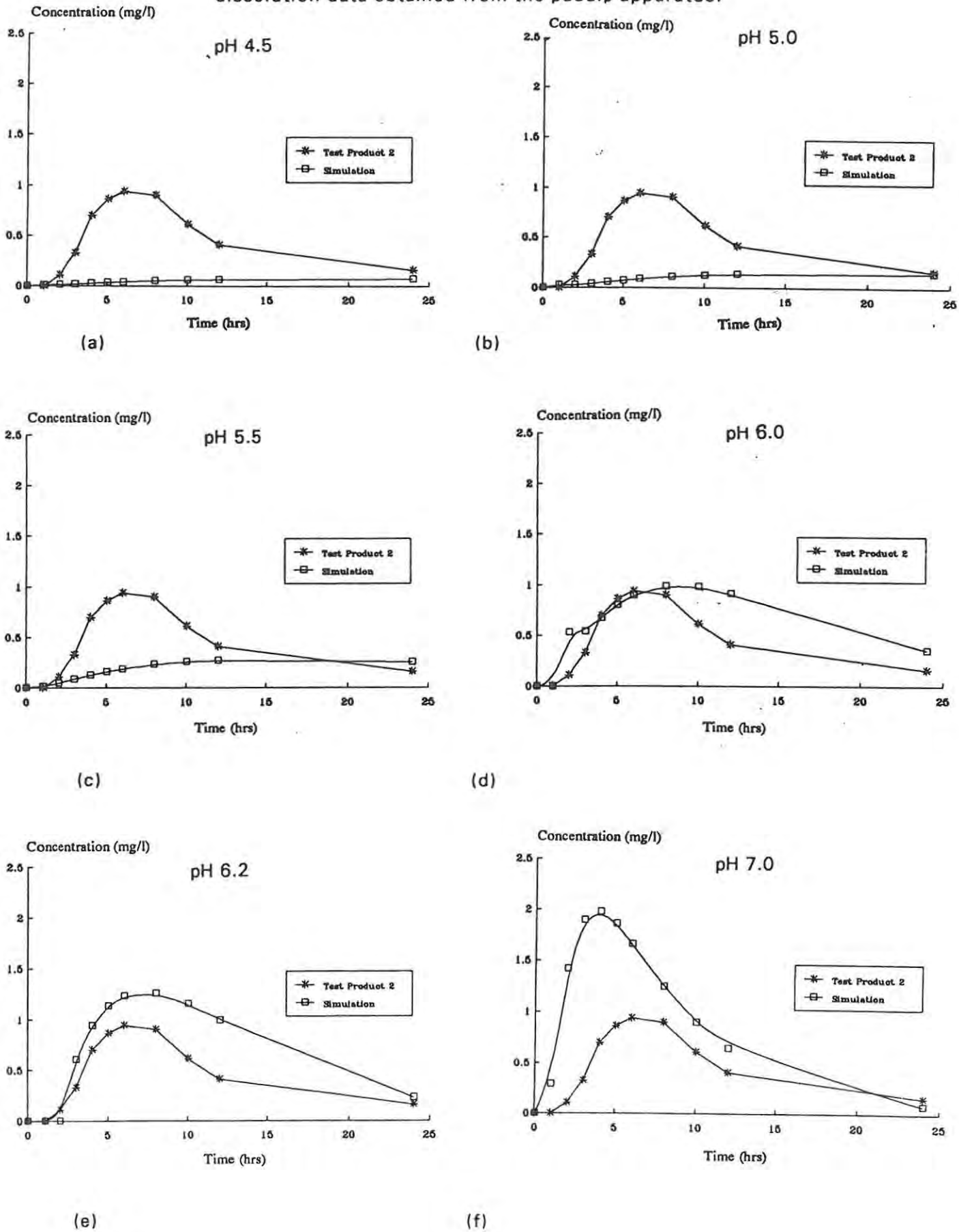



(d)




(e)

Figure 4.14 Simulated serum concentration profiles for Test Product 2 utilising dissolution data obtained from the paddle apparatus.



(ii) Indocid R

Simulations were also performed to determine the expected serum concentration-time curve following the administration of a single 75mg capsule of Indocid R. Simulations were performed using data obtained from dissolution studies conducted utilising the basket apparatus (Figures 4.15 a - f) and the paddle apparatus (Figures 4.16 a - f). Results of the simulations are reported in Tables B4.7 and B4.9 (Appendix B).

The simulations employing dissolution data from the basket apparatus are again not truly reflective of the *in vivo* situation. The profiles obtained at pH 6.2 and 7.0 are perhaps the most predictive, however, the predicted $t_{max}$ values are longer although the $C_{max}$ values of the actual profile and the simulation are comparable.

Inspection of the profiles obtained using data derived from the dissolution studies conducted utilising the paddle apparatus, indicated that only at pH 6.2 and 7.0 the predictions are fairly reflective of the *in vivo* situation. Considering these profiles it is evident that the predicted and actual absorption rates are very similar since the absorption phase of the profiles are superimposable. The values for $t_{max}$ of 4.5h and 5h for the actual and predicted profiles respectively, are in close agreement. The values for $C_{max}$ of 1.5mg/l are highly comparable. However, the elimination phase of the predicted profiles are slower, resulting in predicted elevated serum concentrations. In all cases, the simulated profiles fail to provide a accurate indication of the actual elimination rate. This may cause problems in the prediction of profiles following multiple dose therapy, giving rise to steady state concentrations above those than which would be expected *in vivo.*

Figure 4.15 Simulated serum concentration profiles for Indocid R utilising dissolution data obtained from the basket apparatus.

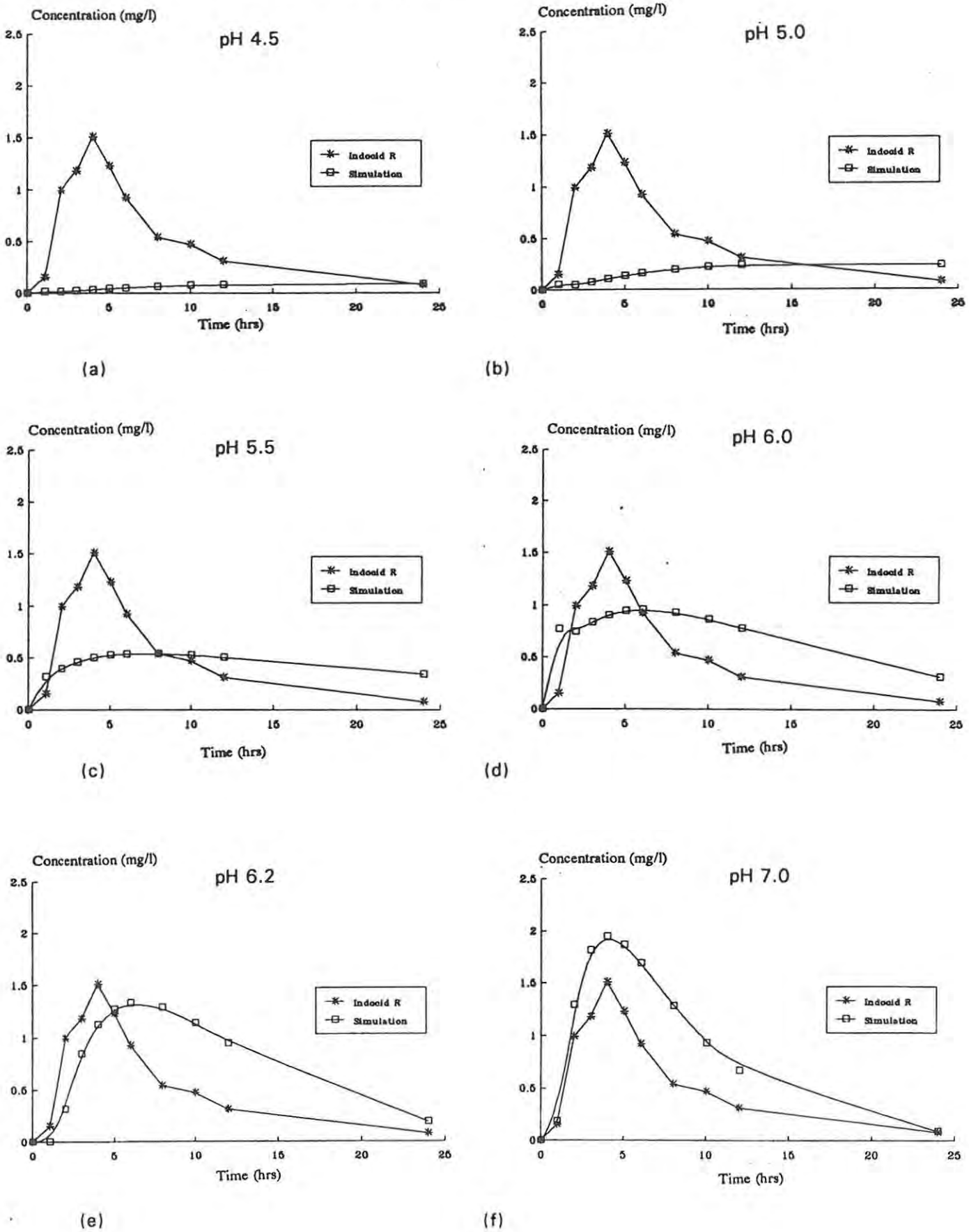
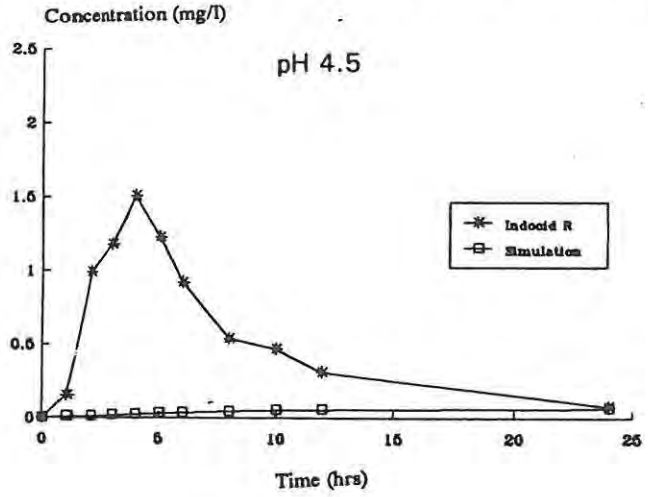
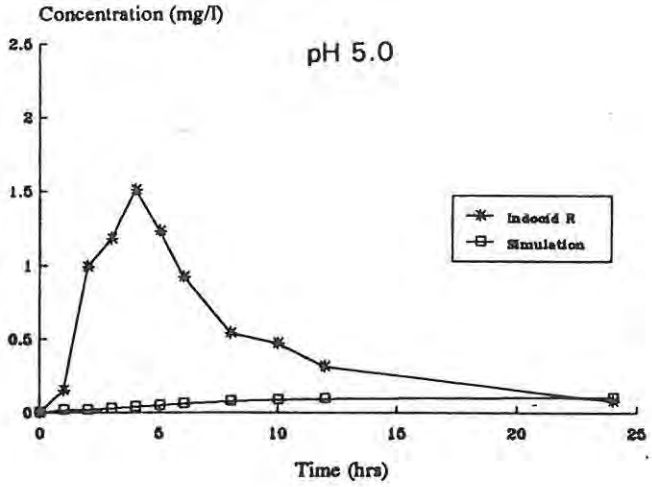


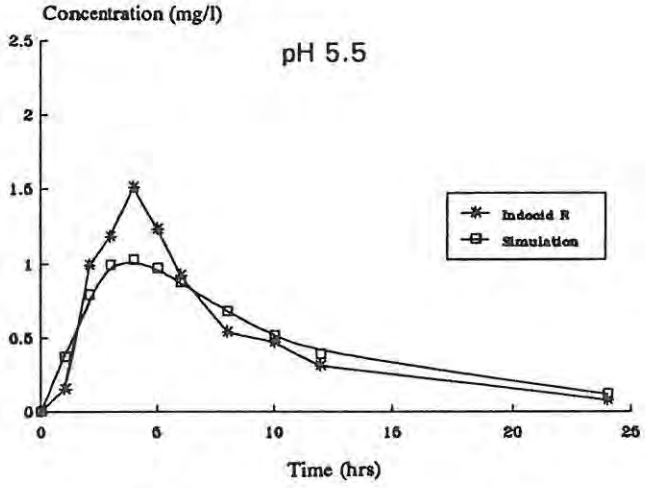
Figure 4.16 Simulated serum concentration profiles for Indocid R utilising dissolution data obtained from the paddle apparatus.



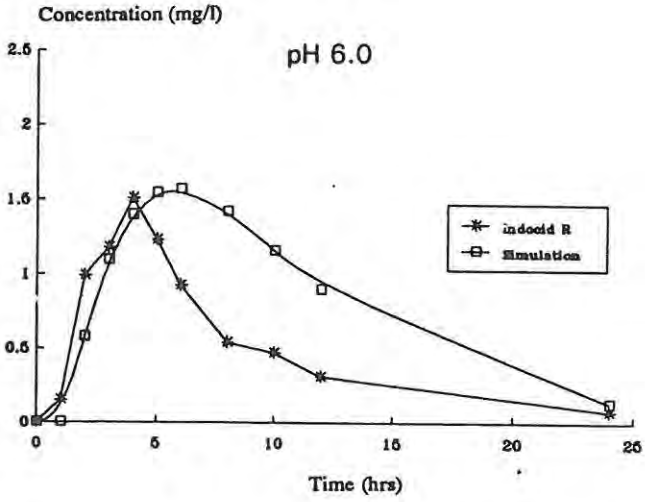

(a)



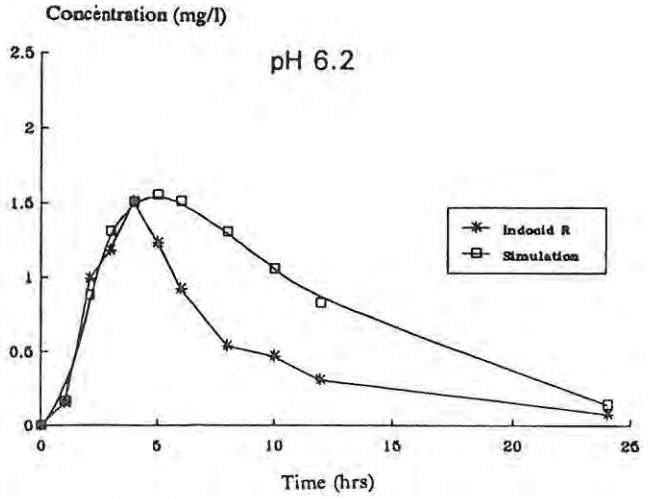

(b)



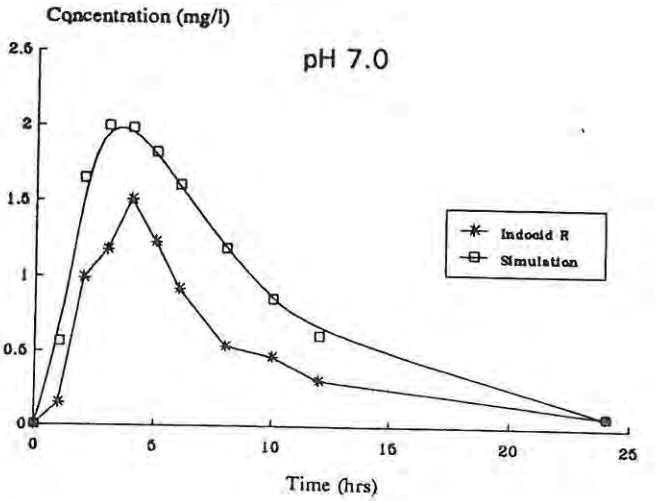

(c)




(d)




(e)




(f)

4.2.3.6 Weibull Distribution Analysis

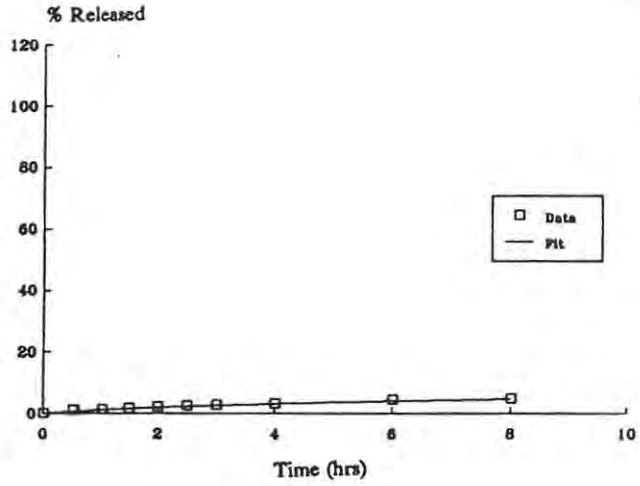
(i) Analysis of dissolution data

Dissolution rate data for both products, obtained from the basket and paddle methods, were plotted and subsequently fitted to the Weibull equation. The observed dissolution rate data and the predicted values calculated according to the Weibull equation are depicted in Figures 4.17 and 4.18 for the Test Product and Figures 4.19 and 4.20 for the reference product. The Weibull function again proved to be robust and versatile as it enabled the reasonable description of all the dissolution profiles. However, the values for the sum of least squares were higher than those found for the theophylline data sets.

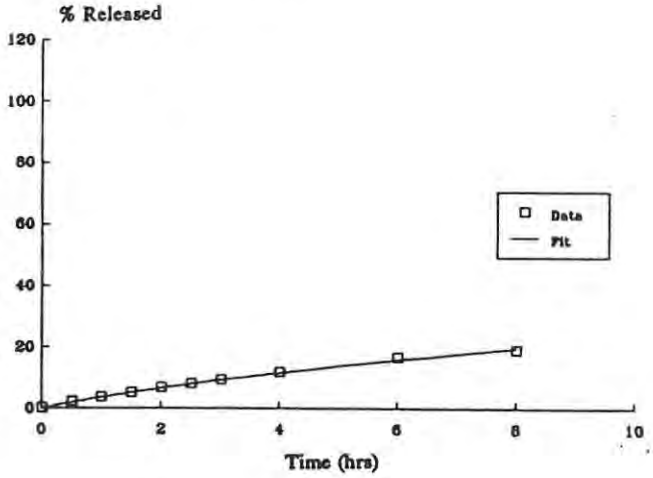
The results indicate that the mean dissolution time, $t_d$, was faster for Indocid R at all the pH's studied when compared to the values obtained for the Test Product. It also appears that the function was unable to fully describe the dissolution profiles for Indocid R obtained using the paddle apparatus even thought the fits to the data appear to be very good. However, when the $t_d$ values are considered at the lower pH's it is evident that these are significantly shorter than those found for the Test Product and Indocid R using the basket apparatus. Bearing in mind that the $t_d$ value is representative of the time to reach 63.2% release it is unlikely that Indocid R will reach this point in 2 hours at the lower pH's.

The detailed parameter values from the Weibull function analysis of the two products are depicted in Tables 4.8 - 4.11. In Figures 4.17 - 4.20 the symbols represent the experimental data whilst the solid line is the best fit according to the Weibull equation.

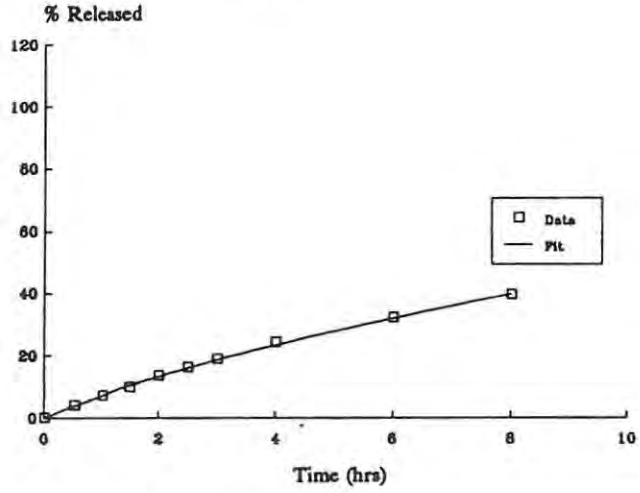
Figure 4.17 Dissolution profiles and Weibull fits for Test Product 2 obtained from dissolution tests employing the basket apparatus.



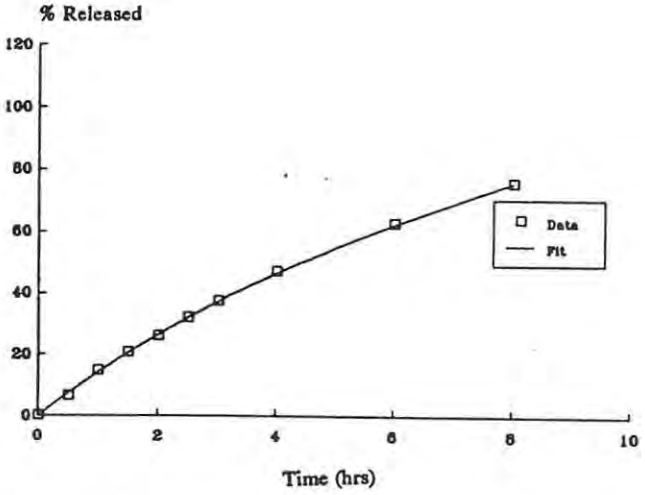

(a) pH 4.5



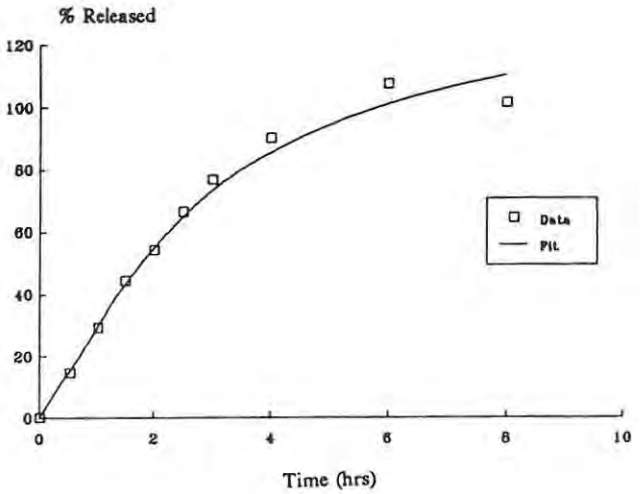

(b) pH 5.0



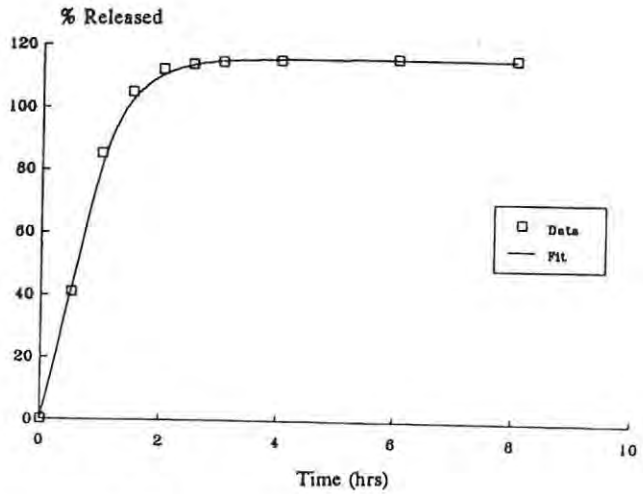

(c) pH 5.5




(d) pH 6.0

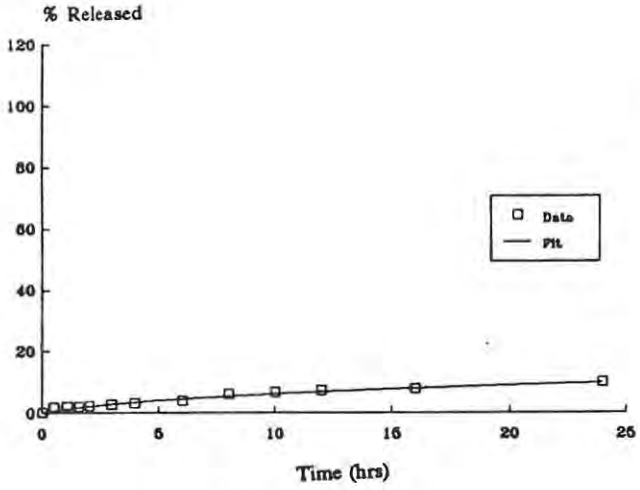



(e) pH 6.2

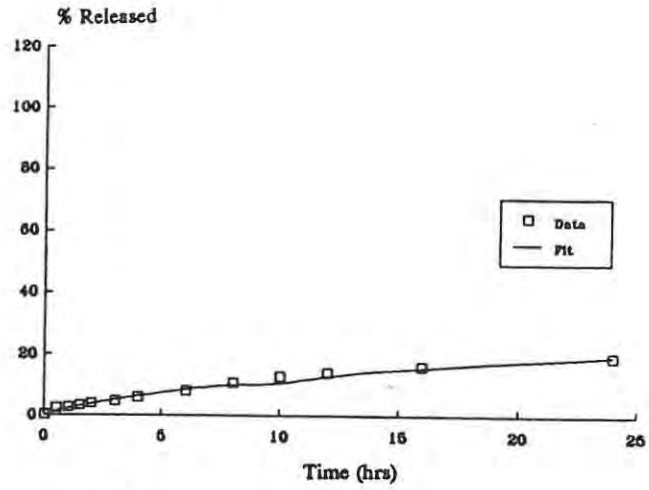



(f) pH 7.0

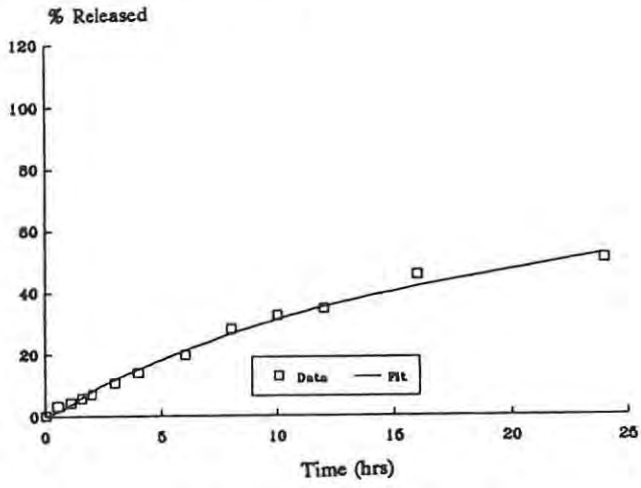
Figure 4.18 Dissolution profiles and Weibull fits for Test Product 2 obtained from dissolution tests employing the paddle apparatus.



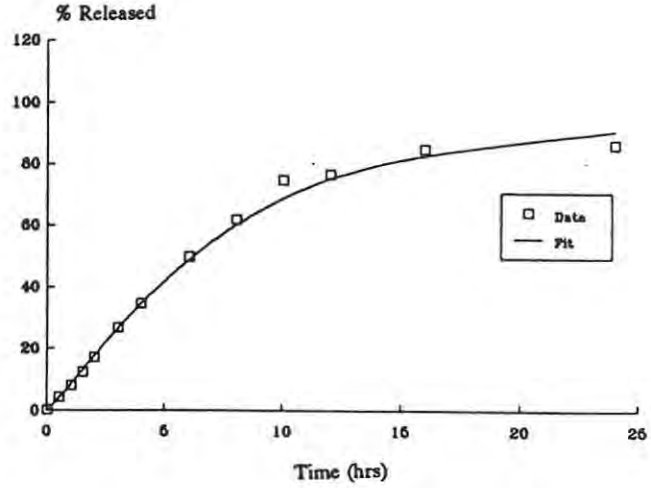

(a) pH 4.5



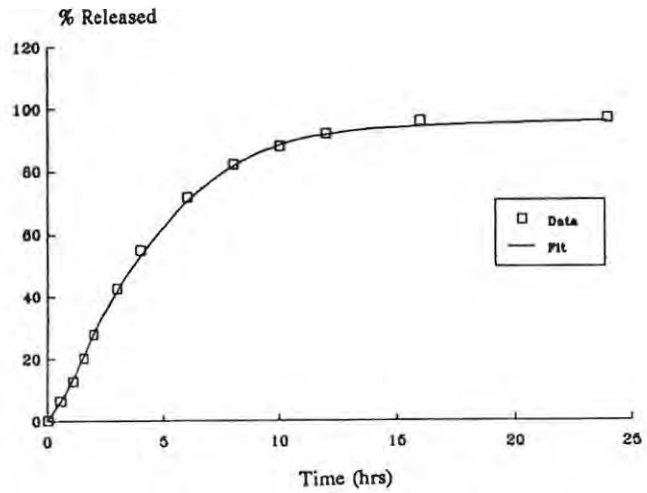

(b) pH 5.0



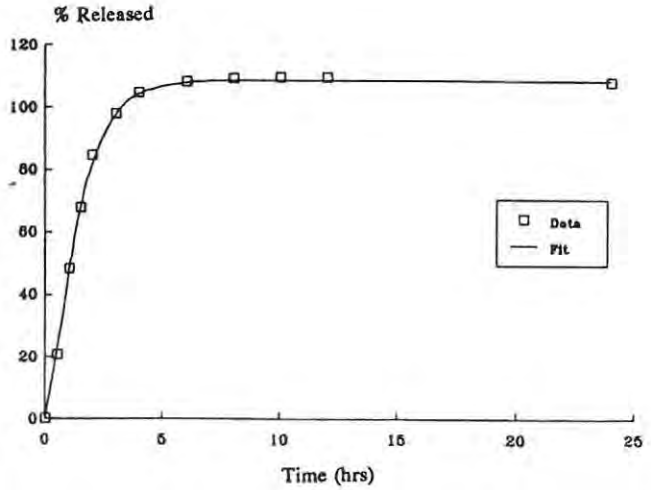

(c) pH 5.5




(d) pH 6.0

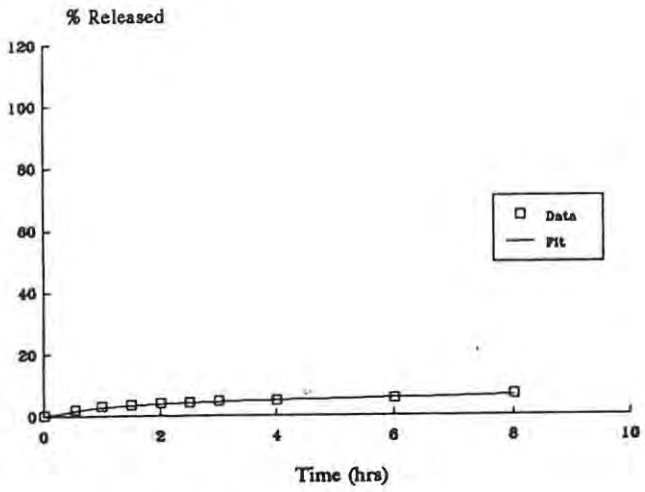



(e) pH 6.2

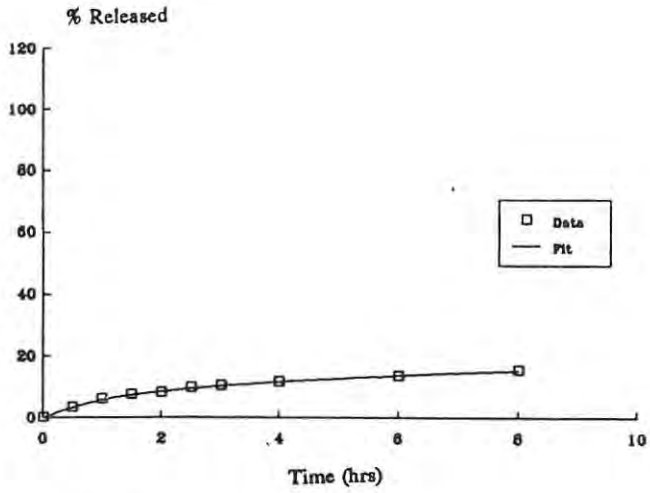



(f) pH 7.0

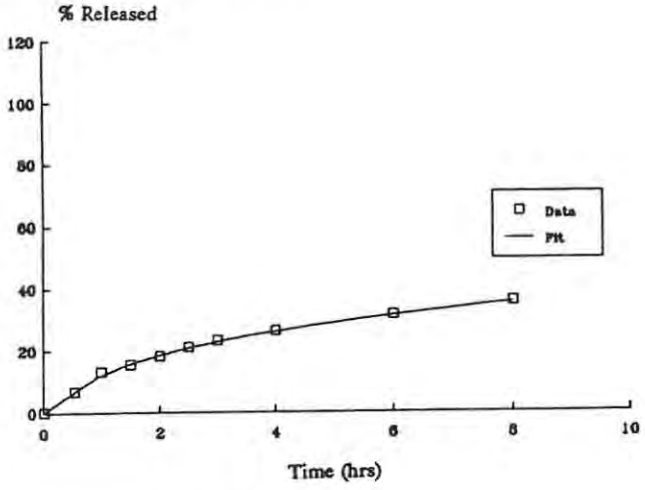
Figure 4.19 Dissolution profiles and Weibull fits for Indocid R obtained from dissolution tests employing the basket apparatus.



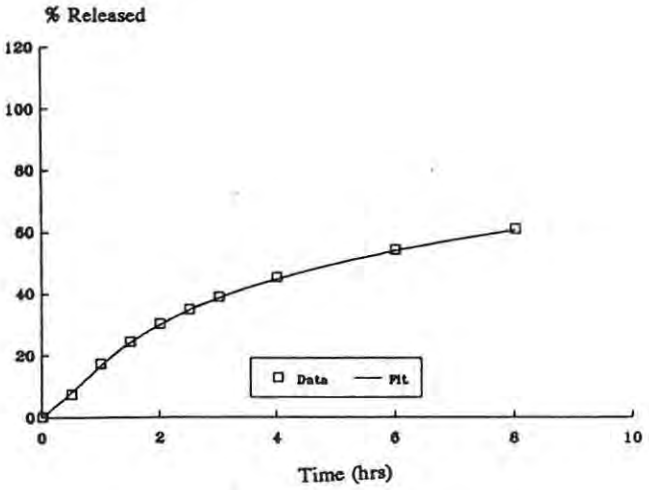

(a) pH 4.5



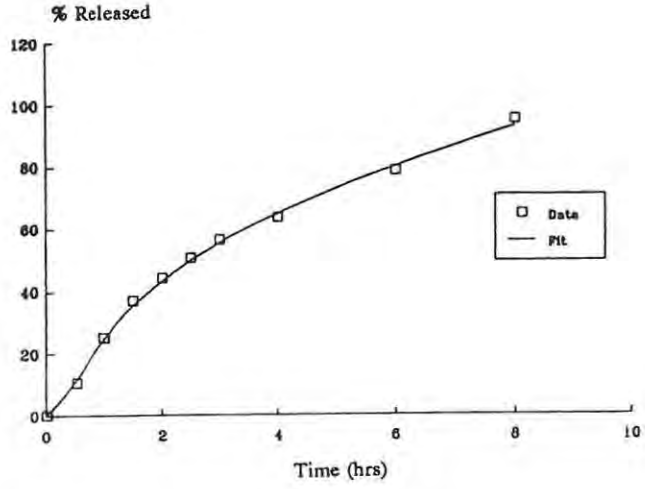

(b) pH 5.0



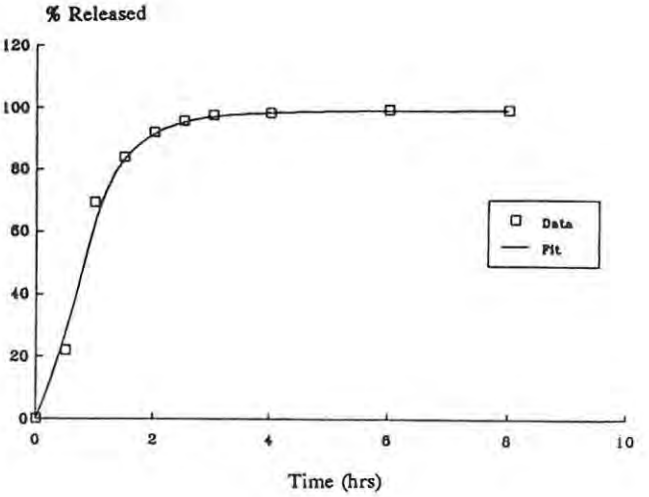

(c) pH 5.5




(d) pH 6.0

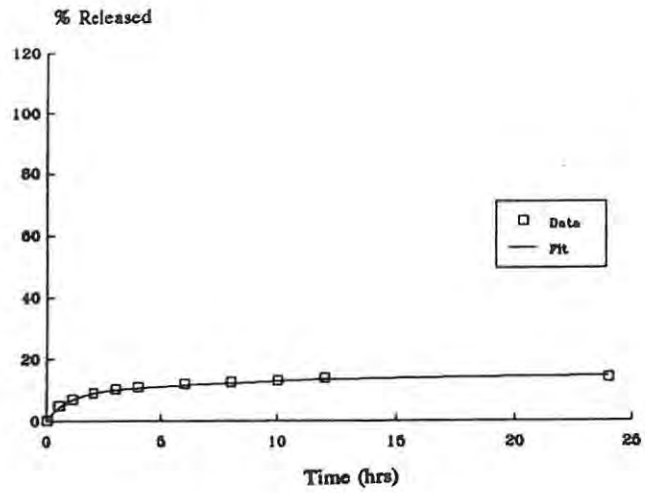



(e) pH 6.2

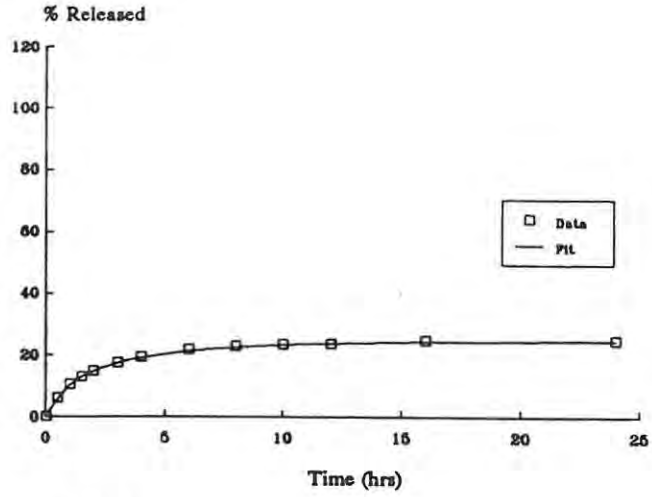



(f) pH 7.0

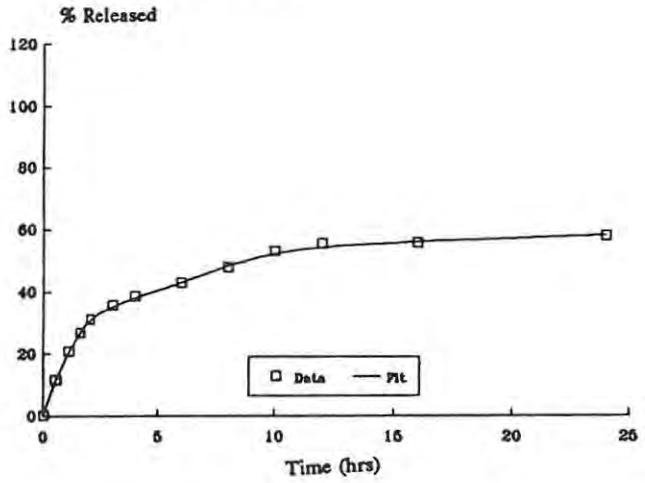
Figure 4.20 Dissolution profiles and Weibull fits for Indocid R obtained from dissolution tests employing the paddle apparatus.



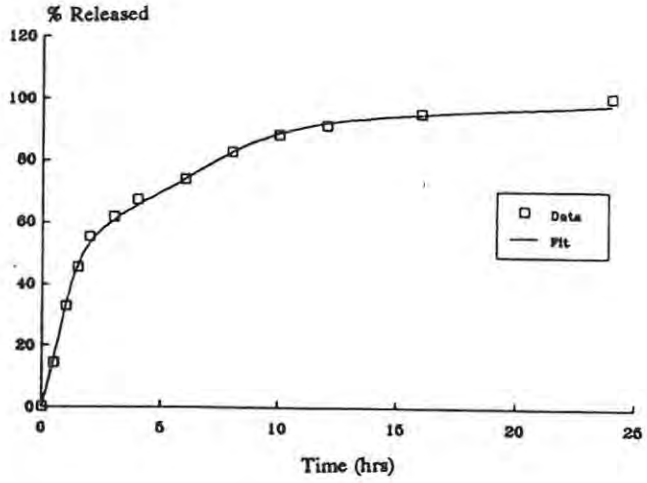

(a) pH 4.5



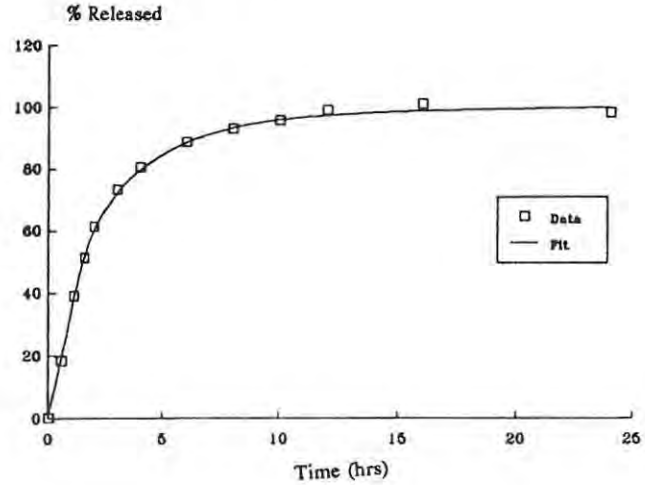

(b) pH 5.0



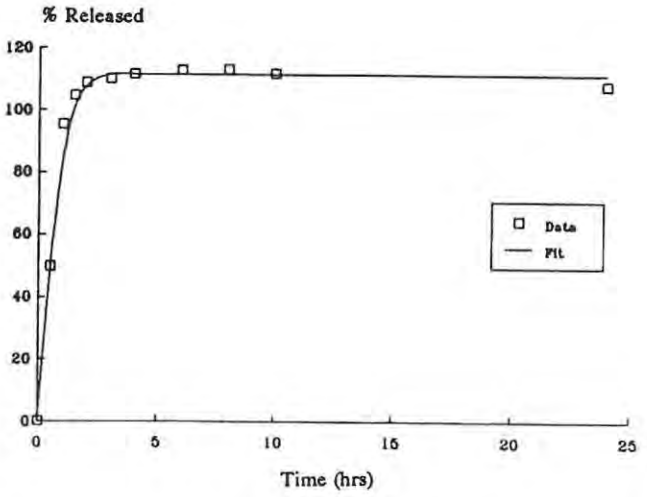

(c) pH 5.5




(d) pH 6.0




(e) pH 6.2




(f) pH 7.0

Table 4.8 Weibull function analysis of dissolution data for Test Product 2 (basket apparatus).

| Parameter | Dissolution pH | | | | | |
|---|---|---|---|---|---|---|
| | 4.5 | 5.0 | 5.5 | 6.0 | 6.2 | 7.0 |
| $t_0$ (h) | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.42 |
| $t_d$ (h) | 20.20 | 20.00 | 20.00 | 8.86 | 3.00 | 0.51 |
| ß | 0.71 | 0.89 | 0.89 | 0.98 | 1.11 | 0.5 |
| $F^{\infty}$ (%) | 11.93 | 55.07 | 111.5 | 127.23 | 116.23 | 126,76 |

Table 4.9 Weibull function analysis of dissolution data for Test Product 2 (paddle apparatus).

| Parameter | Dissolution pH | | | | | |
|---|---|---|---|---|---|---|
| | 4.5 | 5.0 | 5.5 | 6.0 | 6.2 | 7.0 |
| $t_0$ (h) | 0.00 | 0.00 | 0.31 | 0.00 | 0.00 | 0.11 |
| $t_d$ (h) | 24.37 | 24.49 | 19.97 | 7.64 | 4.74 | 1.40 |
| ß | 0.77 | 0.82 | 0.89 | 1.17 | 1.27 | 1.22 |
| $F^{\infty}$ (%) | 15.59 | 31.46 | 76.44 | 92.61 | 95.82 | 108.60 |

Table 4.10 Weibull function analysis of dissolution data for Indocid R (basket apparatus).

| Parameter | Dissolution pH | | | | | |
|---|---|---|---|---|---|---|
| | 4.5 | 5.0 | 5.5 | 6.0 | 6.2 | 7.0 |
| $t_0$ (h) | 0.20 | 0.26 | 0.26 | 0.30 | 0.33 | 0.40 |
| $t_d$ (h) | 16.00 | 23.59 | 26.07 | 4.74 | 10.00 | 0.50 |
| ß | 0.45 | 0.49 | 0.53 | 0.74 | 0.65 | 0.82 |
| $F^{\infty}$ (%) | 12.36 | 34.84 | 86.44 | 79.72 | 162.14 | 99.48 |

Table 4.11 Weibull function analysis of dissolution data for Indocid R (paddle apparatus).

| Parameter | Dissolution pH | | | | | |
|---|---|---|---|---|---|---|
| | 4.5 | 5.0 | 5.5 | 6.0 | 6.2 | 7.0 |
| $t_0$ (h) | 0.20 | 0.21 | 0.21 | 0.37 | 0.30 | 0.00 |
| $t_d$ (h) | 2.05 | 2.10 | 2.80 | 2.52 | 1.86 | 0.78 |
| ß | 0.47 | 0.65 | 0.68 | 0.65 | 0.72 | 1.30 |
| $F^{\infty}$ (%) | 14.72 | 25.07 | 60.42 | 102.41 | 99.69 | 112.24 |

**(ii) Weibull Fits to Wagner-Nelson Absorption plots**

Wagner (128) has reported that the Wagner-Nelson Absorption method can be successfully applied to the two-compartment open model. Wagner has also demonstrated that the Wagner-Nelson method can be applied not only to estimate $k_a$ but also $k_{21}$, $k_{12}$ and $k_e$ and that this method can be more accurate than the classical feathering or back-projection method.

The mean concentration-time data from the Test Product and Indocid R were transformed using the Wagner-Nelson method to obtain the absorption plots. The method could, however, not be successfully applied to the individual subject data due to the lack of sufficient data points in the terminal elimination phase. The lack of sufficient data points in this region led to the inability to calculate the terminal rate constant due to the non-linearity of the log data. The absorption plots of the mean profiles were subsequently fitted to the Weibull equation. The plots for the mean profiles are depicted in Figures 4.21 a and b.

The Weibull function appeared to be robust enough to enable a description of the absorption rate data, even though the values for the least squares summation were very large. The Weibull parameters obtained for the analysis of the data are summarised in Table 4.12.

Table 4.12 Weibull function analysis of absorption data for Test Product 2 and Indocid R.

| Parameter | Mean data | |
|---|---|---|
| | Test Product 2 | Indocid R |
| $t_0$ (h) | 1.50 | 0.50 |
| $t_d$ (h) | 3.51 | 1.43 |
| ß | 2.40 | 1.86 |
| $F^{\infty}$ (%) | 104.51 | 112.25 |

The time for 63.2% of the drug to be absorbed *in vivo*, $t_d$, was markedly longer for the Test Product than for the reference product. The curves showed sigmoidicity which is in accordance with the high values for ß. It must, however, be pointed out that the data shown are based on the mean serum concentration profile and not on individual data sets.

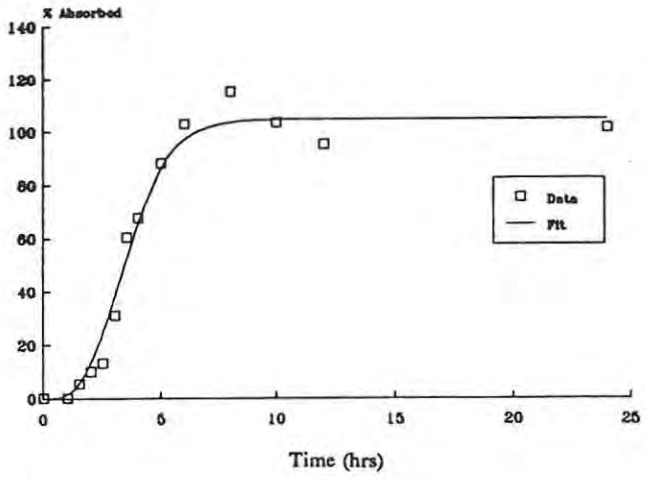



Figure 4.21(a) Mean Wagner-Nelson plot of serum concentration-time curve after administration of Test Product 2. The solid line represents the Weibull fit to the data.

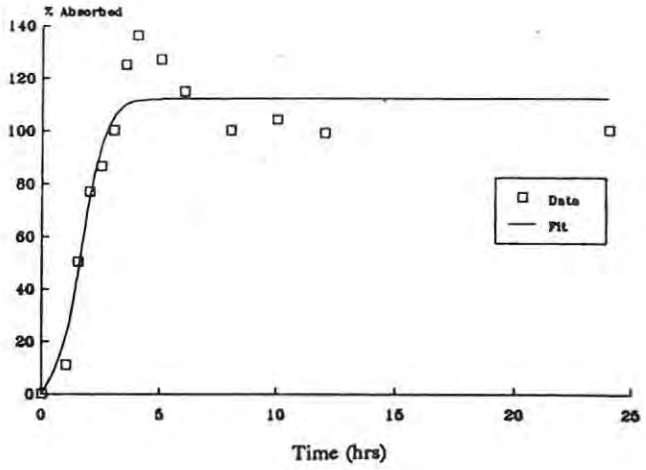



Figure 4.21(b) Mean Wagner-Nelson plot of serum concentration-time curve after administration of Indocid R. The solid line represents the Weibull fit to the data.

4.2.3.7 Moment analysis

The lack of sufficient points in the elimination phase of the individual subject profiles did not allow for the calculation of the parameters for these data sets. The parameters could, however, be estimated for the mean subject profiles. Results of the moment analysis of the mean data for Test Product 2 and Indocid R are summarised in Table 4.13.

The long values for MRT are indicative that the formulations are CMRD's as long values for this parameter are usually an indicator of the efficiency of the dosage form as a sustained release product. It is evident that the MAT is considerably longer for the Test Product than for Indocid R, and is in agreement with the longer $t_{max}$ for the Test Product. The MRT is also longer for the Test Product when compared to that of Indocid R.

Table 4.13 Pharmacokinetic and Moment analysis parameters for Test Product 2 and Indocid R.

| Parameter | Mean Data | |
|---|---|---|
| | Test Product 2 | Indocid R |
| $K_e$ ($h^{-1}$) | 0.10 | 0.12 |
| $AUC_\infty$ (mg/l.h) | 11.53 | 11.50 |
| $AUMC_\infty$ (mg/l.$h^2$) | 153.88 | 103.08 |
| MRT (h) | 13.34 | 8.96 |
| MAT (h) | 3.40 | 0.69 |

### 4.2.4 Test Product 3

#### 4.2.4.1 Objectives

The failure of Test Product 2 to provide *in vivo* results similar to those of Indocid R prompted the modification of the product, with the purpose of designing it to mimic Indocid R more closely. As mentioned previously, Indocid R consists of two components, namely a 25mg instant release portion in combination with a 50mg sustained release portion. Test Product 2 consists solely of a sustained release portion. It was therefore decided to develop a dosage form which contained a 25mg instant release fraction and a 50mg sustained release fraction based on Indocid 25mg capsules and Test Product 2.

#### 4.2.4.2 Dosage form development

Dosage form content assays conducted on Test Product 2 indicated that the capsules contained approximately 287mg of pellets per 75mg of indomethacin. To

obtain a capsule which contained the correct proportions of indomethacin, the contents of a single Indocid 25mg capsule was combined with 193mg (representing 50mg indomethacin) of the pellets of Test Product 2. The material was filled into size 00 capsules (Elanco, USA). A content uniformity assay conducted on three capsules indicated that the capsules contained 80.90 ± 1.41mg indomethacin.

4.2.4.3 Dissolution Rate Studies

Dissolution rate studies were conducted as set out in section 4.2.2.2. Drug concentrations were determined spectrophotometrically. The mean dissolution profile obtained at pH 6.2 is depicted in Figure 4.22. The profile represents the mean of three replicate determinations.

Inspection of the dissolution profile indicates that the profile closely approximates that of Indocid R. The dissolution rate was determined to be $0.170h^{-1}$.The results suggest that the slower dissolution rate of Test Product 2 may have been due to the lack of the 25mg instant release portion. The dissolution profile obtained at pH 6.2 also suggests that Test Product 3 may be expected to behave similarly to Indocid R *in vivo*.

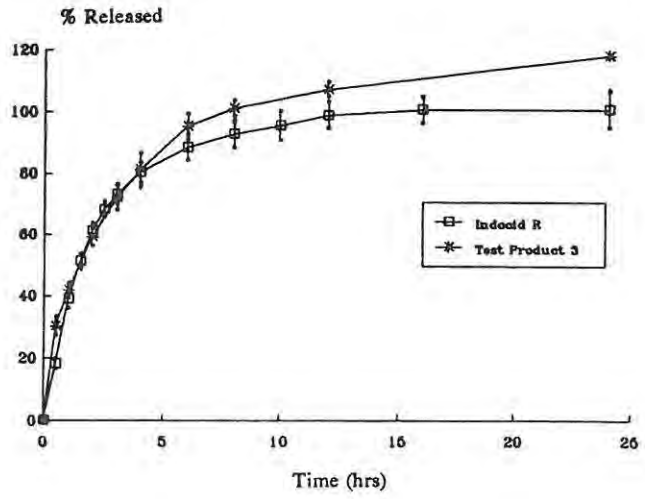



Figure 4.22 Mean dissolution profile of Test Product 3 using the paddle method in phosphate buffer of pH 6.2.

4.2.4.4 Simulated Serum Profile

In order to establish the expected *in vivo* serum concentration-time profile for Test Product 3, simulations were conducted according to the methods set out in section 4.2.3.5. Simulations were conducted over the range of pH's tested and the results are depicted in Table B4.11 (Appendix B). The predicted profile obtained at pH 6.2 was selected as the profile on which to predict the outcome of a bioavailability study, and is depicted in Figure 4.23.

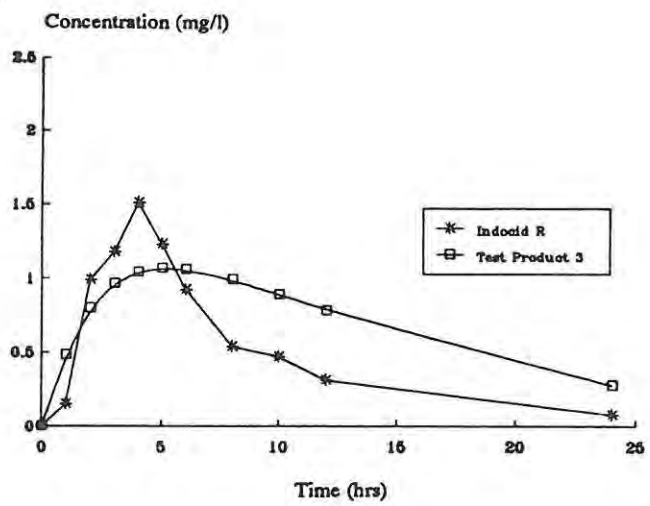



Figure 4.23 Predicted serum profile for Test Product 3 obtained at pH 6.2.

Inspection of the profile suggests that the time course for the Test Product would approximate that of Indocid R. The predicted $t_{max}$ of 4h is similar to that of the reference product although the expected $C_{max}$ value is lower.

4.2.5 Conclusions

The results from the UV and HPLC analysis correlated well and it was therefore decided to perform all routine analysis of the dissolution samples using UV spectrophotometric methods. The release of indomethacin beyond 100% of labelled

claim can possibly be ascribed to excess indomethacin content even though a capsule content uniformity test indicated that the capsules contained only a marginal excess. However no reason was found to explain the release of indomethacin beyond 110% which is the USP upper limit for capsule content.

From the dissolution studies undertaken employing the basket apparatus it is evident that the results do not reflect the *in vivo* findings. The results indicated that the Test Product should have behaved similarly to the reference product, with the Test Product possibly having a faster absorption. The basket method was, however, able to verify the existence of the immediate release fraction of Indocid R even though only a maximum of 25% was accounted for. This is marginally less than the 33% which is reported in the literature.

Dissolution rate studies employing the paddle apparatus allow for a more accurate prediction of the *in vivo* behaviour of Test Product 2 and Indocid R. The paddle method successfully differentiated between the faster Indocid R and the slower Test Product. It therefore appears that the paddle method should perhaps replace the prescribed basket apparatus as the method of choice for the dissolution rate testing of extended-release indomethacin capsule formulations. However, it remains to be established whether the paddle method would be able to differentiate between other formulations of indomethacin before any conclusive decision can be made. The paddle method in contrast to the basket method, was, however, not able to confirm the presence of the immediate release fraction of Indocid R.

The method of representing dissolution rate data in the form of three dimensional topographs was able to successfully highlight differences between Test Product 2 and Indocid R. The topographs constructed from dissolution data obtained with the paddle method indicated that Test Product 2 shows a decreased release rate at the lower pH's studied when compared to Indocid R. Topographs constructed with data obtained from the basket method indicated that the two products had similar release

profiles. The following conclusions can therefore be made: (1) the official USP dissolution test for extended-release indomethacin formulations, and specifically for the two products tested, is not sensitive enough to predict product differences; (2) the basket method in conjunction with pH profiling was also unable to predict the *in vivo* findings, and (3) the use of the paddle method with pH profiling could indicate possible *in vivo* bioavailability problems.

Using pharmacokinetic parameters obtained from mean data, predictions were possible for both Test Product 2 and Indocid R. Simulations based on dissolution data obtained with the paddle apparatus yielded more accurate predictions for both products. These findings support the suggestion that the paddle apparatus should be employed to evaluate the dissolution characteristics of extended-release capsule formulations of indomethacin. Although the simulated profiles are not as predictive as those determined for theophylline, they do provide an indication of the *in vivo* response expected from the two dosage forms tested. It, however, must be established whether the methods demonstrated for these two formulations are also able to predict the expected *in vivo* response for other extended-release formulations of indomethacin. It is also apparent that the predicted profiles could have forewarned the outcome of the *in vivo* bioavailability study that was undertaken on the formulations.

It is suggested that on the basis of the dissolution profile obtained at pH 6.2 and the simulated profile obtained from this, that the test formulation, Test Product 3, could be expected to provide an *in vivo* response similar to that of Indocid R. These findings would however need to be substantiated with a pilot scale bioavailability study in order to establish the accuracy of the predictive serum profiles.

4.2.6 Summary

The failure of the official compendial method for the dissolution testing of extended-release indomethacin capsules was demonstrated for two formulations. The conversion to the alternative USP dissolution method, namely the paddle method, allowed for the accurate prediction of the *in vivo* behaviour of the two dosage forms tested. It is suggested that the basket apparatus be replaced with the paddle apparatus for the routine dissolution testing of indomethacin CMRD's although the utility of the method has only been demonstrated for two formulations. In order to substantiate these suggestions, additional dosage forms would have to be assessed using this method.

The utility of dissolution topographs was again demonstrated with success. The topographs allowed for the identification of a product which had been shown to exhibit a delayed *in vivo* absorption profile. It appears that the routine use of profiling CMRD's over a range of pH may forewarn of any possible bioavailability problems.

The use of predictive or simulated serum concentration-time profiles was successfully demonstrated for Test Product 2 and Indocid R. The simulations were able to give an approximation of the expected outcome of a bioavailability study. Furthermore, simulations based on the dissolution data of an "in-house" formulation indicated that this product would behave similarly to Indocid R.

The use of predictive methods provide for an alternative to expensive pilot bioavailability studies. It must, however, be borne in mind that these methods will unlikely be able to completely replace *in vivo* methods but they are of use as an adjunct and aid in the development of new CMRD's

Table B4.1 Dissolution rate data for Indocid R using basket apparatus

| Time (Hrs) | pH | | | | | |
|---|---|---|---|---|---|---|
| | 4.50 | 5.00 | 5.50 | 6.00 | 6.20 | 7.00 |
| 0.00 | 0.00 ± 0.00[1] | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 0.50 | 1.89 ± 0.16 | 3.43 ± 0.47 | 6.78 ± 1.16 | 7.34 ± 0.67 | 10.57 ± 1.30 | 22.00 ± 2.34 |
| 1.00 | 2.98 ± 0.49 | 6.17 ± 1.39 | 13.07 ± 2.44 | 17.14 ± 1.31 | 25.11 ± 1.90 | 69.26 ± 1.15 |
| 1.50 | 3.44 ± 0.70 | 7.45 ± 1.74 | 15.33 ± 1.16 | 24.16 ± 1.40 | 36.72 ± 3.40 | 83.94 ± 0.74 |
| 2.00 | 3.94 ± 0.89 | 8.20 ± 1.51 | 18.08 ± 0.86 | 29.90 ± 1.66 | 43.96 ± 3.40 | 91.84 ± 0.96 |
| 2.50 | 4.19 ± 1.13 | 9.77 ± 2.32 | 20.84 ± 1.06 | 34.61 ± 1.31 | 50.41 ± 4.10 | 95.62 ± 1.05 |
| 3.00 | 4.52 ± 1.13 | 10.34 ± 2.60 | 22.96 ± 4.36 | 38.67 ± 1.05 | 56.01 ± 4.80 | 97.38 ± 1.25 |
| 4.00 | 4.91 ± 1.25 | 11.55 ± 2.83 | 26.13 ± 0.97 | 45.12 ± 1.22 | 63.30 ± 5.20 | 98.36 ± 1.24 |
| 6.00 | 5.45 ± 1.35 | 13.46 ± 3.46 | 31.27 ± 1.24 | 53.95 ± 1.28 | 78.35 ± 10.0 | 99.62 ± 1.57 |
| 8.00 | 6.75 ± 1.48 | 15.65 ± 5.16 | 35.55 ± 0.96 | 60.80 ± 1.04 | 94.69 ± 14.4 | 99.59 ± 1.15 |

[1] Mean (± S.D) of three determinations

Table B4.2 Dissolution rate data for Test product 2 using basket apparatus

| Time (Hrs) | pH | | | | | |
|---|---|---|---|---|---|---|
| | 4.50 | 5.00 | 5.50 | 6.00 | 6.20 | 7.00 |
| 0.00 | 0.00 ± 0.00[1] | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 0.50 | 1.03 ± 0.08 | 2.09 ± 0.10 | 4.09 ± 0.15 | 5.05 ± 4.37 | 14.32 ± 0.51 | 40.87 ± 1.96 |
| 1.00 | 1.33 ± 0.20 | 3.54 ± 0.22 | 7.10 ± 1.58 | 14.76 ± 1.53 | 29.23 ± 1.06 | 85.06 ± 5.23 |
| 1.50 | 1.67 ± 0.43 | 4.95 ± 0.28 | 9.88 ± 2.28 | 20.64 ± 1.68 | 44.33 ± 2.63 | 104.89 ± 5.77 |
| 2.00 | 2.06 ± 0.45 | 6.71 ± 0.88 | 13.48 ± 3.77 | 26.10 ± 4.51 | 54.55 ± 2.43 | 112.90 ± 5.00 |
| 2.50 | 2.46 ± 0.40 | 7.86 ± 0.46 | 16.13 ± 4.05 | 32.0 ± 5.89 | 66.36 ± 2.87 | 113.76 ± 5.50 |
| 3.00 | 2.72 ± 0.60 | 9.17 ± 0.54 | 18.91 ± 4.91 | 37.40 ± 6.62 | 76.69 ± 2.61 | 114.66 ± 5.00 |
| 4.00 | 3.11 ± 0.72 | 11.68 ± 0.52 | 24.16 ± 6.23 | 47.07 ± 8.82 | 90.03 ± 1.45 | 115.20 ± 5.20 |
| 6.00 | 4.34 ± 1.03 | 16.60 ± 2.20 | 32.13 ± 8.17 | 62.90 ± 11.24 | 107.43 ± 1.60 | 116.11 ± 4.90 |
| 8.00 | 4.75 ± 1.13 | 19.14 ± 0.83 | 39.45 ± 10.26 | 75.84 ± 16.06 | 101.21 ± 15.2 | 116.26 ± 5.00 |

[1] Mean (± S.D.) of three determinations

Table B4.3 Dissolution rate data for Indocid R using paddle apparatus

| Time (Hrs) | pH | | | | | |
|---|---|---|---|---|---|---|
| | 4.50 | 5.00 | 5.50 | 6.00 | 6.20 | 7.00 |
| 0.00 | 0.00 ± 0.00[1] | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 0.50 | 4.91 ± 0.53 | 6.01 ± 1.27 | 11.53 ± 2.44 | 14.40 ± 2.06 | 18.20 ± 1.62 | 49.61 ± 4.73 |
| 1.00 | 6.91 ± 1.14 | 10.50 ± 0.54 | 20.68 ± 0.75 | 32.60 ± 1.88 | 38.89 ± 2.88 | 95.21 ± 3.61 |
| 1.50 | 8.00 ± 0.65 | 12.96 ± 0.43 | 26.70 ± 2.19 | 45.21 ± 2.29 | 51.11 ± 2.32 | 104.50 ± 3.73 |
| 2.00 | 9.01 ± 0.41 | 14.72 ± 0.62 | 31.00 ± 2.15 | 55.09 ± 2.81 | 61.24 ± 2.45 | 108.60 ± 3.63 |
| 2.50 | 9.81 ± 0.65 | 16.20 ± 1.33 | 35.60 ± 3.65 | 61.61 ± 4.39 | 68.21 ± 2.56 | 110.51 ± 4.01 |
| 3.00 | 10.24 ± 0.45 | 17.44 ± 1.30 | 38.51 ± 3.92 | 67.08 ± 2.49 | 73.11 ± 2.84 | 109.81 ± 3.38 |
| 4.00 | 10.81 ± 0.42 | 19.30 ± 1.26 | 42.83 ± 4.48 | 73.74 ± 2.81 | 80.29 ± 3.23 | 111.20 ± 2.94 |
| 6.00 | 11.75 ± 0.68 | 21.70 ± 1.60 | 47.79 ± 4.89 | 82.73 ± 3.11 | 88.44 ± 4.17 | 112.51 ± 2.96 |
| 8.00 | 12.46 ± 0.36 | 22.71 ± 2.11 | 53.00 ± 5.22 | 88.24 ± 3.11 | 92.71 ± 4.34 | 112.81 ± 3.52 |
| 10.00 | 12.92 ± 0.34 | 23.40 ± 2.31 | 55.25 ± 5.64 | 91.21 ± 2.90 | 95.43 ± 4.77 | 111.50 ± 3.09 |
| 12.00 | 13.29 ± 0.13 | 23.50 ± 2.12 | 55.60 ± 5.45 | 95.10 ± 2.87 | 98.80 ± 4.16 | 112.00 ± 2.38 |
| 16.00 | 13.70 ± 0.48 | 24.60 ± 2.53 | 57.61 ± 4.34 | 100.70 ± 2.67 | 100.50 ± 4.29 | 112.00 ± 2.38 |
| 24.00 | 14.10 ± 0.45 | 24.90 ± 2.07 | 60.00 ± 4.95 | 100.71 ± 2.03 | 100.50 ± 5.78 | 107.81 ± 2.38 |

[1] Mean ((± S.D.) of three determinations

Table B4.4 Dissolution rate data for Test Product 2 using paddle apparatus

| Time (Hrs) | pH | | | | | |
|---|---|---|---|---|---|---|
| | 4.50 | 5.00 | 5.50 | 6.00 | 6.20 | 7.00 |
| 0.00 | 0.00 ± 0.00[1] | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 0.50 | 1.66 ± 0.23 | 2.40 ± 0.62 | 3.09 ± 0.63 | 4.21 ± 1.43 | 6.19 ± 0.40 | 20.81 ± 0.96 |
| 1.00 | 1.93 ± 0.66 | 2.50 ± 0.17 | 4.24 ± 0.79 | 7.90 ± 1.71 | 12.71 ± 1.88 | 48.21 ± 4.64 |
| 1.50 | 1.89 ± 0.39 | 3.10 ± 0.19 | 5.50 ± 0.59 | 12.30 ± 2.26 | 20.09 ± 3.37 | 67.61 ± 6.31 |
| 2.00 | 2.20 ± 0.46 | 3.70 ± 0.36 | 6.89 ± 0.80 | 17.10 ± 2.89 | 27.67 ± 0.68 | 84.41 ± 7.02 |
| 2.50 | 2.37 ± 0.18 | 4.20 ± 0.30 | 8.92 ± 1.06 | 21.89 ± 3.43 | 35.61 ± 6.17 | 94.10 ± 7.92 |
| 3.00 | 2.52 ± 0.26 | 4.64 ± 0.30 | 10.61 ± 1.41 | 26.61 ± 4.05 | 42.31 ± 7.18 | 97.71 ± 7.89 |
| 4.00 | 2.89 ± 0.23 | 5.82 ± 0.30 | 13.94 ± 1.97 | 34.62 ± 5.38 | 54.83 ± 8.55 | 104.51 ± 8.96 |
| 6.00 | 3.80 ± 0.34 | 8.02 ± 0.35 | 19.71 ± 2.83 | 49.59 ± 6.52 | 71.51 ± 9.73 | 107.91 ± 9.69 |
| 8.00 | 5.86 ± 0.51 | 10.70 ± 0.56 | 27.86 ± 3.95 | 61.68 ± 7.84 | 81.93 ± 10.81 | 109.00 ± 9.13 |
| 10.00 | 6.54 ± 0.62 | 12.70 ± 0.90 | 32.30 ± 4.44 | 74.51 ± 9.02 | 87.71 ± 11.02 | 109.50 ± 8.35 |
| 12.00 | 7.06 ± 0.34 | 13.89 ± 0.87 | 34.60 ± 4.97 | 76.41 ± 9.60 | 91.73 ± 10.65 | 109.50 ± 8.35 |
| 16.00 | 7.78 ± 0.33 | 16.00 ± 1.21 | 43.50 ± 5.66 | 84.63 ± 9.59 | 95.81 ± 10.21 | 109.50 ± 8.35 |
| 24.00 | 9.88 ± 0.74 | 19.27 ± 1.76 | 50.70 ± 5.62 | 86.10 ± 10.48 | 96.73 ± 9.31 | 107.90 ± 5.67 |

[1] Mean (± S.D.) of three determinations

Table B4.5 Serum Concentrations of Indomethacin (mg/l) following the administration of one 75mg Indocid R capsule

| Time (Hrs) | Subject | | | | | | | | Mean ± S.D. |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 ± 0.00 |
| 0.50 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 ± 0.00 |
| 1.00 | 0.47 | 0.00 | 0.00 | 0.23 | 0.00 | 0.00 | 0.48 | 0.00 | 0.15 ± 0.22 |
| 1.50 | 2.02 | 0.00 | 0.00 | 0.39 | 0.39 | 0.00 | 2.02 | 0.57 | 0.67 ± 0.86 |
| 2.00 | 2.41 | 0.00 | 0.00 | 0.70 | 1.62 | 0.00 | 2.09 | 1.07 | 0.99 ± 0.97 |
| 2.50 | 1.69 | 0.39 | 0.53 | 0.74 | 2.33 | 0.00 | 1.78 | 0.99 | 1.06 ± 0.80 |
| 3.00 | 1.36 | 1.12 | 0.60 | 0.77 | 2.57 | 0.00 | 1.71 | 1.31 | 1.18 ± 0.77 |
| 3.50 | 1.17 | 1.80 | 1.29 | 1.11 | 2.27 | 0.63 | 1.39 | 1.94 | 1.45 ± 0.53 |
| 4.00 | 0.74 | 1.77 | 3.14 | 1.39 | 1.69 | 0.88 | 0.69 | 1.76 | 1.51 ± 0.80 |
| 5.00 | 0.32 | 1.17 | 2.26 | 1.78 | 1.26 | 1.30 | 0.61 | 0.96 | 1.23 ± 0.58 |
| 6.00 | 0.60 | 0.74 | 1.10 | 1.46 | 0.90 | 1.37 | 0.57 | 0.59 | 0.92 ± 0.36 |
| 8.00 | 0.34 | 0.37 | 0.56 | 0.65 | 0.52 | 1.04 | 0.56 | 0.30 | 0.54 ± 0.24 |
| 10.00 | 0.39 | 0.38 | 0.61 | 0.45 | 0.44 | 0.75 | 0.37 | 0.35 | 0.47 ± 0.14 |
| 12.00 | 0.34 | 0.34 | 0.35 | 0.32 | 0.45 | 0.39 | 0.00 | 0.26 | 0.31 ± 0.14 |
| 24.00 | 0.00 | 0.00 | 0.23 | 0.13 | 0.00 | 2.25 | 0.00 | 0.00 | 0.08 ± 0.11 |

Table B4.6 Serum Concentrations of Indomethacin (mg/l) following the administration of one 75mg capsule of Test product 2

| Time (Hrs) | Subject | | | | | | | | Mean ± S.D |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 ± 0.00 |
| 0.50 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 ± 0.00 |
| 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 ± 0.00 |
| 1.50 | 0.00 | 0.48 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.06 ± 0.17 |
| 2.00 | 0.00 | 0.61 | 0.28 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.11 ± 0.22 |
| 2.50 | 0.00 | 0.58 | 0.40 | 0.12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.14 ± 0.23 |
| 3.00 | 0.34 | 1.09 | 0.56 | 0.21 | 0.00 | 0.00 | 0.00 | 0.24 | 0.33 ± 0.38 |
| 3.50 | 1.01 | 1.83 | 0.55 | 0.31 | 0.24 | 0.24 | 0.56 | 0.43 | 0.65 ± 0.34 |
| 4.00 | 0.71 | 1.56 | 0.49 | 0.30 | 0.61 | 0.48 | 0.56 | 0.86 | 0.70 ± 0.39 |
| 5.00 | 0.50 | 1.24 | 0.80 | 0.35 | 0.94 | 0.94 | 0.61 | 1.49 | 0.86 ± 0.38 |
| 6.00 | 0.62 | 0.83 | 0.90 | 1.08 | 0.67 | 1.70 | 0.89 | 0.81 | 0.94 ± 0.34 |
| 8.00 | 0.66 | 0.39 | 1.90 | 1.76 | 0.75 | 0.77 | 0.43 | 0.57 | 0.90 ± 0.59 |
| 10.00 | 0.76 | 0.32 | 0.73 | 0.80 | 1.00 | 0.54 | 0.36 | 0.33 | 0.61 ± 0.27 |
| 12.00 | 0.55 | 0.34 | 0.43 | 0.46 | 1.18 | 0.34 | 0.00 | 0.00 | 0.41 ± 0.37 |
| 24.00 | 0.00 | 0.00 | 0.34 | 0.29 | 0.34 | 0.28 | 0.00 | 0.00 | 0.16 ± 0.17 |

Table B4.7 Model predicted Indomethacin Serum Concentrations (Using model D [see section 2.2.4.2]) based on dissolution parameters obtained from Indocid R employing the Basket apparatus.

| Time (Hrs) | Dissolution pH | | | | | |
|---|---|---|---|---|---|---|
| | 4.5 | 5.0 | 5.5 | 6.0 | 6.2 | 7.0 |
| 0.00 | 0.00[1] | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.00 | 0.02 | 0.05 | 0.32 | 0.77 | 0.00 | 0.18 |
| 2.00 | 0.02 | 0.05 | 0.39 | 0.74 | 0.32 | 1.29 |
| 3.00 | 0.02 | 0.08 | 0.46 | 0.83 | 0.85 | 1.82 |
| 4.00 | 0.03 | 0.11 | 0.50 | 0.90 | 1.12 | 1.95 |
| 5.00 | 0.04 | 0.13 | 0.52 | 0.94 | 1.27 | 1.87 |
| 6.00 | 0.05 | 0.16 | 0.54 | 0.95 | 1.33 | 1.69 |
| 8.00 | 0.06 | 0.20 | 0.54 | 0.93 | 1.29 | 1.28 |
| 10.00 | 0.07 | 0.22 | 0.53 | 0.86 | 1.14 | 0.93 |
| 12.00 | 0.08 | 0.24 | 0.50 | 0.78 | 0.95 | 0.67 |
| 24.00 | 0.09 | 0.24 | 0.34 | 0.32 | 0.19 | 0.09 |

[1] Predicted serum concentration of indomethacin (mg/l)

Table B4.8 Model predicted Indomethacin Serum Concentrations (Using model D [see section 2.2.4.2]) based on dissolution parameters obtained from Test Product 2 employing the Basket apparatus.

| Time (Hrs) | Dissolution pH | | | | |
|---|---|---|---|---|---|
| | 4.5 | 5.0 | 5.5 | 6.0 | 6.2 |
| 0.00 | 0.00[1] | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.00 | 0.02 | 0.08 | 0.25 | 0.00 | 0.00 |
| 2.00 | 0.02 | 0.08 | 0.19 | 0.00 | 0.83 |
| 3.00 | 0.02 | 0.11 | 0.27 | 0.16 | 1.35 |
| 4.00 | 0.03 | 0.16 | 0.36 | 0.74 | 1.62 |
| 5.00 | 0.04 | 0.20 | 0.45 | 0.99 | 1.70 |
| 6.00 | 0.05 | 0.24 | 0.52 | 1.12 | 1.66 |
| 78.00 | 0.06 | 0.29 | 0.61 | 1.18 | 1.40 |
| 10.00 | 0.07 | 0.33 | 0.66 | 1.11 | 1.09 |
| 12.00 | 0.08 | 0.34 | 0.65 | 0.98 | 0.81 |
| 24.00 | 0.09 | 0.32 | 0.43 | 0.27 | 0.11 |

[1] Predicted serim concentration of indomethacin (mg/l)

Table B4.9 Model predicted Indomethacin Serum Concentrations (Using model D [see section 2.2.4.2]) based on dissolution parameters obtained from Indocid R employing the Paddle apparatus.

| Time (Hrs) | Dissolution pH | | | | | |
|---|---|---|---|---|---|---|
| | 4.5 | 5.0 | 5.5 | 6.0 | 6.2 | 7.0 |
| 0.00 | 0.00[1] | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.00 | 0.01 | 0.02 | 0.37 | 0.00 | 0.16 | 0.56 |
| 2.00 | 0.01 | 0.02 | 0.79 | 0.58 | 0.88 | 1.65 |
| 3.00 | 0.02 | 0.03 | 0.99 | 1.09 | 1.31 | 2.00 |
| 4.00 | 0.02 | 0.04 | 1.02 | 1.40 | 1.51 | 1.98 |
| 5.00 | 0.03 | 0.05 | 0.97 | 1.55 | 1.55 | 1.82 |
| 6.00 | 0.04 | 0.06 | 0.88 | 1.57 | 1.51 | 1.61 |
| 8.00 | 0.05 | 0.08 | 0.68 | 1.42 | 1.30 | 1.19 |
| 10.00 | 0.05 | 0.09 | 0.51 | 1.16 | 1.06 | 0.86 |
| 12.00 | 0.06 | 0.10 | 0.39 | 0.90 | 0.83 | 0.61 |
| 24.00 | 0.07 | 0.11 | 0.12 | 0.13 | 0.14 | 0.08 |

[1] Predicted serum concentration of indomethacin (mg/l)

Table B4.10 Model predicted Indomethacin Serum Concentrations (Using model D [see section 2.2.4.2]) based on dissolution parameters obtained from Test Product 2 employing the Paddle apparatus.

| Time (Hrs) | Dissolution pH | | | | | |
|---|---|---|---|---|---|---|
| | 4.5 | 5.0 | 5.5 | 6.0 | 6.2 | 7.0 |
| 0.00 | 0.00[1] | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.00 | 0.01 | 0.03 | 0.02 | 0.00 | 0.00 | 0.29 |
| 2.00 | 0.01 | 0.03 | 0.05 | 0.53 | 0.00 | 1.42 |
| 3.00 | 0.02 | 0.04 | 0.09 | 0.54 | 0.60 | 1.89 |
| 4.00 | 0.03 | 0.06 | 0.13 | 0.67 | 0.94 | 1.98 |
| 5.00 | 0.04 | 0.07 | 0.16 | 0.80 | 1.13 | 1.86 |
| 6.00 | 0.04 | 0.09 | 0.19 | 0.90 | 1.23 | 1.67 |
| 8.00 | 0.05 | 0.11 | 0.23 | 1.00 | 1.26 | 1.25 |
| 10.00 | 0.06 | 0.12 | 0.26 | 0.98 | 1.15 | 0.90 |
| 12.00 | 0.07 | 0.13 | 0.27 | 0.91 | 0.99 | 0.65 |
| 24.00 | 0.07 | 0.14 | 0.26 | 0.35 | 0.23 | 0.08 |

[1] Predicted serum concentration of indomethacin (mg/l)

Table B4.11 Model predicted Indomethacin Serum Concentrations (Using model D [see section 2.2.4.2]) based on dissolution parameters obtained from Test Product 3 employing the Paddle apparatus.

| Time (Hrs) | Dissolution pH | | | | | |
|---|---|---|---|---|---|---|
| | 4.5 | 5.0 | 5.5 | 6.0 | 6.2 | 7.0 |
| 0.00 | 0.00[1] | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.00 | 0.44 | 0.44 | 0.45 | 0.52 | 0.48 | 0.74 |
| 2.00 | 0.64 | 0.64 | 0.65 | 0.71 | 0.80 | 1.42 |
| 3.00 | 0.68 | 0.69 | 0.71 | 0.79 | 0.96 | 1.79 |
| 4.00 | 0.65 | 0.66 | 0.69 | 0.80 | 1.04 | 1.89 |
| 5.00 | 0.60 | 0.60 | 0.64 | 0.78 | 1.07 | 1.82 |
| 6.00 | 0.52 | 0.53 | 0.57 | 0.74 | 1.06 | 1.66 |
| 8.00 | 0.39 | 0.41 | 0.46 | 0.66 | 0.99 | 1.28 |
| 10.00 | 0.29 | 0.31 | 0.36 | 0.58 | 0.89 | 0.93 |
| 12.00 | 0.21 | 0.23 | 0.30 | 0.51 | 0.78 | 0.67 |
| 24.00 | 0.05 | 0.07 | 0.14 | 0.28 | 0.28 | 0.09 |

[1] Predicted serum concentration of indomethacin (mg/l)

Table B4.12 Dissolution rate data for Test Product 3 obtained using the paddle apparatus.

| Time (Hrs) | pH | | | | | |
|---|---|---|---|---|---|---|
| | 4.50 | 5.00 | 5.50 | 6.00 | 6.20 | 7.00 |
| 0.00 | 0.00 ± 0.00[1] | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 0.17 | 0.00 ± 0.00 | 10.60 ± 1.04 | 10.31 ± 1.47 | 13.10 ± 2.07 | 16.17 ± 2.07 | 15.53 ± 1.00 |
| 0.33 | 0.00 ± 0.00 | 11.80 ± 0.77 | 17.54 ± 1.77 | 18.71 ± 0.49 | 25.23 ± 2.79 | 24.81 ± 0.71 |
| 0.50 | 0.00 ± 0.00 | 12.63 ± 0.78 | 22.92 ± 3.31 | 22.34 ± 0.31 | 30.13 ± 3.11 | 31.27 ± 0.23 |
| 1.00 | 5.92 ± 0.95 | 14.60 ± 0.49 | 29.04 ± 3.32 | 28.50 ± 1.28 | 41.68 ± 2.38 | 47.69 ± 2.42 |
| 2.00 | 5.97 ± 0.80 | 14.84 ± 1.03 | 32.33 ± 3.14 | 33.11 ± 1.72 | 59.00 ± 2.90 | 73.26 ± 6.83 |
| 3.00 | 6.35 ± 0.69 | 16.03 ± 1.18 | 34.32 ± 3.29 | 36.47 ± 1.87 | 72.17 ± 4.22 | 89.00 ± 9.43 |
| 4.00 | 6.93 ± 0.25 | 16.79 ± 1.19 | 35.72 ± 3.26 | 39.88 ± 1.89 | 81.14 ± 5.36 | 95.86 ± 9.28 |
| 6.00 | 7.79 ± 0.61 | 18.41 ± 1.36 | 37.56 ± 3.22 | 44.38 ± 4.20 | 93.09 ± 3.98 | 102.38 ± 7.11 |
| 8.00 | 7.81 ± 0.64 | 18.30 ± 0.95 | 37.11 ± 2.01 | 48.31 ± 2.43 | 100.95 ± 2.43 | 104.46 ± 7.11 |
| 12.00 | 8.38 ± 0.91 | 19.53 ± 1.31 | 39.50 ± 1.20 | 56.97 ± 3.66 | 105.05 ± 2.41 | 106,70 ± 7.48 |
| 24.00 | 9.67 ± 0.65 | 20.05 ± 1.56 | 46.62 ± 2.35 | 71.58 ± 7.30 | 117.80 ± 0.35 | 115.68 ± 1.25 |

[1] Mean (± S.D.) of three determinations

Table B4.13 Inferential statistics for Indocid R and Test Product 2.

| Parameter | Ratio of means | Confidence limit | | |
|---|---|---|---|---|
| | | | Classical | Westlake |
| $AUC_{12}$ | 0.78 | 95% | 63 - 94 | 66 - 135 |
| $AUC_{24}$ | 0.93 | 95% | 78 - 108 | 81 - 120 |
| $C_{max}$ | 0.69 | 95% | 42 - 96 | 47 - 153 |

Table B4.14 Goodness-of-fit data ($R^2$ values) for Dissolution rate determinations obtained form the basket apparatus.

| Dissolution pH | Product | |
|---|---|---|
| | Indocid R | Test Product 2 |
| 4.5 | $K_s$: 0.7574 | $K_s$: 0.9130 |
| 5.0 | $K_s$: 0.9399 | $K_s$: 0.9906 |
| 5.5 | $K_s$: 0.9729 | $K_s$: 0.9987 |
| 6.0 | $K_s$: 0.9878 | $K_s$: 0.9967 |
| 6.2 | $K_s$: 0.9968 | $K_s$: 0.9980<br>$K_f$: 0.9945 |
| 7.0 | $K_s$: 0.9977 | --- |

Table B4.14 Goodness-of-fit data ($R^2$ values) for Dissolution rate determinations obtained form the paddle apparatus.

| Dissolution pH | Product | |
|---|---|---|
| | Indocid R | Test Product 2 |
| 4.5 | $K_s$: 0.7211 | $K_s$: 0.9388 |
| 5.0 | $K_s$: 0.8630 | $K_s$: 0.9601 |
| 5.5 | $K_s$: 0.9032<br>$K_f$: 0.9123 | $K_s$: 0.9734<br>$K_f$: 0.9861 |
| 6.0 | $K_s$: 0.9645<br>$K_f$: 0.9762 | $K_s$: 0.9916 |
| 6.2 | $K_s$: 0.9786<br>$K_f$: 0.8960 | $K_s$: 0.9970 |
| 7.0 | $K_s$: 0.9096 | $K_s$: 0.9827 |

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