BIOPHARMACEUTICS OF PHENYLPROPANOLAMINE

by

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ABSTRACT

Phenylpropanolamine (PPA), a sympathomimetic amine, has been widely used over the past 40 years as a decongestant and, in much larger dosages, as an appetite suppressant. Considerable interest has recently been shown in this drug due to its increasing popularity as an over-the-counter anorectic agent. Much controversy exists concerning the unfavourable side-effects of PPA resulting from the higher doses required for appetite suppression and the potential of this drug for abuse.

A literature search revealed a paucity of information concerning the determination of PPA in biological fluids and, most noticeably, on the pharmacokinetics of this drug. An original method for determining PPA in serum and urine using high performance liquid chromatography (HPLC) which has increased sensitivity over other published HPLC methods is presented here. The simplicity of the extraction from biological fluids and subsequent determination by HPLC, enables concentrations of PPA to be monitored after a single dose of the drug. This method is therefore readily applicable to bioavailability and pharmacokinetic studies.

The dissolution profiles of 4 sustained-release formulations of PPA were determined in a modified USP rotating paddle apparatus and the samples analysed using HPLC. A mathematical equation was applied to these data which are expressed in terms of dissolution parameters. Oral test dosage forms and solutions of PPA were investigated in bioavailability trials using the developed HPLC method to analyse the urine and serum samples.

Linear one body compartment kinetics were assumed and the Wagner-Nelson method used to transform *in vivo* serum data to absorption plots which were then fitted to the well known Weibull equation. In order to more appropriately characterize the kinetic processes of absorption, distribution and elimination, a more complex model was utilized which involved numerical integration of a series of

differential equations. The data were fitted to these models using nonlinear regression techniques. The pharmacokinetics of PPA are shown to exhibit some evidence of nonlinearity. The absorption of the drug appears to be discontinuous and PPA seems to favour a two body compartment model.

INSTRUMENTATION

THE HIGH PRESSURE LIQUID CHROMATOGRAPHIC SYSTEMS

SYSTEM A

This chromatographic system consisted of an M45 solvent delivery system (Waters Associates), a model U6K injector (Waters Associates), a fixed wavelength Uvicord S UV detector Model LKB 2138 and a Perkin-Elmer strip-chart recorder, model 560. Separation was achieved on a 30cm x 4.0mm i.d. μBondapak C_{18} reverse phase column (Waters Associates). Temperature was ambient.

SYSTEM B

A model 6000A constant flow pump (Waters Associates) and a model U6K injector (Waters Associates) were used. The effluent from a 30cm x 3.9mm i.d. μBondapak C_{18} reverse phase column (Waters Associates) was monitored with a variable wavelength Pye Unicam LC3-UV detector to which was attached a Perkin-Elmer strip-chart recorder Model 561. The temperature of the column was controlled by an LC-22 temperature controller and column heater (Bioanalytical Systems Inc.).

SYSTEM C

This system incorporated a model 6000A solvent delivery system (Waters Associates), an automated sample injector, WISP model 710B (Waters Associates) and a variable wavelength Pye-Unicam LC3-UV detector. A data module Model 730 (Waters Associates) was used as well as a Perkin-Elmer strip-chart recorder, model 561. A temperature controller, model LC-22 (Bioanalytical Systems Inc.) was used to maintain the μBondapak C_{18} reverse phase column at the desired temperature.

CHAPTER 1

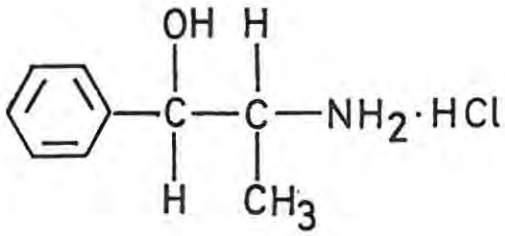
INTRODUCTION

1.1 PHYSICOCHEMICAL PROPERTIES OF PHENYLPROPANOLAMINE

1.1.1 Description

Phenylpropanolamine hydrochloride, sometimes referred to as ± norephedrine (1-5) can also be named in a number of ways :

(a) α-(1-aminoethyl)benzenemethanol hydrochloride
(b) α-(1-aminoethyl)benzyl alcohol hydrochloride
(c) (±)-2-amino-phenylpropan-1-ol hydrochloride
(d) 2-amino-1-phenyl-1-propanol hydrochloride
(e) α-hydroxy-α-aminopropylbenzene hydrochloride
(f) 1-phenyl-2-amino-1-propanol hydrochloride



$C_9H_{13}ClNO$ MM 187.67

FIGURE 1.1 Phenylpropanolamine hydrochloride

The compound is a white crystalline powder with an odour resembling that of crude benzoic acid.

1.1.2 Synthesis

Phenylpropanolamine hydrochloride (PPA.HCl) is prepared by reacting benzaldehyde with nitroethane in 95% ethanol in the presence of sodium hydroxide to form α-(1-nitroethyl)benzyl alcohol and then reducing this nitro-alcohol to the corresponding amino compound. A stream of hydrogen chloride passed into a suitable solution of the base yields the hydrochloride (6).

1.1.3 Solubility

Table 1.1 reflects the solubility of PPA.HCl in various solvents after samples have been sonicated for one minute at ambient temperature.

Solvent	mg/ml	Solubility
Water	>50-<1000	Soluble
Methanol	>50-<1000	Soluble
Isopropanol	>10-<33.3	Sparingly soluble
Diethylether	<0.5	Practically insoluble
Ethyl acetate	<0.5	Practically insoluble
Chloroform	<0.5	Practically insoluble
Benzene	<0.5	Practically insoluble
Carbon tetrachloride	<0.5	Practically insoluble
Acetonitrile	<0.5	Practically insoluble
Acetone	<0.5	Practically insoluble
Cyclohexane	<0.5	Practically insoluble

TABLE 1.1 Solubility of PPA.HCl

1.1.4 Melting Range

Phenylpropanolamine hydrochloride crystals melt at 190-194°C. The free base melts at 101-101.5°C (2).

1.1.5 Specific Rotation

The specific rotation, $[\alpha]_D^{25}$, of PPA.HCl in water is +32° (7).

1.1.6 Crystal Structure

The crystal structure was determined (8) using single crystals of PPA.HCl obtained by slow evaporation of an aqueous solution at room temperature. The intensity data were collected on an automatic four-circle diffractometer using Ni-filtered radiation. Phenylpropanolamine hydrochloride has a monoclinic crystal system with possible space groups of $P2_1$(non-centrosymmetric) or $P2_1/m$ (centrosymmetric). The cell dimensions are a = 7.448Å, b = 9.461Å, c = 14.595Å, β = 103.4° with each asymmetric unit containing two molecules.

1.1.7 Dissociation Constant

The pK_a of PPA.HCl determined potentiometrically at 20°C is 9.44± 0.04 (9).

1.1.8 Infrared Spectrum

The infrared spectrum of PPA.HCl is shown in Fig.1.2. It was obtained from a Nujol mull between KBr plates using a Perkin-Elmer 180 Infrared Spectrophotometer.

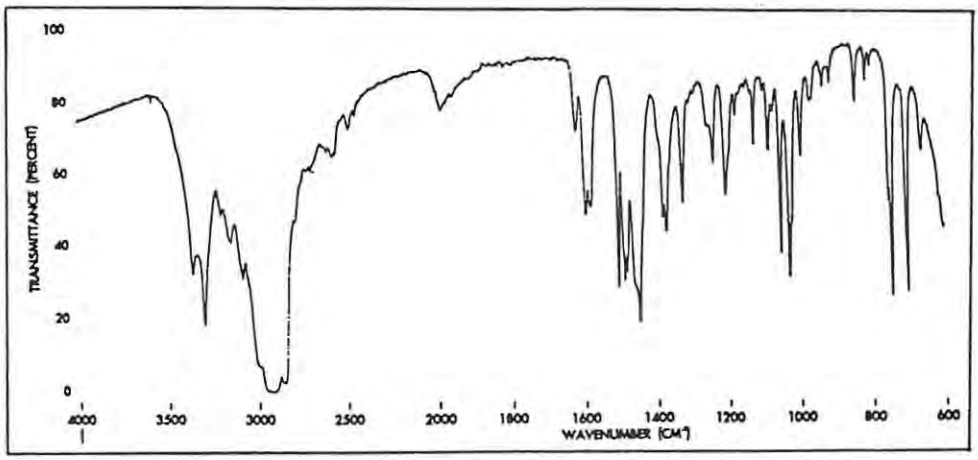


FIGURE 1.2 Infrared spectrum of phenylpropanolamine hydrochloride in Nujol mull.

1.1.9 Proton Magnetic Resonance Spectrum

The 60 MHz proton magnetic resonance spectrum of PPA base was obtained with a Perkin-Elmer Model R-12 Spectrometer. The spectrum in $CDCl_3$ with tetramethylsilane (TMS) as the internal standard is depicted in Fig. 1.3. The integration and multiplicities are consistent with the protein assignments. Chemical shifts (δ) in ppm relative to TMS are:

Proton assignment	Number of protons	J(Hz)	Chemical Shift (δ)	Multiplicity
$-CH_3$	3	9.0	0.94	Doublet
$-NH_2$, -OH	3	-	2.07	Singlet
N-C-H	1	9.0	3.12	Quintuplet
O-C-H	1	9.0	4.51	Doublet
Aromatic	5	-	7.41	Singlet

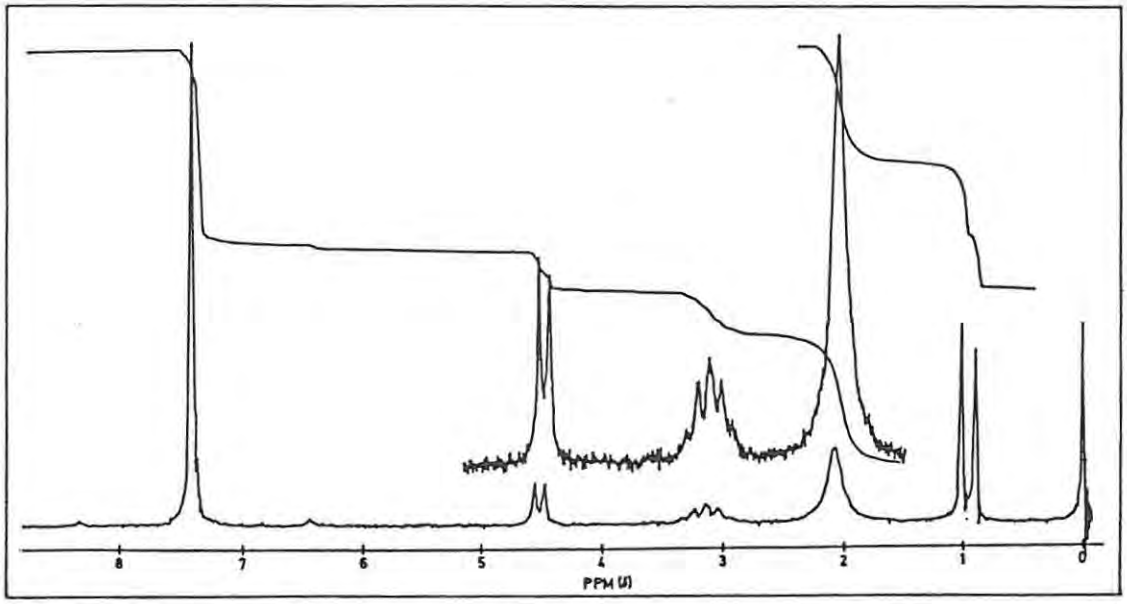


FIGURE 1.3 Proton magnetic resonance spectrum of phenylpropanolamine in $CDCl_3$

1.1.10 Differential Scanning Calorimetry

Phenylpropanolamine hydrochloride was heated from 320 to 520°K at a rate of 20°/minute under an atmosphere of nitrogen in a Perkin-Elmer Model DSC-2 Differential Scanning Calorimeter. The thermogram is depicted in Fig. 1.4. A single endotherm was observed with an onset temperature of 194.5°C which corresponds to the melting point. The heat of transition (ΔH melting) calculated in relation to an indium standard is 168 ± 6 Jg^{-1}.

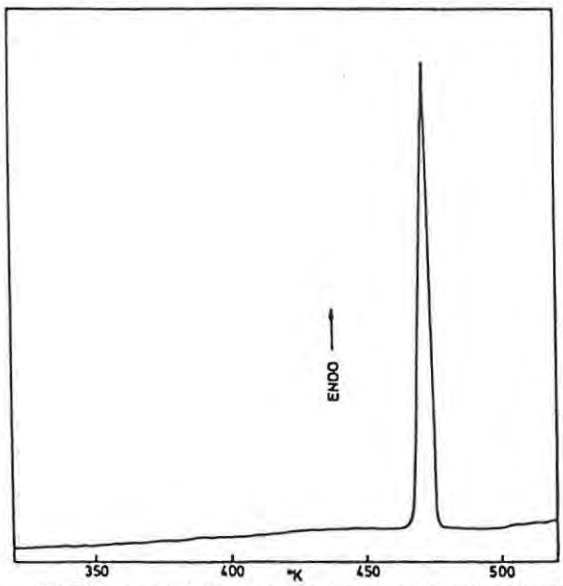


FIGURE 1.4 Differential scanning calorimetry curve of phenylpropanolamine hydrochloride

1.1.11 Ultraviolet Spectrum

The ultraviolet spectra of PPA.HCl in methanol and 0.1M HCl at a concentration of 1 mg/ml were obtained with a Beckman Acta MVI ultraviolet/visible spectrophotometer. The spectrum in methanol is depicted in Fig. 1.5. and shows shoulders at 267, 248 and 243 nm, whilst in 0.1M HCl shoulders occur at 267, 247 and 242 nm.

Solution	Absorption Maxima (nm)	ε
Methanol	264.0	133.63
	258.0	179.04
	252.0	145.44
0.1M HCl	262.8	144.32
	257.0	185.04
	251.0	148.82

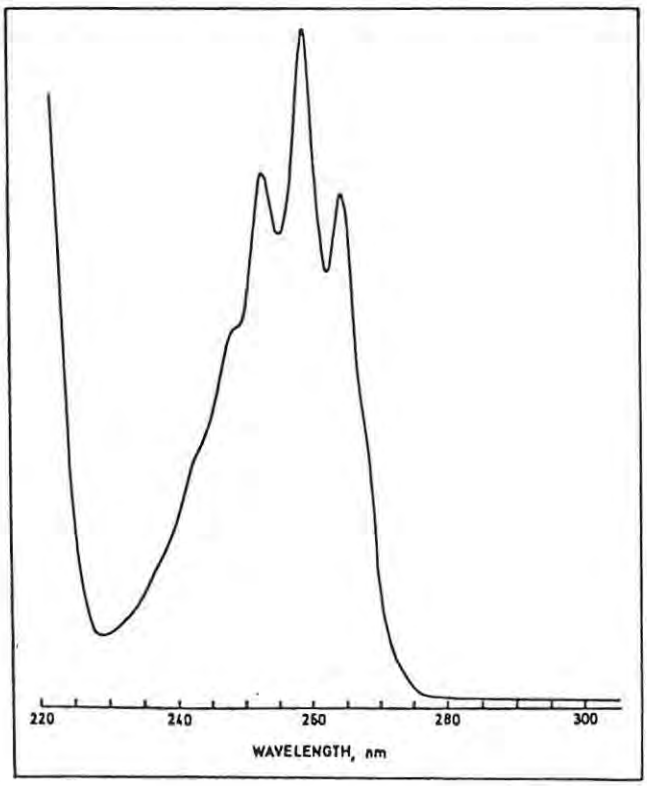


FIGURE 1.5 Ultraviolet spectrum of phenylpropanolamine hydrochloride in methanol.

1.1.12 Mass Spectrum

The low resolution mass spectrum of PPA.HCl is shown in Fig. 1.6.

It was obtained with a Varian MAT CH5-DF mass spectrometer. Direct probe at 80°C into the ion source was used to obtain the mass spectrum. The molecular ion is not observed. The assignments of some of the major ions formed are:

m/e	%	Ion
132	17	$Ph\text{-}CH{=}C(CH_2)NH_2^+$
105	29	$Ph\text{-}C{=}O^+$
91	26	$Ph\text{-}CH_2^+$
77	100	Ph^+

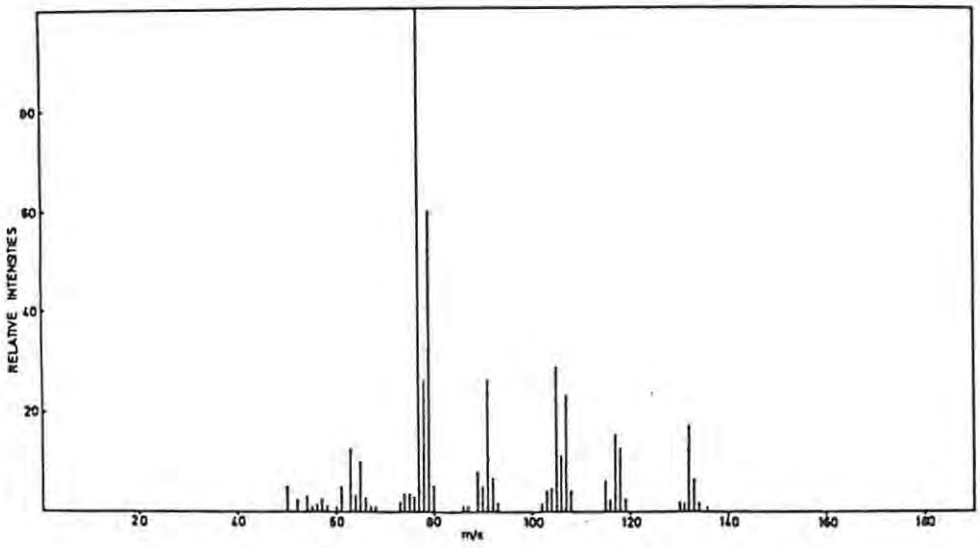


FIGURE 1.6 Mass spectrum of phenylpropanolamine hydrochloride

1.2 MODE OF ACTION OF PHENYLPROPANOLAMINE

Phenylpropanolamine hydrochloride belongs to the sympathomimetic class of drugs. These substances evoke physiological responses similar to those produced by the sympathetic adrenergic nerves in the autonomic nervous system (ANS) or involuntary nervous system. The ANS consists of two large divisions i.e. sympathetic and parasympathetic, that provide innervation to the heart, blood vessels, glands, viscera and smooth muscles. The main transmitter of the adrenergic fibres in the sympathetic nervous system is noradrenaline, others being adrenaline and dopamine .

Adrenaline within the nerve terminal is partitioned into a cytoplasmic mobile pool and intragranular reserve pools. Noradrenaline is discharged rapidly to the exterior from the intragranular pools by the nerve action potential. Following its release and action at adrenoreceptive sites of effector cells, excess noradrenaline is removed from the extracellular region largely by return to the axonal terminal through active transport and to some extent by diffusion and subsequently enzyme inactivation by catechol-O-methyl-tranferase (COMT). Drugs may thus exert their effects by modification of one of the many processes involved in transmitter synthesis, storage and displacement, or enzyme inactivation and activation of effector cells (10).

Adrenaline, noradrenaline and other catecholamines can cause either excitation or inhibition of smooth muscle, depending primarily on the site and, to a lesser extent, on the dose. The terms α and β receptors are now used for adrenoreceptive sites on smooth muscle where catecholamines produce either excitation or inhibition (11).

Phenylpropanolamine hydrochloride is a phenylethanolamine having predominantly an indirect sympathomimetic action. These amines enter the adrenergic neurons usually by an active process and then displace noradrenaline from its binding sites on the storage vesicles (see Fig. 1.7). These substances have no direct action as they lack the necessary groups for interaction with the receptor site (12). Compounds acting mainly indirectly produce effects predominantly on α-receptors and have relatively feeble β-effects except in the heart (13).

The pharmacological effects of PPA.HCl resemble those of ephedrine, the main effect being on the α-receptors of the peripheral vascular tree to cause vasoconstriction. The peripheral vasoconstriction induced by PPA sets in somewhat more slowly than that due to ephedrine, but lasts considerably longer and is not followed by any secondary vascular paralysis. This distinction between the two drugs arises from their different sites of action.

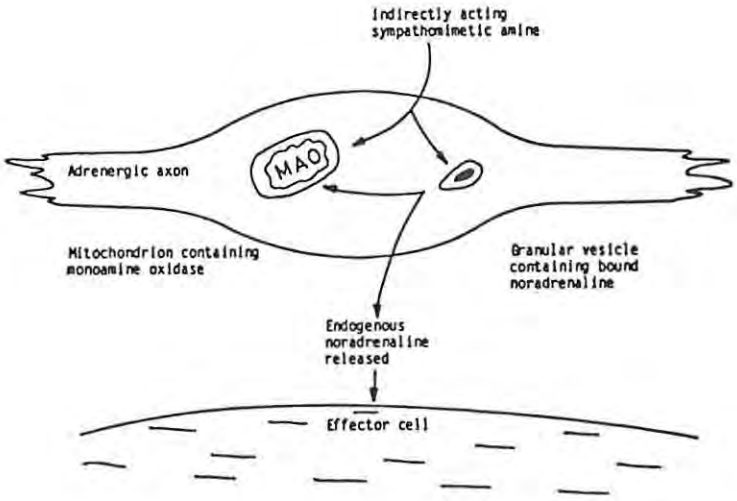


FIGURE 1.7 Action of indirectly acting sympathomimetic amines

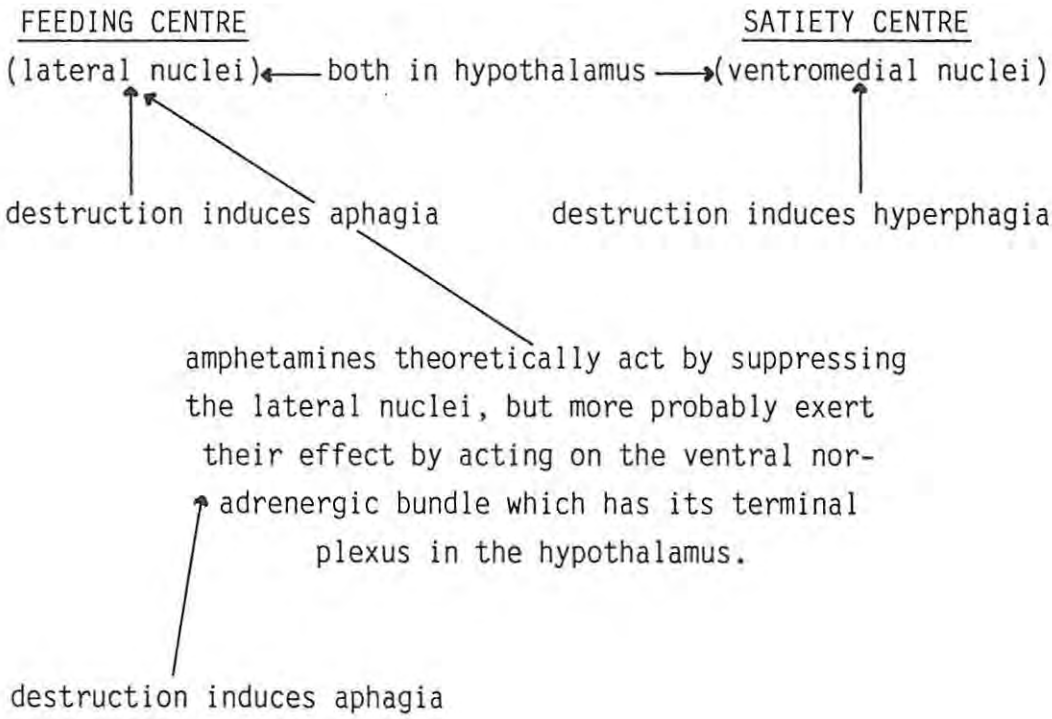
Phenylpropanolamine primarily causes the arterioles to contract and only slightly influences capillary width (14). It is used as a decongestant and its other actions include a moderate inhibition of mucosal secretion and an anti-allergic effect.

Although PPA is less active than ephedrine as a CNS stimulant and does not produce central stimulation to any great degree, it is active enough to be used for controlling the appetite (15). Despite its widespread use in this context the mechanism of appetite suppression of PPA is uncertain. Phenylpropanolamine appears to have an anorectic action within the lateral hypothalamus, but this could be due to a number of actions such as (a) an adrenergic synaptic effect, (b) a direct receptor effect, (c) an indirect receptor effect on glucoreceptors caused by increasing glucose utilization or (d) a combination of these (16).

Resnick *et al.* (17) designed a study to determine whether or not PPA affects the utilization of glucose or alters blood glucose levels, thereby possibly being capable of influencing a glucostatic system involved in feeding. He found that PPA caused a decrease in blood sugar levels in both normal and diabetic rats, with a greater hypoglycaemic effect in diabetic animals. Phenylpropanolamine may facilitate withdrawal of glucose from the blood and transfer into the cells, thereby increasing the amount of

glucose available to the tissues. This is signalled to the CNS which in turn restrains feeding behaviour. Secondly, decreased glucose output from the liver could decrease blood glucose. Feeding would only be affected if the increase in liver stores is signalled to the brain as an anorectic stimulus.

The proposed mode of action of PPA on the CNS as an anorectic is similar to that of amphetamine. A schematic diagram for the feeding-satiety mechanism illustrating how the amphetamines act on the higher nervous centres is as follows (18):



The amphetamines increase impulse formation in the noradrenergic bundle. If the bundle is removed, hyperphagia results.

1.3 PHARMACOKINETICS OF PHENYLPROPANOLAMINE

1.3.1 Absorption and Distribution

After oral administration of PPA, the absorption of the drug is rapid, being completed in less than 2.5 hours (19). Phenylpropanolamine, being a weak base, will not be absorbed readily from the acidic stomach because of ionization. However, in the more

alkaline intestine, absorption does occur at rates determined by the dissociation constant and the lipid solubility of the unionized form (20). Studies with many drugs in unbuffered solutions revealed that in the normal intestine, acids with pKa greater than 3.0 and bases with pKa less than 7.8 are very well absorbed (21). Although the pKa of PPA is 9.4 (9), it is almost completely absorbed in the intestine partly because of the large surface area available for absorption.

Phenylpropanolamine is not highly protein bound in the blood. Because of the large pH difference between the blood and the gastric contents, weak bases such as PPA are, to a small extent, passively concentrated in the stomach. PPA is also highly localized in various organ tissues with negligible localization in body fat. The tissue distribution in dogs 2 hours after administration is kidney > lung > liver > spleen > brain > heart > muscle> plasma > fat > cerebrospinal fluid (22). The pH difference between the intracellular and extracellular fluids is 7.0 vs. 7.4, thus the concentration gradient across the membrane is small and this results in PPA being slightly concentrated inside the cells.

1.3.2 Metabolism

Despite the long and extensive use of PPA (or norephedrine), comparatively little is known about its metabolism and excretion in man.

It has been reported that the metabolism of PPA shows considerable species differences. Sinsheimer *et al.*(23) found that in studies done on the metabolism of PPA in man, the rabbit and the rat, 86% of the dose of PPA in man was excreted in 24 hours unchanged in the urine. Only about 4% occurred as transformation products.

p-Hydroxylation to 4-hydroxynorephedrine (see Fig. 1.8) was only detected in appreciable amounts in one of the three subjects examined.

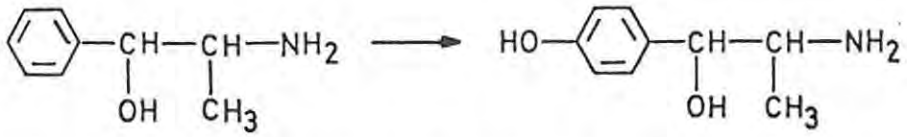


FIGURE 1.8 Hydroxylation of PPA to 4-hydroxynorephedrine

Deamination of the side chain was also low, ranging from 1% to 7% in the three subjects, the main product being hippuric acid (23) the structure of which is shown in Fig. 1.9.

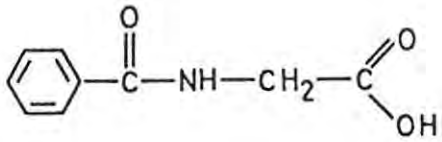


FIGURE 1.9 Hippuric acid

In urine studies done in man, Beckett and Wilkinson (24) and Heimlich *et al.* (25) have found that PPA is rapidly excreted predominantly unchanged. In the rat and the rabbit, 80 to 90% of [^{14}C] of small oral doses of [^{14}C]-PPA is excreted in the urine within 24 hours, with 1 to 2% of the dose being excreted in the faeces. In the rat, 60% occurs as the unchanged drug and 35% as 4-hydroxynorephedrine. Small amounts of 4-hydroxyhippuric acid and 1,2-dihydroxy-1-phenylpropane were also detected. In the rabbit however, 76% is excreted as deamination products consisting of 31% 1,2-dihydroxy-1-phenylpropane, 27% 1-hydroxy-2-oxo-1-phenylpropane and 24% benzoic acid (23).

N-methylation of numerous amines has been reported. Phenylethanolamine-N-methyltransferase (PNMT) methylates phenylethanolamine derivatives (26,27). Norephedrine is one of a number of exogenous primary amines metabolized by PNMT to the corresponding N-methylated metabolite (28,29).

Although catechol-O-methyltransferase (COMT) is involved in the physiologic inactivation of noradrenaline, PPA is not a substrate for COMT (28). Glucuronides of PPA have never been detected and

this lack of conjugation may perhaps be explained by the marked water solubility of PPA (30).

Phenylpropanolamine has been found as a metabolite of amphetamine in the urine of man (31) and as a metabolite of methamphetamine in man and the guinea-pig (32). Possible routes of metabolism of these compounds are shown in Fig. 1.10 below.

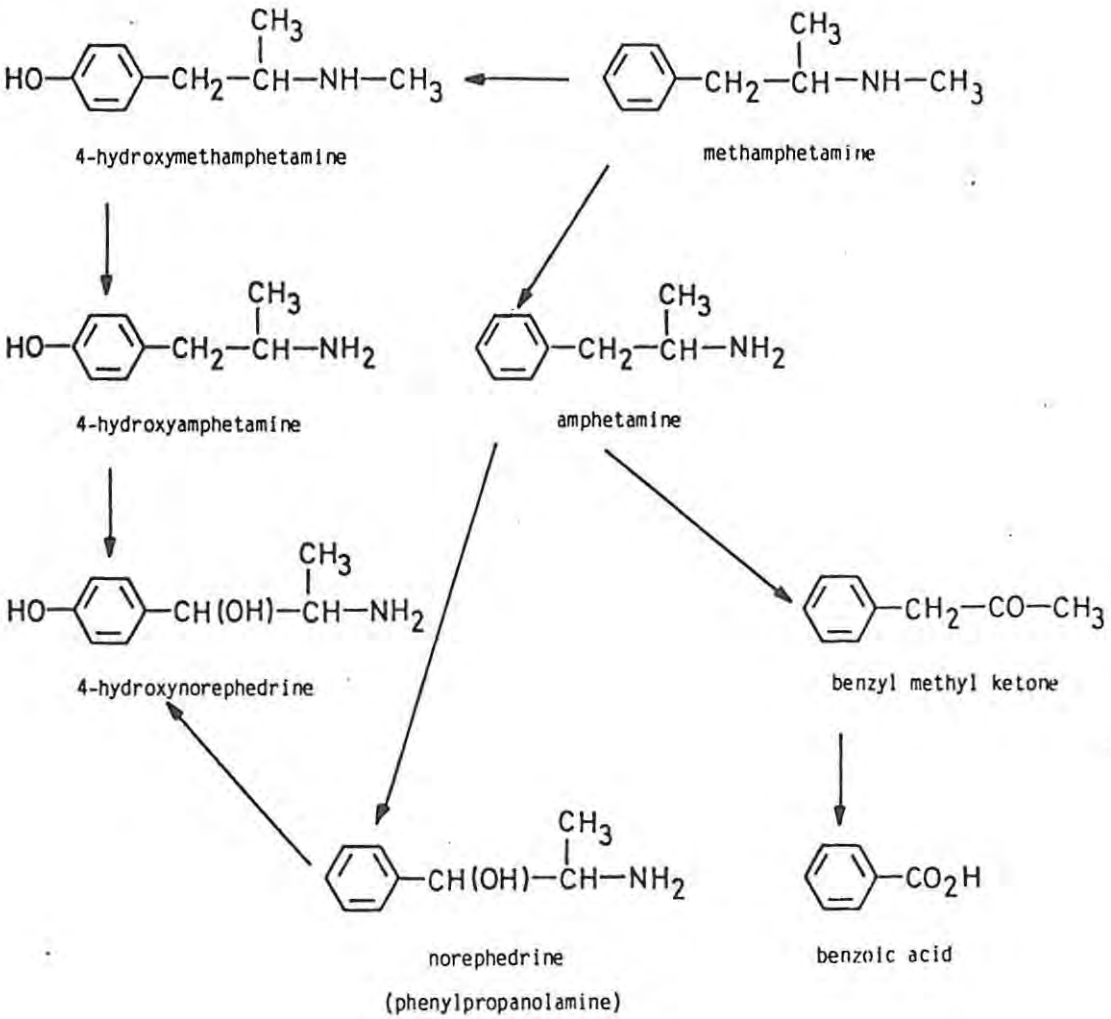


FIGURE 1.10 Probable routes of metabolism of methamphetamine

Within the ephedrine series, successive N-methylation increases the metabolism of the compound i.e. (-)-PPA is eliminated predominantly unchanged, (-)-ephedrine undergoes a small amount of N-demethylation but is also excreted mainly unchanged, while (-)-methylephedrine is extensively metabolized in part to ephedrine

and then to PPA (19,24). This is shown in Fig. 1.11.

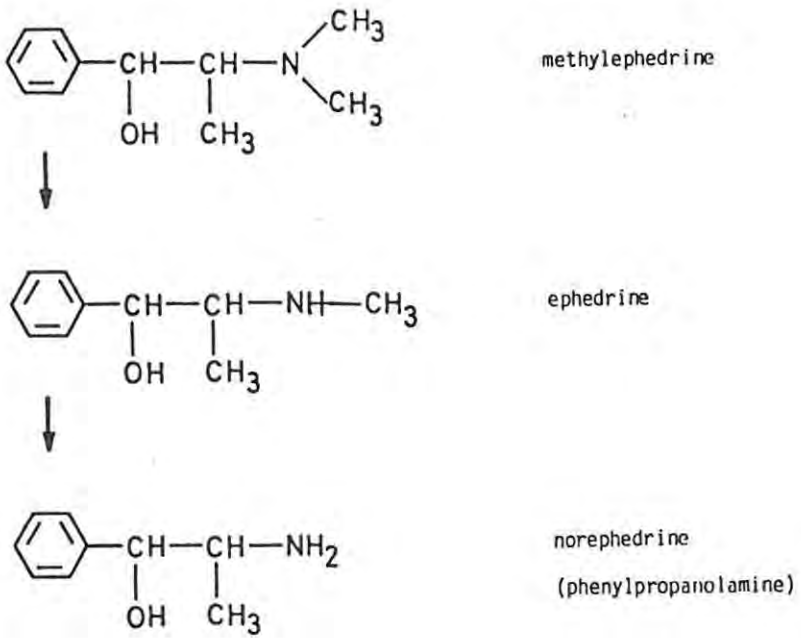


FIGURE 1.11 N-demethylation of methylephedrine

After oral administration of oxyfedrine, PPA was identified in the urine (33). The proposed metabolic pathway of oxyfedrine is shown in Fig. 1.12.

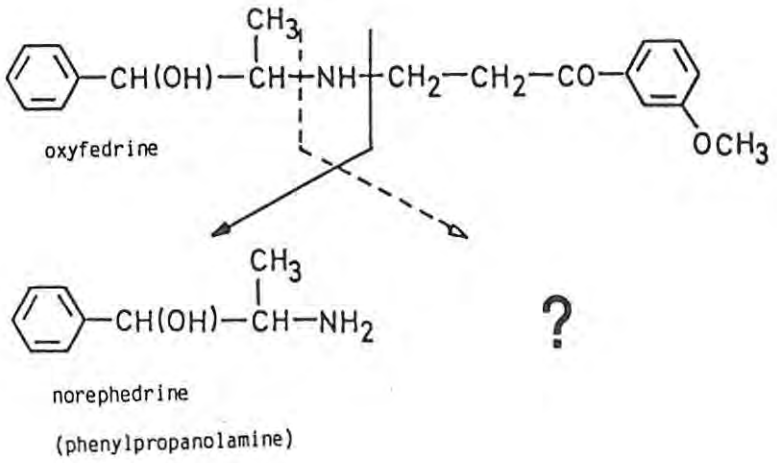


FIGURE 1.12 Proposed metabolic pathway of oxyfedrine

The suspected metabolite containing the propiophenone moeity may undergo further spontaneous chemical transformation. No *p*-hydroxynorephedrine could be detected in urine from man when treated with oxyfedrine (33).

1.3.3 Excretion

The elimination half-life of PPA in man has been reported as 3.9 hours and the elimination rate constant as 0.18 hr^{-1} (25). In all the studies previously mentioned, no attempt was made to control such factors as urinary pH and urine volume output. However it has been shown that these factors play an important role in the kinetics of metabolism and excretion of some basic drugs e.g. (+)- and (-)-amphetamine (34) and (+)- and (-)-methylamphetamine (35). It was proposed that the pH-dependent excretion of these drugs could be explained by passive reabsorption of the unionized drug in the distal tubules in the kidney.

The reported pKa value of PPA is 9.44 at 20°C (9). Recovery of PPA is therefore slightly greater in acidic rather than in alkaline urine, as the more alkaline the urine the lower the fraction of drug in the unionized form and hence the greater the reabsorption.

In a study conducted on the urinary excretion in man of (-)-PPA under extreme acidic and alkaline urinary conditions (19), the excretion rate in subjects with acidic urine (pH 5) reached a maximum about 1.5 to 2.5 hours after administration and then fell exponentially. Urine volume output did not appear to affect the excretion rate significantly. Under alkaline urine control (pH 8) the excretion rate fluctuated throughout the day, corresponding with changes in urine output. A high urine flow rate resulted in an increased excretion of PPA. Fluctuations also occurred which could not be correlated with either changes in urinary output or urinary pH.

An increase in the flow rate has a diluting effect upon the unionized drug concentration and also reduces the time available

for reabsorption back into the body which would result in the availability of more drug for a longer time. However with PPA, reabsorption does not influence the metabolism significantly (19).

1.4 USES OF PHENYLPROPANOLAMINE

The main use of PPA is to relieve congestion of the nasal mucosa and sinuses in the treatment of colds, rhinitis, sinusitis and hayfever (5,36). The drug is administered orally in doses of 12.5 to 50 mg every 3 to 8 hours and is often found in mixtures containing antipyretics, analgesics, sedatives, antihistamines or atropine-like drugs (1).

A number of studies has been reported to determine whether preparations containing PPA are effective in the symptomatic treatment of inflammatory or allergic conditions of the upper respiratory tract, or in reducing the incidence of allergic infectious diseases in patients with asthma and allergic rhinitis (14,36-40). PPA was found to be useful in reducing the number of allergic attacks (35) and in keeping the nasal mucosa and mucous membranes of the lower respiratory passages in a decongested state (14,41), as well as improving air flow in the nasal passages (42-44). Nasal stuffiness of allergic rhinitis may be relieved with the administration of 50-100 mg PPA to adults or 25-50 mg PPA to children, given up to four times daily (45). Phenylpropanolamine, because of its mild stimulatory properties, may be beneficial in combined preparations for allergic rhinitis by decreasing the antihistamine-induced drowsiness (46).

Local topical applications of adrenergic vasoconstrictors in the form of nose drops in oily or aqueous solutions or in a nasal jelly, shrink the engorged mucous membranes thereby promoting drainage, relieving the feeling of stuffiness and diminishing pain. These solutions are usually made isotonic with the addition of sodium chloride, thus protecting the nasal cilia (15). Phenylpropanolamine is used in concentrations varying from 1% to 3% in nasal solutions and jellies (1).

In a trial conducted with patients having increased airway resistance, the effectiveness of phenylephrine and thonzylamine was markedly enhanced by the addition of PPA (47). However, the disadvantage is that their beneficial effect may be followed by rebound decongestion and prolonged use may result in chronic rhinitis (41).

Phenylpropanolamine in dosages of 50 mg twice daily seems to be of some use in relieving or eliminating stress during incontinence due to sphincter weakness by stimulating α-adrenergic receptors at the vesical neck and in the proximal urethra to cause urethral sphincter contraction (48-56). α-adrenergic stimulation with PPA has also been used to correct, at least partially, retrograde ejaculation (57,58) and in heavy menstrual periods PPA has been of some use due to its vasoconstricting effect on the lining of the uterus (59). The drug has been used alone or with an antihistamine in the treatment of urticaria because of its systemic vasoconstrictive effects (60).

Doses of 50 mg PPA have been given by intramuscular injection or by slow intravenous infusion to raise the blood pressure in hypotensive states such as during surgery or spinal anaesthesia (1,61). It has been reported that PPA in combination with paracetamol and phenyltoloxamine citrate is of some benefit in cluster headache (Harton's headache) (62).

Clinically PPA has been used as an anorectic agent for over 40 years and in this time has been subject to both debate and dispute in its use as an adjunct to control obesity (63-65). Many references have been reported in the literature that attest to its efficacy as an appetite suppressant (15,18,63-69). Of eight amphetamines tested for their ability to reduce appetite, PPA ranked seventh, while dextroamphetamine was found to be the most effective (66). However while PPA is weaker in its action, it is relatively safe as it lacks the strong CNS stimulatory effect of amphetamine (67,68) with less likelihood of inducing nervousness or insomnia (69).

In a systematic study on rats, Tainter (68) investigated the actions of PPA in the control of obesity. He found PPA to cause a loss of body weight in white rats comparable to that reported for humans. This loss was not due to energy output changes as measured in revolving cages and no change in metabolism occurred after subcutaneous injection of PPA. However, PPA slowed the rate of passage of food through the gastrointestinal tract. The main clinically applicable effect was diminished food intake, but after approximately a week, tolerance to the drug developed and food comsumption returned to normal. Phenylpropanolamine has also been given to Rhesus monkeys with a resultant significant decrease in food intake and a subsequent weight loss (16). The results of a double-blind evaluation of a formulation containing PPA, caffeine and vitamins, and a placebo in the treatment of exogenous obesity in human volunteers, showed that PPA was both safe and effective as an anorectic agent (18).

Fazekas *et al.* (71) casts doubts on the above assumption as a result of his trial with PPA using mentally deficient patients. He found PPA to be relatively ineffective in reducing appetite when compared to dextroamphetamine, although this is perhaps an unfair analogy as the latter compound is the most powerful of the amphetamines used in the control of the appetite (66). Other reports have stated the inefficacy of PPA as an anorectic agent (72,73). Preparations intended for anorectic effect contain large doses of PPA, 25 to 100 mg being given up to three times daily (18,71,73).

1.5 SIDE EFFECTS OF PHENYLPROPANOLAMINE

Phenylpropanolamine has for many years been the subject of controversial letters, case reports and studies carried out to substantiate its efficacy and its safety for use. On the basis of chemical structure and pharmacological activity it appears that PPA is capable of causing a systemic vasopressor response. Hypertensive episodes have occurred after ingestion of single doses of PPA in previously normotensive subjects (74-78). Similar side

effects have been reported in people who have taken double the recommended dose (79) and three times the recommended dosage for PPA (80). Transient hypertension and cardiac arrythmias developed in a young girl taking 25 mg of PPA three times daily (81).

Horowitz (82,83), in two studies carried out on normotensive volunteers, reported a statistically significant rise in systolic and diastolic blood pressure and demonstrated that in a large group of young, healthy adults, important and sometimes potentially dangerous rises in blood pressure may occur after ingestion of a single capsule containing PPA.

Three patients were reported to have developed clinical evidence of myocardial injury after acute ingestion of PPA (84) and death resulting from the delayed onset of the acute respiratory distress syndrome was attributed to the ingestion of about twelve prolonged action tablets containing PPA with phenylephrine, chlorpheniramine maleate, hyoscyamine, atropine sulphate and scopolamine hydrobromide (85). Two cases were documented in which intracerebral haemorrhage occurred after the ingestion of PPA, although no direct association was proven (75,86).

As a result of the above reports, letters have appeared in various journals condemning the widespread use and promotion of PPA (87-89) and suggesting an increase in restrictions on such preparations. Contrary to these opinions however, researchers have replied to unfavourable articles by stating that in the recommended dosage levels of 25 mg three times a day or 50-75 mg in a sustained-release preparation, PPA is a safe and effective drug (90-92).

Silverman (63) found no increase in systolic or diastolic blood pressure in a controlled study carried out on 37 volunteers after ingestion of a standard dose of 25 mg PPA. He stated that only after excessive dosages were taken or if PPA was taken concurrently with other drugs, did adverse responses occur. Three further studies on normotensive adults revealed no effect on blood

pressure after the ingestion of higher doses of PPA (93-95). A group of hypertensive asthmatic patients were given 25 mg PPA three times a day for one to three weeks. The majority of patients showed no change in their blood pressure readings (90). The formulation of PPA may be an important factor since immediate release tablets seem more likely to precipitate hypertensive episodes than the slow-release preparations (96).

Capsules and tablets containing PPA in combination with caffeine and ephedrine have been named "pseudo-speed" and are commonly abused by adolescents. In combination with alcohol they are reported to produce euphoric effects. Patients have also used these products to enable them to continue working a 60 to 80 hour work week (97). Eleven patients were reported to have neurologic symptoms, acute onset of headache, psychiatric symptoms or seizures after taking "look-alike" pills thought to contain amphetamine but actually containing PPA (98).

Three cases of psychotic episodes were reported after ingestion of preparations containing PPA as an oral decongestant (99). Symptoms such as restlessness, irritability, aggressiveness and insomnia were noted in adults, and children displayed an inability to recognise parents. (100). Hypertension associated with seizures has occurred with normal and elevated doses of the drug (101-103). Wharton (104) reported one case of paranoid psychosis related to the use of PPA as a nasal decongestant. A psychotic episode occurred in a woman taking PPA as an oral diet aid (105) and acute CNS effects ranging from stimulation of the medullary centre to tremor, restlessness, increased motor activity, agitation and hallucinations were reported in 7 women after ingesting a single tablet in an anorectic preparation (106).

Three separate episodes occurred in which patients with bipolar disorder had to be hospitalized after a period of regularly ingesting anorectic preparations containing PPA for a period of more than two weeks (107). Other researchers have reported side effects such as headache, fever, nausea, vomiting, muscle tenderness and

weakness, dyskinesia and slurring of speech (75,108). A 14 year old female, after ingesting 15 to 18 capsules, was reported to have experienced blurred vision, nervousness, tremor, inability to work and serious arrythmia (109).

PPA has been known to cause significant blurring of eyesight and glaucoma in some patients (110). Acute renal failure and rhabdomyolysis was reported to have occurred after ingestion of PPA-containing diet pills (111). Two cases of allergy to PPA have been reported, resulting in symptoms of dyspnea, wheezing, cough, hives and facial swelling in one case, and chest tightness, wheezing and marked fatigue in the second (112). PPA should not be used in hypertensive, hyperthyroid, cardiac, depressed or diabetic patients (87).

1.6 INTERACTIONS OF PHENYLPROPANOLAMINE WITH OTHER DRUGS

The potential for adverse effects following the use of PPA has been the topic of sporadic reports and studies. A number of these reports refer to situations in which PPA has been taken concomitantly with other drugs.

Concurrent administration of an indirectly-acting sympathomimetic amine and a monoamine-oxidase inhibitor (MAOI) can result in a moderate to severe hypertensive crisis characterised by a sharp rise in blood pressure accompanied by tachycardia, chest pains and severe occipital headache. Other symptoms have included neck stiffness and hypertonicity of the limbs, epileptiform convulsions, hyperexia, sweating, flushing, vomiting and unconsciousness (113,114). In some cases the hypertensive crisis has been so severe that it has led to intracranial haemorrhage, cardiac arrythmias and cardiac failure.

The symptoms of the interaction have been attributed to the over-stimulation of sympathetic receptors, in particular those associated with the cardiovascular system. The inhibition of monoamine oxidase within the sympathetic nerve endings permits the

buildup of large amounts of noradrenaline, which, when released by indirectly-acting sympathomimetic amines causes a massive stimulation of the receptors (115).

Several case reports have appeared in the literature describing this type of reaction where the patients were receiving chronic MAOI treatment, and after ingestion of a preparation containing PPA, a number of the above symptoms were manifested (116-121). In an experiment with healthy volunteers, Cuthbert (122) found that severe reactions appear more likely with standard dosage forms of PPA, while the sustained-release preparations are more prone to produce headache, nausea and vomiting. When PPA and tranylcypromine were given together, a rapid and potentially dangerous rise in blood pressure resulted (122). D'Mello (123) showed that PPA is not a substrate for MAO and that other enzymes are involved in its metabolism.

A case has been reported of an interaction of PPA with cheese causing similar symptoms to those described above (124). This could have been due to the high tyramine content of the cheese having an additive effect on the action of another sympathomimetic amine.

There appears to be a mutual antagonism between the effect of the guanidines and the indirectly-acting sympathomimetics (125,126). Patients receiving PPA are likely to be refractory in some degree to the actions of the guanidines. The hypotensive action of bethanidine has been clearly shown in one case to be reversed by PPA (127). The interaction appears to involve competition between the two types of agents for entry into the nerve ending.

Acute rises in blood pressure can be precipitated by the effect of PPA-containing preparations reversing the antihypertensive effect of β-adrenergic neuron-blocking drugs (128-130). In one case the patient was receiving both methyldopa and oxprenolol when the PPA was given. It is postulated that the methyldopa increased the amount of α-methylnoradrenaline in storage sites in the adrenergic

neuron which was released by PPA. The β-effects of the α-methyl-noradrenaline were blocked by oxprenolol, resulting in excessive α-stimulation and thus hypertension (131).

Two reports have appeared in the literature on the appearance of hypertension after concurrent ingestion of PPA and indomethacin (132,133) and it has been suggested that PPA may diminish the effect of anticoagulants (134). In another case, death was attributed to ventricular arrythmia induced by thioridazine in combination with a single capsule containing PPA (135).

There is one report of a positive phentolamine phaeochromocytoma test in hypertension induced by PPA (80). In some over-the-counter products containing caffeine and PPA, the possibility of an additive effect with these two cardiac stimulants should be considered (81).

1.7 ORAL SUSTAINED-RELEASE PHARMACEUTICALS

Over the past 30 years, pharmaceutical manufacturers have introduced a great number of products which, after their administration, are claimed to provide a long-term therapeutic response.

Enteric coatings were used many decades ago to prevent gastric irritation and slow the release of the active ingredient when the tablet had passed from the stomach to the small intestine. In 1938 Lipowski described the construction of an oral dosage form consisting of a number of small beads containing the dose of a drug, with several thicknesses of coating utilized to give a slow and constant release of drug on ingestion. However only in 1952 was the first practical sustained-release type marketed, and since then interest in and manufacture of sustained-release products has increased considerably (136).

1.7.1 Terminology

Owing to the large number of names used to describe long-acting

products, it is desirable to classify them using the following terminology (137,138):

1. Sustained-release. This term describes a formulation in which a drug is initially made available to the body in an amount sufficient to cause the desired pharmacological response. This occurs as rapidly as is consistent with the properties of the drug determining its intrinsic availability for absorption. The formulation also provides for maintenance of activity at the initial level for a desirable number of hours in excess of the activity resulting from the usual single dose of the drug.
2. Prolonged-action. Included in this classification are those products in which a drug is initially made available to the body in an amount either sufficient to, or not dangerously in excess of, the amount needed to cause the desired therapeutic response. These products also provide for replacement of the drug at some rate which gives a measurable increase in the length of time activity for the preparations when compared to the usual single dose.
3. Repeat-action. In these formulations provision is made for the usual single dose of the drug and they are constructed so as to provide another single dose at some time after administration.

1.7.2 Advantages and Disadvantages of Sustained-Release Products

A general advantage of the sustained-release preparation is convenience and improved patient compliance as it reduces frequency of administration and missed doses due to forgetfulness. An economic advantage arises in hospitals as nursing time for drug distribution and administration is minimised, thus releasing nurses for other duties. Cost and time of dispensing is also reduced (138,139). Adequate overnight therapy is provided without having to wake the patient for administration of single doses (140,141).

Therapeutic advantages include control of the level of sleep throughout the entire period of sleep, control of nocturnal seizures associated with epilepsy, control of enuresis, of migraine headache on awakening and of appetite over long periods

of time (137). The variation in drug concentration with high peak levels and sub-therapeutic concentrations commonly associated with periodic dosing is reduced with controlled-release products. As a result a more uniform response to the drug occurs. With a reduction of toxic effects at high drug blood levels, a better control of the condition can be maintained at low blood levels and the severity or frequency of untoward side effects and gastrointestinal irritation may sometimes be reduced (140,142,143).

Undesirable consequences may result such as a lack of precision of dosage which varies between patients depending on rate of excretion and rate of stomach emptying, thus affecting drug absorption. Poor product design may result in incomplete or less efficient release than that from immediate-release forms. The too frequent administration of sustained-release dosage forms may result in undesirably high blood levels of the drug due to repetition of the loading dose. These dosage forms do not permit the prompt termination of the effects of the drug if severe toxic or side effects develop (137,138,141).

1.7.3 Properties of Drugs for use in Sustained-Release Products

The drug should be orally effective and resistant to decomposition in the gastrointestinal tract, have a short duration of action with an elimination half-life of less than 12 hours and be absorbed throughout the gastrointestinal tract. The drug should have a relatively small therapeutic dose as usually two, three or four times the single dosages are incorporated into a sustained-release product with the final product being of a suitable size for ingestion (144,145).

Drugs having a slow onset of action should be formulated with only part of the drug being in the slow-release portion (146). With certain drugs which are inherently long-acting due to their long half-lives, there is little or no need to formulate them into a sustained-action form. Poorly absorbed drugs or drugs with a narrow absorption window should not be used for these products as

they are so formulated to decrease the rate of release of the drug. When dosage is critical *e.g.* hypotensives and anticoagulants, these preparations would be unsuitable due to the difficulty of obtaining a critical or accurate dose (145).

1.7.4 Design of a Sustained-Release Dosage Form

In designing a controlled-release dosage form, the pharmacodynamics and the pharmacokinetics of the active substance must first have been elucidated for a standard dosage form. From a pharmaceutical viewpoint the only factors which can be modified are the strength, disintegration and dissolution of the dosage form (139).

In the determination of the total amount of drug to be incorporated into the preparation, important considerations are knowledge of the initial dose, the total daily dose and the frequency of administration of the drug in order to obtain a satisfactory response (145). An important parameter for the controlled release of active substance is the input rate constant 'k', which is a combination of *in vivo* dissolution and absorption. However if the half-life of elimination is large, the elimination rate constant 'λ' will determine the overall kinetics, and any changes in 'k' will have little effect (139).

With wide variations likely to be encountered in drug elimination time, the design of long-acting products is necessarily concerned with this factor. If the elimination rate constant, k, and the normal dose, A_n, required to produce a satisfactory blood level are known, the maintenance dose, A_m can be calculated (147).

$$A_m = A_n \, k \, h$$

$$= A_n \frac{0.693 \, h}{t_{1/2}} \qquad (1\text{-}1)$$

where 'h' is the number of hours of maintenance desired. The amount in the maintenance portion could range from one to four times the amount in the initial portion.

1.7.5 Evaluation of Sustained-Release Dosage Forms

In the case of true sustained-release products, pharmacological activity may be reflected by blood concentrations. The intensity of a pharmacological effect is related to the concentration of the active substance at some receptor in the organism and in turn this concentration must be correlated in some way with the measurable amount of substance present in the plasma (139).

Modification of the pharmacodynamics by use of a sustained-release dosage form changes the time course of the plasma concentration. Evaluation of sustained-release dosage forms involves bioavailability testing, bioavailability being defined as the measurement of the rate and extent to which the active drug ingredient is absorbed from a drug product and becomes available at the site of action (148).

For the assessment of bioavailability, the concentration of the drug in a selected body fluid can be determined over a period of time after administration of the preparation, and hence an indication of the rate and amount of absorption of the drug from the preparation can be obtained. Assessment of long-acting products should ideally be done by properly designed *in vivo* trials where actual measurements of drug concentration are compared to concentrations resulting from the usual single dose administered in solution or in a rapidly disintegrating tablet where the pharmacokinetics are known (149).

The most commonly used method for determining bioavailability involves the construction of plasma concentration-time curves from which three important parameters can be derived: the peak concentration, the time of peak concentration and the area under the curve which is related to the total amount of drug absorbed from the dose (139,140,148-150). Thus time of onset and duration of effect can be estimated provided the therapeutically effective minimum and maximum blood level concentrations have been determined as seen in Fig. 1.13.

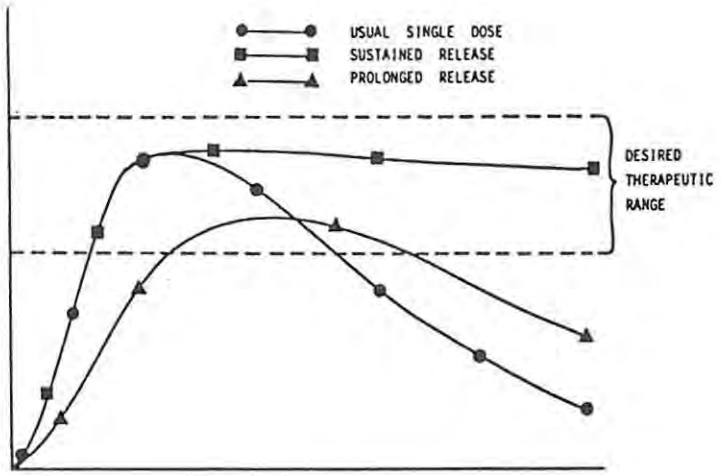


FIGURE 1.13 Relationship between drug activity and time

To maintain a reasonably constant concentration of drug in the blood over an estimated time, the first dose should contain both the rapidly absorbed loading dose and the delayed-release form of the drug, while the second and succeeding doses should contain only the necessary amount of the delayed-release form of the drug (137).

Measurement of the urinary excretion of a drug, or of a drug and its metabolites can also be used in bioavailability studies. The advantages are that samples are more easily obtained , the method is non-invasive and it involves less trauma for the subjects. (139,140,148,150). The concentration of a drug in the urine is usually greater than that in the blood and less sensitive analytical methods can often be used.

However, this procedure is only applicable when predominantly unchanged drug is excreted or when, in the case of a drug modified in the body prior to excretion, the metabolism of the drug is well understood. It must also be shown that, in the same subject, the urinary excretion of the drug is proportional to the plasma concentrations. When the distribution and excretion are more complex, the information gained from urinary measurements can only be used as a supplement to that gained from blood measurements.

Important parameters gained from urinary measurements are the cumulative amount excreted and the rate at which this excretion takes place. The pH of the urine may have to be controlled for those drugs which are partially ionised at physiological pH values and where there is a significant difference in solubility between the ionised and unionised forms.

In the case of PPA, urinary excretion is very well suited to testing different formulations since a relatively large fraction of the absorbed PPA is excreted fairly rapidly in the urine (139). Heimlich *et al.* (25) employed urinary excretion data to evaluate a sustained-release dosage form containing PPA and to compare it with an immediate-release formulation of the drug. Results showed that PPA contained in the sustained-release dosage form was excreted in a manner similar to that of the drug in the conventional dosage form when an equivalent dose of the latter was given at 4-hourly intervals for 3 doses.

1.7.6 Sustained-Release Formulations (137,140,145,151)

1.7.6.1 Coated slow-release beads. Manufacture of formulations of this kind involves the preparation of beads or pellets, groups of which are coated with different materials of varying thicknesses. The total dose can be divided into three to nine parts, one part of which is intended to establish the initial therapeutic level, with the remaining parts prepared for the sustained-release dose. Materials used for coatings are beeswax, carnauba wax or bayberry wax, with glyceryl monostearate or similar fatty acid esters. Dose division increases the probability that the effective dose of the drug will be made available for absorption, hence well-designed long-acting products can be expected to make gastrointestinal absorption more regular and predictable.

1.7.6.2 Tablets with slow-release cores. These consist of a core containing the therapeutically active material evenly mixed with substances of low solubility which permit the drug to dissolve slowly or to be leached from the poorly soluble substance. An outer layer which also contains therapeutically active material as

well as excipients, binders and lubricants is compressed onto the core.

1.7.6.3 Repeat-action tablets. A core containing the repeat dose is prepared in a manner similar to that of an enteric coated tablet so as to delay the release of the drug for a few hours after the outer coat has dissolved. The initial dose is applied by usual techniques to the core.

1.7.6.4 Tablet mixed-release granules. These preparations contain granules made by normal methods carrying the initial dose of the drug, as well as granules containing drug either coated with slowly digestible or poorly soluble materials or mixed with solution-retarding additives such as glyceryl monostearate, glyceryl monopalmitate, beeswax, stearic acid and cetyl alcohol. The two sets of granules are lubricated and compressed into tablets.

1.7.6.5 Multiple-layer tablets. Modern multiple-layer compression machines allow the incorporation into one tablet of two or three separate layers of granulation, one layer providing the initial dose, the second layer for release at an intermediate rate and the third for release at a slower rate.

1.7.6.6 Porous inert carriers. The dosage form is prepared from a small plastic pellet containing a large number of small passsages which are filled with the drug and a "channeling agent" which attracts the fluid of the gastrointestinal tract. Thus the drug dissolves and diffuses from the passages to the body fluids and the insert plastic pellet is excreted in the faeces.

1.7.6.7 Ion-exchange resins. An ionic drug is allowed to diffuse through an ion-exchange resin where it replaces the inorganic ions on the resin and a slow displacement reaction occurs when the drug-resin complex contacts gastrointestinal fluids containing ions. A decrease in the rate of solution is obtained and thus a prolonged action occurs.

1.7.6.8 Slightly-soluble salts or complexes. A prolonged-action compound may result from preparations of salts or complexes of active drugs that are only slightly soluble in gastrointestinal fluids.

CHAPTER 2

ANALYTICAL METHODS

2.1 INTRODUCTION

Chromatography as a separation technique was first described in 1906 by a Russian, Michael Tswett (153,154). Liquid chromatography, the oldest form of chromatography, is a separation process governed by partition, adsorption or ion-exchange phenomena.

High performance liquid chromatography (HPLC) is widely used in the analysis of complex mixtures in the pharmaceutical industry, in food processing and in the chemical industry and has become increasingly popular in the clinical laboratory as it is capable of detecting low physiological concentrations of many medicinal compounds.

Adsorbants such as silica, are widely used with relatively non-polar mobile phases which usually contain a hydroxylic solvent to ensure that the column is at constant activity. In contrast, reverse phase chromatography involves the addition of a hydrocarbon chain to the silica which renders it non-polar, and involves the use of polar mobile phases. The mechanism of separation is based on the interaction of the components in the mixture between the mobile phase and the stationary phase, and compounds which are strongly retained on silica are only slightly retained on a reverse phase and vice versa. The most common alkyl groups are C_2, C_8 and C_{18} which are bonded to various sizes and shapes of silica, imparting varying degrees of lipophilicity to the column.

Ion-exchange chromatography using bonded phases will separate only positive or negative ions whereas paired-ion chromatography involves the use of a silica column with a mobile phase that contains a counter-ion which results in the formation of a lipophilic ion-pair complex. This technique was developed to solve

analytical problems associated with samples containing ionic species and to avoid problems of pH control, reproducibility and short column life, as well as allowing the simultaneous assay of acids, bases and neutral compounds (155).

Reverse phase paired-ion chromatography uses a reverse phase column with a mobile phase containing a counter-ion and thus for ionic compounds that are poorly retained on conventional reverse phase systems, a dramatic increase in retention time is observed in the appropriate paired-ion chromatography system. The reverse phase technique has a number of associated advantages,such as enabling the separation of a large number of compounds using relatively inexpensive solvents. Furthermore, the aqueous solvents used are compatible with biological samples which makes direct analysis possible without the prior necessity of solvent extraction (156,157). Most endogenous compounds in biological fluids are predominantly hydrophilic, therefore, using reverse phase chromatography these compounds will elute first, before the compound of interest.

2.2 ANALYSIS OF PHENYLPROPANOLAMINE ALONE AND IN PHARMACEUTICALS

2.2.1 Ultraviolet Spectrophotometric Analysis

Periodate oxidation of PPA.HCl has been used where the sample to be determined is placed in a separatory funnel with $NaHCO_3$ and $NaIO_4$ and after standing for about 15 minutes, the solution is extracted with hexane after the addition of 1M HCl. The extract is filtered and the absorbance determined at 242 nm in 1 cm cuvettes using hexane as the reference solution. The amount of the oxidation product of PPA.HCl is determined by comparison of the sample absorbance against the absorbance of a PPA.HCl reference standard treated in the same manner (158). Other organic solvents such as ether (25) and chloroform (173) have also been used for the extraction of the derivative. Wallace (159) used alkaline periodate oxidation to form benzaldehyde which was subsequently converted to the semicarbazone derivative, thereby enhancing the sensitivity and specificity of the procedure. The drug has also

been determined after alkaline extraction into an organic solvent followed by back-extraction under aqueous acidic conditions (160).

2.2.2 Colorimetric Analysis

Phenylpropanolamine hydrochloride can be reacted with ninhydrin in a citrate buffer at elevated temperatures and determined colorimetrically at 570 nm. This reaction has been applied to the determination of PPA.HCl in a multicomponent mixture by an automated system which, after phase separation, utilizes the stream-splitting technique to divide the chloroform stream into segments (161). An ion-pair extraction technique using an acidic dye, bromothymol blue, has been utilized and the resulting chloroform exctract determined at 420 nm (162).

2.2.3 Spectrofluorometric Analysis

Phenylpropanolamine hydrochloride has been determined by measuring its fluorescamine derivative, 4-phenylspiro(furan-2(3H),1'-phthalan)-3,3'-dione at 480 nm using an excitation wavelength of 398 nm (163).The reaction favours a pH of 9 for optimal reactivity (164).

2.2.4 Titrimetric Analysis

After extraction of PPA.HCl from an alkaline aqueous solution with chloroform, shaking with saturated NaCl solution and back-extracting with an excess of H_2SO_4, the excess acid is titrated with a standard NaOH solution using methyl red as indicator (165).

2.2.5 Chromatographic Analysis

2.2.5.1 Column Chromatography

A weakly basic anion exchange resin, Amberlite IR-45, was found to be suitable for the separation of PPA.HCl from various dosage forms and yielded a 99.6% recovery of the drug which was then determined titrimetrically (166). Being a nitrogenous base, the drug is retained on a sulphonated polystyrene cation exchange resin. Determination is then effected by measuring the ultraviolet absorption after elution with HCl (167,168). The drug has also been determined by mixing with NH_4OH, eluting with chloroform from a Celite column and measuring the absorbance at 258.5 nm (169).

Separation of PPA.HCl from mixtures of drugs in various dosage forms has also been described (170). The method involves the retention of PPA on the first of four Celite columns followed by elution, addition of NaOH, extraction of the free base with chloroform, back-extraction into H_2SO_4 and subsequent determination at 257 nm using H_2SO_4 as the reference solution. An on-column periodate oxidation of PPA to benzaldehyde has also been described and the derivative determined at 267 nm (171).

2.2.5.2 Paper Chromatography

A descending paper chromatographic technique using Whatman No.1 paper has been reported. The solvent system consisted of a 1:1 mixture of butanol (saturated with 1M HCl) and methanol. After spraying with Dragendorff's reagent, the resulting orange-red spots were quantitatively determined by photoelectric densitometry (172).

2.2.5.3 Thin Layer Chromatography

A number of thin layer chromatographic systems have been described for PPA.HCl and these are listed in Table 2.1. Phenylpropanolamine is detected by spraying with reagents to form a coloured or fluorescent spot, or by overspotting the drug with a derivatizing agent. These procedures are necessary as PPA has poor inherent ultraviolet absorptivity and consequently is difficult to detect directly.

2.2.5.4 Gas Chromatography

Gas chromatography has been extensively used for determining PPA in pharmaceutical preparations and, to a lesser extent, for the determination of the drug in biological fluids. The drug has been chromatographed directly without derivatization and also as the silyl, pentafluorophenyloxazolidine, acetone, butanone, trifluoroacetyltrimethylsilyl, heptafluorobutyryl and the 2,6-dinitro-4-trifluoromethylbenzenesulphonic acid derivatives. The gas chromatographic conditions are listed in Table 2.2.

TABLE 2.1 THIN LAYER CHROMATOGRAPHIC SYSTEMS FOR PHENYLPROPANOLAMINE

Solvent System	Adsorbant	Detection	Rf	Ref
Chloroform layer from a mixture of chloroform/ acetic acid/methanol/ water (85:20:8:20)	Silica Gel G (F254) (60μ)	Sprayed with 0.3% *p*-nitroaniline plus 5% sodium nitrite, heated at 70°C for 15 minutes then sprayed with 20% Na_2CO_3	Not reported	173
Not reported	Slica Gel G (F254) (60μ)	Sprayed with buffer (pH 9.3), over-sprayed with fluorescamine/acetone solution and resprayed with buffer	0.50	164
Benzene/ethyl acetate (70:30)	Gelman ITLC fibre SAF	*p*-nitrobenzoyl chloride applied and heated (100°C)	0.79	174
Benzene/ethyl acetate (30:70)			0.90	
Hexane/ethyl acetate (50:50)			0.76	
Hexane/ethyl acetate (30:70)			0.90	
Ethyl acetate/methanol/ formic acid (69:30:1)	HPTLC Silica Gel 60	Dipped into *o*-phthalaldehyde solution followed by 20% polyethylene glycol in methanol	0.40	175
n-butanol/acetic acid/ water (4:1:5)	DC-cellulose	Ninhydrin reagent	Not reported	33
Heptane/methanol (60:40)	HPTLC Silica Gel 60	Fluorescamine solution	0.45	176
Benzene/methanol (83:17)			0.37	
Benzene/acetone/methanol/ dioxane (40:40:4:5)			0.28	
Ethyl acetate/methanol/ water/ammonia (85:10:3:1)	Silica Gel	Sprayed with 0.3% ninhydrin acid, heated at 100°C for 5 minutes, sprayed iodoplatinate reagent then p-nitroaniline reagent, heavily sprayed with 25% alcoholic NaOH solution	Not reported	177
Chloroform/methanol (4:1)	Silica Gel G	Iodine vapour	0.17	178
Chloroform/methanol (4:1)	Silica Gel G (HF254)	Iodine vapour	0.14	

TABLE 2.2 GAS CHROMATOGRAPHIC SYSTEMS FOR PHENYLPROPANOLAMINE

Drug Source	Column	Carrier Gas	Column Temp(°C)	R_T(mins)	Detector	Injected as	Ref
Tablets, syrup	2.0m x 4.0mm (i.d.) glass 0.1% silicone oil (DC-710) on 60-80 mesh dimethyldichloro-silane treated glass beads.	He	200	2.80	FID	Silyl derivative	179
Syrup	2.4m x 3.2mm (o.d.) Pyrex glass 2% SE-30 on Chromosorb W (HP)	He	180	1.80	FID	Phenylprop-anolamine	180
Tablets	1.8m x 4mm glass 3% OV-17 on Gas Chrom Q	He	230	0.85	FID	Phenylprop-anolamine	181
Tablets, capsules, liquids	1.8m x 6.4mm (i.d.) glass 1% HI-EFF-8BP and 10% SE52 on Gas Chrom Q	N_2	220	2.40	FID	Phenylprop-anolamine hydrochloride	182
	4% HI-EFF-8BP on Gas Chrom Q		220	1.25			
Raw material	1.4m x 4mm (i.d.) glass 3% Poly A 103 on Gas Chrom Q	He	Progr-ammed 70-250	28.00	FID	Trifluoroacet-yltrimethylsi-lyl derivative	183
Raw material	1.1m x 2.5mm glass 18.8% Apiezon N on Diatoport S	N_2	180 138 101	2.55 2.77 3.18	FID	Phenylprop-anolamine	184
Raw material	1.8-2.4m x 3mm (i.d.) glass 1.15% SE-30 on Gas Chrom P	Ar	104	9.10 13.40 123.50	EC	Phenylprop-anolamine Acetone derivative Butanone derivative	185
Raw material	1.8m x 2mm (i.d.) glass 3% OV-1 on Supelcoport	N_2	250	1.38	EC	2,6-dinitro-4-trifluoro-methylbenzene sulphonic acid derivative	186
	0.9m x 2mm (i.d.) glass 3% SP-2250 on Supelcoport		220 230	1.38 0.98			
Raw material	1.2m x 4mm (i.d.) glass 2% SE-30 and 2% Carbowax 20M on Anachrom ABS	N_2	185	1.60	FID	Phenylprop-anolamine hydrochloride	187
Raw material	1.8m x 2mm (i.d.) glass 3% OV-17 on Anachrom ABS	He	Progr-ammed 100-250	8.36	Nitrogen	Phenylprop-anolamine	188
Bio-logical material	1m x 6mm (o.d.) glass 7.5% Carbowax 20M on Chromosorb W	N_2	165	4.10	FID	Phenylprop-anolamine	178
	2.0% Carbowax 20M on Chromosorb G		120	1.10			

2.2.5.5 High Performance Liquid Chromatography

Methods and the associated conditions for the high performance liquid chromatographic determination of PPA alone and in pharmaceutical formulations are listed in Table 2.3. Relatively little information has appeared on the HPLC analysis of PPA in biological fluids (see Section 3.1).

2.3 DEVELOPMENT OF AN HPLC METHOD FOR THE DETERMINATION OF PHENYLPROPANOLAMINE

2.3.1 Methods

2.3.1.1 Column Packing Material

The column used throughout the developmental stages was a reverse phase C_{18} octadecylsilane column. The use of a guard column packed with 20-40 micron LC-18 pellicular packing (Supelco Inc., Pennsylvania) was initiated at various stages during development as biological samples were being injected directly onto the column. The guard column serves to trap particulate matter and high molecular weight sample constituents such as protein, thereby prolonging the life of the analytical column.

2.3.1.2 Detection Wavelength

All determinations in the initial stages were done at 206 nm using a fixed wavelength Uvicord S ultraviolet detector Model LKB 2138. Detection in the later stages was accomplished using a Pye Unicam LC3 variable wavelength ultraviolet detector. After injecting aqueous samples of PPA.HCl (500 ng/ml) at wavelengths ranging from 200 nm to 260 nm, it was found that 220 nm yielded the most stable baseline with a satisfactory response.

2.3.2 Results and Discussion

Phenylpropanolamine hydrochloride absorbs in the ultraviolet wavelength range (200) but its inherent molar absorptivity is poor. The need to detect low concentrations of PPA (15 ng) necessitated the choice of a wavelength where absorbance was relatively high. Although the absorbance of PPA at 206 nm was good, the absorbance of other interfering compounds also increased as the wavelength

TABLE 2.3 HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC SYSTEMS FOR PHENYLPROPANOLAMINE

Drug Source	Column	Mobile Phase	Flow rate (ml/min)	R_T(mins)	Detection	Ref
Tablets, syrup	0.5m x 2.1mm (i.d.) Du Pont Zipax SCX	0.02M $(NH_4)_2HPO_4$ in 36% dioxane/water	1.0	2.2	Stated only as u.v.	189
Syrup	0.3m x 4mm (i.d.) Waters μBondapak C_{18}	0.05M KH_2PO_4 in water containing 13% (v/v) methanol	2.0	3.0	254nm	190
Tablets, capsules, liquid	0.3m x 4mm (i.d.) Waters μBondapak phenyl	Water/methanol/acetic acid (55:44:1) containing 0.005M sodium heptane sulphonate	2.0	Not stated	254nm	191
Tablets, liquid	0.3m x 4mm (i.d.) Waters μBondapak CN	13% acetonitrile in water containing 1.8% acetic acid 13% acetonitrile in water containing 1.8% acetic acid and 0.005M sodium heptane sulphonate	Gradient elution	7.5 8.8	254nm	192
Tablets	0.25m x 4.6mm (i.d.) Whatman Partisil-10-ODS	2.85×10^{-3}M ethylenediamine buffer (pH 7.44)/acetonitrile (1:1)	3.8	3.3	216.5nm	193
Syrup	0.3m x 3.9mm (i.d.) Waters μBondapak phenyl Waters μBondapak C_{18}	Methanol/water (60:40) with 0.004Msodium heptane sulphonate and 1% acetic acid	1.0 1.0	7.4 11.7	254nm	194
Syrup	0.15m x 4.6mm (i.d.) Spherisorb S5W	0.1M KH_2PO_4 in 10% aqueous ethanol	1.0	5.4	198nm	195
Spray	0.3m x 4mm (i.d.) Waters μBondapak C_{18}	Methanol/water (50:50) with PIC reagent B-7		3.1	254nm	196
Syrup	0.25m x 4.6mm (i.d.) Zorbax TMS column	hexanesulphonic acid/acetic acid/water/methanol (0.1:1:68.9:30)	2.5	2.4	254nm	197
Liquid	0.3m x 4mm (i.d.) Waters μBondapak C_{18}	methanol/water/tetrahydrofuran 80% phosphoric acid (67:29:4: 0.1) with 5.8g dioctylsulfosuccinate adjusted to pH 3.8	1.3	7.1	254nm	198
Tablets, capsules, liquid	0.25m x 4mm (i.d.) Waters μBondapak C_{18} 0.25m x 4.6mm (i.d.) Whatman Partisil-10 C_8	Methanol/water (35:62.5) with 2.5ml PIC reagent B-5 Methanol/water (30:67.5) with 2.5ml PIC reagent B-5	2.0 2.0	5.1 5.8	254nm	199

decreased. At 220 nm, the baseline was more stable with an improved signal/noise ratio. At this higher wavelength, less interference occurred from other compounds, hence 220 nm was chosen as the wavelength for the determination of PPA in biological samples and in dissolution media.

A number of compounds were assessed for use as an internal standard. Phenylephrine hydrochloride (PEP.HCl) was chosen as the internal standard for dissolution studies as it eluted before PPA.HCl, peak shape was sharp and symmetrical and resolution between the two peaks was satisfactory. In the analysis of serum and urine samples, endogenous compounds in the biological fluids eluted just before PPA and would have obscured the PEP.HCl peak. Consequently ephedrine hydrochloride (EPH.HCl) which eluted after PPA was chosen as the internal standard.

1-heptanesulphonate sodium, an ion-pairing agent commonly used for bases, was included in the mobile phase. This compound pairs with PPA.HCl to form a neutral, non-polar complex. In preliminary studies, binary mixtures consisting of varying proportions of methanol and a 0.005M solution of HSS were utilized to separate PPA.HCl and PEP.HCl. Baseline resolution was obtained using 35% methanol/65% HSS solution (0.005M), but broad peaks resulted, with long retention times (Fig.2.1). Acidification with acetic acid (0.5%) caused swamping of the detection system at 206 nm and the inclusion of a buffer, dibasic potassium phosphate (0.001M) in a further mobile phase, yielded an extremely unstable baseline.

Since it was necessary to decrease the pH of the mobile phase to reduce tailing of the compounds of interest, hydrochloric acid was included as this did not interfere with detection at 206 nm. The use of halides is, however, not ideal as they are detrimental to the stainless steel components of the HPLC apparatus. Care was thus taken to flush the system thoroughly each night with 50% methanol/50% water. The mobile phase containing 45% methanol/55% HSS solution (0.005M) with 0.2% 1M HCl improved peak shape dramatically but caused a loss in resolution (Fig.2.2).

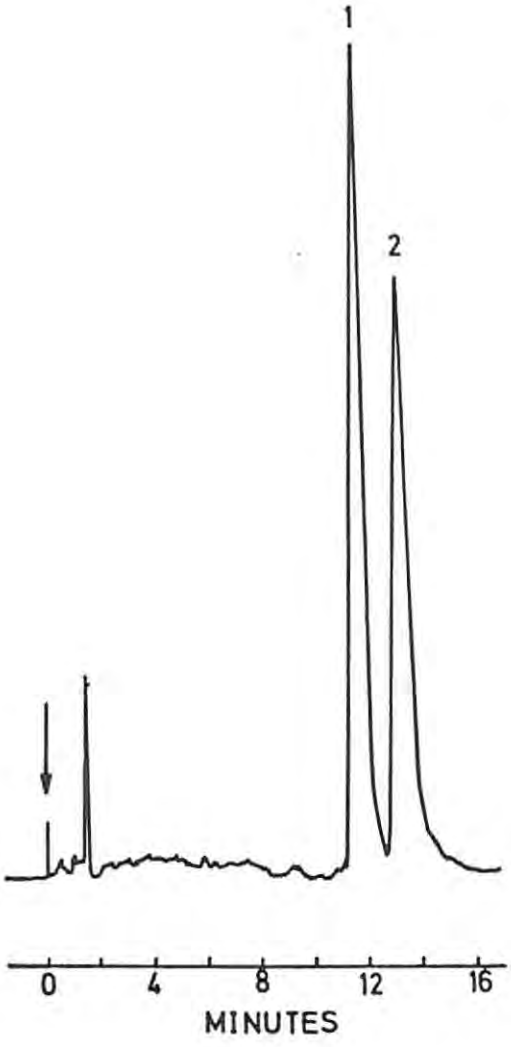


FIGURE 2.1 Chromatogram of PPA (1) and EPH (2)
MOBILE PHASE: 35% methanol/65% HSS solution (0.005M)

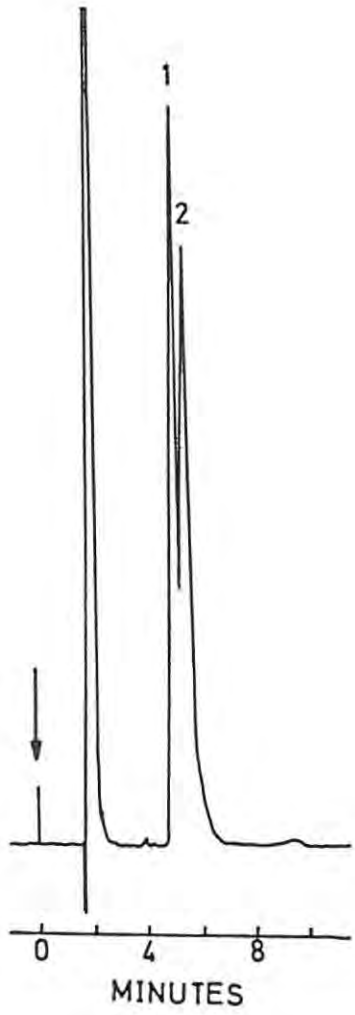


FIGURE 2.2 Chromatogram of PPA (1) and EPH (2)
MOBILE PHASE: 45% methanol/55% HSS solution (0.005M) with 0.2% 1M HCl

Acetonitrile (HPLC grade) was then used in place of the methanol component and temperature control was introduced. An unacidified mobile phase consisting of 30% acetonitrile/70% HSS solution (0.005M) afforded resolution at ambient temperatures, although tailing of both peaks occurred (Fig.2.3). Increasing the temperature to 30°C had little effect on the tailing. The inclusion of tetrahydrofuran in the mobile phase improved peak shape but caused a loss in resolution. A different ion-pairing salt, dodecylsulphate sodium, was included in an acidified mobile

phase at a concentration of 0.005M, but a marked deterioration in peak shape and resolution resulted and an increase in retention time occurred as was anticipated due to the increase in chain length of the ion-pairing agent (Fig.2.4).

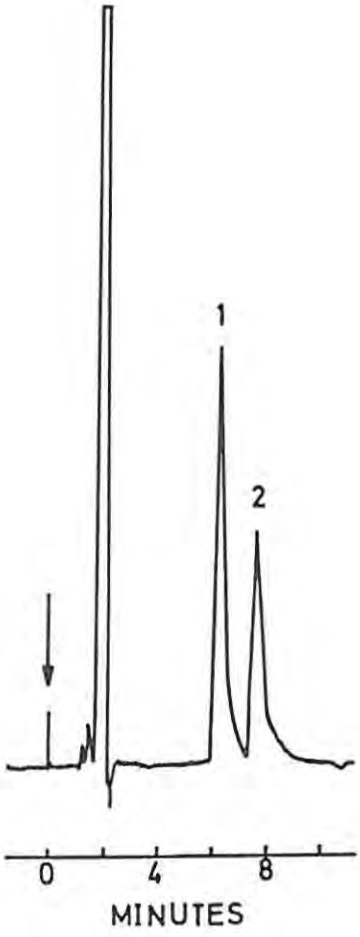


FIGURE 2.3 Chromatogram of PPA (1) and EPH (2)
MOBILE PHASE: 30% acetonitrile/70% HSS solution (0.005M)

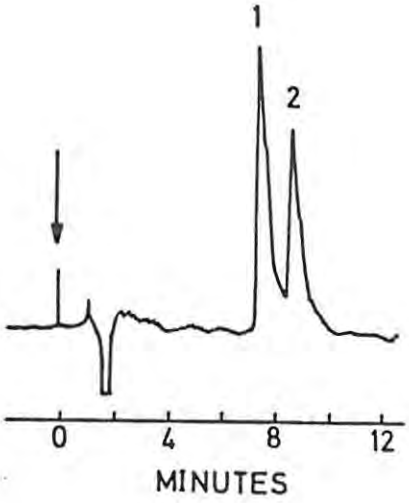


FIGURE 2.4 Chromatogram of PPA (1) and EPH (2)
MOBILE PHASE: 40% acetonitrile/60% dodecylsulphate sodium solution (0.005M) with 0.2% 1M HCl

Acidification of the acetonitrile/aqueous HSS mobile phase was attempted with acetic acid and *o*-phosphoric acid, but these methods were found to be unsatisfactory. The inclusion of 0.2% 1M HCl yielded a pH of 2.5 and gave good peak shape. After varying proportions of acetonitrile/aqueous HSS solution in acidified mobile phases were tested at different temperatures, a final mobile phase consisting of 25% acetonitrile/75% HSS solution (0.005M) with 0.2% 1M HCl was found to be satisfactory at 25°C and

was used for the determination of all serum and urine extracts (Fig.2.5)

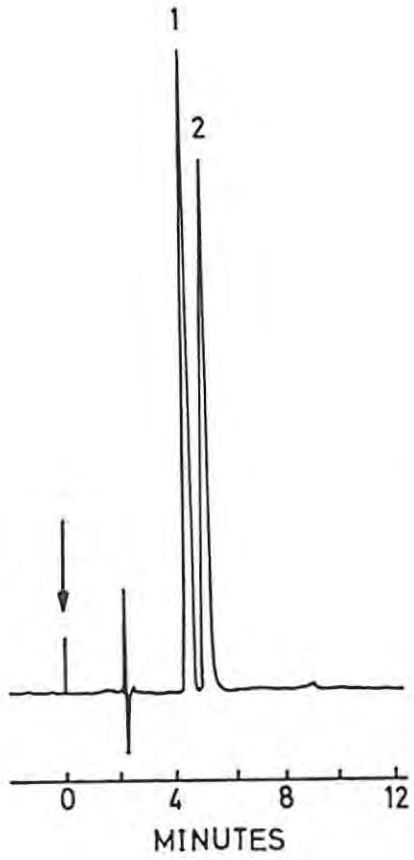


FIGURE 2.5 Chromatogram of PPA (1) and EPH (2)
MOBILE PHASE: 25% acetonitrile/75% HSS solution (0.005M) with 0.2% 1M HCl

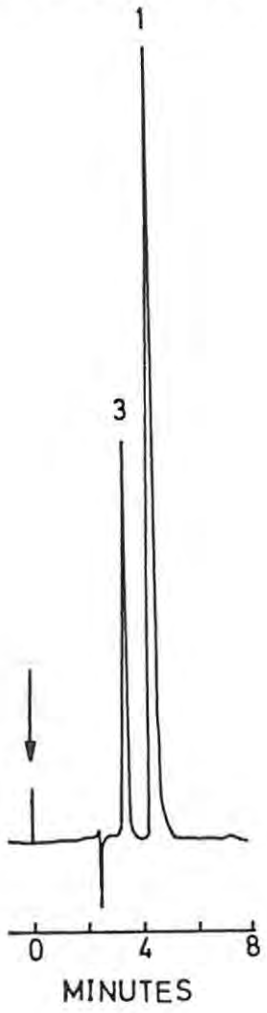


FIGURE 2.6 Chromatogram of PPA (1) and PEP (3)
MOBILE PHASE: 45% methanol/55% HSS solution (0.005M) with 0.2% 1M HCl

The separation of PPA.HCl and PEP.HCl was achieved using 45% methanol/55% HSS solution (0.005M) with 0.2% 1M HCl (Fig.2.6) and this mobile phase was used for all dissolution studies. The mobile phase of 50% methanol/50% HSS solution (0.005M) was utilized for most of the initial stages of development of the urine analysis. After the introduction of phenacetin as the internal standard in the urine analysis, the mobile phase was changed to 45% methanol/55% HSS solution (0.005M) to enable resolution of the internal standard and PPA.HCl (Fig.2.7).

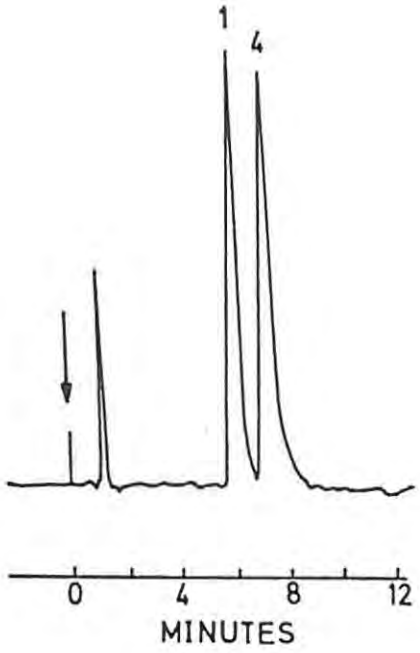


FIGURE 2.7 Chromatogram of PPA (1) and PHEN (4)

MOBILE PHASE: 45% methanol/55% HSS solution (0.005M)

CHAPTER 3

DETERMINATION OF PHENYLPROPANOLAMINE IN BIOLOGICAL FLUIDS

3.1 INTRODUCTION

The direct UV detection of PPA at the low concentrations found in biological fluids after therapeutic doses of the drug is extremely difficult due to its low inherent molar absorptivity. Hence methods in which the drug has been derivatized or concentrated to increase specificity and sensitivity have been developed.

An early method for quantitatively determining PPA in plasma and urine was developed by Axelrod (22), involving extraction into benzene and assay by the methyl orange reaction. Heimlich (25), using periodate oxidation, determined PPA in urine by converting the drug to its corresponding aryl aldehyde, benzaldehyde. The formation of this derivative affords sensitivity three to four times greater than that for UV spectroscopy of unconverted PPA. Wallace (159) subsequently converted the benzaldehyde to its semicarbazone derivative, thereby significantly enhancing the sensitivity and specificity of the procedure. However, relatively large volumes of the biological fluid are required for the assay and the method is time-consuming, involving many manipulations. Limits of sensitivity are not given, although the method is reported to be applicable to the determination of the drug in blood, serum and urine, whereas only the higher concentrations found in urine could be determined with the other methods.

Chromatographic techniques have been increasingly used for the determination of drugs in biological fluids with a resultant increase in specificity and decrease in interference from other drugs, metabolites or endogenous substances. Phenylpropanolamine has been separated and concentrated in urine by passing a buffered urine specimen through a column of Amberlite XAD-2 resin, a styrene divinylbenzene copolymer that absorbs a wide variety of water soluble organic compounds, and then eluting the drug with an

appropriate solvent which is evaporated. The residue is applied to thin layer plates, developed in a suitable solvent system and the drug detected qualitatively by sequential spraying (177,201). After chloroform extraction of PPA from serum and urine, the drug has been reacted with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole to produce a highly fluorescent compound which can be measured with a spectrofluorimeter. The extract is then evaporated and the residues subjected to thin-layer chromatography for qualitative identification of the drug (202).

Neelakantan and Kostenbauder (203) described a procedure for gas chromatography with electron capture detection (GC-ECD) for the determination of PPA in plasma based on derivatization with pentafluorobenzaldehyde without prior extraction of the drug, to yield an electron-capturing derivative which is then readily extracted into hexane. The limits of sensitivity of this method are 1.1 ng/3 ml. Cummins and Fourier (204) used benzene to partially purify the serum by acid extraction, re-extracted with benzene after alkalinization of the aqueous phase and then formed the heptafluorobutyryl derivative which was detected by GC-ECD. However the method is a tedious one as it involves several extractions both before and after derivatization and at least 4 hours is required for the derivative to form. GC with a nitrogen-selective detector has been used to determine PPA after ether extraction from alkaline urine (33,205).

Very few reports on the HPLC analysis of PPA in plasma, serum or urine have appeared in the literature. An HPLC procedure described by Mason and Amick (206) involved extraction of PPA from serum with 10% v/v 1-butanol in butyl chloride and back-extraction into a small volume of 1% acetic acid. This aqueous layer was then derivatized with *o*-phthalaldehyde and the derivative measured using fluorescence detection.

HPLC using detection in the visible range (450 nm) has been used to determine PPA in urine (207). Large volumes of alkaline urine (50 ml) were extracted three times with 50 ml of hexane after

which HCl was added to convert the free amine into the hydrochloride salt. Detection in the UV range resulted in interference from urinary constituents. Noggle (208) extracted PPA from alkaline urine with methylene chloride and then formed the phenylisothiocyanate derivative which was detected using HPLC and a wavelength of 254 nm.

3.2 METHODS OF EXTRACTION AND DETERMINATION OF PHENYLPROPANOLAMINE IN SERUM

3.2.1 Extraction Procedures

Generally, for each solvent assessed, 1 ml of an aqueous solution of PPA.HCl (5 µg/ml) or spiked serum (500 ng/ml) was extracted. A specified volume of basifying agent (0.05 - 1.0 ml) was added together with, when stated, an inorganic salt. The organic solvent (2-5 ml) was added to the contents in the test tube which was then stoppered and either shaken for 20 min or vortexed for 1 minute. The tubes were centrifuged at 3000 rpm for 10 min after which an aliquot of the organic phase was transferred to a tapered centrifuge tube. Subsequent treatment was of two types, involving either evaporation of the extract at 40°C under a gentle stream of nitrogen, washing down the sides of the tube with methanol during drying and reconstituting with 100 µl of mobile phase, or back-extraction of the organic extract into a small volume of acetic acid.

The following extraction procedures were assessed:

I. Ethyl acetate/sodium hydroxide/sodium chloride. An aqueous solution of PPA.HCl and spiked serum samples were basified with NAOH and enough NaCl was added to saturate the aqueous phase. Ethyl acetate was added and after shaking and centrifuging the clear upper phases were transferred to a tapered centrifuge tube.

II. Hexane/sodium hydroxide/sodium chloride. The serum samples and aqueous PPA.HCl samples were treated as in extraction I, using hexane in place of ethyl acetate.

III. Hexane/pH 11 buffer. A pH 11 buffer was added to a Clin Elut CE 1003 column (Analytichem International, Inc., Harbor City, CA) and allowed to saturate the column. The serum sample and hexane were added to the top of the column. A further two aliquots of hexane were added to the column and the eluent collected in a tapered tube.

IV. Ether/sodium hydroxide/sodium chloride. The aqueous PPA.HCl solution and serum samples were both treated as in extraction I, except that ether was used as the organic solvent.

V. Acetonitrile/sodium hydroxide/sodium chloride. An aliquot of the aqueous PPA.HCl solution and a number of spiked serum samples were treated as in extraction I, with acetonitrile being used in place of ethyl acetate.

VI. Acetonitrile/sodium hydroxide/sodium chloride/silica column. A serum sample was extracted with acetonitrile as in extraction V to yield a clear supernatant. A column was prepared consisting of a pasteur pipette packed with 0.5 g silica gel. The supernatant was added to the top of the column, and after most of the liquid had passed through the column, fresh acetonitrile was added to wash the column. A gentle stream of nitrogen was used to blow the acetonitrile off the column and the eluent was collected in a tapered tube. In a further attempt, the serum sample was initially added to the top of the column, blown through with a gentle stream of nitrogen and the eluent basified and extracted with acetonitrile.

VII. Solvent Mixtures. The serum samples were basified with NaOH, and NaCl and the solvent mixture were added to the tube and the contents shaken for 20 min, then centrifuged. An aliquot of the organic phase was transferred to a tapered tube. The following solvent mixtures were assessed:

(a) chloroform/ethyl acetate (4:1)
(b) acetonitrile/ethyl acetate (1:1)
(c) chloroform/propan-2-ol (4:1)

(d) cyclohexane with 2% isoamyl alcohol
(e) chloroform/methanol (3:1)
(f) ethyl acetate/methanol (2:1)
(g) ethyl acetate/propan-2-ol (2:1)
(h) cyclohexane/methanol (2:1)

VIII. Ethylene chloride/sodium hydroxide. The aqueous solution of PPA.HCl and spiked serum samples were basified with NaOH and extracted with 2 and 6 ml of ethylene chloride respectively. After centrifuging, the extracts were transferred to a tapered tube.

IX. Dichlormethane/sodium hydroxide. Serum samples and an aqueous solution of PPA.HCl were treated as in extraction I, using dichlormethane instead of ethyl acetate.

X. Dichlormethane/pH 11 buffer. The method used for extraction of the serum sample was similar to that described in extraction III with the dichlormethane being used in place of hexane.

XI. Butanol (10% v/v in butyl chloride)/ammonia. After basifying with ammonia, the serum samples were extracted with 6 ml butyl chloride containing butanol (10% v/v). Half the organic extracts were treated as in extraction I, the other half were back-extracted into 300 µl of 1% acetic acid by vortexing for 1 minute. An aliquot of the acetic acid extract was injected onto the column.

X11. Chloroform/sodium hydroxide. The aqueous PPA.HCl solution and serum samples were treated as in extraction I using chloroform in place of ethyl acetate.

XIII. Chloroform/sodium hydroxide/amyl alcohol. The serum sample was basified with NaOH, 10 drops of amyl alcohol were added and extraction attempted with a larger volume (6 ml) of chloroform. The tube was vortexed and centrifuged and the lower organic layer transferred to a tapered tube.

XIV. Chloroform/sodium carbonate/acetic acid. The aqueous PPA.HCl solution was basified with Na_2CO_3, extracted by vortexing with chloroform and centrifuged. The lower layer was transferred to a tapered tube containing 300 µl acetic acid (1%) and vortexed for 1 minute to initiate back-extraction. The chloroform was then removed down to about 200 µl and discarded and an aliquot of the acetic acid extract injected onto the column.

3.2.2 Chromatographic Conditions

The following chromatographic conditions were used after the extraction procedures I-VII:

Column:	reverse phase µBondapak C_{18}
HPLC system:	A (see Instrumentation)
Detection wavelength:	206 nm
Sensitivity:	0.01 AUFS
Flow rate:	1.5 ml/min
Pressure:	2300 psig
Temperature:	ambient
Recorder input:	100 mV
Mobile phase:	50% methanol/50% HSS solution (0.005M) with 0.2% 1M HCl

For the remainder of extraction procedures, the chromatographic conditions used are listed below:

Column:	reverse phase µBondapak C_{18}
HPLC system:	B (see Instrumentation)
Detection wavelength:	220 nm
Sensitivity:	0.01 AUFS
Flow rate:	1.3 ml/min
Pressure:	2500 psig
Temperature:	25 - 30°C
Recorder input:	20 mV
Mobile phase:	acetonitrile/HSS solution (0.005M) in varying proportions with 0.2% 1M HCl

3.2.3 Results and Discussion

In the HPLC analysis of drugs in serum, it is the protein and other macromolecules which present the greatest potential problem as their presence in the injected sample results in a rapid increase in back-pressure and a deterioration in column performance and column life. Interference with the drug to be determined may also occur (209-211). The compound of interest must therefore be obtained free from protein without loss of any protein-bound moeity. Dissociation of the latter may be favoured by diluting the plasma at the outset (212).

The problem of removal of protein and other macromolecules can be approached in three different ways:
(i) precipitation of the undesired species.
(ii) solvent extraction.
(iii) selective adsorption of the drug of choice onto a suitable substrate.

Addition of an appropriate reagent to the serum denatures the protein which is precipitated and can be removed by centrifugation. An acidic pH will encourage precipitation and the use of acidic precipitants such as perchloric acid, trichloroacetic acid and tungstic acid followed by sulphuric acid has been described (212,213). Water-miscible organic solvents e.g.methanol, ethanol, isopropanol, acetone and acetonitrile can be used (214) provided that the organic/aqueous ratio is not less than 2:1 (156). These solvents, although water-miscible, can be separated from the aqueous phase by the addition of sufficiently high concentrations of inorganic salts such as anhydrous Na_2CO_3, NaCl and $(NH_4)_2SO_4$ (214). This salting-out approach enables the organic phase to be removed and concentrated.

Phenylpropanolamine, being a weak base with a pKa of 9.4 is most readily extracted by making it alkaline prior to its extraction into an organic, water-immiscible solvent, since the unionised form is more lipophilic than the ionised form. Guidelines have been suggested relating to the ideal properties of an organic

solvent (214). Drugs can therefore be purified and then concentrated by the removal of low boiling point organic solvents at elevated temperatures under nitrogen. However, care must be taken that the drug itself is not removed by evaporation, volatilization or lost during the extraction process (209,213).

Separation may also be achieved by adsorption onto solid materials which can either be added to the extract or packed into a column. The compound of interest must be preferentially retained on the column and then eluted with a solvent of suitable polarity, or it may pass through the column, with the interfering substances being retained. However there exists the risk that a small amount of the compound to be determined may be retained, resulting in inaccuracies (212).

Concentrations of PPA found in serum after the ingestion of doses of the drug between 30 and 150 mg range from 10 to 500 ng/ml. These low levels make sample concentration essential to enable direct UV detection of the drug.

Solvent purity of ethyl acetate can be a problem as inadvertent exposure to light and air has been found to adversely affect the solvent and the spectrophotometric determination of various drugs extracted with the solvent (212). Ethyl acetate (extraction I) was found to yield a fair recovery for PPA (75%), but interfering peaks constituted the main problem. The initial peak resulting from endogenous substances in the serum (serum blank) was not high as the extract was reconstituted with 1 ml mobile phase. However, further extractions using 6 ml ethyl acetate and reconstituting in 100 µl mobile phase resulted in a concentration of PPA and the endogenous compounds extracted from the serum. The latter caused a high serum blank to occur with peaks which interfered with the drug of interest.

Hexane is one of the most non-polar organic solvents and the resulting serum extract (extraction II) was free of interfering compounds. Hexane has been used in large volumes to extract PPA

(207) and its pentafluoroxazolidine derivative from biological fluids (203). Recovery of PPA from both serum and water was found to be inadequate. The Clin Elut CE 1003 column used in extraction V is a 3 ml disposable extraction column purified for use in sample preparation for liquid chromatography. After passing the spiked serum sample and hexane through the column, no PPA was detected in the reconstituted eluent. The drug may have been totally adsorbed to the packing material with hexane being an inadequate solvent for its desorption from the column.

Ether has been used to extract PPA directly from body fluids (25,159) and also after its conversion to benzaldehyde (205). A 50% recovery was achieved after single extraction (extraction V) which was insufficient to enable measurement at low concentrations of PPA. The use of ether has associated advantages as evaporation is rapid, it is less prone to emulsion formation than chloroform (212) and it yielded a clean serum extract with no interfering peaks. Subsequent attempts using multiple ether extractions did cause a slight increase in PPA recovery but also resulted in the simultaneous extraction of interfering compounds.

Addition of acetonitrile to the serum denatures and precipitates the protein. After centrifugation, the precipitated protein formed a disc which adhered to the bottom wall of the tube, yielding a clear supernate. Acetonitrile, being a water-miscible solvent, was separated from the aqueous phase by the addition of an excess amount of NaCl. Varying amounts of NaOH were added to try and establish the optimum pH for extraction. However, the tendency to form an emulsion increased with the addition of larger volumes of NaOH and care had to be taken with vortexing as the resultant emulsion did not crack on centrifuging.

Extraction V resulted in a 44% recovery of PPA when reconstituted with mobile phase. On reconstitution with methanol, a decrease in peak height and deterioration of peak shape resulted. The use of acetonitrile yielded a large serum blank with an interfering peak appearing on the leading edge of the PPA peak. Passing the extract

through the silica column (extraction VI) did not eliminate interference. Extraction VI was repeated, this time extracting twice with acetonitrile and then passing the extract through the column. Recovery increased substantially to 80%, but although further extractions were attempted using the column method to try and remove a larger proportion of serum constituents, interference with the PPA peak occurred in all cases.

A variety of solvent combinations were assessed in an attempt to obtain a better recovery for PPA and a cleaner extract. It was thought that the combination of a protein precipitant, acetonitrile, with ethyl acetate might yield adequate recovery of the drug (extraction VII (b)) but this was not the case. Extraction VII(a) yielded only 50% recovery of PPA.

The presence of a second solvent, usually an alcohol, is commonly recommended (214) as this reduces the likelihood of adsorption to glass or protein and can also sometimes overcome problems of inefficient and variable extraction. A mixture consisting of chloroform/propan-2-ol (4:1) has been used for drug-screening studies at an alkaline pH (212). However, extractions VII (c)-(h) proved unsuccessful as none yielded a good recovery.

Ethylene chloride initially appeared very promising as it yielded a 90% recovery from an aqueous solution (extraction VIII). Two problems did arise however, one being concerned with the formation of a solid gelatinous emulsion intractable to centrifuging, a phenomenon which has been described (212). The other problem was the high serum blank which occurred, especially after extracting with a larger volume of ethylene chloride. Interference resulted even after the injection of extremely low volumes of extract, thus the use of this solvent for the low concentrations likely to be encountered in this work was not practical.

Dichlormethane yielded much cleaner extracts than ethylene chloride with about 70% recovery (extraction IX). The solvent exhibited a tendency to form an emulsion on vigorous vortexing, so

care had to be taken with agitation. No PPA was detected after the spiked samples had been passed through the Clin Elut column (extraction X) probably due to complete adsorption of the drug onto the column.

The solvent combination of butanol (10% v/v) in butyl chloride has been used for the extraction of PPA from serum (206), but in this study (extraction XI) it was found to yield extremely poor recovery when the extract was evaporated and reconstituted. Back-extraction into acetic acid improved recovery of the drug, although only 40% recovery was obtained.

The final solvent to be assessed was chloroform, the specific density of which can be a disadvantage in the manipulations since it is heavier than water and forms the lower layer. Initially, recovery was found to be about 45% (extraction XII). When extracting from serum with chloroform using NaOH as the basifying agent, there was a tendency to form emulsions which were difficult to crack even after centrifugation. With the increase in volume of NaOH from 0.1 ml to 1 ml, this tendency increased, so great care had to be taken if the sample was vortexed. The use of a more concentrated solution of NaOH (20%) did not appear to have any advantageous effect on recovery.

In extraction XIII the volume of chloroform was increased to 6 ml and amyl alcohol was added in the hope that it would reduce emulsion formation. It did help in preventing the formation of intractable emulsions and recovery was increased to 60%, possibly as a result of the increase in organic/aqueous ratio. The method used in extraction XIII was repeated, extracting twice with 6 ml of chloroform each time. Recovery increased to 80%, but interfering peaks were observed. NH_4OH was tried as the basifying agent and although recovery was not affected, it did seem to yield a cleaner extract.

A recurrent problem encountered throughout the extraction method development was the lack of reproducibility, which was eventually

traced to the evaporation and reconstitution steps. Loss of drug seemed to be occurring during evaporation, possibly through volatilization of the free base form (205). Adsorption to the glass may have prevented complete solution of the drug in the mobile phase on reconstitution. It has been found with ephedrine, a drug structurally similar to PPA, that nitrogen evaporation of pentane extracts of derivatized or underivatized drug caused perceptible loss of derivative and even greater loss of free drug (215). Attempts were therfore undertaken to eliminate the evaporation step.

A method similar to extraction XI was developed in which chloroform was used as the extracting solvent and a back-extraction was substituted for the evaporation and reconstitution steps (extraction XIV). Results seemed promising with a 75% recovery of the drug and an extract which was relatively free from interfering peaks. This result was compared with the recovery obtained after extraction with 6 ml chloroform, evaporating and then reconstituting with 300 µl acetic acid (1%). Recovery was a mere 20%, confirming that PPA was indeed lost in the evaporation and reconstitution stages.

The saturated Na_2CO_3 solution used for basification did not result in irreversible emulsion formation as was the case when using NaOH. Amyl alcohol was retained in the extraction procedure, until it was discovered that its presence was impairing recovery. It seemed to cause a percentage of the PPA to be retained in the chloroform presumably through a partitioning process, thus preventing the drug form diffusing into the acetic acid.

The effect of multiple extractions on recovery was investigated. A 72% recovery obtained after a single extraction was increased to 81% on extracting the same sample twice, but thereafter the slight increase did not justify the extra number of manipulations involved. There was a need to concentrate PPA further to enable detection at the lower concentrations. Back-extraction into 100 µl of 1% acetic acid was found to yield satisfactory results. The

percentage of the acetic acid solution was varied, with a 5% v/v solution being finally selected.

The use of phenacetin as the internal standard was unsuccessful as the drug did not back-extract into the acetic acid phase. Ephedrine hydrochloride (EPH.HCl) was introduced as the internal standard, after which the mobile phase was modified to allow separation of PPA.HCl and EPH.HCl. The final mobile phase consisted of 25% acetonitrile/75% HSS solution (0.005M) with 0.2% 1M HCl and a pH of 2.5.

It was found that an extremely large peak eluted at about 13 min. The initial assessment was that it may have been due to an impurity in the acetic acid or a component that was extracted by the acetic acid from the rubber stopper of the Vacutainer tube during vortexing. An aliquot of a 5% solution of acetic acid was injected onto the column, but this did not yield a similar peak, nor did a solution that had been vortexed in such a manner so as to make contact with the rubber stopper. A small volume of chloroform was then injected onto the column and the peak reappeared at 13 min. It was concluded that a small percentage of the chloroform dissolves in the acetic acid which is then injected, accounting for the peak eluting at 13 min.

During chloroform extraction of drugs and metabolites with amine substituents, chloroform contaminants, phosgene and ethyl chloroformate were found to form carbamoyl chlorides and carbamates (216). With previously opened bottles and prior exposure of the chloroform to air, the amount of artifact formation rose substantially. Consequently, appropriate analytical controls must be included for the detection and elimination of chemical artifacts.

Problems occurred during extraction with chloroform when inserting the pasteur pipette through the serum to the lower chloroform layer, as water-soluble endogenous impurities were unavoidably transferred to the acetic acid in which they dissolved, resulting

in a cloudy extract. On injection these impurities often interfered with the peaks to be determined. The procedure was modified by removing the serum layer above the semi-solid disc that formed between the two layers on centrifuging. The disc could then be pushed aside and all the chloroform removed. The pipette was rinsed with about 2 ml of fresh chloroform which was added to the original tube, vortexed and centrifuged. The chloroform washings were added to the 6 ml chloroform and the acetic acid. In this way, the transfer of water-soluble impurities was minimised and chromatographically clean extracts were obtained.

The acetic acid (100 µl) floated on top of the chloroform and appeared as globules adhering to the walls of the tube. After back-extraction, the chloroform was removed down to about 200 µl and discarded, thus allowing the small volume of acetic acid to form a single, continuous upper layer from which aliquots for injection could be removed. The final method used is described in the following section.

3.3 ANALYSIS OF PHENYLPROPANOLAMINE IN SERUM

3.3.1 Experimental

3.3.1.1 Solutions

Phenylpropanolamine hydrochloride serum stock solution. Five mg PPA.HCl was dissolved in 100 ml water and 1 ml of this solution made up to 100 ml with blank serum. The resultant concentration in serum was 500 ng/ml. Appropriate dilutions were then made.

Ephedrine hydrochloride stock solution. Forty mg EPH.HCl was dissolved in 1000 ml water to yield 40 µg/ml. This was used as the internal standard solution.

3.3.1.2 Chromatographic Conditions

Column:	reverse phase µBondapak C_{18}
HPLC system:	C (see Instrumentation)
Detection wavewlength:	220 nm
Sensitivity:	0.01 AUFS

Flow rate:	1.2 ml/min
Pressure:	2200 psig
Temperature:	25°C
Recorder input:	20 mV
Mobile Phase:	25% acetonitrile/75% HSS solution (0.005M) with 0.2% 1M HCl

3.3.1.3 Extraction Procedure

One millilitre of serum, 1 ml EPH.HCl solution (0.24 µg/ml) and 50 µl of a saturated solution of Na_2CO_3 which adjusted the pH of the mixture to 10, were vortexed for 15 sec in a test tube. Five millilitres of chloroform were added, the tube stoppered, vortexed for 1 min and centrifuged at 3500 rpm for 5 min. A disc formed between the aqueous and organic phases and the aqueous layer above this disc was removed by aspiration and discarded. A pasteur pipette was used to transfer the chloroform extract to a tapered centrifuge tube containing 100 µl of 5% v/v acetic acid. The pipette was rinsed with 2 ml chloroform which was then added to the original test tube, vortexed for 30 sec and centrifuged at 3500 rpm for 5 min. The chloroform washings were added to the tapered centrifuge tube and centrifuged at 1000 rpm for a further minute. The chloroform layer was reduced by aspiration to about 200 µl and discarded and the tube re-centrifuged at 3500 rpm for 10 min. Aliquots of between 15 and 30 µl of the acetic acid extract were injected directly onto the column.

3.3.1.4 Calibration Curve

The serum stock solution was diluted to yield five different concentrations, the stock solution itself providing the sixth. Concentrations ranged from 25 to 500 ng/ml and each concentration was assayed in triplicate. The calibration curve was constructed by plotting the ratio of the peak height of PPA to that of the internal standard, EPH.HCl versus the respective PPA concentration. A straight-line fit of the data was made by least-squares linear regression analysis.

3.3.1.5 Precision

Within-run precision was assessed by extracting six spiked serum samples each of the highest PPA concentration (500 ng/ml) and the two lowest PPA concentrations (25 and 50 ng/ml) determined in the calibration curve.

3.3.1.6 Extraction Efficiency

For this study, in which the analytical recovery of PPA from serum was assessed, spiked serum samples were assayed in triplicate at four different concentrations. A similar extraction procedure to the one described in section 3.3.1.3 was applied to all the samples with one modification. The internal standard was omitted at the start of the procedure and instead 1 ml water was added to the serum. The EPH.HCl was dissolved in the acetic acid and was thus not carried through the extraction procedure.

Standard solutions of PPA.HCl were made up with the internal standard solution in concentrations corresponding to those extracted above. The internal standard therefore accounted for variations in sample volumes injected. To determine percentage recovery, the ratios obtained from the serum extracts were compared to those resulting from the relevant standard solution of equivalent concentrations.

3.3.2 Results and Discussion

Linearity was established for the range of concentrations studied (25 - 500 ng/ml). The curve had a slope of 0.00387, a y-intercept at 0.0509 and the correlation coefficient was 0.9997. The calibration curve of PPA in serum is shown in Fig.3.1.

Chromatograms of a blank serum extract and a serum extract containing PPA and EPH are depicted in Fig.3.2. The retention time of PPA was 4.4 min and that of EPH was 5.6 min.

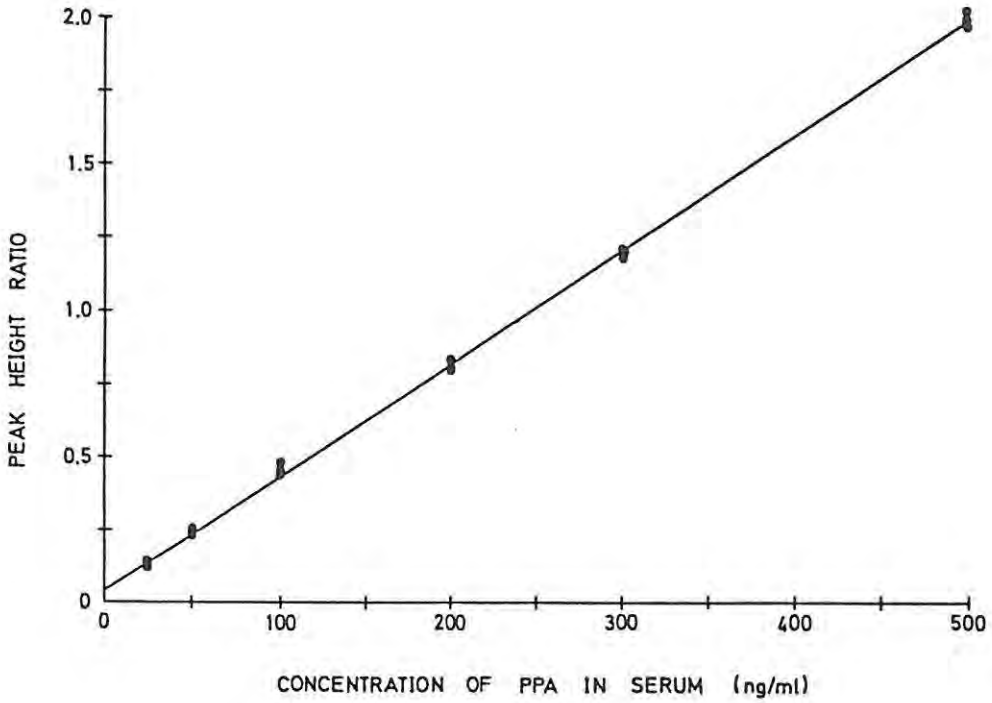


FIGURE 3.1 Calibration curve of phenylpropanolamine in serum

TABLE 3.1 Within-run precision study on serum assay

SPIKED CONC (ng/ml)	CONCENTRATION MEASURED (ng/ml) SAMPLE NUMBER 1	2	3	4	5	6	MEAN ± SD	CV %
25	23.31	24.60	25.38	22.79	25.64	22.26	24.34 ± 1.26	5.18
50	53.36	46.05	45.82	47.89	51.51	49.18	48.97 ± 3.01	6.15
500	490.16	510.07	494.22	501.72	511.39	506.90	502.41 ± 8.68	1.73

TABLE 3.2 Analytical recoveries of PPA from serum

SERUM CONC (ng/ml)	% RECOVERY SAMPLE NUMBER 1	2	3	MEAN ± SD
50	79.66	83.32	82.04	81.67 ± 1.86
100	78.87	82.36	80.83	80.69 ± 1.75
250	80.15	79.35	80.58	80.03 ± 0.62
500	80.20	79.96	78.04	79.40 ± 1.18

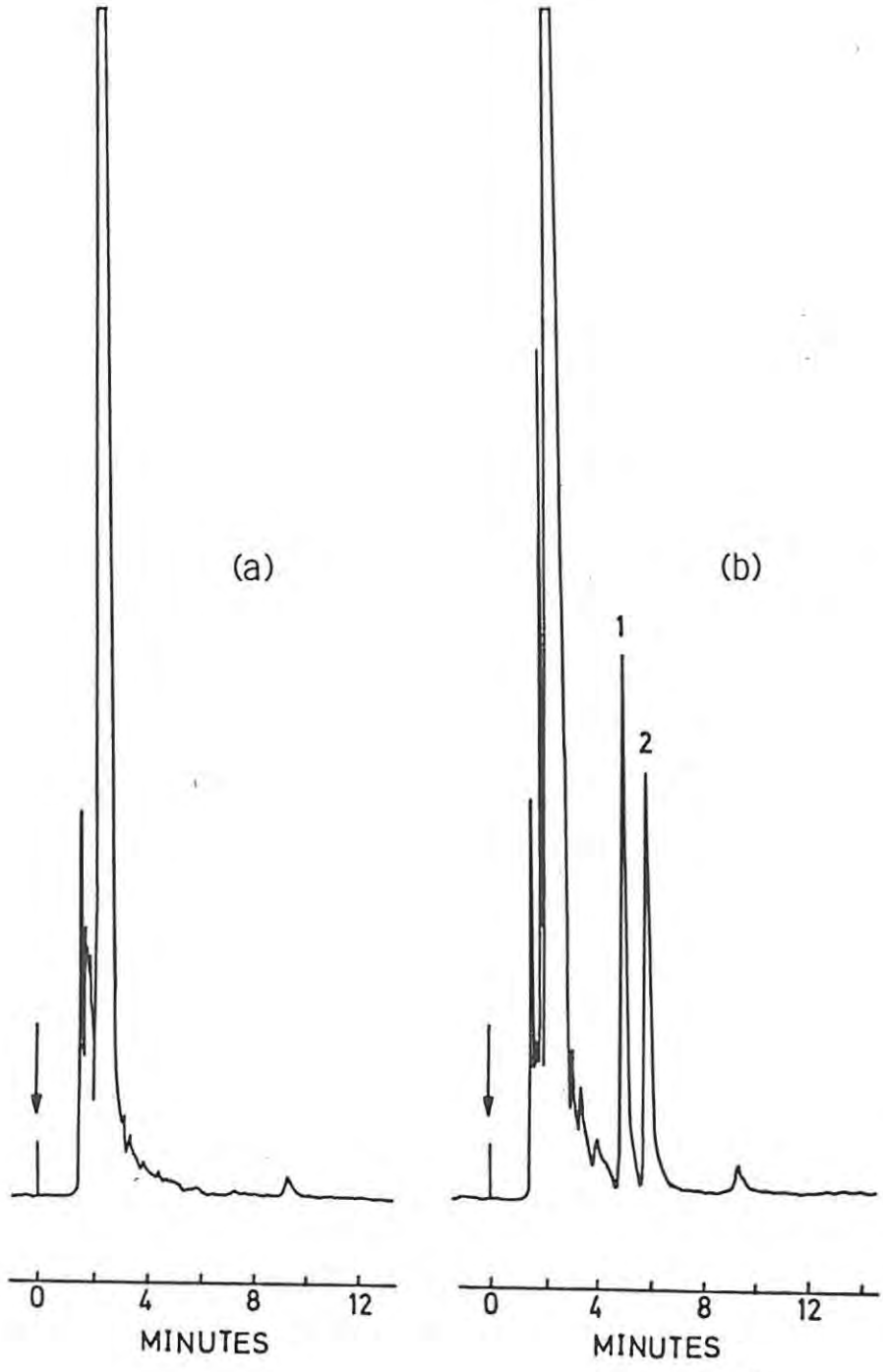


FIGURE 3.2 Chromatogram of (a) a blank serum extract and (b) a serum extract containing PPA (1) and EPH (2).

3.3.2.1 Precision

Results of the within-run precision study are depicted in Table 3.1. Coefficients of variation at all 3 concentrations studied fall within the accepted limits for drug determinations. The method, as indicated by the above results, was found to be reproducible.

3.3.2.2 Extraction Efficiency

Table 3.2 shows the analytical recoveries of PPA from serum. Results indicate that the recovery of PPA is constant and quantitative over the range of concentrations studied. Mean recovery was 80.4%.

3.3.2.3 Background Interference

The technique of back-extraction eliminated a significant amount of interfering endogenous substances which had been extracted into the chloroform. The acetic acid elutes at the void volume and peaks from serum constituents did not interfere with the peaks of interest. Occasionally a small volume of the aqueous phase was removed with the chloroform and would dissolve in the acetic acid. On injection, this resulted in a large absorbance due to the serum constituents and caused interference with the PPA peak.

A small unidentified peak eluted at 7.6 min and was present in all serum samples, but did not interfere with the peaks of interest. The large peak at 13 min was due to a trace amount of chloroform which dissolved in the acetic acid and was then injected.

3.3.2.4 Stability of Samples on Storage

Spiked serum samples were stored at -20°C and were assayed periodically over 6 months. The PPA content remained constant with the standard deviations falling within the acceptable limits.

3.4 METHODS OF EXTRACTION AND DETERMINATION OF PHENYLPROPANOLAMINE IN URINE

3.4.1 Extraction Procedures

Method I

Spiked urine (2 ml) was pipetted into a 15 ml Pyrex test tube which had a ground glass stopper. Three drops of 1M NaOH and 2 ml organic solvent were added. The tubes were shaken for 20 min, centrifuged at 3000 rpm for 10 min and 1 ml of the organic extract transferred to a tapered centrifuge tube. This was evaporated at 40°C under a gentle stream of nitrogen and the dried extract reconstituted with 100 µl mobile phase containing the internal standard, PEP.HCl. Between µl and 5 µl was injected onto the column. Three solvents were assessed , chloroform, dichlormethane and ether. The mobile phase consisted of 50% methanol/50% HSS solution (0.005M).

Method II

A direct dilution and precipitation technique was assessed. A spiked urine sample (2 ml) was pipetted into a test tube and the organic solvent added. The tube was stoppered, vortexed for 30 sec and centrifuged at 3000 rpm for 10 min. An aliquot of the supernatant (5 to 10 µl) was injected onto the column. The following precipitants were assessed:

(i) water/methanol (2 ml:1 ml)
(ii) water/acetonitrile (1 ml:2 ml)
(iii) water/acetonitrile (1 ml:2 ml) with 0.4g NaCl
(iv) methanol/3.5% perchloric acid (2 ml:1 ml)
(v) methanol (2 ml)
(vi) ethanol (2 ml)
(vii) isopropanol (2 ml)

The mobile phase was 50% methanol/55% HSS solution (0.005M).

Two internal standards were assessed, PEP.HCl and phenacetin (PHEN). Each was dissolved in methanol (60 µg/ml) and 2 ml of this solution was used as the sole precipitant. Calibration curves with concentrations ranging between 10 and 200 µg/ml PPA.HCl were

established using both internal standards. The mobile phase for PEP.HCl consisted of 40% methanol/60% HSS solution (0.005M) and that for PHEN contained 45% methanol/55% HSS solution (0.005M).

Method III

A spiked urine sample (2 ml) was pipetted into a test tube and 2 ml methanol containing EPH.HCl was added. The contents of the tube were vortexed for 30 sec, centrifuged at 3000 rpm for 10 min and an aliquot of the supernatant injected onto the column. The mobile phase consisted of 45% methanol/55% HSS solution (0.005M) and temperature control was introduced.

Method IV

A modification of the final method used for serum was assessed (see extraction XIV on method development). The only modification was an increase in the volume of acetic acid used in the back-extraction with 1 ml instead of 100 µl being used. The mobile phase was 25% acetonitrile/75% HSS solution (0.005M) with 0.2% 1M HCl. (For the final method used see section 3.5).

3.4.2 Chromatographic Conditions

The following conditions were used for analysis after Methods I and II:

Column:	reverse phase µBondapak C_{18}
HPLC system:	A (see Instrumentation)
Detection wavelength:	206 nm
Sensitivity:	0.02 AUFS
Flow rate:	1.5 ml/min
Pressure:	2500 psig
Temperature:	ambient
Rocorder input:	100 mV
Mobile phase:	see in each method

For Methods III and IV the conditions were changed as follows:

Column:	reverse phase µBondapak C_{18}
HPLC system:	B (see Instrumentation)

Detection wavelength:	220 nm
Sensitivity:	0.04 AUFS
Flow rate:	1.3 ml/min
Pressure:	2500 psig
Temperature:	20 - 30°C
Recorder input:	100 mV
Mobile phase:	see in each method

3.4.3 Results and Discussion

Problems associated with the determination of drugs in urine mainly involve the removal of inorganic salts or small molecular weight components which may have similar chromatographic properties as the compounds being assayed (154). Removal may be achieved by precipitation of the undesired species or by solvent extraction (210). The use of the former method depends to a large extent on the presence of adequately high concentrations of drug in the urine. General guidelines for the development of solvent methods and general extraction methodology have appeared in the literature (212,214). The concentrations of PPA anticipated in the urine after administration of test dosage forms range from 10 to 200 µg/ml. Sensitivity did not constitute a problem as these concentrations are easily detected in the UV range.

Emulsions are better avoided rather than broken up later (214) and the addition of inorganic salts has been reported to help avoid emulsion formation and facilitate extraction into an organic solvent (212). In method I a gelatinous emulsion intractable to centrifuging formed on vortexing, so the contents were instead shaken gently for 20 min. Ether, the most non-polar solvent of the three, was not efficient in extracting PPA, whereas chloroform gave a better recovery, but a high urine blank. The dichlormethane, being less polar than chloroform, caused fewer endogenous impurities to be extracted and also gave a fair recovery of PPA. The addition of an inorganic salt, NaCl, did not improve recovery and also resulted in a high urine blank, probably due to the more efficient transfer of impurities from the urine into the organic solvent.

A study was carried out to attempt to ascertain the optimum pH for extraction by adding varying amounts of 1M NaOH to the urine. It was found that results were non-reproducible, with recoveries ranging from 10% to 80%. This was caused by loss of PPA during shaking due to leakage of the volatile organic phase from the tube, as sealing between the tube and ground glass stopper was inadequate. Losses could also have occurred during evaporation and reconstitution, due to volatilization of the base and incomplete solution in the solvent used for reconstitution.

Direct dilution and precipitation methods as described in method II were then assessed. An advantage of this method is the decrease in the number and complexity of manipulations involved, which enhances the precision and reproducibility of the assay. This simplified technique will often suffice for HPLC analysis, although the endogenous urinary constituents may interfere with the compound to be determined. The most efficient precipitant was methanol. Perchloric acid was added in an attempt to sharpen peak shape and enable more efficient precipitation. Peak shape did sharpen slightly but a much higher urine blank resulted. An acidified mobile phase was tried, but although peak shape improved dramatically, the urine blank was high.

Linear relationships were shown to exist for both calibration curves which were constructed with correlation coefficients of 0.9997 for the method using PEP.HCl as the internal standard, and 0.9999 when using PHEN as the internal standard. Interference with the PEP peak occasionally occurred as it eluted before PPA and in these cases, quantitation was difficult. A pilot trial was carried out to determine the excretion of PPA in urine after the ingestion of a 50 mg PPA.HCl solution in water. Serum and urine samples were assayed using PHEN as the internal standard. After 30 hours, 88% of the drug was recovered.

After several months column characteristics were found to have altered, and neither PEP.HCl nor PHEN could be used as the internal standard. Samples obtained using method III were

injected, but results and retention times varied considerably. Aliquots from a standard aqueous solution of PPA.HCl and EPH.HCl were injected over the course of three days. Retention times and resolution between the two peaks differed after each day and results were inconsistent and unreliable.

The method developed for the serum analysis was then successfully applied to the determination of PPA in urine (method IV). The final method used was simple, reproducible and yielded an analytically clean extract.

3.5 ANALYSIS OF PHENYLPROPANOLAMINE IN URINE

3.5.1 Experimental

3.5.1.1 Solutions

Phenylpropanolamine hydrochloride urine stock solution. Twenty mg PPA.HCl was dissolved in 100 ml blank urine to yield a solution of 200 µg/ml. Dilutions were made from this solution.

Ephedrine hydrochloride stock solution. Forty mg EPH.HCl was dissolved in 1000 ml water to yield a 40 µg/ml solution, which was then used as the internal standard solution.

3.5.1.2 Chromatographic Conditions

Column:	reverse phase µBondapak C_{18}
HPLC system:	C (see Instrumentation)
Detection wavelength:	220 nm
Sensitivity:	0.04 AUFS
Flow rate:	1.2 ml/min
Pressure:	2500 psig
Temperature:	25°C
Recorder input:	20 mV
Mobile phase:	25% acetonitrile/75% HSS solution (0.005M) with 0.2% 1M HCl.

3.5.1.3 Extraction Procedure

To 1 ml of urine in a test tube, 1 ml EPH.HCl aqueous solution (40 µg/ml) was added and 50 µl of a saturated solution of Na_2CO_3 to adjust the pH to 10. The tube was vortexed for 15 sec, 5 ml chloroform was added, the tube stoppered and vortexed again for 1 min. After centrifugation at 3500 rpm for 5 min, the chloroform extract was transferred with a pasteur pipette to a tapered centrifuge tube containing 1 ml of 5% v/v acetic acid. The pipette was rinsed with 2 ml chloroform which was then added to the original tube, vortexed for 30 sec and centrifuged at 3500 rpm for 5 min. The chloroform washings were added to the centrifuge tube, vortexed for 1 min and centrifuged at 3500 rpm for 10 min. Aliquots of between 1 and 8 µl of the upper acetic acid extract were injected onto the column.

3.5.1.4 Calibration Curve

The urine stock solution was diluted to yield five different concentrations, with the stock solution providing the sixth. Concentrations ranged from 10 to 200 µg/ml and each concentration was determined in triplicate. A calibration curve was constructed as in section 3.3.1.4.

3.5.1.5 Precision

Within-run precision was assessed in a similar manner to that described in section 3.3.1.5.

3.5.1.6 Extraction efficiency

The method used for assessing extraction efficiency is described in section 3.3.1.6.

3.5.2 Results and Discussion

The calibration curve (see Fig.3.3) was found to be linear over the range of concentrations studied (10 - 200 µg/ml) with a slope of 0.0265, a y-intercept at 0.0386 and a correlation coefficient of 0.9999.

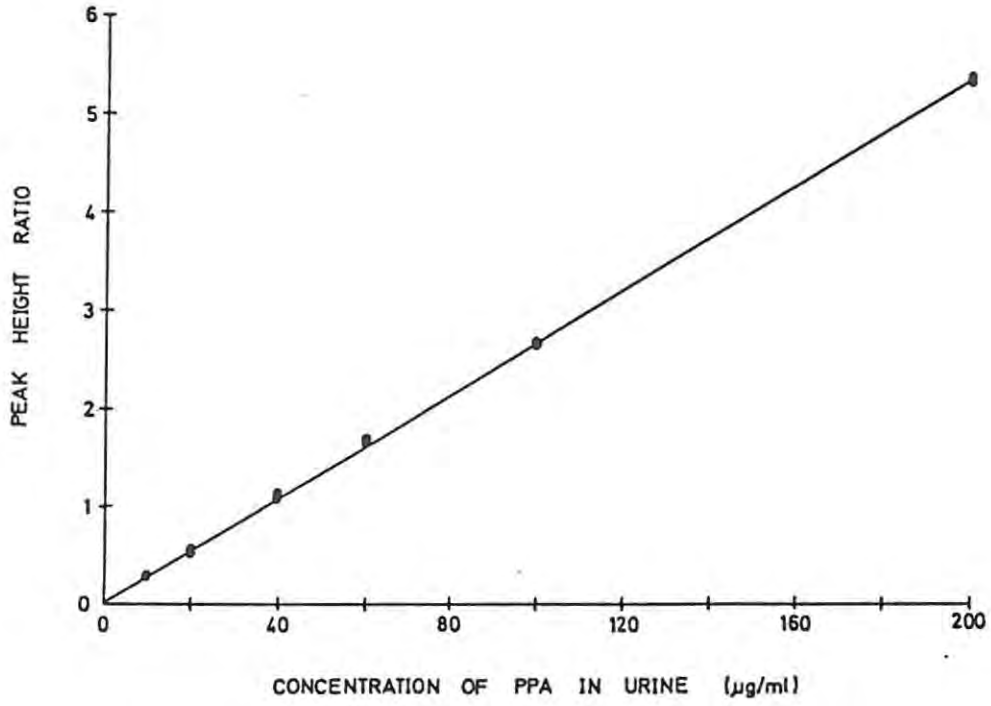


FIGURE 3.3 Calibration curve of phenylpropanolamine in urine

TABLE 3.3 Within-run precision study on urine assay

SPIKED CONC (µg/ml)	CONCENTRATION MEASURED (µg/ml) SAMPLE NUMBER 1	2	3	4	5	6	MEAN ± SD	CV %
10	9.27	9.24	9.46	9.12	9.54	9.39	9.34 ± 0.16	1.66
100	99.05	99.12	99.20	98.71	98.82	98.33	98.87 ± 0.32	0.33

TABLE 3.4 Analytical recoveries of PPA from urine

URINE CONC (µg/ml)	% RECOVERY SAMPLE NUMBER 1	2	3	MEAN ± SD
10	80.88	82.76	79.94	81.19 ± 1.44
40	79.63	79.22	81.58	80.14 ± 1.26
60	77.95	78.83	80.66	79.15 ± 1.38
100	80.84	79.68	80.42	80.31 ± 0.59

Relevant chromatograms are shown in Fig.3.4. The retention time of PPA was 4.4 min and that of EPH was 5.6 min.

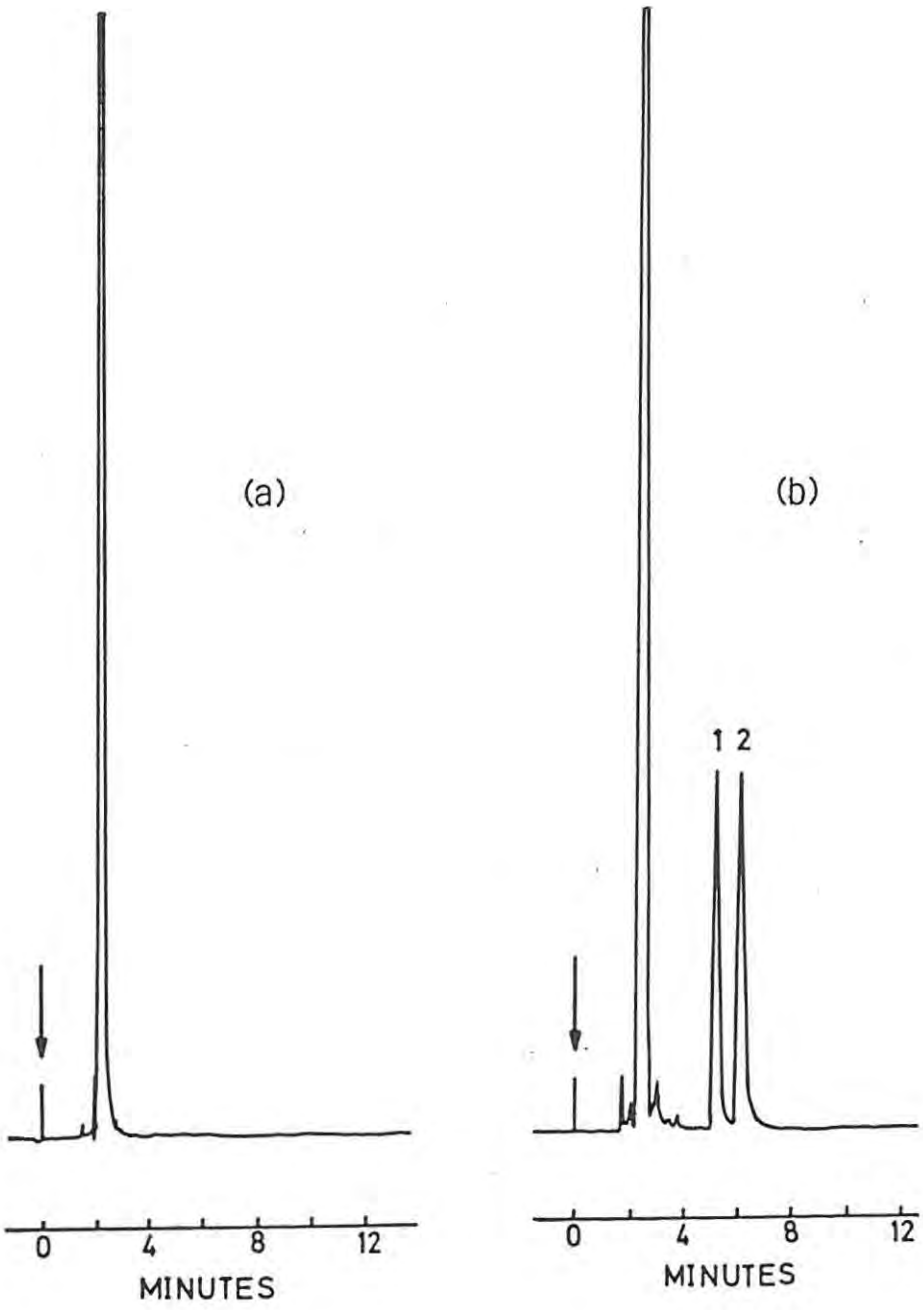


FIGURE 3.4 Chromatograms of (a) a blank urine extract and (b) a urine extract containing PPA (1) and EPH (2).

3.5.2.1 Precision

Results of the within-run precision study on urine are shown in Table 3.3. Results indicate the good reproducibility of this method of extraction of PPA from urine.

3.5.2.2 Extraction Efficiency

The analytical recoveries of PPA from urine are shown in Table 3.4. The recoveries were constant over the entire concentration range studied, with a mean recovery of 80.20%.

3.5.2.3 Background Interference

The acetic acid and endogenous urinary constituents elute near the void volume and no interference with the PPA or EPH peaks was encountered. The extract was stable for at least 5 days if stored in the refrigerator at 4°C, with no change in the size of the peaks or chemical degradation of the compounds occurring.

3.5.2.4 Stability of Samples on Storage

Spiked urine samples, stored at -20°C, were assayed over a 6 month period and were found to be constant with respect to PPA content, with standard deviations falling within the acceptable range.

CHAPTER 4

IN VITRO DISSOLUTION RATE DETERMINATIONS

Oral sustained-release dosage forms have become increasingly popular recently and assuming that there is some relationship between the blood level of a drug and its pharmacological response, an orally administered drug must be absorbed in a sufficient quantity and at a sufficient rate to attain and maintain therapeutically active blood levels over a prolonged period. The drug delivery system can result in alteration of the onset and intensity of the pharmacological response by altering the absorption rate of the drug. In many instances, absorption rate may be directly related to the dissolution rate of the drug and consequently a reliable method of monitoring the release rate of an active drug from the dosage form is necessary.

Assessment of bioavailability should ideally be carried out on all drug products to measure the amount of drug available at the site of action. However, the investigation of bioequivalence by exhaustive *in vivo* testing is seldom practical and has a number of associated disadvantages (218,219). The tests are time-consuming, require the use of large numbers of human volunteers, trained personnel and sophisticated analytical equipment. In addition these tests are affected by many variables.

In vitro dissolution tests have been developed as an indication of bioavailability. However, conditions for *in vitro* testing must be adjusted and optimized to yield reliable data which may be correlated with the *in vivo* situation.

4.1 METHODS AVAILABLE FOR DISSOLUTION RATE DETERMINATIONS

Numerous methods of determining *in vitro* dissolution rates of solid, oral dosage forms have appeared in the literature (220-230). Dissolution rate methods should meet certain criteria. The apparatus should be economically practical and flexible as over

the last decade, the increase in literature concerning *in vitro* dissolution testing has highlighted problems and deficiencies in the methods used. The more flexible the standard is, the more easily changes can be made to accommodate the new findings. The method should be reproducible and yield accurate, meaningful results that can be quantitatively related to theoretical dissolution rate equations and thirdly, they should be sensitive enough to detect small differences among various drugs and also among different formulations of the same drug (220).

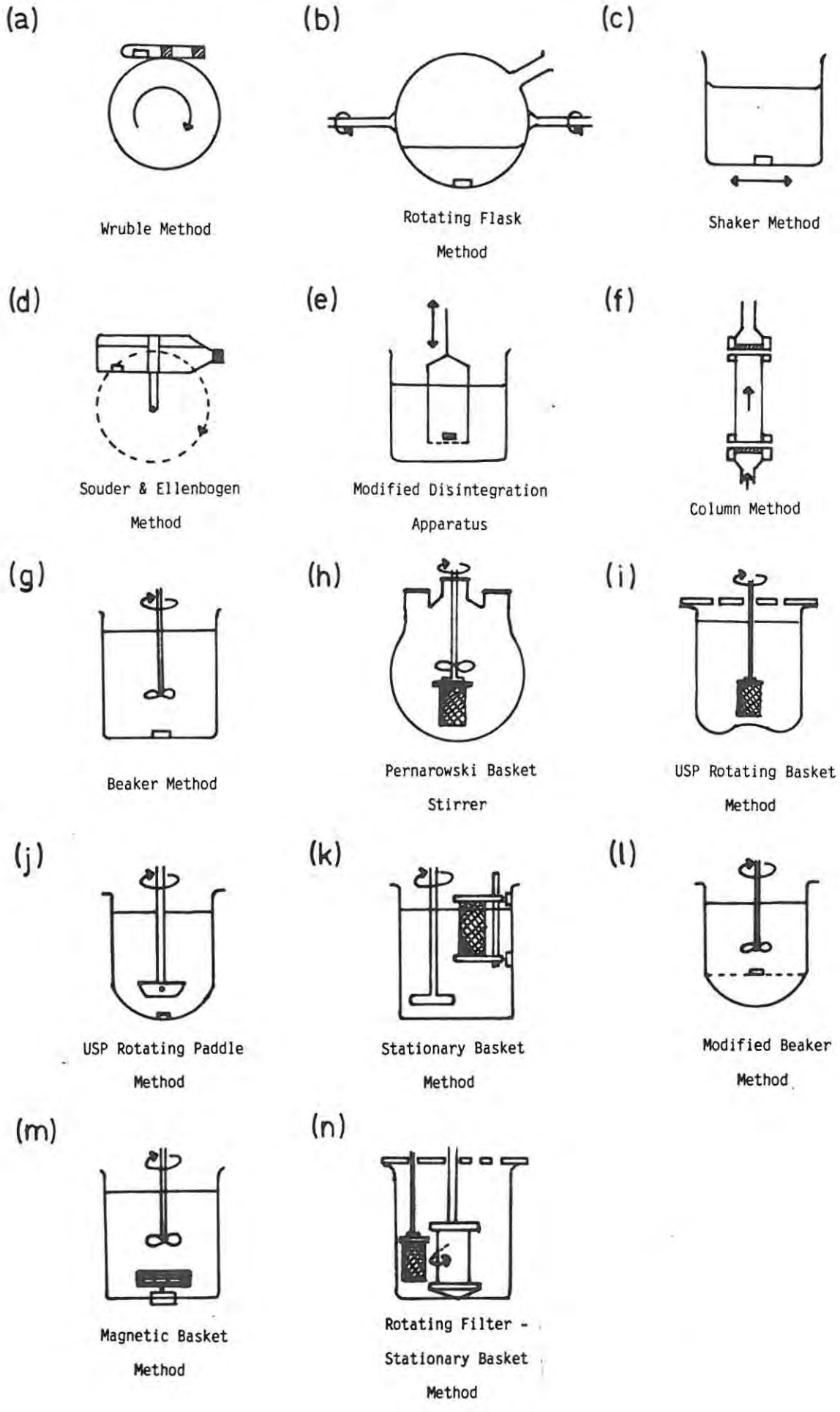
Dissolution rate methods can be classified according to various criteria such as geometry of the apparatus, degree of agitation within the system, the mode in which the solvent enters the dissolution process and the achievement of sink or non-sink conditions. Under non-sink conditions, a significant increase of concentration in the dissolution medium occurs until the solubility limit of the solute is approached. This may influence the dissolution rate of undissolved drug. Under sink conditions, drug concentration is much lower than the equilibrium solubility of the drug. Dissolution rate-limited absorption implies that there is very little build-up of drug concentration in the gastro-intestinal fluids *i.e.* the fluids function as a perfect sink, with the drug concentration remaining much lower than the solubility of the drug. Consequently it is thought that maintenance of sink conditions improves the chance of achieving good *in vitro - in vivo* correlations (220,221,231).

In this instance, classification has been attempted on the basis of the relative agitation within the system.

4.1.1 Agitation of the Dissolution Vessel

Included in this classification are methods such as the Wruble method (221,232,233), the rotating flask apparatus (234,235), the "shaker" method (236) and the Souder and Ellenbogen method (221,223,237) (see Fig. 4.1 a,b,c,d). In the Souder and Ellenbogen dissolution test procedure, the method of agitating the tablet or capsule is similar to that described in the tablet disintegration

FIGURE 4.1 Methods of *in vitro* dissolution testing



apparatus of the British Pharmacopoeia 1948 (238) and 1953 (239).

The rotating bottle apparatus and official test procedure for sustained-release preparations in the National Formulary XII, 2nd supplement (240) are based on the apparatus and method of Souder and Ellenbogen (237). The dosage form is sealed with the dissolution medium in cylindrical bottles which are rotated end over end at a fixed rate in a water bath at 37°C. Variations of this procedure which included varied bottle size, fluid volumes and rotation rates, were used by Campbell and Theivagt (241), Nessel *et al.* (242), Blythe (243), Krueger and Vliet (244), Shenoy (245) and Chaudhry and Saunders (246). This method is gradually falling into disuse as all new sustained-release dosage forms are being tested by the compendial dissolution methods. A serious drawback of the rotating bottle apparatus is the difficulty involved in removing samples without interrupting the dissolution test and the fact that it does not lend itself to automated testing. Automation of dissolution testing is particularly desirable in the longer runs required for assessing sustained-release dosage forms.

4.1.2 Movement of the Dosage Form through the Dissolution Medium

Many investigators have used the modified USP/NF disintegration apparatus for standard as well as sustained-release tablets (see Fig. 4.1 e). The major modifications are the replacement of the 10-mesh screen by a 40-mesh screen and the elimination of the plastic discs (247-256). Advantages of this method include information on tablet disintegration obtained during the dissolution process and automation of sampling (225). However the apparatus has only one oscillation speed and the effective degree of agitation is high but is difficult to define. Agitational intensity, which depends upon the volume of dissolution medium and the dimensions of the container, influences results obtained with this apparatus. Dissolution results have not correlated well with *in vivo* results and as such it is doubtful that this method will enjoy widespread use.

4.1.3 Flow of Dissolution Medium past the Dosage Form

The flow-through cell dissolution apparatus consists of a cell which is cylindrical in shape, constructed of glass or other appropriate material and immersed in a water bath to maintain the contents at 37°C. A bottom barrier of either a porous glass plate or a bed of glass beads should be capable of suitably dispersing the solvent to provide constant laminar flow in the chamber. A filter is situated at the top of the cell and direction of fluid flow is upwards (see Fig. 4.1 f). The apparatus can be run either as a closed (226,257) or an open system (220,258-261). A modified version consisting of a cascade barrier bed has been used (262).

A major advantage of this method is the maintenance of sink conditions for drugs that form saturated solutions in volumes in excess of 10%-20% of the 900 ml normally used in the basket or paddle methods. "Perfect" sink conditions can be obtained with the open flow-through method as pure solvent is continuously presented to the surface of the solid during the test. In the closed system, pure solvent is presented to the dosage form only once and gradually changes as the drug is dissolved.

The flow-through system has built-in filtration , it allows the convenience of changing the pH during dissolution testing and fewer external variables need to be controlled to yield reproducible results. A further advantage is that the position of the dosage form may be fixed, making it simpler to maintain a constant and reproducible fluid-flow pattern around the dosage form (230).

The filter can cause problems, since the built-in unidirectional filter-flow is inherently susceptible to clogging. Dissolution characteristics may change if particulate matter adheres to the filter with turbulence at the solid/liquid interface. The parameters of flow-rate and its ripple effect must be more carefully studied and understood and finally, the cost of the equipment should be reduced to enable comparison with the cost of compendial methods (229,230).

4.1.4 Tank Reactor with a Mechanical Agitator

This group encompasses a large number of methods such as the Levy and Hayes beaker method (263-270), the Pernarowski basket stirrer assembly (271), the USP rotating basket assembly (272) and the USP rotating paddle method (272). Other methods include the stationary basket method (225,273), the magnetic basket method (269,274) and the rotating-filter stationary basket apparatus (227,275) (see Fig. 4.1 g,h,i,j,k,l,m). Nash and Marcus (276) developed the Buchner funnel apparatus for sustained-release preparations and Goodhart *et al.* (228,277) developed and evaluated an apparatus for testing disintegration and dissolution of capsules and tablets. These methods have been used to test a wide range of drugs, are fairly simple to use, a number of determinations can be run concurrently and they can be readily adapted to automated sampling (278-280).

The above methods have a number of shortcomings and results are affected by many variables. At low agitation intensities, the particles or granules form a mound at the bottom of the beaker which may lead to reduced surface area being presented to the dissolution medium (235). Researchers have substituted round-bottomed flasks for the traditional beaker to ensure mound formation in the centre of the bottom of the vessel (281,282), as it has been reported that the location of the mound within the dissolution vessel influences results (283,284).

In a study of the hydrodynamic conditions in the Levy beaker vessel, tablet positioning within the vessel was shown to strongly influence dissolution results (264). The marine propeller and the straight-bladed turbine impeller produce mainly rotational and radial currents. Much longer mixing times were found for marine type propellers and mixing times appeared to be dependent on firstly the angle of the propeller blades from the horizontal plane and secondly the direction of rotation of the propeller (285). Consequently, to centre the dosage form, straight-bladed impellers used in round-bottomed vessels were recommended as a modification (264).

A further problem is presented by capsules as they tend to float and consequently are not wetted on all sides which may alter dissolution characteristics. Researchers have ensured submersion of capsules in the dissolution medium in a number of ways (286-288).

The two official methods (272), the rotating basket method and the rotating paddle method wll be discussed in greater detail.

4.1.4.1 The Rotating Basket Apparatus

In 1970 a dissolution apparatus known as the rotating basket method appeared in the USP based on the Pernarowski basket stirrer assembly (271). This apparatus, mainly because of its official status, has been widely used. It has also been adapted for use in the dissolution testing of sustained-release dosage forms (267,289,290). The official apparatus consists of a 1000 ml dissolution vessel made of glass with a slight concave bottom and sides which are flanged near the top. A fitted cover with sufficient openings to allow insertion of a thermometer and withdrawal of samples may be used to retard evaporation. The medium in the flask is kept at a constant temperature of 37 ± 0.5°C by means of a suitable water bath. All significant vibration or agitation must be eliminated. A cylindrical 40-mesh stainless steel basket consists of two parts, the top of which is attached to the stainless steel shaft which rotates smoothly and without significant wobble in the dissolution medium. A speed-regulating device is used that allows the shaft rotation speed to be selected and maintained at a specified rate. The distance between the inside bottom of the vessel and the basket is maintained at 2.5 ± 0.2 cm during the test.

Modifications to this method have been made to enable the dissolution testing of sustained-release tablets and microencapsulated particles. Baskets with 80-mesh and higher screening have been selected for testing of microencapsulated particles as the particles will be retained in the basket, yet solvent can penetrate without clogging (230).

Dissolution media used in the testing of sustained-release products include USP simulated gastric fluid for one to two hours which is then replaced with USP simulated intestinal fluid and release of active drug measured over eight hours. Some researchers feel that it is necessary to maintain the temperature at 37°C as temperature has an effect both on diffusion and on the physical parameters producing the sustained release of the drug. However, Nash (276) felt that for control purposes, neither body temperature nor simulated pH changes are mandatory. Fremstad *et al.*(290) showed that variation in the pH of the medium from pH 1 to pH 7 had no effect on the dissolution of procainamide from a sustained-release formulation. The release characteristics of PPA.HCl in sustained-release formulations have been assessed in a variety of media (277,278).

Although today a large majority of *in vitro* dissolution studies are being performed using the USP rotating basket apparatus, this method has attracted much criticism and has been shown to be adversely affected by a number of variables.

Flow pattern consistency which is essential for reproducible and reliable dissolution rate data, is affected by the geometry of the stirring device, its alignment, external vibration and speed of rotation. Researchers have cautioned against the permanent insertion of sampling probes and thermometers into the dissolution fluid as they may disturb flow patterns and adversely affect mixing (291). Flow pattern has also been found to be affected by slight differences in smoothness of the round bottom of the flask and by variations in other dimensions of the flask. This phenomenon will be dependent not only on the hydrodynamics in the bulk of the solution, but also on the concavity at the base of the flask where the granules tend to accumulate and lie relatively undisturbed (292). These factors emphasize the lack of rigorous specifications for the official vessel.

Withey and Bowker (283) found undesirable mixing and flow characteristics within the agitated solvent in all dissolution methods

examined. Mixing time was found to be dependent upon the hydrodynamics of the dissolution medium and the density and viscosity differences caused by fast dissolving drugs and excipients (285). The return of substantial amounts of the dissolution medium to the vessel may result in the disturbance of the flow pattern if the temperature of the fresh medium is lower than that of the medium in the flask. The effect of the length of the stirring shaft on dissolution was reported by Carstensen *et al.* (293) who showed that the longer shaft length gave higher dissolution results.

Variation in sampling positions within the dissolution vessel should be avoided as poor reproducibility will occur especially at low rotational speeds of the basket (283,285,294). This appears to be due to a vertical concentration gradient resulting from inadequate mixing within the system. The USP XX/NF XV (272) recommends that samples be removed at approximately half the distance from the bottom of the basket and surface of the media and not closer than 1 cm to the side of the flask. The BP (295) specifies half the distance from the basket and surface of the flask at the middle of the basket.

Vibration is a common variable resulting in significant change in dissolution (296) as it has the effect of changing the flow patterns of the liquid and of introducing unwanted energy to the dynamic system (230). Beyer and Smith (297), investigating the effects of vibration, reported a six-fold increase in the dissolution rate when using apparatus rated "slightly rough" according to a vibration severity chart.

Positioning of the tablet in the basket and vessel has been shown by Lerk (264) to influence dissolution rates. Highest dissolution rates were found to occur in the centre of the vessel floor and these rates decreased as the periphery of the vessel floor was approached. In the basket itself, tablets against the wall have higher dissolution values than those positioned in the centre of the basket.

It was shown by Mattock *et al.*(298) that corrosion of the basket occurred after exposure to compendial dissolution fluid for 40 hours, making the basket unsuitable for further use. This can be minimised by replacing the dissolution fluid with either a less concentrated HCl solution or by using a buffered dissolution medium. The 40-mesh wire screen of the basket is susceptible to clogging by the granules which consequently impairs visual observation of the behaviour of the tablets during dissolution (299). As the basket itself serves as a stirring device, there may be excessive abrasion and wear of the tablet due to its mechanical impact with the container surface, thereby adversely influencing its micro-environment (275).

The dissolution medium may be the source of variability in results. Dissolved gases must be removed from the dissolution medium if they affect the test. Gas may significantly change the pH of the medium although this is less likely in a buffered medium (230). However the major influence of gas seems to be physical. Flow patterns may be altered if bubbles are disturbed and rise to the surface. Bubbles may associate with aggregate particles, resulting in random concentrations of the particles in the solvent stream and they may collect at the screen on the basket thereby changing mesh porosity. The surface area of dosage forms exposed to the solvent stream may be reduced by bubbles becoming attached to the dosage forms thus altering the disintegration process.

Variability in results has occurred due to entrapment of certain dosage forms in an air-bubble at the top of the basket assembly. A conical surface on the drive plug has been proposed which imparts enough of a slope to permit an entrapped air bubble to escape from the basket assembly and allow the tablet to sink to the bottom of the basket (300).

High variability in data generated by different laboratories has raised doubts about the reproducibility of dissolution testing. Fusari *et al.* (301), in an effort to improve reproducibility, identified samples appropriate for use as dissolution rate

calibrators and also established acceptance limits for a non-disintegrating 300 mg salicylic acid tablet and a disintegrating 50 mg prednisone tablet. It was agreed that careful definition of operating parameters such as basket dimensions and limits on rotational speed, along with the use of standard samples as calibrators will improve reproducibility of dissolution data.

However it was found by Prasad *et al.*(302) that no single standard can predict the suitability of the basket or paddle dissolution apparatus, as after tests using the USP prednisone calibrator and a prednisone standard from the National Centre for Drug Analysis (NCDA), the former was found to be sensitive to perturbation by the basket method but not to perturbations by the paddle method. The opposite was found for the NCDA prednisone calibrator.

Problems in the use of calibrators include the effect of storage conditions on the dissolution rate of the calibrators, the broad acceptance range and the infrequency of validation runs. These runs yield little information on performance between the checks or on variations due to the operator. Mazuel *et al.*(303) demonstrated the use of standardization in *in vitro* dissolution assays. The standard, which is a preselected lot of the same formulation as the one being tested, is run at the same time and adequately compensates for small variations in pH and temperature of the dissolution medium, and large variations in stirring speed. Equipment performance can therefore be monitored during every dissolution test.

Cox *et al.* (304) identified many critical factors in dissolution testing using the USP basket method and in an extensive review suggested guidelines for conducting dissolution tests. Specifications include the position and method of withdrawal of aliquots, adjustment and centering of the vessel, elimination of significant wobble, individual adjustment of basket height 2 cm from the bottom of the vessel and position of tablets and capsules in the basket.

4.1.4.2 Rotating Paddle Apparatus

The second official method in the USP XX/NF XV (272) which is gaining in popularity is the rotating paddle apparatus (287,305,306). As for the rotating basket apparatus, the assembly consists of a 1000 ml vessel made of glass, and a variable-speed drive, except that a paddle formed from a blade and a shaft is used as the stirring element.

The stirring blade passes through the diameter of the shaft so that the bottom of the blade is flush with the bottom of the shaft. A distance of 2.5 ± 0.2 cm between the blade and the inside bottom of the vessel is maintained during the test. The metallic blade and shaft comprise a single entity that may be coated with a suitable fluorocarbon polymer. The dosage unit is allowed to sink to the bottom of the vessel before rotation of the blade is initiated.

If the dosage form is one that will not sink, a small, loose piece of non-reactive material such as wire or glass helix may be attached to it. The practice has been criticised for a number of reasons. The helix may become clogged with gummy excipients resulting in inconsistent release of particles and changes in the physical form of individual dosage forms. The metallic wire may react with the dissolution medium. The helix characteristics are not precisely defined and this leads to significant differences in the physical dimensions of individual dosage forms which results in random positioning and movement of the dosage form in the vessel (230). Some researchers have modified the method to include a dosage form holder (307,308).

Similar guidelines as those discussed by Cox *et al.*(304) for the rotating basket method are applied to this method. Large variations in dissolution rates have been traced to minor variations in the alignment of the apparatus as the flow rates generated in different sections of the vessel are controlled not only by the rotational rate of the paddle, but also by the location of the paddle in the vessel. To minimize error, the

following adjustments must be made as precisely as possible: the base of the apparatus must be horizontal, each shaft must be positioned along the vertical axis of each vessel and the paddles must be set at a standardized depth in the vessels (309). Differences in the bottom curvature of dissolution vessels were found to cause bias in the dissolution results (310).

Studies have shown that large sampling probes caused significant hydrodynamic disturbances, resulting in changes in the dissolution rate (311,312). Probe location within the vessel was also shown to requre careful control. The use of a small capillary probe is recommended as this reduced hydrodynamic disturbances (311).

Carstensen *et al.* (305) and Braae (307) found that the rotating paddle apparatus gave good homogeneity with a lower mean deviation than the rotating basket method. When this method was compared with the rotating filter-stationary basket system (287) it was found to cause significantly less variability.

4.2. EXPERIMENTAL

4.2.1 Chromatographic Conditions

Column:	reverse phase μBondapak C_{18}
HPLC system:	B (see Instrumentation)
Detection Wavelength:	220 nm
Sensitivity:	0.04 AUFS
Flow rate:	1.5 ml/min
Pressure:	2500 psig
Temperature:	25°C
Recorder input:	100 mV
Mobile phase:	45% methanol/55% HSS solution (0.005M) with 0.2% 1M HCl

4.2.2 Dosage Form Purity

Four sustained-release dosage forms containing PPA.HCl were tested for their dissolution characteristics:

(i) Product BS (150mg PPA.HCl) - a conical-shaped tablet

consisting of a wax matrix core.

(ii) Product DT (75mg PPA.HCl) - a capsule containing microencapsulated particles.

(iii) Product LC (75mg PPA.HCl) - a capsule containing microencapsulated particles.

(iv) Product CO (75mg PPA.HCl) - a capsule containing microencapsulated particles.

No official monographs on sustained-release PPA.HCl dosage forms appear in the BP or USP, thus no methods are specified for their analysis. For Product BS, ten tablets were weighed, finely crushed and a mass of powder approximately equivalent to the weight of one tablet was accurately weighed and dissolved in methanol containing 60 mg/100 ml PEP.HCl as the internal standard. The suspension was made up to 100 ml, shaken and filtered twice through Whatman No.40 filter paper. An aliquot of the filtrate was injected onto the HPLC column. The tablet assay was carried out in duplicate.

Limited numbers of the remaining three products prevented a comprehensive assay of PPA content. At the end of each dissolution run, the particles were crushed and dissolved in the dissolution medium. Six tests were therefore carried out in this way on each product. An aliquot of the dissolution medium was injected onto the HPLC column and the total amount of PPA.HCl contained in that dosage form was calculated.

4.2.3 Rotating Basket Method

The basic apparatus used is described in section 4.1.4.1. The vessel was mounted in a clear perspex bath in which water was heated by a Thermomix-1480 circulating pump mounted in such a way that vibrations were not transmitted to the rest of the apparatus. A multiple spindle apparatus (Hanson Research Corp., USA) was used for rotation of the baskets. The vessel was covered with a perspex lid containing four openings, a central one for the shaft and three peripheral openings for sampling and fluid replacement (see Fig.4.2)

Each beaker held 500 ml of the dissolution fluid which was maintained at 37°C. A single tablet or two capsules were placed in the basket which was then immersed in the dissolution fluid 2 cm from the vessel bottom. Rotation was initiated immediately at 100 rpm.

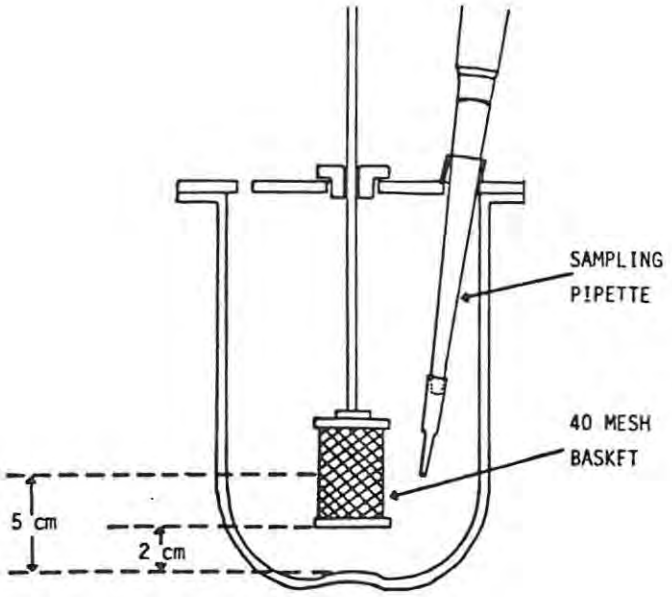


FIGURE 4.2 The USP Rotating Basket Apparatus

In the preliminary testing using this apparatus, aliquot withdrawal was achieved with a clean grade A, 1 ml pipette, care being taken to ensure that all samples were removed from the same point within the vessel. There was no replacement of the medium but the volume removed was compensated for in the calculation of results. Sampling times were 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 hours after initiation of rotation. Samples were stored in a cool place until analysis.

For subsequent dissolution testing, one peripheral opening in the perspex lid was modified to enable the insertion and exact positioning of a micropipette whch was used to withdraw 50 µl aliquots 5 cm from the bottom of the vessel and 2 cm from the basket. The number of early sampling times was increased as follows: 1, 2, 4, 6, 8, 10, 12.5, 15, 20, 25, 30 and 45 minutes,

then 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9 and 10 hours after initiation of rotation. As such small volumes were removed, no replacement of fluid was necessary.

4.2.4 Rotating Paddle Method

The apparatus used was a modification of that described in section 4.1.4.2. It consisted of a 2000 ml cylindrical Pyrex beaker fitted with a perspex lid which had peripheral openings to allow for the housing of a dosage holder and to provide for sampling. The lid was modified to allow for the insertion of a micropipette in exactly the same position each time. The stainless steel stirrer shaft of the perspex paddle passed through the centre of the lid and was rotated by a multiple spindle drive apparatus (Hanson Research Corp., USA). The water in the clear perspex water bath was heated by a Thermomix-1480 circulating pump.

After the preliminary dissolution testing, the perspex dosage holder was substituted with a cylindrical stainless-steel 40-mesh basket to retain the microcapsules within the dosage form holder (see Fig.4.3).

Each beaker held 1500 ml of dissolution medium which was maintained in equilibrium with the temperature of the water bath at 37°C. Either one tablet or two capsules were placed in the dosage holder which was then immersed in the dissolution fluid so that the bottom of the dosage holder was 2 cm above the paddle, and rotation at 100 rpm was immeditely initiated. Aliquots of 50 µl were withdrawn at the same time intervals as specified above. No replacement of fluid was necessary as less than 2 ml in total was removed which, in a volume of 1500 ml, is negligible.

4.2.5 Dissolution Media

(i) Simulated gastric fluid (GF). Hydrochloric acid (1M, 236 ml) and 1M NaOH (168 ml) were added to a 2 litre volumetric flask. Eight millilitres of the internal standard solution (12.5 mg PEP.HCl/ml) were pipetted into the flask and distilled water was added to dilute to volume. The resultant pH was 1.5 ± 0.05.

(ii) Simulated intestinal fluid (IF). One hundred and twenty five ml of monobasic potassium phosphate (3.48 g/100 ml) was measured into a 5 litre volumetric flask. 1M NaOH (190 ml) was added and the solution diluted to about 4000 ml with water. Twenty millilitres of the internal standard solution (12.5 mg PEP.HCl/ml) was pipetted into the flask and the pH of the fluid adjusted to 7.2 ± 0.05 with 1M HCl. The final solution was made up to 5 litres with distilled water.

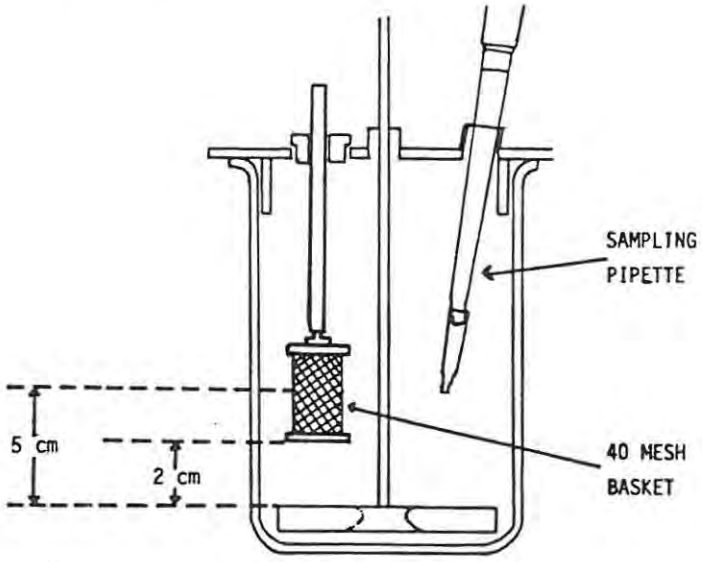


FIGURE 4.3 The USP Rotating Paddle Apparatus

4.3 DISSOLUTION TESTING

4.3.1 The Rotating Basket Apparatus

Test I. Initially three sets of media conditions were evaluated for their effect on the release of PPA.HCl from Product BS:

(i) 500 ml IF for 10 hours

(ii) 500 ml GF for 1 hour, then 500 ml IF for the remaining 9 hours

(iii) 500 ml GF for 1 hour, 500 ml IF for the second and third hours, then 500 ml fresh IF for the remaining 7 hours.

Two dissolution runs were carried out using each set of conditions.

Test II. To determine the dissolution characteristics of Product BS, tests on 6 tablets were carried out using simulated IF for the full 10 hours and the first sampling schedule described in section 4.2.3 was followed.

Test III. A subsequent series of tests on Product BS using the same apparatus included an increased number of sampling times in the first hour to enable a fuller characterization of the initial part of the curve and to give some indication of any lag time shown by the tablets.

4.3.2 The Rotating Paddle Apparatus with Perspex Holder

Test I. Release of PPA.HCl from Product BS was determined in IF using this apparatus with the latter sampling schedule and compared with the results obtained from the other apparatuses.

Test II. The effect of dissolution medium pH on the dissolution characteristics of Product LC was assessed. Two dissolution tests were run, one in GF and the other in IF for the full 10 hours.

4.3.3 The Rotating Paddle Apparatus with Wire Holder

The release characteristics of Products BS (Test I), LC (Test II), CO (Test III) and DT (Test IV) were determined in IF using the above apparatus and the second sampling schedule. An additional test (Test V) was run on Product DT using GF for 1 hour and IF for the remaining 9 hours.

4.4 RESULTS AND DISCUSSION

Analysis of dissolution samples by HPLC was rapid, accurate and precise. The HPLC column (μBondapak C_{18}) remained stable throughout the series of tests and no increase in back-pressure was observed. A guard column packed with 20-40 micron LC-18 pellicular packing (Supelco Inc., Pennsylvania) was placed in line directly before the HPLC column and served to remove any particulate matter which may have been injected into the system.

The PPA.HCl content for Products BS, LC, CO and DT were found to be 98%, 115%, 98% and 97% respectively. Phenylephrine hydrochloride was chosen as the internal standard because of its favourable elution time. Standards made up with the dissolution medium containing the internal standard were injected throughout each run to monitor the stability and reproducibility of the system. Incorporation of the internal standard into the dissolution medium had many associated advantages. The same internal standard solution was used for the preparation of standards for the calibration curve and for all dissolution fluids used. Its presence compensated for any loss in the total volume of dissolution fluids due to evaporation during the dissolution run and evaporation from the sample removed for determination.

Greater accuracy is associated with the incorporation of the internal standard in the dissolution fluid than with the addition of an internal standard after sampling. The dissolution characteristics of PPA were not affected by the presence of the internal standard, as identical profiles were obtained when internal standard was added after sampling. No pretreatment such as extraction or derivatization was necessary, thus allowing the sample to be injected directly onto the column.

Sampling with a micropipette enabled rapid, accurate removal of the medium from precisely the same point within the beaker. Only 50 µl was withdrawn each time resulting in total decrease in volume of less than 1.5 mls from the 1500 mls over the 10 hours. Replacement of the medium was therefore unnecessary.

No *in vitro* dissolution rate parameters have been specified for sustained-release formulations of PPA.HCl, but it has been suggested that a tablet or capsule that contains the equivalent of three single doses should release 25% to 40% of the active principle within one hour after administration; after that release should proceed gradually so that another 25% to 30% is released within 4 hours and the remaining quantity within the time indicated on the label (313).

It has also been found that freely soluble drugs with a pKa of 8 to 10 have produced satisfactory *in vivo* responses if their *in vitro* release rates approximated the following: 20% - 40% release within the first 30 minutes, 40% - 60% within 2 hours, 60% - 80% up to 4.5 hours and more than 80% in 7 hours (25).

The *in vitro* release characteristics of PPA from a wax matrix timed-release tablet were studied by Goodhart *et al.* (277). The tablets showed a relatively fast initial drug release during the first hour ranging from 33% to 40%. This was followed by a slower dissolution rate until 80% of the drug was released in 6 to 7 hours.

The dissolution curves resulting from Test I using the rotating basket apparatus with different dissolution media were very similar and are depicted in Fig. 4.4. The rate of release of PPA in the first hour of immersion seemed to be slightly higher in GF than in IF and was comparable for all three methods up to 4 hours. Thereafter release of PPA in the test using IF only was nominally higher than in the other methods used. This method was selected for further dissolution testing as only a single dissolution fluid had to be prepared and the number of manipulations could be reduced to a minimum.

The average dissolution curves from Product BS (Tests II and III) using the rotating basket apparatus can be seen in Fig. 4.5 and the results from Test III in Appendix 1, Table A1.1. Test III was conducted about 1.5 years after Test II with the rate of release and the total amount of PPA released during the tests decreasing from 93% to 80% of the stated amount. Reasons for this are unknown and can only be postulated such as unfavourable storage conditions, instability of the drug due to ageing, changes in the wax matrix affecting release of the drug or changes in the excipients and/or binders preventing PPA release from the tablet to the surrounding medium.

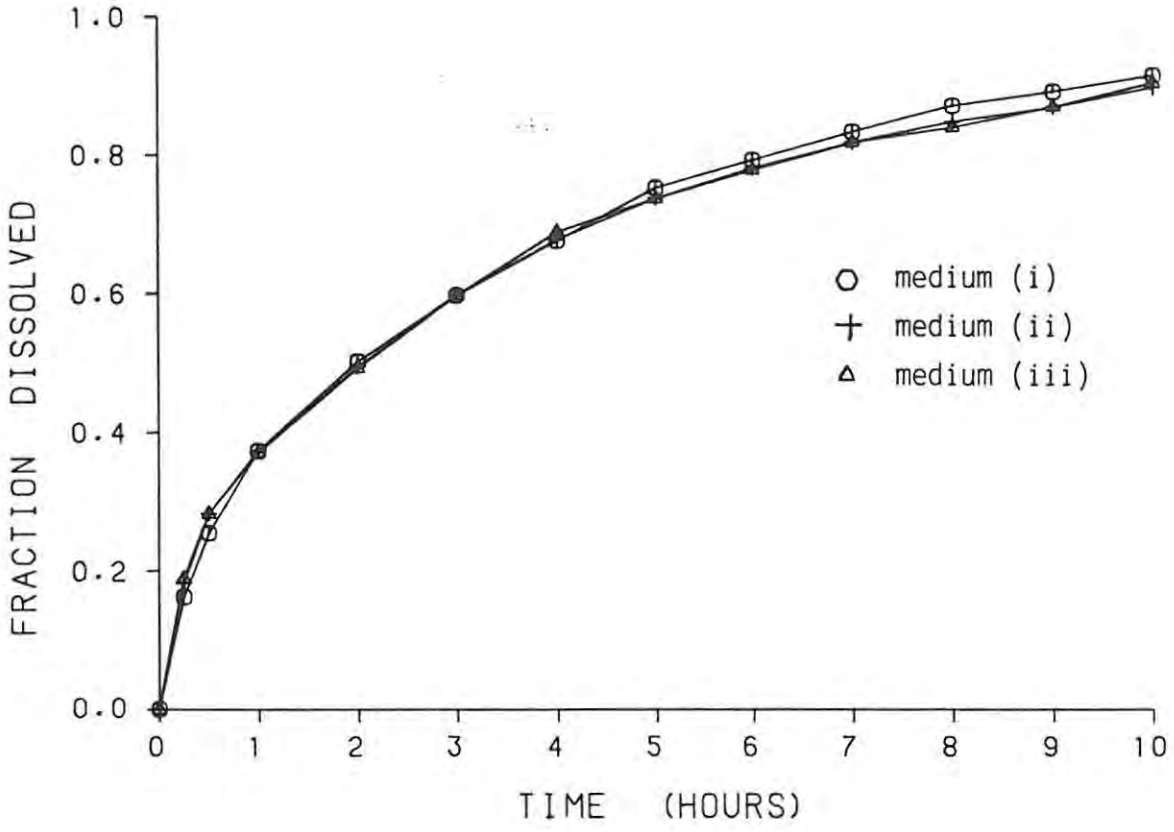


FIGURE 4.4 Dissolution profiles of Product BS in different dissolution media

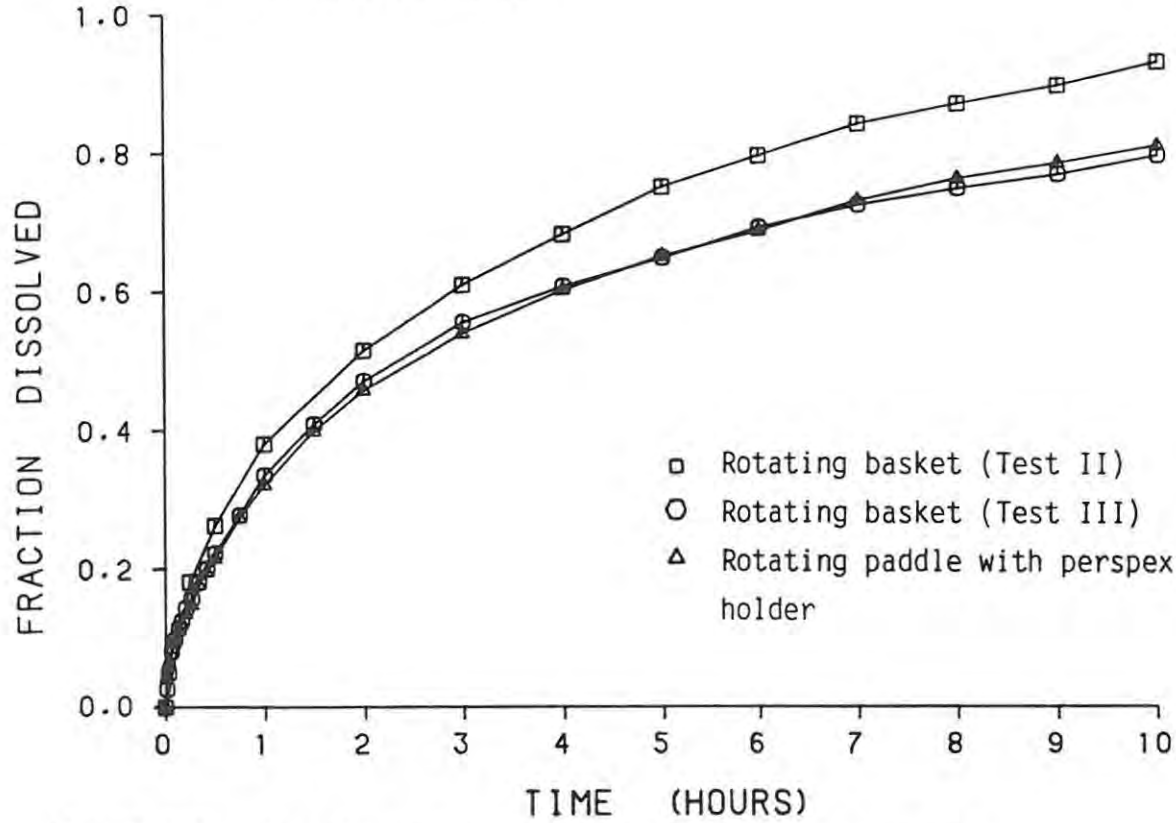


FIGURE 4.5 Dissolution profiles of Product BS using two different dissolution apparatuses

The rotating paddle apparatus with the perspex holder yielded almost identical dissolution curves (Fig. 4.5, Table A1.2) with a slightly higher total release than that obtained using the rotating basket apparatus. The latter method seems to indicate an inferior stirring mechanism when compared with the paddle and this can result in improper mixing and the formation of concentration gradients which can cause inhomogeneity in samples removed from varying positions with the beaker. Between-run variability was found to be approximately equal for these apparatuses.

The dissolution characteristics of Product LC were then assessed in different dissolution media to evaluate the effect of pH on dissolution of the capsule using the rotating paddle with the perspex dosage holder. The curves are depicted in Fig. 4.6.

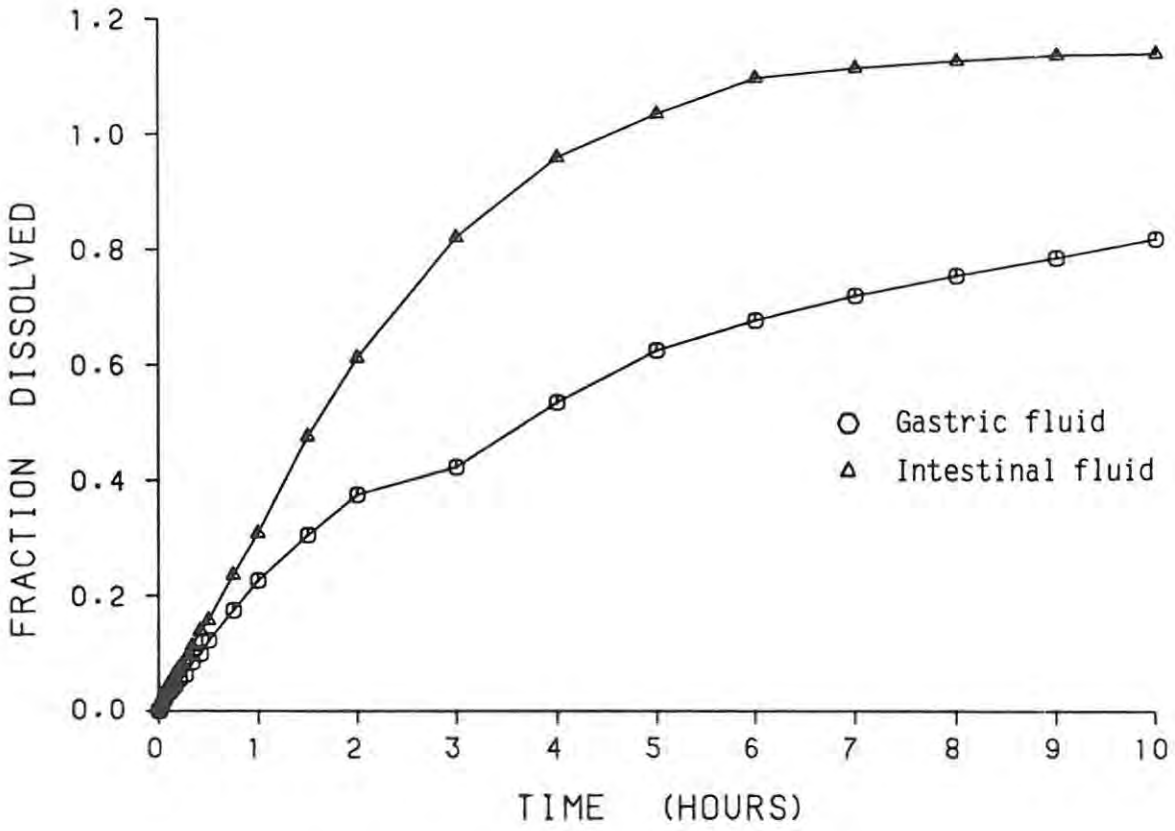


FIGURE 4.6 Dissolution profiles of Product LC in different dissolution media.

Substantially greater and more rapid release of PPA occurred in the IF. The acidic environment encountered in the GF did not affect the release of PPA from the microencapsulated particles to any great extent as might have been expected. However, as the products are all sustained-release preparations, the majority of time will be spent in the intestine rather than in the stomach, so the method using the IF only was chosen for all subsequent dissolution rate determinations.

The gelatin capsule disintegrated within 1 to 2 minutes and the microencapsulated pellets fell out of the large openings in the perspex dosage holder and swirled around the bottom of the vessel. The majority of particles collected in a mound directly below the paddle as the stirring rate was insufficient to suspend the particles in the moving stream. It was felt that dissolution within the mound was not reproduced within each unit which could have resulted in large within-run variation. The 40-mesh stainless steel wire baskets were then modified to enable them to be used with the rotating paddle apparatus. With this method the pellets were retained in the basket and were exposed to a more constant shear rate. An advantage of the rotating paddle apparatus is that the dosage holder remains stationary, thus eliminating wear and abrasion of the dosage form due to its mechanical impact with the surface of the dosage form holder as it rotates.

Dissolution results of Products BS, LC, CO and DT using the rotating paddle appararus with the wire dosage form holder are tabulated in Tables A1.3, A1.4, A1.5 and A1.6 respectively. The average curves are depicted in Figs. 4.7-4.10. Product BS consisted of a wax matrix core which did not disintegrate but retained its conical shape throughout the run. The only observable difference was a discolouration of the tablet. Phenylpropanolamine release was initiated almost immediately on immersion into the medium as positive readings were obtained for all 1 minute sampling times. Fifty percent of the drug was released within 3 hours and a total of 78% was released after 10 hours. Results using the wire dosage form holder were generally slightly lower

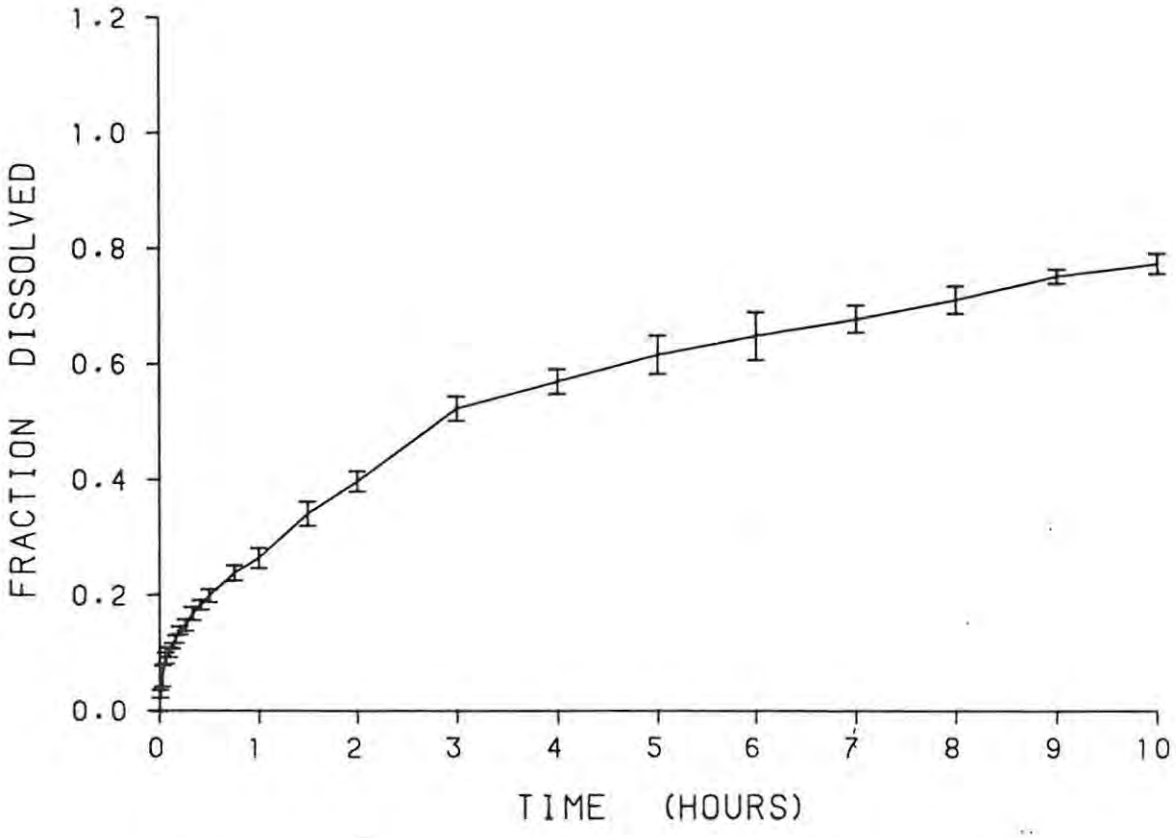


FIGURE 4.7 Average dissolution profile (±SD) of Product BS using the rotating paddle apparatus with the wire holder

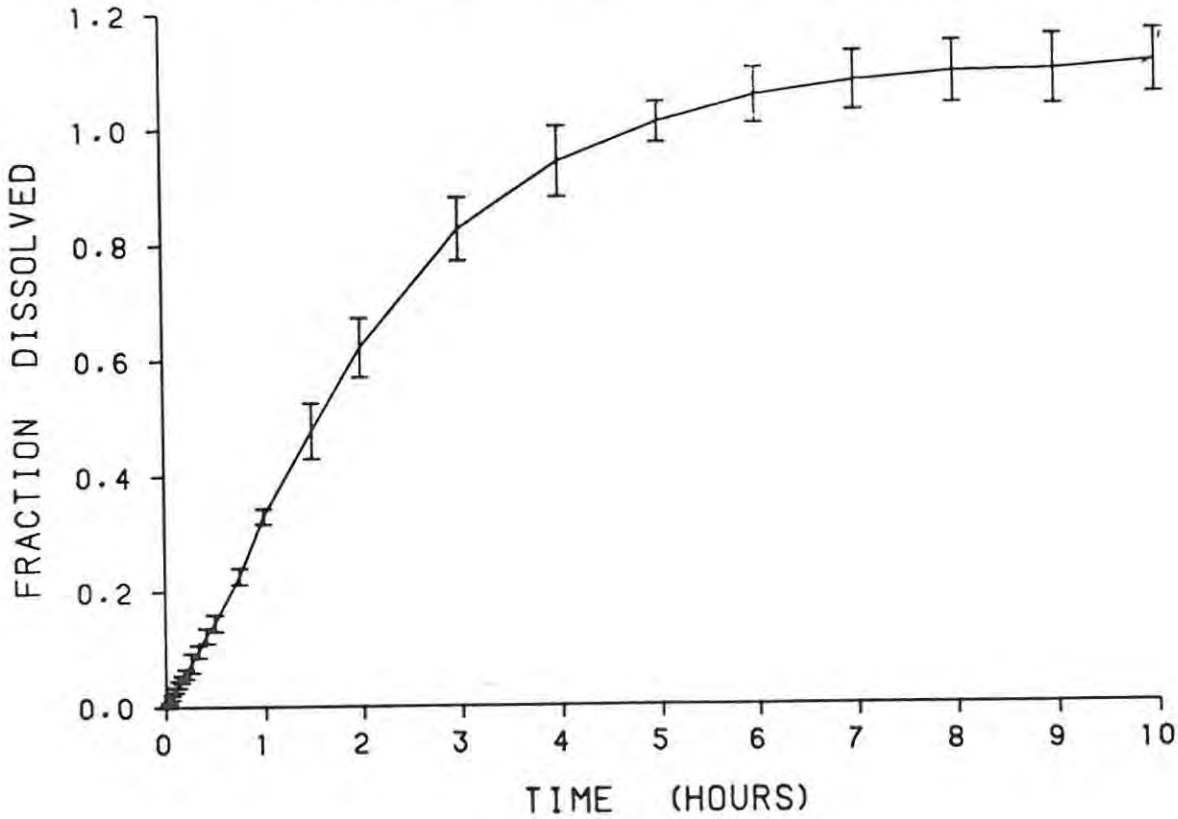


FIGURE 4.8 Average dissolution profile (±SD) of Product LC using the rotating paddle apparatus with the wire holder

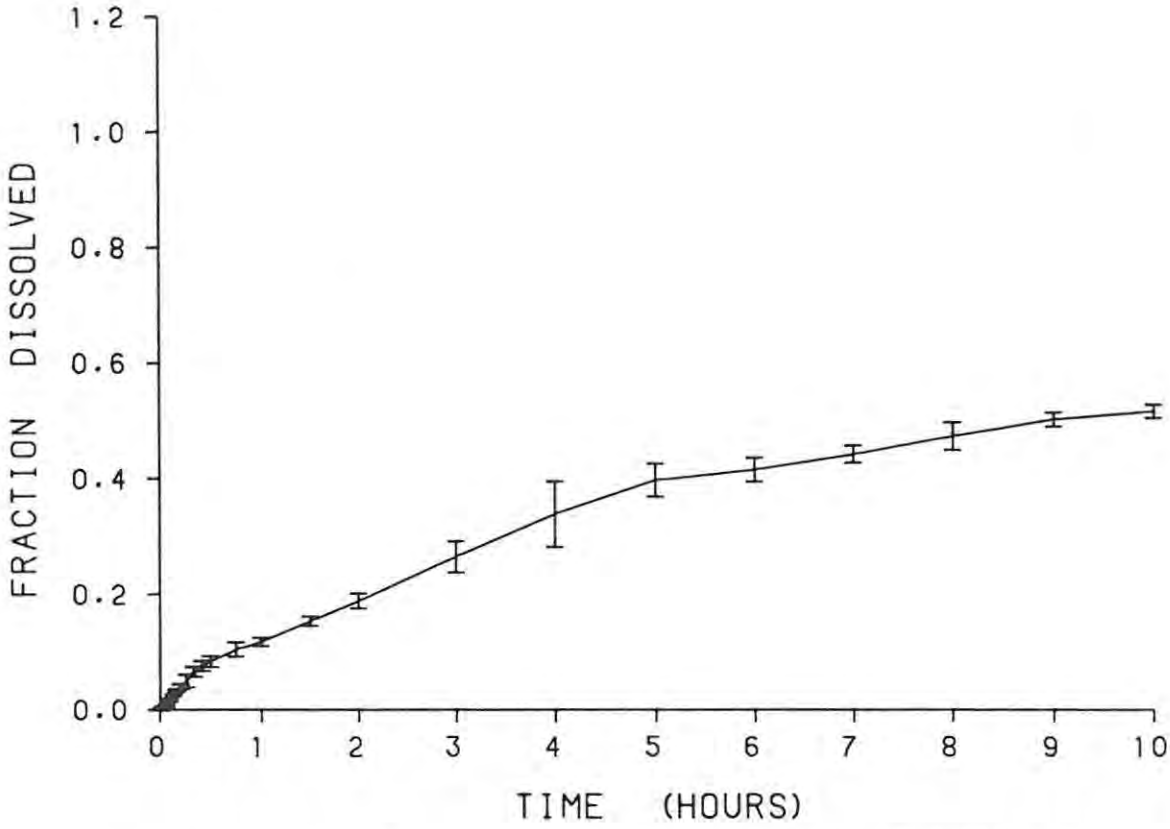


FIGURE 4.9 Average dissolution profile of Product DT (±SD) using the rotating paddle apparatus with the wire holder

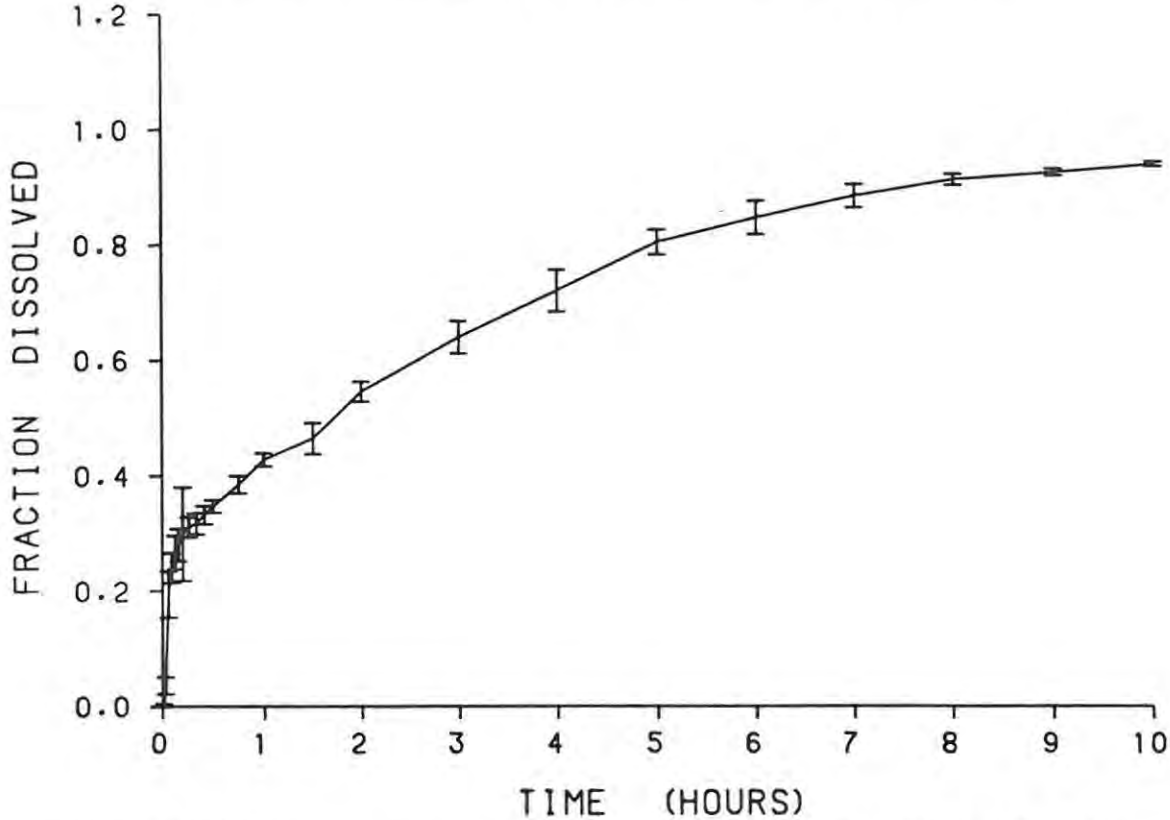


FIGURE 4.10 Average dissolution profile of Product CO (±SD) using the rotating paddle apparatus with the wire holder

than those obtained using the perspex dosage form holder. This may have been due to the larger openings in the sides of the latter holder, therefore allowing a relatively unhindered flow of medium around the tablet, whereas the wire basket could have presented a slight obstacle to flow over the tablet, resulting in a lower release rate.

Product LC displayed a more pronounced lag time than BS. Disintegration of the capsule occurred 1.5 minutes after immersion into the medium. Release of PPA during the first 45 minutes was lower than that from BS, but increased rapidly in the following 4 hours. Rate of release thereafter was similar to that from the other formulations, although total PPA release was higher.

In contrast, Product CO did not display any lag time and disintegration commenced 1 minute after immersion into the medium. The initial slope of the curve up to 12.5 minutes was extremely steep, with 30% release having been achieved by this time. Thereafter rate of PPA release was similar to that exhibited by BS. Product DT, after displaying a lag time similar to that of LC, released PPA extremely slowly with a total release of only 52% after 10 hours.

It was thought that a lower pH may have been required to initiate the dissolution of the pellets before being immersed in a more alkaline medium. This theory was tested, but the different dissolution media did not have a significant effect on the release characteristics. Release in GF and IF in the first hour was similar, but after immersion in the IF an 8% increase in dissolution was observed at 1½ hours. The release rates thereafter were similar.

On superimposing the graphs it was noted that in spite of the different slopes in the first 4 hours, the release rates thereafter of all four products ran almost parallel to one another. Amounts of between 17% and 20% of the drug were released in the last 6 hours of the dissolution tests.

4.5 INTERPRETATION OF DISSOLUTION RATE DATA

4.5.1 Review of Methods Used

In vitro drug release incorporates complex kinetic processes and it is therefore illogical to presume that *in vitro* dissolution should fit a specific zero or first order equation. The quantitative interpretation of dissolution rate data is greatly facilitated by the application of a general mathematical function, where the entire curve is described in terms of meaningful parameters.

Gibaldi and Feldman (314) proposed a first order exponential distribution to describe dissolution kinetics and Wagner (315) introduced the log-normal presentation for this purpose. Kitazawa *et al.*(316) proposed an equation to elucidate the relation between hardness, disintegration time and dissolution rate and in a later paper (316) attempted to discover if, theoretically and experimentally, a relation existed between this theory and that of Wagner (315). Although the original treatment for surface area of drug available for dissolution was quite different for the two theories, the dissolution rate constants obtained were in fair agreement.

An equation derived from physical chemical theory which required a special computer program for solution was proposed by Pedersen (318). Lippman (319) has utilized several other empirical approaches for slowly disintegrating systems.

The Weibull function, which is more versatile, was first proposed by Rosin in 1933 and later reproposed by Weibull (the Rosin-Rammler-Sperling-Weibull, RRSW distribution) (320). It has been applied to a large variety of distributions such as yield strength of fibres and steels, size of beans and insects and failure rate of electronic components. Langenbucher (321) applied this function to the linearization of dissolution curves and also discussed its application to dissolution curves (322). The Weibull function has been used by various other researchers to describe dissolution

curves and express dissolution rate data (323-326).

Christensen *et al.* (327) interpreted the parameters in the distribution function for drug release from controlled release dosage forms. Riegelman and Upton (328) described the application of the function to *in vitro* dissolution rate data and *in vivo* absorption rate data obtained after administration of the same dosage form to a panel of human volunteers. When the Weibull function was compared with the equation proposed by Pedersen (318) both equations seemed to fit the data equally well (329).

The Weibull function is shown in equation 4.1:

$$F = F^{\infty}\left[1 - e^{-((t - t_o)/t_d)^{\beta}}\right] \qquad (4\text{-}1)$$

F is the dependent variable and represents the fraction of the administered dose which is dissolved at time t. The fundamental form of this equation is not defined for data points within the lag time 0 to t_o and an ordinate value F=0 is uniquely assigned in this range.

F^{∞} gives an indication of the amount of active ingredient released at infinite time under the given experimental conditions and characterizes the actual drug content of the dosage form. Any analytical or method error will affect F^{∞} such as incorrect calibration, dilution, volume or flow rate and interaction or degradation in solution.

The lag time, t_o, describes any preliminary process before the actual onset of dissolution *e.g.* removal of a tablet layer or disintegration of a tablet or capsule. The time parameter, t_d, represents a scale factor of the time axis with two curves differing only in t_d appearing as being stretched or shortened along the time axis. When $t-t_o=t_d$, $F=1-e^{-1}=1-0.368=0.632$ *i.e.* t_d represents the time required for 63.2% of the drug to dissolve. If $F^{\infty}=1$, t_d corresponds with 63.2% of the actual end plateau F^{∞} rather than the labelled amount.

β defines the shape of the curve and is a non-dimensional number ranging from 0, when the system simulates zero order dissolution, to 1 when a simple first order exponential results. $\beta=1$ is characteristic for a slower initial rate followed by an accelerated approach to the final plateau, described by a sigmoid cumulative curve. For $\beta \rightarrow \infty$ the curve degenerates to a step function. Decreasing β corresponds with a steeper initial slope followed by a flattened tail in the final part.

4.5.2 Weibull Analysis of the Data

The data from the dissolution tests (Sections 4.3.2 and 4.3.3) were fitted to the Weibull equation, and the values for the four parameters t_o, t_d, β and F^∞ automatically optimized using a modified method of steepest descent (330). The iterations were carried out on a CDC Cyber 179 series 825 computer using a generalised fitting program where the form of the model and the fitting criteria are defined by the user.

4.5.3 Results and Discussion

The observed dissolution rate data and the predicted values as calculated from the Weibull equation are depicted in Figs. 4.11-4.16 . The Weibull function was shown to be a robust and versatile function as it enabled description of all the curves. It was least efficient in describing the dissolution curves of Product CO, where the correlation coefficients ranged from 0.9698 to 0.9908. For all other products, the correlation coefficients were greater than 0.99.

The detailed parameter values from the Weibull function analysis of the six series of curves are summarised in Tables 4.1-4.6. Means of the fitted Weibull parameters are summarised in Table 4.7 to enable direct comparison of the fits.

The lag time, t_o, was greatest for Product LC, which was evident from the dissolution tests as this product took longer than the other two capsules to disintegrate. As noted earlier, Product BS displayed a negligible lag time before release of PPA was

FIGURE 4.11 Dissolution profiles and Weibull function fits for Product BS using the rotating basket apparatus.
The solid curve represents the Weibull fit to the data.

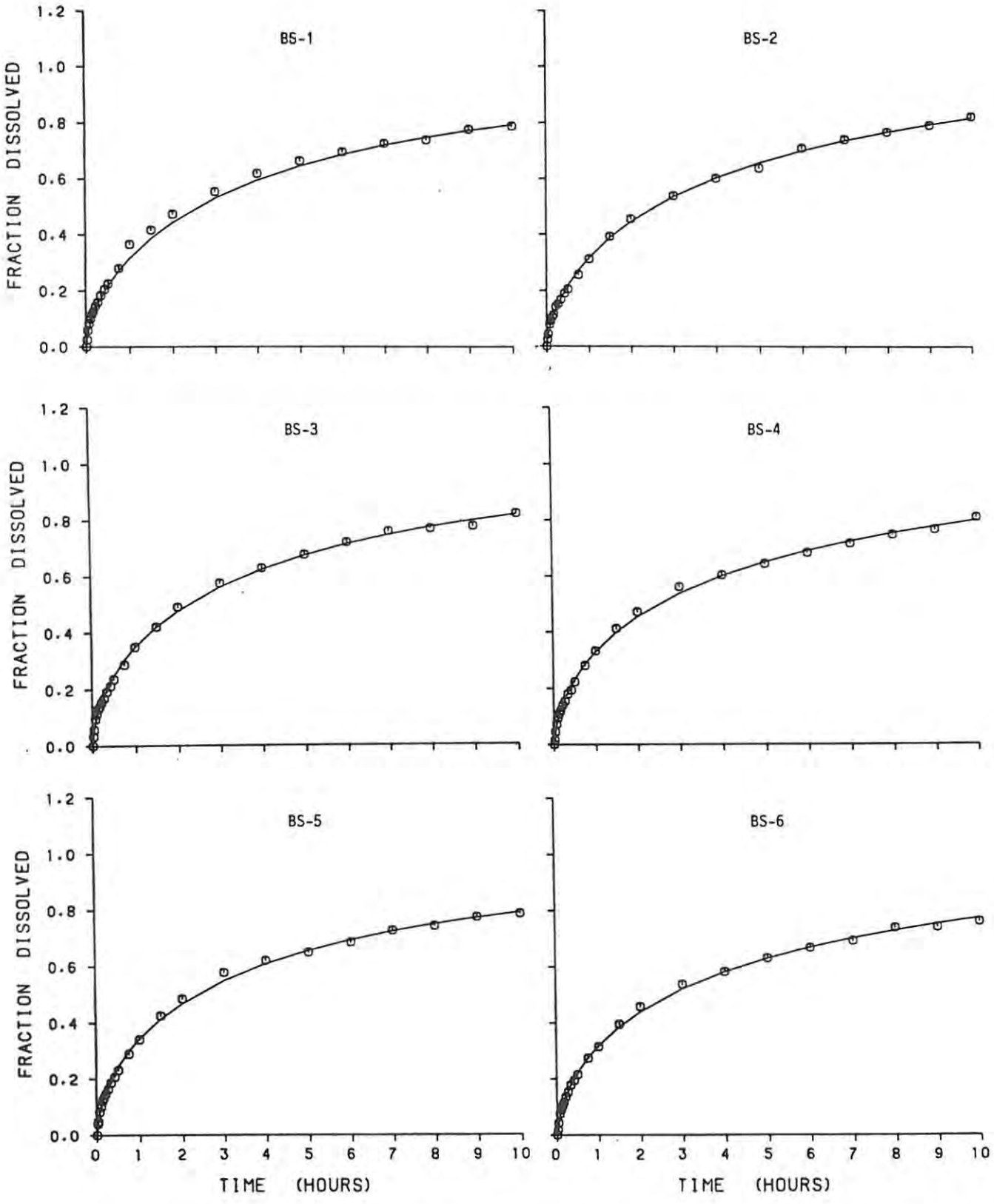


FIGURE 4.12 Dissolution profiles and Weibull function fits for Product BS using the rotating paddle apparatus with perspex holder. The solid curve represents the Weibull fit to the data.

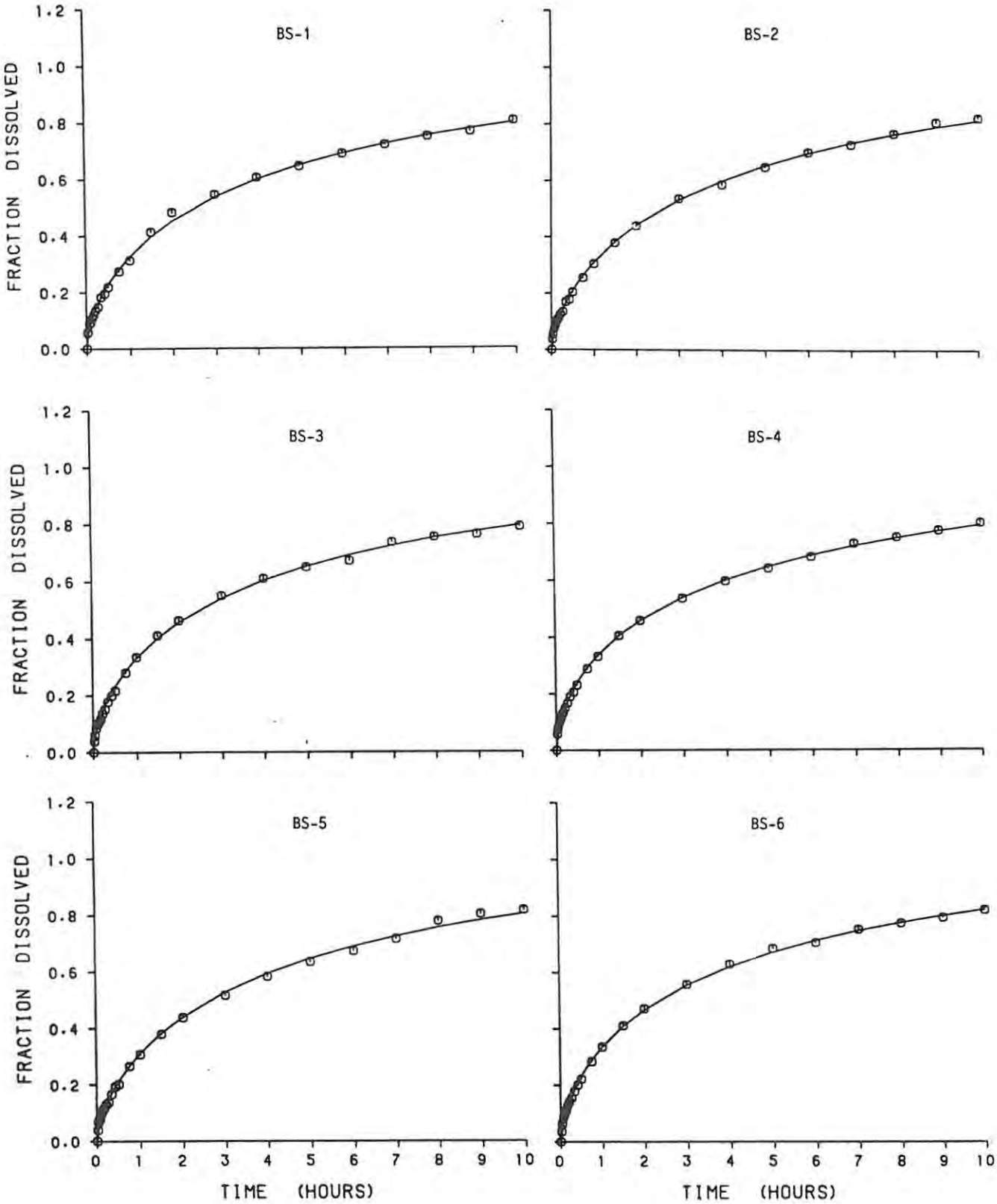


FIGURE 4.13 Dissolution profiles and Weibull function fits for Product BS using the rotating paddle apparatus with wire holder. The solid curve represents the Weibull fit to the data.

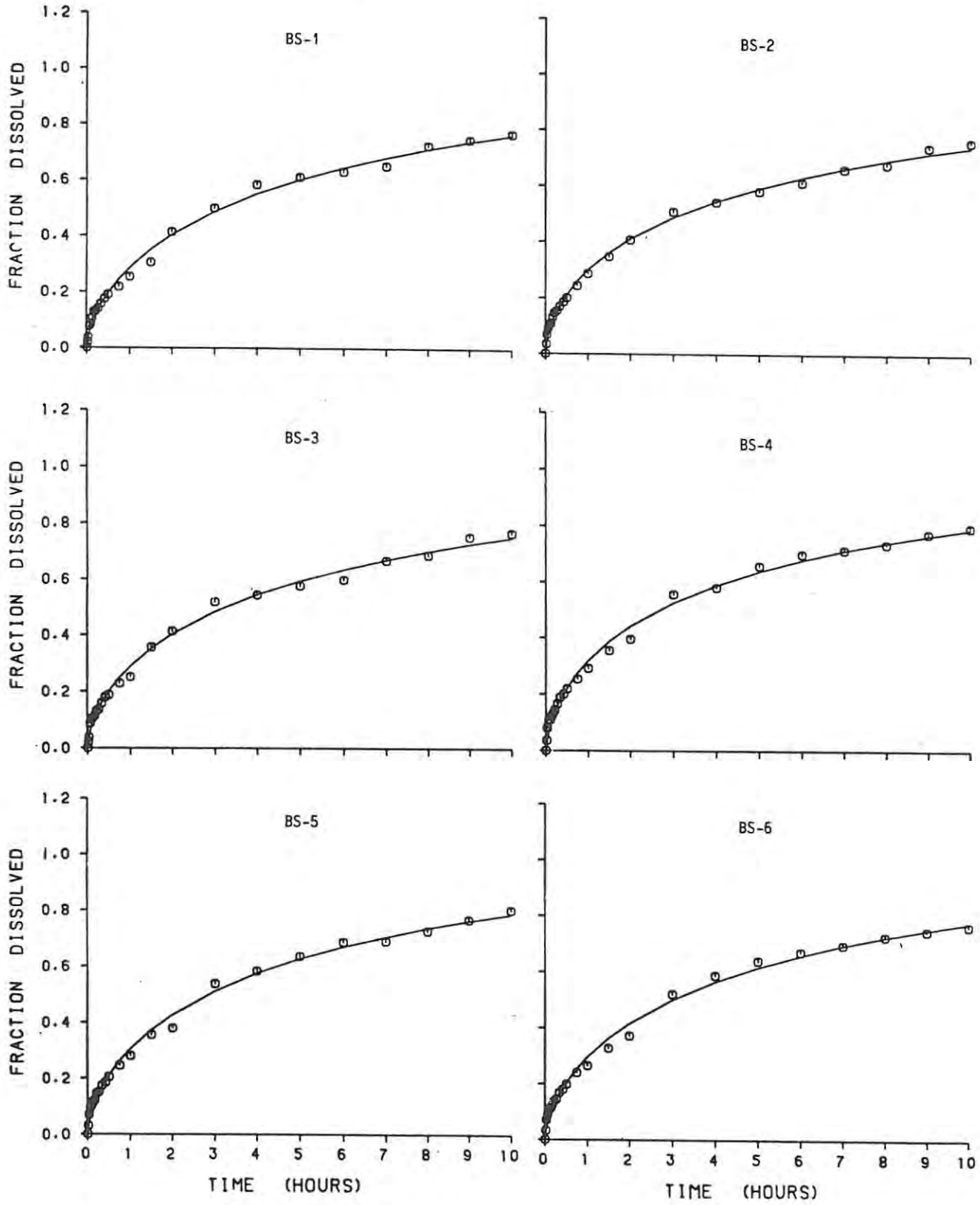


FIGURE 4.14 Dissolution profiles and Weibull function fits for Product LC using the rotating paddle apparatus with wire basket. The solid curve represents the Weibull fit to the data.

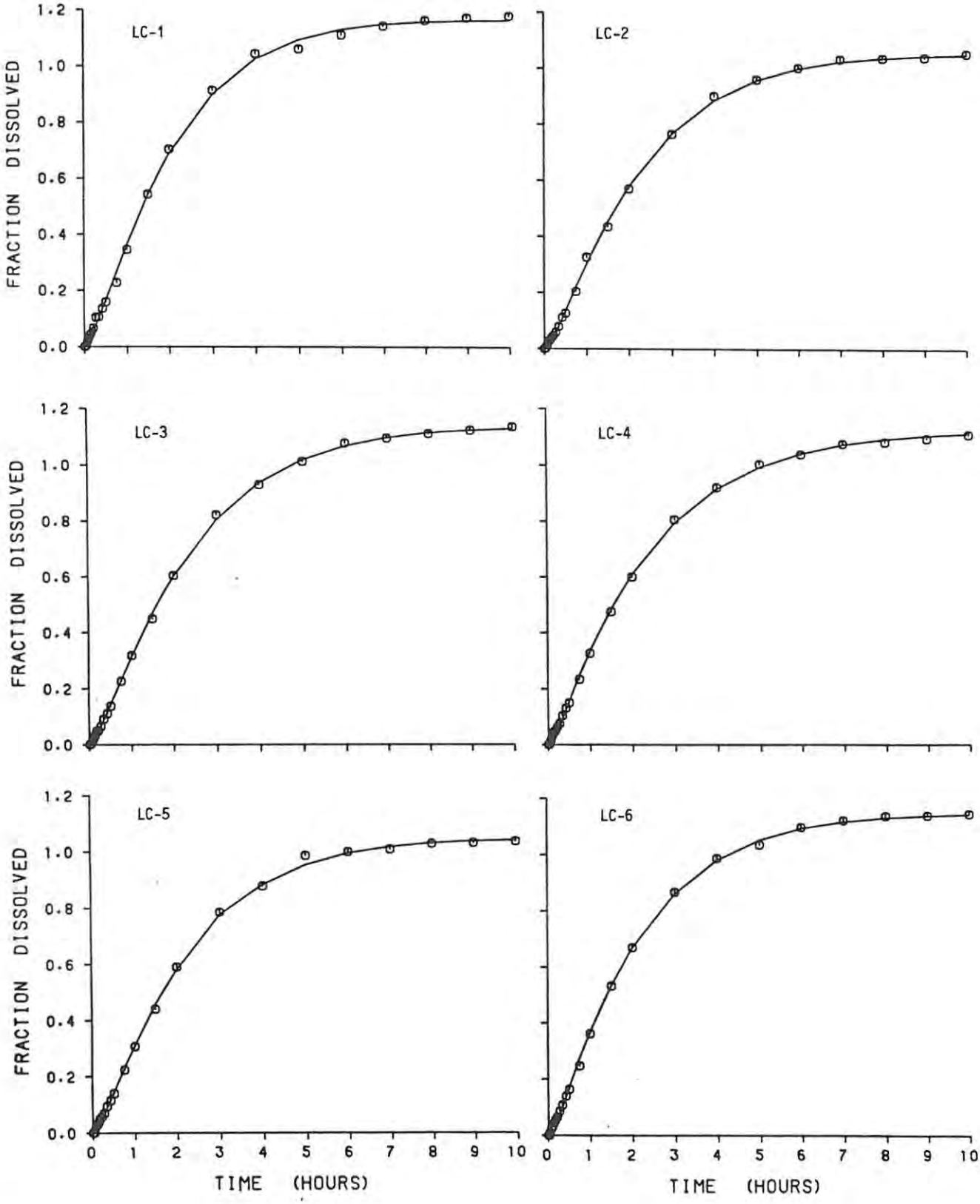


FIGURE 4.15 Dissolution profiles and Weibull function fits for Product DT using the rotating paddle apparatus with wire basket. The solid curve represents the Weibull fit to the data.

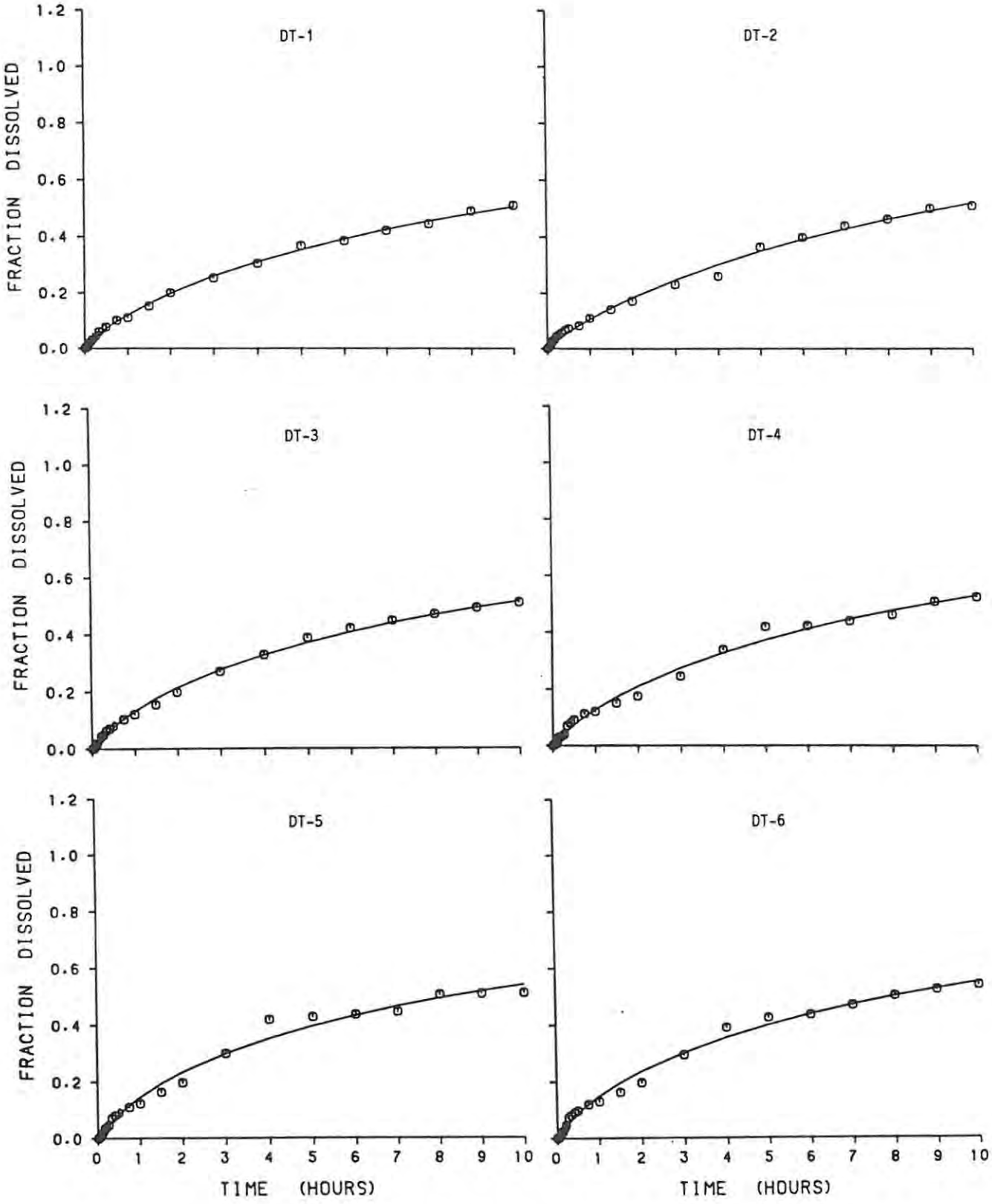


FIGURE 4.16 Dissolution profiles and weibull function fits for Product CO using the rotating paddle apparatus with wire basket. The solid curve represents the Weibull fit to the data.

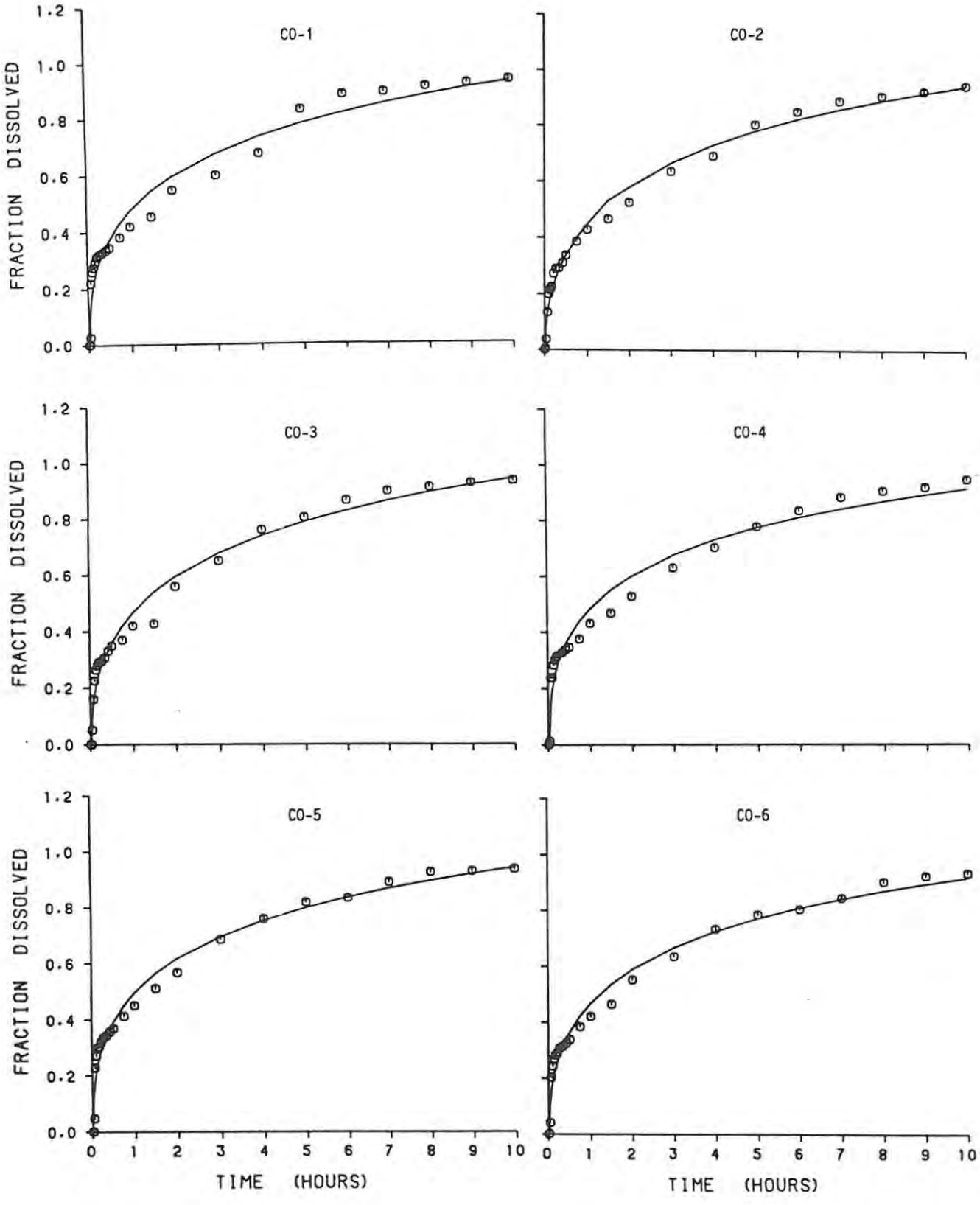


TABLE 4.1 Weibull function analysis of dissolution data for Product BS
Rotating basket apparatus used for dissolution testing

PARAMETERS	BS-1	BS-2	BS-3	BS-4	BS-5	BS-6	MEAN	CV%
t_o	0.010	0.010	0.001	0.010	0.009	0.009	0.008	44.31
t_d	4.392	5.171	4.156	4.772	4.040	4.866	4.566	9.66
β	0.586	0.622	0.598	0.594	0.590	0.594	0.597	2.14
F^∞	0.992	1.051	1.000	1.008	0.968	0.985	1.001	2.82

TABLE 4.2 Weibull function analysis of dissolution data for Product BS
Rotating paddle apparatus with perspex dosage form holder used for dissolution testing

PARAMETERS	BS-1	BS-2	BS-3	BS-4	BS-5	BS-6	MEAN	CV%
t_o	0.008	0.010	0.010	0.009	0.010	0.008	0.009	10.92
t_d	4.822	4.788	4.453	4.835	4.948	4.447	4.716	4.51
β	0.600	0.647	0.603	0.573	0.631	0.615	0.612	4.22
F^∞	1.016	1.022	0.990	1.012	1.021	1.021	1.014	1.20

TABLE 4.3 Weibull function analysis of dissolution data for Product BS
Rotating paddle apparatus with wire dosage form holder used for dissolution testing

PARAMETERS	BS-1	BS-2	BS-3	BS-4	BS-5	BS-6	MEAN	CV%
t_o	0.012	0.009	0.008	0.009	0.004	0.000	0.007	61.28
t_d	6.380	6.465	6.506	4.857	5.703	5.598	5.918	11.04
β	0.614	0.569	0.595	0.606	0.608	0.622	0.602	3.09
F^∞	1.041	1.025	1.031	1.002	1.038	1.016	1.026	1.43

TABLE 4.4 Weibull function analysis of dissolution data for Product LC
Rotating paddle apparatus with wire dosage form holder used for dissolution testing

PARAMETERS	LC-1	LC-2	LC-3	LC-4	LC-5	LC-6	MEAN	CV%
t_o	0.036	0.078	0.036	0.098	0.027	0.093	0.061	52.29
t_d	2.160	2.341	2.486	2.389	2.354	2.160	2.315	5.63
β	1.225	1.170	1.195	1.071	1.192	1.098	1.159	5.22
F^∞	1.166	1.057	1.132	1.122	1.044	1.150	1.112	4.50

TABLE 4.5 Weibull function analysis of dissolution data for Product DT
Rotating paddle apparatus with wire dosage form holder used for dissolution testing

PARAMETERS	DT-1	DT-2	DT-3	DT-4	DT-5	DT-6	MEAN	CV%
t_o	0.021	0.017	0.081	0.047	0.092	0.075	0.056	57.07
t_d	9.000	9.528	8.301	9.027	7.609	9.000	8.744	7.77
β	0.793	0.865	0.756	0.783	0.739	0.719	0.776	6.64
F^∞	0.760	0.802	0.756	0.799	0.767	0.835	0.787	3.92

TABLE 4.6 Weibull function analysis of dissolution data for Product CO
Rotating paddle apparatus with wire dosage form holders used for dissolution testing

PARAMETERS	CO-1	CO-2	CO-3	CO-4	CO-5	CO-6	MEAN	CV%
t_o	0.022	0.017	0.019	0.033	0.011	0.017	0.020	36.94
t_d	7.939	7.204	7.353	7.227	7.030	7.491	7.374	4.30
β	0.420	0.469	0.446	0.401	0.412	0.425	0.429	5.77
F^∞	1.399	1.374	1.393	1.339	1.379	1.358	1.374	1.63

initiated. Values of t_o for the other two products ranged between the above values.

TABLE 4.7 Weibull function analysis of dissolution data
Means of the fitted parameters for the products are shown

PARAMETERS	BS[2]	BS[3]	BS[4]	LC	DT	CO
t_o	0.008 (44%)[1]	0.009 (11%)	0.007 (61%)	0.061 (52%)	0.056 (57%)	0.020 (37%)
t_d	4.566 (10%)	4.716 (5%)	5.918 (11%)	2.315 (6%)	8.744 (8%)	7.374 (4%)
β	0.597 (2%)	0.612 (4%)	0.602 (3%)	1.159 (5%)	0.776 (7%)	0.429 (6%)
F^{∞}	1.001 (3%)	1.014 (1%)	1.026 (1%)	1.112 (5%)	0.787 (4%)	1.374 (2%)

1 Interindividual differences expressed as coefficients of variation.
2 Rotating basket apparatus used for dissolution testing.
3 Rotating paddle apparatus with perspex dosage form holder used for dissolution testing.
4 Rotating paddle apparatus with wire dosage form holder used for dissolution testing.

Product LC exhibited the fastest mean dissolution time, t_d, requiring only 2.3 hours for 63% dissolution. The effect of change in the apparatus on the t_d values of Product BS can be seen. Using the rotating paddle apparatus with the wire dosage form holder, an extra hour was required for 63% of the drug to be released.

The values for the beta exponents are concordant with the slopes and shapes of the curves. Product LC, with β greater than 1, and a sigmoidal cumulative curve, exhibited a slower initial rate of release of PPA, followed by an accelerated release up to the plateau. As β decreased from Product DT to BS to CO, a steeper initial slope resulted, followed by a flattened tail in the final part. The F^{∞} values give some indication of the total amount of drug dissolved, with Product DT showing a decreased release.

CHAPTER 5

THE *IN VIVO* EVALUATION OF PHENYLPROPANOLAMINE IN DIFFERENT DOSAGE FORMS

In vivo trials using human volunteers were undertaken to enable the elucidation of the pharmacokinetic parameters of PPA.HCl and compare the bioavailability of two sustained-release formulations containing PPA.HCl with that of the pure powder.

5.1 PILOT TRIAL

A pilot trial using one volunteer was conducted to establish various parameters such as frequency and times of sampling, PPA concentrations which could be expected in serum and urine, the concentration of heparin in saline necessary for prevention of clotting of the blood in the needle and butterfly sets and the necessity for monitoring blood pressure.

The volunteer, a healthy 24 year old male weighing 82 kg was a non-smoker, a moderate drinker and had no previous history of hypertension. Blood and urine samples collected prior to the trial were subjected to clinical pathology tests and were found to be normal. The volunteer received a typed copy of trial protocol and signed a consent form. He received an honorarium for participating in the trial and practised the same standardization procedures as described in section 5.2.3.

The test dose consisted of 50 mg PPA.HCl dissolved in 200 ml water ingested at 0, 4 and 8 hours. Blood was drawn prior to the trial for the blank and at 0.25, 0.5, 1, 1.75, 2, 3, 4, 5, 8, 9, 12 and 24 hours after ingestion of the initial dose of the drug. Urine was collected before the trial for the blank and at 2, 4, 6, 8, 12 and 24 hours. Urine voided at times other than those specified above was collected and the times and volumes recorded. Blood pressure readings were taken at 0, 4, 9, 12 and 24 hours after ingestion of the test dose.

The insertion of the needle and subsequent withdrawal of blood samples in the early stages of the trial presented no problems, but between 1 and 1.5 hours clotting occurred in the butterfly set. Additional flushing cleared the clot and thereafter the butterfly was flushed every 30 minutes. The flushing solution was a 10 u/ml heparin solution in normal saline and approximately 0.5 ml was used each time. The concentration of this solution was increased to 50 u/ml for further trials.

The blood pressure readings seemed to indicate an increasing trend after ingestion of the drug, so blood pressure was monitored in subsequent trials to enable early detection of any possibly dangerous rise in blood pressure. Extra blood sampling times were introduced to improve characterisation of the serum curve, one at 6 hours and another at 10 hours which yielded extra information concerning peak concentrations of PPA.

5.2 CLINICAL TRIALS

5.2.1 Volunteers

Six normal, healthy adults who were non-smokers and moderate drinkers participated in the trial. Hypertension, heart disease, asthma, diabetes mellitus and treatment with antidepressant drugs were criteria for exclusion from the trials. Individual details of the volunteers are given in Table 5.1.

Volunteer No.	Code name	Age	Body mass (kg)	Sex
1	JM	21	67	male
2	MK	23	77	male
3	DD	20	71	male
4	PS	21	79	male
5	MM	21	54	female
6	SR	28	62	male

TABLE 5.1 Details of volunteers

Volunteers were chosen on the basis of interview, physical health and appropriate clinical pathology laboratory tests. The tests included the following:

Haematology: haemaglobin, haematocrit, WBC, platelet count, differential count, RBC.

Blood chemistry: serum creatinine, serum urea, blood sugar, serum bilirubin, serum alkaline phosphatase, SGPT and SGOT.

Urinalysis: specific gravity, bile, pH, protein, glucose, RBC, WBC, epithelial cells, granular casts.

All the volunteers received a typed copy of the protocol and signed a consent form. Volunteers were paid an honorarium for participating in the trial. A copy of the consent form can be found in Appendix 2.

5.2.2 Treatments

Trial 1 - 50 mg pure PPA.HCl powder dissolved in 200 ml water was ingested at 0 hours.

Trial 2 - 100 mg pure PPA.HCL powder dissolved in 200 ml water was ingested at 0 hours

Trial 3 - a sustained-release tablet containing 150 mg PPA.HCl (Product BS) was ingested at 0 hours.

Trial 4 - A sustained-release capsule containing 75 mg PPA.HCl (Product DT) was ingested at 0 hours.

Trial 5 - 50 mg pure PPA.HCl powder dissolved in 200 ml water was administered at 0, 4 and 8 hours.

5.2.3 Standardization Procedure

All volunteers had to conform to the following restrictions:

(a) No drugs, including over-the-counter preparations, were allowed for at least a week before the trial and for the duration of the trial.

(b) No alcohol was to be consumed for at least 48 hours before the trial and for the duration of the trial.

(c) No caffeine-containing food or drinks were to be ingested for at least 48 hours before the trial and for the duration of the trial. This included coffee, tea, chocolate and cola drinks.

(d) No food or drink was to be ingested for 10 hours before the start of the trial up until the serving of a standardized breakfast during the trial.

The standardized breakfast consisted of toast with margarine and jam and 250 ml orange juice. The same lunch was given to all volunteers during the trials. Volunteers were allowed to move about freely during the trial and were encouraged to resume normal activities, but had to refrain from engaging in any strenuous activities.

5.2.4 Sampling Schedules for the Trials

The sampling schedules for the trials are given in Table 5.2.

5.2.5 Collection and Storage of Blood Samples

An indwelling 0.8 mm butterfly catheter (21G, Medispo (Pty) Ltd.,Industria, S.A.) was inserted into a suitable vein in the forearm and securely strapped into position with Micropore surgical tape (3M Medical Products Division, JHB,S.A.) to allow complete mobility of the arm.

A 10 ml blood sample was withdrawn from the butterfly through a sterile hypodermic needle (0.8 x 40LB, Medispo (Pty) Ltd., S.A.) by syringe aspiration. The butterfly was then flushed with about 1 ml of sterile saline solution containing heparin (50 u/ml).

Immediately prior to the withdrawal of a further blood sample, the butterfly was cleared of the heparin solution by the withdrawal of about 1 ml of blood which was discarded. In a fresh syringe, 10 ml of blood was collected and transferred to a labelled Vacutainer tube. The tube was stoppered and allowed to stand for the blood to clot. Samples were centrifuged at 3000 rpm for 10 minutes and the upper layer (serum) transferred to a clean, labelled Vacutainer tube. In a few cases the serum contained fibrin clots, which were squeezed with the aid of an orange stick to free the serum. These samples were then recentrifuged to separate the serum. The serum samples were stored at -20°C for a maximum of 4 weeks until

Table 5.2 Sampling Schedule for trials 1 - 5

SAMPLING INTERVAL	TIME	TRIAL [3] 1		TRIAL 2		TRIAL 3		TRIAL 4		TRIAL 5	
		BLOOD	URINE	BLOOD	URINE	BLOOD	URINE	BLOOD	URINE	BLOOD	URINE
-20 min		B	U	B	U	B	U	B	U	B	U
0 [1,2]	8.00 am										
10 min	8.10 am	B									
15 min	8.15 am			B							
20 min	8.20 am	B									
30 min	8.30 am	B		B		B		B		B	
40 min	8.40 am	B									
45 min	8.45 am			B							
50 min	8.50 am	B									
1 hr	9.00 am	B		B	U	B		B		B	
1½ hr	9.30 am	B		B		B		B		B	
2 hr	10.00 am	B		B	U	B	U	B	U	B	U
B R E A K F A S T											
2½ hr	10.30 am			B							
3 hr	11.00 am	B		B	U	B		B		B	
4 hr [2]	12 noon	B		B		B	U	B	U	[1] B	U
5 hr	1.00 pm				U	B		B		B	
L U N C H											
6 hr	2.00 pm	B		B		B	U	B	U	B	U
7 hr	3.00 pm				U			B			
8 hr [2]	4.00 pm	B		B		B	U	B	U	[1] B	U
9 hr	5.00 pm				U	B		B		B	
10 hr	6.00 pm	B		B		B		B	U	B	
11 hr	7.00 pm				U						
S U P P E R											
12 hr	8.00 pm	B		B		B	U	B	U	B	U
13 hr	9.00 pm				U						
14 hr	10.00 pm	B		B				B			
15 hr	11.00 pm				U						
16 hr	12 midnight	B									
18 hr	2.00 am	B									
24 hr	8.00 am	B		B	U	B	U	B	U	B	U

1 Test dose ingested at these times

2 Blood pressure readings were taken at these times

3 Urine was collected in this trial over 48 hours although no times were specified for collection Exact times and volume of urine voided were accurately recorded

analysis. Spiked serum samples were stored for up to 6 months and tested periodically to ascertain PPA content and determine stability on storage.

5.2.6 Collection and Storage of Urine Samples

The collection and accurate recording of details were the responsibility of each volunteer and were not subject to the same rigid control as the collection of blood samples. The importance of strict adherence to sampling times and the accurate recording of volume voided was thus stressed verbally and in the protocol.

Volunteers were asked, for each sampling time, to void the urine into large collection jars, emptying the bladder completely each time. The total volume of urine was measured and a representative sample of about 40 ml transferred to a labelled, glass, screw-capped bottle and the rest of the urine was discarded. Urine samples were frozen immediately after collection and stored at -20°C for a maximum of 4 weeks before analysis. Spiked urine samples were kept for up to 6 months and assessed throughout that time for PPA content to determine stability of the drug in urine on storage.

Urine voided at times other than those specified in the protocol were collected in the same way as above. The exact time of voiding was recorded as well as total urine volume.

5.3. ANALYSIS OF BIOLOGICAL SAMPLES

5.3.1 The Analysis of Serum Samples

Serum samples were analyzed in randomized order to avoid sequential effects. They were brought to room temperature and mixed thoroughly on a vortex mixer before being analysed. The method of sample preparation and chromatographic conditions are described in section 3.3. Samples were analysed in duplicate and between 15 and 30 µl of the final extract was injected onto the column.

5.3.2 The Analysis of Urine Samples

Urine samples were brought to room temperature and the bottle shaken thoroughly to mix the urine. Each urine sample was analysed in duplicate and in random order to avoid sequential effects. Samples were extracted in preparation for chromatography according to the method described in section 3.5 and 1 to 10 µl of the final extract was injected onto the column.

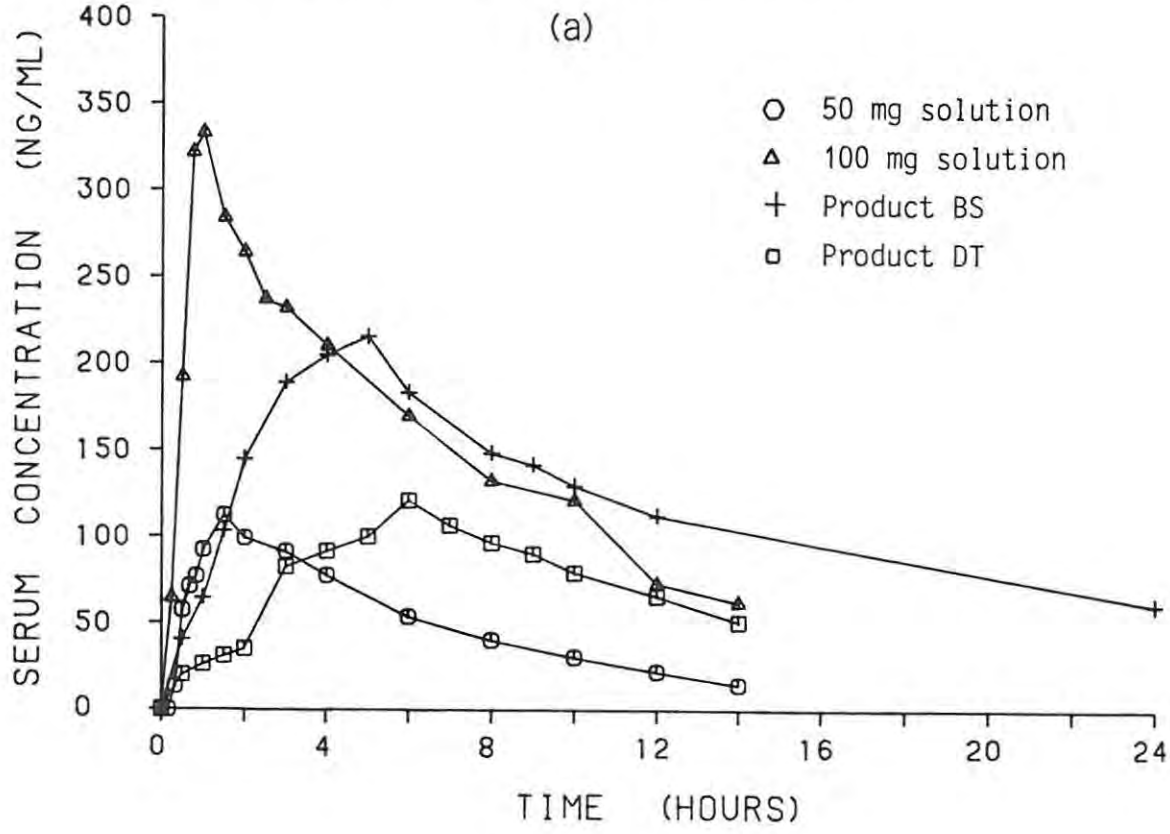
5.4 RESULTS AND DISCUSSION

The serum and urine concentrations from trials 1-5 are presented in Tables A3.1 - A3.10 and the curves from trials 1-4 depicted in Figs.5.1 - 5.6. The mean serum concentration and urinary excretion profiles from 6 subjects after the ingestion of a 50 mg PPA.HCl solution at 0, 4 and 8 hours are shown in Fig.5.7. Each reading represents the average of duplicate determinations.

In the 50 mg single dose solution study, absorption of the drug was rapid with peak blood concentrations occurring 50 minutes to 2 hours after ingestion of the solution and the concentrations ranging between 110 and 185 ng/ml of serum. In a study reported by Mason and Amick (207), the mean peak level occurred 2 hours after ingestion of a 25 mg PPA.HCl solution. Subject DD exhibited an extremely rapid absorption rate with a serum concentration of 105 ng/ml after 20 minutes, whereas the mean concentration of the other 5 subjects was 30 ng/ml at the same time. This discrepancy could only be attributed to individual variability. After 14 hours, serum concentrations of PPA could not be accurately quantitated since they were found to be below 10 ng/ml.

Three subjects, JM, MK and SR participated in the 100 mg PPA.HCl solution study. Peak concentrations occurred 1 to 2 hours after ingestion and ranged between 250 and 420 ng/ml. Subject JM exhibited a more rapid absorption rate than in the 50 mg solution study, with the time of peak concentration differing by 1 hour. Conversely, absorption was slower by 1 hour for subject SR during this trial.

FIGURE 5.1 Serum concentrations (a) and urinary excretion profiles (b) from Subject JM after the ingestion of 4 test doses of phenylpropanolamine



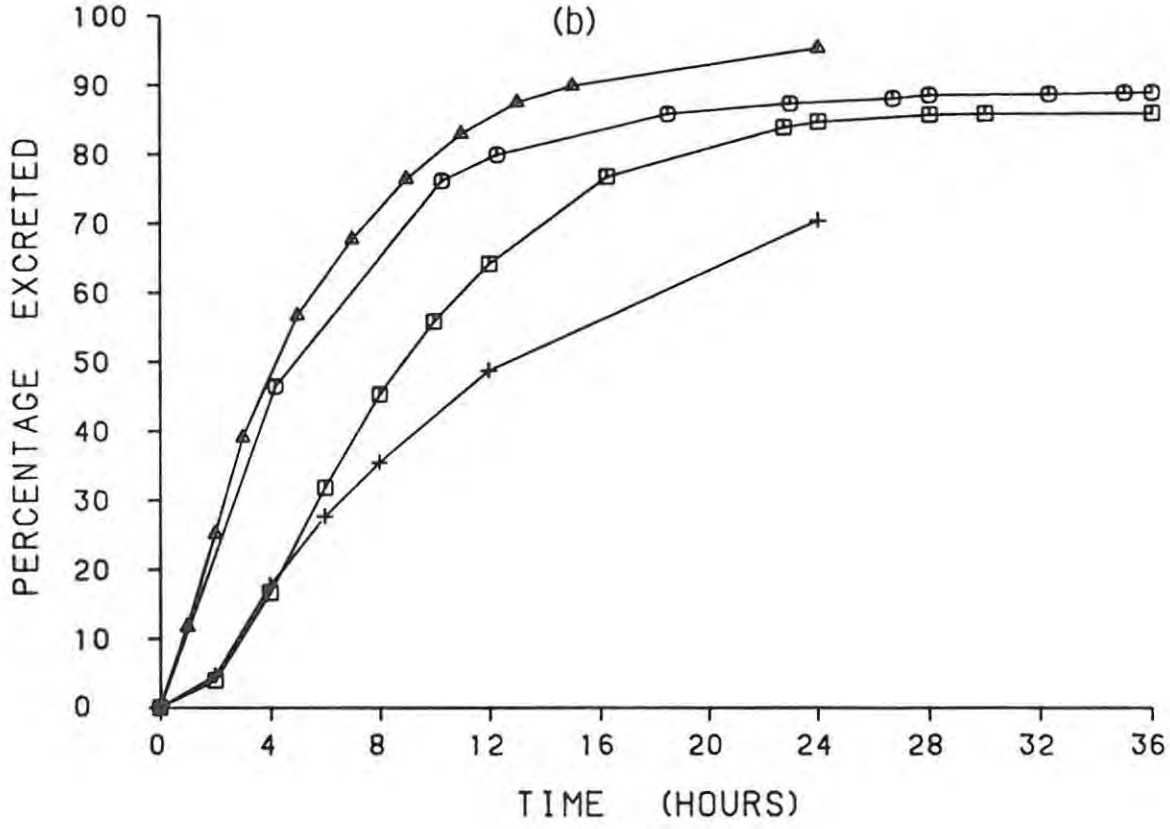
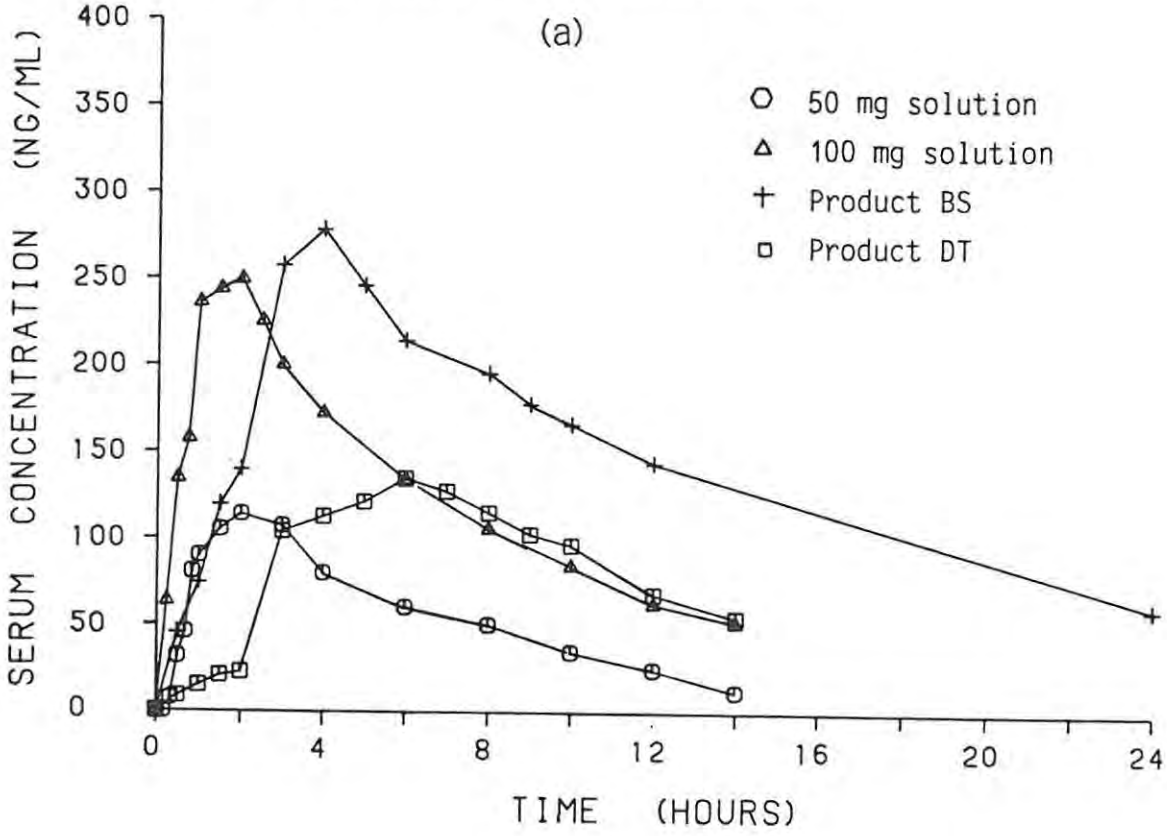


FIGURE 5.2 Serum concentrations (a) and urinary excretion profiles (b) from Subject MK after the ingestion of 4 test doses of phenylpropanolamine



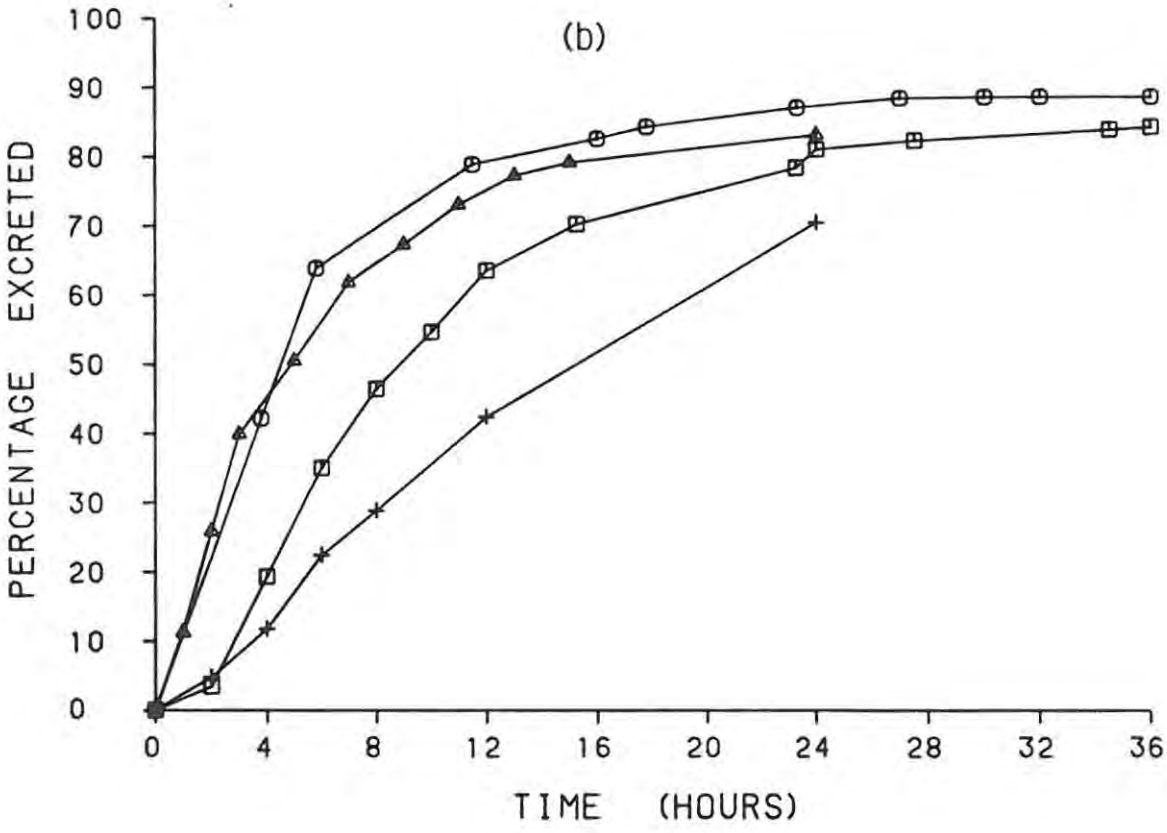


FIGURE 5.3 Serum concentrations (a) and urinary excretion profiles (b) from Subject SR after the ingestion of 4 test doses of phenylpropanolamine

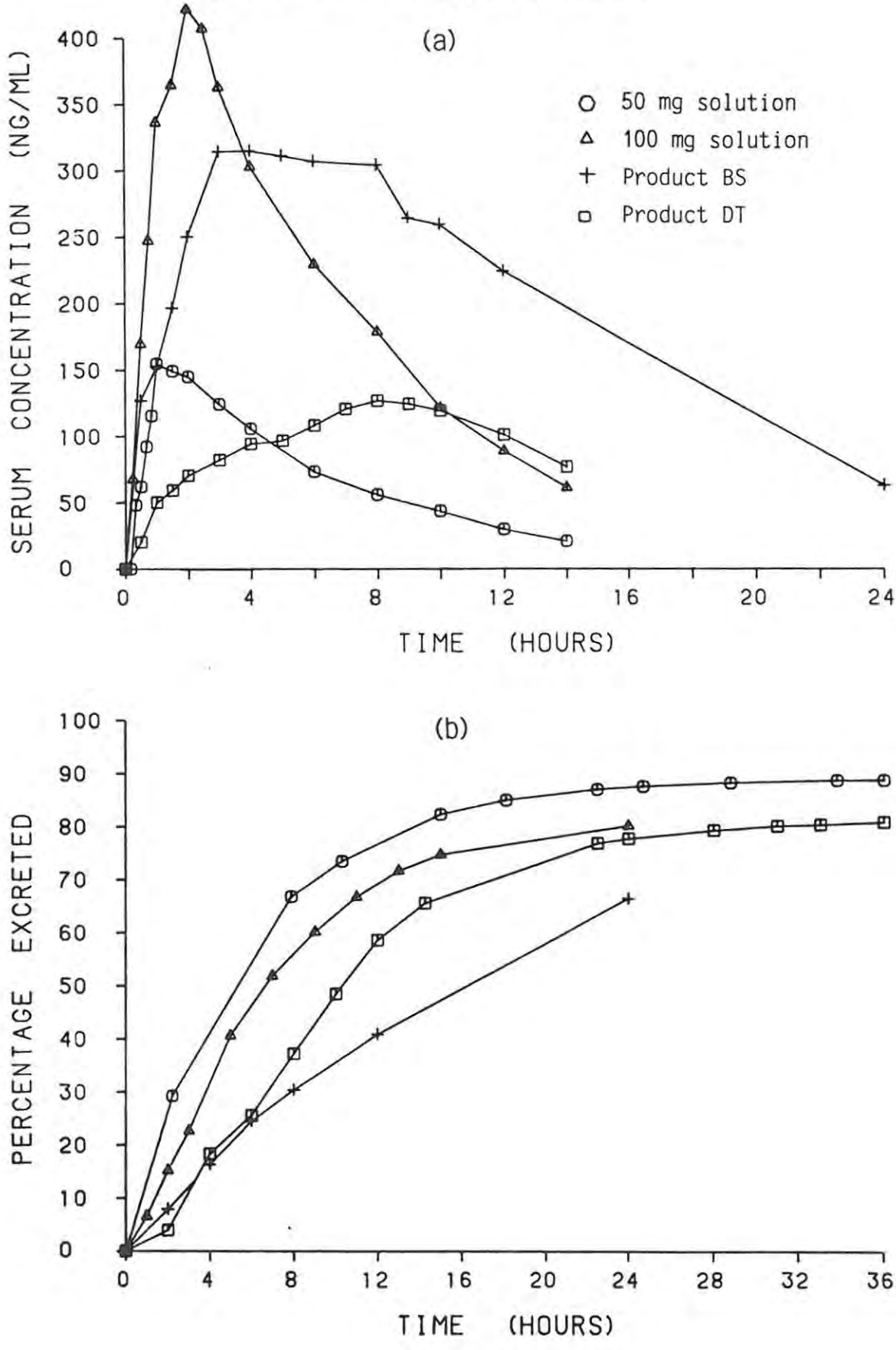
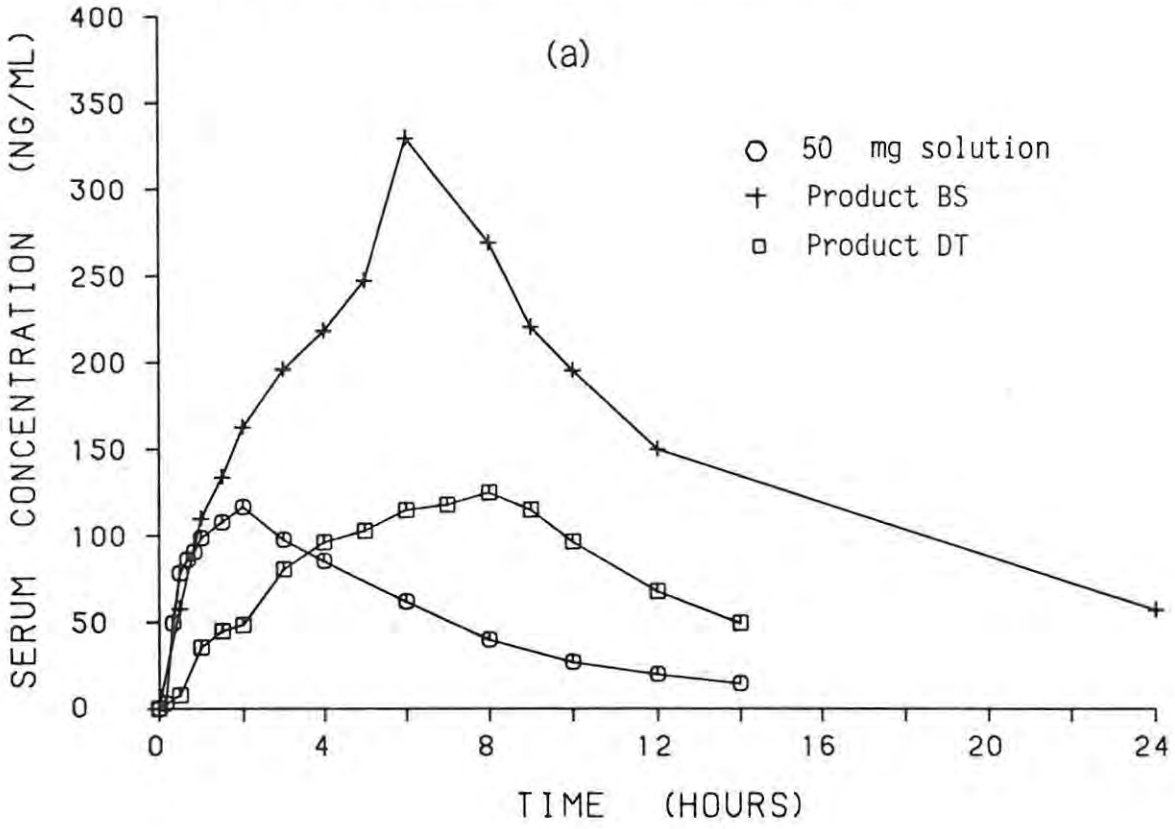


FIGURE 5.4 Serum concentrations (a) and urinary excretion profiles (b) from Subject PS after the ingestion of 3 test doses of phenylpropanolamine



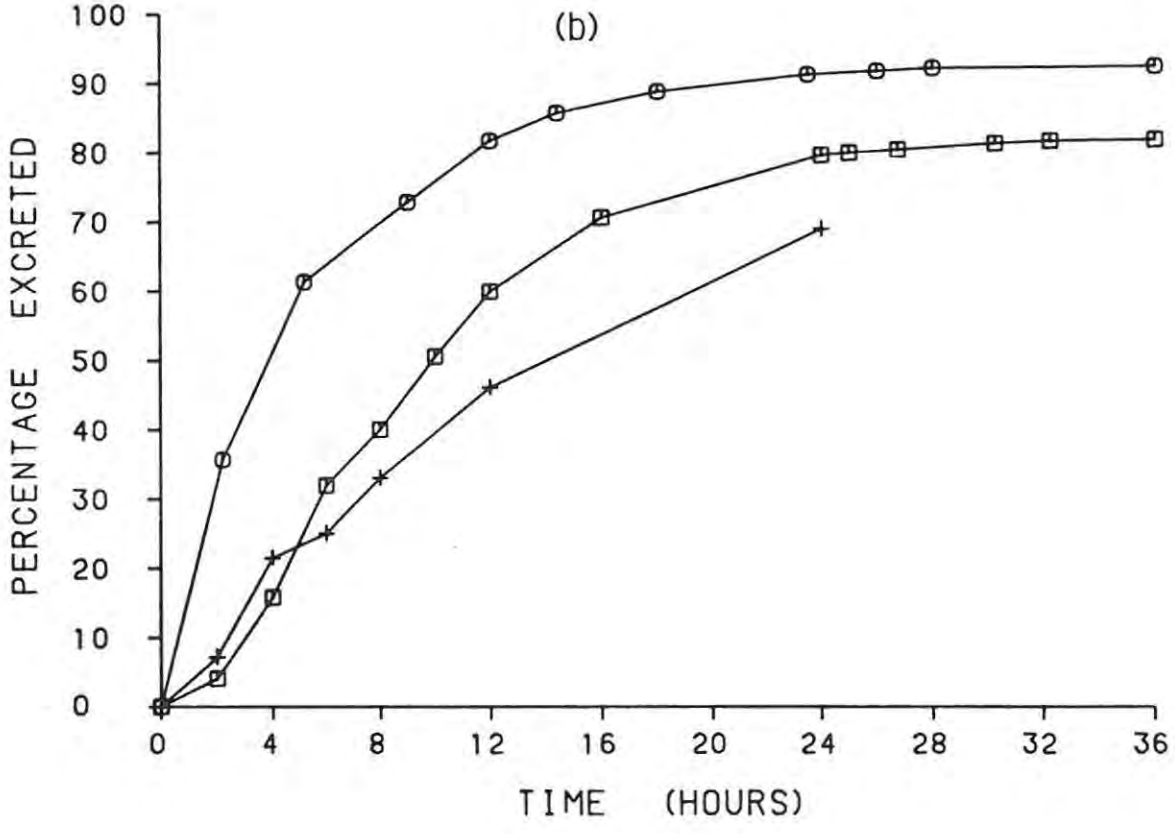


FIGURE 5.5 Serum concentrations (a) and urinary excretion profiles (b) from Subject DD after the ingestion of 3 test doses of phenylpropanolamine

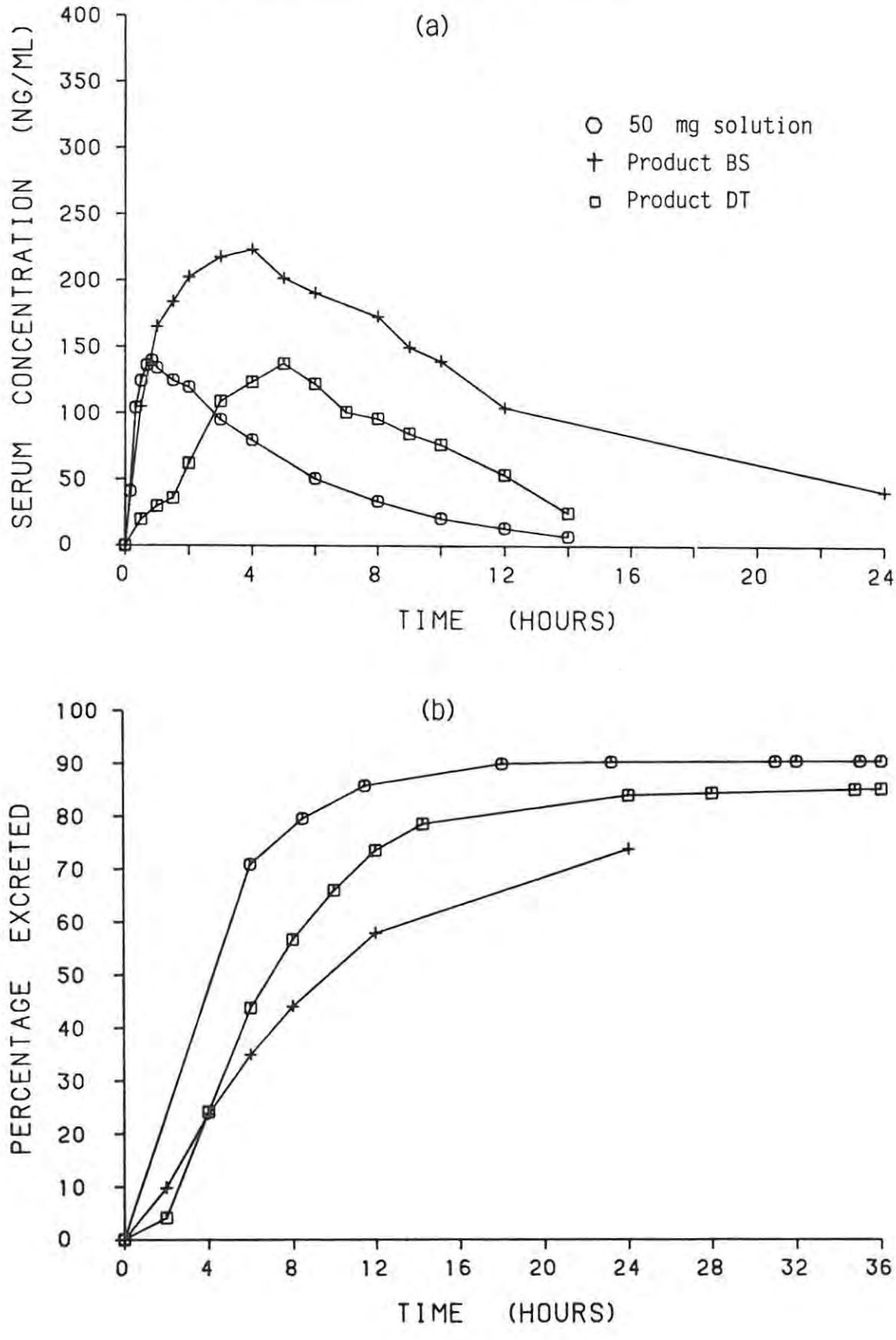
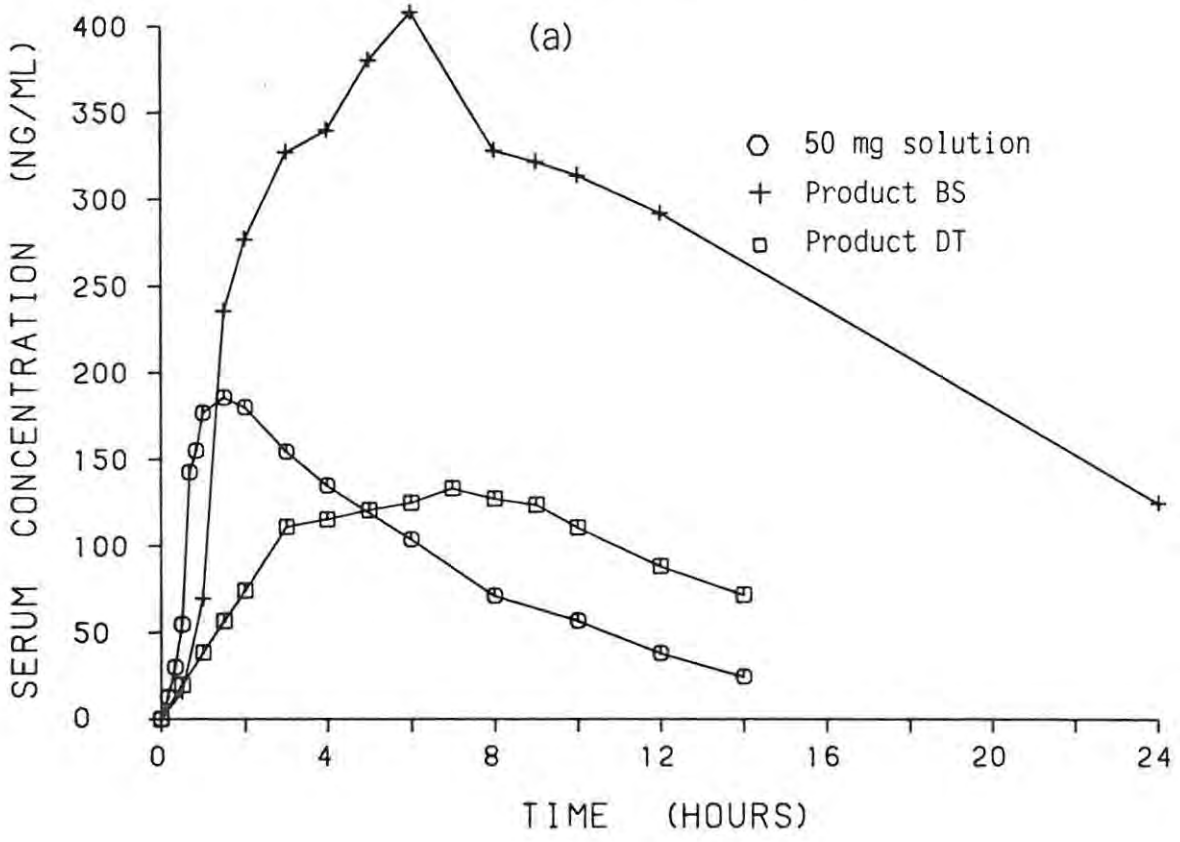


FIGURE 5.6 Serum concentrations (a) and urinary excretion profiles (b) from Subject MM after the ingestion of 3 test doses of phenylpropanolamine



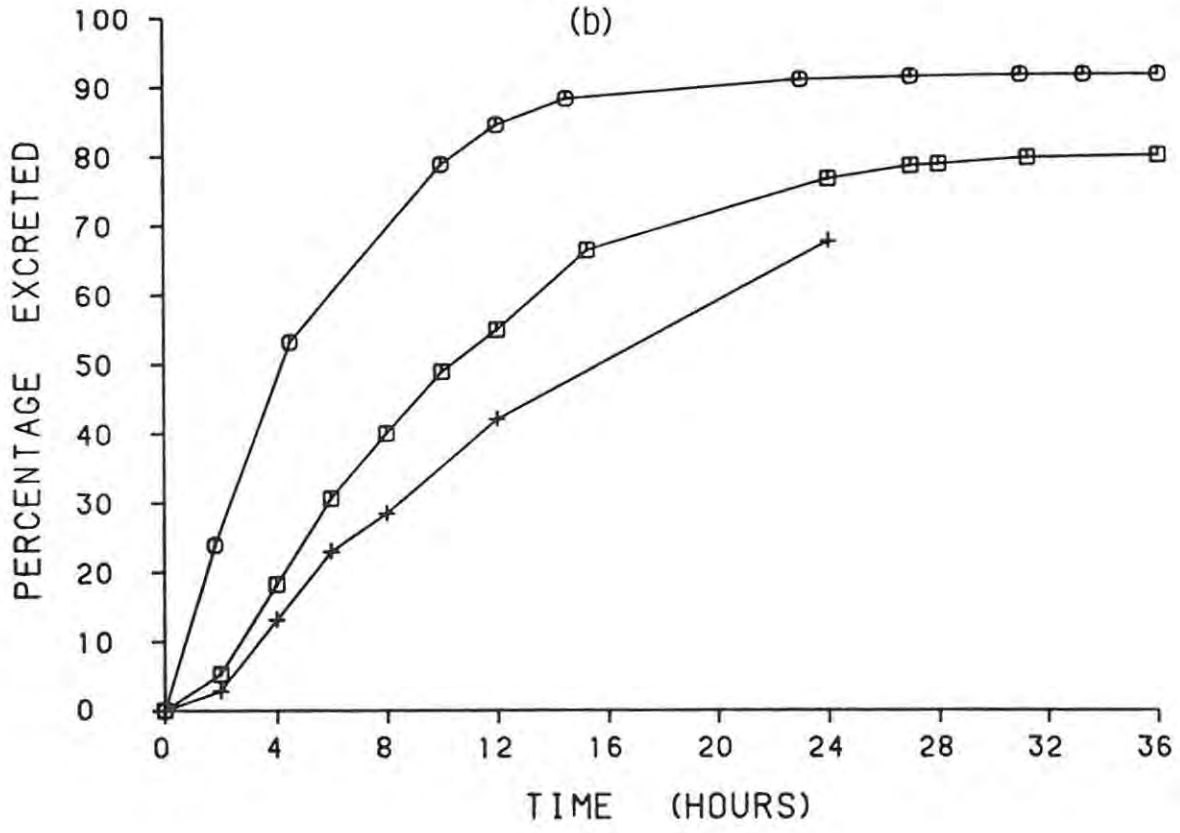
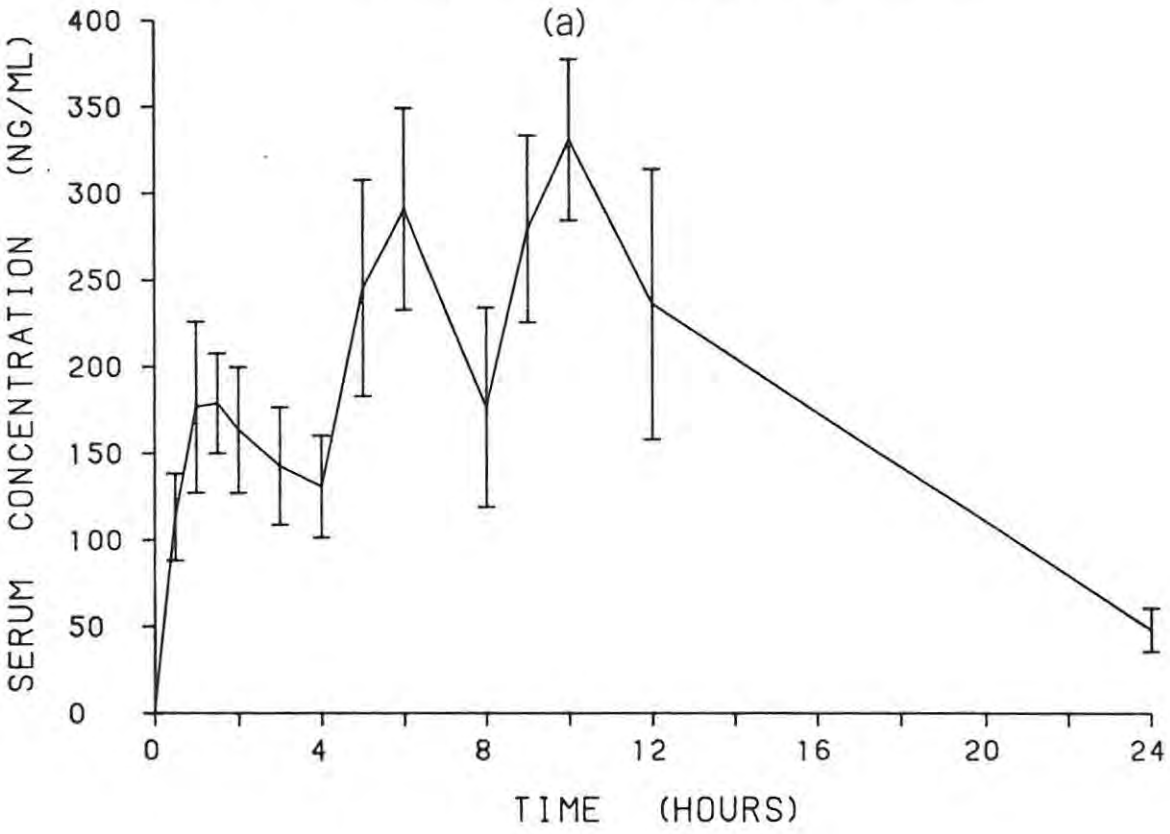
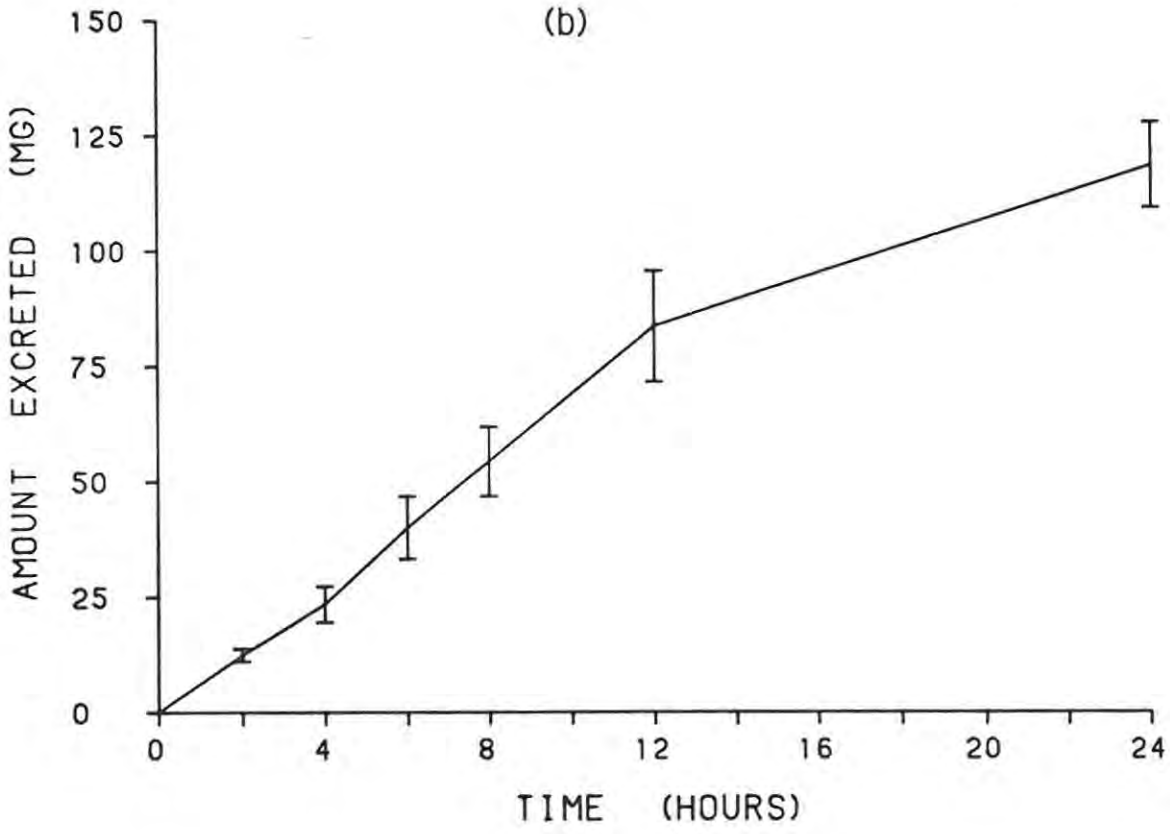


FIGURE 5.7 Mean serum concentrations (a) and urinary excretion profiles (b) ± SD of 6 subjects after the ingestion of a 50 mg PPA.HCl solution at 0, 4 and 8 hours.





Product BS. Recovery could have been greater if urine had been collected for a longer time as PPA was still clearly detectable in the blood after 24 hours. Heimlich (25), after testing two spansule formulations containing 150 mg PPA.HCl reported recoveries after 24 hours of between 64% and 92% for one formulation and between 36% and 61% for a slower-release formulation. These values increased after 36 hours to between 82% and 96% recovery for the former and between 49% and 70% recovery for the latter.

Recovery of PPA from the urine after ingestion of Product DT was monitored for 36 hours with between 80% and 86% of the unchanged drug being recovered. Larger discrepancies were encountered in the percentage of PPA excreted after ingestion of the solution at 0, 4 and 8 hours. 69% - 86% recovery was found after 24 hours and this correlates with literature values (23,24). Heimlich (25) reported 69 - 94% recovery from the urine after 24 hours following a similar dosage regimen of PPA.HCl.

Limited facilities restricted the size and scope of the trials undertaken. Availability of volunteers constituted a further problem as only non-smokers were used. Ages of the volunteers varied between 20 and 28 years and weights ranged from 54 to 80 kg. Blood pressure was monitored throughout the trials. No general trends were observed, although some volunteers did show a slight increase in systolic and diastolic blood pressure during the trials. However, no significant rises in blood pressure were encountered.

The method of blood withdrawal using butterfly sets was convenient as relatively little pain or trauma occurred on the initial insertion of the needle and after securing the butterfly with tape, the arm could be moved freely. Less trauma is associated with this method than with multiple venopuncture.

HPLC analysis of the samples provided a rapid, accurate method for determining the concentration of PPA in serum and urine. THe

extraction was relatively simple with the back-extraction step eliminating the need for evaporation of the organic solvent, thus decreasing total sample preparation time significantly. Reproducibility of the method was monitored by the preparation and injection of standards during each run.

CHAPTER 6

PHARMACOKINETIC ANALYSIS OF SOLUTION DATA

6.1 INTRODUCTION

The originator of pharmacokinetics is considered to be Torsten Teorrell, a physiologist and medical biophysicist (331,332). The word "pharmacokinetics" means the application of kinetics to *pharmacon*, the Greek word for drugs and poisons and was defined as "the science of the quantitative analysis between organism and drug". Pharmacokinetics is the study of the time course of drug and metabolite concentrations in biological fluids, tissues and excreta and of the mathematical relationships required to develop models to interpret such data (333).

The problem of finding an adequate method to interpret *in vivo* data frequently leads to the use of compartment models. These pharmacokinetic compartment models are only an approximation of a biological system and reflect reality in a simplified way, for they characterise only the rate limiting steps out of the many processes occurring during a drug-biological system interaction. The model is the equation or set of equations used to describe and interpret data obtained by experimentation. Appropriately chosen models permit simulations and prediction of pharmacokinetic data.

The behaviour of a drug in a biological system can usually be described in terms of a compartmental model, either a one, two or multi-compartmental model. These classical pharmacokinetic models are based upon linear systems of differential equations, kinetic linearity being defined as direct proportionality of transfer rates to either drug concentrations or concentration differences. Kinetic linearity also implies that the elimination of the drug obeys first order kinetics. In a linear pharmacokinetic system, the total area under the plasma concentration-time curve should be a linear function of the dose administered. The assumption is also made that the concentration of drug in each tissue at a given time

after intravenous injection is a linear function of dose and that tissue concentrations are far below the saturation level.

6.2 LINEAR COMPARTMENT MODELS

6.2.1 The One Compartment Model

The one compartment model considers the body being represented by a single compartment with volume, V_1. This model is used for drugs which distribute rapidly between the blood and other body fluids or tissues upon entry into the body. Changes in the plasma concentration are therefore assumed to quantitatively reflect changes occurring in tissue drug levels and loss from the body is assumed to be first order. Drug elimination can occur by many processes including renal and biliary pathways, biotransformation and excretion in expired air with the apparent first order elimination rate constant being the sum of the rate constants of a number of individual processes.

Definition of symbols:

- D = dose of the drug
- C_p = plasma concentration at time t
- C_o = plasma concentration at time t = 0, $C_o = D/V$
- V_1 = volume of the body
- k_{el} = first order elimination rate constant
- A_b = amount of drug in the body at time t
- A_o = amount of drug in the body at time t = 0, $A_o = D$
- A_a = amount of drug at the site of absorption
- k_a = first order absorption rate constant
- F = bioavailability

6.2.1.1. Intravenous Bolus Injection (Single Dose)

This is schematically represented as follows:

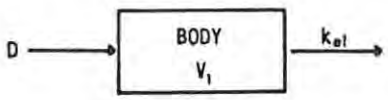


Figure 6.1 One compartment model with intravenous bolus dose

The differential equation for amount of drug in the body at time t is:

$$\frac{dA_b}{dt} = -k_{el} A_b \qquad (6\text{-}1)$$

Integrating: $A_b = A_o e^{-k_{el} t}$ (6-2)

Since $A_o = D$ and $C = A/V$

$$C = C_o e^{-k_{el} t} \qquad (6\text{-}3)$$

6.2.1.2 First Order Absorption (Single Dose)

This model is represented as follows:

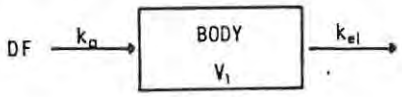


Figure 6.2 One compartment model with first order absorption.

For a drug that enters the body by an apparent first order absorption process, is eliminated by a first order process and distributes in the body according to a one compartment model the following differential equation applies:

$$\frac{dA_b}{dt} = k_a A_a - k_{el} A_b \qquad (6\text{-}4)$$

The amount of drug in the body can be obtained from:

$$A_b = \frac{k_a F D}{k_a - k_{el}} (e^{-k_{el} t} - e^{-k_a t}) \qquad (6\text{-}5)$$

6.2.2 The Two Compartment Model

Most drugs entering the body do not instantly distribute between the blood and other body fluids or tissues which they eventually reach. Body fluids or tissues which are in equilibrium with the circulatory system form the central compartment which is accessible through blood sampling. The levels of drug associated with this compartment should decline more rapidly during the distributive phase than during the post-distributive phase. In contrast, levels of drug in body fluids or tissues into which the drug distributes slowly (peripheral compartment) will increase to

a maximum and then begin to decline during the distributive phase until eventually a steady state will be reached which terminates the distribution phase.

There are 3 possible types of two compartment models;

(a) the one in which elimination occurs only from the central compartment ,

(b) the one in which elimination occurs only from the peripheral compartment and

(c) the one in which elimination occurs from both the central and peripheral compartments.

Only model (a) will be considered here.

Definition of terms:

k_{12} = first order rate constant for transfer of drug from central compartment to peripheral compartment 2

k_{21} = first order rate constant for transfer of drug from peripheral compartment to central compartment

k_{10} = first order rate constant for elimination of drug by all processes from central compartment

A_1 = amount of drug in central compartment

A_2 = amount of drug in peripheral compartment

CC = central compartment

PC = peripheral compartment

6.2.2.1 Intravenous Bolus Injection (Single Dose)

The two compartment model is shown schematically in Fig.6.3.

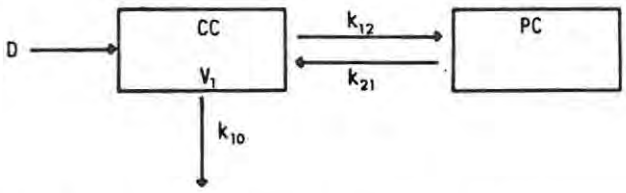


Figure 6.3 Two compartment model with intravenous bolus dose

The rate of change in the amount of drug in the central compartment following rapid intravenous injection of a drug that distributes in the body according to a two compartment model with

elimination occurring from the central compartment, can be described by the following differential equation:

$$\frac{dA_1}{dt} = k_{21}A_2 - k_{12}A_1 - k_{10}A_1 \qquad (6\text{-}6)$$

The differential equation describing rate of change in the amount of drug in the peripheral compartment is:

$$\frac{dA_2}{dt} = k_{12}A_1 - k_{21}A_2 \qquad (6\text{-}7)$$

6.2.2.2 First Order Absorption (Single Dose)

Schematic representation can be seen in Fig.6.4.

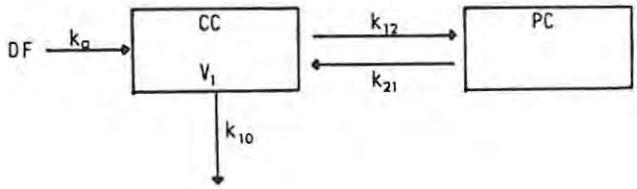


Figure 6.4 Two compartment model with first order absorption

The rate of change in amount of drug in the central compartment following oral administration of the drug can be described by the following differential equation:

$$\frac{dA_1}{dt} = k_aFDe^{-k_at} + k_{21}A_2 - k_{12}A_1 - k_{10}A_1 \qquad (6\text{-}8)$$

and the differential equation describing rate of change in the amount of drug in the peripheral compartment is:

$$\frac{dA_2}{dt} = k_{12}A_1 - k_{21}A_2 \qquad (6\text{-}9)$$

6.3 BIOAVAILABILITY

Bioavailability is defined as the rate and the extent to which the active drug ingredient is absorbed from a drug product and becomes available at the site of action (334). The availability of a drug to its site of action is controlled by 3 main factors:

(1) the rate and extent of release of the drug from the dosage

form containing the drug and its subsequent absorption from the solution state;

(2) the first-pass effect whereby, when a drug is administered orally, only a certain fraction presented to the gastrointestinal wall and the liver reaches the system intact and

(3) the combined processes of drug distribution to various body fluids, plasma protein binding, tissue binding, metabolism and excretion. In bioavailability studies, therefore, an attempt is made to quantitatively assess the magnitudes of (1) and/or (2).

In all situations except intravenous administration, the drug must be released from the dosage form which acts as a drug delivery system. Any changes to the dosage form may alter the rate at which the drug is released *in vivo* and the ratio of the amount released to the total amount of the drug in the dosage form. This causes differences in onset, intensity and duration of pharmacological or clinical effect. These dosage form effects will be manifest principally in differences in rate and/or efficacy of absorption.

In testing bioavailability, *in vivo* study protocol must include a true cross-over design to exclude physiologically based modifications of bioavailability such as age, sex, physical state of the patient, body weight, type and amount of food, liver function and kidney function.

Four possible approaches are available to determine bioavailability and involve using blood level data, urinary excretion data, pharmacological data and clinical data. Blood level studies are preferable since most information can be obtained from these data and should be conducted wherever possible, although urinary excretion studies can be used as a substitute. When neither of these methods is feasible, as in the situation where drug concentrations cannot be assayed accurately in body fluids, pharmacological data may be used. In a case where a pharmacological response is difficult to quantify, clinical studies in patients are conducted.

6.4 ASSESSMENT OF EXTENT OF ABSORPTION

Absorption may be interpreted as the first step of the process involving delivery of the drug or its active moiety to its site of action. As the drug must, in most cases, reach the general circulation before exerting its pharmacological effect, absorption may then be described as the process of delivery of the drug to the general circulation (335).

Methods of assessing the extent of absorption which do not involve determination of the absorption rate, rely on fewer assumptions and are easier to apply to experimental data than methods for assessing the absorption rate. However, knowledge of merely the extent of absorption yields incomplete information on the absorption process as rate of absorption should be known to enable complete characterisation of the process. Calculation of the fraction, F, of the dose, D, which is absorbed and reaches the general circulation reflects the extent of absorption.

Estimation of the area under the concentration curve is usually involved in assessment of the extent of absorption. Area is given by the integral

$$AUC = \int_0^\infty C(t)\,dt \qquad (6\text{-}10)$$

The most frequently used method for estimating area under the curve (AUC) is the linear trapezoidal method:

$$AUC = \left[\frac{C_n + C_{n-1}}{2}\right](t_n - t_{n-1}) \qquad (6\text{-}11)$$

Error is introduced through the linear interpolation and approximation to the data between successive data points. Significant errors arise if sampling intervals are long relative to the half-life of the drug and if the function to be assessed shows marked curvature. The use of the linear trapezoidal method results in considerable under-estimation of area during the absorption phase where the curve is increasing to a maximum, and over-estimation of area during the post-absorption phase, where

the curve decreases in an approximately exponential manner. Chiou (336) and Yeh and Kwan (337) investigated errors associated with trapezoidal integration. Chiou (336) has reported that for a curve decreasing monoexponentially, error is related to the ratio of the sampling interval to the half-life of the exponential function.

The log trapezoidal method, which employs an exponential rather than a linear approximation to the data between successive points, has been applied to the post-absorption phase where the function declines monoexponentially. The log trapezoidal equation is shown below:

$$AUC = \frac{C_n - C_{n-1}}{(1/t_n - t_{n-1}) \ln (C_n/C_{n-1})} \qquad (6\text{-}12)$$

However, for a curve ascending to a maximum, the log trapezoidal method yields very large errors.

The use of cubic polynomials for interpolating between data points has been investigated by Yeh and Kwan (337). The estimate of the area for each interval is obtained by analytical integration of the approximating polynomial and the total area is given by the sum of the estimates for the individual intervals. The aim of this method is to obtain better approximation to the curvature of the function for an ascending curve in the vicinity of the maximum. For the monoexponential decline period, the log trapezoidal method gives the best estimation.

To determine AUC_∞, the assumption is made that concentration C(t) decreases monoexponentially after the last data point. When elimination is by a first order process, this decline must be measured for a sufficient time prior to the last data point to enable estimation of λ_z, the slope of the terminal portion of a semilogarithmic plot of concentration versus time. This extrapolation technique may also be applied to Michaelis-Menten elimination, since at low concentrations, the Michaelis-Menten process is approximately first order.

Kwan and Till (338) proposed a method for assessing the extent of absorption based on estimating the renal clearance, plasma clearance and urinary excretion of unchanged drug. The method is model-independent and does not require a complete definition of the time course of change in plasma concentrations, thus lending itself to a more flexible sampling schedule.

6.5 ASSESSMENT OF RATE OF ABSORPTION

A fuller understanding of the absorption process requires information about the time course of the absorption process. However, in some cases simple measures such as peak concentration and time of peak concentration may be adequate. Time dependence of the absorption rate or amount absorbed at various times either as a mathematical function or expressed numerically is the ideal representation. Numerous methods for determining absorption rates have been developed and reviewed (335,339-363).

6.5.1 Single Value Parameters

6.5.1.1 C_{max} and t_{max}

The maximum blood drug concentration, C_{max}, and the time at which the maximum occurs, t_{max}, give a crude indication of the absorption rate and are estimated directly from the serum concentration versus time data. A disadvantage is that these parameters do not refer solely to the absorption process but depend partly on the disposition of the drug. Although the rate of absorption controls t_{max}, absorption which continues after t_{max} has no influence on this parameter.

6.5.1.2 Statistical Moments

Statistical moments were first applied to pharmacokinetic analysis by Yamaoka *et al.* (362) and Cutler (363). Riegelman and Collier (364) subsequently reviewed and clarified the moment concept. In the statistical moment theory, absorption is regarded as a stochastic process. Since statistical moments are characteristic of the shape of the statistical distribution curve, such as the time course of serum concentration following a single dose of the

drug, they are only dependent on the observed time course data and are independent of the pharmacokinetic compartment model. This approach allows separation of the absorption from the disposition phase and although it does not enable a complete description of the time course of the absorption process, it does summarise in a single figure the salient features of the absorption rate.

The zero moment represents the AUC_∞ , which has been previously discussed. The area under the first moment of the curve is defined as the area under the curve of the product of time, t, and serum concentration, C_p, from zero time to infinity. Using this approach the mean residence time (MRT) can be calculated as follows:

$$MRT = \frac{\int_0^\infty t C_p \, dt}{\int_0^\infty C_p dt} = \frac{AUMC_\infty}{AUC_\infty} \qquad (6\text{-}13)$$

The MRT can be defined as the mean time for intact drug molecules to transit through the body and it yields significant information on all kinetic processes including *in vivo* release from the dosage form, absorption into the body and all disposition processes. The MRT for a non-instantaneous input (n.i.v.) involves a mean absorption time (MAT) which refers to the mean time involved in the *in vivo* release and absorption processes as they occur in the input compartment.

$$MRT_{n.i.v.} = MAT + MRT_{i.v.} \qquad (6\text{-}14)$$

To evaluate the exact value of the MAT, the $MRT_{i.v.}$ should be subtracted from the $MRT_{n.i.v.}$. However MAT can be approximated in cases where the concentration-time curves yield a terminal log-linear slope from which the terminal rate constant, λ_z , can be defined. The reciprocal of λ_z is subtracted from the MRT to yield MAT_{uncorr} , the uncorrected mean absorption time.

$$MAT_{uncorr} = MRT_{n.i.v.} - \frac{1}{\lambda_z} \qquad (6\text{-}15)$$

The above method results in an error in MAT when multi-compartment kinetics are involved. If, however, a solution and solid dosage form were evaluated in the same subject whose disposition parameters were constant between studies, the error term would be

constant between studies. For an extravascular dose:

$$MAT_{true} - MAT_{uncorr} = \frac{1}{\lambda_1} - \frac{1}{k_{21}} \qquad (6\text{-}16)$$

In the absence of intravenous data, MAT offers significant advantages over the use of t_{max} as a means of comparing absorption rates, as the latter depends on disposition as well as absorption, whereas MAT is independent of the disposition of the drug.

6.5.2 Mass Balance Methods

These methods are based on the assumption of a compartmental model and amounts absorbed at any time are calculated from the sum of the amounts in each compartment plus that which has been eliminated.

6.5.2.1 Wagner-Nelson Method

The Wagner-Nelson method (340) assumes a one compartment open model and linear pharmacokinetics. Wagner, (341) however, has successfully applied the method to a two compartment open model in certain instances and obtained a good approximation to the absorption profile.

Assuming first order elimination

$$\text{Amount absorbed} = \text{Amount in body} + \text{amount eliminated}$$

$$A_{abs}(t) = VC_p + CL\ AUC_0^t$$

$$A_{abs}(\infty) = CL\ AUC_0^\infty \qquad (6\text{-}17)$$

Fraction absorbed, (Fa), to time, t, is equal to

$$F_a = \frac{C_p + k\ AUC_0^t}{k\ AUC_0^\infty} \qquad (6\text{-}18)$$

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

The Wagner-Nelson method has the advantages of not requiring intravenous data or a prior estimate of the volume of

distribution, V, and no limitations are placed on the order or the nature of the absorption process. Although the method is convenient, a number of shortcomings exist in determining the absorption profile without intravenous data. It is not possible to determine whether disposition follows one or two compartment pharmacokinetics with an extravascular dose alone but a minority of drugs exhibits one compartment kinetics. Ronfield and Benet (342) showed that a drug which appeared to follow one compartment kinetics after oral dosing, actually displayed two compartment distribution following intravenous dosing.

There may be doubt about the validity of the estimate of the elimination rate constant from the terminal phase data as absorption is relatively slow. In the "flip-flop" model, where $k_a < k$, the true absorption profile will not be recovered as the terminal slope represents k_a rather that k. The main application of this method would therefore be in studying absorption profiles of drugs in which one compartment kinetics have been shown to exist.

6.5.2.2. Loo-Riegelman Method

The Loo-Riegelman method (343) uses the two compartment model to estimate the amount of drug in the body which includes drug in both compartments. The disposition parameters k_{12}, k_{21}, k_{10} and V can be determined with an intravenous dose study and parameters are assumed to be constant between studies.

A linear approximation for C_p is used over each time interval in the original method with a two term Taylor series to simplify the final equation, although this has been shown by Boxenbaum and Kaplan (344) to be a potential source of error. A cubic spline interpolation procedure prior to the application of the Loo-Riegelman equation has been proposed by Wagner (345).

Assuming a two compartment model, the amount absorbed is equal to the amount in the central compartment plus the amount in the peripheral compartment plus the amount eliminated.

$$A_{abs}(t) = V_1 C_1(t) + A_2(t) + CL\ AUC_0^t \qquad (6\text{-}19)$$

Assuming that C_1 can be represented by a linear segment between any two times, t_{i-1} and t_i,

$$A_2(t_i) = A_2(t_{i-1})\, e^{-k_{21}\Delta t} + \frac{k_{12}}{k_{21}} V_1 C_1(t_{i-1})(1 - e^{-k_{21}\Delta t}) \qquad (6\text{-}20)$$

$$+ \frac{k_{12}}{k_{21}} V_1 \Delta C_1 - \frac{k_{12}}{k_{21}} V_1 \frac{\Delta C_1}{\Delta t} (1 - e^{-k_{21}\Delta t})$$

Fraction, F_a, absorbed to time t is calculated as follows:

$$F_{a_t} = \frac{C_1(t) + k_{10} AUC_0^t + A_2(t)/V_1}{k_{10} AUC_0^\infty} \qquad (6\text{-}21)$$

In the Loo-Riegelman method it is assumed that elimination occurs solely from the central compartment. However, Vaughan and Dennis (346) and Wagner (345) have shown that the Loo-Riegelman method is model independent providing input is into the central compartment, and the central compartment is the one sampled. Recently, Wagner (347) proposed an exact absorption equation when drug disposition is characterised by two exponential terms and showed that it is an exact Loo-Riegelman equation, but is simpler and easier to use than the latter.

A disadvantage of the Loo-Riegelman method is its limitation to linear systems with monotonically decreasing characteristic response where the reference dose must be an intravenous injection. Oral solution data cannot be used as a reference to determine release rates from an oral dosage form.

6.5.3 Deconvolution Methods

These methods provide another approach to the assessment of absorption rates. The body is considered to be a linear system with respect to drug disposition, no assumptions are made concerning the detailed stucture of the system and the methods do not require specification of the form of the input function in most cases.

6.5.3.1 Finite Differences Methods

These methods for numerical deconvolution, although the simplest, are unstable in the presence of data noise (348). An approximation is found for the absorption rate over the nth time interval in terms of approximations already derived from previous intervals. Different methods arise depending on the approximation used for the absorption rate over each interval.

A known method for numerical deconvolution is the area-area method described by Rescigno and Segre (349), but this method seems to be ambiguous with some confusion about its exact mathematical basis. The point-area method proposed by Vaughan and Dennis (350) appears to be the most rigorous of these methods. In a method by Chiou (351) which can be regarded as a deconvolution method,the input rate is approximated by a train of impulse functions. The entire amount absorbed during an interval is considered to be delivered as a pulse at the mid-point of the interval.

Vaughan (352) derived the relationship between the empirical method of Chiou (351) and the point-area deconvolution method (350) and showed that the instantaneous midpoint-input method is an approximation of the point-area method when the analytical integration of the characteristic response is approximated by a rectangular function. He concluded that such an approximation can result in large errors in the cumulative drug input functions and should be avoided.

6.5.3.2 The Least-Squares Method

Cutler (353,354) reported a new approach to the numerical treatment of data based on the least-squares criterion. It is essentially a curve-fitting approach in which the least squares iteration operates only on the input rate. This method requires prior assumptions concerning the mathematical form of the input function. In numerical examples, an exponential function and a function derived from the cube-root dissolution law were used to describe the input function. When the input function is unknown, a polynomial function may be used, since polynomials are

sufficiently flexible to approximate a wide range of functions.

6.5.3.3 Exact Methods

The methods used by Smolen (355) and Pedersen (356) are carried out using a digital computer. They utilise response data such as plasma concentration-time curve after extravascular input. These data require "smoothing" in some way by using a function generator or by using spline polynomials.

6.5.4 Curve-Fitting Methods

Curve-fitting methods involve fitting the plasma concentration-time data to an analytical function into which parameters accounting for the absorption and disposition processes are incorporated. This can be done either by the method of residuals or by nonlinear least-squares regression. The parameters in the function referring to the absorption process must be identified to enable elucidation of the absorption process. This problem can be overcome if intravenous data are also available.

A compartmental model or sums of exponentials can be used as the analytical function. A problem arises as the number of exponential coefficients needed to describe extravascular data increases. This is due to the non-uniqueness of the exponential coefficients fitted to the experimental data (357).

6.5.5 Nonparametric Methods

A disadvantage of nonlinear regression techniques is their failure to converge under certain circumstances. Consequently, Shelver and Farris (396) developed a nonparametric method for the estimation of parameters in nonlinear problems. In order to apply this method, appropriate numerical techniques for solving simultaneous nonlinear equations must be developed for the model under examination. The nonparametric method was found to be superior to nonlinear regression techniques if the assumptions for the error structure of the regression were not true.

6.5.6 First Order Rate Constant Methods

These methods asssume that drug absorption is a first order process. Curve-fitting of the entire curve is unnecessary as simple curve parameters are utilised. The method derived by Vaughan *et al.* (358,359) requires no specific knowledge of drug distribution or elimination and allows for multiexponential or multicompartmental disposition. Other methods (360,361) assume a one compartment open model and require that both drug absorption and disposition are monoexponential processes which in practice is seldom true.

6.6 NONLINEARITY IN PHARMACOKINETICS

The theoretical and physiological basis of dose linearity was presented by Dost (365) at the inception of pharmacokinetics as a science. Evidence of nonlinearities in pharmacokinetics go back as far as the linear kinetic theory (366,367). Most nonlinearities result from interactions of low molecular weight compounds with endogenous macromolecular structures such as enzymes or carrier proteins when substrate concentrations exceed critical levels and saturable kinetics occur. All deviations from first order kinetics can be classed as nonlinear, although the underlying mechanisms responsible for the nonlinearity may remain unknown. Examples of some well known drugs exhibiting nonlinear kinetics are phenytoin (368), theophylline (369), salicylate (370), riboflavin (371), ethyl alcohol (372) and warfarin (373). Wagner (366) summarized evidence for nonlinearites in drug absorption, distribution, metabolism, renal excretion of the drug and its metabolites, biliary excretion and in pharmacokinetics of drug action.

6.6.1 Causes of Nonlinear Pharmacokinetics

Nonlinear pharmacokinetics may be due to the operation of Michaelis-Menten or some other nonlinear elimination kinetics, saturable tissue-binding in the nonlinear region of binding or plasma-protein binding upon the administration of doses which are large enough to enter into the nonlinear region (367).

Drug absorption in linear systems may be described by one or two first order processes where diffusion of drug through membranes is the rate limiting step. Nonlinear absorption phenomena arise as soon as some other step becomes rate limiting. These may result from low solubility of the drug, from a low rate of dissolution, from many sustained-release preparations and from saturable active absorption processes (374). Some drugs require the presence of food in the GI tract to be absorbed, intestinal blood flow may fluctuate thereby influencing absorption and the change in pH of luminal contents may affect the absorption of an acidic, basic or amphoteric drug (375). Delay in gastric emptying which may be caused by food in the stomach, enteric-coated tablets and anticholinergic agents will also result in nonlinearity of the absorption process.

During distribution in linear systems, the ratio of drug concentrations in two compartments is dependent solely on the ratio of the distribution rate constants and is independent of the total amount of drug involved. Therefore, the volume of distribution is dose-independent. Reversible binding of drug to plasma protein is one of the most frequent causes of nonlinear distribution. Saturable binding to other tissue constituents, or carrier system involvement in drug transport may also be responsible for nonlinearity in drug distribution depending upon the total binding capacity and the affinity of the drug for the system concerned. When the binding becomes saturated, further dose increments will result in a rapid increase of the fraction of free drug and consequently in more extensive distribution.

Elimination mechanisms in some drugs are essentially nonlinear and are only apparently linear in certain concentration ranges. Total clearance is the sum of contributions from metabolic and renal pathways and occasionally from other pathways (lung, sweat, bile, faeces). The renal excretion of drugs in urine is governed by a number of processes (376,377):

(a) Glomerular filtration is a passive process during which blood flowing through the glomuruli is ultrafiltered at about 125

ml filtrate per minute.

(b) Renal tubular secretion is assumed to be an active process and appears to be linear for most drugs under clinical conditions and may be described by Michaelis-Menten kinetics. This is limited by a certain fixed transport value and is susceptible to competition by various compounds secreted by the same mechanism.

(c) Passive tubular back-diffusion which is pH-dependent, involves reabsorption of non-ionized molecules from the renal tubular lumen back into the blood. Reabsorption of weak acids and bases varies with the pH of the urine. For a basic nonpolar drug with a pKa of between 6 and 12, the extent of reabsorption varies from negligible to almost complete with changes in pH. Renal clearance is only pH sensitive for an acid whose pKa lies between 3 and 7.5 (366). Renal clearance of drugs such as theophylline (378) that are extensively reabsorbed will be affected most by urine flow.

(d) Active tubular reabsorption may also occur during which some drugs are actively reabsorbed from the urine into the capillaries around the renal tubule. This active process is saturable and susceptible to competition phenomena.

Only at low drug concentrations can the active processes (b) and (d) be represented by constant concentration-independent parameters, as at higher concentrations nonlinearity occurs.

Drugs in the urine exist in the unbound form. The influence of protein binding on the renal clearance of a drug depends upon its extraction ratio (379). If the extraction ratio is high, renal clearance depends upon blood flow and not on protein binding. Conversely if the extraction ratio is low, protein binding has a marked influence on renal clearance.

Pickup *et al.* (380) related an apparent increase in volume of distribution and total body clearance with increasing dose to protein binding. Prednisolone appears to distribute more rapidly than it is eliminated and consequently the concurrent changes in volume of distribution and clearance result in a half-life that

apparently increases with dose.

6.6.2 Tests for Nonlinearity

In testing for nonlinearity, a complete investigation of the concentrations in blood and/or urine at several dose levels is required for a sound decision. Intravenous data are preferred to extravascular data.

After administration of a drug at several doses, the following pharmacokinetic tests may be applied:

(a) Divide plasma concentration by the dose or normalized dose and plot the ratio versus time.

(b) Calculate the area under the concentration-time curve and compare results.

(c) After intravenous administration, extrapolate the concentration in the central compartment at time zero from the first data points.

(d) Determine the steady state plasma concentrations after constant infusions at different rates.

(e) Determine the total amount of parent compound and/or any metabolite excreted into the urine at infinity.

(f) Fit each set of concentration-time data to what appears to be the appropriate linear pharmacokinetic model and observe if the magnitude of the parameters change with dose.

(g) If Michaelis-Menten kinetics are applicable;

- (i) the percentage metabolized by the Michaelis-Menten path will decrease with increase in dose
- (ii) the AUC will increase disproportionately with an increase in dose
- (iii) the AUC will be a nonlinear function of the rate of absorption and
- (iv) semilogarithmic plots of blood levels versus time will curve inwards.

6.6.3 Curve-Fitting Nonlinear Models

When nonlinear kinetics prevail, explicit equations of the type $C=f(t)$ generally used in curve-fitting procedures cannot be

obtained. The original differential equations can then be used, numerically integrated and the parameter values optimized by some iterative regression procedure. The possibility of using explicit equations of the type t=f(C) for curve-fitting purposes has been described (377).

6.7 RESULTS AND DISCUSSION

6.7.1 Bioavailability Parameters

The 50 and 100 mg solution data were initially treated using model-independent methods. The parameters C_{max} and t_{max} were obtained from the individual serum concentration versus time data. Area under the curve was calculated as described in section 6.4. The terminal rate constant, λ_z , was calculated from the terminal slope of a semilog plot of serum concentration versus time. From these data, the elimination half-life, $t_{1/2}$, was calculated where

$$t_{1/2} = \frac{0.693}{\lambda_z} \qquad (6\text{-}22)$$

TABLE 6.1 Bioavailability parameters for 50 and 100 mg solution data.

TEST DOSE	SUBJECT	PARAMETERS				
		t_{max} (hrs)	C_{max} (ng/ml)	AUC_∞ (ng/ml.hr)	λ_z (hr^{-1})	$t_{1/2}$ (hrs)
50 mg PPA.HCl SOLUTION	JM	1.50	112.21	809.01	0.160	4.33
	MK	2.00	114.10	858.62	0.177	3.92
	SR	1.00	154.97	1135.39	0.157	4.41
	PS	2.00	116.70	839.57	0.180	3.85
	DD	0.83	139.62	773.40	0.232	2.99
	MM	1.50	185.68	1143.83	0.163	4.25
	MEAN	1.31	137.21	926.64	0.170	3.96
	± SD	0.63	29.16	167.50	0.028	0.53
100 mg PPA.HCl SOLUTION	JM	1.00	333.26	2741.45	0.118	5.87
	MK	2.00	249.42	2177.94	0.123	5.63
	SR	2.00	421.91	3260.61	0.159	4.36
	MEAN	1.67	334.86	2726.00	0.130	5.29
	± SD	0.58	86.26	541.49	0.020	0.81

For the 100 mg solution, peak concentrations were more than double those for the 50 mg solution and the AUC_∞ values were nearly 3 times those of the 50 mg solution. Both these phenomena raised doubts concerning the supposed linear kinetics of the drug in the body.

Of the 3 subjects JM, MK and SR who completed both trials, SR had the highest peak serum concentration and AUC_∞ values for both studies. The λ_z values for all subjects in both trials did not differ to any great extent except for Subject DD, who displayed a larger λ_z and consequently a shorter half-life than the other subjects in the 50 mg trial. Interestingly, the t_{max} for DD in the same trial was the shortest. Rapid absorption may partially have accounted for the higher rate of elimination occurring in this subject. The AUC_∞ value for DD was the lowest of the 6 subjects, partly due to the rapid elimination of the drug.

6.7.2 Weibull Fits to Wagner-Nelson Absorption Plots

The solution data were then treated assuming a linear one compartment model with first order absorption. The Wagner-Nelson equation was applied to the serum concentration-time data to yield absorption plots of fraction absorbed versus time. These transformed data were subsequently fitted to the Weibull equation and the resultant fits can be seen in Figs. 6.5 and 6.6. The absorption rate constants were calculated from the slope of the semilog plot of fraction of dose remaining to be absorbed versus time and are shown in Table 6.2.

TABLE 6.2 Values of k_a for the 50 and 100 mg solution studies

TEST DOSE	SUBJECTS						MEAN ± SD
	JM	MK	SR	PS	DD	MM	
50 mg	1.721	1.430	1.671	1.220	2.930	1.604	1.762 ± 0.601
100 mg	2.204	1.592	1.496	-	-	-	1.764 ± 0.384

For the 50 mg solution study, Subject DD exhibited a much larger absorption rate than the other subjects which is consistent with the short t_{max} from this subject.

FIGURE 6.5 Wagner-Nelson absorption plots of serum concentration-time curves after administering the 50 mg PPA.HCl solution. The solid curve represents the Weibull function fit to the data.

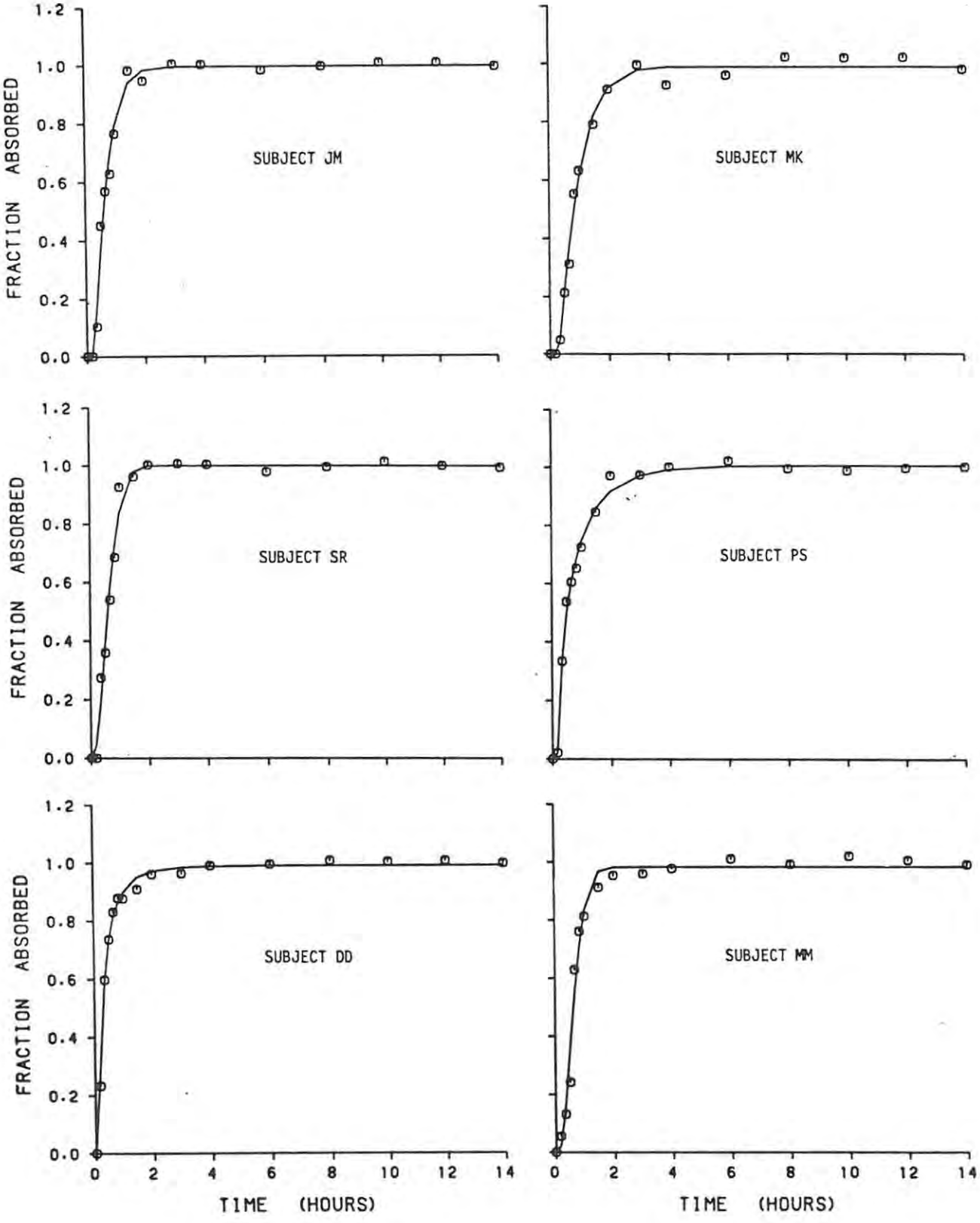
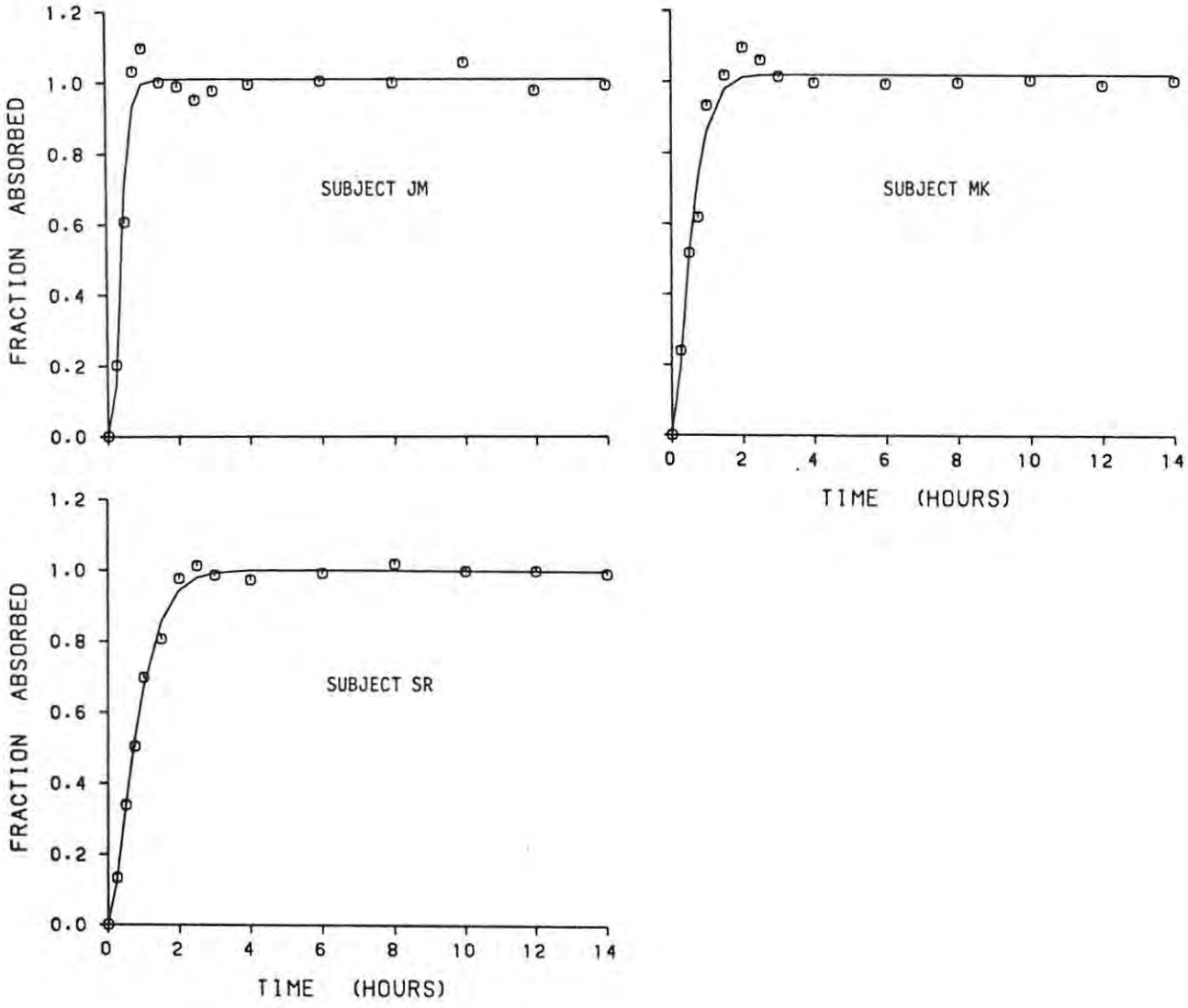


FIGURE 6.6 Wagner-Nelson absorption plots of serum concentration-time curves after administering the 100 mg PPA.HCl solution. The solid curve represents the Weibull function fit to the data.



The Weibull fits to the 50 mg data in all but one subject had correlation coefficients (r) greater than or equal to 0.99, indicating the applicability of the Weibull equation to the transformed absorption data. Subjects MK and SR had the greatest and smallest t_o values respectively. The t_d value for DD was far smaller than those for the other 5 subjects, indicating the rapid absorption of 63.2% of the drug in this subject. Two subjects, PS and DD, had β values less than 1, with the curves exhibiting a steeper initial slope followed by a flattened tail in the final section of the curve. F^{∞} values for all 6 subjects were close to 1.00.

TABLE 6.3 Weibull function analysis of absorption data for the 50 mg PPA. HCl solution

	SUBJECTS						MEAN	MEAN OF	
PARAMETERS	JM	MK	SR	PS	DD	MM	DATA[1]	PARAMETER[2]	CV %[3]
t_o	0.182	0.271	0.069	0.167	0.128	0.093	0.123	0.152	47.65
t_d	0.576	0.761	0.655	0.557	0.242	0.639	0.601	0.572	30.90
β	1.289	1.173	1.700	0.741	0.674	1.799	1.162	1.229	38.16
F^{∞}	0.997	0.989	1.002	1.008	0.990	0.983	0.995	0.995	0.93
r	0.992	0.993	0.990	0.996	0.996	0.987	0.999		

1 Weibull fit to mean data of the six subjects

2 Arithmetic mean of the fitted parameters from the six subjects

3 Interindividual differences expressed as coefficients of variation

TABLE 6.4 Weibull function analysis of absorption data for the 100 mg PPA. HCl solution

	SUBJECTS			MEAN	MEAN OF	
PARAMETERS	JM	MK	SR	DATA[1]	PARAMETER[2]	CV %[3]
t_o	0.173	0.092	0.071	0.075	0.112	48.09
t_d	0.293	0.551	0.860	0.596	0.568	49.98
β	1.385	1.240	1.283	1.340	1.303	5.72
F^{∞}	1.011	1.021	1.003	0.990	1.016	0.89
r	0.974	0.979	0.996	0.997		

1 Weibull fit to mean data of the six subjects

2 Arithmetic mean of the fitted parameters from the six subjects

3 Interindividual differences expressed as coefficients of variation

The Weibull fits to the 100 mg data for JM and MK showed greater scatter, with lower correlation coefficients. In this study, JM exhibited a much faster absorption rate which is exemplified by the small t_d value found for this subject. The presence of food in the gastrointestinal tract and any changes in the pH of the intestinal contents may have an influence on the absorption of PPA, a weakly basic drug. Being an α-mimetic, PPA is a vasoactive drug and may therefore cause fluctuations in intestinal blood flow, affecting the absorption of the drug.

The discrepancy between the rates of absorption for JM in the 2 solution studies cannot be easily explained, as the same dietary restrictions were practised prior to both trials. As the 2 trials were held 6 months apart, differences in the physical state of the subject could have contributed to the observed different rates of absorption. The β values were all very similar, with the mean of 1.3 describing an overall sigmoidal curve.

6.7.3 Renal Clearance

Elimination of drugs occurs mainly through the liver and the kidney and depends on both the blood flow through these organs and the extraction efficiency (500). Rate of renal excretion is generally proportional to the concentration of the drug in the blood entering the kidney, thus:

$$\frac{dA_e}{dt} = CL\ C \qquad (6\text{-}23)$$

where dA_e/dt is the amount of drug (mg) eliminated per unit time (hr), CL is the renal clearance (ℓ/hr) and C is the concentration of drug in the serum/plasma (ng/ml). Linear kinetics occur only when clearance is constant, with the rate of elimination being directly proportional to concentration. Linear elimination may also be termed supply limited elimination.

Clearance depends on factors such as blood flow, drug concentration, degree of protein binding and the condition of the organs and is therefore not always constant (382). The elimination

of certain drugs cannot be described by a set of linear differential equations. These drugs exhibit dose-dependent kinetics which are frequently due to the limited capacity of an enzyme system involved in the metabolism or excretion of the drug and the elimination is said to be capacity limited. Drugs may also exhibit simultaneous first order and capacity limited kinetics.

The capacity limited process may be described by the Michaelis-Menten equation. The differential equation describing the rate of change in concentration of the drug, C, in the body, with respect to time, t, is:

$$\frac{dC}{dt} = \frac{V_m\ C}{K_m + C} \qquad (6\text{-}24)$$

V_m is the maximum velocity of the capacity limited process in concentration per unit time and is indicative of the capacity of the enzyme system. K_m represents the Michaelis-Menten constant, which is equivalent to the concentration when the velocity of the reaction is half maximal, and is in concentration units.

Equation 6-24 may be numerically integrated to obtain C,t data, or the following integrated form may be solved numerically:

$$\log (C(t)/C_o) + (C(t) - C_o)/K_m + \beta t = 0 \qquad (6\text{-}25)$$

where $\beta = V_m/K_m$

An explicit solution to Eq.6-24 was presented by Beal (383) in which the exponential function is not involved. Instead, the solution involves another simple function for which a table was presented. A program to solve the Michaelis-Menten equation for concentration, operable on a programmable pocket calculator or a microcomputer with facilities for BASIC was described by Collier (384). Wagner (385) discussed the Michaelis-Menten equation, its properties and its application to various pharmacokinetic models.

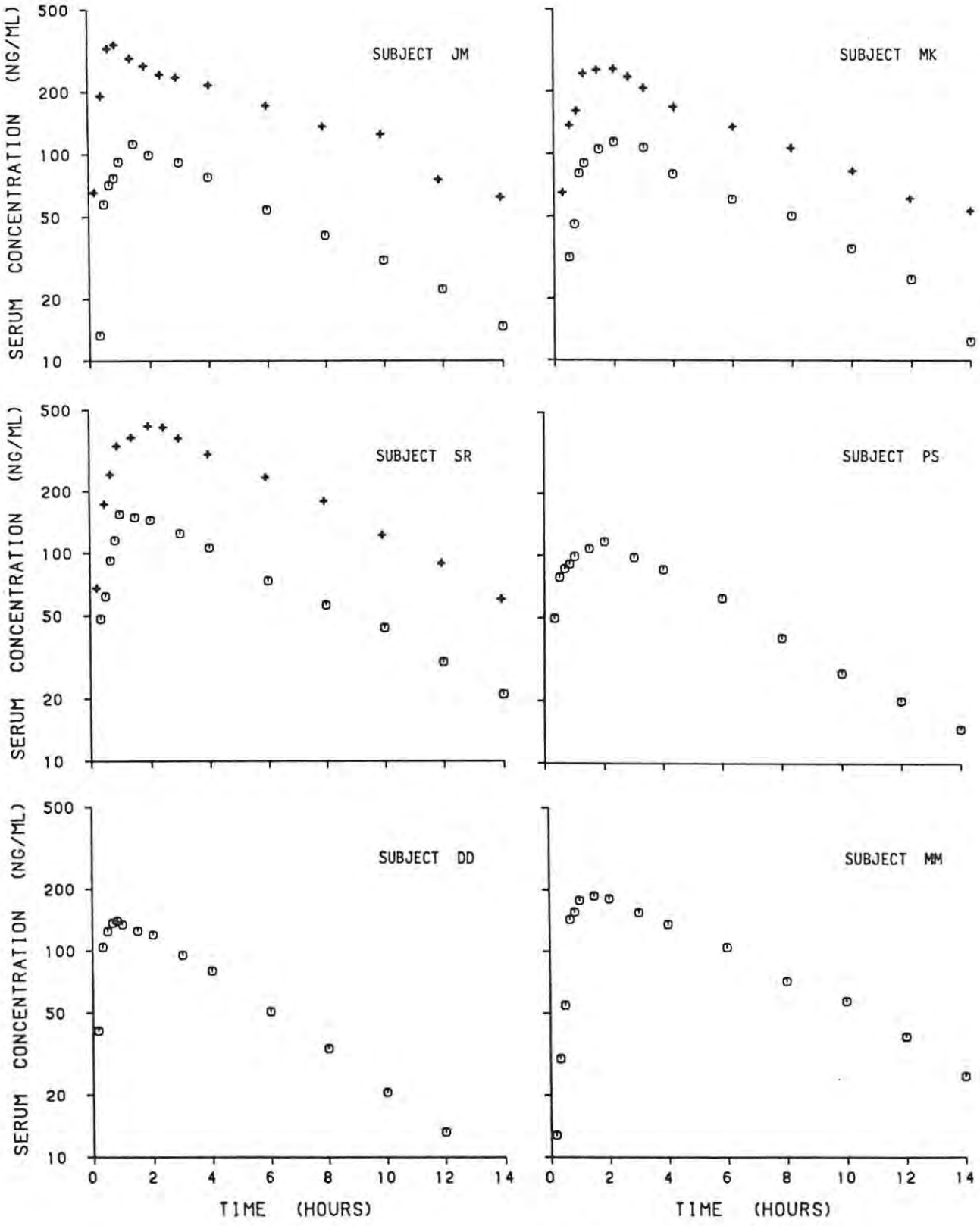
Terminal serum/plasma concentration-time data obeying Eq.6-24 display characteristic curves. Initially there is an apparently linear segment and then a sharp break in the curve, with the whole curve then taking on a "hockey stick" shape. The integrated form of the Michaelis-Menten equation always gives a pseudo-linear portion over about two-thirds of the range or more. When the same data are plotted on semilogarithmic graph paper, the graph takes on a convex shape, curving inwards towards the end.

The semilog plots of serum concentration versus time for the 50 and 100 mg solution studies are shown in Fig.6.7. From these curves it is difficult to extract any definite characteristics. The 50 mg curves all curve inwards, but this only occurs at low concentrations where the accuracy of the assay may be questionable. Looking at the 100 mg curves, it is possible that if sampling had been continued for longer, the graphs may have displayed an inward curve. However, no predictions concerning the presence of Michaelis-Menten kinetics could be made from these graphs.

Fitting of the terminal serum concentration-time data was performed by numerical integration of Eq.6-24 using the nonlinear regression program NONLIN (386) on a CDC Cyber 170 series 825 computer to obtain the least squares estimates of the parameters and their standard deviations. The 50 and 100 mg terminal serum data were initially fitted simultaneously, resulting in an overestimation of the serum concentrations in the 100 mg study and an underestimation in the 50 mg study for all 3 data sets evaluated. The large systematic deviations indicated that the model did not adequately describe these data.

When the data were fitted separately, good fits were obtained for both the 50 and 100 mg solution data with correlation coefficients (r) of about 0.99. However, wide variability between the fitted parameters for each subject at a particular dose, large inter-dose variability and the extremely large standard deviations for these parameters stressed the inadequacy of the model. No

FIGURE 6.7 Semilogarithmic plots of serum concentration versus time after the ingestion of a 50 mg (⊙) and 100 mg (+) solution of PPA.HCl.



trends were noted for Vm and Km with an increase in dose.

TABLE 6.5 Clearance values from the 50 mg solution study

JM		MK		SR		PS		DD		MM	
TIME [1]	CL	TIME	CL	TIME	CL	TIME	CL	TIME	CL	TIME	CL
2.1	56.40	1.9	50.46	1.1	43.32	1.1	81.10	3.0	62.21	0.9	41.44
7.2	53.41	4.8	77.71	5.1	36.67	3.7	46.52	7.3	41.95	3.2	36.20
11.3	36.60	8.7	36.47	9.1	28.29	7.1	30.20	10.0	49.05	6.6	27.89
15.4	43.36	13.8	29.29	12.7	35.83	10.5	59.60	13.3	35.56	9.4	29.67
-	-	-	-	16.6	48.33	13.2	51.88	-	-	11.0	31.09
-	-	-	-	-	-	16.2	71.67	-	-	13.3	26.21
GRAPH [2]	56.03		48.70		38.24		43.86		50.00		30.56

1 Time taken as mid-point of the urine collection interval (hrs)

2 Clearance calculated graphically from the slope of the urinary excretion rate versus serum concentration at the mid-point

TABLE 6.6 Clearance values from the 100 mg solution study

TIME [1] (hrs)	CLEARANCE (ℓ/hr)		
	JM	MK	SR
0.5	61.15	83.99	38.66
1.5	47.17	59.97	23.74
2.5	58.81	62.49	18.27
4.0	41.89	30.61	29.70
6.0	32.47	42.33	24.36
8.0	32.90	25.63	23.32
10.0	27.03	34.38	27.10
12.0	30.47	33.42	27.44
14.0	-	18.51	25.54
GRAPH [2]	32.87	37.39	24.00

1 Time taken as mid-point of the urine collection interval (hrs)

2 Clearance calculated graphically from the slope of the urinary excretion rate versus serum concentration at the mid-point

Renal clearance was calculated for each time interval using Eq.6-23. The results can be seen in Table 6.5-6. The graph of urinary excretion rate versus the serum concentration at the mid-point of the urine collection interval was then plotted, the slope of which gave renal clearance over the entire duration of the trial (see

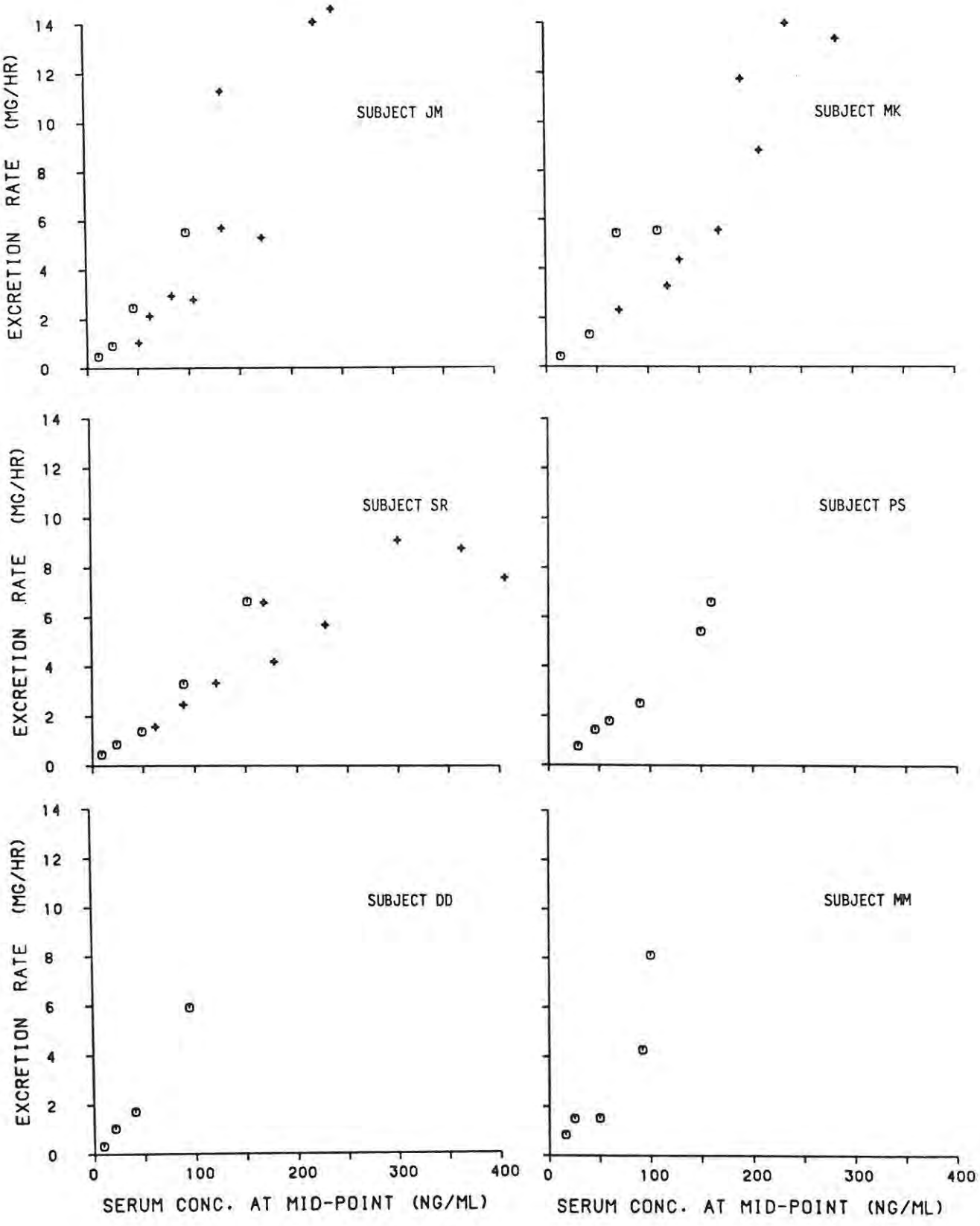
Fig.6.8) The 50 mg solution study was not designed to enable clearance calculations and as such the serum values at the mid-point were obtained by interpolation and only a small number of data points were available. The 100 mg solution study on the other hand was designed specifically to accomodate such calculations.

As can be seen from Table 6.5, large inter-individual differences in clearance occurred during the trials in every subject. The 50 mg curves showed greater linearity than the 100 mg curves. Estimation of clearance from the slopes of the curves showed that this parameter fell by approximately 35% when the oral dose of PPA was increased from 50 to 100 mg. This reduced renal clearance would contribute to the nonlinear increase in AUC with the 100 mg dose. During the trial, however, higher clearance values tended to occur at the higher concentrations of the drug. This is contrary to what would normally be anticipated as the result of saturation of an enzyme system. In the 100 mg study, clearance during the first collection interval (0.5 hr) was high for all 3 subjects, then declined and seemed to reach a steady state.

Flow rate and urine pH have been shown to have an effect on the clearance of certain drugs (378,387). Clearance in this study was shown to be unaffected by these factors. Urine pH was monitored throughout one trial and was found to fluctuate between 5.5 and 7.5. Phenylpropanolamine, being a weak base with a pKa of 9.4 would not undergo a significant change in tubular reabsorption at these pH values.

Renal clearance data indicated that PPA clearance (>500 ml/min) exceeded the glomerular filtration rate (125 ml/min) , possibly implying tubular secretion of the drug. Another possibility to try and account for the higher clearance values at higher concentrations may be the occurrence of saturable tubular reabsorption, as occurs with riboflavine (388). In this case, at increased concentrations of the drug in the renal tubules, a smaller fraction will be reabsorbed and will therefore yield higher renal clearance values with increasing concentrations.

FIGURE 6.8 Plots of urinary excretion rate versus serum concentration at the mid-point of the urine collection interval after the ingestion of a 50 mg (⊙) and 100 mg (+) solution of PPA.HCl.



Increasing clearance with higher concentrations has also been attributed to a concentration-related change in plasma protein binding of the drug (389,390). Phenylpropanolamine has been reported to be 20% protein bound (22), although no recent studies have been reported to support this figure. The influence of binding on the clearance of the drug is more pronounced when the rate of dissociation of the drug from protein is slow. Any capacity limited process will only have an influence on the plasma concentration curve if its contribution to the total clearance is rather greater than 20% (377).

The relationship between renal clearance and serum concentrations in this study was determined under dynamic conditions, as serum concentrations of PPA were changing rapidly. Usually, relatively constant serum concentrations are maintained by continuous infusion of the drug. The point arises whether clearance is then an artifact due to distribution effects. Unfortunately volume could not be calculated due to the lack of intravenous data. Distribution volumes and clearance, however, are usually separate and non-interacting pharmacokinetic elements.

The pharmacokinetic clearance concepts have been criticised by Keller and Scholle (391) who argued that elimination half-life and volume of distribution are the most important clinical pharmacokinetic parameters. Although renal clearance can be calculated from the elimination half-life and volume of distribution, a knowledge of the value for clearance does not enable one to determine whether a change in this parameter is due to an alteration in volume or in half-life and provides no information about the amount of drug eliminated by the kidneys. Renal clearance is merely a relative measure of the patient's kidney function, and in a clinical situation clearance of a reference substance like creatinine is used for this purpose. Drug clearance therefore provides no further information on bioavailability or distribution of the drug.

6.7.4 Computer Fitting of the Data

The nonlinear regression program, NONLIN (386) was used to attempt the various fits, with the DFUNC subroutine being modified for each function. Differential equations were employed to describe each model and although they require more computer time than integral equations, the writing of DFUNC subroutines for complex models is greatly simplified when differential equations are used.

In order to characterise the entire serum concentration-time curve and the cumulative urine curve, the processes of absorption, distribution and elimination had to be taken into account. One data set containing serum and urine data was selected and fitted to the various models.

The differential equation for the rate of change of drug concentration in the body should include a bioavailability term, F. In the absence of intravenous data absolute bioavailability cannot be determined. However, as PPA is metabolised only to a very small extent with the majority of unchanged drug being excreted in the urine, a fairly accurate estimate of bioavailability may be obtained from the proportion of the dose recovered in the urine at time infinity. From the 50 mg study, this was found to be 90%. In all the following equations describing rate of change in drug concentration in the body, the dose of the drug, D, was reduced by a factor of 0.9 to compensate for the bioavailability term.

Symbols are defined as follows:

dC_1/dt = rate of change of drug concentration in the central compartment

dA_2/dt = rate of change in amount of drug in the peripheral compartment

k_a = first order absorption rate constant

k_{10} = elimination rate constant

C_1 = concentration in the central compartment

C_2 = concentration in the peripheral compartment

A_2 = amount in the peripheral compartment

D = dose of the drug

V_1 = volume of the central compartment

6.7.4.1 One compartment model, 1 k_a, linear elimination.

The first model incorporated a one body compartment model (1BCM), a first order absorption rate constant and linear elimination. The differential equations describing the model are shown below:

$$\frac{dC}{dt} = k_a \frac{D}{V} e^{-k_a t} - k_{10} C_1 \qquad (6\text{-}27)$$

The urine curve was described by:

$$\frac{dA}{dt} = k_{10} C_1 V_1 / 1000 \qquad (6\text{-}28)$$

The values of r for the serum and urine fits were 0.947 and 0.995 respectively. The predicted fit to the entire curve was poor and the model was unable to account for the rapid increase to the peak concentration and the rapidly decreasing values tailing off to a gentle slope in the elimination phase. The predicted fit increased more gradually and exhibited a more rounded peak with a steeper gradient in the terminal phase. The fit to the urine data displayed a completely different slope resulting in a poor characterisation of the curve.

6.7.4.2 Two compartment model, 1 k_a, linear elimination.

The data were then fitted to a 2BCM with continuous absorption and linear elimination. The 2BCM was included in an attempt to account for the observed rapid decrease in concentration which occurred just after the peak. The differential equations describing the rate of change of drug in the peripheral and central compartments are shown in Eqs.6-29 and 6-30 respectively.

$$\frac{dA_2}{dt} = k_{12} C_1 V_1 - k_{21} A_2 \qquad (6\text{-}29)$$

$$\frac{dC_1}{dt} = k_a \frac{D}{V_1} e^{-k_a t} + k_{21} \frac{A_2}{V_1} - k_{12} C_1 - k_{10} C_1 \qquad (6\text{-}30)$$

The urine curve was described by Eq.6-28. The fit, as indicated by the values for r, (0.953 for serum and 0.993 for urine) was better than the previous one, with an improved fit to the latter part of the curve. The absorption phase, however, was still very poorly characterised with an initial large over-estimation of concentration followed by an inability to attain the high peak concentrations. The predicted urine curve exhibited a systematic deviation resulting in considerably lower values than the observed ones. The statistic, r, is not sensitive to systematic error between 2 data sets and consequently does not reflect this effect. It should be noted that NONLIN uses the sum of squares and not the correlation coefficient as a fitting criterion.

6.7.4.3 Two compartment model, 2 k_a's, Michaelis-Menten elimination

On closer observation of the absorption phase of the serum concentration-time plots for the 6 subjects, the absorption process appeared to consist of a series of steps, representing discontinuous rather than continuous absorption. Discontinuous processes in relation to linear pharmacokinetic models have been reported by Süverkrup (392) who derived integrated equations for hybrid flow/compartmental models (HFCM) for application to single amd multicompartment drugs exhibiting 2 special cases of discontinuous absorption. The usefulness of these HFCM equations was demonstrated for a number of drugs for which less exact fits had been obtained when continuous absorption was assumed.

Zimmerman (393) replaced the HFCM-integral equations with a system of differential equations in which sequential sets of equations were used to describe the absorption profile from time zero to infinity. Metzler's NONLIN program was used for implementing these methods and it was shown that good fits for some unusually shaped absorption profiles of griseofulvin, buformin and sulfisoxazole were obtained.

As the single absorption rate constant was not able to account for the absorption phase in this data set, the DFUNC subroutine was modified to include 2 absorption rate constants, both of which

were parameters to be optimised. Zimmerman (393) entered the first time value as a constant, using the time for the first blood sample after drug administration. The other times were entered as parameters to be optimised. In this study, the times at which the absorption rates changed were estimated by studying the graphs of serum concentration-time plots and the times entered as constants. If these had been entered as parameters to be optimised, this would have resulted in too large a number of unknown parameters. These time values were altered to try and improve subsequent fits. The Michaelis-Menten equation was introduced to characterise the elimination phase.

Eq.6-29 describes the change in amount of drug in the peripheral compartment and the differential equation describing the concentration of drug in the central compartment is shown below:

$$\frac{dC_1}{dt} = k_{a_x} \frac{D}{V_1} e^{-k_{a_x} t} + k_{21} \frac{A_2}{V_1} - k_{12} C_1 - \left[\frac{(V_m / V_1) C_1}{K_m + C_1} \right] \quad (6\text{-}31)$$

where k_{a_x} describes either the first or second absorption rate constant. Eq.6-32 describes the urine curve:

$$\frac{dA}{dt} = - \frac{V_m C_1}{K_m + C_1} \Big/ 1000 \quad (6\text{-}32)$$

The inclusion of 2 absorption rate constants improved the fit with r values of 0.981 and 0.994 for serum and urine curves respectively. The first predicted value in the absorption phase was good, but again an over-estimation of the next value in the absorption profile and an under-estimation of the peak value occurred. The fit to the urine data was similar to the previous one.

6.7.4.4 Two compartment model, 3 k_a's, Michaelis-Menten elimination

The majority of the serum concentration-time plots seemed to exhibit more than 2 rate changes in the absorption process, thus a

third k_a was included in the DFUNC subroutine and the same data set fitted to the modified program. A noticeable increase in goodness of fit was observed, with values of r for serum and urine being 0.997 and 0.991 respectively. The allowance for 3 different absorption rates enabled the different parts of the absorption process to be more closely characterised. The disposition phase was accounted for by the transfer rate constants to and from the peripheral compartment and a good fit was obtained in the terminal phase with the use of the Michaelis-Menten equation.

6.7.4.5 One compartment model, 3 k_a's, Michaelis-Menten elimination

Intravenous data are necessary for the elucidation of the disposition characteristics of a drug. As the only data available in this study were extravascular data, the disposition of PPA into a 1BCM or a 2BCM could only be postulated. The previous series of fits indicated that the data required a 2BCM. However, to enable a more confident prediction, the same data were fitted to a similar model as the one described in section 6.7.4.4, except that a 1BCM was used instead of a 2BCM.

The overall fits for serum and urine as indicated by the values for r were 0.985 and 0.988 respectively. Although these figures do not suggest an extremely poor fit, certain salient features in the observed curve were inadequately characterised. The predicted peak concentration was lower than the observed. The subsequent disposition and elimination phases included higher and lower concentrations respectively than the observed values. From this fit, it appeared that loss of drug by elimination alone could not account for the rapid decrease in concentration after the peak. Transfer of drug into the peripheral compartment as well as elimination accounted much more accurately for the observed drug concentrations.

In the terminal elimination phase, the slope of the fit of the curve to the 1BCM was too steep, thus predicting a faster elimination than was observed. However, with the 2BCM, 2 processes are accounted for in this latter section of the curve, elimination

as well as transfer of drug back into the central compartment from the peripheral compartment.

6.7.4.6 Two compartment model, 3 k_a's, linear elimination.

A similar model with linear elimination was proposed and the same data fitted to the model. The results were almost identical to the fits obtained using Michaelis-Menten elimination. However, as evidence for nonlinearity in elimination had been presented, the final model included a 2BCM with 3 k_a's and Michaelis-Menten elimination in an attempt to account for the observed nonlinearity.

6.7.4.7 Comparison of fits.

All serum and urine data sets for the 50 and 100 mg solutions were then fitted to the following 3 models:

(1) 2BCM with 3 k_a's and Michaelis-Menten elimination
(2) 2BCM with 3 k_a's and linear elimination
(3) 2BCM with 1 k_a and linear elimination.

The observed values and the resultant fits to models (1) and (3) can be seen in Figs. 6.9 and 6.10. In Appendix 4, the observed and predicted values for serum data from the 50 and 100 mg solution studies resulting from the fits to model 3 are shown in Table A4.1 and A4.2. The parameter values are depicted in Table A4.3.

FIGURE 6.9 Observed data and predicted fits to the 50 mg (a) serum and (b) urine data using models 1 (——) and 3 (-----).

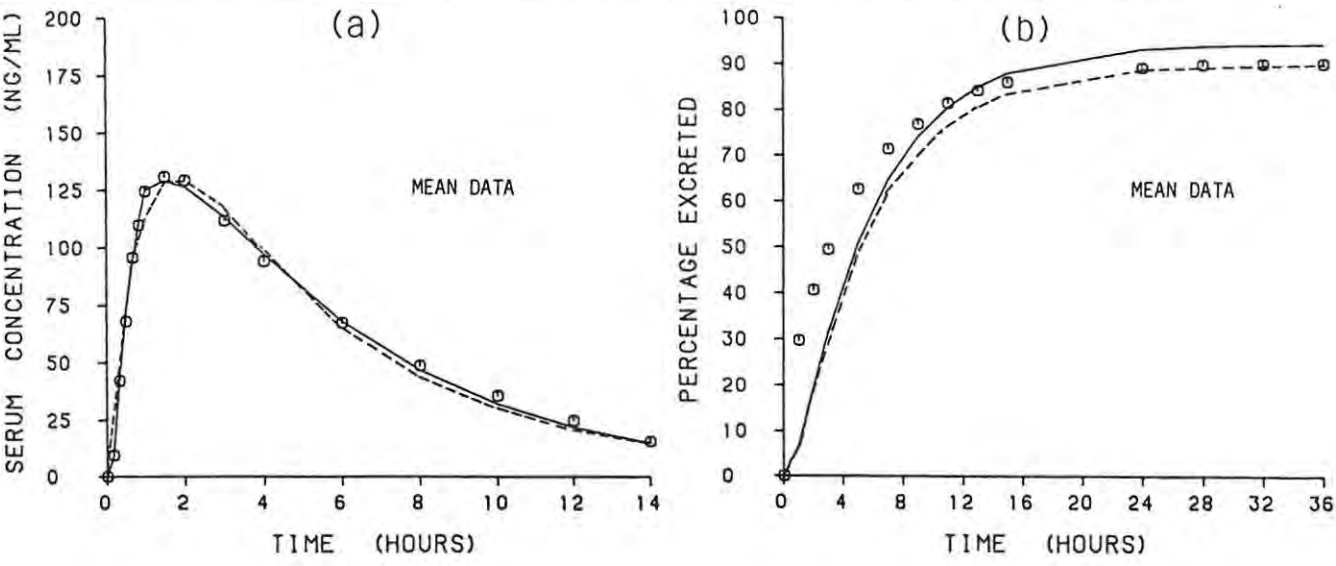


FIGURE 6.9 (continued)

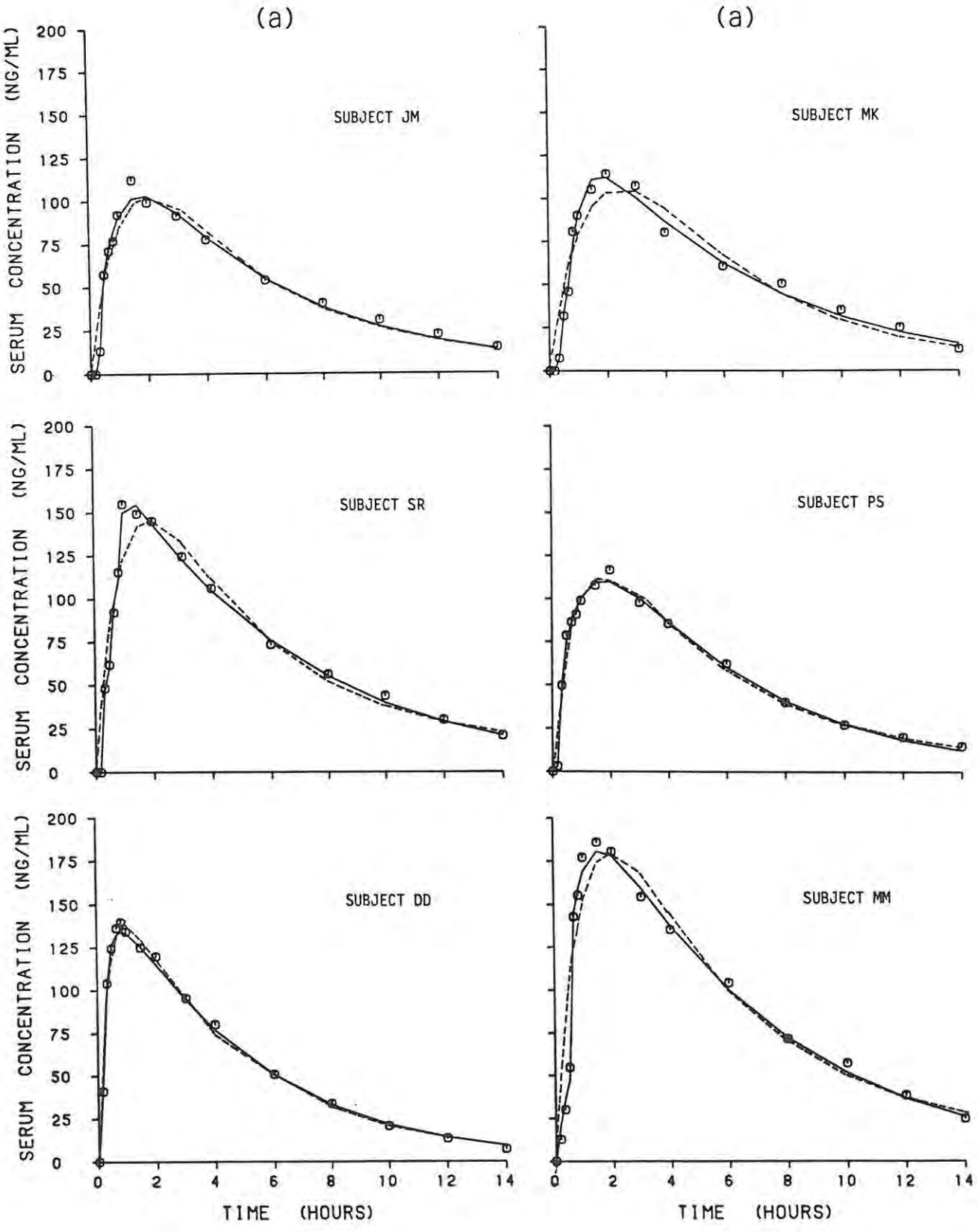
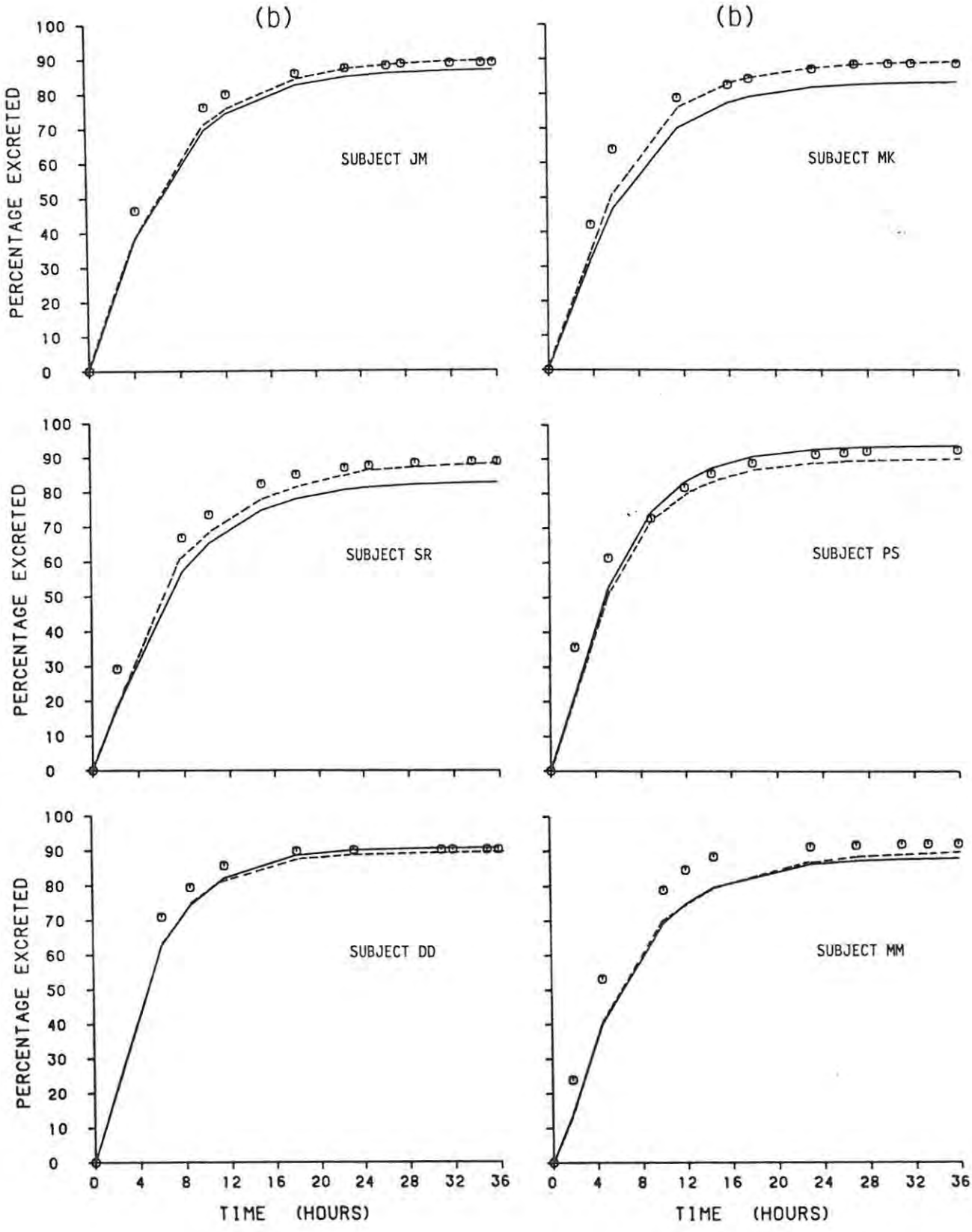


FIGURE 6.9 (continued)



Models (1) and (2) differed only in the characterisation of the elimination process. The fits to these 2 models were extremely similar and graphically superimposable, consequently only the fit to models (1) and (3) were plotted. The fits to the model incorporating only one absorption rate constant did not appear, from visual inspection, to differ dramatically from the fit to that model employing discontinuous absorption. However, in the majority of cases, the early absorption phase was poorly characterised using only one absorption rate constant, and the peak was either distorted or was substantially lower than the observed peak concentration.

FIGURE 6.10 Observed data and predicted fits to the 100 mg (a) serum and (b) urine data using models 1 (——) and 3 (- - - -).

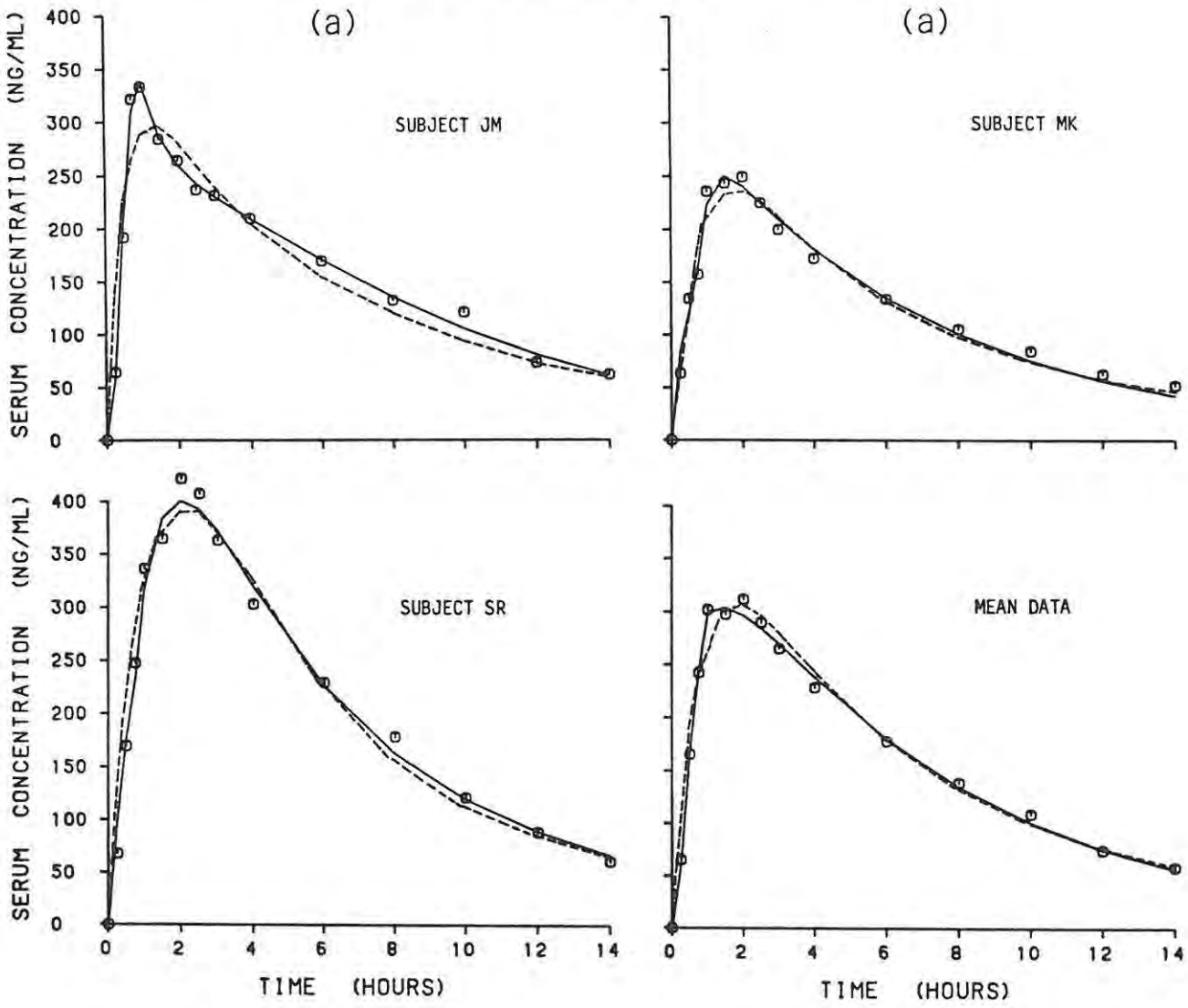
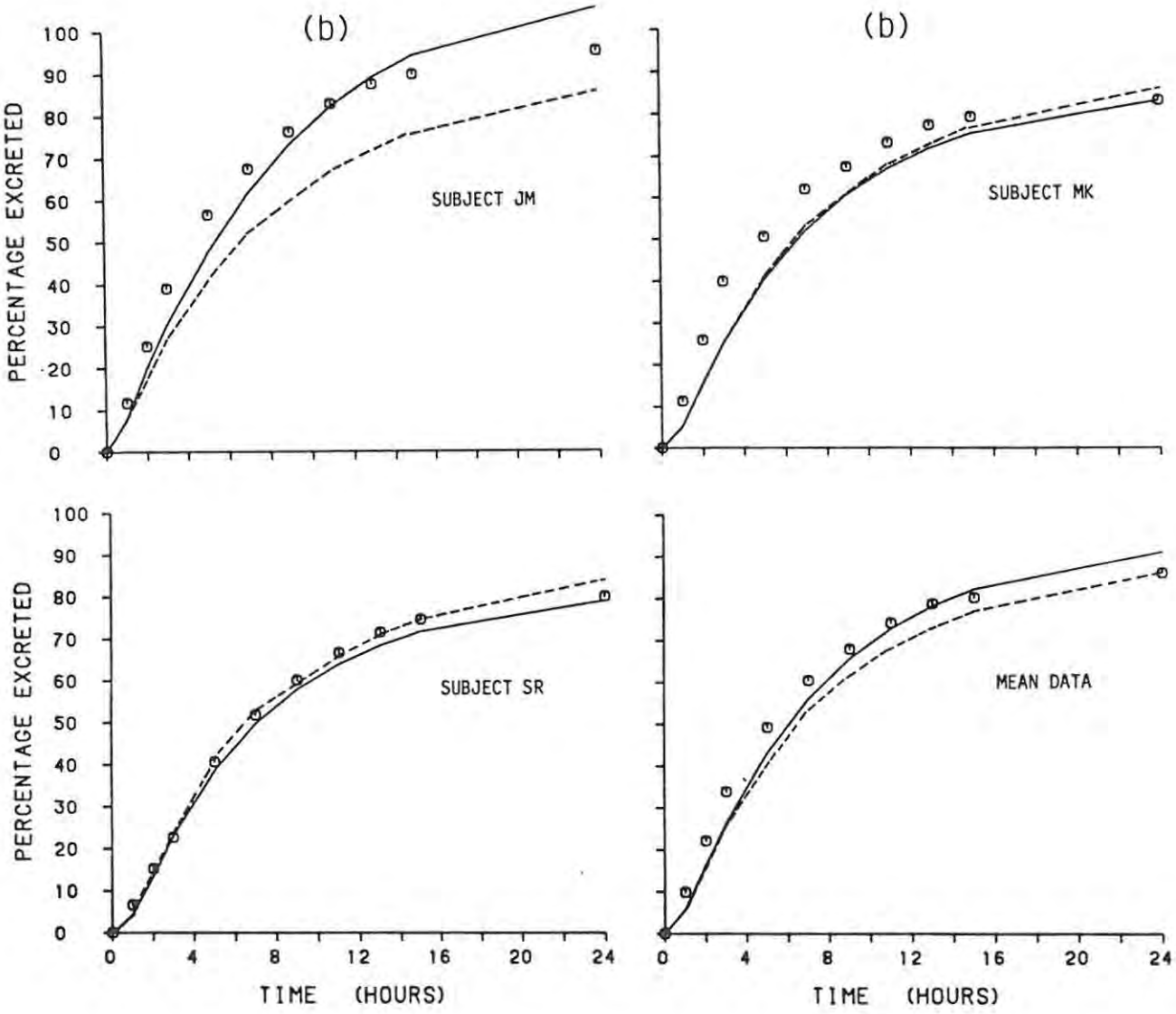


FIGURE 6.10 (continued)



6.7.4.8 Simultaneous Fitting of Data Sets

The simultaneous fitting of multiple data sets has been proposed, as statistically the results are more meaningful due to the increased degrees of freedom. The 50 and 100 mg serum and urine data were then fitted simultaneously using the model incorporating a 2BCM with 3 absorption rate constants and linear elimination. The observed serum and urine data and the predicted fits are shown in Figs 6.11 and 6.12.

FIGURE 6.11 Observed data and predicted fit to the 50 mg (a) serum and (b) urine data using model 2.
The predicted fits resulted from the simultaneous fitting of the 50 and 100 mg solution data

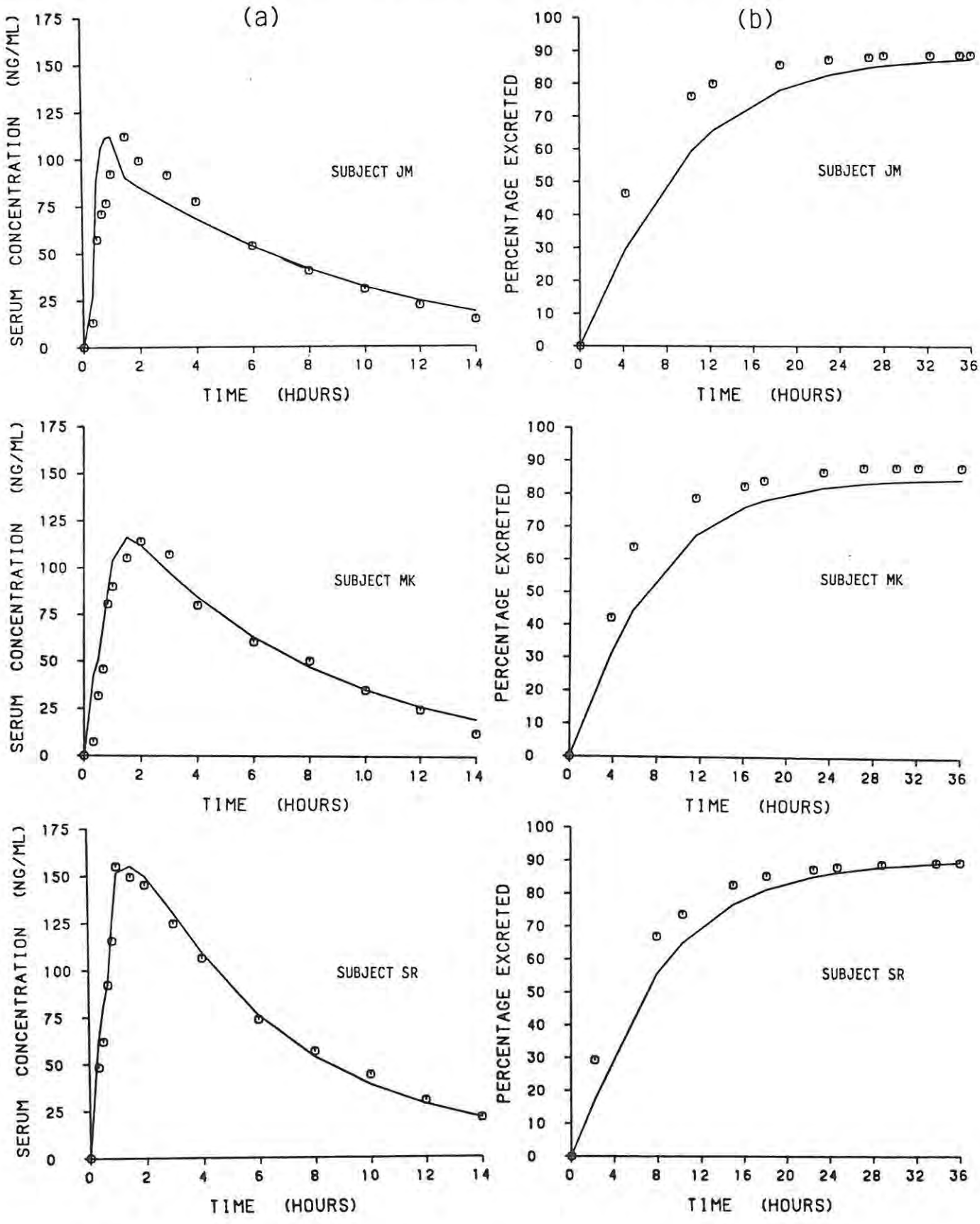
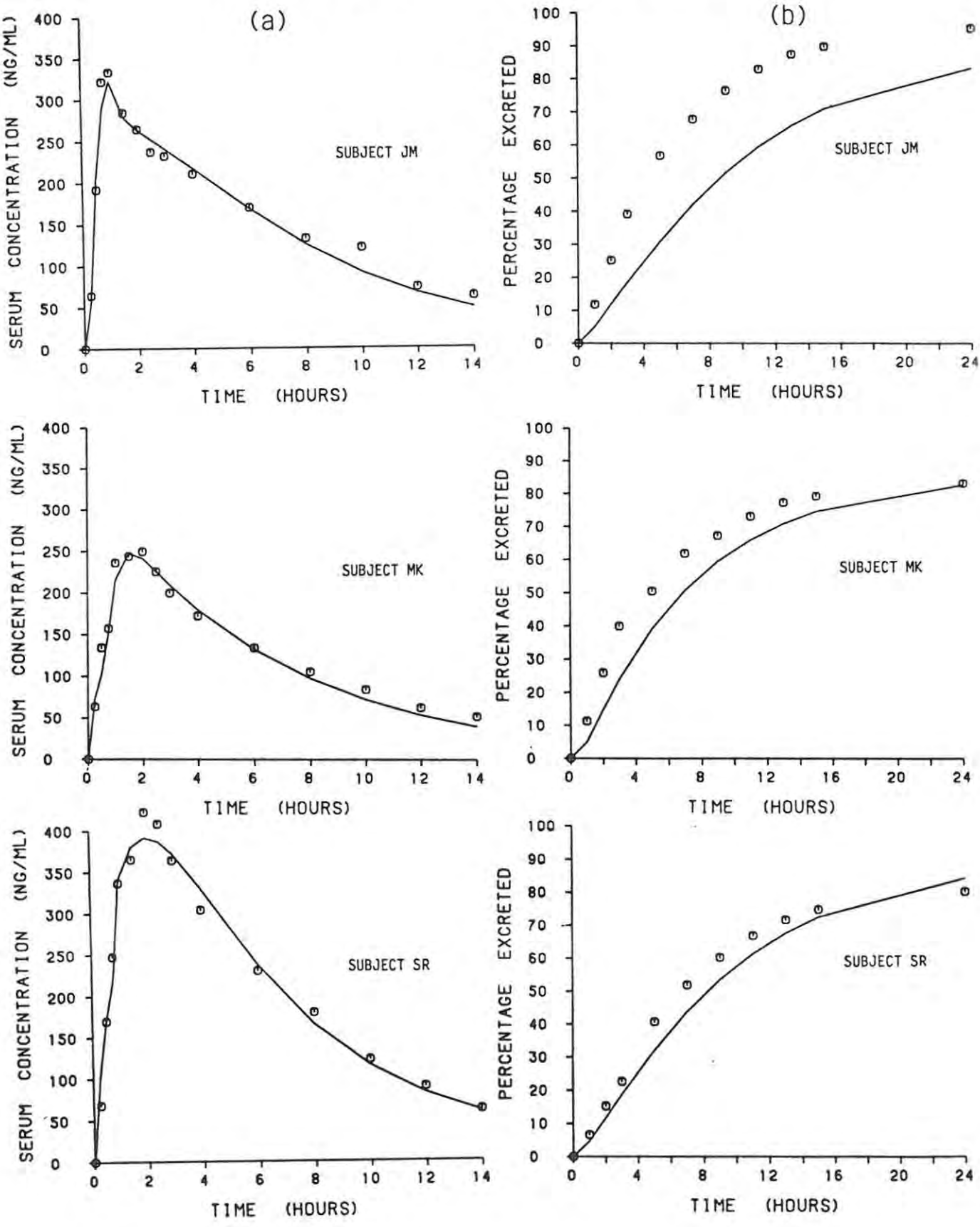


FIGURE 6.12 Observed data and predicted fits to the 100 mg (a) serum and (b) urine data using model 2.
The predicted fits resulted from the simultaneous fitting of the 50 and 100 mg solution data.



Problems arose in the simultaneous fitting of these data sets as the absorption for the 3 subjects differed considerably between studies. From the serum data for JM (Fig.5.1) it is apparent that absorption rates in the 2 solution studies differed widely, as did the times at which the different absorption rates appeared to occur. The main discrepancy in these fits occurred in the absorption phases and at the peak concentrations where predicted values were distorted in an attempt to account for both observed data sets. Values for r were smaller in all cases for the simultaneous fits than for the corresponding separate fits.

The advantages of fitting serum and urine data simultaneously have been considered (394). Fitting of cumulative urine data was advocated despite the smoothing tendency of integrated functions, as the calculation of a true measurement time for a urine sampling period is not very practical. Simultaneous fitting of serum and urine data again includes statistical considerations, with increased degrees of freedom. In some cases where the assay lacks sensitivity, fitting of the blood level data alone may not reveal certain important characteristics of the curve, whereas fitting with the urine data may reveal processes occurring when blood concentrations are too low to measure.

The serum data were then fitted separately and extremely good fits were obtained. However, as has been found previously (394) separate fits lead to very different parameter values from those obtained with simultaneous fitting. The best approach, therefore, would appear to be that of initial separate fits followed by simultaneous fits. This should reveal whether the proposed model is a satisfactory one. The parameter values from the simultaneous fits may then be considered as the final values.

6.7.5 Discussion on Pharmacokinetic Modelling

The problems of nonlinear regression analysis have been discussed at length (386). Complex nonlinear models are often characterised by response surfaces with multiple minima. Only the absolute minima of the response surface should be used to compare pharmaco-

kinetic models for the goodness of fit. The possibility exists, when fitting large numbers of parameters (>5) with this program, that a local minima may be reached and the program, judging this to be the best fit to the observed data, terminated there. This would result in inadequate parameter estimates, and goodness of fit criteria may not accurately describe the ability of the model to fit the observed data.

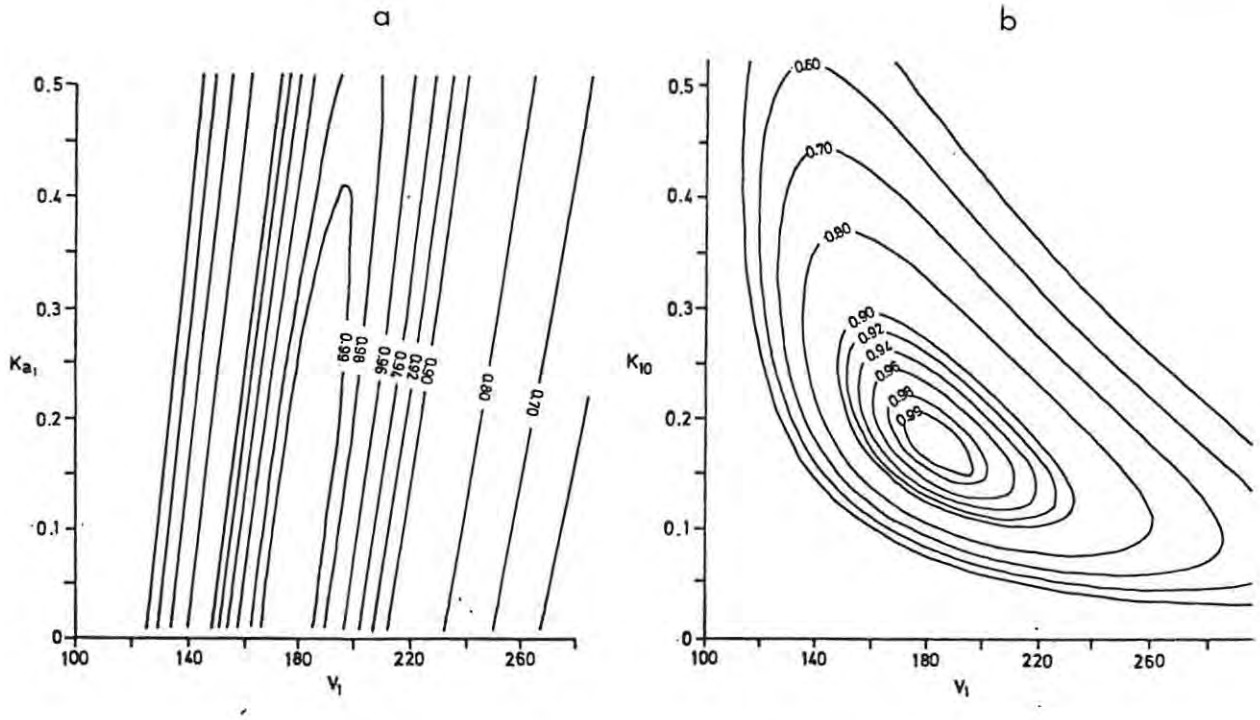
The understanding of any mathematical model is enhanced by a knowledge of the sensitivity of the model fitting criteria to changes in the size of the model parameters. Response surface diagrams are contour plots representing the variation in size of an objective function for a range of values of a pair of parameters. Such diagrams will reveal not only individual parameter sensitivity but also the level of interdependence between a pair of parameters. Low sensitivity is indicated by small changes in the value of the objective function over a range of values for a parameter. A high degree of interdependence between two parameters will result in a response surface which is aligned obliquely to the parameter axes, while surfaces aligned with one axis indicated low interdependence.

Fig.6.13 shows two response surfaces for the 2BCM model, with 3 absorption rate constants and linear elimination, where the mapped fitting criterion is the coefficient of efficiency (Eq.6-33).

$$E = 1 - \left[\frac{\sum_{i=1}^{n} (\text{observed}_i - \text{predicted})^2}{\sum_{i=1}^{n} (\text{observed}_i - \text{observed mean})^2} \right] \qquad (6\text{-}33)$$

While the surfaces for all possible pairs of the 7 parameters included in this model were computed, it was evident that the 3 absorption rate constants and 2 transfer rate constants (k_{12} and k_{21}) were relatively insensitive and had a low level of interdependence over the entire range of values considered. k_{10} however, was found to be far more sensitive but still showed a low interdependence with the other rate constants.

FIGURE 6.13 Response surface diagrams for the 2BCM, 3 k_a's and linear elimination, (a) k_{a_1} versus V_1 and (b) k_{10} versus V_1.



Volume was found to be an extremely sensitive parameter, although interdependence with all rate constants was weak, with this relationship remaining fairly consistent over the range of parameter values evaluated. The highest degree of interdependence was found between k_{10} and volume. This relationship was inconsistent with the k_{10} parameter being far more sensitive than V_1 at high values for volume. At lower volume values, however, sensitivity of V_1 increased with a concomitant substantial decrease in sensitivity of k_{10}.

Another possible source of error is the estimation of final parameter values for different sets of empirically obtained initial estimates (395). To check this, the 3 sets of 100 mg serum and urine data were fitted to a 2BCM with 3 k_a's and Michaelis-Menten elimination using 2 completely different sets of initial parameter estimates. In all cases, the fits were equally good and the final parameter estimates did not differ substantially from one another.

Weighting of data is a consideration when using mathematical models of this sort. The observations should be weighted inversely proportional to their variance estimates. If the variances are assumed to be constant, the weights should be 1, which would effectively mean unweighted data. Weighting of data should not be used in mathematical modelling to obtain a better fit; there must be a sound basis for the weighting used. Poland (394) reported some considerations in the weighting of data. In this study data were unweighted (W=1).

In the 50 mg solution study, a lag time was observed for some of the subjects. This was accounted for in the DFUNC subroutine resulting in good fits in the early absorption phase. The flexibility of the fit introduced by discontinuous absorption models makes these models more vulnerable to influences of experimental error than the more rigid alternative of continuous absorption. The ease of improving the fit merely by including another absorption segment supplements this problem, thus caution must be exercised in handling the data using this method. In the results presented here, each absorption profile was individually studied to ascertain the presence or absence of discontinuous absorption, and in every case an improved fit resulted when using more than one absorption rate constant.

CHAPTER 7

PHARMACOKINETIC ANALYSIS OF PRODUCT DATA

7.1 INTRODUCTION

The introduction onto the market of increasingly effective but potentially dangerous drugs emphasizes the need, firstly, to develope safe, effective and reliable dosage forms and secondly, to develop and apply *in vitro* drug tests that are rapid, convenient and reliable in reflecting the *in vivo* behaviour of a particular drug dosage form.

Numerous reports have shown that different formulations of the same drug, although conforming to compendial requirements, possess altered bioavailability characteristics which can only be detected by extensive *in vivo* testing. While compendial assay procedures may indicate that different products exhibit chemical and physical properties that are within acceptable limits, the rate and extent of absorption of the drug from the dosage form following oral administration may differ significantly from product to product (397). Alternately, perfectly acceptable blood profiles have been found for tablets which have failed to meet compendial requirements with no useful correlations being observed between the *in vitro* and *in vivo* results (398-401).

Before decisions concerning *in vitro-in vivo* correlations relative to the therapeutic efficacy of a drug can be made, rate limiting mechanisms operative in the gastrointestinal tract during the absorption process must be understood. The rate limiting step for determining rate and/or extent of absorption is often the release of drug from the dosage form into solution in the gastrointestinal fluids. In other cases, the rate limiting step may be the transport of drugs across the intestinal barrier as is the case with polar, water-soluble drugs. For some drugs, change in the total amount absorbed rather than in the rate of absorption may be reflected by differences in dissolution rate. Gastric emptying of the dissolved drug rather than the *in vivo* dissolution rate may govern the rate of absorption (402). The longer the biological

half-life, the less evident the effect of dissolution rate on the blood level profile (403)

Reports have suggested that *in vitro* to *in vivo* correlations can be improved through systematic adjustment of *in vitro* test conditions such as composition and physical properties of the dissolution medium, agitation or flow rates, dimensions or geometry of the apparatus, temperature, permeability of membranous barriers and sink conditions and related volume (404-407). Apart from the importance of *in vitro* dissolution testing in the quality control of pharmaceuticals, another objective of *in vitro* dissolution testing is to enable prediction of *in vivo* behaviour of a dosage form, as well as predicting the resulting blood concentrations following oral administration (405).

The majority of *in vitro* to *in vivo* correlations thus far have involved single and multiple point quantitative correlations. Dakkuri and Shah (402) published a fairly extensive review on correlation of *in vitro* rate of dissolution with *in vivo* bioavailability and Stricker (408) described different types and sources of error in the correlation of non-analagous data.

Linear correlations have been established for various drugs between *in vivo* serum/plasma data or parameters obtained from these data and *in vitro* dissolution data. Some examples include prednisone (409,410), digoxin (411-414), sulfadiazine in trisulfapyrimidine suspensions (415), warfarin (416), oxazepam (417), griseofulvin (418-420), spironolactone (421) and erythromycin (422). Plasma data have also been correlated with the logarithm of *in vitro* burst time of soft gelatin capsules (414) and with percent absorbed *in vitro* using an absorption simulator (423). Correlations have also been drawn between *in vivo* absorption data and *in vitro* dissolution data for drugs such as aspirin (424-427), warfarin (416), oxazepam (417) and aminorex (428).

Linearity has been found to exist between urinary excretion data and *in vitro* dissolution data for aspirin (429,430), salicylamide

(431), digoxin (413), chloramphenicol (432), methenamine (433), hydrochlorothiazide (434), reserpine (435) and nitrofurantoin (436). No useful correlations were established between *in vivo* excretion and *in vitro* dissolution for sulfasoxizole (437) and nitrofurantoin (397). Other *in vivo* data which have been correlated with *in vitro* dissolution data are clinical efficacy data for prednisone (438,439) and saliva levels for lithium carbonate (440).

Smolen (441,404) has described two approaches to enable the prediction of the time course of pharmacological response or concentration of the drug in body fluids from *in vitro* dissolution testing. In the first method (441) drug dissolution-time data are mathematically transformed into computationally predicted *in vivo* drug input or response output profiles using a previously determined weighting function. The second approach (404) involves the calibration of a computer-controlled continuous flow-through type of dissolution apparatus with *in vivo* bioavailability data. Once calibration is accomplished, *in vivo* pharmacological responses, blood concentrations, urinary recovery or input versus time profiles can be predicted for the same panel of human subjects from whom the *in vivo* data were obtained which were originally used to calibrate the apparatus.

Von Hattingberg and Brockmeier (442) and Voegele *et al.* (443) used the additivity of mean times as a method for *in vitro - in vivo* correlations. The additivity of mean times in a series of biopharmaceutical sub-systems defines the linear relationship between the mean time of *in vitro* dissolution and the mean time of the drug in the total *in vivo* system for a given pharmaceutical formulation. The mean *in vivo* dissolution time was used to predict total concentration-time curves.

In another report (444) dissolution profiles were shown to be superimposable by a simple linear transformation of the time base and an efficient method for testing the equivalence was proposed (445). The parameters used in the transformation of the time base

can be evaluated from the statistical moments of the residence times. The moments themselves are calculated from the cumulative frequency functions. The principles can be applied to the comparison of *in vitro* and *in vivo* dissolution profiles.

Riegelman and Upton (328) described the application of the Weibull equation to dissolution and absorption data and attempted to draw correlations between the *in vivo* and *in vitro* parameters. The advantages of employing statistical moments and Weibull parameters for establishing *in vitro-in vivo* correlations were investigated by Eckert and Erni (446).

7.2 RESULTS AND DISCUSSION

7.2.1 Bioavailability Parameters

Both products were sustained-release formulations, Product BS (150 mg PPA.HCl) being a tablet with a wax matrix core and Product DT (75 mg PPA.HCl) consisting of a capsule containing microencapsulated particles. The bioavailability parameters of the two formulations are shown in Table 7.1. A pronounced *in vivo* sustained-release effect was observed from the serum data of both products, as the t_{max} values were much larger than those from the solution studies and increased concentrations were sustained for a much longer period. The mean peak time for Product DT occurred almost 2 hours after that for BS and was less than half the concentration.

The elimination rate constant was calculated as before from the terminal slope of a semilog plot of concentration versus time. The values obtained were much lower than the corresponding values for the solution studies and are not an accurate reflection of the true elimination rate constant of the drug. This is possibly due to the fact that elimination is occurring while the drug is still being released over a prolonged period from the dosage form and is being absorbed into the body at a rate which is limited by the *in vivo* dissolution of the dosage form.

TABLE 7.1 Bioavailability parameters for Products BS and DT.

TEST DOSE	SUBJECT	PARAMETERS				
		t_{max} (hrs)	C_{max} (ng/ml)	AUC_∞ (ng/ml.hr)	λ_z (hr^{-1})	$t_{½}$ (hrs)
PRODUCT BS (150 mg PPA.HCl)	JM	5.00	215.58	3870.00	0.055	12.60
	MK	4.00	278.38	4214.07	0.071	9.76
	SR	4.00	315.43	5344.28	0.098	7.07
	PS	6.00	329.66	4251.49	0.092	7.53
	DD	4.00	223.34	3309.46	0.087	7.97
	MM	6.00	408.31	7957.80	0.064	10.83
	MEAN	4.83	295.12	4825.52	0.078	9.29
	±SD	0.98	72.36	1673.03	0.017	2.16
PRODUCT DT (75 mg PPA.HCl)	JM	6.00	120.79	1533.41	0.108	6.42
	MK	6.00	134.72	1616.15	0.128	5.41
	SR	8.00	127.02	2146.78	0.097	7.14
	PS	8.00	125.05	1451.91	0.169	4.10
	DD	5.00	134.72	1235.92	0.219	3.16
	MM	7.00	133.59	2036.90	0.110	6.30
	MEAN	6.00	129.32	1670.18	0.139	5.42
	±SD	1.21	5.88	351.99	0.047	1.52

With the solution, however, the total amount of drug is available for absorption immediately after ingestion. This results in a faster absorption rate, a sharper peak at the maximum and a larger value for λ_z. The elimination of drug from the body was more rapid following the ingestion of Product DT than the rate of elimination of Product BS. Consequently the half-lives varied between the products. Caution must be used in the calculation of λ_z as it is well known that in some cases the terminal slope of the semilog plot of drug concentration versus time following oral administration may not reflect the first order elimination rate constant. If absorption is rate limiting then the terminal slope approaches k_a (447), a situation known as the flip-flop phenomenon. However, Byron and Notari (448) demonstrated the frailty of the assumption that the slope reflects the absorption rate constant for both one and two compartment models.

Evidence for nonlinearity was observed in the AUC_∞ values. If the kinetics of PPA were assumed to be linear, then the predicted AUC_∞ value for BS would be approximately double that for DT. However,

AUC_∞ for BS was shown to be significantly greater than double AUC_∞ for DT. A possible over-estimation in AUC_∞ for BS could have occurred in the extrapolation of the terminal slope to infinity as no samples were collected between 12 and 24 hours and at the last sampling time (24 hours) serum concentrations were relatively high, with a mean of 68 ng/ml. The inclusion of extra sampling times between 12 and 24 hours may have had a noticeable effect on the λ_z value which would then, in turn, have affectd the AUC_∞ value.

7.2.2 Weibull Fits to Wagner-Nelson Absorption Plots

The serum concentration-time data from Products BS and DT were transformed using the Wagner-Nelson method assuming a one compartment model and linear kinetics to obtain the absorption rate plots which were subsequently fitted to the Weibull equation. The plots may be seen in Figs.7.1 and 7.2.

The absorption rate constants were calculated as before. Values of k_a for Product BS were:

JM - 0.478 hr^{-1}
MK - 0.335 hr^{-1}
SR - 0.311 hr^{-1}
PS - 0.295 hr^{-1}
DD - 0.758 hr^{-1}
MM - 0.481 hr^{-1}

Linearity could not be established for Product DT in the semilog plot of fraction remaining to be absorbed versus time, possibly as a result of the sustained-release mechanism of the capsule formulation. As was noted in the solution data, Subject DD again had the largest k_a value, which agrees with the short t_{max} of 5 hours as compared with the other t_{max} values for BS.

The Weibull function appeared to be robust enough to enable a good description of the absorption rate data, albeit with larger standard errors of the estimates than for *in vitro* dissolution rate data. The Weibull parameters obtained from the analysis of the data are shown in Tables 7.2 and 7.3. The mean values and the

FIGURE 7.1 Wagner-Nelson plots of serum concentration-time curves after administering Product BS. The solid curve represents the Weibull function fit to the data.

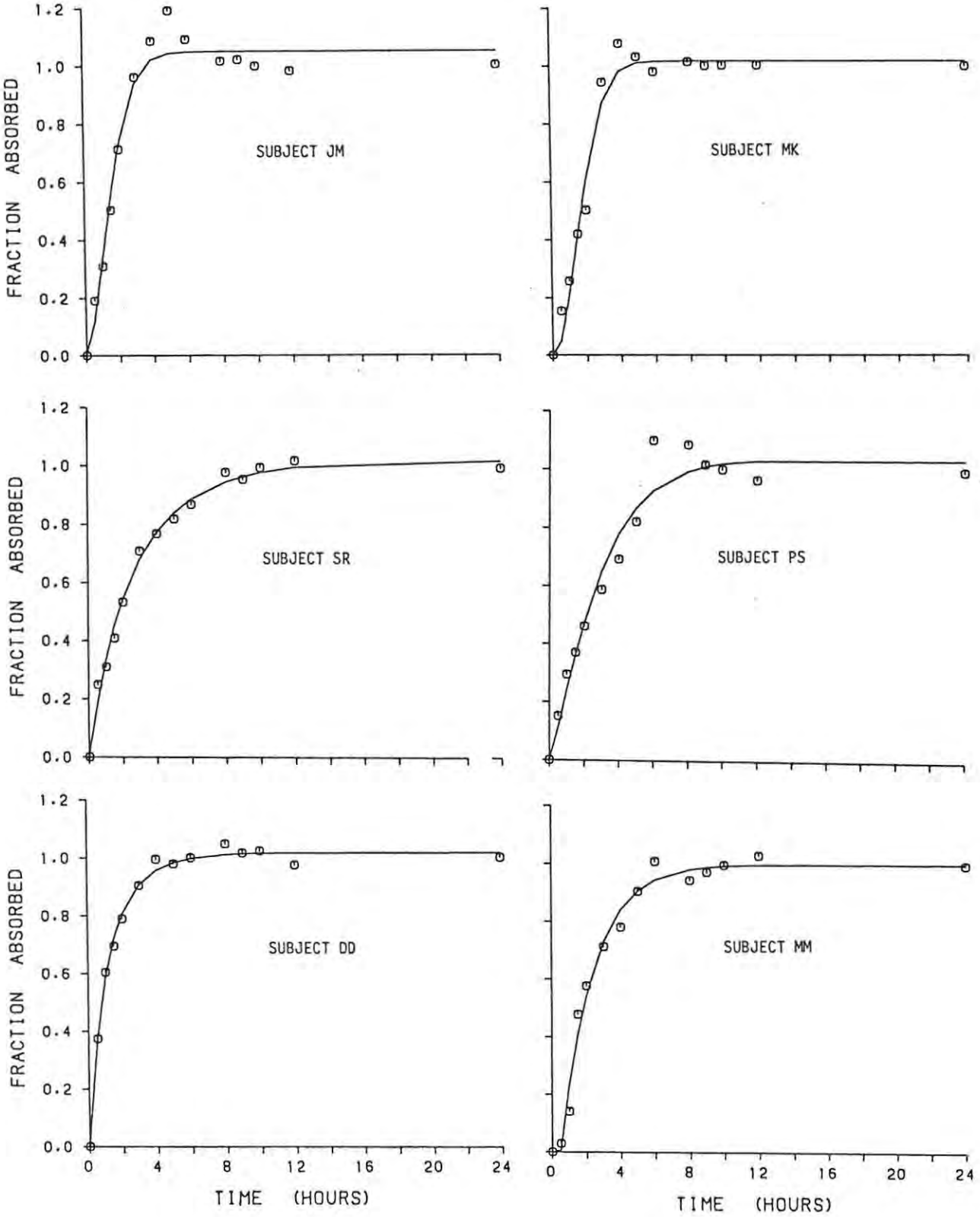
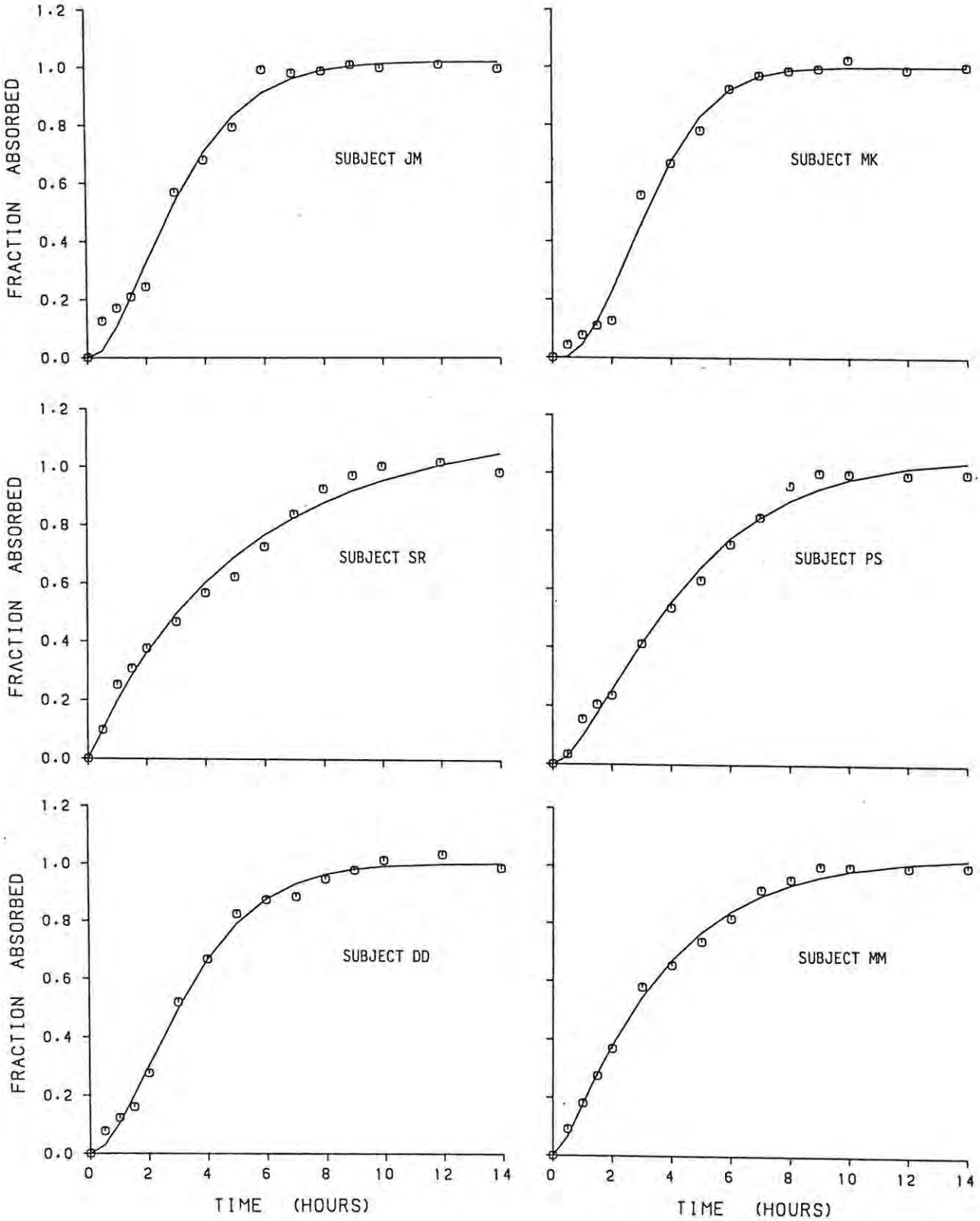


FIGURE 7.2 Wagner-Nelson plots of serum concentration-time curves after administering Product DT. The solid curve represents the Weibull function fit to the data.



coefficients of variation expressing interindividual differences are also listed in the tables. The lag time for absorption, t_o, was slightly larger for Product DT which is in agreement with the longer t_{max} of DT and also with the Weibull parameters obtained from the *in vitro* dissolution results.

TABLE 7.2 Weibull function analysis of absorption data for Product BS

PARAMETERS	SUBJECTS JM	MK	SR	PS	DD	MM	MEAN DATA[1]	MEAN OF PARAMETER[2]	CV %[3]
t_o	0.030	0.139	0.094	0.019	0.084	0.485	0.071	0.142	122.38
t_d	1.790	1.966	2.646	3.043	1.112	1.934	2.142	2.082	32.60
β	1.597	1.789	0.873	1.192	0.818	1.013	1.148	1.214	32.77
F^∞	1.052	1.017	1.023	1.042	1.019	1.000	1.009	1.026	1.82
r	0.975	0.981	0.993	0.967	0.995	0.986	0.998		

1 Weibull fit to mean data of the six subjects

2 Arithmetic mean of the fitted parameters from the six subjects

3 Interindividual differences expressed as coefficients of variation

TABLE 7.3 Weibull function analysis of absorption data for Product DT

PARAMETERS	SUBJECTS JM	MK	SR	PS	DD	MM	MEAN DATA[1]	MEAN OF PARAMETER[2]	CV %[3]
t_o	0.223	0.332	0.092	0.187	0.117	0.187	0.091	0.190	44.79
t_d	3.391	3.461	5.358	4.753	3.669	3.673	3.998	4.051	19.97
β	1.511	1.884	1.946	1.339	1.551	1.115	1.363	1.558	20.36
F^∞	1.019	1.001	1.151	1.058	1.000	1.039	1.023	1.044	5.43
r	0.984	0.988	0.982	0.991	0.994	0.995	0.997		

1 Weibull fit to mean data of the six subjects

2 Arithmetic mean of the fitted parameters from the six subjects

3 Interindividual differences expressed as coefficients of variation

The time for 63.2% of the drug to be absorbed *in vivo*, t_d, was markedly less from BS. This was again in accordance with the t_d values for *in vitro* dissolution which were 5.918 hours (Product BS) and 8.744 hours (Product DT). A slight increase in sigmoidicity was observed from the β value for Product DT, although both β values were greater than 1. The infinity value, F^∞, obtained from the analysis of absorption rate plots, is not a true estimate of bioavailability since it is expressed relative to the AUC for that preparation. The values for both products were close to 1.000.

Riegelman and Upton (328) compared plots of the Weibull fit to the mean absorption data of a theophylline preparation, to the curve generated from the mean parameters obtained from 5 individual curves. The latter curve began to deviate above 60% absorption and displayed a more rapid apparent absorption than is seen in the curve derived from the mean absorption data. This is a phenomenon common to all averaged *in vivo* and *in vitro* data and therefore caution must be exercised in interpreting mean data.

7.2.3 Moment Analysis

In vitro dissolution and *in vivo* drug release followed by the absorption of the drug are complex kinetic processes. Prior to the *in vivo* absorption of released drug, the tablet has to be wetted, and, if the tablet is coated or the drug is formulated as a capsule, the shell must disintegrate to allow dissolution of the dispersed drug into gastrointestinal contents.

Disintegration and dispersion into granules and particles are influenced by conditions in the mucous environment of the stomach such as pH, surface tension, viscosity, the volume of the fluids and gastric motility. Stomach contents also affect gastric emptying and delivery of the drug to the intestine which is the main absorption site. The particles may be coated with a mucous film which could change the diffusion layer thickness. All these complexities occurring in the dissolution and absorption processes create difficulties in attempting to characterise these processes with a specific zero or first order equation. Model independent methods such as the Moment Analysis method are therefore preferred to methods in which a specific model is assumed.

A mean residence time, MRT, can theoretically be calculated for any concentration-time curve, even one displaying saturable kinetics. However, unless linear kinetics apply, the subsequent interpretation of the data is somewhat limited. The MRT and MAT are calculated as follows:

$$MRT = \frac{\int_0^\infty t C_p \, dt}{\int_0^\infty C_p dt} = \frac{AUMC_\infty}{AUC_\infty} \qquad (7\text{-}1)$$

$$MAT = MRT - \frac{1}{\lambda_z} \qquad (7\text{-}2)$$

The administration of a drug formulated as a suspension, a capsule or a tablet involves an *in vivo* dissolution step prior to the absorption of the drug from solution. The mean *in vivo* dissolution time for a solid dosage form (MDT_{prod}) enables the contribution of the dissolution process to be separated from the apparent absorption and disposition processes and may be estimated as follows:

$$MDT_{prod} = MAT_{prod,uncorr} - MAT_{soln,uncorr} \qquad (7\text{-}3)$$

where $MAT_{prod,\,uncorr}$ and $MAT_{soln,\,uncorr}$ are the uncorrected mean absorption times for the product and the solution of the drug respectively.

Results of moment analyses of the product data are shown in Table 7.4. The efficacy of the sustained-release formulation as shown by MRT values was more pronounced for BS than for DT. In all except one subject, the MRT for Product BS was longer than that for DT, with the mean value for BS exceeding that for DT by almost 4 hours. The longest transit time of the drug through the body was encountered in Subject JM. This can be partially explained by the low elimination rate of the drug in this subject. Conversely, Subject DD has the largest λ_z for Product DT and the shortest MRT.

TABLE 7.4 MRT, MAT and MDT of Product BS and DT

		SUBJECTS						
	PRODUCT	JM	MK	SR	PS	DD	MM	MEAN ± SD
MRT	BS	19.214	15.741	12.672	13.350	12.730	18.007	15.286 ± 2.834
	DT	12.337	11.205	14.013	9.917	7.945	12.421	11.306 ± 2.139
MAT	BS	1.032	1.656	2.468	2.480	1.236	2.382	1.876 ± 0.655
	DT	3.078	3.392	3.704	4.000	3.379	3.330	3.481 ± 0.323
MDT	BS	0.319	0.553	1.865	1.737	0.721	1.600	1.131 ± 0.674
	DT	2.365	2.289	3.092	3.257	2.864	2.548	2.736 ± 0.397

From the t_{max} values for Products BS (4.8 hours) and DT (6.7 hours) it was evident that BS was absorbed more rapidly and consequently a shorter MAT would be predicted for the product. The MAT for DT was found to be almost double that for BS with JM exhibiting the shortest MAT for both products.

The analysis of the MDT may be used to determine whether or not the absorption of a solid dosage form of a drug is dissolution rate limited, by determining any significant differences between MAT_{prod} and MAT_{soln} . If no difference is detected, the dosage form probably dissolves in the gastric fluids at a rapid rate, significantly faster than the subsequent absorption step. Where dissolution is a rate limiting step, as is the case with Products BS and DT, evaluation of the MDT gives some indication of the *in vivo* equivalent to the time required *in vitro* for 63.2% of the drug to dissolve. This method of estimation of MDT *in vivo* is independent of the extent of bioavailability of the product.

From the *in vitro* dissolution results, a lag time was observed for Product DT, whereas BS started releasing PPA almost immediately on immersion into the dissolution medium, which is concordant with the *in vivo* MDT values. These values were substantially larger for DT than for BS and could be attributed to the capsule formulation of DT as the gelatin capsule initially had to disintegrate to enable dispersion of the microencapsulated particles. These in turn had to dissolve before release of PPA could occur.

The use of moment analysis is subject to many potential errors, with the risk of error increasing with the magnitude of the extrapolation required (362). If sampling time is extended, the precision and accuracy of the assay must be carefully evaluated, particularly at the low drug concentrations. However, unless appropriate data are available and log linearity in the terminal phase is assured to allow accurate calculation of λ_z, results from moment analyses are unreliable and open to challenge.

7.2.4 Correlation of Dissolution and Absorption Profiles

The correlation of non-analagous data has been shown to be of limited value and contain many sources of error (408). In order, therefore, to establish a meaningful relationship between *in vitro* and *in vivo* data, the most analagous data obtainable should be used.

Riegelman and Upton (328) reported on the use of the Weibull equation to describe the *in vivo* absorption process and the *in vitro* dissolution process. The mean absorption or dissolution time for processes which may be described by the Weibull equation occur when 63.2% of the process is complete. However, distribution describing complex sustained-release products may exhibit a mean time intermediate between 50 and 63.2%. A zero order process will have the mean at 50%. Correlations were drawn between *in vivo* and *in vitro* lag times (t_0) and mean absorption or dissolution times (t_d). Correlations from parameters of individually fitted data were far superior to those correlations obtained using fits to mean data, again indicating the drawbacks inherent in using mean data.

The evaluation of the MDT from *in vivo* studies where the dissolution step is a significant component in the process enables the resultant *in vivo* parameter value to be compared with the *in vivo* derived time for 63.2% of the drug to dissolve. Von Hattingberg *et al.* (442) described a method of calculating MDT *in vitro* from dissolution curves:

$$MDT_{in\ vitro} = AUC/D \qquad (7\text{-}4)$$

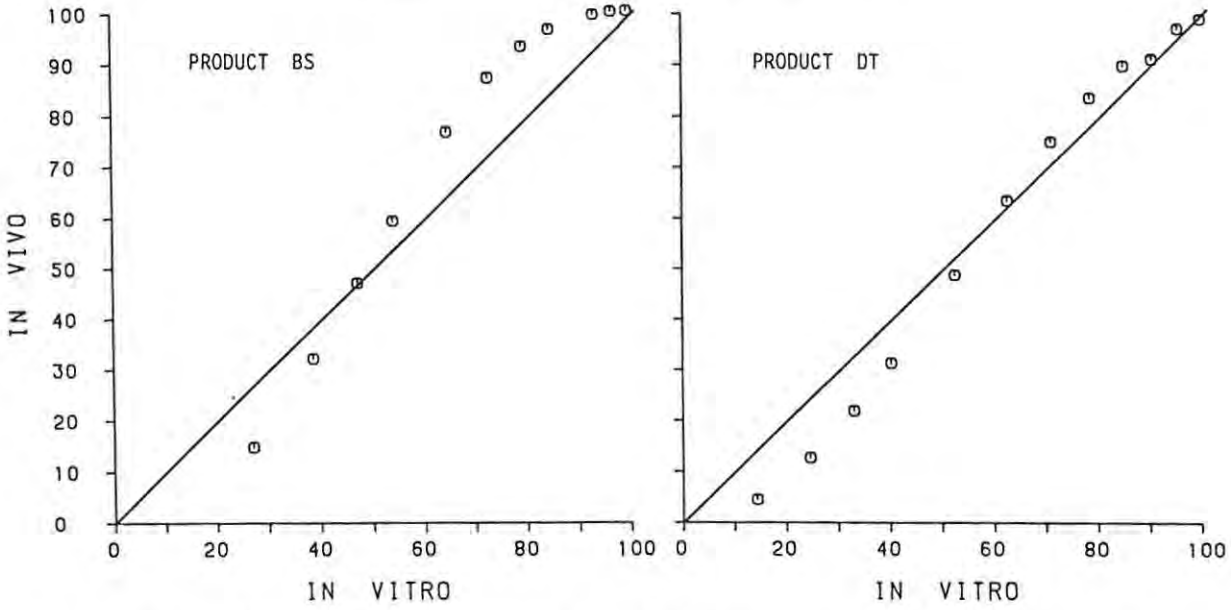
This yields values corresponding to MDT *in vitro* for first and zero order dissolution as calculated by the method of Riegelman and Collier (364).

Collier (449) found that when the shape parameter, β, was equal to 1 or 2, the percentage dissolution at MDT *in vivo* corresponded well with that at MDT *in vitro*, with minor differences being explained from measurement of AUMC and AUC. Poor correlation

resulted at $\beta = 0.5$ due to a longer mean dissolution time (t_d) when an increased risk of a flip-flop situation develops and a change in clearance between doses can no longer be compensated for.

Eckert and Erni (446) correlated the Weibull function fit to *in vitro* and *in vivo* data over the total time period and showed 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.Similar curves were plotted, using the Weibull fits to *in vitro* and *in vivo* data from Products BS and DT (see Fig.7.3).

FIGURE 7.3 Correlation of the Weibull function for *in vitro* and *in vivo* data over the total time period



The curve for Product DT agreed with this assumption as the curve from correlated data crossed the straight line at 63% for both *in vivo* and *in vitro* data. In this case, t_d is the best parameter to use for analagous correlations. However, the curve for Product BS appeared to cross the straight line at a value of about 49%. At 63% absorption *in vivo*, only approximately 58% of the drug was dissolved *in vitro*, thus t_d would not give a good indication of correlation of the data. Data from at least 4 dosage forms are necessary to establish *in vivo-in vitro* correlations. However, in

this study limited data were available as only 2 dosage forms were studied, thus no meaningful conclusions could be drawn.

The dissolution rates of Product BS and DT were shown to have an effect on the *in vivo* absorption of the drug. These differences were evident from the Wagner-Nelson plots and from the bioavailability parameters such as t_{max}, C_{max}, MAT, MRT and t_d. However, the unusually low dissolution rate of Product DT, from which only 52% PPA.HCl was released over the entire 10 hours of the study was not reflected in the *in vivo* bioavailability of the drug. Although absorption *in vivo* was only completed in 10 hours as compared with 6 hours for Product BS, the percentage recovery in the urine after 24 hours was 10% higher for DT than for BS. It appears, therefore, that the decreased *in vitro* dissolution of the drug did not affect the *in vivo* dissolution of the drug and subsequent absorption into the body.

CHAPTER 8

DISCUSSION AND CONCLUSIONS

There is a noticeable lack of pharmacokinetic studies on PPA reported in the literature, possibly due to the difficulties inherent in the determination of PPA especially at the low concentrations encountered in the blood following administration of therapeutic doses. The extraction method which was developed and the subsequent direct determination of PPA by HPLC and UV detection without the relatively time-consuming and expensive procedure of derivatization makes this method readily applicable to pharmacokinetic studies of the drug.

A large difference in dosage size of phenylpropanolamine exists when the drug is used as a decongestant (25 mg three times a day) or as an appetite suppressant (100 - 150 mg daily). Throughout the course of this study, an increasing number of papers appeared on the undesirable side effects when used as an anorexigenic. In many letters and editorial comments the continued use of PPA without restrictions has been questioned. However, the results of a recent well-controlled study on the hypertensive effects of high doses of PPA showed that the drug had no significant effect on the blood pressure (450). Blood pressure was monitored throughout the trials undertaken in this study and no significant increases were reported.

A number of methods of calculating absorption rates have been previously discussed and a few of the techniques have been applied to the data obtained in the *in vivo* trials. The use of noncompartmental methods, which does not require the assumption of a particular compartment model, has grown in popularity as there has been a shift away from the curve-fitting of data on a computer after the assumption of a certain model. Results obtained from noncompartmental methods are more physiologically significant than those results obtained from methods using empirically derived models.

Statistical moment analysis was applied to the dosage form data and results yielded pertinent information on the *in vivo* dissolution and absorption of PPA from the two sustained-release formulations. The tablet, Product BS, had a much faster *in vivo* dissolution time than DT and consequently was absorbed much more rapidly, although the transit time of PPA in this formulation through the body was much longer. Product DT, the capsule, took longer to dissolve in the body and was absorbed more slowly, but it appeared to exhibit a superior sustained-release effect, with no sharp peaks in the serum concentrations as had occurred in some subjects for Product BS.

The release of PPA from sustained-release dosage forms *in vitro* was determined using a modified USP rotating paddle apparatus. Although 4 sustained-release dosage forms were tested in this way, only 2 were chosen for bioavailability trials, Product DT being chosen on the basis of its extremely low *in vitro* dissolution. In the *in vivo* trials, DT showed no evidence of unusually low bioavailability and the sustained-release effect and serum concentration profile were satisfactory. An average of 83% of the drug was recovered in the urine, providing conclusive evidence that the capsules, although exhibiting poor *in vitro* dissolution characteristics, dissolved readily *in vivo*, enabling complete absorption of PPA.

Bioavailability parameters were calculated from both the *in vivo* solution and product data. Subject DD consistently displayed a rapid absorption rate, generally associated with a faster elimination rate than occurred in other subjects. Subject DD was of an average age and weight for this study and the discrepancies can only be attributed to individual variation. The serum AUC_∞ values calculated for subjects SR and MM were, in all trials, relatively high. Both volunteers were small in stature (MM was the only female) and had the lowest body weights, thereby possibly accounting for this phenomenon.

Serum AUC_{∞} values did not appear to increase in proportion to the increasing dose administered for all 4 trials. This deviation from linearity was also noted in the C_{max} values. Renal clearance of the drug after administration of the solutions was also investigated. Contrary to a relatively constant renal clearance which would normally be expected throughout a trial, the renal clearance fluctuated , with higher values occurring at higher serum concentrations of the drug. The average renal clearance decreased when the dose was increased from 50 to 100 mg of the drug.

Unfortunately, no reliable protein binding data appear in the literature for PPA , protein binding of drug being a phenomenon which could account for higher clearance values at increased concentrations. The lower overall clearance for the 100 mg study could be due to the saturation of an enzyme system involved in the elimination of PPA. As the renal clearance exceeds the glomerular filtration rate, active tubular secretion is a possible mechanism by which additional PPA may be excreted. Since most of a given oral dose of PPA is recovered unchanged in the urine, hepatic contributions are unlikely.

PPA is an α-mimetic with a vasocoactive effect, therefore it is likely that vasoconstriction in the renal arteries caused by PPA may result in a reduced renal blood flow. As PPA has a relatively high extraction ratio, a decrease in renal blood flow could account for a decreased renal clearance, which would be more noticeable at higher concentrations of the drug (451).

The solution data were fitted to compartmental models which were characterised by a series of differential equations. The inability of the 1BCM to describe the kinetics of PPA in the body was immediately apparent. The use of a two 2 compartment model improved characterisation of the curve in the distribution phase, immediately after the maximum concentration. However, the absorption phase was still poorly characterised and the predicted peak was far lower than the experimentally observed value.

The incorporation of a discontinuous absorption phase into the model was discussed and appeared to enable a more accurate characterisation of the absorption phase. Elimination via linear as well as nonlinear (Michaelis-Menten) kinetics was studied. The elimination of both the 50 and 100 mg solution was equally well described by both processes.

A major drawback in these studies was the lack of intravenous data. The possibility of undertaking an intravenous study was investigated, but permission could not be obtained from the Medicines Control Council of South Africa. In addition to this, lack of back-up facilities added to the risks inherent in a trial of this sort.

The availability of intravenous data would enable the calculation of the absolute bioavailability of the drug, the volume of distribution, and the total clearance from the body. An intravenous serum concentration versus time profile would also allow improved characterisation of the 2BCM. In the computer modelling, far more accurate initial estimates would be available for the parameters to be optimised and volume could be incorporated as a constant, decreasing the number of parameters to be optimised. The parameter values which were obtained from the computer fits have no real physiological significance and may not be applicable for use in predicting serum concentrations following oral administration of the drug.

The existence of nonlinear mechanisms for the elimination of PPA could unfortunately not be confirmed. The most practical approach to investigate the existence of nonlinearity would be to use animal models. The intravenous administration of different doses of the drug and the application of the various tests for nonlinearity discussed previously would probably shed more light on the pharmacokinetics of PPA.

TABLE A1.1 Dissolution results from Product BS using the rotating basket apparatus

TIME (HRS)	PRODUCT BS						MEAN ± SD
	BS-1	BS-2	BS-3	BS-4	BS-5	BS-6	
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000 ± 0.0000
0.02	0.025	0.025	0.033	0.015	0.039	0.017	0.026 ± 0.0092
0.03	0.058	0.043	0.058	0.046	0.048	0.042	0.049 ± 0.0072
0.07	0.083	0.078	0.095	0.073	0.082	0.075	0.081 ± 0.0079
0.10	0.100	0.091	0.112	0.095	0.100	0.090	0.098 ± 0.0081
0.13	0.117	0.104	0.130	0.109	0.121	0.102	0.114 ± 0.0110
0.17	0.126	0.112	0.138	0.121	0.131	0.116	0.124 ± 0.0097
0.21	0.144	0.142	0.153	0.139	0.145	0.134	0.143 ± 0.0064
0.27	0.158	0.151	0.167	0.153	0.162	0.152	0.157 ± 0.0064
0.33	0.183	0.167	0.191	0.179	0.185	0.177	0.180 ± 0.0082
0.42	0.205	0.189	0.211	0.193	0.206	0.194	0.200 ± 0.0088
0.50	0.225	0.204	0.235	0.222	0.230	0.214	0.222 ± 0.0112
0.75	0.279	0.256	0.286	0.279	0.288	0.273	0.277 ± 0.0115
1.00	0.365	0.312	0.348	0.331	0.339	0.314	0.335 ± 0.0204
1.50	0.416	0.392	0.419	0.410	0.424	0.394	0.409 ± 0.0133
2.00	0.472	0.455	0.489	0.469	0.484	0.457	0.471 ± 0.0138
3.00	0.552	0.538	0.574	0.559	0.578	0.536	0.556 ± 0.0177
4.00	0.618	0.601	0.627	0.601	0.620	0.581	0.608 ± 0.0169
5.00	0.662	0.637	0.674	0.641	0.649	0.629	0.649 ± 0.0167
6.00	0.694	0.709	0.717	0.679	0.685	0.666	0.692 ± 0.0190
7.00	0.724	0.741	0.756	0.712	0.727	0.691	0.725 ± 0.0226
8.00	0.737	0.768	0.765	0.743	0.743	0.738	0.749 ± 0.0138
9.00	0.776	0.792	0.773	0.761	0.774	0.740	0.769 ± 0.0175
10.00	0.788	0.823	0.817	0.804	0.786	0.760	0.796 ± 0.0232

TABLE A1.2 Dissolution results from Product BS using the rotating paddle apparatus with a perspex dosage form holder

TIME (HRS)	PRODUCT BS						MEAN ± SD
	BS-1	BS-2	BS-3	BS-4	BS-5	BS-6	
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000 ± 0.0000
0.02	0.055	0.037	0.039	0.057	0.039	0.036	0.044 ± 0.0095
0.03	0.059	0.055	0.060	0.076	0.068	0.063	0.064 ± 0.0075
0.07	0.088	0.076	0.086	0.096	0.078	0.085	0.085 ± 0.0072
0.10	0.092	0.088	0.098	0.110	0.095	0.097	0.097 ± 0.0075
0.13	0.107	0.104	0.106	0.122	0.108	0.110	0.110 ± 0.0064
0.17	0.120	0.112	0.116	0.133	0.116	0.126	0.121 ± 0.0077
0.21	0.134	0.126	0.135	0.148	0.128	0.140	0.135 ± 0.0081
0.27	0.147	0.134	0.151	0.166	0.136	0.154	0.148 ± 0.0119
0.33	0.182	0.169	0.177	0.189	0.165	0.177	0.177 ± 0.0087
0.42	0.194	0.178	0.198	0.205	0.192	0.200	0.195 ± 0.0093
0.50	0.217	0.204	0.216	0.230	0.200	0.221	0.215 ± 0.0111
0.75	0.273	0.255	0.279	0.287	0.264	0.283	0.274 ± 0.0121
1.00	0.312	0.305	0.334	0.331	0.306	0.335	0.321 ± 0.0143
1.50	0.413	0.379	0.411	0.404	0.378	0.411	0.399 ± 0.0164
2.00	0.482	0.440	0.463	0.457	0.437	0.471	0.458 ± 0.0175
3.00	0.545	0.537	0.550	0.535	0.516	0.557	0.540 ± 0.0143
4.00	0.606	0.588	0.610	0.595	0.583	0.629	0.602 ± 0.0168
5.00	0.646	0.650	0.650	0.639	0.635	0.685	0.651 ± 0.0178
6.00	0.689	0.703	0.672	0.679	0.673	0.706	0.687 ± 0.0149
7.00	0.721	0.731	0.737	0.726	0.716	0.753	0.731 ± 0.0132
8.00	0.750	0.770	0.756	0.747	0.779	0.775	0.763 ± 0.0136
9.00	0.767	0.810	0.765	0.771	0.804	0.795	0.785 ± 0.0200
10.00	0.805	0.826	0.792	0.797	0.817	0.822	0.810 ± 0.0139

TABLE A1.3 Dissolution results from Product BS using the rotating paddle apparatus with a wire basket.

TIME (HRS)	PRODUCT BS						MEAN ± SD
	BS-1	BS-2	BS-3	BS-4	BS-5	BS-6	
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000 ± 0.0000
0.02	0.020	0.034	0.021	0.036	0.031	0.033	0.029 ± 0.0069
0.03	0.036	0.068	0.037	0.080	0.070	0.071	0.060 ± 0.0189
0.07	0.078	0.091	0.087	0.107	0.095	0.090	0.091 ± 0.0096
0.10	0.086	0.101	0.102	0.108	0.105	0.107	0.101 ± 0.0081
0.13	0.107	0.108	0.109	0.120	0.114	0.112	0.112 ± 0.0048
0.17	0.127	0.130	0.112	0.131	0.122	0.123	0.124 ± 0.0070
0.21	0.130	0.145	0.130	0.143	0.146	0.138	0.139 ± 0.0073
0.27	0.139	0.153	0.136	0.164	0.151	0.144	0.148 ± 0.0103
0.33	0.156	0.168	0.158	0.188	0.173	0.167	0.168 ± 0.0116
0.42	0.174	0.184	0.179	0.199	0.185	0.179	0.183 ± 0.0086
0.50	0.189	0.199	0.187	0.218	0.204	0.197	0.199 ± 0.0113
0.75	0.217	0.243	0.228	0.253	0.245	0.239	0.238 ± 0.0130
1.00	0.253	0.286	0.251	0.291	0.280	0.264	0.264 ± 0.0172
1.50	0.304	0.347	0.356	0.354	0.355	0.327	0.341 ± 0.0209
2.00	0.413	0.407	0.413	0.395	0.379	0.372	0.397 ± 0.0177
3.00	0.498	0.508	0.518	0.556	0.537	0.521	0.523 ± 0.0208
4.00	0.582	0.543	0.542	0.580	0.582	0.585	0.570 ± 0.0211
5.00	0.609	0.582	0.575	0.658	0.634	0.639	0.616 ± 0.0332
6.00	0.629	0.614	0.597	0.699	0.685	0.670	0.649 ± 0.0414
7.00	0.650	0.662	0.666	0.714	0.688	0.695	0.679 ± 0.0239
8.00	0.723	0.679	0.685	0.735	0.724	0.726	0.712 ± 0.0237
9.00	0.745	0.741	0.750	0.772	0.765	0.746	0.753 ± 0.0124
10.00	0.768	0.760	0.764	0.794	0.801	0.763	0.775 ± 0.0178

TABLE A1.4 Dissolution results from Product LC using the rotating paddle apparatus with a wire basket.

TIME (HRS)	PRODUCT LC						MEAN ± SD
	LC-1	LC-2	LC-3	LC-4	LC-5	LC-6	
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000 ± 0.0000
0.02	0.000	0.000	0.000	0.000	0.000	0.000	0.000 ± 0.0000
0.03	0.003	0.003	0.003	0.005	0.000	0.008	0.004 ± 0.0024
0.07	0.017	0.013	0.013	0.018	0.022	0.023	0.016 ± 0.0043
0.10	0.031	0.026	0.023	0.034	0.028	0.032	0.029 ± 0.0041
0.13	0.042	0.029	0.033	0.044	0.038	0.045	0.039 ± 0.0064
0.17	0.052	0.037	0.048	0.049	0.049	0.054	0.048 ± 0.0059
0.21	0.065	0.045	0.049	0.061	0.057	0.065	0.057 ± 0.0084
0.27	0.103	0.057	0.065	0.075	0.069	0.086	0.076 ± 0.0165
0.33	0.104	0.078	0.091	0.103	0.094	0.108	0.096 ± 0.0110
0.42	0.134	0.110	0.110	0.130	0.115	0.139	0.123 ± 0.0129
0.50	0.158	0.125	0.137	0.149	0.139	0.163	0.145 ± 0.0142
0.75	0.227	0.203	0.226	0.233	0.222	0.246	0.226 ± 0.0141
1.00	0.345	0.326	0.317	0.326	0.306	0.361	0.330 ± 0.0132
1.50	0.542	0.434	0.448	0.474	0.439	0.532	0.478 ± 0.0477
2.00	0.702	0.570	0.603	0.599	0.589	0.669	0.622 ± 0.0516
3.00	0.913	0.766	0.821	0.805	0.784	0.867	0.826 ± 0.0549
4.00	1.043	0.903	0.927	0.919	0.877	0.987	0.942 ± 0.0612
5.00	1.060	0.962	1.010	0.002	0.985	1.035	1.009 ± 0.0350
6.00	1.110	1.004	1.075	1.037	0.998	1.098	1.054 ± 0.0478
7.00	1.142	1.036	1.092	1.074	1.007	1.122	1.079 ± 0.0511
8.00	1.163	1.039	1.108	1.080	1.027	1.138	1.093 ± 0.0540
9.00	1.172	1.042	1.120	1.092	1.012	1.140	1.096 ± 0.0605
10.00	1.179	1.056	1.132	1.106	1.035	1.146	1.109 ± 0.0549

TABLE A1.5 Dissolution results from Product CO using the rotating paddle apparatus with a wire basket.

TIME (HRS)	PRODUCT CO						MEAN ± SD
	CO-1	CO-2	CO-3	CO-4	CO-5	CO-6	
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000 ± 0.0000
0.02	0.002	0.006	0.000	0.002	0.001	0.002	0.002 ± 0.0020
0.03	0.027	0.038	0.051	0.012	0.047	0.041	0.036 ± 0.0144
0.07	0.202	0.134	0.160	0.237	0.228	0.203	0.194 ± 0.0398
0.10	0.261	0.199	0.225	0.238	0.271	0.243	0.240 ± 0.026
0.13	0.275	0.214	0.263	0.282	0.299	0.268	0.267 ± 0.0288
0.17	0.292	0.226	0.278	0.301	0.302	0.283	0.280 ± 0.0283
0.21	0.312	0.272	0.289	0.312	0.319	0.290	0.299 ± 0.0182
0.27	0.320	0.289	0.293	0.322	0.334	0.307	0.311 ± 0.0176
0.33	0.326	0.291	0.305	0.326	0.342	0.313	0.317 ± 0.0180
0.42	0.336	0.309	0.330	0.337	0.357	0.325	0.332 ± 0.0158
0.50	0.345	0.337	0.348	0.346	0.368	0.338	0.347 ± 0.0112
0.75	0.382	0.385	0.369	0.375	0.412	0.384	0.385 ± 0.0148
1.00	0.420	0.427	0.420	0.431	0.450	0.421	0.428 ± 0.0116
1.50	0.454	0.465	0.427	0.468	0.511	0.464	0.465 ± 0.0272
2.00	0.548	0.524	0.560	0.527	0.567	0.552	0.546 ± 0.0174
3.00	0.601	0.636	0.652	0.629	0.686	0.635	0.640 ± 0.0281
4.00	0.677	0.691	0.763	0.701	0.759	0.733	0.721 ± 0.339
5.00	0.835	0.804	0.808	0.776	0.819	0.785	0.805 ± 0.0217
6.00	0.888	0.852	0.869	0.833	0.835	0.805	0.847 ± 0. 293
7.00	0.896	0.891	0.903	0.881	0.892	0.846	0.885 ± 0.0203
8.00	0.914	0.909	0.917	0.903	0.928	0.904	0.913 ± 0.0094
9.00	0.926	0.926	0.932	0.916	0.931	0.926	0.926 ± 0.0057
10.00	0.937	0.947	0.941	0.943	0.939	0.935	0.940 ± 0.0043

TABLE A1.6 Dissolution results from Product DT using the rotating paddle apparatus with a wire basket.

TIME (HRS)	PRODUCT DT						MEAN ± SD
	DT-1	DT-2	DT-3	DT-4	DT-5	DT-6	
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000 ± 0.0000
0.02	0.001	0.003	0.001	0.003	0.000	0.000	0.001 ± 0.0014
0.03	0.005	0.005	0.001	0.004	0.003	0.000	0.003 ± 0.0021
0.07	0.006	0.010	0.008	0.005	0.006	0.007	0.007 ± 0.0018
0.10	0.019	0.019	0.014	0.013	0.015	0.018	0.016 ± 0.0027
0.13	0.023	0.028	0.025	0.028	0.024	0.023	0.025 ± 0.0023
0.17	0.030	0.032	0.034	0.030	0.034	0.035	0.033 ± 0.0022
0.21	0.038	0.043	0.042	0.032	0.038	0.046	0.040 ± 0.0049
0.27	0.046	0.048	0.048	0.039	0.045	0.072	0.050 ± 0.0114
0.33	0.060	0.056	0.062	0.069	0.070	0.080	0.066 ± 0.0086
0.42	0.072	0.066	0.070	0.079	0.080	0.091	0.076 ± 0.0090
0.50	0.077	0.072	0.080	0.091	0.089	0.097	0.084 ± 0.0095
0.75	0.101	0.083	0.103	0.112	0.109	0.119	0.105 ± 0.0124
1.00	0.110	0.109	0.120	0.120	0.122	0.129	0.118 ± 0.0076
1.50	0.152	0.141	0.154	0.150	0.162	0.162	0.154 ± 0.0079
2.00	0.199	0.171	0.198	0.174	0.195	0.195	0.189 ± 0.0127
3.00	0.252	0.230	0.270	0.244	0.298	0.293	0.265 ± 0.0273
4.00	0.304	0.260	0.328	0.337	0.418	0.389	0.339 ± 0.0572
5.00	0.367	0.364	0.387	0.418	0.427	0.424	0.398 ± 0.0288
6.00	0.384	0.398	0.421	0.421	0.436	0.434	0.416 ± 0.0206
7.00	0.421	0.439	0.447	0.438	0.446	0.468	0.443 ± 0.0153
8.00	0.445	0.463	0.470	0.460	0.505	0.501	0.474 ± 0.0239
9.00	0.489	0.501	0.492	0.507	0.508	0.522	0.503 ± 0.0120
10.00	0.509	0.511	0.510	0.523	0.510	0.538	0.517 ± 0.0116

APPENDIX 2

CONSENT TO ACT AS A RESEARCH SUBJECT

1. Phenylpropanolamine hydrochloride (PPA.HCl) has been used for many years as a decongestant and appetite suppressant. The purpose of this study is to determine the amount of phenylpropanolamine (PPA) in the body when given sustained-release formulations and to compare these data with similar data obtained after ingesting the pure powder.

2. To participate in this study, I must be in good health on the basis of physical examination, interview and blood and urine tests.

If I agree to be a subject, the following will occur:
I will arrive at the Pharmacy Department, Rhodes University, at 8.30 a.m. on the appointed day having fasted since 10.00 p.m. the previous night and will not have had breakfast. I will have followed all previous instructions regarding drug, alcohol and dietary restrictions.

An indwelling needle will be placed in one of the veins of my arm and blood samples will be drawn from this needle over a period of 12 hours. Thereafter the needle will be removed and I may go home. I must also collect urine at times specified in the protocol and I will be totally responsible for the accurate recording of time and volume of urine voided. If I void urine at times other than those specified in the protocol, I will record the exact time and volume of urine voided. I must return at 9.00 a.m. the following day for a final blood and urine collection. The total volume of blood drawn will not exceed 120 ml.

I will be given ..(dosage form to be studied).. at ..(time).

3. Phenylpropanolamine hydrochloride is a widely used drug that should not cause any problems in the concentrations used in this

study. However, any drug has the potential to cause an adverse reaction. This drug has been reported to cause an increase in blood pressure in susceptible patients.

The insertion of the needle for drawing blood can be painful and a small bruise may develop on my arm and, very rarely, a localized infection may occur. To prevent blood from clotting in the needle when it remains in the vein for a prolonged period of time, the needle will be flushed with small doses of heparin which will help prevent clot formation.

4. These procedures will have no direct benefit for me, but additional information may be learned about the way this drug is released from these dosage forms and distributed throughout the body.

5. This information was explained to me by Miss Dowse. I understand that she will answer any questions I may have concerning this investigation. If I have any other questions I may ask Professor Kanfer.

6. I will be paid R25.00 for participating in each study.

7. Particiaption in this research is voluntary. I have the right to refuse to participate or may withdraw at any time without jeopardy to my standing whatsoever.

_____

SIGNATURE

_____

DATE

APPENDIX 3

TABLE A3.1 Phenylpropanolamine Serum concentrations after the administration of 50 mg PPA HCl dissolved in 200 ml of water.

TIME	CONCENTRATION OF PPA IN SERUM (ng/ml)						
(HRS)	JM	MK	SR	PS	DD	MM	MEAN ± SD
0	0	0	0	0	0	0	0 ± 0
0.17	0	0	0	3.08	40.87	12.76	9.45 ± 16.17
0.33	13.24	7.33	48.20	49.61	103.95	30.01	42.06 ± 34.95
0.50	57.41	31.66	61.90	78.43	124.26	54.58	68.04 ± 31.38
0.67	71.11	45.68	92.14	86.23	136.07	142.69	95.65 ± 37.54
0.83	76.78	80.56	115.52	90.72	139.62	155.21	109.74 ± 32.54
1.00	92.14	90.01	154.97	98.77	133.95	176.94	124.46 ± 36.51
1.50	112.21	105.21	149.30	107.73	124.73	185.68	130.81 ± 31.38
2.00	99.22	114.10	145.05	116.70	119.54	180.25	129.14 ± 29.10
3.00	91.43	107.25	124.53	97.80	95.21	154.26	111.75 ± 23.97
4.00	77.49	80.09	106.07	85.52	79.85	135.05	94.01 ± 22.67
6.00	53.87	60.48	73.47	62.28	50.64	103.95	67.45 ± 19.55
8.00	40.50	50.34	56.23	40.17	33.55	71.35	48.69 ± 13.73
10.00	30.72	34.97	43.71	27.17	20.56	56.94	35.68 ± 12.98
12.00	22.21	24.81	29.99	20.10	13.24	38.28	24.77 ± 8.62
14.00	14.65	12.29	20.92	14.65	7.09	24.61	15.70 ± 6.24

TABLE A3.2 The cumulative urinary excretion (Ae) of PPA after the administration of 50 mg PPA.HCl dissolved in 200 ml of water.

JM		MK		SR		PS		DD		MM	
TIME (HRS)	Ae (mg)	TIME (HRS)	Ae (mg)	TIME (HRS)	Ae (mg)	TIME (HRS)	Ae (mg)	TIME (HRS)	Ae (mg)	TIME (HRS)	Ae (mg)
0	0	0	0	0	0	0	0	0	0	0	0
4.2	23.21	3.8	21.07	2.2	14.62	2.2	17.84	6.0	35.43	1.8	11.94
10.3	38.08	5.8	31.95	7.9	33.43	5.2	30.67	8.5	39.72	4.5	26.58
12.3	39.96	11.5	39.44	10.3	36.75	9.0	36.41	11.5	42.80	10.0	39.42
18.5	42.91	16.0	41.30	15.0	41.20	12.0	40.87	18.0	44.86	12.0	42.28
23.0	43.69	17.8	42.18	18.1	42.55	14.4	42.87	23.2	45.02	14.5	44.17
26.7	44.05	23.3	43.55	22.5	43.57	18.0	44.41	31.0	45.05	23.0	45.55
28.0	44.31	27.0	44.25	24.7	43.89	23.5	45.67	32.0	45.07	27.0	45.77
32.3	44.40	30.0	44.33	28.8	44.25	26.0	45.92	35.0	45.08	31.0	45.91
35.0	44.46	32.0	44.35	33.8	44.51	28.0	46.15	36.0	45.09	33.3	45.95
36.0	44.49	36.0	44.36	36.0	44.52	36.0	46.30			36.0	45.97

TABLE A3.3 Phenylpropanolamine Serum concentrations after the administration of 100 mg PPA.HCl dissolved in 200 ml of water.

TIME (HRS)	CONCENTRATION OF PPA IN SERUM (ng/ml)			
	JM	MK	SR	MEAN ± SD
0	0	0	0	0 ± 0
0.25	64.42	63.38	67.76	65.19 ± 2.29
0.50	192.06	134.29	169.54	165.30 ± 29.12
0.75	321.79	157.23	247.33	242.12 ± 82.40
1.00	333.26	235.86	336.39	301.84 ± 57.16
1.50	284.23	243.58	364.76	297.52 ± 61.67
2.00	264.23	249.42	421.91	311.85 ± 95.60
2.50	237.11	225.43	407.51	290.02 ± 101.92
3.00	231.90	199.99	363.30	265.06 ± 86.56
4.00	210.21	172.46	303.44	228.70 ± 67.42
6.00	169.75	134.29	229.61	177.88 ± 48.18
8.00	132.41	105.72	178.92	139.02 ± 37.05
10.00	121.15	84.44	121.78	109.12 ± 21.38
12.00	73.18	62.75	89.03	74.99 ± 13.23
14.00	61.92	51.86	61.23	58.34 ± 5.62

TABLE A3.4 The cumulative urinary excretion (Ae) of PPA after the administration of 100 mg PPA.HCl dissolved in 200 ml of water.

TIME (HRS)	AMOUNT OF DOSE (mg) EXCRETED			
	JM	MK	SR	MEAN ± SD
0	0	0	0	0 ± 0
1.0	11.74	11.28	6.56	9.86 ± 2.87
2.0	25.15	25.89	15.22	22.09 ± 5.96
3.0	39.10	39.97	22.66	33.91 ± 9.75
5.0	56.71	50.53	40.68	49.31 ± 8.09
7.0	67.73	61.90	51.87	60.50 ± 8.02
9.0	76.44	67.32	60.21	67.99 ± 8.14
11.0	82.99	73.12	66.81	74.31 ± 8.16
13.0	87.58	77.32	71.70	78.87 ± 8.05
15.0	89.96	79.24	74.83	81.34 ± 7.78
24.0	95.42	83.20	80.33	86.32 ± 8.01

TABLE A3.5 Phenylpropanolamine Serum concentrations after the administration of Product BS (150 mg PPA.HCl)

TIME (HRS)	CONCENTRATION OF PPA IN SERUM (ng/ml)						MEAN ± SD
	JM	MK	SR	PS	DD	MM	
0	0	0	0	0	0	0	0 ± 0
0.5	40.37	45.16	127.06	57.72	104.94	15.00	65.04 ± 42.43
1.0	64.34	74.17	152.68	109.73	165.18	69.88	109.11 ± 49.50
1.5	103.41	119.23	196.67	133.79	183.85	235.74	162.12 ± 51.33
2.0	144.64	139.60	250.48	162.77	202.52	277.13	196.19 ± 57.47
3.0	188.98	257.53	314.65	195.89	217.58	327.33	250.33 ± 59.86
4.0	204.56	278.38	315.43	218.14	223.34	340.01	263.31 ± 56.42
5.0	215.58	245.95	311.55	247.38	201.52	380.37	267.06 ± 67.23
6.0	183.33	214.28	307.41	329.66	190.42	408.31	272.24 ± 90.56
8.0	148.71	195.45	304.82	269.37	172.61	328.36	336.55 ± 74.38
9.0	141.76	177.95	264.97	220.42	149.72	321.90	212.79 ± 70.60
10.0	129.54	166.45	260.06	195.37	139.27	314.13	200.80 ± 72.70
12.0	112.52	144.16	224.61	150.09	104.33	292.14	171.31 ± 72.90
24.0	60.99	60.99	63.16	57.21	41.28	124.99	68.10 ± 28.98

TABLE A3.6 The cumulative urinary excretion of PPA after the administration of Product BS (150 mg PPA.HCl)

TIME (HRS)	AMOUNT OF THE DOSE (mg) EXCRETED						MEAN ± SD
	JM	MK	SR	PS	DD	MM	
0	0	0	0	0	0	0	0 ± 0
2.0	6.885	7.193	11.82	10.74	14.67	4.27	9.26 ± 3.82
4.0	26.506	17.667	24.46	32.27	35.63	19.70	26.04 ± 6.98
6.0	41.523	33.652	36.75	37.62	52.46	34.38	39.40 ± 6.98
8.0	53.143	43.265	45.60	49.60	66.01	42.66	50.05 ± 8.77
12.0	73.063	63.592	61.30	69.14	86.72	63.10	69.49 ± 9.51
24.0	105.675	105.724	99.86	103.59	110.50	101.66	104.50 ± 3.72

TABLE A3.7 Phenylpropanolamine Serum concentrations after the administration of Product DT (75 mg PPA.HCl)

TIME	CONCENTRATION OF PPA IN SERUM (ng/ml)						
(HRS)	JM	MK	SR	PS	DD	MM	MEAN ± SD
0	0	0	0	0	0	0	0 ± 0
0.5	20.07	8.59	20.22	7.85	19.66	19.74	16.02 ± 6.05
1.0	26.29	14.90	50.31	35.52	29.57	38.34	32.49 ± 11.98
1.5	31.21	20.56	59.40	44.82	35.88	56.62	41.42 ± 15.07
2.0	35.15	22.77	70.47	48.34	61.94	74.16	52.14 ± 20.41
3.0	82.43	103.99	82.27	80.63	108.99	111.03	94.89 ± 14.56
4.0	91.53	112.76	94.40	96.12	123.57	115.46	105.64 ± 13.30
5.0	99.89	121.11	96.86	102.84	137.42	120.87	113.17 ± 15.87
6.0	120.79	134.72	108.50	114.80	122.18	125.21	121.03 ± 8.97
7.0	106.61	127.34	120.95	118.08	100.87	133.49	117.89 ± 12.32
8.0	96.77	115.21	127.02	125.05	95.95	127.51	114.59 ± 14.81
9.0	90.30	102.68	124.72	115.21	84.65	123.98	106.92 ± 17.13
10.0	79.40	96.45	119.48	96.61	76.37	110.85	96.53 ± 16.92
12.0	65.78	68.25	101.28	67.93	53.59	88.50	74.22 ± 17.36
14.0	50.80	54.73	76.86	49.65	24.82	71.78	54.77 ± 18.53
24.0	5.64	8.92	10.15	13.76	2.45	19.25	10.03 ± 5.95

TABLE A3.8 The cumulative urinary excretion of phenylpropanolamine after the administration of Product DT (75 mg PPA.HCl)

TIME	AMOUNT OF THE DOSE (mg) EXCRETED						
(HRS)	JM	MK	SR	PS	DD	MM	MEAN ± SD
0	0	0	0	0	0	0	0 ± 0
2.0	2.97	2.60	2.95	3.03	3.07	3.92	3.09 ± 0.44
4.0	12.47	14.51	13.77	11.82	18.09	13.69	14.06 ± 2.20
6.0	23.87	26.27	19.21	23.98	32.79	22.97	24.85 ± 4.52
8.0	33.96	34.84	27.90	30.01	42.44	29.99	33.19 ± 5.24
10.0	41.88	41.00	36.36	37.92	49.40	36.65	40.54 ± 4.90
12.0	48.13	47.66	44.02	44.97	55.04	41.22	46.84 ± 4.75
24.0	63.58	60.85	58.41	59.99	62.83	57.58	60.54 ± 2.37
28.0	64.34	61.99	59.62	60.52	63.14	59.18	61.47 ± 0.02
36.0	64.51	63.31	60.85	61.49	63.69	60.16	62.34 ± 1.74

TABLE A3.9 Phenylpropanolamine Serum concentrations after the administration at 0, 4 and 8 hours of 50 mg PPA.HCl dissolved in 200 ml water.

TIME	CONCENTRATION OF PPA IN SERUM (ng/ml)						
(HRS)	MM	SR	MS	PS	DM	RS	MEAN ± SD
0	0	0	0	0	0	0	0 ± 0
0.5	138.71	109.73	146.73	87.22	86.70	110.76	113.31 ± 25.18
1.0	237.03	148.80	233.41	145.44	116.46	179.85	176.83 ± 49.50
1.5	219.95	154.75	197.18	169.76	189.42	141.81	178.81 ± 28.89
2.0	206.50	188.39	179.33	165.62	129.39	111.51	163.46 ± 36.30
3.0	195.37	160.18	143.11	144.92	104.55	108.44	142.76 ± 33.81
4.0	172.34	150.61	133.02	134.05	93.17	103.00	131.03 ± 29.40
5.0	345.96	294.99	212.71	196.67	235.22	187.35	245.48 ± 62.43
6.0	373.90	334.57	259.28	272.99	297.83	208.57	291.19 ± 58.21
8.0	226.94	200.29	173.12	241.69	118.78	100.16	176.83 ± 57.50
9.0	353.20	285.41	306.63	300.16	217.11	215.55	279.68 ± 54.05
10.0	400.55	336.38	344.15	348.29	284.12	273.77	325.21 ± 55.28
12.0	323.45	294.21	244.28	265.23	176.48	113.87	236.25 ± 78.00
24.0	48.93	65.48	45.30	53.84	47.89	27.19	48.11 ± 12.50

TABLE A3.10 The cumulative urinary excretion of phenylpropanolamine after the administration at 0,4 and 8 hours of 50 mg PPA.HCl in 200 ml water

TIME	AMOUNT OF THE DOSE EXCRETED (mg)						
(HRS)	MM	SR	MS	PS	DM	RS	MEAN ± SD
0	0	0	0	0	0	0	0 ± 0
2.0	14.68	11.20	13.05	10.88	12.48	12.40	12.45 ± 1.37
4.0	30.15	19.63	23.62	19.77	24.40	23.16	23.46 ± 3.85
6.0	51.83	33.43	42.83	33.91	40.10	37.91	40.00 ± 6.82
8.0	66.49	49.72	57.10	46.85	58.10	48.15	54.40 ± 7.55
12.0	96.83	79.98	92.72	66.91	92.04	73.42	83.65 ± 12.04
24.0	117.49	117.70	128.39	129.07	114.92	104.12	118.62 ± 9.28

TABLE A4.1 Observed and model-predicted serum concentrations of phenylpropanolamine (using model No.3) following administration of a 50 mg solution of the drug.

TIME	JM		MK		SR		PS		DD		MM		MEAN DATA	
(HRS)	OBS.[1]	PRED.[2]	OBS.	PRED.	OBS.	PRED.	OBS.	PRED.	OBS.	PRED.	OBS.	PRED.	OBS.	PRED.
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.17	0	0.00	0	0.00	0	0.02	3.08	3.08	40.87	41.93	12.76	20.69	9.45	8.42
0.33	13.24	13.44	7.33	7.26	48.20	46.59	49.61	49.75	103.95	100.89	30.01	35.43	42.06	42.48
0.50	57.41	57.72	31.66	32.80	61.90	60.41	78.43	75.08	124.26	126.30	54.58	47.28	68.04	71.53
0.67	71.11	72.93	45.68	50.30	92.14	97.04	86.23	86.37	136.07	132.28	142.69	144.40	95.65	94.40
0.83	76.78	82.32	80.56	73.48	115.52	110.95	90.72	94.24	139.62	133.90	155.21	159.08	109.74	111.23
1.00	92.14	89.96	90.01	90.15	154.97	149.79	98.77	100.40	133.95	133.28	176.94	168.97	124.46	124.94
1.50	112.21	101.55	105.21	110.66	149.30	154.47	107.73	109.35	124.73	125.43	185.68	180.50	130.81	129.04
2.00	99.22	103.06	114.10	112.12	145.05	143.49	116.70	109.99	119.54	115.00	180.25	178.02	129.14	126.47
3.00	91.43	93.37	107.25	100.66	124.53	122.85	97.80	100.50	95.21	94.56	154.26	159.29	111.75	113.36
4.00	77.49	79.31	80.09	86.67	106.07	104.93	85.52	86.55	79.85	76.99	135.05	137.57	94.01	97.21
6.00	53.87	55.00	60.48	62.61	73.47	76.14	62.28	60.31	50.64	50.60	103.95	100.15	67.45	68.21
8.00	40.50	38.36	50.34	44.58	56.23	55.36	40.17	40.89	33.55	33.15	71.35	72.09	48.69	46.94
10.00	30.72	27.13	34.97	31.51	43.71	40.04	27.17	27.52	20.56	21.70	56.94	51.51	35.68	32.15
12.00	22.21	19.38	24.81	22.19	29.99	29.16	20.10	18.48	13.24	14.19	38.28	36.59	24.77	21.97
14.00	14.65	13.93	12.29	15.59	20.92	21.10	14.65	12.40	7.09	9.28	24.61	25.86	15.70	14.99

1 Observed serum concentration (ng/ml)

2 Predicted serum concentration (ng/ml)

TABLE A4.2 Observed and model-predicted serum concentrations of phenylpropanolamine (using model No.3) following administration of a 100 mg solution of the drug.

TIME	JM		MK		SR		MEAN DATA	
(HRS)	OBS.[1]	PRED.[2]	OBS.	PRED.	OBS.	PRED.	OBS.	PRED.
0	0	0	0	0	0	0	0	0
0.25	64.42	58.70	63.38	84.55	67.76	100.96	65.19	62.80
0.50	192.06	205.49	134.29	120.49	169.54	176.95	165.30	168.53
0.75	321.79	308.87	157.23	163.94	247.33	233.61	242.12	245.69
1.00	333.26	338.42	235.86	223.55	336.39	319.28	301.84	299.21
1.50	284.23	288.46	243.58	250.20	364.76	384.02	297.52	303.80
2.00	264.23	260.68	249.42	240.97	421.91	400.97	311.85	296.68
2.50	237.11	243.63	225.43	225.64	407.51	392.98	290.02	284.54
3.00	231.90	231.06	199.99	210.05	363.30	372.68	265.06	269.87
4.00	210.21	210.18	172.46	181.54	303.44	320.40	228.10	238.30
6.00	169.75	171.63	134.29	135.53	229.61	227.17	177.88	180.41
8.00	132.41	136.25	105.72	101.21	178.92	163.66	139.02	135.24
10.00	121.15	105.93	84.44	75.96	121.78	120.44	109.12	101.25
12.00	73.18	81.14	62.75	56.47	89.03	89.70	74.99	75.82
14.00	61.92	61.48	51.86	42.19	61.23	67.20	58.34	56.80

1 Observed serum concentration (ng/ml)

2 Predicted serum concentration (ng/ml)

TABLE A43 Parameter estimates from computer modelling of the 50 and 100 mg solution data

MODEL	PARAMETER	SUBJECTS JM	MK	SR	PS	DD	MM	MEAN DATA
50 MG SOLUTION STUDY								
2BCM 3 k_a's M-M elim.	ka_1	0.241	0.030	0.057	0.082	0.677	0.077	0.063
	ka_2	4.163	0.114	0.100	3.006	2.137	3.849	0.320
	ka_3	0.443	0.178	0.160	0.859	1.241	0.208	0.203
	V_1	113.13	24.88	5.004	206.93	107.50	24.77	55.94
	k_{12}	0.414	0.188	0.198	0.439	1.823	0.323	0.055
	k_{21}	0.361	0.494	0.582	1.308	1.370	0.273	0.244
	V_m	458158	323831	234195	573467	320000	192400	623259
	K_m	8702	6535	6228	9936	5429	6027	12362
	r^1	0.995	0.994	0.998	0.998	0.999	0.997	0.999
2BCM 3 k_a's linear elim.	ka_1	0.241	0.029	0.105	0.078	0.756	0.066	0.063
	ka_2	4.155	0.113	0.176	0.934	2.957	4.339	0.320
	ka_3	0.441	0.182	0.253	0.401	1.326	0.189	0.201
	V_1	113.15	24.10	12.14	123.18	119.52	20.67	56.08
	k_{12}	0.413	0.305	1.589	0.269	1.602	0.225	0.034
	k_{21}	0.363	0.505	0.283	0.334	1.509	0.296	0.241
	k_{10}	0.487	1.999	3.000	0.464	0.493	1.503	0.901
	r	0.995	0.995	0.997	0.998	0.999	0.998	0.999
2BCM 1 ka linear elim.	ka	0.621	0.448	0.653	0.933	1.902	0.649	0.749
	V_1	197.71	172.74	137.53	225.05	192.69	113.81	165.58
	k_{12}	0.156	0.077	0.193	0.141	0.458	0.182	0.176
	k_{21}	0.308	0.205	0.277	0.714	1.358	0.368	0.466
	k_{10}	0.282	0.302	0.279	0.243	0.299	0.272	0.289
	r	0.965	0.955	0.968	0.982	0.993	0.954	0.984
100 MG SOLUTION STUDY								
2BCM 3 k_a's M-M elim.	ka_1	0.223	0.072	0.361				0.124
	ka_2	1.484	0.100	1.209				0.259
	ka_3	0.266	0.146	0.558				0.156
	V_1	70.68	12.97	70.19		———		38.61
	k_{12}	0.763	0.002	0.389				0.064
	k_{21}	0.427	0.195	0.397				0.152
	V_m	60.760	184400	246400				820631
	K_m	14340	43490	9747				22942
	r	0.997	0.992	0.994				0.998
2BCM 3 k_a's linear elim.	ka_1	0.218	0.077	0.330				0.118
	ka_2	1.374	0.106	1.118				0.246
	ka_3	0.270	0.152	0.490				0.150
	V_1	68.31	14.08	64.90		———		36.95
	k_{12}	0.815	0.118	0.390				0.026
	k_{21}	0.445	0.191	0.353				0.147
	k_{10}	0.615	2.991	0.387				0.959
	r	0.997	0.992	0.994				0.998
2BCM 1 k_a linear elim.	k_a	1.238	0.781	0.518				0.858
	V_1	161.62	181.66	94.46				153.92
	k_{12}	0.429	0.268	0.166		———		0.239
	k_{21}	0.700	0.440	0.200				0.516
	k_{10}	0.214	0.237	0.270				0.214
	r	0.953	0.985	0.990				0.988

1 Correlation coefficient as an indication of the goodness of fit

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