AN ABSTRACT OF THE THESIS OF

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Title: New Product Formulation and Multiple dose Pharmacokinetics of Acetaminophen.

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Abstract approved: _____

Four different treatments of acetaminophen were administered to eight healthy volunteers in multiple doses (five doses). Saliva acetaminophen concentration-time profiles were determined. Non-compartmental pharmacokinetic parameters for the first and last dose were calculated and compared to determine whether acetaminophen exhibited linear or dose dependent pharmacokinetics. Dose corrected area under the curve, mean residence time, half-life, and apparent clearance were not significantly different among treatments. Plots of saliva acetaminophen concentration vs time normalized to a 325 mg dose were superimposable, indicating linearity of kinetics.

Acetaminophen is a prime candidate for sustained release dosage forms (chapter II) due to its relatively short half-life. Acetaminophen pellets were spray coated with sustained release and/or enteric coats and incorporated into suspension formulations. A non-aqueous semisolid suspension was also investigated using coated pellets. These formulations were aged for a period of one year and

in-vitro dissolution studies conducted. Time to 50% drug release after 6 days, 3 months, 9 months, and 12 months aging were similar to each other (about 5 hours) and did not change significantly. Bioavailability studies of some suspension formulations were conducted in two subjects and compared to an immediate release commercial product. Suspension containing 5/10/5 coated acetaminophen pellets produced sustained saliva acetaminophen concentrations over 24 hour period, especially for a formulation with immediate release portion of drug. Non aqueous formulations did not appear promising as the in-vitro dissolution was too rapid following aging. It may be possible to keep the pellets and non aqueous formulations separated in unit dose packages and mix prior to administration of the dose.

NEW PRODUCT FORMULATION
AND
MULTIPLE DOSE
PHARMACOKINETICS OF ACETAMINOPHEN

by Chandrahas G. Sahajwalla

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hands join
towards the one who can
not be touched ___
but who is ___
___ that a space
divides ___

Dedicated in fond

Memory of my Mother,

KAMLA G. SAHAJWALLA (1926 -1986),

who would have cherished this day.

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My deepest gratitude goes to my father, Gope K. Sahajwalla whose understanding, encouragement and love were of great value during my graduate work.

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NEW PRODUCT FORMULATION AND MULTIPLE DOSE PHARMACOKINETICS OF ACETAMINOPHEN.

INTRODUCTION

Acetaminophen (APAP) is a widely used non-narcotic analgesic and antipyretic with a relatively short half-life. Since APAP has a short half-life it is a prime candidate for a sustained release product. The drug is extensively metabolized and excreted largely in urine as glucuronide and sulfate conjugates . Acetaminophen is incompletely available to systemic circulation after oral administration. Indications from Borin (1985) show that APAP may obey non-linear kinetics. The relative absence of multiple dosing studies in the literature, and interest in developing a sustained release product instigated this research. First chapter of the thesis describes multiple dose pharmacokinetics of APAP after multiple dosing and chapter 2 deals with sustained release supensions.

NEW PRODUCT FORMULATION

AND

MULTIPLE DOSE PHARMACOKINETICS

OF

ACETAMINOPHEN

CHAPTER I

MULTIPLE DOSE PHARMACOKINETICS OF ACETAMINOPHEN

AFTER ORAL ADMINISTRATION

INTRODUCTION

Acetaminophen (Paracetamol, N-acetyl paraminophenol) is a widely used non-narcotic analgesic with a relatively short half-life. Acetaminophen was first used in medical therapy by Vonmering in 1893 (Forrest, Clements and Prescott, 1982). It is a white, odorless, bitter, crystalline powder with a melting point of 169-170.5°C (Humeida A., El-obeid and Abdullah A. Al-Badr 1985). It is a moderately water soluble (1g in 70 ml of water) and lipid soluble weak organic acid with pKa of 9.5 which is unionized at physiological pH. A saturated solution has a pH of about 6.0.

Its antipyretic and analgesic actions are similar to those of aspirin (Beaver 1966). Acetaminophen has only weak anti-inflammatory action. The analgesic and antipyretic mode of action is unclear for both aspirin and acetaminophen. It has been suggested that inhibition of prostaglandin synthesis in the central nervous system (CNS) is responsible for the action, but experimental evidence is unsatisfactory since inhibition of prostaglandin biosynthesis occurs only at doses or concentrations rarely reached in therapy. It has also been reported that acetaminophen reduces fever by inhibiting the actions of endogenous pyrogens on the hypothalmic heat regulation center (Clark and Moyer 1972), and analgesic action may be central. The pharmacokinetics and analgesic effects in post operative dental patients have been studied by Rawlins (Seymour and Rawlins 1981). No significant analgesic effect was observed up to 90 minutes. Significant analgesia was noted between 90 and 240 minutes, but no clear relationship between analgesia and acetaminophen concentrations

in either the central or peripheral compartment existed when considering only a two compartment open pharmacokinetic model. Data presented support the hypothesis that the receptor site for analgesic effect of acetaminophen (APAP) is in a "deep" or slowly penetrated tissue compartment which cannot be observed pharmacokinetically with the data collected. A comparative efficacy study of aspirin and acetaminophen in the antipyresis of children (Walker et al. 1986) indicated that therapeutic differences in the antipyretic activities may exist at higher body temperatures. At higher temperatures, the duration of effect of acetaminophen was shorter than that for aspirin.

Acetaminophen is rapidly absorbed and distributed after oral administration with peak concentrations obtained within 40 to 60 minutes. Pharmacokinetics after oral and intravenous administration are best described by a two compartment open model, with a rapid distribution phase and an elimination half life of 2 to 3 hours in the usual dose range (Albert et. al., 1974; Rawlins et. al., 1977; Clements et. al., 1978; Levy, 1981; Ameer et. al., 1983). The absorption of acetaminophen from orally administered solid dosage forms (tablets or capsules) is affected by gastric emptying rate (Heading et. al., 1973; Clements et. al., 1978) and by release characteristics from the dosage form. Absorption is primarily from the small intestine by passive transport (Bagnall et. al., 1979). Factors such as any drug, disease or other conditions which alter gastric emptying influence the rate of acetaminophen absorption (Nimmo, 1976). Food delays the rate of drug absorption although total amount of drug absorbed remains unchanged (Jaffe et. al., 1971; McGilverary and Mattok 1972).

Acetaminophen is relatively uniformly distributed throughout most body tissues and fluids in appreciable concentrations reaching a tissue to plasma concentration ratio of about unity except in fat and cerebrospinal fluid (Brodie and Axelrod, 1949; Gwilt et. al., 1963). Binding of acetaminophen to plasma protein is variable, being negligible at usual therapeutic concentrations (Wagner 1975) as binding does not occur with plasma concentrations of less than 60 µg/ml. After toxic doses of acetaminophen, up to 43% is bound to plasma protein (Gazzard et. al., 1973).

Acetaminophen is incompletely available to systemic circulation after oral administration, since it is partly metabolized during absorption, primarily to pharmacologically inactive products. Present studies suggest that about 10% of acetaminophen is subject to this presystemic biotrans-formation at doses of 1g or more and the percentage may be higher (~ 40%) at lower doses (Chiou, 1975; Rawlins et. al., 1977; Perucca and Richens, 1979; Ameer et. al., 1983). About 25% of the acetaminophen dose has been reported to be metabolized by a first pass effect (Clements et. al., 1978). The drug is extensively metabolized and excreted largely in urine as various conjugates; 45-55% as glucuronide conjugates, 20-30% as sulfate and 15-55% as cysteine and mercapturic acid conjugates. About 2-4% of unchanged drug is excreted in urine, which may increase to as much as 10-14% after overdosage (Slattery et. al., 1979). A minor fraction of acetaminophen is converted by cytochrome p-450 dependent hepatic mixed function oxidase to a highly reactive alkylating metabolite, which is probably n-acetyl-p-benzo-quinoneimine (Miner and Kissenger, 1979). This

metabolite is usually rapidly inactivated by conjugation with reduced glutathione and excreted in urine as cysteine and mercapturic acid conjugate. Overdoses of acetaminophen cause acute hepatic necrosis due to saturation of conjugation pathways, and glutathione stores become depleted due to covalent binding of the excessive reactive metabolites (possibly an epoxide) to vital cell constituents (Mitchell et, al., 1973, 1974). This toxic metabolite is believed to combine irreversibly with hepatocyte constituents causing serious cell damage (Andrews et. al., 1976; Davis et. al., 1976).

A useful antidote for acetaminophen toxicity is N-acetylcysteine. Treatment with N-acetylcysteine is warranted if the half-life of acetaminophen exceeds 4 hours (Prescott et. al., 1971; Peterson and Rumach, 1977) and should be admininstered within the first 10 hours to be effective. N-acetylcysteine is believed to inactivate the toxic metabolite intermediate of acetaminophen as a glutathione analog (Peterson and Rumack, 1977).

DEFINITION OF PROBLEM AND OBJECTIVES

Based on available clinical and pharmacokinetic data it can be summarized that acetaminophen is a widely used non-narcotic analgesic with a relatively short half life. Although it is relatively safe analgesic in high doses and over prolonged periods, its metabolism may produce a quantitatively minor metabolite of drug which can destroy the enzyme systems responsible for its reduction. This could paralyze the capacity of the liver to reduce other toxic substances in addition to the drug itself.

Recently Borin and Ayres (1985) conducted a study with 15 human subjects by administering five different single doses (up to 2000 mg) of acetaminophen and collected saliva samples for 16 hours post dosing. They concluded that for single doses up to 2000 mg, acetaminophen pharmacokinetics were dose dependent based on statistically significant differences in elimination, half life, mean residence time and AUC/D.

Multiple dosing pharmacokinetics of acetaminophen have not been well defined in the literature. One study of multiple rectal administration has been reported (Ebel et. al., 1980) in which acetaminophen (300 mg) and salicylamide (200 mg) in combined repitative (three times, every 4h) administration of suppositories was studied in 10 male volunteers. This study concluded that acetaminophen pharmacokinetic behavior conformed to literature values for single dose and was described by a two compartment open model. Kinetics of acetaminophen following single strength and double strength administration to febrile children (Nahata and Powell 1982) have been studied and found to be linear, but no multiple dose study has been conducted in adults.

Since acetaminophen has a short half-life, it is a prime candidate for sustained release dosage forms (Chapter II). Indications from Borin and Ayres (1985) are that acetaminophen may obey non linear kinetics. The relative absence of multiple dosing studies in the literature, and interest in developing a sustained release product instigated this research. The objective of this study was to determine whether acetaminophen exhibits dose dependent pharmacokinetics when comparing single dose data to steady state data. If dose dependency, that is, a deviation from linearity in pharmacokinetics exists, there should be accumulation of drug at steady state.

MATERIALS AND METHODS

STUDY DESIGN

The present study was designed with eight healthy volunteers who were administered four different treatments (325 mg, 650 mg, 825 mg, or 1000 mg of acetaminophen). Each treatment was administered orally in five multiple doses with dosing intervals of six hours each. Saliva samples were collected for 36 hours after administration of the first dose.

One blood sample was also collected at a specified time after the last dose and analyzed using HPLC.

Eight (8) healthy volunteers between 22 and 29 years of age participated in this study (Table 1). Each participant signed an informed written consent. Four different treatments i.e., 325 mg, 650 mg, 825 mg and 1000 mg of commercial acetaminophen (Tylenol^{R}) were administered to each subject in the following manner.

A. 325 mg Tylenol tablets, Lot No. AFR318 12/87 (McNeil, Fort Washington, PA) were administered every 6 hours at 0, 6, 12, 18, and 24 hours.

B. 650 mg Tylenol (two 325 mg tablets) were administered every 6 hours at 0, 6, 12, 18 and 24 hours.

C. 825 mg Tylenol, one each of 325 mg and 500 mg Tylenol Extra strength, Lot No. ADF986 5/86 (McNeil, Fort Washington, PA) administered every 6 hours at 0, 6, 12, 18 and 24 hours.

D. 1000 mg Tylenol (two 500 mg extra strength tablets) administered every 6 hours at 0, 6, 12, 18, and 24 hours.

TABLE I.1. Vital Statistics of the Subjects Participating in the Study

Subject No.	Sex	Age (yrs)	Weight (Kg)	Max Dose (mg/kg)
1	F	22	59.00	16.95
2	M	25	55.00	18.18
3	M	27	68.18	14.67
4	M	25	62.00	16.13
5	M	27	63.64	15.71
6	F	29	53.64	18.64
7	M	29	79.55	12.57
8	F	24	58.15	17.19
Mean		26	62.40	16.26
Standard Deviation		2.45	8.37	1.97
Range		22-29	53.64-79.55	12.57-18.64

These treatments were administered on four separate occasions separated by at least a week according to a randomized block design (Snedecor and Cochran, 1980a; Peterson, 1986). Subjects fasted at least 12 hours prior and one hour after the first dose in each phase. Subjects were also required to fast 2 hours prior to and 1 hour after each dose for subsequent doses during each phase. No alcohol was permitted during the study. Treatments were taken with six fluid ounces of water and immediately after swallowing the tablets and fluid, the mouth was rinsed with 20 ml of Scope^{R} mouthwash (Proctor and Gamble, Cincinnatti, OH) followed by a water rinse to remove drug that was adsorbed to the buccal mucosa. Saliva samples were collected by chewing on parafilm (American Can Co., Greenwich, CT) squares of size one inch by one inch, for one minute with continuous spitting into a 12 ml glass centrifuge tube. All subjects were provided with an alarm watch to remind them of sample time.

Saliva was centrifuged (Beckman Model TJ-6 Centrifuge, Palo Alto, CA) at 3000 rpm for 30 minutes to remove mucous and particulate matter. Salivary supernatant was transferred to a polypropylene container with a lock cap and frozen at -20^{0} C until analyzed.

A blood sample was withdrawn from each subject for each treatment at a specified time by personnel at Good Samaritan Hospital Laboratory, centrifuged at 3000 rpm, and supernatant plasma transferred to polypropylene containers with a lock cap and frozen at -20^{0}C until analyzed. Blood samples were collected at 24.5 h, 25 h, 25.5 h and 26 h after the first dose from subjects 3 and 4, 1 and 6, 2 and 7, 5 and 8, respectively. This pattern of collecting blood samples was

followed to obtain a wide range of concentrations for correlation with saliva acetaminophen concentrations.

All participants in the study were taking no other medication during and one week prior to each phase. Criterion for exclusion from the study: History of chronic disease, therapy with an enzyme inducing agent within the previous 30 days of the study, recent myocardial infarction, allergy to acetaminophen, and subjects with bleeding gums.

STANDARD SOLUTIONS

Stock solutions containing 20, 50, 100, 200, 400, 500, 600, or 1000 µg/ml of acetaminophen (USP reference standard, USP, Inc., Rockville, MD) were prepared in distilled, deionized water. The internal standard was 2-acetamiodophenol (Aldrich Chemical Co., Inc., Milwaukee, WI) in deionized water (60 µg/ml). Standards were prepared by spiking 500 µl of blank saliva with 25 µl of stock solutions. 100 µl of the standard or unknown was combined with 100 µl of internal standard in 250 µl polyethylene centrifuge tubes and vortexed for fifteen seconds.

ACETAMINOPHEN HPLC ASSAY

An HPLC system consisting of a delivery pump (M-6000 A, Waters Associates, Milford, MA), automatic sample injector, (WISP 710 B, Waters Associates, Milford, MA), 30 cm reverse phase µ-Bondapak C18 column, (Waters Associates, Milford, MA), variable wave length UV detector (Lambda-max model 480, Waters Associates, MA), with wave length set at 254 nm and dual pen (Soltec company, Encino, CA) recorder

were used. A guard column (14 cm long and packed with C18 packing powder) was used to protect the column. The mobile phase used for HPLC was composed of methanol (25% v/v) in distilled water. Flow rate of the HPLC system was 1.4 ml/min and chart speed 4 inches/hour. Ten microliters of sample was injected at 0.02 AUFS sensitivity for concentrations below 20 µg/ml whereas for higher concentrations the sensitivity used was 0.05 AUFS. Retention times were four minutes for acetaminophen and six minutes for 2-acetamidophenol (internal standard used in concentrations of 60 µg/ml).

Linearity of peak height ratio versus acetaminophen concentration was excellent having correlation coefficients of greater than 0.995 with coefficients of variation ranging from 1 to 3%.

NONCOMPARTMENTAL ANALYSIS

Noncompartmental pharmacokinetic parameters for first and last dose were calculated and compared to determine whether acetaminophen exhibited linear or dose dependent pharmacokinetics. Half-lives were calculated using the slopes of the terminal phase of acetaminophen concentratons vs time curves.

ANOVA were performed using SIPS (Statistical Interactive Programming System for Cyber 70/73, Department of Statistics, Oregon State University) for statistical analysis for dose corrected AUC, half-life, terminal slopes, and mean residence times among treatments and subjects. Daily secretion of saliva normally ranges between about 800 and 1500 milliliters (Guyton 1986). Hence, less than 1% of the dose would be recirculated due to swallowing of saliva containing drug.

This negligible amount was ignored in all pharmacokinetic analyses. Saliva acetaminophen concentrations were determined using an HPLC procedure developed by Borin and Ayres (1985) which was a modification of an assay used by Gwilt (1984).

Pharmacokinetic parameters were calculated for the first dose, fifth dose (assuming steady state), corrected fifth dose (assuming steady state not reached) and fourth dose. Area under the curve (AUC) and area for the first moment (AUMC) were calculated using trapezoidal rule (Yamaoka 1979, Riegelman 1980). AUC and AUMC for the first dose (0-6 h) were extrapolated to infinity, whereas for the steady state (24-30 h) saliva acetaminophen concentrations areas were not extrapolated to infinity (assuming steady state has been reached by 24 hours). AUC and other pharmacokinetic parameters were calculated for fourth dose (18-24 h) in similar fashion as for steady state. AUC and AUMC for the fifth dose were recalculated (corrected parameters for fifth dose) assuming that steady state may not have been reached. This correction was achieved by extrapolating the terminal monoexponential decline in saliva APAP concentrations of the fourth dose to 30 h, and subtracting these from observed fifth dose concentrations, since saliva acetaminophen concentrations for the fifth dose represent the residual concentrations remaining from the fourth dose plus concentrations produced by the fifth dose. AUC and AUMC were calculated from 24-30 h extrapolated to infinity. Ratio of AUMC to AUC gave mean residence time (MRT) Riegelman and Collier (1980).

Clearance is a function of intrinsic ability of certain organs like liver, kidney, etc., to metabolize or excrete a drug, and blood flow

rates to these organs. As amount of drug eliminated after intravenous (IV) admininstration would equal the dose given, clearance (CL) is calculated as IV dose/AUC. Complete bioavailability is not assured when drug is given orally hence the ratio of dose to AUC is termed apparent clearance (CLapp) (Gibaldi 1986). Apparent clearance for each treatment was calculated.

Time to peak (tp) and maximum saliva APAP concentrations (Cmax) were obtained from concentration time data of individual subjects. Steady state volume of distribution (Vdss) after multiple dosing can be calculated from MRT (Hayton 1985). Because doses administered were oral in this research, apparent Vdss was calculated as (CLapp) (MRT).

Absolute bioavailability could not be calculated. However, bioavailability relative to the 1000 mg dose was calculated as ratio of average AUC for each dose to average AUC for the 1000mg dose.

RESULTS AND DISCUSSION

Eight subjects between the ages of 22 and 29 participated in the study. All subjects complied with the protocol and completed the study. Three subjects reported having a headache a few hours after the last dose. They did not report a headache after each treatment and there was no association between the dose and the reported headache. Two subjects reported headache at the lower dose whereas the third subject was at a higher dose. One subject was female and the other two were males. This side effect could be attributed to a disturbed sleep pattern due to the fact that subjects had to wake up in the middle of the night to collect samples.

Mean saliva acetaminophen concentrations have been reported to be proportional to serum concentrations (Glynn and Bastain 1973; Ahmed and Enever 1981; Aditan and Thangam 1982; Muclow 1982). The amount of drug present in saliva can be predicted theoretically knowing the pKa of the drug, saliva pH, plasma pH and fraction of drug bound to saliva and plasma protein (Mucklow 1982). Theoretical saliva to plasma ratio of APAP (pKa 9.5, drug unbound to plasma protein, assuming plasma pH of 7.4) would be 1.00 in the physiological range of saliva pH of 6, 7 or 8 (Mucklow 1982).

FIRST DOSE VS STEADY STATE

Mean saliva acetaminophen concentration versus time curves for all four treatments (325 mg, 650 mg, 825 mg, 1000 mg) are presented in Figure I.1. Peak acetaminophen concentration for the first dose and the last dose (steady state) were reached within 30 to 60 minutes

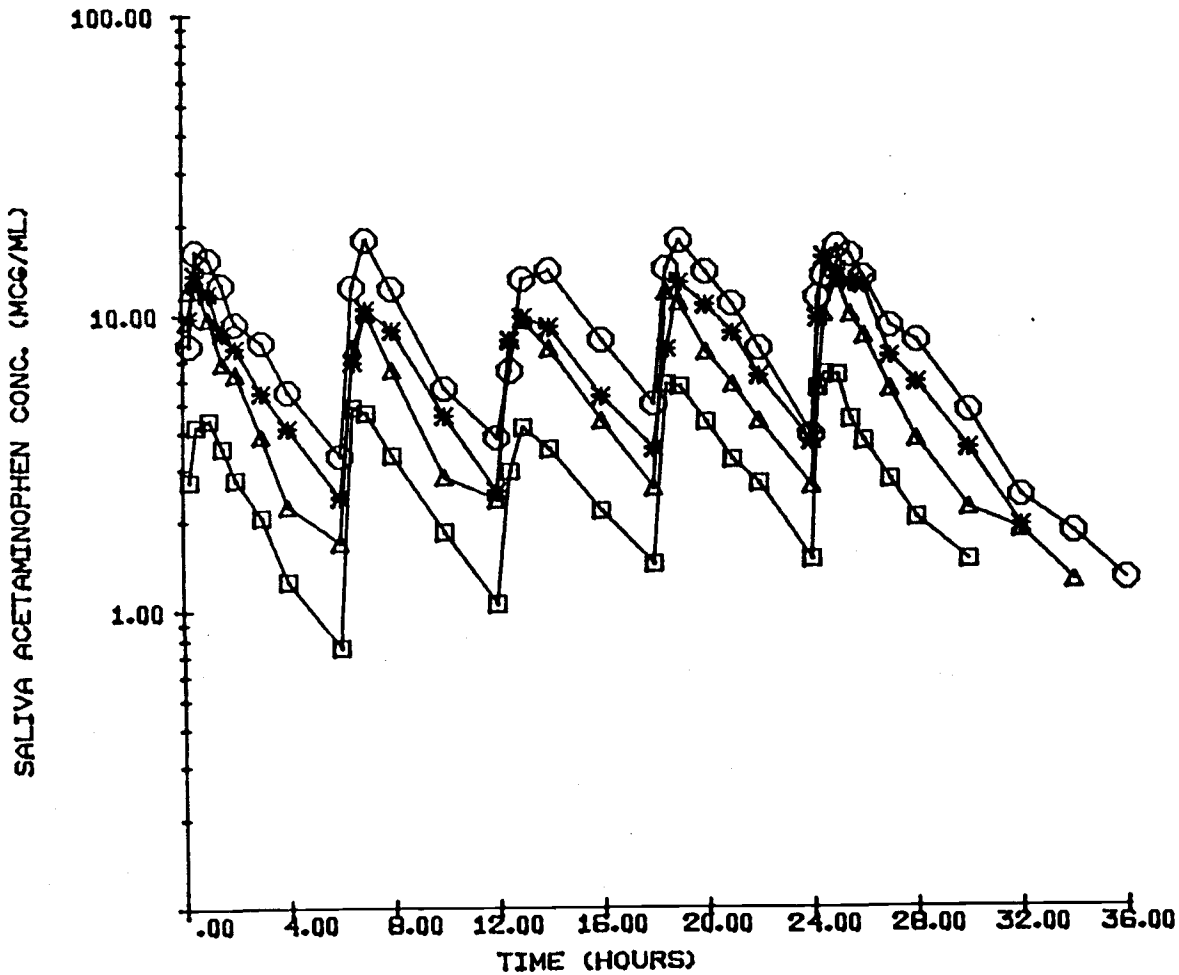

Figure I.1 Mean saliva acetaminophen concentration versus time curve for eight human subjects following multiple oral administration of commercial APAP tablets at 0, 6, 12, 18, and 24 hours. Key (□) 325 mg APAP; (△) 650 mg APAP; (✦) 825 mg APAP; (◯) 1000 mg APAP.

indicating rapid absorbtion and distribution. Saliva acetaminophen concentration time profiles for individual subjects are presented in appendix A (Figures A.1-A.8).

Pharmacokinetic parameters for the four treatments are presented in Tables I.2-I.5. Mean pharmacokinetic parameters are presented in Tables I.6a and I.6b (first and fifth dose).

The elimination half life ($T_{1/2}$) was estimated by least square regression of the saliva concentration - time curves from data lying in the terminal portion (2-6h) of log-linear plots. A non compartmental approach was used to calculate model independent parameters. The trapezoidal rule was utilized to calculate AUC and AUMC over the dosing intervals. AUC ($\int_0^\infty Cpdt$) following the administration of single dose equals the AUC ($\int_{t_1}^{t_2} Cpdt$) during a dosing interval at steady state (Leon 1985).

Dose dependency could be established if there is significant change among treatments or between the first and the last dose (due to accumulation of drug on multiple dosing) for $T_{1/2}$, dose corrected AUC, MRT, nonlinear plot of AUC vs dose.

Dose corrected AUC ANOVA indicated no significant differences among treatments ($P \leq .05$) for the first dose or the last dose. No statistically significant differences were noted among treatments for MRT (suggesting linearity of kinetics; Yamaoka et. al. 1978), or terminal slopes and $T_{1/2}$ for the first or the last dose. However average $T_{1/2}$ for the first dose ranged from 1.85 h (lower doses) to 2.2 h (for higher doses), but did not increase at steady state compared to the first dose for a particular treatment. Plots of AUC vs dose for

TABLE I.2. Pharmacokinetic Parameters Following Oral Administration of 325 mg Acetaminophen Tablets.

	First dose[a]					Fifth dose[b]				
Subject No.	β[c] hr^{-1}	$t_{\frac{1}{2}}$[d] hr	MRT hr	AUC[e] µg-hr/ml	CLapp[f] ml/min	β[c] hr^{-1}	$t_{\frac{1}{2}}$[d] hr	MRT hr	AUC[e] µg-hr/ml	CLapp[f] ml/min
1	0.36	1.93	3.08	15.57	347.8	0.35	1.98	2.13	20.60	263.0
2	0.53	1.31	2.09	14.37	377.0	0.42	1.65	3.20	14.25	380.2
3	0.39	1.78	3.34	10.24	529.0	0.36	1.93	2.10	17.21	314.8
4	0.38	1.82	3.22	15.49	349.7	0.41	1.69	1.97	16.47	328.8
5	0.27	2.31	3.81	22.49	240.8	0.30	2.57	2.34	28.50	190.0
6	0.32	2.17	2.29	15.46	350.3	0.31	2.24	3.38	23.48	230.7
7	0.36	1.93	2.37	13.60	398.2	0.38	1.82	2.80	17.15	315.8
8	0.39	1.78	2.30	10.37	522.3	0.38	1.82	1.71	14.25	380.2
Average	0.37	1.848	2.81	14.70	389.4	0.364	1.904	2.45	18.99	300.4
S.D.	0.075	---	0.63	3.83	95.8	0.043	---	0.61	4.94	68.1

[a] First dose taken at 0 hour, and all calculations are performed using the saliva acetaminophen concentrations obtained from 0 to 6 hours.
[b] Fifth dose, the dose taken at 24 hour, and all calculations are performed using the saliva acetaminophen concentrations obtained from 24 to 30 hours.
[c] Is the terminal slope for 0 to 6 hour or 24 to 30 hours
[d] Average of half-life is calculated as 0.693/average of β.
[e] Calculated using linear trapazoidal method.
[f] Apparent clearance dose/AUC

TABLE I.3. Pharmacokinetic Parameters Following Oral Administration of 650 mg Acetaminophen Tablets.

	First dose[a]					Fifth dose[b]				
Subject No.	β[c] hr^{-1}	$t_{\frac{1}{2}}$[d] hr	MRT hr	AUC[e] µg-hr/ml	CLapp[f] ml/min	β[c] hr^{-1}	$t_{\frac{1}{2}}$[d] hr	MRT hr	AUC[e] µg-hr/ml	CLapp[f] ml/min
1	0.3	2.31	3.40	41.96	258.2	0.3	2.31	2.51	45.09	240.3
2	0.53	1.31	2.21	27.54	393.3	0.48	1.44	2.27	27.06	400.3
3	0.32	2.17	3.24	33.84	320.2	0.35	1.98	2.19	28.72	377.2
4	0.43	1.61	2.38	33.76	320.8	0.34	2.04	2.33	44.10	245.7
5	0.38	1.78	3.43	28.64	378.3	0.36	1.95	2.46	31.77	341.0
6	0.41	1.69	2.62	38.68	280.0	0.42	1.65	2.69	37.07	292.2
7	0.38	1.82	2.68	30.74	352.5	0.35	1.87	2.99	37.27	290.7
8	0.30	2.31	3.51	38.47	281.7	0.35	2.01	2.32	31.40	345.0
Average	0.381	1.819	2.93	34.20	323.1	0.364	1.878	2.47	35.31	316.6
S.D.	0.078	---	0.52	5.16	48.8	0.056	---	0.28	6.75	58.8

[a] First dose taken at 0 hour, and all calculations are performed using the saliva acetaminophen concentrations obtained from 0 to 6 hours.
[b] Fifth dose, the dose taken at 24 hour, and all calculations are performed using the saliva acetaminophen concentrations obtained from 24 to 30 hours.
[c] Is the terminal slope for 0 to 6 hour or 24 to 30 hours
[d] Average of half-life is calculated as 0.693/average of β.
[e] Calculated using linear trapazoidal method.
[f] Apparent clearance. Dose/AUC

TABLE I.4. Pharmacokinetic Parameters Following Oral Administration of 825 mg Acetaminophen Tablets.

Subject No.	First dose[a] β[c] hr^{-1}	$t_{\frac{1}{2}}$[d] hr	MRT hr	AUC[e] µg-hr/ml	CLapp[f] ml/min	Fifth dose[b] β[c] hr^{-1}	$t_{\frac{1}{2}}$[d] hr	MRT hr	AUC[e] µg-hr/ml	CLapp[f] ml/min
1	0.24	2.89	3.53	71.59	192.0	0.25	2.77	2.50	60.73	226.3
2	0.47	1.47	2.28	40.22	341.8	0.40	1.73	2.81	49.91	275.5
3	0.28	2.48	3.74	33.15	414.8	0.31	2.34	2.24	38.77	354.7
4	0.29	2.39	3.55	59.38	231.5	0.33	2.10	2.26	57.70	238.3
5	0.35	1.93	3.09	30.99	443.7	0.36	1.98	2.20	45.55	301.8
6	0.39	1.78	2.84	41.95	327.7	0.35	1.98	3.00	55.75	246.7
7	0.30	2.29	2.98	42.38	324.5	0.38	1.93	2.81	45.23	304.0
8	0.36	1.93	3.00	39.45	348.5	0.32	2.17	2.70	39.30	349.8
Average	0.335	2.069	3.13	44.89	328.1	0.338	2.050	2.57	49.12	287.2
S.D.	0.073	---	0.47	13.73	84.0	0.047	---	0.31	8.32	49.1

[a] First dose taken at 0 hour, and all calculations are performed using the saliva acetaminophen concentrations obtained from 0 to 6 hours.
[b] Fifth dose, the dose taken at 24 hour, and all calculations are performed using the saliva acetaminophen concentrations obtained from 24 to 30 hours.
[c] Is the terminal slope for 0 to 6 hour or 24 to 30 hours
[d] Average of half-life is calculated as 0.693/average of β.
[e] Calculated using linear trapazoidal method.
[f] Apparent clearance. Dose/AUC

TABLE I.5. Pharmacokinetic Parameters Following Oral Administration of 1000 mg Acetaminophen Tablets.

	First dose[a]					Fifth dose[b]				
Subject No.	β[c] hr^{-1}	$t_{\frac{1}{2}}$[d] hr	MRT hr	AUC[e] µg-hr/ml	CLapp[f] ml/min	β[c] hr^{-1}	$t_{\frac{1}{2}}$[d] hr	MRT hr	AUC[e] µg-hr/ml	CLapp[f] ml/min
1	0.31	2.34	3.53	63.54	262.3	0.28	2.48	2.53	62.53	266.5
2	0.30	2.31	3.50	80.45	207.5	0.24	2.89	5.40	70.03	238.0
3	0.28	2.44	3.54	48.95	340.6	0.26	2.67	2.45	57.86	288.0
4	0.31	2.24	3.36	48.03	347.0	0.34	2.04	2.27	65.00	256.3
5	0.29	2.17	3.23	50.09	332.7	0.32	2.39	2.27	63.34	263.2
6	0.27	2.56	4.25	94.67	176.0	0.31	2.24	3.82	69.03	241.5
7	0.39	1.78	2.86	42.11	395.8	0.37	2.10	3.14	46.97	354.8
8	0.37	1.87	2.61	42.60	391.2	0.39	1.78	3.04	39.42	422.8
Average	0.315	2.200	3.36	58.81	306.6	0.314	2.207	3.16	59.27	291.4
S.D.	0.043	---	0.49	19.30	82.4	0.052	---	1.07	10.81	64.7

[a] First dose taken at 0 hour, and all calculations are performed using the saliva acetaminophen concentrations obtained from 0 to 6 hours.
[b] Fifth dose, the dose taken at 24 hour, and all calculations are performed using the saliva acetaminophen concentrations obtained from 24 to 30 hours.
[c] Is the terminal slope for 0 to 6 hour or 24 to 30 hours
[d] Average of half-life is calculated as 0.693/average of β.
[e] Calculated using linear trapazoidal method.
[f] Apparent clearance. Dose/AUC

TABLE I.6a. Mean Pharmacokinetic Parameters[a] (for the first dose 0 to 6 h) Following Oral Administration of Acetaminophen in Multiple Dosing to Eight Subjects.

	First dose								
Dose in mgs.	β[b] hr^{-1}	$t_{\frac{1}{2}}$[c] hr	MRT hr	AUC µg-hr/ml	AUC/d[e] mg-hr/ ml/mg	Relative[i] Bioavail	tp[g]	Cmax	CLapp[f] ml/min
325	0.375	1.848	2.45	18.99	0.058	0.79	0.75	5.4	389.4
	(0.075)		(0.061)	(4.94)	(0.0152)		(0.44)	(1.24)	(95.84)
650	0.381	1.819	2.47	35.31	0.054	0.91	0.44	14.91	323.1
	(0.078)		(0.28)	(6.75)	(0.010)		(.120)	(3.10)	(48.77)
825	0.335	2.069	2.57	49.12	0.060	0.94	0.50	15.28	328.1
	(0.073)		(0.31)	(8.32)	(0.010)		(0.23)	(5.00)	(83.97)
1000	0.315	2.200	3.16	59.27	0.059	1.00	0.59	(19.14)	306.5
	(0.043)		(1.07)	(10.81)	(0.011)		(0.27)	(3.75)	(82.36)

[a] Values in parenthesis are standard deviations
[b] Slope of the terminal portion of the saliva acetaminophen concentration-time curves.
[c] $0.693/\beta$
[d] Area under the curve for steady state calculated between 24 to 30 hours, not extrapolated to infinity.
[e] Area under the curve divided by dose (D).
[f] Apparent clearance = Dose/AUC
[g] tp is time to peak concentration
[h] steady state volume of distribution Vd_{SS} = (CLapp) (MRT)
[i] Relative bioavailability is calculated as bioavailability for each dose relative to 1000 mg dose. Average AUC for a dose/average AUC for 1000 mg dose.

TABLE 6b. Mean Pharmacokinetic Parameters[a] (for steady state 24 to 30 h) Following Oral Administration of Acetaminophen in Multiple Dosing to Eight Subjects

	Steady State									
Dose in mgs.	β[b] hr^{-1}	$t_{\frac{1}{2}}$[c] hr	MRT hr	AUC µg-hr/ml	AUC/d[e] mg-hr/ml/mg	Relative[i] Bioavail	tp[g]	Cmax	CLapp[f] ml/min	Vd_{SS} l/Kg
325	0.364 (0.043)	1.904	2.81 (0.68)	14.70 (3.83)	0.0452 (0.012)	0.99	0.50 (0.23)	7.48 (1.31)	300.4 (68.07)	0.846
650	0.369 (0.056)	1.878	2.93 (0.52)	34.20 (5.16)	0.053 (0.008)	0.93	0.72 (0.31)	13.59 (1.64)	316.6 (58.80)	0.953
825	0.338 (0.047)	2.050	3.13 (0.47)	44.89 (13.73)	0.054 (0.017)	1.01	1.06 (0.68)	16.74 (4.12)	287.2 (49.05)	0.864
1000	0.314 (0.052)	2.207	3.36 (0.49)	58.81 (19.30)	0.059 (0.019	1.00	0.97 (0.67)	18.10 (4.24)	291.4 (64.7)	0.941

[a] Values in parenthesis are standard deviations
[b] Slope of the terminal portion of the saliva acetaminophen concentration-time curves.
[c] $0.693/\beta$
[d] Area under the curve for steady state calculated between 24 to 30 hours, not extrapolated to infinity.
[e] Area under the curve divided by dose (D).
[f] Apparent clearance = Dose/AUC
[g] tp is time to peak concentration
[h] steady state volume of distribution Vd_{SS} = (CLapp) (MRT)
[i] Relative bioavailability is calculated as bioavailability for each dose relative to 1000 mg dose. Average AUC for a dose/average AUC for 1000 mg dose.

the first and the fifth dose are presented as Figure I.2, it does not exhibit curvature, and area under the saliva concentration time curve increases proportionately with dose, hence indicates linearity of pharmacokinetics. Significant differences in T1/2 and MRT were noted among subjects, which would be expected due to intersubject biological variations.

Cmax (peak acetaminophen conc.) among treatments increased with increasing dose as would be expected. Tmax (time to peak concentration) for the first dose ranged from 0.44h to 0.70h and at steady state ranged from 0.5 h to 1.06 h. Although statistically not significant, mean Tmax for the fifth dose (650, 825, and 1000 mg) was higher than for the first dose suggesting that the rate of absorption may be dose dependent. Dose dependency of absorption has also been suggested by other researchers (Rawlins 1979, Ameer 1982, Wilson et.al. 1982).

Saliva acetaminophen concentrations for individual subjects were normalized and dose corrected to 325 mg. Average dose corrected concentration - time curves are presented in Figure I.3 and are superimposable, indicating linear pharmacokinetic behavior of acetaminophen. Dose corrected concentration - time curves for individual subjects are presented in appendix A Figures A.17-A.24.

Dose and weight corrected AUC for each subject and treatment are presented in Table I.7. This data were generated by dividing AUC by dose in mg/kg (Table A.1, appendix A provides dose in mg/kg for each subject). Mean values of AUC (Table I.7) are not significantly different suggesting that APAP follows linear kinetics.

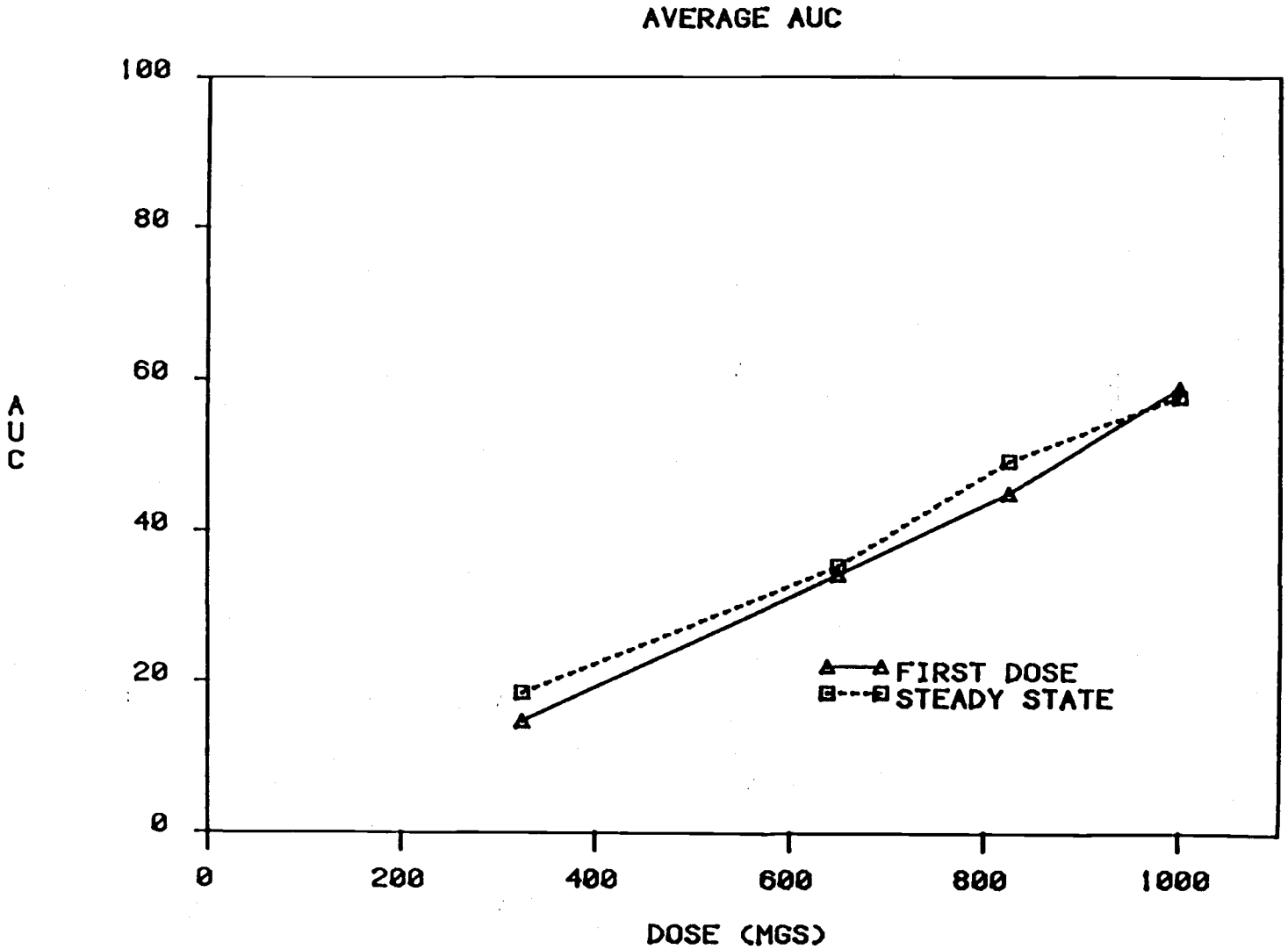

Figure I.2 Mean area under the saliva APAP concentration -time curve versus dose for eight subjects.

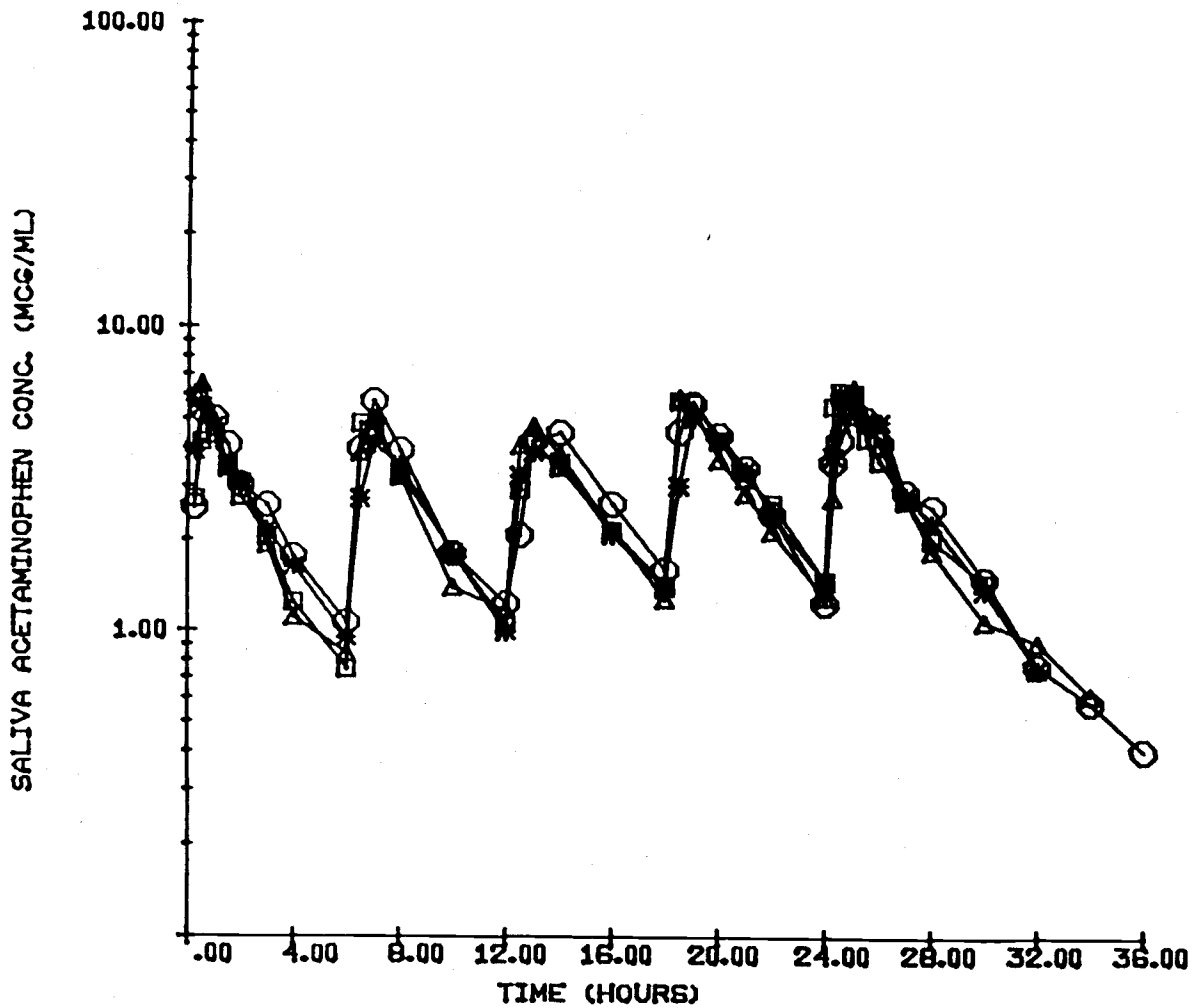

Figure I.3 Saliva acetaminophen concentration versus time curve for eight human subjects normalized to 325 mg dose following multiple oral administration of commercial APAP tablets at 0, 6, 12, 18, and 24 hours. Key (□) 325 mg APAP; (△) 650 mg APAP; (✦) 825 mg APAP; (◯) 1000 mg APAP.

TABLE I.7. Dose and Weight Corrected[a] Area Under the Curves For Each Treatment.

Subject No.	First Dose (0 to 6 h) 325 mg	650 mg	825 mg	1000 mg	Steady State (24 to 30 h) 325 mg	650 mg	825 mg	1000 mg
1	2.83	3.81	5.12	3.75	3.74	4.09	4.34	3.69
2	2.43	2.33	2.68	4.42	2.41	2.29	3.33	3.85
3	2.15	3.55	2.74	3.34	3.61	3.13	3.20	3.94
4	2.96	3.22	4.46	2.98	3.14	4.21	4.34	4.03
5	4.40	2.81	2.39	3.19	5.58	3.12	3.52	4.03
6	2.57	3.21	2.75	5.11	3.9	3.08	3.65	3.80
7	3.24	3.69	4.00	3.30	4.08	4.47	4.27	3.66
8	1.86	3.44	2.78	2.48	2.55	2.81	2.77	2.29
Average	2.81	3.26	3.37	3.57	3.63	3.40	3.68	3.66
S.D.[b]	0.78	0.49	1.02	0.84	0.99	0.77	0.59	0.57
Relative[c] Bioava.	0.79	0.91	0.94	1.00	0.99	0.93	1.01	1.00

[a] Area under the curve obtained was divided by dose in mg/kg hence the values represent (AUC)/(mg/kg).
[b] Standard deviation.
[c] Relative bioavailability - calculated as bioavailability for each dose relative to 1000 mg dose. (Average AUC for the dose/average AUC for 1000 mg dose).

Rate of absorption (Ka) could not be calculated as there were not enough data points prior to peak concentration of the drug. APAP is reported to follow two compartment model pharmacokinetics and is hypothesized to be following multicompartmental model (Rawlins 1981). Since enough data were not available to determine Ka and other microconstant reliably, a non compartmental approach was preferred to a compartmental approach. Estimation of Ka during multiple dosing is difficult since the residual drug from the previous dose superimposes on the dose that follows (Leon 1985). Residual concentrations of previous doses were subtracted from the fifth dose (corrected fifth dose; presented later in this section) but still there points prior to peak concentrations to calculate Ka reliabil. Ka cannot be calculated from MRT as such calculations are model dependent (Reigelman 1980). MRT = 1/ka + 1/ke (one compartment), MRT = 1/ka + (K12 + K21)/keK21 (for two compartment model) and MRT = (kb + ke)/kake + 1/ke (for one compartment with enterohepatic cycling where K12, K21 are first order partition rate constants out of and back to the centeral and peripheral compartments and kb is the first order rate constant of bile excretion). Hence, it is evident from the above that ka cannot be calculated from MRT until compartmental microconstants are known. However, a longer Tmax for higher doses at steady state (Table I.6) is indicative of dose dependent rates of absorption.

It has been reported that with some drugs, drug-induced malabsorption syndrome can alter the percentage of drug absorbed, decreasing the bioavailability after administration of repeated doses due to a decrease in F (fraction of the dose absorbed) or due to an

increase in the total body clearance ($AUC_0^\infty = FD/CL$). Rawlins (1979) in his study reported that bioavailability decreases from 0.9 at 1000 mg and 2000 mg to 0.63 at 500 mg for oral doses. This has been attributed to presystemic biotransformation or metabolism in the epithelium of the GI tract or a combination of such processes (George 1981; Ameer et. al. 1983). AUC at steady state for some subjects (Table I.2-I.5) was lower than AUC for the first dose. This observation of decreased AUC at steady state could be attribued to presystemic biotransformation or drug induced malabsorption syndrome.

Absolute bioavailability cannot be calculated since no APAP was administered via the intravenous (IV) route. However, bioavailability relative to 1000 mg dose was calculated (Table I.6). Relative bioavailability for the first dose of 325 mg was about 80% compared to 91 and 94% for 650 and 825 mg doses respectively. Bioavailability relative to 1000 mg dose at steady state was 93% to 99% for all treatments indicating that at steady state the amount of drug absorbed from lower doses (325 mg) is not substantially different from that absorbed from higher doses (650 mg - 1000 mg). However, for the first dose, relative bioavailability from the lower dose (325 mg) was considerably (20%) lower than the higher dose (1000 mg), which is in agreement with that reported by Rawlins (1979). It should be noted that Rawlins calculated absolute bioavailability.

Apparent clearance (dose/AUC; Table I.6) although not significantly different, decreases as the dose is increased. Apparent clearance also decreases at steady state compared to the first dose and ranged between 290 ml/min and 325 ml/min (with exception of 325 mg

dose; Table I.6). Decreased relative bioavailability for 325 mg (first dose) could be attributed to increased apparent total body clearance. Total body clearance reported by Rawlins (1979) is 352±40 ml/min and is close to the apparent clearance calculated (Table I.6).

Steady state apparent volumes of distribution (Vdss) calculated as (CLapp) (MRT) are presented in Table I.6. Apparent Vdss ranged from 0.84L/kg to 0.95L/kg and indicate that apparent Vdss is independent of the dose (maximum difference in app. Vdss among treatments being about 12%). Vd_{β} reported for 1000 mg injections and 12 mg/kg injections are 0.9 L/kg and 0.96 L/kg, respectively (Forrest et. al. 1982).

Dose normalized plots for all subjects except subject No. 2 were superimposable (Figure I.4). Figure I.4 indicates that the 1000 mg dose normalized to 325 mg is not superimposable, indicative of nonlinear pharmacokinetics being followed in subject No. 2. Nonlinearity for subject is also indicated by AUC vs dose (Figure I.5) which shows curvature with increased dose. Subject 2 received a maximum of 18.18 mg/kg body weight dose (1000 mg; Table A.1) whereas other subjects received doses lower than 18 mg/kg. This indicates that APAP follows nonlinear kineitcs for doses greater than 18 mg/kg, however, it cannot be substantiated until an organized study administering doses greater than 18 mg/kg is conducted. A study conducted with children (Nahata and Powell 1982) in which multiple doses of 24 to 30 mg/kg were administered every 8 hours for 72 hours did not indicate non linear pharmacokinetics for APAP.

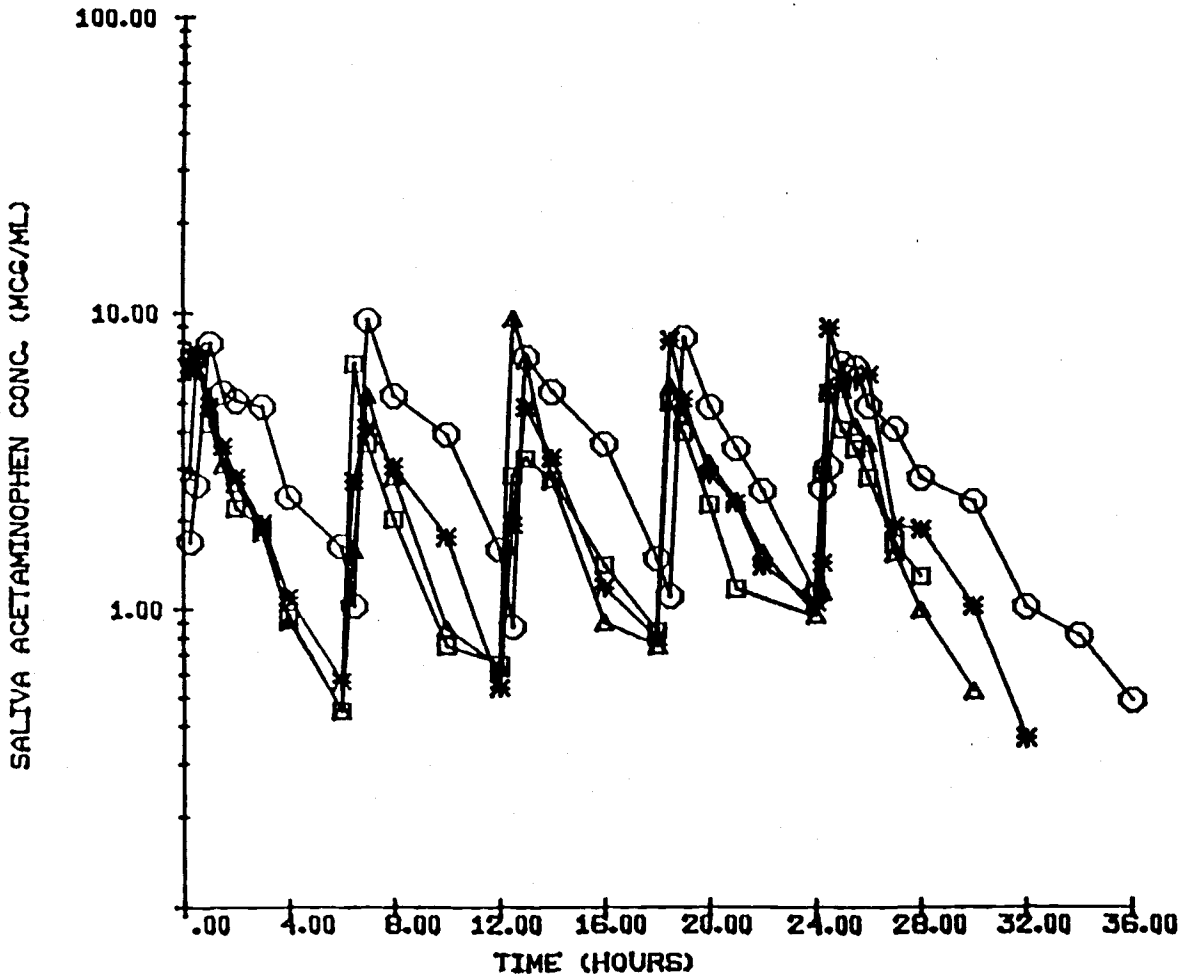

Figure I.4 Saliva acetaminophen concentration versus time curve for subject no. 2 each dose normalized to 325 mg dose. Key (□) 325 mg APAP; (△) 650 mg APAP; (✦) 825 mg APAP; (◯) 1000 mg APAP.

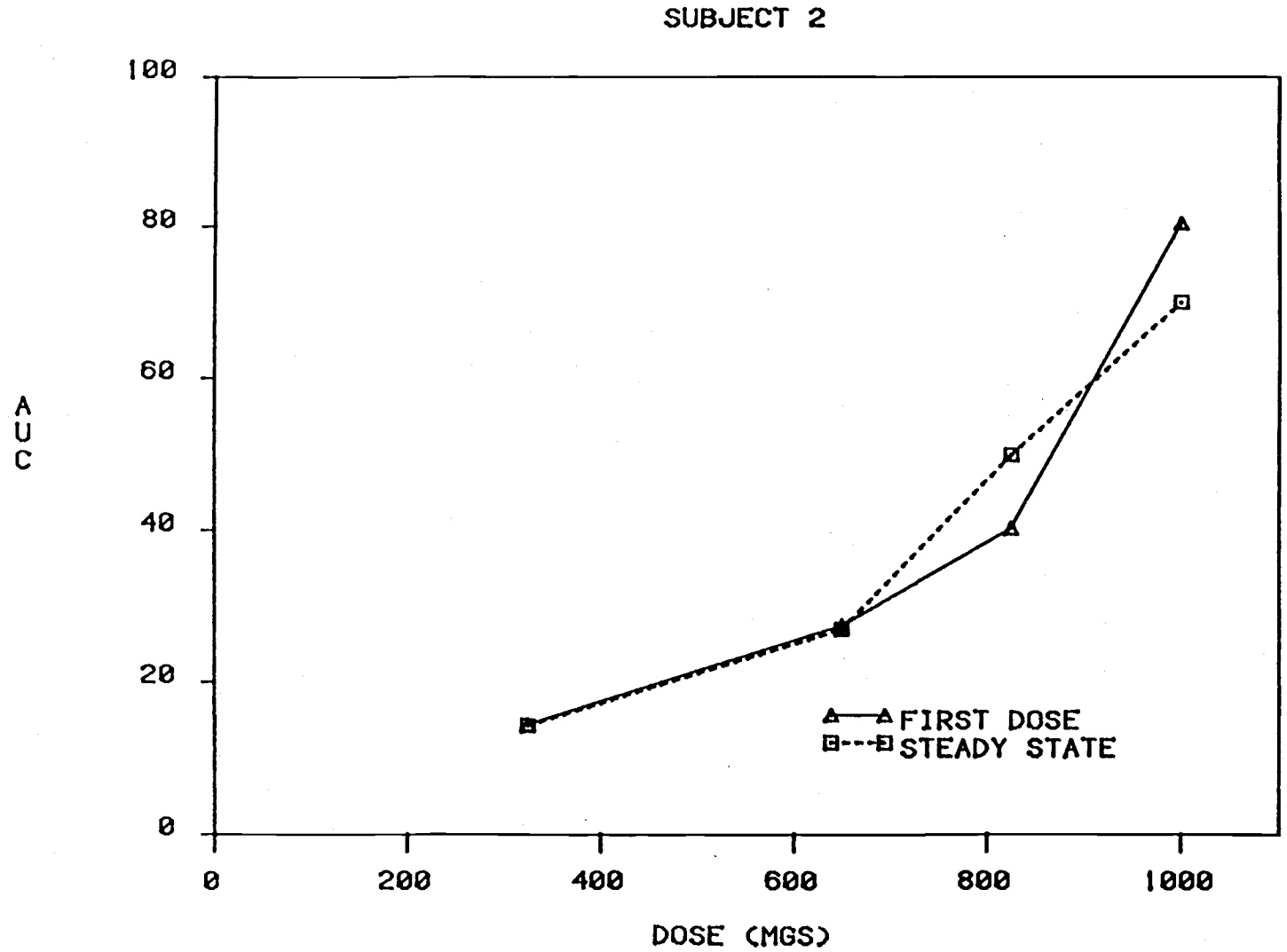

Figure I.5 Area under the saliva APAP concentration - time curve versus dose for subject No: 2.

FIFTH DOSE CORRECTED

In calculations of the above discussed parameters, steady state was assumed, AUC and AUMC were calculated without extrapolating to infinity. AUC and AUMC for the fifth dose were recalculated (corrected fifth dose; as explained in materials and methods) assuming that steady state may not have been reached and these pharmacokinetic parameters are presented in Tables I.8-I.11, and averages are summarized in Table I.12. Dose corrected AUC, MRT, apparent clearance and $T_{1/2}$ were not significantly different among treatments and neither were these parameters different compared to parameters calculated assuming steady state (Table I.6b). This indicates that the assumption that steady state was reached by the fifth dose was corrected as expected.

FOURTH DOSE

Pharmacokinetic parameters calculated for the fourth dose (18-24 h) for individual subjects are presented in tables I.13-I.16 and mean parameters in Table I.17. Steady state was assumed while calculating these parameters. No significant differences were noted in dose corrected AUC, apparent clearance, or half life among treatments. MRT for 325 mg, 650 mg, or 825 mg were not significantly different from that of steady state MRT (Table I.6b). However, MRT for the fourth dose for 1000 mg was significantly different from that at steady state. Half-life for the fourth dose were slightly higher than those at steady state, although β (terminal slopes) were not significantly different among treatments. Parameters for the fourth dose may not be reliable as there were only six data points during dosing interval which could give incorrect estimates of AUC and terminal slopes.

TABLE I.8. Pharmacokinetic Parameters Calculated for the Fifth Dose[a] (corrected) Following Oral Administration of 325 mg Acetaminophen in Multiple Doses.

Subject No.	β hr^{-1}	$t_{\frac{1}{2}}$[b] hr	MRT hr	AUC[c] µg-hr/ml	CLapp[d] ml/mim
1	0.38	1.82	2.97	17.11	316.5
2	0.41	1.69	4.02	18.61	291.0
3	0.38	1.82	2.81	16.30	332.3
4	0.41	1.69	2.63	12.69	426.8
5	0.25	2.77	4.31	29.03	186.5
6	0.23	3.01	4.65	29.05	186.5
7	0.38	1.82	2.80	17.04	317.8
8	0.40	1.73	1.70	12.42	435.5
Average	0.355	1.952	3.24	19.03	311.6
S.D.	0.072	---	0.99	6.54	93.2

a Residual concentrations from fourth dose were subtracted from fifth dose observed concentrations and above parameters were calculated.
b Average $t_{\frac{1}{2}}$ calculated as 0.693/average β.
c Area under the curve is calculated by linear trapazoidal method, between 24 to 30 h (corrected concs) and extrapolated to infinity.
d Apparent Clearance Dose/AUC

TABLE I.9. Pharmacokinetic Parameters Calculated for the Fifth Dose[a] (corrected) Following Oral Administration of 650 mg Acetaminophen in Multiple Doses.

Subject No.	β hr-1	$t_{\frac{1}{2}}b$ hr	MRT hr	AUC[c] µg-hr/ml	Clapp[d] ml/min
1	0.31	2.2	2.61	34.46	314.3
2	0.57	1.22	2.27	24.94	434.2
3	0.41	1.69	2.77	22.57	480.0
4	0.35	1.98	3.67	51.17	211.7
5	0.44	1.58	2.55	26.24	412.8
6	0.43	1.61	2.69	33.27	325.7
7	0.35	1.98	2.99	34.03	318.3
8	0.33	2.10	3.09	34.27	316.2
Average	0.399	1.737	2.83	32.62	351.7
S.D.	0.084	---	0.42	8.89	85.4

a Residual concentrations from fourth dose were subtracted from fifth dose observed concentrations and above parameters were calculated.
b Average $t_{\frac{1}{2}}$ calculated as 0.693/average β.
c Area under the curve is calculated by linear trapazoidal method, between 24 to 30 h (corrected concs) and extrapolated to infinity.
d Apparent Clearance Dose/AUC

TABLE I.10. Pharmacokinetic Parameters Calculated for the Fifth Dose[a] (corrected) Following Oral Administration of 825 mg Acetaminophen in Multiple Doses.

Subject No.	β hr-1	$t_{\frac{1}{2}}b$ hr	MRT hr	AUC[c] µg-hr/ml	CLapp[d] ml/min
1	0.27	2.57	4.03	54.34	253.0
2	0.41	1.69	2.81	53.11	258.8
3	0.32	2.17	3.61	28.42	483.8
4	0.35	1.98	3.23	52.04	264.2
5	0.35	1.98	3.68	47.95	286.8
6	0.36	1.93	3.01	50.17	274.0
7	0.38	1.82	2.81	42.67	322.2
8	0.33	2.10	4.07	36.57	376.0
Average	0.346	2.003	3.41	45.66	314.9
S.D.	0.042	---	0.51	9.15	79.6

a Residual concentrations from fourth dose were subtracted from fifth dose observed concentrations and above parameters were calculated.
b Average $t_{\frac{1}{2}}$ calculated as 0.693/average β.
c Area under the curve is calculated by linear trapazoidal method, between 24 to 30 h (corrected concs) and extrapolated to infinity.
d Apparent Clearance Dose/AUC

TABLE I.11. Pharmacokinetic Parameters Calculated for the Fifth Dose[a] (corrected) Following Oral Administration of 1000 mg Acetaminophen in Multiple Doses.

Subject No.	β hr^{-1}	$t_{\frac{1}{2}}$[b] hr	MRT hr	AUC[c] µg-hr/ml	CLapp[d] ml/min
1	0.31	2.24	4.02	53.40	312.2
2	0.39	1.78	5.35	102.69	162.3
3	0.41	1.69	3.09	64.43	258.7
4	0.37	1.87	2.97	58.62	284.3
5	0.31	2.24	3.38	56.35	295.8
6	0.30	2.31	3.82	66.61	252.2
7	0.37	1.87	3.17	48.44	344.0
8	0.39	1.78	4.22	42.17	395.2
Average	0.356	1.950	3.75	61.57	288.1
S.D.	0.043	---	0.79	18.42	68.9

a Residual concentrations from fourth dose were subtracted from fifth dose observed concentrations and above parameters were calculated.
b Average $t_{\frac{1}{2}}$ calculated as 0.693/average β.
c Area under the curve is calculated by linear trapazoidal method, between 24 to 30 H (corrected concs) and extrapolated to infinity.
d Apparent Clearance Dose/AUC

TABLE I.12. Mean Pharmacokinetic Parameters for the Fifth Dose[a] (corrected) For Residual Concentrations of Fourth Dose Following Oral Administration of Acetaminophen in Multiple Doses.

Dose mgs	β[b] hr^{-1}	$t_{\frac{1}{2}}$[c] hr	MRT hr	AUC[d] µg-hr/ml	AUC/d[e] µg-hr/ml/mg	CLapp[f] ml/min
325	0.355 (0.072)	1.952	3.24 (0.99)	19.03 (6.54)	0.059 (0.020)	311.6 (93.2)
650	0.399 (0.084)	1.737	2.83 (0.42)	32.62 (8.89)	0.050 (0.014)	351.7 (85.4)
825	0.346 (0.042)	2.003	3.41 (0.51)	45.66 (9.15)	0.055 (0.011)	314.9 (79.6)
1000	0.356 (0.043)	1.950	3.75 (0.79)	61.57 (18.42)	0.062 (0.018)	288.1 (68.9)

a Values in parentheses are standard deviation.
b Slopes of the terminal portion of the saliva acetaminophen concentration-time curves between 24 to 30 hours.
c $0.693/\beta$.
d Area under the curve obtained from corrected acetaminophen concentrations for 24 to 30 hours and extrapolated to infinity.
e Area under the curve divided by dose.
f Apparent clearance Dose/AUC

TABLE I.13. Pharmacokinetic Parameters Calculated for the Fourth Dose (18 to 24 h)[a] Following Oral Administration of 325 mg Acetaminophen in Multiple Doses.

Subject No.	β[b] hr^{-1}	$t_{\frac{1}{2}}$[c] hr	MRT hr	AUC[d] µg-hr/ml	CLapp[f] ml/min
1	0.25	2.77	2.37	23.96	226.0
2	0.58	1.19	2.13	12.12	447.0
3	0.31	2.24	2.31	16.39	330.5
4	0.25	2.77	2.35	21.71	249.5
5	0.26	2.66	2.41	30.04	180.3
6	0.34	2.04	2.22	26.74	202.5
7	0.37	1.87	2.65	15.56	348.2
8	0.37	1.87	2.18	14.18	382.0
Average	0.341	2.032	2.33	20.09	295.8
S.D.[e]	0.108	---	0.16	6.46	95.1

a Calculated from saliva acetaminophen conc. vs time curve between 18 to 24 h.
b Slope of the terminal portion.
c $0.693/\beta$, average $t_{\frac{1}{2}}$ calculated as 0.693/average β.
d Area under the curve calculated using linear trapazoidal method.
e Standard deviation.
f Apparent clearance Dose/AUC

TABLE I.14. Pharmacokinetic Parameters Calculated for the Fourth Dose (18 to 24 h)[a] Following Oral Administration of 650 mg Acetaminophen in Multiple Doses.

Subject No.	β[b] hr^{-1}	$t_{\frac{1}{2}}$[c] hr	MRT hr	AUC[d] µg-hr/ml	CLapp[f] ml/min
1	0.26	2.67	2.37	47.88	226.3
2	0.36	1.94	2.19	30.25	358.2
3	0.22	3.15	2.61	29.76	364.0
4	0.36	1.94	2.00	26.47	409.3
5	0.23	3.01	2.58	29.65	365.3
6	0.34	2.04	2.16	46.99	230.5
7	0.26	2.67	2.26	37.64	287.8
8	0.57	1.22	2.47	37.38	289.8
Average	0.325	2.132	2.32	35.75	316.4
S.D.[e]	0.114	---	0.215	8.19	67.7

a Calculated from saliva acetaminophen conc. vs time curve between 18 to 24 h.
b Slope of the terminal portion.
c $0.693/\beta$, average $t_{\frac{1}{2}}$ calculated as 0.693/average β.
d Area under the curve calculated using linear trapazoidal method.
e Standard deviation.
f Apparent clearance Dose/AUC

TABLE I.15. Pharmacokinetic Parameters Calculated for the Fourth Dose (18 to 24 h)[a] Following Oral Administration of 825 mg Acetaminophen in Multiple Doses.

Subject No.	β[b] hr^{-1}	$t_{\frac{1}{2}}$[c] hr	MRT hr	AUC[d] µg-hr/ml	CLapp[f] ml/min
1	0.20	3.47	2.52	57.26	240.2
2	0.47	1.47	2.03	40.77	337.3
3	0.28	2.48	2.40	39.95	344.2
4	0.24	2.89	2.39	53.11	258.8
5	0.29	2.39	2.67	37.75	364.2
6	0.32	2.17	2.39	71.10	193.3
7	0.29	2.39	2.30	46.89	293.2
8	0.30	2.31	3.02	36.16	380.3
Average	0.299	2.318	2.46	47.87	301.4
S.D.[e]	0.079	---	0.289	12.01	66.1

a Calculated from saliva acetaminophen conc. vs time curve between 18 to 24 h.
b Slope of the terminal portion.
c $0.693/\beta$, average $t_{\frac{1}{2}}$ calculated as 0.693/average β.
d Area under the curve calculated using linear trapazoidal method.
e Standard deviation
f Apparent clearance Dose/AUC

TABLE I.16. Pharmacokinetic Parameters Calculated for the Fourth Dose (18 to 24 h)[a] Following Oral Administration of 1000 mg Acetaminophen in Multiple Doses.

Subject No.	β[b] hr^{-1}	$t_{\frac{1}{2}}$[c] hr	MRT hr	AUC[d] µg-hr/ml	CLapp[f] ml/min
1	0.23	3.01	2.48	77.13	216.2
2	0.29	2.39	2.38	62.19	268.0
3	0.38	1.82	2.61	47.36	351.8
4	0.31	2.24	2.14	60.54	275.3
5	0.24	2.89	2.28	54.68	340.8
6	0.32	2.17	2.45	90.25	184.7
7	0.43	1.61	2.29	49.90	334.0
8	0.37	1.87	2.20	45.62	365.3
Average	0.321	2.159	2.35	60.96	292.0
S.D.[e]	0.069	---	0.155	15.60	66.8

a Calculated from saliva acetaminophen conc. vs time curve between 18 to 24 h.
b Slope of the terminal portion.
c $0.693/\beta$, average $t_{\frac{1}{2}}$ calculated as 0.693/average β.
d Area under the curve calculated using linear trapazoidal method.
e Standard deviation.
f Apparent clearance

TABLE I.17. Mean Pharmacokinetic Parameters for the Fourth Dose[a] (18 to 24 h) Following Oral Administration of Acetaminophen in Multiple Doses

Dose mgs	β[b] hr^{-1}	$t_{\frac{1}{2}}$[c] hr	MRT hr	AUC[d] µg-hr/ml	AUC/d[e] µg-hr/ml/mg	CLapp[f] ml/min
325	0.341 (0.108)	2.032	2.33 (0.160)	20.09 (6.48)	0.062 (0.021)	295.8 (95.1)
650	0.325 (0.114)	2.132	2.32 (0.215)	35.75 (8.19)	0.055 (0.0126)	316.4 (67.7)
825	0.299 (0.079)	2.318	2.46 (0.289)	47.87 (12.01)	0.058 (0.015)	301.4 (66.1)
1000	0.321 (0.069)	2.159	2.35 (0.155)	60.96 (15.60)	0.061 (0.016)	292.0 (66.1)

a Values in parentheses are standard deviation.
b Slopes of the terminal portion of the saliva acetaminophen concentration-time curves between 18 to 24 hours.
c $0.693/\beta$.
d Area under the curve obtained from acetaminophen concentrations for 18 to 24 hours and not extrapolated to infinity.
e Area under the curve divided by dose.
f Apparent clearance Dose/AUC

CONCLUSIONS

Four treatments of acetaminophen were administered to eight human subjects in multiple doses and saliva samples collected over a period of 36 hours. All subjects followed the protocol and completed the study successfully. Pharmacokinetic parameters for the first dose and steady state were compared. Peak concentrations for all treatments were reached within 1 h indicating rapid absorption and distribution. Tmax, although statistically non-significant, tended to increase at higher doses at steady state suggesting a dose dependent rate of absorbtion. Cmax was significantly different for treatments as would be expected with the increasing doses. Dose corrected AUC, MRT, $T_{1/2}$, and apparent clearance were not significantly different among treatments. Plots of saliva acetaminophen concentrations vs time normalized to a 325 mg dose were superimposable, indicating linearity of kinetics being followd by APAP. Based on peak and trough levels and comparing first dose and steady state parameters, no accumulation of drug was noted over the study period of treatments. This is in agreement with the results of Nahata and Powell (1982) who reported no accumulation of drug in children receiving 24-30 mg/kg doses every eight hours for a period of 72 hours. However one subject receiving greater than 18 mg/kg dose exhibited non linear kinetics based on a curved plot for AUC vs dose. APAP may follow (in adults) non linear dose dependent kinetics for doses greater than 18 mg/kg; or, it is possible that this subject had impaired metabolism of the drug compared to other subjects. Bioavailability relative to the 1000 mg dose at steady state was comparable for high and low doses. However, for the

first dose, the 325 mg dose was only 80% bioavailable compared to the 1000 mg first dose. This and the fact that Tmax increases with dose are indicative that bioavailability and rate or absorption may be dose dependent and would also support the finding of Rawlins (1979) that lower doses had lower bioavailability, but in multiple dosing when steady state is reached, bioavailability is comparable for the doses studied.

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CHAPTER II

SUSTAINED RELEASE ACETAMINOPHEN SUSPENSIONS: A USEFUL MODEL?

INTRODUCTION

Acetaminophen (APAP) pellets prepared at Warner-Lambert Co., have been studied at Oregon State University College of Pharmacy. The original objective was to develop a crushable, chewable, controlled sustained release tablet. A Ph.D. thesis on this subject was completed and a report submitted to Warner Lambert (Borin 1985). One aspect of the research included absorption from pellets which were not compressed into tablets. This comparison was necessary to evaluate the effects of compression. An additional research effort was directed towards preliminary development and evaluation of an oral controlled and sustained release suspension formulation. Such a product avoids compression forces and may be useful for pediatric and elderly patients. If successful, the acetaminophen formulation can be considered a "model" from which modified formulations may be prepared for other drugs.

One of the triple-layered pellet formulations which was available after completion of initial tablet research was prepared in a pH adjusted suspension vehicle to test release from non-compressed pellets. The pellets consisted of acetaminophen on a sugar core support, and were coated with a "sandwich" membrane of 5% ethylcellose/15% eudragit L30D (enteric coat)/5% ethylcellose. It was hypothesized that the pellets might be formulated in an acidic suspension without release of drug, but then would release drug when taken orally. After eleven days of storage there was no release of acetaminophen from the pellets into the suspension vehicle.

To carry out further studies, a fresh suspension using the same

vehicle formulation (see Materials and Methods) was prepared, but using APAP pellets coated with 5/10/5 (ethylcellulose/enteric coat/ethylcellulose). The 5/10/5 coat was used rather than 5/15/5 because more 5/10/5 pellets remained from the earlier studies. Evaluation included dissolution testing of suspension formulations on days 1, 28, 90, 270 (9 months) and 355 (approx. one year). These suspension formulations containing APAP (5/10/5)were also administered to two human subjects and sustained saliva acetaminophen concentrations vs time profiles obtained (see Results and Discussion Section).

5/5/5 pellets (ethylcellulose/endragit L30D (enteric coat)/ethylcellulose) were also suspended in the vehicle. A dissolution profile at the end of 3 months was obtained and is presented in the Results section, but study at different times could not be performed due to a limited supply of 5/5/5 pellets. Additional pellets were not requested from Warner Lambert since this was still part of a preliminary investigation and the "best" pellet coating for further research had not been determined.

Formulation with a negligible amount of water was also considered desirable. A semisolid vehicle (hence forth referred to as a "non-aqueous formulation") was combined with APAP pellets coated with 2% ethylcellulose or 2% ethylcellulose/Eudragit, and 10% ethylcellulose coated granules of APAP (for immediate release). These pellets were selected since Borin reported (Borin 1985) that the time to 50% dissolution of acetaminophen was about 12 and 14.5 hours respectively. Release patterns of APAP from pellets aged in this formulation are also included in the Results and Discussion section.

MATERIALS AND METHODS

Acetaminophen pellets were spray coated with sustained release coating materials at Warner-Lambert Company in Morris Plains, New Jersey. Aquacoat (FMC Corp., Philadelphia, PA) was used as ethylcellulose coat and Eudragit L30D (Rohur Pharma, Weiterstadt, West Germany), an enteric coating material of polymethacrylic acid and acrylic acid esters were used for spray coating acetaminophen pellets. Detailed spray coating procedure has been described by Borin and Ayres (Borin 1985).

AQUEOUS VEHICLE

The suspension formulation initially contained 8 gms of pellets (5/15/5) which was 4.48 gm (56%) acetaminophen in 40 ml of (% in w/v):

Methyl cellulose 1%
Sodium stearate 0.1%
Methyl and Propylparaben 0.01% each
Mannitol 10%
Kaolin 2%
Lecithin 0.8%
Flavor (raspberry) 0.25%
Color (saffaranin) 1 drop (optional)
Sodium saccharin 0.3%
Saccharin 0.03%

The pH of the suspension was adjusted to either 4.0 or 4.5 with citric acid, stored at room temperature or refrigerated and shaken occasionally. After 11 days one teaspoonful was placed in a test tube, and the pellets washed with 100 ml of simulated gastric fluid. The gastric fluid was divided into four equal portions, followed by vortexing, allowing the pellets to settle, and removing the supernatant. Washed pellets were then placed on a filter paper and

further washed with 100 ml of gastric fluid and then with 100 ml of deionized water (using vaccum on the filter). Pellets used for controls (reference pellets) which had been stored dry were subjected to similar washings. All pellets were then oven dried at 40°C for 2 hours and 150 mg of the oven dried pellets were then placed in a 100 ml volumetric flask with 10 ml of HPLC grade methanol. Pellets in methanol were sonicated until the pellet structure was gone and all drug dissolved. The mixture was then brought to volume with simulated intestinal fluid. One ml of the total was collected and diluted to 50 ml with distilled water. The final solution was evaluated on a spectrophotometer to obtain the absorbance at 244 nm. Absorbance was converted to concentration and total amount of drug present using a standard curve (Fig. II.1).

APAP pellets coated with 5/10/5 ethylcellulose/eudragit/ ethylcellulose) were also used in the preparation of suspensions. Bulk product was prepared in a 100 ml glass bottle with 80 ml of suspension and 16 g of triple coated pellets. Suspension formulations were also prepared in three glass vials with 3.3 gm (62.5% acetaminophen) of 5/5/5 pellets in 15 ml vehicle, and dissolution tests conducted in triplicate at the end of three months. All these suspension formulations were refrigerated. Dissolution tests using the United States Pharmacopeia XX (USP) basket method were performed (in duplicate or triplicate) on the 5/10/5 formulations at day 1, 6 and 28 and also at 3, 9 and 12 months, and for the 5/5/5 formulation at 3 months only. The bottle containing 80 ml of suspension formulation plus pellets was well shaken and one teaspoonful of

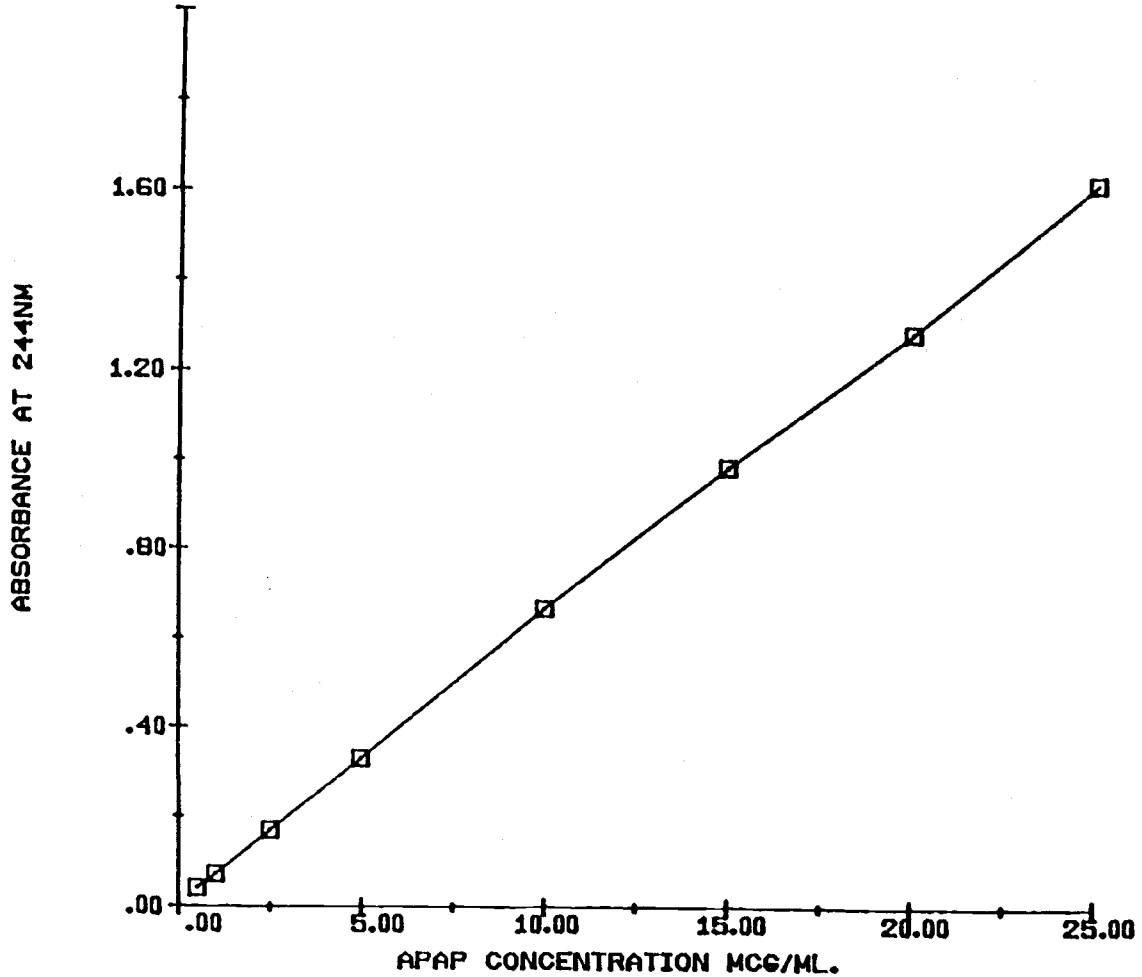

Figure II.1 Absorbance vs acetaminophen concentration. Standard curve used for one of the dissolution studies.

formulation removed, and placed in a dissolution apparatus basket to perform the dissolution study. For the 5/5/5 formulation the contents of the vial were emptied into the basket and the dissolution test performed.

A non-aqueous semisolid suspension was also investigated using single coated pellets. The coating was 2% ethylcellulose or 2% ethylcellulose/eudragit E30D (50/50). One and one half gram of pellets containing 1.05 gm (70%) acetaminophen were combined with 2 gm of vehicle and dissolution of the active ingredient was determined after storage for 5 days and 3.5 months.

NON-AQUEOUS VEHICLE

Corn syrup	25 ml	25%
Powdered sugar	34 g	34%
Mannitol	6.0 g	6%
Glycerine	12.5 ml	12.5%
Hydrogenated Vegetable Oil (Crisco)	9.0 g	9%
Vanilla flavor	12.5 ml	12.5%
Menthol	.05 g	--
Koolaid (cherry flavor)	1.0 g	1%
Tween 80	0.5 g	

Corn syrup was heated to about 70°C and powdered sugar and mannitol added while heating, until sugar and mannitol dissolved. Crisco, tween 80 and menthol were added and mixed until a homogenous mix was obtained. Vanilla and Koolaid (General Foods Company, New York) were added and the pH adjusted to 4.5. using 0.2 N sodium hydroxide solution.

To 2 g of the non-aqueous vehicle was added 1.5 g of 2% ethylcellulose coated APAP pellets (containing 70% acetaminophen) or 2% ethylcellulose/ eudragit coated APAP pellets and 360 mg of very

small APAP granules coated with 10% ethylcellulose for immediate release. The 10% ethylcellulose on small granules could mask the initial bitter taste of APAP, but earlier dissolution tests indicated an immediate release in gastric fluid. Unit doses were prepared and stored in covered petri dishes at room temperature. Dissolution was conducted after 5 days and 3.5 months, and compared to control (no vehicle, 1.5 g pellets plus 360 mg immediate release as 10% coated ethylcellulose). All dissolution tests were conducted at least in duplicate.

DISSOLUTION TEST

Dissolution of APAP from suspension or non-aqueous formulations was conducted using the United States Pharmacopeia XX (USP) basket with enzyme free simulated gastric fluid for the first two hours, followed by transfer to simulated intestinal fluid. Simulated gastric fluid was prepared by dissolving 6g sodium chloride in 21 ml hydrochloric acid and adding 3 liters of water. pH was adjusted to 1.4 $\pm$ 0.1 with hydrochloric acid. Water used for simulated gastric or intestinal fluid was deionized, degassed by boiling, and cooled prior to use. Phosphate buffer (simulated intestinal fluid) was prepared by diluting 750 ml of potassium biphosphate (27.2 g dissolved in 1000 ml) and 586 ml of sodium hydroxide (8 g dissolved in 1000 ml) to 3000 ml, pH adjusted to 7.4 $\pm$ 0.1. Nine hundred milliliters of gastric fluid was placed in each dissolution vessel. Formulations were placed in the rotating basket and rotated at 50 revolutions/min. Temperature was maintained at 37°C$\pm$0.5°C throughout dissolution. In the case of

bulk suspensions the amount of dosage form was 1 teaspoonful which had a volume of about 6.4 ml, whereas in the case of unit doses vials (5/5/5 formulation) the contents of one vial was poured into each basket. While pouring, the basket was held over the vessel and care was taken that suspension which passed through the basket should fall into the vessel. Since non-aqueous formulations were semisolid, they did not pass through the basket. At the end of two hours the baskets were removed from the shaft, and the gastric fluid filtered to recover any undissolved particles which may have escaped from the basket into the fluid. The filter paper containing recovered particles (if any) was put into the vessels and 900 ml of temperature equilibrated intestinal fluid added. The basket was then replaced at the end of the shaft and rotation at 50 revolutions/min resumed. Switching from gastric to intestinal fluid took about 15 minutes. Not counting the time involved in switching, the samples (3.0 ml) were collected at 15 m, 30 m, 45 m, 1 h, 1.5 h, 2 h (switch), 2.25, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 12, 24, 36, and 48 h after starting dissolution. In some cases samples were collected only until 36 h. Samples were replaced with temperature equilibrated dissolution liquid (gastric or intestinal). Samples were filtered, appropriately diluted and assayed for APAP content using a UV spectrophotometer (Beckman) at 224 nm. Absorbance obtained was converted to concentration and total amount of drug present using a standard curve. One typical standard curve is given as Fig. II.1.

Dissolution for acetaminophen pellets (5/10/5) aged one year was modified slightly. After 36 hours of sampling, the basket containing

residual pellets was removed from the apparatus and the pellets dried overnight at room temperature. These pellets were weighed and then transferred to a 50 ml volumetric flask, 10 ml of HPLC grade methanol added, and the contents sonicated five minutes which cracked the pellets and dissolved the contents. To this was added 40 ml of distilled water. Spectrophometric absorbance was used to determine the residual content of acetaminophen in the pellets. The reference solution for absorbance reading was a mixture of 10 ml HPLC grade methanol plus 40 ml water.

In another experiment, one teaspoonful of suspension (aged 12 months) was poured into a 250 ml volumetric flask and the contents washed with about 30 ml of simulated gastric fluid. Washings were transferred into another 250 ml volumetric flask. To both volumetric flasks, (one containing supernatant and washings, the other containing just the pellets) was added 20 ml of HPLC grade methanol, then sonicated for five minutes, and then distilled water was added to 250 ml. These were also assayed separately for acetaminophen content.

A mixture of ethylcellulose (325 mg), Eudragit L30D (325 mg), dibutyl sebecate (DBS 33 mg) and sugar pellets (900 mg) was placed in a 250 ml volumetric flask followed by 20 ml methanol (HPLC grade) and distilled water up to 250 ml. Spectrophotometric absorbance of this solution was obtained at 244 nm and found not to interfere with the APAP assay.

BIOAVAILABILITY

Suspension was administered to two subjects and saliva samples

collected over a period of twenty-four hours. Subject No. 1 received suspension on two separate occasions. One product contained 3.57 g of 5/15/5 triple coated APAP pellets (2 gm acetaminophen) as freshly prepared suspension (12 hours old), and another formulation contained 2 gm of 5/10/5 triple coated APAP pellets (1-1/2 teaspoonfuls of suspension, 1.2 gm acetaminophen) nineteen days old. Neither of these contained immediate release APAP. Subject No. 2 received a ninety day old suspension containing 1360 mg APAP from 2.3 gm pellets (5/10/5) and 240 mg immediate release APAP. The immediate release was Tylenol^{R} tablets crushed and weighed (289.37 mg) to contain 240 mg APAP in a capsule.

Suspensions were spread on culture plates and found to be free of any microbial growth before administration. Both subjects fasted 12 hours prior and 2 hours post drug administration. Both subjects weighed about 78 kg. Treatments were taken with six fluid ounces of water and immediately after swallowing the dose, the mouth was rinsed with 20 ml of Scope^{R} mouthwash followed by a water rinse to decrease APAP adsorption to oral mucosa. Saliva samples were collected by chewing parafilm squares of size one inch by one inch, for one minute with continuous spitting into 12 ml glass tubes. Saliva was centrifuged (Beckman Model TJ-6 centrifuge) at 3000 rpm for 30 minutes, salivary supernatants transferred to polypropylene containers with lock caps and refrigerated. Samples were collected at 0, 15 m, 30 m, 45 m, and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 12, 16, and 24 h's and analyzed within 24 hours of completion of the study. Samples were refrigerated until analysis.

STANDARD SOLUTIONS

Stock solutions containing 20, 50, 100, 200, 400, 500, 600, and 1000 µg/ml of acetaminophen (USP reference standard, USP, Inc., Rockville, MD) were prepared in distilled, deionized water. The internal standard was 2-acetamiodophenol (Aldrich Chemical Co., Inc., Milwaukee, WI) in deionized water (60 µg/ml). Standards were prepared by spiking 500 µl of blank saliva with 25 µl of stock solutions. 100 µl of the standard or unknown was combined with 100 µl of internal standard in 250 µl polyethylene centrifuge tubes and vortexed for fifteen seconds.

Saliva acetaminophen concentrations were determined using high pressure liquid chromatography (HPLC). The sensitivity of HPLC assay was 1 µg/ml.

ACETAMINOPHEN HPLC ASSAY

A HPLC system consisting of a delivery pump (M-6000 A, Waters Associates, Milford, MA), automatic sample injector, (WISP 710 β, Waters Associates, Milford, MA), 30 cm reverse phase C18 column (µ-Bondapak C18 column, Waters Associates, Milford, MA), variable wave length UV detector (Lambda-max model 480, Waters Associates, MA), with wave length set at 254 nm and dual pen (Soltec company, Encino, CA) recorder were used. A guard column (14 cm long and packed with C18 packing powder) was used to protect the column. The mobile phase used for HPLC was composed of methanol (25% v/v) in distilled water. Flow rate of the HPLC system was 1.4 ml/min and chart speed 4 inches/hour. Ten microliters of sample was injected at 0.02 AUFS sensitivity for

concentrations below 20 µg/ml whereas for higher concentrations, the sensitivity was 0.05 AUFS. Retention times were four minutes for acetaminophen and six minutes for 2-acetamidophenol (internal standard, used in concentrations of 60 µg/ml).

Linearity of peak height ratio versus acetaminophen concentration was excellent with correlation coefficients of greater than 0.95 and coefficients of variation ranging from 1 to 3% for standard curves.

RESULTS AND DISCUSSION

Results for "leakage" of acetaminophen from pellets coated with 5% ethylcellulose/15% eudragit L30D/5% ethylcellulose and suspended for eleven days are presented in Table II.1. The average acetaminophen recovered from 150 mg pellets (Table II.1) is 95.62% ± 4.97 (mean ± standard deviation) indicating that the variation of drug recovered is within ± 5%. Based on this single study, storing at room temperature or refrigeration for 11 days at pH 4.0 or 4.5 did not result in "leakage" or release of APAP from pellets into the aqueous vehicle. Uncoated acetaminophen was also added to some preparations since free acetaminophen would have to be added for an immediate release portion. The presence of free acetaminophen did not influence release from coated pellets.

DRUG RELEASE FROM 5/10/5 COATED PELLETS

Uncorrected dissolution profiles of acetaminophen pellets triple coated with 5% ethylcellulose/10% eudragit L30D/5% ethylcellulose and aged in aqueous suspension for 1, 6, or 28 days, or 3, 9, or 12 months are presented in Figure II.2. Percent acetaminophen released was calculated as percent of acetaminophen expected to release from a teaspoonful if the suspension had a uniform distribution of pellets per ml and a teaspoonful was accurate at 6.4 ml (approx. 800 mg acetaminophen). With these assumptions, the rate and amount of drug released after three months and six month storage are lower than the rate and amount of drug released after 28 days storage. However, rate and amount of release after 9 months and 12 months storage were higher

TABLE II.1. Results After 11 Days in Suspension

	Suspension Storage Conditions	Color of Suspension[1]	Absorption	Concentration (µg/ml)	Total Drug in 150 mg Pellets[2]
1.	150 mg pellets[3]	White	1.202	18.0 µg/ml	90 mg
2.	Room Temp. pH 4.5	Pink	1.239	18.5 µg/ml	92.5 mg
3.	Room Temp. pH 4.5	White	1.235	18.5 µg/ml	92.5 mg
4.	Refrigeration pH 4.5	White	1.237	18.5 µg/ml	92.5 mg
5.	Room Temp. pH 4.0	Pink	1.202	18.0 µg/ml	90.0 mg
6.	Refrigeration pH 4.0	Pink	1.330	20.5 µg/ml	102.5 mg
7.	APAP[4], Room Temp. pH 4.5	White	1.306	19.5 µg/ml	97.5 mg
8.	APAP[4], Room Temp. pH 4.5	White	1.250	19.0 µg/ml	95 mg
9.	APAP[4], Room Temp. pH 4.0	Pink	1.330	20.25µg/ml	101.2 mg
10.	APAP[4], Refrigeration	Pink	1.332	20.5 µg/ml	102.5 mg

[1]Colored suspensions contained 1 drop of saffarin. White suspensions did not contain color. Results show the color did not interfere with the assay reliability.

[2]Data indicate that little or no drug was released from the pellets in suspension.

[3]Control: Not suspended, but stored dry.

[4]125 mg/5 ml of uncoated acetaminophen was added to the suspension. Results indicate that the washing process removed any adsorbed free drug from the pellets.

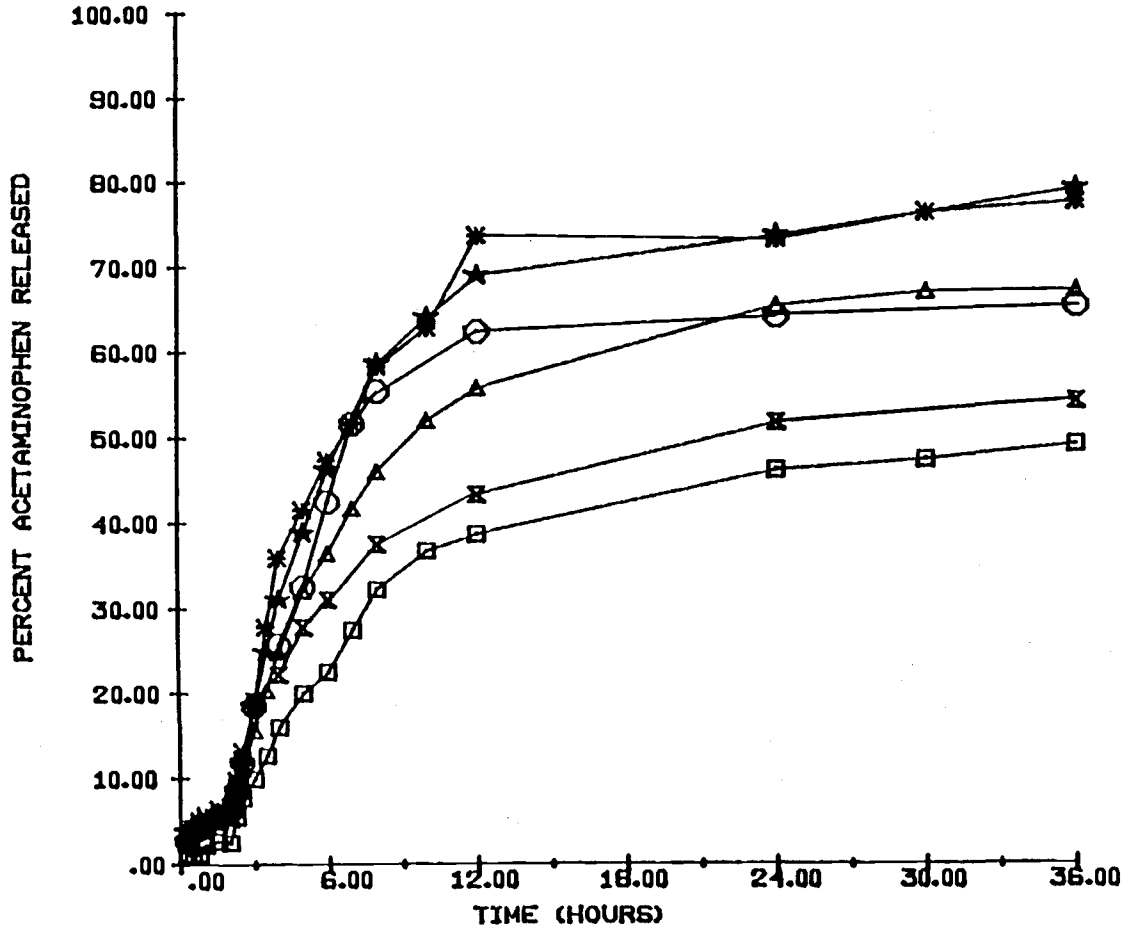

Figure II.2 In-Vitro dissolution of spray coated acetaminophen pellets, triple coated with 5% ethylcellulose/10% eudragit L30D/5% ethylcellulose; aged in aqueous suspension. Key (□) 1 day old; (Δ) 6 days old; (*) 28 days old; (⧗) 3 months old; (○) 9 months old; (★) 12 months old. Each point is the mean of two to three replications.

than that of 3 months old suspension. The variation is apparently due to the incorrect assumption that pellets were uniformly suspended as indicated below.

Replicates of dissolution profiles for samples stored for 1 and 3 days or 9 months were comparable, whereas replicates of samples stored 6 or 28 days (run in duplicate) were not comparable. Percent release at 36 hours in one vessel was 57.3 compared to 76.5 in the second dissolution vessel for 6 day storage data. Similarly for 28 day old suspension, release at 36 hours from two dissolution vessels was 65.6% and 89.0%. In all cases, the release curve was "flat" for the last two or three sample times indicating that all or nearly all drug present had been released. Further, total drug present in suspension aged for 12 months when evaluated in triplicate (based on drug dissolved at 36 hours plus residual drug in the pellets) was 720, 740 and 561 mgs, whereas the theoretically expected acetaminophen was about 800 mgs. One teaspoonful (weight 6.9 gm) of 12 month old suspension analyzed for acetaminophen content contained 43 mg in the supernatant and 814 mg in the pellets (total of 857 mg). Thus, teaspoonfuls varied in drug content from 561 to 857 mg compared to an expected amount of 800 mg. There was no interference in the assay from coating materials and sugar pellets as the contribution of such material was only about 1.33 mg, i.e. error of only 0.17% in the assay. Intact pellet shells remained in the basket after dissolution studies were completed.

Taking the above factors into consideration, it was assumed that at the end of 36 hours the acetaminophen present in the dissolution

vessel was 100% of drug originally present for dissolution in each case. The acetaminophen released at each time point was then calculated as a percent of the total released at 36 hours. These corrected dissolution profiles are presented in Figure II.3 and are quite similar indicating that the release pattern of acetaminophen from these pellets is stable after very different storage periods up to one year. The relatively constant time to 50% release of acetaminophen (d50%) from aged suspension was very encouraging and is presented in Figure II.4 and Table II.2. d50% for 1 day old suspension was 7.1 hours which reduced to 5.3 hours at 6 days and remained about 5 hours for 3, 9 and 12 month old suspensions. Borin[a] (Ph.D. thesis pg. 160, 1985) found d50% release from triple coated pellets stored dry (same batch) to be 46.7 $\pm$ 2.9 hours. It was not expected that there would be such a change in d50% release (from 46 hours to 5 hours) from suspension formulations, and it was surprising that when such a change occurred, that the d50% release stabilized and remained almost constant for 3, 9, and 12 month storage periods (Figure II.4).

DRUG RELEASE FROM 5/5/5 COATED PELLETS

In-vitro dissolution results from spray coated acetaminophen pellets, triple coated with 5% ethylcellulose/5% eudragit L30D/5% ethylcellulose, aged in suspension for 3 months are presented in Figure II.5. Total drug released at 48 hours was comparable to the theoretically expected. The study was in duplicate and data from both dissolution vessels were in agreement.

Time to 50% dissolution was calculated as 12.11 hours. It is

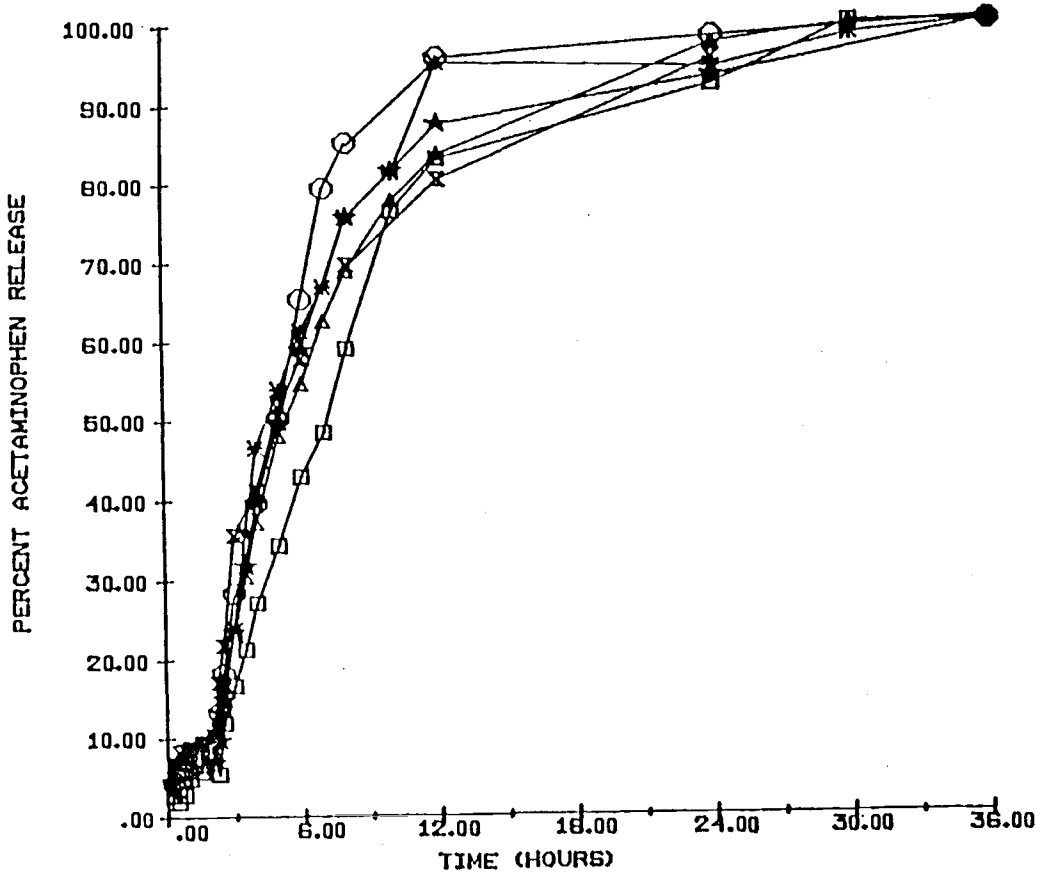

Figure II.3 In-Vitro dissolution of spray coated acetaminophen pellets, triple coated with 5% ethylcellulose/10% eudragit L30D/5% ethylcellulose; aged in aqueous suspension. Key (□) 1 day old; (Δ) 6 days old; (*) 28 days old; (⧖) 3 months old; (○) 9 months old; (✱) 12 months old. Differs from Figure II.2 in that the amount released at the end of 36 hours is considered to be 100% in each case and values at other time points are a percent of the total amount of acetaminophen released at 36 hours.

TABLE II.2. Time to 50% dissolution of spray coated acetaminophen pellets in suspension formulations.

Storage Time (Days)	Time to 50% release d50% (Hour)
1[a]	7.12
6[a]	5.29
28[a]	4.47
90[a]	5.22
270[a]	4.99
355[a]	5.11
5/5/5 (3.5 month)	12.00
*5/10/5	46.7±2.9
*5/5/5	48

[a] 5/10/5 pellets in suspension formulations

* Time to 50% release from pellets coated with ethylcellulose/eudragit L30D/ethylcellulose - data obtained from Borin (1985).

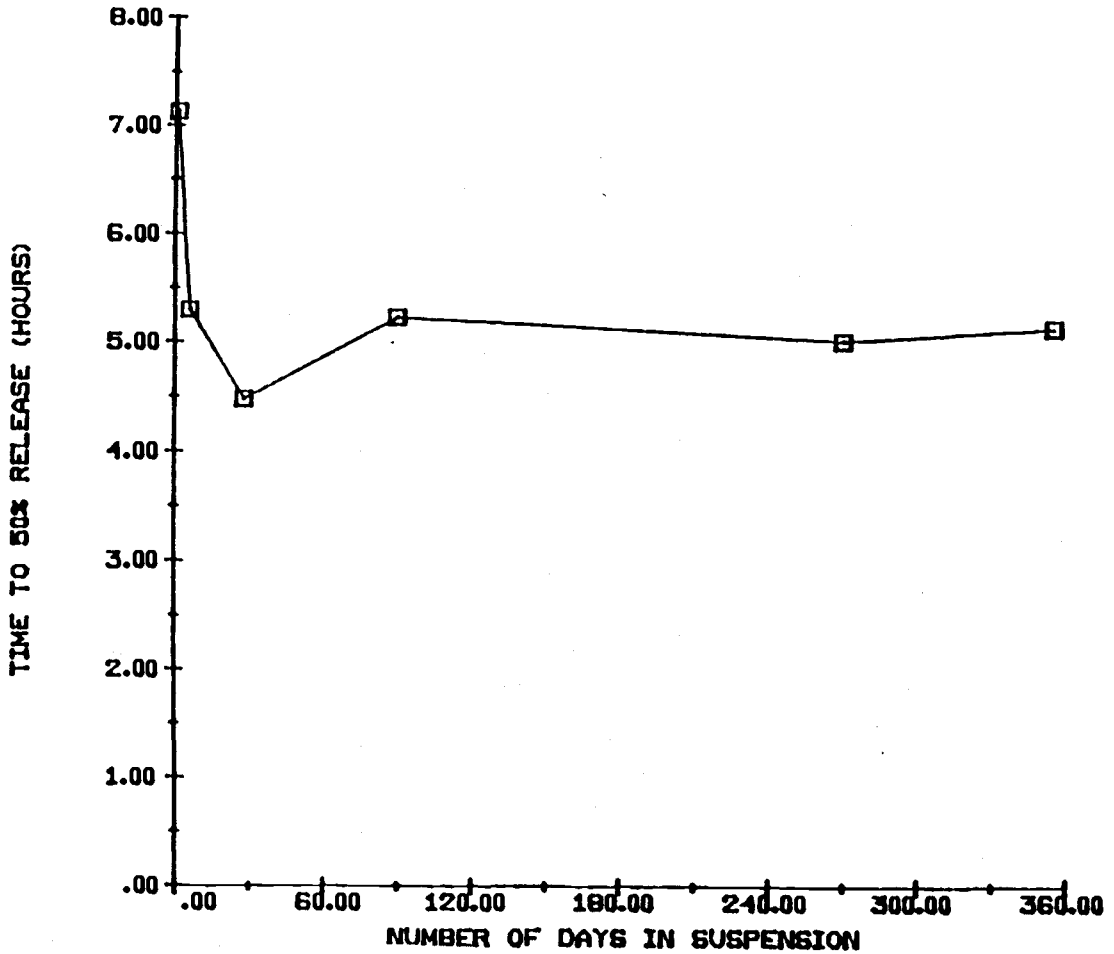

Figure II.4 Time to 50% dissolution of 5% ethylcellulose/10% eudragit L30D/5% ethylcellulose pellets aged in aqueous suspension. Based on data in Figure II.3.

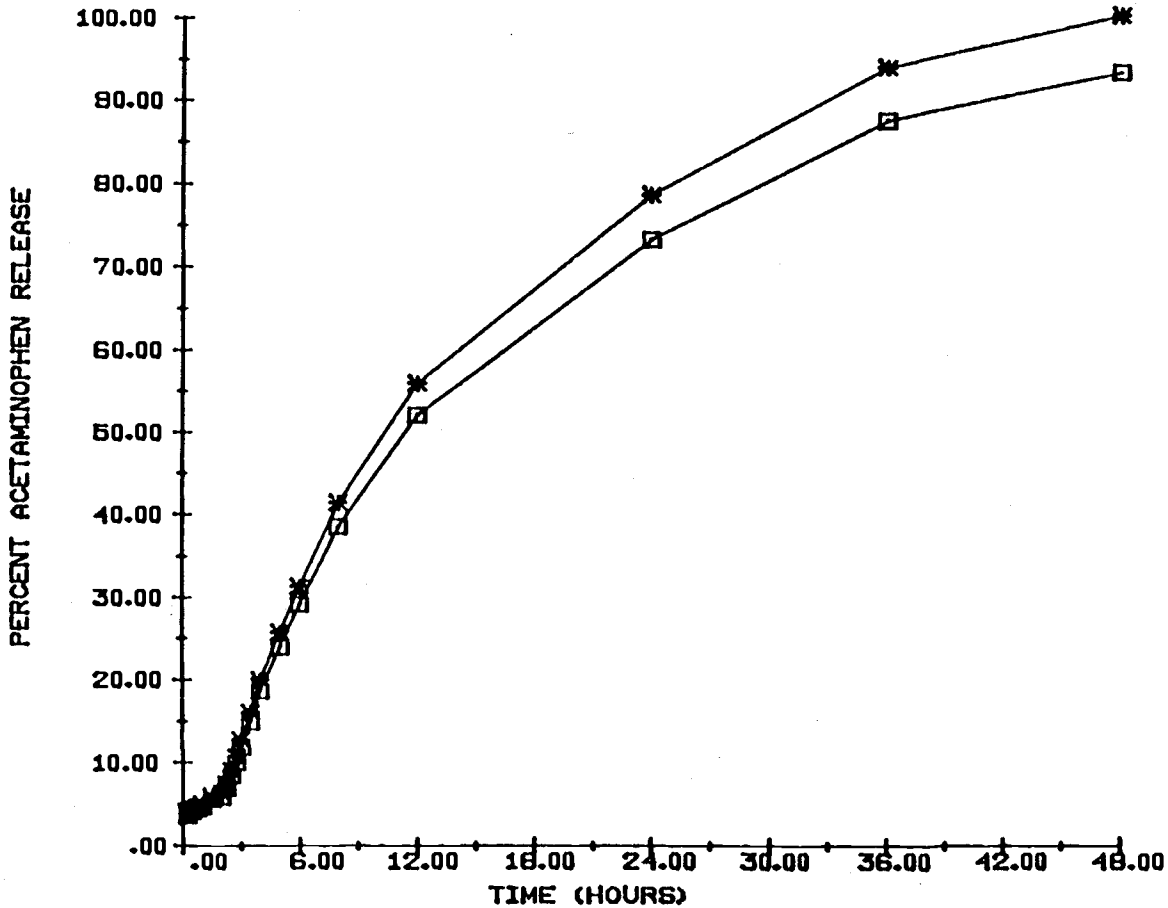

Figure II.5 In-Vitro dissolution of spray coated acetaminophen pellets, triple coated with 5% ethylcellulose/5% eudragit L30D/5% ethylcellulose; aged in suspension formulation for 3 months. Key (□) percent of labeled content released; (*) percent of amount released at 48 hours.

surprising to note that the time to 50% dissolution for 5/5/5 pellets is longer (12 hours) than for pellets with a thicker coat (5 hours for 5/10/5). Time to 50% dissolution for pellets which had not been stored in suspension was observed by Borin (Table II.2) and showed d50% for 5/5/5 to be greater than 48 hours.

BIOAVAILABILITY OF SUSPENSIONS

Freshly prepared suspension (12 hours old) of 5/15/5 pellets containing about 2 g of acetaminophen did not produce measurable saliva acetaminophen concentrations within 24 hours. This suggests that release from 5/15/5 pellets is too slow to be a useful sustained release product, which is to be expected based on the data of Borin (1985).

Results of oral administration of 19 day old acetaminophen 5/10/5 suspension administered as 1.5 teaspoonfuls (1200 mg acetaminophen) to Subject 1 are presented in Figure II.6. Saliva acetaminophen concentrations remained between 2 and 2.85 µgs/ml up to 12 hours with the peak concentration of 2.85 µgs/ml observed at 5 hours. The suspension did not contain any immediate release APAP but saliva concentrations of 2.01 µgs/ml were observed at 15 minutes. This initial result was probably due to drug which remained adsorbed to buccal mucosa despite washings with mouthwash. It could also be the result of some initial rapid release from the pellets but such a hypothesis is not supported by the dissolution data which shows little release during the first two hours in gastric fluid (Figure II.3). Drug did not diffuse or "leak" from the pellets into the aqueous

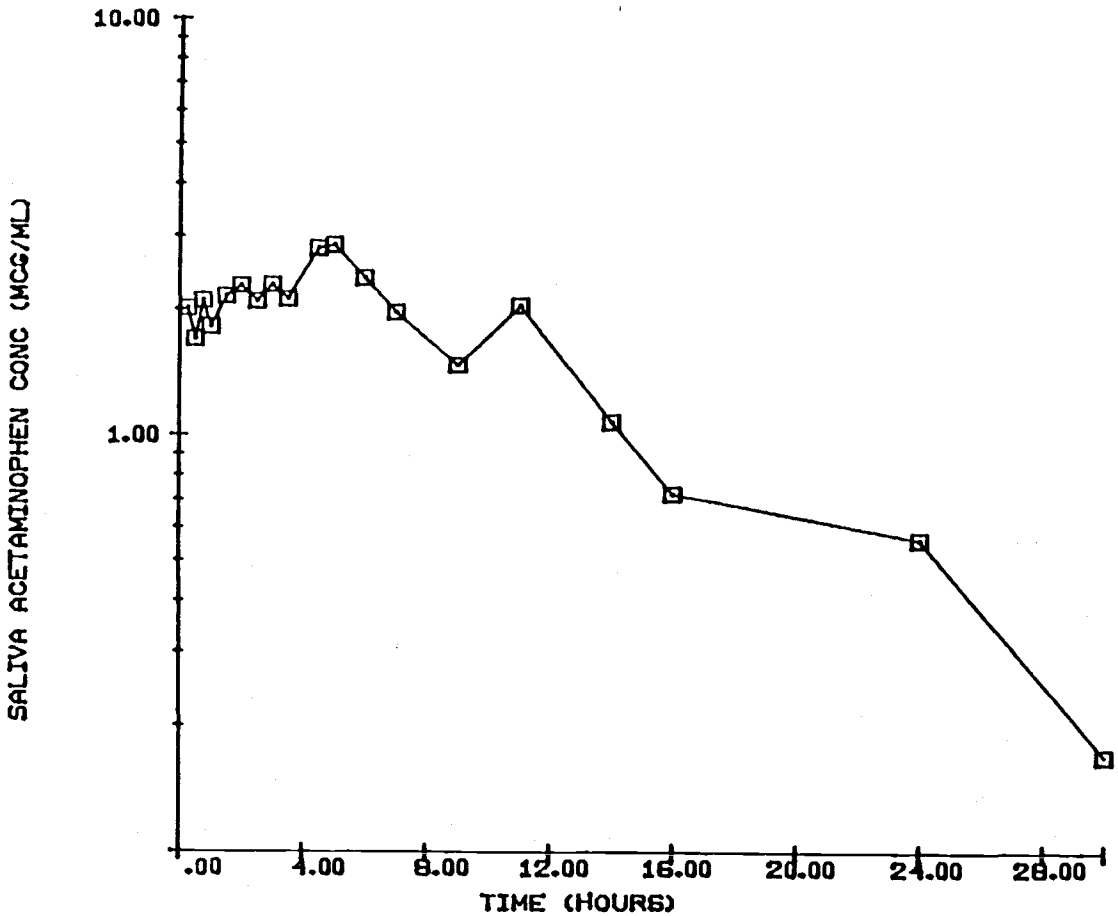

Figure II.6 Saliva acetaminophen concentration in subject one following administration of 1.5 teaspoonfuls of 19 day old suspension formulation containing 1.2 g acetaminophen (approx. 2 g pellets) coated with 5% ethylcellulose/10% eudragit L30D/5% ethylcellulose.

solution to give free drug available for rapid immediate release (see earlier discussion). Saliva APAP concentrations were maintained above 2 µgs/ml for over 12 hours, indicating that controlled release was achieved. Earlier studies indicate that any false high concentration due to adsorbed drug is removed by 90 minutes (Borin 1985).

Saliva acetaminophen concentrations versus time for subject No. 2 (who received 1360 mg APAP accurately weighed as 5/10/5 pellets from 3 month old suspension which also contained 240 mg APAP as immediate release (total APAP 1600 mg) are presented in Figure II.7. Drug concentrations rose steadily reaching a maximum concentration of 5.15 µgs/ml at 45 minutes with sustained concentrations of 3.8 to 2.8 µgs/ml up to 12 hours. Release of drug from pellets continued up to at least 24 hours, maintaining saliva APAP concentrations of about 1.6 µgs/ml.

Sustained release of APAP was achieved up to 24 hours in both subjects. Subject No. 2 received controlled release pellets in suspension plus an immediate release portion with concomitant higher APAP concentrations. It may be necessary to administer even higher doses of APAP with a larger immediate release portion to adults to achieve higher concentrations. Such increased doses would not, of course, be necessary for children and may or may not be necessary for the elderly.

Both subjects had participated in earlier single dose pharmacokinetic (Borin 1985) studies, and subject No. 1 had also participated in a multiple dose pharmacokinetics study of commercially available acetaminophen (Tylenol^{R}) conducted at Oregon State

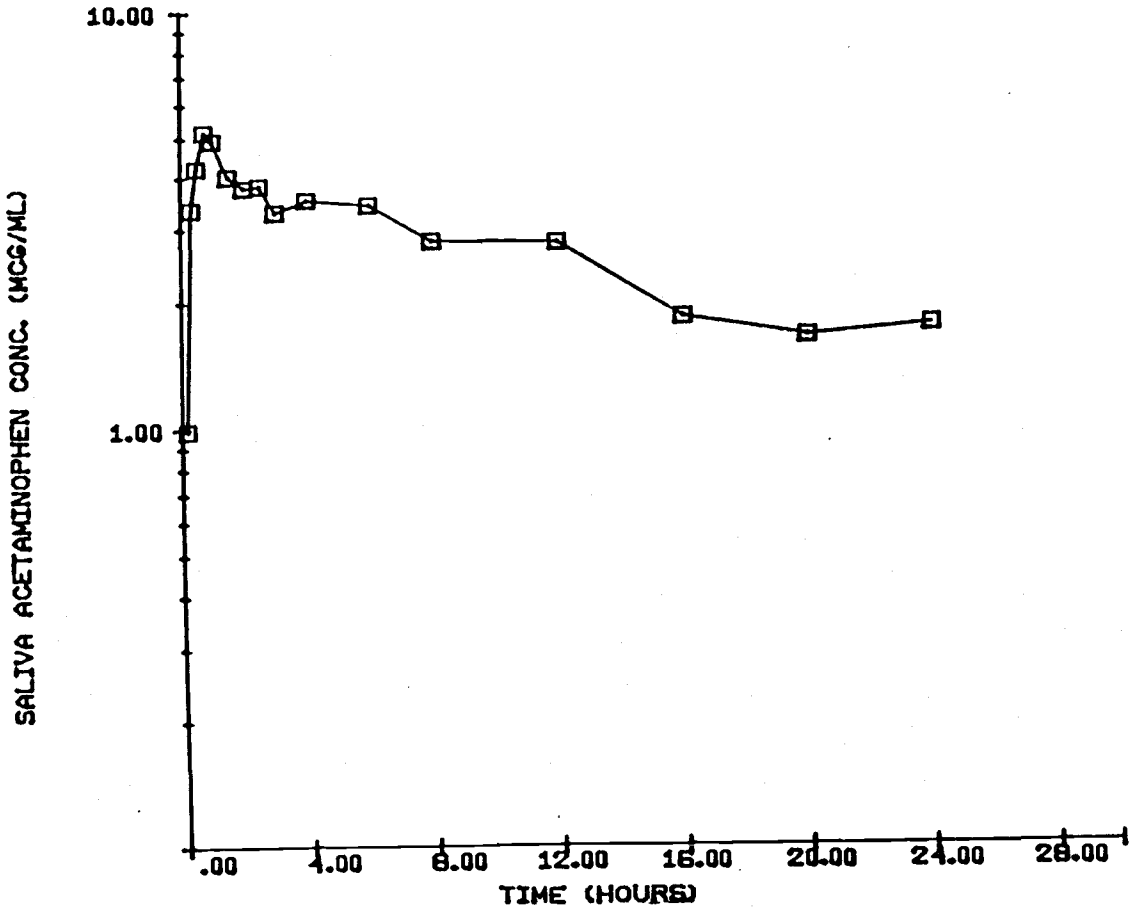

Figure II.7 Saliva acetaminophen concentration in subject two following administration of 3 month old suspension containing 1360 mg acetaminophen from 2.3 g pellets (5/10/5) and 240 mg immediate release acetaminophen in a capsule. (Tylenol was crushed and filled in a capsule for immediate release acetaminophen).

University.

Saliva APAP concentrations versus time profiles of both subjects obtained with suspension were compared to those from the above mentioned different studies. Relative bioavailability was also determined compared to data obtained from the single dose study (Borin 1985). Saliva APAP concentrations were compared in several ways. For subject No. 1 comparisons were made as follows:

1. Concentrations from controlled release suspension were corrected to a 1500 mg dose and compared to concentrations from a single dose of 1500 mg Tylenol (Figure II.8). Concentrations from the single dose were higher than from the controlled release suspension up to 10 hours. Peak concentrations from a single dose of 1500 mg APAP were 32 µg/ml compared to peak concentrations of only 2.85 µg/ml for the suspension, which is expected for immediate release vs. sustained release.

2. Suspension data corrected to a 1300 mg dose were compared to data obtained from a multiple dose study (325 mg every 6 hours for 24 hours) and are presented in Figure II.9. Saliva APAP concentrations were higher for the controlled release suspension from 1 to 6 hours and from 10 to 12.5 hours.

3. Suspension administered every 12 hours is predicted by superposition to give more uniform APAP concentrations compared to 650 mg administered every 6 hours (Figure II.10). Adding some free APAP for immediate release into the suspension would shift the concentrations higher.

Subject No. 2 had participated only in a single dose

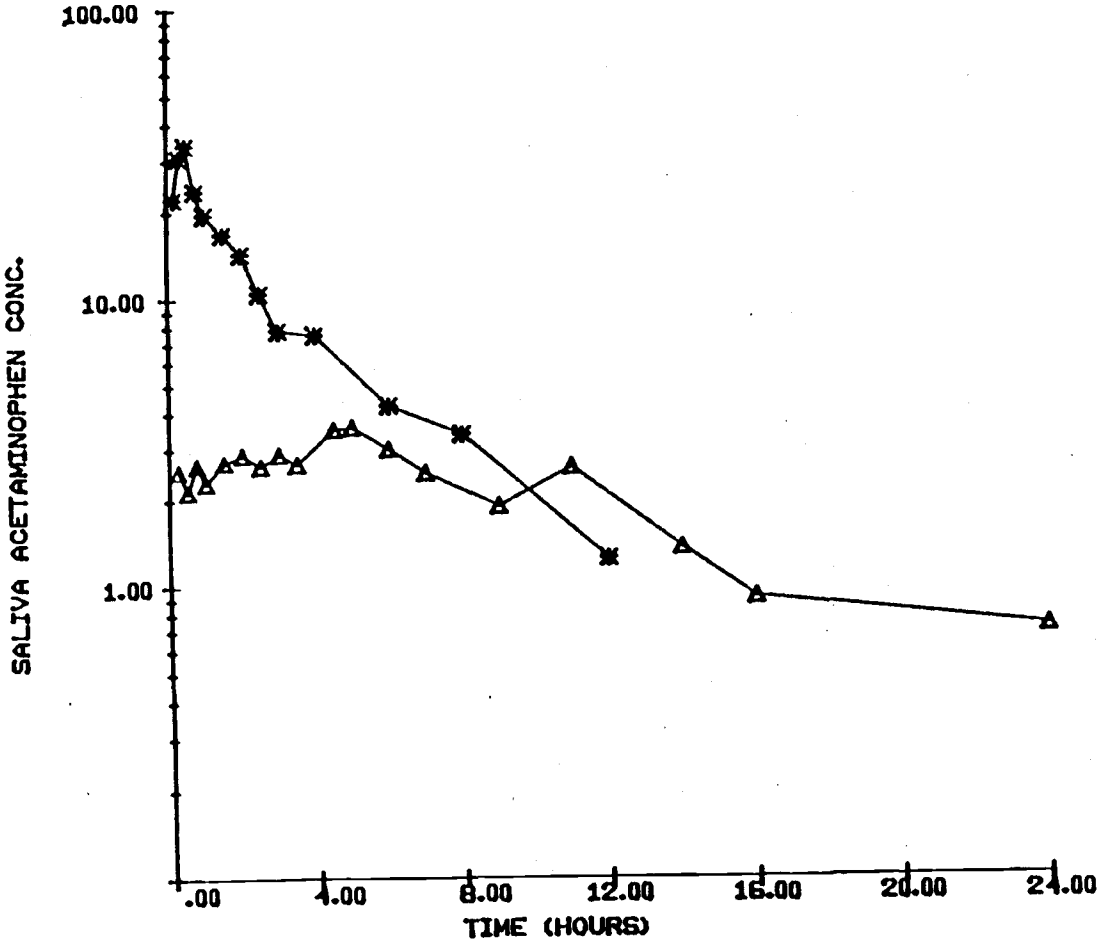

Figure II.8 Saliva acetaminophen concentration in subject one. Key (Δ) following administration of 1.5 teaspoonfuls of 19 day old suspension formulation containing 1.2 g acetaminophen as 5/10/5 pellets and APAP concentrations corrected to 1500 mg. (*) single dose[a] of 1500 mg Tylenol (different study).

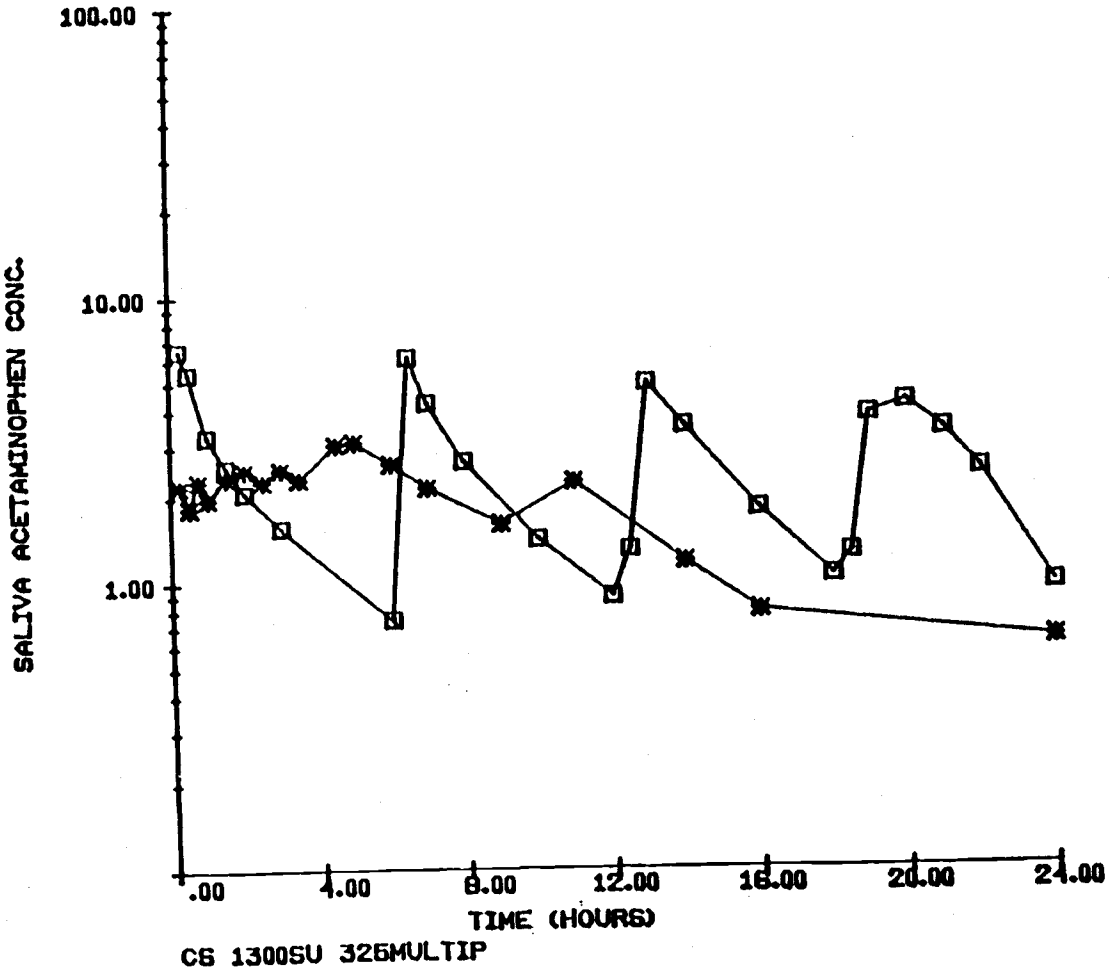

Figure II.9 Saliva acetaminophen concentration in subject one. Key (*) concentraitons from controlled release suspension corrected to 1300 mg; (□) 325 mg Tylenol administered every 6 hours for 24 hours (different study).

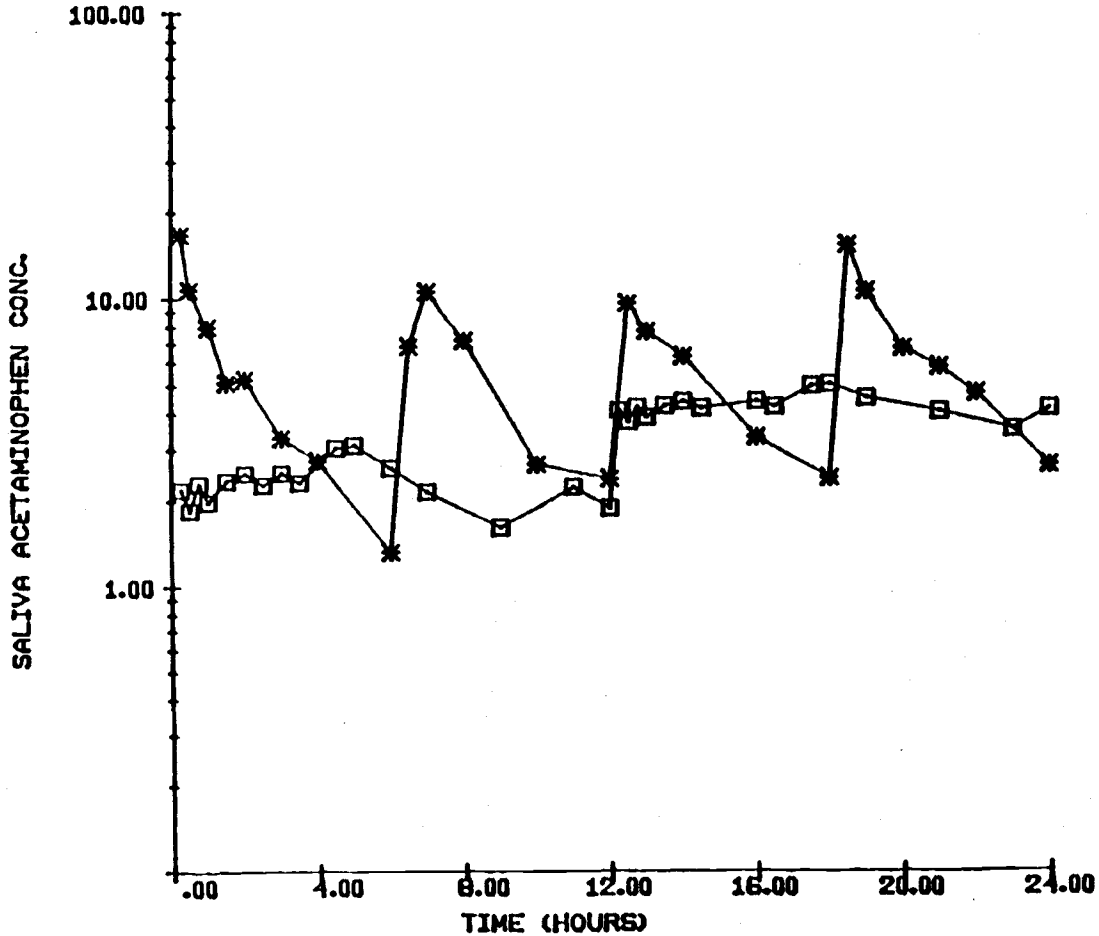

Figure II.10 Saliva acetaminophen concentrations for subject one. Key (□) 1300 mg APAP from controlled release suspension administered every 12 hours predicted by superposition; (*) 650 mg Tylenol administered every 6 hours (different study).

pharmacokinetic study (Borin 1985). Data were compared across studies as follows:

1. Saliva APAP concentrations were corrected to 1500 mg and plotted with data from 1500 mg APAP administered as single dose (Figure II.11). Controlled release from the suspension is apparent compared to the immediate release tablet.

2. Theoretical data for a second dose of 1500 mg APAP were generated using the superposition method from single dose data and are presented in Figure II.12. The results are impressive in indicating that the suspension will produce much more uniform sustained concentrations of APAP than immediate release tablets.

3. Theoretical saliva APAP concentrations for multiple dose were generated from actual single dose data for 325 mg or 500 mg to compare with controlled release suspension data and are presented in Figures II.13 and 11.14. 1500 mg APAP in suspension is predicted to give sustained saliva drug concentrations for 24 hours compared to 325 mg APAP administered every 6 hours (Figure 11.13). Controlled release suspension administered every 12 hours (Figure II.14) is predicted to produce APAP concentrations of 5 to 7 µg/ml which are sustained with less fluctuation than with 500 mg APAP (Extra Strength Tylenol) administered every 6 hours.

Relative bioavailability of the suspensions may be estimated by comparing area under the curves of acetaminophen concentrations versus time profiles ($AUC_{0\rightarrow\infty}$). Area under the curve was calculated by the trapezoidal method up to the last point and extrapolated to infinity using the last saliva concentration divided by the elimination rate

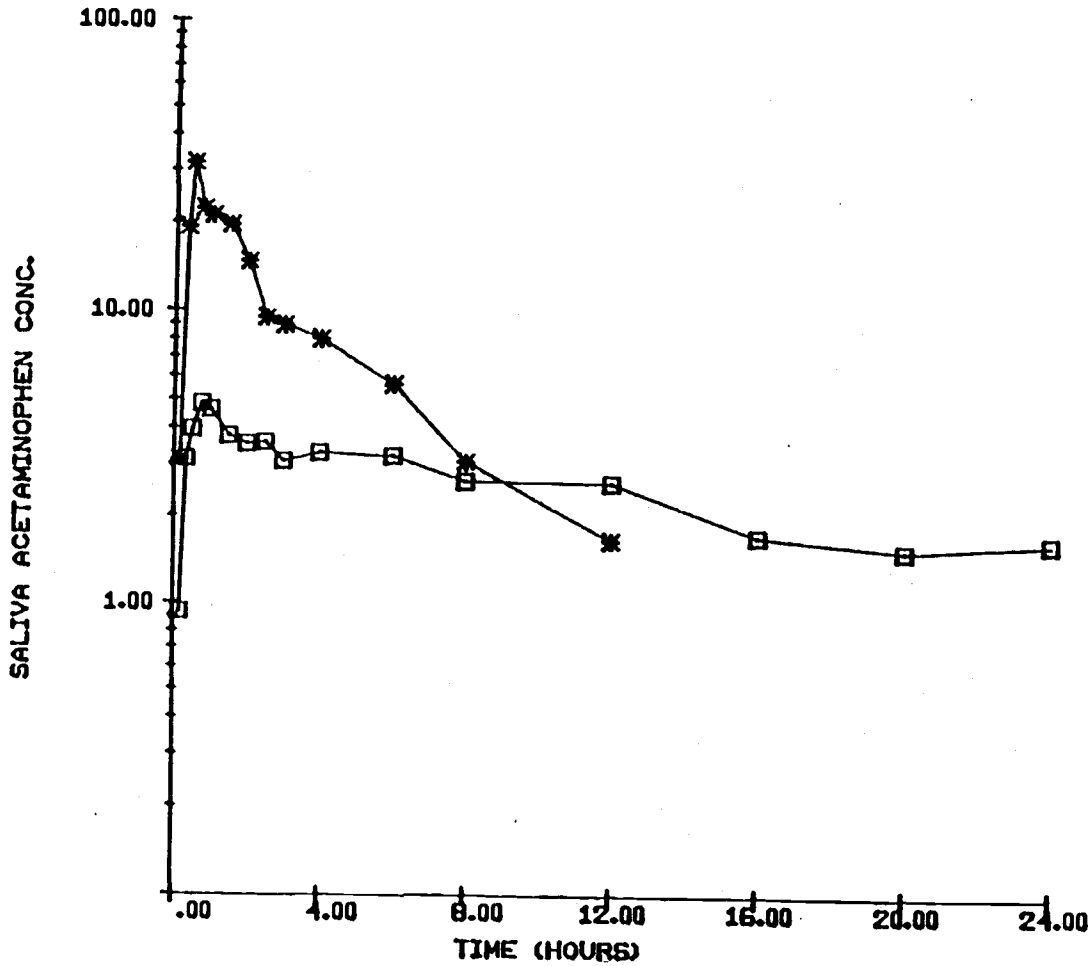

Figure II.11 Saliva acetaminophen concentrations in subject two. Key (□) administration of 1600 mg APAP from controlled release suspension, corrected to 1500 mg; (*) single dose[a] of 1500 mg Tylenol (different study).

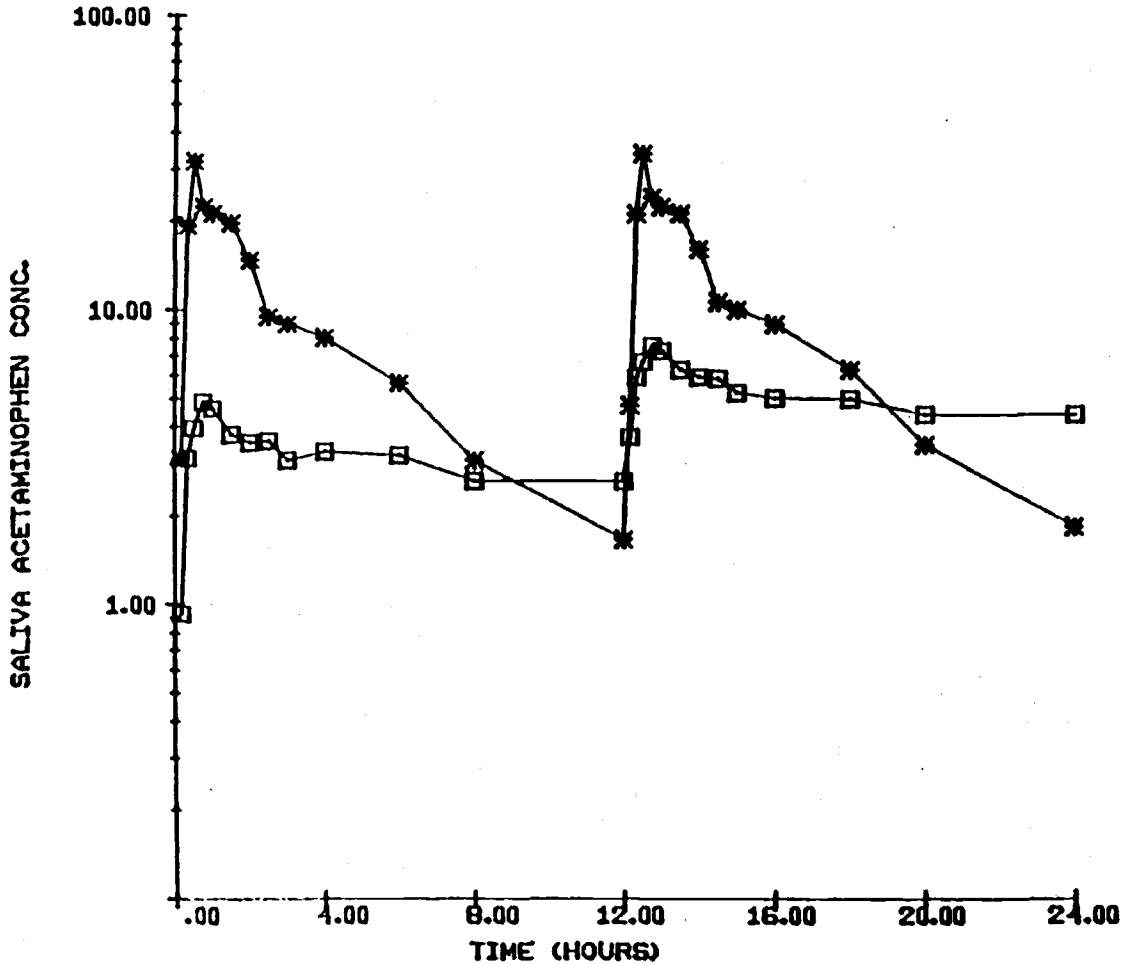

Figure II.12 Saliva acetaminophen concentrations for subject two. Key (□) 1500 mg APAP from suspension every 12 hours predicted by superposition of suspension data; (*) 1500 mg APAP administered every 12 hours generated using superposition method from single dose data.[a]

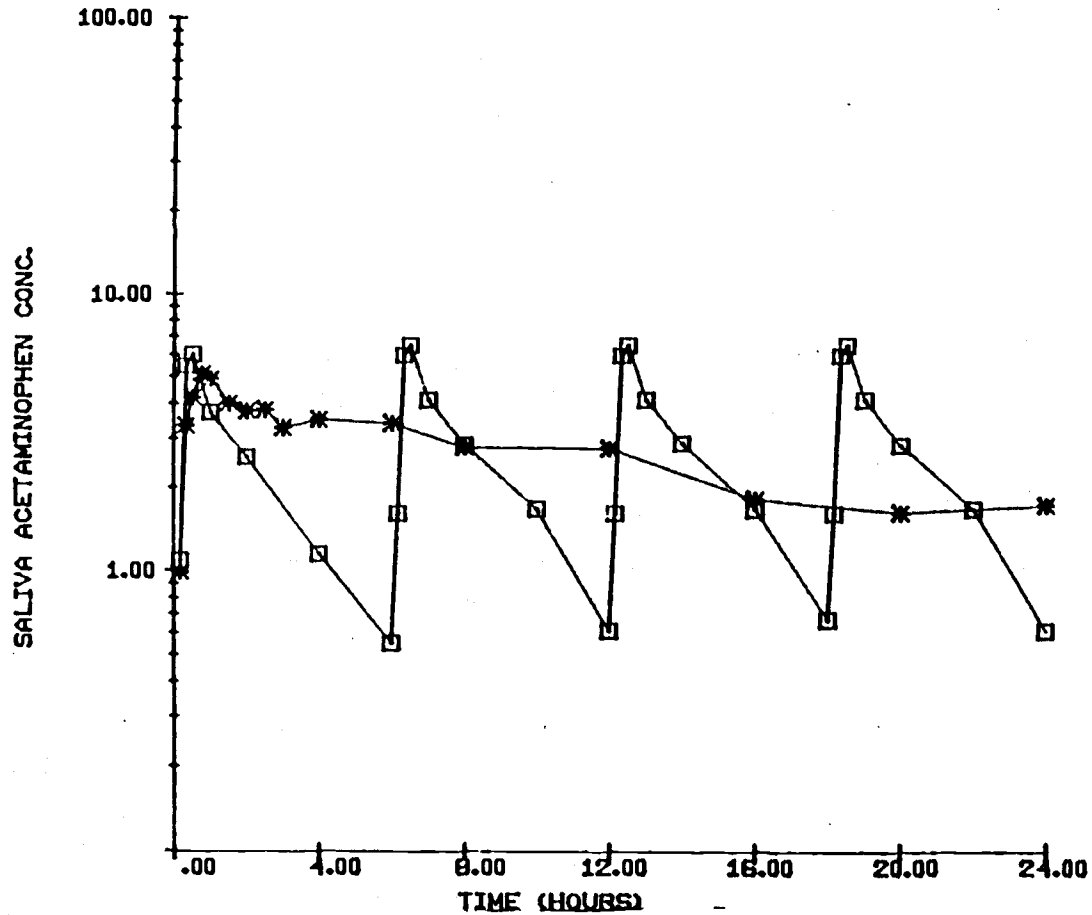

Figure II.13 Saliva acetaminophen concentrations for subject two. Key (□) 1600 mg acetaminophen from controlled release suspension (240 mg immediate release) corrected to 1500 mg, total dose. (*) 325 mg APAP administered every 6 hours generated using superposition method from single dose[a] data.

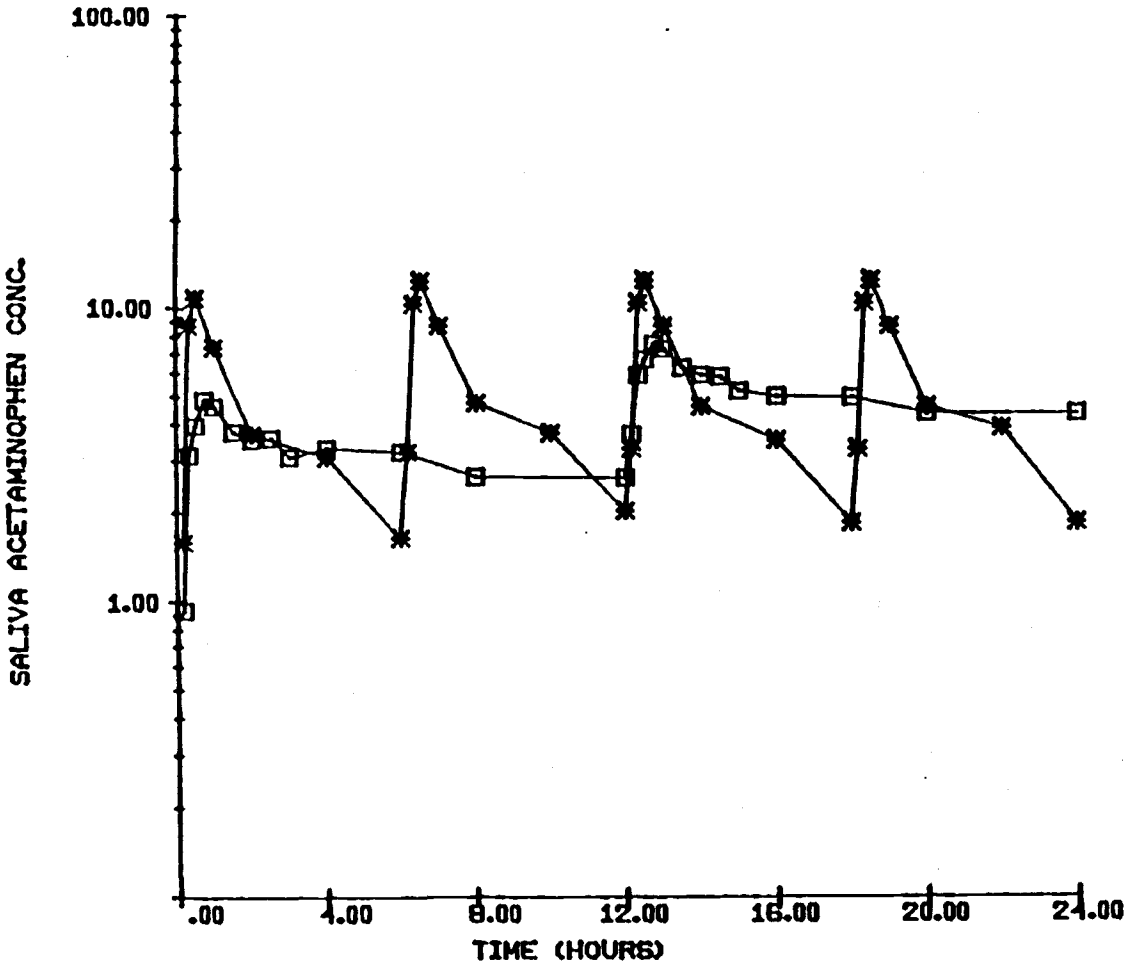

Figure II.14 Saliva acetaminophen concentrations for subject two. Key (□) 1500 mg APAP from controlled release suspension administered every 12 hours as predicted by superposition of suspension data; (*) 500 mg APAP administered every 6 hours generated using superposition of single dose[a] data.

constant obtained from the single dose study.[a] Elimination rate constants were 0.245 and 0.209 for subjects 1 and 2, respectively. Area under the curve was calculated as 36.30 and 69.96 for subjects 1 and 2, respectively. Area under the curve from the 325 mg tablet for both the subjects was corrected to 1200 mg and 1600 mg doses, respectively to obtain relative bioavailability. Area under the curve of the 325 mg dose was selected for correction for both subjects although the APAP administered in suspension was 1200 mg and 1600 mg because the rate of input from suspension would be comparable to (or lower than) that from a 325 mg tablet (peak concentration obtained from 325 mg APAP for the 2 subjects was 6.8 µgs/ml and 6.00 µgs/ml). Relative AUC's for subjects 1 and 2 were calculated to be 65% and 103%, respectively.

Decreased relative AUC for subject No. 1 could be because the assumption that one and a half teaspoonfuls contained 1200 mg APAP was incorrect, which was supported by a previously described experiment. Increased relative metabolism due to slower absorption could account for the decrease in AUC, but other bioavailability experiments in our laboratories make this explanation unlikely. It is also possible that comparing AUC from the two different studies has little meaning due to inter-study variation. It is obvious that a properly designed bioavailability study will be needed once the preliminary research is complete and some "preferred" formulations have been selected.

To maintain an average concentration of 10 µg/ml, 15 µg/ml, or 20 µg/ml (for a 70 Kg person) a dose of about 2400 mg, 3600 mg, or 4800 mg of acetaminophen, respectively, needs to be administered every 12

hours based on a literature reported volume of distribution of 0.91 L/kg and an elimination rate constant of 0.32 hr^{-1}. These doses are greater than recommended by FDA. Unexpectedly, however, data from our laboratories indicate that multiple dosing with a lower dose of a controlled release suspension will provide the "target" drug concentrations. Further research and data analysis are also needed in this area.

DISSOLUTION OF NON-AQUEOUS FORMULATION

In-vitro dissolution of spray coated acetaminophen pellets (1.5 g), coated with 2% ethylcellulose aged in a non-aqueous formulation plus 360 mg acetaminophen granules coated with 10% ethylcellulose are presented in Figure II.15. Although this formulation is referred to as non-aqueous, it has some water present from corn syrup, vanilla flavor (solution), and 0.2N NaOH which was used to adjust pH.

Dissolution rate increased with age of the formulation. The control was 2% coated pellets plus immediate release acetaminophen with no vehicle. Dissolution from control pellets showed 50% release after 12 hours, indicating these pellets to be a good candidate for a sustained release product. Five day old formulation released 50% within 6 hours and 3.5 month old formulation released 50% in about 1 hour. Total drug dissolved was about 93% of the labeled content after 48 hours. A powdery material (and no intact pellets) remained in the dissolution basket at the end of 48 hours which is different from what was observed from the aqueous suspension formulations containing 5/10/5 or 5/5/5 pellets.

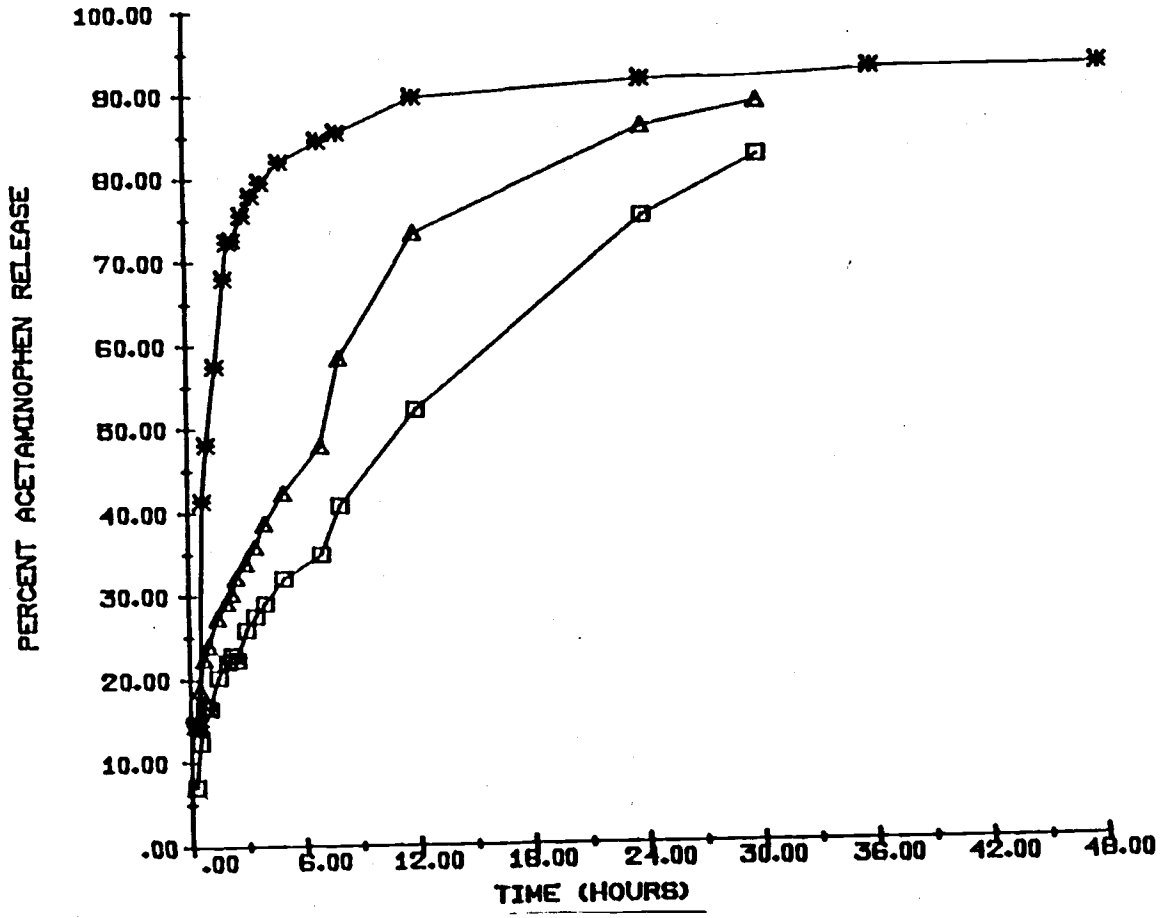

Figure II.15 In-Vitro dissolution of spray coated acetaminophen pellets, coated with 2% ethylcellulose (3.4 g pellets) aged in a non-aqueous formulation plus 360 mg acetaminophen granules coated with 10% ethylcellulose. Key (□) control - no formulation, contained pellets and granules of acetaminophen; (Δ) pellets and granules of acetaminophen aged in the formulation for 5 days; (*) pellets and granules of acetaminophen aged for 3.5 months in the formulation.

In-vitro dissolution data for spray coated acetaminophen pellets coated with 2% ethylcellulose/eudragit mixture aged in non-aqueous formulation for 5 days and 3.5 months are presented in Figure II.16. Fifty percent of the acetaminophen dissolved from the pellets within 11, 5 and 0.25 hours for control, 5 days old and 3.5 month old formulations, respectively. As noted for 2% ethylcellulose pellet formulations, these formulations also do not seem useful to store the pellets in the formulation until administered. The non-aqueous vehicle could be used for unit dosage forms by keeping the pellets and vehicle separated until administration of the dose, or may be useful with multiple coated pellets.

RELEASE PATTERNS OF APAP PELLETS

In-vitro dissolution profiles did not exhibit zero order release, therefore dissolution data was plotted as log of percent acetaminophen unreleased vs time (Figures II.17 and II.18) to determine whether acetaminophen release could be described as a first order process. Figure II.17 values are based on theoretically expected percent released and II.18 are values considering the total amount at 36 hours being 100% released. Plots of percent acetaminophen released vs square root time are also presented in Figures II.19 and II.20. Acetaminophen release from the aqueous suspension appear to be first order up to 12 hours as indicated by plots of percent unreleased versus time Figure (II.17 and II.18). Deviation from linearity is observed for the 12 to 36 hours release pattern. The relationship between percent release and square root of time is not linear as

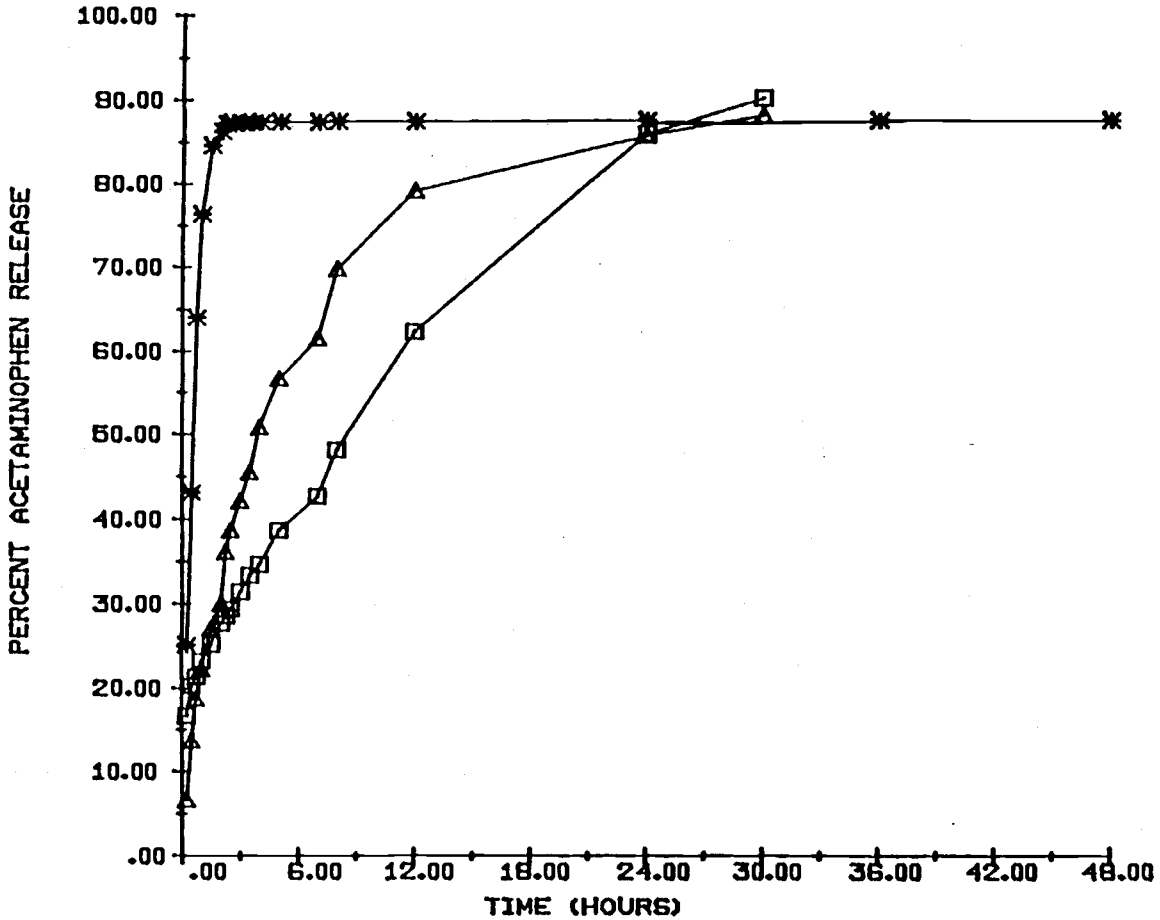

Figure II.16 In-Vitro dissolution of spray coated acetaminophen pellets, coated with 2% ethylcellulose/ eudragit (3.4 g pellets) aged in a non-aqueous formulation plus 360 mg acetaminophen granules coated with 10% ethylcellulose. Key (□) control - no formulation, contained pellets and granules of acetaminophen; (Δ) pellets and granules of acetaminophen aged in non-aqueous formulation for 5 days; (*) pellets and granules of acetaminophen aged for 3.5 months in the formulation.

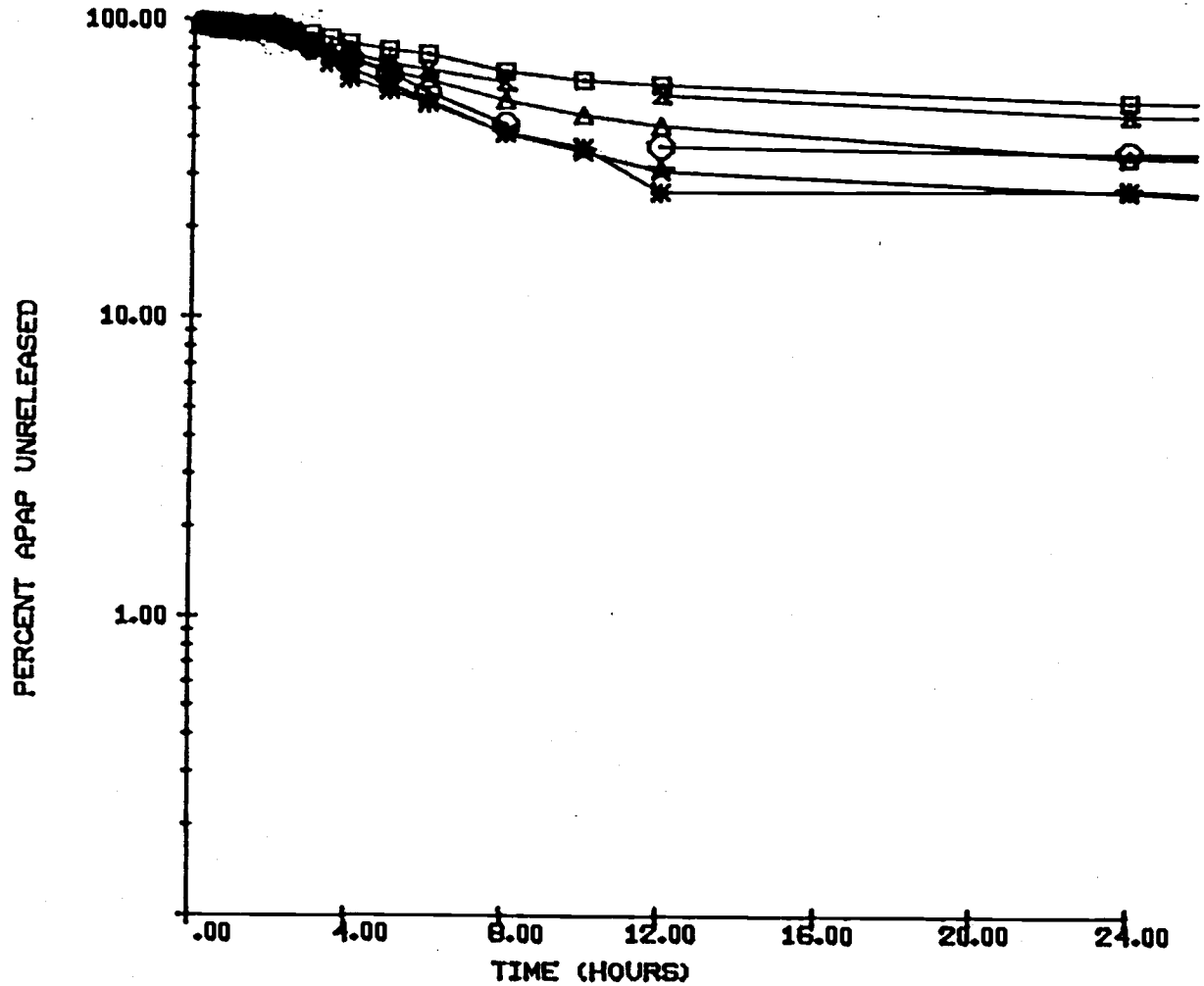

Figure II.17 In-Vitro dissolution of triple coated acetaminophen pellets 5/10/5) aged in suspension. Percent acetaminophen unreleased vs time. Key (□) 1 day old; (◁) 6 days old; (✱) 28 days old; (⧖) 3 months old; (✗) 9 months old○12 months old. Data from Figure II.2.

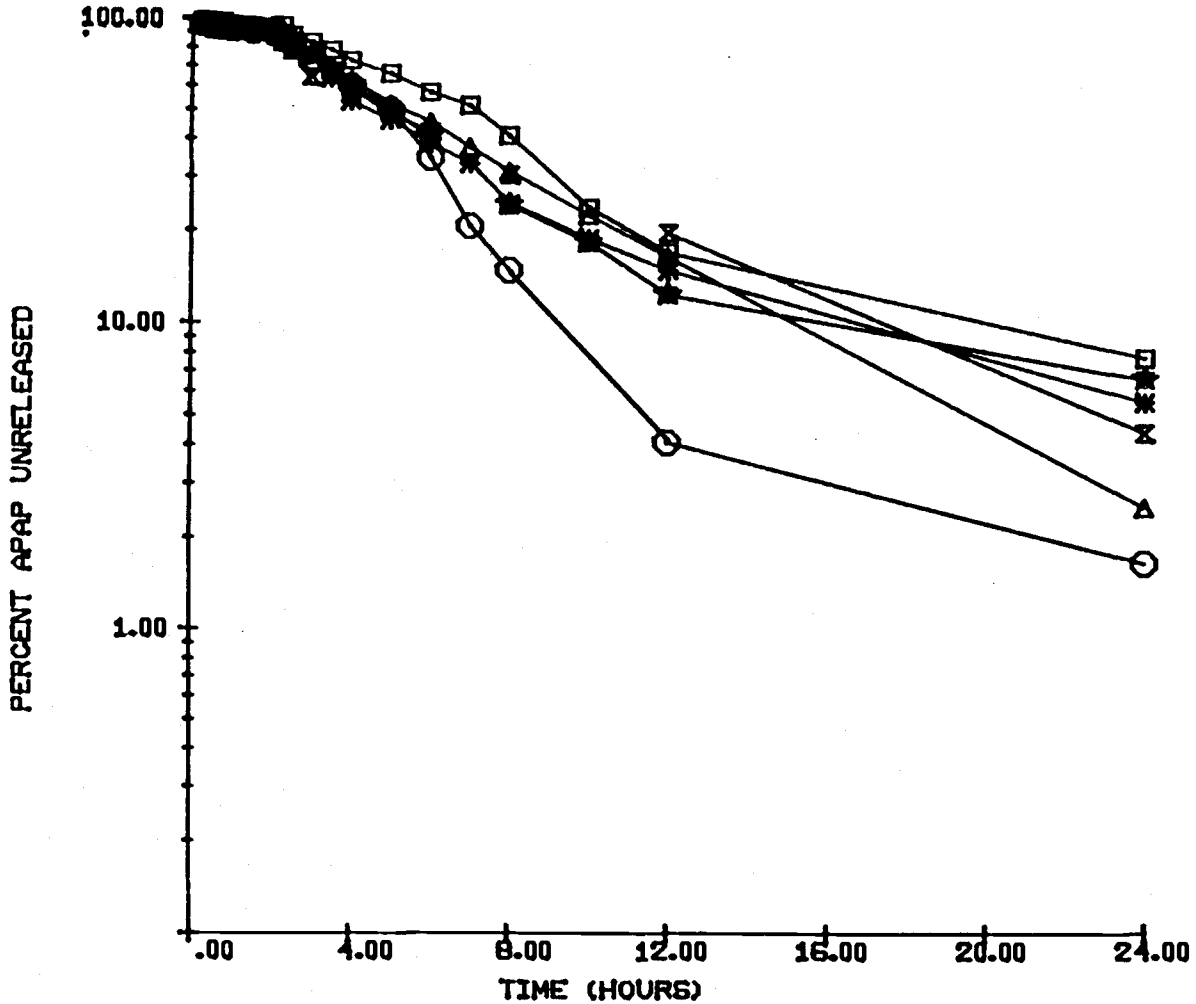

Figure II.18 In-Vitro dissolution of triple coated acetaminophen pellets 5/10/5) aged in suspension. Percent acetaminophen unreleased vs time. Key (□) 1 day old; (Δ) 6 days old; (✱) 28 days old; (⧖) 3 months old; (○) 9 months old; (★) 12 months old. Data from Figure II.3.

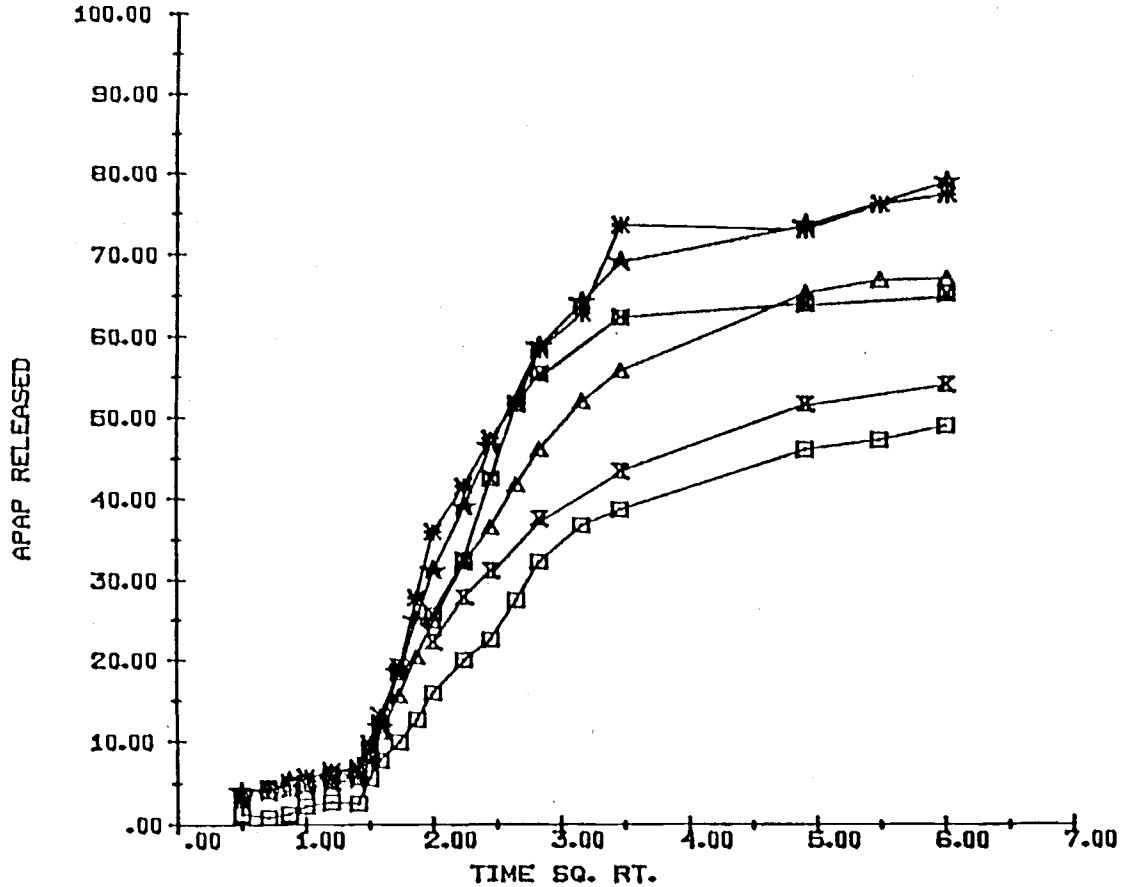

Figure II.19 In-Vitro dissolution of triple coated acetaminophen pellets 5/10/5) aged in suspension. Percent acetaminophen released vs square root of time in hours. Key (□) 1 day old; (Δ) 6 days old; (✳) 28 days old; (✖) 3 months old; (⊠) 9 months old; (★) 12 months old. Data from Figure II.2.

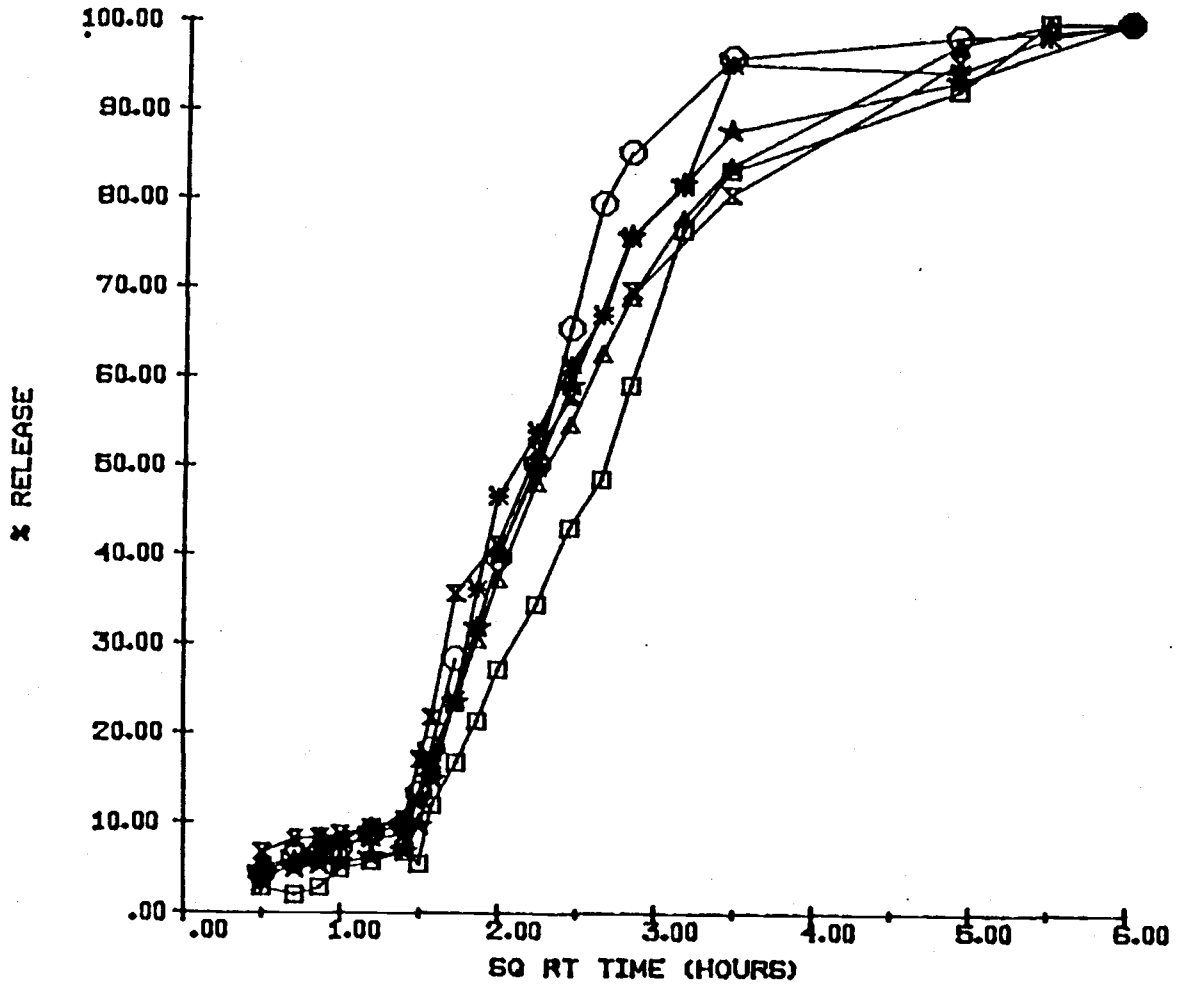

Figure II.20 In-Vitro dissolution of triple coated acetaminophen pellets 5/10/5) aged in suspension. Percent acetaminophen released vs square root of time in hours. Key (□) 1 day old; (△) 6 days old; (✱) 28 days old; (⧗) 3 months old; (○) 9 months old ☆12 months old. Data from Figure II.3.

indicated by Figures II.19 and II.20. It was expected that linear relationship between percent release vs square root of time would not exist, since this type of relationship exists for monolithic devices and not pellets. However, it has been reported by Kydonicus (1980) that if cracks or pores are present in microcapsule walls, this type of relationship may exist. Since the pellets had been wetted for a long time, there was a possibility that the coating may have swelled and developed pores or cracks in the coating wall. It is interesting to note that dissolution profiles obtained by Borin (1985) for triple coated pellets 5/10/5 and 5/5/5 produced a zero order release pattern for release of the first 78% of drug over 72 hours.

Plots of percent acetaminophen unreleased versus time (Figure II.21) and percent released versus square root of time (Figure II.22) are presented. Figure II.21 indicates a linear relationship suggesting that release from 5/5/5 pellets in suspension followed first order kinetics.

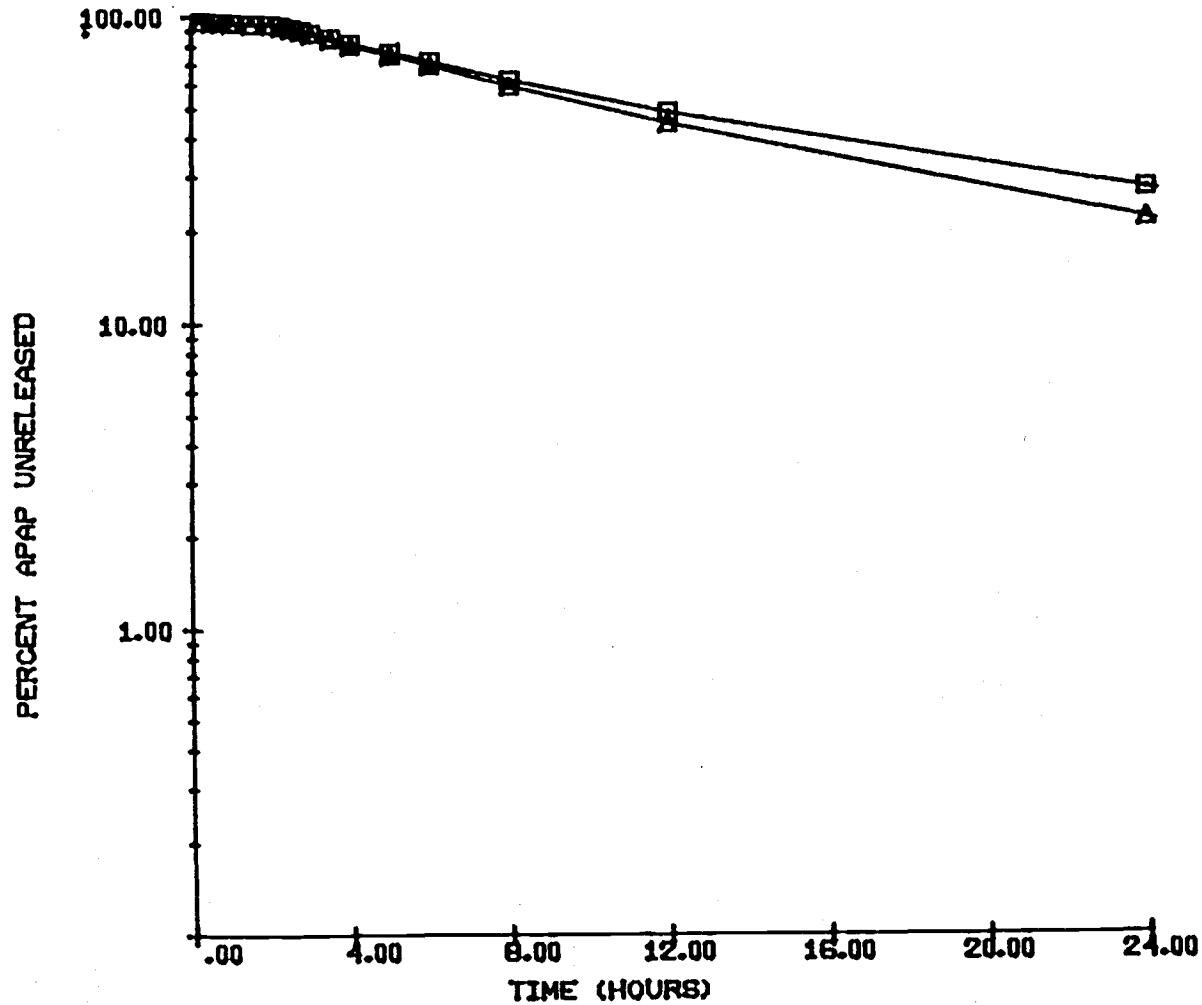

Figure II.21 In-Vitro dissolution of triple coated acetaminophen pellets (5/5/5) aged in suspension for 3 months. Percent acetaminophen unreleased vs time.

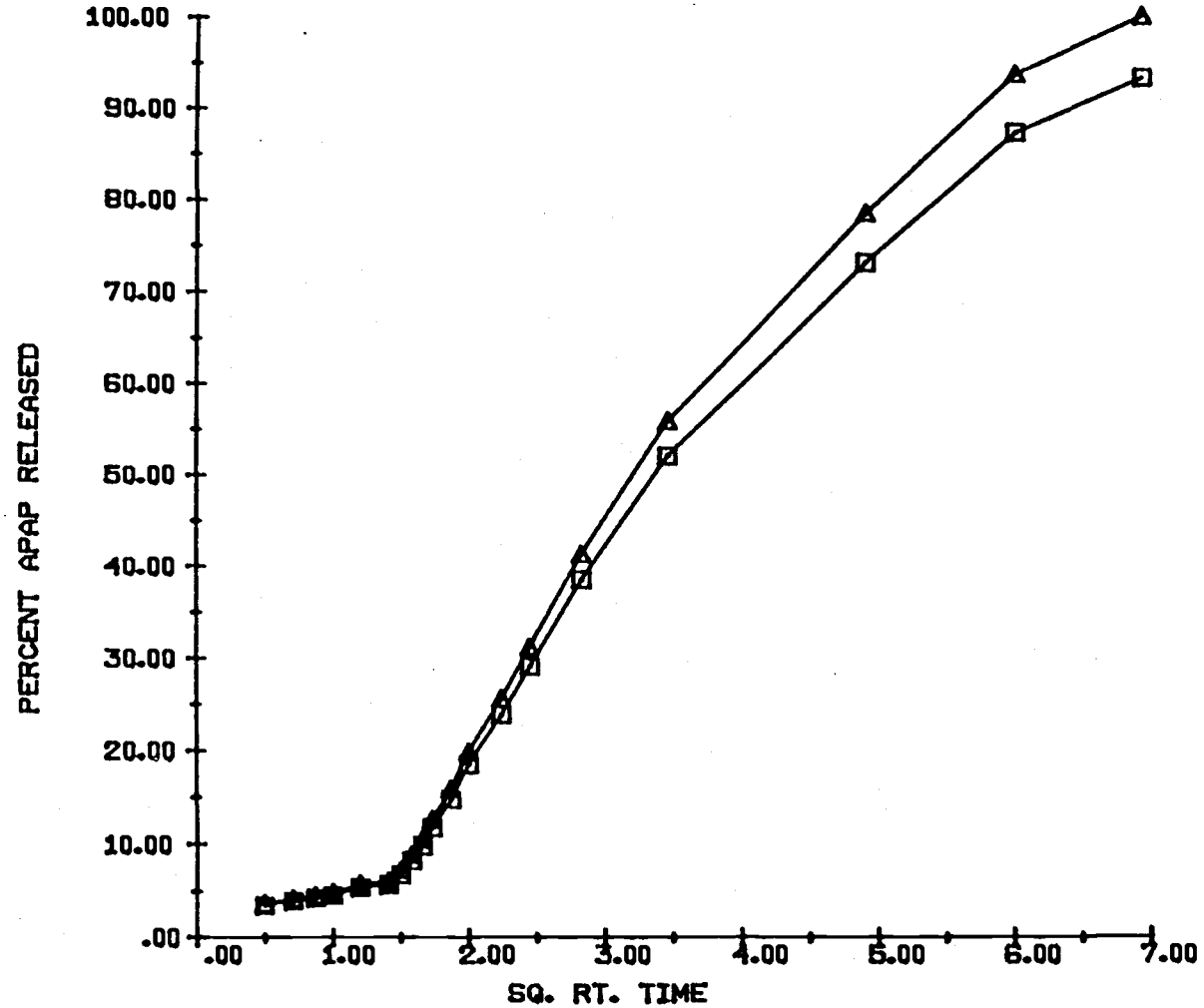

Figure II.22 In-Vitro dissolution of triple coated acetaminophen pellets (5/5/5) aged in suspension for 3 months. Percent acetaminophen released vs square root of time in hours.

CONCLUSIONS

Pellets of drugs coated with multiple layers of sustained release and pH dependent materials can be formulated into controlled sustained release suspensions. Acetaminophen pellets triple coated with ethylcellose/eudragit (enteric)/ethylcellulose are a useful model and demonstrate the feasibility of such formulations as they do not "leak" the drug during storage, and do produce uniform, sustained amounts of drug in the body when administered. More or less coating layers may be effective. Potential dosage forms might include both pre-mixed and reconstitutable oral, injectable (intramuscular) or rectal products.

Aqueous suspensions of triple-coated acetaminophen pellets were prepared and dissolution profiles obtained after aging up to one year. The amount of acetaminophen present in a teaspoonful was variable, indicating non-uniform suspension of pellets. Time to 50% release for 5/10/5 coated APAP pellets changed considerably from control (46.7 h) to 1 day old (7.12 h) suspension. Time to 50% release after 6 days, 3 months, 9 months and 12 months aging were similar to each other (about 5 hours) and did not change significantly. It was not expected that pellets would change their dissolution after initial wetting in suspension but would not be further affected by aging in the suspension. A three month old suspension of 5/5/5 coated pellets had d50% release of about 12 hours compared to about 5 hours for 5/10/5 coated pellets. Suspension containing 5/10/5 coated APAP pellets produced sustained saliva acetaminophen concentrations over 24 hours, especially for a formulation with an immediate release portion.

Non-aqueous formulations were prepared with 2% ethylcellulose or

2% ethylcellulose/eudragit coated pellets which were mixed with vehicle along with 10% ethylcellulose coated immediate release acetaminophen. Neither formulation was promising as a sustained release product as dissolution was too rapid following aging. It may be possible to keep the pellets and formulation separated in unit dose packages, and mix immediately prior to administration of the dose, or to use multiple coated pellets.

AREAS FOR FUTURE RESEARCH

1. Expand the approach to other drugs and dosage forms.
2. Modify the suspension vehicle by increasing viscosity so that pellets would be suspended more uniformly for a longer time.
3. Use smaller pellets.
4. Age formulations in unit dose containers, containing accurately weighed pellets and vehicle
5. Conduct the study at three different temperatures; room temperature, refrigerator and about 35°C.
6. Identify other coating combinations which should be investigated.
7. Use scanning electron microscopy (SEM) to study changes that take place due to aging in the formulation.
8. Dispense the formulation as a semisolid form, perhaps using carbopol or other appropriate viscosity increasing agent and dispense with an ointment pump.
9. After identifying the "right" drug and pellets with the "right" release pattern and a suspension with the "right" viscosity, conduct bioavailability studies of the formulation for different ages.
10. Evaluate aqueous and non-aqueous formulations using pellets with the same coatings.
11. Continue work with unit dosage form packages in which pellets and vehicle are kept separated, and mixed immediately prior to administration of the dose.

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APPENDICES

APPENDIX A

SALIVA ACETAMINOPHEN CONCENTRATION - TIME CURVES FOR INDIVIDUAL SUBJECTS

TABLE A.1. Each Dose Administered to the Subjects in mg/kg

Subject No.	weight in kg	325 mg tablet mg/kg	650 mg tablet mg/kg	825 mg tablet mg/kg	1000 mg tablet mg/kg
1	59.00	5.51	11.02	13.98	16.95
2	55.00	5.91	14.82	15.01	18.2
3	68.18	4.77	9.53	12.10	14.67
4	62.00	5.24	10.48	13.31	16.13
5	63.64	5.11	10.21	12.96	15.71
6	53.64	6.02	12.04	15.28	18.52
7	79.55	4.3	8.33	10.58	12.82
8	58.18	5.59	11.12	14.18	17.19

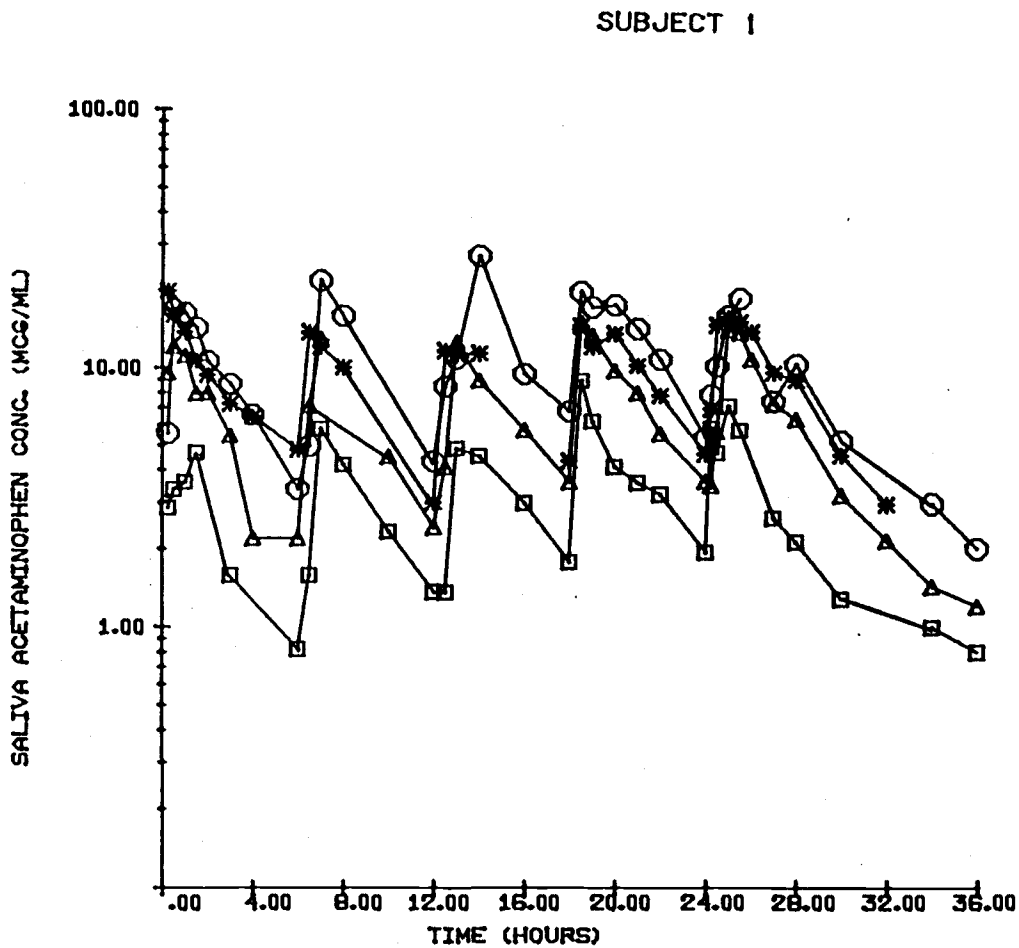

Figure A.1 Saliva acetaminophen concentration versus time curve for subject No. 1 following multiple oral administration of commercial APAP tablets at 0, 6, 12, 18, and 24 hours. Key (□) 325 mg APAP; (△) 650 mg APAP; (✦) 825 mg APAP; (◯) 1000 mg APAP.

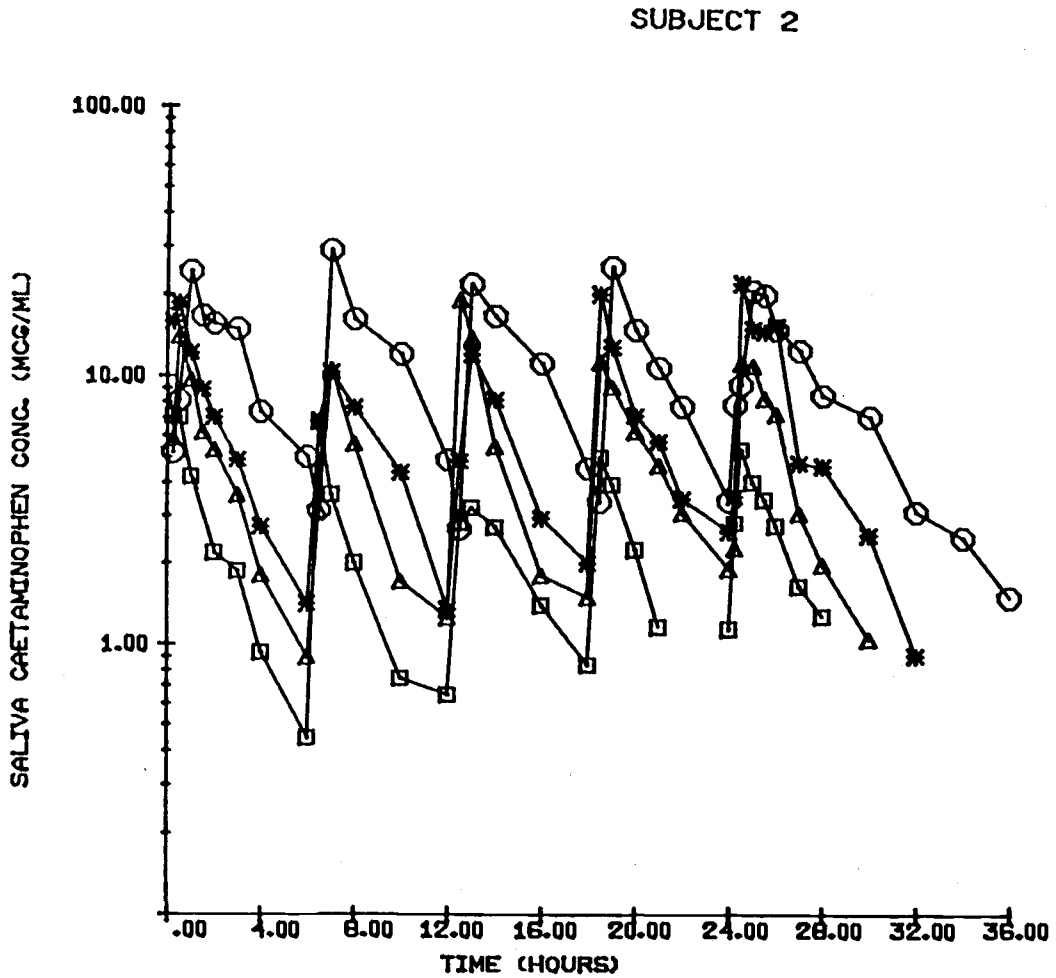

Figure A.2 Saliva acetaminophen concentration versus time curve for subject No. 2 following multiple oral administration of commercial APAP tablets at 0, 6, 12, 18, and 24 hours. Key (□) 325 mg APAP; (△) 650 mg APAP; (✦) 825 mg APAP; (◯) 1000 mg APAP.

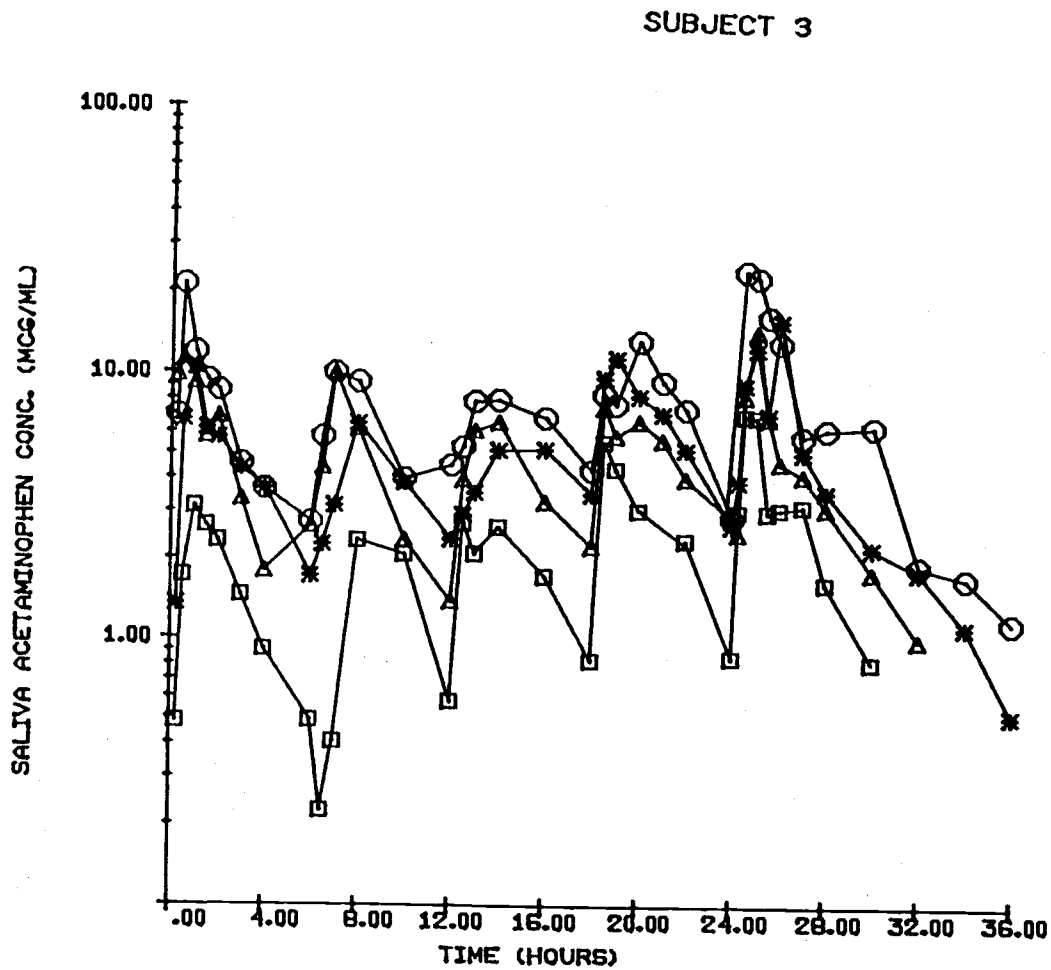

Figure A.3 Saliva acetaminophen concentration versus time curve for subject No. 3 following multiple oral administration of commercial APAP tablets at 0, 6, 12, 18, and 24 hours. Key (□) 325 mg APAP; (△) 650 mg APAP; (✦) 825 mg APAP; (○) 1000 mg APAP.

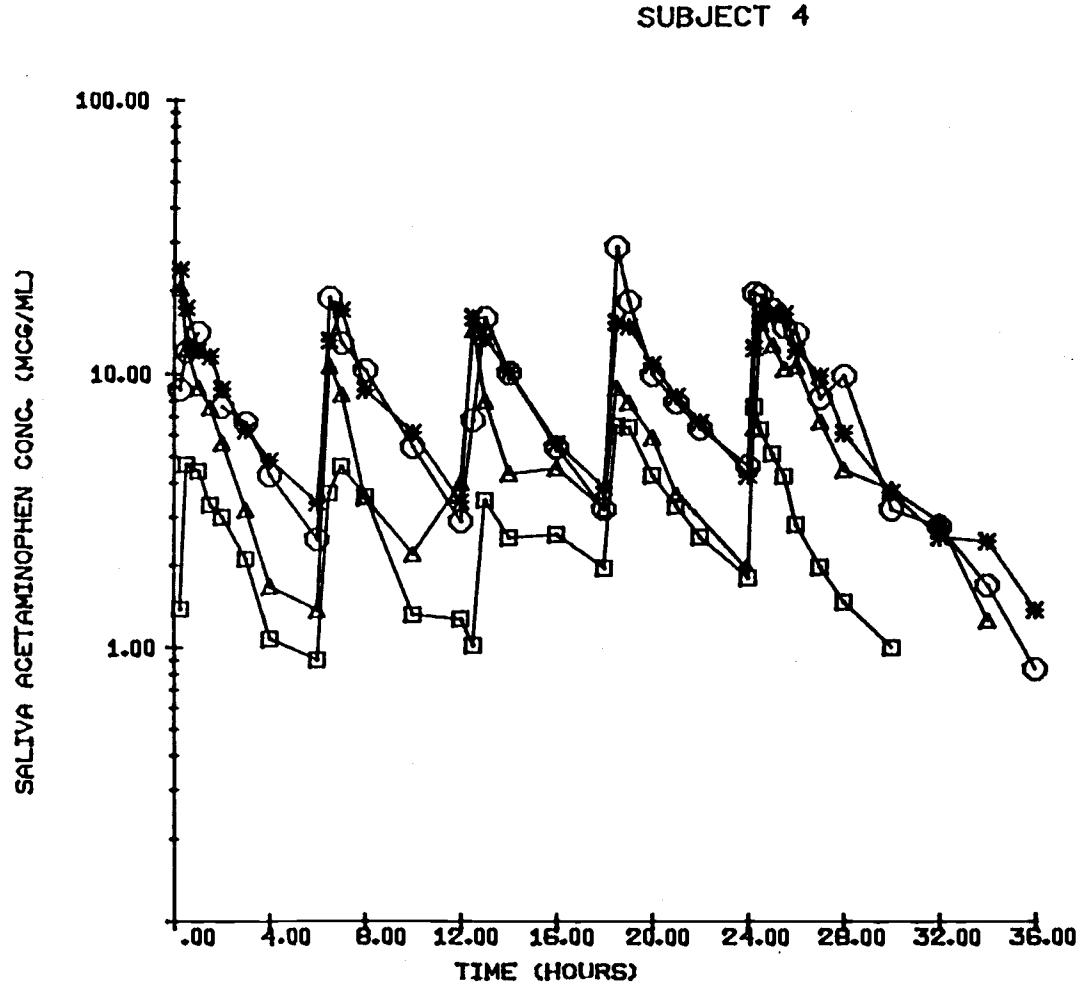

Figure A.4 Saliva acetaminophen concentration versus time curve for subject. No. 4 following multiple oral administration of commercial APAP tablets at 0, 6, 12, 18, and 24 hours. Key (□) 325 mg APAP; (△) 650 mg APAP; (✦) 825 mg APAP; (◯) 1000 mg APAP.

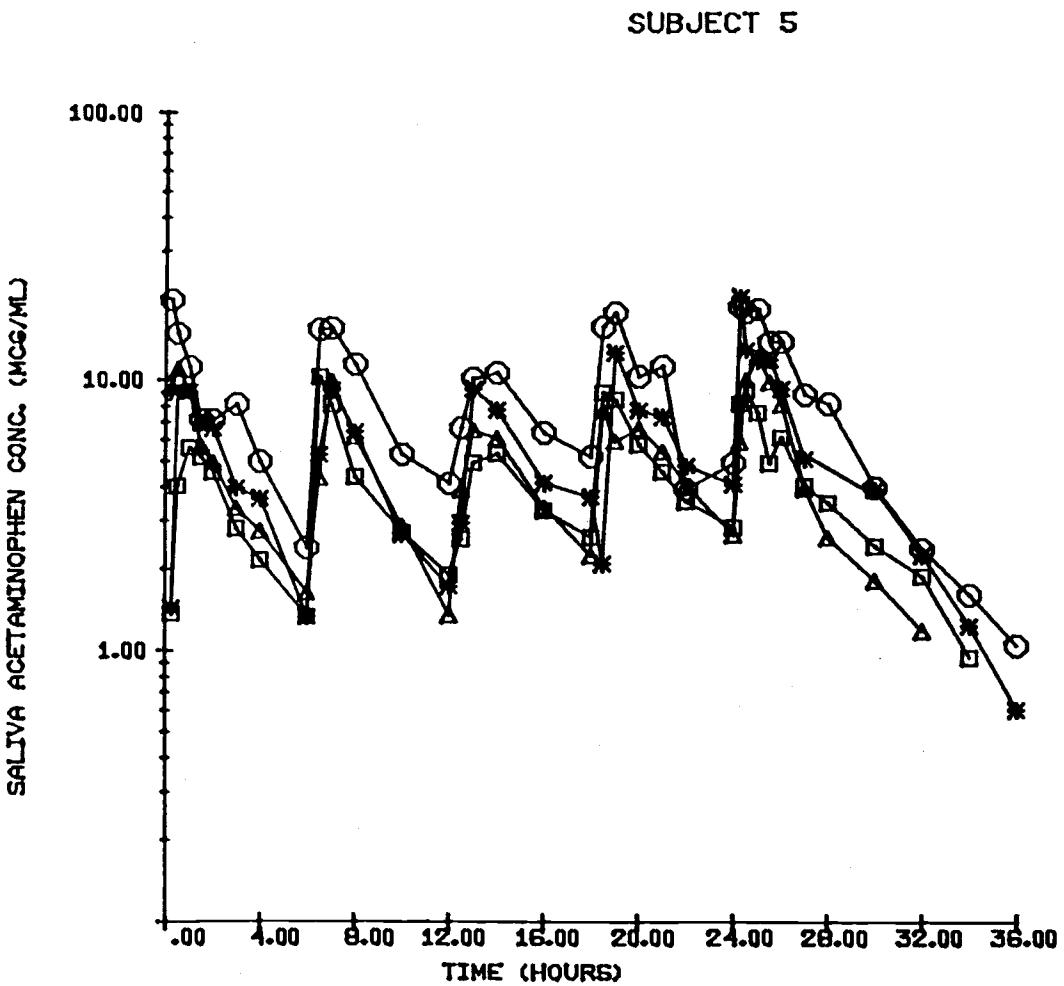

Figure A.5 Saliva acetaminophen concentration versus time curve for subject No. 5 following multiple oral administration of commercial APAP tablets at 0, 6, 12, 18, and 24 hours. Key (□) 325 mg APAP; (△) 650 mg APAP; (✦) 825 mg APAP; (○) 1000 mg APAP.

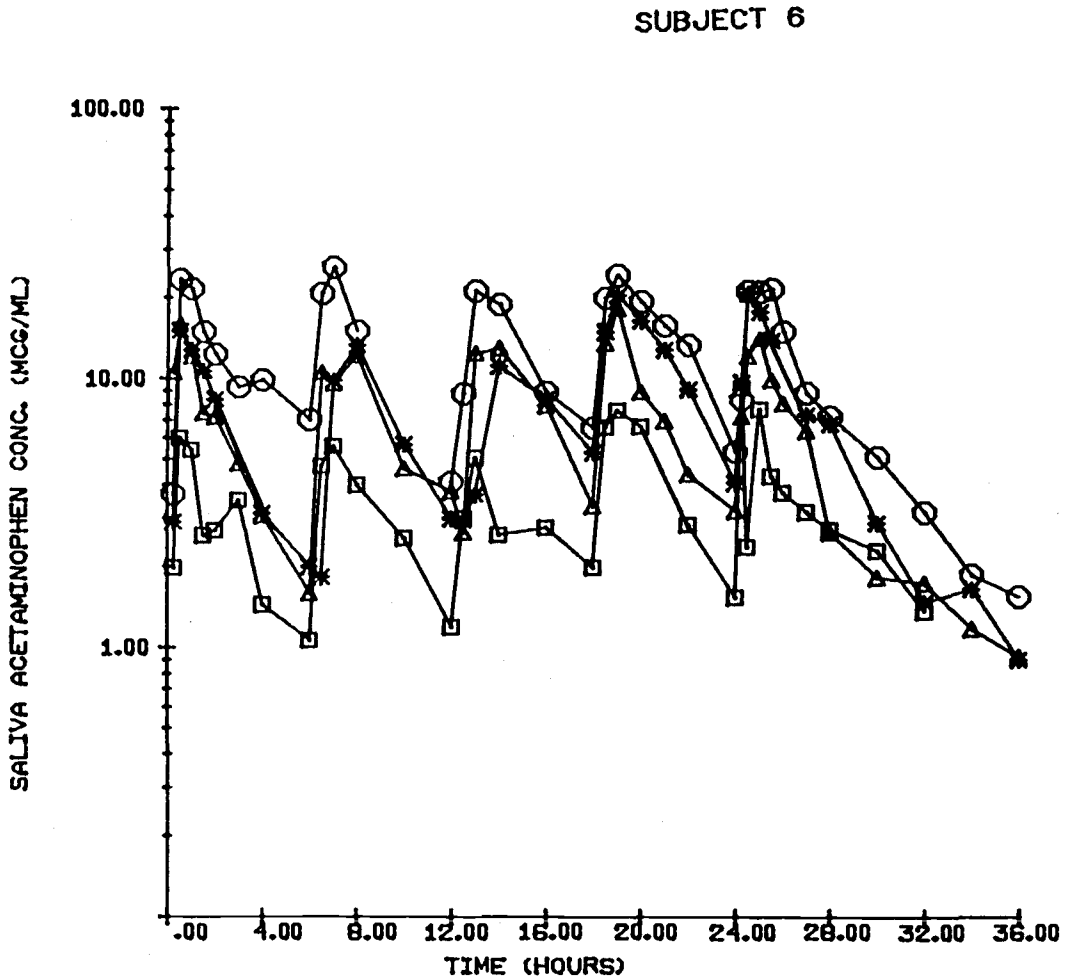

Figure A.6 Saliva acetaminophen concentration versus time curve for subject No. 6 following multiple oral administration of commercial APAP tablets at 0, 6, 12, 18, and 24 hours. Key (□) 325 mg APAP; (△) 650 mg APAP; (♦) 825 mg APAP; (○) 1000 mg APAP.

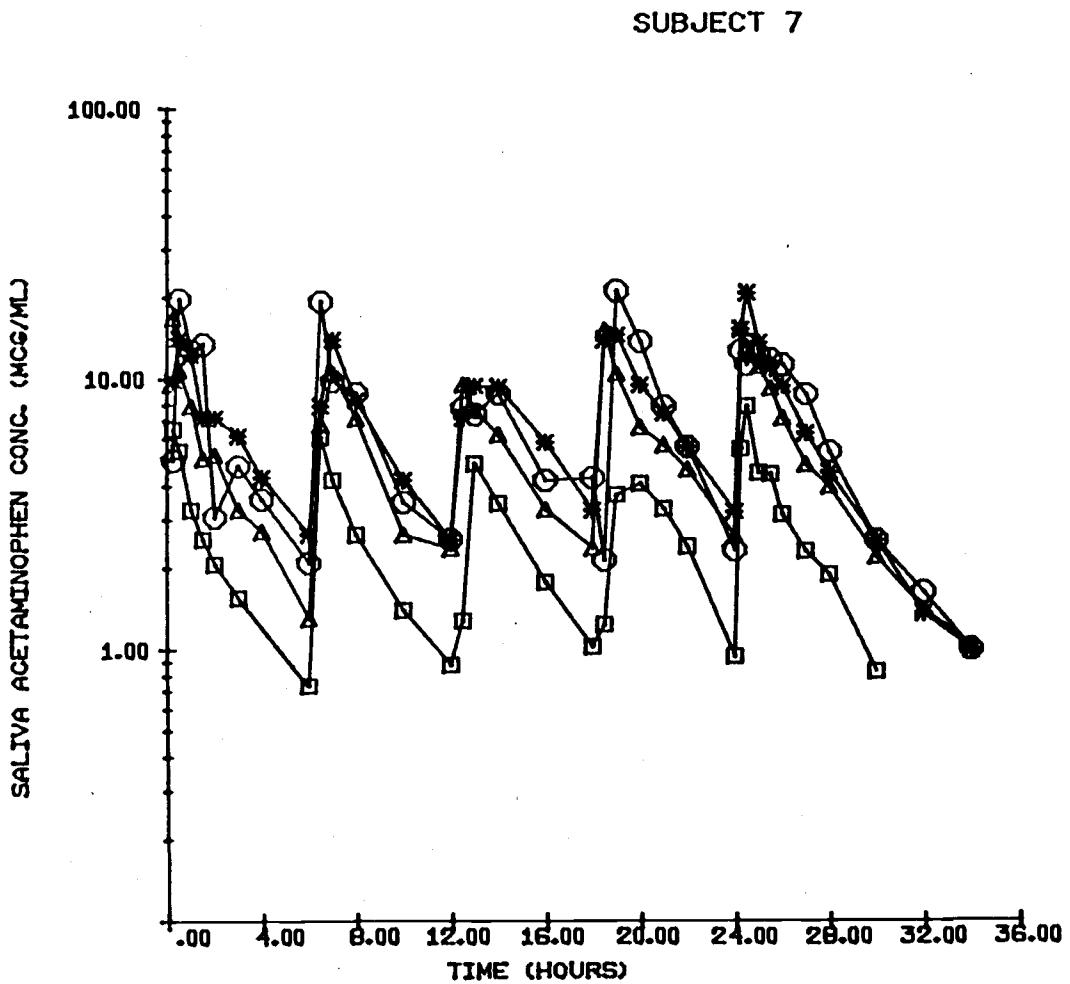

Figure A.7 Saliva acetaminophen concentration versus time curve for subject No. 7 following multiple oral administration of commercial APAP tablets at 0, 6, 12, 18, and 24 hours. Key (□) 325 mg APAP; (△) 650 mg APAP; (✦) 825 mg APAP; (○) 1000 mg APAP.

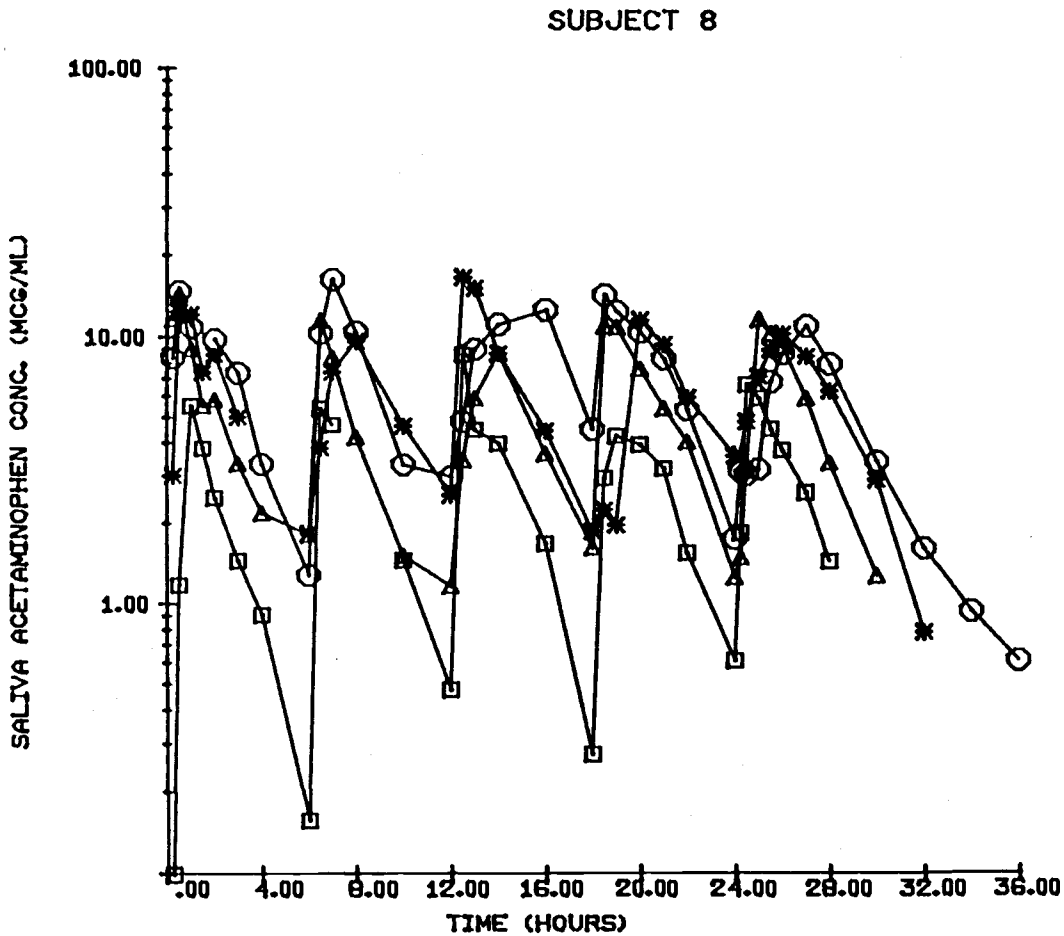

Figure A.8 Saliva acetaminophen concentration versus time curve for subject No. 8 following multiple oral administration of commercial APAP tablets at 0, 6, 12, 18, and 24 hours. Key (□) 325 mg APAP; (△) 650 mg APAP; (✦) 825 mg APAP; (○) 1000 mg APAP.

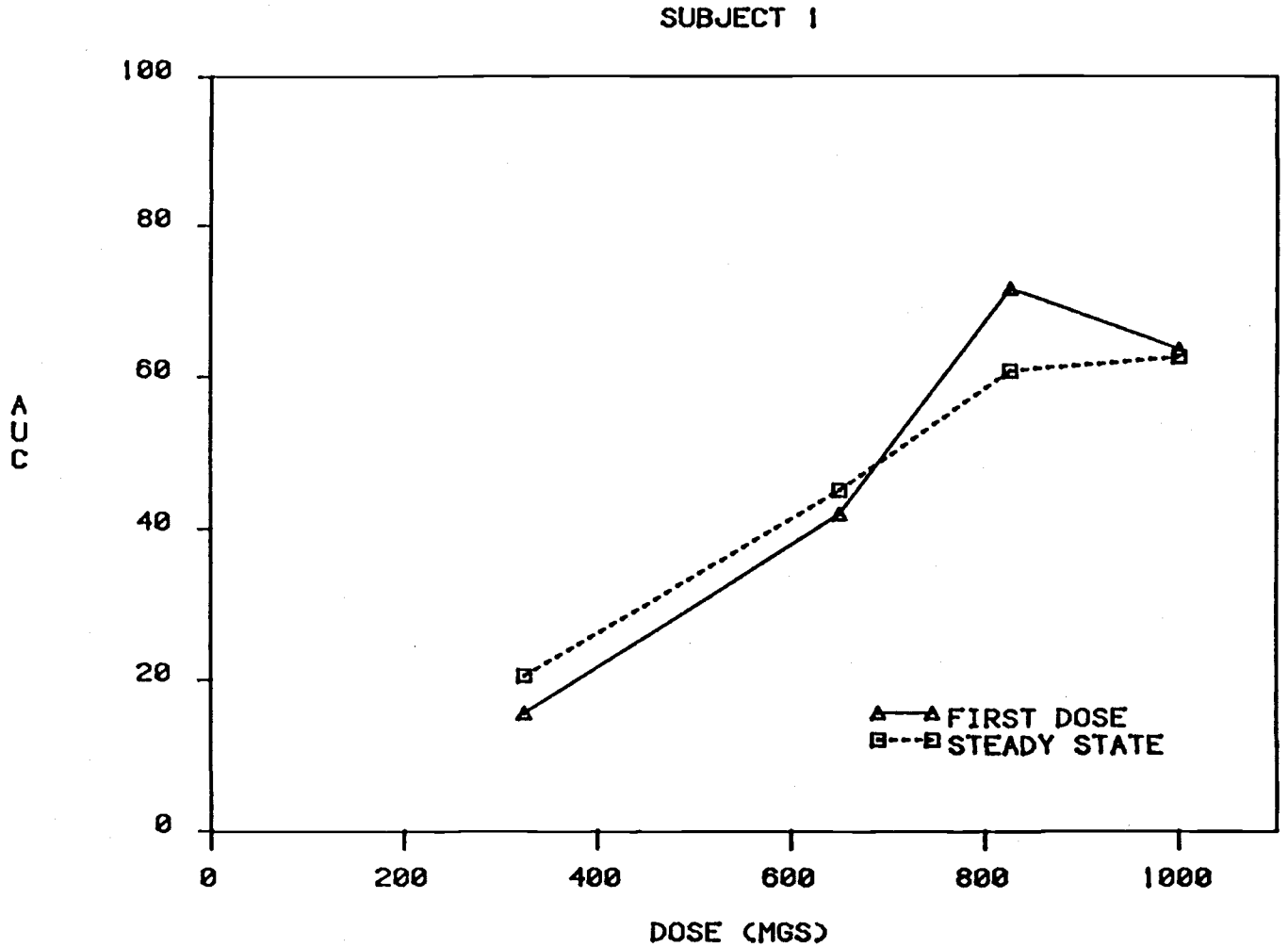

Figure A.9 Area under the salliva APAP concentration -time curve versus dose for subject No. 1.

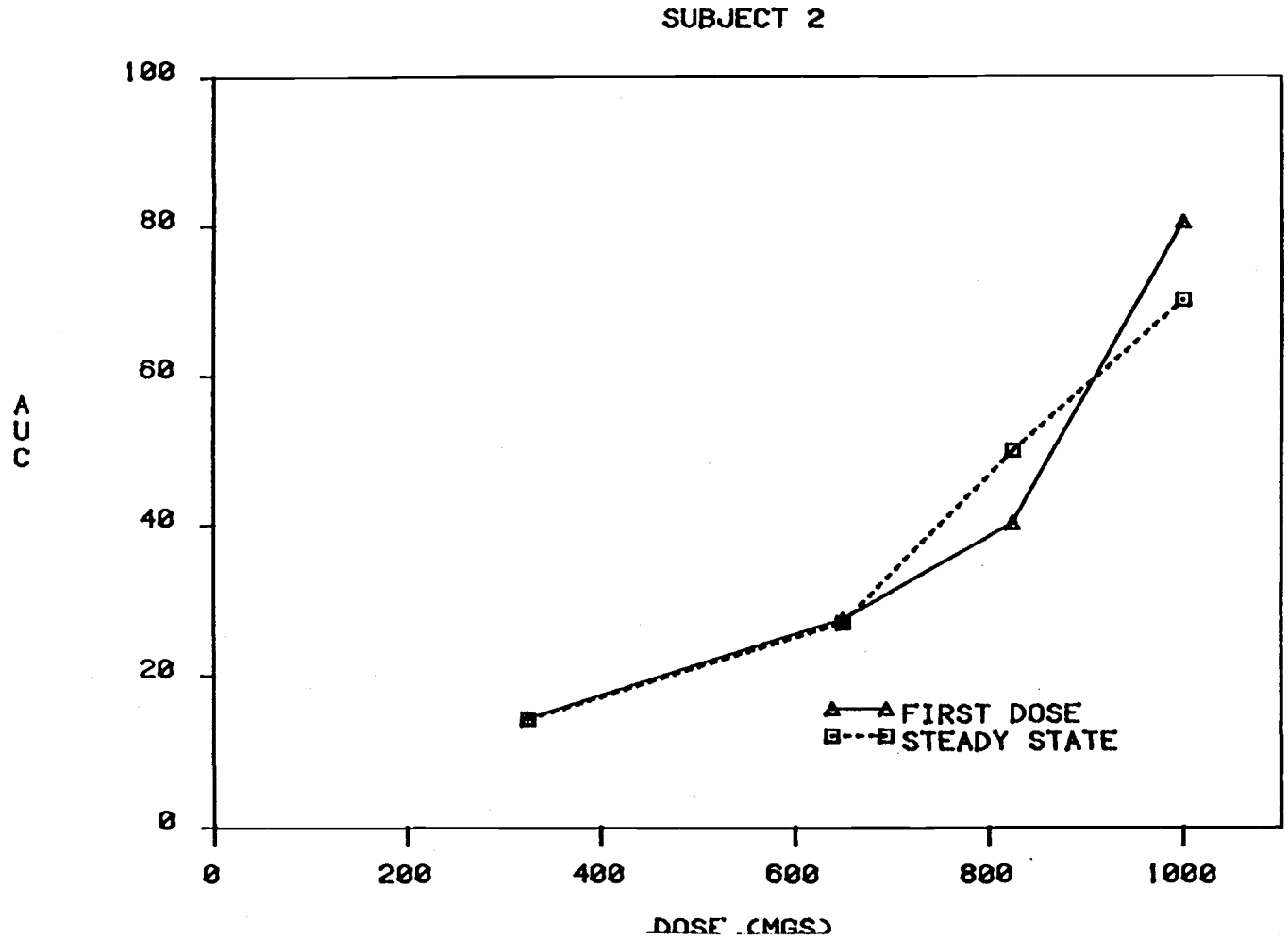

Figure A.10 Area under the saliva APAP concentration -time curve versus dose for subject No. 2.

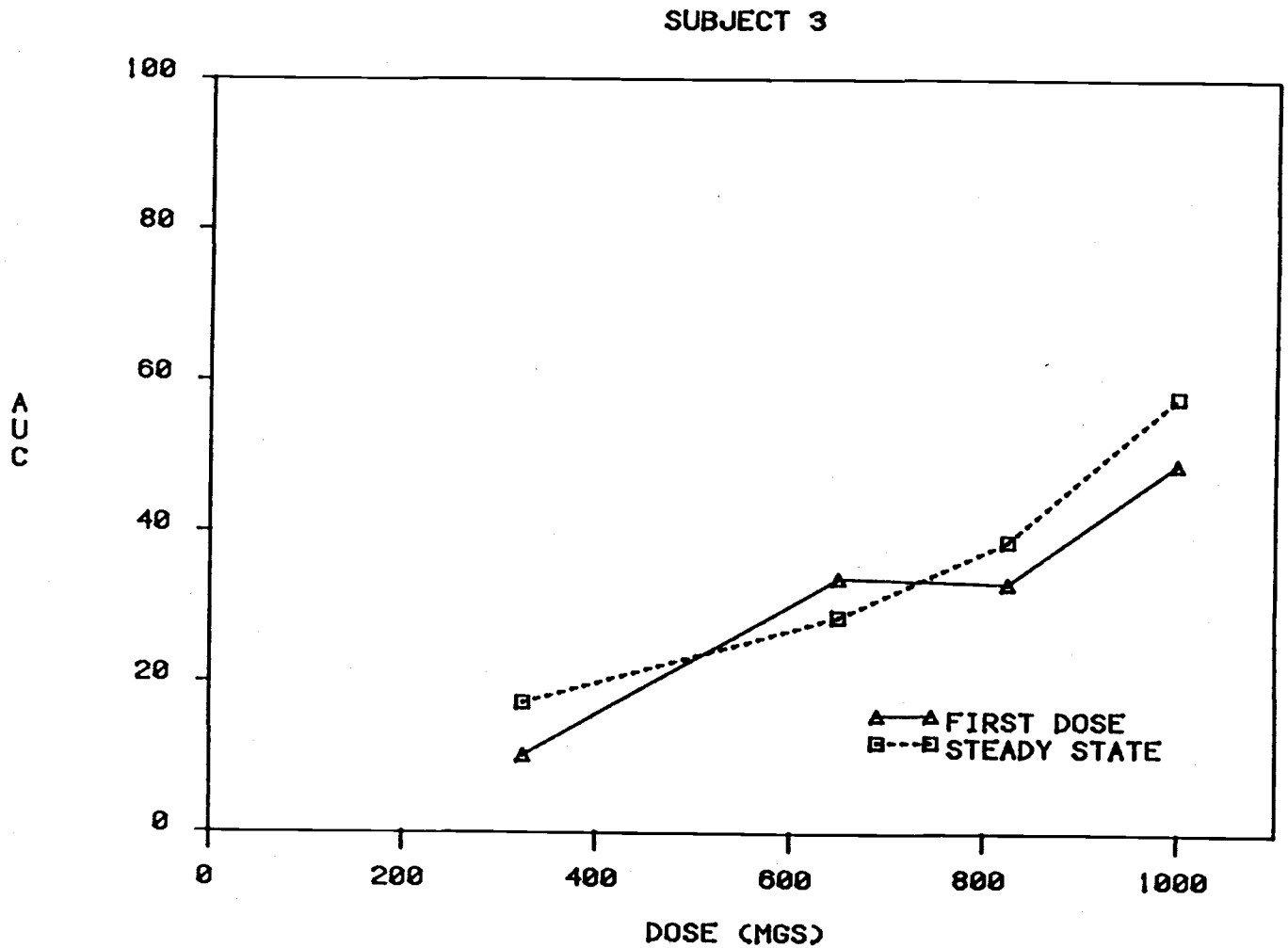

Figure A.11 Area under the saliva APAP concentration -time curve versus dose for subject No. 3.

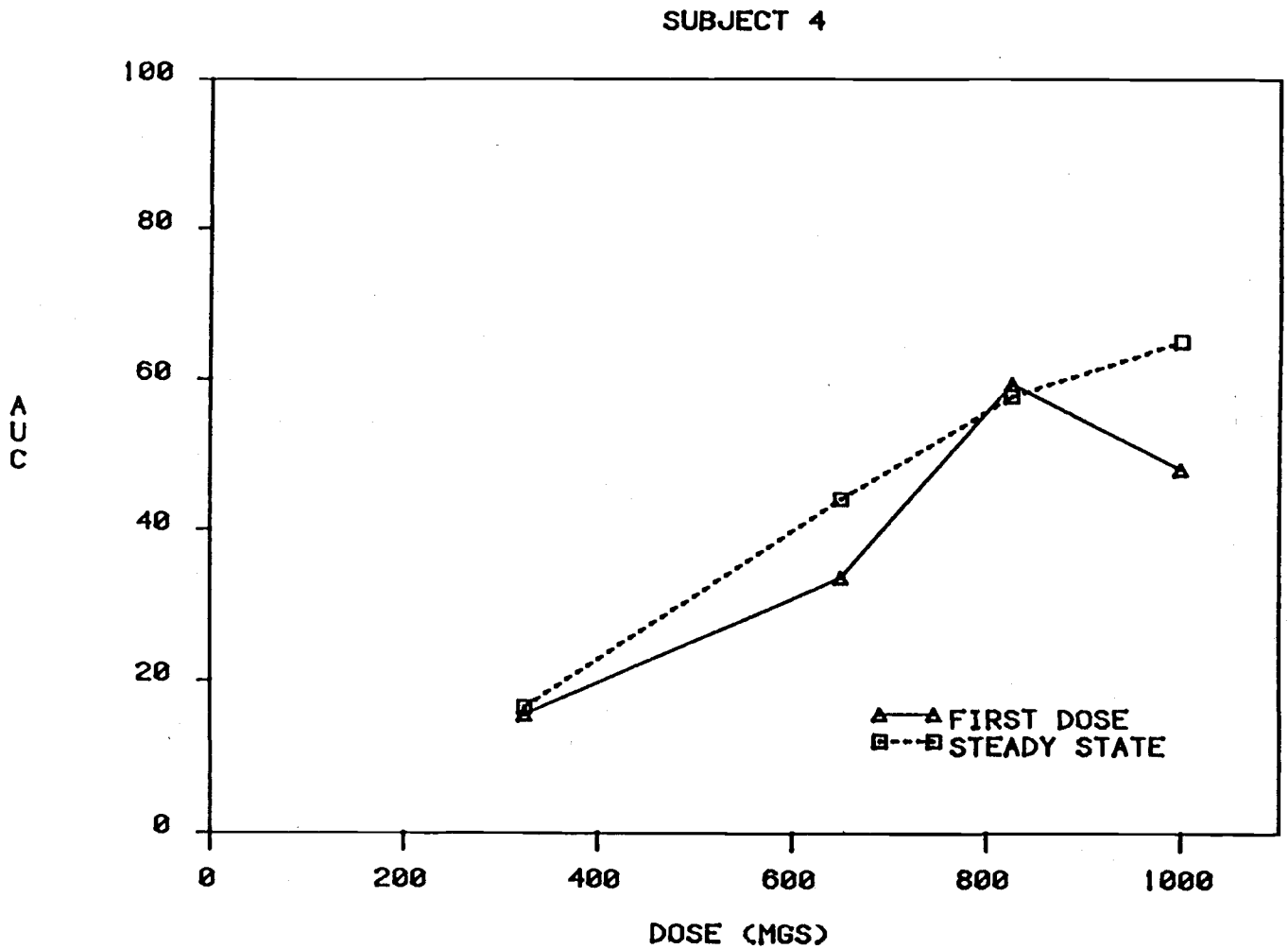

Figure A.12 Area under the saliva APAP concentration -time curve versus dose for subject No. 4.

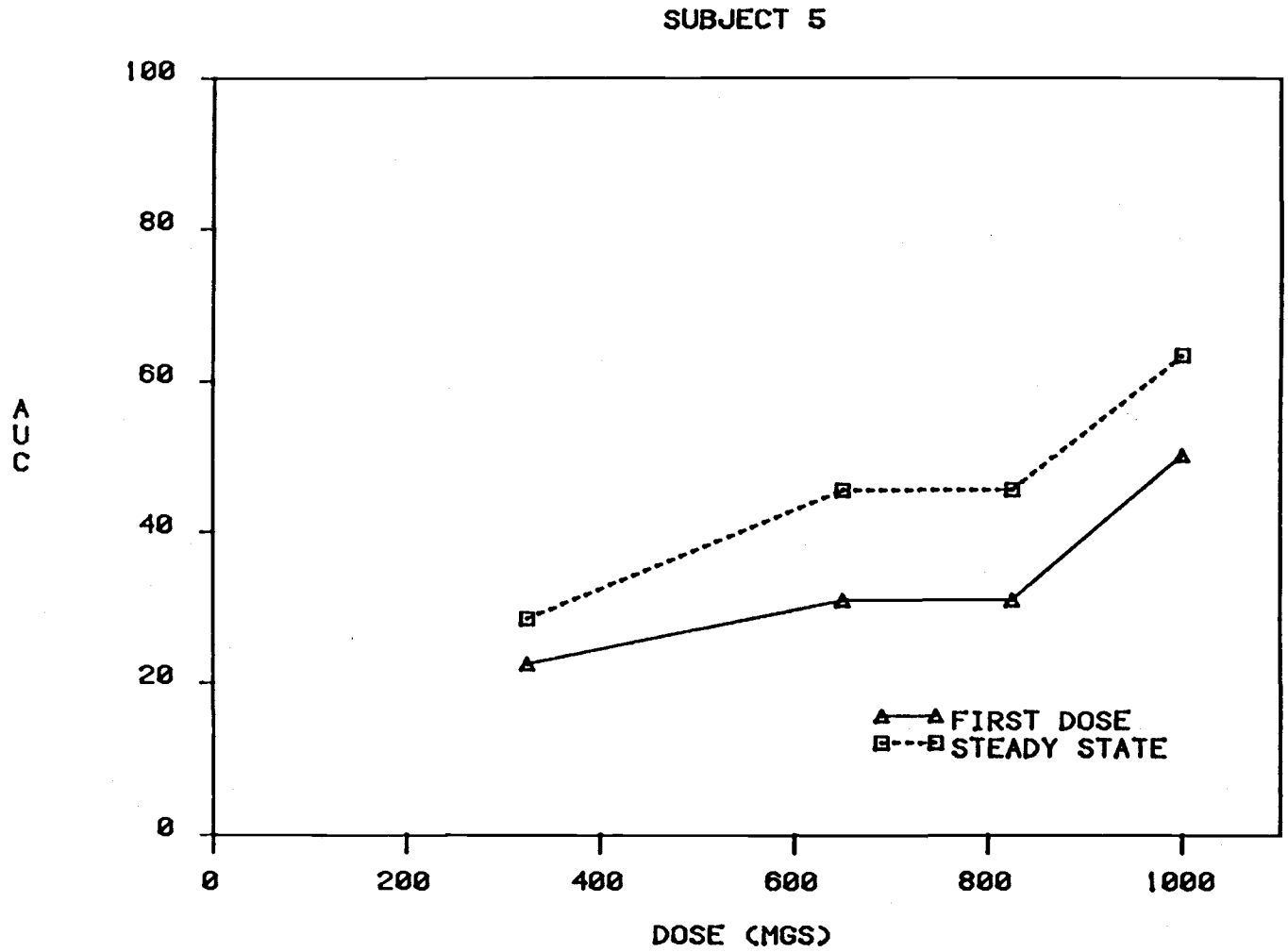

Figure A.13 Area under the saliva APAP concentration -time curve versus dose for subject No. 5.

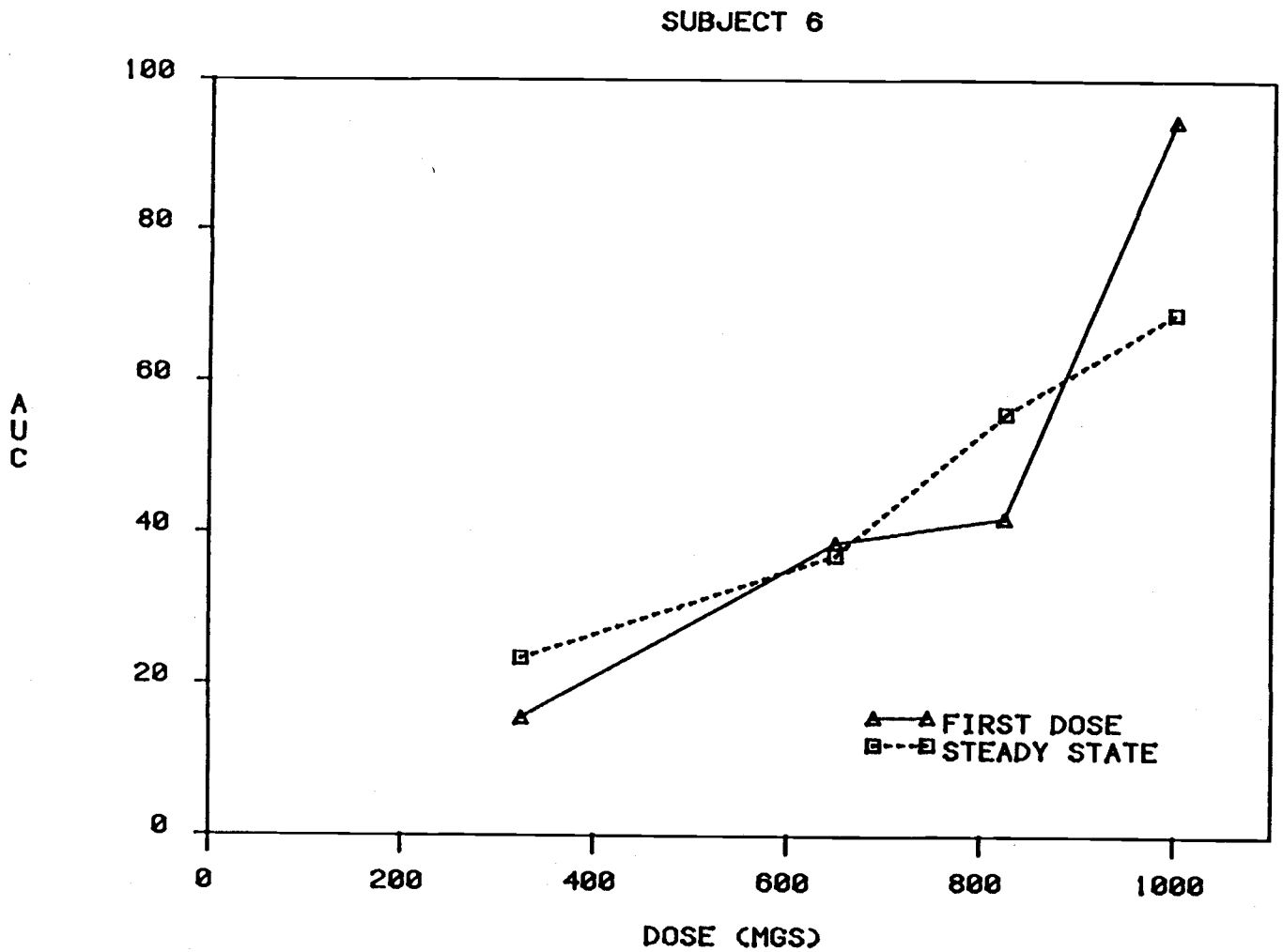

Figure A.14 Area under the saliva APAP concentration -time curve versus dose for subject No. 6.

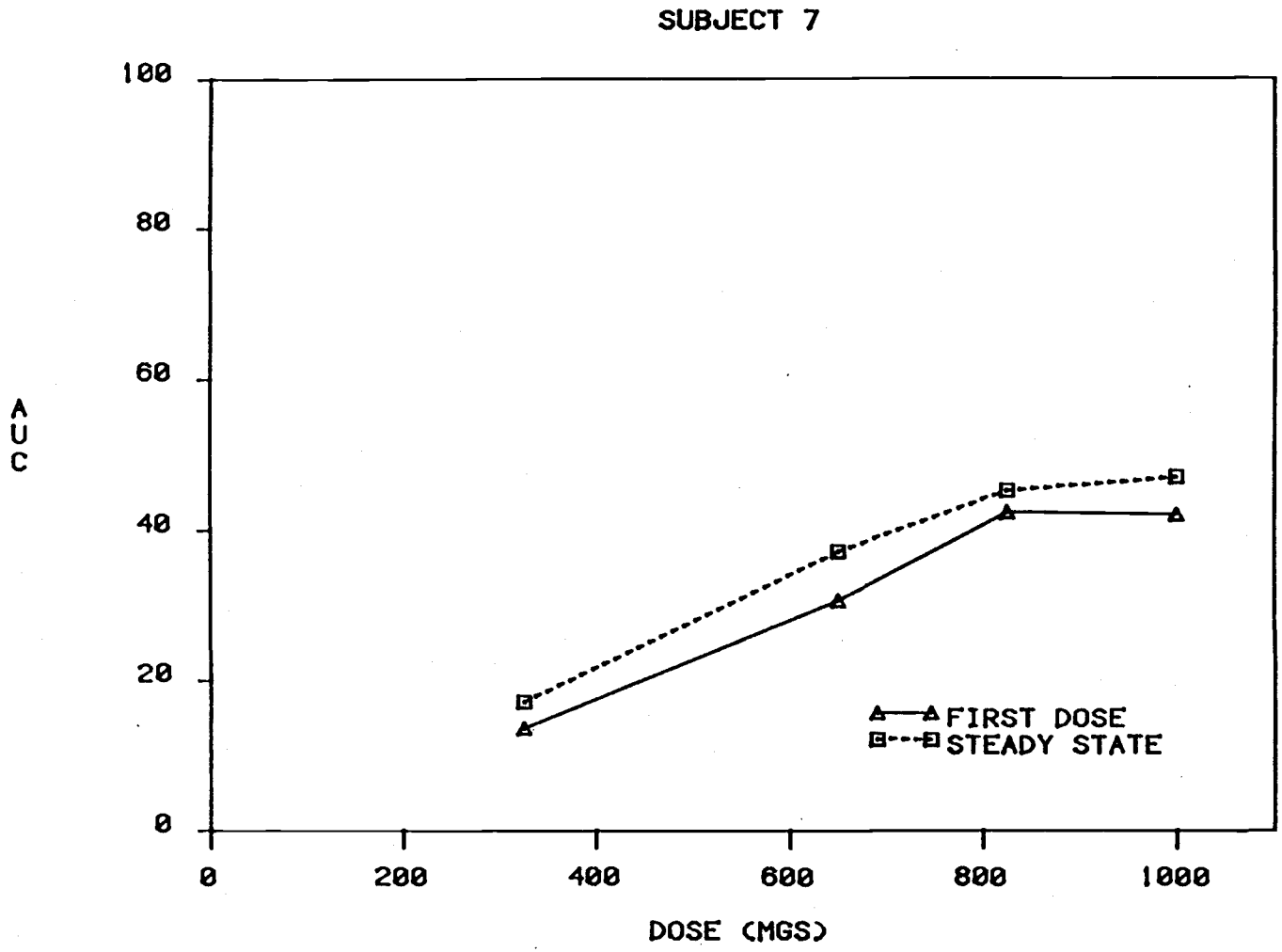

Figure A.15 Area under the saliva APAP concentration -time curve versus dose for subject No. 7.

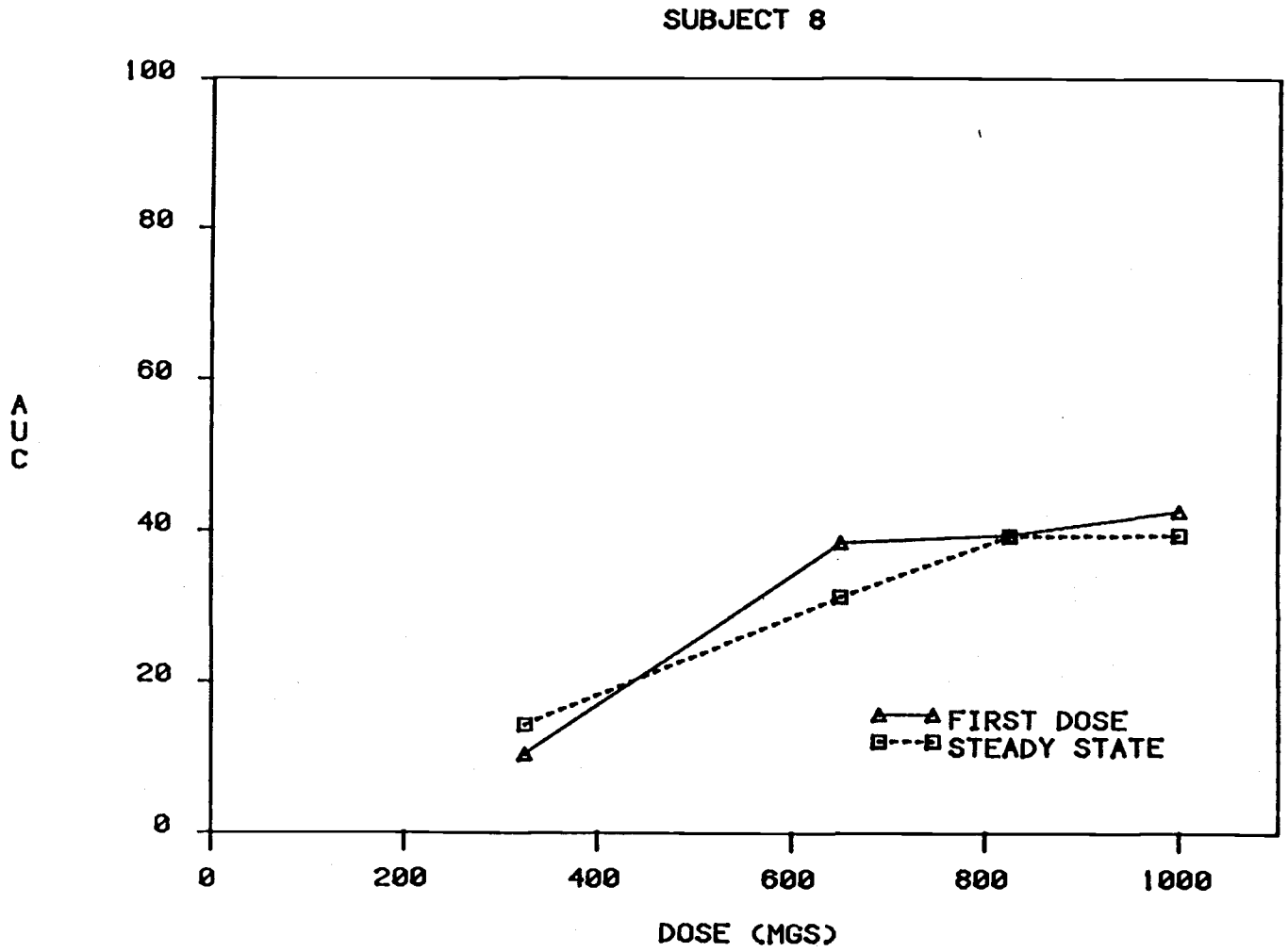

Figure A.16 Area under the saliva APAP concentration -time curve versus dose for subject No. 8.

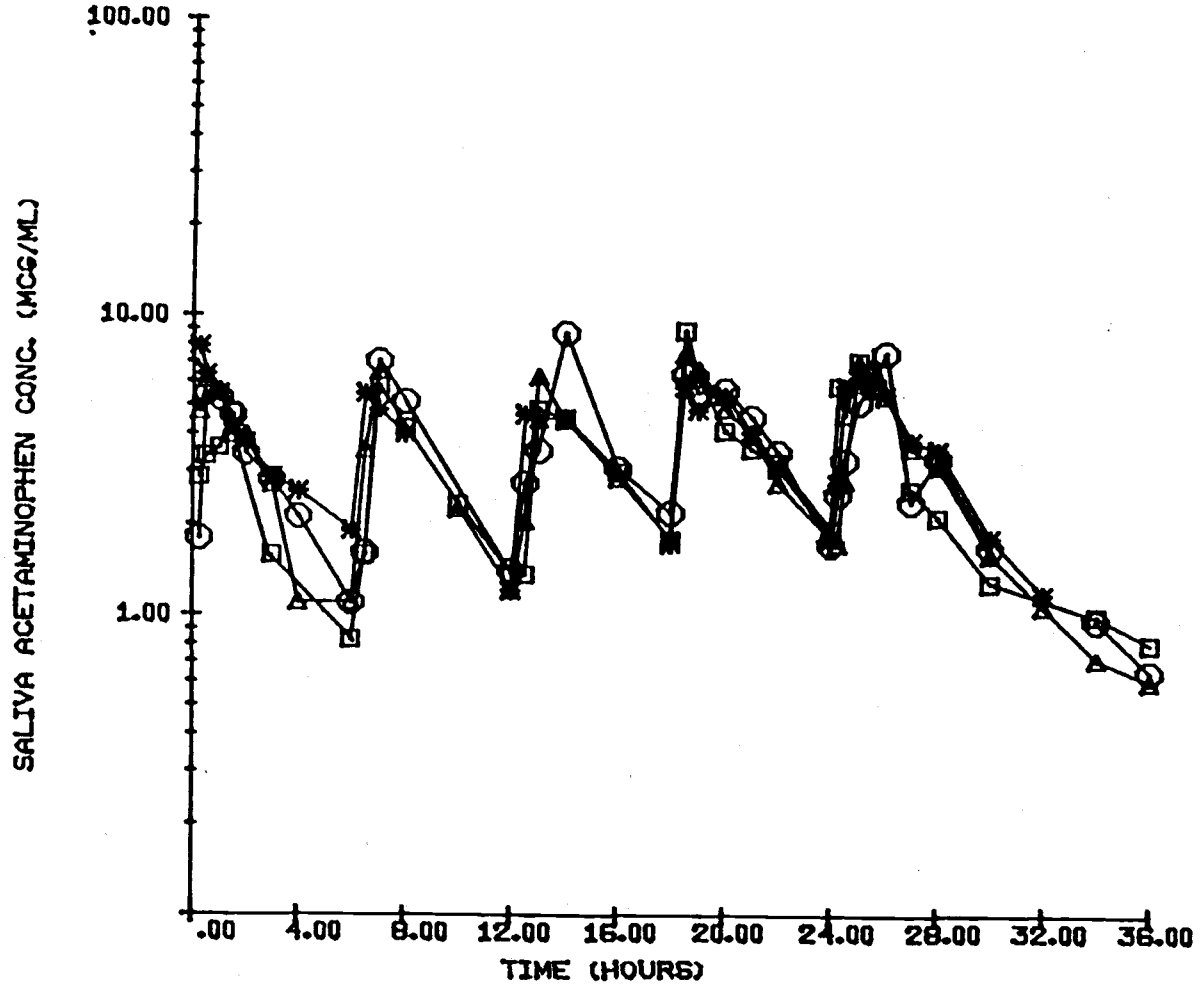

Figure A.17 Saliva acetaminophen concentration versus time curve for subject No. 1. Each dose normalized to 325 mg dose. Key (□) 325 mg APAP; (△) 650 mg APAP; (✱) 825 mg APAP; (○) 1000 mg APAP.

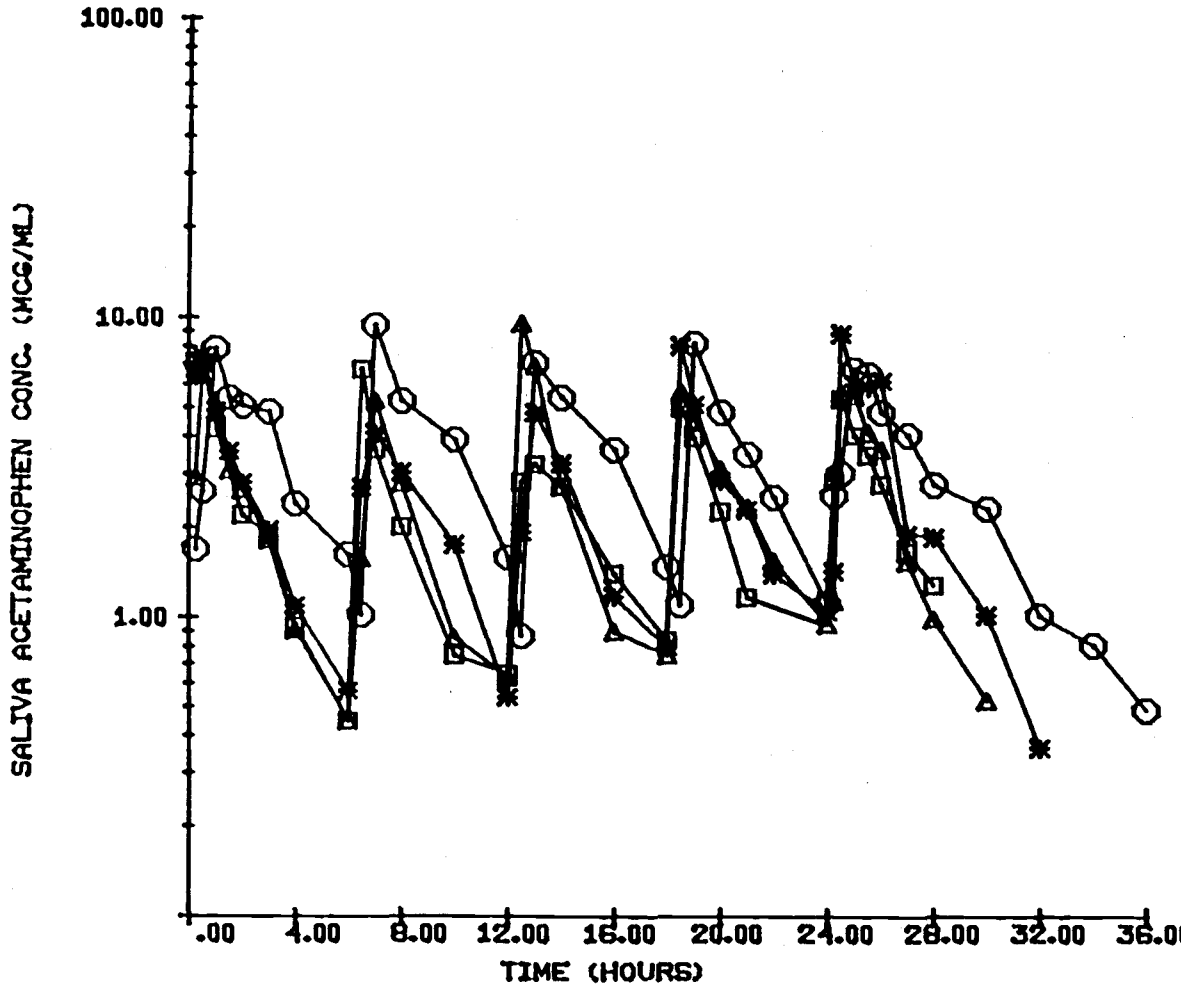

Figure A.18 Saliva acetaminophen concentration versus time curve for subject No. 2. Each dose normalized to 325 mg dose. Key (□) 325 mg APAP; (△) 650 mg APAP; (✱) 825 mg APAP; (○) 1000 mg APAP.

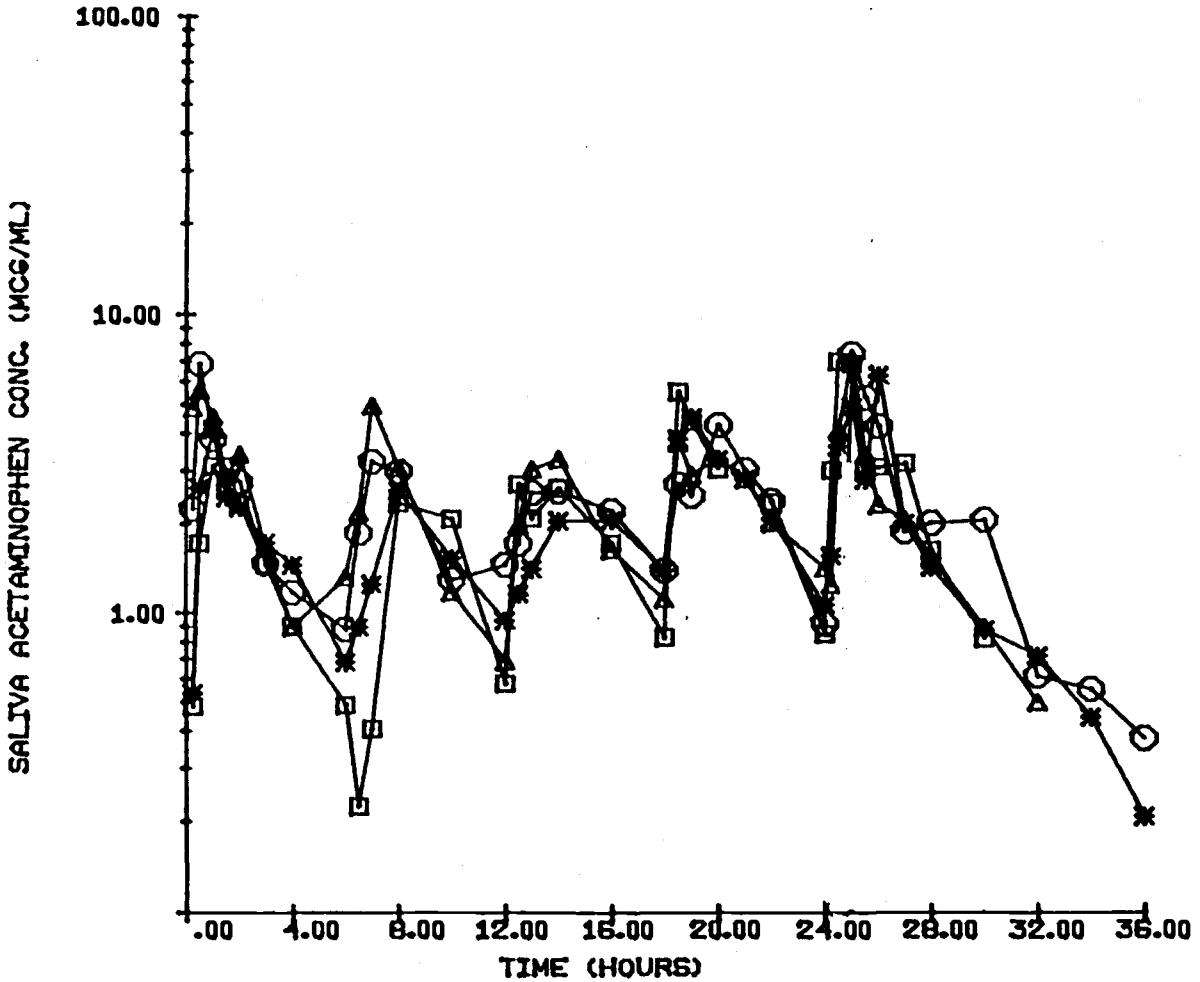

Figure A.19 Saliva acetaminophen concentration versus time curve for subject No. 3. Each dose normalized to 325 mg dose. Key (□) 325 mg APAP; (△) 650 mg APAP; (✱) 825 mg APAP; (○) 1000 mg APAP.

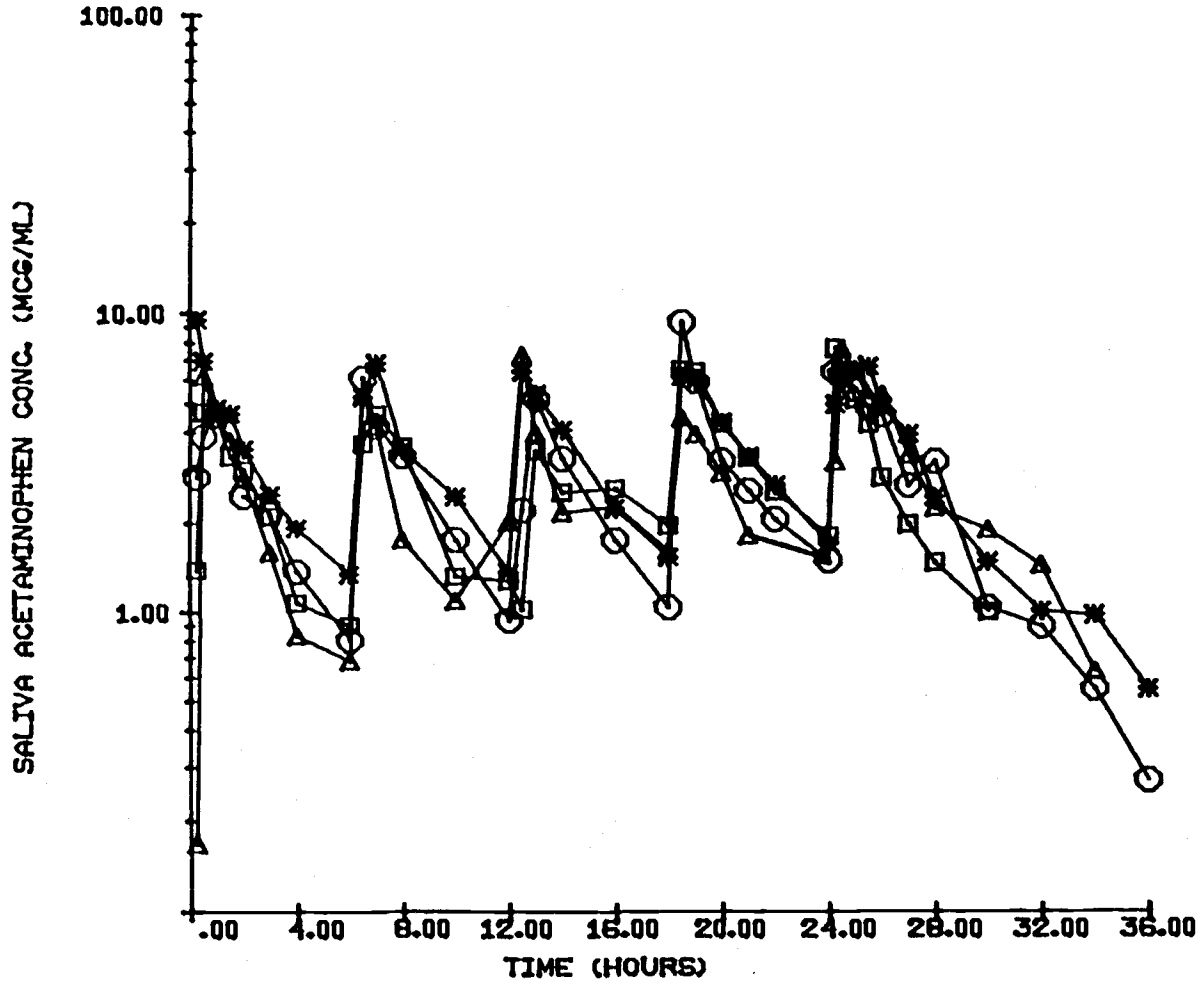

Figure A.20 Saliva acetaminophen concentration versus time curve for subject No. 4. Each dose normalized to 325 mg dose. Key (□) 325 mg APAP; (△) 650 mg APAP; (✱) 825 mg APAP; (○) 1000 mg APAP.

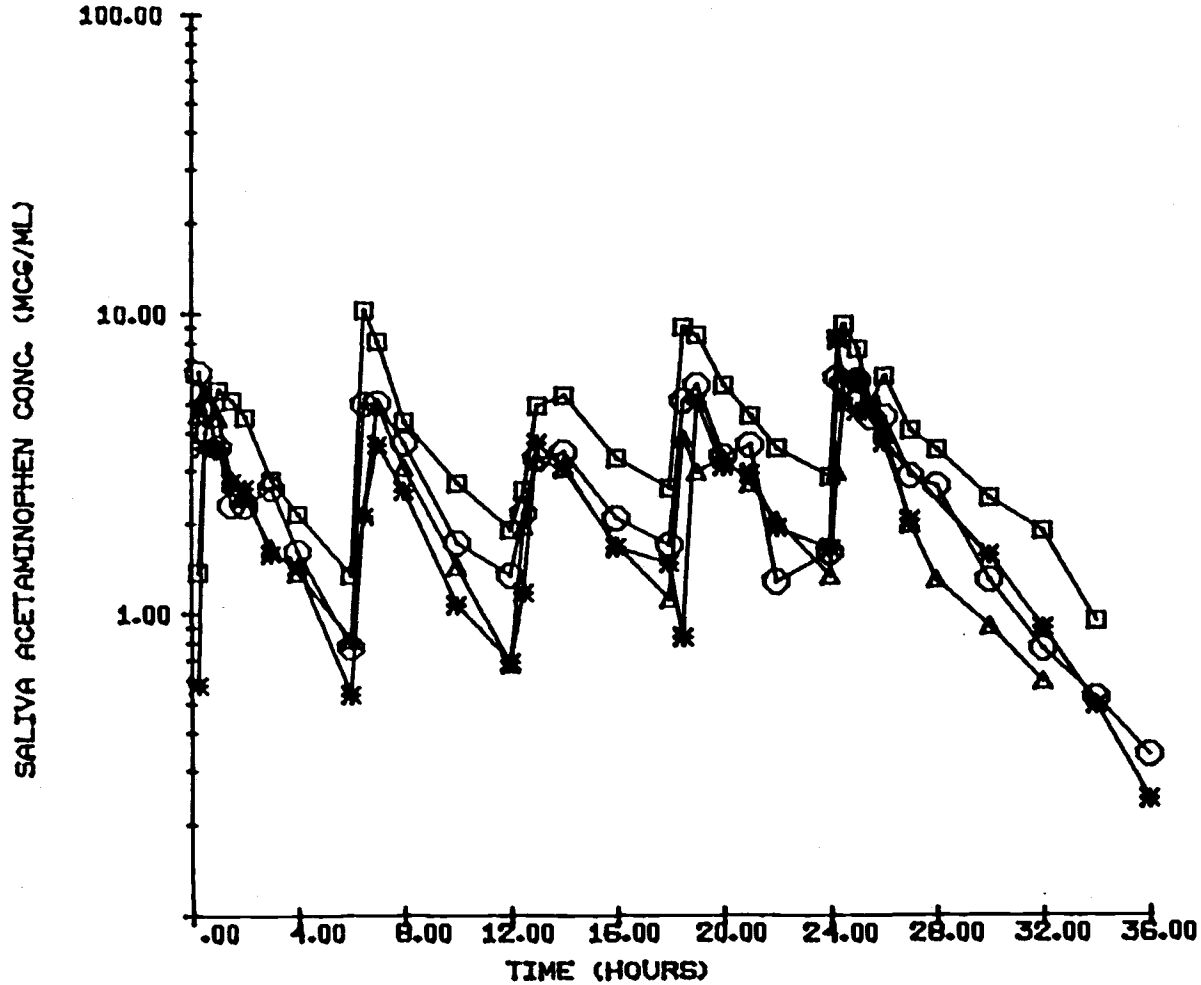

Figure A.21 Saliva acetaminophen concentration versus time curve for subject No. 5. Each dose normalized to 325 mg dose. Key (□) 325 mg APAP; (△) 650 mg APAP; (✱) 825 mg APAP; (○) 1000 mg APAP.

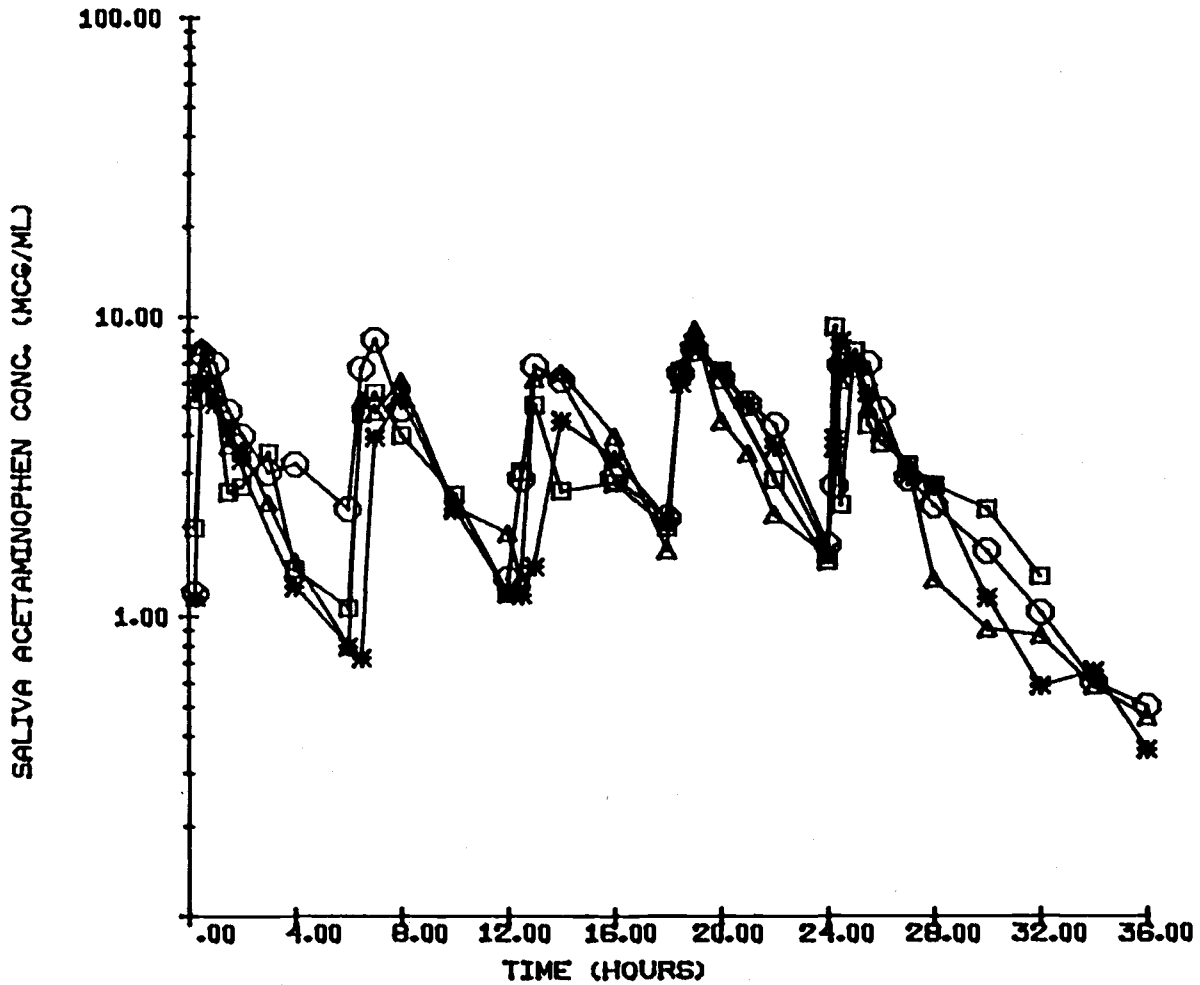

Figure A.22 Saliva acetaminophen concentration versus time curve for subject No. 6. Each dose normalized to 325 mg dose. Key (□) 325 mg APAP; (△) 650 mg APAP; (✱) 825 mg APAP; (○) 1000 mg APAP.

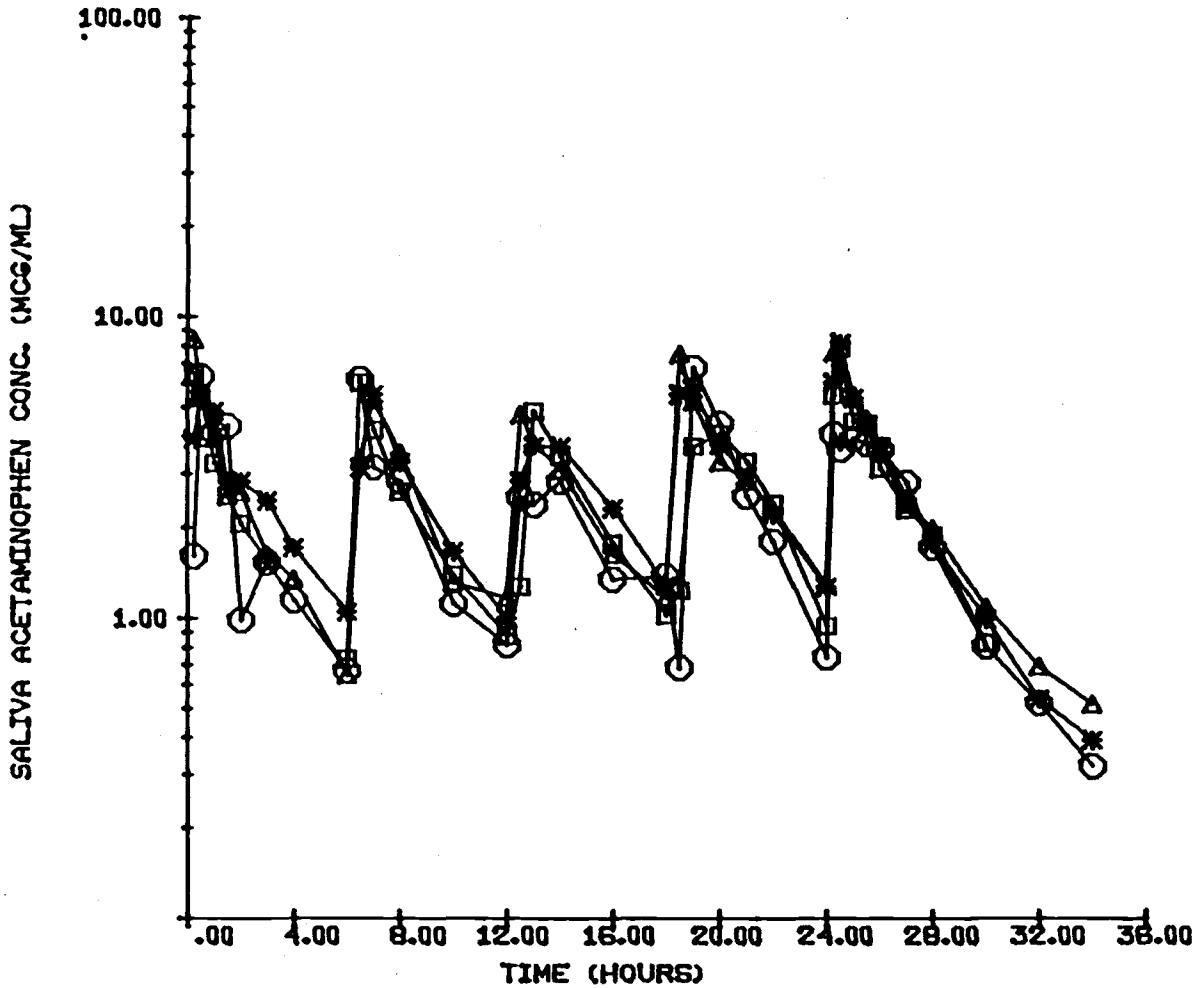

Figure A.23 Saliva acetaminophen concentration versus time curve for subject No. 7. Each dose normalized to 325 mg dose. Key (□) 325 mg APAP; (△) 650 mg APAP; (✱) 825 mg APAP; (○) 1000 mg APAP.

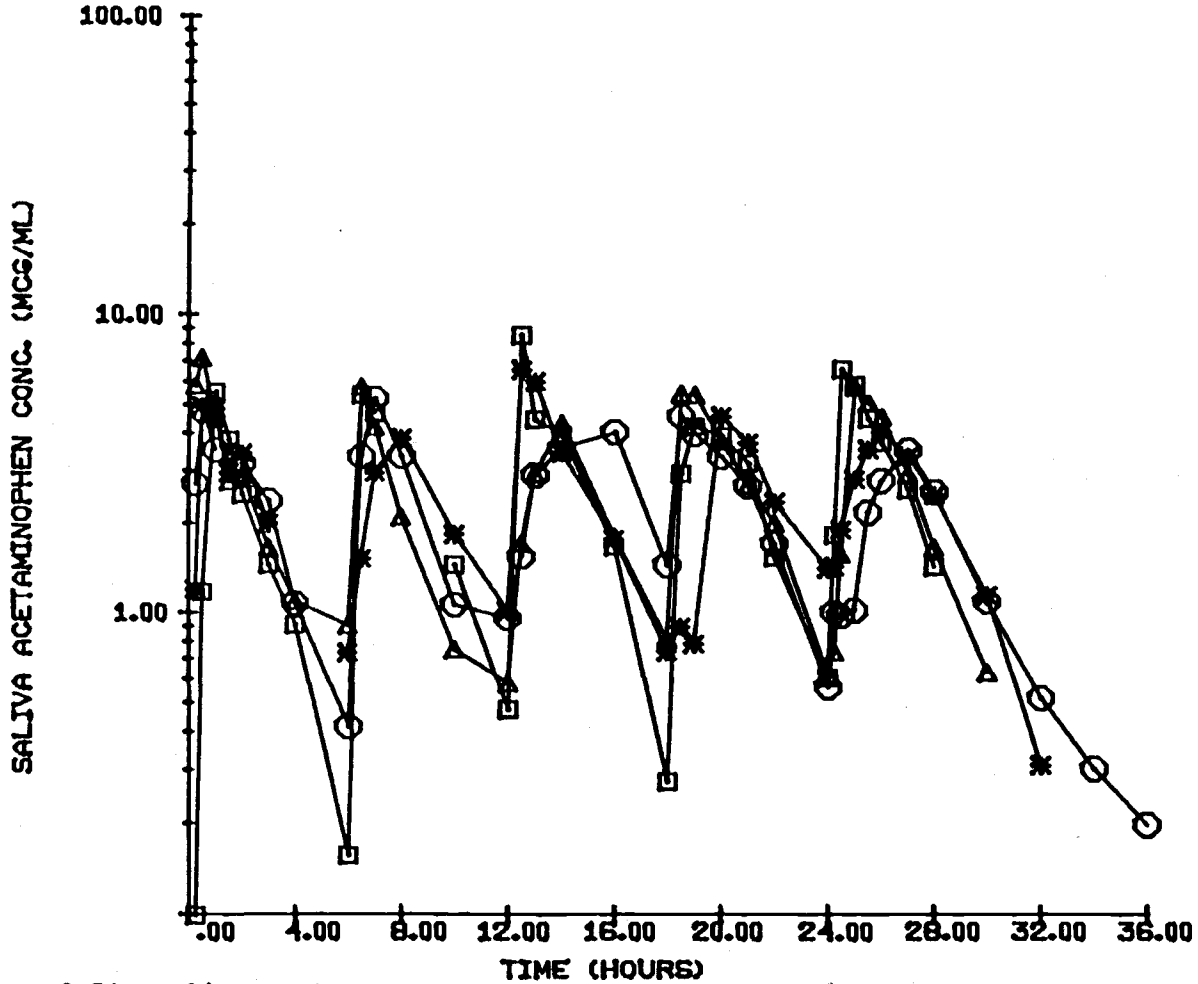

Figure A.24 Saliva acetaminophen concentration versus time curve for subject No. 8. Each dose normalized to 325 mg dose. Key (□) 325 mg APAP; (△) 650 mg APAP; (✱) 825 mg APAP; (○) 1000 mg APAP.