THE GASTRIC EMPTYING AND DRUG ABSORPTION FROM LIQUID FORMULATIONS OF 4-AMINOSALICYLIC ACID

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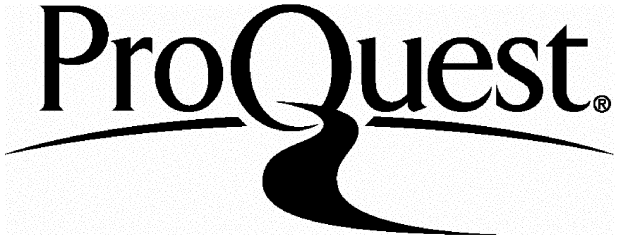

ABSTRACT

The fate of a 500mg dose 4-aminosalicylic acid (4ASA) liquid formulation was investigated in humans. Buffered pH3 and 7 oral liquid formulations were designed to provide adequate evaluation of the gastric emptying using applied potential tomography (APT). The gastric luminal pH profiles were monitored using a pH-sensitive radiotelemetry capsule and blood was sampled over 8 hours. In a further series of experiments, the gastric luminal acid secretion was restricted by ranitidine pre-treatment, to test the performance of APT and to observe the influence on the absorption profile of 4ASA. The content of 4ASA and its metabolites in the blood were analysed by HPLC assay. An intravenous administration of 4ASA was used as a reference to calculate the pharmacokinetic profiles with a deconvolution technique based on maximum entropy theory and a mass balance technique based on Wagner-Nelson compartmental theory.

The gastric emptying parameters were determined using statistical moments to provide a mean gastric residence time. The results showed that the performance of APT was not affected by physiological acid secretion. The ranitidine pre-treatment gave a full acid suppression until after the gastric emptying process of the oral liquid was completed. The shape of the blood level curves of 4ASA liquid formulations was influenced by the gastric emptying rate, which in turn was controlled by the duodenum acid feedback mechanism. The gastric emptying rate of pH3 4ASA formulation was delayed and hence an extended 4ASA mean absorption time was observed. The elimination rate of 4ASA was rapid when compared to its major metabolite, N-acetyl-4ASA (AASA). The bioavailability of 4ASA and AASA of the different formulations was restricted, with a mean average value ranging from 61 to 85% depending on treatment.

The results derived from the maximum entropy approach differed from those of the Wagner-Nelson method. The limited intravenous data points restricted the calculations. The 4ASA absorption rate distribution profiles, calculated using the maximum entropy approach, in some cases displayed a double peak phenomenon, which demonstrated the complexity in disposition even for of a simple oral liquid formulation of 4ASA. This phenomenon was not observed with the Wagner-Nelson method. The clearance and rate constant values of the current study differed from previous reported cases, where much higher doses and different dosage forms had been used.

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4ASA	4-aminosalicylic acid
5ASA	5-aminosalicylic acid
AASA	N-acetyl-4-aminosalicylic aid
ACh	Acetylcholine
APT	Applied potential tomography
AUC	Area under the curve
AUEC	Area under the gastric emptying curve
C_o	Plasma drug concentration at time zero after an intravenous bolus dose
CCK	Cholescytokinin
CGRP	Gastric releasing peptide
Cl	Clearance
df	Degree of freedom
EIT	Electric impedance tomography
ENS	Enteric nervous system
F	Variance value
F_a	Absolute bioavailablity
F_R	Relative bioavailablity
GABA	Gamma amino butyric acid
GE_{50}	Gastric emptying half time
GMRT	Gastric mean residence time
5HT	5-Hydroxytrytamine
IV dose	Intravenous route
K_a	Absorption rate constant

K_f	Formation rate constant
K_{GE}	Gastric emptying rate constant
K_{el}	Elimination rate constant
Lagtime	Time before onset of gastric emptying process
Liquid A	pH3 4ASA formulation without ranitidine treatment
Liquid AA	pH3 4ASA formulation with ranitidine treatment
Liquid B	pH7 Na4ASA formulation without ranitidine treatment
Liquid BA	pH7 Na4ASA formulation with ranitidine treatment
MAT	Mean absorption time
MAXENT	Maximum entropy
MMC	Migrating motor complex
MRI	Magnetic resonance imaging
MRT	Mean residence time
Na4ASA	Sodium 4-aminosalicylic acid
NANC	Non adrenergic non cholinergic
p	Probability level
r_s	Spearman's correlation coefficient
Sig.	Significant level
$t_{½}$	Half life
V_d	Apparent volume of distribution
VGRT	Variance of GMRT
VIP	Vasoactive intestinal polypeptide
Z	Rank value

Chapter 1

Introduction

1.1 The physiology of stomach

1.1.1 The structure and role of the stomach

The stomach can be broadly divided into four different regions that possesses unique functions. This includes the fundus, body, antrum and pylorus. The gastric body consists of the parietal cells that produce and secrete acid especially over the first hour in the post-prandial period. The fundus, antrum and pylorus regulate the transit of the non-nutrient liquid and nutrient particulate by a co-ordinated mechanism (Collins et al 1991, Camilleri et al 1994, Kelly 1981). Two sphincters found within the stomach prevent the food from either reflux back to the oesophagus or enter prematurely into the duodenum.

The main roles of stomach are to store food temporarily and release the digested contents into the duodenum in a control manner. The gastric acid activates the pepsinogen into pepsin to digest protein. It also sterilises the food contents. Although the food materials are not vastly absorbed within the stomach, vitamin B_{12} and some drugs are absorbed here (Johnson 1997).

1.1.2 The electrophysiology of the stomach

The smooth muscle of the stomach exhibits a series of cyclical changes in the resting membrane potentials called the 'slow wave' electrical activity. The initiation of the slow wave is via the multiple discrete loci consisting of the pacesetter activities. These loci are located within the mid-corpus of the gastric body where a group of closely associated cells called the interstitial cell of cajal have been identified (Bauer et al 1985, Christenten 1992, Kelly et al 1971). In humans, the slow wave propagates aborally along the gastric smooth muscle with a typical frequency of 3 cycles per minute (Malagelada 1991). The depolarisation of the resting membrane potential beyond the threshold action potential generates a series of smooth muscle contractions that is coupled by a complex network of neurohumoral system (Sarna 1985). Gastric emptying is a sophisticated process co-ordinated by this motor activity. A fine-tuning in temporal and spatial organisations is observed in the rhythmic pulsatile contraction patterns across the antrum and duodenum to optimise this transpyloric flow (Heddle et al 1993, Houghton et al 1988).

1.1.3 Neuronal and humoral controls of the gastric motility

The stomach consists of a complex network of nervous system and receptors that response to hormone and neurotransmitters, which modulate the gastric motility tone (Dockray 1994). A brief explanation is presented here as it involves a vast yet complex interaction that is beyond the discussion of the scope of current study.

The myenteric plexus of the stomach consists of a network of neurones known as the enteric nervous system (ENS), which regulates the basal electrical function of the smooth muscle as discussed earlier. These neurones synapse to the extrinsic and other intrinsic neuronal systems to generate the local reflex actions. Together with the humoral system, the gastric motility tone is modulated.

Two extrinsic neuronal pathways, which modulate the gastric motility tone, are the parasympathetic and the sympathetic systems. The parasympathetic system that acts via the vagal nerve efferent supplies to the stomach, are the low threshold cholinergic fibres and high threshold non-adrenergic non-cholinergic (NANC) fibres. The low threshold cholinergic fibres are capable of stimulating gastric contractions while the NANC fibres inhibit the gastric contractions. Hence, the vagal nerve system is excitatory and inhibitory depending on the degree of stimulation (Roman et al 1981). The afferent vagal fibres convey the sensory stimulation into the autonomic and central output to modulate the gastric motility tone (Wingate 1993).

The sympathetic pathway to the stomach is supplied from the neurones at the pre-ganglionic level of the spinal cord. Sympathetic fibres are inhibitory in nature. They synapse with neurones in the ENS to modulate the gastric motility tone (Gershon 1991).

Many endogenous neurotransmitters and hormones, which circulate the blood or secrete locally, modulate the gastric motility by acting directly on the nerve synapse and gastric smooth muscle receptors. The major group of neuromodulators are substances of non-adrenergic non-cholinergic (NANC) in nature. Several amines including dopamine, 5-Hydroxytryptamine (5HT) and gamma-amino butyric acid (GABA) have been identified to inhibit the gastric muscle tone. Neuropepetides and hormones such as motilin, gastrin

releasing peptide (CGRP), cholescytokinin (CCK), gastrin, galanin, substance P, somatostatin, vasoactive intestinal polypepetide (VIP), secretin, glucagon and pepetides YY are examples of the active substances that modulate the gastric tone in either excitatory or inhibitory manner (Burks 1994). Other endogenous substances such as opiate peptides, prostaglandins and nitric oxide have been identified to influence the control pathway (Schuurkes et al 1994).

1.1.4 Gastric motility in the fasted and fed states

The small intestine is the main absorption and assimilation site for the food and drugs. It consists of a large surface area with massive blood supply network, the special carrier systems and numerous digestive enzymes. The small intestine absorption is influenced by the rate of arrival of the materials from the stomach. The gastric emptying pattern is diet dependence and there is a great difference in the motility pattern between the fed and fasted states.

1.1.4.1 Gastric motility in the fasted state

When the stomach is empty, the gastric motility is characterised by a distinctive pattern of cyclical fluctuations in the contractions, refers to as the migrating motor complex (MMC). The MMC initiates in the antrum and propagates distally along the intestinal tract (Kellow et al 1986). The length and direction of the MMC are variable and they are disrupted by the ingestion of food. The cycle can be divided into four phases and lasts approximately between 90 and 120 minutes. Phase I is a period of quiescence with little or no motor activity that lasts up to one hour. Phase II is a period of intermittent motor contractions, which lasts for 30 minutes. The contractions in phase II increase gradually in magnitude and frequency as they progress into phase III. Phase III, which is often referred as the 'house keeping wave', is characterised by a series of high frequency intensive contractions that last between 5 and 15 minutes. During this phase, all the materials including the non-digestible materials are propelled into the duodenum. The phase IV is a transition period that follows shortly after the phase III where the contractions diminish and return to phase I. Clinically, it is difficult to distinguish the phase IV. Materials that are administered during phase II/III empty faster as compared to phase I especially for the non-nutrient liquids. Hence, the absorption of the materials during phase I is expected to be greater than any other phases (Code et al 1975).

1.1.4.2 Gastric motility in the fed state

The stomach can be functionally divided into two regions by the presence of a transverse mid-band during the fed state. The function of the transverse mid-band is unclear. However, many reports have postulated the presence of the mid-band to be related to the redistribution of food between the proximal and distal stomach (Collins et al 1988, Moore et al 1986).

The proximal stomach regulates the gastric emptying process by the intra-abdominal pressure difference. Immediately after swallowing food, the pressure within the proximal stomach drops in response to the vagal reflex to accept food materials. This mechanism is known as receptive relaxation. The arrival of food in the stomach raises the intra-gastric pressure to a threshold value where the intra-gastric pressure will remain the same. This accommodation property protects against the food reflux, and is described as the adaptive relaxation mechanism (Cannon 1911). The proximal stomach therefore acts as a food reservoir where materials are redistributed into the distal stomach by a gradual increase in the tonic contractions of the smooth muscle.

In the distal stomach, a series of co-ordinated contractions is activated, which propel the food towards the pylorus. On arrival at the pylorus, the pylorus opening shuts to retro-propel the food back into the distal stomach. In this way, the solid nutrient foods are ground and mixed before emptying into the duodenum (Malagelada 1991).

During the fed state, the MMC cycle is disrupted. The motor events can be described as a series of intermittent, non-cyclical contractions similar to the phase II activity. Delay in the gastric emptying is observed and the duration of disruption depends on the compositions of the food (Grimes et al 1977, Hunt 1963). The liquid and solid particles, which are less than 2mm in diameter, are emptied immediately but the larger solid particles are retained (Meyer et al 1981, Meyer et al 1988). The large particles distribute in the antrum are ground and mixed by a rhythmic antral pyloric contractions described above into the appropriate size before transiting to the duodenum for further digestion. The pylorus functions as a sieve to filter of materials according to their sizes (Horowitz et al 1991). Recent studies using the ultrasound technique suggested that the pylorus is in fact open for a longer period of times

than it is close during the fed state (King et al 1984), which may account for passage of the large size particles into the duodenum.

The volume of administration affects the gastric motility via the duodenum feedback mechanism. A large fluid volume distends the proximal stomach by generating a pressure difference across the gastric duodenum junction, which is big enough to induce rapid emptying rate during the initial phase. Then a constant fraction of volume is emptied following the initial phase, which can either be exponential or linear depending on the type of fluids ingested (Hunt et al 1954). The gastric emptying rate and onset of the small fluid volume are also affected by the MMC cycle (Oberle et al 1990).

A constant energy density of the contents is delivered each time through to the duodenum (Phillips et al 1991, McHugh et al 1979). Lin et al (1989) has reported an initial surge of glucose in the duodenum, which results in a dose and intestinal length dependence inhibition. Fatty materials delay the gastric emptying process via the fatty acid receptors that are located in the small intestine (Annegers et al 1947). The degree of delay depends on the degree of saturation and the carbon-chain length of the fatty acid. Carbon-chain lengths between 12-18 carbons of the unsaturated fatty acids exert the greatest effect on slowing the gastric emptying whereas chain lengths of between 2-10 carbons have no effect (Hunt et al 1968). Tryptophan, which is an essential amino acid in the body, is known to inhibit the gastric emptying process via the tryptophan receptors located along the small intestine (Stephens et al 1975).

The arrival of chymes into the duodenum stimulates the osmo-receptors to regulate the gastric emptying rate. The isotonic contents tend to empty faster than the hypertonic and hypotonic contents. Deflation of the osmo- receptors in responds to the arrival of the hypertonic contents triggers a feedback mechanism to inhibit the gastric peristalsis. Conversely, when the osmo-receptors are inflated, gastric emptying rate increases (Hunt 1956).

Previous studies have shown that a constant amount of acid titratable to pH6 is transferred to the duodenum in unit time. Hence, if the luminal pH of the duodenum is less than this value, the gastric emptying process is inhibited (Hunt et al 1962). Increase in the concentration of the acid content in the meal delays the gastric emptying process more. Those weak acids having a larger molecular weight slow the gastric emptying rate less effectively, as the

presence of large anionic couterions hinder interaction between the hydrogen ion and the acid sensitive chemoreceptors. The inhibition on gastric emptying rate is proportional to the inverse square root of the molecular weight of the acid contents (Hunt et al 1969). The strong acids however, have a greater inhibition on the gastric emptying rate with the increase in their molecular weights (Hunt et al 1972). Furthermore, the neutralisation of the chymes in the duodenum depends upon the bicarbonates buffering capacity from the pancreatic and duodenum secretions (Youngberg et al 1987).

The optimum temperature for gastric emptying of a meal is 37°C. Several reports show that any deviation from the body temperature delays the onset of gastric emptying especially when the volume of administration is large (Sun et al 1988, Bateman 1982). The viscosity of the meal has little effect on the gastric emptying process.

1.1.5 Physiological factors affecting gastric emptying process

Many physiological factors as described above affect the gastric emptying process. Other endogenous factors such as the posture, body mass index, gender, age, psychological stress and the type of routine diets also influence this process (Barkin et al 1988, Madsen 1992). The findings are often conflicting. Hunt (1963) demonstrated in subjects who ingested a 750ml solution either in head down or head up position, the amount of solution remaining in the stomach measured at a given interval is significantly greater with the head down position. The liquid preparations that do not activate the braking mechanism are emptied faster when the subject lies on the right side. Ingestion of the food when upright compares to the supine position favours the gastric emptying by the assistance of the gravitational force (Rainbird et al 1987). A study using the alginated antacid has shown that the antacid emptied faster than the meal when the subjects were lying on their left than right side (Bennett et al 1984).

The gender-related gastric emptying rate difference is most probably arises from the cyclical variations in the female sex hormone, progesterone (Datz et al 1987). Age related delays in the gastric emptying rates in both solid and liquid meals are subjective (Fich et al 1989, Horowitz et al 1984, Moore et al 1983). The gastrointestinal response to stress is adaptive, hence, the effect on the gastric emptying rate is often controversial (Cann et al 1983,

Thompson et al 1983). As inter and intra-subject variations in the gastric emptying rate are large, comparison studies can be conflicting especially when the population is small.

1.1.6 Pathophysiological and pharmacological factors affecting gastric emptying process

Gastric motility can be altered by the disease states. The disturbance in the contractions of the stomach and the small intestine may result in either a delayed or an accelerated gastric emptying and is usually presented as a series of common symptoms such as nausea, vomiting, belching, fullness, early satiety, upper abdominal pain, heartburn, anorexia and sometime weight loss. Diseases such as diabetes mellitus, non-ulcer dyspepsia, anorexia nervosa, gastric and duodenum ulcers, connective tissue disorders, chronic idiopathic pseudo-obstruction and myotonic dystrophy share some forms of gastrointestinal motility dysfunction. Surgeries including gastric resection, vagotomy and gastrectomy, which are the solution to many diseases, may create new forms of motility disorders that can be either temporary or permanent (Smout et al 1994).

Many pharmacological interventions are used to control the gastrointestinal motility dysfunction. Delay in gastric motility can be improved with thé prokinetic drugs. The dopamine-2 receptor antagonists including the metoclopromide and domperidone are found to be useful in enhancing the gastric emptying rate and small bowel motility. Domperidone, which can not cross the blood brain barrier to cause extrapyramidal side effects via acetylcholine (ACh) receptors in contrast to metoclopamide, is more acceptable (McCallum et al 1983). Cisapride, which behaves like $5HT_4$ agonists, acts by stimulating the release of acetylcholine in the myenteric plexus increasing the gastrointestinal motility tone, is amongst the most effective motility promoter (Fraser et al 1994[a]). Many cytotoxic drugs including all the alkylating agents cause nausea and vomiting as the classical side effects to chemotherapy treatment. The $5HT_3$ antagonists such as granisetron and ondensetron are powerful anti-emetic agents that inhibit the vagal afferent fibres, which possess promotility activities (Grundy et al 1994, Talley et al 1990). Recently, the antibiotic erythromycin has been discovered to possess a strong prokinetic property by binding to the motilin receptors in the gut (Peeters et al 1994, Tack et al 1992).

Accelerated gastric emptying process although less prevalent clinically, has no known drug treatment. A small frequent meal with low carbohydrate is advised to reduce the gastric emptying rate. Low dose opiates such as the morphine and loperamide are used commonly in treating diarrhoea (Borody et al 1985, Kachel et al 1986).

The use of gastric acid suppressers including histamine-2 receptor antagonists and proton pump inhibitors may affect the gastric digestion and motility. The effect of histamine-2 receptor antagonists such as raniditine and cimetidine on gastric motility is conflicting (Parikh et al 1994).

1.2 Techniques for monitoring gastric emptying process

1.2.1 Direct methods

1.2.1.1 Gastric intubation

The gastric dye dilution technique uses intubation of a nasogastric tube for sampling gastric contents by aspiration, has first been documented by Hunt et al (1954) to understand the physiology of gastric emptying process. A test meal is drunk either before or after ingestion of known concentration phenol red, which is a non-absorbable water-soluble marker, is added. After mixing with several aspirations, a further known concentration of phenol red is added before the gastric contents are sampled. The gastric volume can be estimated by using the spectrophotometric determination of the dye concentration in the gastric aspirates (Hurwitz 1981).

The technique is based on the assumption that the dye attains a rapid distribution and equilibrium within the gastric contents. No gastroduodenal reflux occurs during sampling. The procedure itself may result in the alteration of the gastric motility. Due to the complexity, discomfort and invasiveness, its research and clinical usage is limited.

The gastric emptying of the meals measured with the dye dilution technique is comparable to the gamma scintigraphy technique (Beckers et al 1992). The method has shown to be accurate

and repeatable (Sheiner 1975) and can be used to estimate the emptying of both solid and liquid meals (Akkermans et al 1991).

1.2.1.2 Radiology

The commonly used radio-opaque marker is barium sulphate, which can be incorporated into both solid and liquid meals for the sequential X-ray imaging to monitor the gastric emptying process. The normal gastric emptying rate of the barium meal is 2-3 hours. Prolongation beyond this time period indicates possible gastric stasis. The technique is semi-quantitative as it can only estimate the total gastric emptying time. Although barium sulphate is not a physiological marker, it provides a good estimation for the gastric emptying process resembling the solid indigestible meal (Feldman et al 1984). Recently, barium sulphate has been incorporated into capsules where emptying of the capsules from the stomach reflect the first occurrence time for the antral obliterating contractions (Chang et al 1997).

This technique is non-invasive, accurate and reproducible data can be obtained (Hebbard et al 1995). The exposure to a high dose radiation has limited the number of repeat measurements, and it is not suitable in children, pregnant women and women of child bearing age (Corazziari et al 1993). The studies utilise standard equipment and experienced radiographers.

1.2.1.3 Ultrasonography

Serial real time ultrasound, which scans parallel to the long axis of the stomach by placing an ultrasound probe over the abdomen at the level of the epigastrum post-prandially, allows the dynamic imaging of the stomach. The method provides a quantification of the gastric volume after ingestion of a test meal and monitors the gastric motility in the antrum and pylorus (Bateman et al 1982, Berstad et al 1994).

The technique is relatively inexpensive and completely non-invasive. It allows physiological monitoring of the gastric emptying rate of both solid and liquid meals at bedside. Ultrasonography has been shown to correlate with the gamma scintigraphy technique and is repeatable (Gilja et al 1997). The operating procedure is however highly dependent upon the operator. The technique has also been described as time-consuming and cumbersome. The

accuracy can be disrupted by the presence of gastric air, which distorts the ultrasound image. Even though both the solid and liquid meals can be use, it is not yet possible to distinguish between the contents. Experienced ultrasonographer is required for identification of the anatomical structures and image interpretation during gastric emptying studies (Bolondi et al 1985).

1.2.1.4 Magnetic resonance imaging

The magnetic resonance imaging (MRI) has many clinical uses and current advance in technology further extends its application into the dynamic monitoring of gastric emptying and gastric motility in a non-invasive manner. The meal is labelled with a non-toxic and non-absorbable physiological marker that provides a contrasting medium for MRI. Schwizer et al (1992) first described the MRI in monitoring gastric emptying using a liquid phase marker [Gd]-DOTA. The subject was placed in a magnetic field generated by the MRI magnetic scanner in a supine position with restrained movement and posture. By performing the multiple transaxial T1-weighted sections, the three-dimensional images of the meal and stomach can be constructed. The gastric emptying curve is obtained by performing an area of interest mapping. The technique has shown a good correlation with the double indicator technique involving intubation and the gamma scintigraphy technique.

The echo-planar MRI currently available, with snapshot images requiring a data acquisition time of only 64-128 msecs, can also monitor the gastric motility using a fast coronal scan that allows simultaneous recording of the gastric volume and gastric contraction of the whole stomach. This is not possible with the ultrasonography (Fraser et al 1994[b], Evans et al 1993).

As there is no exposure to any forms of radiation and other health hazards, the prolonged monitoring is not under ethical concerns. In fact, since the gastric volume and the meal volume are calculated, by subtracting these volumes, we are able to describe the gastric secretion process. With the advance in computer technology, it may possible in the future to distinguish the meal contents, especially the fatty components.

To date, the clinical usage is limited due to the cost of the equipment. The technique is monitored with the subject at the supine position and only gastric emptying of the liquid meal

has been demonstrated as there is no suitable solid marker, which confines its use in research at present. Further development and justification are yet required to explore the full potential of this powerful tool (Schwizer et al 1994).

1.2.1.5 Gamma scintigraphy

Gamma scintigraphy is currently the gold standard for evaluating the transit of meals and different dosage forms along the gastrointestinal tract. The incorporation of the gamma-emitting radionuclide into a test meal allows a non-invasive monitoring of the gastric emptying process by the external gamma scintigraphic equipment. The standard radionuclides are non-absorbable, non-toxic and relatively cheap with half lives long enough to cover the gastric phase transition time. Radionuclides such as ^{99m}Tc, ^{131}I and ^{111}In have low radiation burden. They are adsorbed specifically and distributed homogeneously in the test meal (Thomforde et al 1985). More than one radionuclide can be scanned simultaneously in the same subject to determine the gastric emptying of both solid and liquid meals. These nuclides emit different photon energy, which is specific to each radioactive nuclide (Malagelada et al 1984, Davis et al 1986, Mitchell 1997).

Both single and dual headed gamma cameras are used in the current practice, a triple headed rotating camera will be available in the near future can create a three dimensional visualisation of the gastric motor activity. The accuracy of the image acquisition depends on the position and distance of the detector head. A radiolabeled marker is placed on the iliac crest to position detector head accurately. Errors arise from the gamma ray attenuation is due to the nuclei decays, movement of the radiolabeled food and the subject. This can be counteracted by either calculating the geometrical mean radioactive value using a dual headed detector camera or by application of the correction factors derived from a lateral image of the stomach (Akkernams et al 1994, Collin et al 1984, Hardy et al 1985).

If two or more radionuclides with the photon energy levels which overlap, are scanned simultaneously, interference from both the low and high energy windows may arise that result in deflection of the radiation. This interference can be minimised by using a small amount of the high-energy isotope on a separate occasion to demonstrate the background interference. Septal penetration is a phenomenon where the gamma emission ray enters the detector at an

angle instead of between the parallel head septal of the detector. This effect is not significant provided the deflection is constant throughout the study period (Loo et al 1984).

The technique allows the dynamic and quantitative monitoring of the gastric emptying of a wide range of physiological meals as well as pharmaceutical dosage forms in both supine and erect postures. Once the system has been set up, it is easy to use. This method is accurate, reproducible and correlates well with other techniques. However, day to day variations in gastric emptying rates of the same type of meals were demonstrated intra and inter-individually in the liquid and solid meals, respectively (Brophy et al 1986).

Gamma scintigraphy equipment is costly and repeat measurements are limited due to the exposure to the radiation. Monitoring with gamma scintigraphy is not suitable in children, women of childbearing age and pregnant women. The use of the radionuclides introduces expense of handling and difficulties in manufacturing radioactive dosage forms that are appropriate for administration and maintenance of their radioactive labels. In addition, although the gated gamma scintigraphy allows assessment of regional variations in the antral contractions, it does not permit the variations of individual contraction to be evaluated due to the long acquisition time (Stacher et al 1987, Jacobs et al 1982). As the volume of the gastric secretion can not be accessed, the radionuclide may dilute to an unknown degree especially for the liquid phase marker. When comparing to radiology, computerised tomography, ultrasound and magnetic resonance imaging, the scintigraphic image's resolution is inferior. In subjects with large stomach, the overlapping with the bowel may result in difficulties in mapping the region of interest (Wolverson et al 1982).

1.2.1.6 Electrical impedance tomography

Electric impedance tomography (EIT) is a technique that images the distribution of resistivity of the tissues within a body region (Jongschaap et al 1994, Dijkstra et al 1993). It began for practical purposes just over a decade ago, where a prototype system, which is suitable for clinical use, has been developed. Two types of EIT have been used to monitor gastric emptying.

1.2.1.6.1 Impedance epigastrography

Epigastric impedance is a portable device, which consists of a combined monitor and computer that can be connected to a chart recorder or tape recorder. Four electrodes are used and an alternating current of 4mA at 100kHz is applied through two input electrodes. The changes in the tissue resistivity are measured as the changes in the potential difference across the four electrodes. Epigastric impedance gives a direct reading of the impedance with data stores in the computer and output signal records on the chart or tape recorder (McClelland et al 1985).

1.2.1.6.2 Applied potential tomography

Applied potential tomography (APT) on the other hand consists of a data collection module, visual display unit, computer and the printer. It uses 16 electrodes, which are placed in a circular array around the upper abdomen. An alternating current of 5mA at 50kHz is passed between one pair of adjacent electrodes and the potential different between the remaining 13 electrodes are measured. When one pair of electrodes acts as the drive electrodes, one cycle has been performed. One data set consists of 150 cycles. The initial data set is recorded and the subsequent data sets are back projected against the initial set to produce an image of change in resistivity in the plane of electrodes. To follow the gastric emptying process, several pre-prandial images are obtained over short time intervals as the baseline reading.

As the electric impedance tomography measures the change in the gastric impedance, it is expected that the gastric acid secretion and bile acid reflux can alter the electrolyte balance and the ionic strength of the test materials. Gastric acid secretion may prolong the gastric emptying half-life of the conducting meal whilst reducing the gastric emptying half-life of the non-conducting meal. Acid secretion can be inhibited by the ingestion of histamine-2 receptor antagonists such as cimetidine and ranitidine at approximately 1 to 1½ hours prior to the start of the test to improve the reproducibility of the measurement. Recently, APT has been used to evaluate the basal acid output due to its sensitivity to acid secretion (Sarker et al 1997). Ideally, electrodes should be placed over the body of the stomach to reflect the emptying from the whole stomach. However, as the position and the shape of the stomach in humans vary, the location of the electrodes may not be at an optimum position for imaging. In addition, movement of the subject may influence the measurement of the impedance especially with

the epigastric impedance technique. The 16 electrodes that are placed midway between xiphisternum and costal region in the APT measure the changes in resistivity over a band of approximately 8cm wide, which overcome the above problem when epigastric impedance technique is used. Another advantage of the APT over epigastric impedance is that it can measure the gastric emptying of both semi-solid and liquid meals. Epigastric impedance has only been used to measure liquid meals (Mangnall et al 1988). Recent developments in EIT systems have brought about the possibility of a real-time impedance measurement (Smallwood et al 1993). This has opened the potential for monitoring and quantifying gastric motility.

Although there is a good correlation between measurement with electric impedance tomography and other techniques, there is a generally lack of accuracy and resolution (Avill et al 1987). It also does not distinguish between the liquid and the solid meals or specific dosage forms such as tablet or capsule. However, due to its non-invasiveness, quick data analysis, repeatable and cheap properties, it is attractive in clinical diagnosis and research experiment especially with infants, breast-feeding and pregnant women (Nour et al 1995).

1.2.2 Indirect methods

Two indirect methods have been used to estimate the gastric emptying rate. The absorption of the orally administered drugs such as paracetamol depends on the rate of delivery of the drug to the small intestine. Blood or salivary concentration of the drug can indirectly estimate the gastric emptying rate (Clements et al 1978, Heading et al 1973, Yuen et al 1997). The estimation based on pharmacokinetic of the drug is less accurate as many other factors also influence the absorption process.

Recently, a radiolabeled CO_2 breath test has been developed to measure the gastric emptying process. The solid and liquid test meals are incorporated with the C-radiolabeled octanoic acid and acetate as markers, respectively. Rapid breakdown of the test meal occurs in the duodenum releases the marker. This marker is quickly absorbed and transported to the liver where it is preferentially oxidised to CO_2. The rate of the CO_2 excretion in the breath is correlated with the emptying of the meal (Meas et al 1994, Mossi et al 1994). The breath test is based on the assumption that the gastric emptying rate is the rate-limiting step for CO_2

excretion. Good correlation with other standard methods such as gamma scintigraphy and double detector technique has been reported (Cummings et al 1996). The method is non-invasive, reproducible, cheap and without discomfort. No radiation is required, therefore it is repeatable and can be performed on children and pregnant women. Field-testing is feasible as breath samples can be collected away from the analyst centre.

1.3 Quantification of the gastric emptying process

The gastric emptying process can be characterised by both graphical and mathematical approaches. The common description of gastric emptying process is the gastric emptying half time (GE_{50}) which is the time for 50% of the test sample to be emptied from the stomach. This value can be taken from the graphical presentation of the percentage of the test sample remaining in the stomach effectively using only two sampling time points. Hence, the value represents only very crude picture of the whole gastric emptying process. The GE_{50} value can also be difficult to determine when the gastric emptying curve is irregular in shape. Furthermore, the time interval between the measurements affects the smoothness of the gastric emptying curve and is therefore another crucial point for the accuracy in the value derived.

Grimes and Goddard (1977) introduced the 'emptying index' to describe gastric emptying curve. The emptying index can be calculated using the simple mathematical expression as given below:

$$\text{'index'} = (1-f_T)/A$$

where f_T = fraction of the initial volume remaining in the stomach at the last observation time and A = area under the normalised emptying curve. Although the whole gastric emptying profile is taken into consideration, the incorporation of a non-dimensional value of 1.0 always considers complete emptying regardless of the shape of the emptying profiles. Furthermore, if the f_T value changes proportionally with A value, different emptying profiles can have the same index value.

Podczeck et al (1995[b]) described a novel mathematical approach based on the statistic moments to characterise the gastric emptying profile. The gastric mean residence time (GMRT) and its variance (VGRT) can be calculated with the expressions below:

$$GMRT = \frac{\int_0^{tmax} t \,.\, P_t \, dt}{AUC}$$

$$VGRT = \frac{\int_0^{tmax} t^2 \,.\, P_t \, dt}{AUC} - GMRT^2$$

where P_t = % remaining in the stomach at time t and AUC = area under the gastric emptying curve. The GMRT is defined as the value of the centre of gravity of a triangle that is equal in area to the gastric emptying curve. Hence, the whole gastric emptying curve is taken into account.

A good correlation between GMRT and GE_{50} values of gastric emptying profiles of theophylline pellets using gamma scintigraphy has been demonstrated by Podczeck et al (1995[b]). However, the correlation was not 100% as the calculation approaches differ.

1.3.1 Gastric emptying analysed by the Moore and Wallis trend analysis

The gastric emptying curves that show considerable fluctuation, their onset and completion can be difficult to detect. Such measures influence the time at which the AUC should be measured. The calculation of gastric emptying characteristics using the statistical moments analysis, can be improved by incorporating a significance test based on the Moore and Wallis (1943) trend analysis which identifies the filling and emptying phases in the gastric emptying process. The analysis uses sequences in direction of movement and a series of time intervals known as the 'phase' can be constructed based on the changes in the signs. It is a sign test, which requires no assumption about the form of the population unless, the expected frequency distribution of the phase duration is to be calculated.

The advantages are speed, simplicity of application and freedom from dependence upon complex mathematical methods such as least squares. The method is also adaptable to the cases where the sequences are either abnormally long or short. However, the analysis can occasionally lack of the sensitivity to detect the primary trend and this can be resolved by comparing it to other rank correlation techniques for confirmation. The secondary fluctuation within a sequence is more likely to be concealed especially in the case of gradual movement.

1.4 pH throughout the gastrointestinal tract

1.4.1 Acid secretion in the stomach

The stomach secretes acid and components such as intrinsic factor and mucus that form the main constituents in the gastric juice. As early as the 1820s, Beaumont had described the presence of gastric juice and its functions. Acid secretion is generated by the K^+/H^+ APTase pump located within the parietal cells in responses to various chemicals such as gastrin, acetylcholine, histamine, enteroxyntic and circulating peptides. Blockage of the H^+/K^+ APTase pump by chemicals such as omeprazole and lansoprazole are used in clinical treatment of both peptic and duodenum ulceration.

The acid secretion is mainly stimulated during the gastric phase (control mechanism from the stomach) as a result of the stomach distension by the meal. The cephalic phase (higher brain control) accounts for approximately 30% of the acid secretion depending on the nature of the meals and on a non-physiological process known as the sham-feeding. As the meal arrives at the small intestine, small amount of acid secretion is stimulated in the stomach. The intestinal phase (control mechanism from the small intestine) however, is mainly involved in the release of cholescytokinin, which opposes the action of gastrin and results in the inhibition of acid secretion from the stomach (Johnson 1997).

1.4.2 Interluminal pH along the gastrointestinal tract

The pH values of the luminal contents vary along the gastrointestinal tract and range between 1.5 and 7.5 (Meldrum et al 1972, Evans et al 1988). The parietal cells produce acid that maintains a mean pH value of 2 during the fasted state. As the parietal cells locate mainly in

the gastric fundus, the luminal pH is expected to be the lowest in this region. The gastric luminal pH is therefore not one entity and it shows regional (Fisher et al 1997) as well as circadian variations (Stein et al 1994). A 24-hour ambulatory monitoring on gastric body and antral using the healthy volunteers has shown that the pH value in the gastric body increases in response to a meal whereas the pH values in gastric antral rises during the night time due to duodenum reflux (Mclauchlan et al 1989). The luminal contents of the small intestine on the other hand range between 5.5 and 7.5 with mean pH value of 6.6 at the proximal small intestine and the value progressively increases to mean pH of 7.4 at terminal ileum. The steepest pH changes of at least 2 units are observed at the proximal duodenum due to the bicarbonate ion secretion from the pancreas and intestinal mucosa and the buffering effect of the bile salts produced in the liver (Ovesen et al 1986). The luminal pH value drops to 5.5 at the caecum and then increases gradually from the right to the left colon to a final pH of 7.4.

Acid secretion is at its highest level during the first hour of post-prandial state. The foods that enter the stomach act as a buffer to temporarily neutralise the secreted acid and increase the pH from 2.0 to 6.0-6.5. The pH value then gradually returns to fasting pH within 2 hours. In the small intestine, the return to basal pH value is observed only after 4 hours of ingestion. No difference has been found in the pH ranges between gender and no correlation has been established between the gastric and duodenum pH values in both fasted and fed states (Dressman et al 1990). However, age affects the acid secretion process. Ageing has demonstrated to be associated with an increase in the gastric acid secretion especially in man.

The variation of pH along the gastrointestinal tract has profound effect on the stability, absorption and dissolution of the weak acids and bases. The luminal pH changes in the stomach may alter the rate of degradation of the acid labile materials and the bioavailability of enteric coated and slow released formulations (Youngberg et al 1987). According to the pH partition hypothesis, the absorption of weak acids and bases depend on the dissociation constants and pH of the medium (Shore et al 1956). Weak acids and their salts are unionised in the stomach where they can be readily absorbed whereas weak bases and their salts are ionised and can only be absorbed at the small intestine where the pH is more favourable (Schanker et al 1957). The solid contents depend on the luminal pH for its disintegration and dissolution. A liquid content on the other hand may precipitate at certain luminal pH value depending on its nature.

As stomach is not the major organ for absorption, the weak acids and theirs salts despite their ionisation states in the small intestine are mainly taken up due to the large surface area for the absorption process (Hogben et al 1959). The pH of the microclimate of the stomach and intestinal mucosa determine the ionisation ratio and the permeability of the contents through the mucosa. The microclimate pH is regulated by an ion exchange mechanism and is less affected by the luminal pH value.

1.5 Assessment of gastric pH and secretion

1.5.1 Monitoring gastric pH profile by pH sensitive radiotelemetry capsule

Radiotelemetry is a device used to measure *in vivo* the value of pH, temperature, redox potential and pressure by radio transmission (Evans 1993). The pH-sensitive radiotelemetry device consists of a pH sensitive capsule, a radio detector and the chart recorder. The capsule acts as a transducer with electric circuitry that converts the measurement in a radio frequency and transmits the signal to an external radio receiver via an aerial detector system. Two types of electrode are built to the capsule for pH detection. The pH sensitive radiotelemetry capsule consists of a hydrogen sensitive glass electrode and an in-built sodium chloride reference electrode to sense the pH changes (Colson et al 1980). The Heidelburg capsule on the other hand composes of either a poly- or monocrystalline antimony sensor (Noller 1962). Both types are commercially available.

The capsule can be swallowed freely and allowed to pass through the gastrointestinal tract by the force of gravity and peristaltic contractions. The location of the capsule in the gastrointestinal tract can be sensed by a sudden change in pH values as the capsule transits from stomach to the duodenum, from ileum to the colon and then from ilea fossa to the rectal. The capsule can also be tethered singly or in multiples along the whole length of gastrointestinal tract in order to measure pH at a specific location. Practically, the maximum numbers of capsules that can be tethered are three as this is limited by the frequency separation necessary to allow for clear transmission of the signal (Morris et al 1987). The free fall radiotelemetry capsule is more vulnerable to signal loss than the tethered capsule (Branicki 1982). Recently, the pH-sensitive radiotelemetry capsule has also been implanted

surgically into the stomach wall for a long term pH monitoring. Most of the work has been performed on animals and the only human-based experiment is by incorporating with an endoscopically controlled stapling device that implants the capsule at gastric body (Swain et al 1992). The implant stays intact within the stomach for three months without causing complications.

Radiotelemetry capsule has several clinical and research applications. For example, the tethered radiotelemetry capsule has been used to measure the lower oesophagus sphincter competent in hiatus hernia patient. It is also used as an indicator in the diagnosis for the gastro-oesophagus reflux diseases by a 24 hours ambulatory oesophagus pH monitoring (DeMeester et al 1976, Fehr et al 1966, Vitale et al 1984). The gastric and duodenum pH values can be monitored using either a free fall or a tethered radiotelemetry capsule (Watson et al 1965, Wiliamson et al 1969). The circadian pH pattern has also been measured for information in relationship to acid suppression by the acid blocking drugs (Patel et al 1992, Reynolds et al 1987). Transit time of ingested materials across the gastrointestinal tract can be monitored with a free fall radiotelemetry capsule in a non-invasive manner as compared to the gamma-scintigraphy (Coupe 1991, Hardy et al 1991, Mojaverian et al 1989). Pathological features related to abnormality in gastrointestinal pH such as cystic fibrosis, after gastrointestinal surgery and pouchitis can also be diagnosed (Chattopadhay et al 1990, Gilbert et al 1988, Watson et al 1966). Its pharmaceutical usage includes the evaluation the novel drug targeting such as the delivery of enteric coated and slow released preparations (Hardy et al 1987, Alioth et al 1993).

The pH-sensitive radiotelemetry capsule is more accurate when compared to the conventional methods. It retains contact with gastric juice for a long period of time and consists of a large surface area for pH sensing. The capsule is also less likely to be contaminated and affected by the presence of food. Tethered radiotelemetry capsule can be placed at any site along the gastrointestinal tract without X-ray screening. Reynolds et al (1986) has shown that the ambulatory pH monitoring using the nasogastric tube and the radiotelemetry are comparable.

In summary, the radiotelemetry capsule is non-invasive yet durable for measuring pH along the gastrointestinal tract of the human.

1.6 Background on 4-Aminosalicylic acid

After first being synthesised in the German laboratory in 1889, 4-aminosalicylic acid (4ASA), also known as para-aminosalicylic acid came into notice as a safe anti-tuberculotic agent given at a relatively high dose, in the late 1940s. The emergence of the resistant strain of *Mycobacterium tuberculosis* and the gastrointestinal intolerance to 4ASA together with the introduction of ethambutol in 1960s reduced the demand of 4ASA. The removal from the market came at the same time as a rise in frequency of the multi-drug-resistant strain *M. tuberculosis,* which frequently necessitates the use of 4ASA in the cocktail therapy. In the late 1970s, scientists discovered 5-aminosalicylic acid (5ASA) to be the active moiety of sulfasalazine, which is used in the treatment of inflammatory bowel disease (Van Hees et al 1980). 4-Aminosalicylic acid, which is an isomer of 5ASA, has been tested to possess a comparable anti-inflammatory action. This discovery has lead to re-establish the potential medicinal usage of 4ASA.

Since the 1980s, a series of clinical studies have been organised throughout the world to investigate the potential of this new candidate for the treatment of inflammatory bowel disease (IBD). Gandolfo et al (1987), Ginberg et al (1992) and Selby et al (1984) compared the effect of 4ASA to placebo either in acute or short-term maintenance therapy up to 12 weeks in the ulcerative colitis patients, significant improvements in clinical, histological and sigmoidoscopic variables were concluded. Schreiber et al (1994) compared 4ASA with 5ASA in the patients in remission from either ulcerative colitis or Crohn's disease, the results reported were similar to those published by Prantera et al (1992) using a different formulation of 5ASA. Campieri et al (1984) and Nagy et al (1989) compared the effect of 4ASA with either sulphasalazine or 5ASA in an acute episode of ulcerative colitis with enemas whilst a proportion of the patients in the Campieri study were also on maintenance therapy with salphasalazine throughout the trial. Whether the drugs were given for treatment of an acute episode or for maintaining patients, the results were the same among the different drug treatments; they were equally efficacious. Marteau et al (1995) compared 4ASA with 5ASA in the moderately active ulcerative colitis and also reported equivalent efficacy of both the drugs. In addition, the gastrointestinal tolerance was claimed to be much better in the 4ASA treatment group. A study by O'Donnell et al (1992) attempted to establish the efficacy of 4ASA relative to the conventional therapy using topical steroids i.e. hydrocortisone and

prednisolone, respectively. The results revealed no significant differences between the activities of the two treatment groups. The 4ASA also approached a statistical significant with regards to the physician's overall evaluation. Beeken et al (1997) examined the oral uncoated 4ASA tablet in mild to moderate ulcerative colitis. A 6 grams daily dose was found to be more effective in patients with more than 60cm inflammation from the distal anus but not in those whose inflammations was less than 60cm. As uncoated tablets released early in the upper gastrointestinal tract, the brief contact with the distal inflammation loci only came at defecation.

In vitro stability studies on 4ASA and 5ASA preparations have shown that despite their structural similarity, the two compounds undergo different degradation pathways. 5ASA is preferentially oxidised to give a series of related compounds of 5ASA-quinoneimine. The oxidation process is greatly increased at high pH values and in the presence of Fe^{3+}, which acts as the catalysing agent under hydrogen peroxide experimental condition (Palsmeier et al 1992). Under low pH condition, the decarboxylation process dominates but the product of decomposition is the minimum. In contrast, 4ASA is not oxidised but degrades into m-aminophenol at low pH values and the rate of degradation is maximum at the isoelectric point of 4ASA i.e. pH 2.7. At pH values above 6, the decarboxylation process is negligible (Rekker et al 1956, Jivani et al 1985). The m-aminophenol is a known hepatotoxin and the administration of uncoated 4ASA preparation orally may result in its formation in the stomach acidic environment. An enteric-coated formulation can be used to protect against this degradation process.

The schematic presentation of the *in vivo* metabolism pathways of 4ASA is shown in figure 1.1. In plasma, N-acetyl-4ASA and para-aminosalicyuric acid, which is a glycine conjugated 4ASA, constitute 90% of the metabolites of 4ASA. Of the 90% metabolites composition, approximately 75% and 25% are N-acetyl-4ASA and para-aminosalicyuric acid, respectively (Wan et al 1974). The conversion of 4ASA into para-aminosalicyuric acid follows a first order kinetic and is independent of the dose (Lauener et al 1957). The metabolism by acetylation however, occurs pre-systematically at the intestinal mucosa and in the liver systematically (Hassan et al 1981). A study using 5ASA enema has shown that the pre-systemic metabolism by bacteria flora in the faeces is minimal but acetylation is rapid and

completed at the mucosal site (Allgayer et al 1989). The rate of acetylation is pH dependent with the maximum rate at pH7.

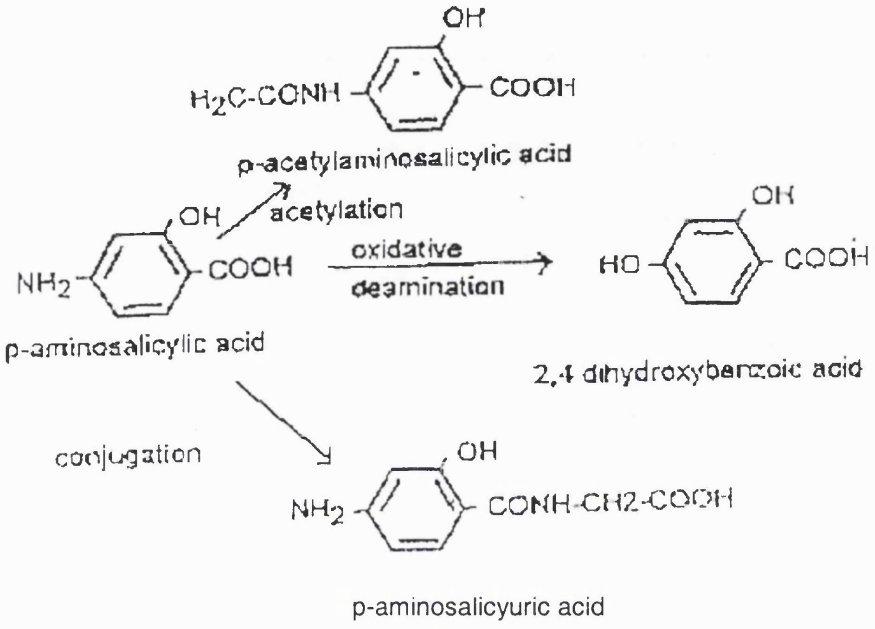


Figure 1.1: The schematic presentation of the 4ASA metabolism pathways *in vivo*

The effect of genetic acetylator phenotype on the rate of acetylation by N-acetyl-transferase is conflicting. The acetylation rates were not different between the volunteers and IBD patients and no correlation between N-acetyl-transferase and acetylator phenotype status had been demonstrated (Allgayer et al 1989). Fischer et al (1979) nevertheless has shown a strong correlation of the acetylator phenotype in the patients with IBD after the oral treatments with salicylazosulfapyridine and sulfapyridine. The slow acetylator phenotype results in a high drug concentration, increases the tendency to cause toxicity.

N-acetyl-4ASA is an inactive metabolite that accumulates in the body with a long elimination half-life. The kinetics of N-acetyl-4ASA formation in either the oral or intraperitoneal route has been found to be absorption rate limited i.e. the formation of the metabolite is dependent on the absorption of the parent drug (Houston et al 1982). By administration of the radioactive labelled d3-acetyl-5ASA as rectal suppository, no d0-acetyl-5ASA was detected, confirming that the deacetylation process is minimum (Meese et al 1984). Pieniaszek et al (1979) explored the possible dose-dependent characteristics of the 5ASA metabolism by

acetylation using rat. Although the urinary recovery of total 5ASA was independent of the dose and the administration routes, it was subjected to capacity limited pre-systemic and systemic acetylation. As there is no studies on the dose dependent acetylation of the 4ASA, this report serves as an indication of the 4ASA behaviour *in vivo*.

Rasmussen et al (1982) confirmed that the elimination of N-acetyl-5ASA was mainly by tubular secretion rather than the glomerular filtration as the highly protein bound N-acetyl-5ASA was not filtered through the glomerulus and the compound clearance was much higher than the creatinine clearance. Similarly, the 4ASA can be excreted as an unchanged drug and the polar metabolites by glomerular filtration and tubular secretion, respectively. In urine, glucuronic acid conjugated 4ASA has also been found.

Absorption of 4ASA is rapid from the gastrointestinal tract. Way et al (1948) demonstrated that in rat the highest level was attained in kidney, then the lung and the liver after an intravenous dose. The level fell quickly and within four hours practically, no 4ASA was detected in any tissues except for the gastrointestinal tract due to re-secretion back into the gastrointestinal tract to a small extent. The drug and its acetylated metabolites are protein bound to approximately 60 and 83% respectively, presumably to the albumin.

The disposition of 4ASA is dosage form dependent. Different formulations of 4ASA show a considerable variation in the pharmacokinetic behaviours. Wan et al (1973, 1974) formulated the 4ASA and its salts into a solution, a suspension and the uncoated tablets to establish the pharmacokinetic behaviour of 4ASA and its major metabolite, N-acetyl-4ASA in humans. The studies concluded that the absorption of the drug was rapid and complete in all the formulations. The absorption rate of the acid was the slowest whereas its sodium salt was the fastest, due to the poor dissolution of the acid in the aqueous environment of the gastrointestinal tract. A much lower concentrations of the N-acetyl-4ASA in the urine for the salt forms were also reported when compared to the acid, suggesting that the conversion into the inactive N-acetyl-4ASA was capacity limited with a 4 grams dose. The peak concentration of 4ASA was attained within one hour after the oral ingestion, indicating that the absorption occurred in the upper gastrointestinal tract, which involved the stomach and small intestine. In a study using a 4 grams enteric-coated granules preparation, the peak concentration of 4ASA occurred only after 4 hours of the drug ingestion (Peloquin et al

1994). However, once the drug reached the systemic circulation, it followed the kinetic pattern of the uncoated preparation as mentioned above. However, the C_{max} value was found to be much smaller for the equivalent dose.

1.7 Maximum entropy theory for pharmacokinetic analysis

Quantified maximum entropy is a deconvolution technique, which utilises the Bayesian theorem and maximum entropy as the framework to perform pharmacokinetic analysis. The input rate; f(t), which describes the absorption rate model is derived as a smooth continuous function that requires only the assumption of the magnitude of the data errors from the experiment. It is constructed from two hypothetical functions, the Intrinsic Correlation Function (ICF) and h(x) function, which are without any physiological significance, according to the equation below:

$$f(t) = \int_0^\infty c(t,x)\, h(x)\, dx \text{ , where } c(x,t) \text{ is the ICF function}$$

The ICF is a simple diffusion model which ensures the smoothness of the f(t) by introducing the correlations between adjacent portions of the input rate. Its width is specified by parameter, *w*. The h(x) function is a positive and additive distribution that describes absorption kinetics and needs not to be smooth.

The raw data concentration; c(t), is related to the absorption and disposition models by the equation below:

$$c(t) = R(t) * f(t)$$

The impulse response function, R(t), is used to calculate the disposition kinetic model, which in turn is assumed to be linear and time invariant. The approach considers a distribution of peripheral volumes as a function of the return rate constant of the drug from these volumes. Theoretically, the number of these volumes is unlimited but practically, it is restricted to 90 different peripheral volumes for the reasons of computing. As these peripheral volumes are the 'equivalent' to the peripheral compartments in the classical pharmacokinetic analysis, the

unphysiologic restriction of the body fluids to be only two or three of the similar nature as those in the one or two compartment model, has been removed. A return rate constant, which describes the rate of return of the drug from the associated peripheral volume can be reconstructed whenever possible. Therefore, the disposition model can be considered as an extension of the standard compartmental system, in which the drug may distribute into a range of different type of tissues, with a continuous spectrum of different characteristics. The detail of the mathematical principles were described by Charter et al (1991, 1987) and Podczeck et al (1995[a]).

The calculation can be carried out using the MADAME software package (version 2.01, 1993, Maximum Entropy Data Consultants Ltd., Cambridge). The method and program are mathematically complex. However, there are some advantages. No interpretation of the numbers of fixed compartments, which is non-physiological, is required. The disposition model can be described in the absence of any absorption and this allows the use of both intravenous bolus injection and oral preparation as the weighting function, provided the length of the terminal phase is sufficient. The program is also able to calculate one or more data set simultaneously provided the ICF values used for smoothing the input rate are not too different. Several runs of the program are usually required to optimise the width of the ICF functions, before the most probable absorption rate function can be found. This is a time-consuming process as the Lagrange function used to solve the mathematical problem is complex, and it is not always possible to find a solution. However, no assumption about the absorption rate function in advance apart from the error input from the experiment is required, hence, the solution is model free and smoothes without curve fitting. Furthermore, it is free from spurious oscillation yet still showing small-scale structure when there is evidence for it in the data set. The program does not require equal distant time intervals between the concentration measurements and the absorption rate model can be described as cumulative rate profile as well as a distribution function.

The Bayesian probability theory also allows the consistent and rational treatment of uncertainties in the original data, arising from the measurement errors. The consequence is that the uncertainties in the derived quantities are available as part of the analysis which differs from other pharmacokinetic practice, where the only error estimates available are based on intra and inter-subject variability.

1.8 Study aims and objectives

Applied potential tomography will be used to establish the effect of administering a non-nutrient buffer liquid on the gastric emptying process. A series of *in vitro* tests will be performed to evaluate the suitability of this liquid for the APT monitoring. Then, the administered fluid volume and pH of the buffer liquids on gastric emptying rate will be investigated in the human subjects in the physiological fasted state. This will serve a control study to provide a comparison to the second study that uses a model drug.

The second study aims to investigate the oral absorption of a model weak acid, 4-amonisalicylic acid, which is formulated into a suspension of buffer pH3 value and a solution of buffer pH7 value at physiological fasted state and an elevated gastric luminal environment using ranitidine treatment in humans. The fate of these fluids in the stomach will be monitored simultaneously after ingestion, with a pH-sensitive radiotelemetry capsule and the applied potential tomography. Blood will be sampled at fix time intervals and analysed to assess the concentration of the drug and its metabolite. The pharmacokinetic parameters will be calculated with the Maximum Entropy approach, a deconvolution technique and by the Wagner-Nelson method. It is hope to be able to correlate the gastric emptying rate, the gastric luminal pH with the pharmacokinetic characterisations of 4-aminosalicylic acid and its metabolite.

Chapter 2

Formulation of 4-Aminosalicylic Acid Liquid Dosage Forms

2.1 Introduction

The model drug, 4-aminosalicylic acid (4ASA) which has been chosen, is formulated into a suspension and a solution at specific pH values. A fairly low concentration of 2mg/ml 4ASA suspension may be formulated at the pH values between 1.9 and 3.5, as the lowest solubility of 4ASA is at its isoelectric point that is pH 2.7 (Forbe et al 1995). The pH3 value has been chosen for formulating the suspension to avoid possible mucosa injury using lower pH value. The solution is formulated with the sodium salt of 4ASA (Na4ASA) as the salt has a high solubility in water (1 in 2 parts of water).

To obtain a satisfactory imaging using the electrical impedance tomography (EIT) system, the formulations need to be conducting. According to Holder (1993) a conductivity of 6.5mS/cm, which is equivalent to 0.9% sodium chloride (NaCl) solution, is the standard reference for good quality imaging. A buffer system is incorporated to achieve this goal and to maintain the pH values of the formulations. The McIlvaine buffer system serves the purpose here as it has a buffer pH range between 1 and 8 and is safe to be ingested (Shah et al 1989).

Although the formulations are designed for oral route of administration, a bulk volume consisting of very low drug concentration will be consumed. It is therefore essential to maintain the isotonicity to the body fluid if not a hypo-osmolar state is preferred, as the gastric emptying process will be less affected. When the body is in a hyper-osmolar state, a large volume of water is drawn into the gastrointestinal tract, which may cause symptoms such as distention, cramps, nausea, vomit and even in severe cases as shock. Since the serum has an iso-osmolar value of 300mOsmo/kg, the formulations are adjusted with addition of tonicity modifier such as sodium chloride (NaCl) to this value (Siegel 1990).

As discussed above, factors such as the ionic strength, buffer capacity, osmolarity, pH and solubility profile are crucial keys to successful formulations of the appropriate criteria. Minor alteration in the formulations may lead to changes in the dissolution profiles and the ionisation states of the drug (Iga et al 1996). A theoretical approach using the acid-base theory, as described below, provides a basic prediction for formulating a stabilised multi-component system (Perrin et al 1993).

2.2 Theory for calculating multi-component system

The fundamental parameter for the mathematical calculation is the pKa values of each component, which are listed in table 2.1.

Table 2.1: The pKa values of the components for computer program. Sources: Citric acid and phosphoric acid from Smith et al 1989, 4-aminisalicylic acid from Moffat 1986

Citric acid	thermodynamic pKa values at 25°C
pK1	3.128
pK2	4.761
pK3	6.396
Phosphoric acid	
pK1	2.148
pK2	7.198
pK3	12.325
4-aminosalicylic acid	
pK1	1.8
pK2	3.6

As the system is in a thermodynamic equilibrium, the true dissociation or the concentration of each component can be calculated with an additional term, the activity coefficient constant. In a diluted system, the activity terms of each parameter can be calculated by a limited Debye-Huckle equation as below:

$$\log(g_i) = -A*z*z*I^{1/2} / (1+B*I^{1/2}) \qquad (1)$$

where A and B are the activity coefficients and are equal to 0.509 and 1.5, respectively, z is the charge of the dissociated ions, I is the ionic strength of the system and g is the activity terms.

The activity terms vary with the temperature and here we assume the temperature of the system to be 25°C. The classical dissociation constant, K, of each component can be calculated as below:

$$K_1 = 10^{-pK1} / g1 * g1 \qquad (2)$$

$$K_2=10^{-pK2}/g2 \quad (3)$$

$$K_3=10^{-pK3}/g1*g3 \quad (4)$$

As the components in the system are made of the weak acids and their salts, the concentration of the dissociated and neutral components can be calculated using the Henderson-Hasselbalch equation and the thermodynamic pKa values. The equations for determining the concentration of each component are shown below with the assumptions based on the ionic equilibrium, electric neutrality and mass balance.

On ionic equilibrium:

For citric acid:

$$[H_2Cit^-]\,[H^+]/[H_3Cit] = 10^{-pK1} \quad (5)$$

$$[HCit^{2-}]\,[H^+]/[H_2Cit^-] = 10^{-pK2} \quad (6)$$

$$[Cit^{3-}]\,[H^+]/[HCit^{2-}] = 10^{-pK3} \quad (7)$$

For phosphoric acid:

$$[H_2PO_4^-]\,[H^+]/[H_3PO_4] = 10^{-pK1} \quad (8)$$

$$[HPO_4^{2-}]\,[H^+]/[H_2PO_4^-] = 10^{-pK2} \quad (9)$$

$$[PO_4^{3-}]\,[H^+]/[HPO_4^{2-}] = 10^{-pK3} \quad (10)$$

For 4ASA:

$$[^-OOC\text{-}DOH\text{-}NH_3^+]\,[H^+]/[HOOC\text{-}DOH\text{-}NH_3^+]=10^{-pK1} \quad (11)$$

$$[^-OOC\text{-}DOH\text{-}NH_2]\,[H^+]/[^-OOC\text{-}DOH\text{-}NH_3^+]=10^{-pK2} \quad (12)$$

On mass balance:

$$C_{Na2HPO4} = [H_3PO_4]+[H_2PO_4^-]+[HPO_4^{2-}]+[PO_4^{3-}] \quad (13)$$

$$C_{H3Cit} = [H_3Cit]+[H_2Cit^-]+[HCit^{2-}]+[Cit^{3-}] \quad (14)$$

$$C_{4ASA} = [^-OOC\text{-}DOH\text{-}NH_2]+[HOOC\text{-}DOH\text{-}NH_3^+]+ [^-OOC\text{-}DOH\text{-}NH_3^+]+[HOOC\text{-}DOH\text{-}NH_2] \quad (15)$$

On electric neutrality:

$$[Na^+]+[H^+]+[HOOC\text{-}DOH\text{-}NH_3^+] = [H_2Cit^-]+2[HCit^{2-}]+3[Cit^{3-}] +[H_2PO_4^-]+2[HPO_4^{2-}]+3[PO_4^{3-}] +[^-OOC\text{-}DOH\text{-}NH_2]+[OH^-] \quad (16)$$

As water dissociates into hydrogen ions and hydroxyl ions, the concentration of these ions can be calculated by the following equations:

$$[H^+] = 10^{-pH}/g_1 \quad (17)$$

$$[OH^-] = 10^{-pKw}/(g_1*10^{-pH}) \quad (18)$$

After deriving the concentration of each component in the system, the ionic strength of each component can be calculated using equation (1). The amount of the tonicity adjuster can be determined by subtracting the initially fixed ionic strength of the system from the ionic strength of each individual component. The isotonicity of the system can also be estimated by summing the mass balance equations. Hence, the formulations can be estimated provided that the system is kept to a total charge balance of zero. Practically, this is not possible to achieve, however, the estimated charged balance of below 10^{-5} gives a good approximation to electric neutrality.

2.2.1 Computer program for predicting the liquid formulations

There have been many reports on the methods and computer programs to calculate the pH and capacity of the buffer system (Cutler 1986, Kipp et al 1995, Ventura et al 1980). Okatomo et al (1997) reported a computer Window version to calculate the pH values of the multi-component system with prediction of other factors such as ionic strength and temperature. Here, a similar system, which runs on the Window Excel workspace, based on equations (1)-(18), using the McIlvaine buffer and 4ASA, was written. The osmolarity values of the system were also estimated.

To start the computation, the desired 4ASA concentration, pH and the ionic strength were entered. The concentrations of the buffer components were then entered and adjusted manually to fit the desired criteria of the system.

Two examples of the calculated formulations (formulations 1 and 2) were shown in **Appendix 1** and by altering the pH values in these hypothetical formulations, the changes in other parameters, which were crucial for the formulations, can be observed.

Figures 2.1-2.4 illustrate the effect of varying the pH values on the overall balance of the formulation system. The results served as a mean for estimating the effect of the stomach acid secretion on the system. As the formulation 1 (**Appendix 1**) possesses a higher starting ionic strength, the overall charge balance and the resultant ionic strength due to the pH variation are less affected (figures 2.1 & 2.2). When the concentration of each individual species of the 4ASA at different pH values was estimated, the concentration of the neutral species was most abundant at approximately pH3, which coincided with the occurrence of the lowest solubility of the 4ASA, and was therefore chosen for formulating the suspension (figure 2.3).

The calculated osmotic values of the system altered when the pH value was changed. By lowering the pH value in the system, the osmotic pressure increased. The reverse was observed by elevating the pH value (Figure 2.4).

Figure 2.1: The effect of changing the pH values on the overall ionic strength of the formulations using the theoretical approach

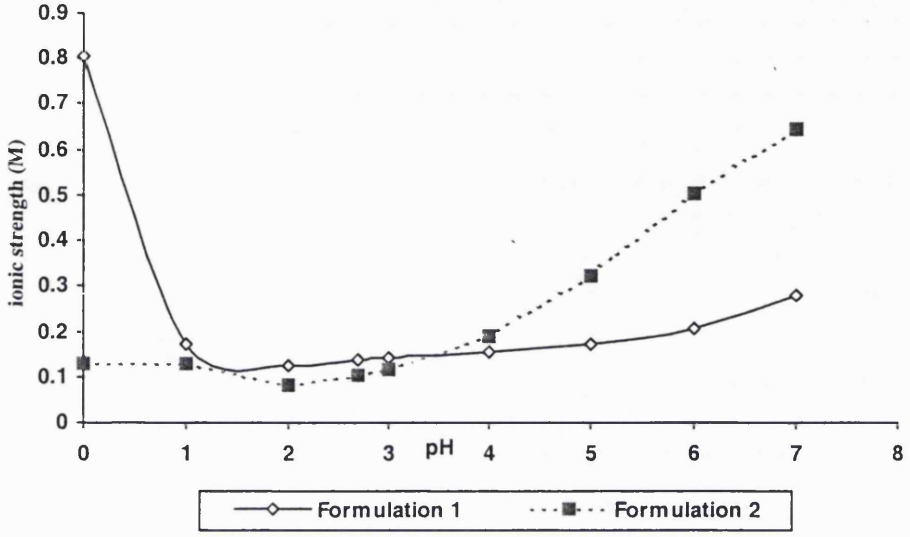


Figure 2.2: The effect of changing the pH values on the overall charge balance of the formulations using the theoretical approach

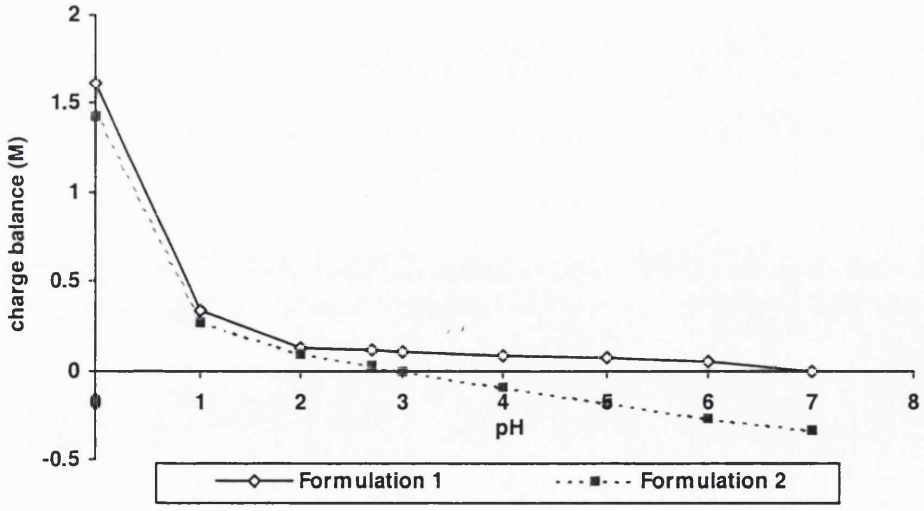


Figure 2.3: The effect of changing the pH values on the concentration of the drug species of the formulations using the theoretical approach

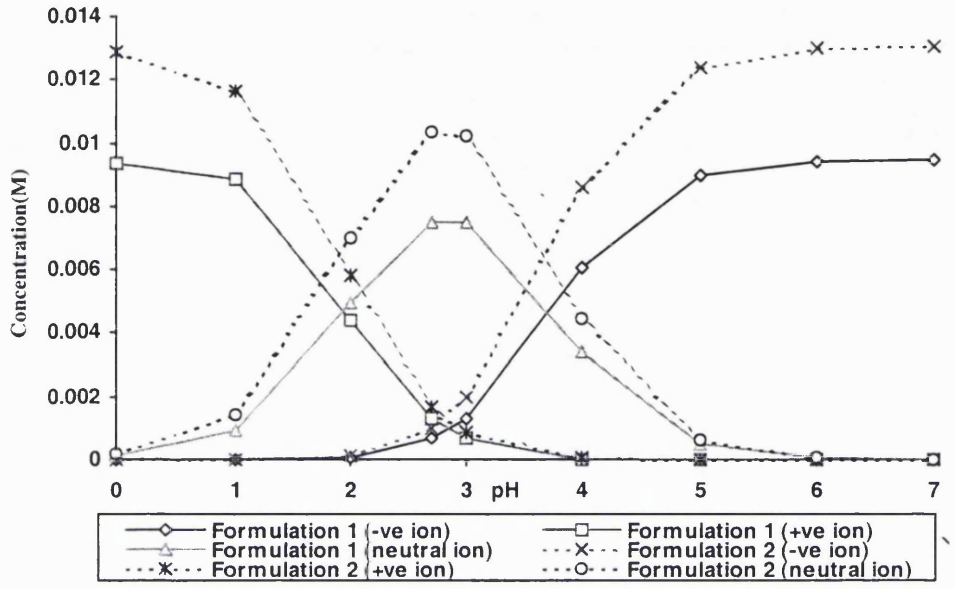
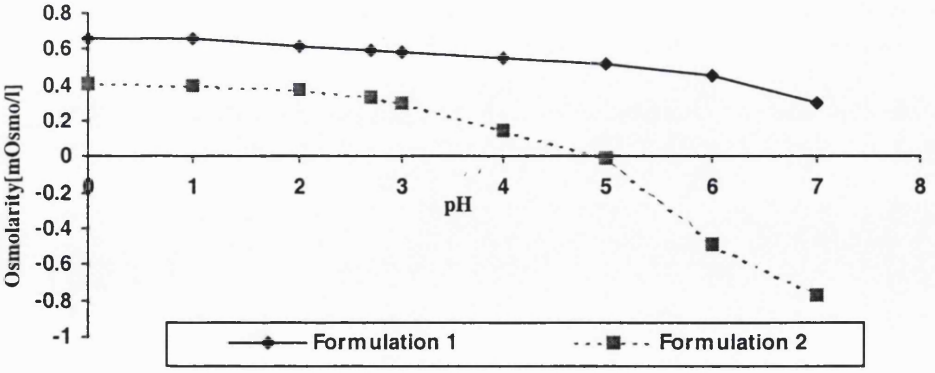


Figure 2.4: The effect of changing the pH values on the osmolarity of the formulations using the theoretical approach



2.3 Evaluation of the raw materials

2.3.1 Particle characterisations

Materials: 4ASA (Jacobus Pharmaceutical Inc, USA), Na4ASA (Norgine Pharmaceutical Inc, Uxbridge, UK).

Methods:

1. Light microscopy:
Dry powder materials were placed on the slides and visual at the magnifications of 100, 200 and 400x by Olympus BX50F light microscope (Olympus Optical Co, LtD, Japan).

2. Laser light diffraction:
The Malvern Instruments series 2600c Droplet and particle sizer (Malvern, England) was used. From the literature, the solubility of 4ASA in water is 1 in 500 ratio. The 4ASA was therefore suspended in small amount of water and measured using a filtered saturated 4ASA solution as background. The Na4ASA was suspended in chloroform and measured using chloroform as background. Repeat measurements were performed over a 20 minutes interval to ensure no changes in the particle size due to stirring and dilution.

Results:

Under light microscope, the appearance of the 4ASA powders was rod shape crystals. Repeat measurements using the laser light diffraction method gave a mean particle size of 25μm (plates 2.1.1-2.1.2 and figure 2.5).

The appearance of Na4ASA was flat with irregular shape crystals. Repeat measured using the laser light diffraction method gave a mean particle size of 27μm (plates 2.1.3- 2.1.4 and figure 2.6).

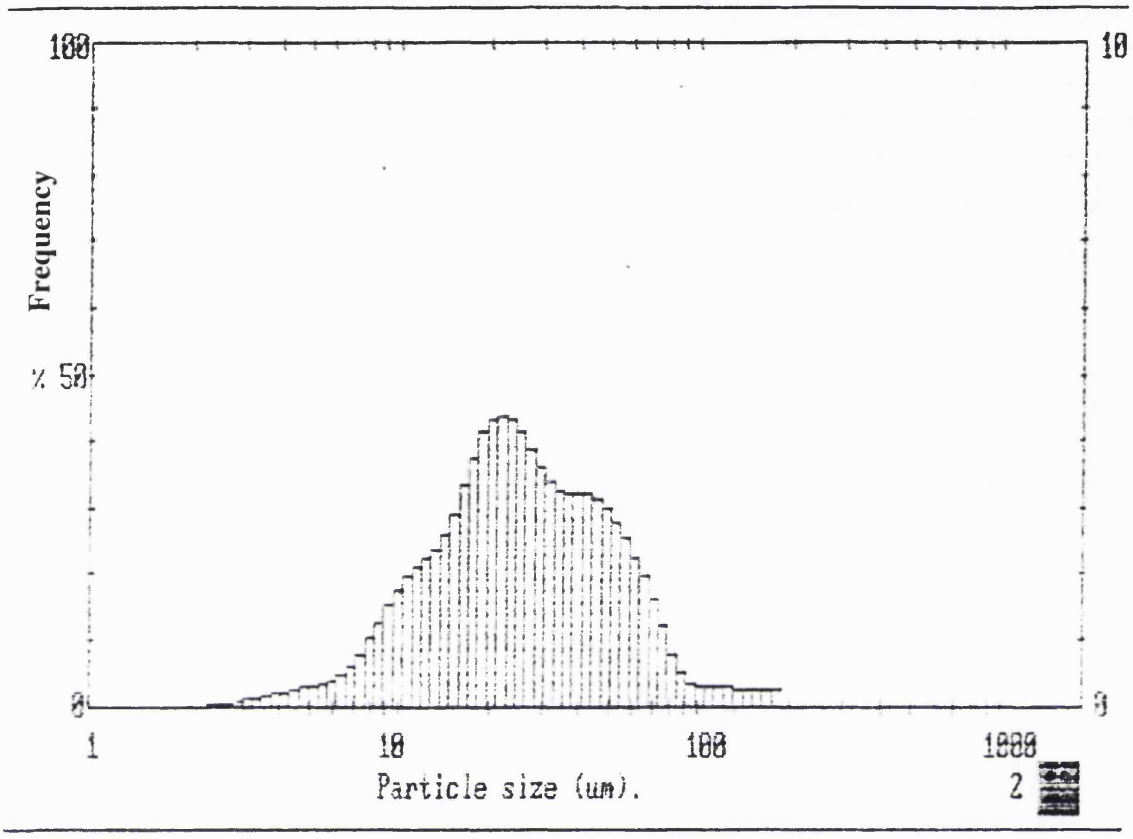


Figure 2.5: The particle size of 4ASA measured using the Malvern particle Sizer

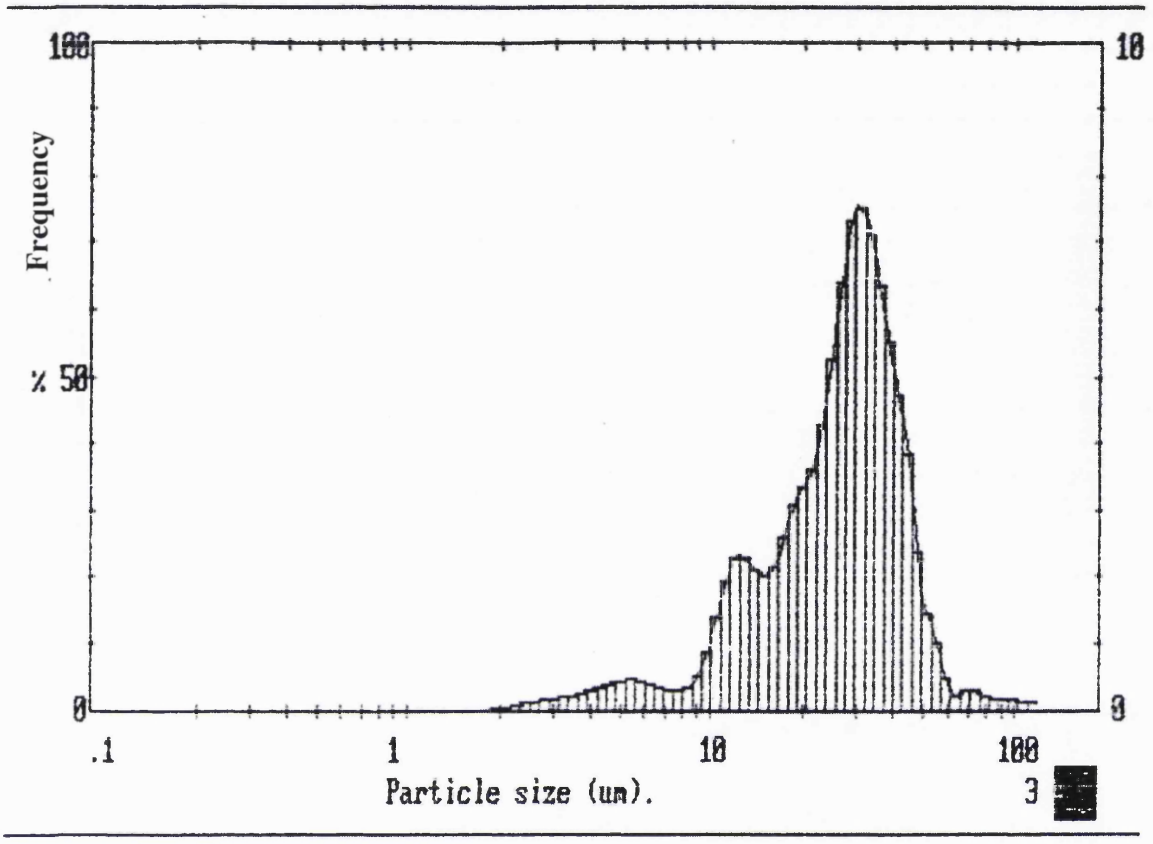


Figure 2.6: The particle size of Na4ASA measured using the Malvern particle Sizer

Plate 2.1.1

Plate 2.1.2

Plates 2.1.1-2.1.2: The particle appearances of 4ASA under light microscope. Magnification: 200x

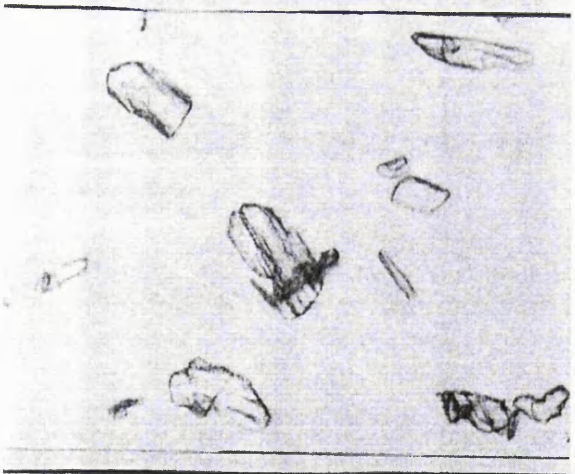


Plate 2.1.3

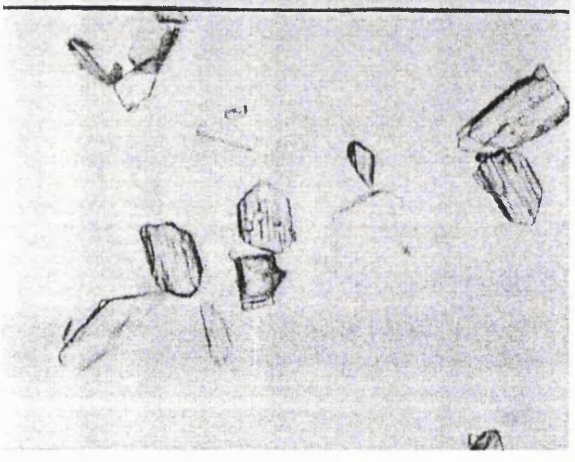


Plate 2.1.4

Plates 2.1.3-2.1.4: The particle appearances of Na4ASA under light microscope. Magnification: 100x

2.3.2 Solubility test

Materials: 4ASA (Jacobus Pharmaceutical Inc, USA), sodium hydroxide and hydrochloric acid (BDH, UK)

Method:

A 500mg of 4ASA was dissolved into a 10ml of water and adjusted to a desired pH value by titration with either a 1M sodium hydroxide or a 5M hydrochloride solutions. Ten duplicate samples of different pH values were prepared. The samples were agitated for 2 hours in 20°C water bath. They were then centrifuged for 20 minutes at high speed (3000rpm), and the supernatant collected from each sample was filtered through a 0.45μm filter. The pH values of the filtrates were measured. A small sample was diluted with a mixed phosphate buffer pH 6.8 BP and the concentration of the diluted sample was measured with the Perkin Elmer UV spectroscopy model 554 at 300nm. The final concentrations were determined from the calibration curve in **Appendix 2.**

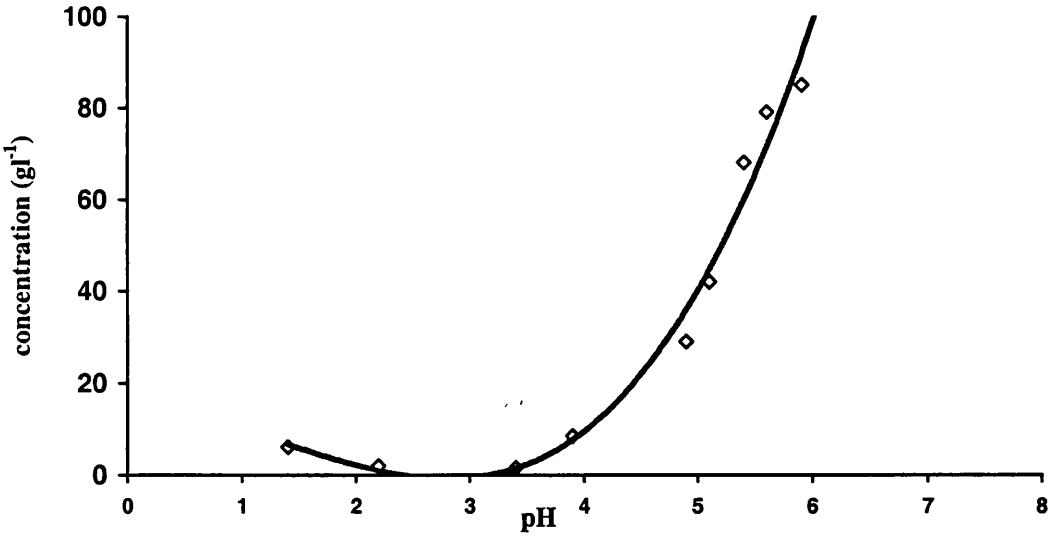


Figure 2.7: The solubility profile of 4ASA

The solubility of the 4ASA was the lowest at the pH values around 3 (figure 2.7), which was close to 1.25mgml^{-1}, and was similar to the literature value of 1.66mgml^{-1} (Moffat 1986).

2.4 Experimental evaluation of the properties of the 4ASA liquid formulations

The operating system Microsoft Windows (version 3.1, Microsoft Inc.) and Microsoft Windows Excel (version 5.0, Microsoft Inc.) were installed in a personal computer for calculating the formulations. The empirical formulations were derived with the properties and compositions in table 2.2.

Ingredients & Formulations	1	2	3	4	5	6	7	8
0.5M Citric acid (ml)	2.72	3.20	1.70	5.48	3.00	8.75	41.70	58.50
0.5M Sodium hydrogen orthophosphate (ml)	20.00	23.45	12.37	45.15	21.85	82.00	20.00	27.65
1M Sodium chloride (ml)	5.2	0.0	0.0	0.0	0.0	0.0	16.5	0.0
Sodium 4-aminosalicylate (g)	0.5	0.5	4.0	0.5	1.0	0.5	0.0	0.0
4-aminosalicylic acid (g)	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5
7.5%w/v HPMC (ml)	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3
Water to (ml)	250	250	250	250	250	250	250	250
ionic strength (M)	0.15	0.15	0.15	0.28	0.15	0.50	0.15	0.115
Estimated osmolarity (mOsmo/l)	186	166	229	300	175	529	350	297
The pH value	7	7	7	7	7	7	3	3
Presence of the precipitation process	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve

+ve = precipitation observed -ve = no precipitation observed

Table 2.2: The compositions and properties of the buffer 4ASA liquid formulations

The chemicals, Analar grade, used were citric acid monohydrate, sodium hydrogen othophasphate and sodium chloride purchased from BDH Chemical Industries (Poole, UK), the drug component, Na4ASA (Norgine Pharmaceutical Inc, Uxbridge, UK) and 4ASA (Jacobus Pharmaceutical Inc, USA).

Hydroxypropylmethylcellulose (HPMC), which has no ionic charge and remains stable over a pH range from 2 to 11, is chosen as the suspending agent for the 4ASA formulations. The commercial product, Methocel E15-LV Premium EP (Colorcon Ltd, Dartford, UK), which dissolves in water in all proportions and exhibits fairly low viscosity but high clarity, is selected (Dow Chemical Company 1993). Having minimal taste, odour and is not metabolised, it is useful as an additive for oral preparation where calorific value needs is minimum. The final concentrations of the Methocel E15-LV Premium EP between 0.45 and 1%w/w in a formulation is safe for human consumption. An excessive oral consumption can have a laxative effect (Wade et al 1994). The suspending agent was formulated into a 7.5%

w/v liquid preparation to ease pouring from a container. A 1.0% w/v Methocel E15-LV Premium EP in the suspension pH3 4ASA formulation 8, sediment was observed after 30 minutes on standing and could be resuspended with 10 turnings.

The pH values of all the formulations were measured with a pH-meter connected to a Gelplas combination electrode (BDH, Poole, UK) at 25°C. Two commercially available reference standards of pH values 1 and 7 were used to calibrate the pH probe before any measurements were performed. The buffer effects of the formulations were measured by titration with either hydrochloric acid (HCl) or sodium hydroxide (NaOH) under constant stirring condition using a magnetic stirrer.

The changes in the solubility of the drug component within the formulation were monitored as the changes in both the drug concentration and particle appearances. A 1ml sample was removed from the formulations at unit interval changes of the pH value and was divided into two parts. One part was filtered through the 0.45μm filter and the collected filtrate sample was diluted into a 50ml mixed phosphate buffer pH 6.8 BP and measured with the Perkin Elmer UV spectroscopy at 300nm. A drop of the sample was drawn from the second portion onto a microscope slide and the changes of particle appearances were monitored using a light microscopy.

2.4.1 Buffer capacity study

As the McIlvaine buffer is incorporated into the formulations to maintain the pH values, the buffer effect can be described in terms of the buffer capacity, which can either be calculated mathematically based on existing theory or directly by acid base titration (Van Slyke 1922). Although intuitive reasoning may lead to simplifications, it may lead to inaccurate results. Therefore, measurement using a direct stepwise acid base titration, which is quick and simple, was chosen.

Buffer capacity (β) for the formulations can be calculated with the expression below:

$$\beta = dB/dpH$$

where **dB** is the increment of strong acid or base added to the buffer and **dpH** is the resultant increment in the pH.

Figure 2.8 shows that the stronger the ionic strength in the pH7 Na4ASA formulations, the greater is the buffer capacity as demonstrated by formulations 4 and 6. This is expected as the concentrations of the buffer species increase, more HCl or NaOH will require to shift the equilibrium maintained by these buffer species. Although the pH3 4ASA formulations 7 and 8 were formulated with a lower ionic strength, a strong buffer resistance was observed when compared to pH7 Na4ASA formulations. The drug component, 4ASA, which is also a weak acid, improves the buffering capacity in the multi-component system especially when the pH value of formulations is close to the pKa values of 4ASA (table 2.1). This effect was much smaller as observed in the pH7 Na4ASA formulations 3 and 5. The buffer capacity of pH7 Na4ASA formulation 3 was poor, despite the incorporation of a high concentration of Na4ASA, as the concentration of the buffer components were low (table 2.2). When the ionic strength of the formulations was kept constant, the buffer value was the greatest at the minimum concentration of the tonicity modifier, as illustrated by the pH7 Na4ASA formulations 1 and 2.

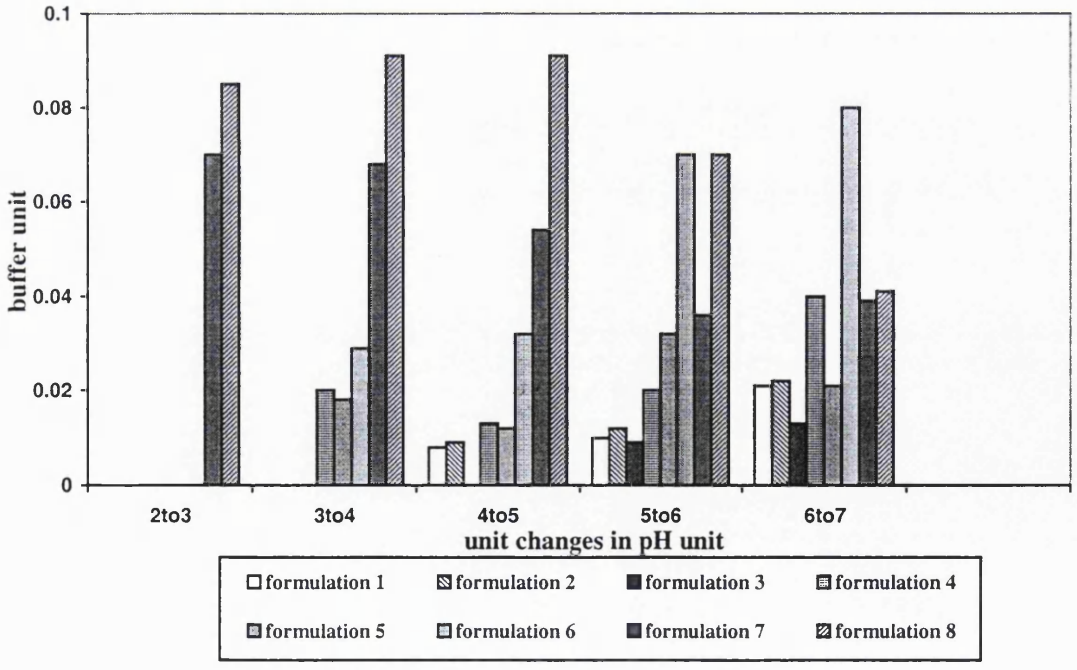


Figure 2.8: The buffer capacity profiles of the 4ASA buffer formulations measured by a stepwise acid base titration

2.4.2 Particle appearances

The changes in the particle appearances of the 4ASA and Na4ASA of the formulations 3 and 8 were observed at unit changes in pH value with the light microscopy (Olympus BX50F, Olympus Optical Co, LtD, Japan). The alteration of size and shape of the particles of these formulations are shown in plates 2.2.1- 2.2.11 and 2.3.1-2.3.7.

For the pH3 4ASA formulation 8, the 4ASA existed as rod-like particles. The acid and alkaline titration processes increase the dissolution of the drug particles. At pH above 6.0, all the drug particles were in solution (plates 2.2.1-2.2.11). The pH7 Na4ASA formulation 3 started to precipitate at pH5 value into the needle-like crystal structure. When the pH of the formulation was adjusted to a value of pH1, the drug particles transformed into a plate-like structure as 4ASA hydrochloride was formed at the low pH environment (plates 2.3.1-2.3.7).

The precipitation process of the low dose pH7 Na4ASA formulations depends on the concentration of Na4ASA incorporated rather than the ionic strength of the buffer system. For the 2mg/ml Na4ASA pH7 formulations, no precipitation of 4ASA was observed at the ionic strength as high as 0.5, as shown by the result of pH7 Na4ASA formulation 6 (table 2.2). The precipitation process is influenced by the equilibrium between the formation of uncharged neutral species and its zwitterion counterpart of the 4ASA, at very diluted concentration the zwitterion species formation was favoured to hinder precipitation process. By doubling the concentration to 4mg/ml as in pH7 Na4ASA formulation 5, the precipitation process was observed.

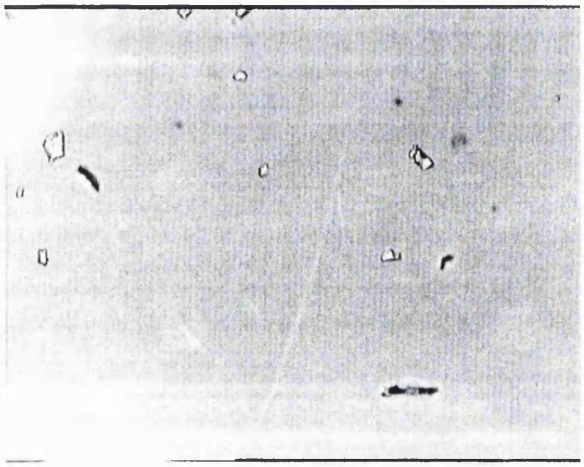


Plate 2.2.1: pH1 Magnification: 200x

Plate 2.2.2: pH1.5 Magnification: 200x

Plate 2.2.3: pH2 Magnification: 100x

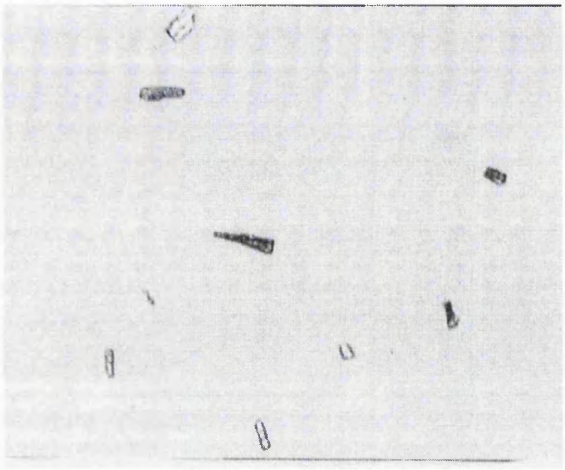


Plate 2.2.4: pH2.5 Magnification: 100x

Plate 2.2.5: pH3 Magnification: 100x

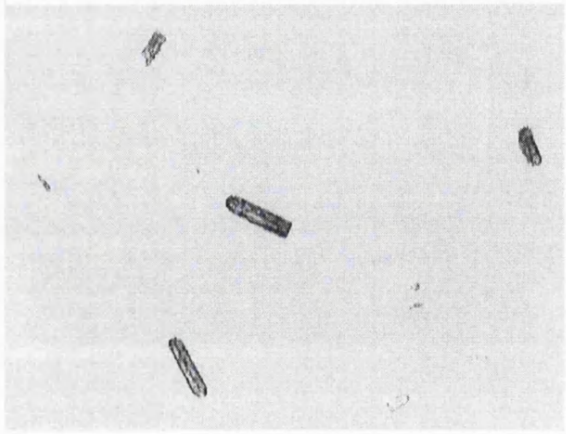


Plate 2.2.6: pH3.5 Magnification: 100x

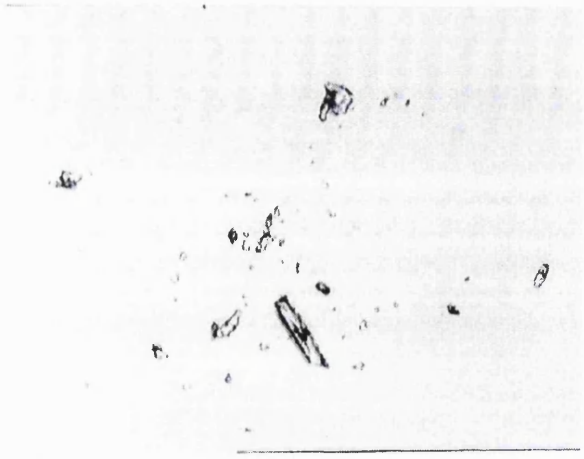


Plate 2.2.7: pH4 Magnification: 200x

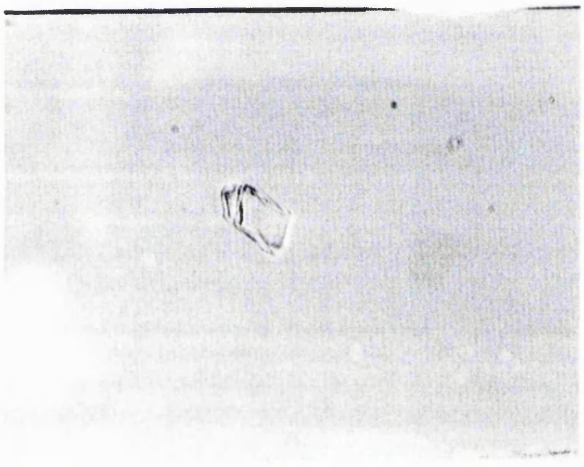


Plate 2.2.8: pH4.5 Magnification: 200x

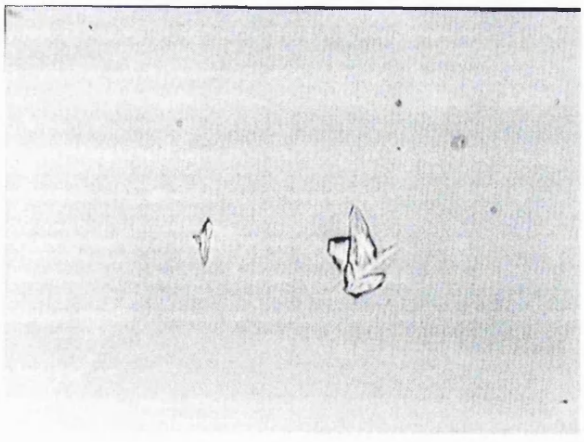


Plate 2.2.9: pH5 Magnification: 400x

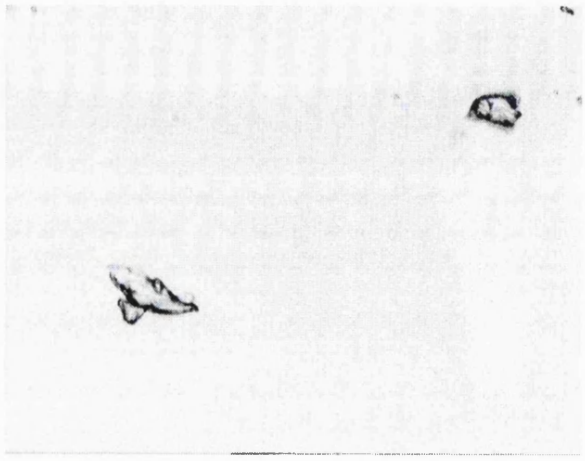


Plate 2.2.10: pH5.5 Magnification: 400x

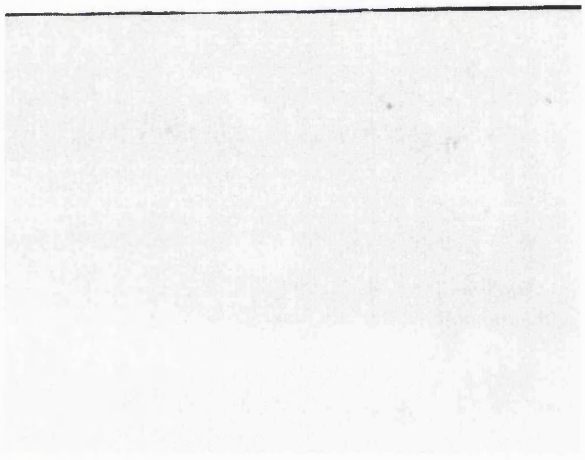


Plate 2.2.11: pH6 All particles dissolved

Plates 2.2.1-2.2.11: The changes in the particle appearances with pH values of pH3 4ASA formulation 8 under light microscope. Magnifications: 100x, 200x and 400x

Plate 2.31: pH1

Plate 2.3.4: pH2.8

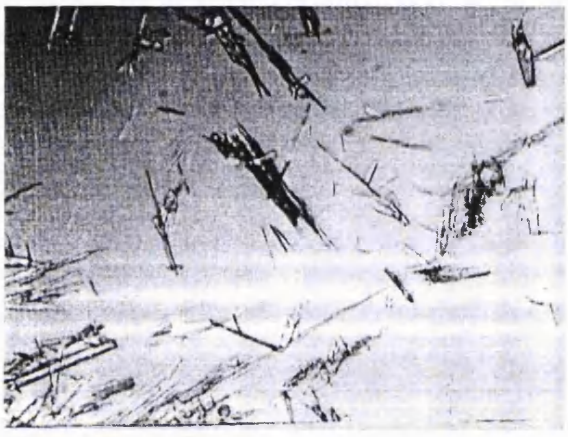


Plate 2.3.2: pH 1.3

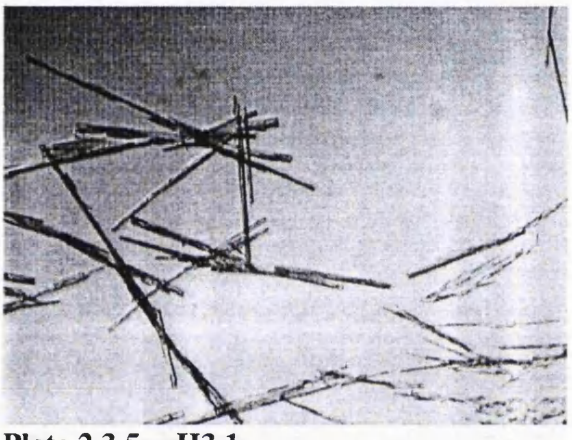


Plate 2.3.5: pH3.1

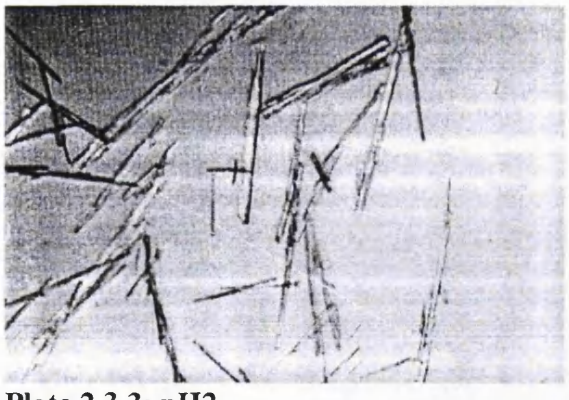


Plate 2.3.3: pH2

Plate 2.3.6: pH4.2

Plate 2.3.7: pH4.7

Plate 2.3.1-2.3.7: The changes in the particle appearances with pH values for pH7 Na4ASA formulation 3 measured under light microscope. Magnification: 100x

2.4.3 Effect of pH variation on the drug concentration profiles in the formulations

Figure 2.9 shows the pH concentration profiles of the filtered samples of pH7 Na4ASA formulations 2, 3 and 4. The 2mg/ml pH7 Na4ASA formulations 2 and 4 showed a minimum changes in overall drug concentration over the pH range as no precipitation of the drug was observed in these formulations, which favoured the formation of the 4ASA zwitterion. The pH7 Na4ASA formulation 3, which was formulated with 16mg/ml Na4ASA, in contrast demonstrated a decrease in the 4ASA dissolution with the acid titration. The precipitation process due to formation of neutral species described in *section 2.4.2* is confirmed by the alteration of the pH concentration profile of this formulation.

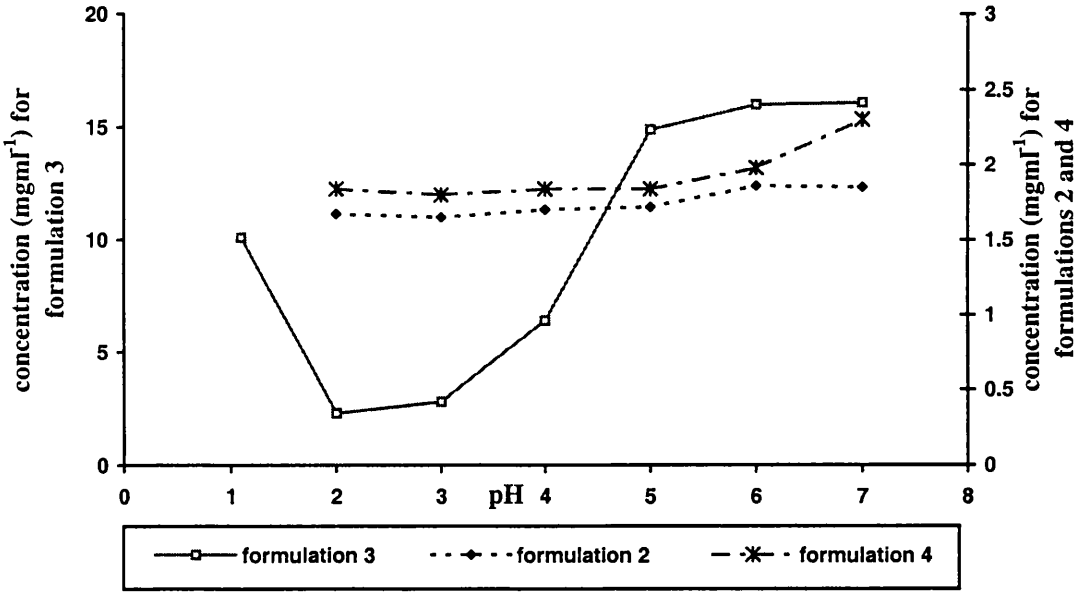


Figure 2.9: The 4ASA pH-concentration profiles of pH7 Na4ASA formulations

2.4.4 Stability test for the formulations

The stability of the pH7 Na4ASA formulation 4 and pH3 4ASA formulation 8 were studied. Each formulation was divided into two parts and placed in a 37°C water bath for a period of two weeks. A 0.1ml of the sample was drawn at fixed time intervals. The samples were diluted into a 50ml of mixed phosphate buffer pH 6.8 BP and the concentrations for these samples were measured with the Perkin Elmer UV spectroscopy at 300nm.

Figure 2.10 shows that the pH7 Na4ASA formulation 4 is more stable when compared to pH3 4ASA formulation 8. This result agreed with other published reports (Rekker et al 1956, Vetuschi et al 1988). Unlike its related compound, 5-aminosalicylic acid (5ASA) which degrades via the decarboxylation and oxidation processes, the breakdown of 4ASA is predominately via the decarboxylation of the carboxylic group into m-aminophenol. Vetuschi et al (1988) showed that the rate of the decarboxylation process is pH dependent. The profile is an approximation of the bell-shaped, with the maximum decarboxylation rate at its apparent isoelectric pH 2.7. The decarboxylation process is negligible at pH 0.1 and pH above 6 as the amount of m-aminophenol formation is small. The pH7 Na4ASA formulation 4 and pH3 4ASA formulation 8 turned brown after 24 and 48 hours, respectively. The intensity of browning was less in the pH3 4ASA formulation 8. The browning of the formulations is thought to be independent of the formation of the m-aminophenol. It has been suggested that the browning was due to the 4ASA molecule itself and there is no parallel between the intensification of the brown colour and the increased in toxicity on standing as described by Rekker et al (1956). The presence of light, oxygen and heat also intensified the browning effect.

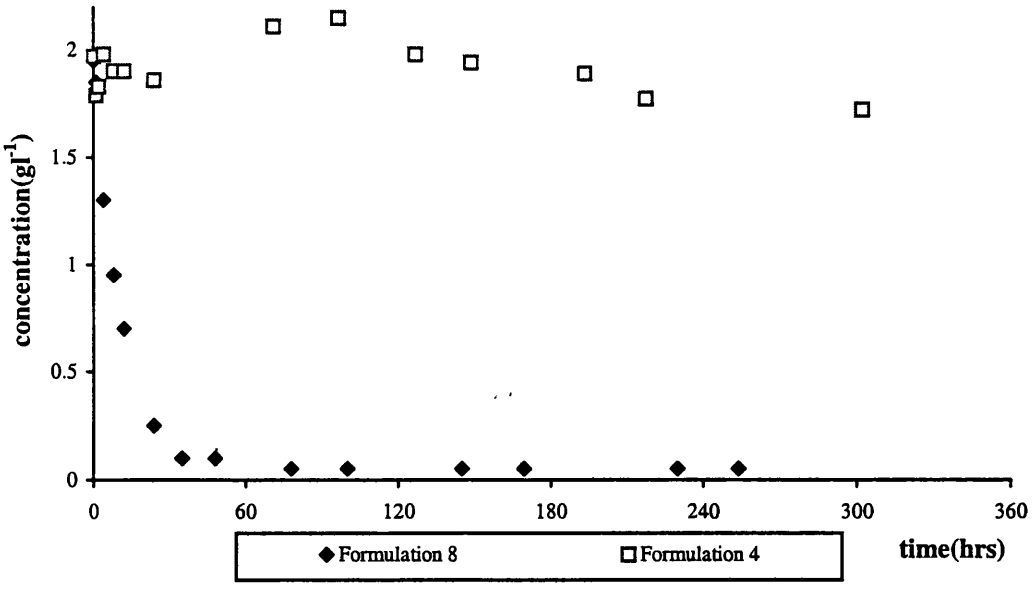


Figure 2.10: The *in vitro* stability test for the 4ASA formulations over a 2-weeks period

2.4.5 Osmolarity of the formulations

The osmolality of pH7 Na4ASA formulation 4 and pH3 4ASA formulation 8 were measured by the Roebling Micro-osmometer Type 13-Autocal (UK). The device determines the freezing point depression of the formulations by comparing to the pure water and calculates the osmotic concentration automatically. The system was calibrated with water followed by 500mOsmo/kg normal saline before the samples were measured. The samples were measured in triplicate.

The measured osmolarity values of pH7 Na4ASA formulation 4 and pH3 4ASA formulation 8 were 266.7 and 255.3 mOsmo/kg, respective. These values are smaller than the estimated values using the computer program. The unit of calculation used in the computer system was mOsmo/l instead of mOsmo/kg, results in minor differences, as the densities of the formulations and pure water differ. The incorporation of the HPMC into the formulations, which is not taken into account during the computation, have also resulted in less satisfactory outcome. However, as mentions earlier, hypo-osmolar formulations can be accepted as they affect the gastric emptying process less.

2.4.6 Conductivity of the formulations

The conductivity of the pH3 4ASA formulation 8 was estimated in voltage term with a standard pre-calibrated pH-meter probe (Gelplas combination electrode, BDH, Poole, UK) using 0.9% sodium chloride as the standard reference. The potential difference of the pH3 4ASA formulation 8 was 224mV. This value is much greater than the OXO soup (115mV) which is used in routine clinical measurement using EIT system (Mitchell 1997). The conductivity of the pH7 4ASA formulation 4 was not tested but it would be much higher as the ionic strength was adjusted to 0.28 as compared to pH3 4ASA formulation 4 (table 2.2).

2.5 Conclusions

The calculated formulations show a close approximation to the practical system in terms of the pH and solubility profiles. The maximum precipitation occurred at pH3 where the concentration of either the zwitterion or neutral species is the highest. Both the pH7 Na4ASA formulation 4 and pH3 4ASA formulation 8 were chosen for future study using the EIT system as they were adjusted to the maximum buffer capacity with acceptable tonicity and were conducting. The stability of the pH3 4ASA formulation 8 is poor, as the degradation into m-aminophenol is high under the acid condition. The decarboxylation of pH7 Na4ASA formulation is small, however, the formulation turned brown after 24 hours at 37°C. Hence, the formulations will be prepared extemporaneously and used within 10 minutes of preparation. Details of the final compositions of the formulations are tabulated in table 2.3.

Table 2.3: The compositions of the 4ASA formulations for EIT study

Ingredients/formulations	pH3	pH7
Sodium 4-aminosalicylate (g)	0.00	0.50
4-aminosalicylic acid (g)	0.50	0.00
0.5M Citric acid (ml)	58.50	5.48
0.5M Sodium hydrogen orthophosphate (ml)	27.70	45.15
7.5% w/v Methocel E15-LV premium EP (ml)	33.33	33.33
water to(ml)	250.00	250.00

Chapter 3

The Volume And pH Effects Of The Administered Liquids Monitored With EIT System

3. 1 Introduction

Early work using electrical impedance tomography (EIT) has prove to be a useful non-invasive clinical technique for the measurement of gastric emptying process with a selection of test meals (Holder 1993, Mitchell 1997). As discussed in *section 1*, many factors affect the gastric motility, EIT is used to test some of the factors that affect gastric emptying rate.

The EIT system measures the changes in tissue impedance, as the physiological hydrogen ion secretion and bicarbonate ion reflux into the stomach affect the image acquisition, this can be interpreted as dynamic changes within the stomach. The present study aims to monitor the effect of administering liquid of different volumes and pH values in the fasted state under the normal physiological conditions using EIT. The gastric emptying curves obtained will be observed qualitatively for smoothness, emptying trends or large fluctuation. Furthermore, whenever possible the emptying curves will be characterised by calculating the values of gastric emptying half time (GE_{50}), lag-time, area under gastric emptying curve (AUEC), gastric mean residence time (GMRT) and variance of GMRT (VGRT).

3.2 Study aims

This preliminary study aims to investigate the effects of administering fluids of different volumes and pH values on the gastric emptying process using EIT without pharmacological intervention.

The findings from this preliminary study will be utilised in future study.

3.3 Materials and methods

The applied potential tomography (APT) system utilises EIT for non-invasive dynamic monitoring of gastric emptying by generating tomographic images of local tissue resistivity. As a test liquid of lower or higher conductivity than the surrounding tissues enters and leaves stomach, the distribution of resistivity in the upper abdomen changes. Sequential images of the tissue resistivity can be used to derive the gastric filling and emptying profiles.

3.3.1 Electrical impedance tomography

The APT Mark I (IBEES, Lodge Moor Hospital, Sheffield, UK) consists of a data acquisition unit, personal computer and visual display monitor. Sixteen pre-gelled silver/silver ECG electrodes (Medicotest Ltd, UK) are placed in a ring around the upper abdomen at the costal margin level and connected to the data acquisition unit. A small alternating current of 1mA at 50kHz is applied to a pair of adjacent electrodes known as driving electrodes and the potential differences across the rest of the electrodes are measured. Each pair of electrodes, in turn, becomes the driving electrodes as the cycle's repeated. A total of 208 measurements are made during each cycle (1 minute), and each data set is made up of several cycles. A reference data set is established initially and subsequent data sets are back projected against the reference data set to produce an image of the change in tissue resistivity.

3.3.2 Test liquids

A non-nutrient buffer liquid, which is conducting and adjusted to the desired pH value for gastric emptying monitoring, is chosen. The test liquids are listed as below:

Citric Phosphate buffer pH7 liquid

Citric phosphate buffer pH3 liquid

The compositions of the test liquids were described in *section 2* with the substitution of the drug component with the appropriate quantity of the tonicity modifier, sodium chloride.

3.4 Study protocol

3.4.1 Design of the study

Ten subjects participated in an open randomised study. Each study day was separated by at least three days interval. Gastric emptying process was to be measured by the EIT.

3.4.2 Study procedures

The study took place over six months at the discretion of Prof M Newton (School of Pharmacy, University of London) and Dr D Evans (Royal London Hospital, Gastrointestinal Science Research Unit).

3.4.2.1 Study times

Each subject was assigned to four study days. Subjects were asked to arrive at the Gastrointestinal Science Research Unit, Royal London Hospital, Whitechapel, by 9.00 am after an overnight fast. A test liquid was given to the subject and the gastric emptying process was monitored for a maximum of two hours.

3.4.2.2 Subject restrictions

Subjects were required to fast for 8 to 12 hours prior to the study day and abstained from alcohol and spicy foods for 24 hours.
Subjects were required to sit upright with minimum movements during the monitoring period.

3.4.2.3 Gastric emptying monitoring

The skin around the upper abdomen of the subject was cleaned with alcohol swabs and dried with paper towels. Sixteen disposable pre-gelled electrodes were placed in rosette at the mid-point of the clean band and attached to the APT system. The subject was asked to sit upright maintaining a quite position with minimum movement and speech, as much as possible. Baseline abdominal tissue impedance was recorded for 10 minutes. The subjects were then given at separated occasions 150, 250 and 450ml Citric phosphate buffer pH7 liquids and a

250ml Citric phosphate buffer pH3 liquid. Gastric impedance was measured using APT system for up to 2 hours. Images were acquired every minute.

3.4.2.4 Subject withdrawal criteria

Subjects who wish not to continue the study or become ill and require any form of medication during the study period.

3.4.2.5 Subject screening

A subject demography and medical history was recorded in the form of a questionnaires, before the study commenced (**Appendix 3**).

3.4.3 Subject selection

Ten healthy male subjects were recruited into the study.

3.4.3.1 Inclusion criteria

a) Healthy male subjects aged between 18 to 65 years old
b) Subjects who are available for the whole set of the study
c) Free from cardiac, hepatic, gastrointestinal, haematological, neurological, renal, pulmonary and psychiatric diseases as determined by medical history and physical examination

3.4.3.2 Exclusion criteria

a) Subjects who have been on regular medication during the previous two weeks prior to the study are excluded
b) Subjects with a body weight index that deviates from ideal weight by 15% are excluded
c) Drug abusers and alcoholic were excluded from the study
d) Those who have participated in a volunteer or clinical study involving drug administration within the previous three months
e) Subjects who regularly drink more than 4 units of alcohol per day

3.4.4 Ethical aspects

3.4.4.1 Written Informed Consent

Subjects received a full explanation of the nature and purposes of the study from the investigator and volunteer information sheet (**Appendix 4**). Each subject gave his written informed consent on forms complying with the ethical committee requirements before participation in the study. The subjects were given a copy of the consent form to keep (**Appendix 4**). The subjects should be fully informed and understood that they were free to withdraw from the study at any time without prejudice.

3.4.4.2 Confidentiality

All information obtained during the study concerning the volunteers' state of health was regarded as confidential and agreement must be obtained from the subject prior to disclosure of such information to a third party.

3.4.4.3 Payment of subjects

Payment of the subjects for time and inconvenience of the study was at the discretion of the investigators. Subjects not completing the study for whatever reasons were paid on a pro rata basis.

3.4.4.4 Study approval

The protocol was submitted to the East London and The City Health Authority Research Ethics Committee for approval, and was received in writing before the starting of the study. Any changes required approval from the committee.

3.4.4.5 Compensation

The subjects must understand that this study was for research purposes only and was not expected to provide any therapeutic benefit to the individual. No alternative procedures were admissible within the strict confines of the study.

3.5 Results and discussion

3.5.1 Data processing

Regions of interest were created from the APT system by totalling the images of approximately 10 image frames, and marking the position of the stomach with the cursor on the APT Mark I program. The change in the impedance within the region of interest was calculated for that image and subsequent images. A profile of gastric emptying was derived from the values of the impedance expressed as a percentage change in value from the initial level measured on ingestion of the liquid.

Abdominal impedance fell from the baseline in the stomach region of interest as the stomach was filled with a relatively conductive liquid. Following this drop, there was a gradual rise in impedance back towards the baseline as the conductive liquid left the stomach region allowing EIT to monitor the gastric emptying process dynamically. The gastric emptying profiles of each subject were showed in figures 3.1.1 - 3.1.10.

Figures 3.1.1-3.1.10: The gastric emptying profiles of the buffer liquid formulations measured using EIT in the healthy subjects

Figure 3.1.1: Subject AA

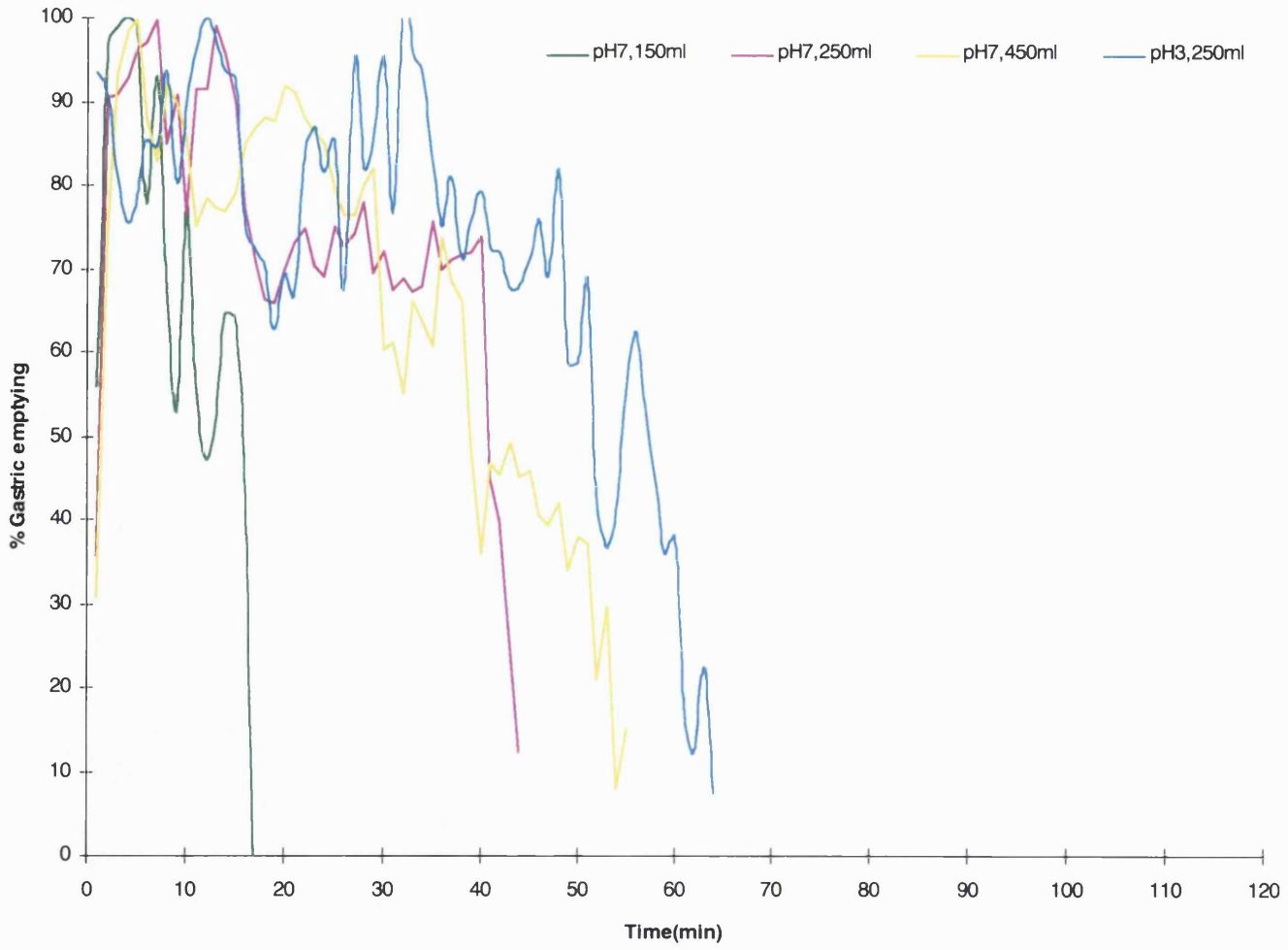


Figure 3.1.2: EIT for Subject BB

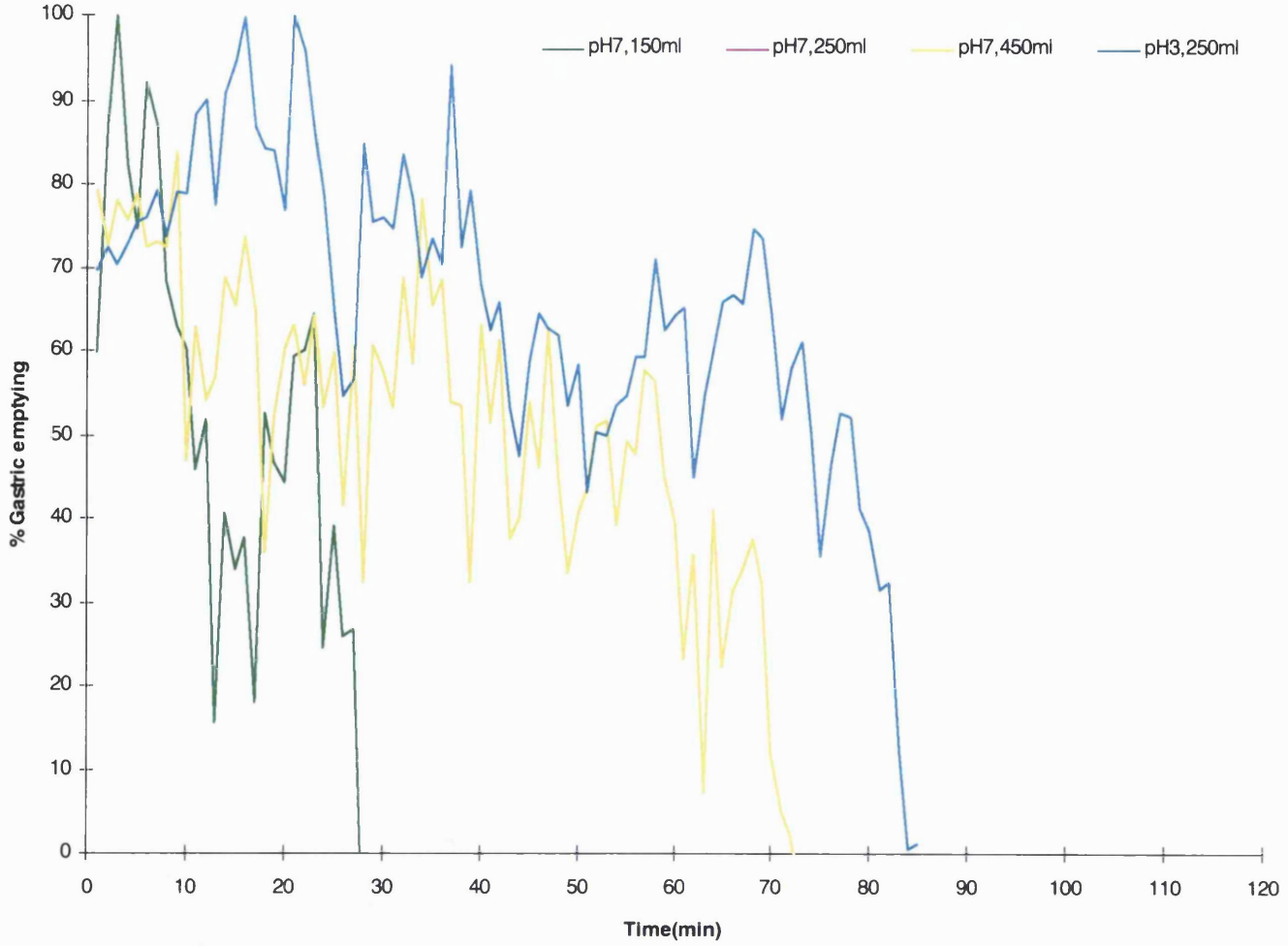


Figure 3.1.3: EIT for Subject CB

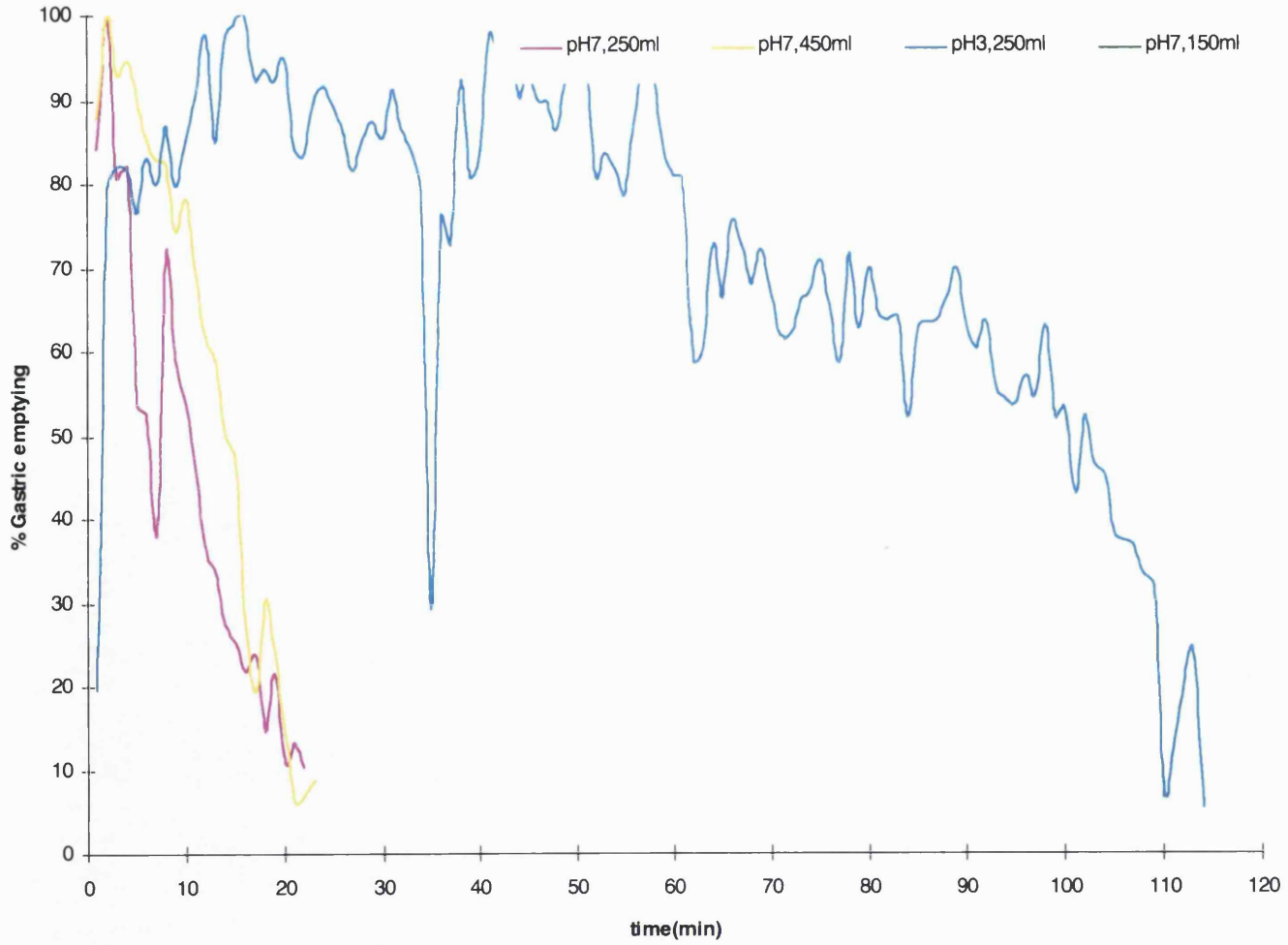


Figure 3.1.4: EIT for Subject DB

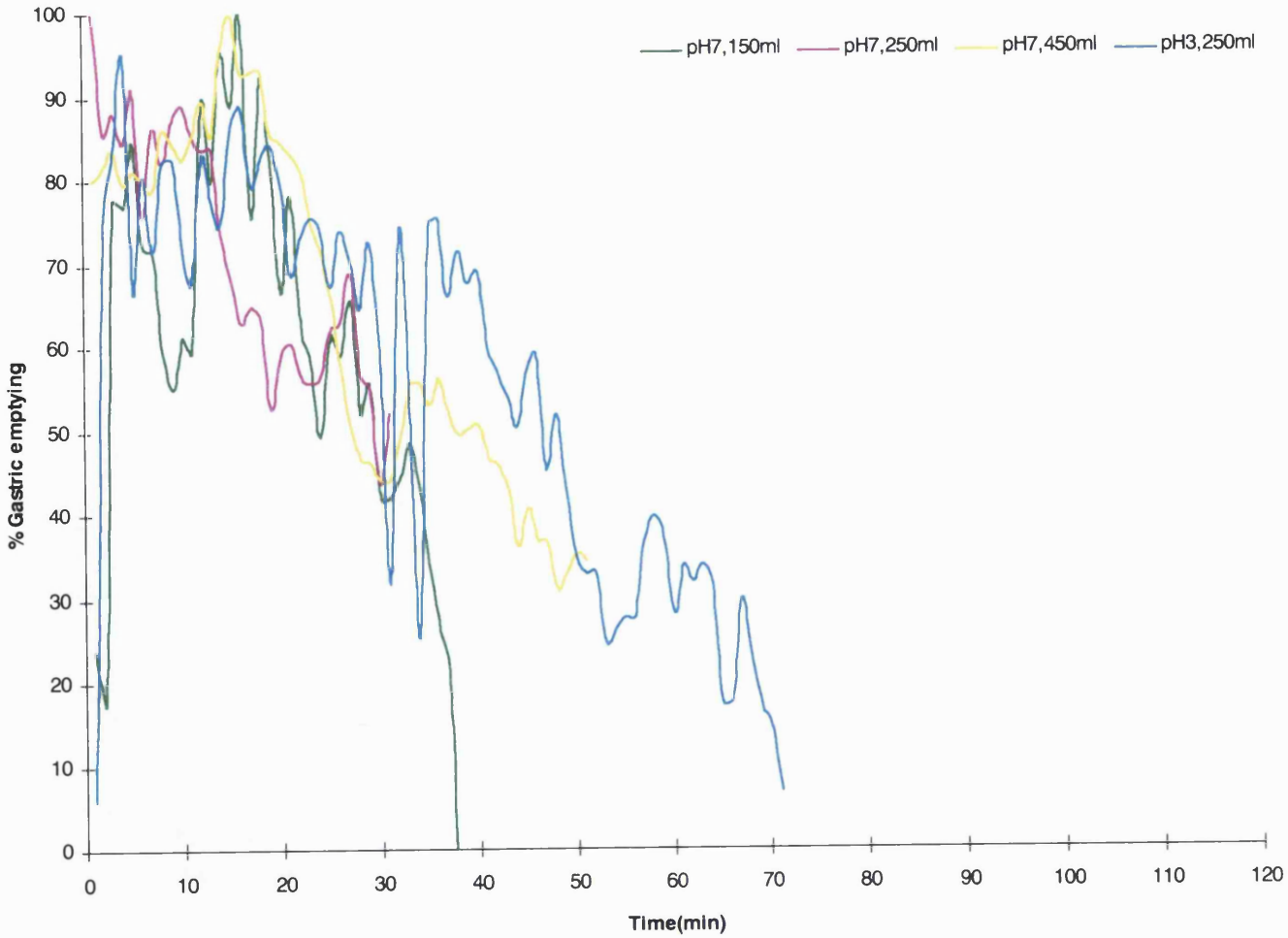


Figure 3.1.5: EIT for Subject DM

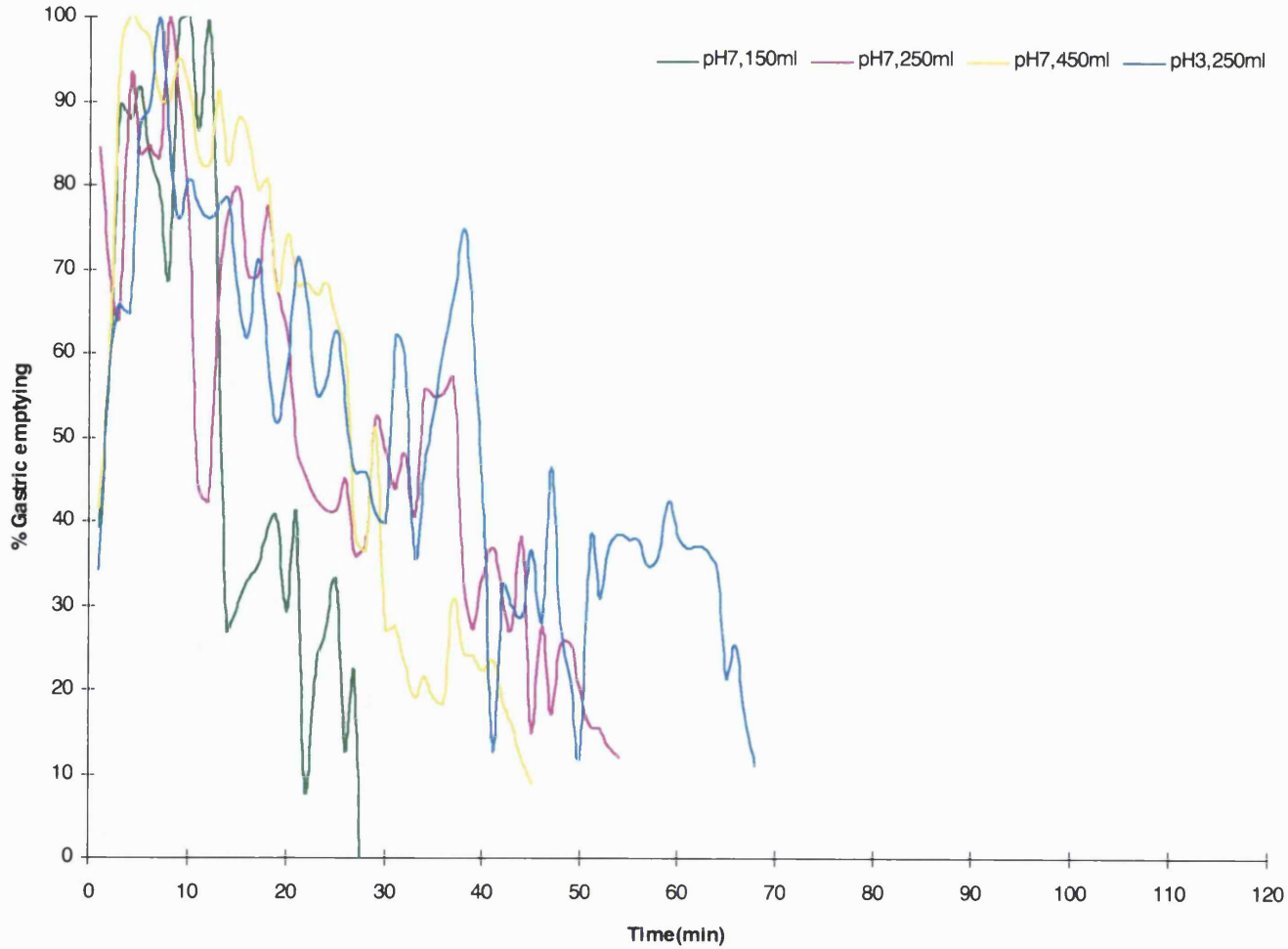


Figure 3.1.6: EIT for Subject EY

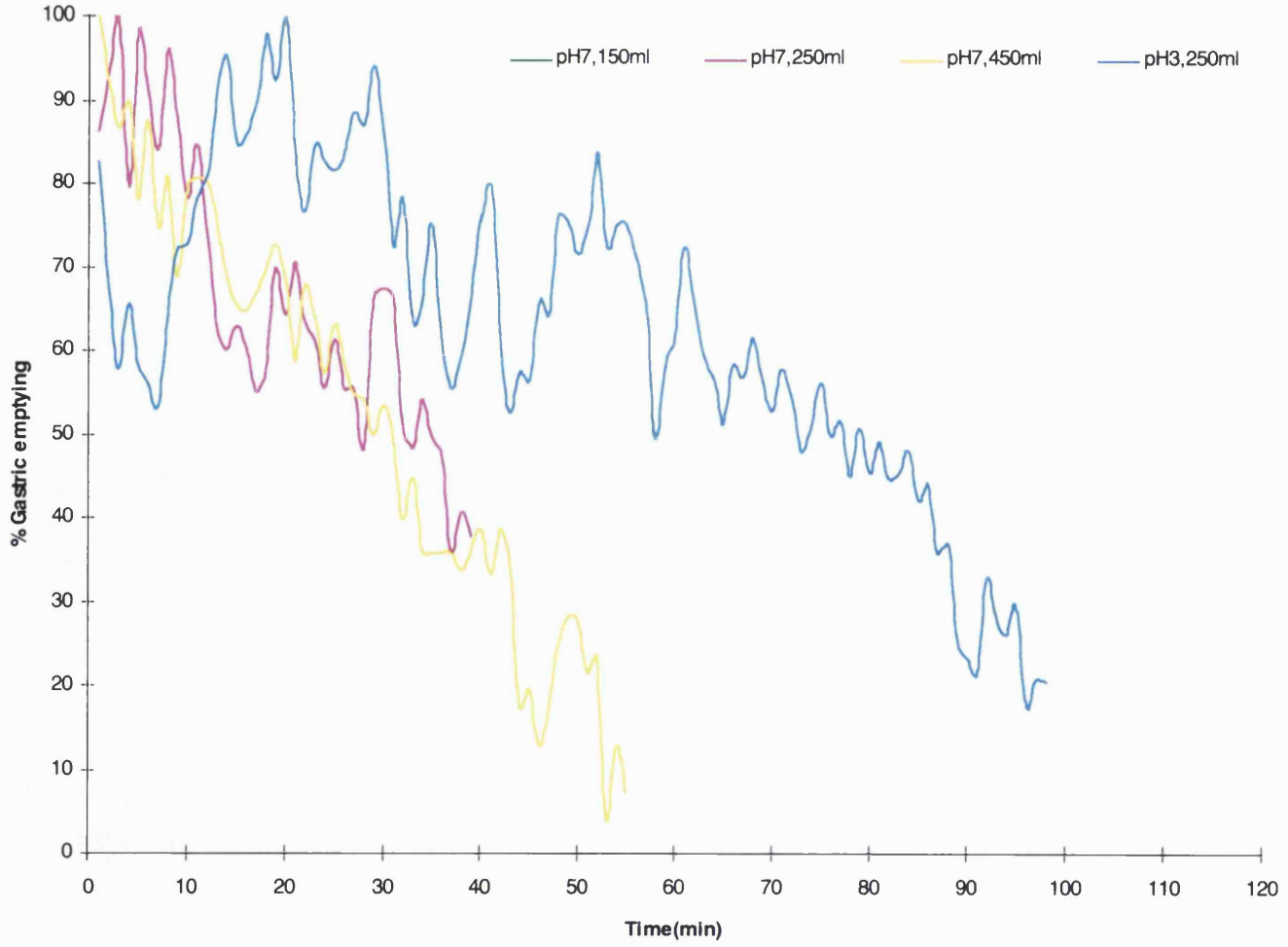


Figure 3.1.7: EIT for Subject OC

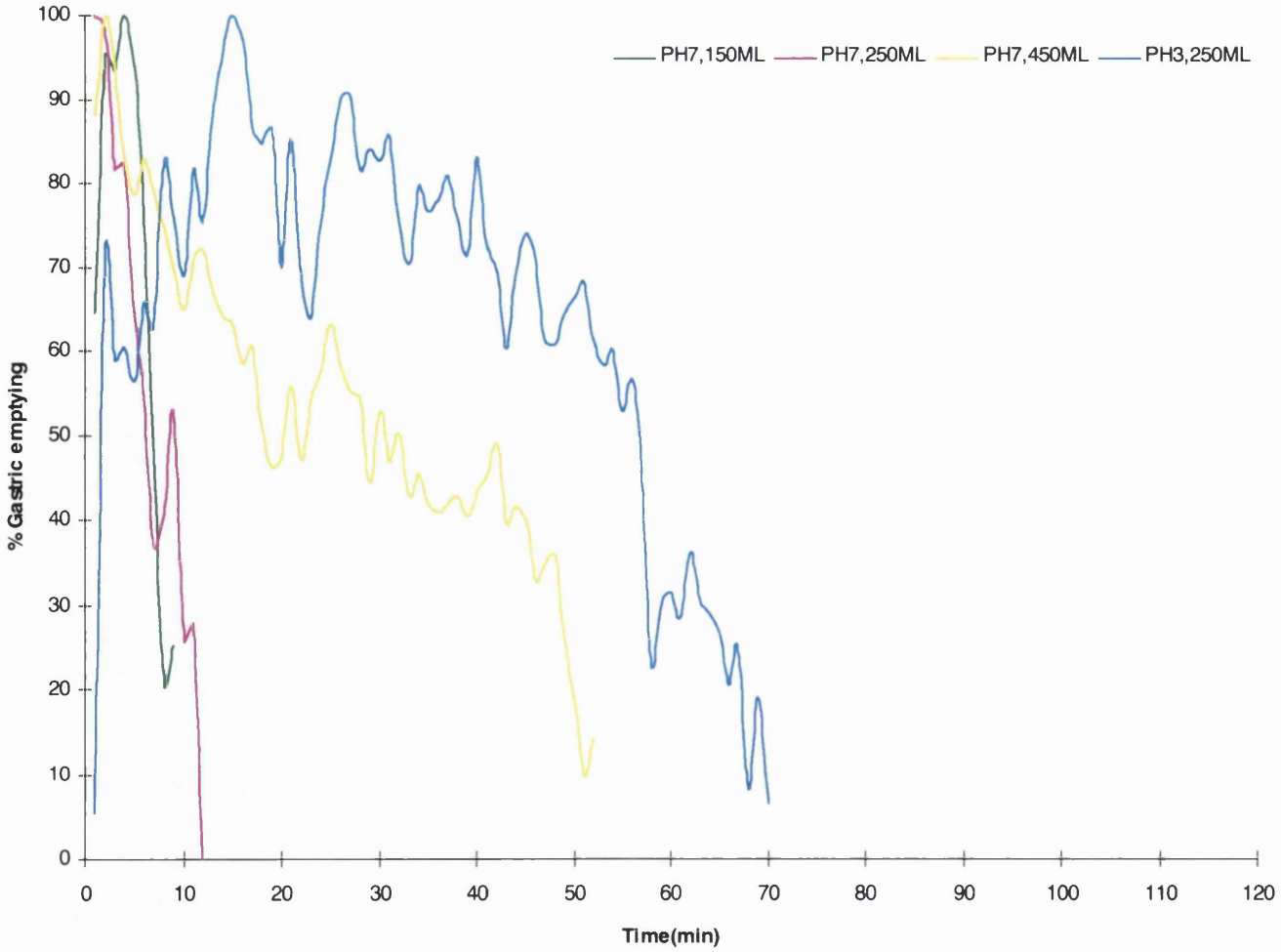


Figure 3.1.8: EIT for Subject TN

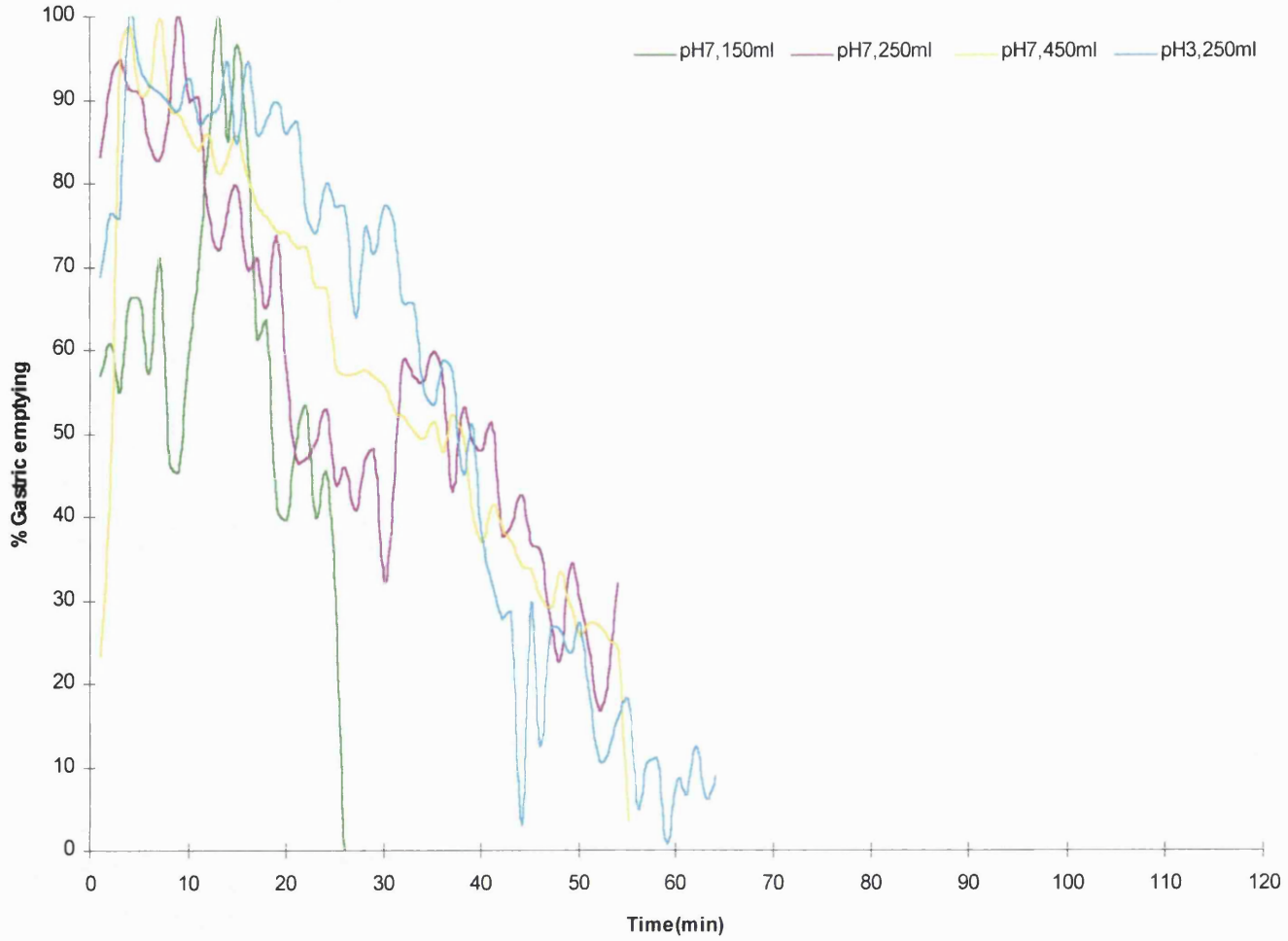


Figure 3.1.9: EIT for Subject WB

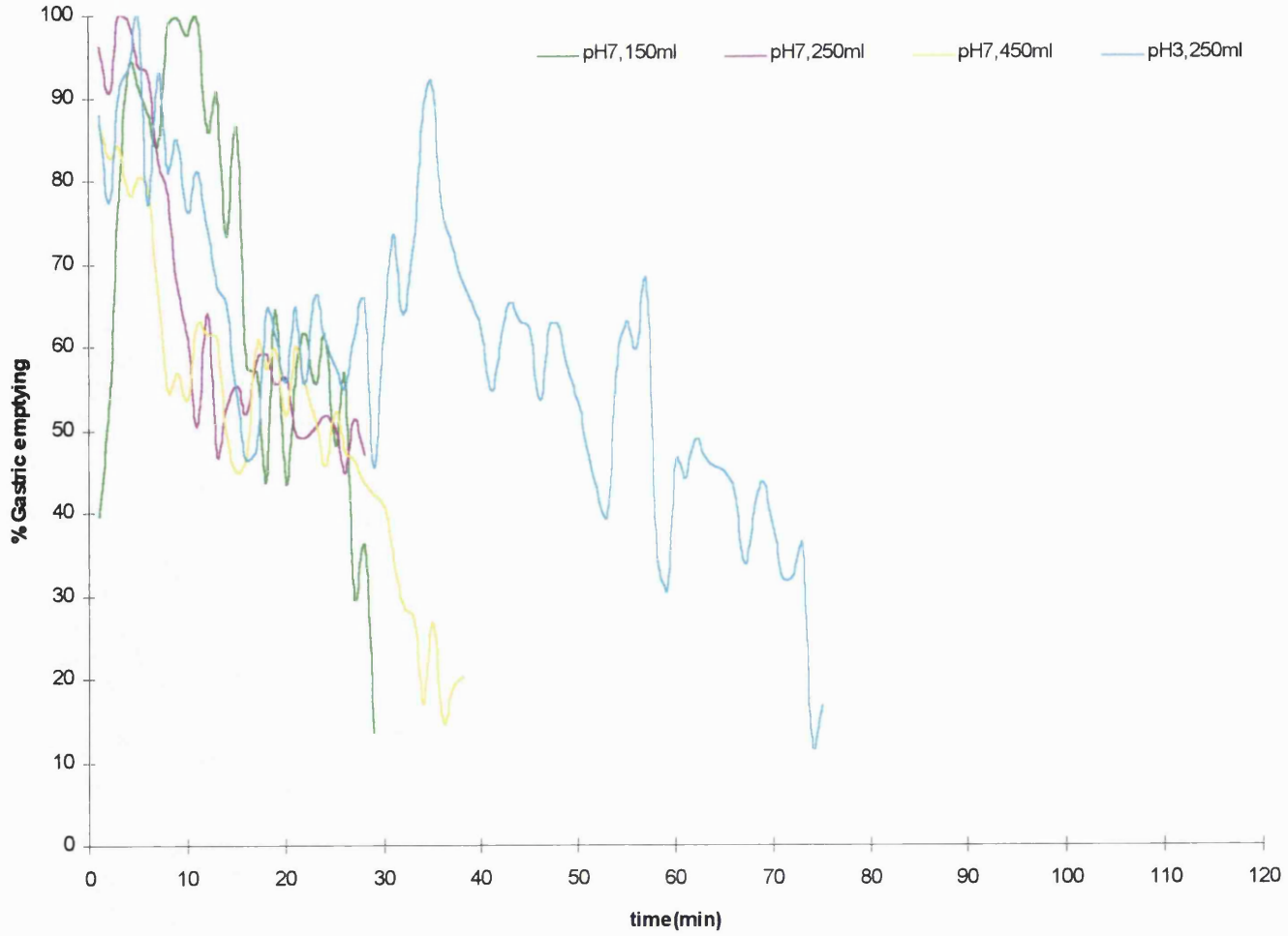
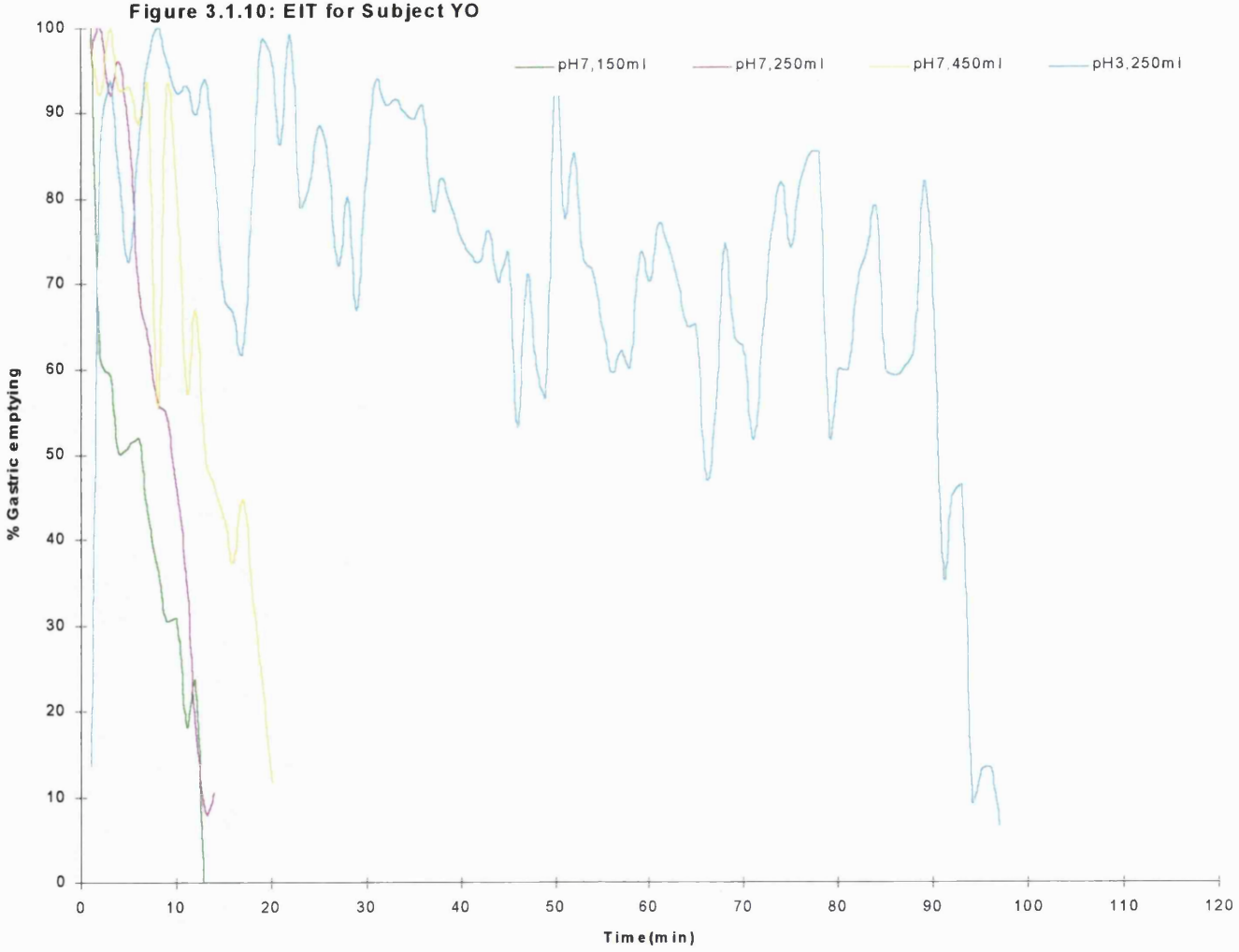


Figure 3.1.10: EIT for Subject YO



3.5.2 Determination of gastric emptying parameters

The gastric emptying parameters were determined both graphically and mathematically. Gastric half emptying time, GE_{50}, can be derived from slope of the gastric emptying curve when the percentage filling dropped to 50% of its original volume. Gastric mean residence time (GMRT), lag times and its variance (VGRT) can be calculated mathematically by the statistical moment and due to the irregularity of some curves, Moore and Wallis trend analysis as described in *section 1.3.1* was performed whenever possible. Tables 3.1 and 3.2 show the gastric emptying parameters of different test liquids in all the subjects.

The results were analysed using analysis of variance from SPSS statistical software package on a personal computer.

Subject	GE_{50} of Different liquids (min)			
	150ml Citrate phosphate buffer pH7	250ml Citrate phosphate buffer pH7	450ml Citrate phosphate buffer pH7	250ml Citrate phosphate buffer pH3
AA	26.0	29.0	41.0	55.0
BB	16.0	-	12.0	87.0
CB	-	10.0	15.0	40.0
DB	25.0	10.0	32.0	53.0
DM	18.0	36.0	27.0	-
EY	-	30.0	30.0	43.0
OC	-	8.0	30.0	49.0
TN	22.0	21.0	36.0	46.0
WB	29.0	19.0	27.0	49.0
YO	6.0	10.0	15.0	75.0
Mean	**20.3**	**19.2**	**26.5**	**55.2**
s.d.	**7.8**	**10.5**	**9.6**	**15.6**

Table 3.1: The gastric emptying half time derived graphically for different test liquids using the gastric emptying profiles from EIT measurements in the healthy subjects.

Test Liquids	150ml Citrate Phosphate buffer pH7				250ml Citrate phosphate buffer pH7				450ml Citrate phosphate buffer pH7				250ml Citrate phosphate buffer pH3			
Subject	GMRT (min)	VGRT (min^2)	AUEC (%min)	lag-time (min)	GMRT (min)	VGRT (min^2)	AUEC (%min)	lag-time (min)	GMRT (min)	VGRT (min^2)	AUEC (%min)	lag-time (min)	GMRT (min)	VGRT (min^2)	AUEC (%min)	lag-time (min)
AA	29.3	109.0	195.8	12.0	-	-	-	-	31.7	87.2	190.9	18.0	34.6	169.3	370.5	13.0
BB	15.6	48.2	91.0	5.0	-	-	-	-	15.4	91.2	176.4	3.0	52.6	369.1	505.4	23.0
CB	-	-	-	-	9.9	29.9	25.1	3.0	10.0	23.2	134.0	3.0	67.1	478.8	408.6	32.0
DB	20.0	38.3	78.4	11.0	8.3	21.3	92.2	3.0	25.3	223.6	361.2	3.0	42.5	163.3	333.7	23.0
DM	11.6	36.3	75.6	3.0	23.8	193.5	108.1	3.0	17.9	108.3	262.4	3.0	53.3	67.6	155.5	39.0
EY	-	-	-	-	19.2	106.4	149.2	4.0	21.0	158.7	268.3	3.0	44.2	583.6	542.6	6.0
OC	-	-	-	-	6.2	5.0	10.4	3.0	22.8	197.0	222.0	3.0	45.0	84.1	143.5	31.0
TN	25.8	68.0	94.9	14.0	26.7	51.9	158.7	10.0	18.0	189.4	1061.9	3.0	29.4	107.5	341.3	15.0
WB	-	-	-	-	14.6	58.9	283.3	3.0	18.0	92.7	338.9	3.0	40.4	266.1	309.0	13.0
YO	6.0	3.6	8.1	3.0	6.5	5.9	54.6	3.0	8.9	15.0	119.4	3.0	58.3	388.9	329.9	26.0
Mean	**18.1**	**50.6**	**90.6**	**8.0**	**14.4**	**59.1**	**110.2**	**3.9**	**19.5**	**118.6**	**313.5**	**4.5**	**46.7**	**267.8**	**344.0**	**22.1**
s.d.	**8.8**	**35.5**	**60.4**	**4.9**	**8.0**	**63.7**	**88.2**	**2.3**	**7.0**	**71.6**	**274.8**	**4.7**	**11.3**	**179.3**	**128.1**	**10.3**

Table 3.2: The gastric emptying characteristics calculated by the statistical moment for different test liquids using the gastric emptying profiles from EIT in healthy subjects. GMRT (gastric mean residence time); VGRT (variance of GMRT); AUEC (area under gastric emptying curve); lag-time (time before onset of gastric emptying).

Table 3.3 shows the F values and their significance levels from the analysis of variance. The F values show that the pH effect is significant in all the gastric emptying parameters. This result is consistent with earlier reports using other methods suggesting that the gastric emptying rate is sensitive to the administered acid content in the non-nutrient meals (*section 1.1.4.2*). Lin et al (1990) has shown that the duodenum feedback mechanism responds to the acid sensitive chemoreceptors along the length of duodenum to mid-jejunum. When the acid chymes reach the duodenum, bicarbonate ion is secreted into the lumen to neutralise the in coming acid. Exhaustion of the bicarbonate ion to neutralise the excess acid induces the tightening of the pylorus sphincter, reduces the gastric muscle contractility and delays further transit of materials from the stomach to duodenum. There were two types of inhibitions on the gastric emptying process as described by Lin et al (1990). The fast response inhibition is achieved by a local reflex action and through the feedback mechanism from the chemoreceptors in the proximal duodenum. The slow but persistent response inhibition is triggered when chemoreceptors from the distal length of the duodenum and up to 150cm of the jejunum are activated. The neutral pH7 buffer liquid emptied faster than acidic pH3 liquid, as the pH value was closer to the luminal pH of the duodenum, which is around pH6. In addition, this liquid lacks an acid component and the pH values of the small intestine proceed towards neutral pH7 distally, no feedback inhibition is exerted to slow the gastric emptying process.

Effect	GE_{50} (F)	GMRT (F)	VGRT (F)	AUEC (F)	Lag-time (F)
pH	40.51***	41.42***	11.50**	7.90***	26.45***
Volume	1.28	0.43	0.50	4.66*	0.28
Subject	0.76	0.22	0.56	1.12	0.52

* is $p<0.05$ **is $p<0.01$ *** is $p<0.001$

Table 3.3: Analysis of variance for gastric emptying parameters of the buffer liquid formulations. The F values and their significance.

When the stomach is filled with liquid, the pressure builds up within the proximal region. The pressure difference due to expansion from the filling process controls the gastric emptying rate (*section 1.1.4.2*). Many reports have shown statistical difference with the volume administered to either animals or humans (Oberle et al 1990, Erskine et al 1981). The volumes used in this study were 150, 250 and 450ml buffer liquids. No statistical significance is evidence for the volume effect as the volume of administration is relatively small. Although the subject effect is not significantly different, figure 3.2 shows that the inter-individual variation is large. This large

variation is due to the administration of the buffer liquids at different motility phases of the stomach. Oberle et al (1987) reported that the liquid emptying from the small volume was affected by the MMC whereas the large volume emptied in response to filling pressure. Only six cases were included for the 150ml buffer liquid due to the irregularity of the raw data, which could not be processed further. As the APT system measures the changes in impedance over a band of area, noise and artefacts are collected during the data acquisition. This has influenced the impedance profiles especially when the administered volume is small. In addition, gastric acid secretion and bicarbonate reflux into the stomach can account for the large fluctuation as no pharmacological intervention has been used to regulate these events.

3.6 Conclusions

The study has shown that the pH differences in the non-nutrient buffer liquids affect the gastric emptying rate. This effect is primary in the regulatory pathway of the gastric emptying process. Although other methods have shown significant difference in the gastric emptying rate of administered fluid volume, the APT system is less sensitive to small volume of administration. This result is restrictive as the MMC, which regulates the gastric emptying rate for small volume is not measured.

Future experiment will use a 250ml volume to standardise the conditions, as it is large enough to be monitored but not too large to induce the pressure effect.

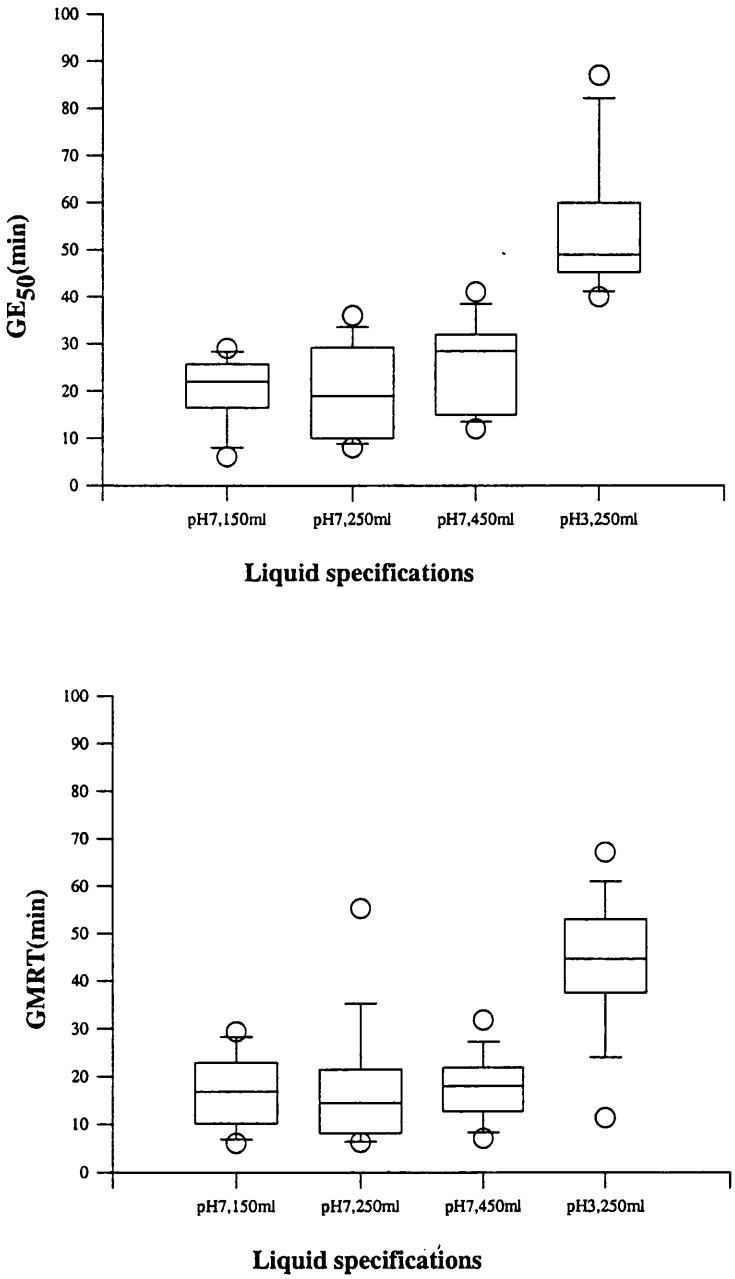


Figure 3.2: The gastric emptying half time (GE_{50}) and gastric mean residence time (GMRT) distributions of the subjects derived from the gastric emptying profiles of the buffer liquid formulations. The lower boundary of the box indicated 25th percentile, a line within the box marked the median, the upper boundary indicated the 75th percentile. The error bar above and below the box indicated the 10th and 90th percentiles. In addition, the symbol o showed the outlying points.

Chapter 4

Evaluation Of 4-Aminosalicylic Acid Liquids Dosage Forms: Pharmacokinetic, Gastric Emptying and pH Profiles

4.1 Introduction

Drug absorption in the gastrointestinal tract is influenced by many factors: for example gastrointestinal physiology, the physico-chemical properties of the drug and specific dosage forms administered (Aulton 1988).

The stomach is responsible for the breakdown of materials into more digestible acidic chymes and also acts as a reservoir for temporary storage (Kelly 1981). The small intestine on the other hand has a large surface area and special features such as the carrier systems and extensive blood supply that allow most absorption to take place (Gibaldi 1977). Uptake of a liquid or solid drug, which dissolves readily in the gastrointestinal tract, may be limited by the rate at which it arrives at the duodenum and the physico-chemical properties such as the dissociation constant and partition coefficient (Amidon et al 1995).

Liquids are emptied in the manner, which can be quantified by either an exponential or a linear expression as supposed to solids that need to be broken into smaller sizes before they are emptied from the stomach (Davis et al 1986). Although emptying of liquids from the stomach is less affected by the digestive state, studies with gamma-scintigraphy have shown that a non-nutrient liquid has a shorter stomach residence time when compared to a nutrient liquid. Food delays gastric emptying by altering the gastric motility pattern through disrupting the fasted state MMC process (*section 1.1.4*). The time of the disruption is governed by factors such as meal composition, caloric load and particle size. Food also changes the pH of the stomach environment by its buffering capacity (Bueno et al 1993). Delay in gastric emptying lengthens the gastric residence time, which may have significant effects especially on sustained released preparation and acid labile drugs. A weak acidic drug and its salt, such as 4-aminosalicylic acid may precipitate under the acidic environment of the stomach (Shore et al 1956). In the fasted state, the gastric emptying rate of liquid depends on the volume of administration and gastric motility phases (Oberle et al 1987).

The aim of this study is to correlate the effect of gastric emptying and pH in the fasted state to the pharmacokinetic profiles of a weak acid formulated into liquid dosage forms of different pH values. The gastric emptying characterisations and the gastric luminal pH are monitored using a non-invasive technique known as the applied potential tomography (Avill et al 1987) and pH sensitive radiotelemetry capsule (Evans 1993), respectively. In addition, studies are to be

performed to investigate the effect of incorporating ranitidine, a histamine-2 receptor acid inhibitor on the rate of gastric emptying and pharmacokinetic of the model drug.

4.2 Study aims

1. To monitor the gastric emptying process of liquids by applied potential tomography (APT)

2. To monitor the intraluminal pH of the stomach by pH sensitive glass radiotelemetry capsule

3. To evaluate the influence of administrating the 4-aminosalicylic acid (4ASA) oral liquid formulations of different pH values on the pharmacokinetic profiles

4. To evaluate the influence of acid inhibition on the gastric emptying and pharmacokinetic of different 4-aminosalicylic acid oral liquid formulations

5. To estimate the absolute bioavailability using an intravenous bolus injection of 4-aminosalicylic acid

6. To establish any possible correlation between gastric emptying, gastric luminal pH and the pharmacokinetic profile of 4-aminosalicylic acid in oral liquid dosage forms

4.3 Materials and methods

4.3.1. Electrical impedance tomography

4.3.1.1 Description of applied potential tomography system (APT)

APT Mark I system (IBEEES, Logde Moor Hospital, Sheffield, UK) as described in *section 3.3.1* which measured the change in tissue resistivity was employed to monitor the gastric emptying process.

4.3.1.2 Test liquids

The conductivity of the test liquids affected the image quality attained with the APT system. The test liquids consisted of the same components as those used in *section 3.3.2* with the replacement of the sodium chloride, which was the tonicity modifier, with the model drug component, 4-aminosalicylic acid.

4.3.2 pH sensitive radiotelemetry capsule

4.3.2.1 Description of pH sensitive radiotelemetry capsule

The pH sensitive radiotelemetry capsule Type 7036 (Remote Control Systems Ltd, Amersham, England) comprises a transducer, a silver oxide battery and a radio transmitter sealed in a non-toxic glass capsule. One end of the capsule known as the reference end must be fitted with a tethered reference cap that incorporates a black conductive membrane and switches the capsule transmitter on when the cap is screwed firmly in place. The tethered reference cap is normally discarded after use. A Type 7036 pH-sensitive radiotelemetry capsule transmits a low power radio signal, the frequency of which is controlled by the voltage developed across a pH sensitive ion electrode. This voltage is referred to a silver/silver chloride electrode located in the reference cap. The capsule has a pickup range of about 0.5 metres using a double loop aerial and data lodger receiver system (Oakfield Instrument Ltd, UK), but does not have sufficient power to interfere with radio, television or other domestic appliances.

4.3.2.2 Procedures for the pH sensitive radiotelemetry capsule

The pH sensitive radiotelemetry capsule is suitable for measurement of the pH values in the range of 1-9 at +5 to 55°C. Prior to the use, the capsule is immersed in a standard buffer solution pH 7.0 (Oakfield Instrument Ltd, UK) at 37°C overnight to stabilise. The calibration of the capsule is performed the next day using the same buffer and another standard buffer pH 1.0 (Oakfield Instrument Ltd, UK) according to the procedure set out in the handbook covering the receiver. The step response time of the capsule after stabilisation and calibration procedure is accessed to ensure performance. If the capsule does not achieve within the reference step response time of ten seconds, re-calibration is required.

For routine studies of oesophageal or gastric pH, absolute sterility of the gastric capsule is not expected. Care should be taken with patients who have dangerous viruses or other diseases, which can be passed on through saliva.

After use, the tethered cap is disposed off and the capsule is washed in soapy water at room temperature. Any surface deposits are removed. The capsule then is soaked without a reference cap fitted for at least 2 hours in 2% buffered gluteraldehyde (Cidex®) finally to eliminate the bacteria and viruses on the surface of the capsule and is thoroughly rinsed under running tap water. The threaded compartment must be dried completely prior to returning the capsule to storage. This is because the capsule performance may deteriorate if this compartment is exposed to moisture for prolonged periods.

4.3.3 Determination of serum drug concentration by High Performance Liquid Chromatography

4.3.3.1 Description of the HPLC assay

The HPLC assay is based on previously published reports (Honigberg et al 1980, Patel 1993) with minor modifications due to the low drug concentration in the serum. The blood samples were centrifuged within 30 minutes of collection and stored at -70°C before analysis. Both the levels of drug and its major metabolite, N-acetyl-4-aminosalicylic acid; were determined. An internal standard, N-Propionyl-4-aminosalicylic acid 10μg/ml in methanol was used to monitor conditions during the assay of the sample. The drug and its metabolites were

extracted directly with methanol and concentrated by evaporation of the solvent before reconstitution with the mobile phase.

The assay procedure was as follows: Samples were allowed to thaw at room temperature and vortex mix before removing the samples. Two 250µl aliquots were taken from each sample, 20µl of the internal standard and 500µl of the methanol were added. The mixtures were vortex periodically and let to stand for one hour before centrifuged at 1400rpm twice using Wifug Haemicrofuge (Lab Centrifuge, Parry Lane, Bradford, England). A 500µl of aliquot was drawn from each mix and evaporated to dryness with a gentle current of nitrogen gas at 40°C using a SC-3 sample concentrator (Techne, Dri-block, DB-3, UK). The residual was reconstituted in a minimal amount of mobile phase to improve the sensitivity of the assay.

The HPLC assay was performed using the following equipment: a Gilson model 303 pump, a Manometric Module Gilson International model 802C, an Applied Biosystems 980 programmable Fluorescence detector, a TC 1980 HPLC temperature controller (ICI instrument, UK) and a Hewlett-Packard 3393A Integrator. The column was a Waters Spherisorb C18 S5ODS2 (4.6 x 100.0 mm ID) column attached to a Waters Spherisorb C18 S5ODS2 pre-column to filter off residual impurities and to prolong the column life. The mobile phase used was HPLC grade methanol-Phosphate buffer pH 7.4 (20:80) containing 1mM tetrabutyl-ammonium hydroxide as the ion-pair modifier. The flow rate was set at 1.0ml/min. Fluorescence intensity was monitored at excitation and emission wavelengths of 245 and 345nm, respectively.

4.3.3.2 Analysis of serum 4-aminosalicylic acid and N-acetyl-4-aminosalicylic acid concentrations

As 4-aminosalicylic acid is amphoteric in nature, its extraction from the biological fluid matrix such as serum, urine is limited unless the component is derivatised into a more lipophilic form (Fischer et al 1979). A direct injection of the serum sample using non-extractive preparation into a reversed phase C18 column presents a simple and yet quick procedure for especially very low concentration samples. Based on a published report, serum containing 4-aminosalicylic acid and its major metabolite, N-acetyl-4-aminosalicylic acid can be analysed directly after precipitation of the serum protein with equal volume of absolute

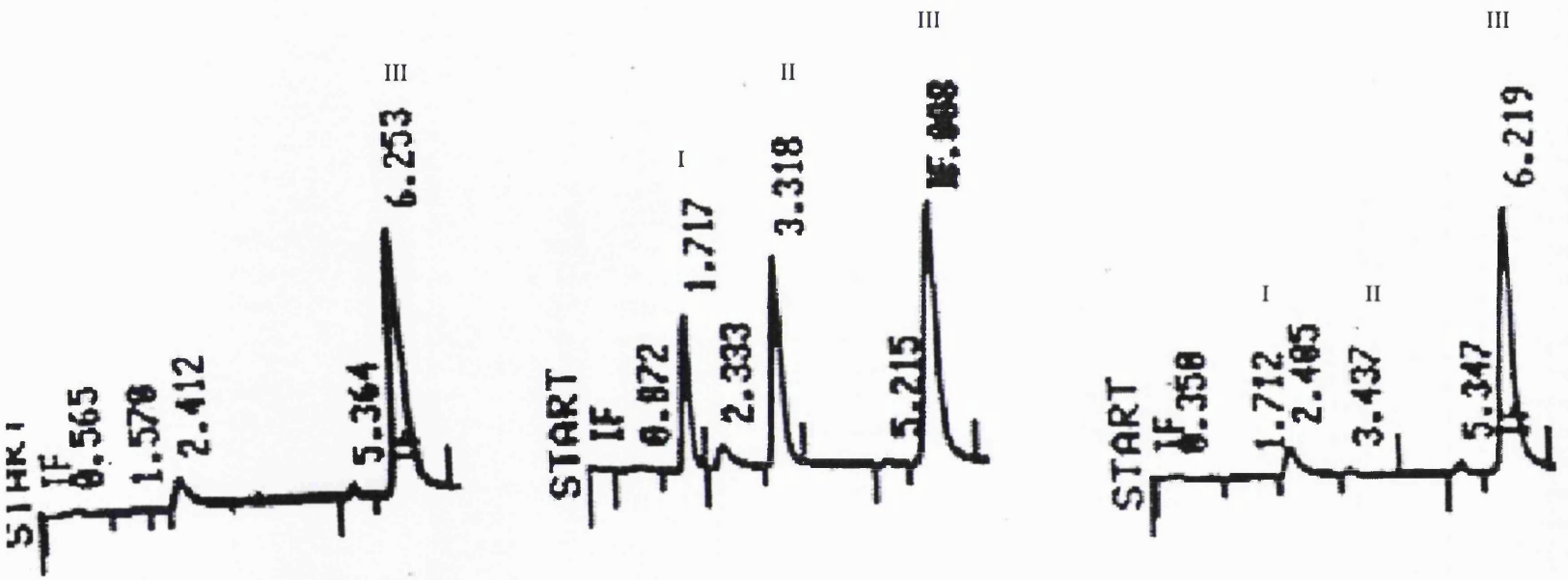
methanol (Honigberg et al 1980). Blanchard (1981) reported that with equal and double volumes of methanol, the protein precipitation were 70 and 98%, respectively. Hence, to ensure satisfactory minimum interference resulting from incomplete precipitation, a double volume of absolute methanol is added.

As the serum drug level is very low, an additional concentration step is essential for achieving a drug concentration within the detection limits. The methanol extracted sample is concentrated by evaporation to dryness by a gentle current of nitrogen gas on a sample concentrator with the temperature set at 40°C. The residue is reconstituted in a minimal amount of mobile phase, which adds to the sensitivity limit of the assay.

A mobile phase mixture made of methanol and phosphate buffer at pH 7.4 (20:80) containing 1mM tetrabutyl-ammonium hydroxide provides satisfactory separation of the components of interest. By stabilising the temperature of the analytical condition at 30°C, the retention times of the components can be analysed within 10 minutes' time interval. Under the chromatographic conditions used, endogenous serum constituents, the metabolites and the internal standard do not interfere with the assay. Furthermore, the spiked plasma containing ranitidine does not interfere with the assay (figure 4.1).

Using spiked serum samples, the precision of the method and the percentage recovery on extraction with methanol was evaluated for both the drug and its metabolite. The recovery values of more than 90% were obtained for both the drug and its metabolites. Satisfactory coefficients of variation were obtained both within day and between day assays (tables 4.1-4.4). Furthermore, over the range of concentration determined, the detector response and the standard curve are both linear (regression coefficient = 0.99). The standard calibration curves derived from the spiked drug and metabolites free serum are shown in **Appendix 5**.

Figure 4.1: Chromatogram from HPLC assay. I is 4ASA; II is AASA; III is PASA (Propionyl-4ASA). The numerical values are the times in minutes for the emergence of the peaks.



(A) Drug and metabolite free spike blood serum

(B) Blood sample at 30 minutes collection

(C) Blood sample at 8 hours collection

Table 4.1: Recovery of 4-aminosalicylic acid and N-acetyl-4-aminosalicylic acid for oral samples

	4-aminosalicylic acid			N-acetyl-4-aminosalicylic acid		
Concentration (μg/ml)	**5.0 (n=5)**	**20.0 (n=5)**	**50.0 (n=3)**	**5.0 (n=4)**	**20.0 (n=5)**	**50.0 (n=4)**
Percentage recovery	95.5	97.6	99.9	90.4	95.3	95.0
Coefficient of variation	2.3	0.9	2.7	7.1	5.6	4.2

Table 4.2: Precision of 4-aminosalicylic acid and N-acetyl-4-aminosalicylic acid for oral samples

	4-aminosalicylic acid						N-acetyl-4-aminosalicylic acid				
	within day			between days			Within day			Between days	
Concentration (μg/ml)	5^{+}	20^{+}	50^{*}	$5^{\wedge}$	$20^{\wedge}$	40^{a}	$5^{\#}$	20^{+}	$50^{\#}$	$5^{\wedge}$	$20^{\wedge}$
Coefficient of variation	2.3	0.9	2.7	7.6	3.6	3.4	7.1	5.6	4.2	7.7	4.7

Detection limit = 0.1μg/ml * is n=3 $^{\#}$ is n=4 $^{+}$ is n =5 ^{a} is n=6 $^{\wedge}$ is n = 7

Table 4.3: Recovery of 4-aminosalylic acid and N-acetyl-4-aminosalicylic acid for intravenous samples

	4-aminosalicylic acid				N-acetyl-4-aminosalicylic acid			
Concentration (μg/ml)	**0.5 (n=3)**	**1.0 (n=4)**	**2.0 (n=4)**	**5.0 (n=5)**	**0.5 (n=3)**	**1.0 (n=5)**	**2.0 (n=4)**	**5.0 (n=5)**
Percentage recovery	92.9	90.2	97.6	90.8	96.3	96.2	97.2	95.7
Coefficient of variation	8.0	4.3	5.2	0.1	11.4	5.8	6.8	2.1

Table 4.4: Precision of 4-aminosalicylic acid and N-acetyl-4-aminosalicylic acid for intravenous samples

	4-aminosalicyic acid		N-acetyl-4-aminosalicylic acid	
	within day	**between days**	**within day**	**between days**
Concentration(μg/ml)	1.0	5.0	1.0	5.0
Coefficient of variation	5.6	5.2	3.2	7.1

n = 6 for all cases Detection limit = 0.1μ g/ml

4.4. Study protocol

4.4.1 Design of the study

Ten healthy male subjects took part in a five-study days open randomised trial. The gastric emptying process and gastric luminal pH were measured simultaneously by APT system and pH sensitive radiotelemetry capsule, respectively. Blood was sampled before dosing and at fixed time intervals after administration of the formulations.

4.4.2 Study procedures

4.4.2.1 Study times

The study took place over one year at the discretion of Prof M Newton (London School of Pharmacy) and Dr D Evans (Royal London Hospital).

Each subject was assigned five study days, each day separated by at least one-week interval. The subject was requested to arrive fasted at the Gastrointestinal Science Research Unit, Royal London Hospital, Whitechapel, by 9.00 am. The pharmaceutical preparations were given in a random order. All the study days with administration of oral preparations incorporated simultaneous monitoring of gastric emptying and gastric luminal pH for up to two hours in addition to blood collection.

On two occasions the subjects were asked to take a 300mg ranitidine (Zantac®) tablet with plain water an hour before arriving at the hospital to suppress the gastric acid secretion.

A standard lunch was provided after the completion of pH and gastric emptying monitoring. For the study using intravenous drug preparation, the subjects were given lunch four hours after the drug administration. The subjects were allowed to drink water *ad.lib.* after lunch.

4.4.2.2 Subject restrictions

Subjects were asked to fast for 8 to 12 hours prior to the study day and for up to four hours after drug administration. Subjects were requested to abstain from alcohol and spicy foods for 24

hours prior to each study day. Subjects were also required to maintain a quite sitting position during pH and gastric emptying monitoring, restricting movement and speech, as much as possible.

4.4.2.3 Gastric emptying measurement

Gastric impedance was measured using the APT Mark I system for up to two hours as described in *section 3.4.2.3.* Images were acquired every minute. Baseline tissue impedance was established in the 10 minutes prior to test liquid administration.

4.4.2.4 Gastric luminal pH measurement

Each subject swallowed a pre-calibrated, tethered pH sensitive radiotelemetry capsule with a minimum amount of plain water so that it resided in the liquid of the stomach. Intraluminal pH was measured and converted into radio signal which was detected by a dual-loop aerial band (Oakfield Instrument Ltd, UK) worn around the abdomen. The detected signal was recorded on to a portable solid state Medilog 1000 receiver (Oakfield Instrument Ltd, UK). The pH was monitored for 30 minutes before the administration of the formulation, and was recording continued up to two hours after administration.

At the end of the study period, the pH sensitive radiotelemetry capsule was pulled out from the stomach via the oesophagus and sterilised.

4.4.2.5 Serum drug concentration sampling

Thirteen 6ml blood samples (including pre-dose) were collected by drawing blood from a tube containing heparin through a Teflon cannula inserted to the forearm vein of the subject by a qualified doctor. Blood samples were collected in to 10ml plain tubes (red top) over eight hours' periods at fix intervals of 0.00, 0.15, 0.30, 0.45, 1.00, 1.30, 2.00, 2.30, 3.00, 4.00, 5.00, 6.00 and 8.00 hours. Samples were promptly centrifuged; the serum harvested was frozen at -70°C in the designated freezer until they were analysed by HPLC assay as described in *section 4.3.3.*

The cannula was removed after the collection of the last blood sample and the subject was allowed to go home.

4.4.2.6 Subject withdrawal criteria

The following were used as the withdrawal criteria:

a) Subjects wishing not to continue the study e.g., unavailability, intolerance of the study procedure.

b) Subjects become ill and require any form of medication during the study period.

4.4.2.7 Subject screening

A subject demography and medical history were recorded in the form of a questionnaire, before the study commenced (**Appendix 3**).

4.4.3 Pharmaceutical preparations

The following products were prepared by the Department of Pharmaceutics, The School of Pharmacy, University of London.

a) 250ml of 2mg/ml 4-aminosalicylic acid pH3 oral suspension (*section 2*)
b) 250ml of 2mg/ml 4-aminosalicylate Sodium pH7 oral solution (*section 2*)
c) 50mg/2.2ml 4-aminosalicylate Sodium intravenous bolus injection (PAS-Fatol N®, Fatol Arzneimittel GmbH, Germany)
d) 150mg Ranitidine tablet (Zantac®, Glaxo-Wellcome, UK)

4.4.4 Subject selection

Ten male subjects participated in the study.

4.4.4.1 Inclusion criteria

a) Healthy non-smoking male volunteers aged between 18 to 65 years old
b) Subjects who are available for the whole set of the study

c) Free from cardiac, hepatic, gastrointestinal, haematological, neurological, renal, pulmonary and psychiatric disease as determined by medical history and physical examination.

4.4.4.2 Exclusion criteria

a) Subjects who have been on regular medication during the previous two weeks prior to the study were excluded
b) Subjects with the previous history of hypersensitivity to salicylates and similar drugs were excluded
c) Subjects with a body weight that deviated from the ideal weight by 15% were excluded.
d) Drug abusers and alcoholic were excluded from the study.
e) Those who have participated in a subject or clinical study involving drug administration within the previous three months
f) Subjects who regularly drink more than 4 units of alcohol per day

4.4.5 Ethical aspects

4.4.5.1 Study approval

The protocol was submitted to the East London and The City Health Authority Research Ethics Committee for approval, and was received in writing before the starting of the study. No major changes were made to the study protocol during the study.

4.4.5.2 Written informed consent

Subjects received a full explanation of the nature and purposes of the study from the investigator and volunteer information sheet (**Appendix 6**). Each subject gave written informed consent on forms complying with the ethical committee requirements before participation in the study. The subject was given a copy of the consent form to keep (**Appendix 4**). The subject should be fully informed and understand that he was free to withdraw from the study at any time without prejudice.

4.4.5.3 Confidentiality

All information obtained during the study concerning the subject's state of health was regarded as confidential and agreement must be obtained from the subject prior to disclosure of such information to a third party.

4.4.5.4 Compensation

The subjects must understand that this study was for research purposes only and was not expected to provide any therapeutic benefit to the individual.
No alternative procedures were admissible within the strict confines of the study.

4.4.5.5 Payment of subjects

Payment of subjects for time and inconvenience of the study was at the discretion of the investigators. Subjects not completing the study for whatever reasons were paid on a pro rata basis.

4.5 Results and discussion

4.5.1 Data processing and analysis

The data from APT, pH radiotelemetry and blood monitoring were processed and analysed as described in the following sections.

4.5.1.1 Applied potential tomography

During the investigation the gastric emptying profiles of the oral liquid formulations were noted from APT tomographic images by creating a region of interest on the APT Mark I program as described in *section 3.5.1.*

The gastric emptying characterisation is calculated graphically as gastric emptying half time and mathematically based on statistic moments analysis as described in *section 3.5.2.* The results are further analysed statistically using the SPSS software package. Details will be described in *sections 4.5.4* and *4.5.5.*

4.5.1.2 pH sensitive radiotelemetry

The luminal pH profile acquired from the radiotelemetry capsules which was stored in the Medilog receiver was replayed on a dedicated microprocessor controlled replay unit to give a hard copy of pH against time. This data was then transferred to a mainframe computer where it was converted into a readable form by a converter device program developed by the Gastrointestinal Science Research Unit, Royal London Hospital before further analysis by a standard software package.

For the pH analysis, the data was divided into pre-dose and post-dose periods. For the post-dose period, the following parameters were calculated: 1) the median, 25th quartile and 75th quartile of each half an hour period of the recording and 2) the pH distribution (the length of time the pH is within various pH ranges). The median, 25th quartile and 75th quartile of each half an hour period of recording of the pre-dose was calculated when ever possible. The entire analysis of the gastric pH record was performed with Flexilog II pH analysis program (Oakfield Instrumental Ltd, UK). The results were shown in **Appendix 7.**

The pHtime value is denoted as the gastric time interval where the pH value of the stomach was ±0.5 of the pH value of the administered liquid. This is calculated by summing the time values at specific pH values from the pH distribution table (**Appendix 7**). The pHtime value is used for statistical analysis described in *sections 4.5.4* and *4.5.5.*

4.5.1.3 Pharmacokinetic analysis by Maximum Entropy method

The measured blood concentration levels of 4-aminosalicylic acid (4ASA) and its metabolite, N-acetyl-4-aminosalicylic acid (AASA) were obtained using the HPLC assay described in *section 4.3.3.* The average numerical value of the blood concentration levels of each individual was tabulated in **Appendix 8**. These data were treated using the MADAME software program (Cambridge, UK). The principle is based on the maximum entropy theory (MAXENT) described in *section 1.7.* A detailed explanation of the theory was also reported by Podczeck et al (1995[a]).

The 4ASA concentration sets obtained from the liquid formulations were compared with the 4ASA concentration sets from intravenous (IV) preparation of each subject. The 4ASA concentrations from the collected blood samples for the IV preparation of subject VM was below detection limit of the HPLC assay. Therefore, only the liquid formulations were compared.

A set of criteria needs to be specified before the MADAME software program calculates the data sets. The output criteria including the α(abs), α(dis), α.S, Ω(abs), good(dis), good(abs), logprob and the test values determine the likelihood of the convergence of the calculation at a specific ICF value, which is the smoothing function for the absorption model. Theoretically, the arbitrarily chosen numerical values α(abs), α(dis), and α.S should be as close to zero as possible. The test and the good(abs) values approached 0.0 ±0.05 and 1.0 ±0.05, respectively, where ±0.05 is the tolerance factor, utol. The correct ICF value can be chosen by optimising the 'logprob' value at its local maximum. An example of the MAXENT output report is shown in **Appendix 9**.

In all cases, the entropy of the disposition model collapsed indicated by good (dis) = 0, hence, there was no evidence of a distribution of 4ASA and its metabolite in other than the central compartment. The kinetics of 4ASA has always been treated as being not more than one compartment model (Peloquin et al 1994, Wan et al 1973). This is one of the properties that is shared with 5ASA, an anti-inflammatory agent having similar structure to 4ASA (Yu et al 1990). The further data treatment was undertaken by suppressing the disposition feature of the MADAME program.

For 4ASA data sets, the maximum logprob values were drawn at optimum ICF width between 0.3 and 0.7. Subjects' BB, DB, TN and WB model fittings were less satisfying. The test values were more than 0.0 ±0.05, showing that the matching of the hypothesis and the likelihood were more difficult with these subjects. This was because the fluctuations in the raw data sets were substantial and to establish these fluctuations with greater certainty, more data points would be required. Furthermore, the intravenous models were fitted less satisfactorily to the measured concentration points. It has to be borne in mind, however, that for the intravenous blood levels only four data points were available, the first being 15 minutes after injection, thus presumably beyond the half life of the drug. As the intravenous blood level was used as weighing function in the mathematical analysis, larger deviations from ideal fit must be acknowledged. Refer **Appendix 10** for the concentration and rate profiles generated by MADAME program.

Before any pharmacokinetic parameters can be reported, the posterior probability distributions that describe the total absorption process need to be generated. As this is a multivariate Gaussian distribution based on a Bayesian model, it is particularly suitable to be determined numerically in its marginal distribution by Monte Carlo statistic. The Monte Carlo sampling also provides the possibility of describing the parameters estimated as distribution based on their variability (Podczeck et al 1995[a]). In this study, 100 Monte Carlo samples were generated for each subject and the results were pooled. The cumulative absorption profiles were calculated for 4ASA. Other pharmacokinetic parameters include the volume of distribution (V_d), clearance (Cl), rate constant (K), and absolute bioavailability (F_A) for 4ASA. Details will be described in *section 4.5.2*.

With the data available, the MAXENT approach is not able to process the metabolite, AASA data sets as their formation profiles can not be determined solely from a single dose of intravenous 4ASA injection. As described in *section 1.6*, conversion of 4ASA into AASA involves both pre-systemic and systemic acetylation by N-acetyl-transferase. Saturation of the enzyme capacity is also possible. The metabolism hence can only be described with non-linear pharmacokinetic models. In principle, MAXENT provides also here an interesting approach. However, the pharmacokinetic characterisation in such instances can only be derived if different doses of 4ASA are administered to study the enzyme capacity activity and if possible by comparing additionally to a direct intravenous injection of AASA.

4.5.2 Relationship between gastric emptying, gastric luminal pH and pharmacokinetic profiles

The gastric emptying, pH and the pharmacokinetic profiles processed in *section 4.5.1* will be discussed as follows:

1. Discussion based on the first 2 hours after liquid administration where simultaneous pH, gastric emptying and blood levels were monitored. Individual cases based on specific test conditions will be considered here to establish possible relationships from the 3 profiles. Combined graphical presentations including the gastric emptying profiles from APT studies, pH profiles from radiotelemetry studies, the measured blood levels of AASA, the measured blood levels as well as the model fits concentration levels and the absorption rate profiles for 4ASA derived from MADAME program are shown in figures 4.2.1-4.2.10.

2. Discussion of overall effects based on the test conditions with statistical analysis whenever necessary. Refer **Appendix 10** for the 8 hours concentration and rate profiles of 4ASA and AASA.

The pharmacokinetic results are tabulated in tables 4.5-4.8. Cross-referencing is required and inevitable.

Subject	% Cumulative absorption profile of 4ASA					MAT values & their
Liquid A	**10**	**25**	**50**	**75**	**90**	**Inter-quartile range**
AA	4.49	11.22	22.88	68.61	143.55	49.44 (43.05-55.19)
BB	3.23	8.06	16.94	32.20	64.95	27.94 (25.88-29.48)
CB	8.36	20.72	36.73	66.92	148.78	63.73 (50.91-73.61)
DB	7.69	19.65	35.28	47.24	100.98	45.69 (39.91-50.30)
DS	7.98	19.31	37.02	69.96	112.61	52.71 (47.30-57.84)
OC	1.61	4.02	8.04	12.06	14.48	14.74 (11.83-17.27)
TN	7.25	16.68	26.01	65.18	230.60	76.13 (65.59-84.44)
VM	3.01	7.53	15.27	33.37	41.81	22.18 (20.00-24.50)
WB	6.04	15.27	39.00	113.17	143.40	68.99 (60.09-77.14)
YO	3.69	9.23	21.28	43.18	159.44	50.99 (45.21-60.11)
MEAN	**5.34**	**13.19**	**25.85**	**55.19**	**116.06**	**47.30 (40.98-52.94)**
Liquid B						
AA	2.88	7.20	14.40	23.30	28.65	17.80 (14.22-21.53)
BB	2.31	5.77	11.55	27.98	66.35	23.62 (22.02-25.19)
CB	3.63	9.09	19.82	36.37	79.33	34.61 (26.36-43.94)
DB	2.83	7.08	14.16	36.99	81.93	33.85 (25.51-39.76)
DS	4.28	10.69	23.22	41.11	70.52	31.40 (28.76-33.19)
OC	3.52	8.80	20.50	39.90	84.36	34.14 (27.80-39.97)
TN	1.61	4.03	8.07	12.10	14.52	12.80 (10.63-15.41)
VM	2.05	5.12	10.24	18.77	72.88	25.34 (22.33-28.32)
WB	5.92	14.80	31.91	50.78	74.89	39.87 (38.71-40.64)
YO	3.25	8.13	19.28	60.72	114.97	41.62 (37.46-45.25)
MEAN	**3.23**	**8.07**	**17.32**	**34.80**	**68.84**	**31.36 (25.38-33.32)**
Liquid AA						
AA	5.86	14.65	24.29	55.35	144.26	52.33 (45.91-59.99)
BB	9.42	21.61	35.08	44.24	97.79	45.03 (42.25-48.54)
CB	12.58	24.73	42.85	121.80	314.20	106.81 (89.94-120.89)
DB	5.18	12.95	26.22	43.36	111.14	42.63 (37.45-46.64)
DS	8.00	19.26	36.73	65.40	102.46	50.10 (38.58-62.00)
OC	3.59	8.99	29.26	112.10	300.92	88.80 (72.47-110.34)
TN	3.79	9.48	19.15	29.01	182.16	51.93 (44.00-57.71)
VM	2.57	6.41	12.83	24.28	39.12	20.35 (17.91-23.27)
WB	12.77	24.76	40.23	69.77	116.89	55.34 (49.98-60.40)
YO	4.97	12.43	29.76	86.71	283.69	82.44 (70.04-96.00)
MEAN	**6.87**	**16.20**	**29.64**	**65.20**	**169.26**	**59.58 (50.85-68.58)**
Liquid BA						
AA	3.00	7.50	15.01	23.88	29.20	21.64 (18.81-25.69)
BB	3.56	8.90	18.54	30.36	42.50	24.56 (22.94-26.03)
CB	2.44	6.10	12.20	29.14	96.31	40.13 (21.17-56.03)
DB	15.61	19.45	25.34	35.65	74.40	38.28 (32.16-43.42)
DS	4.85	12.13	22.52	35.80	74.54	33.13 (27.23-38.90)
OC	4.41	11.03	34.45	44.61	120.89	48.46 (41.31-54.30)
TN	3.03	7.56	15.14	22.91	27.76	17.05 (13.58-19.71)
VM	2.44	6.11	12.22	35.75	42.07	20.55 (17.71-22.85)
WB	3.07	7.69	15.96	39.22	75.87	37.42 (28.88-44.94)
YO	5.13	12.81	22.03	32.70	64.27	28.16 (25.89-30.85)
MEAN	**4.77**	**9.93**	**19.34**	**33.00**	**64.78**	**30.94 (24.97-36.27)**

Table 4.5: The cumulative absorption profiles of 4ASA calculated by MAXENT approach for the oral liquid formulations. Liquid A (pH3 4ASA formulation without ranitidine treatment); Liquid AA (pH3 4ASA formulation with ranitidine treatment); Liquid B (pH7 Na4ASA formulation without ranitidine treatment); Liquid BA (pH7 Na4ASA formulation with ranitidine treatment); MAT (mean absorption time).\ Tabulated values are in minutes and represent the times to give 10, 25, 50, 75 and 90% absorption of 4ASA.

Subject	AUC (μgminml^{-1})					MRT (min)					K_a(min^{-1})			
Dosage Forms	Liquid A	Liquid AA	Liquid B	Liquid BA	IV dose	Liquid A	Liquid AA	Liquid B	Liquid BA	IV dose	Liquid A	Liquid AA	Liquid B	Liquid BA
AA	1469.61	1358.67	832.12	726.07	169.15	69.10	71.59	41.63	45.66	19.63	0.030	0.029	0.048	0.046
BB	1661.12	1708.82	1343.39	999.62	144.70	57.60	61.84	41.29	41.40	16.27	0.041	0.020	0.060	0.037
CB	1462.87	1360.74	1009.56	812.75	133.30	82.18	121.49	54.20	66.10	18.49	0.019	0.016	0.035	0.057
DB	1866.95	1752.90	970.93	947.90	127.00	61.54	56.91	48.01	52.89	15.74	0.020	0.026	0.049	0.027
DS	2068.18	1545.15	1461.45	1097.60	179.29	60.36	59.54	39.64	42.07	9.69	0.019	0.019	0.030	0.031
OC	1768.63	1597.25	944.11	669.74	128.36	34.06	108.89	51.36	67.39	18.02	0.086	0.037	0.034	0.020
TN	1531.83	1857.02	1215.74	848.63	127.03	92.49	71.11	33.65	39.49	18.73	0.027	0.036	0.086	0.046
VM	1090.17	1411.35	774.28	591.15	N/A	41.42	40.79	42.44	40.94	N/A	0.045	0.054	0.068	0.057
WB	2121.89	2425.89	1713.67	1515.88	167.82	83.29	71.75	55.55	55.48	16.03	0.018	0.016	0.022	0.043
YO	2065.36	1794.19	1282.47	949.97	133.44	70.92	98.55	57.21	46.32	16.53	0.032	0.023	0.036	0.032
Mean	**1710.70**	**1681.21**	**1154.70**	**916.10**	**145.44**	**65.30**	**76.25**	**46.40**	**49.78**	**16.56**	**0.034**	**0.026**	**0.047**	**0.039**
s.d.	**331.52**	**317.84**	**300.04**	**262.40**	**20.87**	**18.37**	**25.39**	**7.90**	**10.34**	**2.91**	**0.021**	**0.012**	**0.020**	**0.012**

Table 4.6: The AUC, MRT and K_a values for 4ASA calculated by MAXENT approach. AUC (area under the concentration curve); MRT (Mean residence time); K_a (absorption rate constant); Liquid A (pH3 4ASA formulation without ranitidine treatment); Liquid AA (pH3 4ASA formulation with ranitidine treatment); Liquid B (pH7 Na4ASA formulation without ranitidine treatment); Liquid BA (pH7 Na4ASA formulation with ranitidine treatment); IV dose (intravenous route).

Subject	Mean & inter-quartile range for F_A values			
Liquids	**A**	**AA**	**B**	**BA**
AA	0.57 (0.52-0.64)	0.53 (0.48-0.60)	0.44 (0.37-0.50)	0.39 (0.34-0.44)
BB	0.87 (0.80-0.93)	0.83 (0.74-0.90)	0.90 (0.81-0.97)	0.67 (0.63-0.72)
CB	0.75 (0.70-0.79)	0.68 (0.62-0.75)	0.71 (0.63-0.81)	0.54 (0.49-0.62)
DB	1.04 (0.92-1.13)	0.97 (0.87-1.07)	0.74 (0.67-0.80)	0.71 (0.64-0.77)
DS	0.82 (0.73-0.91)	0.60 (0.54-0.66)	0.76 (0.69-0.84)	0.60 (0.53-0.65)
OC	0.94 (0.86-1.05)	0.83 (0.74-0.93)	0.69 (0.54-0.80)	0.48 (0.44-0.52)
TN	0.82 (0.74-0.90)	0.99 (0.89-1.10)	0.90 (0.81-0.98)	0.63 (0.54-0.69)
VM	0.78 (0.66-0.88)	1.02 (0.87-1.11)	0.79 (0.68-0.87)	0.60 (0.53-0.65)
WB	0.88(0.82-0.93)	0.99 (0.94-1.05)	0.95 (0.91-1.05)	0.84 90.78-0.91)
YO	1.05 (0.98-1.17)	0.94 (0.86-1.01)	0.93 (0.84-1.01)	0.67 (0.62-0.74)
Mean	**0.85 (0.77-0.93)**	**0.84 (0.76-0.92)**	**0.78 (0.70-0.87)**	**0.61 (0.55-0.67)**
s.d	**0.14 (0.13-0.16)**	**0.18 (0.16-0.19)**	**0.15 (0.16-0.16)**	**0.13 (0.12-0.13)**

Table 4.7: The absolute bioavailability values (F_A) for 4ASA calculated by MAXENT approach. Mean values and inter-quartile ranges. Liquid A (pH3 4ASA formulation without ranitidine treatment); Liquid AA (pH3 4ASA formulation with ranitidine treatment); Liquid B (pH7 Na4ASA formulation without ranitidine treatment); Liquid BA (pH7 Na4ASA formulation with ranitidine treatment).

Subject	V_{d4ASA}	Cl_{4ASA}	K_{el4ASA}
AA	4.8 (4.0-6.1)	0.912 (0.172-0.217)	0.040
BB	4.4 (3.6-5.2)	0.241 (0.223-0.271)	0.055
CB	5.7 (4.8-7.4)	0.252 (0.228-0.272)	0.044
DB	4.6 (3.5-6.3)	0.269 (0.229-0.316)	0.058
DS	1.7 (1.3-2.4)	0.193 (0.163-0.220)	0.114
OC	5.6 (5.1-6.1)	0.260 (0.241-0.283)	0.046
TN	6.3 (5.1-6.9)	0.265 (0.238-0.290)	0.042
VM	7.5*	0.361 (0.323-0.389)	0.048
WB	3.5 (3.4-3.8)	0.199 (0.193-0.218)	0.057
YO	4.7 (4.1-5.5)	0.259 (0.238-0.281)	0.055
Mean	**4.88 (3.88-5.52)**	**0.361 (0.225-0.276)**	**0.056**
s.d.	**1.58 (1.17-1.56)**	**0.050 (0.045-0.052)**	**0.021**

* = fixed volume used as no intravenous data available

Table 4.8: The distribution volume, clearance and the elimination rate constant of 4ASA calculated by MAXENT approach. V_d (volume of distribution in liter); K_{el} (elimination rate constant in min^{-1}); Cl (clearance in $lmin^{-1}$). Mean and inter-quartile ranges.

Figure 4.2

Combined graphical presentation of gastric emptying, pH and pharmacokinetic profiles for the first two hours after administration of 4ASA liquid formulations

The following abbreviations are used to identify the curves in figures 4.2.1-4.2.10 (a-d):

apt	(+)	Gastric emptying profile from APT Mark I program
pH	(-)	pH profile from pH-sensitive radiotelemetry
c34	(■)	Measured 4ASA blood concentration for pH3 formulation without ranitidine treatment
c34a	(■)	Measured 4ASA blood concentration for pH3 formulation with ranitidine treatment
c3a	(×)	Measured AASA blood concentration for pH3 formulation without ranitidine treatment
c3aa	(×)	Measured AASA blood concentration for pH3 formulation with ranitidine treatment
c34am	(—)	MAXENT model for 4ASA blood concentration of pH3 formulation with ranitidine treatment
c34m	(—)	MAXENT model for 4ASA blood concentration of pH3 formulation without ranitidine treatment
c74am	(—)	MAXENT model for 4ASA blood concentration of pH7 formulation with ranitidine treatment
c74m	(—)	MAXENT model for 4ASA blood concentration of pH7 formulation without ranitidine treatment
c74	(■)	Measured 4ASA blood concentration for pH7 formulation without ranitidine treatment
c74a	(■)	Measured 4ASA blood concentration for pH7 formulation with ranitidine treatment
c7a	(×)	Measured AASA blood concentration for pH7 formulation without ranitidine treatment
c7aa	(×)	Measured AASA blood concentration for pH7 formulation with ranitidine treatment
r34	(....)	MAXENT model for 4ASA absorption rate of pH3 formulation without ranitidine treatment
r34a	(....)	MAXENT model for 4ASA absorption rate of pH3 formulation with ranitidine treatment
r74	(....)	MAXENT model for 4ASA absorption rate of pH7 formulation without ranitidine treatment
r74a	(....)	MAXENT model for 4ASA absorption rate of pH7 formulation with ranitidine treatment

Figure 4.2.1-10.a: Administration of 4ASA at pH7 without ranitidine treatment

Figure 4.2.1-10.b: Administration of 4ASA at pH7 with ranitidine treatment

Figure 4.2.1-10.c: Administration of 4ASA at pH3 without ranitidine treatment

Figure 4.2.1-10.d: Administration of 4ASA at pH3 with ranitidine treatment

Where 1-10 are the ten subjects

Figure 4.2.1.a:
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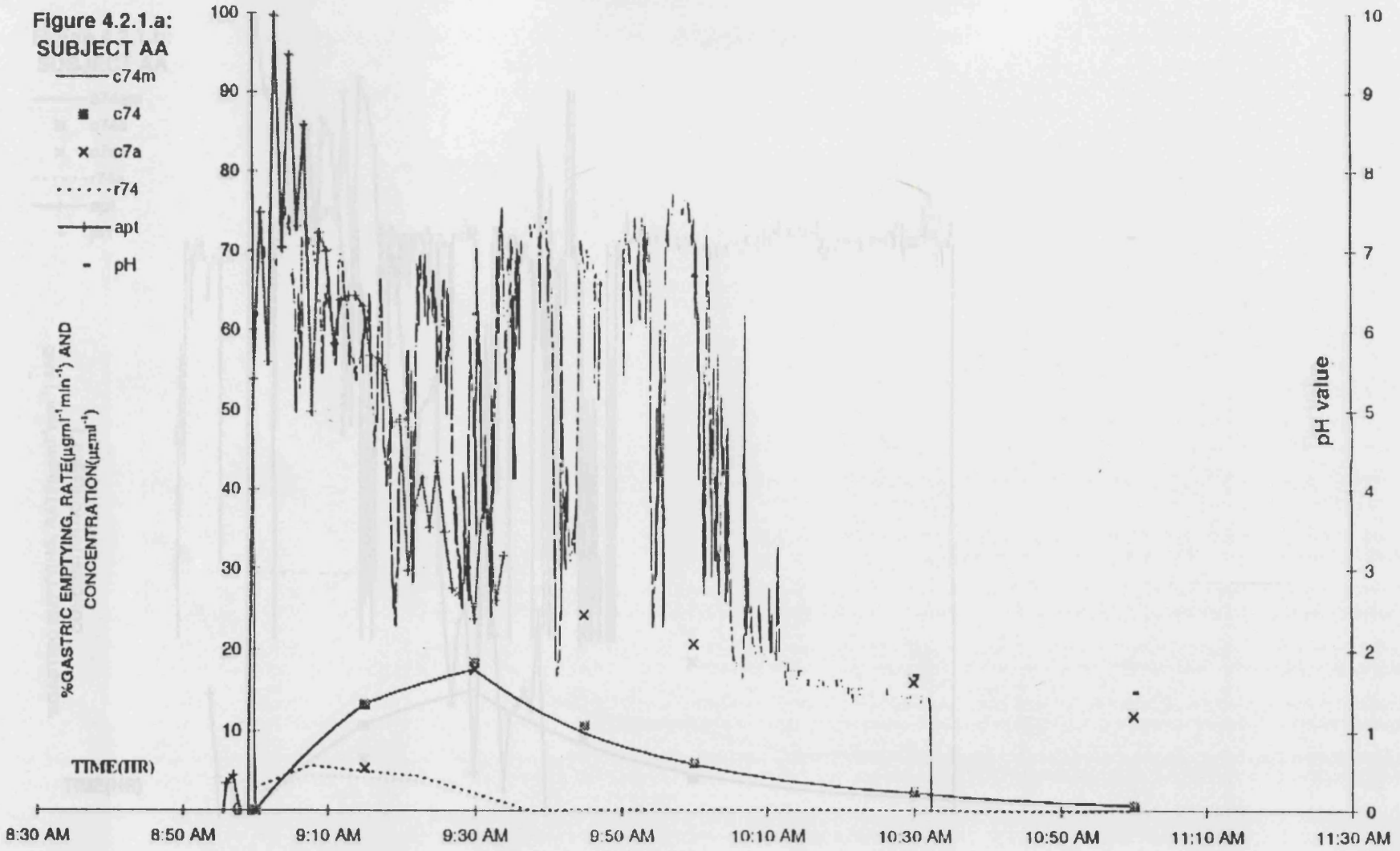

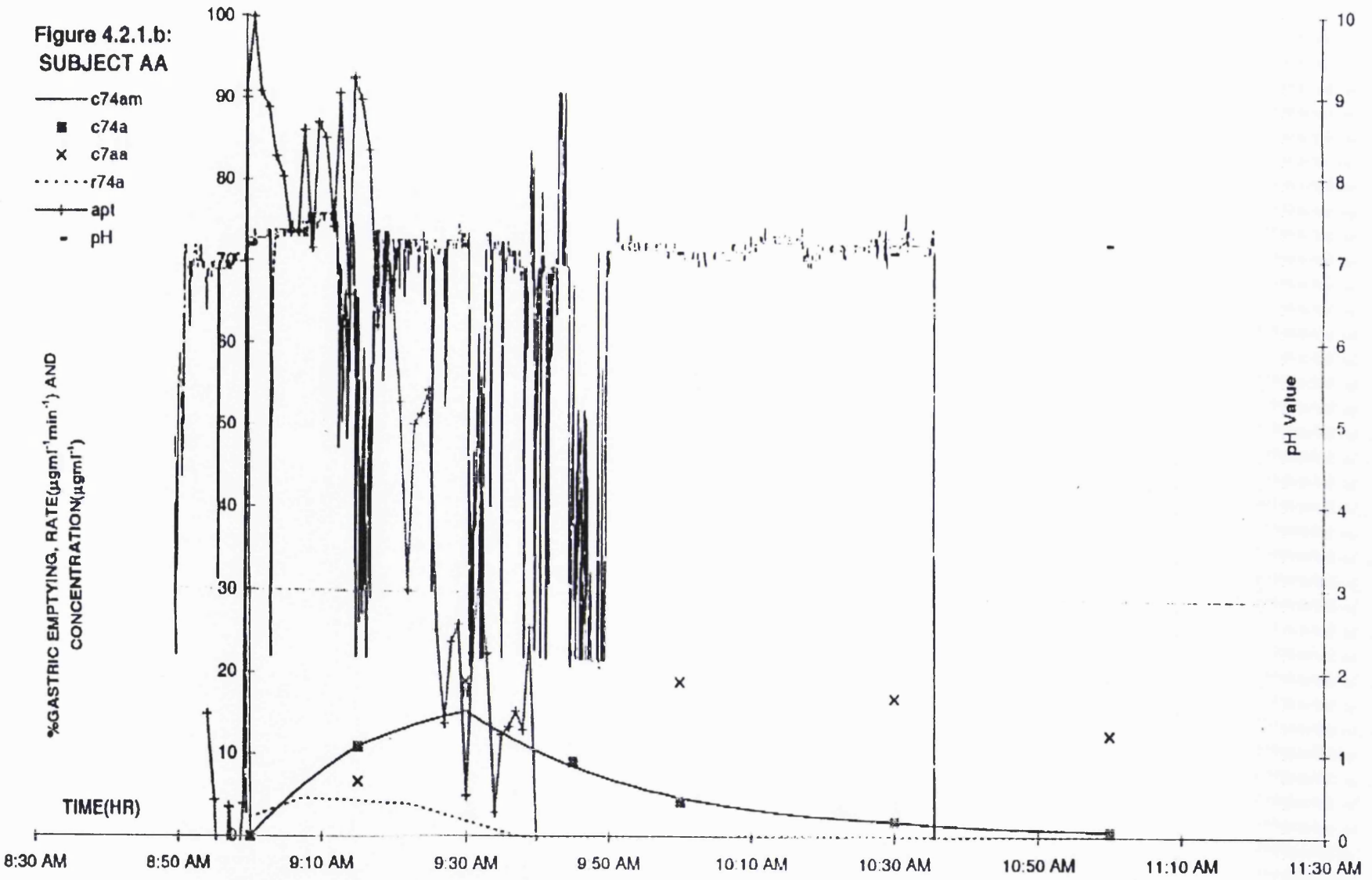
Figure 4.2.1.b:
SUBJECT AA
c74am
c74a
c7aa
r74a
apt
pH
%GASTRIC EMPTYING, RATE(µgml⁻¹min⁻¹) AND
CONCENTRATION(µgml⁻¹)
pH Value
TIME(HR)
100
90
80
70
60
50
40
30
20
10
0
10
9
8
7
6
5
4
3
2
1
0
8:30 AM
8:50 AM
9:10 AM
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10:10 AM
10:30 AM
10:50 AM
11:10 AM
11:30 AM

Figure4.2.1.c:
SUBJECT AA

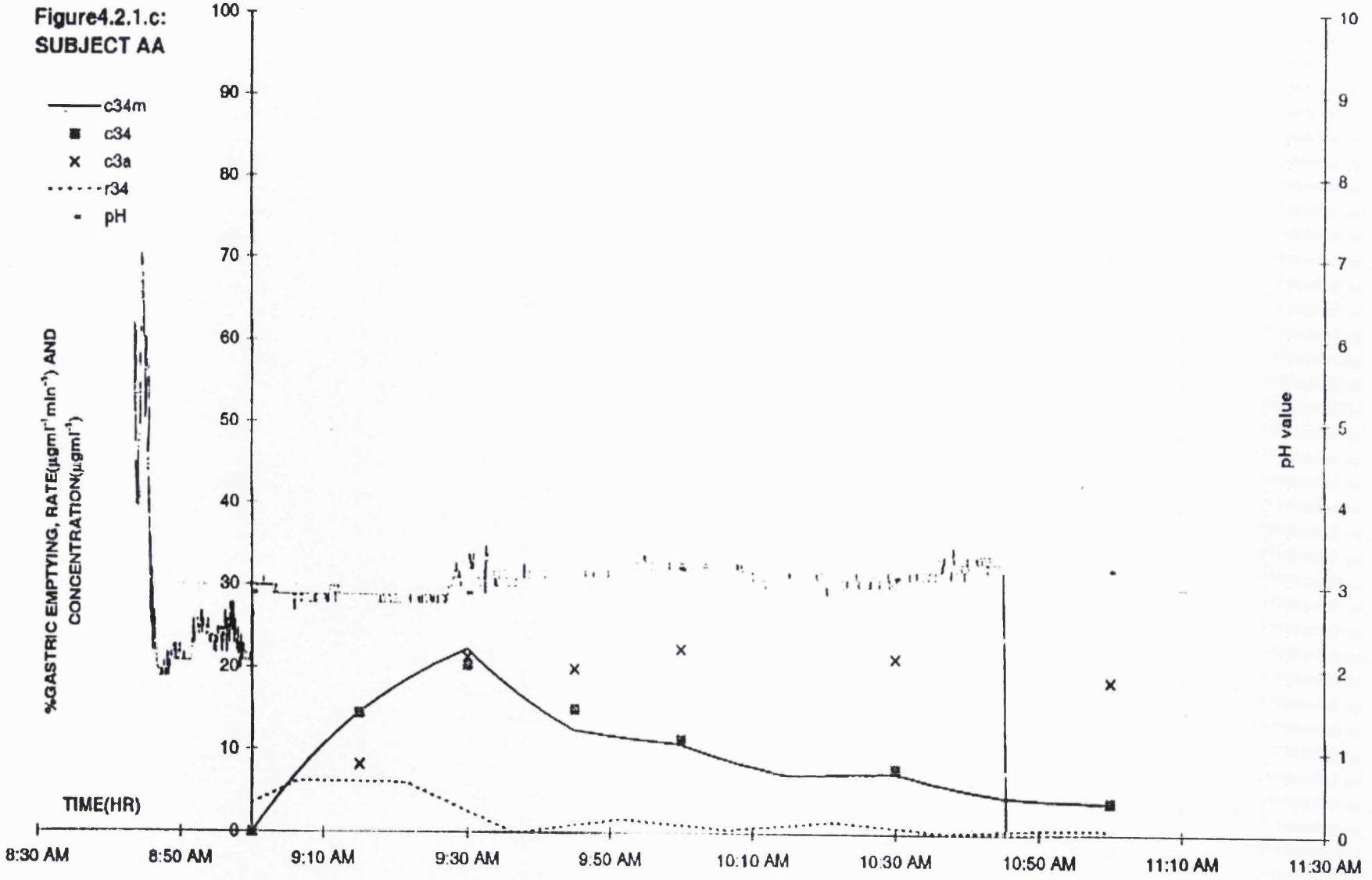

Figure 4.2.1.d:
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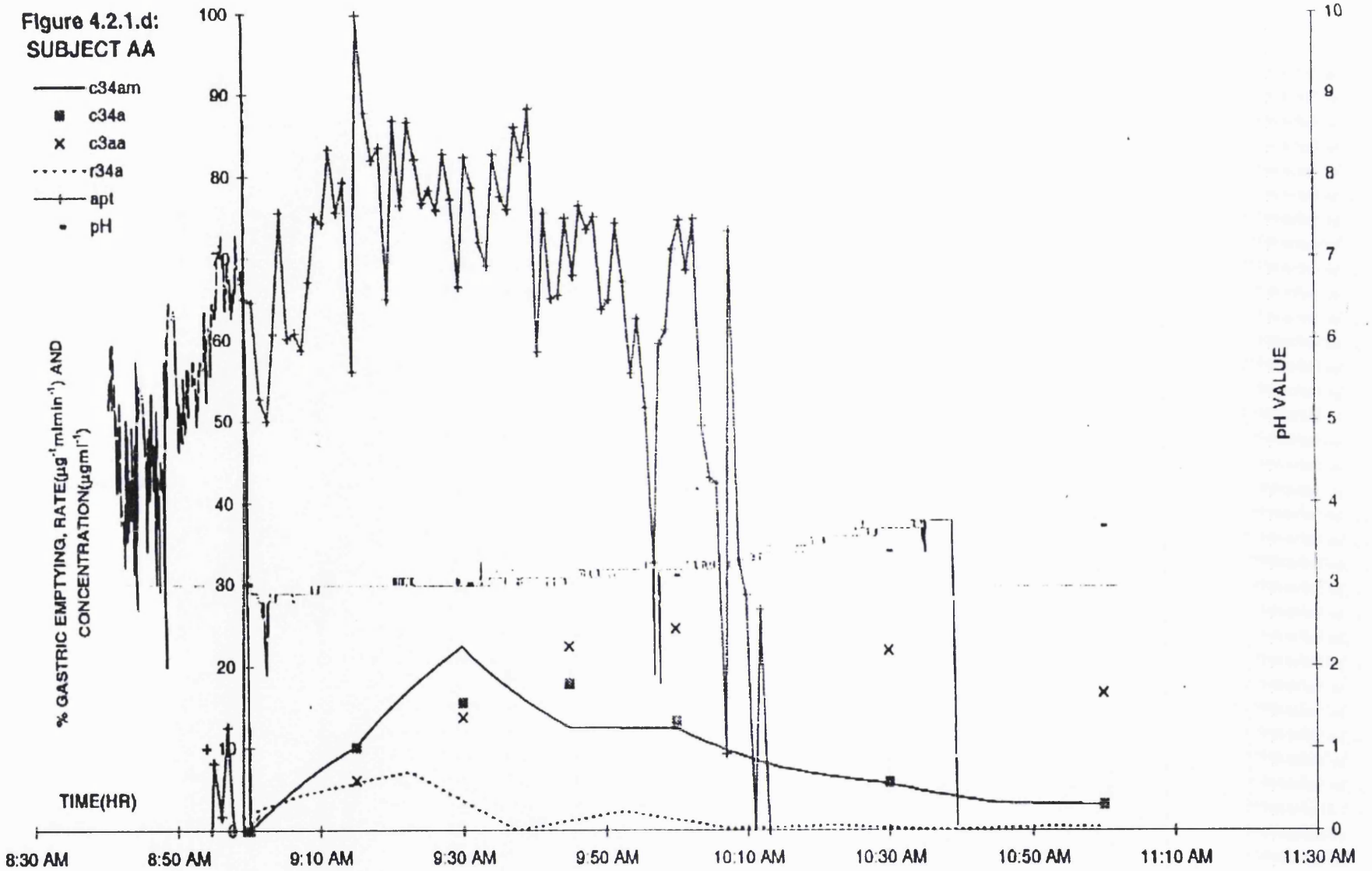
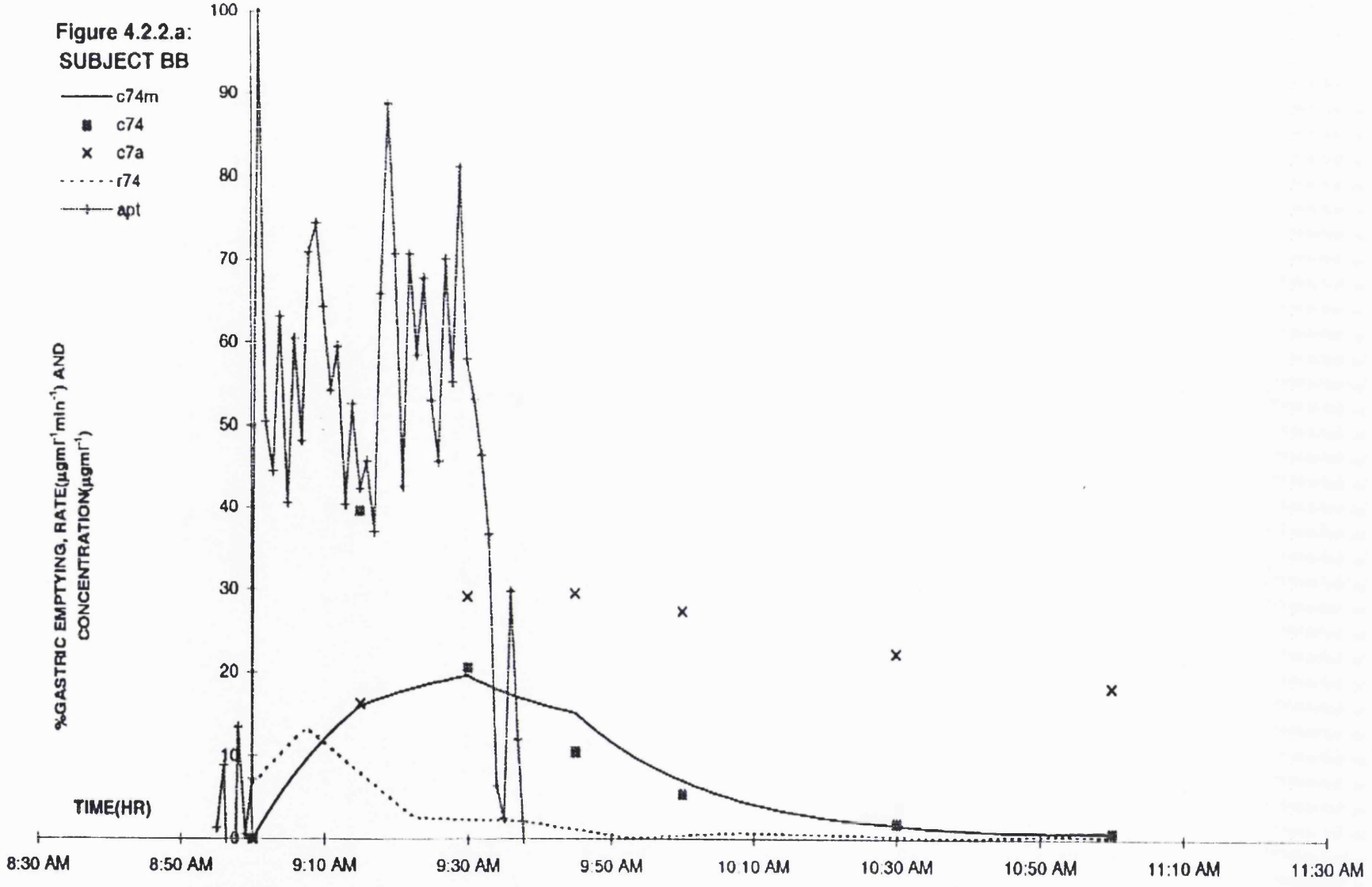


Figure 4.2.2.a:
SUBJECT BB



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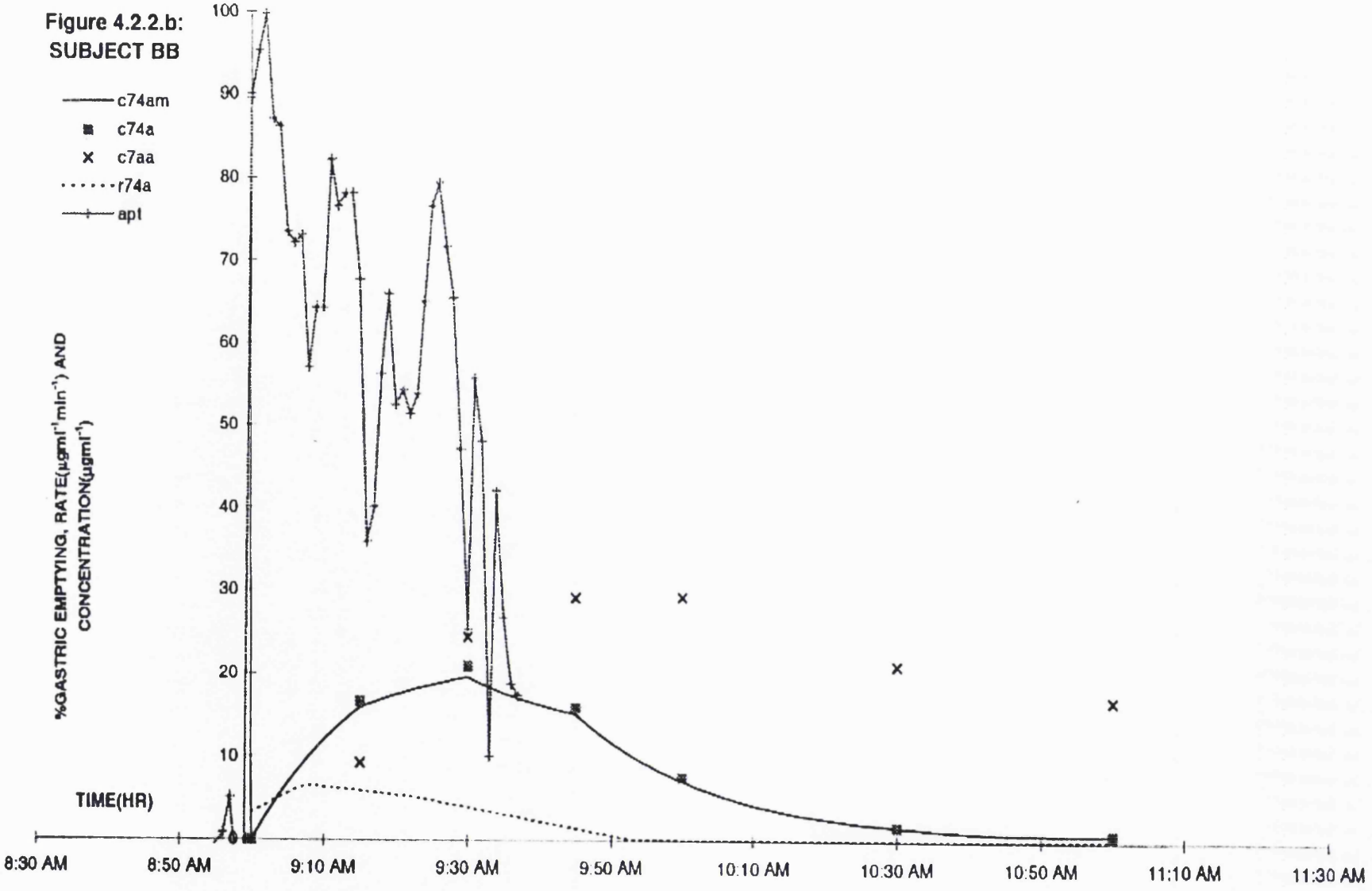

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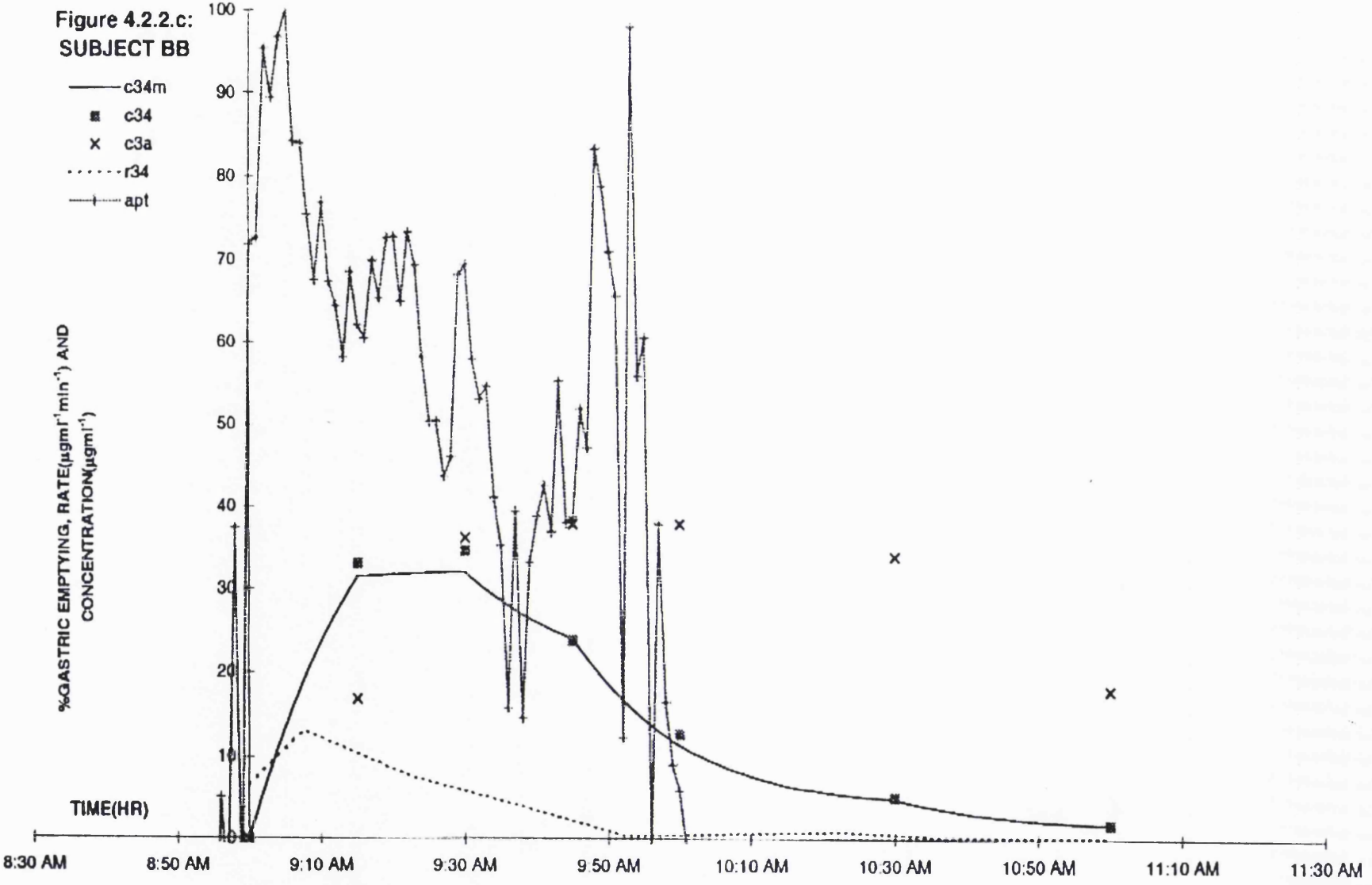

Figure 4.2.2.d:
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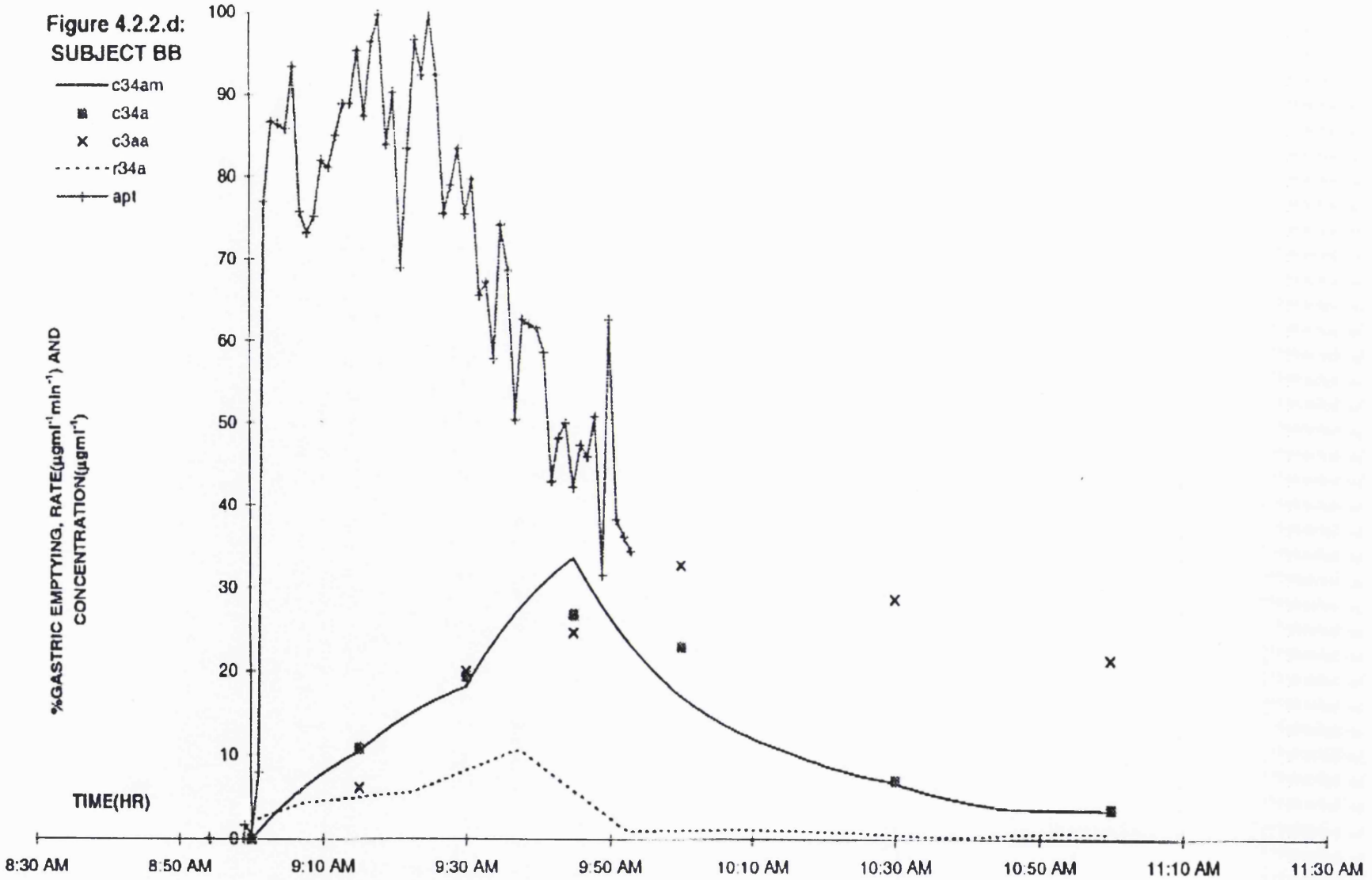

Figure 4.2.3.a:
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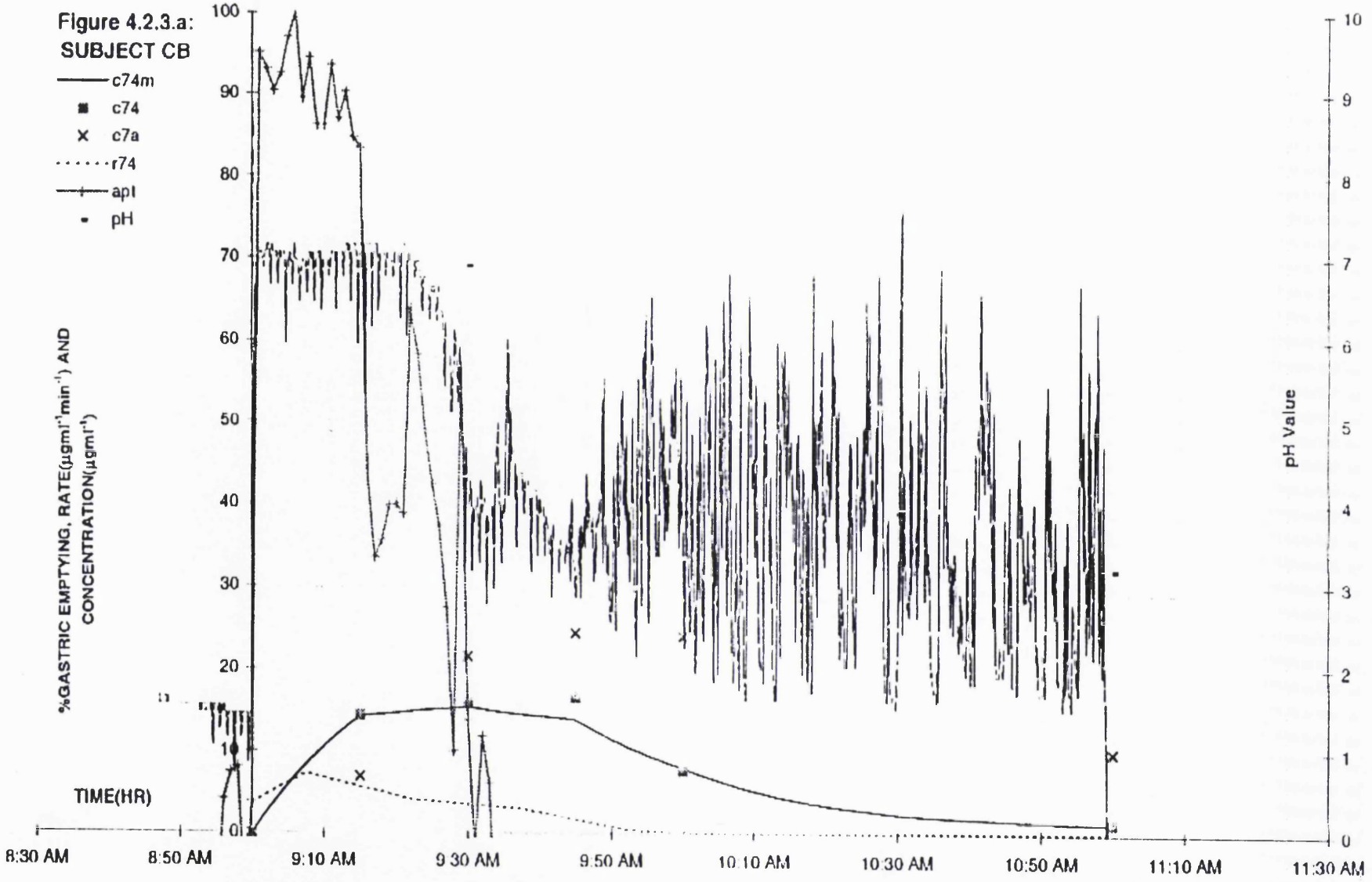

Figure 4.2.3.b:
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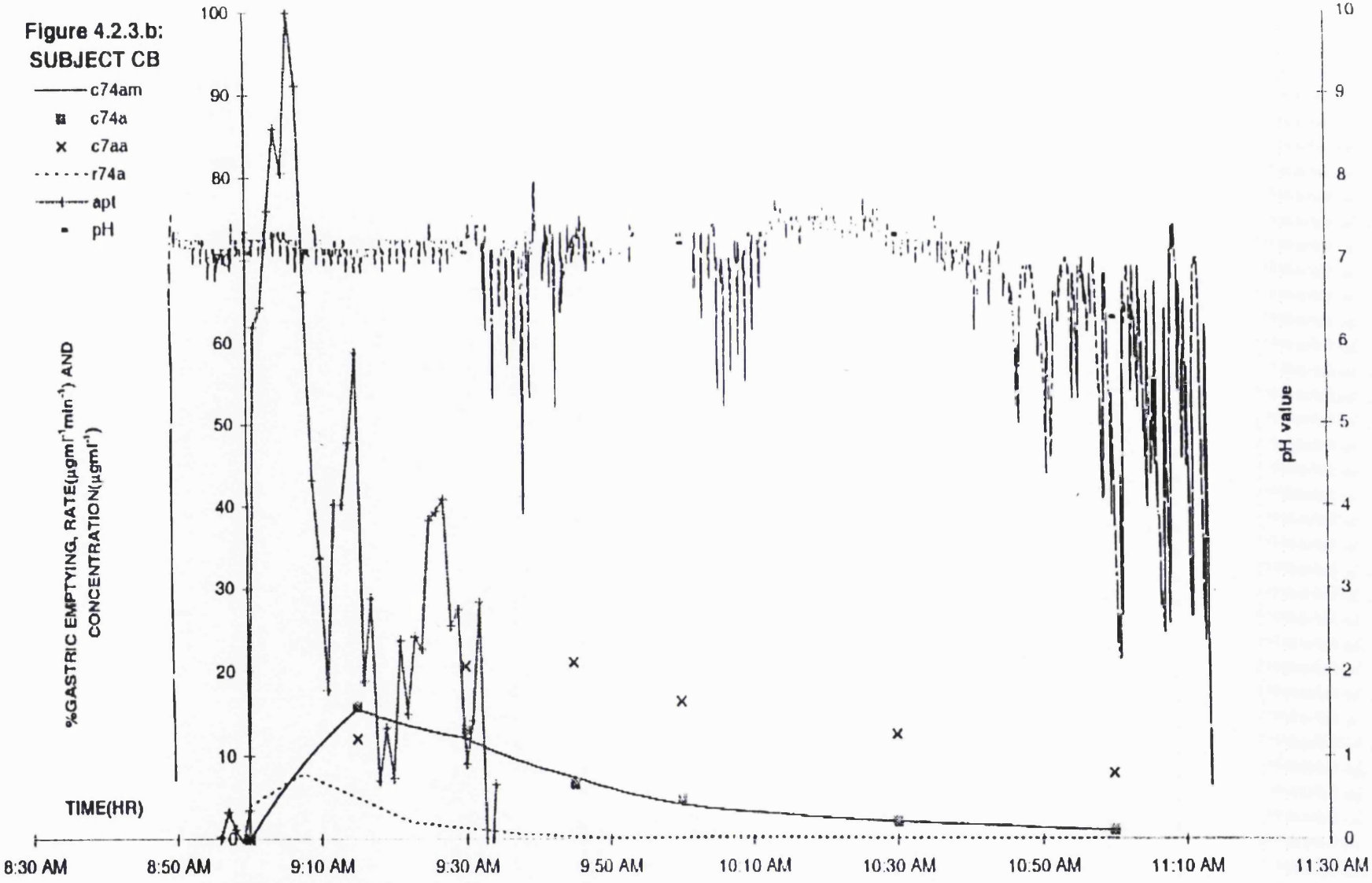

Figure 4.2.3.c:
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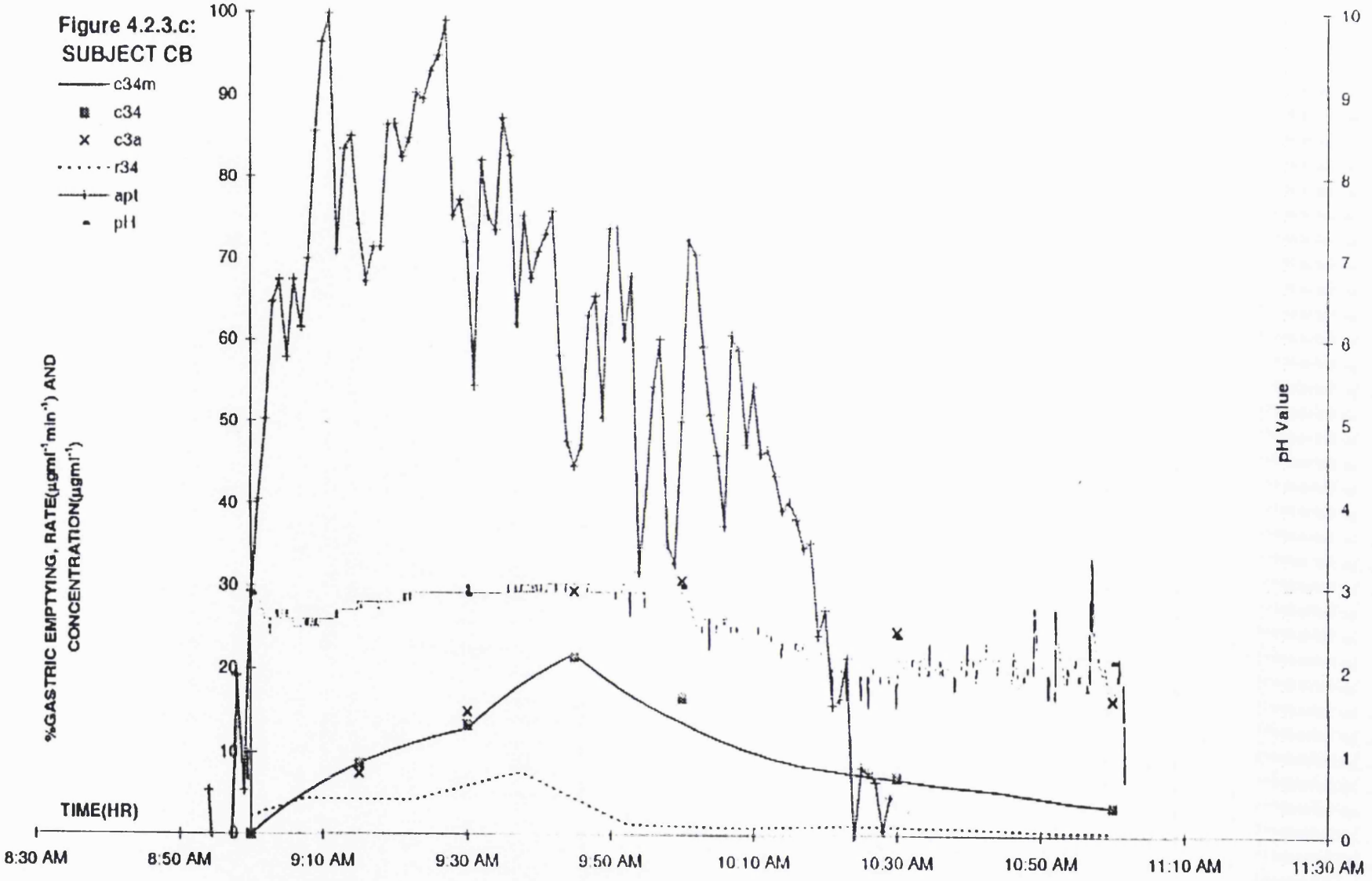

Figure 4.2.3.d: SUBJECT CB

Figure 4.2.4.a:
SUBJECT DB

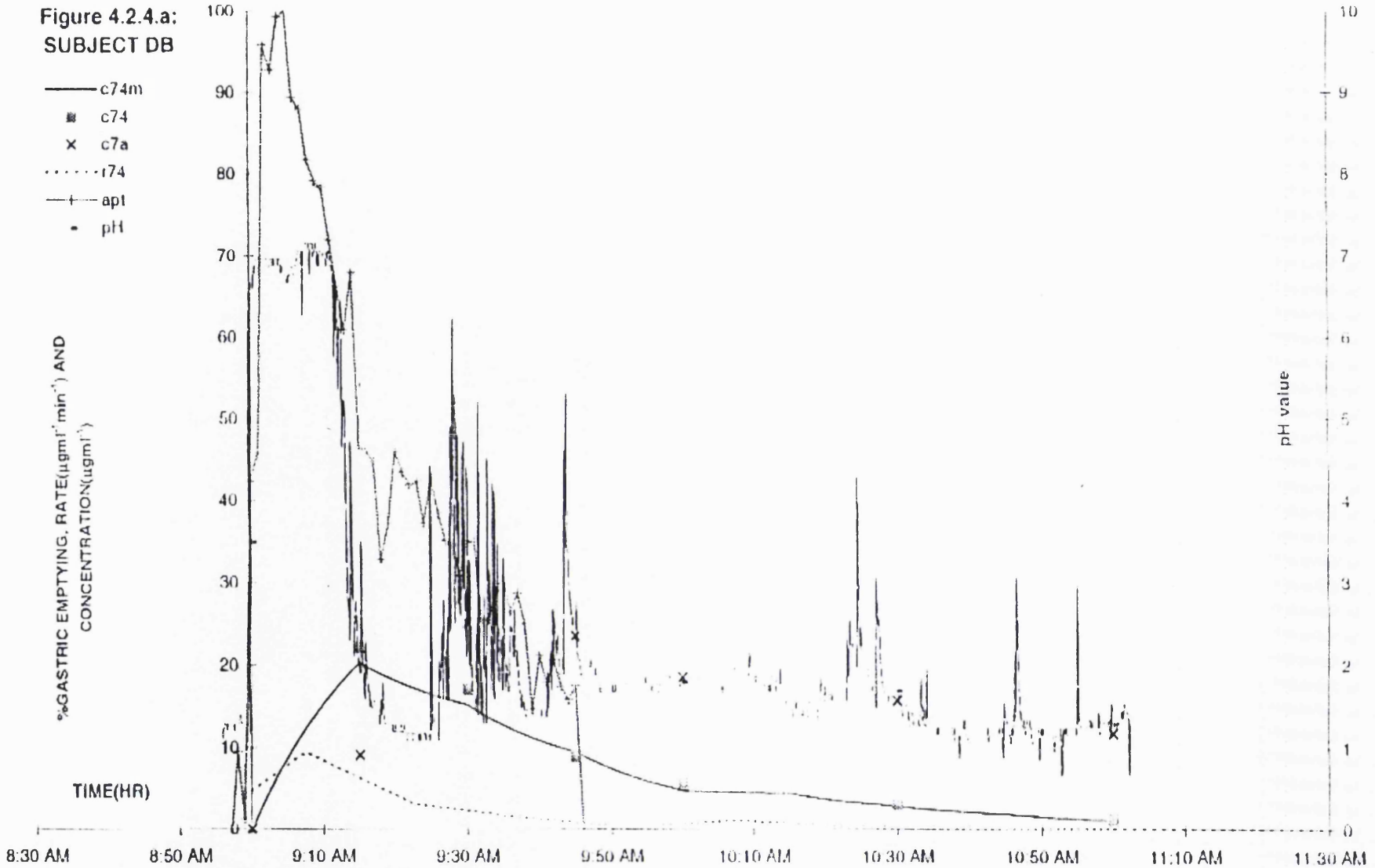

Figure 4.2.4.b:
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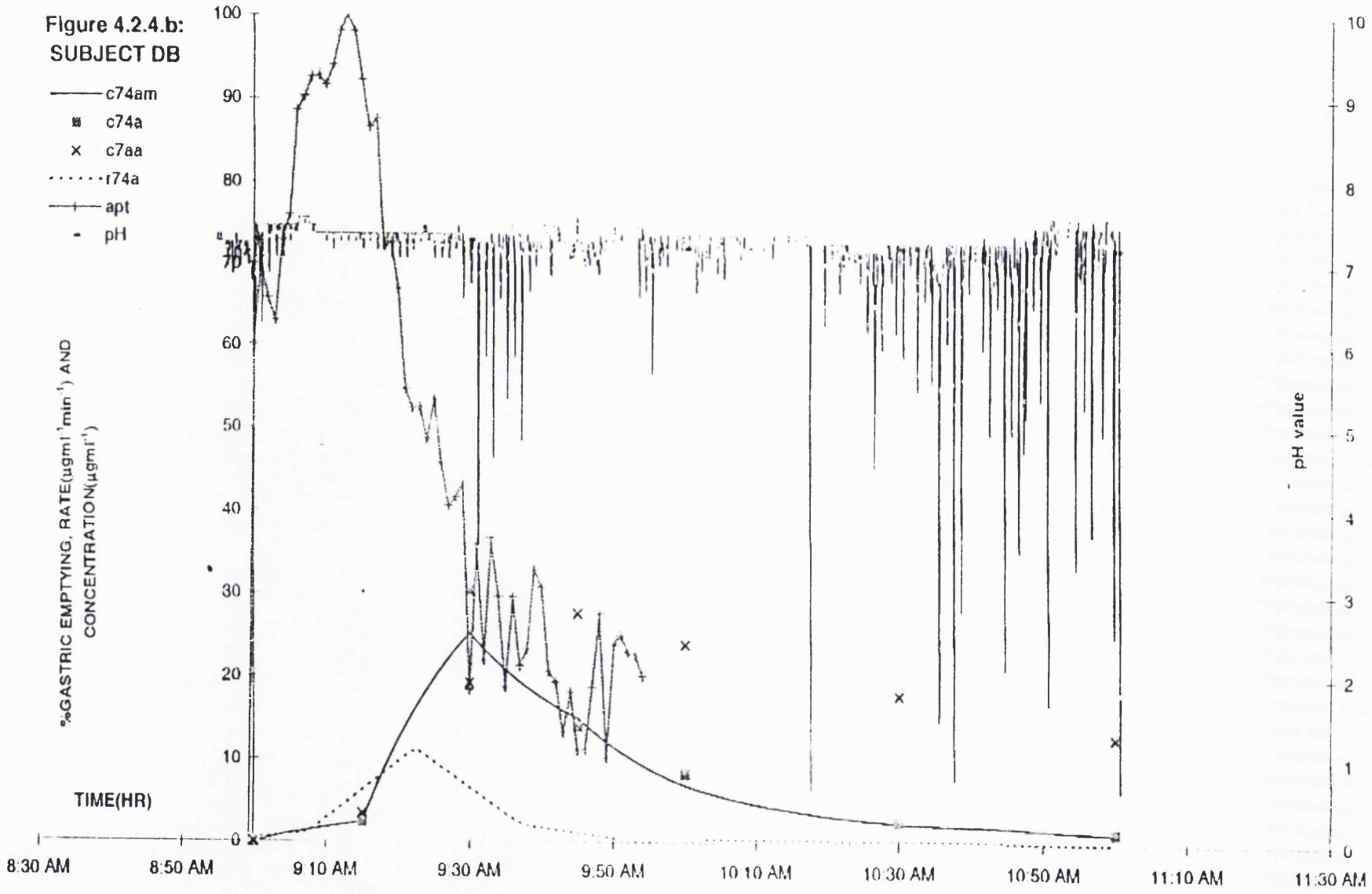

Figure 4.2.4.c:
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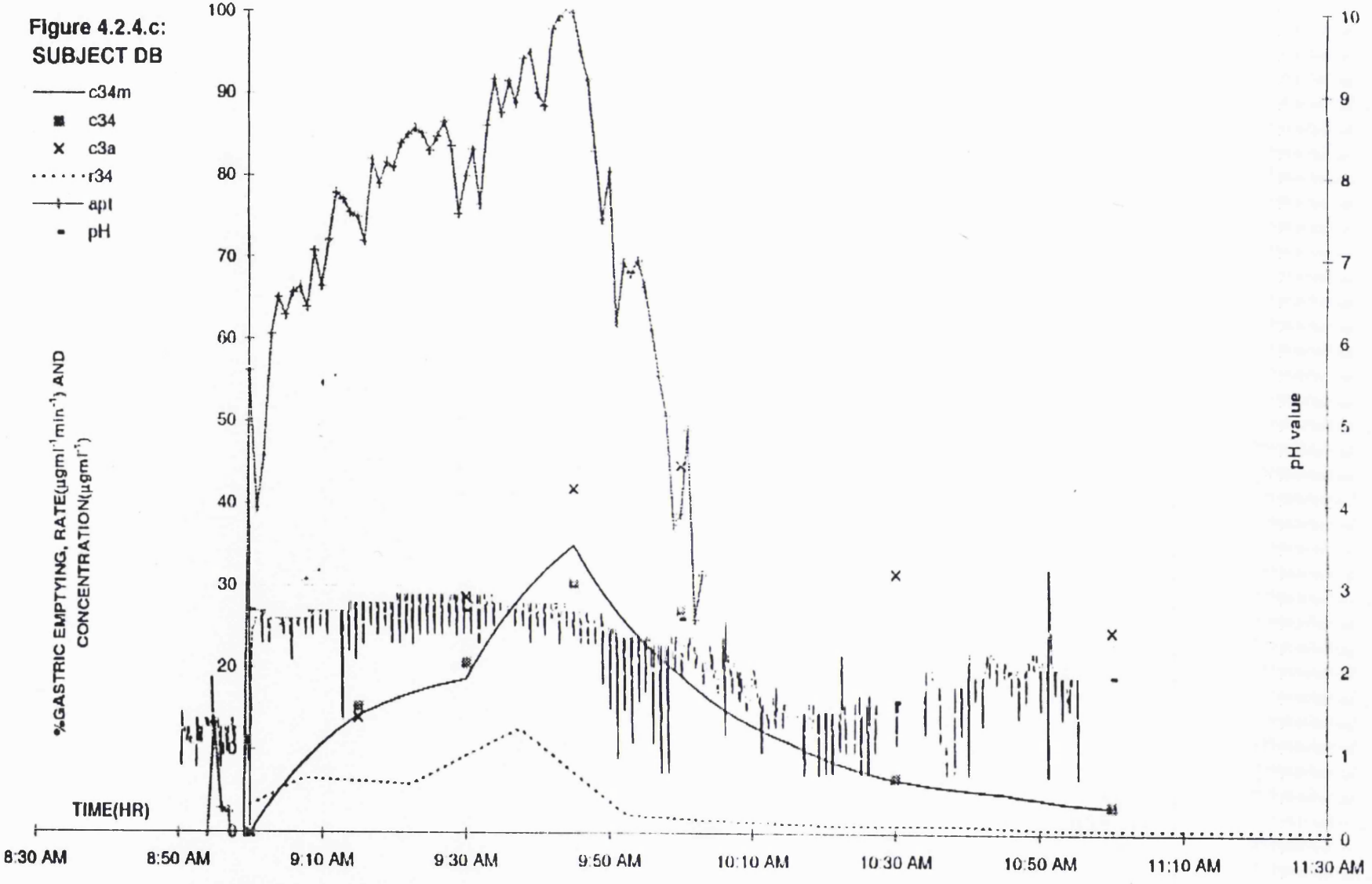

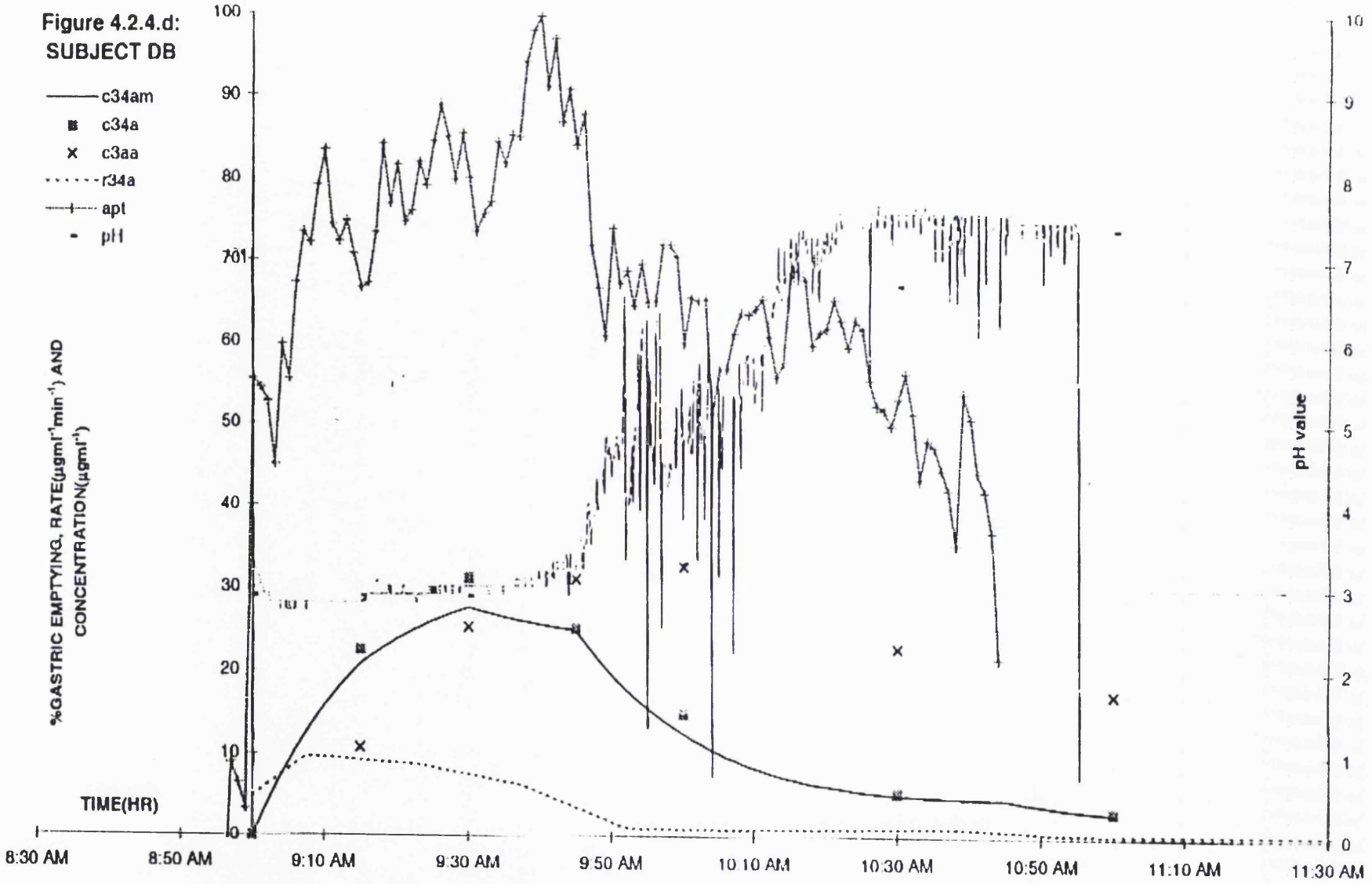
Figure 4.2.4.d:
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c34a
c3aa
r34a
apt
pH
%GASTRIC EMPTYING, RATE(µgml⁻¹min⁻¹) AND CONCENTRATION(µgml⁻¹)
pH value
TIME(HR)
8:30 AM
8:50 AM
9:10 AM
9:30 AM
9:50 AM
10:10 AM
10:30 AM
10:50 AM
11:10 AM
11:30 AM
0
10
20
30
40
50
60
70
80
90
100
0
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2
3
4
5
6
7
8
9
10

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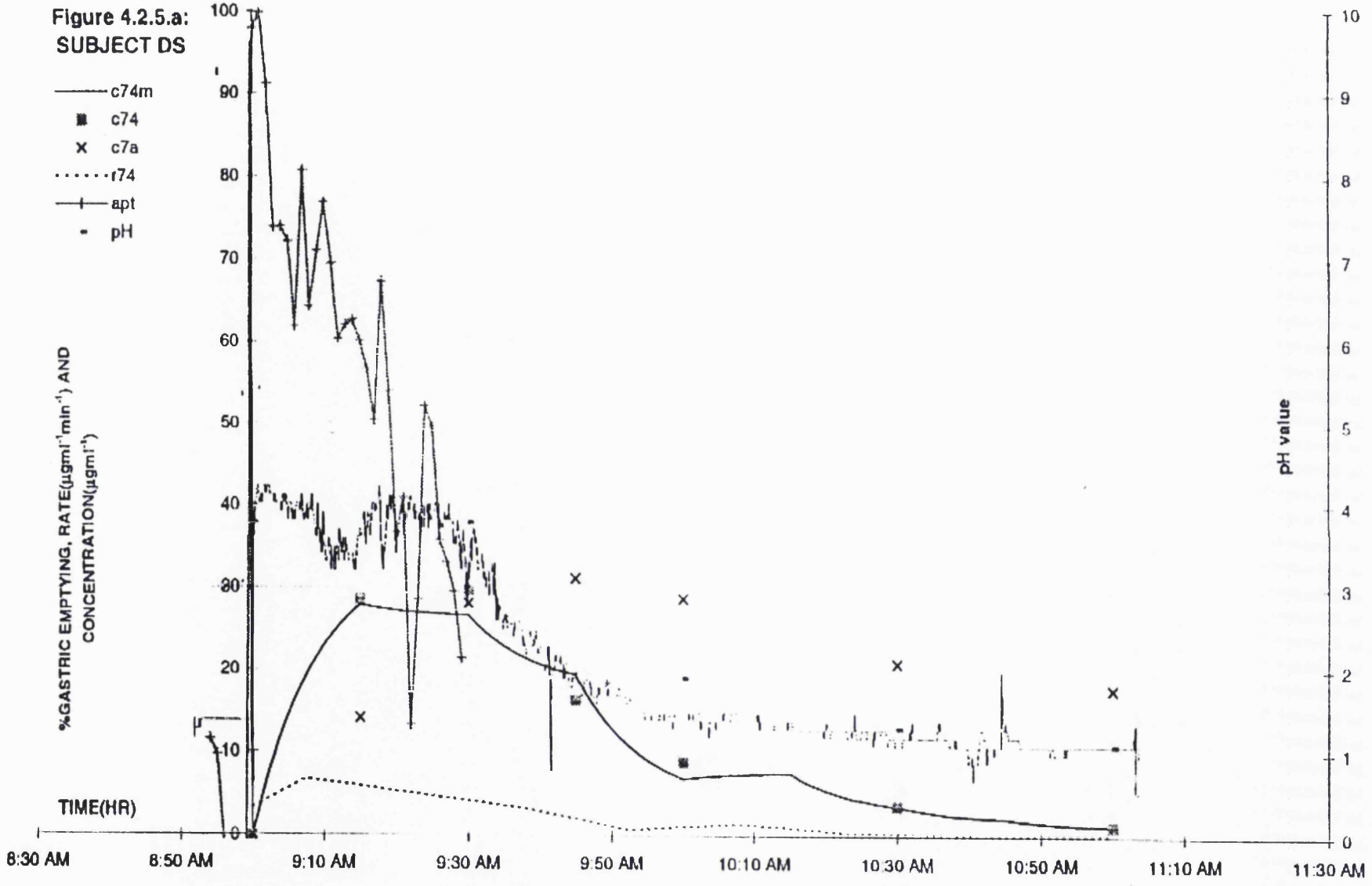

Figure 4.2.5.b:
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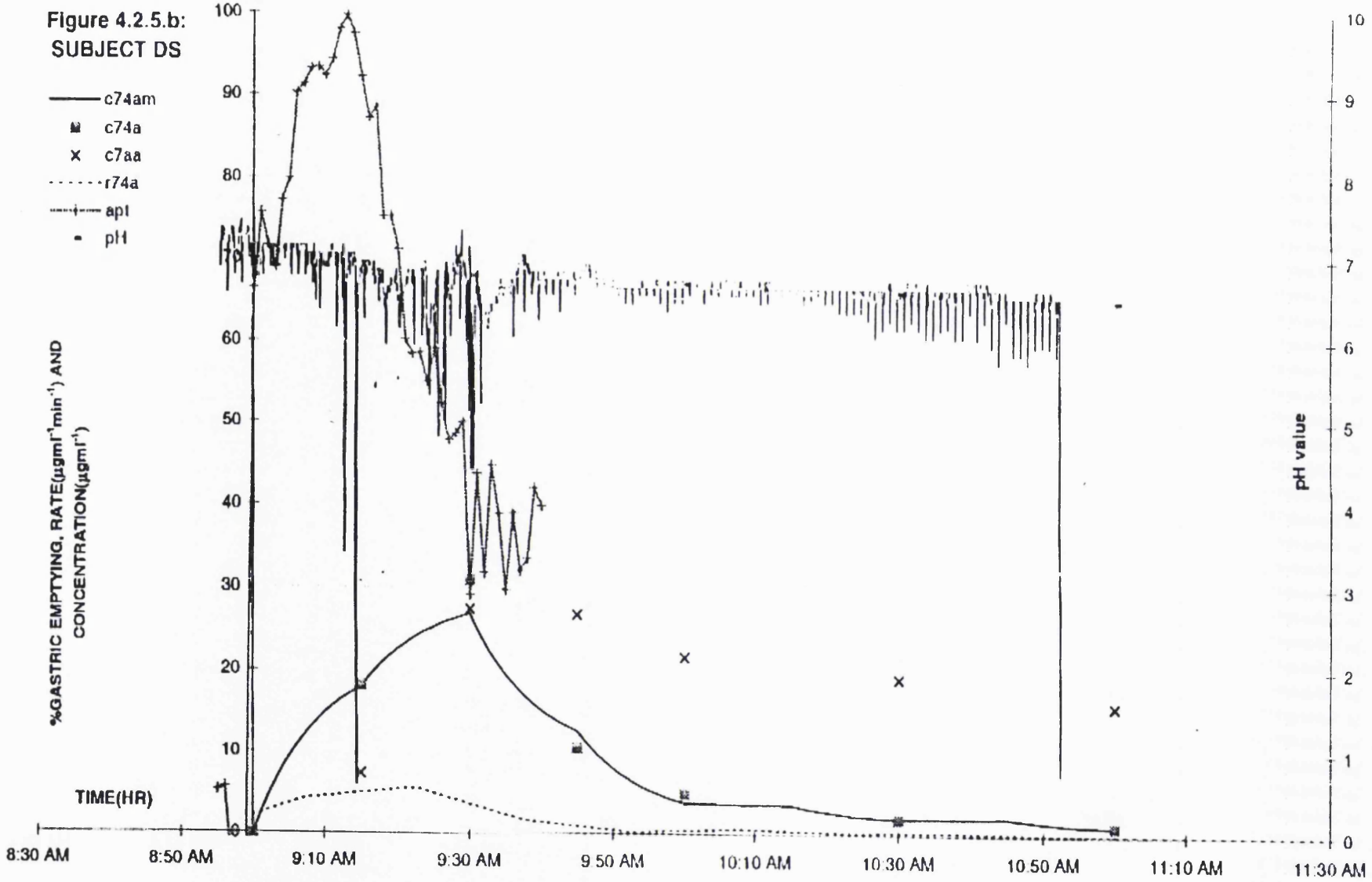

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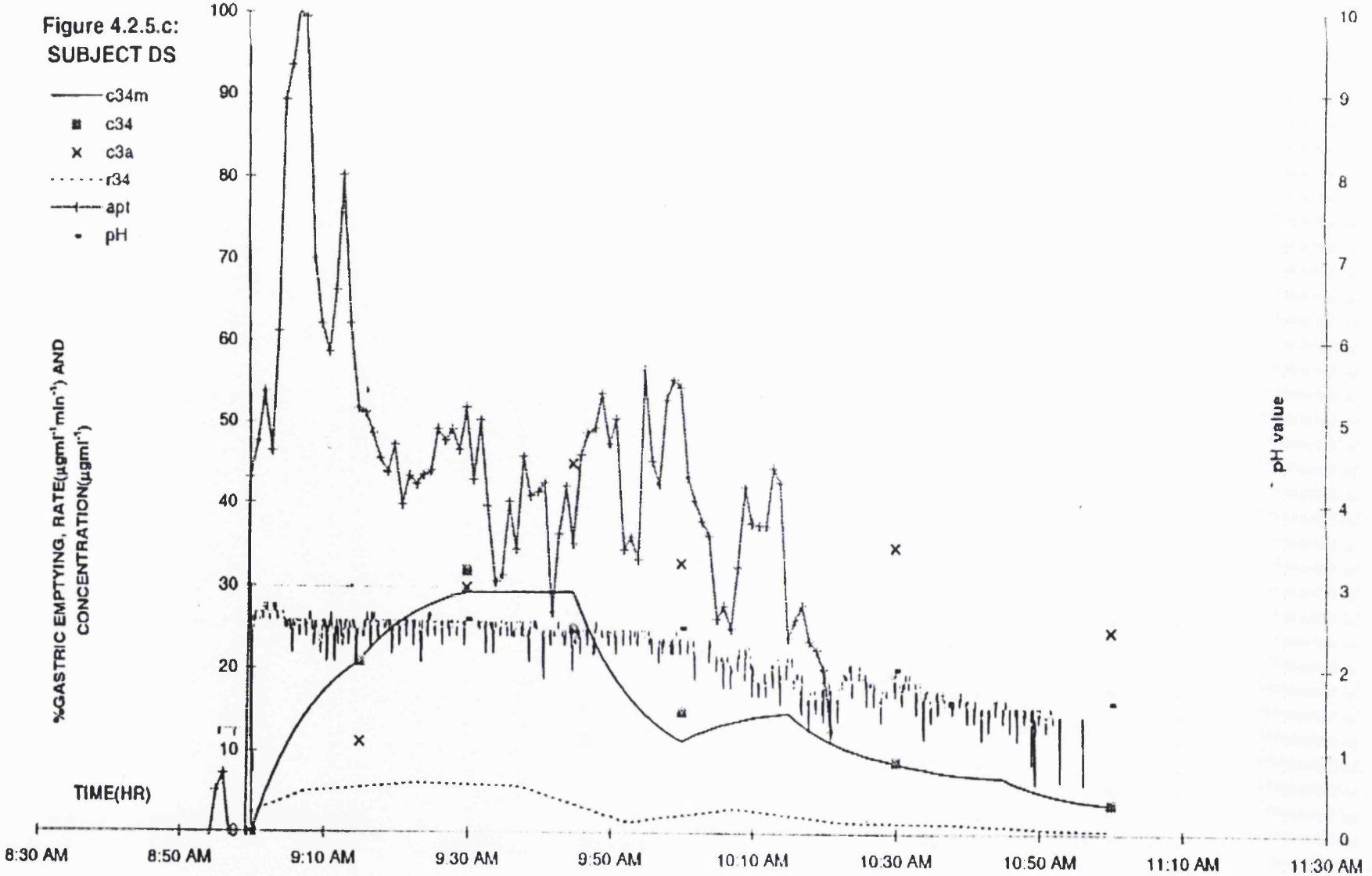

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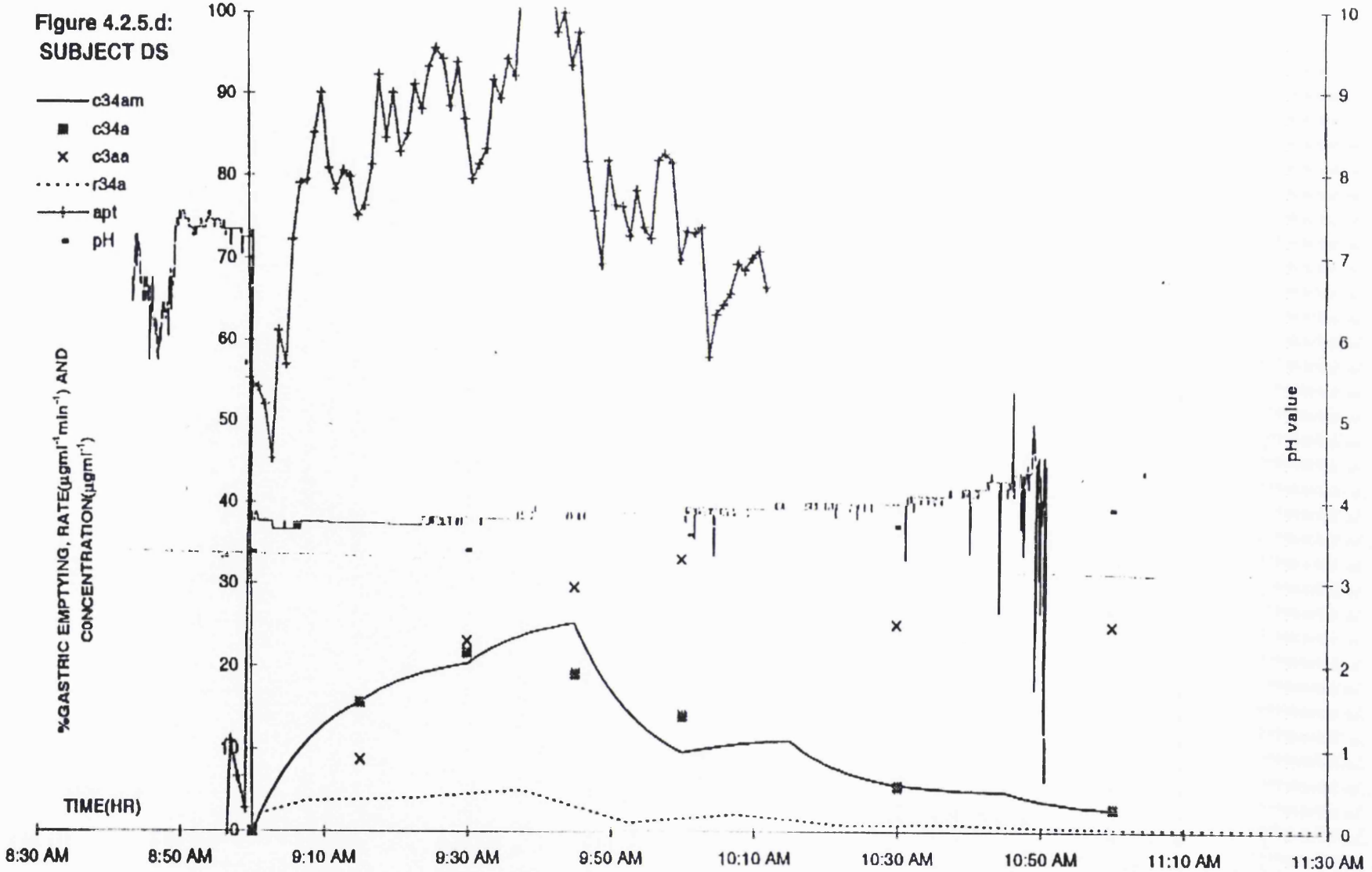

Figure 4.2.6.a:
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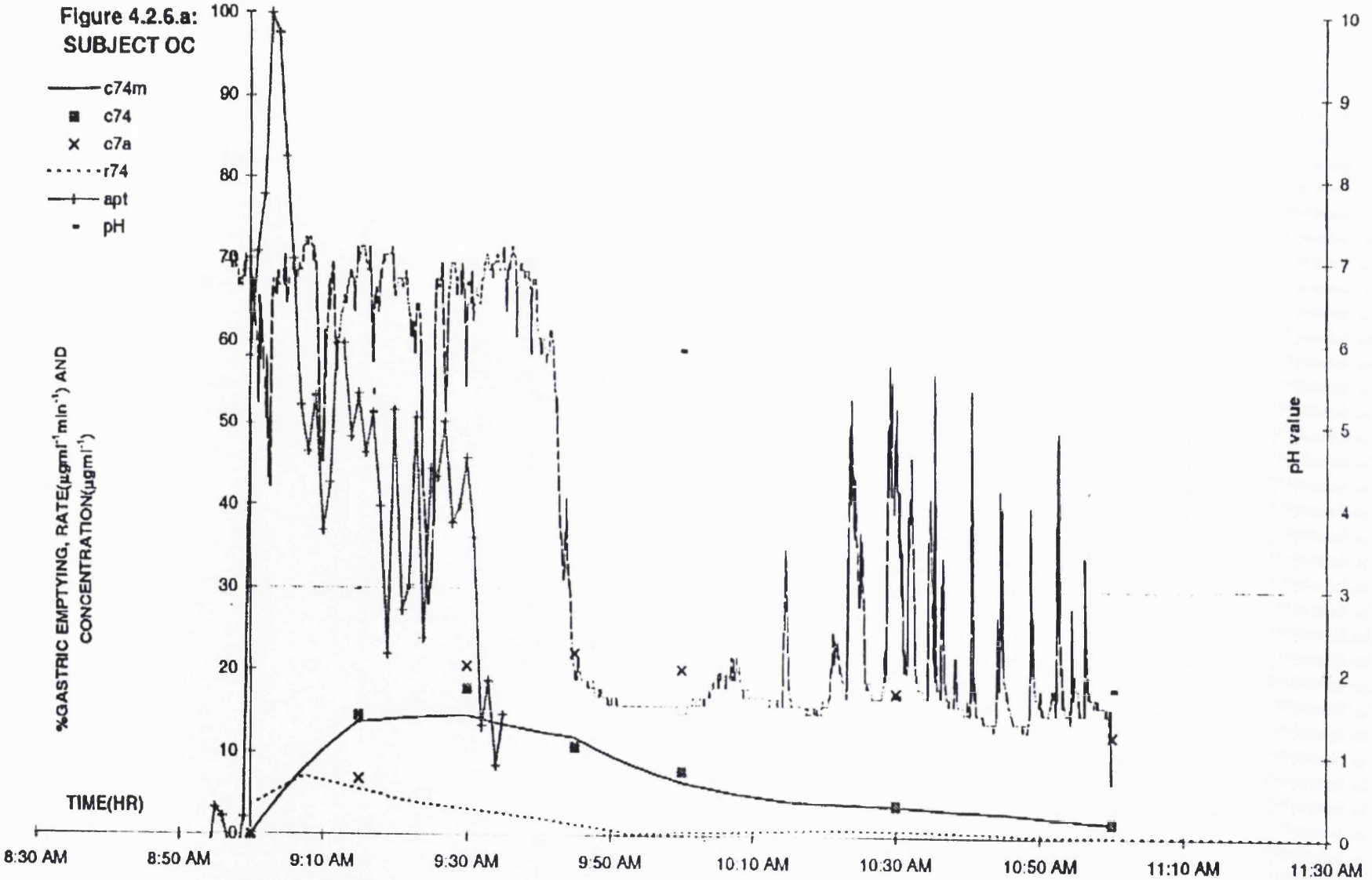

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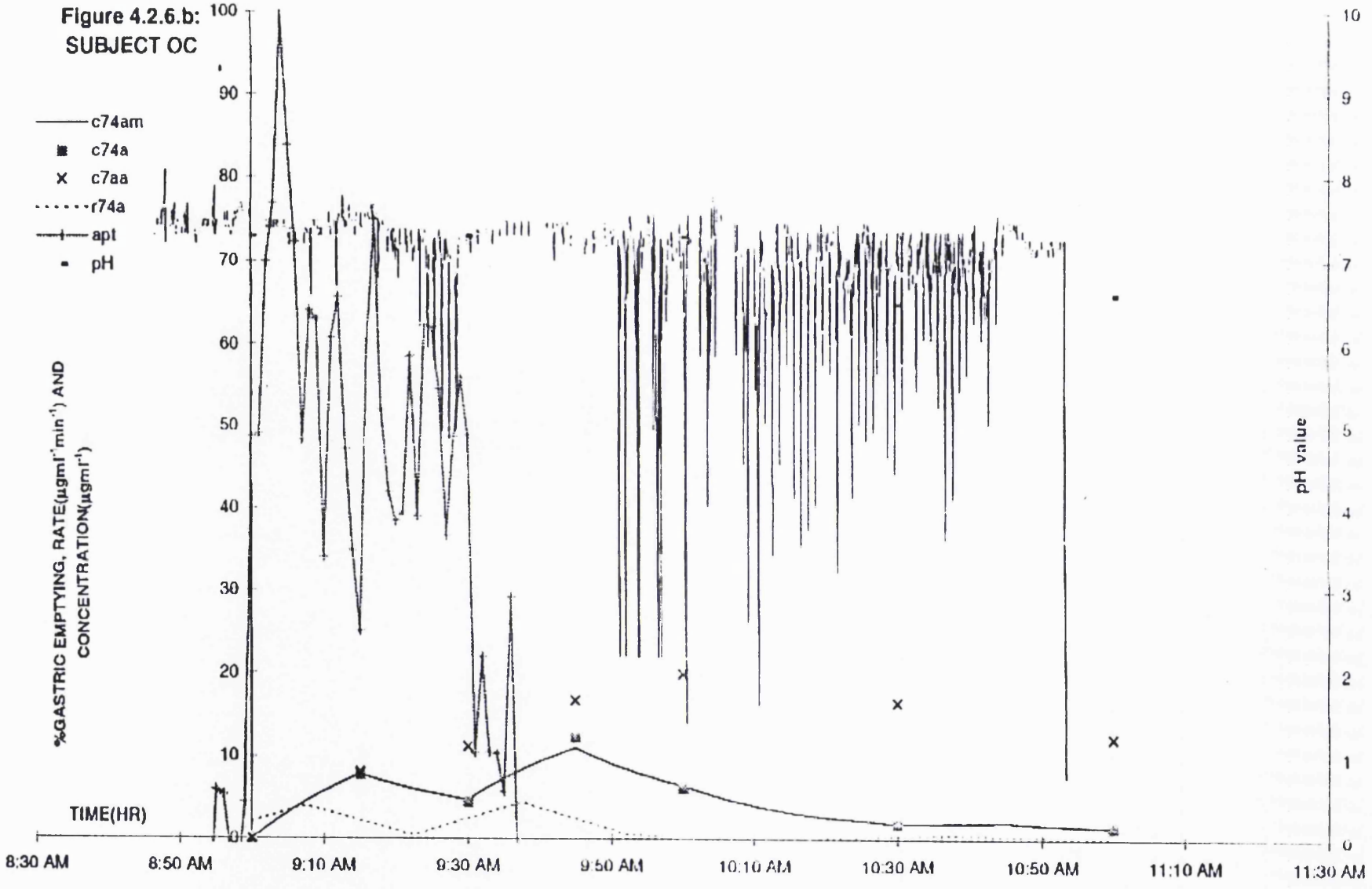

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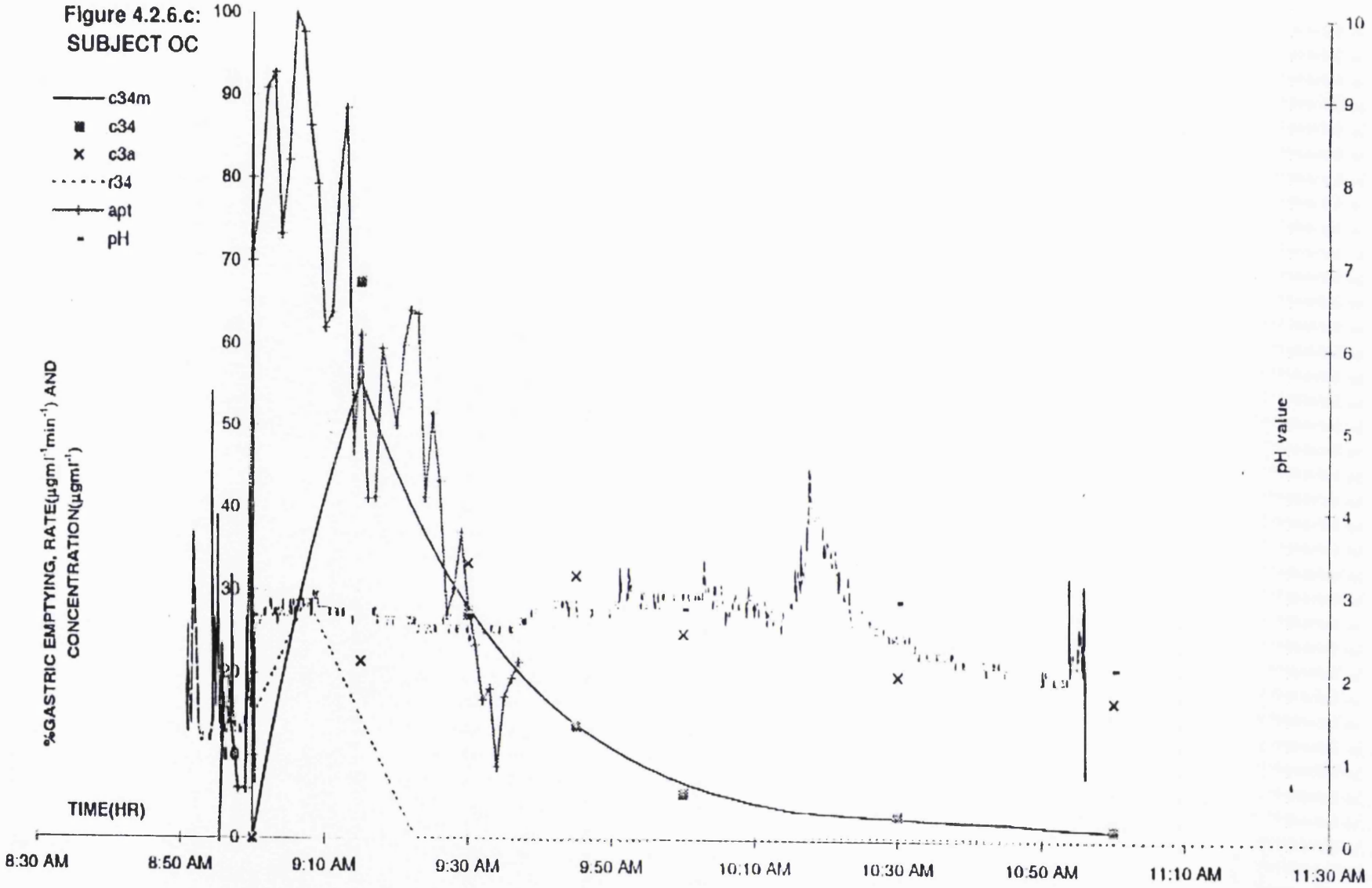

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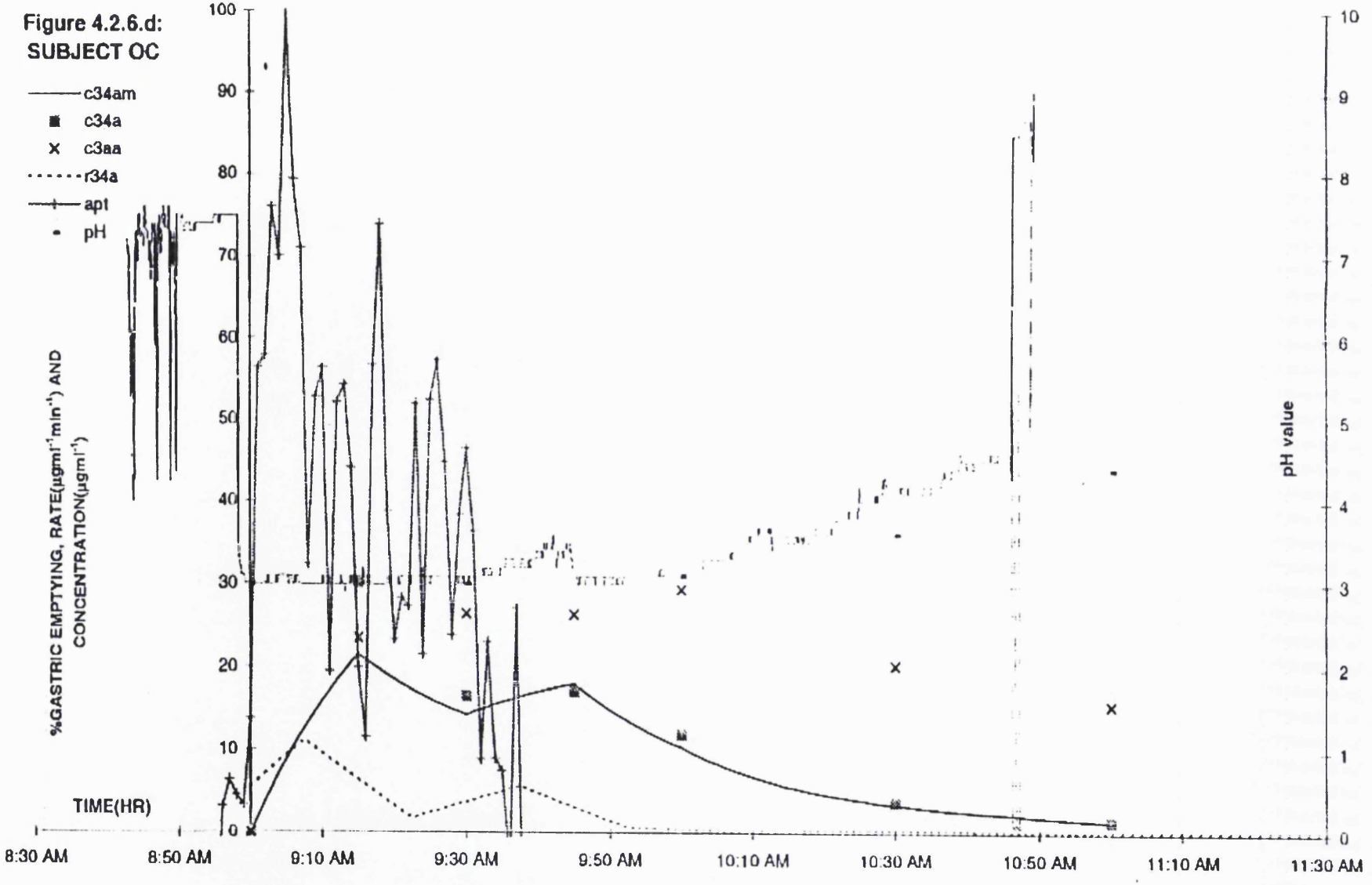


Figure 4.2.7.a:
SUBJECT TN

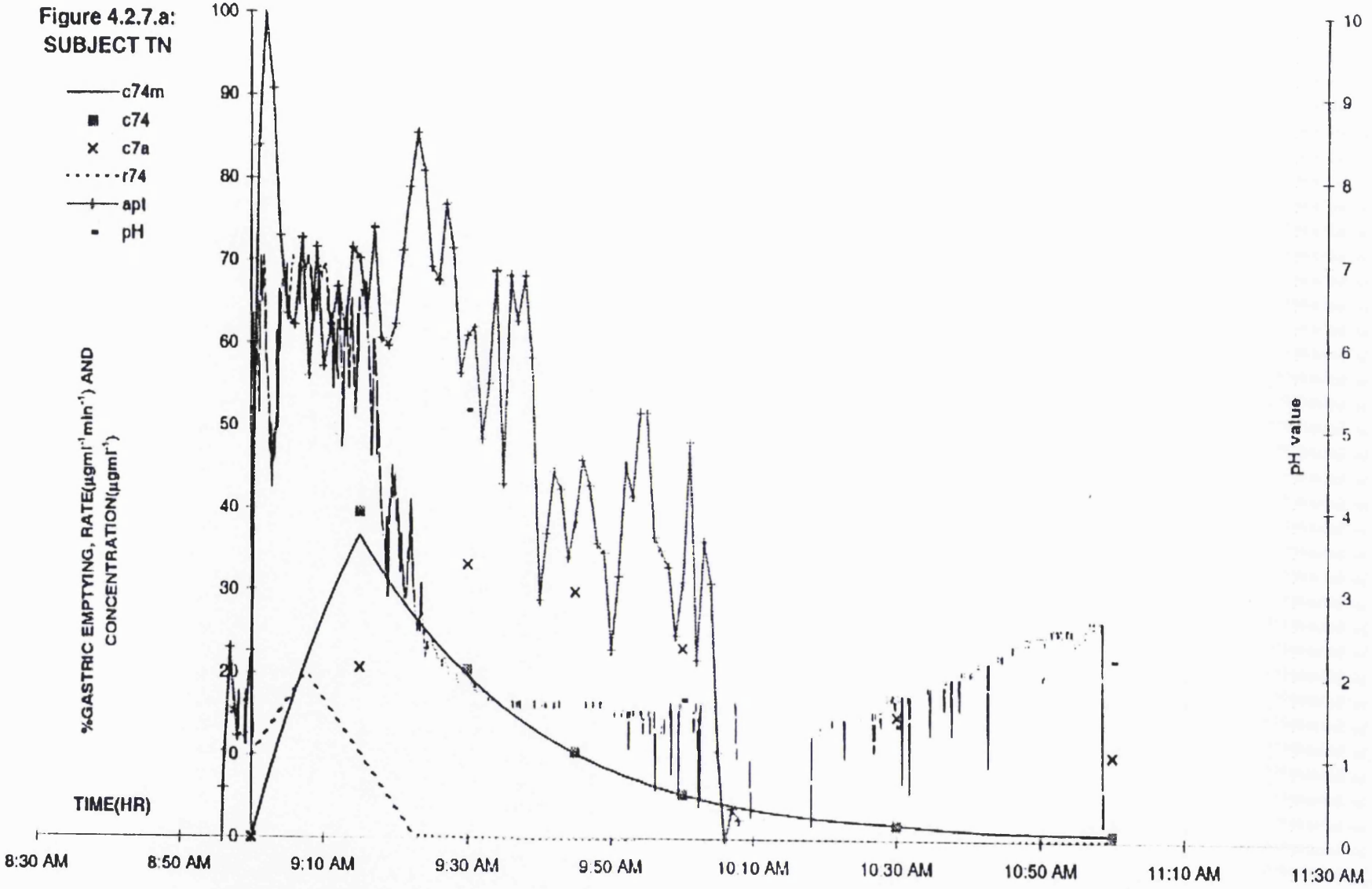


Figure 4.2.7.b:
SUBJECT TN

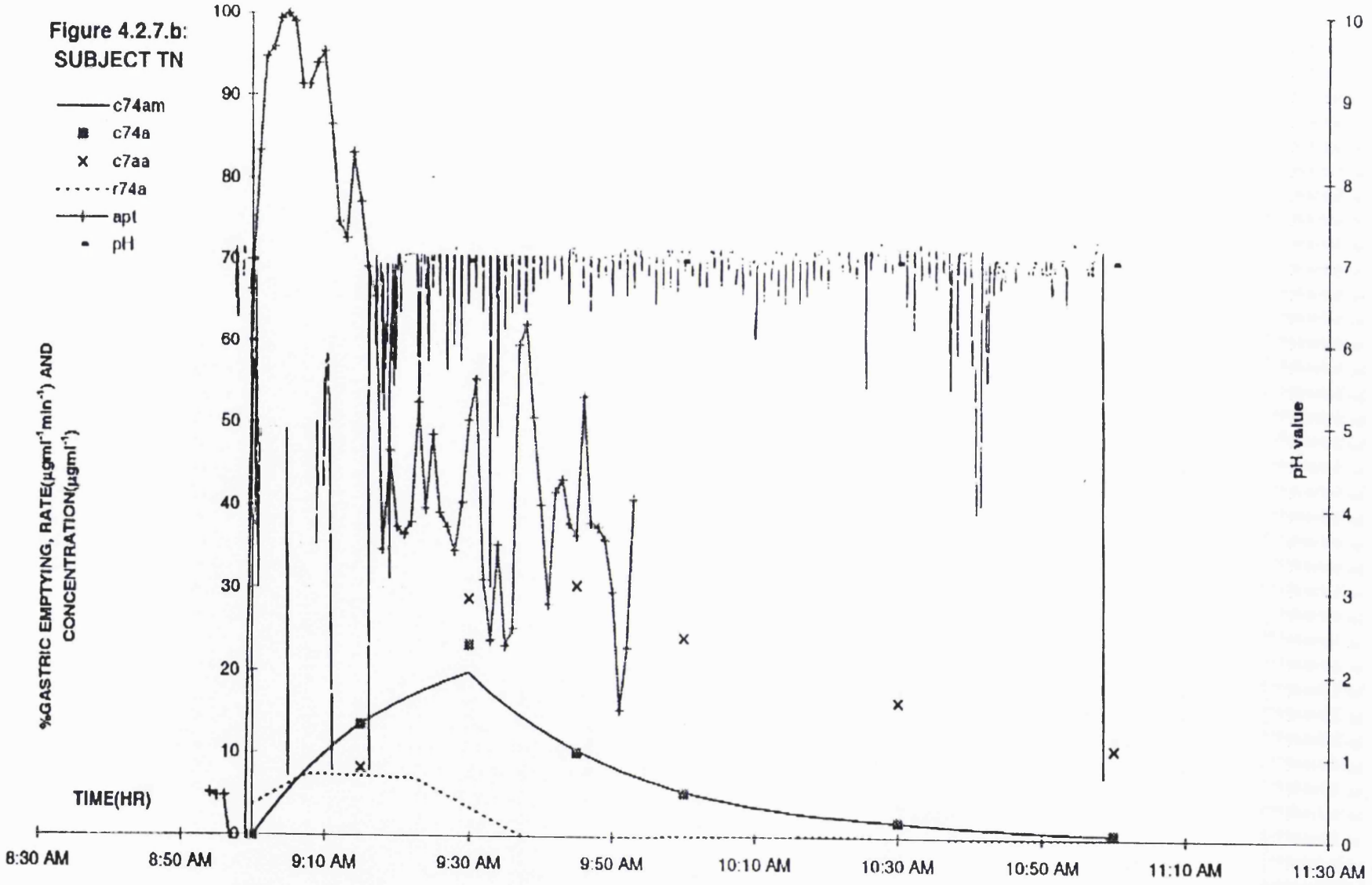


Figure 4.2.7.c:
SUBJECT TN

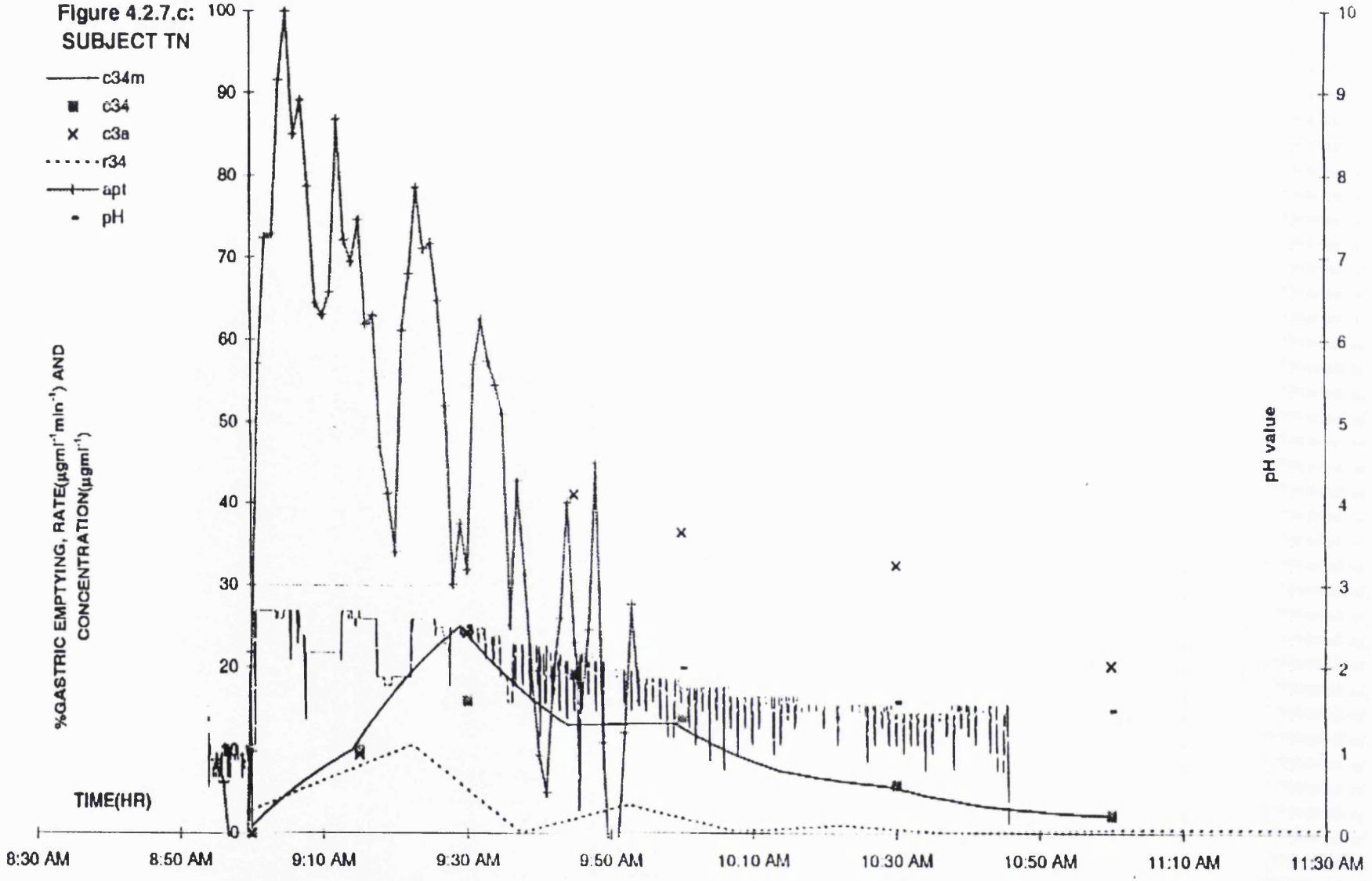


Figure 4.2.7.d:
SUBJECT TN

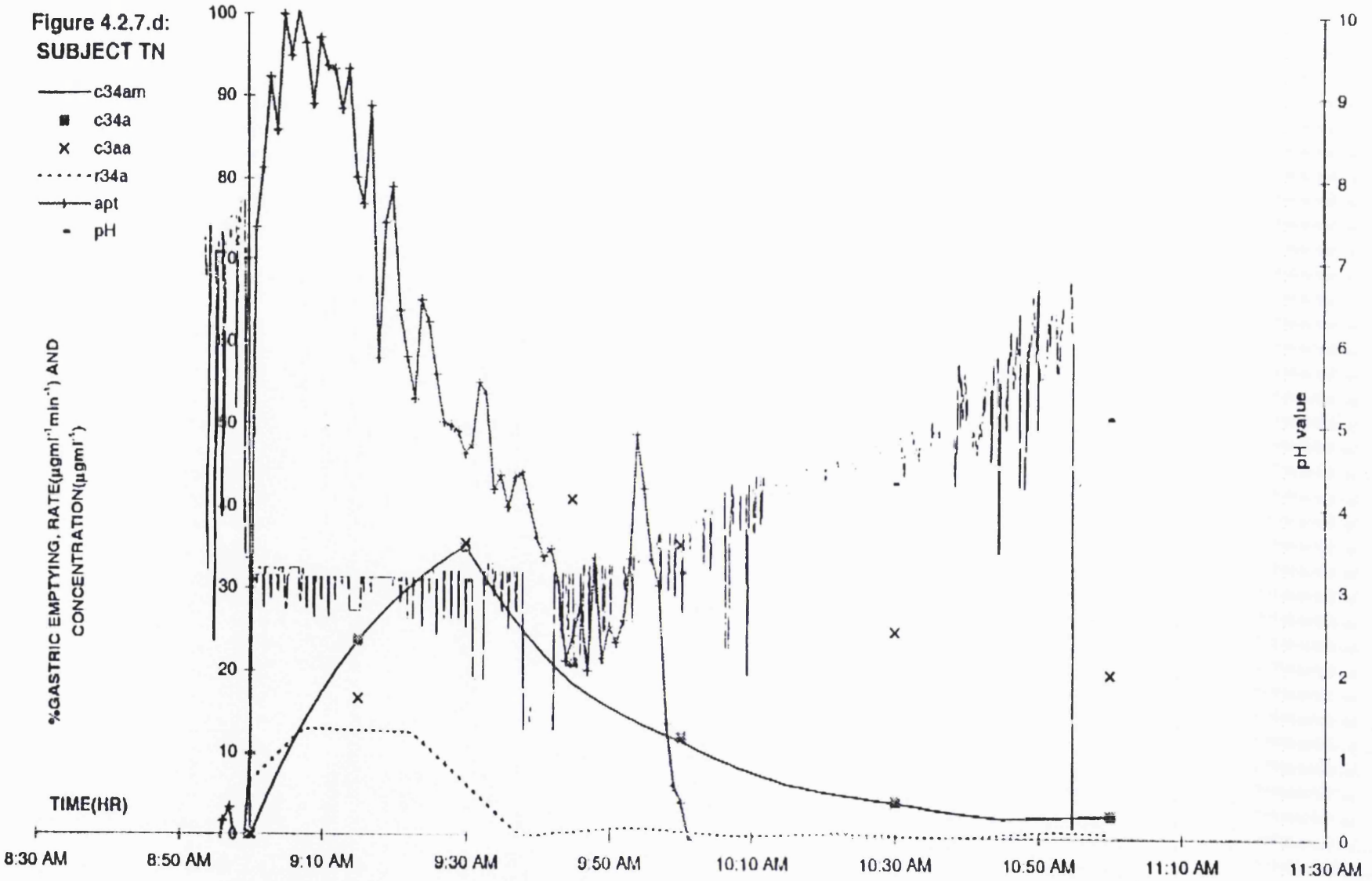
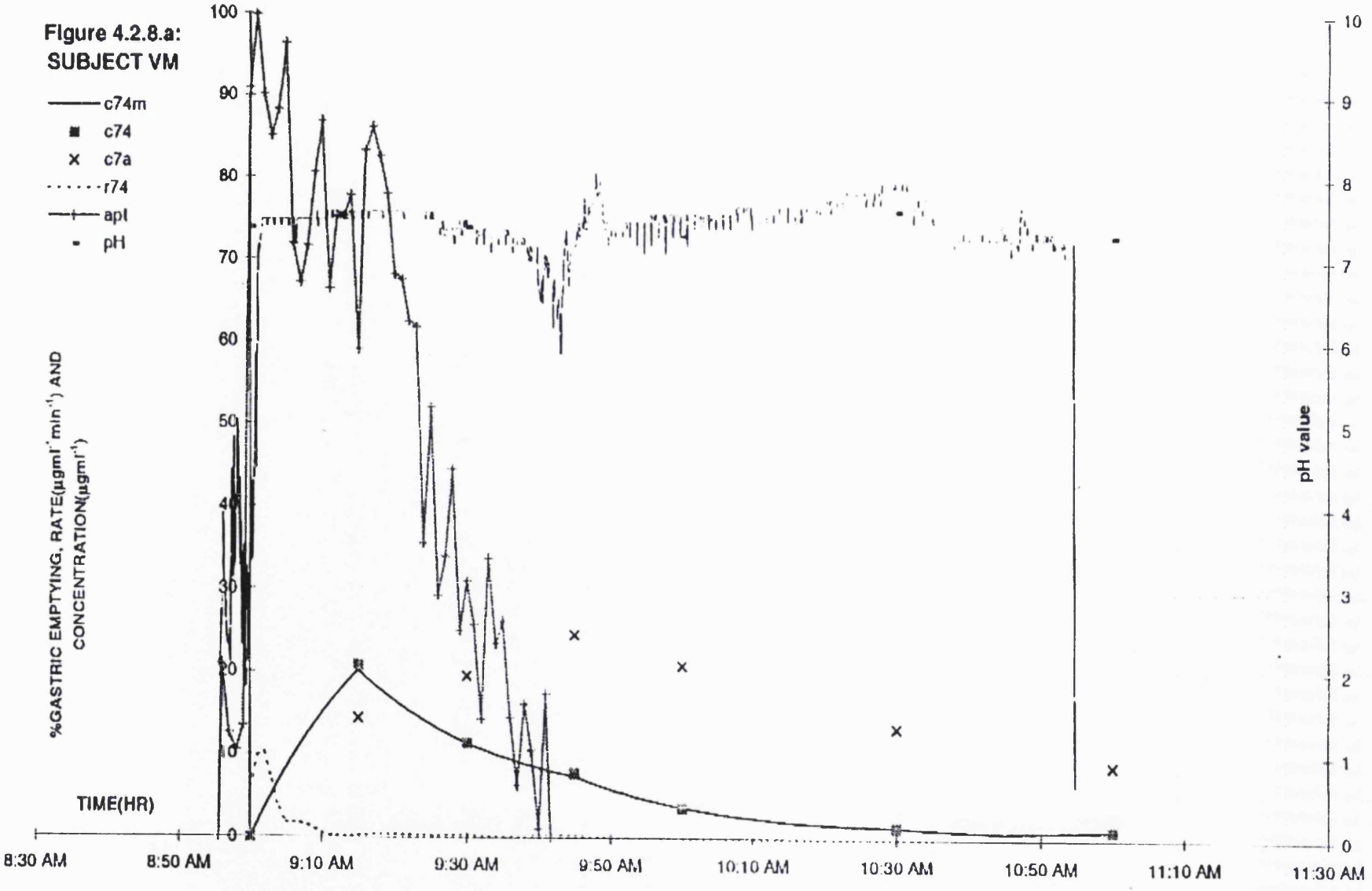


Figure 4.2.8.a:
SUBJECT VM



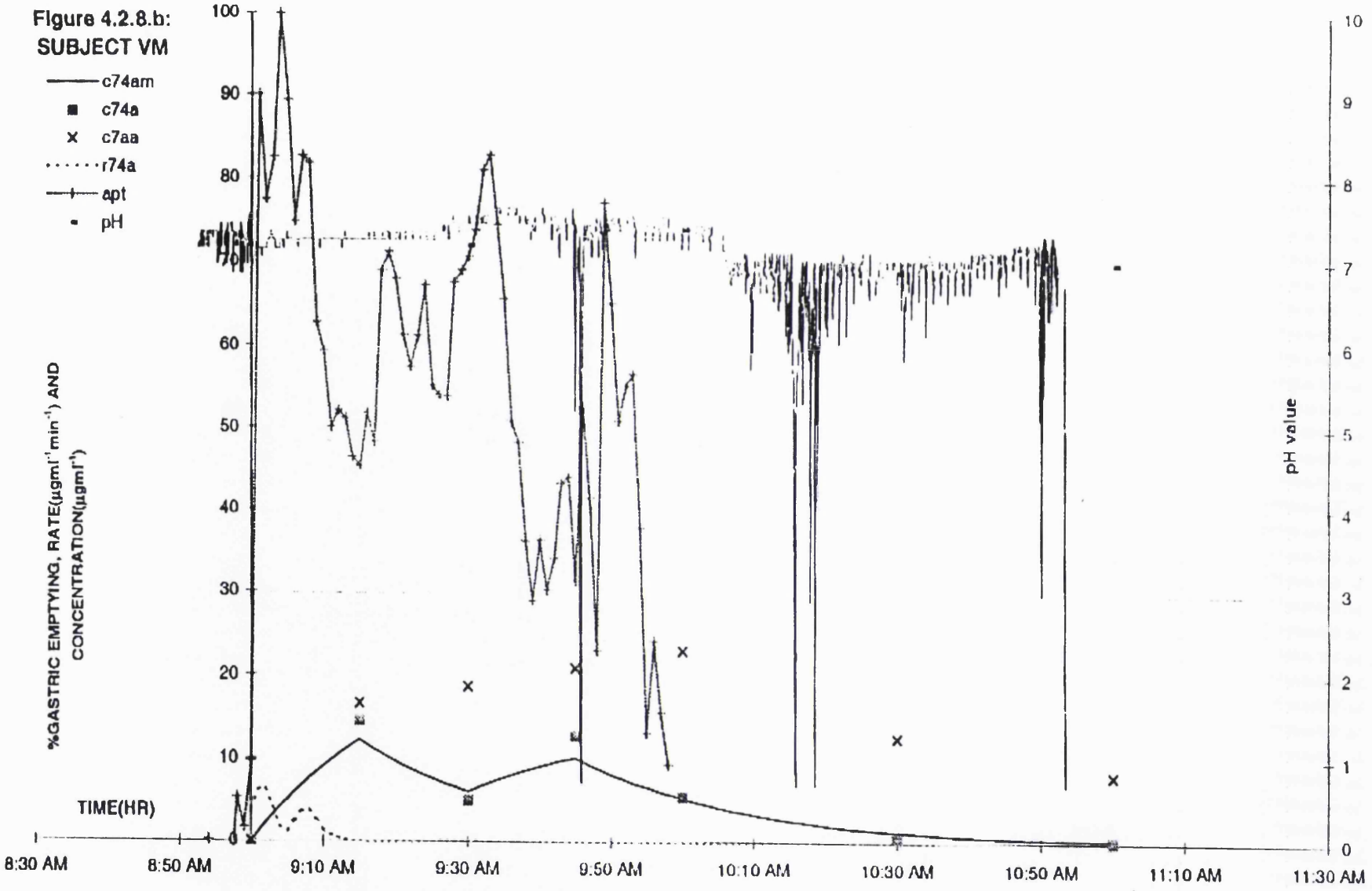


Figure 4.2.8.b:
SUBJECT VM
c74am
c74a
c7aa
r74a
apt
pH
%GASTRIC EMPTYING, RATE(µgml⁻¹min⁻¹) AND
CONCENTRATION(µgml⁻¹)
pH value
TIME(HR)
100
90
80
70
60
50
40
30
20
10
0
10
9
8
7
6
5
4
3
2
1
0
8:30 AM
8:50 AM
9:10 AM
9:30 AM
9:50 AM
10:10 AM
10:30 AM
10:50 AM
11:10 AM
11:30 AM

Figure 4.2.8.c:
SUBJECT VM

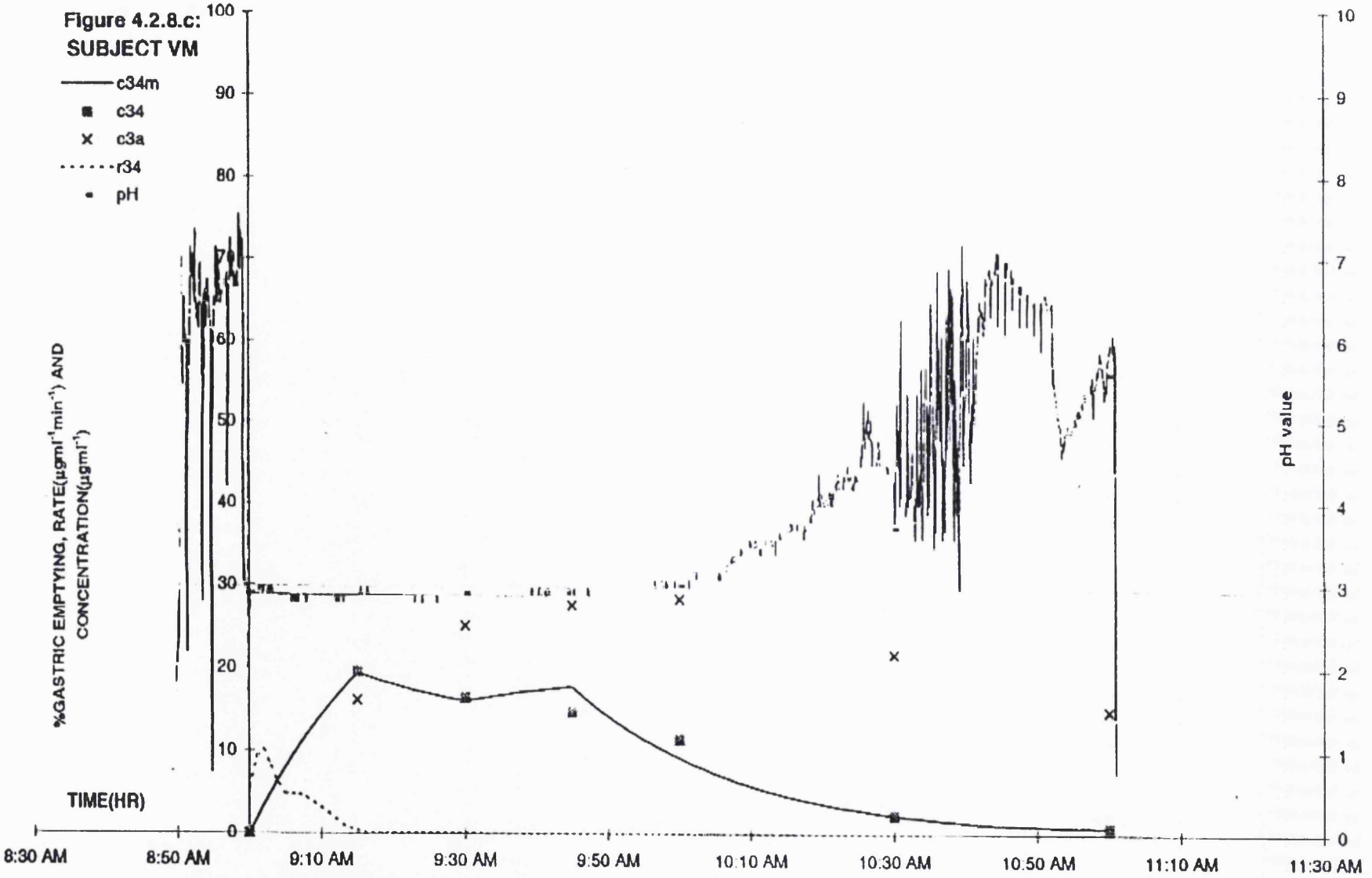


Figure 4.2.8.d:
SUBJECT VM

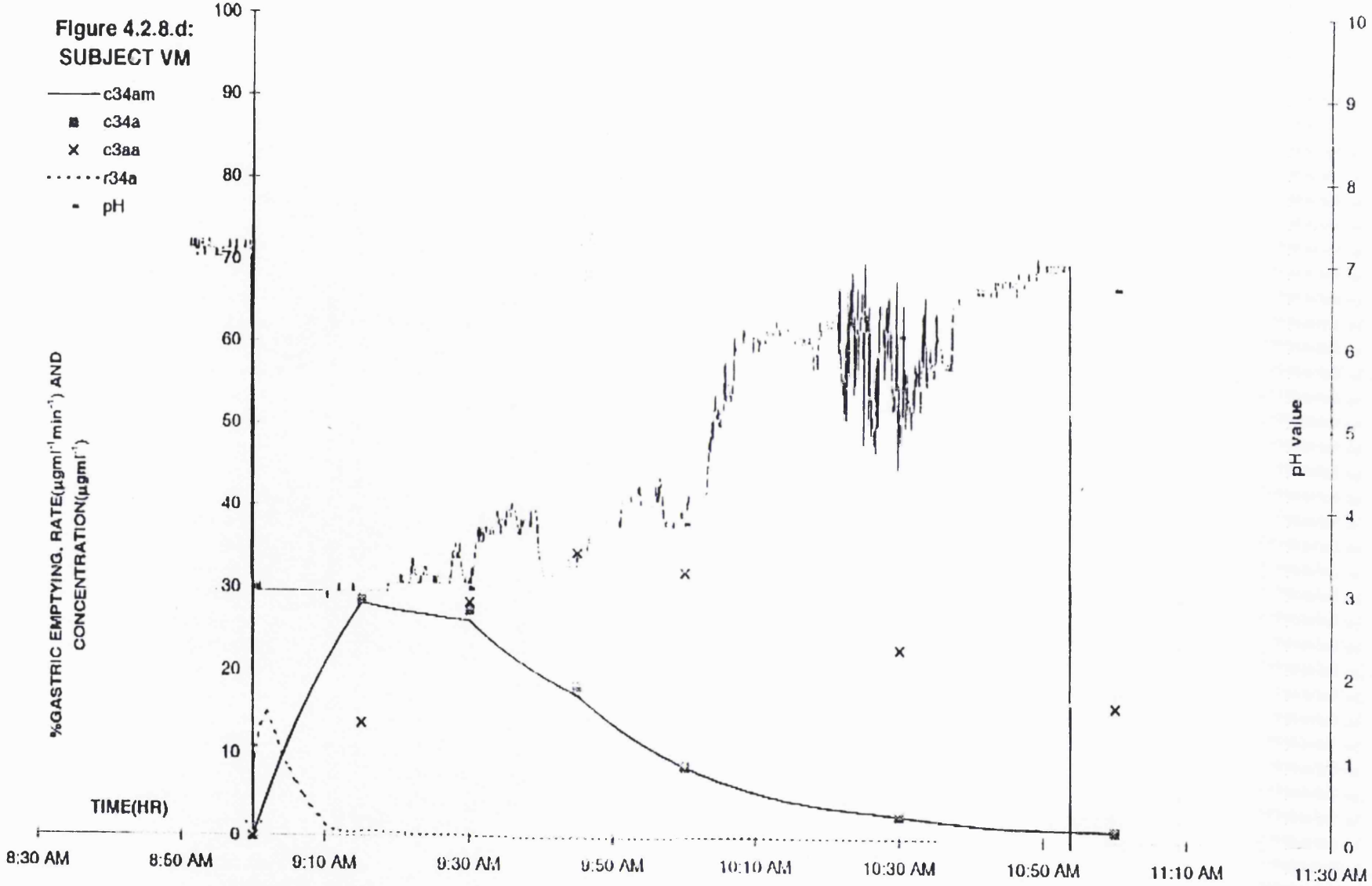


Figure 4.2.9.a:
SUBJECT WB

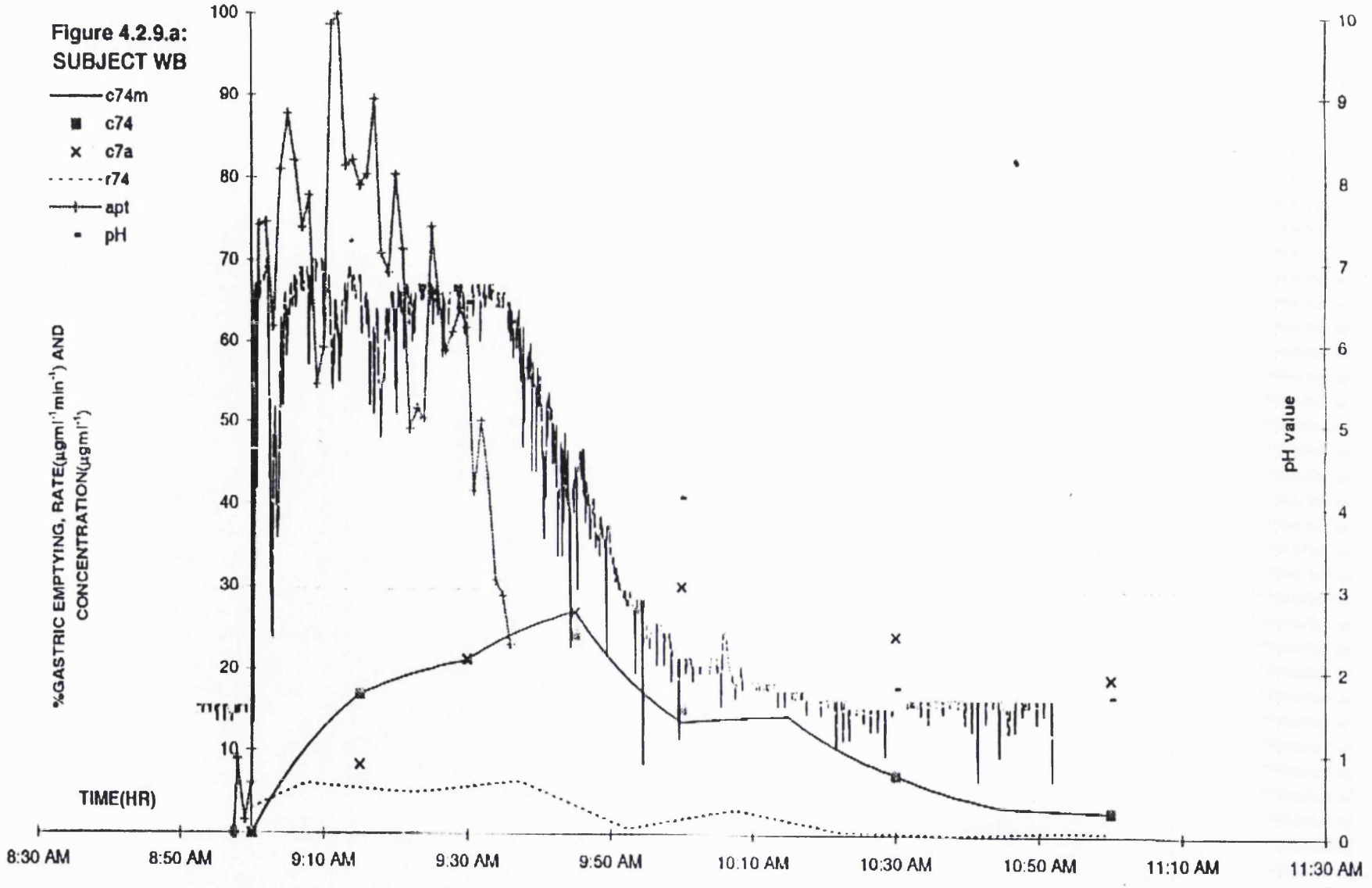


Figure 4.2.9.b:
SUBJECT WB

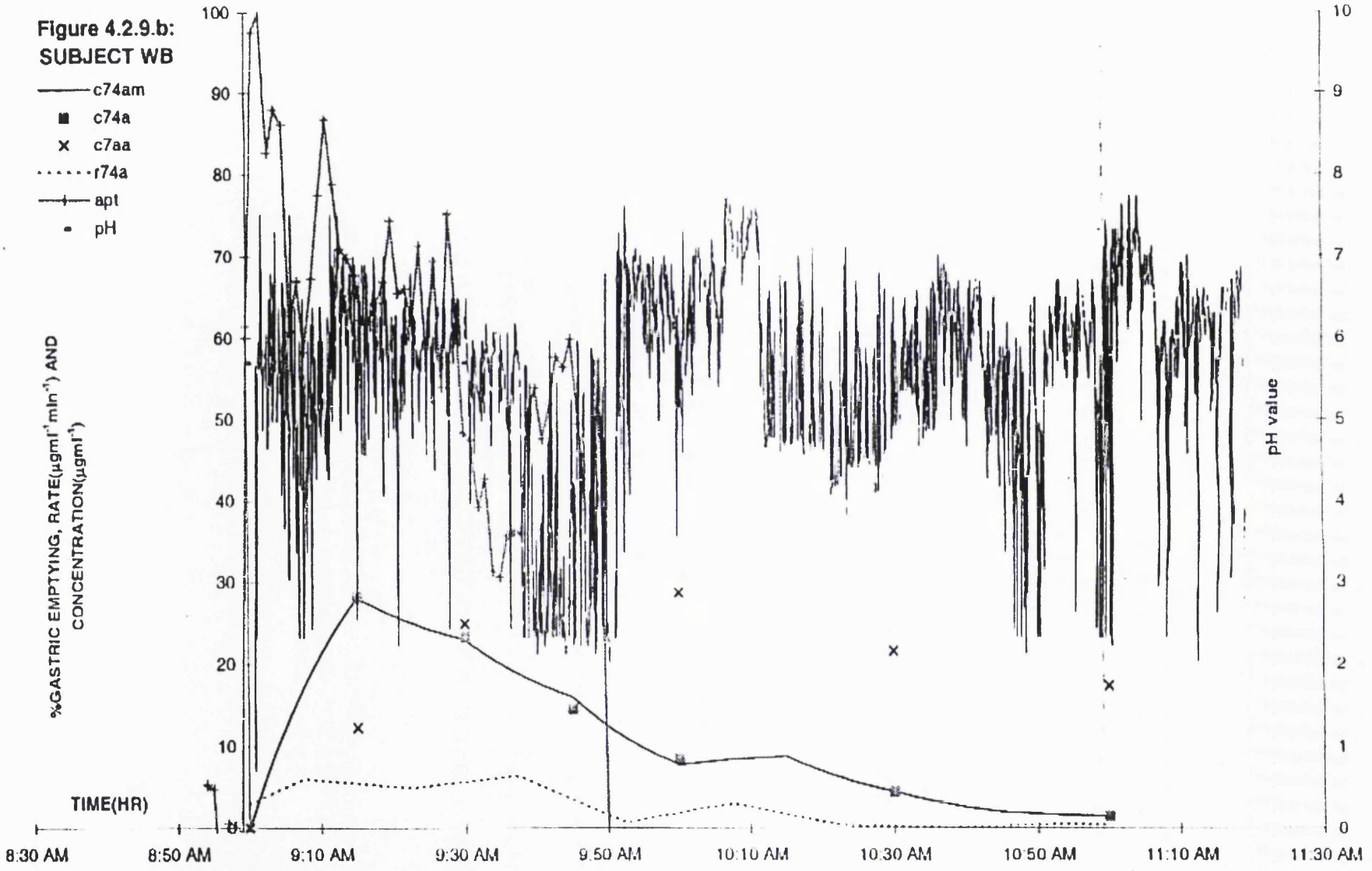


Figure 4.2.9.c:
SUBJECT WB

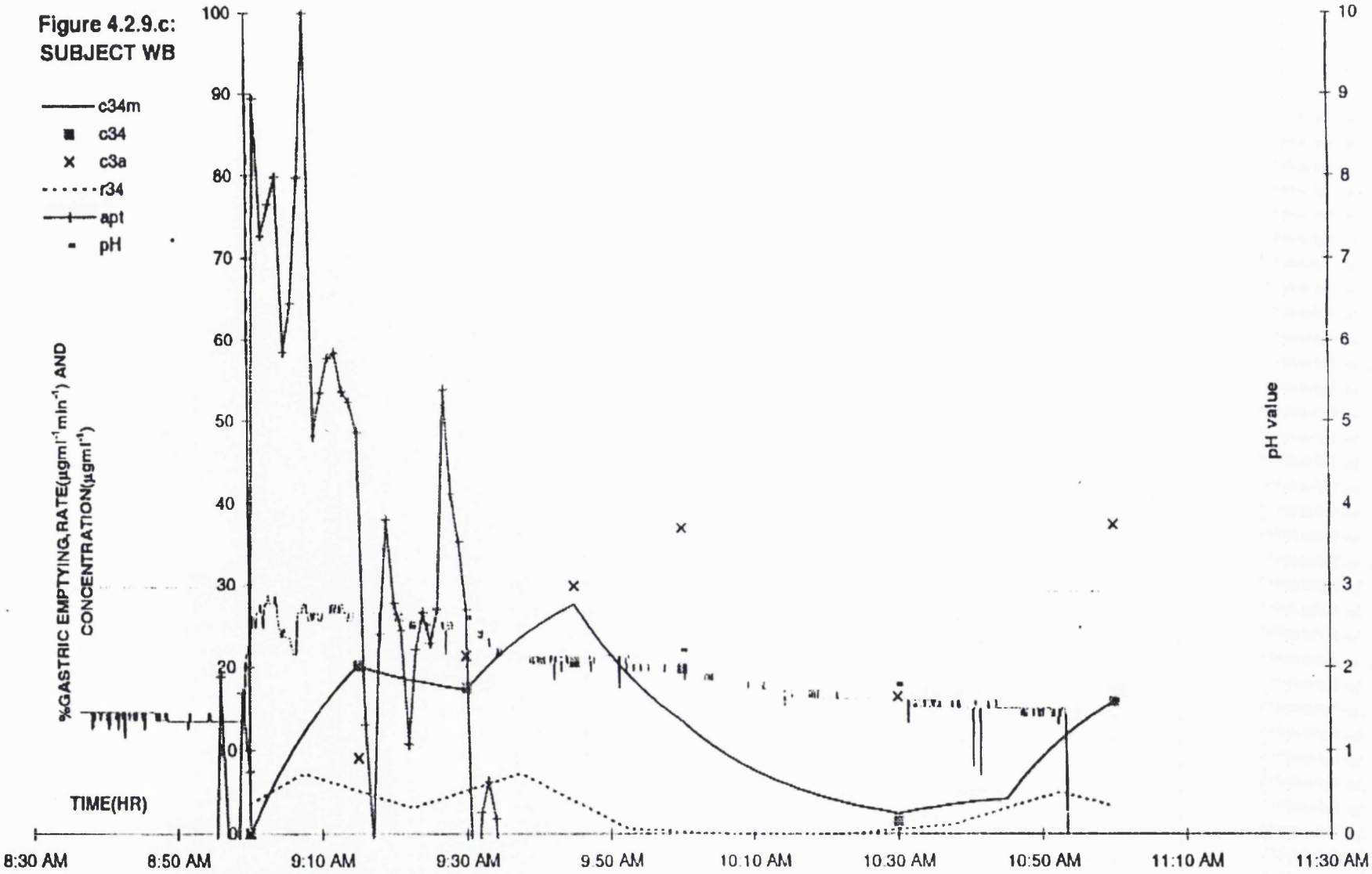


Figure 4.2.9.d:
SUBJECT WB

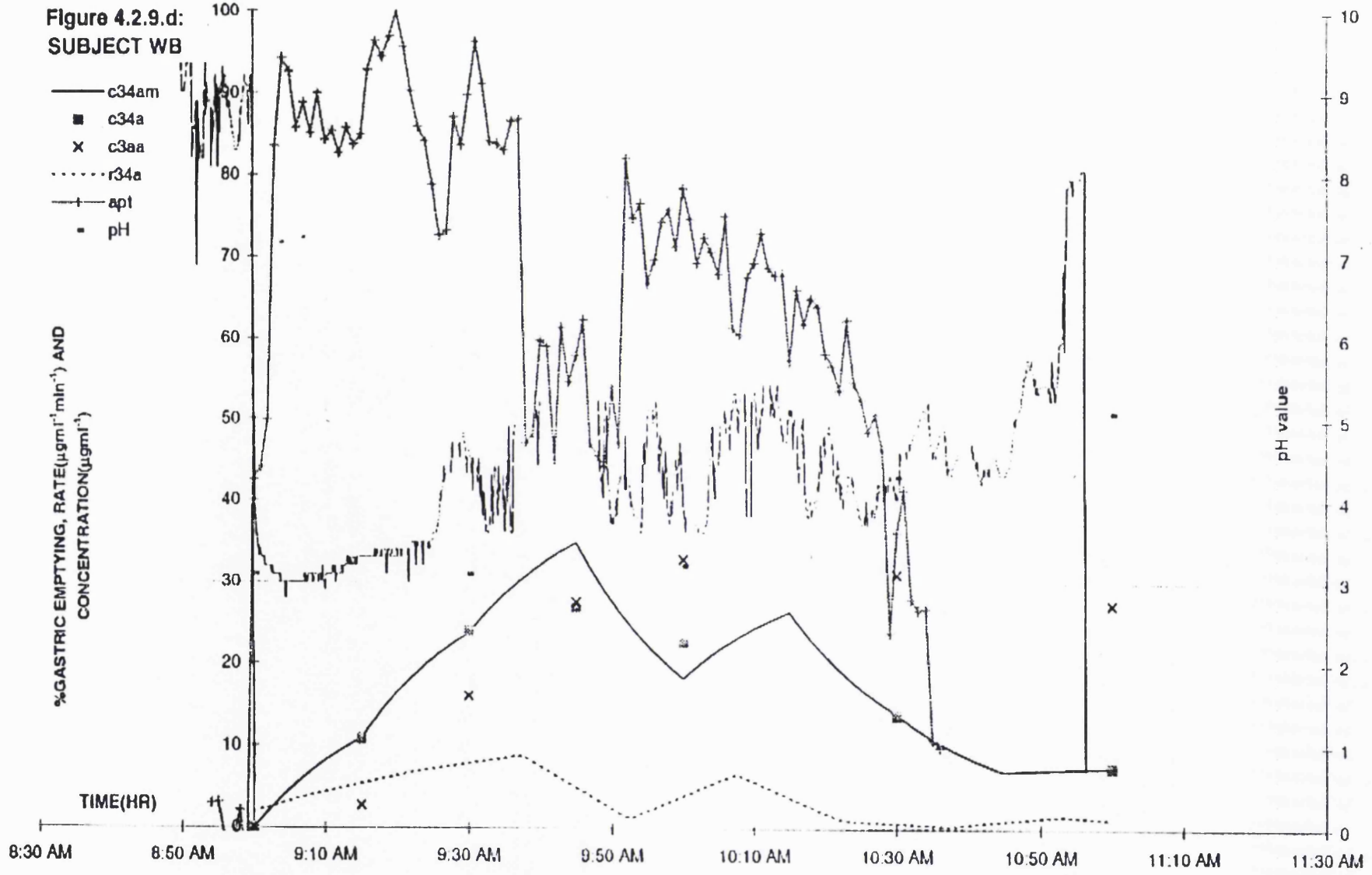


Figure 4.2.10.a:
SUBJECT YO

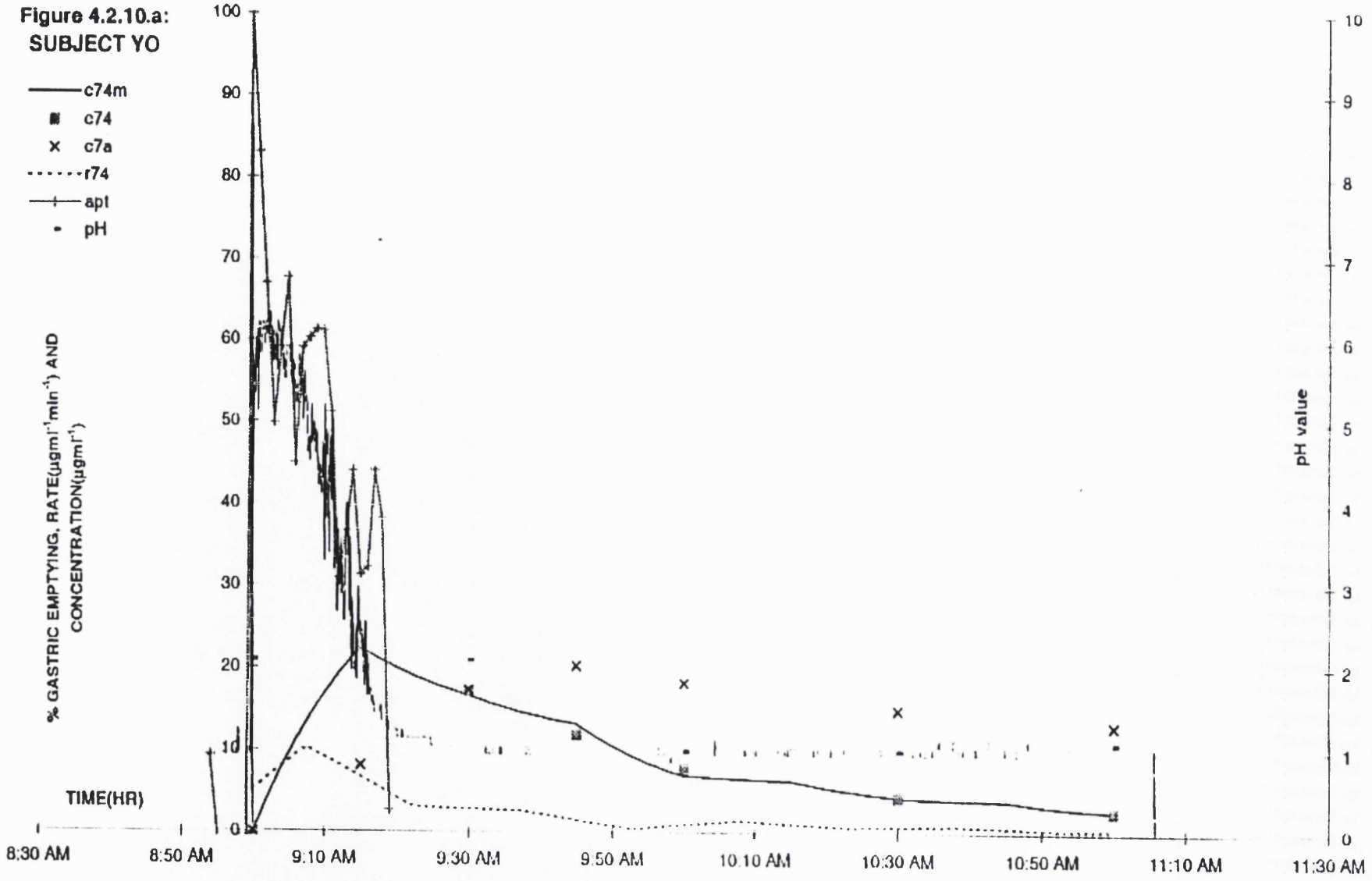


Figure 4.2.10.b:
SUBJECT YO

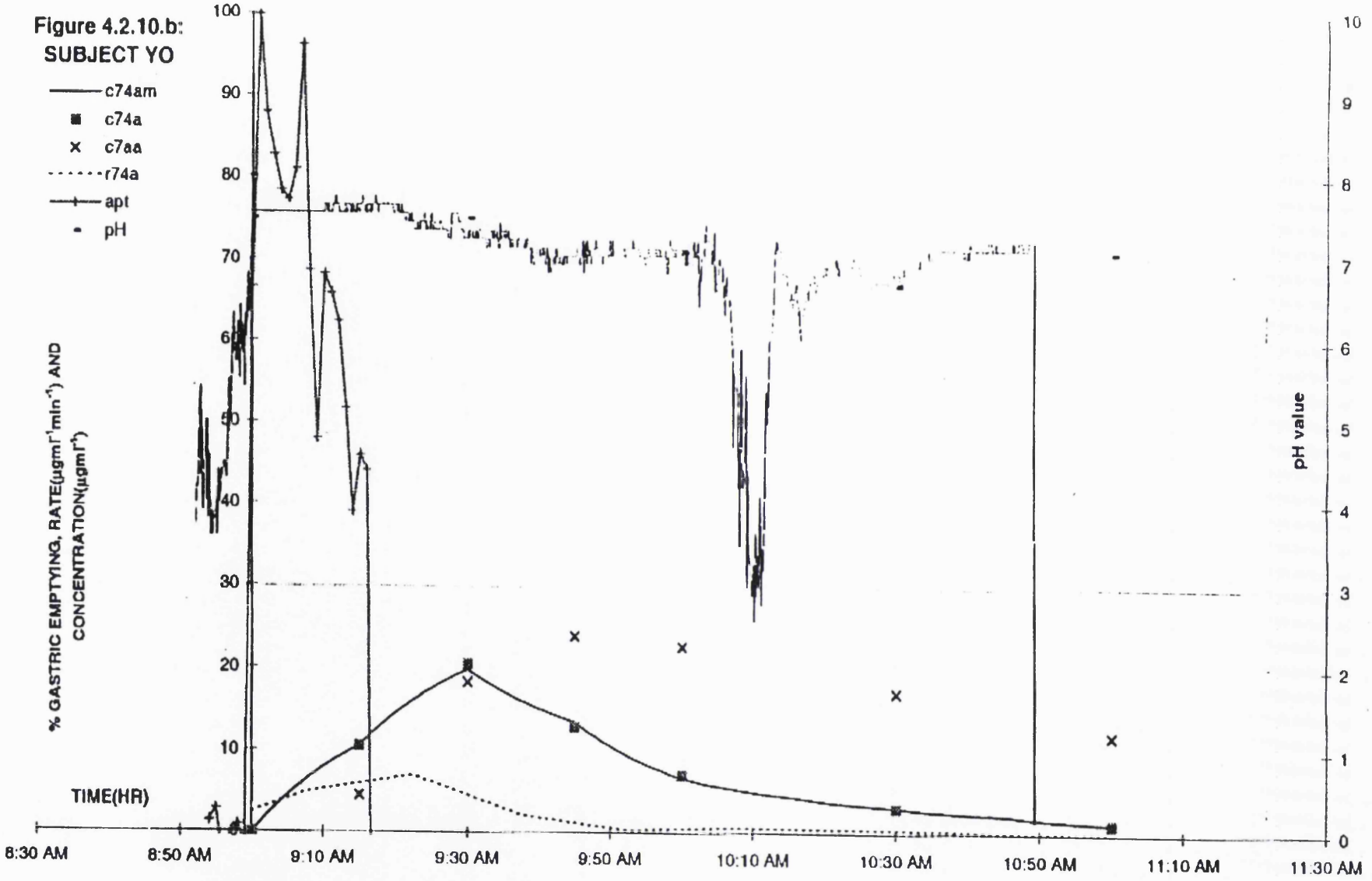
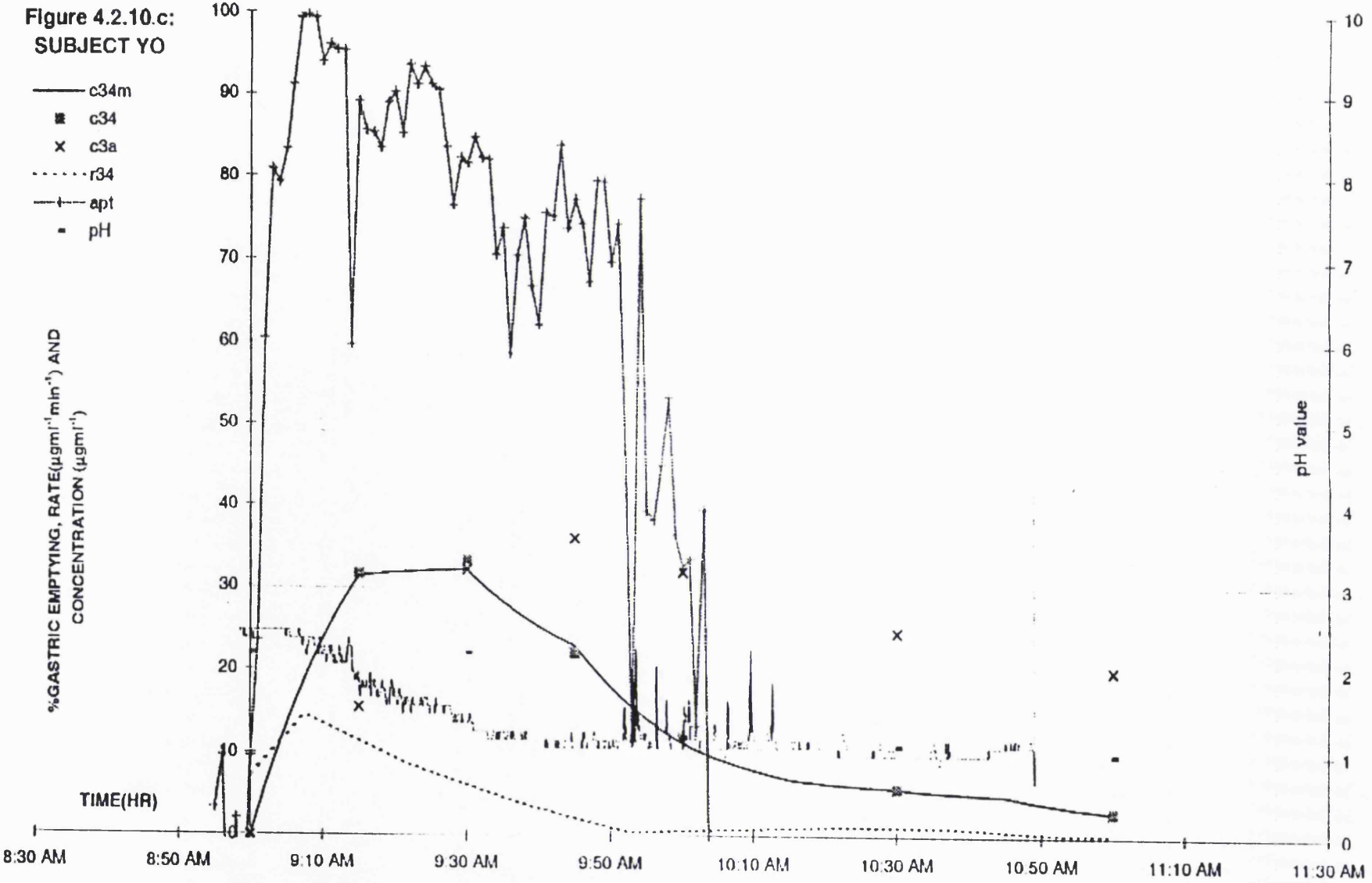


Figure 4.2.10.c:
SUBJECT YO



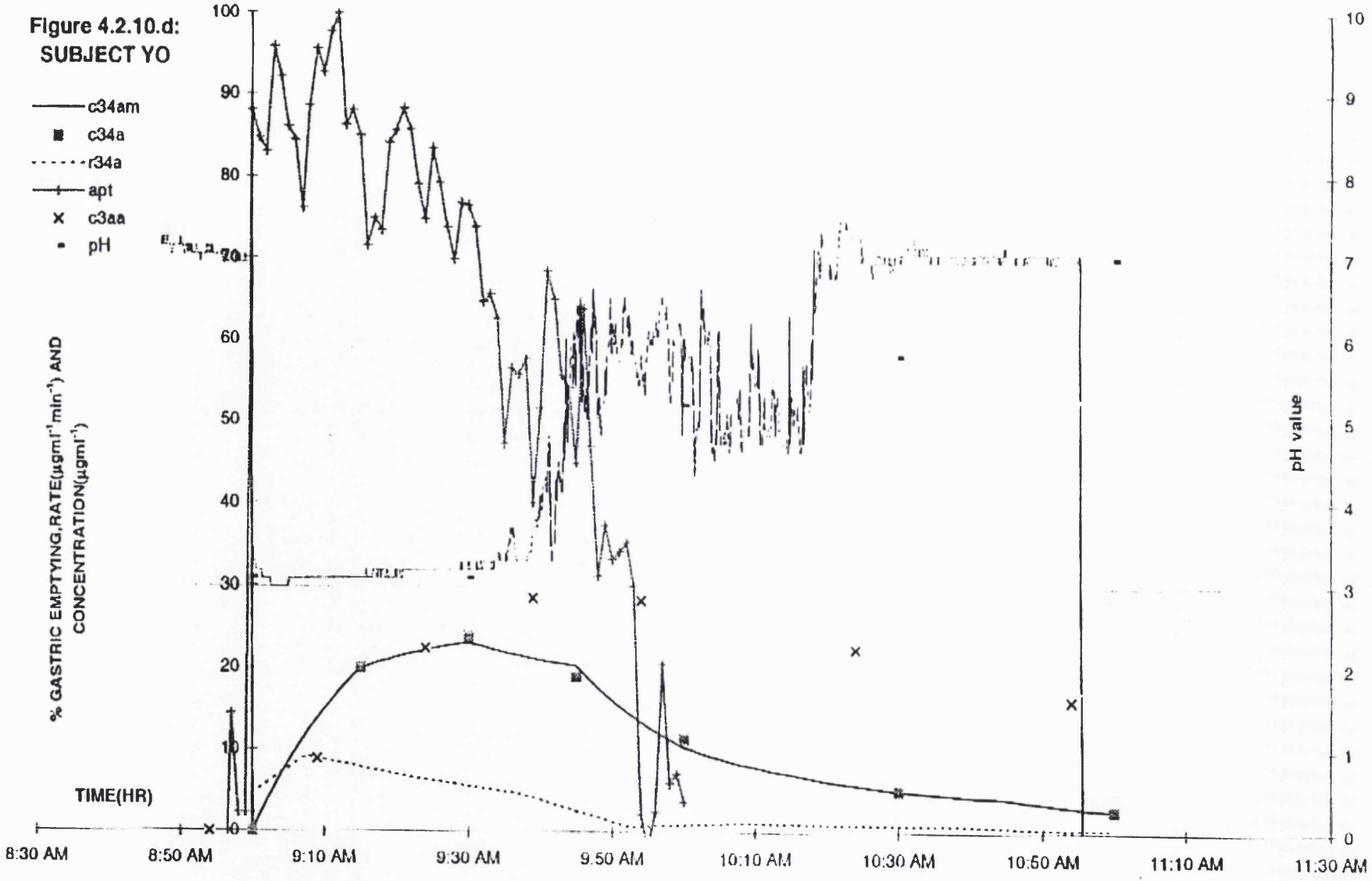


Figure 4.2.10.d:
SUBJECT YO
c34am
c34a
r34a
apt
c3aa
pH
% GASTRIC EMPTYING,RATE(µgml⁻¹min⁻¹) AND CONCENTRATION(µgml⁻¹)
pH value
TIME(HR)
100
90
80
70
60
50
40
30
20
10
0
10
9
8
7
6
5
4
3
2
1
0
8:30 AM
8:50 AM
9:10 AM
9:30 AM
9:50 AM
10:10 AM
10:30 AM
10:50 AM
11:10 AM
11:30 AM

4.5.2.1 Results and discussion based on first 2 hours monitoring on each subject

The gastric emptying, pH and the pharmacokinetic profiles of the drug and its metabolite for the first two hours after liquid administration were compared simultaneously. The profiles from subject VM were not compared, as they were not reliable. The APT data acquisitions from this subject were poor as he had a large body mass index. The baseline pH values of this subject were found to be much higher as the pH radiotelemetry capsule positioning was not optimised. The pH profiles of subject BB were ignored as he had difficulties in swallowing the capsule. The comparison for subject BB relied merely on the gastric emptying and pharmacokinetic profiles. In addition, the gastric emptying and pH profiles from subject AA and OC did not show good correlation graphically in some of the test conditions. This can be explained by the variation in gastric acid secretion, bicarbonate reflux, salivation and the psychological state of each individual during those specific test periods.

As mentioned above, the gastric emptying and pH profiles from subject AA were less well correlated especially for the pH7 formulation without ranitidine treatment (figures 4.2.1.a-4.2.1.d). This was the first set of the study performed on this particular individual and the subject felt nausea after swallowing the tethered radiotelemetry capsule. The degree of salivation was high as spikes of increased pH values were observed (figure 4.2.1.a). The gastric emptying profile of the pH3 formulation without ranitidine treatment could not be constructed due to the irregularity of the image area. The 4ASA model fittings for pH3 formulation were less satisfying especially for the data set with ranitidine treatment. The second absorption peak can be ignored as it represents the noise generated by the MAXENT approach (figures 4.2.1.c & 4.2.1.d). The absorption of 4ASA in all formulations proceeded mainly in the small intestine after the liquid left the stomach. The mean absorption time (MAT) values were extended for the pH3 formulation (table 4.5). The absolute bioavailability values, F_A confirmed these findings (table 4.7).

Figures 4.2.2.a-4.2.2.d show that in subject BB, the absorption of 4ASA increased as the liquid formulations were emptied into the small intestine. The 4ASA model fittings for pH7 formulation without ranitidine treatment and pH3 formulation with ranitidine treatment were less satisfactory (figures 4.2.2.a & 4.2.2.d). The test value from the MAXENT report was beyond the designated range confirming the less likelihood in the matching of these data sets. The F_A values were comparable except for pH7 formulation with ranitidine treatment (table

4.7). The pH3 formulation was more bioavailable as the MAT values were prolonged and overestimation was possible due to poor model fittings (table 4.5).

Figures 4.2.3.a-4.2.3.d show that the gastric emptying profiles fit the pH profiles reasonably well and the model fittings for 4ASA were good in all cases with subject CB. The absorption of 4ASA in the formulations without ranitidine treatment initiated in the stomach as indicated by the presence of two peak absorption rates (figures 4.2.3.a & 4.2.3.c). This resulted in higher F_A values when compared to those with ranitidine treatment where the absorption of 4ASA increased as the liquid was emptied into the small intestine (table 4.7). The reason for the large MAT value for pH3 formulation with ranitidine treatment is unclear (table 4.5).

There were good correlations between gastric emptying and pH profiles of subject DB as shown in figures 4.2.4.a-4.2.4.d. The presence of two absorption rate peaks in the pH3 formulation without ranitidine treatment was due to poor model fittings as the concentration profiles differed among the 4 data sets (figure 4.2.4.c). The absorption increased as the liquid formulations emptied into the small intestine. The two F_A values for the pH3 formulations were comparable and larger than the pH7 formulation as a prolonged gastric emptying process and MAT values were observed (tables 4.5 & 4.7).

As with subjects CB and DB, the gastric emptying and pH profiles of subject DS fit one another well (figures 4.2.5.a-4.2.5.d). The 4ASA model fittings showed 2 absorption rate peaks in all data sets except pH7 formulation with ranitidine. The secondary absorption rate peak in these data sets has to be analysed with care, as its presence is partly due to difficulties in constructing the model fittings. As with subject CB, the F_A values were higher in the formulations without ranitidine treatment when compared to those with ranitidine treatment (table 4.7). The presence of the secondary absorption rate peak indicates the possible initiation of 4ASA absorption within the stomach. For the pH3 formulation, the MAT values were prolonged, as the gastric emptying profiles were delayed (table 4.5).

The subject OC showed poor comparison between the gastric emptying and pH profiles (figures 4.2.6.a-4.2.6.d). The gastric emptying profiles were similar in all cases. As the subject has a large body mass index, the data acquisitions of small volume formulations were less accurate. The 4ASA pharmacokinetic profiles were different in all 4 cases but the model fittings were satisfactory. The formulation with ranitidine treatment showed 2 concentration

peaks indicating possible uptake of 4ASA in the stomach (figures 4.2.6.b & 4.2.6.d). The formulations without ranitidine treatment had no secondary concentration peak and absorption rate peak (figures 4.2.6.a & 4.2.6.c). The absorption rate peak of the pH3 formulation without ranitidine treatment was twice that of other data sets. Hence, despite having a very short MAT value, the F_A value is high (tables 4.5 & 4.7). The reason for the unusual kinetic patterns of the 4 tests in this subject was unclear.

The comparison between gastric emptying and pH profiles in subject TN was good except for the pH7 formulation without ranitidine treatment (figures 4.2.7.a-4.2.7.d). The gastric emptying profiles were similar for all the cases to those of subject OC. The 4ASA model fittings for pH3 formulation without ranitidine treatment was poor and the presence of the secondary peak should be ignored (figure 4.2.7.c). By comparing the 3 profiles, systemic uptake of 4ASA increased as the liquid formulation left the stomach. The initial high absorption rate observed in the pH7 formulation without ranitidine treatment, which was twice that of the other sets, resulting in much higher F_A value (table 4.7). The MAT value of the pH3 formulation without ranitidine treatment was extended but the F_A values was smaller than that with ranitidine treatment, indicating possible pre-systemic degradation by acid in the stomach (tables 4.5 & 4.7). The 4ASA decarboxylates into m-aminophenol at low gastric luminal pH provides an explanation for the higher F_A value for the pH3 formulation with ranitidine treatment. Barditch-Crovo et al (1998) reported that by elevating the gastric pH, the bioavailability of orally administered foscarnet increased as the decarboxylation process was minimised. In addition, the decarboxylation process has been demonstrated in an *in vitro* study in *section 2.4.4.*

Only the pharmacokinetic profiles were considered in subject VM (figures 4.2.8.a-4.2.8.d). The pH and gastric emptying profiles were omitted as no conclusive outcome could be derived. The pH values measured were abnormally high and APT data acquisition was poor as explained at the beginning of this section. However, the 4ASA model fittings were reasonably good, except for pH3 formulation without ranitidine treatment (figure 4.2.8.c). The F_A value of 4ASA for the pH3 formulation with ranitidine treatment was the highest as the absorption rate was the greatest (figure 4.2.8.d, table 4.7). Decarboxylation of 4ASA was at a minimum at the neutral pH value, which coincided with the observation in subject TN. The pH7 formulation with ranitidine treatment showed two concentration peaks indicating

possible absorption in the stomach (figure 4.2.8.b). However, without the APT and pH profiles, it is difficult to make such comment, as the MAT value is small (table 4.5).

Figures 4.2.9.a-4.2.9.d show the pharmacokinetic, pH and gastric emptying profiles of the four tests for subject WB. The gastric emptying and pH profiles were comparable except for the pH7 formulation without ranitidine treatment (figure 4.2.9.a). Due to the large fluctuation in the blood level of pH3 formulation without ranitidine treatment, the 4ASA model fittings for other data sets were affected (figure 4.2.9.c). The double peak phenomenon should be ignored except for the pH3 formulation without ranitidine treatment. As the fluctuation was large in this data set, the kinetic profile of AASA was affected by showing two concentration peaks. The subject experienced great difficulties in swallowing the radiotelemetry capsule, as this was the first attempt to monitor his gastric pH profile. The psychological stress experienced during the test increased the gastric emptying rate and could have contributed to this exceptional case. The MAT value was large despite the short gastric emptying profile (table 4.5). The F_A values are less reliable in this subject due to the poor model fittings (table 4.7).

Finally, the three profiles of subject YO were compared (figures 4.2.10.a-4.2.10.d). The gastric emptying profiles corresponded well with the pH profiles except for the pH3 formulation without ranitidine treatment (figure 4.2.10.c). The pH profile for this data set was less reliable as the basal pH values were very acidic. The rapid gastric emptying profiles for the pH7 formulation indicated that absorption of 4ASA was mainly in the small intestine. The 4ASA model fittings were good in all cases. The F_A values were similar in three cases except for the pH7 formulation with ranitidine treatment, which was smaller (table 4.7). The reduced availability in 4ASA in this particular set is probably due to rapid pre-systemic acetylation in the small intestine. The MAT values were greater in the pH3 formulation, as the gastric emptying process was delayed (table 4.5).

In summary, the pharmacokinetic, pH and gastric emptying profiles for each study condition of each individual are different yet similarity in the pattern still exists. Absorption of 4ASA occurred predominantly in the small intestine with some uptake initiated in the stomach as described above. The F_A values of 4ASA showed that the drug given without ranitidine treatment seems to be more bioavailable than those with ranitidine treatment, with some exceptions described above. The pH7 formulation with ranitidine treatment seems to be the

least bioavailable, as N-acetyl-transferase activity is optimised at neutral pH environment (*section 1.6*). The MAT values are larger in the pH3 formulation as the gastric emptying rates are delayed.

4.5.2.2 Results and discussion based on group effects

The group effects according to the test liquids and conditions were investigated. The statistical analysis was performed whenever, possible using the SPSS statistical software package. The tests include analysis of variance, test of normality and Wilcoxon Signed-Ranks test (tables 4.9-4.11).

4.5.2.2.1 Statistical analysis of the pharmacokinetic parameters

Test of normality in all the subjects shows that the K_{el} and Cl values are significantly deviated from the normal distribution but not for the V_d value (table 4.9). As the data sets for subject VM were not compared to the intravenous route, a fixed volume is designated to calculate the other pharmacokinetic parameters. The calculated V_d value is much smaller in subject DS, therefore, by removing these out lying values, which are subjects VM and DS for Cl and K_{el} values, respectively, the Kolmogorov-Smirnov statistics indicated that the test of normality for the data distributions improved. Table 4.8 shows that the Cl and K_{el} value of 4ASA are not comparable to the previously reported cases (Peloquin et al 1994, Wan et al 1973, Norgine Brochure). The K_{el} value from the published reports is approximately $0.014min^{-1}$ which is much smaller than the value from the current study. As mentioned in *section 4.5.1*, there were fewer than five measured data points for the intravenous route due to the detection limit of the assay, inadequate blood collection time points and the rapid acetylation into AASA. In previous studies at least 2 grams of 4ASA were administered, which is four fold the concentration used in the current study plus neither of these reports outlined an intravenous preparation, which could also explain the difference in the K_{el} values calculated.

Tables 4.10-4.11 compares the pharmacokinetic parameters based on analysis of variance and Wilcoxon Signed-Ranks test for K_a (absorption rate constant), MAT, MRT (mean residence time), F_A values and AUC (area under the curve). The test for normality mainly shows no

significant deviation from the normal distribution in these pharmacokinetic parameters (table 4.9).

The MAT and K_a values of 4ASA are significantly shorter for the pH7 formulation indicating the absorption is rapid and the amount of time for the uptake of 4ASA is lower (table 4.10). Administration of ranitidine does not affect the MAT and K_a values. The mean cumulative absorption rate profiles and the results from the Wilcoxon Signed-Ranks test confirm these findings (figure 4.3, tables 4.5 & 4.11). The mean residence time (MRT) values of 4ASA are significant different in the 2 oral formulations but not affected by ranitidine treatment (tables 4.10 & 4.11). The large MRT values for the pH3 formulation are related to the delayed gastric emptying process, which results in prolonged MAT values.

The analysis of variance shows a significant difference in AUC and F_A values in the 2 oral liquid formulations and the ranitidine treatment. As inter-subject variation is also significant, the results are restricted and serve as indicative only. A larger sample size would be required to evaluate these effects more precisely. There is a trend where the AUC and F_A values are significantly greater for the pH3 formulation (tables 4.10 & 4.11). This is because the actual administered 4ASA concentration in the pH7 formulation is lower than the pH3 formulation, as an equivalent by weight of the sodium salt has been used (the ratio of 4ASA to Na4ASA is 0.73). Furthermore, the pH3 suspension formulation is administered in a bulk, which increased the dissolution of the 4ASA despite the buffer effect to keep the formulation in the minimum dissolution's state. This is confirmed by the emergence of an absorption peak, which is much faster compared to the findings by Wan et al (1973) where the t_{max} of the 4ASA suspension is much slower (approximately 1 hour). For the pH7 formulation, possible precipitation of the salt in the stomach and higher acetylation of the drug into its metabolite could explain the small F_A values calculated especially in those with ranitidine treatment. The Wilcoxon Signed-Ranks test shows that ranitidine treatment does not affect pH3 formulation but a significant difference is observed in pH7 formulation (table 4.11) for both AUC and F_A values. Again, the explanation lies on the favourable acetylation process at elevated pH condition of the stomach and rapid emptying of the pH7 formulation into the small intestine.

The fact that absorption profiles were not smooth even with the simple oral liquid formulations has demonstrated the complexity of the disposition process of low dose 4ASA.

This observation was not demonstrated with other pharmacokinetic techniques such as those used by Wan et al (1973) and Peloquin et al (1994) with a 4 grams dose of 4ASA.

		Kolmogorov-Smirnov		
		Statistic	Df	Sig.
MAT	Liquid A	0.174	10	>0.200
	Liquid AA	0.270	10	0.038
		0.210	6	>0.200
	Liquid B	0.180	10	>0.200
	Liquid BA	0.131	10	>0.200
F_A values	Liquid A	0.136	10	>0.200
	Liquid AA	0.217	10	0.199
	Liquid B	0.182	10	>0.200
	Liquid BA	0.159	10	>0.200
K_a	Liquid A	0.231	10	0.138
		0.233	9	0.171
	Liquid AA	0.221	10	0.182
		0.144	9	>0.200
	Liquid B	0.205	10	>0.200
	Liquid BA	0.147	10	>0.200
AUC	Liquid A	0.107	10	>0.200
	Liquid AA	0.190	10	>0.200
	Liquid B	0.185	10	>0.200
	Liquid BA	0.175	10	>0.200
	IV dose	0.280	9	0.040
MRT	Liquid A	0.103	10	>0.200
	Liquid AA	0.270	10	0.038
		0.222	7	>0.200
	Liquid B	0.198	10	>0.200
	Liquid BA	0.232	10	0.136
		0.221	8	>0.200
	IV dose	0.162	9	>0.200
Clearance (Cl)		0.245	10	0.091
		0.237	9	0.155
Elimination rate constant (K_{el})		0.361	10	0.001
		0.234	9	0.167
Distribution volume (V_d)		0.181	10	>0.200
		0.193	8	>0.200

Table 4.9: Test of normality for the pharmacokinetic parameters of 4ASA calculated by MAXENT approach. Liquid A (pH3 4ASA formulation without ranitidine treatment); Liquid AA (pH3 4ASA formulation with ranitidine treatment); Liquid B (pH7 Na4ASA formulation without ranitidine treatment); Liquid BA (pH7 Na4ASA formulation with ranitidine treatment).

Effect (F)	MAT (n=40)	MRT (n=40)	AUC (n=40)	F_{A4ASA} (n=40)	K_a (n=40)
Route	21.51***	23.90***	197.93***	29.01***	7.25*
Acid Block	1.83	2.36	8.15**	10.97**	2.65
Subject	2.02	0.40	13.78***	9.01***	2.00

***is p<0.005 **is p<0.01 *is p<0.05

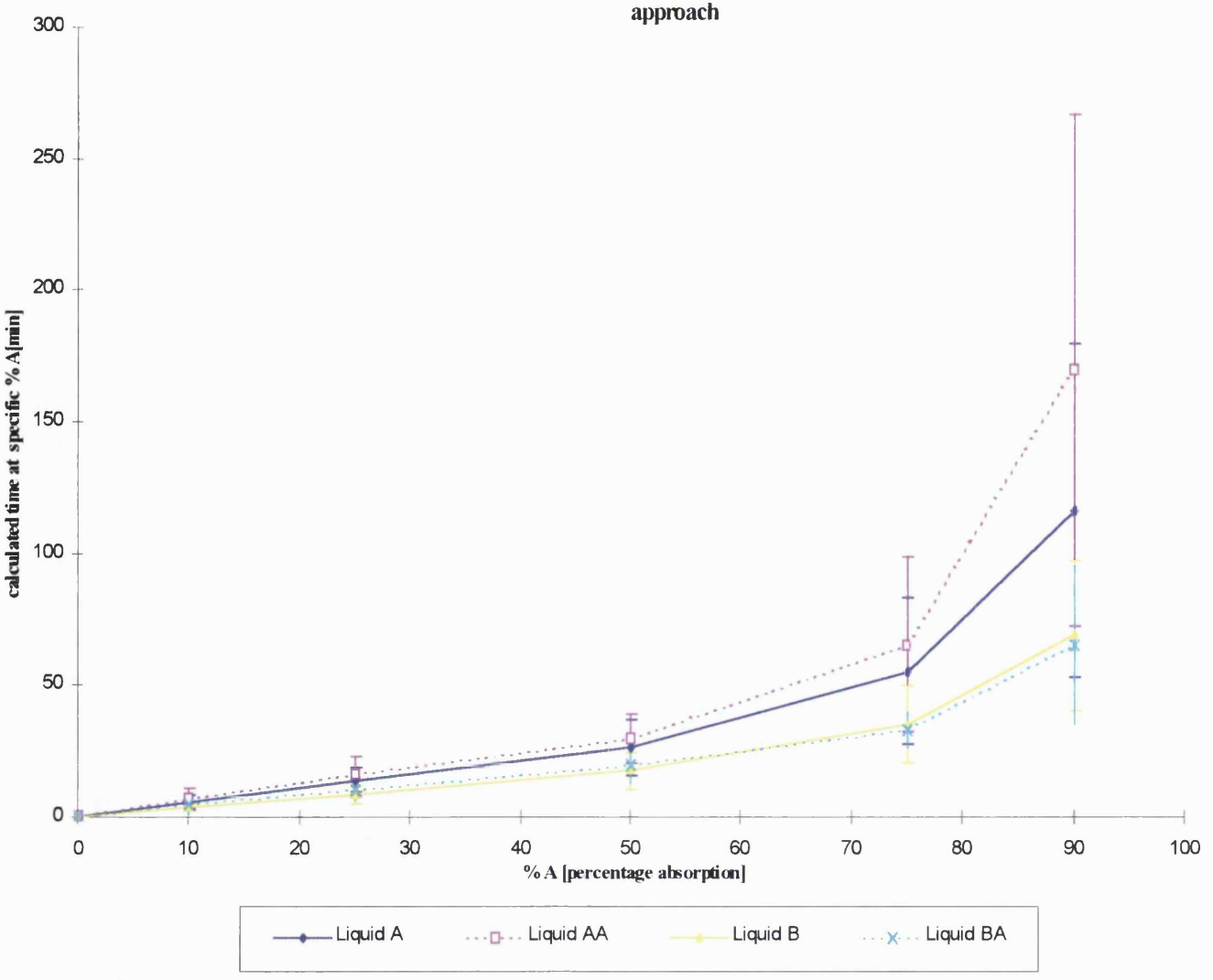
Table 4.10: Analysis of variance for the pharmacokinetic parameters of oral 4ASA formulations calculated by MAXENT approach. The F values and their significance. Route (type of administered formulations); Acid block (with or without ranitidine treatment).

Comparison	Z values and their significance				
	AUC	F_A	MRT	MAT	K_a
Liquid A to liquid AA	-0.255	-0.357	-0.663	-0.867	-0.764
Liquid A to liquid B	-2.803**	-1.376	-2.192*	-2.192*	-1.887
Liquid A to liquid BA	-2.803**	-2.666**	-1.886	-1.886	-1.478
Liquid AA to liquid B	-2.803**	-1.376	-2.701**	-2.701**	-2.808**
Liquid AA to liquid BA	-2.803**	-2.803**	-2.701**	-2.701**	-2.497*
Liquid B to liquid BA	-2.803**	-2.803**	-1.580	-0.968	-1.376

***p<0.001 **is p<0.01 *is p<0.05

Table 4.11: Wilcoxon Signed-Ranks test comparing the pharmacokinetic parameters of 4ASA oral liquid formulations calculated by MAXENT approach. The Z values and their significance. Liquid A (pH3 4ASA formulation without ranitidine treatment); Liquid AA (pH3 4ASA formulation with ranitidine treatment); Liquid B (pH7 Na4ASA formulation without ranitidine treatment); Liquid BA (pH7 Na4ASA formulation with ranitidine treatment).

Figure 4.3: The mean cumulative absorption profiles of the 4ASA liquid formulations calculated with the MAXENT approach



4.5.3 Pharmacokintic analysis of 4ASA and AASA using Wagner-Nelson method

The blood concentrations were further analysed with the Wagner-Nelson method as an alternative to the maximum entropy method. The Wagner-Nelson method is one of the most commonly used mass balance methods for pharmacokinetic analysis. The analysis is based upon a compartmental distribution and intravenous reference is mandatory (Tucker et al 1988). The method does not use complex mathematical functions and no input function is prescribed. However, the results rely strongly on the accurate intravenous blood level, which has not been achieved due to the limited number of blooding sampling immediately after injection of 4ASA.

4.5.3.1 Results for kinetic analysis on 4ASA

One compartment model is used to calculate the pharmacokinetic parameters such as Cl, C_o, $t_{½}$ and V_d values (Clark et al 1993). The AUC and MRT values are determined by the linear trapezoidal rule. The MAT values were determined by subtracting from the MRT values of the oral formulation, that for the intravenous preparation. The F_A values was calculated as $[(AUC_{oral} \times dose_{iv})/(AUC_{iv} \times dose_{oral})]$. The results are tabulated in tables 4.12-4.13. The F_A values of 4ASA calculated using Wagner-Nelson method are larger than 1 in some cases as the intravenous data is limited (table 4.12). Underestimation of the intravenous 4ASA concentration also calculated a larger value of the other pharmacokinetic parameters as compared to those using the MAXENT approach. As the MAXENT approach has shown that the absorption rate profiles of 4ASA are complex even after suppressing the disposition model, the F_A values are not reliable when calculated using the one-compartment model.

Figure 4.4 shows the cumulative absorption profiles of 4ASA. The absorption of the pH7 formulation is faster than that of the pH3 formulation confirming the findings by the maximum entropy method. Test of normality shows that all the pharmacokinetic parameters are normally distributed (table 4.14). The results from analysis of variance and Wilcoxon Ranked-sign test are consistent with those calculate using the MAXENT approach (tables 4.15 & 4.16). With the significant inter-subject difference for F_A and AUC, the results from the statistical evaluation are restricted.

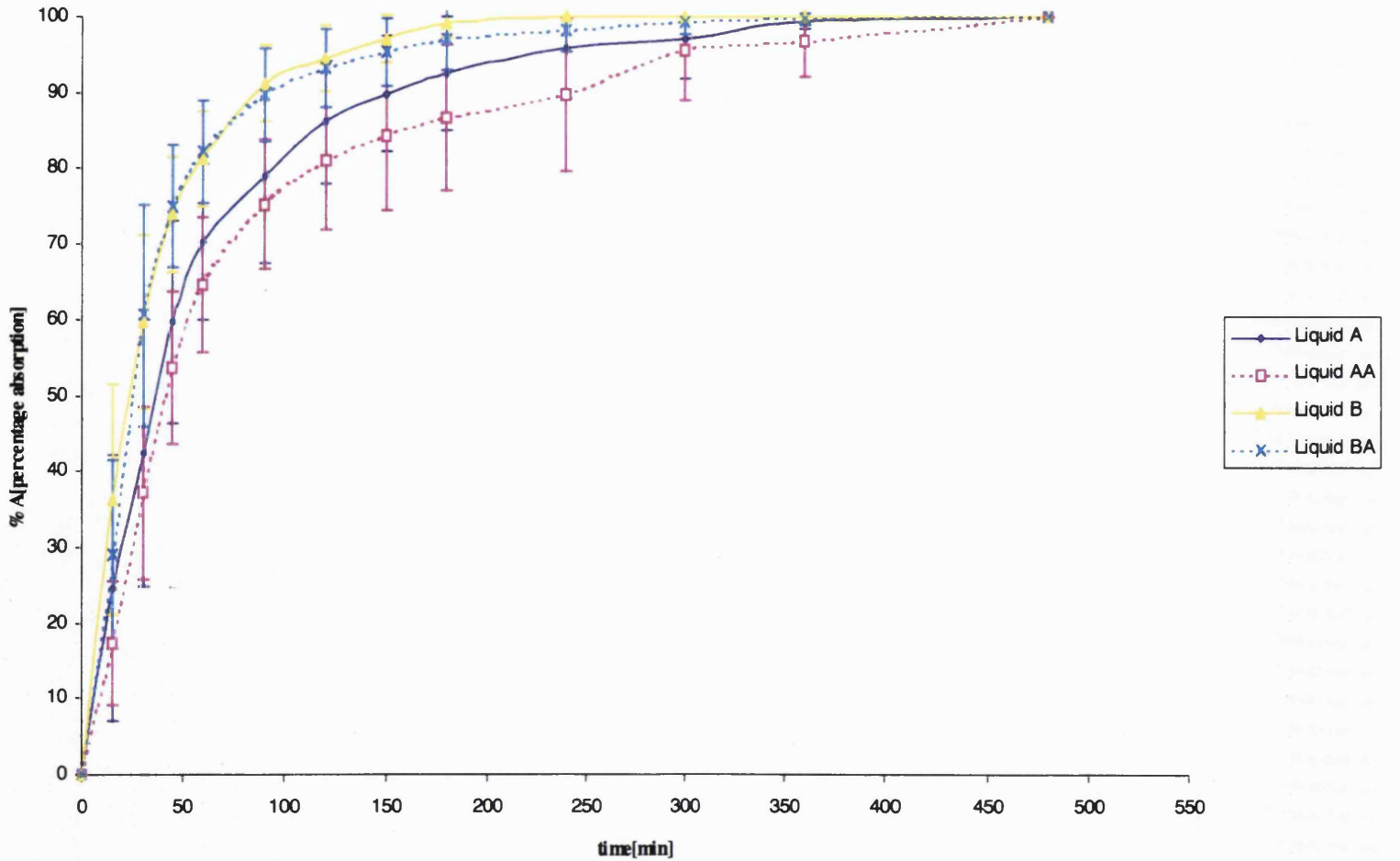
Subject	Pharmacokinetic parameters					F_A values			
	K_{el} (min^{-1})	$t_{½}$ (min)	c_o (μgml^{-1})	V_d (l)	Cl ($lmin^{-1}$)	Liquid A	Liquid AA	Liquid B	Liquid BA
AA	0.0786	8.13	7.92	4.58	0.3500	1.03	0.97	0.79	0.69
BB	0.1121	6.18	17.49	2.07	0.2320	0.76	0.72	0.78	0.59
CB	0.0800	8.67	7.21	5.03	0.4024	1.20	1.18	1.21	0.89
DB	0.0952	7.28	7.96	4.56	0.4341	1.66	1.55	1.15	1.20
DS	0.1534	4.52	17.87	2.03	0.3114	0.92	0.72	0.91	0.69
OC	0.0918	7.55	7.47	4.85	0.4452	1.53	1.50	1.12	0.75
TN	0.0622	11.14	5.49	6.60	0.4105	1.36	1.55	1.37	0.97
VM	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
WB	0.0594	11.67	10.05	3.61	0.2144	0.94	0.95	0.94	0.86
YO	0.0781	8.87	9.02	4.02	0.3140	1.20	1.07	0.99	0.74
Mean	**0.0901**	**7.11**	**10.05**	**4.15**	**0.3471**	**1.18**	**1.13**	**1.03**	**0.82**
s.d	**0.0271**	**2.97**	**4.24**	**1.45**	**0.0283**	**0.30**	**0.33**	**0.20**	**0.18**

Table 4.12: The pharmacokinetic characterisations of 4ASA calculated by Wagner-Nelson method. K_{el} (elimination rate constant in min^{-1}); $t_{½}$(half life in minutes); C_o (calculated concentration at time zero); V_d (apparent volume of distribution); Cl (clearance in $lmin^{-1}$); F_A (absolute bioavailability); Liquid A (pH3 4ASA formulation without ranitidine treatment); Liquid AA (pH3 formulation with ranitidine treatment); Liquid B (pH7 Na4ASA treatment without ranitidine treatment); Liquid BA (pH7 formulation with ranitidine treatment); N/A (not applicable).

Subjects	AUC of different formulations					MRT of different formulations					MAT of different formulations			
	Liquid A	Liquid AA	Liquid B	Liquid BA	IV dose	Liquid A	Liquid AA	Liquid B	Liquid BA	IV dose	Liquid A	Liquid AA	Liquid B	Liquid BA
AA	1581.81	1486.97	876.05	770.77	111.26	85.94	100.33	52.43	55.08	21.11	64.83	79.22	31.32	33.97
BB	1935.25	1836.33	1432.50	1079.44	184.11	55.34	77.27	52.69	52.69	18.61	36.73	58.66	34.08	34.08
CB	1601.70	1580.95	1174.25	864.59	97.12	102.49	170.01	79.29	86.51	21.49	81.00	148.52	57.80	65.02
DB	2092.95	1948.72	1049.71	1097.34	91.23	76.62	70.09	59.94	69.53	20.32	56.30	49.75	39.62	49.21
DS	2081.86	1621.80	1487.66	1133.37	163.84	75.14	93.84	50.14	51.92	16.63	58.15	77.21	33.51	35.29
OC	1936.57	1908.03	1034.46	694.41	92.07	42.34	131.59	62.14	80.85	19.36	22.98	112.23	42.78	61.49
TN	1753.92	2005.03	1282.44	906.25	93.52	150.93	89.45	42.70	49.53	22.61	128.32	66.84	20.09	26.92
VM	1124.71	1476.49	801.81	640.05	N/A	53.56	51.26	52.62	48.68	N/A	N/A	N/A	N/A	N/A
WB	2319.97	2337.10	1678.80	1530.87	178.19	106.33	88.71	68.02	75.25	23.38	82.95	65.33	44.64	51.87
YO	2170.09	1927.79	1291.22	969.90	130.95	91.75	132.13	70.48	57.66	21.24	70.51	110.89	49.24	36.42
Mean	**1860.00**	**1812.85**	**1210.90**	**968.60**	**126.89**	**74.81**	**100.47**	**59.04**	**62.78**	**20.52**	**66.87**	**85.39**	**39.22**	**43.79**
s.d	**352.47**	**271.39**	**277.81**	**260.14**	**38.73**	**38.36**	**34.94**	**11.08**	**14.03**	**2.08**	**30.15**	**32.00**	**11.03**	**13.50**

Table 4.13: The area under the curve, mean residence time and mean absorption time of 4ASA calculated by Wagner-Nelson method. MRT (mean residence time in min); AUC (area under the concentration curve in $\mu gminml^{-1}$); MAT (mean absorption time in min); Liquid A (pH3 4ASA formulation without ranitidine treatment); Liquid AA (pH3 formulation with ranitidine treatment); Liquid B (pH7 Na4ASA treatment without ranitidine treatment); Liquid BA (pH7 formulation with ranitidine treatment), IV dose (intravenous route); N/A (not applicable).

Figure 4.4: The mean cumulative absorption profiles of 4ASA oral liquid formulations calculated by Wagner-Nelson method



		Kolmogorov-Smirnov		
		statistic	df	Sig.
F_A values	Liquid A	0.1369	9	>0.200
	Liquid AA	0.1335	9	>0.200
	Liquid B	0.1333	9	>0.200
	Liquid BA	0.2036	9	>0.200
MAT	Liquid A	0.2433	9	0.132
		0.1950	8	>0.200
	Liquid AA	0.1852	9	>0.200
	Liquid B	0.1232	9	>0.200
	Liquid BA	0.2634	9	0.072
AUC	Liquid A	0.096	10	>0.200
	Liquid AA	0.159	10	>0.200
	Liquid B	0.119	10	>0.200
	Liquid BA	0.164	10	>0.200
	IV dose	0.224	9	>0.200
MRT	Liquid A	0.129	10	>0.200
	Liquid AA	0.202	10	>0.200
	Liquid B	0.216	10	>0.200
	Liquid BA	0.241	10	0.102
	IV dose	0.097	9	>0.200
Clearance (Cl)		0.135	9	>0.200
Elimination rate constant (K_{el})		0.207	9	>0.200
		0.175	8	>0.200
Half life ($t_{½}$)		0.166	9	>0.200
Distribution volume (V_d)		0.160	9	>0.200

Table 4.14: Test of normality for the pharmacokinetic parameters of 4ASA calculated by Wagner-Nelson method. Liquid A (pH3 4ASA formulation without ranitidine treatment); Liquid AA (pH3 4ASA formulation with ranitidine treatment); Liquid B (pH7 Na4ASA formulation without ranitidine treatment); Liquid BA (pH7 Na4ASA formulation with ranitidine treatment); IV dose (intravenous route).

Effect (F)	F_{A4ASA} (n=36)	AUC (n=40)	MAT (n=36)	MRT (n=40)
Route	27.69***	245.56***	21.44***	8.90*
Acid Block	8.20**	9.23**	2.38	2.69
Subject	12.66**	12.10***	1.45	0.75

***is p<0.005 ** is p<0.01 * is p<0.05

Table 4.15: Analysis of variance (ANOVA) for the 4ASA pharmacokinetic parameters of the oral liquid formulations calculated by Wagner-Nelson method. F values and their significance.

Comparison	Z values and their significance			
	AUC	F_A	MRT	MAT
Liquid A to liquid AA	-0.968	-1.599	-1.274	-1.362
Liquid A to liquid B	-2.803**	-1.400	-1.478	-2.310*
Liquid A to liquid BA	-2.803**	-2.666**	-1.070	-1.718
Liquid AA to liquid B	-2.803**	-1.249	-2.701**	-2.666**
Liquid AA to liquid BA	-2.803**	-2.666**	-2.803**	-2.666**
Liquid B to liquid BA	-2.701**	-2.547*	-1.362	-1.599

*** is p<0.001 **is p<0.01 * is p<0.05

Table 4.16: Wilcoxon Signed-Ranks test comparing the pharmacokinetic parameters of 4ASA oral liquid formulations calculated by Wagner-Nelson method. Z values and their significance. Liquid A (pH3 4ASA formulation without ranitidine treatment); Liquid AA (pH3 4ASA formulation with ranitidine treatment); Liquid B (pH7 Na4ASA formulation without ranitidine treatment); Liquid BA (pH7 Na4ASA formulation with ranitidine treatment).

4.5.3.2 Results on kinetic analysis on AASA

The pharmacokinetic characterisation of the metabolite, AASA, is less well known. As described in *section 1.6*, the formation of AASA is complex, involving both pre-systemic and systemic acetylation depending on the route of administration of the 4ASA, and is enzyme capacity limited. Only the elimination phase kinetic can be estimated using Wagner-Nelson method. It is not possible to characterise the formation profiles unless intravenous data by direct injection of the metabolite is available. Moreover, the enzyme capacity effect can only be demonstrated by using several different doses of 4ASA.

The pharmacokinetic parameters calculated are AUC, K_{el}, F_R, $t_{½}$ (half-life), V_d and Cl values using a non-compartment model based on the statistical moments (Gibaldi et al 1982). There is no need to specify compartmental model but the linear kinetic is assumed. It is suitable when only a single dose of either the drug or metabolite is available. The K_{el} value was determined as the slope of the terminal portion of the natural log concentration-time plot using the linear regression analysis. The AUC from time zero to the last measured concentration was determined by the linear trapezoidal rule and AUC from last time point to

infinity was added to give the total AUC. The relative bioavailability (F_R) values were determined by comparing to the intravenous data by assuming the bioavailability of AASA for intravenous dose to be one and the fraction of AASA in the systemic blood circulation to be equal to the bioavailability of 4ASA. As mentioned in the previous section, the F_A values of 4ASA calculated using the Wagner-Nelson method are less reliable, the F_R values were calculated as $[(AUC_{oral} \times dose_{iv})/(AUC_{iv} \times dose_{oral} \times F_A)]$ using the F_A values derived from MAXENT approach. The Cl and V_d values were calculated as $[(F_A)$ (dose)/AUC] and $[Cl/K_{el}]$, respectively. The results are tabulated in tables 4.17-4.18.

The magnitudes of K_{el} values are smaller with the oral formulations when compare to intravenous dose whilst the reverse is observed with other kinetic parameters. The results indirectly indicate pre-systemic acetylation of the oral formulations within the gastrointestinal tract, which consequently enter the systemic circulation. These parameters are different from previous published reports (Peloquin et al 1994, Norgine brochure), as the dose and dosage form differ. However, the systemic elimination of AASA is slower than that of the drug in the current study and all other reports.

The test of normality was performed and the results showed that all the kinetic parameters were normally distributed (table 4.19). The analysis of variance for the oral formulations shows that the inter-subject variation is significant in all cases and the significant effects of formulations and ranitidine treatment are therefore less certain (table 4.20). The MRT, K_{el} and $t_{½}$ values are not significantly influenced by the formulations and ranitidine treatment. This is consistent with the results using the Wilcoxon Signed-Ranks test at a 0.05 significant level (table 4.21). The effect of formulation dominates other pharmacokinetic parameters except for AUC where the ranitidine effect is also significant (table 4.20). The Wilcoxon Signed-Ranks test shows a mixed outcome with these parameters (table 4.21). The trend indicates a higher availability of pH7 formulation with ranitidine treatment, as the acetylation process is optimised at neutral pH condition. A larger sample size would be required in order to evaluate these effects more precisely.

Subjects	K_{el} of different formulations					$t_{½}$					F_R values			
	Liquid A	Liquid AA	Liquid B	Liquid BA	IV dose	Liquid A	Liquid AA	Liquid B	Liquid BA	IV dose	Liquid A	Liquid AA	Liquid B	Liquid BA
AA	0.0082	0.0082	0.0081	0.0078	0.0129	78.93	84.51	85.56	88.85	53.72	0.58	0.63	0.83	0.97
BB	0.0092	0.0090	0.0084	0.0090	0.0135	75.24	77.26	82.60	77.43	51.33	0.43	0.38	0.48	0.56
CB	0.0089	0.0096	0.0101	0.0095	0.0111	77.60	72.34	68.55	73.02	62.43	0.60	0.42	0.61	0.68
DB	0.0096	0.0087	0.0107	0.0120	0.0110	72.11	79.66	64.65	57.75	63.00	0.43	0.32	0.42	0.46
DS	0.0090	0.0090	0.0083	0.0079	0.0130	76.71	76.74	83.39	87.50	53,31	0.58	0.70	0.70	0.69
OC	0.0110	0.0114	0.0084	0.0103	0.0141	63.00	60.79	82.99	67.28	49.15	0.34	0.39	0.51	0.60
TN	0.0111	0.0109	0.0112	0.0093	0.0110	62.43	63.58	61.88	74.84	63.00	0.44	0.34	0.37	0.52
VM	0.0119	0.0100	0.0112	0.0099	0.0145	58.24	69.30	61.88	70.00	47.79	0.53	0.47	0.52	0.68
WB	0.0091	0.0088	0.0071	0.0096	0.0109	76.15	78.75	97.74	72.41	63.58	0.36	0.26	0.31	0.31
YO	0.0086	0.0091	0.0091	0.0104	0.0162	80.49	75.82	75.82	66.63	42.78	0.42	0.39	0.39	0.54
Mean	**0.0097**	**0.0095**	**0.0093**	**0.0096**	**0.0128**	**72.08**	**73.87**	**76.51**	**73.57**	**55.01**	**0.47**	**0.43**	**0.51**	**0.59**
s.d	**0.0012**	**0.0010**	**0.0014**	**0.0012**	**0.0018**	**7.92**	**7.43**	**11.95**	**9.41**	**7.52**	**0.09**	**0.14**	**0.16**	**0.19**

Table 4.17: The elimination rate constant, elimination half-life and relative bioavailability of AASA calculated by Wagner-Nelson method. K_{el} (elimination rate constant in min^{-1}); $t_{½}$(half life in minutes); F_R (relative bioavailability of the liquid formulation to intravenous route, assuming the F value for IV route to be 1); Liquid A (pH3 4ASA formulation without ranitidine treatment); Liquid AA (pH3 formulation with ranitidine treatment); Liquid B (pH7 Na4ASA treatment without ranitidine treatment); Liquid BA (pH7 formulation with ranitidine treatment); IV dose (intravenous route).

Subjects	Cl	V_d	AUC of different formulations					MRT of different formulations				
	IV dose	IV dose	Liquid A	Liquid AA	Liquid B	Liquid BA	IV dose	Liquid A	Liquid AA	Liquid B	Liquid BA	IV dose
AA	0.0571	4.42	3709.21	3718.65	2943.91	3052.23	809.16	136.50	154.08	143.02	146.77	106.46
BB	0.0433	3.21	5483.88	4602.34	4564.11	4002.20	1066.27	134.13	148.58	143.22	139.72	94.28
CB	0.0641	5.78	4490.32	2882.92	3096.21	2561.62	720.67	153.66	138.79	146.68	139.87	91.39
DB	0.0463	4.21	5879.56	4314.83	2094.56	3249.05	998.26	138.27	140.05	143.77	139.27	100.57
DS	0.0526	4.05	5718.34	5096.58	4676.94	3651.29	877.87	146.24	150.07	158.58	142.1	93.97
OC	0.0507	3.60	4053.12	4039.50	3236.41	2645.81	912.03	126.89	130.02	151.60	132.12	92.94
TN	0.0442	4.02	5186.92	4900.39	3457.87	3444.36	1045.97	144.40	128.91	127.40	139.37	98.80
VM	0.0698	4.81	3808.59	4309.07	2707.26	2691.16	662.15	130.88	137.82	129.31	128.27	78.73
WB	0.0288	2.64	7087.63	5660.05	4766.58	4201.57	1605.51	174.96	155.36	163.45	143.61	120.64
YO	0.0543	3.35	4977.30	4283.46	3055.65	3079.86	851.14	143.37	149.86	148.62	139.04	92.35
Mean	**0.0511**	**4.01**	**5039.50**	**4380.80**	**3560.00**	**3267.00**	**954.90**	**142.74**	**143.37**	**145.60**	**139.53**	**97.02**
s.d	**0.0115**	**0.89**	**1061.75**	**766.26**	**790.26**	**554.77**	**263.90**	**13.71**	**9.58**	**11.30**	**5.87**	**10.97**

Table 4.18: The clearance, distribution volume, area under the curve and mean residence time of AASA calculated by Wagner-Nelson method. Cl (clearance in $lmin^{-1}$); V_d (apparent volume of distribution in litre); MRT (mean residence time in min); AUC (area under the concentration curve in $\mu gminml^{-1}$); Liquid A (pH3 4ASA formulation without ranitidine treatment); Liquid AA (pH3 formulation with ranitidine treatment); Liquid B (pH7 Na4ASA treatment without ranitidine treatment); Liquid BA (pH7 formulation with ranitidine treatment), IV dose (intravenous route).

		Kolmogorov-Smirnov		
		statistic	df	Sig.
F_R values	Liquid A	0.229	10	0.148
	Liquid AA	0.229	10	0.146
		0.179	8	>0.200
	Liquid B	0.185	10	>0.200
	Liquid BA	0.188	10	>0.200
AUC	Liquid A	0.124	10	>0.200
	Liquid AA	0.134	10	>0.200
	Liquid B	0.259	10	0.056
	Liquid BA	0.150	10	>0.200
	IV dose	0.269	9	0.059
MRT	Liquid A	0.252	10	0.072
		0.149	9	>0.200
	Liquid AA	0.134	10	>0.200
	Liquid B	0.125	10	>0.200
	Liquid BA	0.175	10	>0.200
	IV dose	0.249	9	0.115
Cl	IV dose	0.102	10	>0.200
K_{el}	Liquid A	0.272	10	0.035
		0.153	7	>0.200
	Liquid AA	0.242	10	0.100
		0.214	8	>0.200
	Liquid B	0.225	10	0.166
	Liquid BA	0.150	10	>0.200
	IV dose	0.228	10	0.149
$t_{½}$	Liquid A	0.174	10	>0.200
	Liquid AA	0.117	10	>0.200
	Liquid B	0.146	10	>0.200
	Liquid BA	0.148	10	>0.200
	IV dose	0.169	10	>0.200
V_d	IV dose	0.132	10	>0.200

Table 4.19: Test of normality for the pharmacokinetic parameters of AASA calculated by Wagner-Nelson method. Liquid A (pH3 4ASA formulation without ranitidine treatment); Liquid AA (pH3 4ASA formulation with ranitidine treatment); Liquid B (pH7 Na4ASA formulation without ranitidine treatment); Liquid BA (pH7 Na4ASA formulation with ranitidine treatment); IV dose (intravenous route).

Effect (F)	F_{RAASA} (n=9)	AUC (n=10)	MRT (n=10)	K_{el} (n=10)	$t_{½}$ (n=10)
Route	21.31***	93.24***	0.40	0.37	0.84
Acid Block	0.49	12.56**	1.25	0.10	0.07
Subject	15.07**	11.68***	4.48**	3.93*	3.89*

*** is p<0.001 **is p<0.005 * is p<0.01

Table 4.20: Analysis of variance (ANOVA) for the pharmacokinetic parameters of AASA calculated by Wagner-Nelson method. F values and their significance for the oral liquid formulations.

	Z values and their significance				
Comparison	AUC	F_R	MRT	K_{el}	$t_{½}$
Liquid A to liquid AA	-2.293*	-1.274	-0.255	-0.770	-1.007
Liquid A to liquid B	-2.803**	-0.663	-0.561	-0.986	-0.917
Liquid A to liquid BA	-2.083**	-2.344*	-0.255	-0.510	-0.255
Liquid AA to liquid B	-2.599**	-2.521*	-0.408	-0.415	-0.533
Liquid AA to liquid BA	-2.803**	-2.547*	-1.478	-0.296	-0.561
Liquid B to liquid BA	-1.580	-2.381*	-1.682	-0.612	-0.765

***p<0.001 **is p<0.01 *is p<0.05

Table 4.21: Wilcoxon Signed-Ranks test comparing the pharmacokinetic parameters of AASA calculated by Wagner-Nelson method. Z values and their significance for the oral liquid formulations. Liquid A (pH3 4ASA formulation without ranitidine treatment); Liquid AA (pH3 4ASA formulation with ranitidine treatment); Liquid B (pH7 Na4ASA formulation without ranitidine treatment); Liquid BA (pH7 Na4ASA formulation with ranitidine treatment).

4.5.3.3 Comparison of the 4ASA pharmacokinetic parameters using different pharmacokintic analysis approaches

The Wilcoxon Signed-Ranks test is used to compare the two methodologies. Table 4.22 shows the Z values and their significance. The K_{el}, Cl, F_A MAT and MRT values differ between the two methods. The MRT values are calculated from the zero and first moments of

the AUC based on the linear functions, and are often referred to as AUC_{∞} and $AUMC_{\infty}$, respectively. Inconsistency in the calculated $AUMC_{\infty}$, because different formulas were used has been reported by Charter (1989). It is desirable to use different formulas for assessment of MRT value in this case in order to minimise the inaccuracy introduced by the interpolation using the Wagner-Nelson method. The MRT value derived from the maximum entropy results has also been reported to be different when compared with a non-linear curve fitting method in the assessment of pharmacokinetic profiles of teicoplanin (Podczeck et al 1996). Satisfactory correlation is observed with the pooled data in all the kinetic parameters except for the Cl and K_{el} values using Spearman's-Rank correlation regression analysis (table 4.23). By separating the data sets according to the administered formulations and ranitidine treatment, all the data except the F_A value and data from the intravenous dose show good correlations between the two methods. The poor correlations observed in Cl, K_{el} and F_A values can be explained by the gross over estimation of the intravenous dose using Wagner-Nelson method.

	Z values and their significance (n=10)				
	Liquid A	**Liquid AA**	**Liquid B**	**Liquid BA**	**IV dose**
AUC	-2.803**	-2.497	-2.395	-2.803**	-1.481^{+}
MRT	-1.162	-2.803**	-2.803**	-2.803**	-2.666**
MAT	-2.666**	-2.666**	-2.666**	-2.801**	N/A
F_A	-2.310^{*+}	-2.310^{*+}	-2.192^{*+}	-2.310^{**+}	N/A
Cl	-2.547^{*+}				
K_{el}	-2.666^{**+}				
V_d	-1.362^{+}				

***is p<0.001 **is p<0.01 *is p<0.05 $^{+}$is n=9

Table 4.22: Wilcoxon Signed-Ranks test for comparison of the pharmacokinetic analysis by Wagner-Nelson and MAXENT methods. Z values and their significance. Liquid A (pH3 4ASA formulation without ranitidine treatment); Liquid AA (pH3 4ASA formulation with ranitidine treatment); Liquid B (pH7 Na4ASA formulation without ranitidine treatment); Liquid BA (pH7 Na4ASA formulation with ranitidine treatment); IV dose (intravenous route); N/A (not applicable).

	R_s values and their significance (n=10)				
	Liquid A	**Liquid AA**	**Liquid B**	**Liquid BA**	**IV dose**
AUC	0.952***	0.939***	1.000***	0.964***	0.756*
MRT	0.661*	0.867**	0.927***	0.964***	0.417
MAT	0.950***	0.870**	0.929***	0.946***	N/A
F_A	0.370^+	0.432^+	-0.502^+	0.286^+	N/A
	R_s values & their significance		**N (pooled data)**		
AUC	0.990***		40		
MRT	0.853***		40		
MAT	0.961***		36		
F_A	0.512**		36		
Cl	0.700*		9		
K_{el}	0.444		9		
V_d	0.967***		9		

***is p<0.001 **is p<0.01 *is p<0.05 $^+$is n=9

Table 4.23: Spearman's correlation coefficient for correlating the pharmacokinetic analysis using Wagner-Nelson and MAXENT methods. R_s values and their significance. Liquid A (pH3 4ASA formulation without ranitidine treatment); Liquid AA (pH3 4ASA formulation with ranitidine treatment); Liquid B (pH7 Na4ASA formulation without ranitidine treatment); Liquid BA (pH7 Na4ASA formulation with ranitidine treatment); IV dose (intravenous route); N/A (not applicable).

4.5.4 Results and discussion on pH and gastric emptying profiles

The gastric emptying characteristics were calculated from APT and pH monitoring as described in *section 4.5.1*. The gastric emptying rate constant (K_{GE}) can be determined with [1/GMRT] by assuming the gastric emptying profiles of the liquid formulations to be a first order kinetic as described by Pinto et al (1997). The results are shown in table 4.25. The raw data showed that the test liquids of varying pH values emptied from the stomach at different rates. The incorporation of 4ASA into the formulations has no effect on the gastric emptying rate, as the results are comparable to findings using the buffer liquids in the first study (*section 3.5.3*). Table 4.24 shows the F values and their significance by the analysis of variance. The acidic pH3 value delayed gastric emptying at a significant level despite the small volume of administration. No significant subject effect was seen, except for the pHtime value, where the measurement depended upon the basal pH value and the buffering capacity in the stomach of each individual. Furthermore, the incorporation of the tethered pH sensitive radiotelemetry capsule induced different degrees of salivation, which was constantly swallowed by the subject into the stomach. Administration of a 300mg ranitidine (Zantac®) tablet prior to the study for acid suppression had no effect on the gastric emptying profiles, which clearly demonstrated that gastric emptying could be monitored using APT completely physiologically, supporting the findings of Mitchell (1997) and Evans et al (1990). Raniditine also exerted a full acid inhibition over the two hours monitoring by APT system and pH sensitive radiotelemetry capsule (figure 4.5).

Effect	GMRT (F)	GE_{50} (F)	pHtime (F)	AUEC (F)	VGRT (F)	lagtime (F)	K_{GE} (F)
Route	26.01***	17.32***	27.48***	10.18**	9.15**	6.36*	23.13***
Acid block	1.64	2.77	0.06	3.25	0.89	1.01	0.98
Subject	0.97	0.88	7.65**	1.23	1.54	0.93	1.44

*p<0.05 **p<0.01 ***p<0.001

Table 4.24: Analysis of variance for characterisations of the gastric emptying and pH profiles of 4ASA oral liquid formulations derived from APT and radiotelemetry analysis. F values and their significance. Route (the effect of pH value of the test liquid); Acid block (the effect of administrating ranitidine 300mg); Subject (inter-subject variation)

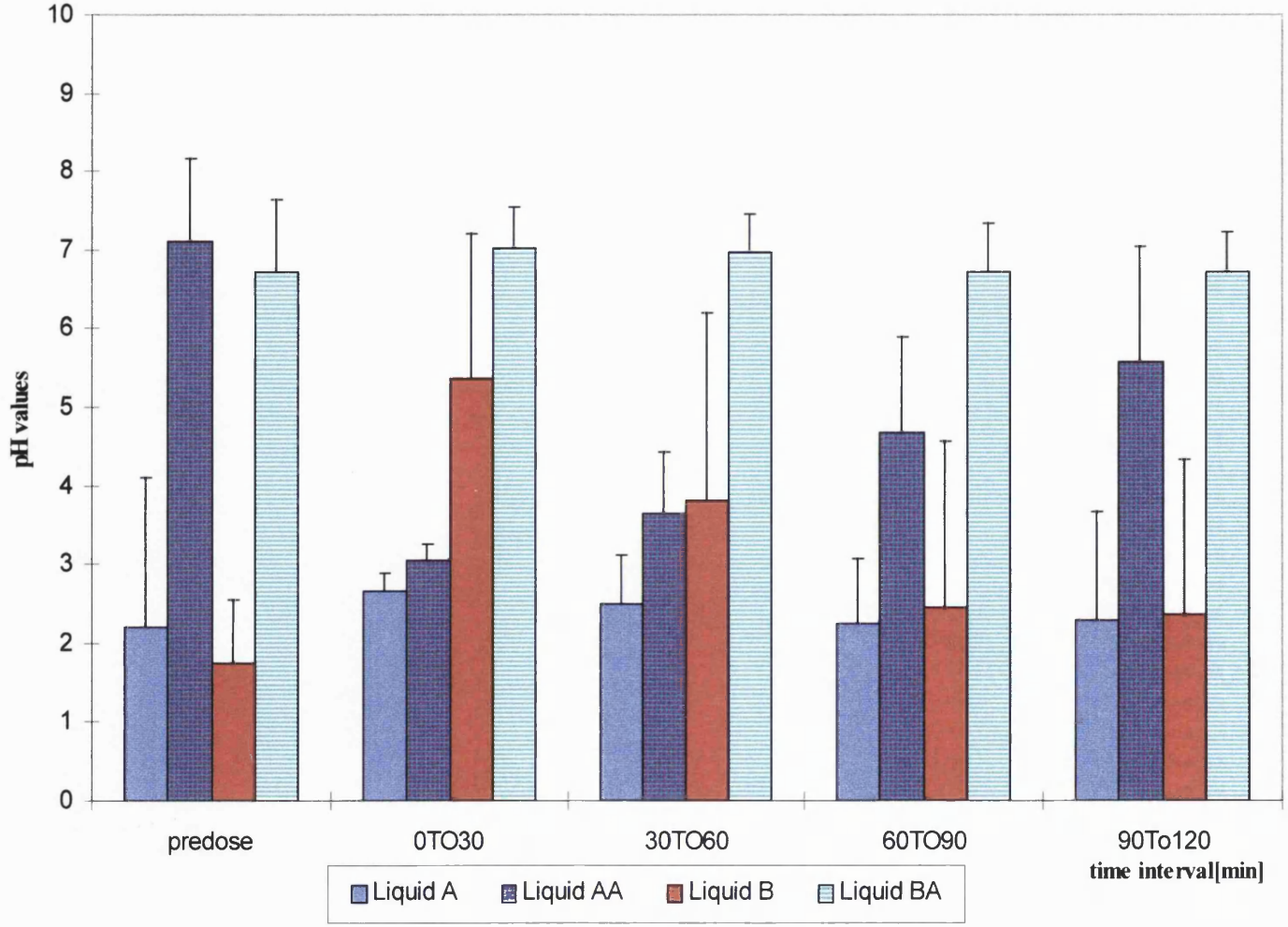
Subject	GMRT (min)	GE_{50} (min)	VGRT (min^2)	AUEC (%min)	Lagtime (min)	K_{GE} (min^{-1})	pHtime (min)
Liquid A							
AA	49.60	-	583.00	-294.20	3.00	0.0202	80.00
BB	29.20	29.00	273.30	-293.60	4.00	0.0342	-
CB	40.30	47.00	53.00	127.40	3.00	0.0248	54.00
DB	39.60	53.00	127.40	-460.20	20.00	0.0253	46.00
DS	49.60	34.00	285.00	-75.90	21.00	0.0202	43.00
OC	25.20*	31.00*	9.00	-36.10	21.00	0.0397	77.00*
TN	27.00	30.00	47.40	-69.30	16.00	0.0370	-
VM	-	-	-	-	-	-	-
WB	21.40	19.00	48.60	-41.60	11.00	0.0467	29.00
YO	31.00	-	223.40	-487.40	7.00	0.0323	22.00
Mean	**34.77**	**34.71**	**233.94**	**-273.09**	**11.78**	**0.0312**	**50.14**
s.d.	**10.43**	**11.56**	**203.88**	**238.17**	**7.86**	**0.0092**	**22.12**
Liquid AA							
AA	40.50	66.00	259.00	-502.80	14.00	0.0247	77.00
BB	23.10	47.00	145.20	-246.60	4.00	0.0433	-
CB	23.90*	27.00*	224.80	-161.70	23.00	0.0418	62.00*
DB	55.10	-	597.00	-875.10	16.00	0.0181	46.00
DS	25.10	36.00	247.40	-213.50	4.00	0.0398	26.00
OC	-	-	-	-	-	-	68.00
TN	25.80	32.00	247.00	-589.30	3.00	0.0388	49.00
VM	-	-	-	-	-	-	38.00
WB	75.20*	82.00*	80.00	-278.60	62.00	0.0139	22.00*
YO	34.70	45.00	127.20	-202.10	14.00	0.0288	38.00
Mean	**37.93**	**47.86**	**240.95**	**-383.71**	**17.50**	**0.0311**	**47.33**
s.d.	**18.63**	**19.71**	**158.32**	**250.43**	**19.32**	**0.0114**	**18.70**
Liquid B							
AA	17.60	29.00	97.20	-83.50	3.00	0.056	-
BB	21.80	33.00	54.50	-130.20	9.00	0.0459	-
CB	20.20	24.00	141.60	-145.20	5.00	0.0495	25.00
DB	18.90	20.00	143.30	-130.20	3.00	0.0529	12.00
DS	13.10	20.00	46.50	-58.10	3.00	0.0763	17.00
OC	-	13.00	-	-	-	-	32.00
TN	-	29.00	-	-	-	-	16.00
VM	24.70	23.00	75.30	-126.60	12.00	0.0405	-
WB	5.90	11.00	3.40	-28.70	3.00	0.1695+	22.00
YO	10.30	10.00	21.10	-124.20	3.00	0.0971+	8.00
Mean	**16.56**	**21.10**	**72.86**	**-103.34**	**5.13**	**0.0537**	**18.86**
s.d.	**6.31**	**7.97**	**51.85**	**41.72**	**3.48**	**0.0124**	**8.13**
Liquid BA							
AA	16.80	25.00	87.60	-187.60	3.00	0.0595	-
BB	18.20	23.00	93.70	-353.40	3.00	0.0549	-
CB	21.80	14.00	54.50	-142.30	3.00	0.0459	-
DB	30.10	33.00	134.10	-208.20	15.00	0.0332	-
DS	14.20	-	74.10	-82.30	3.00	0.0704	-
OC	19.00	17.00	66.60	-234.30	7.00	0.0526	-
TN	25.60	25.00	191.60	-134.80	12.00	0.0391	-
VM	39.20	49.00	632.10	-411.90	3.00	0.0255	-
WB	29.50	30.00	128.20	-105.90	12.00	0.0339	-
YO	10.40	15.00	27.30	-134.50	3.00	0.0962+	-
Mean	**22.48**	**25.67**	**148.98**	**-199.52**	**6.4**	**0.0461**	**-**
s.d.	**8.66**	**10.9**	**176.01**	**107.71**	**4.79**	**0.0145**	**-**

Table 4.25: Gastric emptying characteristics of 4ASA oral liquid formulations derived from APT and pH analysis in the healthy subjects. Liquid A (pH3 4ASA formulation without ranitidine treatment); Liquid AA (pH3 4ASA formulation with ranitidine treatment); Liquid B (pH7 Na4ASA formulation without ranitidine treatment); Liquid BA (pH7 Na4ASA formulation with ranitidine treatment).

+These data were omitted in the calculation for mean, standard deviation and analysis of variance as shown in table 4.24.

***These data were omitted in the statistical comparison by Spearman's Rank correlation analysis and limits of agreement as shown in table 4.26.**

Figure 4.5: The mean of the median pH values distribution of the 4ASA liquid formulations derived from Flexilog II program



4.5.5 Comparison between measurements from pH sensitive radiotelemetry and APT

The gastric emptying and pH profiles were monitored simultaneously with the pH sensitive radiotelemetry capsule and APT system. The pHtime values were compared to the gastric emptying half time (GE_{50}) derived graphically and gastric mean residence time (GMRT) calculated by the statistical moments analysis from the gastric emptying profiles of the APT measurement. The comparison is performed using Spearman's-Rank correlation regression analysis and by calculation of the limits of agreement between the methods (Bland et al 1986). The pHtime value of the pH7 Na4ASA formulation with ranitidine treatment could not be calculated as a fluctuation in pH was not observed. Hence, it was excluded from the comparison. Three data pairs from the rest of the data were not included when comparing the pHtime values to the GMRT and GE_{50} values as the data showed a very large difference between the two methods (table 4.25).

The differences between gastric emptying characteristic values of the two methods were plotted against the corresponding average values, which gave an indication of agreement and possible bias between the methods (figures 4.6 & 4.7). The full limits of agreement data analysis were shown in **Appendix 11.**

Table 4.26 shows the Spearman's-Rank correlation coefficient values (r_s) and the limits of agreement between the two methods. Although the correlation analysis shows that the results have significant levels of correlation, the rank correlation values are poor when the groups are subdivided according to the test liquids and test conditions. As the volume of administration was relatively small, the raw data collected from the APT system presented more noise and artefacts. Some data could not be processed by the statistical moments analysis due to the irregularity of the gastric emptying profiles. On the other hand, the physiological fluid flux and secretion affected the pH data. This accounted for the limited numbers of data included in separate groups' comparison.

The good correlation between the pooled data derived from all tests were not reflected in the corresponding limits of agreement. There were relatively large differences between the measurements from each method for the different test liquids and in the combined test liquids. This suggested that although in some instances, there was a correlation between the pH sensitive radiotelemetry capsule and APT system, the agreements were poor.

Variable	Correlation Coefficient(r_s)	Limits of agreement Mean Difference ± 2SD
All Liquids		
pHgmrt	0.82***	5.36±30.16
pH GE_{50}	0.74**	2.82±34.05
Liquid A		
pHgmrt	0.70	7.08±28.76
pH GE_{50}	0.80	4.75±15.86
Liquid AA		
pHgmrt	0.50	10.96±36.94
pH GE_{50}	0.40	11.40±45.00
Liquid B		
pHgmrt	0.30	-1.03±25.60
pH GE_{50}	0.14	-3.50±31.35
Liquid BA		
pHgmrt	N/A	N/A
pH GE_{50}	N/A	N/A

one-tailed significance: *p<0.05 **p<0.01 ***p<0.001

Table 4.26: The Spearman's correlation coefficient values (r_s) and the limits of agreement between pH and APT measurements of different liquid formulations in the healthy subjects. pHgmrt (comparison between pH measurement and gmrt from APT); pHGE_{50} (comparison between pH measurement and GE_{50} from APT).

Figure 4.6: Graphical limits of agreement between time interval measurements at given pH value by pH radiotelemetry to gastric mean residence time from APT system in all test liquids

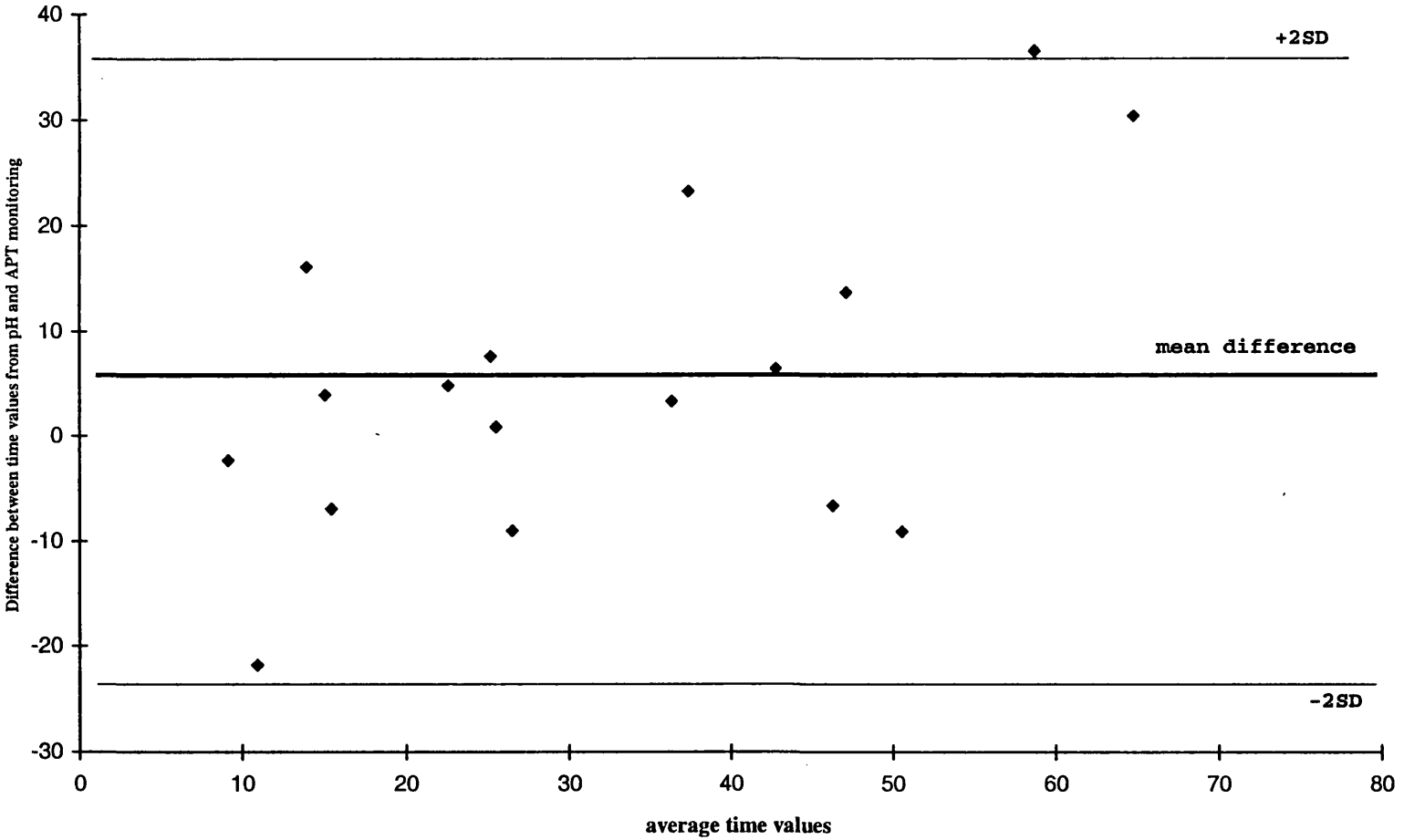
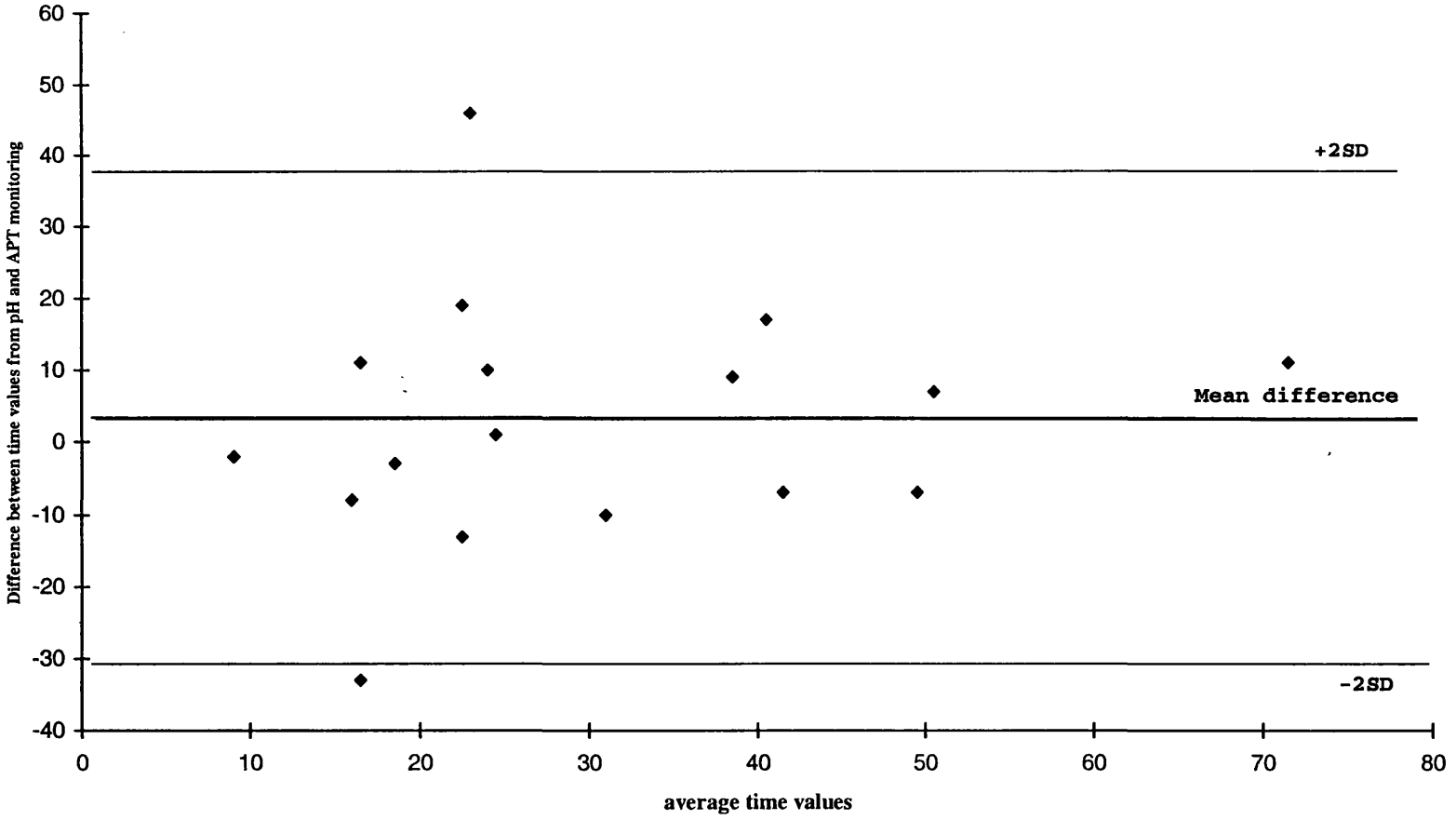


Figure 4.7: Graphical limits of agreement between time interval measurements at given pH value by pH radiotelemetry to gastric emptying half time by APT system in all test liquids



4.5.6 Comparison between the mean residence times derived from gastric emptying and 4ASA pharmacokinetic profiles

As the MRT values are significantly different between the formulations, which in turn is influenced by the gastric emptying rate, Spearman's-Rank correlation analysis is performed to establish possible relationships. Results from the pooled data show significant correlation between the MRT and gastric emptying parameters despite poor correlations when the data are separated by type of formulation and ranitidine treatment (table 4.27). The poor correlations are due to small sample size when the data is separated.

Comparison	r_s values and their significance				
	Liquid A	Liquid AA	Liquid B	Liquid BA	Pooled data
MRT from Wagner-Nelson to GMRT	-0.517 (n=9)	-0.649* (n=10)	-0.417 (n=9)	-0.3333 (n=8)	-0.413* (n=36)
MRT from Wagner-Nelson to GE_{50}	-0.140 (n=10)	-0.895** (n=9)	-0.117 (n=9)	-0.117 (n=9)	-0.439** (n=37)
MRT from MAXENT to GMRT	-0.317 (n=9)	-0.370 (n=10)	-0.533 (n=9)	-0.381 (n=8)	-0.526** (n=36)
MRT from MAXENT to GE_{50}	0.000 (n=10)	0.829** (n=9)	0.183 (n=9)	-0.167 (n=9)	-0.528** (n=37)

*** is p<0.001 ** is p<0.01 * is p<0.05

Table 4.27: The Spearman's correlation coefficient of MRT values between the gastric emptying and 4ASA pharmacokinetic profiles. r_s values and their significance. r_s (Spearman's correlation coefficient); MRT (mean residence time); GMRT (gastric mean residence time); GE_{50} (gastric emptying half time). Liquid A (pH3 4ASA formulation without ranitidine treatment); Liquid AA (pH3 4ASA formulation with ranitidine treatment); Liquid B (pH7 Na4ASA formulation without ranitidine treatment); Liquid BA (pH7 Na4ASA formulation with ranitidine treatment).

4.6 Conclusions

The findings of the current study show that the gastric emptying process is affected by primarily the pH values of the liquid formulations, which in turn is regulated by the duodenum receptor feedback mechanism as described in *sections 1.1.4 & 3.5*. The performance of APT is not affected by physiological acid secretion, as the gastric emptying is not significantly different after ranitidine treatment.

The gastric pH profile shows that the administered of 300mg ranitidine exerted a full acid inhibition over the period when the liquid formulations remained within the stomach. Significant difference in bioavailability of the 4ASA is observed in the different gastric luminal pH and the formulations. The occurrence of the double absorption rate peaks in some cases during the gastric emptying period is possibly due to uptake within the stomach as 4ASA is a weak acid. In contrast to the report by Barditch-Crovo et al (1998), elevated gastric luminal pH does not improve the bioavailability of 4ASA. The rapid emptying of the pH7 formulation into the small intestine allows conversion of the drug into N-acetyl-4ASA by an acetylation process. The rapid depletion of the 4ASA concentration in the intravenous preparation shows that the systemic acetylation process is extensive.

Some positive correlations are calculated when comparing the measurement between the gastric emptying and pH profiles. This is not surprising, as the gastric emptying process is pH dependent. However, as APT system measures changes in the gastric volumes by monitoring change in tissue impedance and the pH-sensitive radiotelemetry capsule measures gastric luminal pH, it is obvious that the limit of agreement between the two methods may be restricted.

The pharmacokinetic analysis of 4ASA using the Wagner-Nelson method provides different values for the pharmacokinetic parameters from the MAXENT approach partially due to the small number of intravenous data points. Underestimation of 4ASA intravenous concentration results in less a reliable outcome using the Wagner-Nelson method. The MAXENT approach has advantages over the Wagner-Nelson method as it describes the kinetic behaviour in terms of an absorption rate distribution. The disposition of 4ASA is complex despite the use of the simple liquid dosage forms, which is not described previously and is not seen in the Wagner-Nelson method.

The use of small volume and dose in the current study has some disadvantages in term of the overall data interpretations. The tethered pH sensitive radiotelemetry capsule induces a form of psychological stress in some subjects especially during the first attempt to swallow the capsule. This would have altered the gastric motility tone via the regulatory pathway at the higher centre in the brain. The pH-sensitive radiotelemetry capsule can be substituted with the pH-sensing probe where the stress induced by difficulty in swallowing the capsule can be eliminated. The gastric emptying rate of a small volume is also affected by the gastric MMC during the fasted state (Oberle et al 1987). Reasonable improvement can hopefully be achieved by introducing a light meal at 2 hours before the start of the test to attempt control the onset of the MMC phase. As the 4ASA preparations undergo extensive yet rapid metabolism resulting in a limited number of useful blood samples for 4ASA analysis, a larger dose and a more frequent blood sampling are essential to provide a better outcome.

Chapter 5

General Discussion

5.1 Measurement of gastric emptying of buffer liquids by EIT

This work has demonstrated that the gastric emptying of a buffer liquid adjusted to various pH values and administered at different volumes in healthy subjects can be measured by EIT. The administration of the non-nutrient small volume liquid has the advantage of not inducing the fed state gastric motility. However, erratic gastric emptying profiles have been observed in some cases for such system due to poor electrode contact, different abdominal size, varying amount of abdominal fat, subject movement and talking, varying degree of gastric acid secretion and gastric antral movement out of the pre-determined region of interest. These erratic gastric emptying profiles which are less reliable, lead to interpretation problems, and in some cases, difficulties in determining what has been measured. With the administration of a 300mg ranitidine, a full acid inhibition of over a 2 hours monitoring periods was provided. The overall gastric emptying rates were not affected after the ranitidine treatment indicating the effect of gastric secretion on the EIT measurement in current study was minimal.

The current work reveals also that the rapid administration of the buffer liquid is not affected by the volume but dominated by the pH effect. The degree of gastric secretion in the stomach and the bicarbonate neutralisation at the duodenum are uncertain. By administering the same buffer over a range of adjusted pH values, the degree of gastric inhibition and secretion could hopefully be established in the future. The effect of gastric MMC on a small volume of liquid has been reported by Oberle et al (1990). The erratic gastric emptying profiles due to antral MMC have not been monitored here. It could be established in the future in a non-invasive manner using a real-time EIT- the EDA system (Smallwood et al 1993) or by inducing the MMC into the same state by a light meal prior to the study.

Although the gastric emptying profiles measured in this work have not been compared with the gold standard 'gamma scintigraphy technique', the positive correlation for the measurement between APT and the pH-sensitive radiotelemetry capsule has indirectly confirmed the validity of the APT measurement.

The baseline variability in the gastric emptying was marked despite strict adherence to the standardised protocol. This variability can be mostly accounted for by consideration of subject differences, gastric residence time varied less when the same subject was studied repeatedly. Such inter-subject variability of gastric emptying has been noted by others using

gamma scintigraphy (Riley et al 1992, Davis et al 1986) and raises the possibility that variability in gastric emptying rate may explain variability in the drug absorption suggested by Nimmo et al (1975) and Hardy et al (1987).

5.2 The disposition of 4ASA oral liquid formulations

The current works has investigated the disposition of low dose 4ASA oral liquid formulations in humans. By incorporating a buffer into the liquid formulations, it allows the simultaneous measurements of the pH and gastric emptying profiles using pH-sensitive radiotelemetry and EIT, respectively, which ultimately offers a physiological explanation for the changes observed in the oral blood-concentration profile of 4ASA and its major metabolite, AASA.

Despite the simplicity of an oral liquid formulation, the 4ASA disposition along the gastrointestinal tract is complex (figures 5.1 & 5.2). In the stomach, where the normal fasted pH value is less than 2, the fate of 4ASA can be determined primarily by 4 physiological processes. Firstly, the oral liquid formulation is prone to the decarboxylation process where the 4ASA can be degraded into m-aminophenol. Second, the fluid flux due to acid secretion, bicarbonate reflux and salivation could alter the dissolution profiles of the 4ASA. An increase in dissolution of 4ASA pH3 suspension and a possible precipitation of the Na4ASA pH7 solution under low pH surrounding using the *in vitro* model have been reported in *section 2*. Thirdly, the emptying of 4ASA into the small intestine, which is regulated by the properties of the oral liquid formulation itself and other physiological factors such as gastric MMC and subject differences. Lastly the conversion of 4ASA into its metabolite, AASA by pre-systemic mucosal acetylation process. With the occurrence of the double peak in the blood concentration profiles in some cases and the selective delayed emptying with the pH3 4ASA suspension formulation as described in *section 4.5*, plausible absorption of 4ASA in the stomach can not be ignored. It would be worthwhile to explore the selective absorption rate of 4ASA from the stomach and small intestine, respectively, by using a perfusion technique described earlier by Merfeld et al (1986) on the pH dependent absorption and uptake of liquid α-methyldopa formulations.

The pre-treatment with ranitidine, which reduced the effect of fluid flux and decarboxylation, did not improve the bioavailability of 4ASA (table 4.7). Early delivery of pH7 Na4ASA

solution with ranitidine treatment into the small intestine favours the formation of AASA (table 4.17). The current study monitored only the blood concentration levels of 4ASA and AASA, from figures 5.1 and 5.2, it is impossible to determine the true elimination rate constant of the AASA from the oral formulation as the pre-systemic acetylation process can not be ruled out. This has provided an explanation for the different elimination rate constant calculated between the oral route and the intravenous route. In addition, the first pass effect and hepatic extraction on the formation of the metabolites has yet to be explored. As mention in *section 4*, the true absorption rate of AASA from the intravenous data can only be determined if the intravenous AASA data is available. The thought of using the residual method to calculate the formation rate constant of AASA by solving the first order kinetic is not possible here. This is not only due to the limited number of measured blood data for the formation phase (maximum 2 data points) but the possibility of that 4ASA metabolises to metabolites other than AASA in the blood circulation.

Section 1.6 has briefly described the conversion of 4ASA into AASA to follow an enzyme capacity limited rate kinetic. However, the current work using a low dose oral 4ASA formulation has not demonstrated this kinetic behaviour, because of a delay in the gastric emptying rate, which in turn extends the absorption of 4ASA in the pH3 formulation, has not resulted in higher level of AASA (tables 4.5, 4.6, 4.17 & 4.25).

The inter-individual variation in the current work is large, suggesting the degree of absorption of 4ASA varies among different subjects. This has been reported earlier using the 5ASA preparations by De Vos et al (1991). The effect of acetylator phenotype, which can be used to explain this finding, is unfortunately not determined. Studies in both rabbits and humans have suggested that the intestinal N-acetyl-transferase is 'monomorphic ' and unlike the liver N-acetylase, which is polymorphic (Stretch et al 1996). Although inter-subject variability coupled with the small sample size have made predictions from this study more tentative, the data have provided useful information into the absorption of 4ASA.

A 4ASA double peak phenomenon for the plasma blood concentration profiles is observed in some cases with the oral liquid preparations but not with the intravenous preparation (refer *section 4.5*). As the peripheral distribution of 4ASA has not been identified by the MAXENT approach, the 'absorption windows' theory, which suggests that a physiological site accumulation leading to the occurrence of the double peaks, is less likely (Williams et al

1992). It is more plausible to explain the double peak phenomenon and the variable bioavailability using gastric emptying rate and gastric MMC as proposed by Oberle et al (1987). Hughes et al (1990) has also demonstrated that by modifying the gastric motility using agents such as propantheline and metoclopramide, the bioavailability of a cephalosporin antibiotic, cefpodoxime was reduced. Simultaneous APT, pH and pharmacokinetic monitoring from the current work has confirmed that the 4ASA absorption is related to the gastric emptying rate even though the gastric MMC was not monitored. It would be interesting in the future to investigate the optimum luminal pH and concentration for 4ASA absorption using either an *in vivo* study or a simulated model as reported by Langguth et al (1994) to predict the kinetic behaviour of 4ASA based on gastric pH and gastric motility phase.

Although the recovery of 4ASA and AASA in the bile has not been investigated, previous reports have excluded the possibility of 4ASA entero-hepatic recycling. However, the potential of the double peak phenomenon due to the gastrointestinal tract resecretion needs to be explored further.

From the blood-concentration profiles of AASA, the systemic appearance of AASA occurs almost at the same time as 4ASA. The disposition of AASA follows a elimination rate limitation model as its elimination half life is much longer while its elimination rate constant is at least 3 times smaller than 4ASA (table 5.1). Others have noted a similar behaviour with 5ASA oral preparations (Meese et al 1984, Houston et al 1982). The fate of the low dose 4ASA oral liquid in the current works is absorption rate limited, as the elimination rate constant is larger than the absorption rate constant. The absorption rate is affected by the gastric emptying rate of the oral liquid because the absorption rate constant accelerates when the gastric emptying rate constant is increased (table 5.1). The rapid systemic absorption of 4ASA in the upper gastrointestinal tract is followed by the conversion into AASA, which reduces the 4ASA availability for local action in the colon. The rapid yet transient blood concentration peak of 4ASA may also accounts for the toxicity and side effects when a large dose is administered. This therefore suggests that treatment of the colon and lower small intestine require the development of the formulation, which delivers and liberates adequate therapeutic moiety to the inflammatory sites but avoids potential risks of the adverse events due to rapid systemic absorption when using a larger dose of 4ASA.

Formulations and pharmacokinetic parameters	AASA	4ASA		
	Mean K_{el}	Mean K_a	Mean K_{GE}	Mean K_{el}
4ASA pH3 suspension without ranitidine treatment	0.0097	0.0340	0.0312	0.0560 (based on MAXENT approach)
4ASA pH3 suspension with ranitidine treatment	0.0095	0.0260	0.0311	
Na4ASA pH7 solution without ranitidine treatment	0.0093	0.0470	0.0537	0.0901 (based on Wagner-Nelson method)
Na4ASA pH7 solution with ranitidine treatment	0.0096	0.0390	0.0461	

Table 5.1: The summary of mean K_{el}, K_a, and K_{GE} values for oral 4ASA liquid formulations. Sources: tables 4.6, 4.8, 4.12, 4.17 & 4.25. The mean k_{el} value for 4ASA is based on intravenous blood concentration using one compartment model. K_{el} (elimination rate constant in min^{-1}); K_a (absorption rate constant in min^{-1}); K_{GE} (gastric emptying rate constant in min^{-1}).

Figure 5.1: The schematic presentation of the disposition of 4ASA oral liquid formulations, without ranitidine treatment, along the gastrointestinal tract. Ds (4ASA in solution state); Dp (4ASA in particulate state); M (AASA); K_{fp} (pre-systemic formation rate constant for AASA); K_{fs} (systemic formation rate constant for AASA); K_{GE} (gastric emptying rate constant); K_{aD} (absorption rate constant for 4ASA); K_{aM} (absorption rate constant for AASA); K_{elD} (elimination rate constant for 4ASA); K_{elM} (elimination rate constant for AASA); INPUT 1 (pH7 solution); INPUT 2 (pH3 suspension); ⟶ (route for 4ASA); ⇢ (route for AASA); ⟶ (acetylation process).

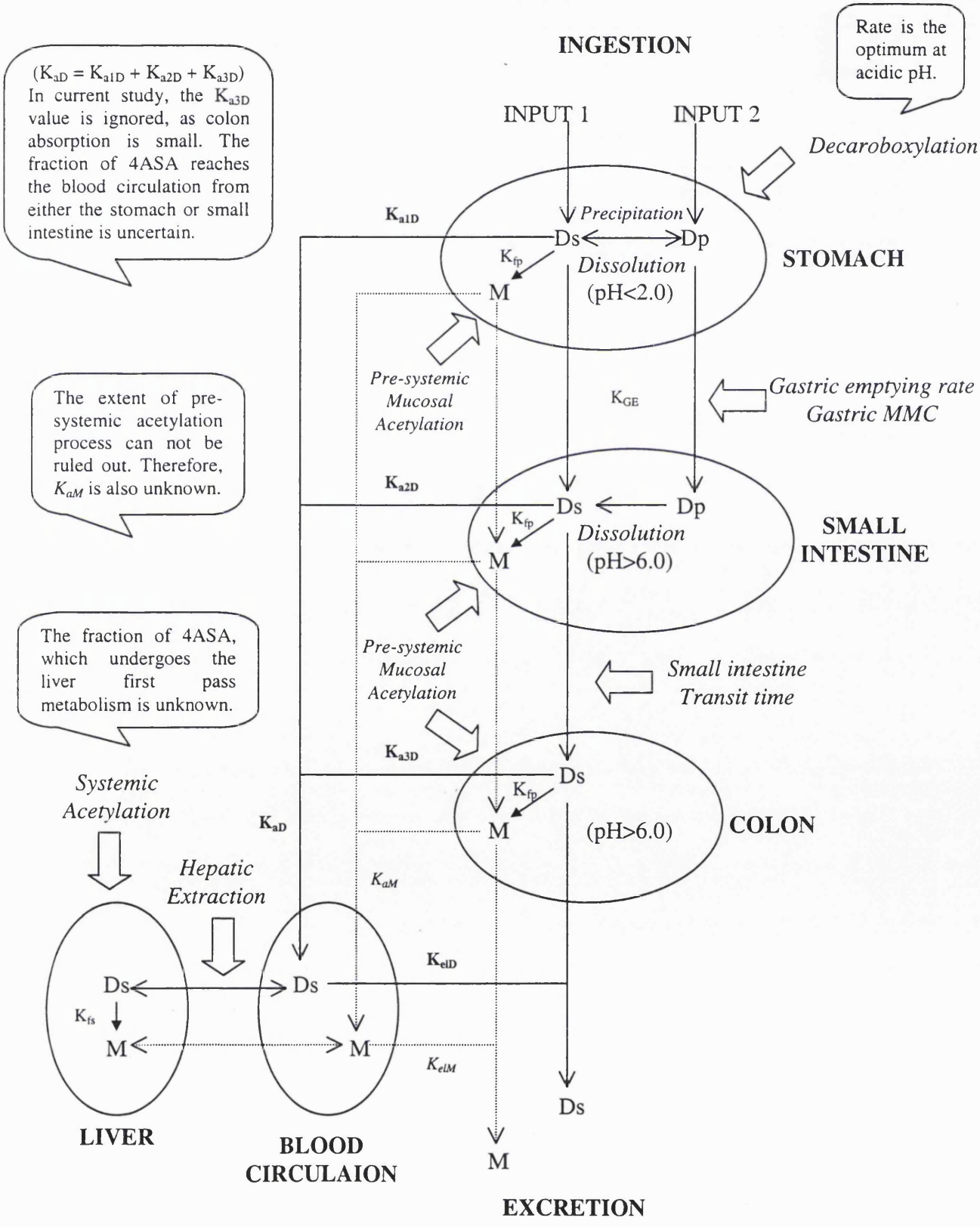
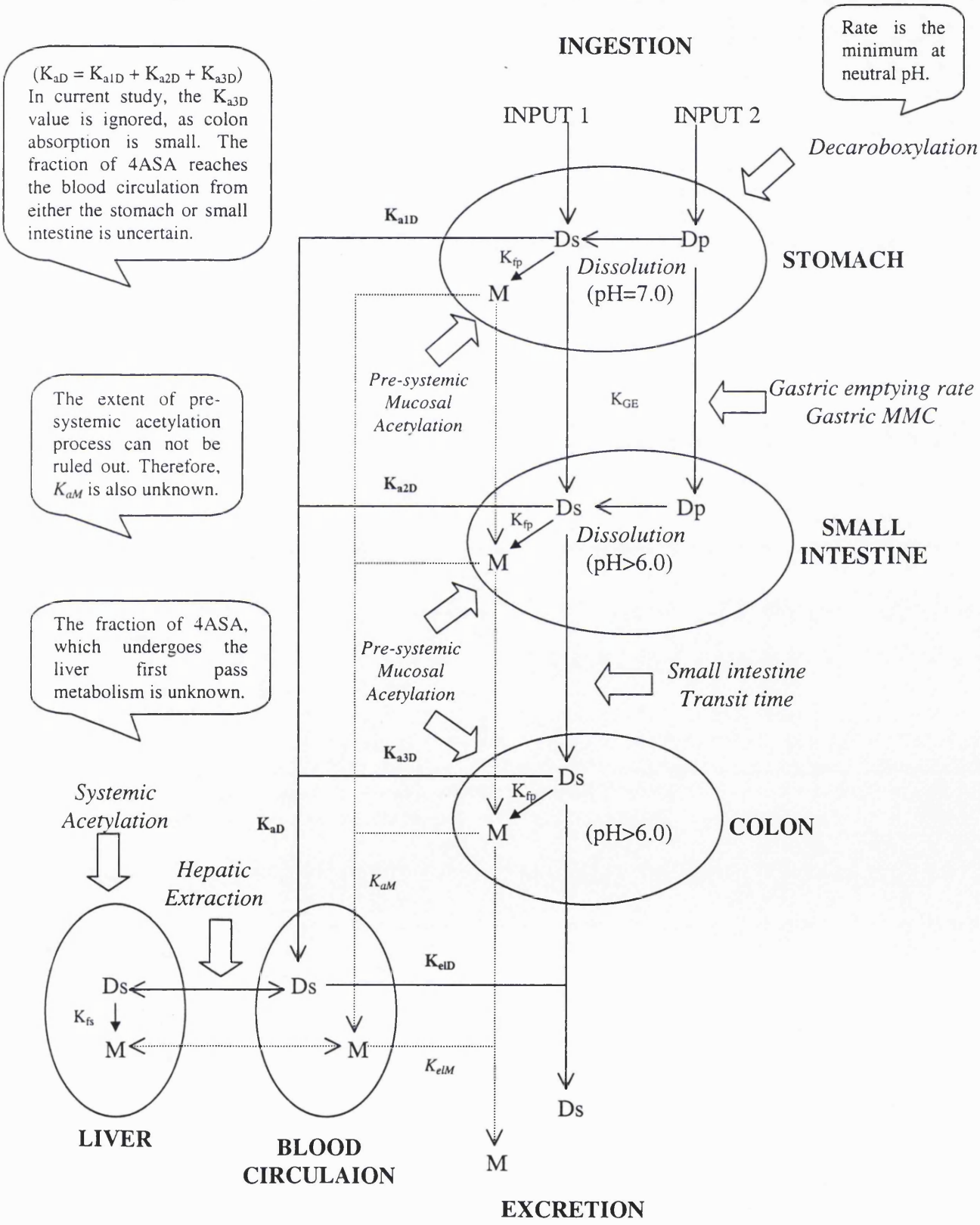


Figure 5.2: The schematic presentation of the disposition of 4ASA oral liquid formulations, with ranitidine treatment, along the gastrointestinal tract. Ds (4ASA in solution state); Dp (4ASA in particulate state); M (AASA); K_{fp} (pre-systemic formation rate constant for AASA); K_{fs} (systemic formation rate constant for AASA); K_{GE} (gastric emptying rate constant); K_{aD} (absorption rate constant for 4ASA); K_{aM} (absorption rate constant for AASA); K_{elD} (elimination rate constant for 4ASA); K_{elM} (elimination rate constant for AASA); INPUT 1 (pH7 solution); INPUT 2 (pH3 suspension); ⟶ (route for 4ASA); ⇢ (route for AASA); ⟶ (acetylation process).



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APPENDIX 1

Excel spreadsheet program for calculating buffer formulations for EIT study

Entered quantities		Calculated quantities				Charge balance	Contribution to I
pH	7	osmolarity	Cations	Anions	Neutral		
Ionic strength	0.28	0.3008663					
Conc. Of citric acid	0.01096	Charge Bal.					
Conc. Of Na2HPO4	0.09031	-2.12E-05					
Conc. Of Drug (Na4ASA)	0.00947	NaCl					
		8.572E-06					
Activity coeff.		IONIC STR					
A	0.509	0.2799914					
B	1.5						
log(g)=-A*z*z*sqrt(I)/(1+B*sqrt(I))							
g1		0.7076926					
g2		0.2508295					
g3		0.0445248					
Hydrogen ion conc.							
(10^(-pH))/g1			1.413E-07			1E-07	7.065E-08
Conc. hydroxide (10^(-pKw))/(g1*10^(-pH))				1.413E-07		-1E-07	7.065E-08
Citric acid							
([H+]^3+[H+]^2*k1+[H+]*K1*K2+K1*K2*K3)		3.433E-13					
[H3C]					9.01E-11		
[H2C(-1)]				9.478E-07		-9E-07	4.739E-07
[HC(-2)]				0.000463		-0.0009	0.0009274
[C(-3)]				0.010495		-0.0315	0.0472292
di-sodium hydrogen phosphate							
([H+]^3+[H+]^2*k1+[H+]*K1*K2+K1*K2*K3)		7.907E-16					
[Na+]			0.18063			0.1806	0.090315
[H3PO4]					3.22E-07		
[H2PO4(-1)]				0.032388		-0.0324	0.0161944
[HPO4(-2)]				0.057924		-0.1158	0.1158487
[PO4(-3)]				1.543E-06		-5E-06	6.948E-06
Drug							
([H+]^2+[H+]*K1+K1*K2)		3.983E-06					
[DH2(+1)]			4.747E-11			5E-11	2.374E-11
[DH(0)]					5.33E-06		
[D(-1)]				0.009466		-0.0095	0.0047333
[Na+]			0.009471			0.0095	0.004736
Totals			0.190102	0.110741	5.65E-06	-2E-05	0.2799914
			cations	anions	neutrals	Charge Balsnce	Ionic strength
		Total	0.300849				
Conc. of NaCl required	8.57E-06		8.572E-06	8.572E-06			

Spreadsheet 1: The computation for pH7 Na4ASA formulation 1 using Excel Window program

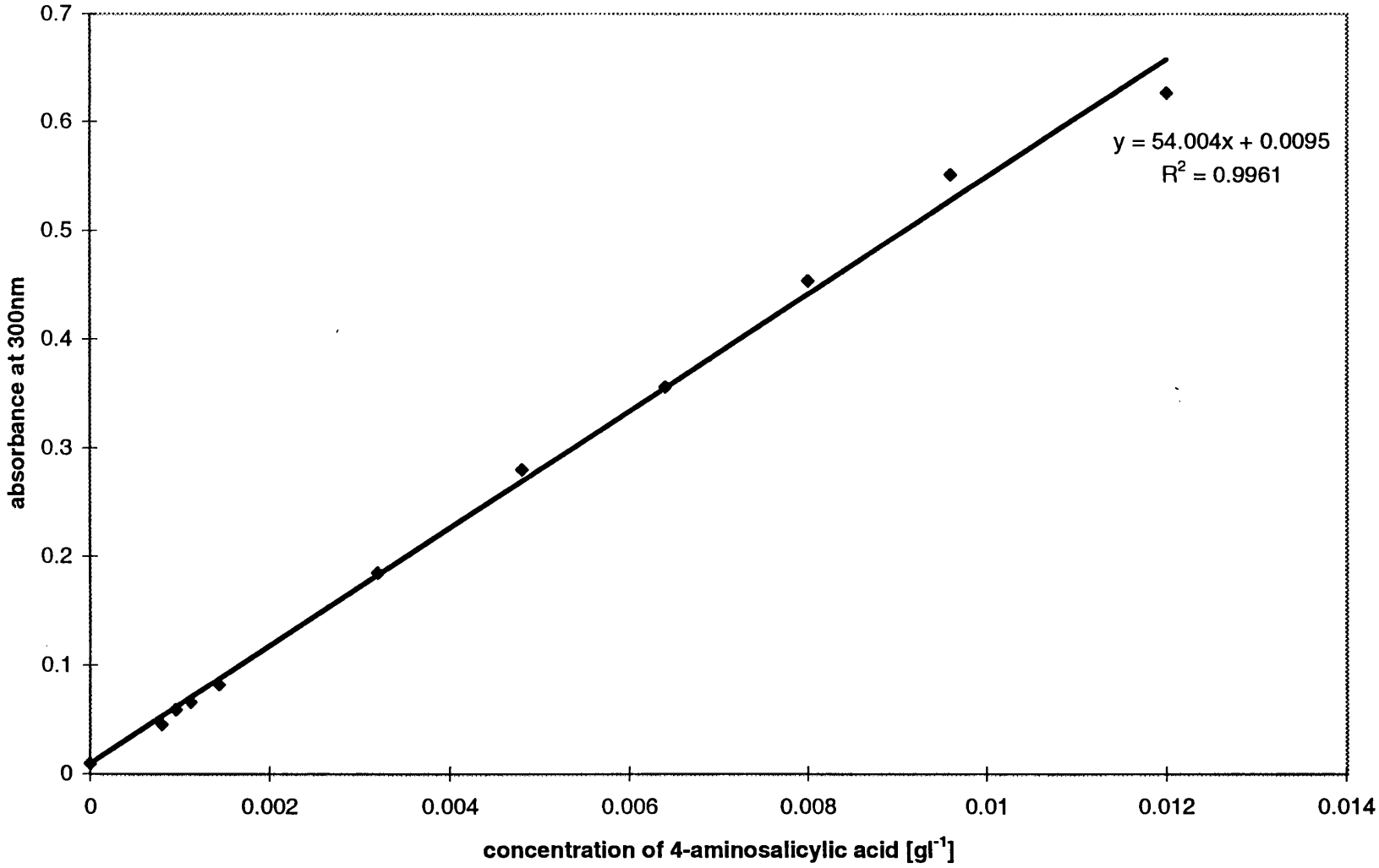
Entered quantities		Calculated quantities				Charge balance	Contribution to I
pH	3	osmolarity	Cations	Anions	Neutral		
Ionic strength	0.115	0.297426					
Conc. Of citric acid	0.117	Charge Bal.					
Conc. Of Na2HPO4	0.0553	-1.2E-07					
Conc. Of Drug(4ASA)	0.01306	NaCl					
		8.07E-05					
Activity coeff.		IONIC STR					
A	0.509	0.114919					
B	1.5						
log(g)=-A*z*z*sqrt(I)/(1+B*sqrt(I))							
g1		0.768401					
g2		0.34862					
g3		0.093388					
Hydrogen ion conc.							
(10^(-pH))/g1			0.001301			0.001301	0.0006507
Conc. hydroxide (10^(-pKw))/(g1*10^(-pH))				1.3E-11		-1.3E-11	6.507E-12
Citric acid							
([H+]^3+[H+]^2*k1+[H+]*K1*K2+K1*K2*K3)		4.42E-09					
[H3C]					0.058317		
[H2C(-1)]				0.05652		-0.05652	0.0282601
[HC(-2)]				0.00216		-0.00432	0.0043199
[C(-3)]				3.24E-06		-9.7E-06	1.458E-05
di-sodium hydrogen phosphate							
([H+]^3+[H+]^2*k1+[H+]*K1*K2+K1*K2*K3)		2.26E-08					
[Na+]			0.1106			0.1106	0.0553
[H3PO4]					0.005391		
[H2PO4(-1)]				0.049902		-0.0499	0.0249508
[HPO4(-2)]				6.97E-06		-1.4E-05	1.394E-05
[PO4(-3)]				1.23E-14		-3.7E-14	5.541E-14
Drug							
([H+]^2+[H+]*K1+K1*K2)		2.63E-05					
[DH2(+1)]			0.000841			0.000841	0.0004206
[DH(0)]					0.010245		
[D(-1)]				0.001977		-0.00198	0.0009887
Totals			0.112743	0.110569	0.073953	-1.2E-07	0.1149193
			cations	anions	neutrals	Charge balance	Ionic strength
		Total	0.297265				
Conc. of NaCl required	8.0E-05		8.07E-05	8.07E-05			

Spreadsheet 2: The computation for pH3 4ASA formulation 2 using Excel Window program

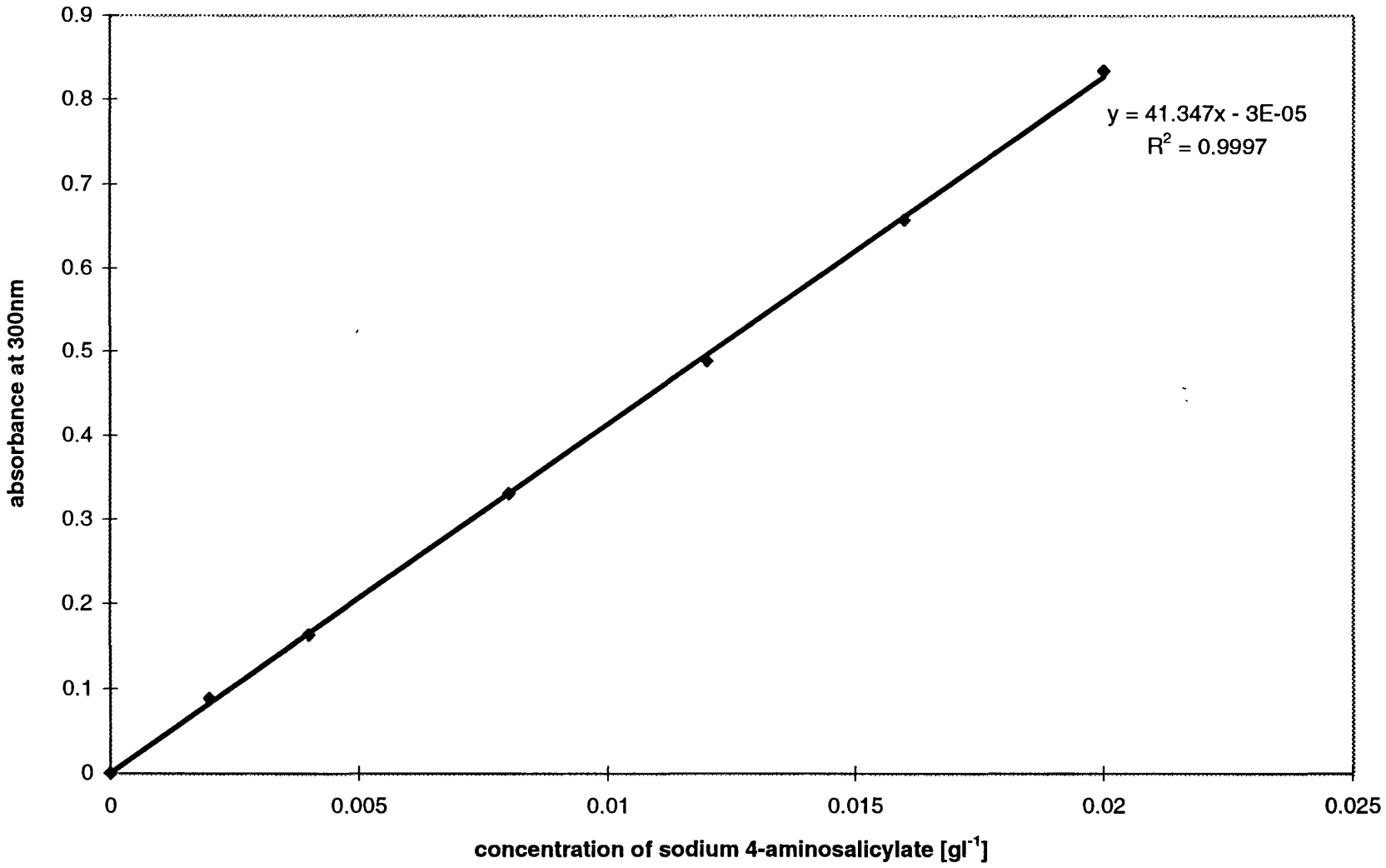
APPENDIX 2

Standard UV calibration curves of 4ASA for *in vitro* study

UV calibration of 4-aminosalicylic acid in mixed phosphate buffer pH 6.8



UV calibration of sodium 4-aminosalicylate in mixed phosphate buffer pH 6.8



APPENDIX 3

Demography and medical health check form

DEMOGRAPHY

CONFIDENTIAL

Subject Reg. Number: _____

Date of Birth : _____

Ethic Group : _____

Sex : _____

Height : _____

Weight : _____

Subjects' initials _____ Date_____

Investigators' signature _____ Date_____

HEALTH CHECK FORM (CONFIDENTIAL)

Have you suffered from any of the following complaints? Please tick Yes or No giving approximate dates.

Complaint	No	Yes	DATE	Still a problem ? & Details
Heart disease	___	___	_____	_____
Tuberculosis	___	___	_____	_____
Asthma	___	___	_____	_____
Allergies	___	___	_____	_____
Skin disease	___	___	_____	_____
Kidney disease	___	___	_____	_____
Liver disease	___	___	_____	_____
Gastrointestinal disease	___	___	_____	_____
Diabetes	___	___	_____	_____
Eye trouble	___	___	_____	_____
Ear trouble	___	___	_____	_____
Respiratory disease	___	___	_____	_____
Any other illness	___	___	_____	_____
Have you ever been admitted to hospital ?	___	___	_____	_____
Do you suffer from any disability or handicap ?	___	___	_____	_____
Do you take any pills, medicines or drugs ?	___	___	Details	_____
Do you smoke ?	___	___	How many ?	_____
Do you drink alcohol ?	___	___	How much ?	____units/week

Subjects' initials _____ Date _____

Investigators' signature _____ Date _____

APPENDIX 4

Volunteer informed consent form and volunteer information sheet: study I

VOLUNTEER CONSENT FORM

CONFIDENTIAL

Title of research proposal:

A STUDY TO EVALUATE THE EFFECT OF pH OF A BUFFER LIQUID ON GASTRIC EMPTYING PROCESS BY SIMULTANEOUS MEASUREMENT USING GAMMA SCINTIGRAPHY AND APPLIED POTENTIAL TOMOGRAPHY

Student investigators: Miss CS Chaw (Dept. of Pharmaceutics, London School of)
Principle investigators: Dr DF Evans (GI Science Research Unit, Royal London Hospital)
Prof. JM Newton (Dept. of Pharmaceutics, London School of Pharmacy)
Pharmacy

REC Number:

Name of Volunteer (Block Capitals):

Address:

Please complete the following: **Delete as necessary**

1. Have you read the information sheet about this study? YES/NO
2. Have you had an opportunity to ask questions and discuss this study? YES/NO
3. Have you received satisfactory answers to all your questions? YES/NO
4. Have you enough information about this study? YES/NO
5. Which investigator have you spoken to about this study? _____
6. Do you understand that you are free to withdraw from this study...

   *at any time

   *without giving a reason for withdrawing
7. Do you agree to take part in this study? YES/NO

Volunteer's Signature: .....

Witness's Name: .....

Witness's Signature: .....

Date: .....

The following should be signed by the Clinician/Investigator responsible for obtaining consent.As the Clinician/Investigator responsible for this research or a designated deputy, I confirm that I have explained to the volunteer named above the nature and purpose of the research to be undertaken.

Investigator's Name: .....

Investigator's Signature: .....

Date: .....

A STUDY TO EVALUATE THE FACTORS THAT INFLUENCE GASTRIC EMPTYING AND pH TO PHARMACOKINETIC PROFILE OF PARA-AMINOSALICYLIC ACID AS A MODEL LIQUID DOSAGE FORM USING RADIOTELEMETRY AND APPLIED POTENTIAL TOMOGRAPHY

EAST LONDON AND THE CITY HEALTH AUTHORITY
Information to Participate in a Research Project for healthy Volunteer

CONFIDENTIAL

Student Investigator : Ms C.S. Chaw
Clinical Investigator : Dr. Taisuke Nomura
Supervising Investigators : Dr. D.F. Evans
Professor D.L. Wingate
Professor M.J. Newton

Study I

We invite you to take part in a research study which we think may be important. The information which follows tells you about it. It is important that you understand what is in this leaflet. It says what will happen if you take part and what risks might be, try to make sure you know what will happen to you if you decide to take part. whether or not you do take part is entirely your choice. Please ask any questions you want to about the research and we will try our best to answer them.

This is an invitation to participate in a research project supervised by the Gastro-Intestinal Science Unit, Royal London Hospital. This is a set of control studies (that is studies which are performance in order to compare with treatment) using Citrate Phosphate buffer as a testing liquid. The study aims to investigate the effect of administered fluid volumes and pHs (that is the acidity) of Citrate Phosphate buffer on the gastric emptying process (that is the rate of liquid passes through your stomach). Gastric emptying process will be monitored by a non-invasive technique known as applied potential tomography (APT). You will be attached to the APT system via seventeen electrodes placed to your upper abdomen whilst in sitting position. The monitoring period will last for upto a maximum of one and a half hour. You will be given the buffer preparations at different pHs and volumes over four separate assigned study days.

You are requested to fast for 12 hours and abstain from alcohol and spicy foods for 24 hours prior to each study day. A standard lunch will be provided after the completion of gastric emptying monitoring.

You don't have to join the study. You are free to decide not to be in this trial or to drop out at any time. If you decide not to be in the study, or drop out, this will not put at risk your ordinary medical care (or course of study if you are a student volunteer). Participation in the study will be paid at the discretion of the investigators. If for what ever reasons you are not able to complete the study or decide to withdraw from the study, you will be paid at the discretion of the investigator, generally on a pro rata basic.

We will take every care in the course of this trial. If through our negligence any harm to you results, you will be compensated. However, a claim may have to be pursued through legal action. Even if the harm is not our fault, The [College, Trust, etc.] will consider any claim sympathetically. If you are not happy with any proposed compensation you may have to pursue your claim through legal action.

If you are willing to participate in this study, please sign the attached consent form and return it to us. All proposals for research using human subjects are reviewed by ethics committee before they are proceed. This proposal was reviewed by East London & The City Health Authority Research Ethics Committee.

If you have any queries with regard to the study, please contact the investigator at the following address:

Name : Ms C.S. Chaw
Address : Dept. of Pharmaceutics, London School of Pharmacy, Brunswick Square, WC1N 1AX.
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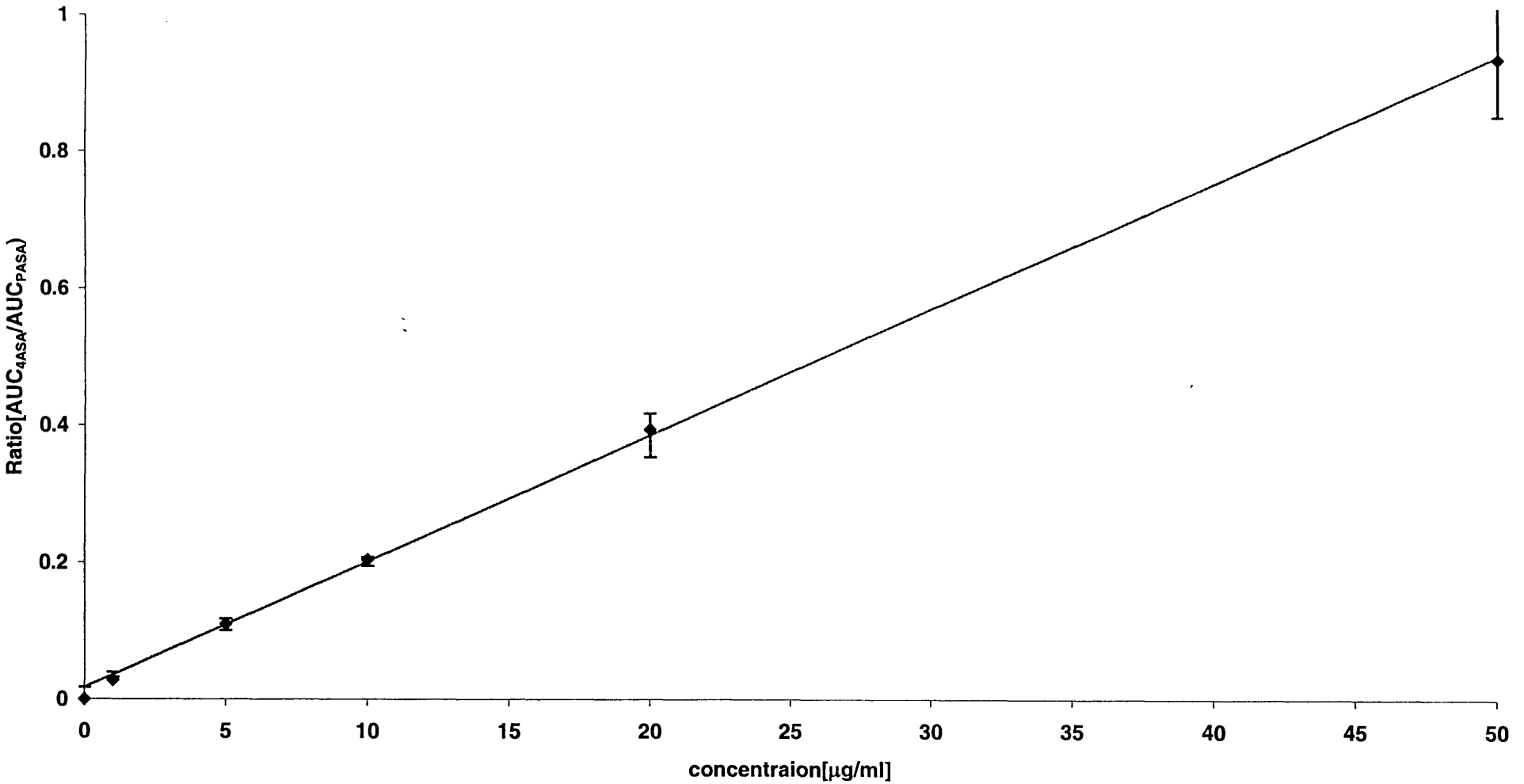
APPENDIX 5

Standard calibration curves of 4ASA and AASA for HPLC assay

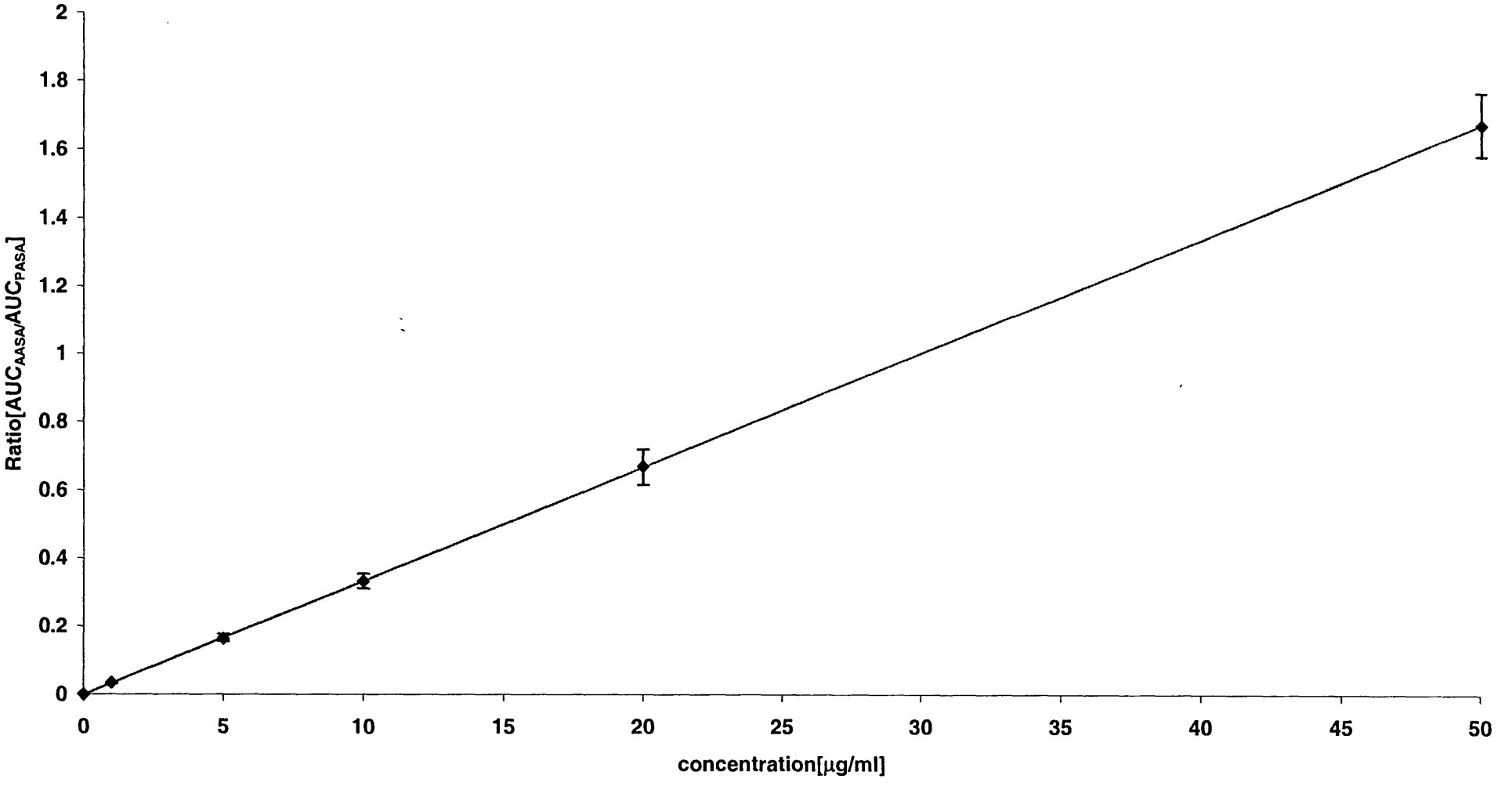
Regression Analysis	4ASA		AASA	
	Oral liquid	IV route	Oral liquid	IV route
Multiple R	0.99569	0.99828	0.99711	0.99374
R adjusted	0.99140	0.99657	0.99423	0.98752
Adjusted R^2	0.99032	0.99614	0.99351	0.98613
Std. Err.	0.03366	0.03854	0.01855	0.08287
F ($p<0.0001$)	921.83991	2321.71343	1379.12821	712.21863
B value	0.018372	0.033385	0.123862	0.190078
Std. Err. For B	0.000605	0.000693	0.003335	0.007122
T ($p<0.0001$)	30.362	48.184	37.137	26.867
Bo value	0.017555	-0.001480	0.010538	0.032955
Std. Err. For Bo	0.014886	0.017045	0.008205	0.034661

Appendix 5: The linear regression analysis derived from SPSS statistical analysis for the standard calibration curves for HPLC assay.

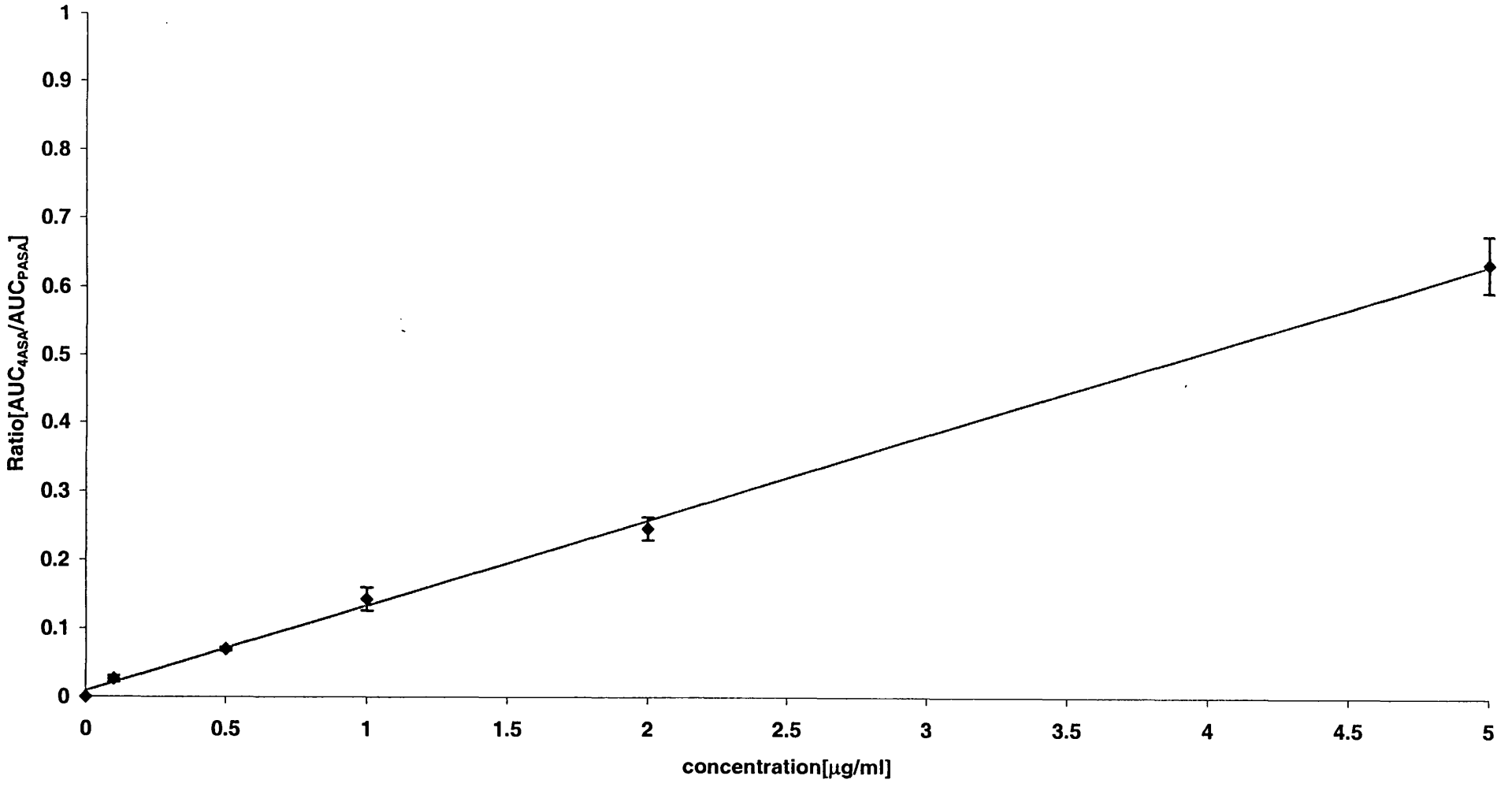
The standard calibration curve of 4ASA for HPLC assay
[For analysis of the blood samples from liquid prepreration)



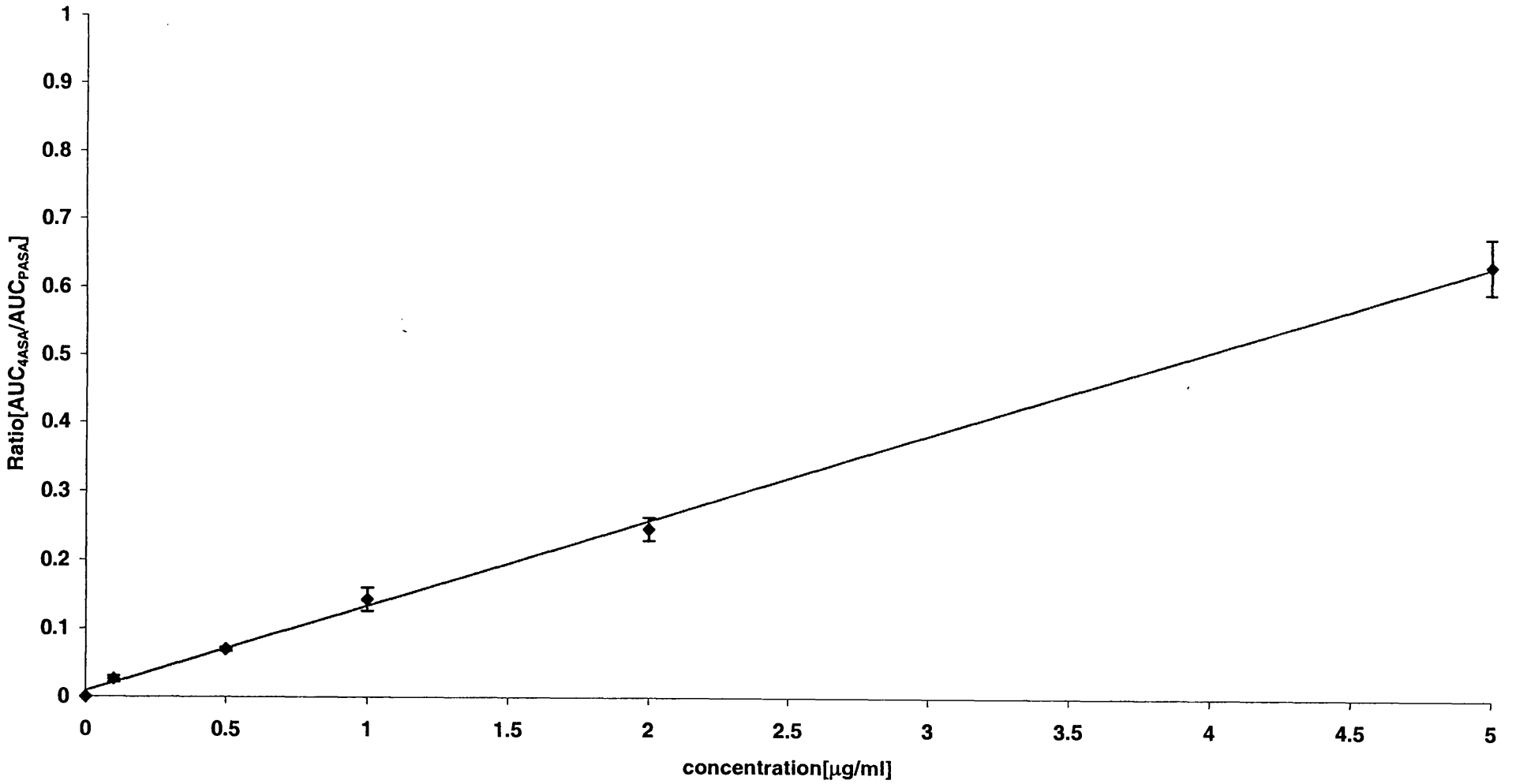
The standard calibration curve of AASA for HPLC assay
[For analysis of blood samples from liquid preparations]



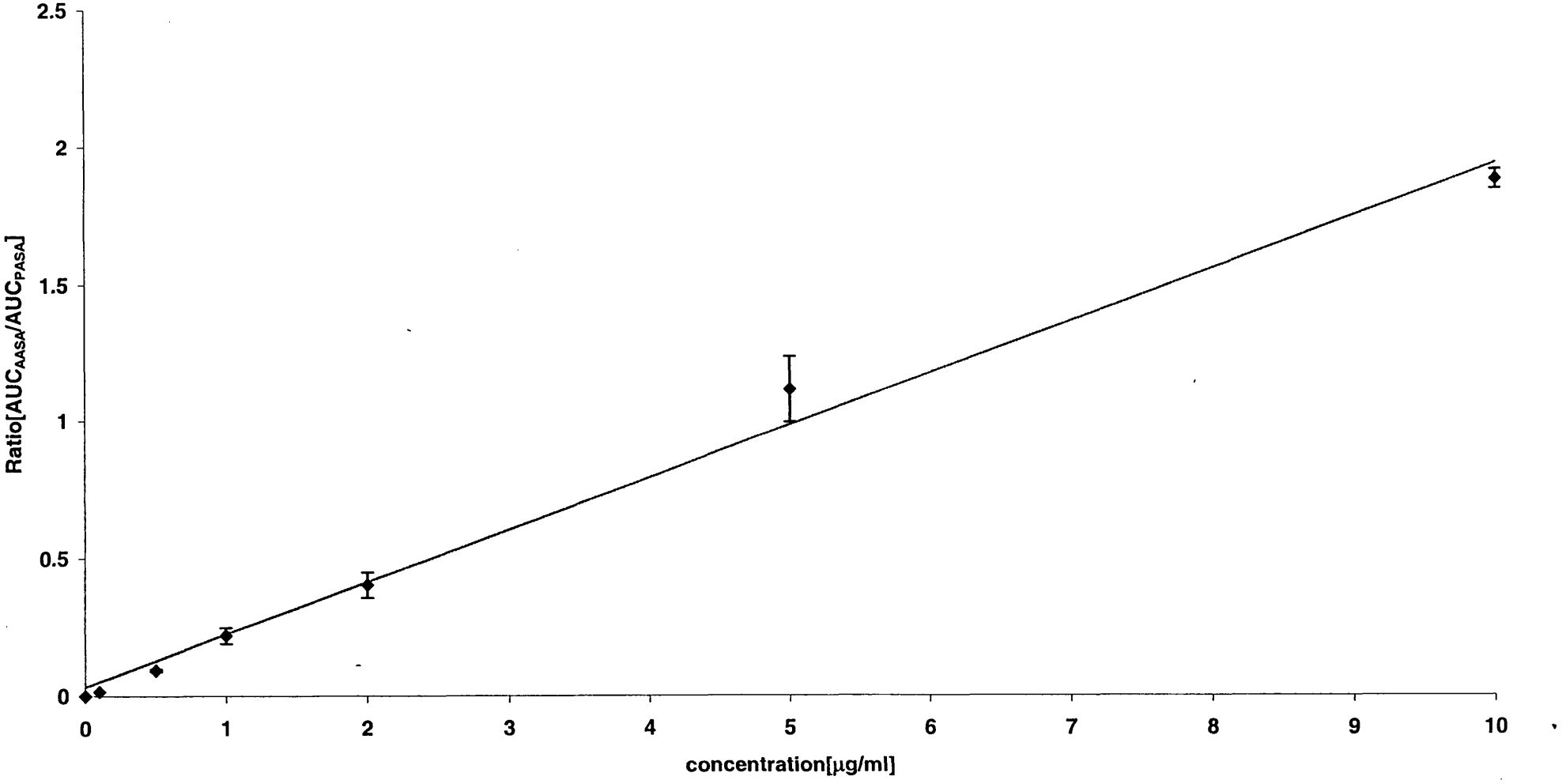
The standard calibration curve of 4ASA for HPLC assay
[For analysis of the blood samples from intravenous route]



The standard calibration curve of 4ASA for HPLC assay
[For analysis of the blood samples from intravenous route]



The standard calibration curve of AASA for HPLC assay
[For analysis of blood samples from intravenous route]



APPENDIX 6

Volunteer information sheet: study II

A STUDY TO EVALUATE THE FACTORS THAT INFLUENCE GASTRIC EMPTYING AND pH TO PHARMACOKINETIC PROFILE OF PARA-AMINOSALICYLIC ACID AS A MODEL LIQUID DOSAGE FORM USING RADIOTELEMETRY AND APPLIED POTENTIAL TOMOGRAPHY

EAST LONDON AND THE CITY HEALTH AUTHORITY
Information to Participate in a Research Project for healthy Volunteer

CONFIDENTIAL

Student Investigator : Ms C.S. Chaw
Clinical Investigator : Dr. Taisuke Nomura
Supervising Investigators : Dr. D.F. Evans
Professor D.L. Wingate
Professor M.J. Newton

Study II

We invite you to take part in a research study which we think may be important. The information which follows tells you about it. It is important that you understand what is in this leaflet. It says what will happen if you take part and what risks might be, try to make sure you know what will happen to you if you decide to take part. whether or not you do take part is entirely your choice. Please ask any questions you want to about the research and we will try our best to answer them.

This is an invitation to participate in a research project supervised by the Gastro-Intestinal Science Unit, The Royal London Hospital. The study aims to investigate factors which influence gastric emptying of stomach (i.e. the rate the liquid passes through your stomach) and drug absorption mechanism by the techniques described below.

In this study, you will be given a drug known as Para-aminosalicylic acid. It shares some properties of salicylate and has been found to be effective for relieving symptoms of Inflammatory Bowel Disease. If you are sensitive to salicylate and its related substances and you have a history of gastrointestinal diseases (such as gastric ulcer, reflux, etc.), you are advised not to take part in this project.

You will be administered either a solution or a suspension preparation of para-aminosalicylic acid at the concentration of 500mg/250ml on four separate occasions. In addition, you will also be administered at one occasion a 50mg/ml intravenous injection by a qualified doctor. For all the five study days, you will have a small needle positioned to your forearm vein in order to withdraw fourteen 10ml blood samples (including pre-dose) over an 8 hour period. This will allow us to assess the drug level in your body in order to elucidate the drug absorption mechanism.

For all the study days except when injection is administered, you will also be attached to a system known as Applied Potential Tomography (APT) which monitors the rate at which the given liquid passes through your stomach. Seventeen electrodes will be attached to your upper abdomen in a ring and a small current will be applied via the APT system to image the changes in your stomach. Furthermore, you will be requested to swallow a tethered pH sensitive radiocapsule (approximately the size of normal antibiotic capsule) so that it resides in the liquid of your stomach to monitor your stomach acidity. Both the procedures will last for up to a maximum of one and a half hour. Throughout the monitoring period, you are requested to remain sitting comfortably in a quiet position with minimum movement.. At

the end of the monitoring, the electrodes will be detached from your abdomen and the radiocapsule will be removed from your stomach by gentle traction.

You are requested to fast for 12 hours and abstain from alcohol and spicy foods for 24 hours prior to each study day. A standard lunch will be provided four hours after the drug administration. On two occasions, you will be asked to take a drug known as Ranitidine (Zantac) tablet 300mg with plain water one hour prior to arrival at the hospital to suppress your stomach acid production.

You don't have to join the study. You are free to decide whether or not to be in this trial or to drop out at any time. if you decide not to be in the study, or drop out, this will not put at risk your ordinary medical care (or course of study if you are a student volunteer). Participation in the study will be paid at the discretion of the investigators. If for what ever reasons you are not able to complete the study or decide to withdraw from the study, you will be paid at the discretion of the investigator, generally on a pro rata basis.

We will take every care in the course of this trial. If through our negligence any harm to you results, you will be compensated. However, a claim may have to be pursued through legal action. Even if the harm is not our fault, The [College, Trust, etc.] will consider any claim sympathetically. If you are not happy with any proposed compensation you may have to pursue your claim through legal action.

If you are willing to participate in this study, please sign the attached consent form and return it to us. All proposals for research using human subjects are reviewed by ethics committee before they are proceed. This proposal was reviewed by East London & The City Health Authority Research Ethics Committee. All the data from this study is confidential and agreement must be obtained from you prior to disclosure of such information to a third party.

If you have any queries with regard to the study, please contact the investigator at the following address :

Name : Ms C.S. Chaw
Address : Dept. of Pharmaceutics, London School of Pharmacy, Brunswick Square, WC1N 1AX.
Telephone number : 0171-837-5800 ext 4857 or 5852

APPENDIX 7

The amount of time at a given pH value derived from Flexilog II program

Appendix 7: The amount of time (min) calculated at given pH values derived from Flexilog II program

Subject	Amount of time (hr) at pH values													
Liquid A	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**
AA	0	0	0	0.24	1.2	0	0	0	0	0	0	0	0	0
CB	0	0.08	0.4	0.15	0.39	0	0	0	0	0	0	0	0	0
DB	0.08	0.3	0.28	0.46	0	0	0	0	0	0	0	0	0	0
DS	0.02	0.37	0.33	0.43	0	0	0	0	0	0	0	0	0	0
OC		0.02	0.23	1.16	0.1	0.03	0	0	0	0	0	0	0	0
TN	0.08	0.52	0.24	0.19	0	0	0	0	0	0	0	0	0	0
VM	0	0	0	0.39	0.3	0.11	0.11	0.06	0.07	0.04	0.03	0.08	0.02	0
WB	0	0.49	0.34	0.29	0	0	0	0	0	0	0	0	0	0
YO	1.17	0.16	0.1	0.08	0	0	0	0	0	0	0	0	0	0
Mean	**0.16875**	**0.21555**	**0.21333**	**0.37666**	**0.22111**	**0.01555**	**0.01222**	**0.00666**	**0.00777**	**0.00444**	**0.00333**	**0.00888**	**0.00222**	**0**
s.d	**0.40607**	**0.20969**	**0.14734**	**0.32096**	**0.39565**	**0.03678**	**0.03667**	**0.02**	**0.02333**	**0.01333**	**0.01**	**0.02667**	**0.00666**	**0**
Liquid AA														
AA	0	0	0	0.09	1.08	0.21	0	0	0	0	0	0	0	0
CB	0	0	0	0.44	0.18	0.11	0.07	0.03	0.01	0	0.02	0.09	0.19	0
DB	0	0	0	0.25	0.21	0.03	0.04	0.01	0.06	0.05	0.03	0.04	0.23	0.15
DS	0	0	0	0	0.26	1.15	0.09	0	0	0	0	0	0	0
OC	0	0	0	0	1.08	0.17	0.14	0.07	0	0	0	0	0	0
TN	0.01	0.05	0.02	0.08	0.41	0.09	0.15	0.18	0.04	0.06	0.04	0.01		0
VM	0	0	0	0	0.35	0.16	0.11	0.01	0.06	0.08	0.17	0.16	0.02	0
WB	0	0	0	0	0.22	0.16	0.24	0.24	0.23	0.02	0	0	0	0.02
YO	0	0	0	0	0.38	0.03	0.02	0.08	0.09	0.09	0.08	0.13	0.25	0
Mean	**0.00111**	**0.00556**	**0.00222**	**0.09556**	**0.46333**	**0.23444**	**0.09556**	**0.06888**	**0.05444**	**0.03333**	**0.03778**	**0.04778**	**0.08625**	**0.01888**
s.d	**0.00333**	**0.01667**	**0.00667**	**0.15349**	**0.35854**	**0.34891**	**0.07465**	**0.08638**	**0.07354**	**0.03701**	**0.05628**	**0.06304**	**0.11488**	**0.04961**

Subject	Amount of time(hr) at pH values													
Liquid B	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**
AA	0.02	0.21	0.04	0.04	0.03	0.03	0.03	0.04	0.03	0.08	0.07	0.14	0.14	0.03
CB	0	0.08	0.09	0.08	0.11	0.17	0.15	0.1	0.07	0.06	0.05	0.13	0.12	0
DB	0.39	0.48	0.11	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.09	0.03	0
DS	0.59	0.17	0.06	0.04	0.07	0.16	0.1	0	0	0	0	0	0	0
OC	0.04	0.49	0.07	0.02	0.03	0.03	0.03	0.02	0.02	0.04	0.09	0.26	0.06	0
TN	0.08	0.51	0.15	0.11	0.02	0.02	0.02	0.02	0.02	0.04	0.04	0.07	0.01	0
VM	0	0	0	0	0	0	0	0	0	0	0.01	0.03	1	0.48
WB	0.02	0.42	0.1	0.06	0.02	0.03	0.03	0.03	0.03	0.04	0.1	0.21	0.01	0
YO	1.42	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.03	0.03	0	0	0
Mean	**0.28444**	**0.26444**	**0.07**	**0.04556**	**0.03333**	**0.05111**	**0.04222**	**0.02667**	**0.02222**	**0.03333**	**0.04444**	**0.10333**	**0.15222**	**0.05666**
s.d	**0.47396**	**0.21131**	**0.04847**	**0.03461**	**0.035**	**0.06548**	**0.04964**	**0.03044**	**0.02102**	**0.02696**	**0.03604**	**0.09083**	**0.32213**	**0.15906**
Liquid BA														
AA	0	0	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.08	1.1	0.03
CB	0	0	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.04	0.07	0.21	1.13	0.06
DB	0	0	0	0	0	0	0	0.01	0.01	0.01	0.01	0.11	1.42	0.03
DS	0	0	0	0	0	0	0	0	0.01	0.02	0.1	1.28	0.1	0
OC	0	0	0.01	0	0	0	0.01	0.01	0.01	0.02	0.03	0.2	1.19	0.04
TN	0	0	0	0	0	0	0	0	0.01	0.01	0.02	0.17	1.15	0
VM	0	0	0	0	0	0			0.01	0.01	0.03	0.19	1.17	0.11
WB	0	0	0.14	0.02	0.03	0.02	0.05	0.1	0.14	0.23	0.23	0.14	0.05	0.01
YO	0	0	0	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.24	0.54	0.22
Mean	**0**	**0**	**0.02333**	**0.00556**	**0.00667**	**0.00556**	**0.01125**	**0.02**	**0.02667**	**0.04111**	**0.06**	**0.29111**	**0.87222**	**0.05555**
s.d	**0**	**0**	**0.04667**	**0.00725**	**0.01**	**0.00725**	**0.01641**	**0.03295**	**0.04302**	**0.07142**	**0.06981**	**0.37427**	**0.50842**	**0.07055**

The time (min) calculated at given pH values by Flexilog II program for pH data (cont). Liquid A (pH3 4ASA formulation); Liquid AA(pH3 4ASA formulation under acid suppress state); Liquid B (pH7 Na4ASA formulation); Liquid BA (pH7 Na4ASA formulation with ranitidine treatment).

APPENDIX 8

Average numerical values of the blood concentration level of 4ASA and AASA of each subject from HPLC assay

Time (hr)	Concentration(μg/ml) for each subject											
	AA	BB	CB	DB	DS	OC	TN	VM	WB	YO	Mean	s.d.
0.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.15	14.4955	33.1386	8.7408	15.3460	20.9178	67.5770	10.1269	19.5823	20.1493	31.6164	24.1691	17.2387
0.30	20.3303	34.6696	13.3229	20.6451	31.8969	27.2094	16.0896	16.4855	17.5477	33.3197	23.1576	7.9272
0.45	15.0273	23.8106	21.4899	30.3956	24.8528	13.8050	19.1897	14.4855	20.6158	22.0738	20.5746	5.2059
1.00	11.3176	12.7579	16.5961	27.0930	14.8836	5.6543	14.1279	11.4591	19.8108	12.1821	14.5882	5.7486
1.30	7.8008	5.1225	7.1415	6.8403	8.8966	3.1883	5.9580	2.3563	1.5119	5.8967	5.4713	2.4257
2.00	3.9517	1.7966	3.6310	3.4497	3.7544	1.6036	2.3089	0.8552	15.9244	3.1299	4.0405	4.3048
2.30	2.4828	1.4814	2.3249	2.7354	2.2576	≤.1000	1.7864	0.5464	6.2832	1.4852	2.2067	1.6086
3.00	2.2341	1.4599	1.4959	1.5892	1.8459	1.0804	1.4186	≤0.1000	3.6070	1.0135	1.7494	0.7880
4.00	1.7258	≤0.1000	0.5683	0.8428	1.7215	≤0.1000	1.4426	≤0.1000	≤0.1000	1.4133	1.2770	0.4479
5.00	≤0.1000	≤0.1000	0.3684	1.2320	1.7945	≤0.1000	1.4986	≤0.1000	1.1308	0.8189	1.3879	0.6770
6.00	2.0646	1.0525	0.4031	1.3760	1.9380	0.8317	≤0.1000	≤0.1000	≤0.1000	0.9521	1.1730	0.5806
8.00	≤0.1000	0.2720	0.3711	1.3093	1.2600	0.2462	1.3680	≤0.1000	1.2400	0.1711	0.7797	0.5541

The 4ASA blood level of the pH3 4ASA formulation without ranitidine treatment

Time (hr)	Concentration(μg/ml) for each subject											
	AA	BB	CB	DB	DS	OC	TN	VM	WB	YO	Mean	s.d.
0.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.15	10.1156	10.8988	4.9893	22.5885	15.6228	30.1917	23.7164	28.6390	10.8923	20.0253	17.7679	8.5450
0.30	15.5904	19.4145	12.6282	31.1885	21.5944	16.6097	35.1157	27.4479	24.0817	23.8082	22.7479	7.0943
0.45	17.9381	26.8341	13.1903	25.1684	19.0599	17.1680	21.1488	18.2596	26.9515	18.9873	20.4706	4.5268
1.00	13.3589	22.9551	12.2946	14.9048	13.9988	11.9499	12.0511	8.7986	22.7383	11.4365	14.4487	4.7176
1.30	5.8589	7.0597	6.8161	5.4502	5.6029	3.7771	4.3523	3.0106	13.7600	5.1411	6.0829	2.9767
2.00	3.1738	3.5972	3.5467	3.2281	2.8533	1.5381	2.8403	1.3105	7.6074	2.7222	3.2418	1.7158
2.30	1.9315	2.3382	1.5193	2.0177	1.2554	1.3770	1.3057	1.1059	3.8345	1.8810	1.8566	0.7992
3.00	1.5980	1.1090	1.4631	1.6093	0.9379	1.6149	1.2327	≤.1000	1.6464	0.8149	1.2062	0.5109
4.00	1.6355	.7624	1.2796	1.1934	≤0.1000	1.5980	1.5756	≤0.1000	0.7857	1.3451	1.2126	0.3693
5.00	1.3826	≤0.1000	≤0.1000	1.5193	≤0.1000	1.4763	1.4800	≤0.1000	0.6441	1.5306	1.3183	0.3190
6.00	1.5025	≤0.1000	1.8735	1.3133	0.8094	1.7442	1.3470	≤0.1000	≤0.1000	1.7873	1.3002	0.4898
8.00	0.8599	≤0.1000	1.3320	≤0.1000	0.7779	1.0940	≤0.1000	≤0.1000	≤0.1000	1.0959	1.0830	0.4478

The 4ASA blood level of pH3 4ASA formulation with ranitidine treatment

Time (hr)	Concentration(μg/ml) for each subject											
	AA	BB	CB	DB	DS	OC	TN	VM	WB	YO	Mean	s.d.
0.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.15	13.2860	34.2122	14.4374	22.2016	28.6328	14.5335	39.5584	20.8231	16.9288	22.6341	22.7248	8.8982
0.30	18.3544	21.8636	15.8779	17.1306	29.7649	17.6914	20.5978	11.4437	21.3420	17.1909	19.1257	4.8040
0.45	10.8462	12.7016	16.6274	8.9645	16.3811	10.7054	10.5553	7.8114	24.2614	11.8892	13.0744	4.8507
1.00	6.0975	8.2989	7.7276	5.7489	8.9305	7.8534	5.5105	3.6917	15.2306	7.8429	7.6933	3.0887
1.30	2.4443	3.7625	≤0.1000	3.0392	3.6472	3.8149	1.9883	1.4353	7.4002	4.3967	3.5476	1.7342
2.00	0.7356	2.1979	1.3436	1.2466	1.3803	1.9385	0.8588	1.1549	3.0077	2.7011	1.6565	0.7723
2.30	0.4106	0.9321	1.2730	0.8247	0.9479	0.9479	0.8247	0.9950	2.2227	1.7446	1.1123	0.5182
3.00	≤0.1000	0.4892	≤0.1000	0.5993	≤0.1000	0.8509	≤0.1000	0.6679	≤0.1000	1.0108	0.9067	0.3590
4.00	≤0.1000	≤0.1000	0.9662	0.5207	≤0.1000	≤0.1000	≤0.1000	0.8719	≤0.1000	0.9426	0.9553	0.2844
5.00	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	0.4054	0.6478	0.3429
6.00	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	1.8677	0.3896	1.1208	0.6042
8.00	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	0.7198	≤0.1000	≤0.1000	1.5478	2.8534	1.5677	0.9214

The 4ASA blood level of pH7 Na4ASA formulation without ranitidine treatment

Time (hr)	Concentration(μg/ml) for each subject											
	AA	BB	CB	DB	DS	OC	TN	VM	WB	YO	Mean	s.d.
0.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.15	10.8742	16.7124	15.9219	2.5404	18.1315	7.8876	13.5532	14.6431	28.1892	10.6152	13.9069	6.8304
0.30	17.6566	20.9589	13.1431	30.5336	30.8574	4.5152	23.2062	5.0332	23.3357	20.4651	18.9705	9.1717
0.45	9.0800	16.0055	6.6547	14.0846	10.5450	12.3094	10.1619	12.8976	14.5730	12.8841	11.9196	2.8131
1.00	4.3403	7.6340	4.7977	8.4272	4.9927	6.2311	5.2841	5.5450	8.5459	7.1295	6.2928	1.5438
1.30	1.9468	1.7903	2.1519	2.6025	2.0062	1.9279	1.9577	0.4794	4.5422	3.1851	2.2590	1.0514
2.00	0.7813	0.8272	1.1104	1.4315	1.1644	1.5556	0.5871	0.2336	1.5584	1.3290	1.0579	0.4417
2.30	0.6356	0.6360	0.6977	0.7624	0.1635	0.7031	0.2175	0.3523	1.1995	0.4252	0.5793	0.3042
3.00	0.5358	0.3281	0.5871	≤0.1000	0.5439	0.7301	≤0.1000	≤0.1000	0.7300	0.1230	0.4825	0.2600
4.00	≤0.1000	≤0.1000	0.3038	0.7463	0.1176	0.3200	≤0.1000	≤0.1000	0.6599	≤0.1000	0.5092	0.2551
5.00	≤0.1000	≤0.1000	0.4306	≤0.1000	0.1905	0.1959	≤0.1000	≤0.1000	0.1257	≤0.1000	0.4463	0.3632
6.00	0.2822	≤0.1000	0.2741	≤0.1000	0.5278	≤0.1000	≤0.1000	≤0.1000	0.5979	≤0.1000	0.5629	0.2570
8.00	≤0.1000	≤0.1000	0.1149	0.6707	0.1527	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	0.4468	0.3690

The 4ASA blood level of pH7 Na4ASA formulation with ranitidine treatment

Time (hr)	Concentration(μg/ml) for each subject											
	AA	BB	CB	DBI4	DSI4	OCI4	TNI4	VMI4	WBI4	YOI4	Mean	s.d.
0.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.15	2.4686	2.4582	1.8987	1.2989	1.8045	1.8512	2.5065	0.4256	4.4446	2.9329	2.2090	1.0613
0.30	0.6954	0.9566	0.5905	0.5000	0.1762	0.4927	0.5736	≤0.1000	1.6995	1.0801	0.7516	0.4431
0.45	0.2578	0.1053	0.3620	0.2944	0.0181	0.1178	0.3479	≤0.1000	0.5454	0.1499	0.2443	0.1633
1.00	0.0675	0.0189	0.0410	0.0133	≤0.1000	≤0.1000	0.1194	≤0.1000	0.3326	0.1142	0.1010	0.1105
1.30	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000
2.00	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000
2.30	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000
3.00	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000
4.00	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000
5.00	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000
6.00	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000
8.00	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000	≤0.1000

The 4ASA blood level of the intravenous formulation

Time (hr)	Concentration(μg/ml) for each subject											
	AA	BB	CB	DB	DS	OC	TN	VM	WB	YO	Mean	s.d.
0.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.15	8.2148	16.8793	7.5109	13.9720	11.2214	21.4694	9.6287	16.1731	9.1335	15.4508	12.9654	4.5497
0.30	21.2767	36.2375	14.9537	28.6476	29.8131	33.3730	24.2228	25.1025	21.3520	32.0995	26.7078	6.5218
0.45	19.8536	37.8974	29.3984	41.7420	44.8816	31.8789	41.0482	27.5386	29.8551	36.0116	34.0105	7.6883
1.00	22.2509	37.8824	30.6984	44.5744	32.7681	24.9690	36.4040	28.2824	36.9278	31.8723	32.6630	6.6047
1.30	21.1399	33.9145	24.5869	31.4732	34.7789	19.9505	32.4272	21.6707	16.8616	24.5490	26.1352	6.4753
2.00	18.4628	17.9085	16.4392	24.4908	24.5825	16.9708	20.2305	14.9492	37.4011	19.9742	21.1410	6.5414
2.30	14.7748	15.5735	14.6682	22.0774	17.0741	10.5744	15.3498	9.7024	32.9720	13.2569	16.6024	6.6917
3.00	8.0619	13.4798	13.6865	11.7057	12.0366	5.8928	10.7094	5.8918	31.1703	8.0950	12.0730	7.2999
4.00	4.5494	4.3869	6.1215	5.4180	5.8197	3.2318	5.1121	3.2146	8.8378	7.2430	5.3935	1.7391
5.00	2.5504	2.5762	2.2836	2.8487	3.6010	2.0627	2.6026	1.8399	6.4332	4.2134	3.1012	1.3670
6.00	1.6095	1.7161	1.8306	2.0342	2.4309	1.3748	≤0.1000	1.3060	3.1837	1.8292	1.9239	0.5809
8.00	≤0.1000	1.0932	1.0980	1.0689	1.2263	0.9841	1.1461	1.0735	2.1525	0.9509	1.1993	0.3665

The AASA blood level of pH3 4ASA formulation without ranitidine treatment

Time (hr)	Concentration(μ g/ml) for each subject											
	AA	BB	CB	DB	DS	OC	TN	VM	WB	YO	Mean	s.d.
0.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.15	6.0497	6.1739	2.8400	10.8026	8.8061	23.5732	16.6662	13.8101	2.8020	8.9092	10.0433	6.4957
0.30	13.7192	19.9858	13.1460	25.2518	23.0207	26.4561	35.5955	28.3591	16.0846	22.4157	22.4035	7.0024
0.45	22.4229	24.6542	19.1764	31.0858	29.4635	26.3583	40.9785	34.3490	27.5143	28.5134	28.4516	6.1619
1.00	24.5504	32.7156	21.7927	32.5782	32.9193	29.3696	35.4652	31.9873	32.8131	28.2162	30.2408	4.2731
1.30	21.8528	28.6417	19.1480	22.8402	24.9608	20.2230	24.9067	22.9668	30.9649	22.2895	23.8794	3.6425
2.00	16.7212	21.3357	13.9258	17.3044	24.6818	15.5189	19.8657	16.2257	27.3320	16.0605	18.8972	4.3471
2.30	11.8235	13.5227	8.4257	11.5580	19.0613	10.7181	13.1939	10.3515	23.7308	13.4864	13.5872	4.5411
3.00	8.4582	11.8654	5.1427	8.6119	12.9225	7.1838	8.3962	7.6601	17.3796	10.0452	9.7666	3.4930
4.00	4.4759	4.4698	3.2920	4.4800	5.6551	3.6015	4.1471	3.4186	10.1645	5.9784	4.9683	2.0269
5.00	2.4849	2.8085	≤0.1000	2.6609	4.2618	2.1407	2.3840	≤0.1000	4.4900	2.5825	2.8315	0.9377
6.00	1.7518	1.8068	1.5411	1.6999	1.6354	1.3690	1.5106	3.1887	3.4519	1.9136	1.9869	0.7224
8.00	1.0922	1.0962	≤0.1000	0.9110	0.8619	0.8010	0.9863	0.3040	≤0.1000	0.9669	1.1193	0.7632

The AASA blood level of pH3 4ASA formulation with ranitidine treatment

Time (hr)	Concentration(μ g/ml) for each subject											
	AA	BB	CB	DB	DS	OC	TN	VM	WB	YO	Mean	s.d.
0.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.15	5.4030	16.2114	6.9245	9.1144	14.1409	6.8419	20.7169	14.3914	8.3826	8.1184	11.0245	5.0208
0.30	17.6134	29.1066	21.4618	25.0763	28.1547	20.4365	33.1545	19.5125	21.2570	17.3035	23.3077	5.3327
0.45	24.3266	29.5743	24.3045	23.5356	31.1283	22.6885	29.9079	24.5125	27.0198	20.2683	25.7266	3.5343
1.00	20.7700	27.3932	23.8750	18.5520	28.6786	20.0321	23.1533	20.8040	30.1189	18.1830	23.1560	4.2735
1.30	16.0491	22.2531	≤0.1000	15.7982	20.7656	17.3197	15.0249	13.2924	24.1761	14.9083	17.7319	3.7570
2.00	11.9805	18.2081	9.9247	11.7768	17.7315	12.2992	10.3556	8.8474	19.1127	13.0444	13.3281	3.6897
2.30	7.3259	15.2670	7.4071	7.7066	13.6759	9.7948	6.7016	4.5087	15.8631	9.2479	9.7499	3.8841
3.00	5.3765	11.1452	5.5889	5.1683	9.0658	5.6303	5.1905	3.8136	10.3526	7.6211	6.8953	2.5000
4.00	2.8913	4.0029	3.0728	2.7880	6.3608	3.2986	2.7954	2.3349	6.0995	3.5421	3.7186	1.4013
5.00	1.9040	2.8942	1.9718	2.1504	≤0.1000	2.8839	1.7667	1.6251	4.1176	2.2331	2.3941	0.7866
6.00	1.3565	1.8066	1.3860	1.7195	2.4456	1.7712	1.5159	1.2001	3.1082	1.2133	1.7523	0.6024
8.00	0.9005	1.2429	1.0466	1.1528	1.5203	1.1676	1.2340	0.7708	1.7638	0.7750	1.1574	0.3139

The AASA blood level for pH7 Na4ASA formulation without ranitidine treatment

Time (hr)	Concentration(μg/ml) for each subject											
	AA	BB	CB	DB	DS	OC	TN	VM	WB	YO	Mean	s.d.
0.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.15	6.6641	9.3090	12.0602	3.4711	7.3785	8.1528	8.2741	16.7105	12.3583	4.5584	8.8937	3.9399
0.30	18.9166	24.4056	20.6749	19.3015	27.4024	11.1766	28.7474	18.6904	24.9522	18.2786	21.2546	5.2085
0.45	22.2819	29.1038	21.1149	27.6556	26.7899	16.7645	30.3139	20.8831	27.5357	23.8035	24.6247	4.3339
1.00	18.7802	29.2341	16.4738	23.9189	21.6379	19.9485	24.1046	22.9364	28.7054	22.5619	22.8302	4.0132
1.30	16.7854	21.0133	12.5874	17.9207	18.9990	16.4984	16.4754	12.6069	21.5840	17.0880	17.1559	3.0013
2.00	12.3673	16.8558	7.9791	12.8495	15.6981	12.2609	10.8216	8.1558	17.3845	12.0289	12.6402	3.2559
2.30	9.0245	13.4261	6.1190	9.3329	8.4239	7.3620	8.2307	5.1934	12.6054	9.2147	8.8933	2.5610
3.00	5.6172	8.7668	5.4450	6.9277	6.7464	5.4195	5.3012	3.7017	8.2666	6.0560	6.2248	1.5007
4.00	3.4935	3.9518	2.4466	3.1955	3.6013	2.7597	3.6433	2.4361	4.6687	3.7930	3.3990	0.7045
5.00	1.8999	1.9793	1.9374	1.6184	3.2868	1.9494	2.9139	1.5855	2.6997	1.7907	2.1661	0.5853
6.00	1.4611	1.5450	1.1781	1.2500	1.7832	1.5165	1.3998	1.0853	1.7547	0.8951	1.3869	0.2858
8.00	0.9924	0.8980	0.9759	0.6629	1.1647	≤0.1000	1.1362	0.7767	1.0628	0.5760	1.0098	0.3542

The AASA blood level of pH7 Na4ASA formulation with ranitidine treatment

Time (HR)	Concentration(μg/ml) for each subject											
	AA	BB	CB	DB	DS	OC	TN	VM	WB	YO	Mean	s.d.
0.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.15	7.8155	11.9346	7.7513	12.1377	10.1484	9.9559	13.6542	10.5740	10.9855	8.0119	10.2969	1.9969
0.30	8.1148	11.5195	8.7531	10.1069	10.0219	9.1115	11.0547	8.6551	13.0036	8.1256	9.8467	1.6138
0.45	7.5222	8.7101	7.5177	6.9998	7.7813	8.3542	8.4839	5.9686	12.1674	8.0075	8.1513	1.6206
1.00	5.1666	8.0856	5.8461	6.9393	5.5340	6.2879	6.9662	3.7848	10.4336	6.5297	6.5574	1.7926
1.30	≤.1000	4.7133	2.8457	4.8104	3.1290	4.1936	4.0079	2.4474	7.9023	4.5392	4.2876	1.6000
2.00	2.6442	2.6963	1.4973	2.6356	2.3496	2.7525	2.4130	1.4833	5.3707	2.7010	2.6544	1.0674
2.30	1.4947	1.8182	0.9646	1.5726	1.7977	1.5991	1.8945	0.8162	4.0712	1.3410	1.7370	0.8928
3.00	0.8783	1.1532	0.5756	1.1514	1.5404	1.2882	1.1827	0.6700	2.6255	0.9883	1.2054	0.5754
4.00	0.4232	0.8341	0.2462	0.6290	0.4532	0.4780	0.6861	0.2820	1.6322	0.4637	0.6128	0.3999
5.00	0.2928	0.2018	≤.1000	0.2866	0.2583	0.2297	0.3681	0.1192	0.8539	0.1865	0.2828	0.2219
6.00	0.1210	0.1010	0.1443	0.2281	0.1261	≤.1000	0.2186	≤.1000	0.3883	≤.1000	0.1526	0.1020
8.00	0.1071	0.0318	0.0699	0.0938	0.1481	≤.1000	≤.1000	≤.1000	≤.1000	≤.1000	0.0909	0.0292

The AASA blood concentration from the intravenous preparation

APPENDIX 9

Example of the MAXENT report

Appendix 9: Example of the output report for subject Oc using MAXENT approach

```
Setting up OPUS .....
        ..... done.

Iteration  1
 Alpha*S === -1.9254E-05              Chisq === 4.5993E+01
                          Scale === 7.3718E-02
 LogProb === -1.1478E+02  Code ===  0
 Ntrans  ===  1142                   Code  === 001011
                  Test(abs)     = .0897
                  Good(abs)     = 5.7335E-04
                  Omega(abs)    = .196335
                  Alpha(abs)    = 1.2437E-01

Iteration  2
 Alpha*S === -1.4892E-03              Chisq === 4.5543E+01
                          Scale === 8.0729E-02
 LogProb === -1.1507E+02  Code ===  0
 Ntrans  ===  1174                   Code  === 000001
                  Test(abs)     = .0216
                  Good(abs)     = 4.5080E-01
                  Omega(abs)    = .999820
                  Alpha(abs)    = 1.2437E-01

Iteration  3
 Alpha*S === -8.2209E-03              Chisq === 4.3867E+01
                          Scale === 8.7789E-02
 LogProb === -1.1532E+02  Code ===  0
 Ntrans  ===  1212                   Code  === 001011
                  Test(abs)     = .0053
                  Good(abs)     = 1.3871E+00
                  Omega(abs)    = .652359
                  Alpha(abs)    = 8.9936E-02

Iteration  4
 Alpha*S === -2.4196E-02              Chisq === 4.0335E+01
                          Scale === 9.2426E-02
 LogProb === -1.1554E+02  Code ===  0
 Ntrans  ===  1268                   Code  === 001011
                  Test(abs)     = .0037
                  Good(abs)     = 3.7661E+00
                  Omega(abs)    = .665638
                  Alpha(abs)    = 7.1744E-02

Iteration  5
 Alpha*S === -4.7455E-02              Chisq === 3.5505E+01
                          Scale === 9.5096E-02
 LogProb === -1.1521E+02  Code ===  0
```

```
Ntrans ===  1336                  Code === 001011
                    Test(abs)     = .0023
                    Good(abs)     = 6.2562E+00
                    Omega(abs)    = .596481
                    Alpha(abs)    = 5.9169E-02

Setting up OPUS .....
          ..... done.

Iteration  6
 Alpha*S === -6.6211E-02                Chisq === 3.2090E+01
                          Scale === 9.7570E-02
 LogProb === -1.1514E+02  Code ===  0
 Ntrans ===  1426                  Code === 001011
                    Test(abs)     = .0021
                    Good(abs)     = 8.3683E+00
                    Omega(abs)    = .601858
                    Alpha(abs)    = 4.8987E-02

Iteration  7
 Alpha*S === -7.2058E-02                Chisq === 3.0237E+01
                          Scale === 9.5618E-02
 LogProb === -1.1223E+02  Code ===  0
 Ntrans ===  1525                  Code === 001011
                    Test(abs)     = .0257
                    Good(abs)     = 1.0168E+01
                    Omega(abs)    = .756749
                    Alpha(abs)    = 3.9792E-02

Iteration  8
 Alpha*S === -7.6762E-02                Chisq === 2.9532E+01
                          Scale === 9.6554E-02
 LogProb === -1.1130E+02  Code ===  0
 Ntrans ===  1639                  Code === 001011
                    Test(abs)     = .0175
                    Good(abs)     = 1.2116E+01
                    Omega(abs)    = .838451
                    Alpha(abs)    = 3.2546E-02

Iteration  9
 Alpha*S === -8.7170E-02                Chisq === 2.7192E+01
                          Scale === 9.6278E-02
 LogProb === -1.1102E+02  Code ===  0
 Ntrans ===  1775                  Code === 001011
                    Test(abs)     = .0112
                    Good(abs)     = 1.4430E+01
                    Omega(abs)    = .836940
                    Alpha(abs)    = 2.7126E-02
```

Iteration 10
Alpha*S === -9.6567E-02 Chisq === 2.5137E+01
Scale === 9.6215E-02
LogProb === -1.1043E+02 Code === 0
Ntrans === 1928 Code === 001011
Test(abs) = .0180
Good(abs) = 1.6240E+01
Omega(abs) = .815584
Alpha(abs) = 2.3046E-02

Setting up OPUS .....
..... done.

Iteration 11
Alpha*S === -1.0514E-01 Chisq === 2.2918E+01
Scale === 9.5450E-02
LogProb === -1.1264E+02 Code === 0
Ntrans === 2132 Code === 001010
Test(abs) = .0176
Good(abs) = 1.8686E+01
Omega(abs) = .833741
Alpha(abs) = 2.0025E-02

Iteration 12
Alpha*S === -1.1693E-01 Chisq === 1.7783E+01
Scale === 9.1039E-02
LogProb === -1.1052E+02 Code === 0
Ntrans === 2342 Code === 001011
Test(abs) = .0734
Good(abs) = 2.0031E+01
Omega(abs) = .800975
Alpha(abs) = 1.7193E-02

Iteration 13
Alpha*S === -1.1751E-01 Chisq === 1.6724E+01
Scale === 8.9597E-02
LogProb === -1.1051E+02 Code === 0
Ntrans === 2573 Code === 001010
Test(abs) = .1867
Good(abs) = 2.1511E+01
Omega(abs) = .828951
Alpha(abs) = 1.5180E-02

Iteration 14
Alpha*S === -1.1942E-01 Chisq === 1.5684E+01
Scale === 8.8759E-02
LogProb === -1.1097E+02 Code === 0
Ntrans === 2832 Code === 001010
Test(abs) = .2518

```
                    Good(abs)     =  2.3148E+01
                    Omega(abs)    =  .854305
                    Alpha(abs)    =  1.3745E-02

Iteration  15
 Alpha*S ===  -1.2175E-01                Chisq ===  1.4732E+01
                              Scale ===  8.8248E-02
 LogProb ===  -1.1127E+02   Code ===  0
 Ntrans  ===  3122                  Code  ===  001010
                    Test(abs)     =  .3002
                    Good(abs)     =  2.4615E+01
                    Omega(abs)    =  .876869
                    Alpha(abs)    =  1.2685E-02

Setting up OPUS .....
          ..... done.

Iteration  16
 Alpha*S ===  -1.2429E-01                Chisq ===  1.3760E+01
                              Scale ===  8.7807E-02
 LogProb ===  -1.1586E+02   Code ===  0
 Ntrans  ===  3473                  Code  ===  000010
                    Test(abs)     =  .3341
                    Good(abs)     =  2.7335E+01
                    Omega(abs)    =  .944290
                    Alpha(abs)    =  1.2685E-02

Iteration  17
 Alpha*S ===  -1.3558E-01                Chisq ===  9.3103E+00
                              Scale ===  8.5968E-02
 LogProb ===  -1.1066E+02   Code ===  0
 Ntrans  ===  3858                  Code  ===  000001
                    Test(abs)     =  .1474
                    Good(abs)     =  2.8375E+01
                    Omega(abs)    =  .967066
                    Alpha(abs)    =  1.2685E-02

Iteration  18
 Alpha*S ===  -1.4033E-01                Chisq ===  9.0142E+00
                              Scale ===  8.7111E-02
 LogProb ===  -1.0988E+02   Code ===  0
 Ntrans  ===  4255                  Code  ===  000000
                    Test(abs)     =  .0125
                    Good(abs)     =  2.8968E+01
                    Omega(abs)    =  .999983
                    Alpha(abs)    =  1.2685E-02

Sample      10 completed.
Sample      20 completed.
```

Sample 30 completed.
Sample 40 completed.
Sample 50 completed.
Sample 60 completed.
Sample 70 completed.
Sample 80 completed.
Sample 90 completed.
Sample 100 completed.

Disposition parameters

Parameter	Lower quartile	Median quartile	Upper
Log(Ke)	-1.42356	-1.34755	-1.26371
Log(Vp)	1.62520	1.71998	1.81274

Absorption parameters for data set number 2

Parameter	Lower quartile	Median quartile	Upper
F	.863373	.935757	1.04695
MAT	11.8342	14.7436	17.2724
t(.10)	1.56005	1.60849	1.67171
t(.25)	3.90012	4.02122	4.17928
t(.50)	7.80024	8.04244	8.35857
t(.75)	11.7004	12.0637	12.5378
t(.90)	14.0404	14.4764	22.6735

Absorption parameters for data set number 3

Parameter	Lower quartile	Median quartile	Upper
F	.736085	.829380	.928633
MAT	72.4662	88.8003	110.339
t(.10)	2.76245	3.59422	5.75405
t(.25)	6.90614	8.98555	14.3851
t(.50)	13.8123	29.2615	40.0153
t(.75)	55.9964	112.095	190.274
t(.90)	237.178	300.921	342.101

Absorption parameters for data set number 4

Parameter	Lower quartile	Median quartile	Upper
F	.542499	.690041	.798065
MAT	27.7963	34.1388	39.9660
t(.10)	2.59226	3.52002	6.98870
t(.25)	6.48066	8.80005	16.6603
t(.50)	12.9613	20.5023	27.0871

t(.75)	27.9545	39.8969	44.3290
t(.90)	70.0000	84.3595	97.7123

Absorption parameters for data set number 5

Parameter	Lower quartile	Median quartile	Upper
F	.436528	.479701	.519589
MAT	41.3062	48.4580	54.2966
t(.10)	3.98303	4.41087	4.79408
t(.25)	9.95759	11.0272	11.9852
t(.50)	32.4598	34.4541	36.3053
t(.75)	43.0918	44.6050	52.0130
t(.90)	101.758	120.886	155.820

Comparison of absorption kinetics for data sets 2 and 3

Parameter	Lower quartile	Median quartile	Upper
F(2) / F(3)	1.00424	1.14892	1.32106
MAT(2) - MAT(3)	-96.1605	-76.2336	-57.8331
t(.10)(2) - t(.10)(3)	-4.15194	-1.98119	-1.11534
t(.25)(2) - t(.25)(3)	-10.3799	-4.95296	-2.78836
t(.50)(2) - t(.50)(3)	-31.8446	-20.8660	-5.57671
t(.75)(2) - t(.75)(3)	-178.365	-99.7110	-43.7462
t(.90)(2) - t(.90)(3)	-313.039	-269.824	-214.947

Comparison of absorption kinetics for data sets 2 and 4

Parameter	Lower quartile	Median quartile	Upper
F(2) / F(4)	1.16013	1.43135	1.66774
MAT(2) - MAT(4)	-26.1396	-20.3861	-14.0912
t(.10)(2) - t(.10)(4)	-5.29545	-1.87283	-.981422
t(.25)(2) - t(.25)(4)	-12.4669	-4.68208	-2.45355
t(.50)(2) - t(.50)(4)	-18.7596	-12.0865	-4.90711
t(.75)(2) - t(.75)(4)	-32.3953	-27.3268	-16.3767
t(.90)(2) - t(.90)(4)	-75.5976	-59.6481	-28.8981

Comparison of absorption kinetics for data sets 2 and 5

Parameter	Lower quartile	Median quartile	Upper
F(2) / F(5)	1.79322	1.93749	2.11303
MAT(2) - MAT(5)	-38.0502	-33.8279	-27.0290
t(.10)(2) - t(.10)(5)	-3.15539	-2.82888	-2.35106
t(.25)(2) - t(.25)(5)	-7.88846	-7.07221	-5.87766
t(.50)(2) - t(.50)(5)	-28.0365	-26.4721	-24.3664
t(.75)(2) - t(.75)(5)	-39.7831	-32.2611	-31.0186

t(.90)(2) - t(.90)(5)	-123.326	-92.9717	-75.0218

Comparison of absorption kinetics for data sets 3 and 2

Parameter	Lower quartile	Median	Upper quartile
F(3) / F(2)	.756983	.870386	.995801
MAT(3) - MAT(2)	57.8331	76.2336	96.1605
t(.10)(3) - t(.10)(2)	1.11534	1.98119	4.15194
t(.25)(3) - t(.25)(2)	2.78836	4.95296	10.3799
t(.50)(3) - t(.50)(2)	5.57671	20.8660	31.8446
t(.75)(3) - t(.75)(2)	43.7462	99.7110	178.365
t(.90)(3) - t(.90)(2)	214.947	269.824	313.039

Comparison of absorption kinetics for data sets 3 and 4

Parameter	Lower quartile	Median	Upper quartile
F(3) / F(4)	.979117	1.23187	1.51531
MAT(3) - MAT(4)	38.9288	52.6266	77.5799
t(.10)(3) - t(.10)(4)	-2.58678	.312029	2.15921
t(.25)(3) - t(.25)(4)	-5.37876	.933985	5.77970
t(.50)(3) - t(.50)(4)	-5.95389	6.96834	20.9877
t(.75)(3) - t(.75)(4)	15.0002	71.5321	156.851
t(.90)(3) - t(.90)(4)	154.355	208.022	255.136

Comparison of absorption kinetics for data sets 3 and 5

Parameter	Lower quartile	Median	Upper quartile
F(3) / F(5)	1.45206	1.68384	1.98496
MAT(3) - MAT(5)	23.4842	40.6170	65.0481
t(.10)(3) - t(.10)(5)	-1.78220	-.829201	1.76666
t(.25)(3) - t(.25)(5)	-4.45550	-2.07301	4.41665
t(.50)(3) - t(.50)(5)	-20.0008	-5.31902	6.59539
t(.75)(3) - t(.75)(5)	7.60154	56.4967	143.937
t(.90)(3) - t(.90)(5)	112.350	168.176	218.549

Comparison of absorption kinetics for data sets 4 and 2

Parameter	Lower quartile	Median	Upper quartile
F(4) / F(2)	.599615	.698644	.861982
MAT(4) - MAT(2)	14.0912	20.3861	26.1396
t(.10)(4) - t(.10)(2)	.981422	1.87283	5.29545
t(.25)(4) - t(.25)(2)	2.45355	4.68208	12.4669
t(.50)(4) - t(.50)(2)	4.90711	12.0865	18.7596
t(.75)(4) - t(.75)(2)	16.3767	27.3268	32.3953
t(.90)(4) - t(.90)(2)	28.8981	59.6481	75.5976

Comparison of absorption kinetics for data sets 4 and 3

Parameter	Lower quartile	Median	Upper quartile
F(4) / F(3)	.659938	.811779	1.02137
MAT(4) - MAT(3)	-77.5799	-52.6266	-38.9288
t(.10)(4) - t(.10)(3)	-2.15921	-.312029	2.58678
t(.25)(4) - t(.25)(3)	-5.77970	-.933985	5.37876
t(.50)(4) - t(.50)(3)	-20.9877	-6.96834	5.95389
t(.75)(4) - t(.75)(3)	-156.851	-71.5321	-15.0002
t(.90)(4) - t(.90)(3)	-255.136	-208.022	-154.355

Comparison of absorption kinetics for data sets 4 and 5

Parameter	Lower quartile	Median	Upper quartile
F(4) / F(5)	1.13268	1.38126	1.65460
MAT(4) - MAT(5)	-21.4739	-12.8252	-4.44606
t(.10)(4) - t(.10)(5)	-1.77485	-.758900	2.62972
t(.25)(4) - t(.25)(5)	-4.44646	-1.89725	5.71982
t(.50)(4) - t(.50)(5)	-21.0430	-14.1242	-6.47756
t(.75)(4) - t(.75)(5)	-21.5984	-10.3676	-.626322
t(.90)(4) - t(.90)(5)	-74.2905	-44.8134	-17.7493

Comparison of absorption kinetics for data sets 5 and 2

Parameter	Lower quartile	Median	Upper quartile
F(5) / F(2)	.473255	.516132	.557658
MAT(5) - MAT(2)	27.0290	33.8279	38.0502
t(.10)(5) - t(.10)(2)	2.35106	2.82888	3.15539
t(.25)(5) - t(.25)(2)	5.87766	7.07221	7.88846
t(.50)(5) - t(.50)(2)	24.3664	26.4721	28.0365
t(.75)(5) - t(.75)(2)	31.0186	32.2611	39.7831
t(.90)(5) - t(.90)(2)	75.0218	92.9717	123.326

Comparison of absorption kinetics for data sets 5 and 3

Parameter	Lower quartile	Median	Upper quartile
F(5) / F(3)	.503790	.593882	.688682
MAT(5) - MAT(3)	-65.0481	-40.6170	-23.4842
t(.10)(5) - t(.10)(3)	-1.76666	.829201	1.78220
t(.25)(5) - t(.25)(3)	-4.41665	2.07301	4.45550
t(.50)(5) - t(.50)(3)	-6.59539	5.31902	20.0008
t(.75)(5) - t(.75)(3)	-143.937	-56.4967	-7.60154
t(.90)(5) - t(.90)(3)	-218.549	-168.176	-112.350

Comparison of absorption kinetics for data sets 5 and 4

Parameter	Lower quartile	Median	Upper quartile
F(5) / F(4)	.604385	.723980	.883088
MAT(5) - MAT(4)	4.44606	12.8252	21.4739
t(.10)(5) - t(.10)(4)	-2.62972	.758900	1.77485
t(.25)(5) - t(.25)(4)	-5.71982	1.89725	4.44646
t(.50)(5) - t(.50)(4)	6.47756	14.1242	21.0430
t(.75)(5) - t(.75)(4)	.626322	10.3676	21.5984
t(.90)(5) - t(.90)(4)	17.7493	44.8134	74.2905

APPENDIX 10

The measured and model fit concentration levels of 4ASA using MAXENT approach plus the measured concentration level of AASA over 8 hours' period. The absorption rate profiles of 4ASA derived using MAXENT approach

The following abbreviations are used to identify the curves in figures 1-10(a-d):

c34	Measured 4ASA blood concentration for pH3 formulation without ranitidine treatment
c34a	Measured 4ASA blood concentration for pH3 formulation with ranitidine treatment
c3a	Measured AASA blood concentration for pH3 formulation without ranitidine treatment
c3aa	Measured AASA blood concentration for pH3 formulation with ranitidine treatment
c34am	MAXENT model for 4ASA blood concentration of pH3 formulation with ranitidine treatment
c34m	MAXENT model for 4ASA blood concentration of pH3 formulation without ranitidine treatment
c74am	MAXENT model for 4ASA blood concentration of pH7 formulation with ranitidine treatment
c74m	MAXENT model for 4ASA blood concentration of pH7 formulation without ranitidine treatment
c74	Measured 4ASA blood concentration for pH7 formulation without ranitidine treatment
c74a	Measured 4ASA blood concentration for pH7 formulation with ranitidine treatment
c7a	Measured AASA blood concentration for pH7 formulation without ranitidine treatment
c7aa	Measured AASA blood concentration for pH7 formulation with ranitidine treatment
r34	MAXENT model for 4ASA absorption rate of pH3 formulation without ranitidine treatment
r34a	MAXENT model for 4ASA absorption rate of pH3 formulation with ranitidine treatment
r74	MAXENT model for 4ASA absorption rate of pH7 formulation without ranitidine treatment
r74a	MAXENT model for 4ASA absorption rate of pH7 formulation with ranitidine treatment

Figure 1.a: The 4ASA and AASA concentration profiles of intravenous preparation for subject AA

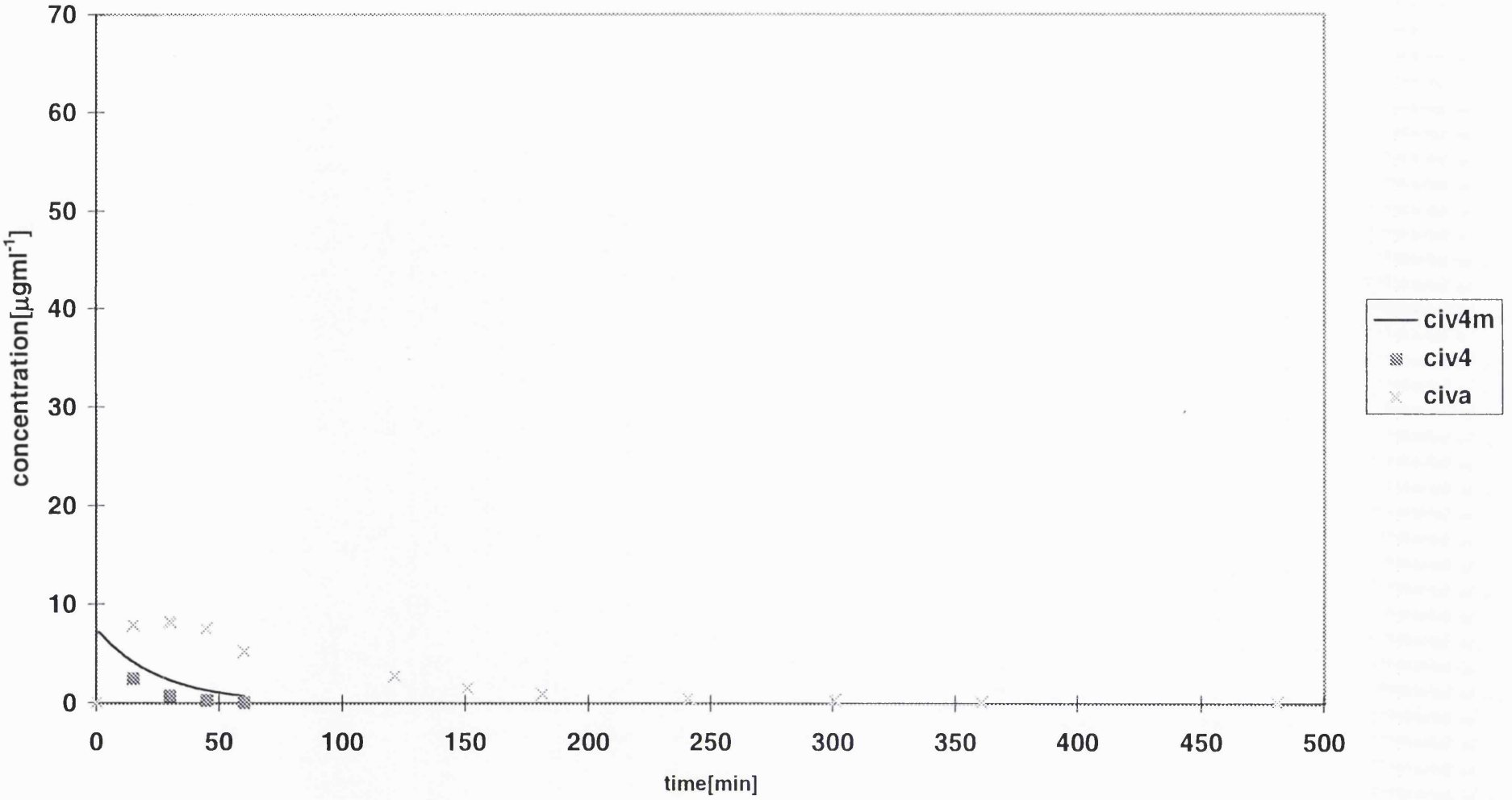


Figure 1.b: The 4ASA and AASA concentration and rate profiles for subject AA

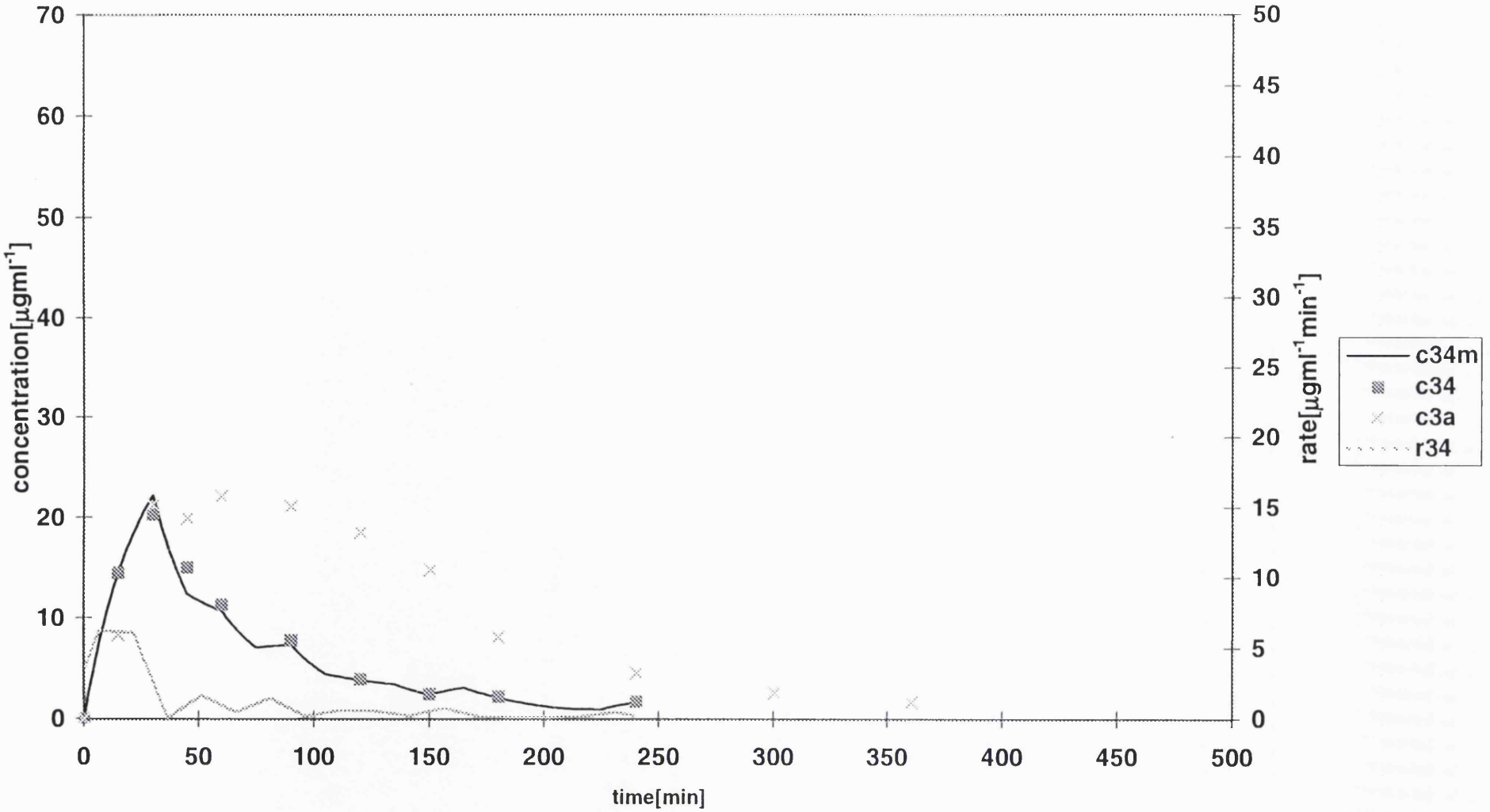


Figure 1.c: The 4ASA and AASA concentration and rate profiles of subject AA

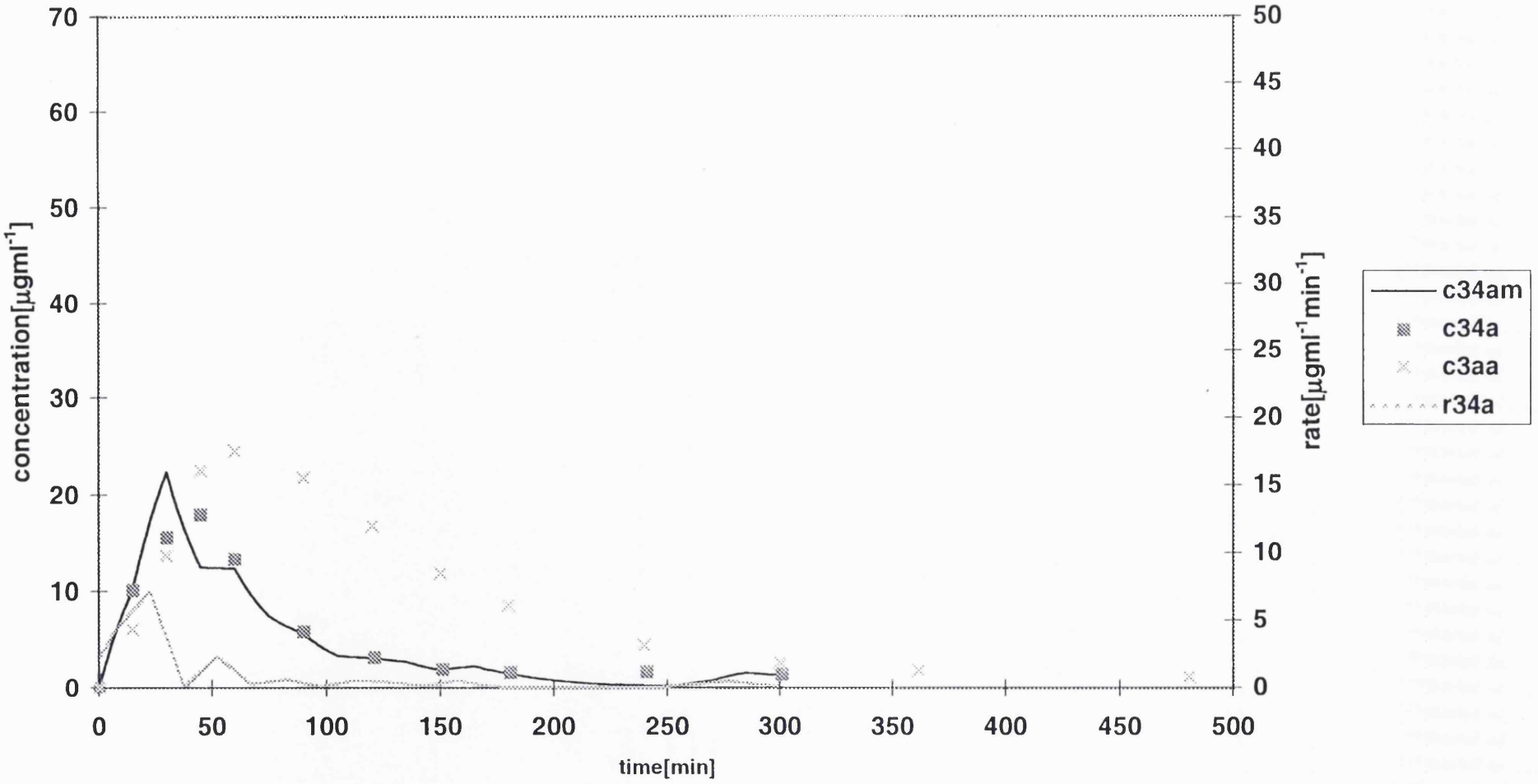


Figure 1.d: The 4ASA and AASA concentration and rate profiles of subject AA

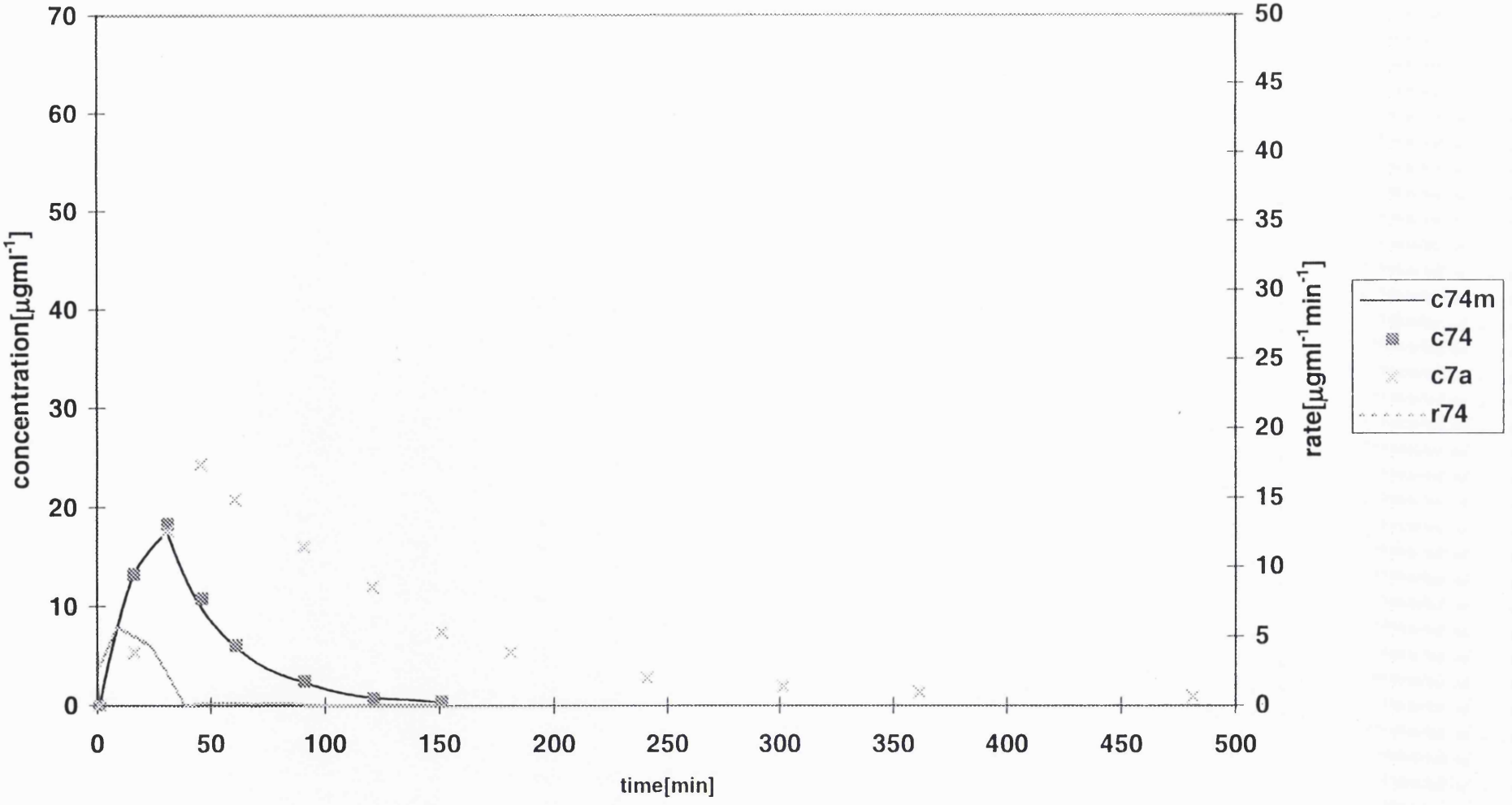


Figure 1.e: The 4ASA and AASA concentration and rate profiles of subject AA

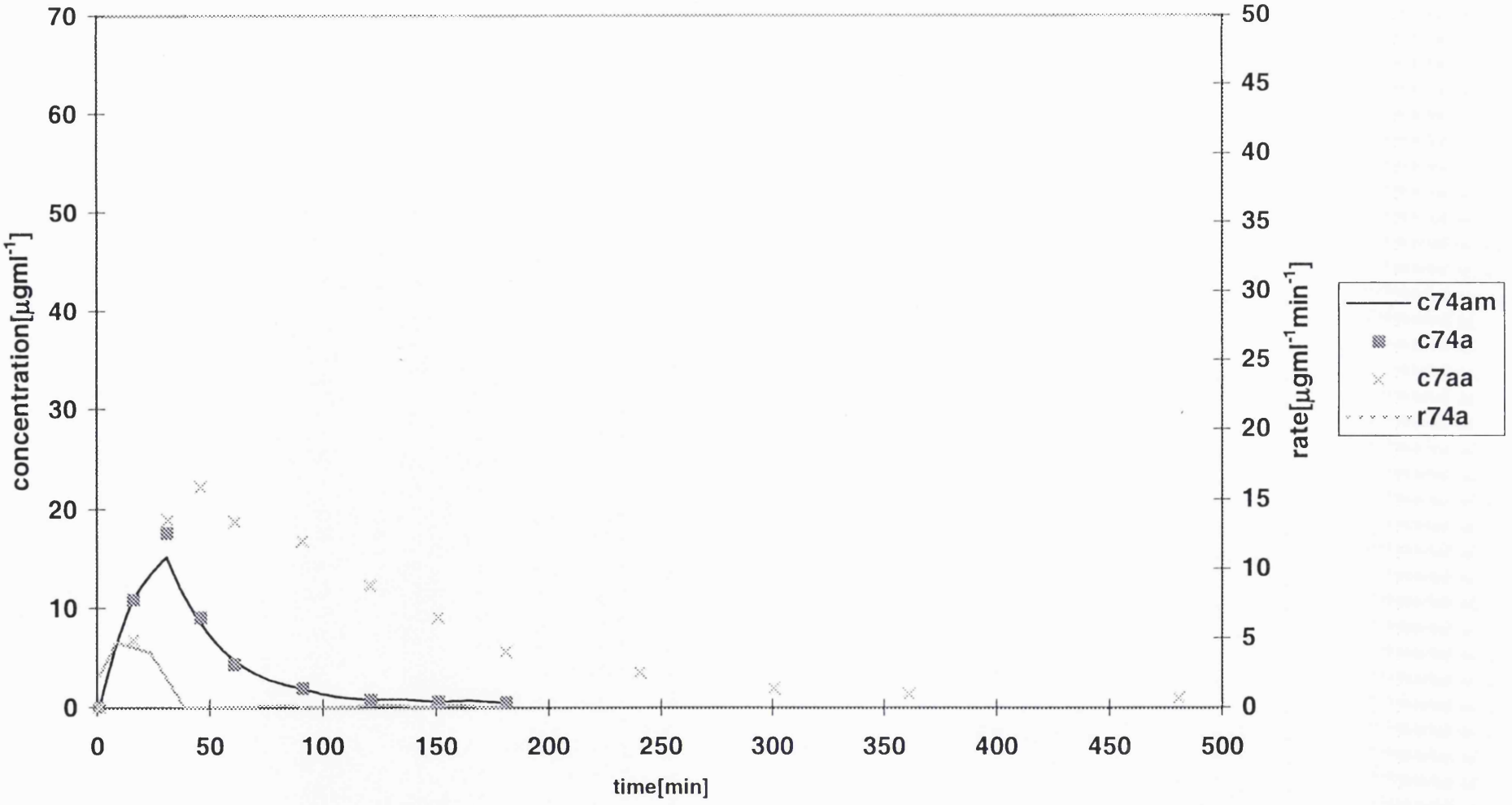


Figure 2.a: The 4ASA and AASA concentration profiles of intravenous preparation for subject BB

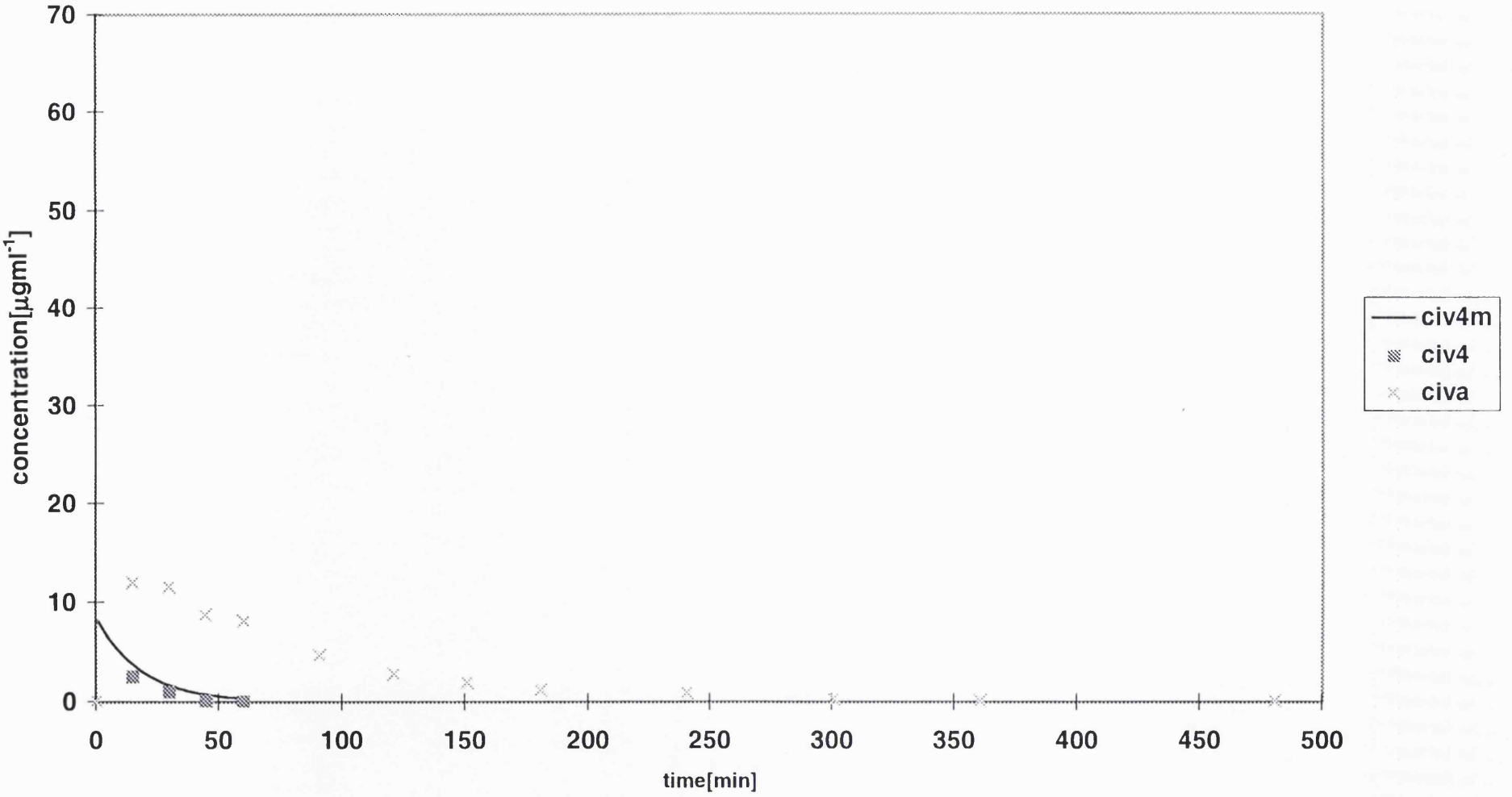


Figure 2.b: The 4ASA and AASA concentration and rate profiles for subject BB

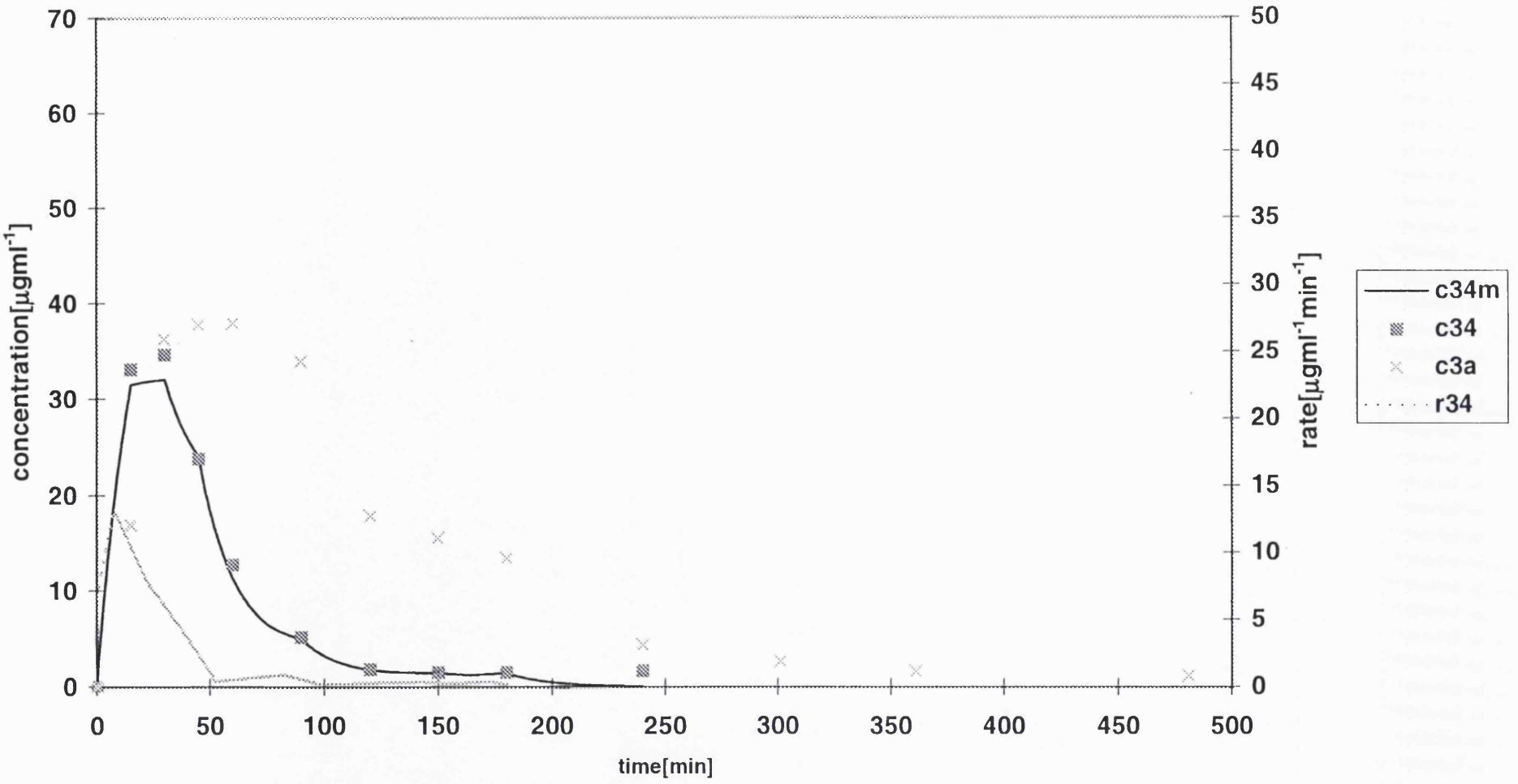


Figure 2.c: The 4ASA and AASA concentration and rate profiles for subject BB

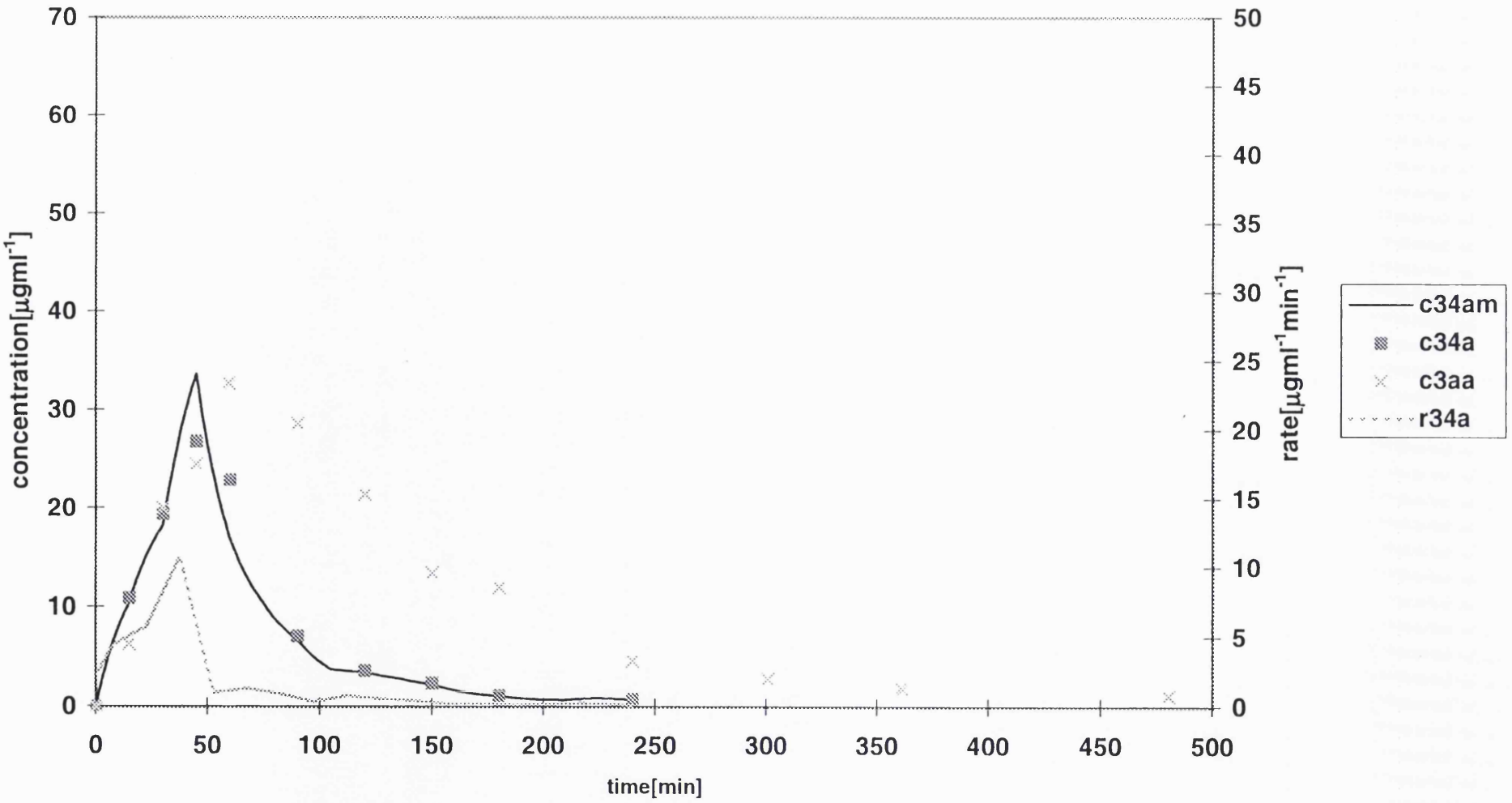


Figure 2.d: The 4ASA and AASA concentration and rate profiles for subject BB

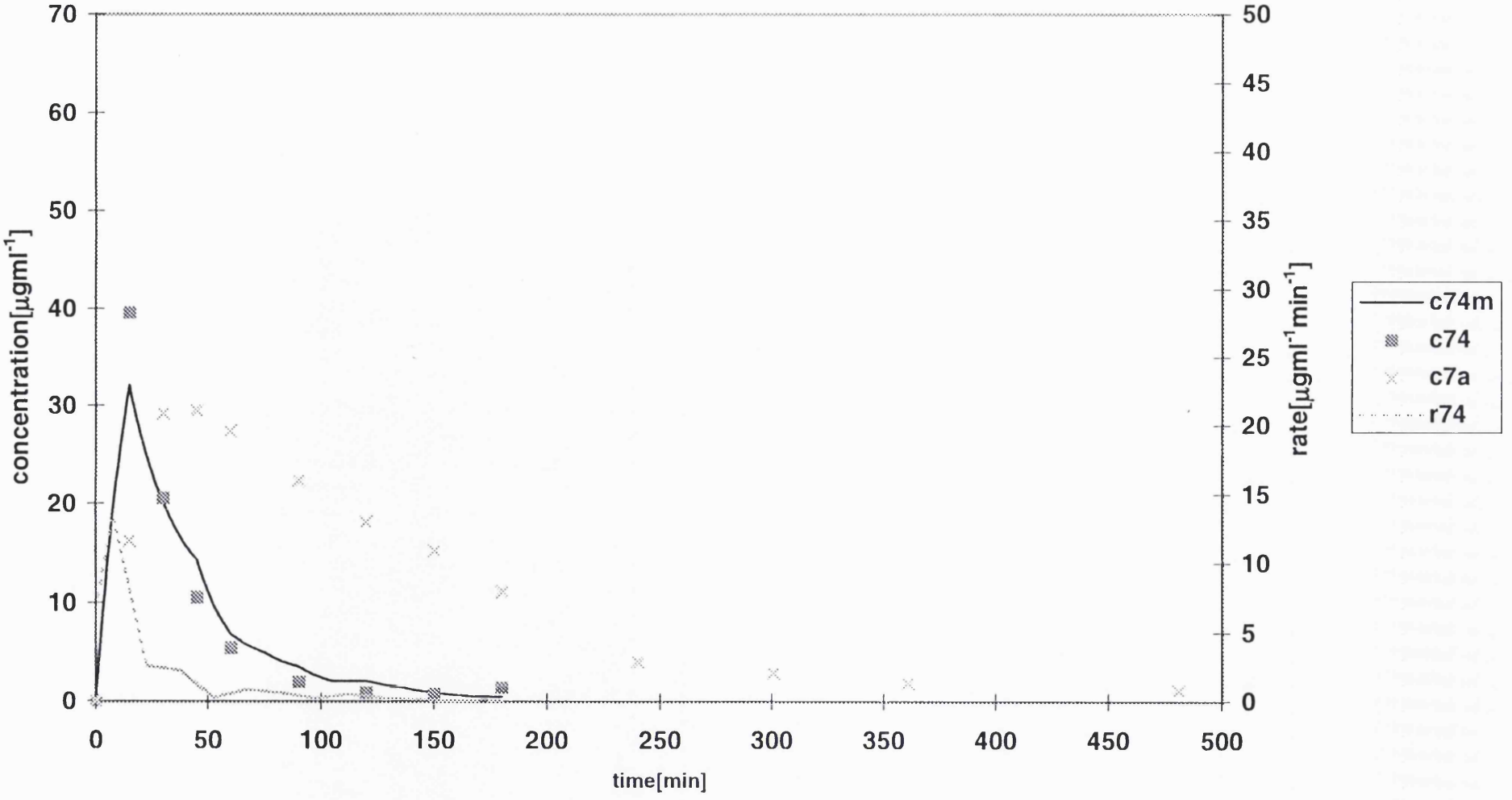


Figure 2.e: The 4ASA and AASA concentration and rate profiles for subject BB

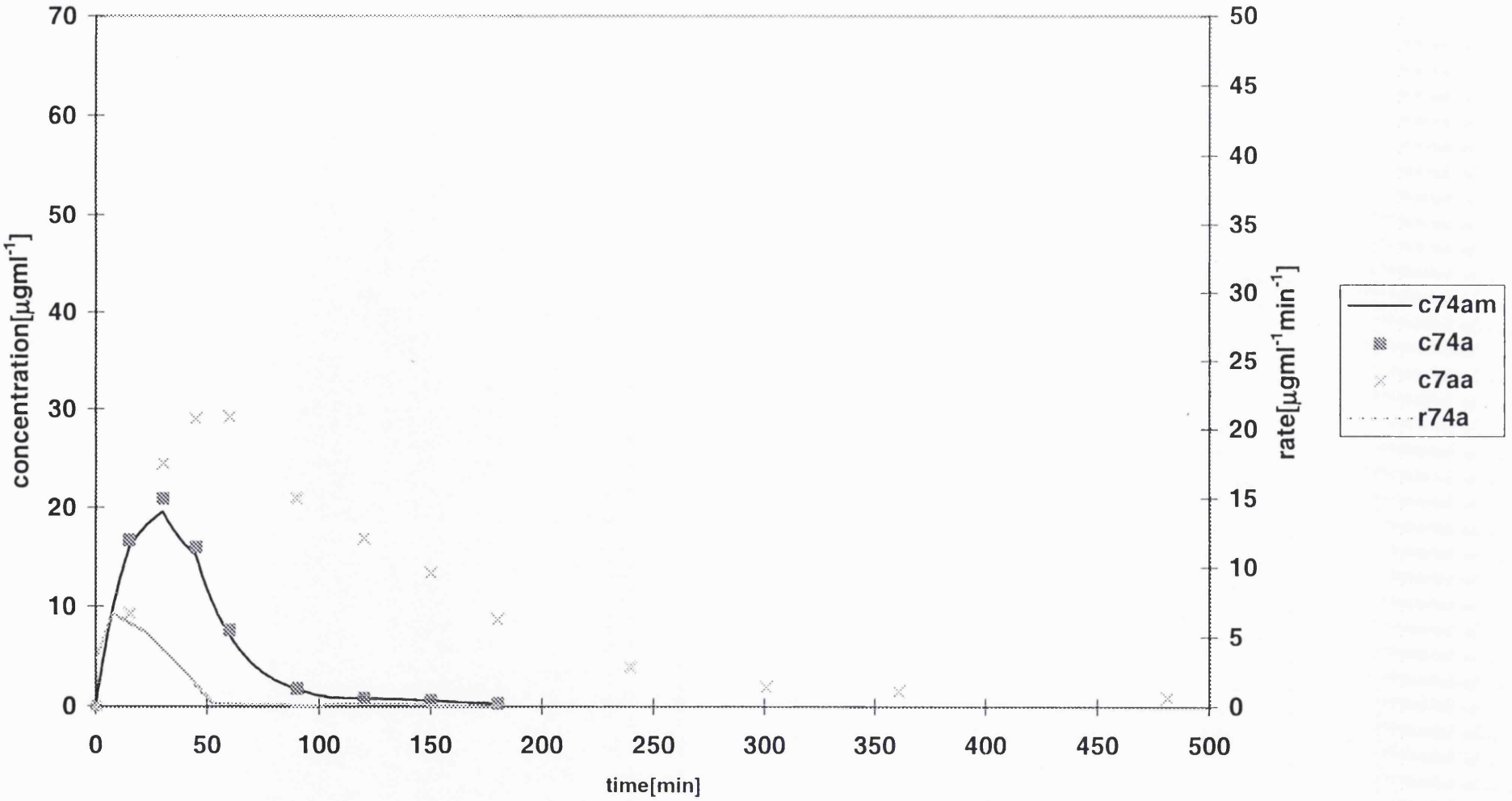


Figure 3.a: The 4ASA and AASA concentration profiles of intravenous preparation for subject CB

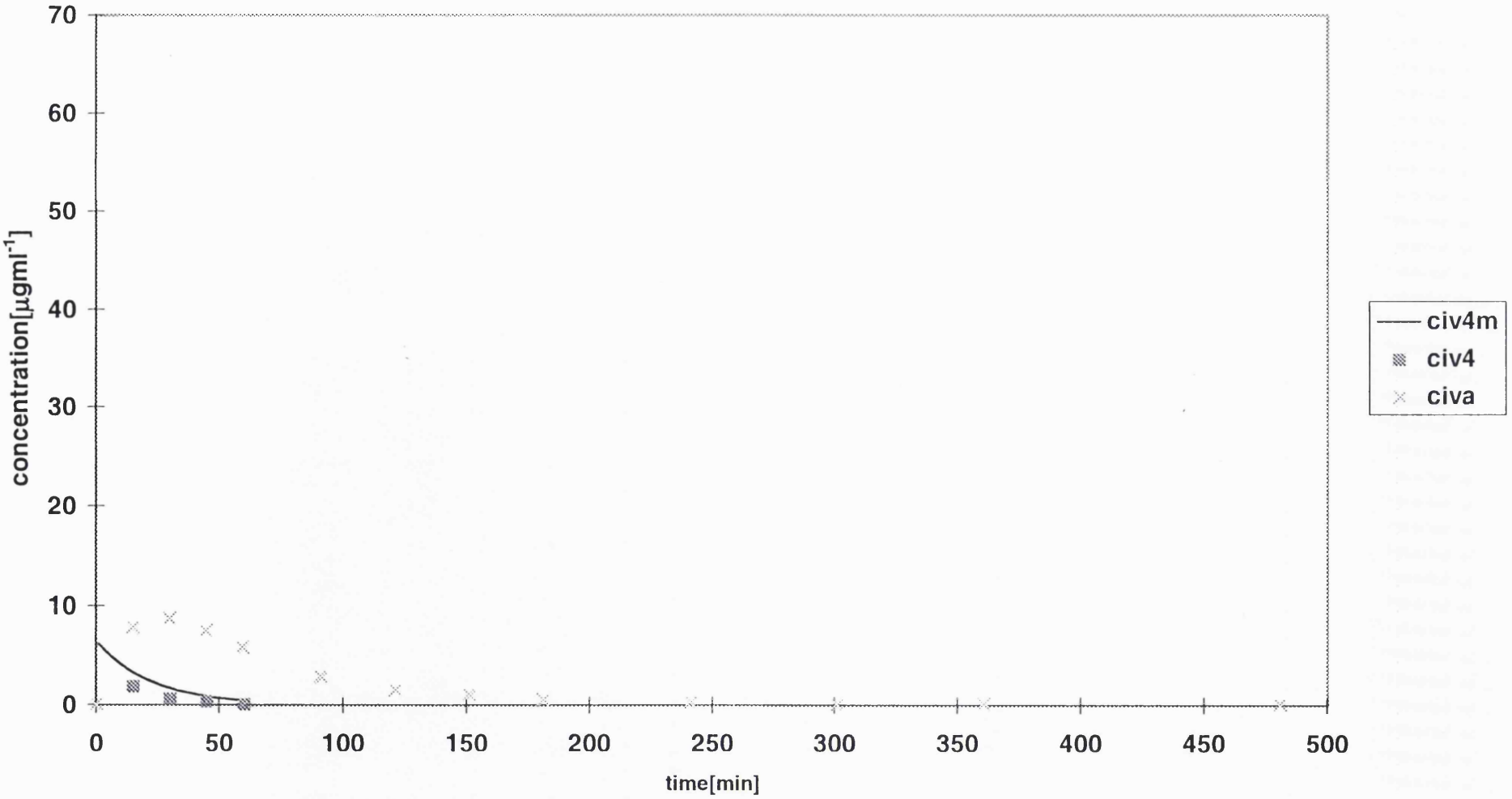


Figure 3.b: The 4ASA and AASA concentration and rate profiles for subject CB

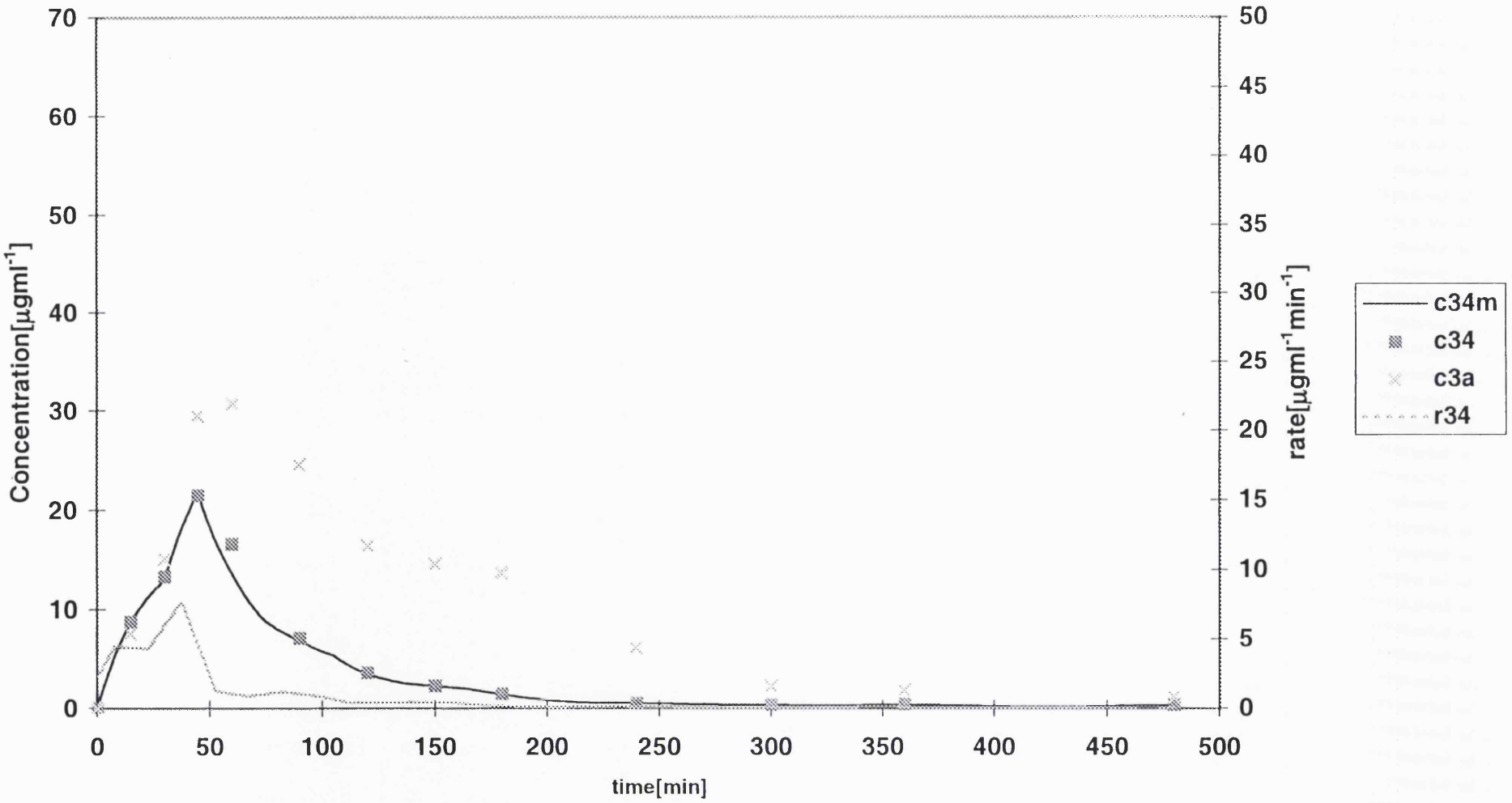


Figure 3.c: The 4ASA and AASA concentration and rate profiles for subject CB

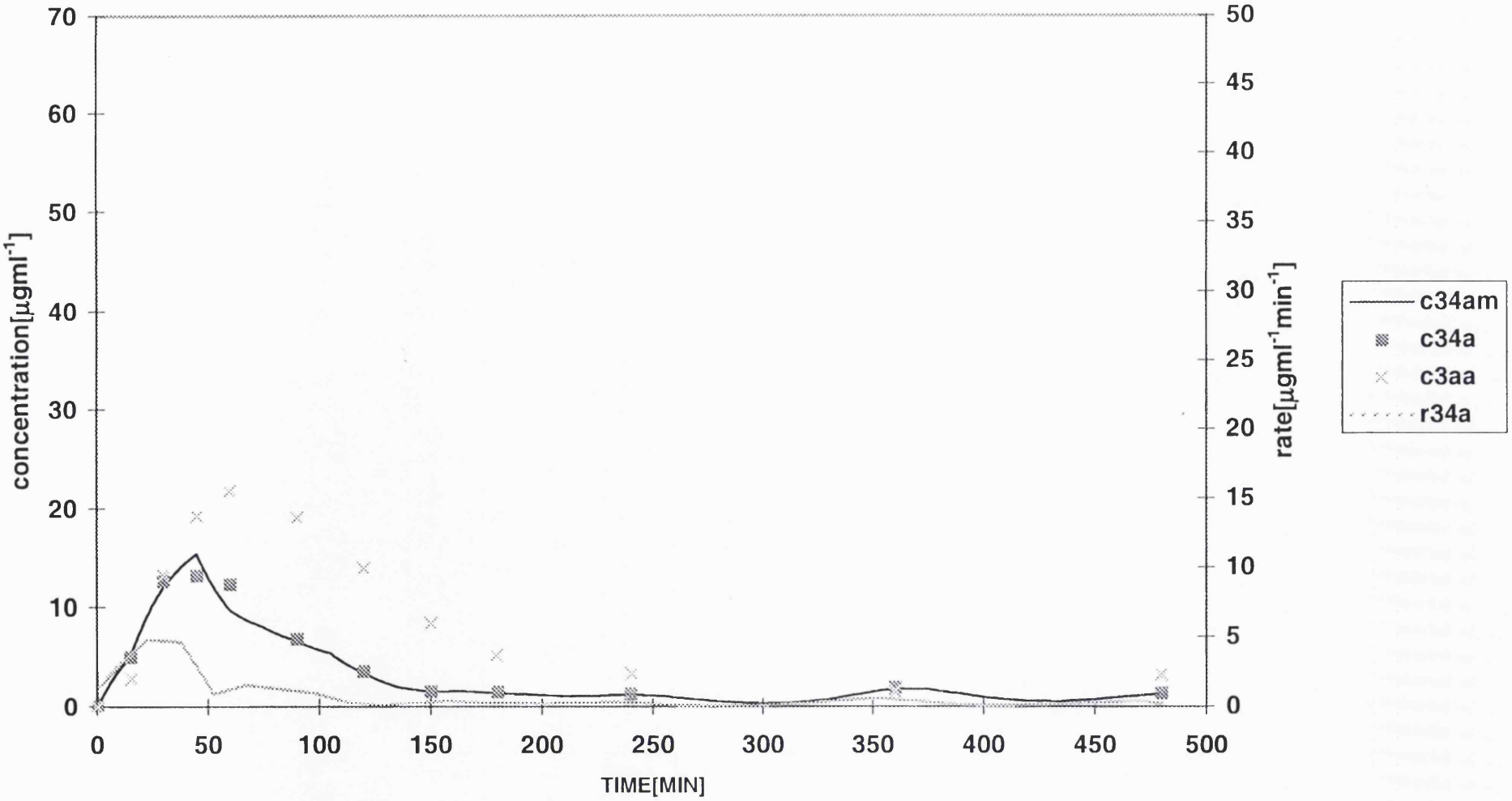


Figure 3.d: The 4ASA and AASA concentration and rate profiles for subject CB

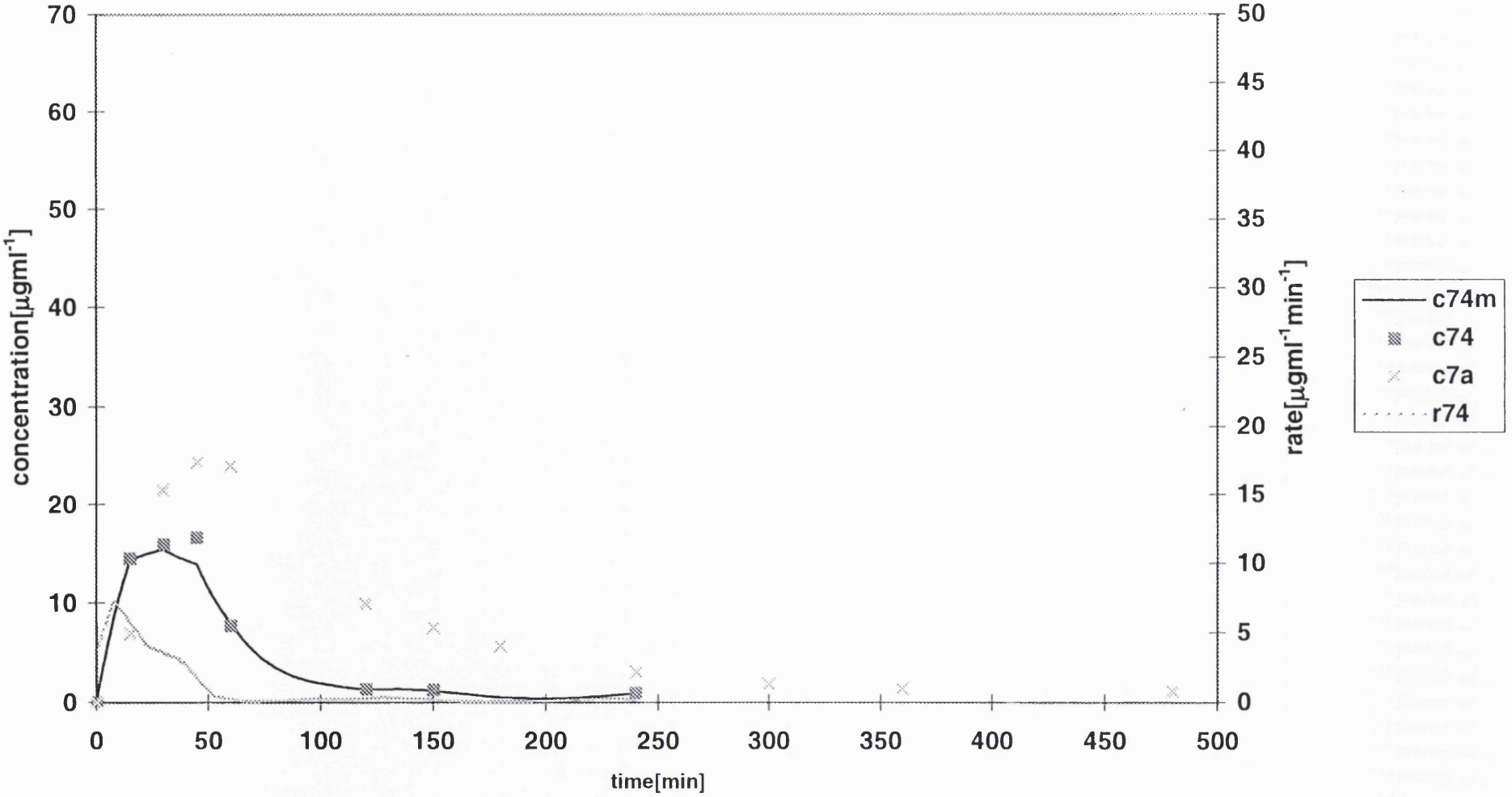


Figure 3.e: The 4ASA and AASA concentration and rate profiles for subject CB

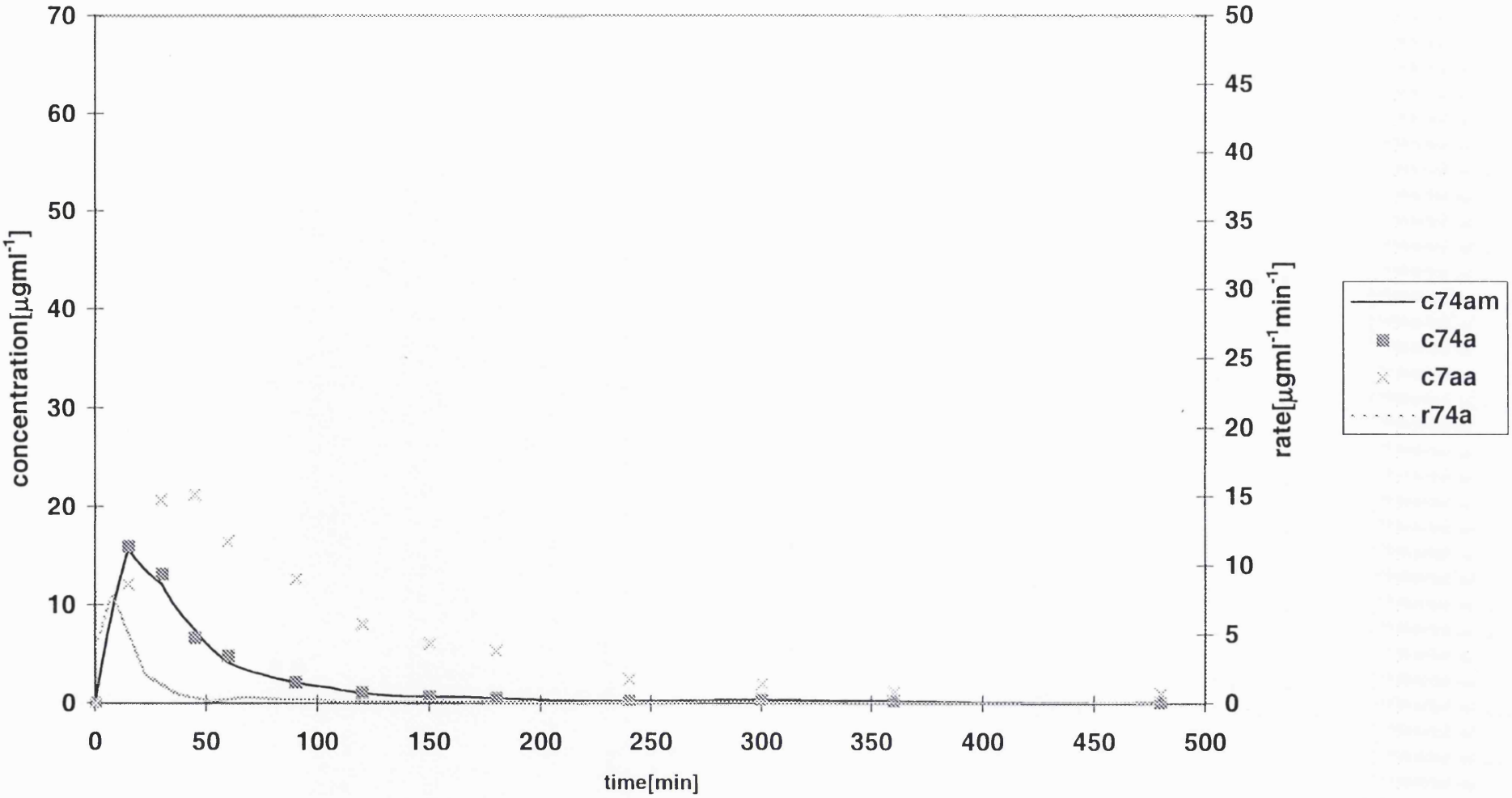


Figure 4.a: The 4ASA and AASA concentration profiles for intravenous preparation for subject DB

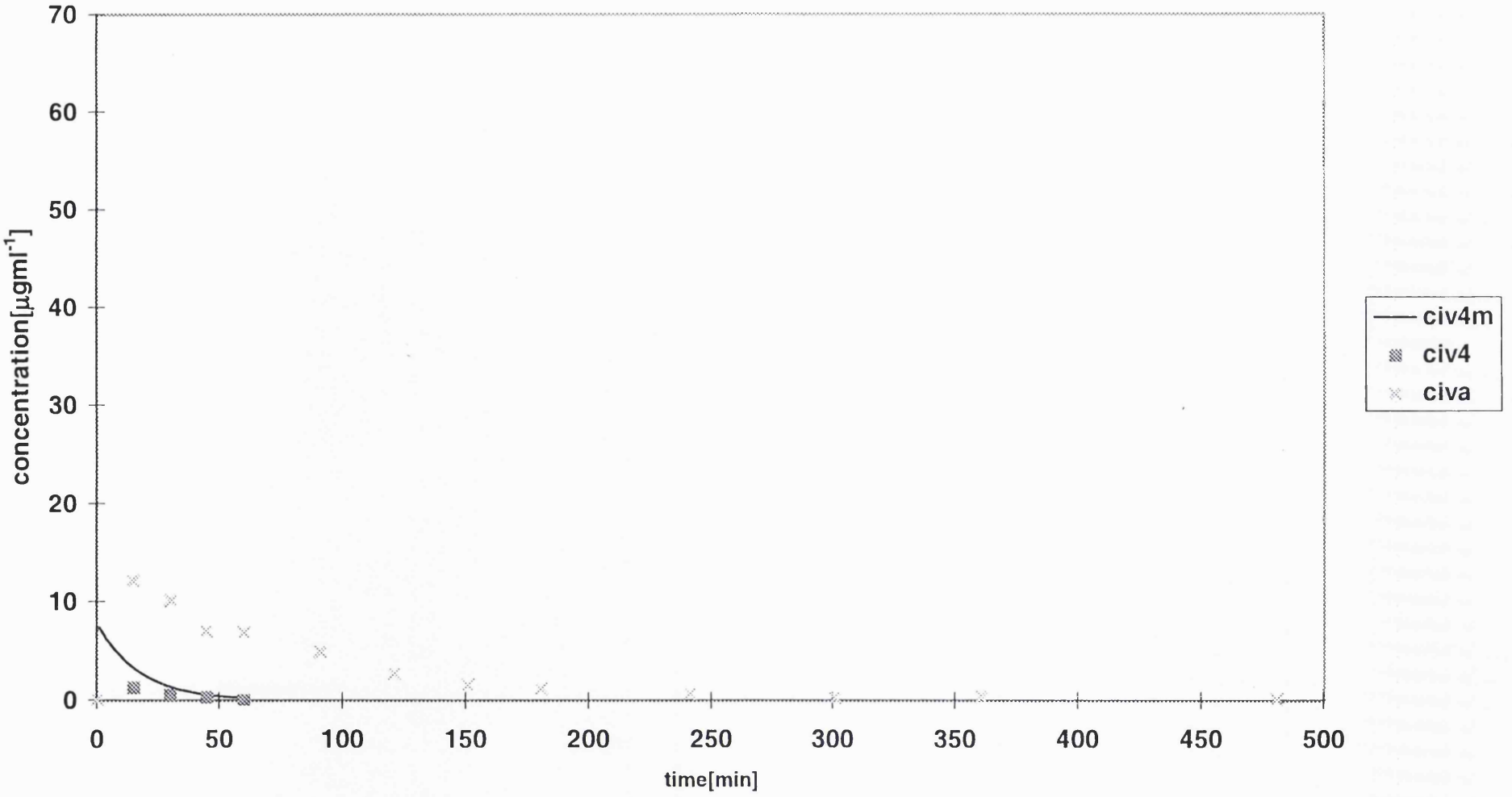


Figure 4.b: The 4ASA and AASA concentration and rate profiles for subject DB

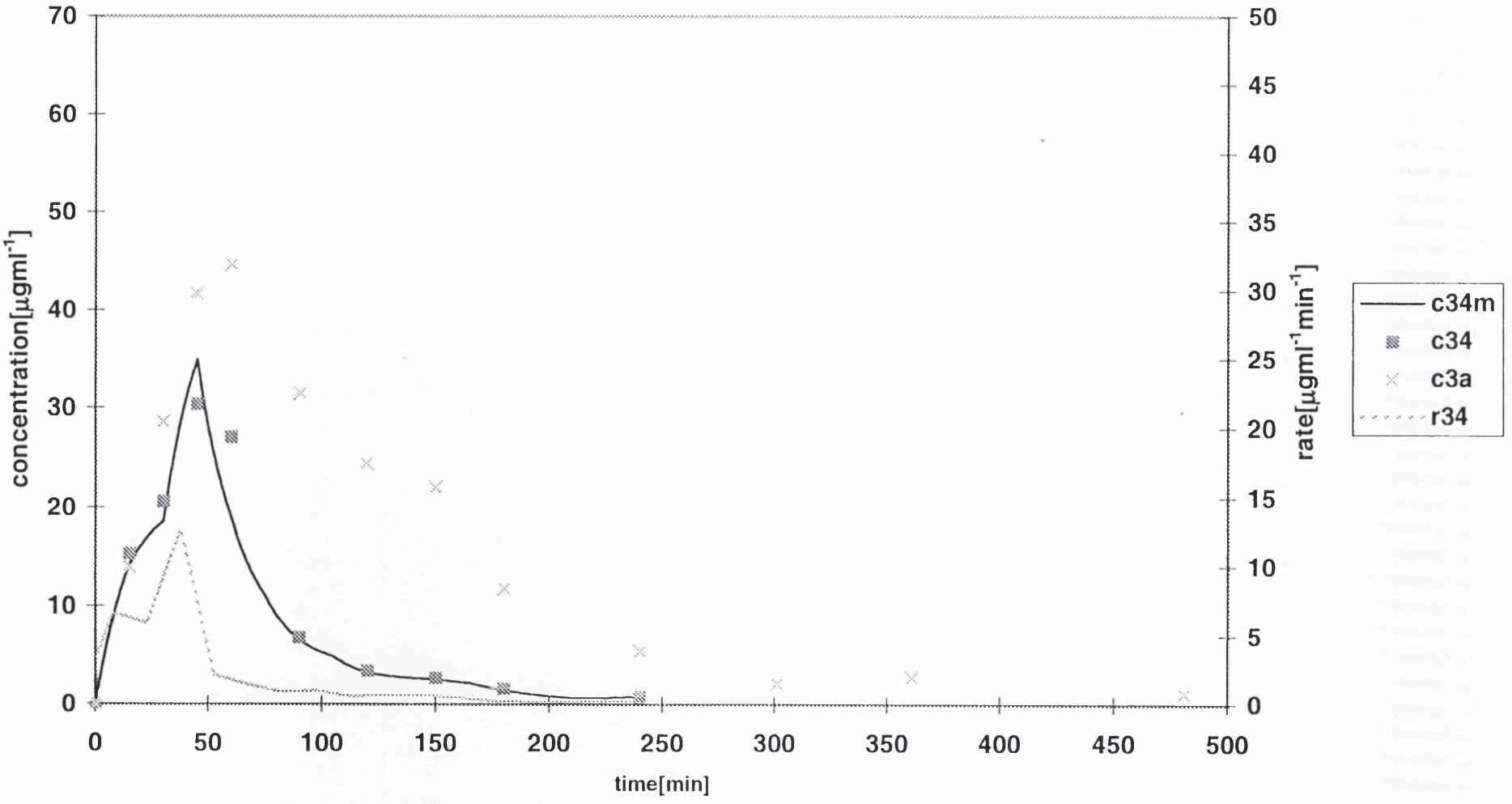


Figure 4.c: The 4ASA and AASA concentration and rate profiles for subject DB

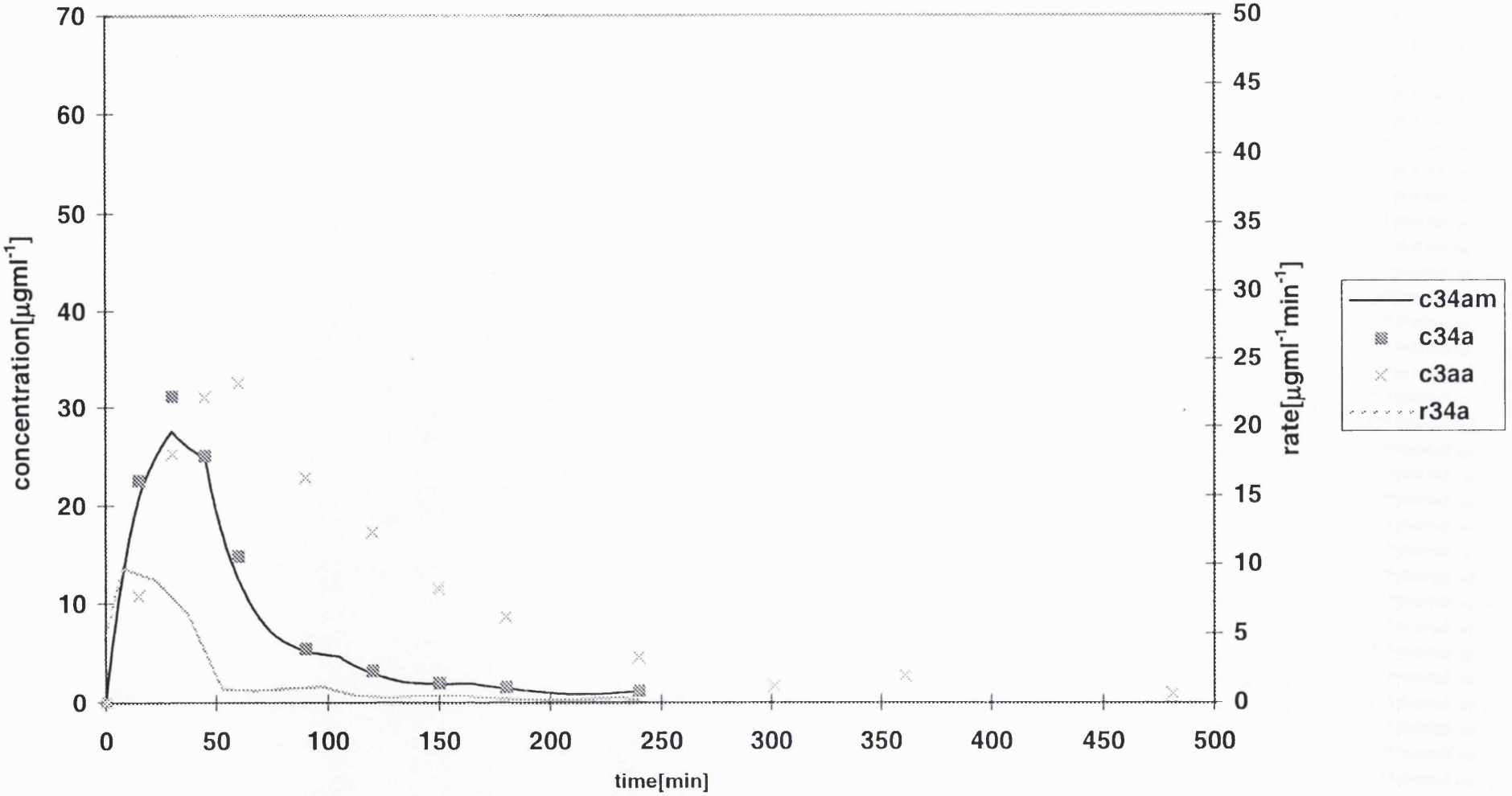


Figure 4.d: The 4ASA and AASA concentration and rate profiles for subject DB

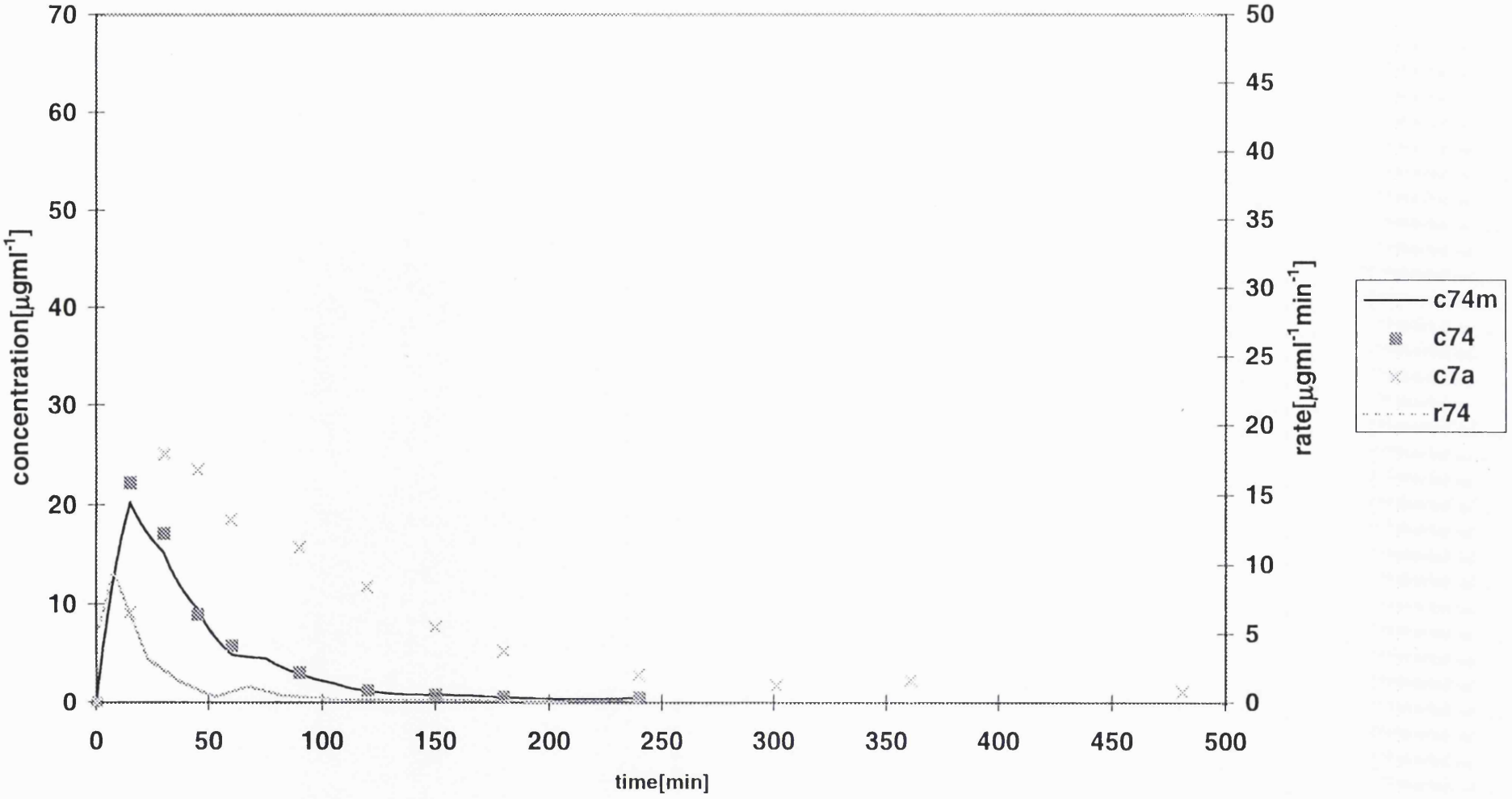


Figure 4.e: The 4ASA and AASA concentration and rate profiles for subject DB

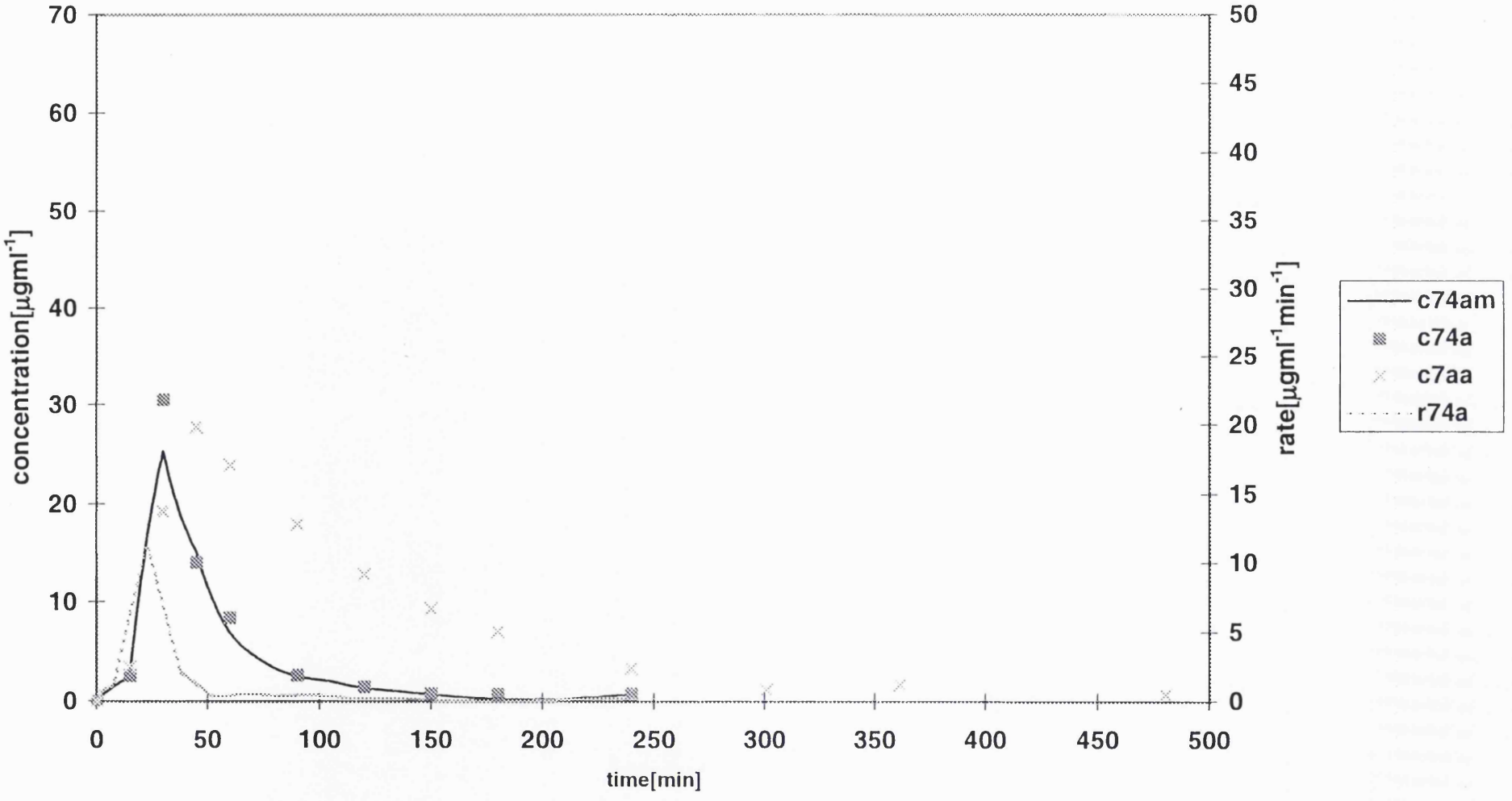


Figure 5.a: The 4ASA and AASA concentration profiles of intravenous preparation for subject DS

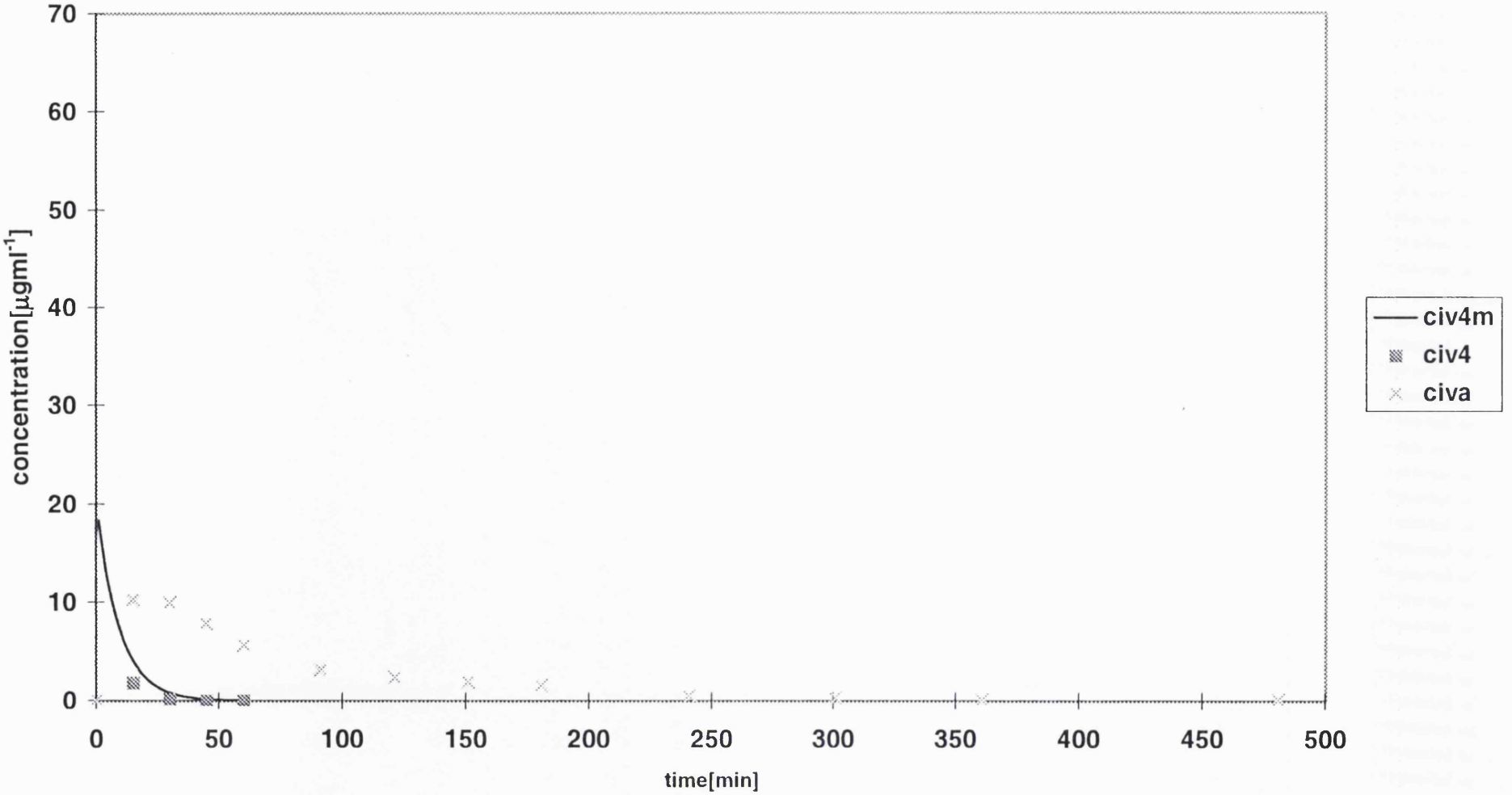


Figure 5.b: The 4ASA and AASA concentration and rate profiles for subject DS

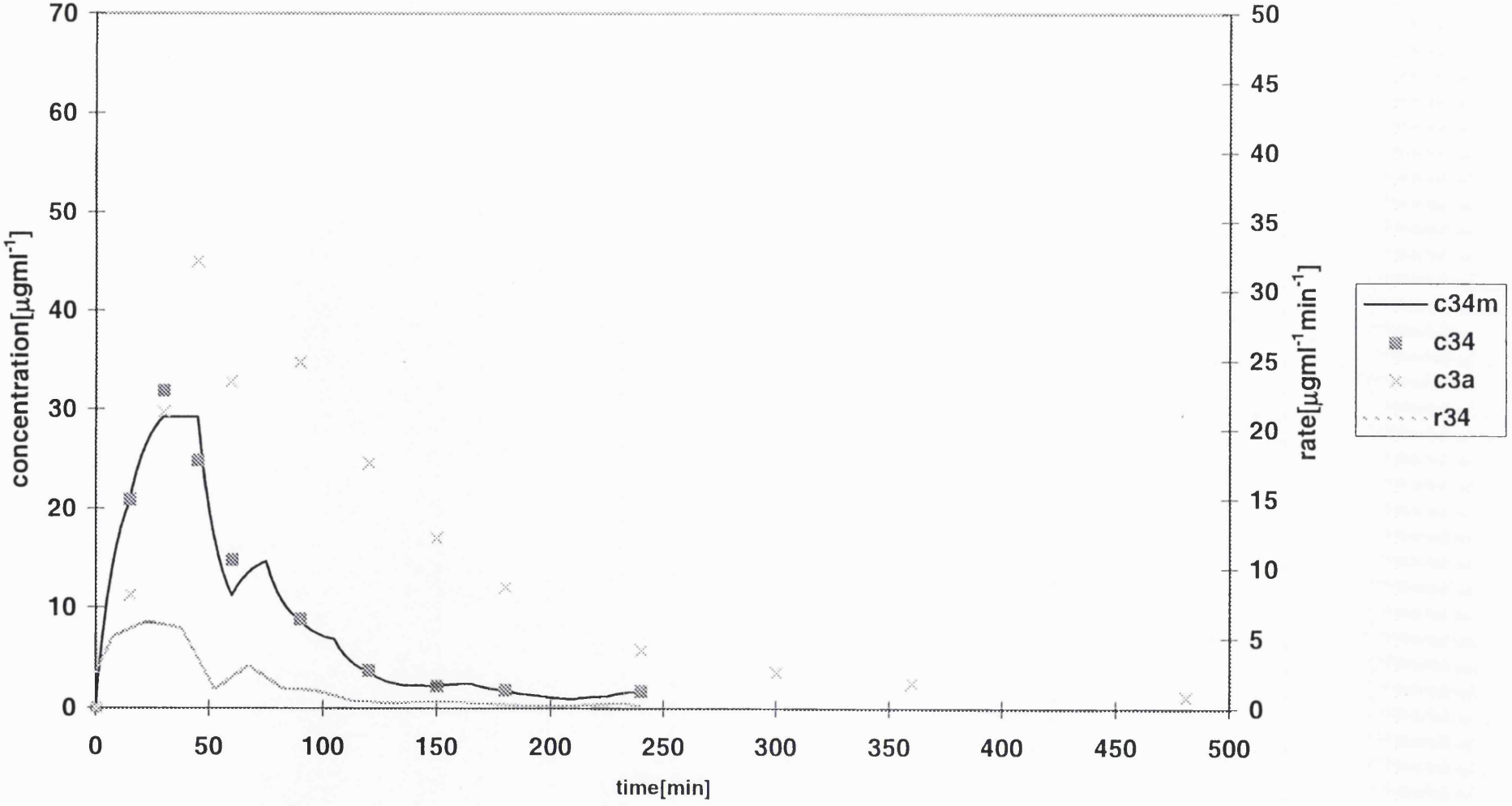


Figure 5.c: The 4ASA and AASA concentration and rate profiles for subject DS

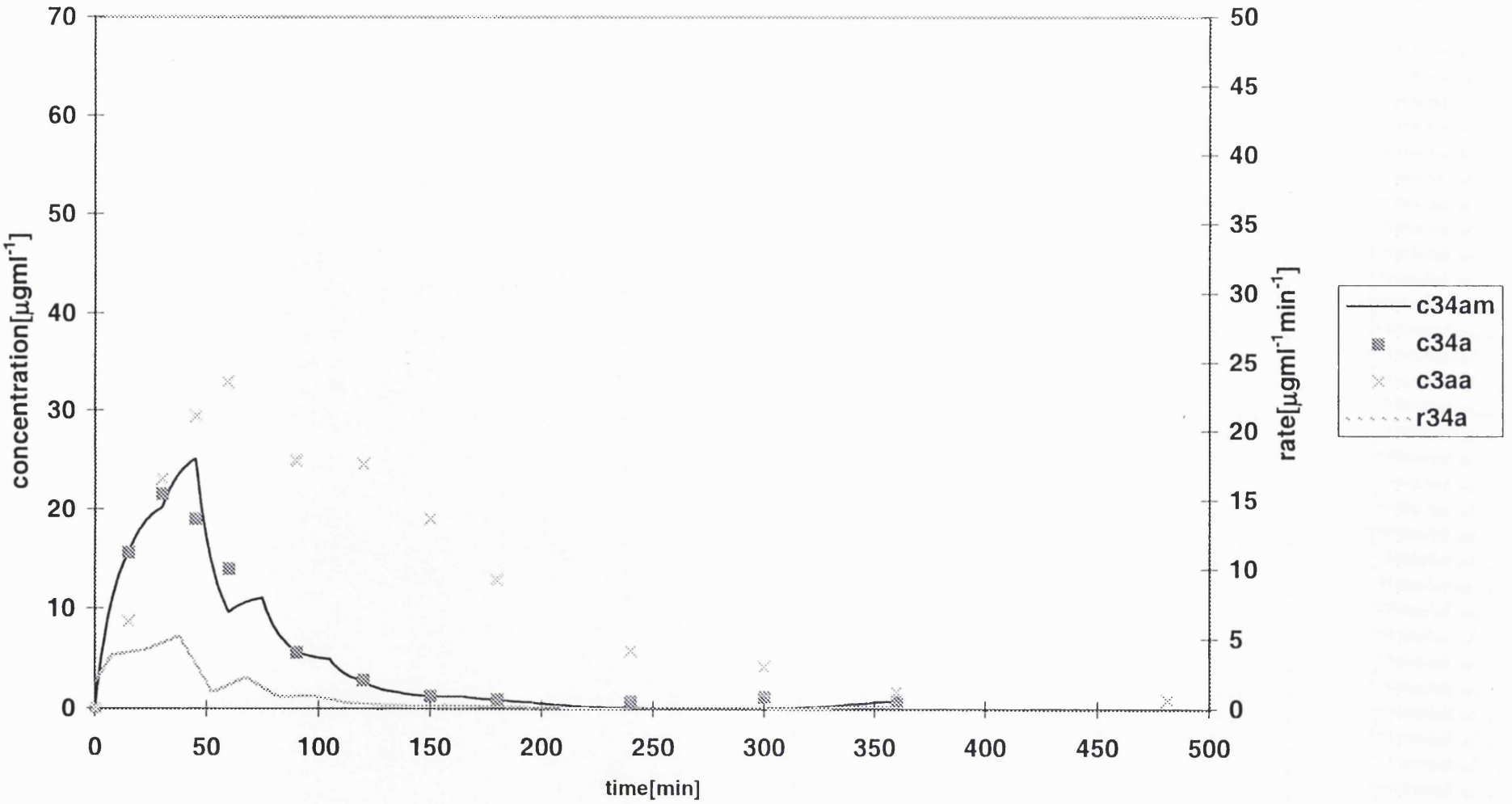


Figure 5.d: The 4ASA and AASA concentration and rate profiles for subject DS

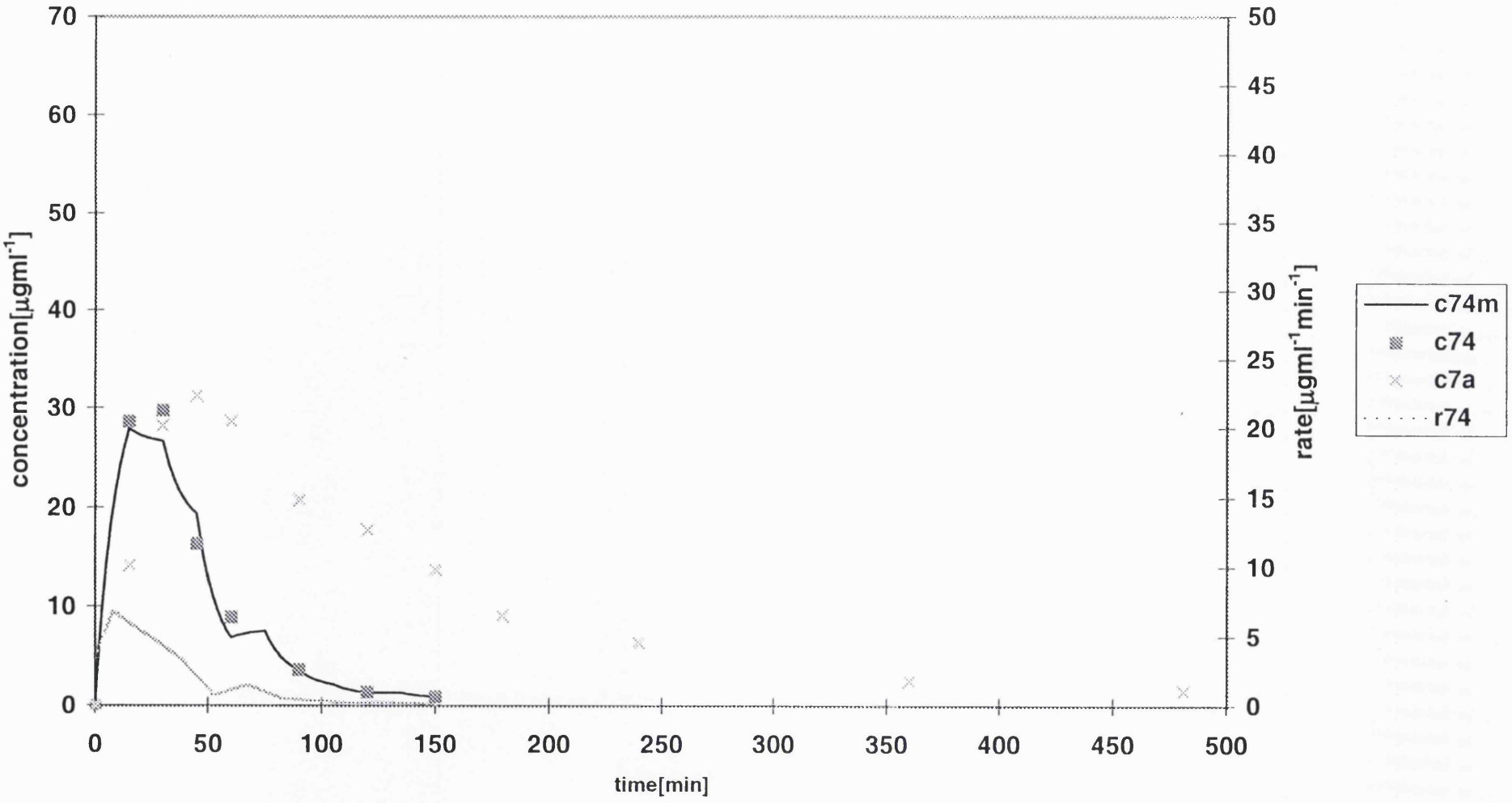


Figure 5.e: The 4ASA and AASA concentration and rate profiles for subject DS

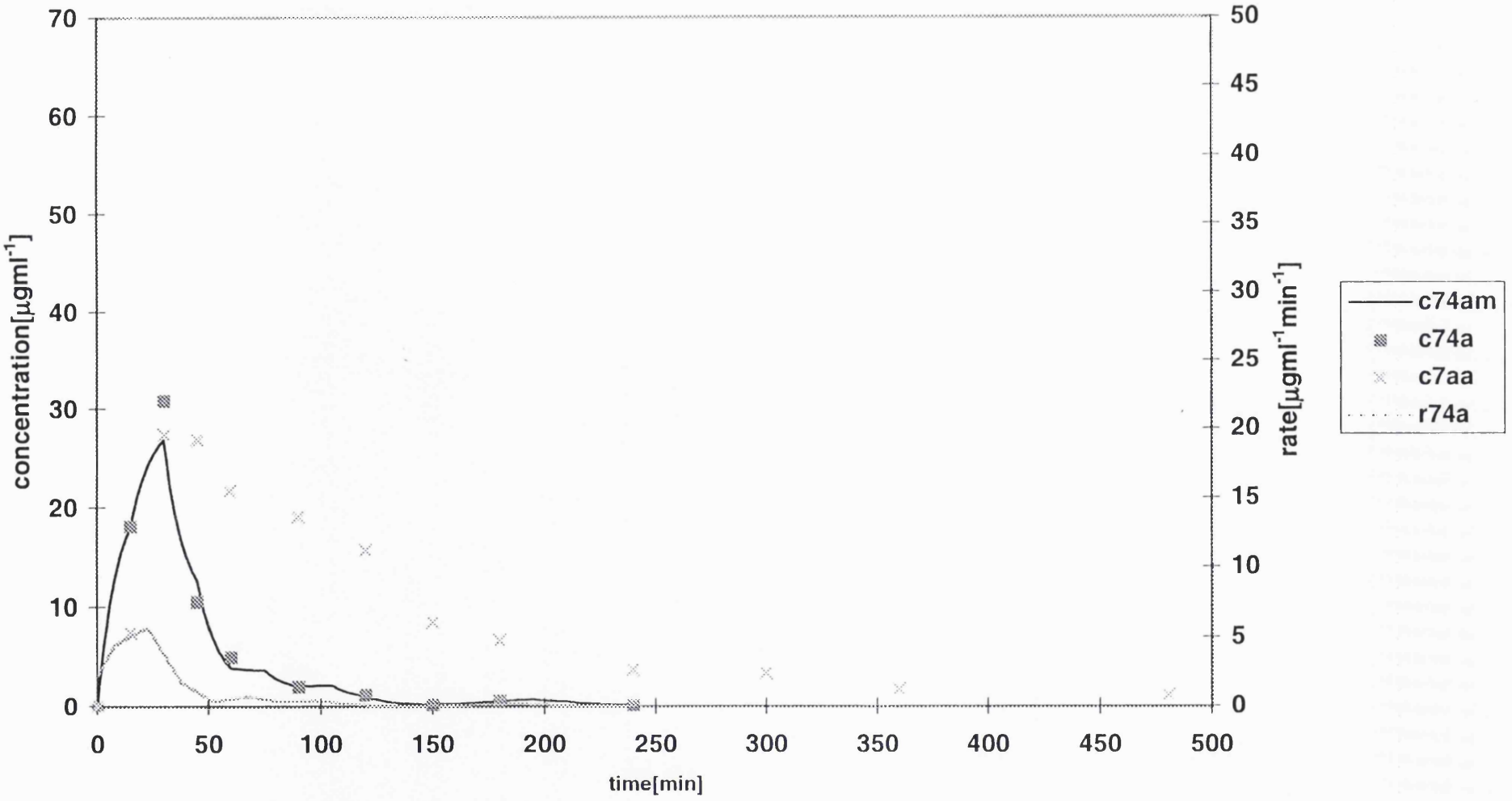


Figure 6.a: The 4ASA and AASA concentration profiles of intravenous preparation for subject OC

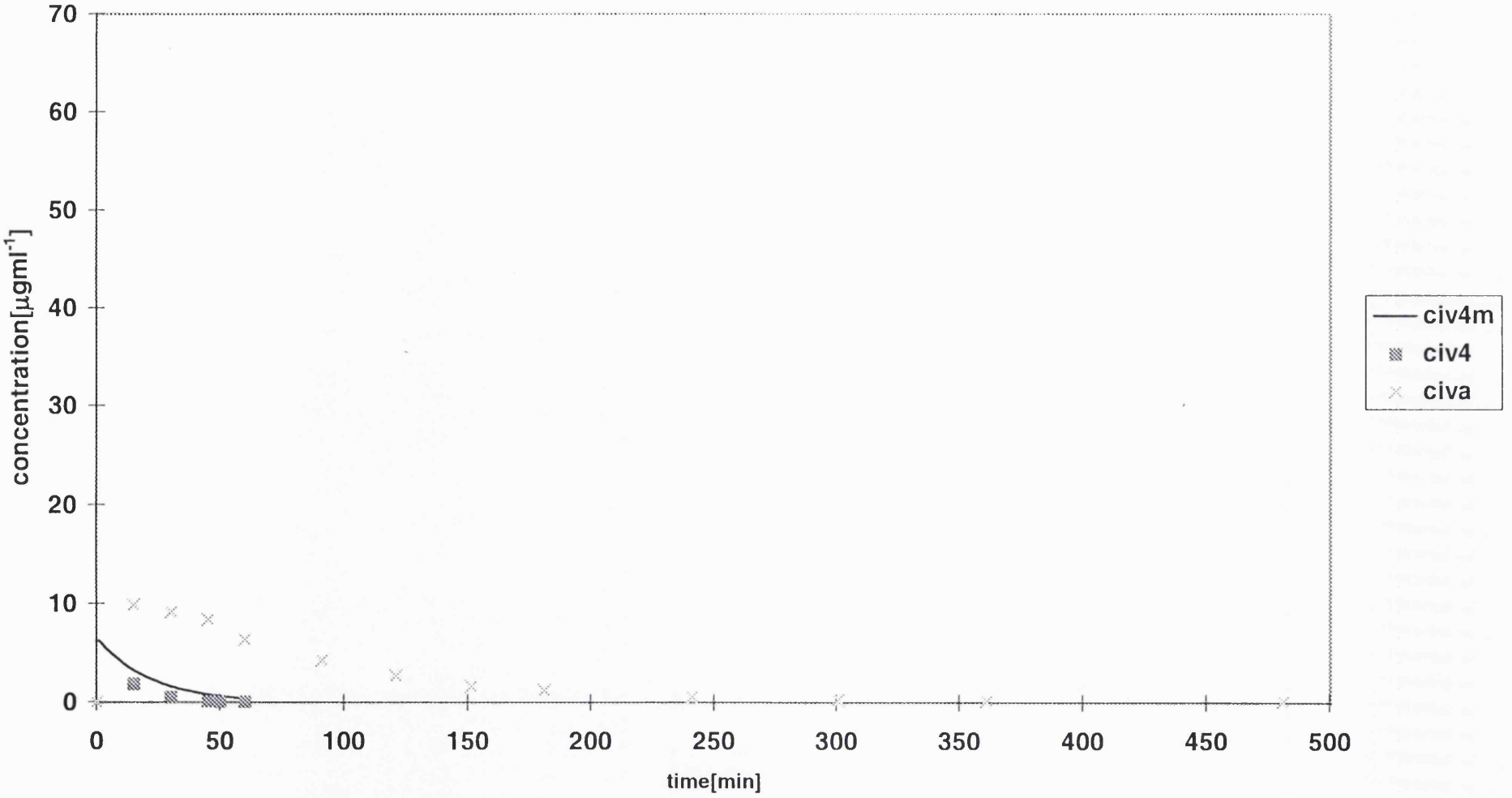


Figure 6.b: The 4ASA and AASA concentration and rate profiles for subject OC

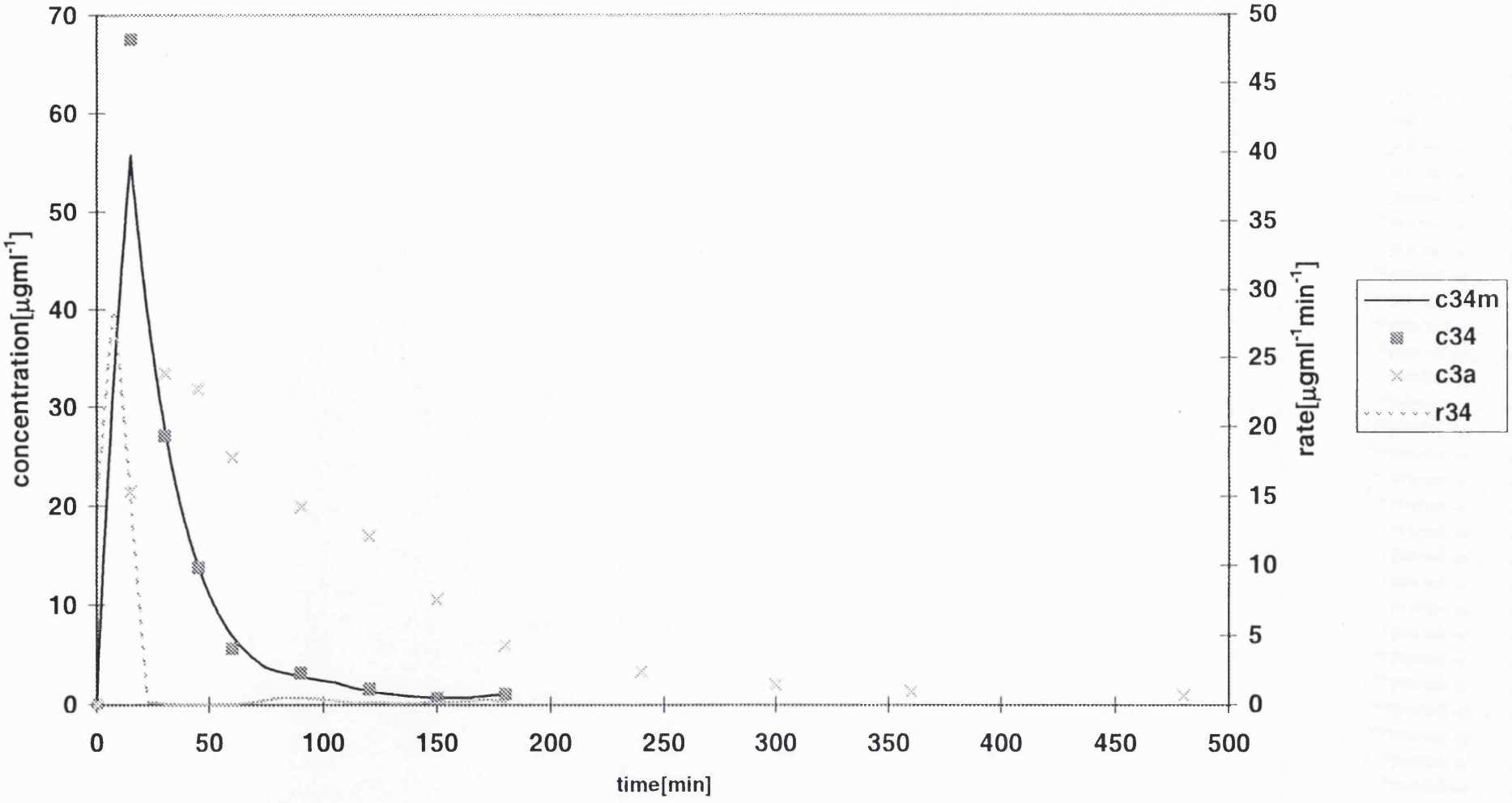


Figure 6.c: The 4ASA and AASA concentration and rate profiles for subject OC

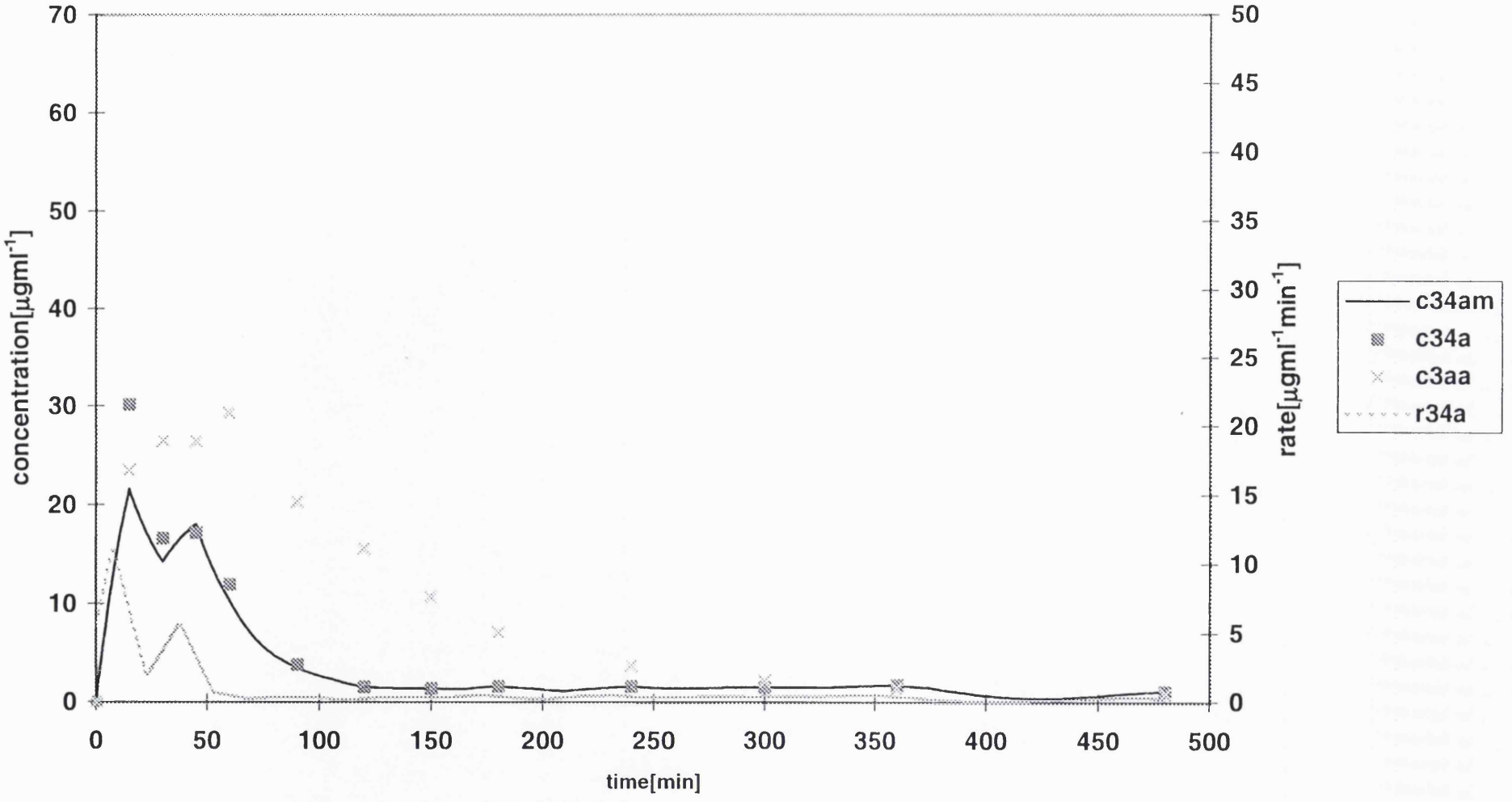


Figure 6.d: The 4ASA and AASA concentration and rate profiles for subject OC

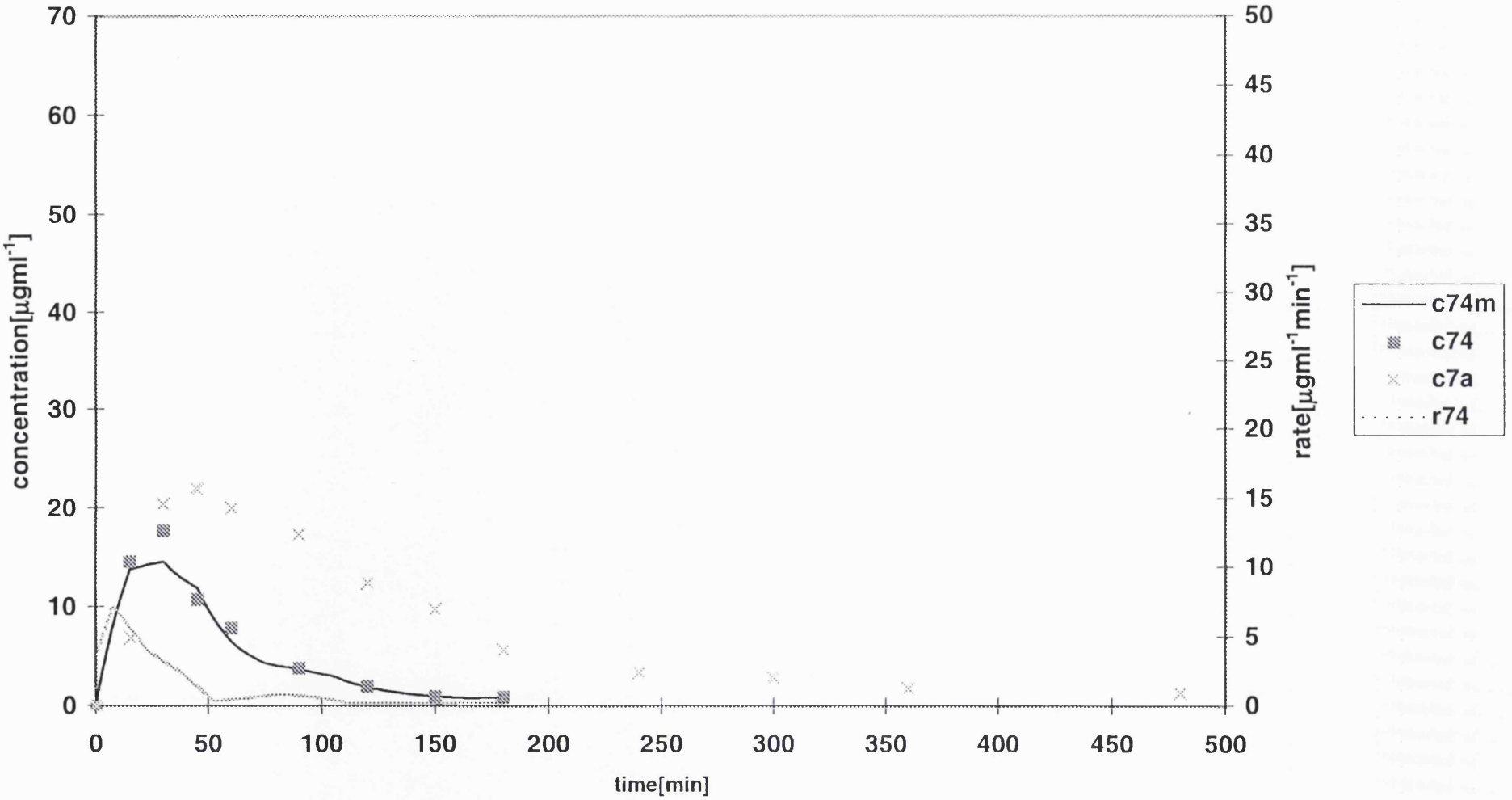


Figure 6.e: The 4ASA and AASA concentration and rate profiles for subject OC

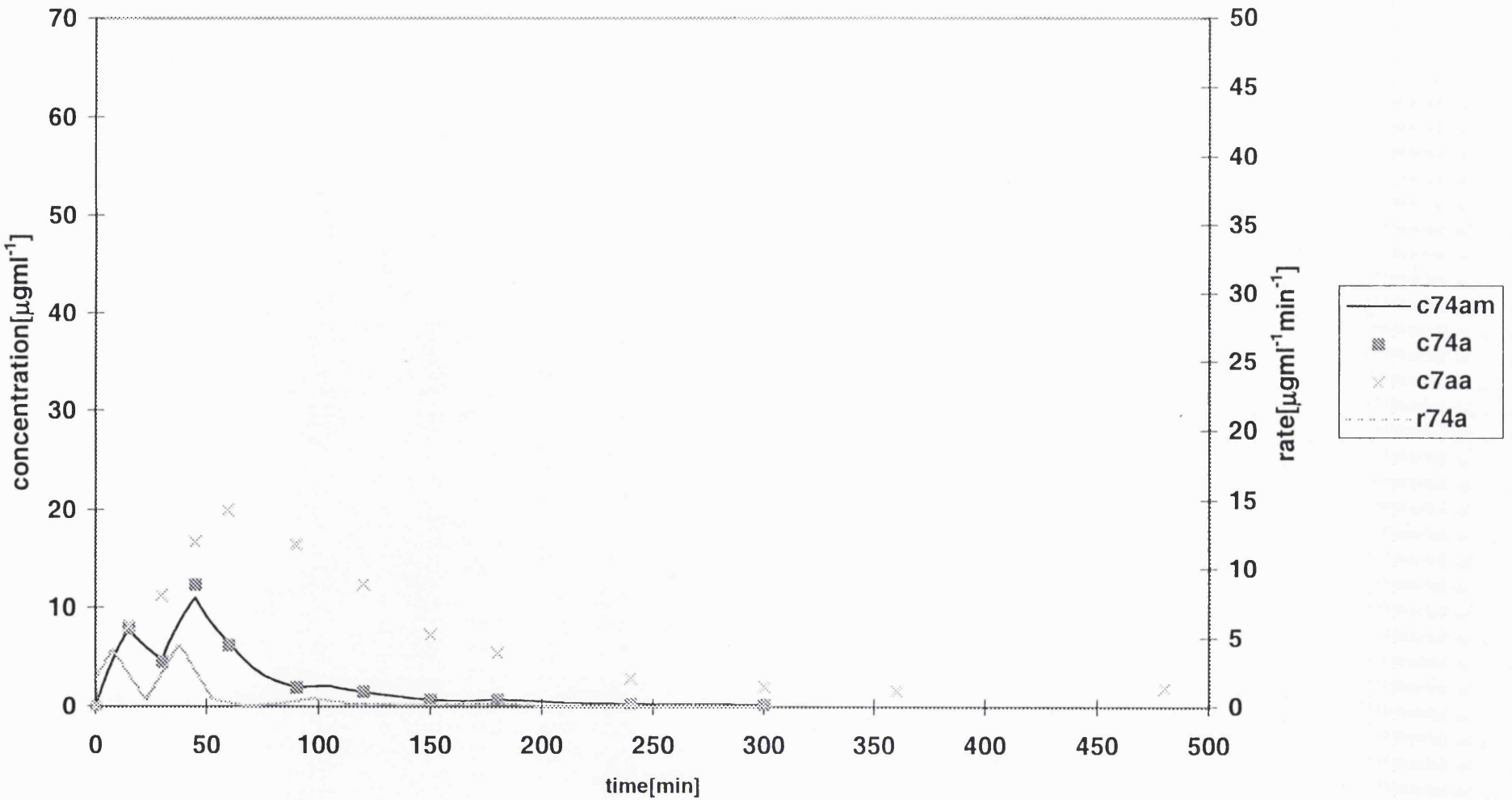


Figure 7.a: The 4ASA and AASA concentration profiles of intravenous preparation for subject TN

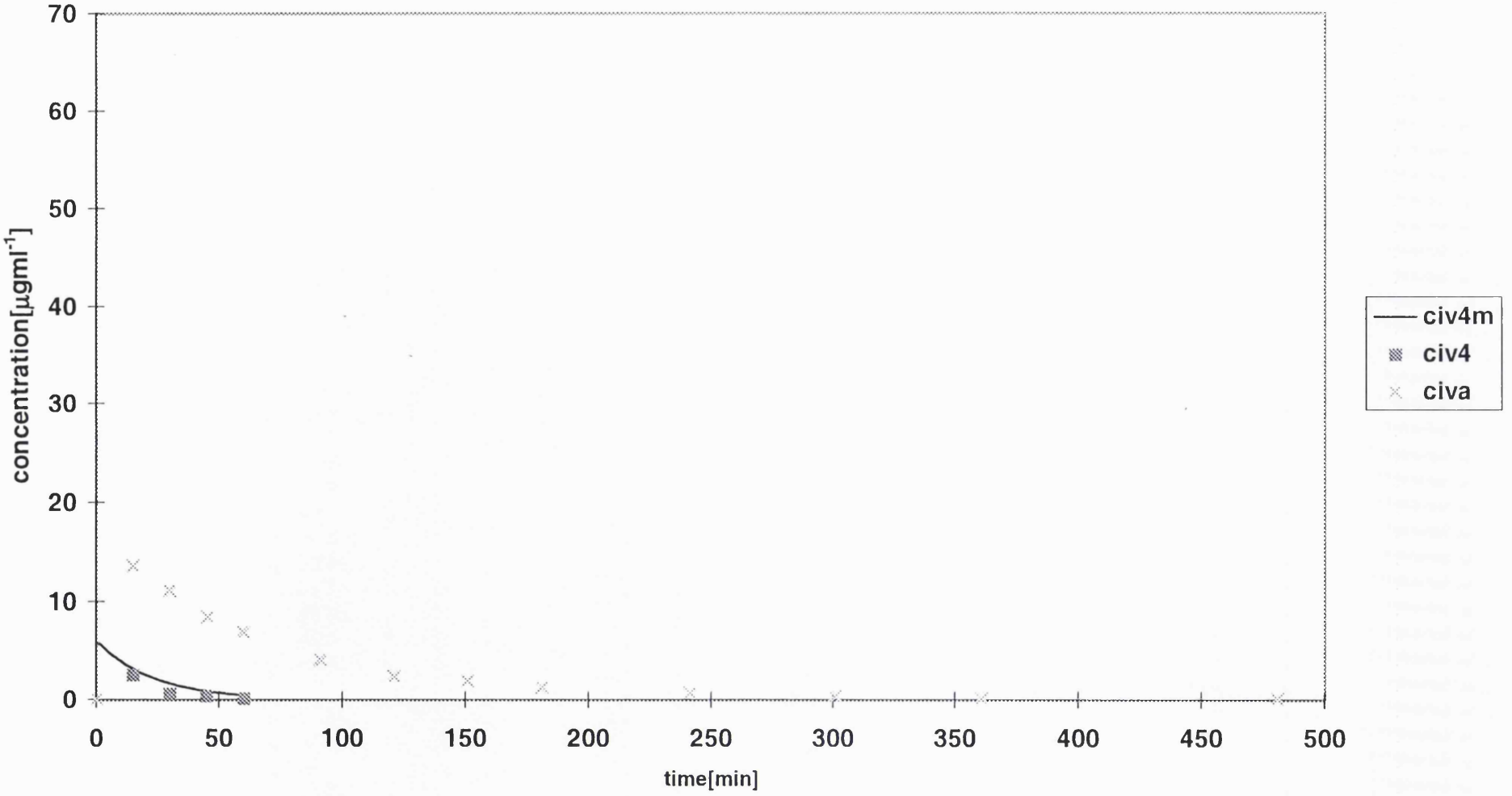


Figure 7.b: The 4ASA and AASA concentration and rate profiles for subject TN

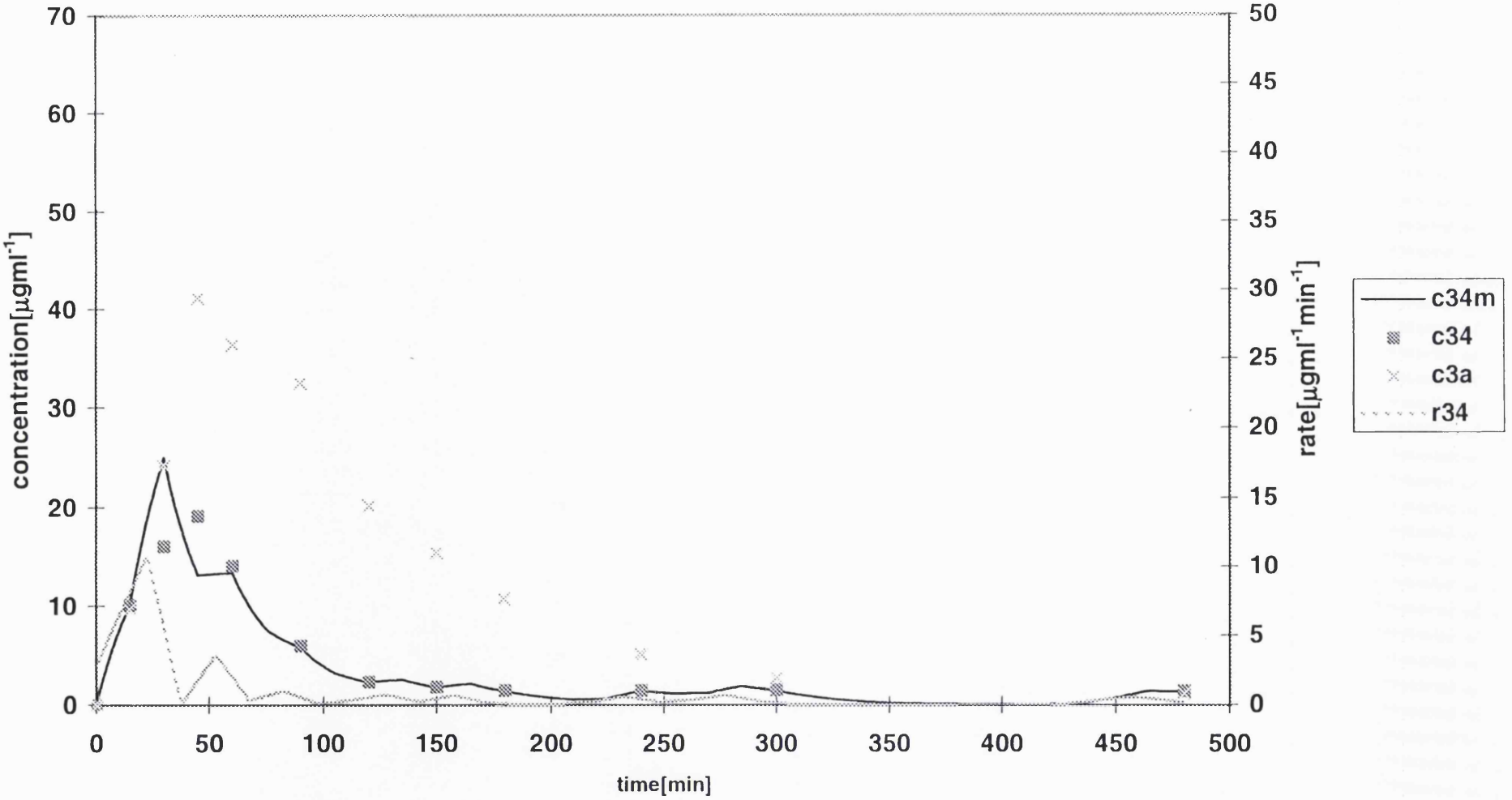


Figure 7.c: The 4ASA and AASA concentration and rate profiles for subject TN

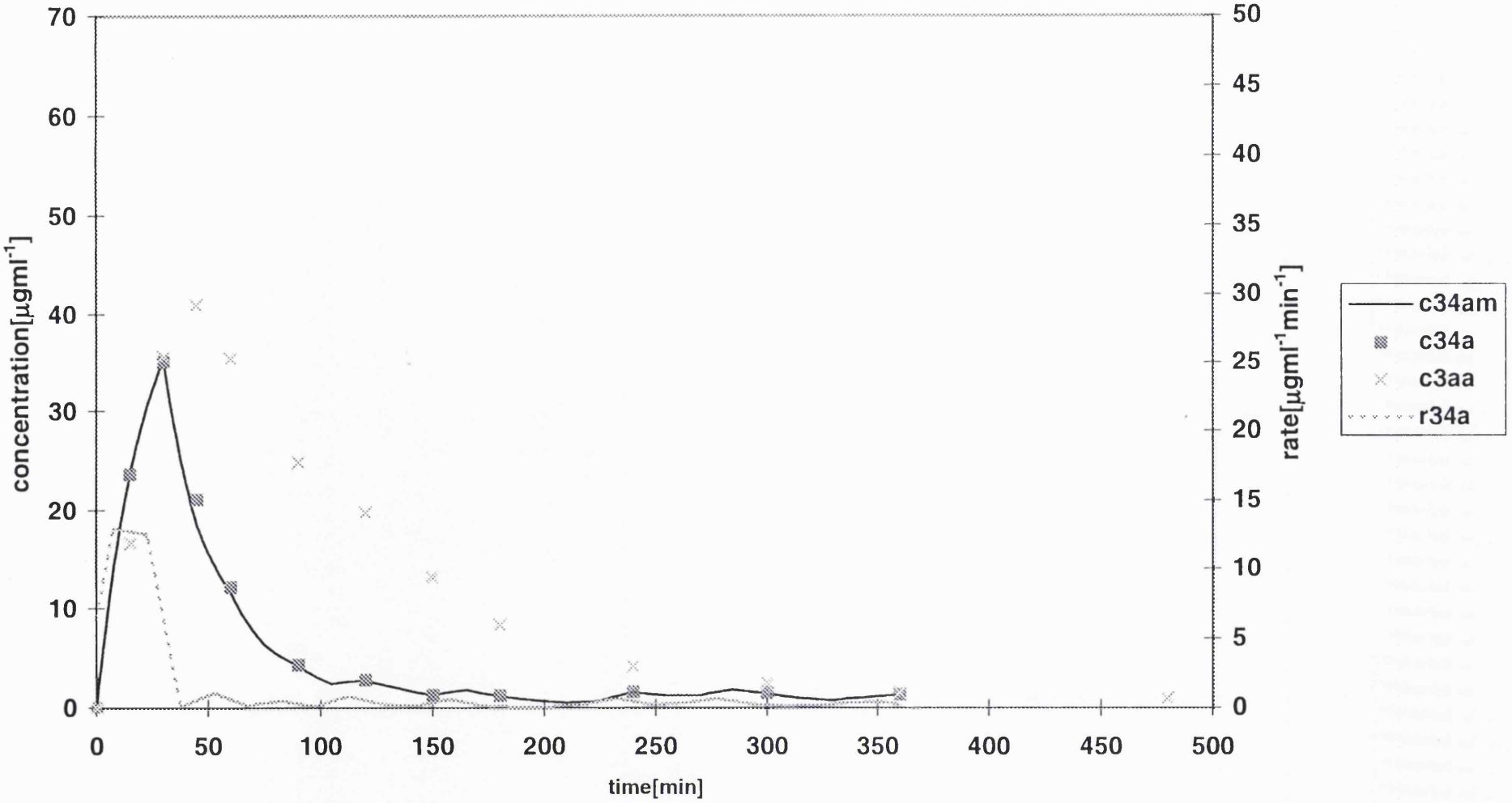


Figure 7.d: The 4ASA and AASA concentration and rate profiles for subject TN

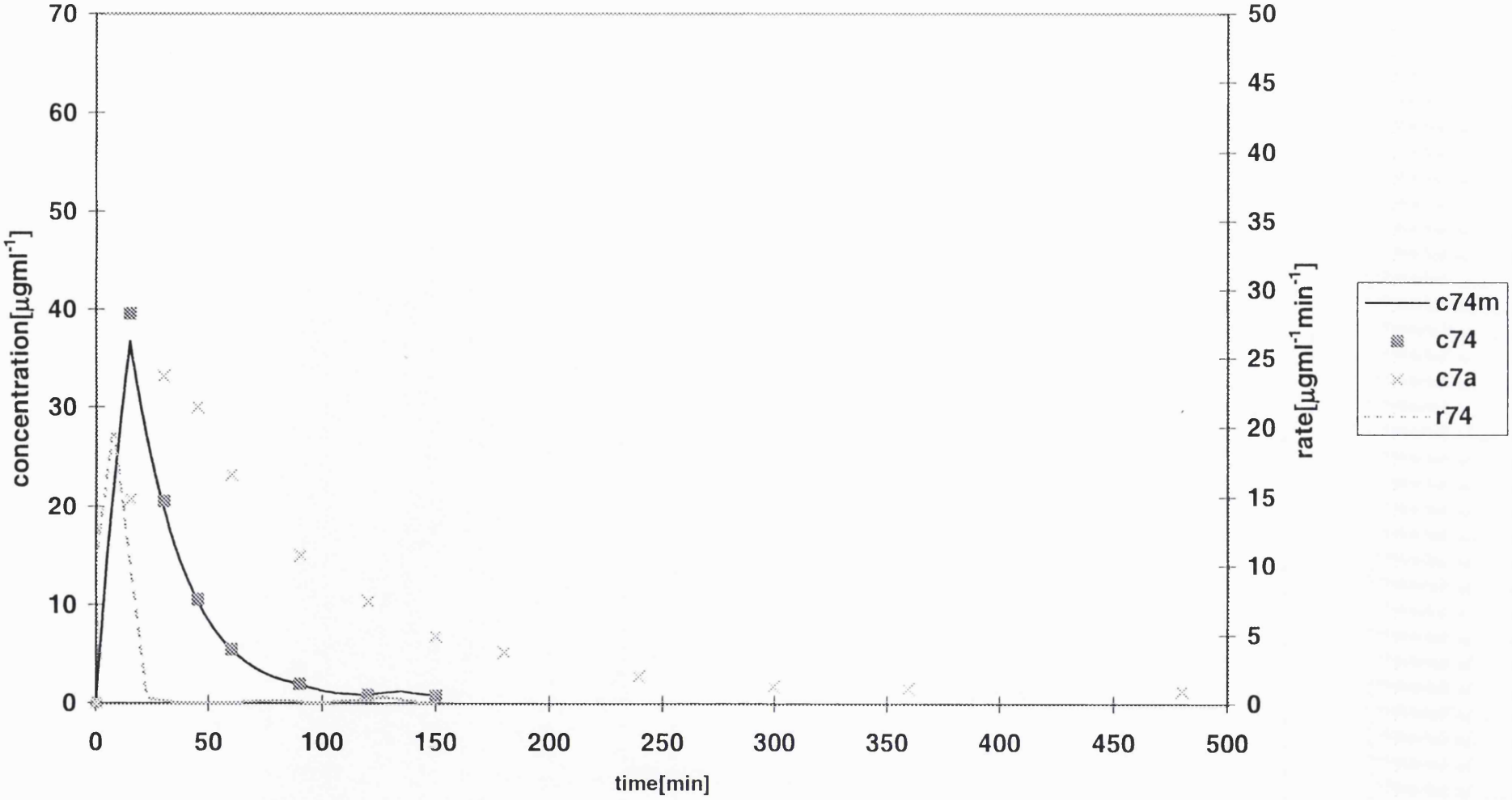


Figure 7.e: The 4ASA and AASA concentration and rate profiles for subject TN

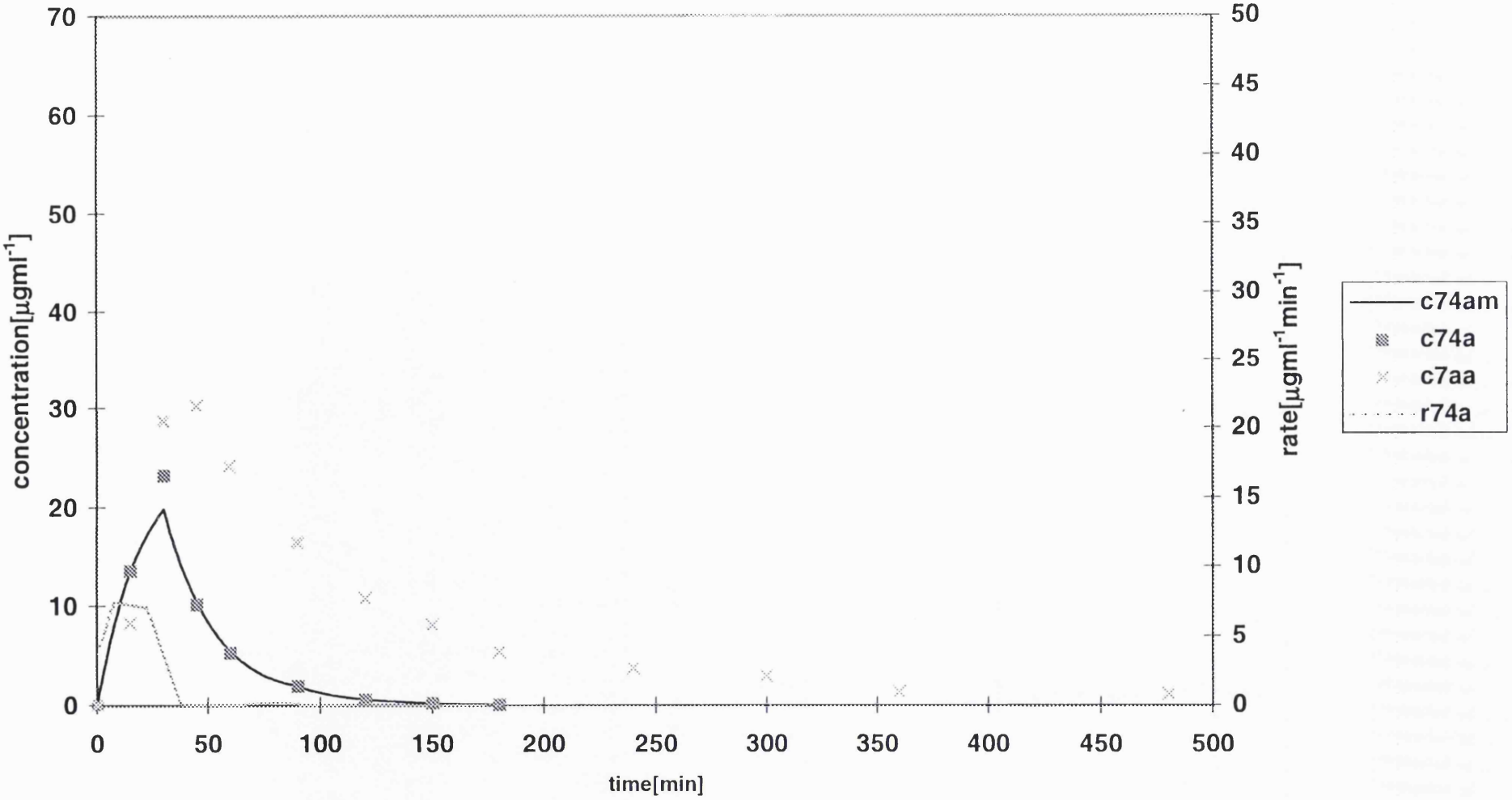


Figure 8.a: The AASA concentration profile of the intravenous preparation for subject VM

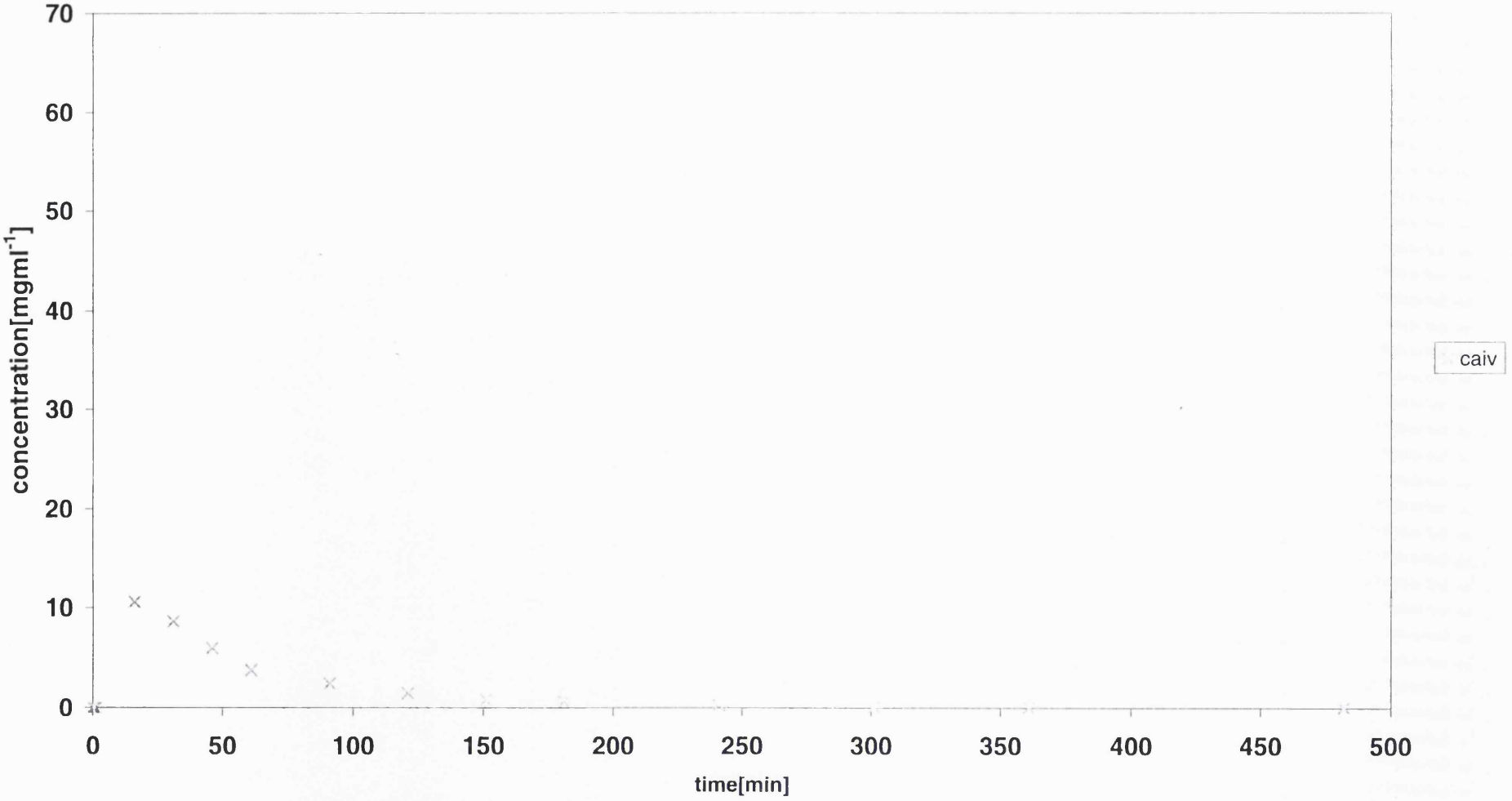


Figure 8.b: The 4ASA and AASA concentration and rate profiles for subject VM

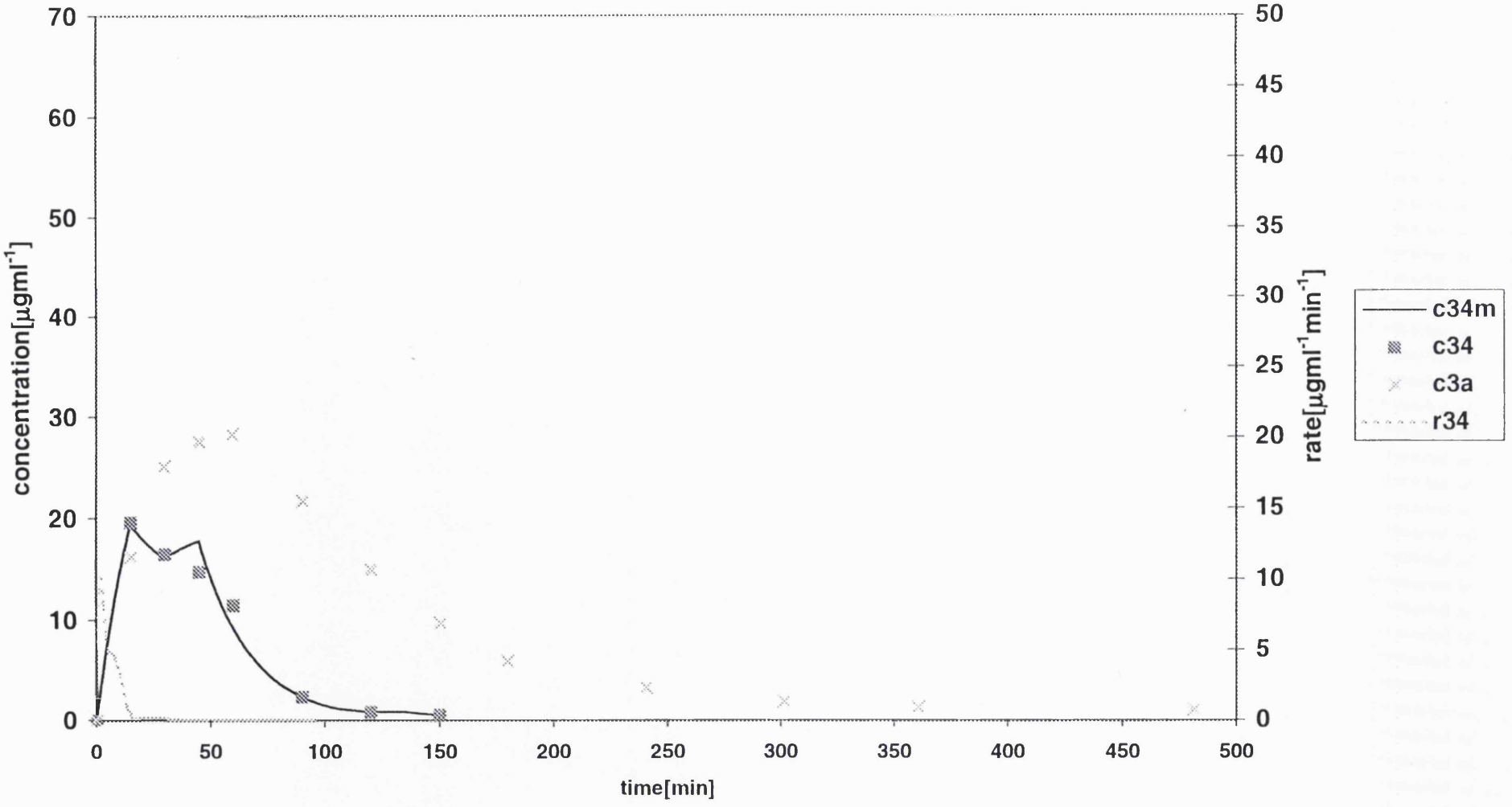


Figure 8.c: The 4ASA and AASA concentration and rate profiles for subject VM

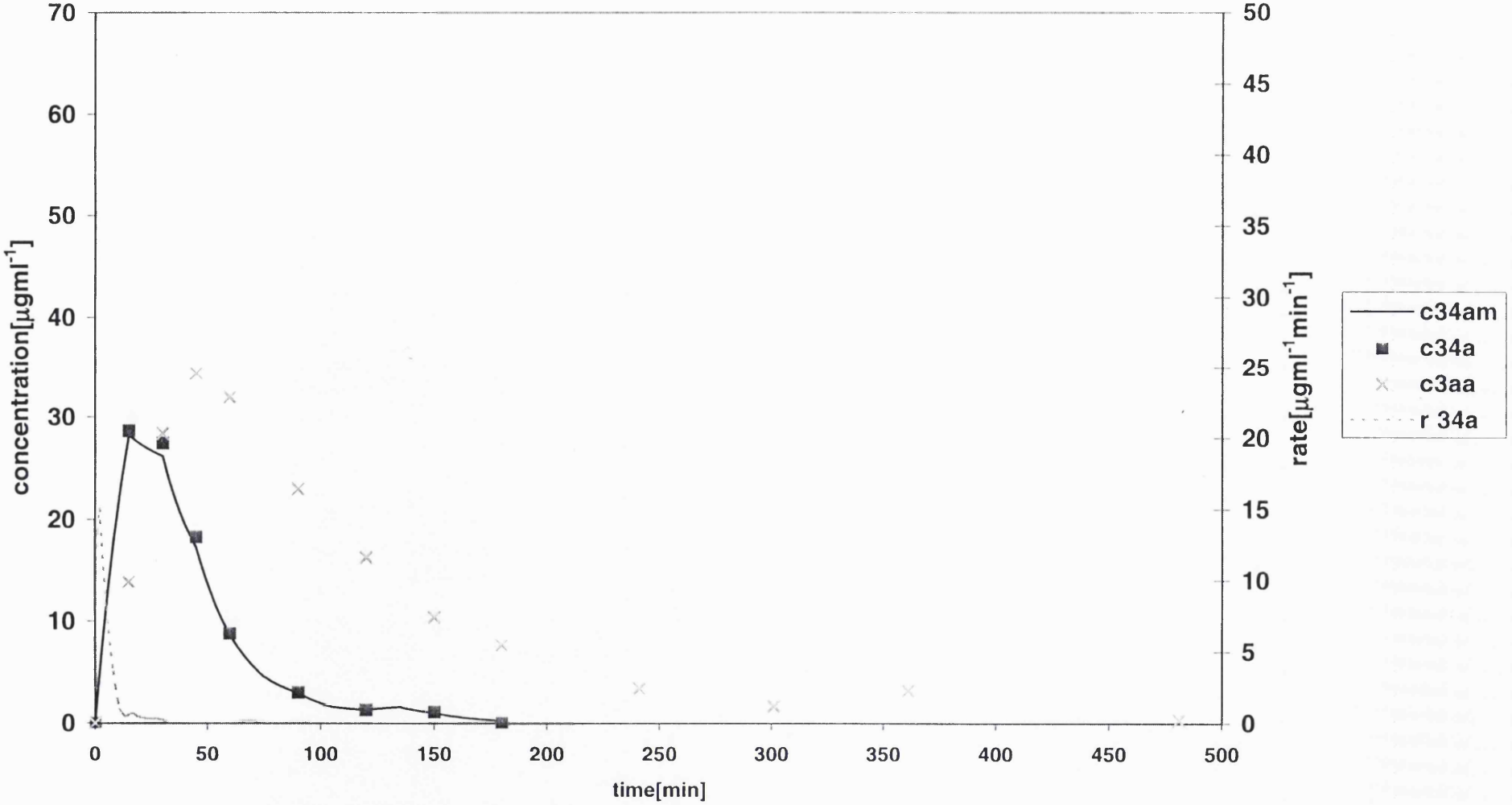


Figure 8.d: The 4ASA and AASA concentration and rate profiles for subject VM

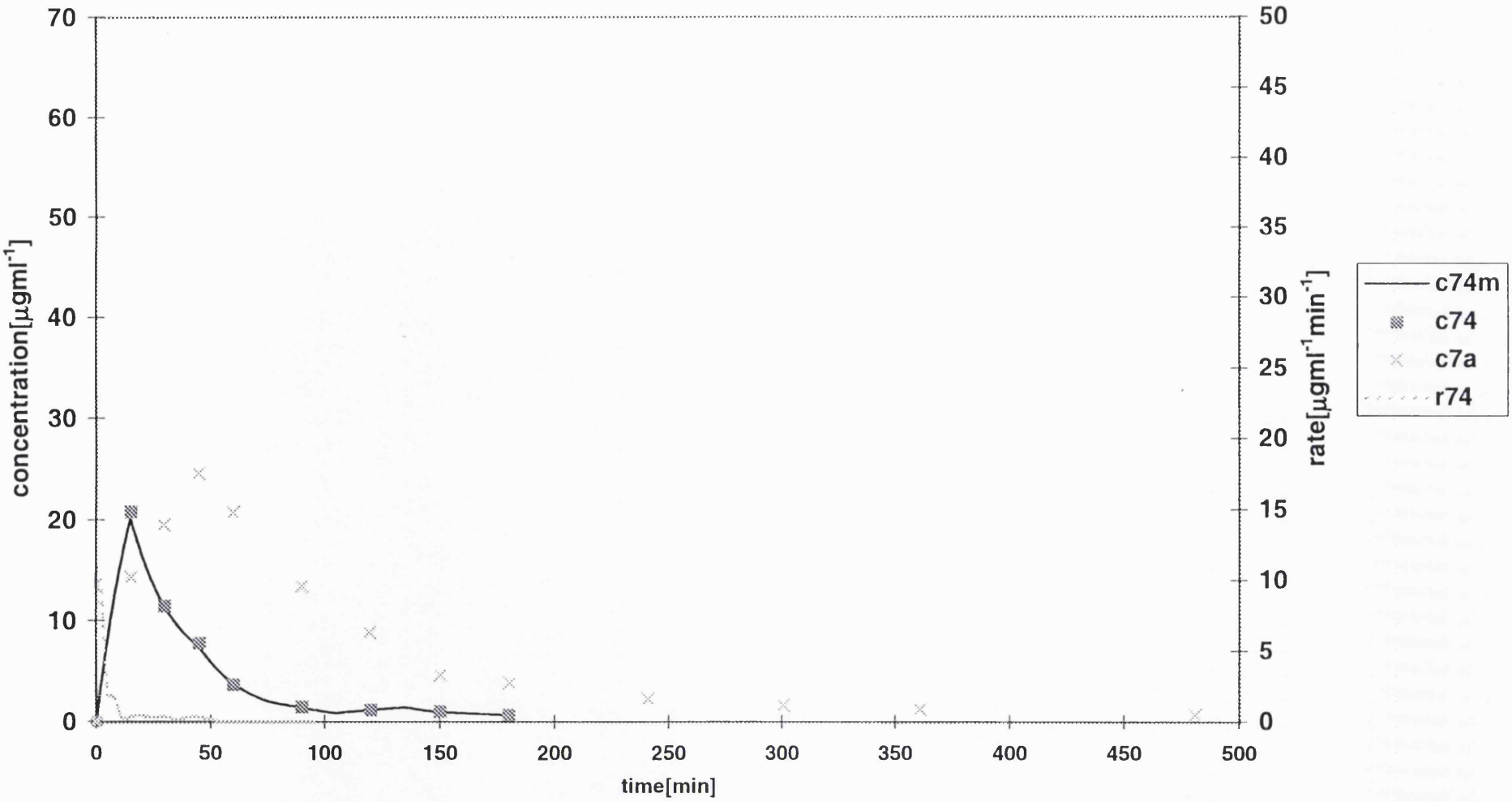


Figure 8.e: The 4ASA and AASA concentration and rate profiles for subject VM

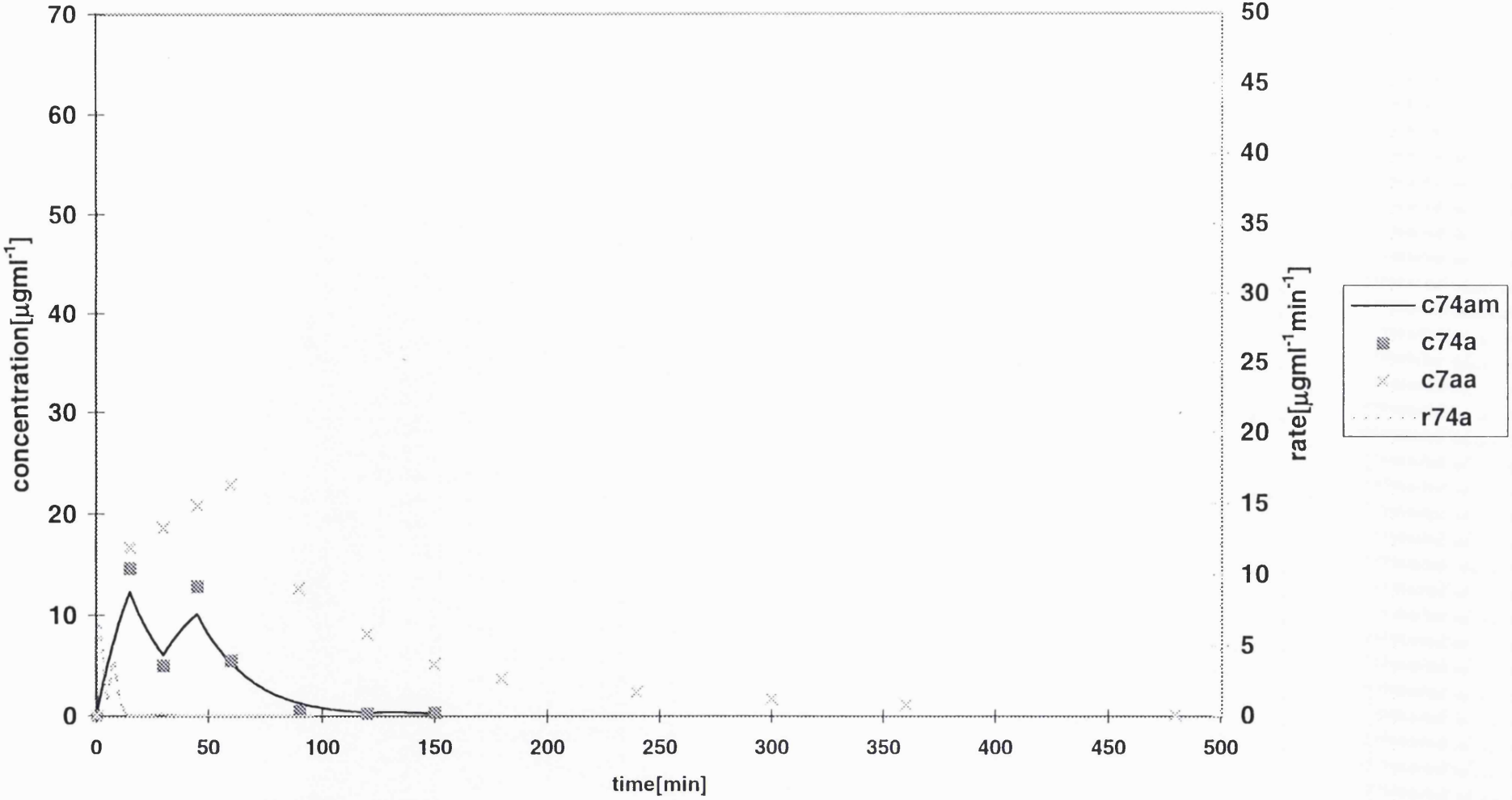


Figure 9.a: The 4ASA and AASA concentration profiles of intravenous preparation for subject WB

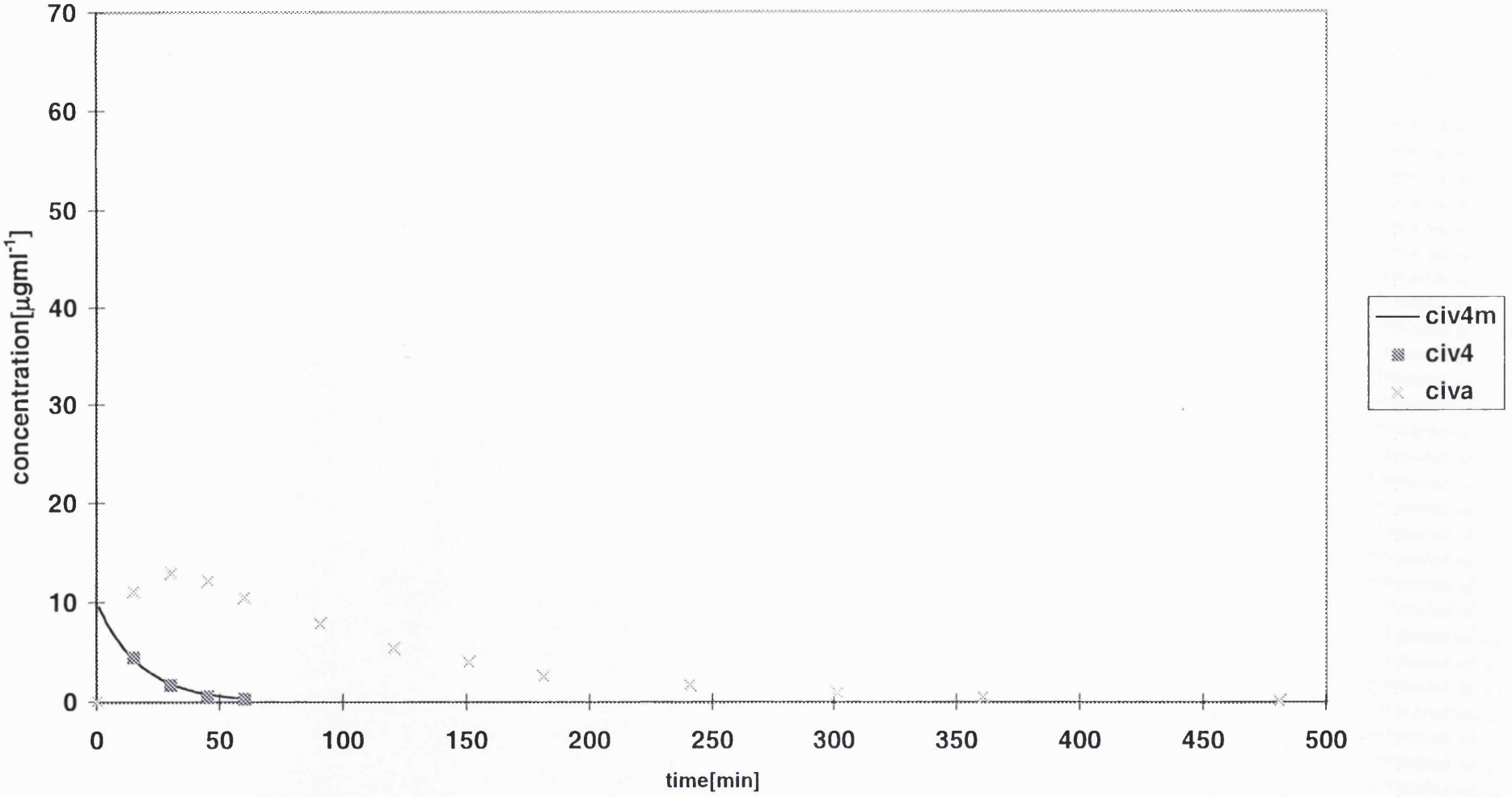


Figure 9.b: The 4ASA and AASA concentration and rate profiles for subject WB

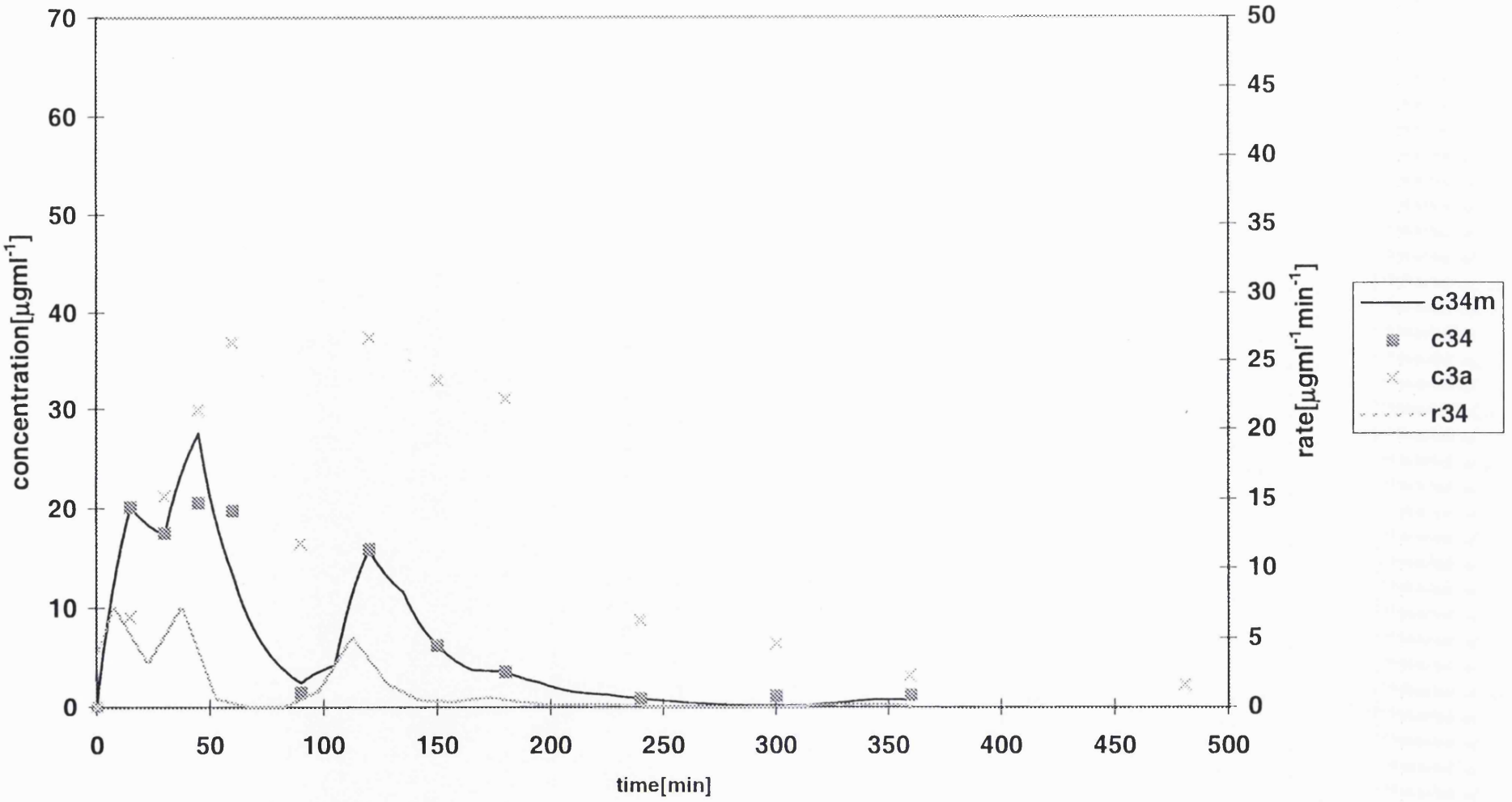


Figure 9.c: The 4ASA and AASA concentration and rate profiles for subject WB

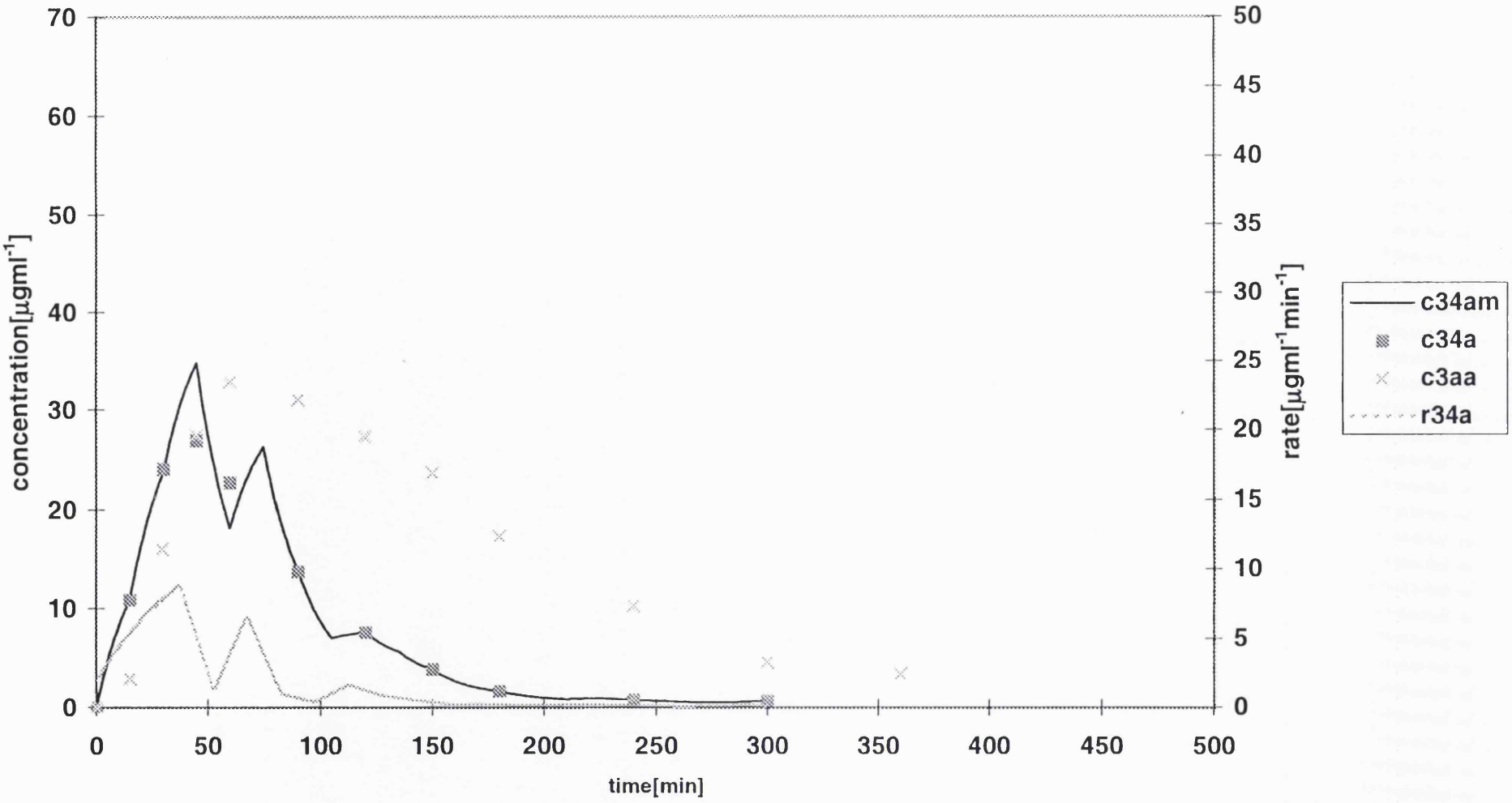


Figure 9.d: The 4ASA and AASA concentration and rate profiles for subject WB

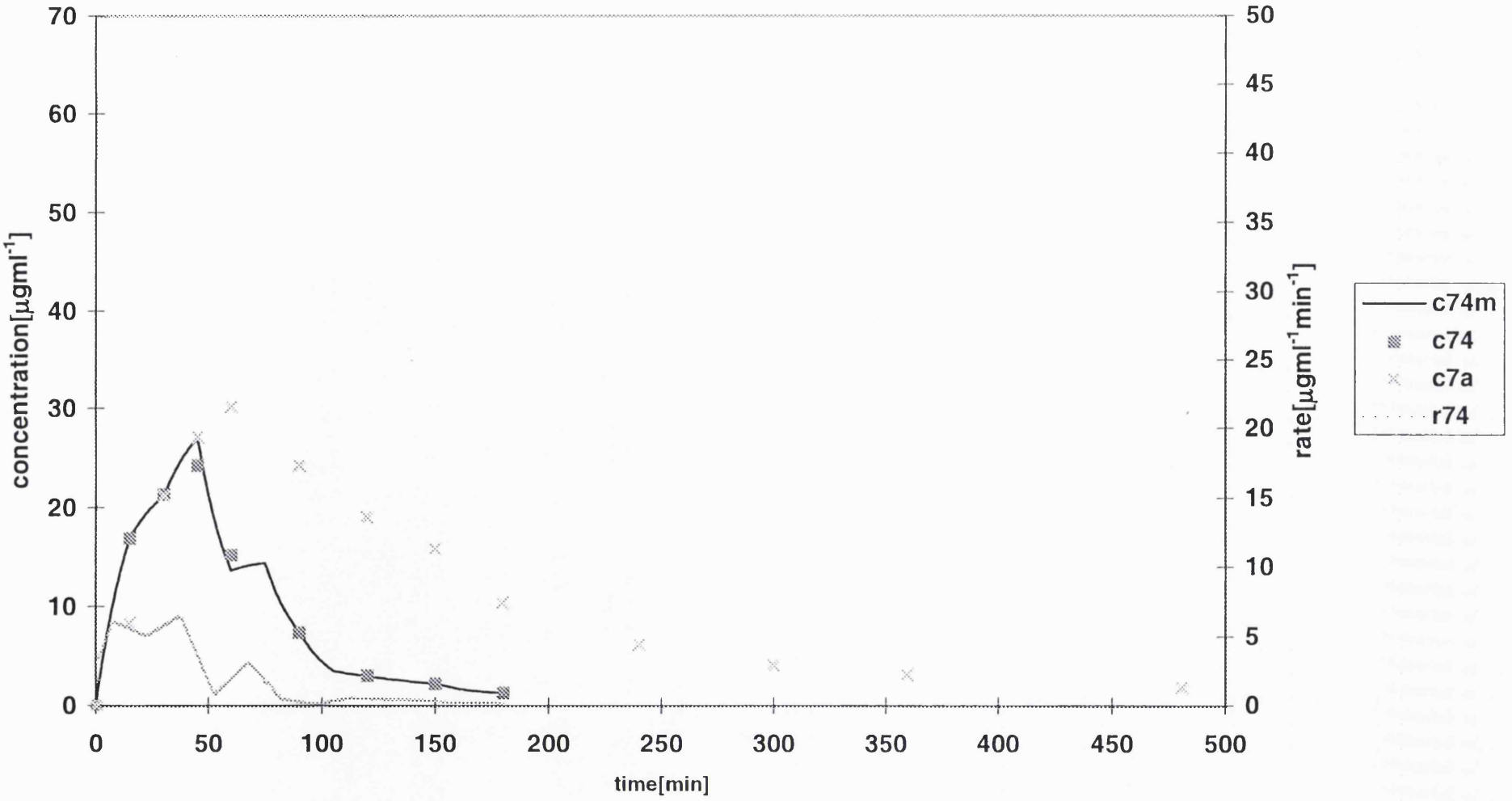


Figure 9.e: The 4ASA and AASA concentration and rate profiles for subject WB

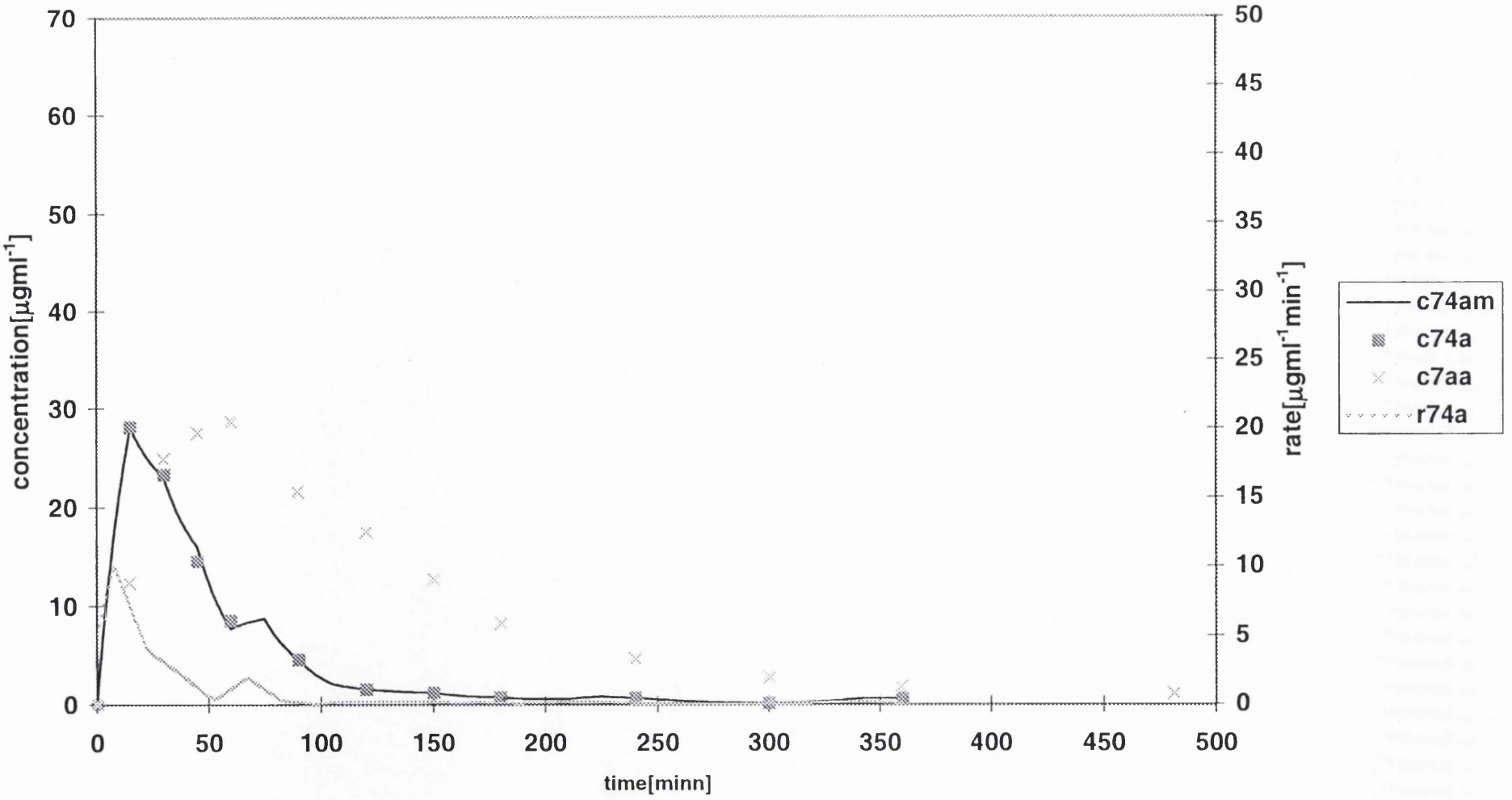


Figure 10.a: The 4ASA and AASA concentration profiles of intravenous preparation for subject YO

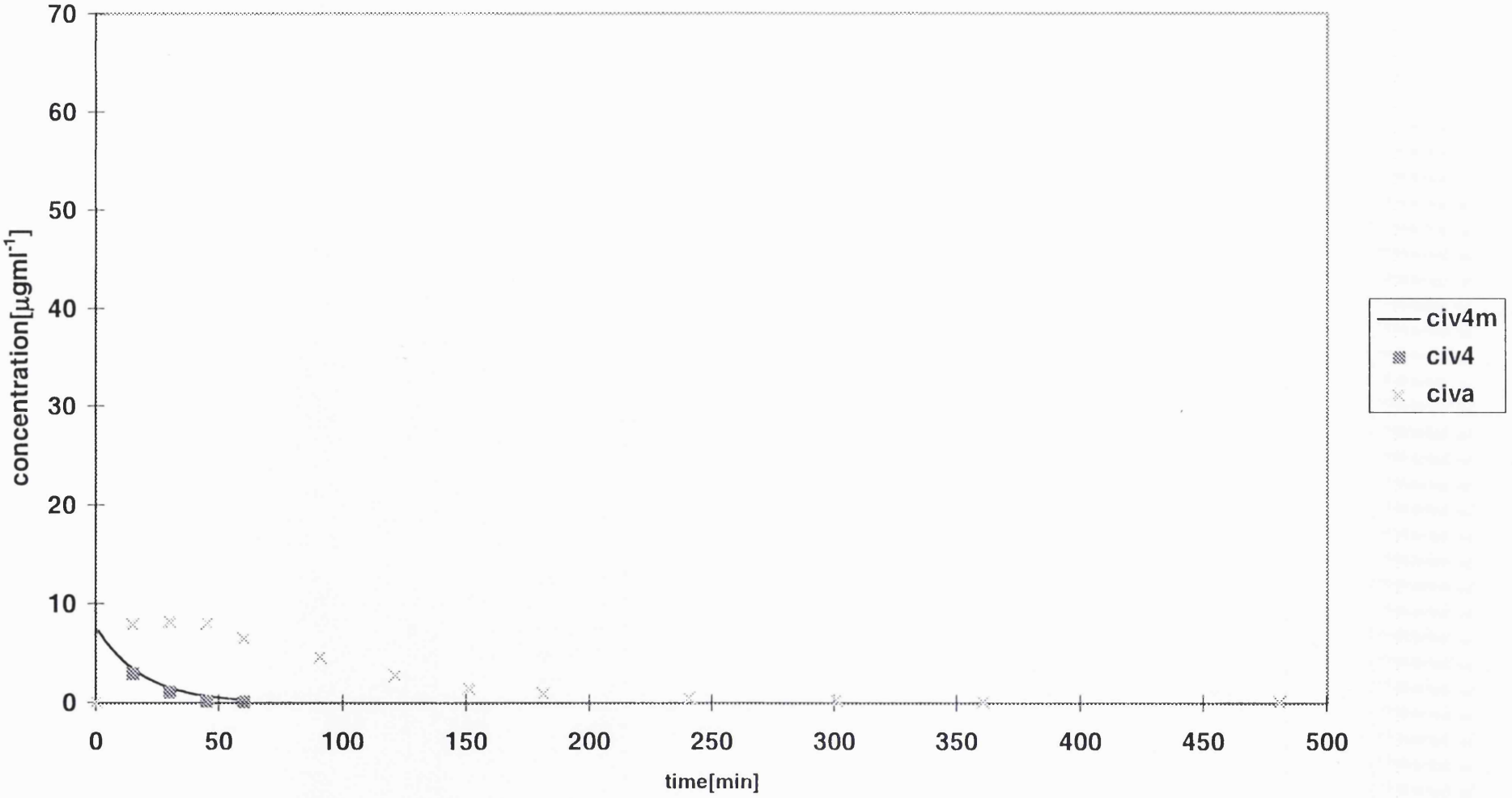


Figure 10.b: The 4ASA and AASA concentration and rate profiles for subject YO

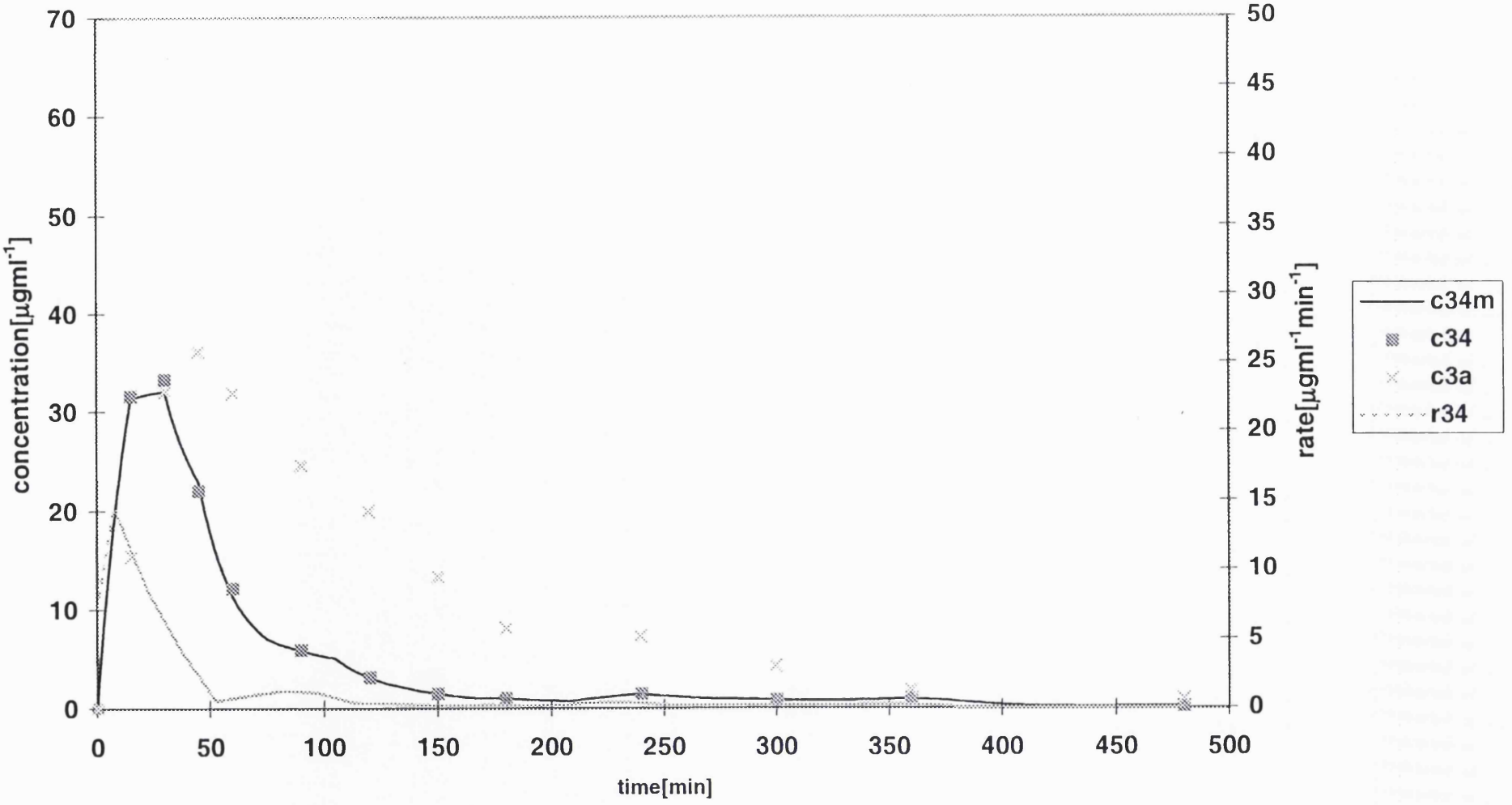


Figure 10.c: The 4ASA and AASA concentration and rate profiles for subject YO

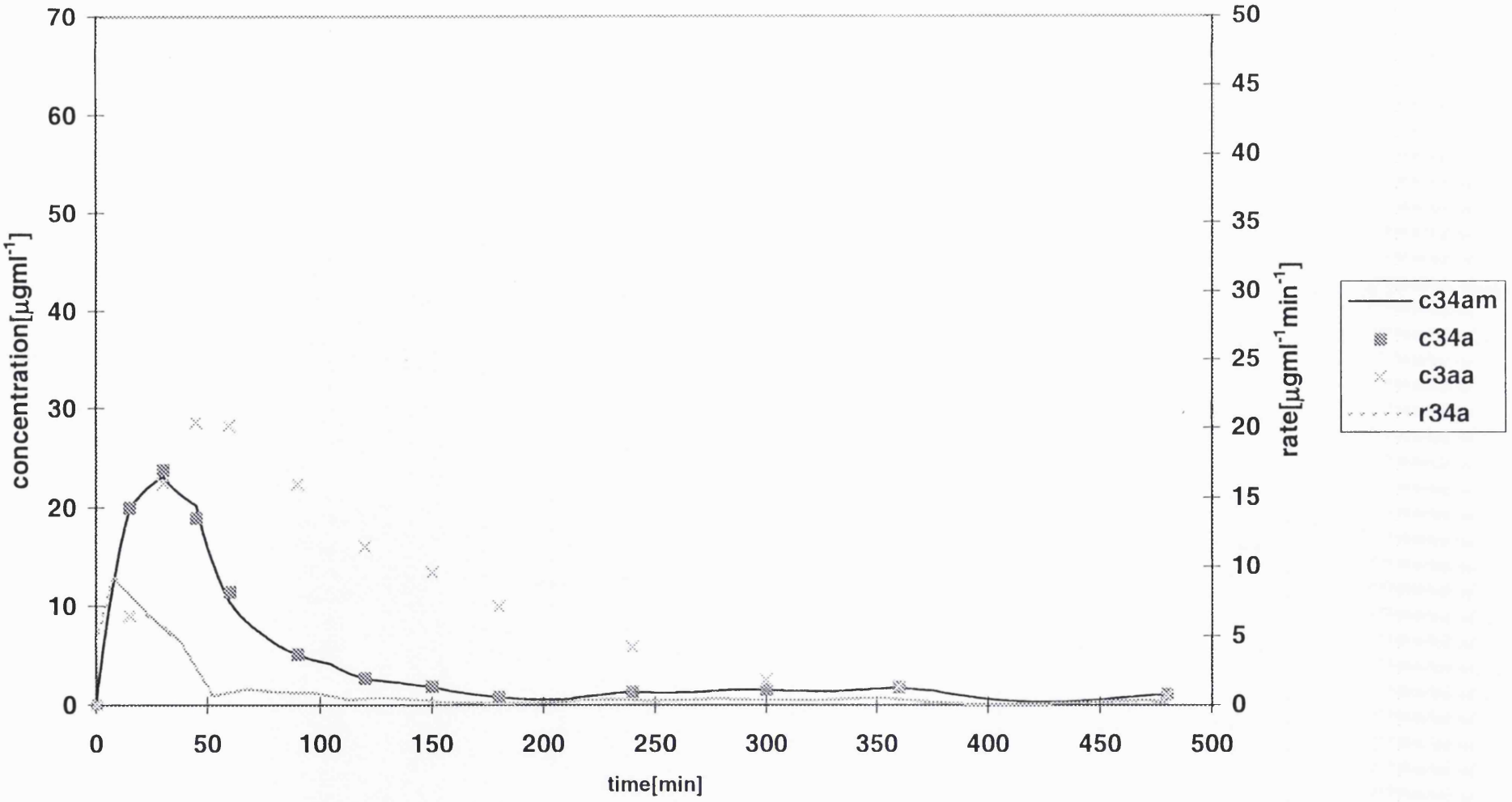


Figure 10.d: The 4ASA and AASA concentration and rate profiles for subject YO

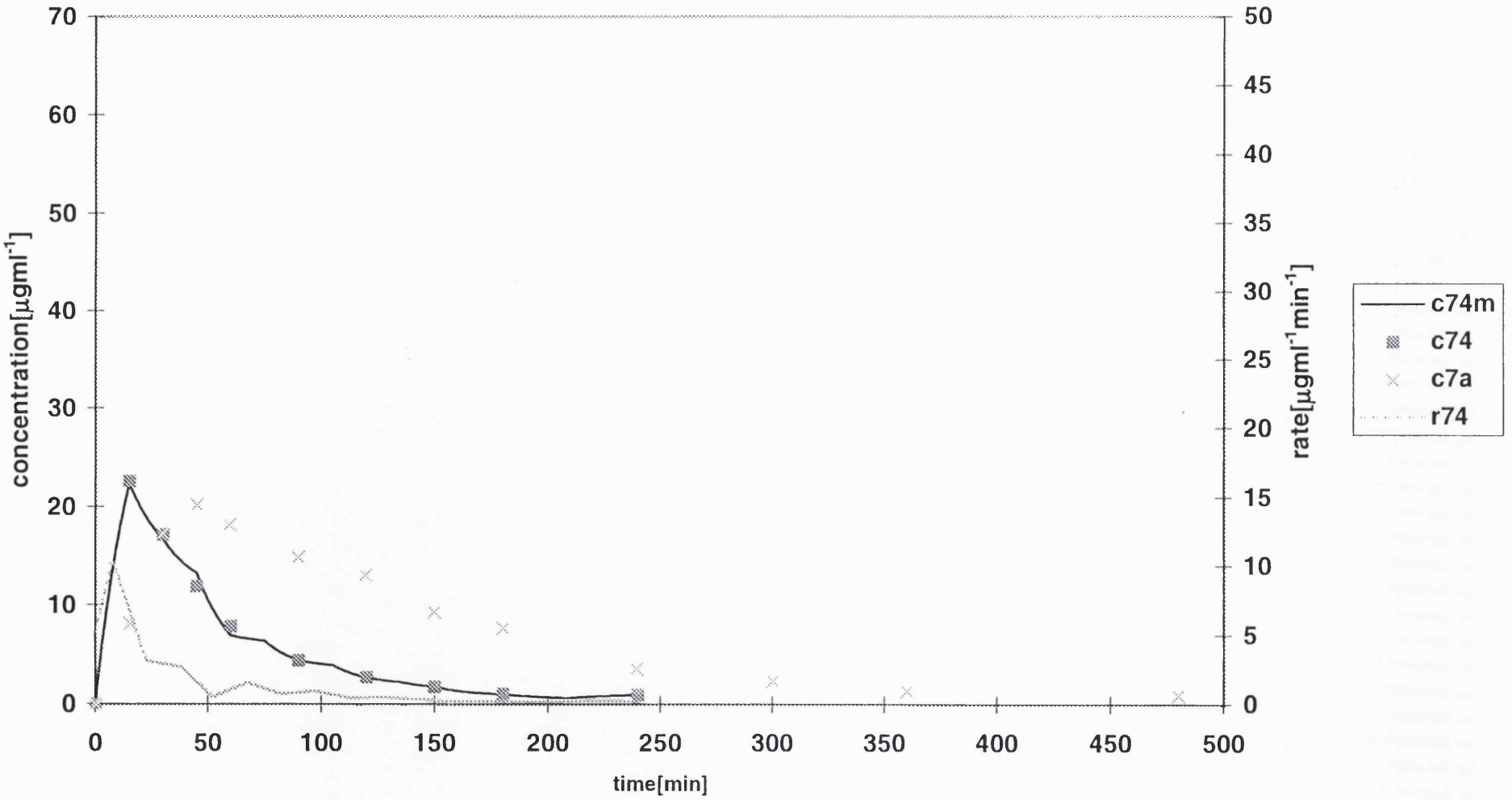
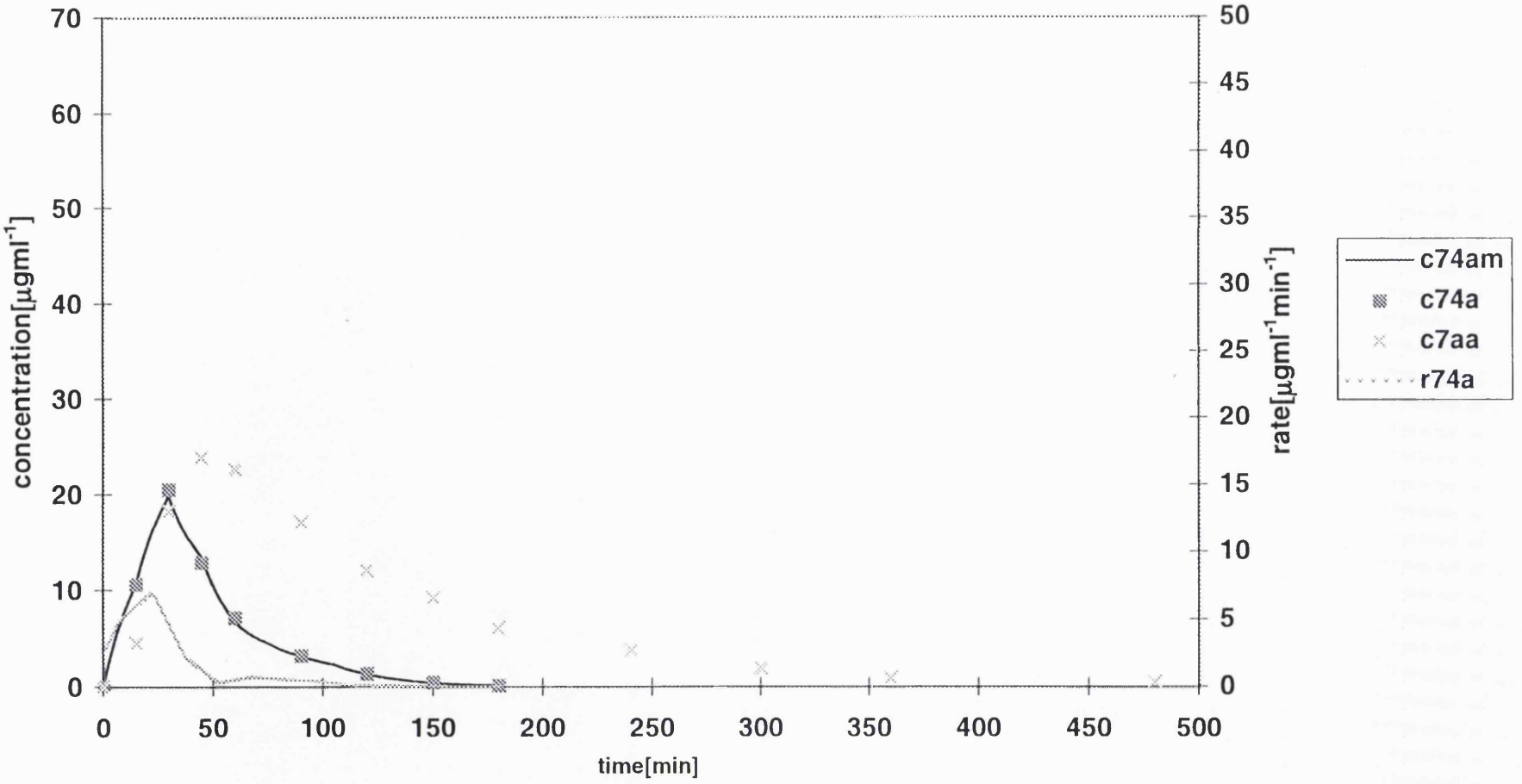


Figure 10.e: The 4ASA and AASA concentration and rate profiles for subject YO



APPENDIX 11

Determination of limits of agreements of the gastric profiles in different liquid formulations

Subject	pHtime (min)	GMRT (min)	GE_{50} (min)	$Phge_{50}$ Ave	pHgmrt ave	pHgmrt diff	$PHGE_{50}$ Diff
Liquid A							
AA	80.00	49.60	-	-	64.80	30.40	-
BB	-	29.20	29.00	-	-	-	-
CB	54.00	40.30	47.00	50.50	47.20	13.70	7.00
DB	46.00	39.60	53.00	49.50	42.80	6.40	-7.00
DS	43.00	49.60	34.00	38.50	46.30	-6.60	9.00
OC	77.00	-	-	-	-	-	-
TN	-	27.00	30.00	38.50	-	-	-
VM	-	-	-	-	-	-	-
WB	29.00	21.40	19.00	24.00	25.20	7.60	10.00
YO	22.00	31.00	-	-	26.50	-9.00	-
Mean	**50.14**	**35.96**	**35.33**	**-**	**-**	**7.08**	**4.95**
s.d.	**22.12**	**10.47**	**12.53**	**-**	**-**	**14.38**	**7.03**
Liquid AA							
AA	77.00	40.50	66.00	71.50	58.80	36.50	11.00
BB	-	23.10	47.00	-	-	-	-
CB	62.00	-	-	-	-	-	-
DB	46.00	55.10		23.00	50.60	-9.10	46.00
DS	26.00	25.10	36.00	31.00	25.55	.90	-10.00
OC	68.00	-	-	-	-	-	-
TN	49.00	25.80	32.00	40.50	37.40	23.20	17.00
VM	38.00	-	-	-	-	-	-
WB	22.00	-	-	-	-	-	-
YO	38.00	34.70	45.00	41.50	36.35	3.30	-7.00
Mean	**47.33**	**34.05**	**45.20**	**-**	**-**	**10.96**	**11.40**
s.d.	**18.7**	**12.27**	**13.18**	**-**	**-**	**18.47**	**22.50**
Liquid B							
AA	-	17.60	29.00	-	-	-	-
BB	-	21.80	33.00	16.50	10.90	-21.80	-33.00
CB	25.00	20.20	24.00	24.50	22.60	4.80	1.00
DB	12.00	18.90	20.00	16.00	15.45	-6.90	-8.00
DS	17.00	13.10	20.00	18.50	15.05	3.90	-3.00
OC	32.00	-	13.00	22.50	-	-	19.00
TN	16.00	-	29.00	22.50	-	-	-13.00
VM	-	24.70	23.00	-	-	-	-
WB	22.00	5.90	11.00	16.50	13.95	16.10	11.00
YO	8.00	10.30	10.00	9.00	9.15	-2.30	-2.00
Mean	**18.86**	**16.56**	**21.20**	**-**	**-**	**-1.03**	**-3.50**
s.d.	**8.13**	**6.31**	**7.97**	**-**	**-**	**12.80**	**15.68**

Appendix 11: Determination of the limits of agreement with different test liquids. pHgmrt ave (average value of pHtime and GMRT values); $pHGE_{50}$ ave(average value of pHtime and GE_{50} values); pHgmrt diff (difference between pHtime and GMRT values), $pHGE_{50}$ (difference between pHtime and GE_{50} values).