

MONITORING OF GENTAMICIN CONCENTRATIONS IN OBSTETRICS &
GYNAECOLOGY PATIENTS AT WINDHOEK CENTRAL HOSPITAL AND
KATUTURA INTERMEDIATE REFERRAL HOSPITAL

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ABSTRACT

The aim of this study was to assess whether the gentamicin dosing strategy as presently performed at Katutura Intermediate Referral Hospital and Windhoek Central Hospital produces gentamicin serum concentrations that are within the recommended therapeutic ranges. This was a quantitative, prospective and observational/non-interventional study. Patients (n=35) Patients who were admitted and receiving gentamicin therapy in the obstetrics & gynaecology wards of the two hospitals (Katutura Intermediate Referral Hospital and Windhoek Central Hospital) were recruited and two blood samples taken from each patient. The first sample was drawn at 1 hour after starting the infusion and a second sample after 6 hours. Serum gentamicin and serum creatinine concentrations were determined using a Thermo Scientific Indiko Plus[®] autoanalyser and plots of serum gentamicin concentration vs time on semi-logarithmic paper were used to deduce the pharmacokinetic characteristics. Data were analyzed using descriptive statistics. The study revealed that 80.0% of non-PID patients received doses that were lower than the 5-7 mg/kg recommended for once daily dosing. In addition, 86.3% of patients had C_{max} values that were below the recommended 17-25 $\mu\text{g/mL}$. All patients treated against PID had their doses falling within the recommended 3-5 mg/kg, but no guiding literature was found to act as a reference for the serum gentamicin levels in PID patients. The results from this study led to the following recommendations: i) discontinue the use of standard dosing of gentamicin, ii) use mg/kg dosing method in determining gentamicin doses, iii) monitor the gentamicin levels to ensure safety and efficacy, iv) studies to evaluate the antimicrobial effectiveness of the 3-5 mg/kg PID dose should be carried out.

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LIST OF ABBREVIATIONS/ACCRONYMS

AUC	area under the serum concentration-time curve
BMI	body mass index
BNF	British National Formulary
CDC	Centres for Disease Control
CEDIA	Cloned Enzyme donor immunoassay
CL	total clearance of the drug
CL_{cr}	creatinine clearance
C_{max}	maximum serum concentrations
C_{max, ss}	maximum steady state concentration
EID	Extensive Interval Dosing
ELISA	Enzyme-linked immunosorbent assay
EMIT	Enzyme multiplied immunoassay technique
f_e	fraction of dose excreted
FPIA	fluorescence polarization Immunoassay
GFR	glomerular filtration rate
HPLC	High Performance Liquid Chromatography
IBW	ideal body weight
IV	intravenous
k₀	infusion rate
KDOQI	Kidney Disease Outcome Quality Initiative

k_e	elimination rate constant
KIRH	Katutura Intermediate Referral Hospital
LSD	Lysergic acid dimethylamine
MDD	Multiple daily dosing
MDRD	modification of diet in renal disease
MIC	Minimum Inhibitory Concentration
mRNA	messenger ribonucleic acid
NBTS	Namibia Blood Transfusion Service
ODD	Once-daily dosing
PAE	post antibiotic effect
PID	Pelvic Inflammatory Disease
SAMF	South African Medicines Formulary
$t_{1/2}$	Half-life
TBW	total body weight
TDM	Therapeutic Drug Monitoring
TI	therapeutic index
V	apparent volume of distribution
WCH	Windhoek Central Hospital

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DEDICATIONS

For Mom & Dad

DECLARATIONS

CHAPTER 1

INTRODUCTION

1.1. Orientation of the study

Gentamicin, together with other aminoglycosides such as kanamycin, amikacin and streptomycin, is a very useful drug in the treatment of severe bacterial infections. Nonetheless, gentamicin along with other aminoglycosides is known to commonly cause nephrotoxicity and ototoxicity due to its low therapeutic index, which makes the monitoring of its serum concentration levels critical (MacDougall & Chambers, 2011). Drugs of a low therapeutic index have a narrow therapeutic window between the minimum effective dose below which there is no desired therapeutic effect and the maximum safe dose above which toxicity occurs. It is not enough to administer the recommended doses for gentamicin as the prevailing practice currently is in Namibia. Plasma concentrations of the drug must be monitored to confirm that the concentrations are within range.

It has been reported that of the patients who receive treatment with an aminoglycoside for a couple of days, approximately 8-26% develop mild renal toxicity (Guthrie, 2008; Smith et al., 1980). Since this renal toxicity is caused by accumulation of the drug in the proximal tubules, the toxicity is aggravated when the patient receiving treatment already has a compromised renal function. Although mild nephrotoxicity is generally reversible, this is not the case with ototoxicity which is almost always irreversible (Guthrie, 2008). Sagwa et al. (2015) carried out a study on amikacin and kanamycin-induced hearing loss in Namibia and reported the incidence of cumulative hearing loss to be 58%. Loss of hearing was mostly bilateral. This evidence is intriguing. In view

of this, it could be of interest to find out what the incidence of nephrotoxicity is among patients that are on other aminoglycosides such as gentamicin in Namibia as this has not been studied. Sagwa et al. (2015) state the importance of Therapeutic Drug Monitoring (TDM) in preventing or detecting onset of such drug toxicity, but lament the lack of availability of such a service in much of the developing world, including Namibia.

Gentamicin dosing as practiced at Katutura Intermediate Referral Hospital and Windhoek Central Hospital

Katutura Intermediate Referral Hospital (KIRH) is one of the three intermediate referral hospitals in Namibia, together with Rundu State Hospital and Oshakati State Hospital. Windhoek Central Hospital (WCH) is the premier and highest referral hospital in Namibia. Both the KIRH and WCH are located in the capital city, Windhoek, in close proximity to one another. These two hospitals are also currently serving as teaching hospitals for the purpose of training student and intern doctors and pharmacists, nurses and other health cadres.

TDM is rarely, if at all practiced at these hospitals in the therapy of gentamicin or any other low therapeutic index drug. There are anecdotal reports that some efforts on provision of TDM service had been attempted by pharmacists in the 1990s but did not continue after they left hospital pharmacy practice. Some of the medical doctors practicing at one of the two hospitals have expressed their intentions and efforts to monitor concentration levels of one of the aminoglycoside drugs that they occasionally have to prescribe. However, TDM practice has not been made a policy in the therapy of any low therapeutic index (TI) drug. It seems prescribers acknowledge the

importance of measuring gentamicin levels and intern doctors are advised by their supervisors to check the gentamicin levels if they see a need for a patient to be put on therapy. Nonetheless, requests for serum concentration levels to be measured are rare, or not done at all.

Due to the risk of side effects of gentamicin such as severe ototoxicity and nephrotoxicity, prescribers seem to be overly cautious with the use of gentamicin. Some doctors altogether avoid the use of gentamicin due to these potentially serious side effects. Although their fears are rational, it is unwise to deny patients from receiving gentamicin therapy as gentamicin is indeed one of the most effective antibiotics available. This fear may effectively be addressed by monitoring the levels of the drug so as to steer clear of toxicity and ensure therapeutic levels.

Those doctors who do prescribe gentamicin, give a standard dose of 240 mg per day, either as a single dose or in three divided doses (80 mg thrice daily). They prefer these standard dosing regimens because the dose is considered low enough to avoid the occurrence of toxicity. Once again, TDM could be vital in ascertaining whether this dose of 240 mg per day is sufficient to achieve the recommended therapeutic concentrations for gentamicin across a range of patients.

It is not clear what the reason could be for the omission of TDM practice either with respect to gentamicin or any other low TI drug. It is most likely not due to the absence of laboratories that can run these tests, as the National Institute of Pathology (NIP) is able to run gentamicin level tests and actually clearly makes it as an option of any of the tests that a doctor may request from their laboratories using their laboratory test

request form. Therefore, the capacity is available for measurement of gentamicin levels and the services are within close location such that test results can be available within a few hours of requesting.

The 240 mg per day gentamicin dosing is certainly of interest as it raises some questions with regard to the attainment of therapeutic concentrations. For instance, will therapeutic levels be achieved in all patients regardless of their differing body weights and heights? This is unlikely to be so as body weight and height are crucial parameters in the dosage calculations of gentamicin. The pharmacokinetics of gentamicin will differ in individuals of varying weights, heights and renal function. In addition, this dose of 240 mg does not seem to be based on any scientific evidence from any published studies or reference books. This study seeks to answer this research question to determine whether the standard dosing regimen of 240 mg daily achieves therapeutic concentrations and make recommendations for a more effective dosing method, if necessary. The findings of this study may help to provide evidence for the need for routine therapeutic drug monitoring of gentamicin in clinical practice.

1.2. Statement of the problem

Gentamicin is a drug with a low therapeutic index. It is notorious for causing kidney and cochlear damage when given for several days. For this reason, it is recommended that the use of gentamicin and other aminoglycosides should always be monitored by measuring the serum concentrations. There are internationally accepted evidence-based guidelines that should be followed when treating patients with gentamicin, these guidelines ensure that the dose administered will be safe and of therapeutic efficacy (Tang et al., 2014). In addition, guidelines aim to protect the patient from the well-

known potential side-effects of kidney and inner-ear damage. These guidelines recommend that blood samples should be collected from the patient at specific times and analyzed in the laboratory to measure the gentamicin concentrations in the serum, which should fall within recommended levels. It seems this practice is not in place at any of the state hospitals in Namibia, of which Windhoek Central Hospital (WCH) and Katutura Intermediate Referral Hospital (KIRH) are the two most important in the country and therefore there is the possibility of patients being exposed to sub- or supra-therapeutic gentamicin concentrations.

1.3. Study objectives

a) Aim

The aim of this study was to assess whether the gentamicin dosing strategy as presently performed at Katutura Intermediate Referral Hospital (KIRH) and Windhoek Central Hospital (WCH) produced gentamicin serum concentrations that were within the recommended therapeutic ranges.

b) Specific objectives:

- i. To identify the gentamicin dosing method(s) adopted at WCH and KIRH
- ii. To determine the peak and trough serum concentrations of patients who are on gentamicin therapy
- iii. To determine the proportions of patients whose gentamicin dose and serum concentrations fell within the recommended ranges

1.4. Significance of the study

This is a feasibility study that seeks to demonstrate the benefits of practicing therapeutic drug monitoring in Namibia. While the focus of this study is gentamicin,

the aim is to demonstrate the value of therapeutic drug monitoring across all drugs of low therapeutic index such as digoxin, phenytoin and theophylline, to mention a few. This evidence-based method of patient-care, if established as conventional, is likely to reduce incidences of adverse drug reactions, improve the quality of life for patients and minimize the cost of healthcare for Namibia. Gentamicin is a valuable drug in the treatment of serious infections; in view of the increasing threat of anti-microbial resistance to many useful drugs, TDM could promote the rational use of gentamicin which will safe-guard the potency of this drug.

1.5. Limitations of the study

Due to the limited resources and the vastness of the Namibian geography, this study could only be carried out at two hospitals located within Windhoek. Furthermore, measured serum concentrations for each patient could not be reported back to the doctors immediately after analysis as this had higher cost implications. For this study, it was more cost-effective to analyze the samples in batches than individually. In addition, we did not have absolute control on the dosage administration method, we might not be completely certain that all the patients received the assumed dose of 240 mg. This could have affected the calculations for the pharmacokinetic parameters such as maximum concentrations achieved (C_{max}), area under the serum concentration-time curve (AUC), volume of distribution (V) and drug clearance (CL). This being a non-interventional study, we were not involved in the determination of the dose of gentamicin and that of any other co-medication that should be given to any of the individual patients.

CHAPTER 2

LITERATURE REVIEW

2.1. Physicochemical properties of gentamicin

Gentamicin is a complex of three structurally-related compounds that belongs to the class of antibiotics referred to as the aminoglycosides (Deck & Winston, 2012). The three gentamicins are: gentamicin C1, gentamicin C1a and gentamicin C2. Gentamicin has a central *2-deoxystreptamine* ring to which two other hexose rings, *garosamine* and *purpurosamine*, are attached by glycosidic bonds. The general structure is given in Figure 1 below. Other aminoglycosides include streptomycin, neomycin, kanamycin, amikacin, tobramycin, netilmicin and sisomicin. Aminoglycosides are highly water-soluble molecules and have a poor lipophilicity profile. They are stable in solution and are more active at alkaline than at acidic pH (Deck & Winston, 2012).

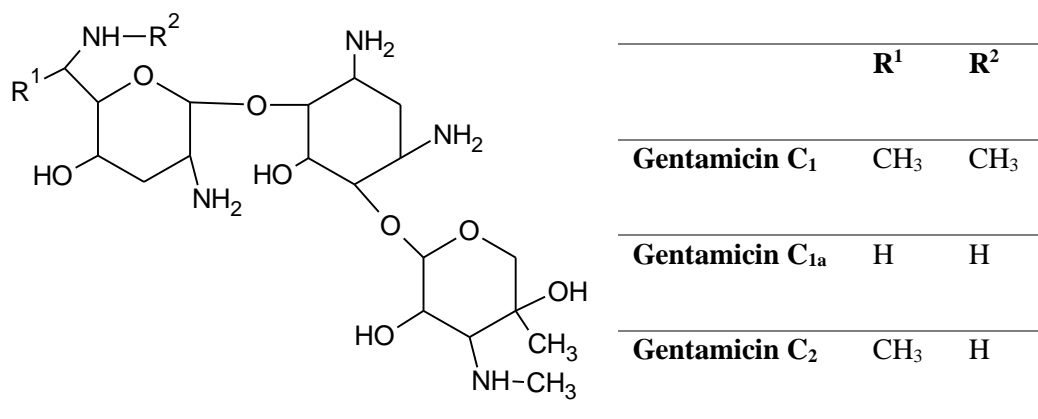


Figure 1 Structure of gentamicin (from Deck & Winston, 2012. Page 822)

2.2. Antimicrobial activity and therapeutic use of gentamicin

Aminoglycosides bind to a specific 30S-subunit of the bacterial ribosome and therefore irreversibly inhibit protein synthesis by either interfering with the initiation complex of peptide formation, misreading of mRNA or breaking-up of polysomes into non-functional monosomes (Deck & Winston, 2012).

Gentamicin is a broad-spectrum antibiotic with bactericidal effect mostly against aerobic gram-negative bacteria. Activity against gram-positive organisms is also marked but not to a great extent. It has no activity against anaerobic microorganisms. Gentamicin is used alone or together with another antibacterial agent in severe infections such as sepsis and pneumonia due to *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus*, *Klebsiella pneumoniae*, *Enterobacter*, *Serratia marcescens*, *Acinetobacter*, *Stenotrophomonas* and other gram-negative bacteria that exhibit resistance to many other antibiotics (Deck & Winston, 2012). In combination with β -lactam antibiotics such as penicillins or cephalosporins, gentamicin is useful in the treatment of gram-positive instigated bacterial endocarditis, most especially due to *Enterococcus*. Although it is known that gentamicin and other aminoglycosides are inactivated by penicillins in-vitro, the reality is different in-vivo. When gentamicin and a penicillin are administered through separate routes there is little or no inactivation that can be demonstrated in patients with normal renal function (Ervin et al, 1975). However, in patients with significant renal failure, this inactivation of gentamicin easily occurs due to reduced clearance of the drugs from the body. A covalent bond is formed between the aminoglycoside and the penicillin which inactivates both of the antibiotic molecules. This inactivation is only most likely seen in patients with renal dysfunction having a creatinine clearance lower than 30 mL/min (Bauer, 2001).

Therapeutic use of gentamicin (or any other aminoglycoside) in combination with another antimicrobial agent is important because of the following reasons: firstly, gentamicin is not effective against anaerobes, therefore combining agents expands the empiric spectrum of activity of the antibiotic regimen to ensure the presence of at least one drug active against a suspected pathogen. Secondly, aminoglycoside monotherapy easily leads to rapid development of bacterial resistance. Lastly, it provides synergistic bacterial killing (specifically in *Pseudomonas aeruginosa* infections) (MacDougall & Chambers, 2011). Gentamicin is indicated for use in the following clinical conditions: septicemia and neonatal sepsis, infective meningitis, biliary-tract infection, acute pyelonephritis, endocarditis, nosocomial pneumonia, listerial meningitis, and specific eye and ear infections (Joint Formulary Committee, 2014).

Gentamicin exhibits what is referred to as *concentration-dependent killing*, in which higher concentrations of the drug are more effective at achieving bactericidal results at a more rapid rate as opposed to what is seen in *time-dependent killing* in which the effectiveness of the agent is a function of time above Minimum Inhibitory Concentration (MIC). This concentration-dependent killing effect is the basis for the use of the once-daily dosing regimen because it brings about high peak concentrations and is regarded to have a lower incidence of toxicities associated with aminoglycosides due to the protracted time the concentrations remain below the toxicity threshold after a dose is administered. Once-daily dosing is also important in dealing with the *adaptive resistance* that bacteria manifest when they are continuously exposed to high concentrations of gentamicin. This type of resistance is not a 'true' resistance because the bacteria revert to their non-resistant state a few hours or days after a period of no exposure.

Gentamicin also has a significant *post-antibiotic effect* (PAE) which describes the time, 10-20 days, in which bacterial growth remains suppressed after the antibacterial concentration is undetectable (Canterbury District Health Board, 2008). This PAE is due to the tissue-bound gentamicin that has a long elimination half-life of 30-700 hours (MacDougall & Chambers, 2011). The three properties mentioned above are what makes a single injection of the entire daily dose preferable in many clinical conditions as opposed to administering it in two or three divided doses. However, in some conditions such as endocarditis, extensive burns of more than 20% of total body surface area, or when creatinine clearance is less than 20 mL/minute, a once-daily dose gentamicin regimen should not be used (Joint Formulary Committee, 2014).

2.3. Clinical pharmacokinetics of gentamicin

2.3.1. Absorption and administration

Absorption of gentamicin from the gastrointestinal tract is poor due to its high water solubility and low lipophilicity. Less than 1% of a dose administered orally or rectally is absorbed. It is however known that aminoglycoside toxicity occurs when applied topically for long periods to large wounds, burns, or cutaneous ulcers, particularly if the patient has renal insufficiency (MacDougall & Chambers, 2011). Gentamicin and all other aminoglycosides are very rapidly absorbed when administered intramuscularly. 30-90 minutes following an intramuscular dose, peak serum concentrations can be reached which are similar to those obtained 60 minutes after the start of a 30-minute infusion. However, absorption from an intramuscular site is known to be reduced in critically ill patients such as those in shock due to compromised perfusion (MacDougall & Chambers, 2011).

When administered intravenously, gentamicin may be given as a slow bolus injection over 3-5 minutes, or by a 30-60 minute intermittent infusion. When giving a dose that aims to achieve a high peak concentration, it is preferable to give a 30-60 minute intermittent infusion as this method has been found to be safer. In addition, a 60-minute infusion simplifies the aminoglycoside pharmacokinetic model as compared to the shorter infusion times because a long enough infusion blurs out the very rapidly occurring distribution phase that is a characteristic feature in bolus or short intravenous infusions (Schentag et al., 2006).

2.3.2. Distribution

Gentamicin is a polar drug and therefore readily distributes into the extracellular fluid compartment as opposed to the intracellular space (Schentag et al., 2006).

The exception to this is in the endolymph and perilymph of the inner ear, and renal cortex where aminoglycoside influx transporters exist. This may explain the ototoxicity and nephrotoxicity that is associated with aminoglycosides. The volume of distribution (V) is 25% of lean body weight and approximates that of extracellular fluid. Aminoglycosides have a very low plasma protein binding (fraction unbound = 0.95). Gentamicin also adequately distributes in other body fluids such as in synovial, peritoneal, ascetic, and pleural fluids but slowly distributes in the bile, feces, prostate and amniotic fluid (Schentag et al., 2006).

The apparent volume of distribution in children > 5years and adults of normal kidney function is in the range of 0.31 ± 0.10 L/kg (Thummel et al., 2011) and the average (0.26 L/kg) is used in calculations (Bauer, 2001). The pharmacokinetic model that gentamicin follows is a two-compartment model which is characterized by an initial

rapidly equilibrating volume of distribution followed by a second apparent, more slowly equilibrating volume of distribution. It takes less than an hour for distribution to complete (Fitzpatrick, 2007). However, it should be noted that a one-compartment model is used to calculate gentamicin pharmacokinetics in clinical settings where the initial phase of distribution is disregarded (Sawchuck et al., 1976; (Schentag & Jusko, 1977; Mendelson et al., 1976; Lynn et al., 1974).

Care should be taken when dealing with obese patients since aminoglycosides distribute poorly into adipose tissue. The use of ideal body weight (IBW) has been found to underestimate and total body weight (TBW) tends to overestimate the volume of distribution. In such patients, a correction factor is used to obtain a more accurate approximation of volume of distribution (Bearden & Rodvold, 2000; Traynor, 1995; Blouin et al., 1985). Adipose tissue contains approximately 40% of the extracellular fluid that is around lean tissue (Korsager, 1980). Therefore the volume of distribution should be adjusted by adding 40% of the obese patient's excess weight to the patient's ideal body weight (IBW) (Bauer et al., 1983). The equations for this approximation are given below:

$$\text{Male IBW (kg)} = 50 + (0.9 \times \text{Height in cm} > 150 \text{ cm}) \quad (1)$$

$$\text{Female IBW (kg)} = 45 + (0.9 \times \text{Height in cm} > 150 \text{ cm}) \quad (2)$$

$$V = (0.26 \text{ L/kg})\text{IBW} + 0.4(\text{TBW} - \text{IBW}) \quad (3)$$

$$\text{Dosing weight} = \text{IBW} + 0.4 (\text{TBW} - \text{IBW}) \quad (4)$$

In conditions such as ascites and edema in which a 'third space' (third compartment) volume is enlarged, the volume of distribution for gentamicin is also increased. In such patients, the volume of distribution can be approximated by increasing the volume by 1 L for each kg of weight gained (Bauer, 2001).

2.3.3. Elimination

Gentamicin is almost completely eliminated through the kidneys unchanged ($f_e = 0.95$ *i.e.* fraction excreted unchanged) by glomerular filtration during the first 24 hours. In patients with normal renal function, the half-life of gentamicin is approximately 2.5 hours. Gentamicin is also known to have a very long terminal half-life of 53 ± 25 hours due to slow release from tissues, this is the reason why gentamicin is excreted in urine until three weeks after a dose (Thummel et al., 2011). The clearance is proportional to the glomerular filtration rate over a wide range of renal function and is given by the equation (in mL/min/kg) (Thummel et al., 2011):

$$CL = 0.82CL_{cr} + 0.11 \quad (5)$$

This means that gentamicin clearance (CL) can be approximated using the Cockcroft-Gault equation for estimation of creatinine clearance (CL_{cr}):

$$\text{Males} \quad CL_{cr} = \frac{(140 - \text{Age}) \times \text{Weight}}{72 \times [\text{Serum Cr}]} \quad (6)$$

$$\text{Females} \quad CL_{cr} = 0.85 \times \frac{(140 - \text{Age}) \times \text{Weight}}{72 \times [\text{Serum Cr}]} \quad (7)$$

In the above equations CL_{cr} is in mL/min, age is in years, $weight$ is in kg, and $serum\ creatinine$ is in mg/dL (Rowland and Tozer, 2011). For obese patients, the adjusted body weight (ABW) is used instead of the TBW (Leader et al, 1994; Pai et al, 2007).

Cockcroft-Gault formula of estimating creatinine clearance is the most commonly used to predict aminoglycoside clearance in an individual patient. However, the disadvantage of this method is that it is inaccurate at low creatinine clearance. More recent methods of estimating glomerular filtration rate such as Modification of Diet in Renal Disease (MDRD) (Aronson, 2007) and the use of cystatin C have been

developed but their use instead of CL_{cr} as estimated by the Cockcroft-Gault formula has not been evaluated yet (Halacova et al., 2008).

In females, GFR can be estimated with the MDRD method by use of the formula below (Pai & Bearden, 2007):

$$GFR = \frac{175 \times 0.742 \times 1.212}{[Serum Cr]^{1.154} \times Age^{0.203}} \quad (8)$$

GFR can be used to define the stage of loss of renal function as described in Table 1.

Table 1 Glomerular filtration rate categories as determinants of kidney function (From: National Kidney Foundation kidney Disease Outcome Quality initiative (KDOQI) Advisory Board, 2012)

GFR category	GFR (mL/min/1.73 m ²)	Terms
G1	≥90	Normal or high
G2	60-89	Mildly decreased
G3a	45-59	Mildly to moderately decreased
G3b	30-44	Moderately to severely decreased
G4	15-29	Severely decreased
G5	<15	Kidney failure

The elimination half-life of a drug depends on the individual patient's volume of distribution (V) and the clearance (CL) and can be calculated using the equation below:

$$t_{1/2} = \frac{0.693V}{CL} \quad (9)$$

2.4. Gentamicin toxicity

Gentamicin is known to cause nephrotoxicity, irreversible ototoxicity and to a lesser extent, neuromuscular blockade (MacDougall & Chambers, 2011). Ototoxicity results

from progressive destruction of vestibular or cochlear sensory cells. Cochlear damage is characterized by auditory dysfunction which clinically presents as a high-pitched tinnitus as the first symptom of toxicity. Tinnitus may persist for several days to two weeks even after gentamicin is withdrawn. Vestibular toxicity clinically presents with a moderately intense headache lasting 1-2 days before labyrinthine dysfunction sets in. Following this, there is an acute period of nausea, vomiting, and difficulty with maintaining balance which continues for 1-2 weeks. Prominent symptoms include vertigo in the upright position, inability to perceive termination of movement and difficulty sitting or standing without visual cues (MacDougall & Chambers, 2011). Afterwards, chronic labyrinthitis starts developing and persists for about 2 months with ataxia as a prominent feature. This stage is gradually superseded by a compensatory stage in which symptoms are latent and appear only when the eyes are closed.

Development of nephrotoxicity is common in patients who receive aminoglycosides for several days (Schentag et al., 1979). It has been published that aminoglycosides accumulate in the kidneys by binding to megalin a multi-ligand, endocytic receptor that is expressed on the epithelial lining of the proximal tubular cells (Nagai & Takano, 2004). Furthermore, the amino groups on aminoglycosides are charged at physiological pH. This makes them interact with the acidic phospholipids on the membrane surfaces and therefore facilitate reuptake into the tubular cells (Nakai & Takano, 2004). This reuptake mechanism is by active transport and is saturable. Therefore, administration of smaller doses at shorter intervals leads to a greater overall accumulation than if the same dose were administered as one daily dose.

Patients develop mild renal impairment (that is mostly reversible) due to accumulation and retention in the proximal tubular cells. This later leads to compromised renal

concentrating ability, mild proteinuria, and appearance of hyaline and granular casts; after several days, there is a reduction in the glomerular filtration rate (MacDougall & Chambers, 2011).

2.5. Therapeutic drug monitoring and its benefits

Monitoring of aminoglycoside plasma concentrations is recommended in order to ensure safety and efficacy (Pai & Rodvold, 2014). Therapeutic Drug Monitoring (TDM) involves the determination of serum or plasma concentrations of drugs (whole blood concentrations are exceptionally used) by use of analytical methods such as antibody based immunoassays or high performance liquid chromatography (HPLC). The measured concentrations (also called levels) are used to adjust the dosage regimen for an individual patient (dose individualization) such that serum or plasma concentrations are maintained within a target range. Dose individualization is accomplished by proper interpretation of the reported serum or plasma values using pharmacokinetic principles and then making appropriate conclusions regarding the drug concentration and dose adjustment. Therefore, the clinical interpretation of the result requires knowledge of the pharmacokinetics, sampling time, drug history and the patient's clinical condition.

For a drug to be suitable for TDM, it is important for it to satisfy certain requirements (Ghiculescu, 2008). Firstly, the drug should have a narrow therapeutic range (or low therapeutic index). The target concentration range must be established, for example peak concentration for multiple daily dosing of gentamicin should be 8-10 $\mu\text{g/mL}$ (Bauer, 2001). When using the once daily dosing regimen there does not seem to be a clear consensus as to what the levels should be. The figures suggested in literature are:

17-25 µg/mL (Begg et al., 1995), 20 µg/mL (Nicolau et al., 1995; Ferriols-Lisart & Alos-Aliminana, 1996). More recent guidelines for once daily dosing suggest levels of 20-25 µg/mL (Stanford Hospitals & Clinics, 2013) and 15-20 µg/mL (Society for Dutch Hospital Pharmacists, 2015). These concentrations are arrived at by multiplying the MIC of the most difficult microorganism (in most cases it is *P. aeruginosa*, median gentamicin MIC = 2 µg/mL) by the desired C_{max}/MIC ratio of 10:1 (Levy & Bauer, 1986; Nicolau et al., 1995). In this study, 17-25 µg/mL as reported by Begg et al. (1995) will be used as the reference as they seem to be the more authoritative in this field. Secondly, there should be a significant inter-subject pharmacokinetic variability. Third, a reasonable relationship between plasma/serum/blood concentrations and clinical effects must exist. Finally, a cost-effective drug assay should be available (Ghiclescu, 2008).

In the practice of TDM, it is relevant to measure the drug concentrations within a timeframe that will allow timely dose adjustments. Ideally, this timeframe should be shorter than the dosing interval. Nonetheless, the aspect of cost usually compels that assays be performed in batches which results in a longer turnaround time.

The benefits of TDM include: identification of non-compliant patients, personalization of dosage, aversion of adverse drug events or toxicity, improvement in patient safety, decreased hospital stay and investigation of non-response of a patient to therapy which may, in general, be due to impaired absorption or genetic variation in drug metabolism (Dasgupta, 2012). A TDM service may be helpful in improving safety, efficacy, and reducing costs. In addition, it has the advantage of promoting rational prescribing and quality use of medicines in an institution offering clinical services (Ghiclescu, 2008).

Fluorescence Polarization Immunoassay (FPIA)

This is one of the several immunoassay methods commonly used in clinical laboratories for TDM purposes. FPIA is a non-enzymatic, fluorescence-based immunoassay as opposed to other enzyme immunoassay methods such as enzyme-linked immunosorbent assay (ELISA), enzyme multiplied immunoassay technique (EMIT) and cloned enzyme donor immunoassay (CEDIA).

A common FPIA method is the homogeneous particle-enhanced turbidimetric immunoassay. This assay is based on competition of drug in the sample against that coated onto a fluorescent molecule or microparticle. Fluorescent light emitted by a small compound, such as a drug, that is conjugated to a fluorescent molecule shows low polarization. However, when the fluorescent molecules binds to a much larger substance, such as an antibody, there is an increase in the degree of polarization because there is a reduction in the rotation of the fluorescent microparticle. (Milone, 2012; Smith & Eremin, 2008).

FPIA is a competitive technique that is based on the ability of the reaction mixtures to fluoresce upon polarization. The reaction mixture contains the sample analyte, fluorescent-labeled tracer, and a specific antibody. When the solution is at a fixed temperature and viscosity, the fluorescence polarization value is directly proportional to the molecular size of the fluorophore. If the molecule is of a small size, such as an unbound tracer, it will possess a fast Brownian rotation in solution and will therefore have a low fluorescence polarization. This is as opposed to the case in larger molecules, such as a tracer bound to antibody, in which the values are higher (see Figure 2). When the analyte is not present in the sample, the tracer will be bound to

the antibody and therefore resulting in a high fluorescence polarization. On the other hand, if the analyte is present in significantly greater amounts than the tracer, the binding site on the antibody will be competitively occupied by the analyte leaving most of the tracer free in the solution. This produces a lower fluorescence polarization.

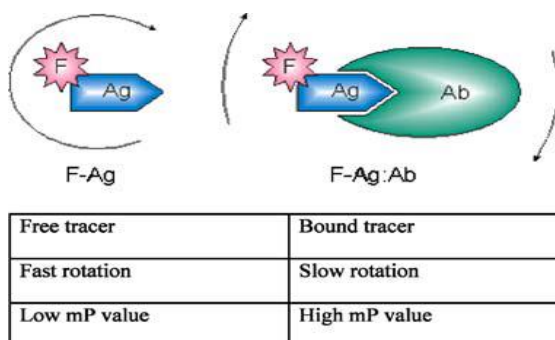


Figure 2 Dependence of fluorescence polarization on molecular size of fluorescent-labeled antigen (Reproduced from Smith, D. S. & Eremin, S. A. *Fluorescence polarization immunoassays and related methods for simple, high-throughput screening of small molecules*. *Anal Bioanal Chem* (2008) 391: 1499-1507

FPIA performance in the laboratory involves serial addition of the serum sample (or standard), tracer, and antibody solution. There is a brief incubation after which the fluorescence polarization is measured and the analyte concentration in the sample is determined by reference to the standard curve. Due to the very rapid time it takes for equilibrium to be attained in the reaction mixture (a few seconds or minutes), the total time for analysis therefore may only be a few minutes when using FPIA (Smith & Eremin, 2008).

The analytical instrument used for this research project was the INDIKO PLUS[®] auto analyzer (Thermo Fisher Scientific, Waltham, MA, USA). In addition to gentamicin, the INDIKO PLUS[®] auto analyzer also measures other classical TDM drugs such as phenytoin, phenobarbitone, theophylline, digoxin, cyclosporine, tacrolimus,

tobramycin, carbamazepine, valproic acid, lithium and vancomycin. The instrument can also measure drugs of abuse such as ethanol, amphetamines, cannabinoids, ecstasy, opiates and lysergic acid dimethylamine (LSD). Furthermore, pH, creatinine and albumin are some of the physiological parameters that can be analyzed using the INDIKO PLUS® (Thermo Scientific, 2014).

2.6. Methods used in determining the gentamicin dosing regimen

Gentamicin dosage regimen can be based on either the once daily dosing (ODD), also known as extended interval dosing (EID) or multiple-daily dosing (MDD) method. The suitable gentamicin dose to be given to an individual patient may be determined by use of either the literature-based dosing method or the pharmacokinetic dosing method.

2.6.1. Use of milligram per kilogram (mg/kg) dosing method

In this method, the gentamicin dose may be determined as recommended by reference material such as the British National formulary (BNF), South African Medicines Formulary (SAMF) or any other source that is regarded to be of repute by the prescribers. Usually the dosing is recommended to be in mg/kg and differs for the respective disease conditions. Below (Table 2) is a summary as to what the BNF and SAMF state regarding gentamicin dosing:

Table 2 Recommended gentamicin dosing strategy as obtained from BNF (vol.68, 2014) and SAMF (2012)

BNF	SAMF
<p>-To avoid excessive dosing in patients who are obese, use IBW to calculate dose and monitor the serum concentrations.</p> <p>-For multiple daily dosing, give the dose by IM or by slow IV injection over at least 3 minutes or by IV infusion, 3-5 mg/kg daily (divided in three 8-hourly doses).</p> <p>-For once daily dosing, give the dose by IV infusion starting with a dose of 5-7 mg/kg and then adjust according to the measured gentamicin serum concentration levels.</p> <p>-In the multiple daily dosing regimen method, the desired 'peak' serum concentration should be 5-10 mg/L (3-5 for endocarditis); whereas the 'trough' concentration should be less than 2 mg/L (less than 1 mg/L for endocarditis).</p> <p>-The BNF refers the practitioner to the local guidelines for monitoring serum gentamicin concentrations (Namibia does not seem to have any such guidelines).</p>	<p>-Due to gentamicin being a drug of low therapeutic index, doses should be individualised based on age, weight and renal function. The efficacy of the prescribed dose should be confirmed by measuring the peak concentration after the second dose.</p> <p>-In adults, the dose may be administered IM or IV (slowly over 3 minutes or infused over 20-30 minutes, may be up to 2 hours. IV dose may be infused diluted in 5% dextrose or 0.9% normal saline solution). The recommended dose is 5-6 mg/kg given once daily (for streptococcal endocarditis it should be 1.5 mg/kg twice daily, in combination with penicillin).</p> <p>-Peak levels should be measured 1 hour after the start of an IV infusion given over 15-30 minutes, or 1 hour after an IM or IV bolus injection.</p> <p>-For once daily dosing, the target peak serum gentamicin concentration should be greater than 8 mg/L and the trough concentration should be less than 1 mg/L.</p>

Both the BNF and SAMF seem to agree in the dose for once daily dosing strategy as they suggest similar doses of 5-7 mg/kg or 5-6 mg/kg, respectively.

2.6.2. Use of nomograms

Several nomograms have been proposed to facilitate the dosage initiation and adjustment of gentamicin, these include: Thomson guidelines (Thomson et al., 1996), Hull-Sarubbi table (Hull & Sarubi, 1976), and rule of eights (Lesar, 1982) for multiple daily dosing; whereas for extended-interval dosing; Hartford nomogram, Barnes-Jewish Hospital nomogram, and the Sanford guide can be used (Lee, et al. 2014). In their study titled '*Predictive performance of gentamicin dosing nomograms*' Lee et al. (2014) found that dosing nomograms performed poorly in attaining the target peak serum levels. Nomograms designed for multiple-daily dosing seemed to predict peak serum gentamicin levels better than the extended-interval dosing nomograms. Nonetheless, all of the nomograms used were able to correctly predict the target trough level in more than 80% of the patients. It was therefore concluded that since the nomograms performed poorly for gentamicin, new ones may be required in patients with infective endocarditis, especially for extensive-interval dosing. In addition to this, Lee et al. (2014) recommended that therapeutic drug monitoring should be done to ensure that target concentrations are achieved. The most commonly used nomogram in the therapeutic drug monitoring guidance of gentamicin dosing is the Hartford high-dose extended interval dosing nomogram (Nicolau, et al., 1995) illustrated in Figure 3 below:

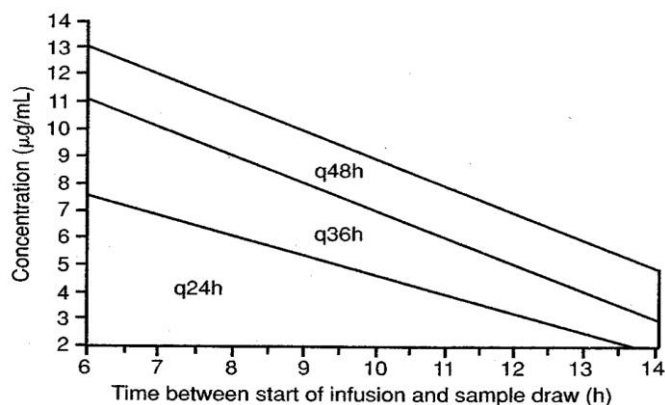


Figure 3 The gentamicin concentration measured 6-14 hours after a 7 mg/kg dose is plotted. The dosing interval is adjusted depending on which area (24 hours, 36 hours or 48 hours) that the plot falls under. (Adapted from Nicolau, et. al., 1995)

The dose in this nomogram is 7 mg/kg and it was designed to achieve the desired gentamicin peak concentration of 20 mg/L (Nicolau et al., 1995). The dosing interval is determined by the degree of renal dysfunction (Table 3) such that the target peak concentration is maintained and a 6 hour drug-free period is achieved in order to avoid accumulation of the drug in the inner ear (Nicolau et al., 1995).

Table 3 Use of creatinine clearance to determine initial dose and interval using the Hartford nomogram (Adapted from Winter, 2010, page 142).

Creatinine Clearance	Initial Dose and Interval
>60 mL/min	7 mg/kg every 24 hr
40-60 mL/min	7 mg/kg every 36 hr
20-40 mL/min	7 mg/kg every 48 hr
<20 mL/min	7 mg/kg, then follow levels to determine time of next dose (level < 1 µg/mL)

The disadvantage of these types of nomograms is that they are designed in such a way that they achieve a fixed peak and trough concentration and therefore do not give allowance for the individualization of dosing regimen with regard to the type of

infection or the fact that it may be a unique patient such as the obese or those with significant third fluid spaces (Winter, 2010).

2.6.3. Pharmacokinetic dosing method

In their publication “*Kinetic model for gentamicin dosing with the use of individual patient parameters*”, Sawchuk et al. (1976) proposed a method for the therapeutic drug monitoring of gentamicin. This was the method used for multiple-daily dosing before once-daily dosing was developed, but the same principles of calculation can be applied for once-daily dosing. They used a one-compartment model for gentamicin kinetics. In that study, multiple-daily dosing infusions were determined for over 80 patients by calculating the different pharmacokinetic parameter values for each individual. The gentamicin elimination rate constant and volume of distribution for each patient were determined from the serum concentration-time data obtained after a single infusion. They used the following equation to calculate the gentamicin volume of distribution:

$$V = \frac{k_0 (1 - e^{-k_e t'})}{k_e (C_{max} - C_0 e^{-k_e t'})} \quad (10)$$

where V is the volume of distribution, k_0 is the zero-order rate of infusion of the first dose infused at a constant rate usually over 60 minutes, k_e is the first-order elimination rate constant, C_{max} is the gentamicin concentration at the time the infusion ends and t' is the duration of infusion. The infusion rate was calculated as the dose divided by the infusion time. It is important to note that the component $C_0 e^{-k_e t'}$ in the equation accounts for the concentration of gentamicin remaining from the previous dose and therefore should not be considered for an initial infusion. It should also not be considered for patients receiving once-daily dosing because in principle no amount of drug should be remaining by the time the next dose is due to be administered. The total body clearance was calculated as:

$$CL = k_e V \quad (11)$$

The selection of the desired maximum and minimum serum concentrations depended on the patient's diagnosis and clinical condition. The desired peak levels ranged from 6-10 µg/ml and the minimum (nadir) levels between 0.5-2 µg/ml. The new dose and dosing interval (τ) were then calculated using a method described in an earlier paper by Sawchuck & Zaske (1976):

$$k_0 = k_e V C_{max,ss} \frac{1 - e^{-k_e \tau}}{1 - e^{-k_e t'}} \quad (12)$$

The infusion time for all the regimens was 1 hour and the calculated dosage interval was adjusted to values such as 4, 6, 8 or 12 hours for the purposes of making the administration of the drug practical. The infusion rate was calculated and approximated to the nearest practical value. It took about 3-5 hours to analyze the gentamicin concentrations and calculate the new dosing regimen after the last blood sample was drawn. This means that the calculated regimen was instituted two to three doses after the initial test dose. Follow-up peak and nadir samples were measured at least after five half-lives of starting the regimen in order to monitor the patient's therapy. Two blood samples were drawn for analysis, the first was taken at the end of the infusion and the second was just before the next infusion.

Bauer (2001) gives a detailed overview of the pharmacokinetic method. To determine the initial dose under this method, the pharmacokinetic parameters of the patient are estimated by use of the average pharmacokinetic parameters measured in other patients with similar disease conditions who had been treated with gentamicin.

The pharmacokinetic parameter that should be estimated first is the elimination rate constant (k_e), which describes the fraction of the dose that is eliminated from the body per given unit of time. The units of the elimination rate constant for gentamicin are

usually expressed as per hour (hr^{-1}). A linear relationship is known to exist between creatinine clearance and aminoglycoside elimination rate, since gentamicin and other aminoglycosides are almost completely eliminated unchanged by the kidneys ($f_e = 0.95$). The elimination rate constant may therefore be approximated by use of the creatinine clearance (CL_{cr}) equations. The gentamicin elimination rate is therefore calculated as:

$$k_e = 0.00293(\text{CL}_{\text{cr}}) + 0.014 \quad (13)$$

The creatinine clearance is given in mL/minute. Considering the patient's renal function when determining the initial aminoglycoside dosing regimen is a critical thing to do. However, the drawback of using this equation is that the elimination rate constant (k_e) is not a primary pharmacokinetic parameter, it is actually a constant whose value may be influenced by both the clearance (CL) and the volume of distribution (V), that is:

$$k_e = \text{CL}/V \quad (14)$$

Clearance and volume of distribution are the primary pharmacokinetic parameters because they are only affected by physiological factors.

Secondly, the volume of distribution is then estimated. A patient without a disease condition, such as obesity or ascites, which may alter the drug's volume of distribution, would have a volume of distribution for gentamicin around the average value, that is, 0.26 L/kg. The patient's weight and height are first used to determine their Body Mass Index (BMI) and their Ideal Body Weight (IBW). If their actual weight is less than their ideal body weight (underweight), actual body weight is used to calculate volume of distribution (this also applies to patients whose actual weight is between their ideal body weight and 30% greater than their ideal body weight). On the other hand, for

those patients whose actual body weight is 30% greater than their ideal body weight, their volume of distribution is estimated thus:

$$V = 0.26 \text{ L/kg}[IBW + 0.4(TBW - IBW)] \quad (15)$$

The final step would then be to compute the gentamicin dose by use of either the equation for intermittent intravenous infusion (when the patient has a creatinine clearance of >30 mL/min) or that for intravenous bolus (see Table 4) in patients who have a critical reduction in kidney function (creatinine clearance \leq 30 mL/min). When the conventional multiple daily dosing regimen is used, severe infections such as pneumonia, septicemia and *P. aeruginosa* that have a MIC of approximately 2 $\mu\text{g/mL}$ may require peak steady-state serum concentrations ($C_{max,ss}$) of 8-10 $\mu\text{g/mL}$, abdominal infections may require 5-7 $\mu\text{g/mL}$ and urinary tract infections only require 3-5 $\mu\text{g/mL}$. The desired trough concentration should be < 2 $\mu\text{g/mL}$.

The alternative once-daily dosing (or extended interval) regimen does not seem to have similarly established target peak serum concentrations. C_{max} of 20-30 $\mu\text{g/mL}$ seems to be the accepted range for serious infections. The trough concentration should be < 1 $\mu\text{g/mL}$.

Table 4 Equations Used to Compute Individualized Dosage Regimens for Various Routes of Administration Used with Aminoglycoside Antibiotics (adapted from Bauer,2010 p.113)

ROUTE OF ADMINISTRATION	DOSAGE INTERVAL (τ), MAINTENANCE DOSE (D OR k_0),AND LOADING DOSE (LD) EQUATIONS
Intravenous bolus	$\tau = (\ln C_{max,ss} - \ln C_{min,ss})/k_e$ $D = C_{max,ss}V(1 - e^{-k_e\tau})$ $LD = C_{max,ss}V$
Intermittent intravenous infusion	$\tau = [(\ln C_{max,ss} - \ln C_{min,ss})/k_e] + t'$ $k_0 = C_{max,ss}k_eV[(1 - e^{-k_e\tau})/(1 - e^{-k_e t'})]$ $LD = k_0/(1 - e^{-k_e\tau})$

It is noteworthy that the above Sawchuk & Zaske method was developed in the 1970s when gentamicin was only given in multiple-daily doses. However, in the 1990s, the once-daily approach was fast becoming preferred to the multiple-daily dosing strategy. The Sawchuk & Zaske method adapted to once-daily dosing remained the basis for gentamicin therapeutic drug monitoring but another approach was suggested by Begg et al. (1995). They suggested that the traditional approach of aiming for target maximum and minimum concentrations was not appropriate for application to once-daily dosing. They proposed a method that uses a target area under the serum concentration-time curve based on the 24 hour AUC that would result with the conventional multiple-daily dosing. This method requires two blood samples one taken 30 minutes after a 30-minute infusion and the second taken 6-22 hours after the start of the infusion (depending on the patient's kidney function). The dose adjustment using the AUC method can be done by use of the target and measured AUCs:

$$\text{Second dose} = \frac{\text{target AUC}}{\text{measured AUC}} \times \text{first dose} \quad (16)$$

Measured AUC is calculated as

$$AUC(0,24) = AUC(\text{end}, 24) + AUC_{\text{infusion}} \quad (17)$$

$$\therefore AUC(0,24) = \frac{C_{\text{end}} - C_{24}}{k_e} + 0.065 \times \frac{C_{\text{end}} - C_{24}}{k_e} \quad (18)$$

Where:

$AUC(0,24)$ = AUC over the entire 24 hour dose interval

$AUC(\text{end}, 24)$ = AUC from the end of the infusion to 24 hours

AUC_{infusion} = AUC during the infusion phase; approximates to 6.5% of the $AUC(0,24)$

C_{end} = concentration at the end of the infusion (peak concentration)

C_{24} = predicted concentration at 24 hr (trough concentration)

And k_e = elimination rate constant

The target AUC is selected from the table 5 below:

Table 5 Selection of starting dose, target AUC and time of second sample (from Begg et al. 1995)

CL _{cr} (ml/s)	Starting dose (mg/kg)	Target AUC	Time of second sample (hr)
>1.1	5,6,7	72, 86, 101	6-14
0.9-1.1	5,6	86, 101	8-16
0.7-0.9	5	101	10-18
0.5-0.7	4	101	12-20
0.35-0.5	3	101	14-22
<0.35	Seek specialist advice		

Table 5 has been simplified and incorporated into a protocol (see Table 6) as published in an article by Chin et al (2013):

Table 6 Extended interval dosing protocol for gentamicin (From Chin et al., 2013)

Initial dose

1. Calculate the patient's creatinine clearance using a modified Cockcroft-Gault formula employing the IBW (or TBW if this is less) as the weight descriptor.

Creatinine clearance (mL/min)	Dose in mg/kg using the IBW (or TBW if this is less)
>66	5-7*
55-66	5-6*
41-54	5
31-40	4
20-30	3
<20	Gentamicin not recommended

2. Doses are administered as infusions over 30 min.
- Subsequent doses
3. Gentamicin concentrations are taken at: 30 min after the end of the infusion and between 6 and 22 h after the end of the infusion depending on degree of renal impairment.
4. Doses are guided using gentamicin population pharmacokinetics, individual characteristics and gentamicin concentration in a pharmacokinetic computer programme.

*Depending on severity of infection. IBW: ideal body weight; TBW: total body weight.

2.7. Studies on gentamicin dosing and pharmacokinetics

Several papers have been published on the pharmacokinetics and therapeutic drug monitoring of gentamicin, particularly focusing on the serum concentration levels attained when using once-daily dosing. One earlier study by Ismail et al. (1990) was on the therapeutic drug monitoring of gentamicin for patients who were on conventional dosing (multiple-daily dosing) being treated for conditions including pneumonia, gynaecological infections, septicaemia, urinary tract infections and pyrexia of unknown origin. The objective of that study was to evaluate whether the method of 'standard dosing' with gentamicin produced adequate desired concentrations. Ismael et al. (1990) also made use of a two-point peak and trough pair concentration method to adjust doses to achieve appropriate concentrations. The study found that only 38% of the patients had serum concentrations in the therapeutic range and a third of patients (30%) had trough concentrations that were above the desired 2 µg/mL. Ismael et al. (1990) reported that after dosage adjustments were performed, the percentage of patients who had serum concentrations falling within the therapeutic concentrations increased from 38% to 68% and the percent with trough concentrations that were above 2 µg/mL fell to 13%. Ismael et al. (1990) also reported that 18% of the patients in whom dosage adjustments were made had been given initial doses that were lower than the minimum recommended dose of 3 mg/kg/day. On the other hand, about 59% were prescribed initial doses that exceeded the recommended maximum dose of 5 mg/kg/day.

A study conducted in New Zealand by Kirkpatrick et al. (1999) sought to first determine the population pharmacokinetics of gentamicin in 957 patients with varying kidney function receiving once-daily dosing. Secondly, to evaluate whether the then

current once-daily starting doses for gentamicin were appropriate and thirdly, to evaluate if creatinine clearance as calculated using an adjusted Cockcroft and Gault method was a better predictor of gentamicin clearance as compared to original Cockcroft and Gault method. Kirkpatrick et al. (1999) used a Bayesian dosing method that gives estimates of gentamicin CL and V that are then used to estimate 24 hour AUC. Depending on the severity of the infection, the target AUC was 70-100 mg L⁻¹ hr. The study found the mean V to be 17.4 (±4.1) L and the mean CL was 4.0 (±1.8) L/hr. Furthermore, it was noted that a reduction in V was directly proportional to reduction in kidney function and volume of distribution was better predicted by a lower dosing weight, whether total body weight or lean body weight. In their conclusion, Kirkpatrick et al. (1999) stated that the usual Cockcroft and Gault method of calculating clearance does not appear to perform well at low values of serum creatinine concentrations. Hence, an adjustment of the Cockcroft and Gault method was proposed.

2.7.1 Gentamicin use in obstetrics & gynaecology

A retrospective review study by Kushner et al. (2016) reported on the frequency and demographics of gentamicin use in several major hospitals in the USA. Kushner et al. (2016) reported that of the 1237 adult patients with ages 18-44 years, 1050 (85%) were female and that for this age group, the 20 most diagnosed conditions were pregnancy-related which include threatened premature labor, urinary tract infection, abdominal pain and post-procedural status. Some patients had an unknown cause of morbidity (Figure 4).

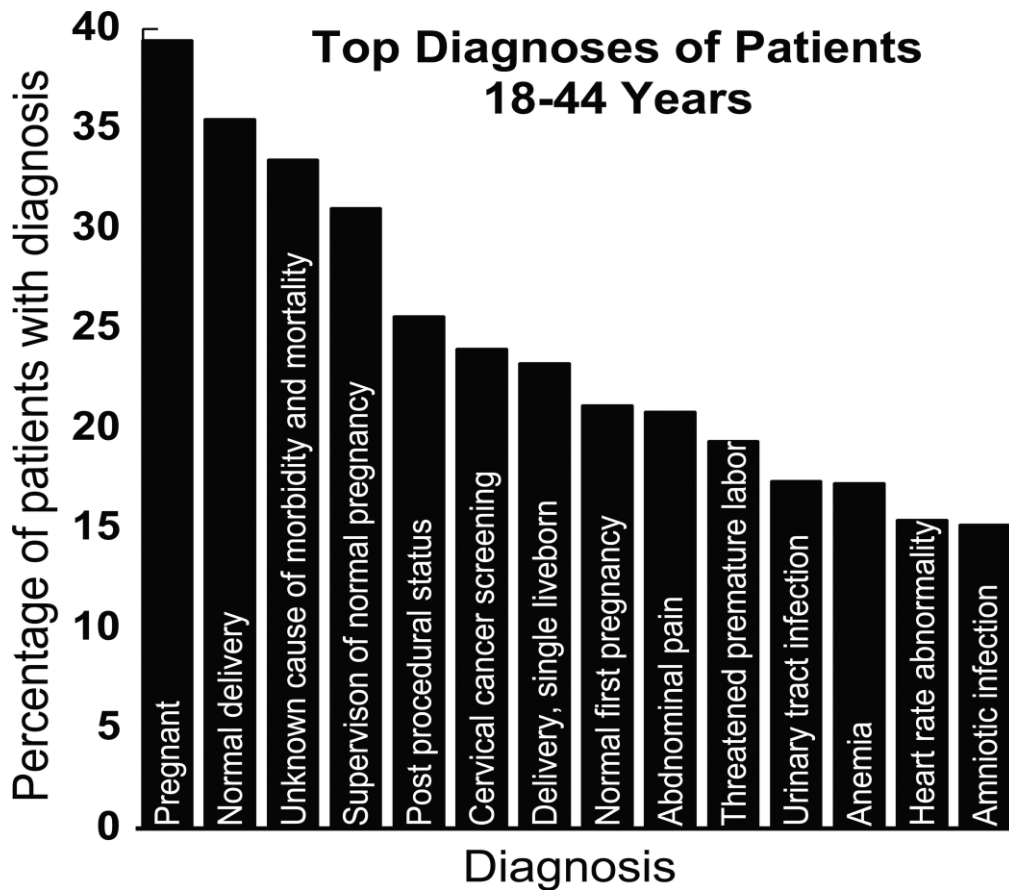


Figure 4 Frequency of pregnancy related disorders in the 18–44 year old age group. Nine (9) of the top 20 most frequent diagnoses associated with patients in this age group were associated with pregnancy. The five (5) most frequent pregnancy related diagnoses involved a normal pregnant or normal delivery status. The most frequent non-pregnancy infectious related diagnosis in this group was a urinary tract infection. (Figure from Kushner et al., 2016).

Pelvic inflammatory disease (PID) is one of the most common infections in non-pregnant women of reproductive age encountered in wards of obstetrics and gynaecology of which the major causative agents have been documented to be *Neisseria gonorrhoeae* and *Chlamydia trachomatis* (Taylor-Robinson et al. 2012) (Figure 5). Even endogenous microorganisms of the vagina and cervix are frequently associated with pelvic inflammatory disease (Sweet, 2011). For this reason, PID is treated with antibiotics which target a wide range of potential pathogens. In PID, infection of the female genital tract leads to local inflammation that often causes

discharge, dysuria, genital discomfort and pain. The infection may also spread to the fallopian tubes and ovaries (Hathorn et al., 2014). PID is known to cause complications such as infertility, chronic pelvic pain and ectopic pain (Aghaizu et al., 2011). The financial implications of PID complications on health care services is considered to be significant (Yeh et al., 2003).

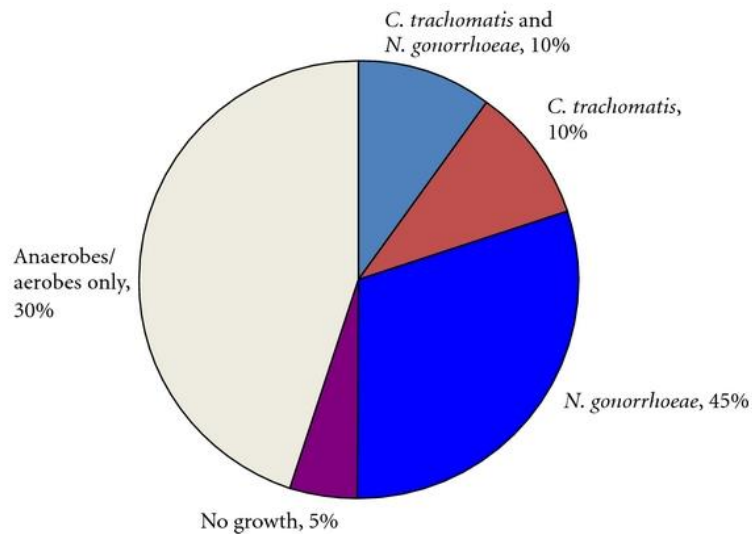


Figure 5 Microbiology of acute pelvic inflammatory disease (Reproduced from Sweet, R. L. *Treatment of acute pelvic inflammatory disease*, 2011)

The recommended parenteral treatment of severe acute PID by Centers for Disease Control (CDC) is outlined in Table 7.

Table 7 Parenteral treatment recommendations for acute pelvic inflammatory disease (adapted from CDC Sexually Transmitted Diseases Treatment Guidelines 2015 MMWR 64(23): 2015 [78-82])

Recommended regimen A	Cefotetan 2 g IV every 12 hours OR Cefoxitin 2 g IV every 6 hours + Doxycycline 100 mg orally or IV every 12 hours
Recommended regimen B	Clindamycin 900 mg IV every 8 hours + Gentamicin loading dose IV or IM (2mg/kg) followed by a maintenance dose (1.5 mg/kg) every 8 hours. A single daily dosing (3-5 mg/kg) can be substituted
Alternative parenteral regimen	Ampicillin/Sulbactam 3 g IV every 6 hours + Doxycycline 100 mg orally or IV every 12 hours

Gentamicin is recommended by CDC in regimen B for treatment of PID in lower doses (3-5 mg/kg) when given as once-daily dosing as compared to the usual recommended once daily dose of 5-7 mg/kg. The CDC guidelines emphasize that the use of once-daily dosing of gentamicin has not been evaluated for the treatment of pelvic inflammatory disease, but it is efficacious in analogous situations. Sweet (2011) reported that the dose of 3-5 mg/kg is effective in achieving both clinical and biological cure of $\geq 92\%$ and $\geq 97\%$ respectively against PID, but noted in the publication that the recommended CDC regimens for the treatment of acute PID were producing suboptimal antimicrobial activity against *M genitalium*. In addition, there does not seem to be any literature that gives the recommended peak concentration when dosing gentamicin 3-5 mg/kg once-daily. Only the recommended peak concentration when giving a gentamicin dose 5-7 mg/kg once daily, and this is stated to be $>20 \mu\text{g/mL}$ (Winter 2010). In general, the literature seems to be lacking on data regarding the use

of once-daily dosing of gentamicin in treatment of pelvic inflammatory disease as stated in the CDC guidelines referred to above. There is therefore a potential gap in the knowledge of what the desired serum peak concentrations should be when using gentamicin to treat PID. However, a review of several studies by Ward & Theiler (2008) on the use of gentamicin in obstetrics and gynaecologic patients with conditions including postpartum endometritis, pyelonephritis and PID recommended that a once-daily dose of 5 mg/kg has been shown to be safe, clinically effective and cost saving. Wiesenfeld & Heine (1998) had earlier also done a review of the literature on the use of once-daily dosing of gentamicin in obstetrics and gynaecology and found that a dose of 5 mg/kg once-daily was both efficacious, safe and more cost-effective in such patients. Sunyecz et al. (1998) did a pharmacokinetic study of once-daily dosing with gentamicin in women with postpartum endometritis in which a single daily intravenous dose of 4.5 mg/kg was given to ten women and the peak and trough levels measured using an automated fluorescence polarization analyser. Nephrotoxicity and ototoxicity were also monitored. Sunyecz et al. (1998) found that the mean elimination rate constant was $0.105 \pm 0.008 \text{ Lhr}^{-1}$ and the mean volume of distribution was $0.34 \pm 0.07 \text{ L/kg}$. In addition, the mean peak serum gentamicin concentrations were greater than 11 mg/L whereas the trough concentrations were $<0.3 \text{ mg/L}$. Although it is not stated what the target serum concentrations were taken to be, Sunyecz et al. (1998) concluded that once-daily dosing with gentamicin in women treated against postpartum endometritis achieves therapeutic peak levels without drug accumulation. A pharmacokinetic study by Liu et al. (1999) that compared two single-daily dosing methodologies of gentamicin for postpartum endometritis had target peak serum concentrations of $>12 \text{ mg/L}$ and prolonged trough levels that were under 2 mg/L. One

group (Group-I) of subjects received an intravenous gentamicin dose of 5 mg/kg over 60 minutes. The other group (Group-II) received a dose that was calculated as:

$$Dose = 14mg/L \times 0.35L/kg \times adjusted\ body\ weight(kg) \quad (19)$$

Where 14 mg/L = target peak concentration

0.35 L/kg = volume of distribution

Serum gentamicin levels were obtained 1 hour after the infusion of the second dose and a second sample was taken 8-12 hours after the same dose. The study found that patients in the first group had significantly higher doses and peak drug concentrations as all had concentrations >12 mg/L but 40% of them had peaks that were greater than 20 mg/L. By comparison, 76% of patients in group II had peak levels that were lower than the target of >12 mg/L. Both groups achieved trough concentrations of less than 2 mg/L for more than 12 hours until the next dose.

Liu et al. (1999) concluded that both methods achieved adequate serum gentamicin levels when compared to an earlier study by Del Priore et al. (1996) which had a target peak serum concentration of >5 mg/L. Del Priore et al. (1996) used the same gentamicin dose of 5 mg/kg and achieved peak serum levels as high as 16.6 mg/L.

The literature seems to suggest that the gentamicin dose for PID should be 3-5 mg/kg while other gynaecological conditions should be treated with a 5-7 mg dose and a recommended peak concentration should be ≥ 20 μ g/mL. There seems to be no recommended peak levels in the literature for gentamicin in PID management.

CHAPTER 3

METHODOLOGY

3.1. Study design

This was a quantitative, prospective and observational/non-interventional study. It was a prospective study in that the data collected was from patients who had been admitted in the wards at the time of the study and were started on gentamicin therapy. These patients were recruited and their blood samples taken for analysis. The study was observational/non-interventional in the sense that there was no interference by the researcher in the decisions that involved diagnosis and therapy, the intent of the study was mainly to obtain blood samples and observe the serum gentamicin concentrations achieved by the current dosing strategy as performed in the study centers. Recommendations for adjustment of the dose for an individual patient was not part of this study.

3.2. Study Setting

The Study was conducted at Katutura Intermediate Referral Hospital (KIRH) and Windhoek Central Hospital (WCH), two of the largest hospitals in Namibia. Both the KIRH and WCH are located in the capital city, Windhoek, in close proximity to one another. These two hospitals are also currently serving as teaching hospitals for the purpose of training student and intern doctors and pharmacists, nurses and other health cadres. The obstetrics & gynaecology wards were chosen as the study sites for this study.

3.3. Study Population

The participants of this study were women aged 18 years and older who were admitted in the obstetrics and gynaecology wards 2W of Windhoek Central Hospital and 3A of Katutura Intermediate Referral Hospital. The patients received gentamicin therapy for various disease conditions as diagnosed by the intern doctors or medical officers practicing in those wards. Prospective participants were selected by convenience sampling, whether they were already on gentamicin therapy or were due to start. It did not matter the duration of treatment for the prescribed gentamicin therapy, nor whether the patient was prescribed to receive once daily or multiple daily therapy, only patients who were less than 18 years of age or refused to consent were excluded from this study. Patients whom the researcher deemed not suitable to participate such as those who were psychologically unstable or were in a condition that could not permit them to give informed consent were also excluded from participation.

3.4. Sample size

Over a period of 5 months (from October 2016 to February 2017) a total of 35 women were recruited as participants in this study. This number was regarded sufficient for the purposes of this study as it was merely a feasibility study making use of descriptive statistics. There were no intentions to make an inference of the findings of this study to a larger population.

3.5. Research ethics

The study was approved by the research ethics committees of the University of Namibia and that of the Ministry of Health & Social Services (see appendix B). Only patients who gave informed, written consent were included in this study.

3.6. Study instruments

The details of the participants including name, age, weight, and height and dosage regimen were captured on the Gentamicin Therapeutic Drug Monitoring request form (appendix D). The request form also captured the diagnosis, details regarding infusion and sample collection times.

The blood samples were analyzed using the INDIKO PLUS[®] auto-analyzer housed at the School of Pharmacy, UNAM.

3.7. Procedure

Prospective participants were identified with the help of ward nurses or by going through individual patient prescription charts that were available on the bed-side. Patients who had been initiated on gentamicin therapy were approached to obtain informed consent (see appendix C). Those patients who could not understand English had the study explained to them in the language they understood best through an interpreter. Patients who consented to participate were asked to sign the consent documents, those who did not wish to participate were excluded from the study. The researcher then, proceeded to document patient details such as name, age, date of birth, weight, height, gentamicin dosage regimen and diagnosis.

In most of the patients (33 out of 35) the gentamicin dose was administered as an intravenous infusion in 50mL or 200 mL 0.9% NaCl targeted to run over 30 minutes. The other 2 patients were mistakenly given bolus doses. The time of starting and completing the infusion were noted. For those on once daily dosing (32 patients), a nurse came to draw the first blood sample from the patient at approximately 60 minutes after the infusion was started. Care was exercised not to draw blood from the arm

through which the infusion was given to avoid collecting excessive normal saline. The blood sample was collected by venipuncture into a yellow-top SST™ II plastic tube that contained no anti-coagulant or clotting agent and immediately inverted 5-6 times before storing it in a cooler box to wait for the second sample. For patients on once daily therapy, the second blood sample was drawn at 6-7 hours after the start time of the gentamicin infusion.

For the 3 patients who received multiple daily therapy, their first sample could only be drawn 30 minutes after their fourth dose (infusion) to allow for steady state to be reached and the second sample was drawn 30-60 minutes before the fifth dose. The samples were then transported to the laboratory where serum was transferred into Eppendorf tubes, labeled with the patient number and frozen at -20 °C to await analysis. Each patient was given a unique identification number upon receipt of samples at the laboratory. The first participating patient was assigned number 1, the second number 2, until patient number 35. Before the frozen serum could be analyzed, it was left to stand at room temperature for a few minutes to thaw.

Reagents, calibration standards and controls used for determination of both gentamicin and creatinine concentrations in serum were all sourced from Thermo Scientific. The reagents for gentamicin were reagent 1 (R1) and reagent 2 (R2). The contents of reagent 1 were stated as <0.1% anti-gentamicin monoclonal antibody (mouse) in ≤0.05% sodium azide, and reagent 2 contained <0.4% gentamicin-coated microparticles in ≤0.05% sodium azide. Reagents for creatinine came as reagent A and reagent B. For gentamicin, a set of six calibration standards A to F with respective concentrations (in µg/mL) of 0.0, 0.5, 1.5, 3.0, 6.0 and 10 were run to produce a six-point standard curve (Figure 6). In the case of creatinine, the standard curve was a two-point straight line (Figure 7) that resulted from running double-distilled water which

gave a reading of 0.0 mg/dL and the sCal standard calibrator (calibrator for serum creatinine by Thermo Fischer) which gave a measurement result of 2.69 mg/dL for enzymatic creatinine tests.

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Calibration results

QMS Gen Version number 3.3

Date	19/12/2016	User	Indiko
Time	09:50:41	Software version: 5.3	

Test	QMS Gen	Coeff. of deter.	0.999066
Status	Accepted	Total factor	-32.566
Accepted	19/12/2016 09:37		
Checked	19/12/2016 09:37	Param A	0.334
User name		Param B	1.575
Comment		Param C	3.265
Errors		Param D	-0.025

Cal/Ctrl	Response	Calc. conc.	Given conc.	Lot	Errors
QMS Gen CalA	0.338	0.000	0.000	72429301	
S Gen CalA	0.335	0.000	0.000	72429301	
QMS Gen CalB	0.309	0.629	0.500	72429302	
QMS Gen CalB	0.318	0.473	0.500	72429302	
QMS Gen CalC	0.249	1.562	1.500	72429303	
QMS Gen CalC	0.257	1.433	1.500	72429303	
QMS Gen CalD	0.169	2.949	3.000	72429332	
QMS Gen CalD	0.169	2.948	3.000	72429332	
QMS Gen CalE	0.070	6.236	6.000	72429333	
QMS Gen CalE	0.071	6.163	6.000	72429333	
QMS Gen CalF	0.028	9.850	10.000	72429335	
QMS Gen CalF	0.030	9.630	10.000	72429335	

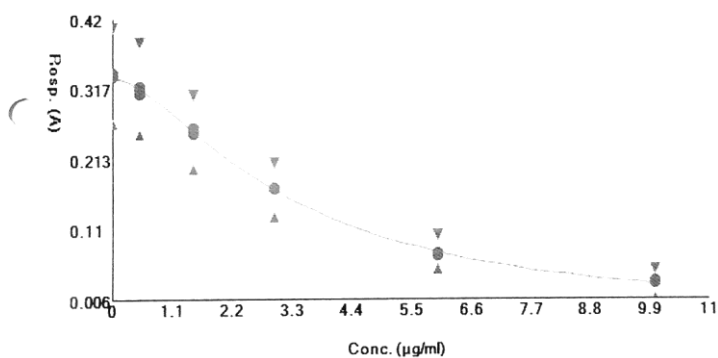


Figure 6 Standard curve for gentamicin obtained after calibration

Calibration results

Crea Enz. Version number 1. 8

Date 19/12/2016 User Indiko
 Time 09:52:56 Software version: 5.3

Test	Crea Enz.	Coeff. of deter.	0.999384
Status	Accepted	Total factor	33.167
Accepted	19/12/2016 09:46		
Checked	19/12/2016 09:46		
User name	Indiko		
Comment			
Errors	Factor limit min Bias limit max		
Factor	33.167		
Bias	0.054		

Cal/Ctrl	Response	Calc. conc.	Given conc.	Lot	Errors
Water	0.053	-0.034	0.000	Default	
Water	0.055	0.034	0.000	Default	
sCal	0.136	2.723	2.690	K396Q	
sCal	0.134	2.657	2.690	K396Q	

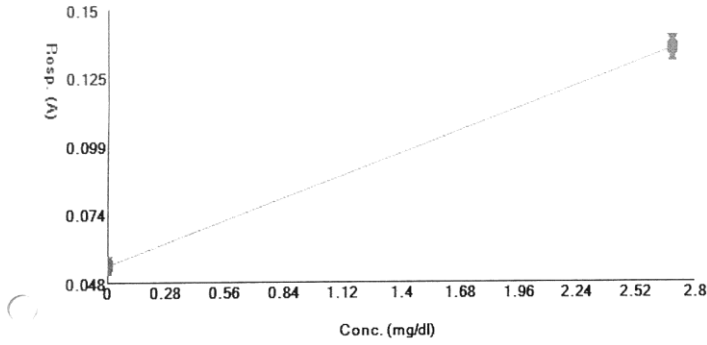


Figure 7 Calibration results for creatinine

MAS[®] ChemTRAK[®] –H liquid assayed chemistry controls, also from Thermo Scientific, were used for the purpose of assuring consistency in the performance of the instrument and reagents. Three controls (1, 2 & 3) of known concentrations and covering the expected concentration ranges were run and their results compared to the given values. These three controls to test for the performance of the gentamicin and creatinine assay were the same so that the performance of both assays was evaluated simultaneously. The control values and the expected ranges are given in Table 8 below:

Table 8 References values for gentamicin and creatinine when performed using MAS[®] ChemTRAK[®] –H liquid assayed chemistry controls.

Control	1		2		3	
	Mean value	Expected range	Mean value	Expected range	Mean value	Expected range
Gentamicin (µg/L)	7.18	5.74-8.61	4.23	3.39-5.08	1.51	1.21-1.81
Creatinine (mg/dL)	0.82	0.66-0.98	4.00	3.20-4.80	7.17	5.73-8.60

Only after the measured control values corresponded to the expected values were the serum samples analyzed for the gentamicin and creatinine concentrations. The highest calibrator had a gentamicin concentration of 10.0 µg/mL. Since the serum concentrations of the first (peak) samples of the once daily dose regimen were all expected to be in ranges of greater than 10 µg/mL, it was necessary to dilute these samples before reanalysis and the obtained serum concentrations were then multiplied by the dilution factor to obtain the actual concentration. In such cases 150 µL of the sample was diluted with 450 µL (dilution factor = 4) of blank human plasma. The

blank human plasma was obtained from the Namibia Blood Transfusion Service (NBTS) and was screened for gentamicin before freezing it in Eppendorf tubes.

The two measured serum gentamicin concentrations were then plotted onto semi-logarithmic paper to yield a serum concentration-time plot. From the straight line, the following information was calculated/deduced (see Figure 8):

- i) the maximum concentration (C_{max}) which was obtained by extrapolation of the line to the time the infusion stopped
- ii) the half-life ($t_{1/2}$) which is the time it takes for the concentration to fall by half
- iii) the elimination rate constant (k_e), that is,

$$k_e = 0.693/t_{1/2} \quad (20)$$

- iv) the volume of distribution (V), that is,

$$V = \frac{k_0 (1 - e^{-k_e t'})}{k_e (C_{max})} \quad (21)$$

- v) the clearance (CL), that is

$$CL = k_e \times V \quad (22)$$

- vi) the area under the serum concentration-time curve (AUC), that is,

$$AUC = Dose/CL \quad (23)$$

- vii) The time it took for the concentration to reach 1 $\mu\text{g/mL}$ was deduced by extrapolating the line to 1 $\mu\text{g/mL}$ line.

The creatinine clearance (CL_{cr}) and Glomerular Filtration Rate (GFR) were calculated using equations 7 and 8, respectively.

The dose for the patient was also calculated in mg/kg . By calculating the BMI, patients who were obese were identified and their weights adjusted accordingly.

Patient 7

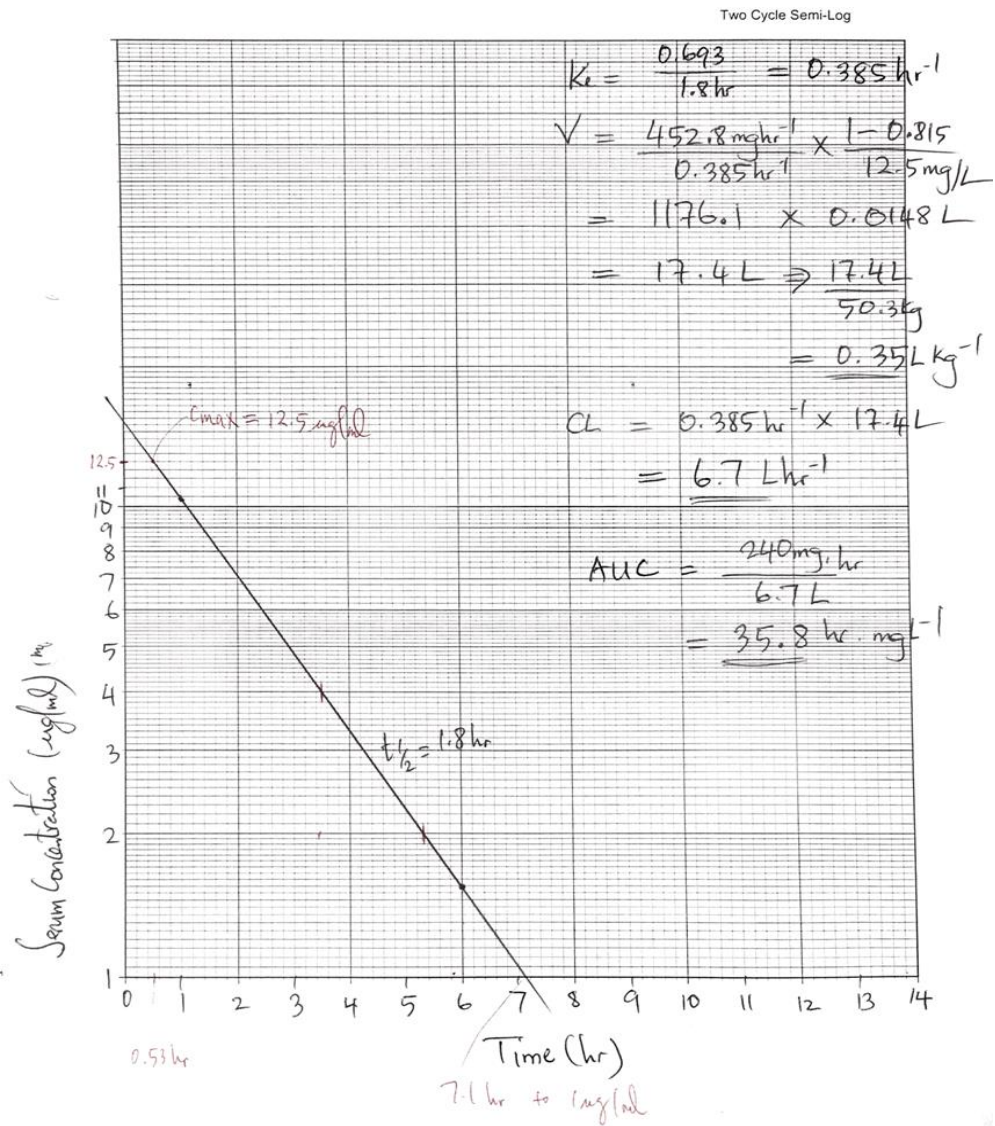


Figure 8 A plot on semi-logarithmic paper. Calculations for various pharmacokinetic parameters are shown

3.8. Statistical analysis

Descriptive statistics (mean, standard deviation, range, coefficient of variation) are used to summarize the results. Using Excel (Microsoft[®]), correlation coefficients between various parameters were tested and a p-value of <0.05 was considered statistically significant.

CHAPTER 4

RESULTS

The study was able to recruit a total of thirty-five (35) participants who were in-patients in the obstetrics & gynaecology wards 2W and 3A of WCH and KIRH, respectively. Sixteen (16) patients were from ward 2W and nineteen (19) were those admitted in 3A.

4.1. Disease profile

All patients were women between the ages of 18 to 49 years who had been initiated on gentamicin therapy for a range of women's health conditions including septic miscarriage and puerperal sepsis, pelvic inflammatory disease, pyelonephritis in pregnancy, miscarriage, ectopic pregnancy and endometritis (Figure 9). Other conditions included pre-eclampsia, abscess and pleural effusion. One of the patients was under treatment after a nephrectomy had been performed.

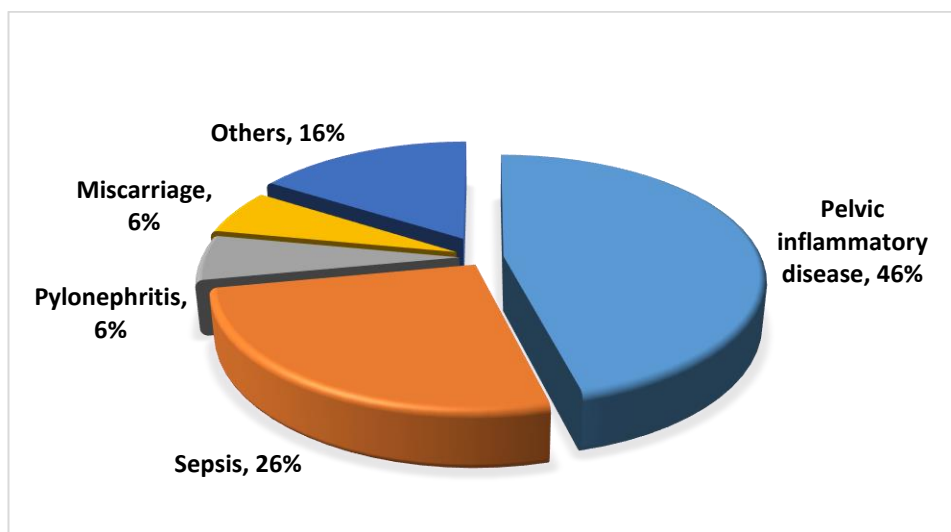


Figure 9 Disease distribution among women receiving gentamicin therapy (n=35)

4.2. Patient population characteristics

The weight for one patient was not measured. Therefore the BMI for this patient could not be determined. The height of the patients was 158 ± 8 cm (range: 136 to 179 cm). Their total body weight was 64.2 ± 18.0 kg. There was a notable variability of individual body weights as they ranged from 35.9 kg to 115.2 kg with a coefficient of variation calculated to be 28.0%. Similarly, the BMI also had a coefficient of variation of 28% with values ranging from 17.3 kg/m^2 to 45.7 kg/m^2 and a mean of $25.9\text{ kg/m}^2 \pm 7.1$. The mean and range for the characteristics of the study participants is summarized in Table 9 below:

Table 9 Patient characteristics

Characteristic	Number of patients	Mean \pm Standard deviation	Range	Coefficient of Variation (%)
Age (years)	35	29 ± 6	18-49	-
Weight (kg)	34	64.2 ± 18.0	35.9-115.2	28.0
Height (cm)	34	158 ± 8	136-179	5.3
BMI (kg/m^2)	34	25.9 ± 7.1	17.3-45.7	27.2

The BMI values reveal that 2 patients were underweight BMI $<18\text{ kg/m}^2$, 16 were of normal weight with a BMI of $18\text{-}24.9\text{ kg/m}^2$, 9 patients were overweight with a BMI of $25.0\text{-}29.9\text{ kg/m}^2$ and 7 patients who had a BMI value equal to or greater than 30 kg/m^2 were classified as obese. The total body weight (TBW) was used to calculate the BMI for the all the patients, including the obese. Figure 10 below depicts this information.

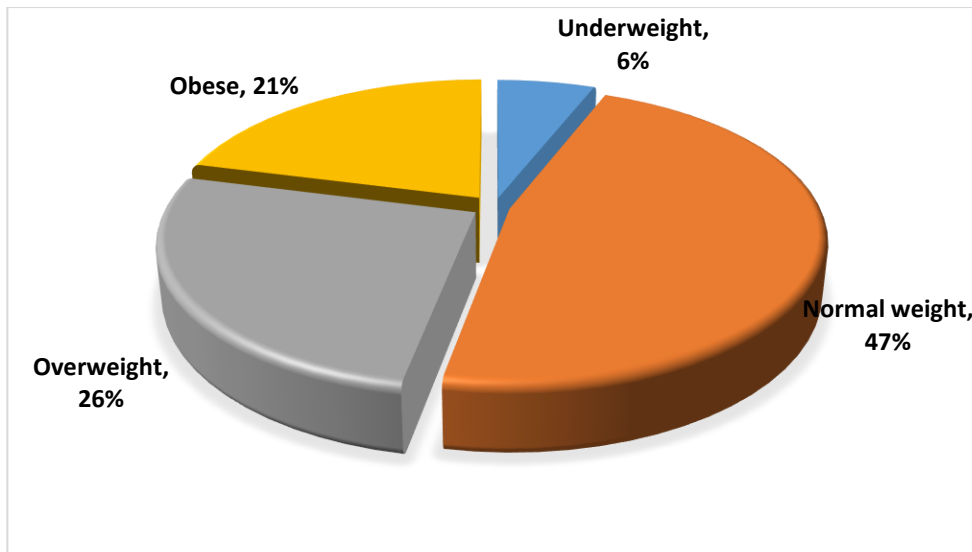


Figure 10 Distribution of BMI values among women receiving gentamicin therapy (n=34)

4.3. Gentamicin doses and serum concentration levels

All patients were given a standard gentamicin dose at both centers of the study. Patients were prescribed to receive either 240 mg gentamicin as a once-daily dose or 80 mg three times a day. The usual mode of administration was by bolus injection, but for this study doses were administered as intermittent intravenous infusions which lasted 36 ± 18 minutes, with a coefficient of variation of 50.7%.

32 of the patients (91%) were on the 240 mg once-daily therapy and only 3 (9%) were on the 80 mg thrice daily regimen, as determined by the physician. Patients were prescribed to be on gentamicin therapy for a duration ranging from 2-14 days, the majority being 2-7 days. Figure 11 is an illustration of the percentages of patients who received therapy for differing periods of time.

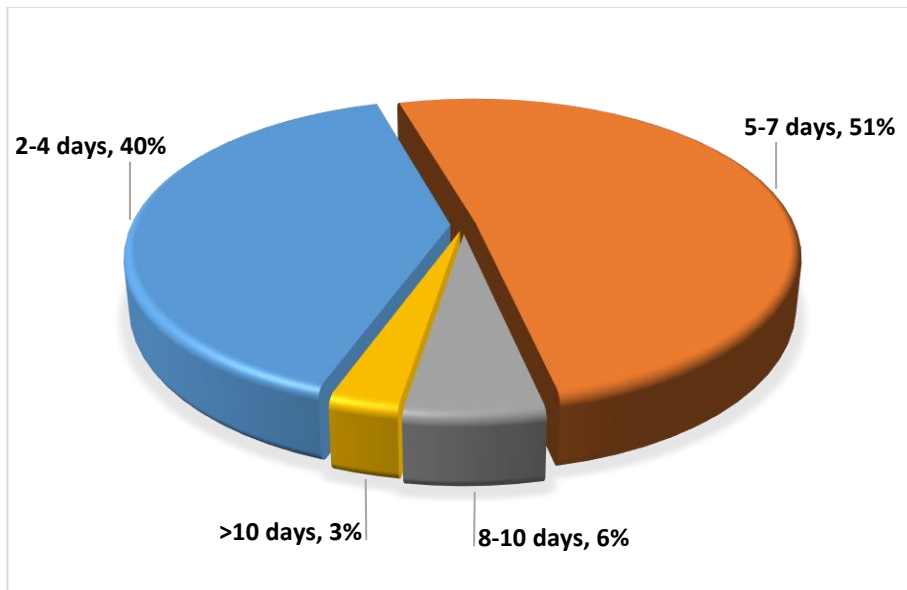


Figure 11 Number of days on gentamicin therapy (n=35)

The dose of gentamicin administered to each patient was calculated in mg/kg by dividing the dose the patient was given with their weight (the adjusted body weight was used in the case of obese patients).

The mean dose for 31 patients on the 240 mg once-daily dose was found to be 4.2 ± 0.8 mg/kg with a coefficient of variation value of 18.0%. The resulting values ranged from 3.1-6.7 mg/kg. The mg/kg doses of the 3 patients on the 80 mg thrice daily dose were 3.3 mg/kg, 4.7 mg/kg and 5.0 mg/kg.

The first and second samples were taken after approximately 1 hour and 6 hours, respectively, following the start of infusion. The C_{\max} values ranged from 7.6-25 $\mu\text{g/mL}$ (see Table 11). The mean C_{\max} was found to be $14.4 (\pm 4.7)$ $\mu\text{g/mL}$ with a coefficient of variation of 32.3%.

Those who received the 240 mg once daily dose and also had complete data for relevant analyses were only 29 (see Table 10). Of these 29 patients, 14 were on treatment for PID and 15 were having non-PID conditions. Only 3 (20.0%) of the non-PID patients had their mg/kg dose falling between 5-7 mg/kg while the rest (80.0%) received doses that were less than the recommended 5-7 mg/kg for once daily dosing (see Figure 14). None of the doses were found to be greater than the recommended 5-7 mg/kg. Figure 12 shows the correlation of serum gentamicin concentrations with respect to total body weight.

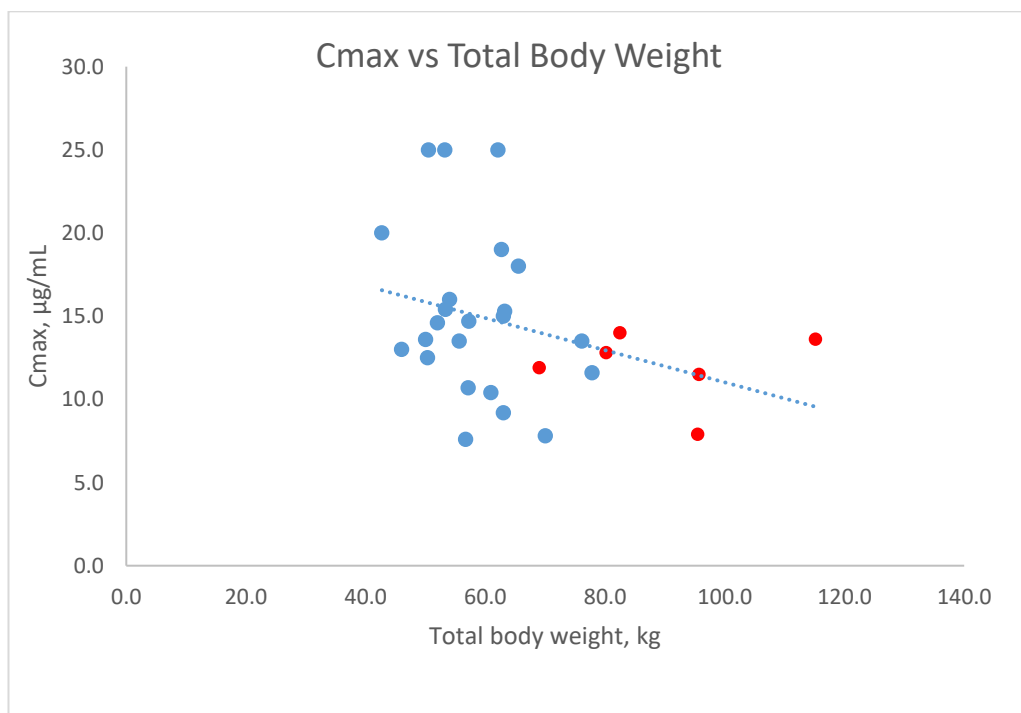


Figure 12 correlation between serum concentration and body weight (obese patients in red) (n=29, $r = 0.34$, $r^2 = 0.11$ and $p = 0.073$)

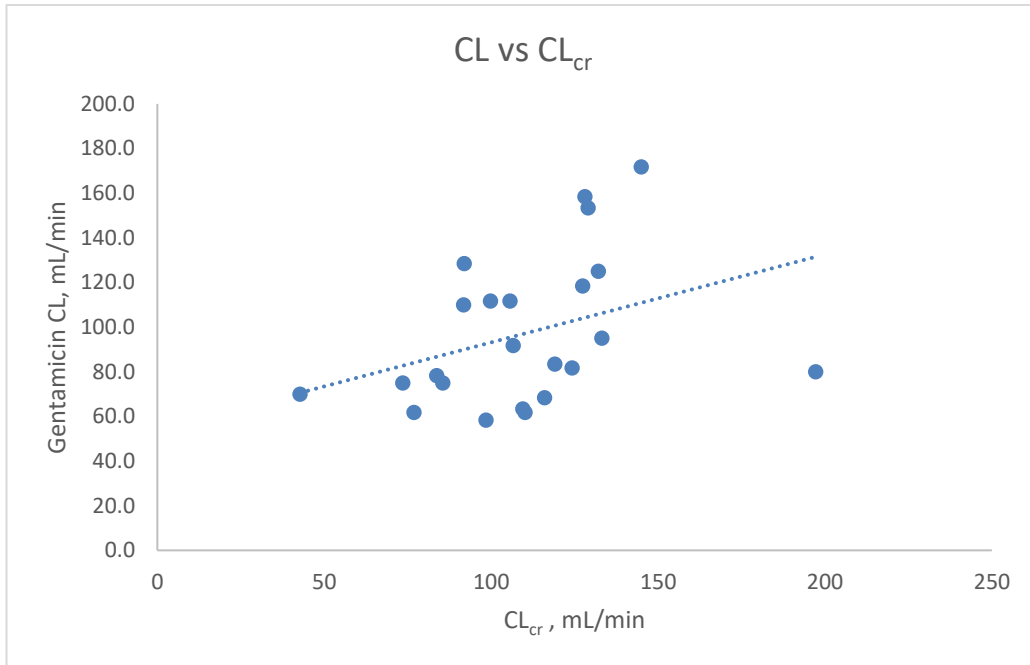


Figure 13 correlation of gentamicin CL with creatinine clearance (n = 23)

Figure 13 Shows the correlation of gentamicin clearance with the creatinine clearance with $r = 0.362$, $r^2 = 0.131$ and a p -value = 0.044.

Table 10 mg/kg doses for 29 patients who received the once daily dose as well as their serum gentamicin concentrations obtained after measurement with the autoanalyser (Only patients who received an infusion, had data of their weight and had a detectable second serum gentamicin concentration were considered).

Patient Number	Diagnosis	Dosage regimen	Dose (mg/kg)	Peak sample Gentamicin ($\mu\text{g/mL}$)	Extrapolated C _{max} ($\mu\text{g/mL}$)	Second sample Gentamicin ($\mu\text{g/mL}$)
4	PID	240 mg OD	4.4	15.7	16.0	1.2
5	PID grade III	240 mg OD	4.8	11.7	13.6	1.6
7	Septic miscarriage	240 mg OD	4.8	10.4	12.5	1.5
8	PID grade II	240 mg OD	3.1	11.6	11.6	1.4
9	Septic miscarriage	240 mg OD	5.2	10.3	13.0	1.1
10	Septic miscarriage	240 mg OD	4.1	9.9	12.8	1.3
11	PID grade II	240 mg OD	4.8	23.7	25.0	2.6
12	Pre-eclampsia	240 mg OD	5.6	18.4	20.0	3.2
13	PID grade II	240 mg OD	3.8	15.1	19.0	2.0
14	Pleural effusion	240 mg OD	3.7	17.5	18.0	3.0
15	PID grade II	240 mg OD	3.8	8.3	9.2	0.5
17	PID grade II	240 mg OD	3.8	12.5	15.0	2.4
18	PID grade II	240 mg OD	3.7	7.0	7.9	2.0
19	PID grade III	240 mg OD	4.4	10.4	11.9	1.3
20	PID grade II	240 mg OD	3.9	19.2	25.0	1.8
21	Puerperal sepsis	240 mg OD	3.2	10.9	13.5	2.2
22	Nephrectomy	240 mg OD	3.6	9.6	11.5	1.5
24	Septic miscarriage	240 mg OD	3.9	11.3	14.0	3.5
25	Pyelonephritis in pregnancy	240 mg OD	4.2	6.0	7.6	1.1
26	Puerperal Sepsis	240 mg OD	3.4	6.5	7.8	1.1
27	Pyelonephritis in pregnancy	240 mg OD	3.9	8.5	10.4	1.4
28	Ectopic Pregnancy	240 mg OD	4.2	8.9	10.7	1.3
29	PID grade II	240 mg OD	2.7	11.4	13.6	2.0
30	PID grade II	240 mg OD	4.5	16.7	25.0	1.4
31	PID grade III	240 mg OD	4.6	13.9	14.6	2.9
32	Threatened Miscarriage	240 mg OD	3.8	12.4	15.3	1.5
33	PID grade II	240 mg OD	4.2	12.7	14.7	2.8
34	Abscess	240 mg OD	4.3	10.0	13.5	1.9
35	Endometritis	240 mg OD	4.5	12.3	15.4	2.8

For the 15 non-PID patients, gentamicin C_{max} concentrations that achieved the recommended levels of 17-25 $\mu\text{g/mL}$, were observed in only 2 of the 15 patients (13.3%). The majority (86.7%) were lower than the target 17 $\mu\text{g/mL}$ (Figure 14).

When the mg/kg doses of the 14 pelvic inflammatory disease (PID) patients who received once daily gentamicin therapy were analyzed separately, all of them (100%) fell within 3-5 mg/kg as recommended by the CDC guidelines. However, there were no guidelines to which the gentamicin concentrations in PID could be compared.

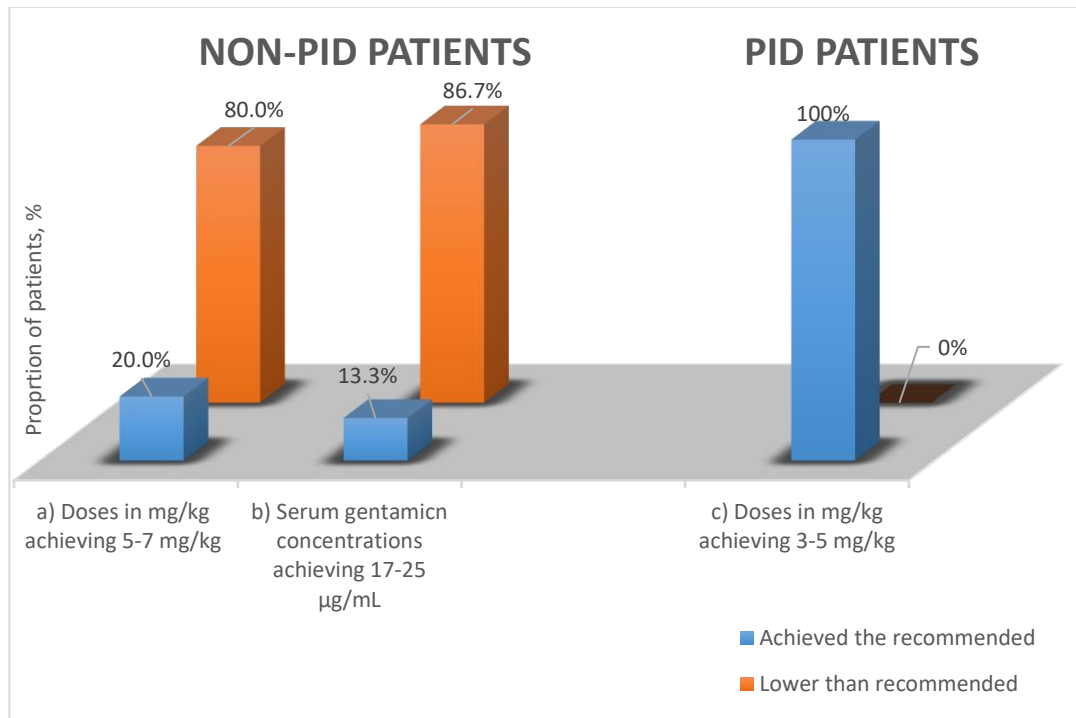


Figure 14 a) Proportion of non-PID patients (n=15) with 240 mg once daily doses falling within 5-7 mg/kg; and b) proportion of non-PID patients with serum gentamicin C_{max} concentrations falling within 17-25 $\mu\text{g/mL}$. c) Shows proportions of PID patients (n=14) who received a dose falling within 3-5 mg/kg. Guidelines for gentamicin peak levels in PID could not be found.

4.4. Pharmacokinetic characteristics

Only 29 of the 32 patients who were on the 240 mg once-daily dose had suitable data to be considered. The C_{max} values ranged from 7.6-25 $\mu\text{g/mL}$. The mean ($\pm\text{SD}$) C_{max} was 14.4 (± 4.7) $\mu\text{g/mL}$ with a coefficient of variation of 32.3%. On average, it took 8.5 hours (range: 5.5-18 hours) for the serum gentamicin concentrations to fall to <1 mg/L . The mean volume of distribution was 16.7 L or 0.29 L/kg. The mean elimination rate constant and half-life were 0.362/hour and 2.0 hours, respectively. The mean creatinine clearance (CL_{cr}) was 101 ± 33 mL/minute. 86% of patients (25) had a CL_{cr} of >66 mL/min. The remaining patients had CL_{cr} values of 59, 56, 43 and 24 mL/min. The mean gentamicin clearance (CL) was 97 mL/min. On average, the AUC was approximately 45 hr.mg/L. Table 11 summarizes this information.

Table 11 Pharmacokinetic parameters of patients on gentamicin once-daily therapy

Parameter	Number of patients	Mean \pm SD	Range	Coefficient of variation (%)
C_{max} ($\mu\text{g/mL}$)	29	14.4 \pm 4.7	7.6-25	32.3
k_e	29	0.362 \pm 0.080	0.231-0.533	23.8
V (L/kg)	29	0.29 \pm 0.11	0.12-0.56	40.3
V (L)	29	16.7 \pm 5.7	8.1-31.6	34.9
$t_{1/2}$ (hr)	29	2.0 \pm 0.4	1.2-3.0	21.9
CL (mL/min)	29	97 \pm 30	3.5-10.3	31.7
AUC (hr.mg/L)	29	45 \pm 13	25-69	29.5

CHAPTER 5

DISCUSSION

Disease profile

Aminoglycosides are considered to be an important class of drugs against obstetrical, gynaecological and postoperative infections, gentamicin being the most commonly used in this clinical settings (Wiesenfeld & Heine, 1998). The results show that the women who participated in this study were receiving gentamicin for treatment against a wide range of conditions such as septic miscarriage and puerperal sepsis, pelvic inflammatory disease, pyelonephritis in pregnancy, miscarriage, ectopic pregnancy and endometritis. Other conditions included pre-eclampsia, abscess, pleural effusion and nephrectomy (see Figure 7). Out of these 11 conditions, 8 (73%) are purely of gynaecological in nature. These findings which seem to suggest a common use of gentamicin in obstetric & gynaecological conditions are similar to what was found in the retrospective review study by Kushner et al. (2016) which reported that of the 1237 adult patients with ages 18-44 years who received gentamicin, 1050 (85%) were female patients who were being treated mostly for gynaecological conditions. The common use of gentamicin as empirical therapy against an array of conditions is due to its low cost and its known efficacy in the treatment of many serious gram-negative bacillary infections.

Gentamicin doses and serum concentrations

There was a minimal variation among patient heights (CV 5.3%, n=35). However, there was a wide range in body weights, 35.9-115.2 kg (n=35), whose coefficient of variation (28%) is close to that of the BMI (27.2%) (Table 8). Over one-quarter (n=35)

of the patients (26%) were classified as obese, this is a substantial number especially with respect to gentamicin dosing which is recommended to be based on body weight. The high proportion of obese patients and the large difference in patient weights as reported in this study suggest that the 240 mg once daily standard dosing of gentamicin may not be appropriate. It would be better to individualize the initial doses using the mg/kg method. All patients classified as obese received sub-therapeutic doses for non-PID conditions as calculated using their adjusted body weight. The usual concern with obese patients is the possibility of overdosing when the dose is approximated using the mg/kg without adjusting for body weight. However, in this case the obese patients were under-dosed because the standard dose of 240 mg was too low. This is evident across all patients of differing body weight classifications.

In addition, only 20% of non-PID patients (n=15) received doses (in mg/kg) that were high enough to achieve the recommended 5-7 mg/kg for once daily dosing. As a result, the percentage of patients whose peak serum gentamicin concentrations fell within the recommended levels of 17-25 $\mu\text{g/mL}$ was also only 13.3% (Figure 13). It is well established that there is an increase in volume of distribution and clearance in obese patients (Blouin et al., 1985). This could have contributed to the low C_{max} values in these patients (Cheymol, 2000; Velissaris et al., 2014).

The mean \pm standard deviation for the C_{max} ($14.4 \pm 4.7 \mu\text{g/mL}$) achieved by the average dose of 4.2 mg/kg is very similar to that of $14.2 \pm 7.7 \mu\text{g/mL}$ obtained by a 4 mg/kg dose as reported by Nicolau et al. (1995) in their ground-breaking study. The trough levels (serum concentrations at 23-24 hours after dose administration) were in essence 0.0 $\mu\text{g/mL}$ since on average, it took 8.5 hours for the serum gentamicin concentrations to fall below 1 $\mu\text{g/mL}$. This means that the gentamicin dose was almost

completely cleared within 9 hours, leaving the post-antibiotic effect to take effect for the next 15 hours. This is the hallmark of the once daily dosing method of gentamicin. This 15 hour drug-free period agrees with the recommended minimum of 4-6 hours (Levy & Bauer, 1986; Winter, 2010). The wide range of C_{max} values observed (7.4-25.0 mg/mL) could be explained by interindividual variability in volume of distribution ($r^2 = 0.756$).

The mean AUC of 45 ± 13 hr.mg/L (n=29) is much lower than the expected 70-100 hr.mg/L for gentamicin (Begg et al., 1995). The AUC is a function of the dose and the clearance (equation 23). Since the gentamicin clearance (5.8 L/hr or 97 mL/min) was found to closely approximate the creatinine clearance (101 mL/min) as expected, it is safe to conclude that the low AUC values were as a result of the low gentamicin dose given. Furthermore, the half-life (2.0 ± 0.4 hours) and the volume of distribution (0.29 ± 0.11 L/kg) were very close to the expected 2-3 hours and 0.31 ± 0.10 L/kg, respectively (Thummel et al., 2011). The correlation between the dose given to the patient and the resulting serum concentrations (Figure 12) therefore demonstrates that the dose that a patient receives determines the systemic concentrations of the drug and therefore the efficacy of therapy. When a patient receives a dose that is sub-therapeutic, the therapeutic systemic concentrations are not achieved and this may lead to ineffective treatment or antibiotic resistance. The rationale for using the 5-7 mg/kg once daily dosing strategy is to achieve peak serum levels more than 10 times greater than 2 µg/mL break-point minimum inhibitory concentration (MIC) for the wide range of gram-negative and some gram-positive microorganisms (Kashuba et al., 1999; Begg et al., 1992; Davis, 1987; Ebert & Craig, 1990; Keating et al., 1979; Moore et al., 1987). This translates to peak serum concentrations ≥ 20 µg/mL (Nicolau et al, 1995). The 5-7 mg/kg dose is therefore proven to be both safe and efficacious. It is also known

that the duration of the post-antibiotic effect of aminoglycosides, which is the ability of the drug to kill bacteria even when the concentrations are below the MIC, is concentration dependent (MacDougal & Chambers, 2011).

PID cases

It is of special interest that all patients (n=14) who received gentamicin therapy against PID had mg/kg doses that were within the CDC recommended 3-5 mg/kg once daily (specifically for PID) (CDC, 2015). All patients improved and were subsequently discharged. The 240 mg dose therefore seems to be appropriate when treating PID patients. Mitchell & Malavika (2013) mention three studies (Hemsell et al., 1994; The European Study Group, 1992; Walters & Gibbs, 1990) that have reported on the efficacy of CDC-recommended treatment regimens (clindamycin 900 mg IV thrice daily + gentamicin 3-5 mg/kg once-daily) for inpatient and outpatient management of PID.

However, there is a lack of literature on the gentamicin peak serum concentrations that are achieved by the 3-5 mg/kg PID dosing. It appears that the serum levels (7.9-25.0 µg/mL) reported in this study for doses between 3-5 mg/kg against PID could be among the very few ever reported. This study does provide some notable evidence that the 240 mg once daily strategy is appropriate for use against PID. However, a study demonstrating the antimicrobial effect of the peak serum concentrations achieved by this dosing strategy and a treatment outcomes study could be useful to validate the use of this regimen. Despite the 240 mg once daily dose falling within the recommended dose range for PID, it remains sub-therapeutic for all the other conditions that make

up 54% of the diagnoses. Therefore, the 240 mg standard dose may be acceptable for PID patients but certainly not for the non-PID patients.

It would have been interesting to measure the serum creatinine of all participants at the end of their therapy to see whether there was any reduction in their kidney functions, especially for those who were on therapy for 5 days and more. The four patients with CLcr of 59, 56, 43 and 24 (in mL/min) who did not have a CLcr of ≥ 66 mL/min translate into GFR values of 89.9, 71.9, 51.4 and 51.7 (in mL/min/1.73 m²), respectively when calculated using MDRD equation (see equation 8). Two of the patients fell into the category mild decrease in GFR (60-89 mL/min/1.73 m²), the other two patients had a kidney function that would be classified as moderate decrease in GFR (30-59 mL/min/1.73 m²) at the beginning of their therapy. There were no patients who would have been categorized as having a severe decrease in GFR (15-29 mL/min/m²) nor kidney failure (<15 mL/min/1.73 m²). These findings are not surprising as the average age of the patients was 29 years with the youngest being 18 and the eldest being 49 years, i.e. all women of child-bearing age who are most likely to be admitted in the gynecology & obstetrics ward. This is not to say that kidney failure is entirely dependent on age, only that it is less common for kidney failure to occur in younger ages than in the elderly.

CHAPTER 6

CONCLUSIONS

The gentamicin dosing strategy employed in the gynaecology & obstetrics wards of the KIRH and the WCH is majorly the use of a 240 mg once daily standard dose. The 80 mg thrice daily dosing is used very rarely. Dosing is done empirically without determining kidney function nor is gentamicin monitoring done. All patients were prescribed a total dose of 240 mg per 24 hours normally administered via IV bolus. Although for the purposes of this study the doses were administered as an IV infusion.

The mean C_{max} was 14.4 ± 4.7 µg/mL with values ranging from 7.6-25 µg/mL, the recommended levels of 17-20 µg/mL were achieved in only 13.3% of patients. In addition, the 5-7 mg/kg dosing range recommended for once daily gentamicin therapy was achieved in only 20% of the non-PID patients, the rest (80%) were sub-therapeutic. The 240 mg once daily regimen was able to achieve the CDC (2015) recommended 3-5 mg/kg once daily for all patients who were on gentamicin therapy against pelvic inflammatory disease. However, there seems to be no specifically recommended peak serum concentrations for the 3-5 mg/kg dosing strategy. This study could be one of the very few to report on the serum gentamicin levels achieved by the use of 240 mg standard dose against PID and other conditions. It would be of benefit for further studies that can evaluate the antibacterial effectiveness of the 3-5 mg/kg dose against PID and also report on the treatment outcomes of this dose.

CHAPTER 7

RECOMMENDATIONS

As a result of this study, the following are recommended:

- a) Discontinue the use of 240 mg standard dosing of gentamicin for all obstetrics & gynaecology patients
- b) Adopt the use of mg/kg dosing strategy when deciding the initial gentamicin dose. 5-7 mg/kg once-daily should be the strategy for non-PID patients. Where possible, serum creatinine should be determined in order to guide the initial dose.
- c) Monitor serum gentamicin peak concentrations to ensure safety and efficacy.
- d) Studies to evaluate the antimicrobial effectiveness and treatment outcomes of the 3-5 mg/kg once daily regimen for PID should be done.

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APPENDICES

APPENDIX A: University of Namibia ethical clearance



STUDENT ETHICAL CLEARANCE CERTIFICATE

Ethical Clearance Reference Number: SOM/107/2016

Date: 12 August, 2016

This Ethical Clearance Certificate is issued by the University of Namibia Research Ethics Committee (UREC) in accordance with the University of Namibia's Research Ethics Policy and Guidelines. Ethical approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the Faculty/Centre/Campus Research & Publications Committee sitting with the Postgraduate Studies Committee.

Title of Project: THERAPEUTIC DRUG MONITORING OF GENTAMICIN IN ADULT PATIENTS AT KATUTURA STATE HOSPITAL AND WINDHOEK CENTRAL HOSPITAL

Nature/Level of Project: MASTERS

Principal Researcher: B.S. SINGU

Student Number : 200308556

Host Department & Faculty: School of Medicine

Main Supervisor : Prof. R. Verbeeck (Main) Mr. M. Mubita (Co)

Take note of the following:

- (a) Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the UREC. An application to make amendments may be necessary.
- (b) Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the UREC.
- (c) The Principal Researcher must report issues of ethical compliance to the UREC (through the Chairperson of the Faculty/Centre/Campus Research & Publications Committee) at the end of the Project or as may be requested by UREC.
- (d) The UREC retains the right to:
 - (i). withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,
 - (ii). request for an ethical compliance report at any point during the course of the research.

UREC wishes you the best in your research.

A handwritten signature in black ink, appearing to read "H. M. Kapenda".

Dr. H. M. Kapenda

Director: Centre for Research and Publications

ON BEHALF OF UREC

APPENDIX B: Ministry of Health & Social Services ethical clearance



REPUBLIC OF NAMIBIA

Ministry of Health and Social Services

Private Bag 13198
Windhoek
Namibia

Ministerial Building
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Tel: 061 – 203 2562
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E-mail: hngombe@gmail.com

OFFICE OF THE PERMANENT SECRETARY

Ref: 17/3/3

Enquiries: Mrs. H. Nangombe

Date: 28 September 2016

Mr. Bonifasius Siyuka Singu
P.O. Box 50989
Bachbreth
Windhoek
Namibia

Dear Mr. Singu

Re: Therapeutic Drug Monitoring of Gentamicin in Adult Patients at Katutura State Hospital and Windhoek Central Hospital.

1. Reference is made to your application to conduct the above-mentioned study.
2. The proposal has been evaluated and found to have merit.
3. **Kindly be informed that permission to conduct the study has been granted under the following conditions:**
 - 3.1 The data to be collected must only be used for academic purpose;
 - 3.2 No other data should be collected other than the data stated in the proposal;
 - 3.3 Stipulated ethical considerations in the protocol related to the protection of Human Subjects should be observed and adhered to, any violation thereof will lead to termination of the study at any stage;

- 3.4 A quarterly report to be submitted to the Ministry's Research Unit;
- 3.5 Preliminary findings to be submitted upon completion of the study;
- 3.6 Final report to be submitted upon completion of the study;
- 3.7 Separate permission should be sought from the Ministry for the publication of the findings.

Yours sincerely,


Andreas Mwoombola
Permanent Secretary



"Health for All"

APPENDIX C: Informed consent form



PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

TITLE OF THE RESEARCH PROJECT:

THERAPEUTIC DRUG MONITORING OF GENTAMICIN IN GYNECOLOGY & OBSTETRICS PATIENTS AT KATUTURA STATE HOSPITAL AND WINDHOEK CENTRAL HOSPITAL

UREC NUMBER: (For Official Use)

THE PRINCIPAL INVESTIGATOR: MR BONIFASIUS SINGU

PO BOX 50989

BACHBRECT

WINDHOEK

+264 61 203 5052/ +264 81 644 3763

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the Research Ethics Committee at The University of Namibia and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and Namibian National Research Ethics Guidelines.

1. What is this research study all about?

This study is being conducted at both the Katutura State Hospital and at Windhoek Central Hospital. The total number of participants that will be recruited for this study is 30 from the two hospitals combined.

The aim of this research is to ascertain that the amount of the medicine that you are being injected with is within the acceptable range. If the levels are not within the range, the doctor will be advised to adjust the dose accordingly. We are interested in ensuring that you receive the right dose for your treatment.

You will be treated as any usual hospital patient. The doctors and nurses will care for you as normal. The doctor has decided to treat you with a medicine called gentamicin. 30 minutes after you receive the gentamicin injection, a nurse will come and draw a blood sample from you. We will then take your blood sample to the laboratory to measure the level of the medicine in your blood. We will need to take at least two blood samples from you in one day. After we have measured the blood levels, we will report back to the doctor to adjust the dose if necessary.

Our study involves any adult patient who has been prescribed by the doctors to be treated with gentamicin for a duration of not less than seven days. We do not expect to have a lot of patients and therefore we will approach every suitable candidate as soon as we identify them.

The gentamicin that you are being treated with was the choice of the doctor who is treating you. We have no influence in the doctor's decision to treat you with this medicine. Our only involvement is to measure the levels in the blood.

2. Why have you been invited to participate?

The interest of this study is to measure the blood levels in patients who are being treated with a medicine called gentamicin. The patients are required to be over the age of 18 years and have been prescribed to be on this medication for five days or more. We have identified you as having met the criteria to be part of the study.

3. What will your responsibilities be?

We do not have much to ask from you, we only request your permission for us to take blood samples from you so we can determine the levels of gentamicin. The blood samples will be taken at specific times only, we therefore request your availability when the time comes for a sample to be taken.

4. Will you benefit from taking part in this research?

The purpose of measuring the levels of the medication in the blood is to ensure you are exposed to a safe dosage of this medicine. It will ensure you the dose is high enough to kill the bacteria but also low enough to prevent any unwanted side effects that are known to be caused by gentamicin. These side effects may include damage to your kidneys and your hearing ability.

5. Are there any risks involved in your taking part in this research?

We have identified no risks that you will suffer as a result of taking part in this study.

6. If you do not agree to take part, what alternatives do you have?

If you disagree to take part in this study, you will still receive your treatment from the hospital. You will not lose any privileges of state health care.

7. Who will have access to your (medical) records?

The information collected will be treated as confidential and protected. If it is used in a publication or thesis, your identity as a participant will remain anonymous. Your name and personal details will not be divulged to any other person.

8. What will happen in the unlikely event of some form injury occurring as a direct result of your taking part in this research study?

We only require to draw blood samples from you, this will be done by a trained nurse.

9. Will you be paid to take part in this study and are there any costs involved?

The study will take place while you remain admitted in the hospital. No payments will be made to you for participating in the study.

10. Is there anything else that you should know or do?

- a) *You should inform your family practitioner or usual doctor that you are taking part in a research study. (Include if applicable)*
- b) *You should also inform your medical insurance company that you are participating in a research study. (Include if applicable)*
- c) *You can contact **Mr Bonifasius Singu** at tel **061 206 5052** if you have any further queries or encounter any problems.*
- d) *You can contact the **Health Research Ethics Committee** at **+264 061 2063061** if you have any concerns or complaints that have not been adequately addressed by your study doctor.*
- e) *You will receive a copy of this information and consent form for your own records.*

11. Declaration by participant/patient

By signing below, I agree to take part in a research study entitled THERAPEUTIC DRUG MONITORING OF GENTAMICIN IN GYNECOLOGICAL & OBSTETRICS PATIENTS AT KATUTURA STATE HOSPITAL AND WINDHOEK CENTRAL HOSPITAL.

I declare that:

- a) I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- b) I have had a chance to ask questions and all my questions have been adequately answered.
- c) I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- d) I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- e) I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) On (*date*) 2016.

.....
Signature of participant/patient

.....
Signature of witness

12. Declaration by doctor/intern

I declare that:

- I explained the information in this document to
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use an interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.*)

Signed at (*place*) On (*date*) 2016.

.....
Signature of doctor/intern

.....
Signature of witness

13. Declaration by interpreter (if necessary)

Ideclare that:

- a) I assisted the doctor/intern (*name*) to explain the information in this document to (*name of participant*) using the language medium of (*Oshiwambo, Otjiherero, Afrikaans, etc.*).....

<p>For Official use</p> <p>Comments:</p> <p>.....</p> <p>.....</p> <p>.....</p> <p>.....</p> <p>.....</p> <p>.....</p>

Original Consent Forms must be made available to the Centre for Research and Publications upon request

APPENDIX D: Data collection tool



Gentamicin Therapeutic Drug Monitoring Request Form

Contact: BS Singu BSc (UNAM) BPharm (Nairobi)
 081 644 3763 | 061 206 5052 | bsingu@unam.na
 School of Pharmacy, University of Namibia

(For lab use only)
TDM request no:

DATE: _____

SAMPLE: BLOOD

PATIENT INFORMATION

SURNAME:		FIRST NAME:	
SEX	Male <input type="checkbox"/>	Female	<input type="checkbox"/>
DATE OF BIRTH:		AGE:	
WEIGHT (kg):		HEIGHT (cm):	
WARD:		HOSPITAL:	

DIAGNOSIS:			
DOSAGE REGIMEN	Dose:	Frequency:	Duration:
DATE AND TIME OF MOST PREVIOUS DOSE			
Date:		Time:	

INFUSION	Time started:	Time completed:
SAMPLE Peak (first sample)	Date taken:	Time taken:
Second sample	Date taken:	Time taken:

NB: Peak sample should be taken 1 hour after starting infusion. Second sample should be taken 6-14 hours after the infusion

REQUESTING DOCTOR	
Name:	Contact number:

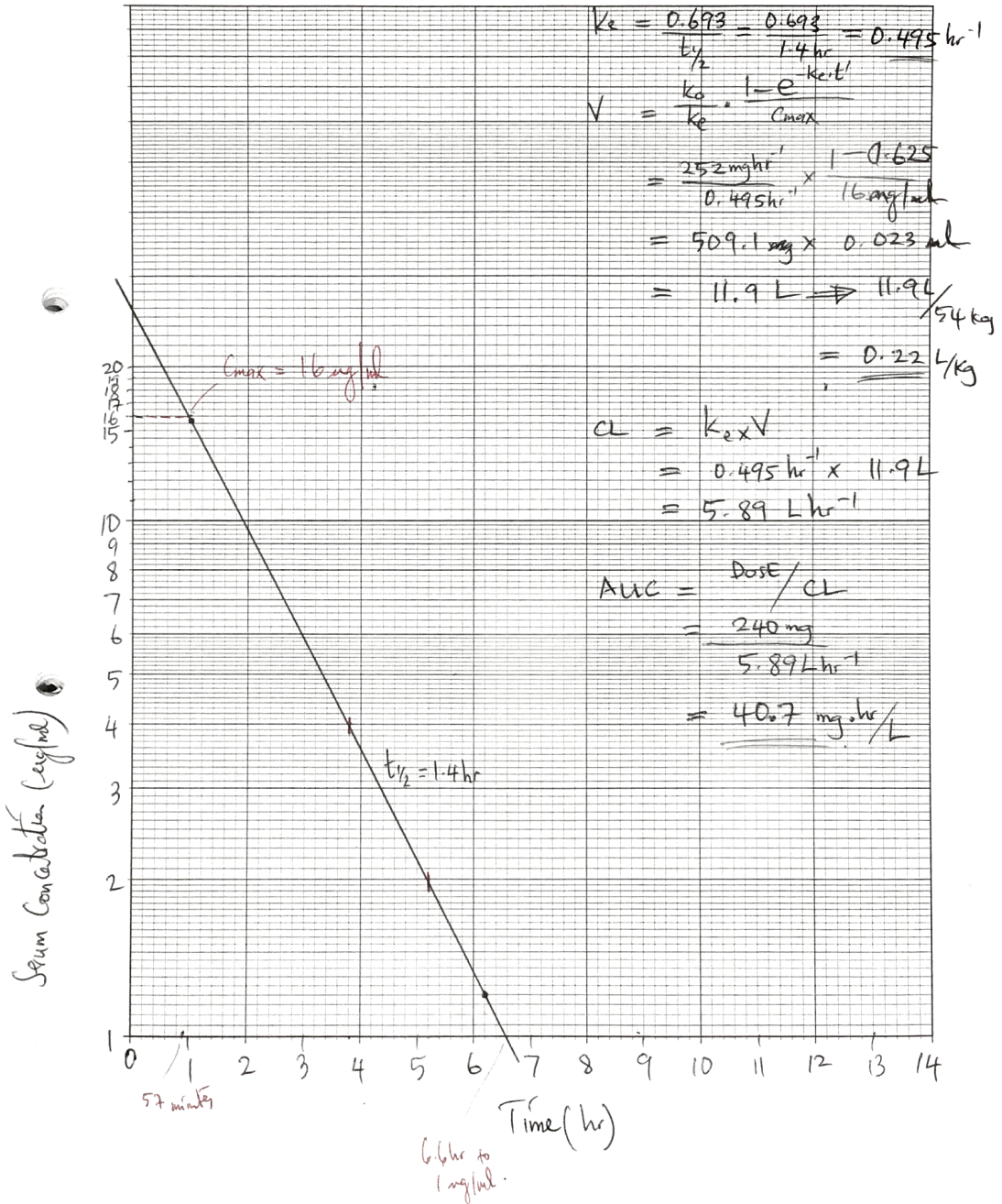
For laboratory use only

Serum Creatinine: _____	Gentamicin concentration: _____
DBW:	
k _e :	
Tau (τ):	
k ₀ :	

APPENDIX E: Semi-logarithmic plots

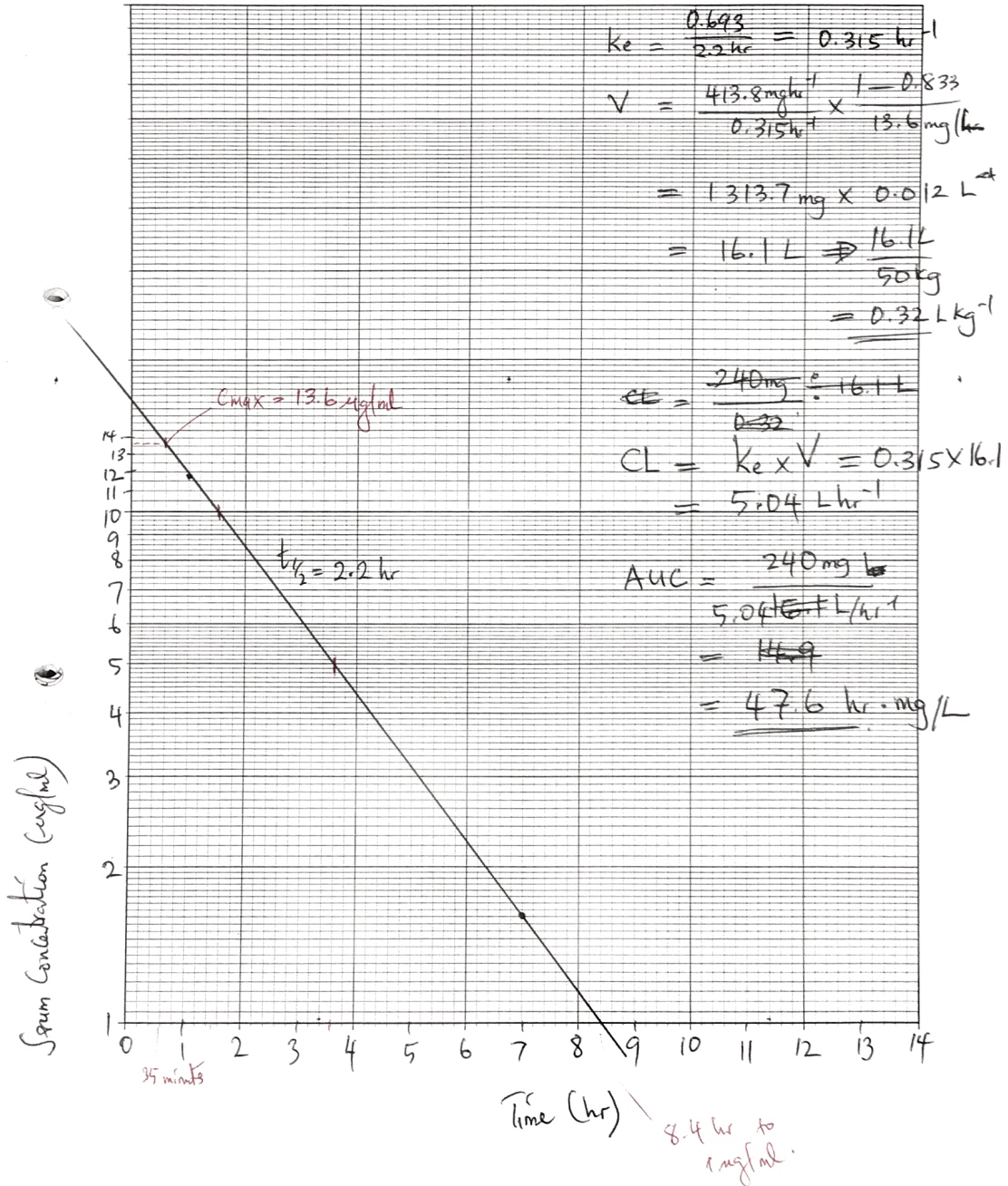
Patient 4

Two Cycle Semi-Log



Patient 5

Two Cycle Semi-Log



$$k_e = \frac{0.693}{2.2 \text{ hr}} = 0.315 \text{ hr}^{-1}$$

$$V = \frac{413.8 \text{ mg hr}^{-1}}{0.315 \text{ hr}^{-1}} \times \frac{1 - 0.833}{13.6 \text{ mg/hr}}$$

$$= 1313.7 \text{ mg} \times 0.012 \text{ L}^{-1}$$

$$= 16.1 \text{ L} \Rightarrow \frac{16.1 \text{ L}}{50 \text{ kg}}$$

$$= 0.32 \text{ L kg}^{-1}$$

$$CE = \frac{240 \text{ mg}}{0.32} = 16.1 \text{ L}$$

$$CL = k_e \times V = 0.315 \times 16.1$$

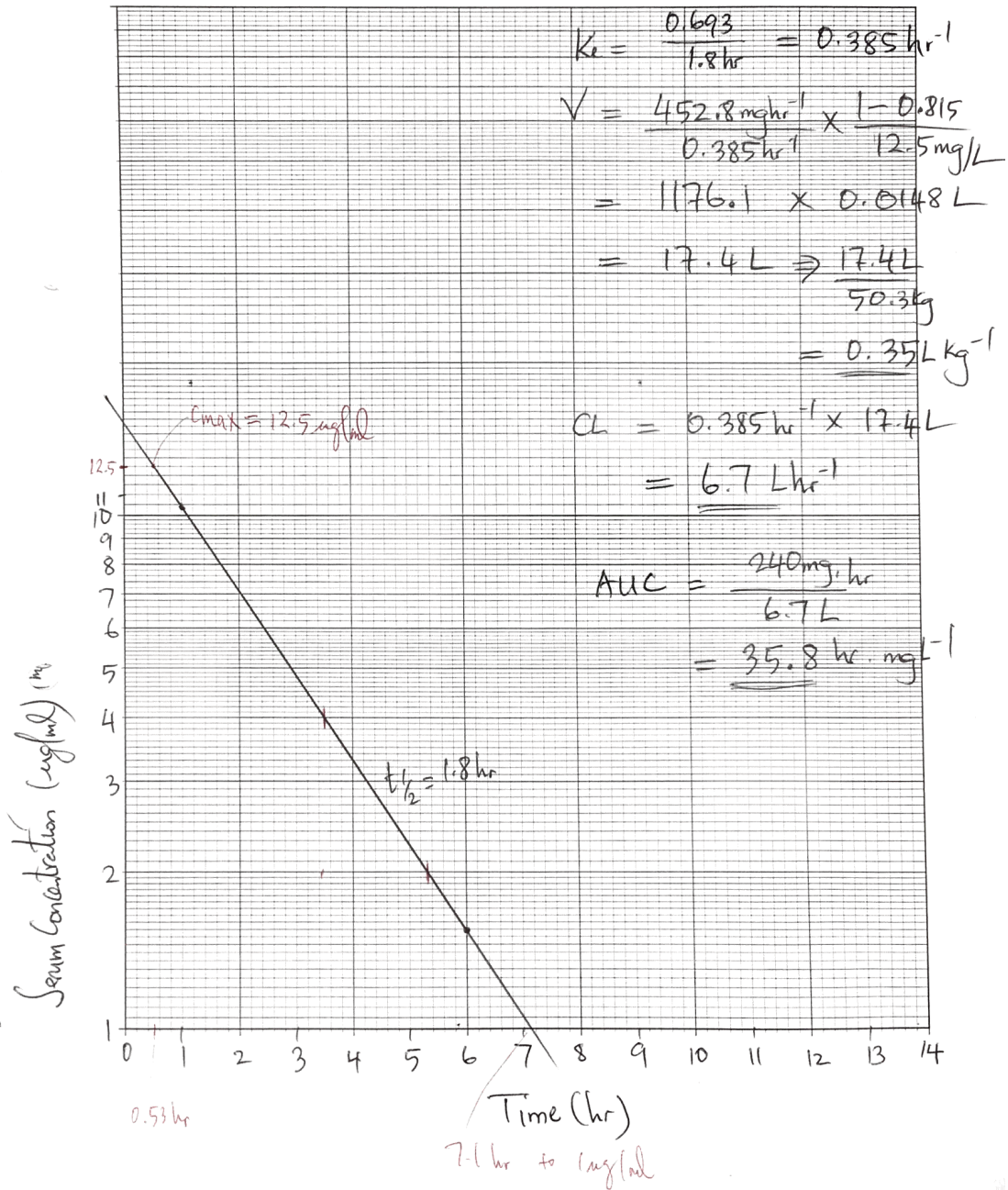
$$= 5.04 \text{ L hr}^{-1}$$

$$AUC = \frac{240 \text{ mg}}{5.04 \text{ L hr}^{-1}}$$

$$= 47.6 \text{ hr} \cdot \text{mg/L}$$

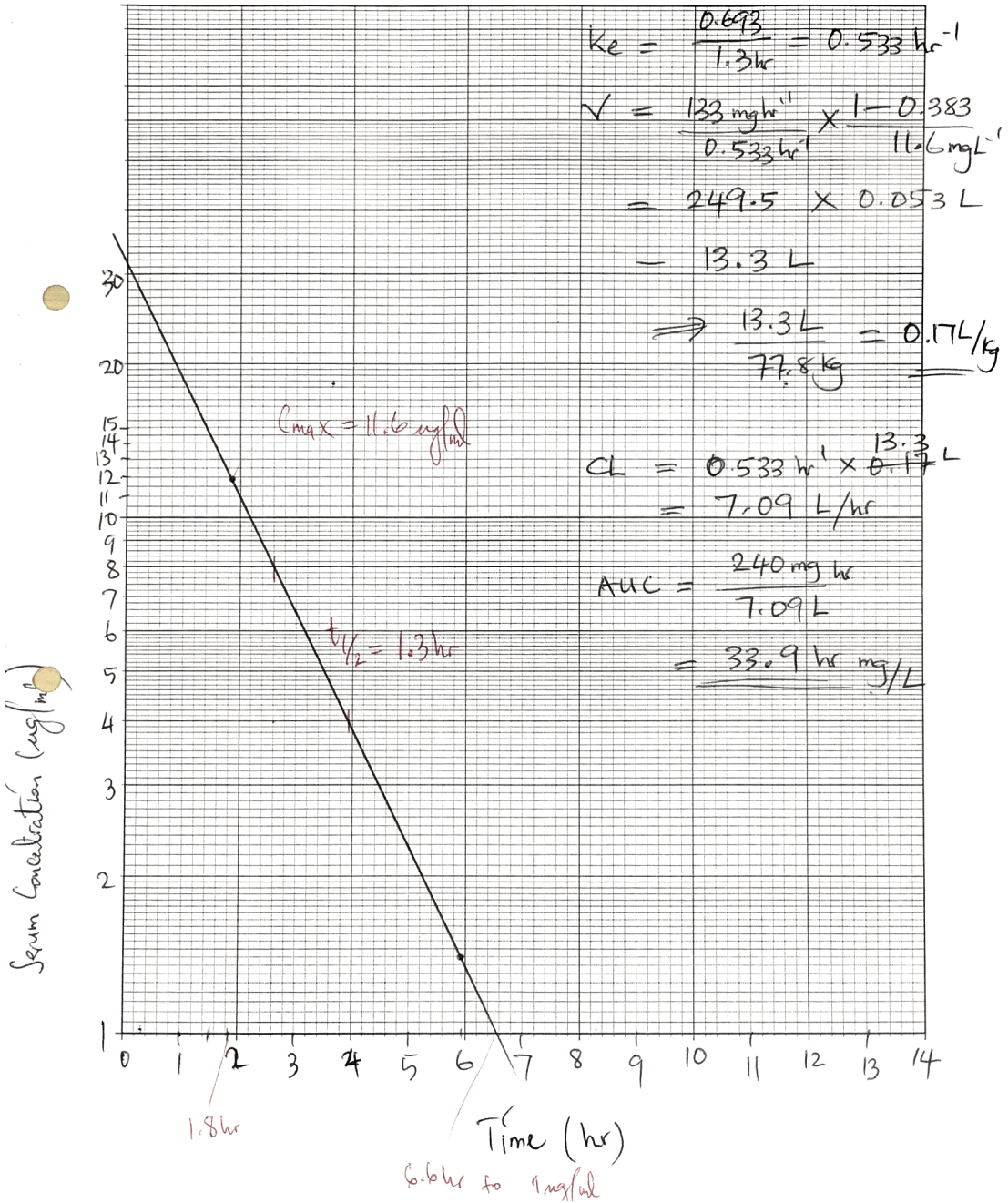
Patient 7

Two Cycle Semi-Log

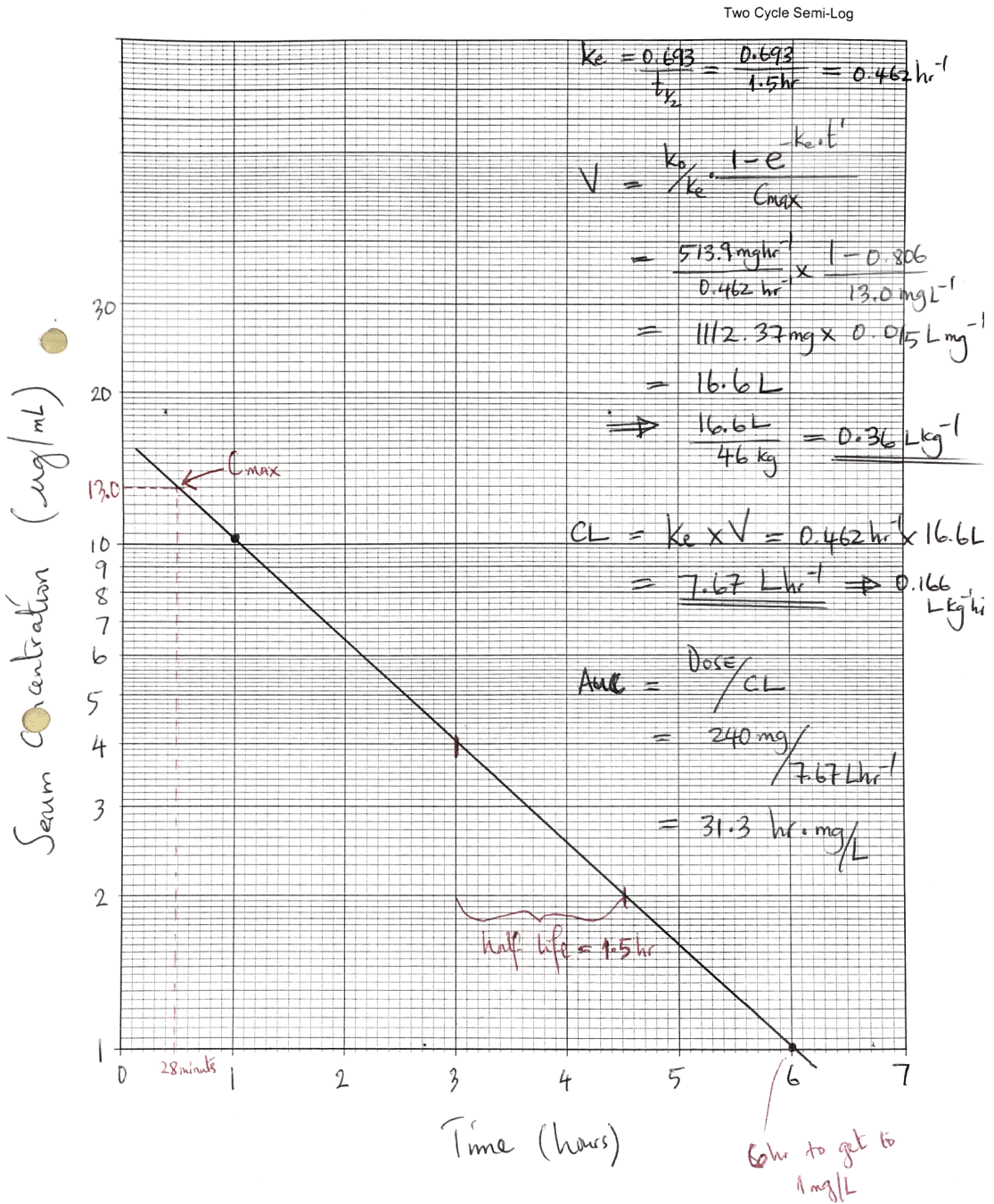


Patient 8

Two Cycle Semi-Log

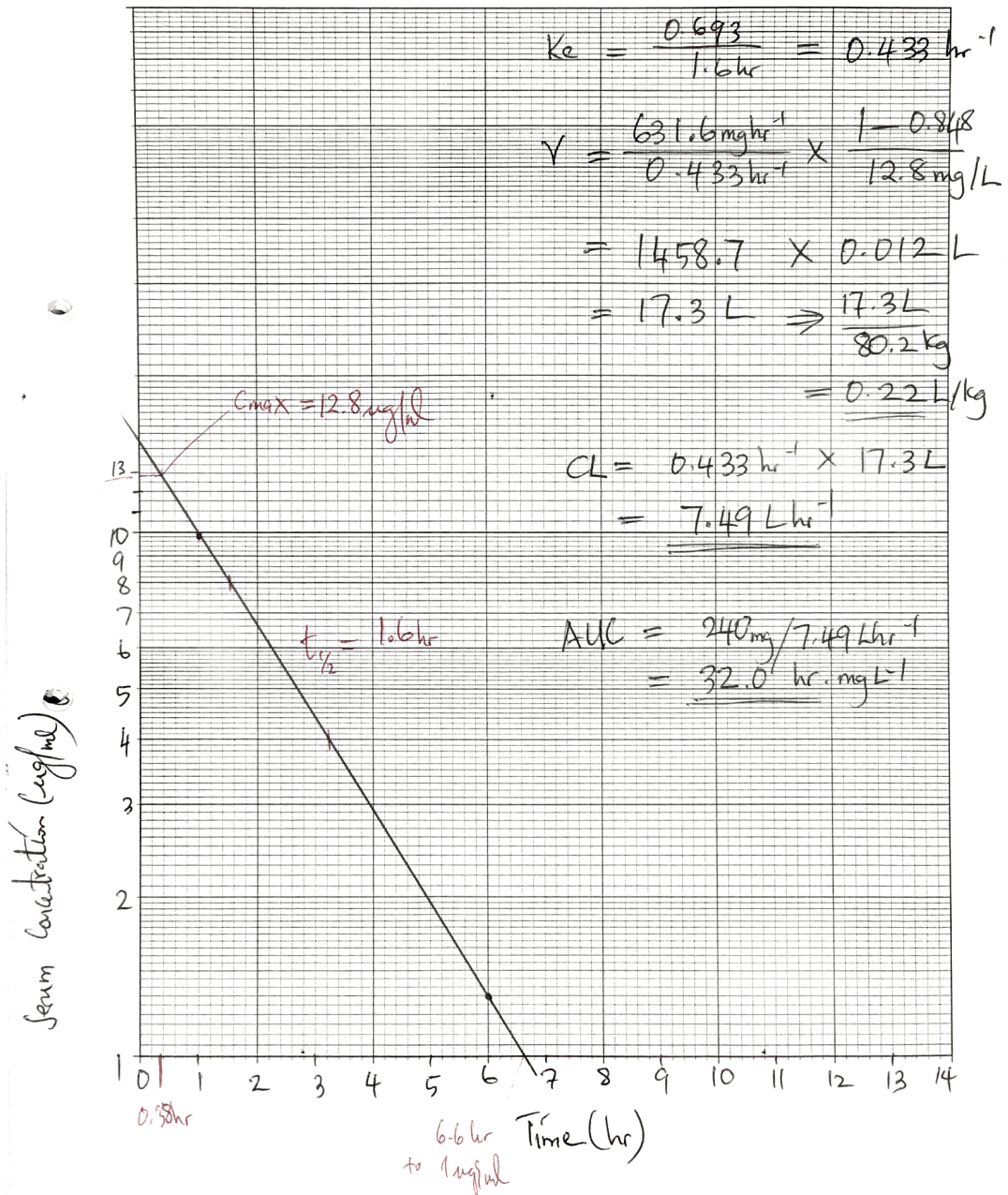


GERUM CONCENTRATION - TIME PLOT FOR PATIENT 9.



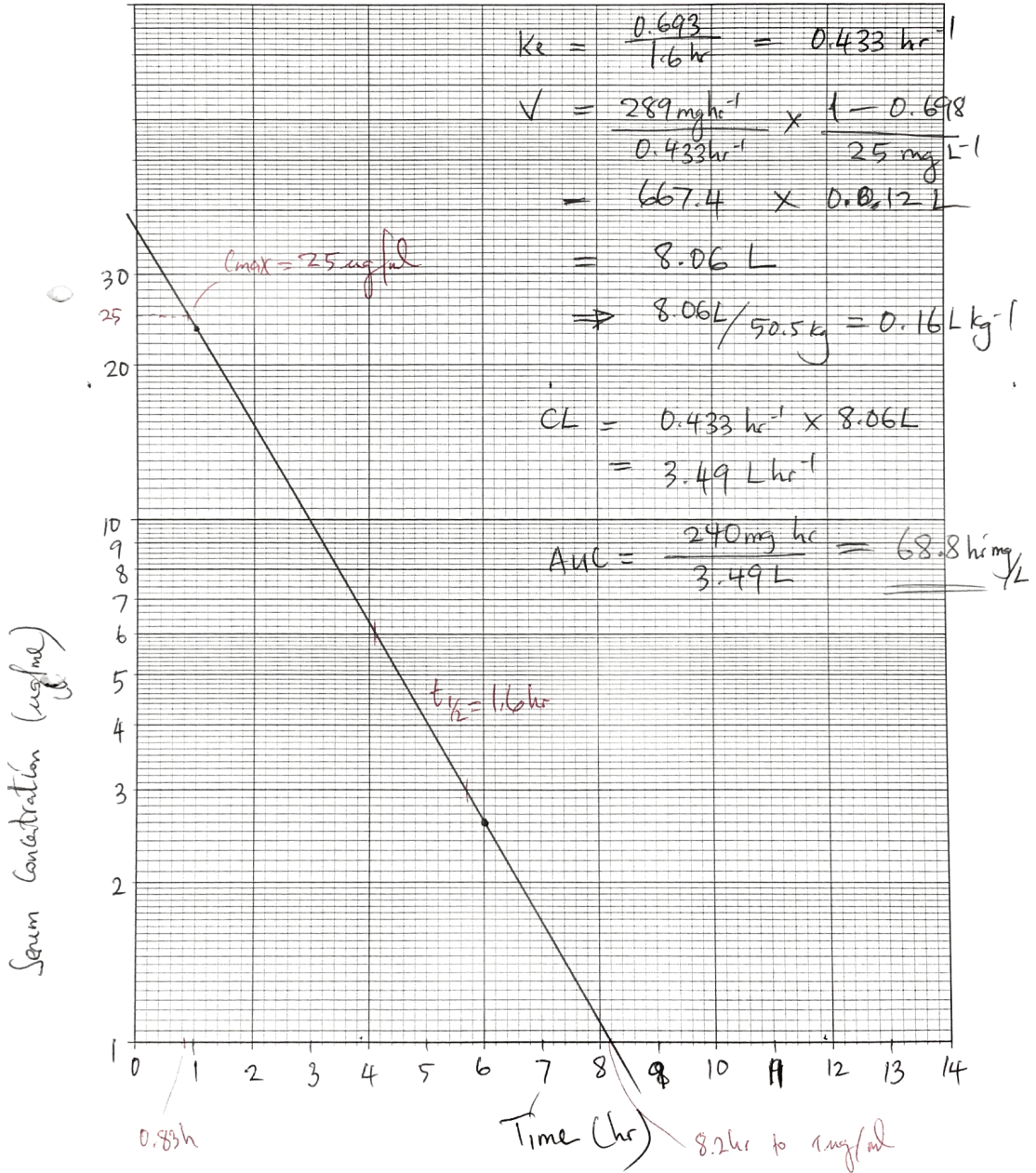
Patient 90

Two Cycle Semi-Log



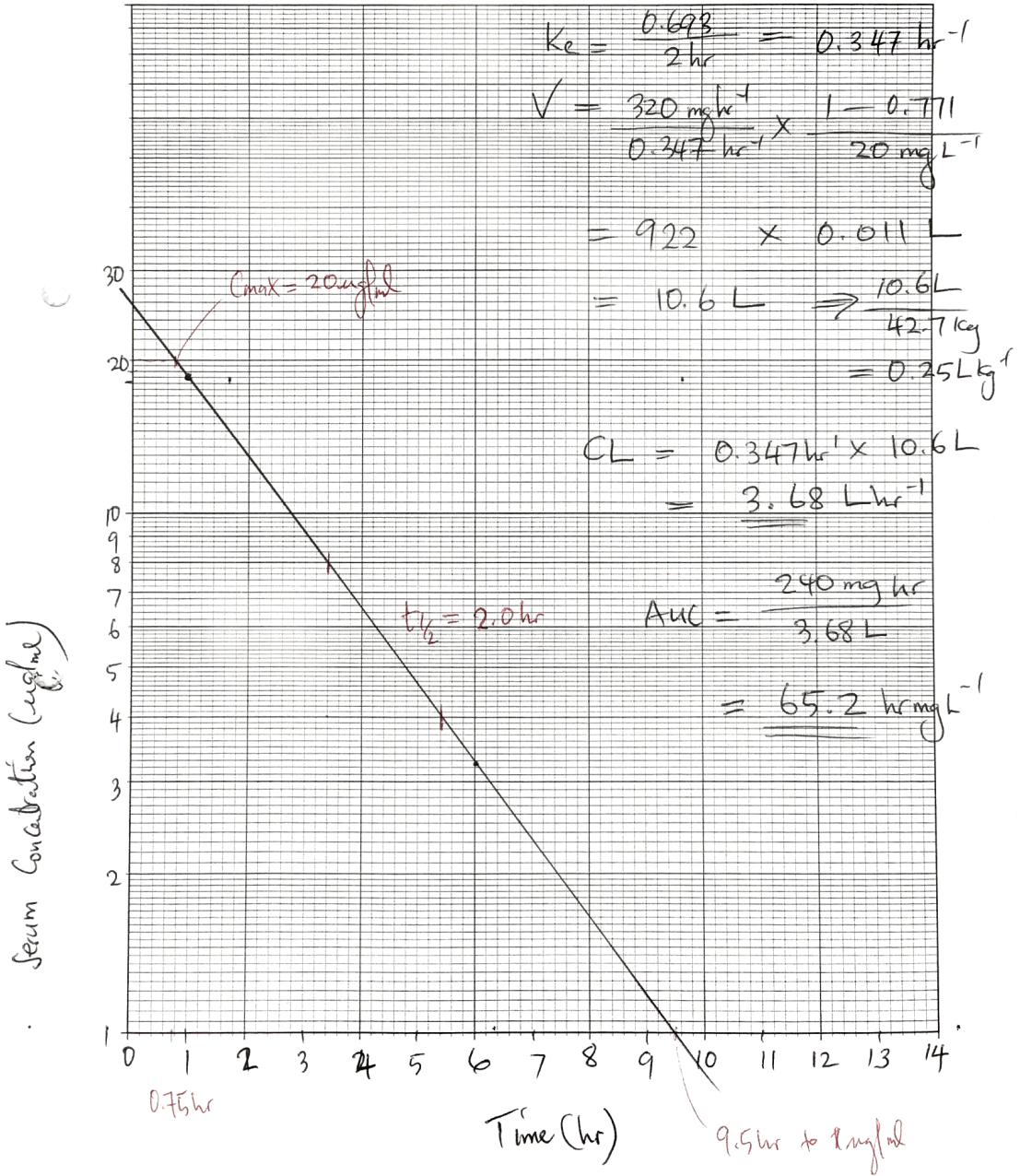
Patient 11

Two Cycle Semi-Log



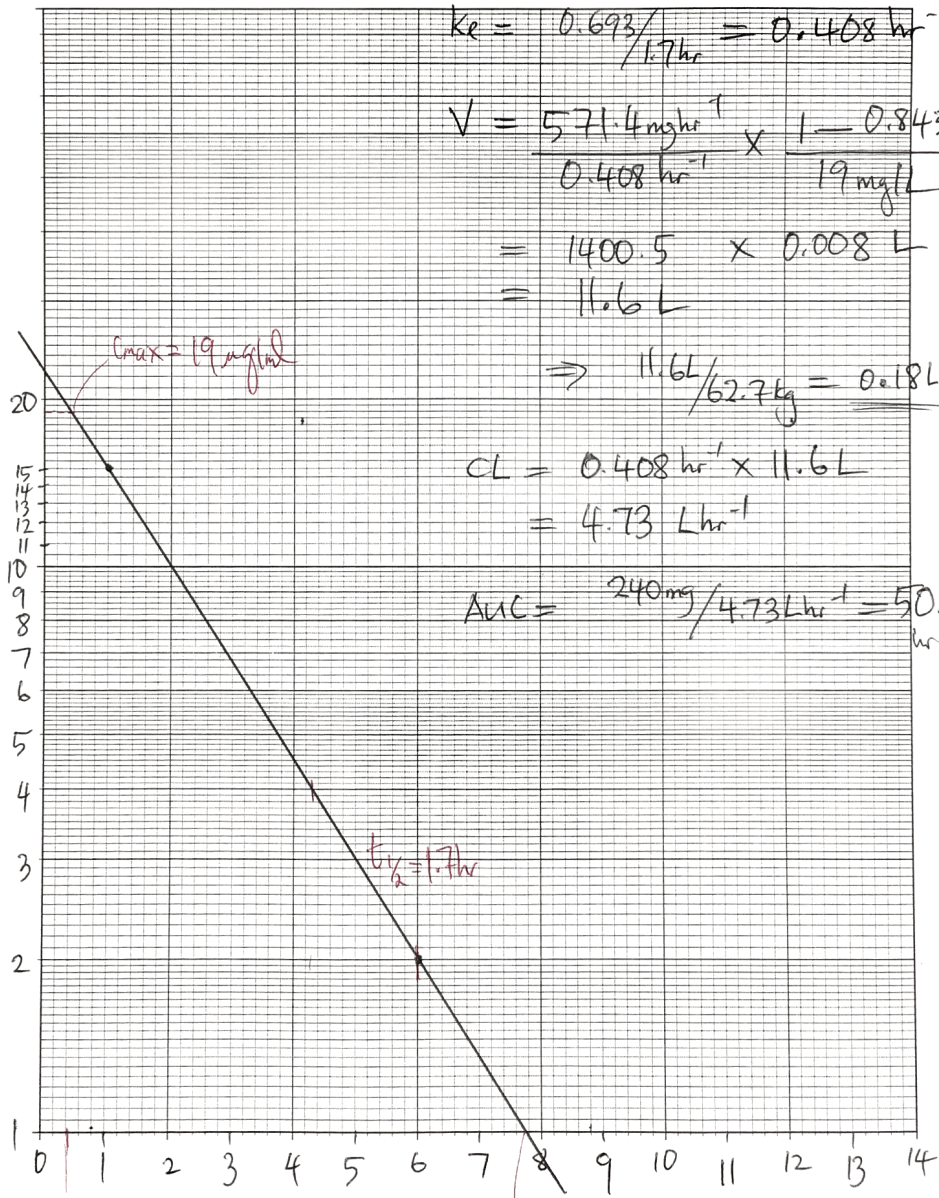
Patient 12

Two Cycle Semi-Log



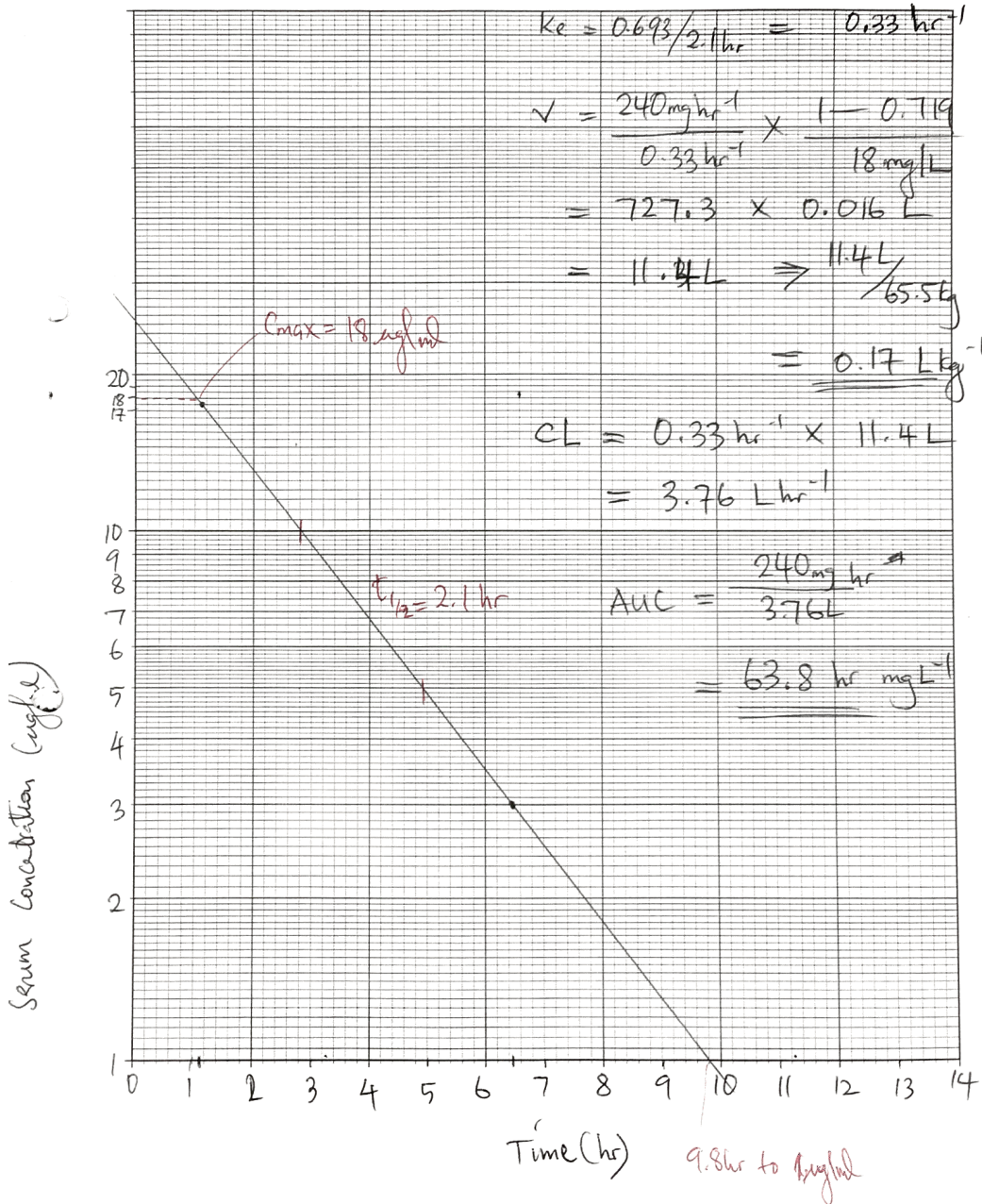
Patient 13

Two Cycle Semi-Log

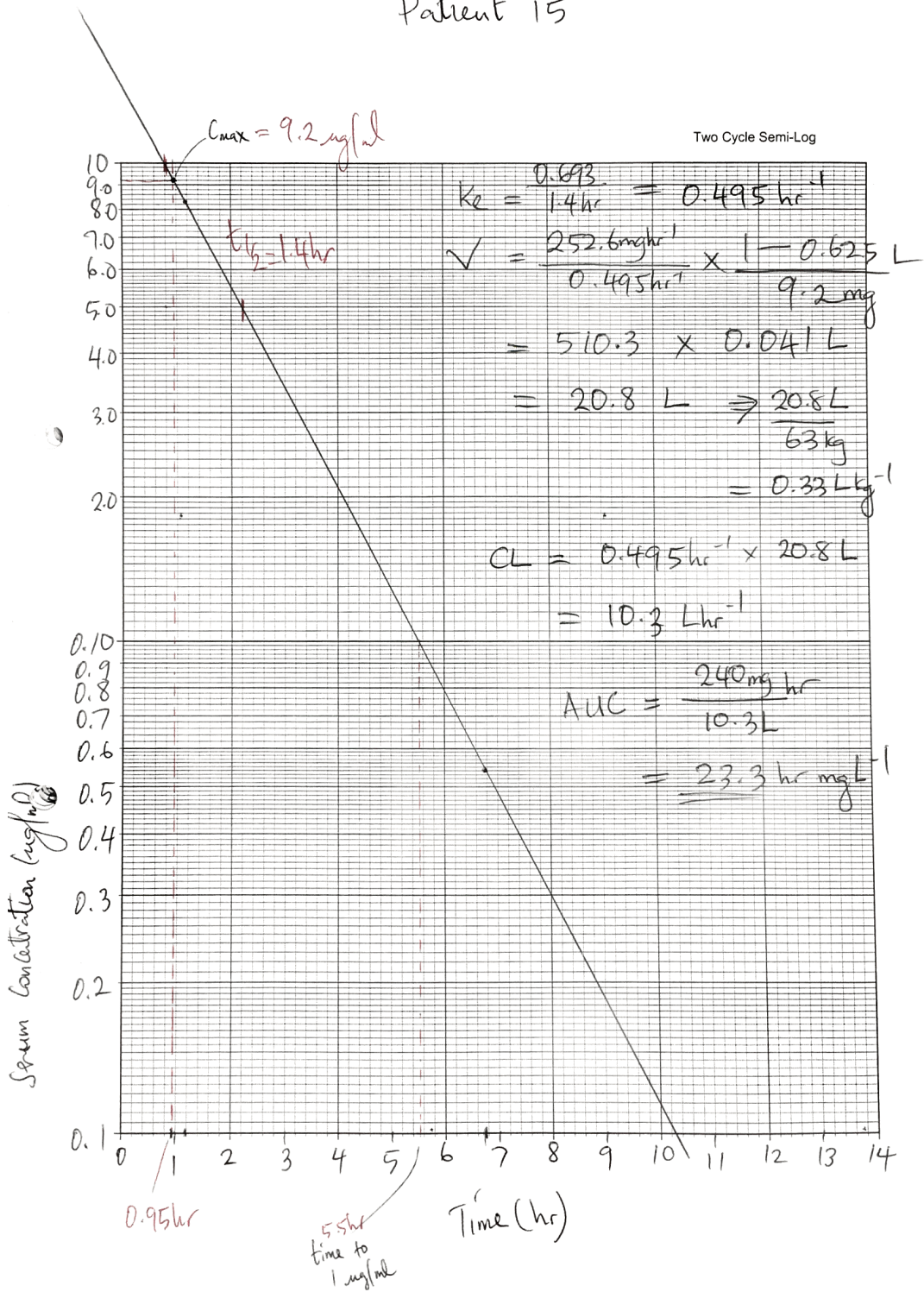


Patient 14

Two Cycle Semi-Log

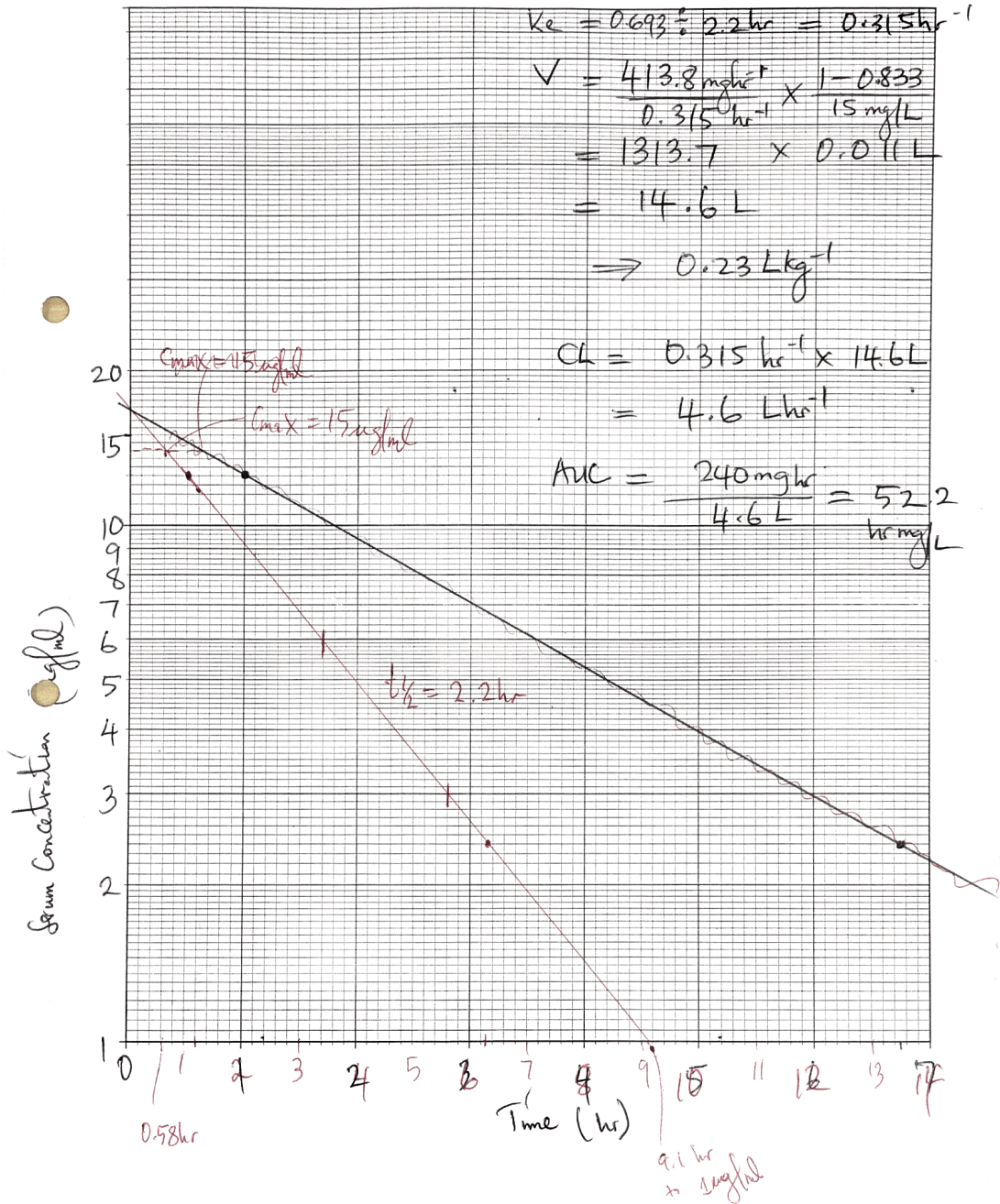


Patient 15

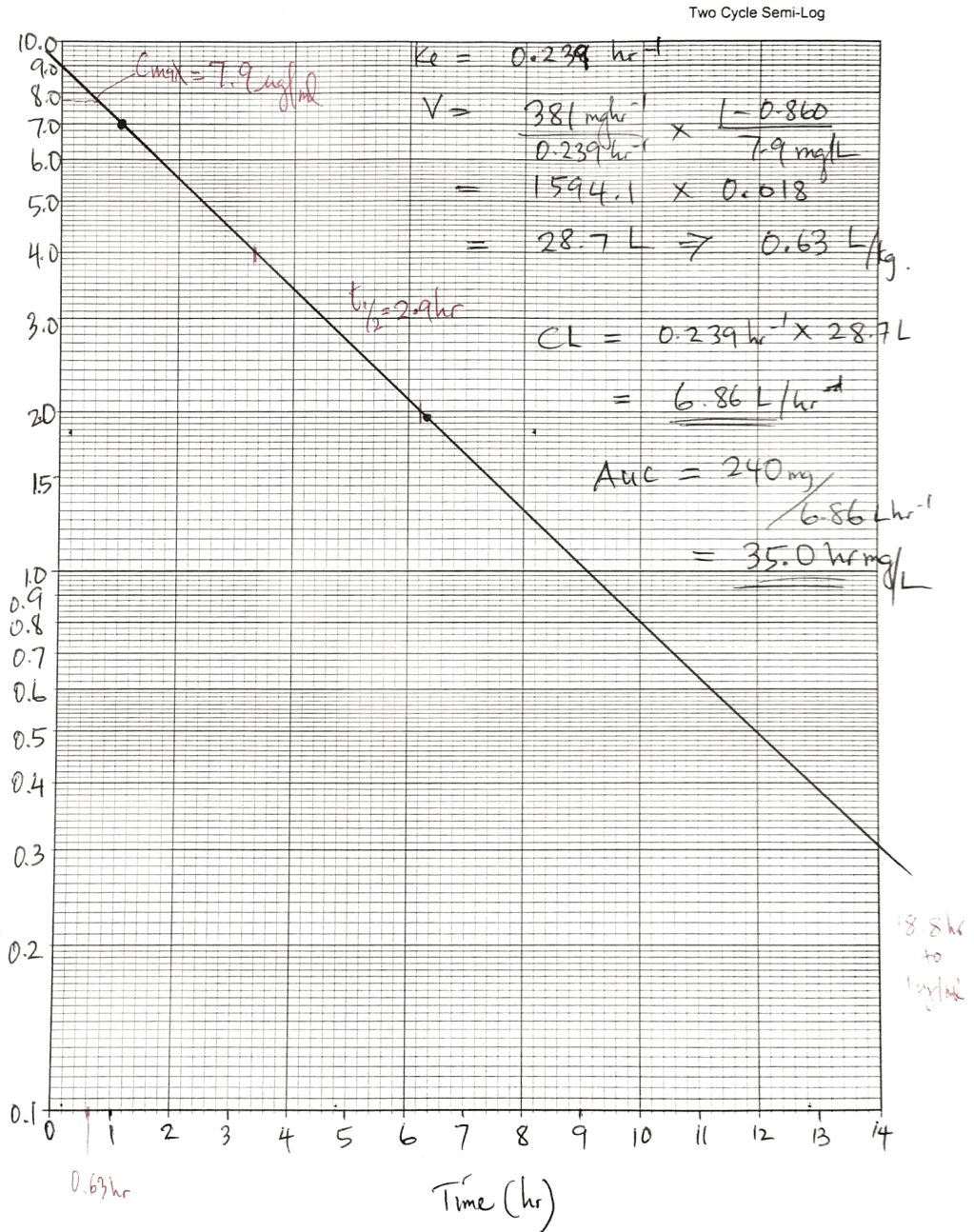


Patient 17

Two Cycle Semi-Log

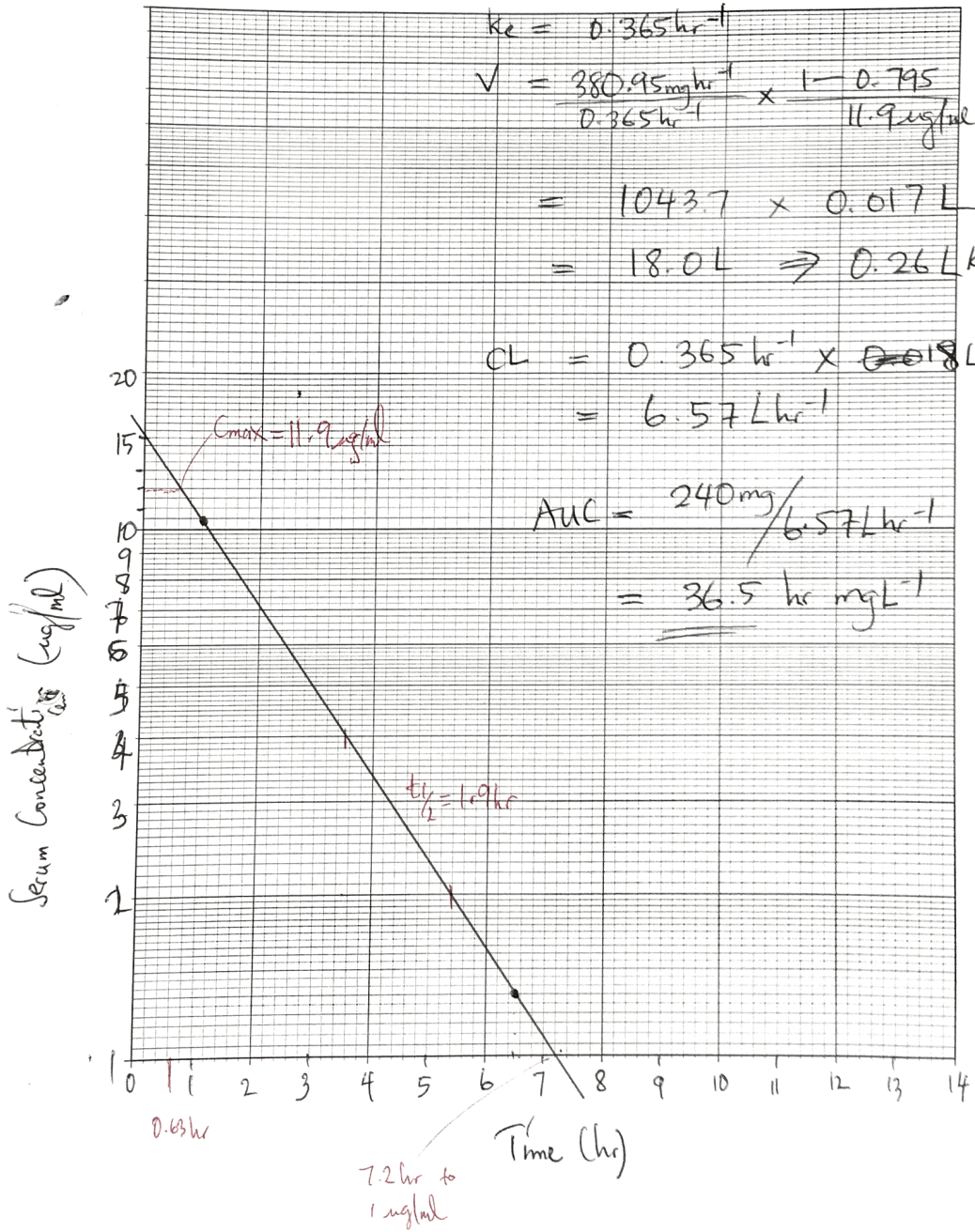


Patient 18



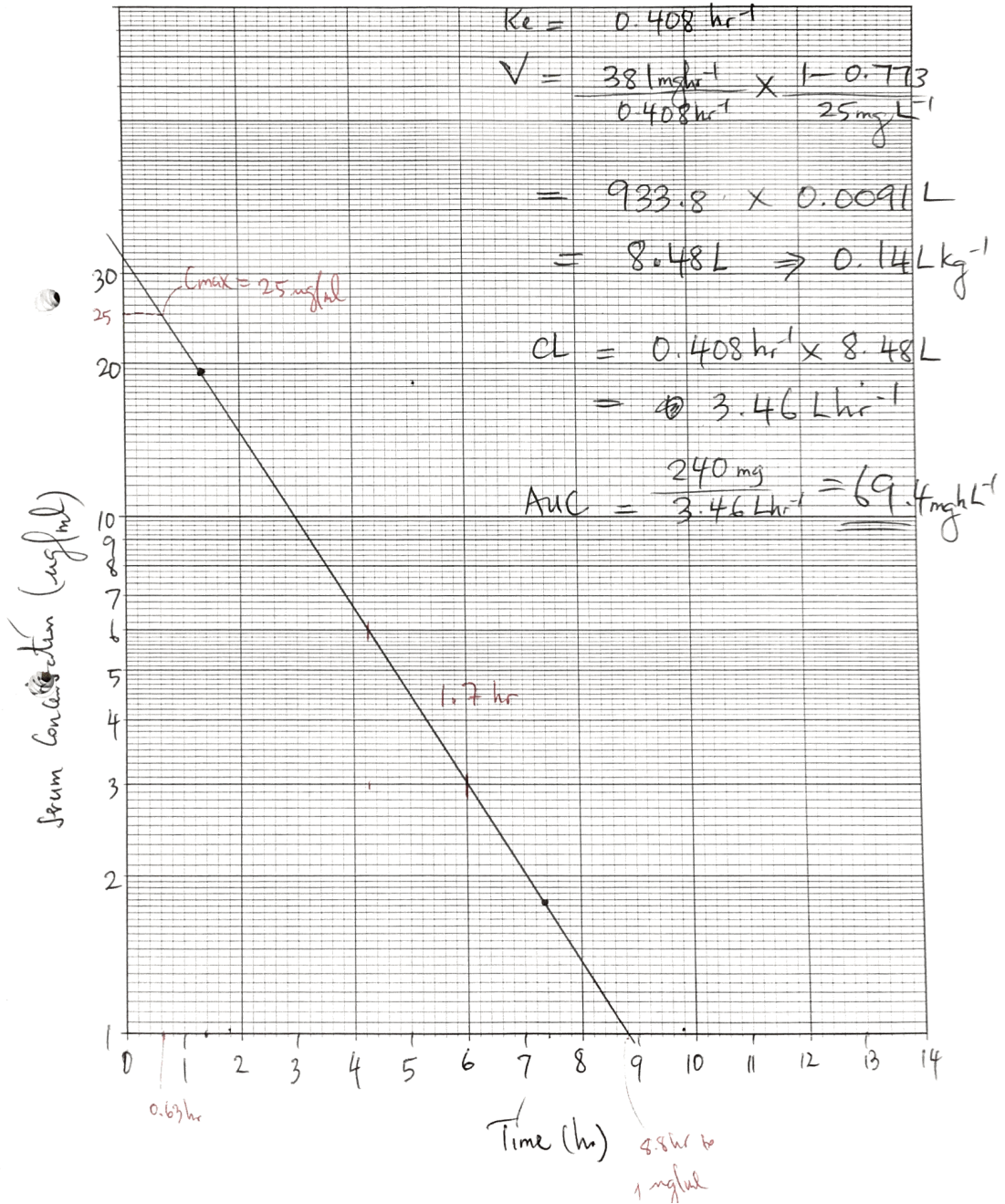
Patient 19

Two Cycle Semi-Log



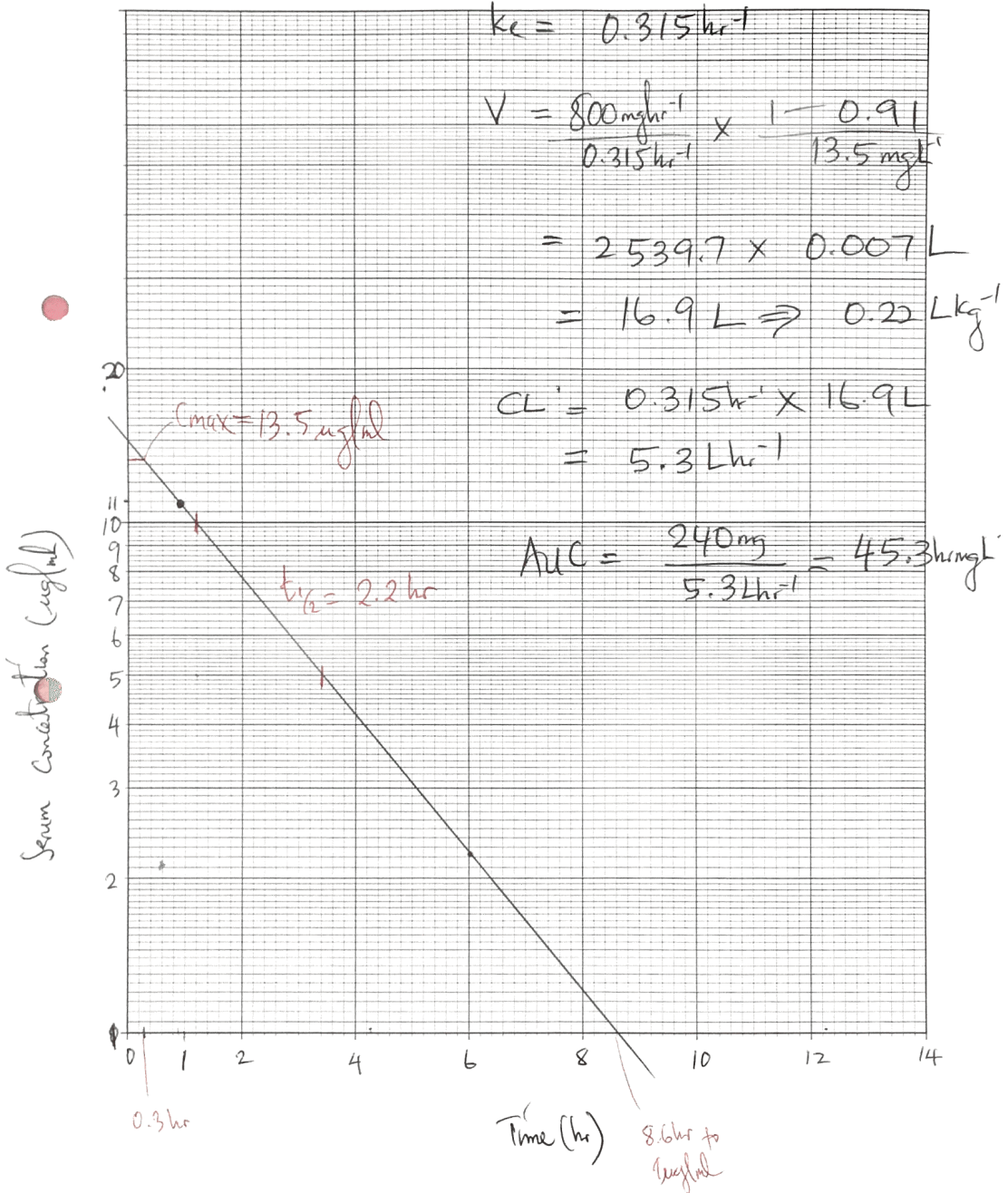
Patient 20

Two Cycle Semi-Log



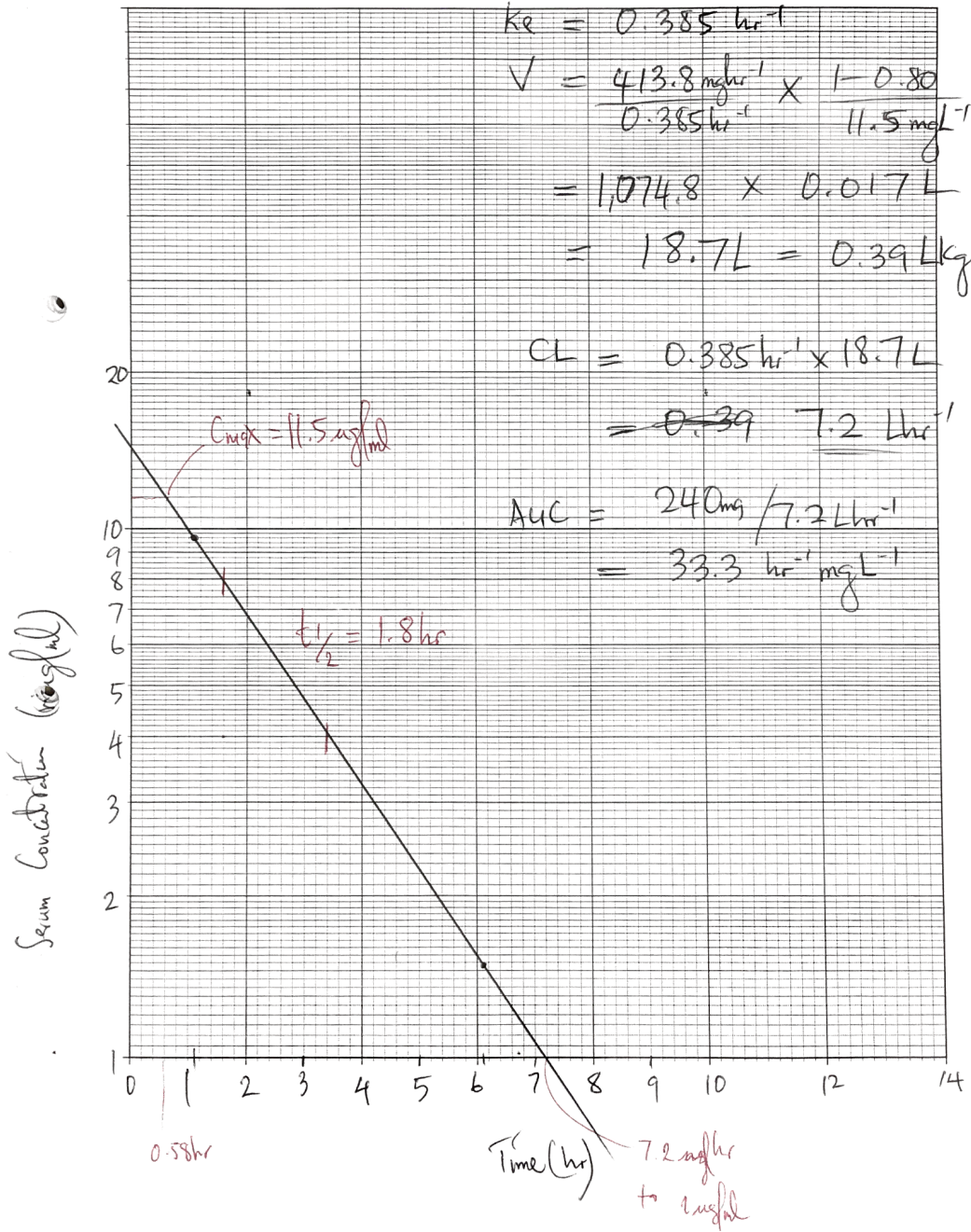
Patient 21

Two Cycle Semi-Log



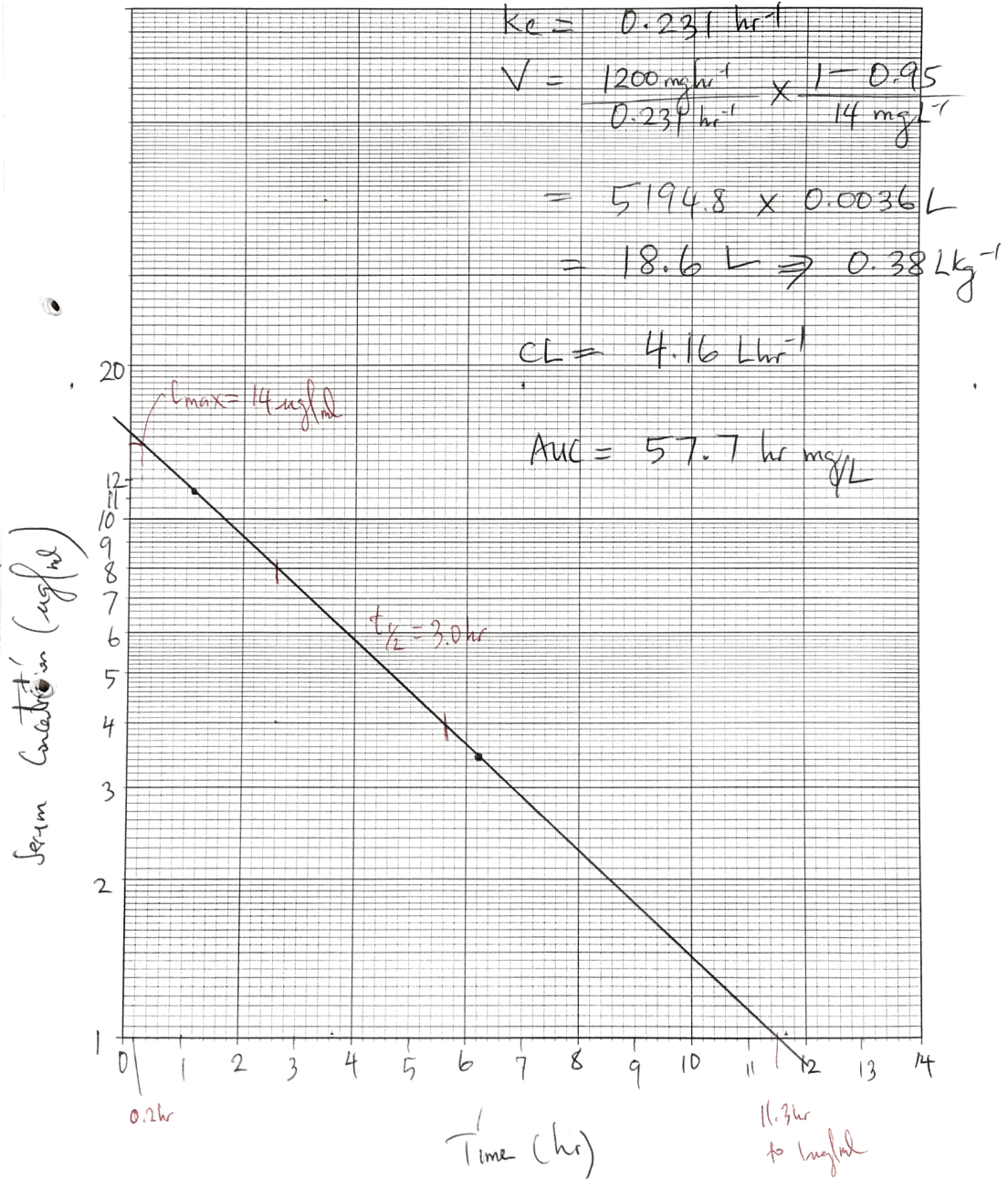
Patient 22

Two Cycle Semi-Log



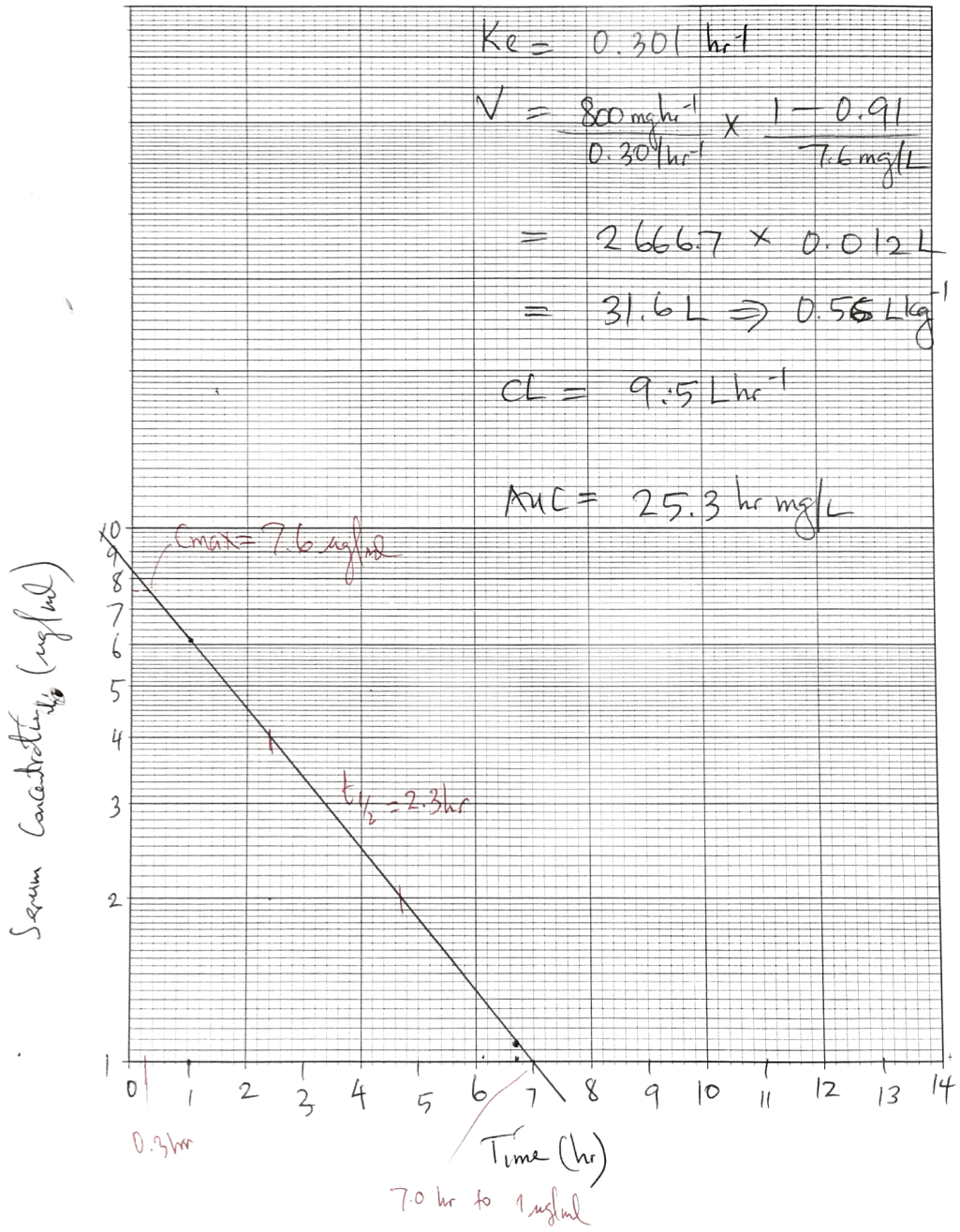
Patient 24

Two Cycle Semi-Log



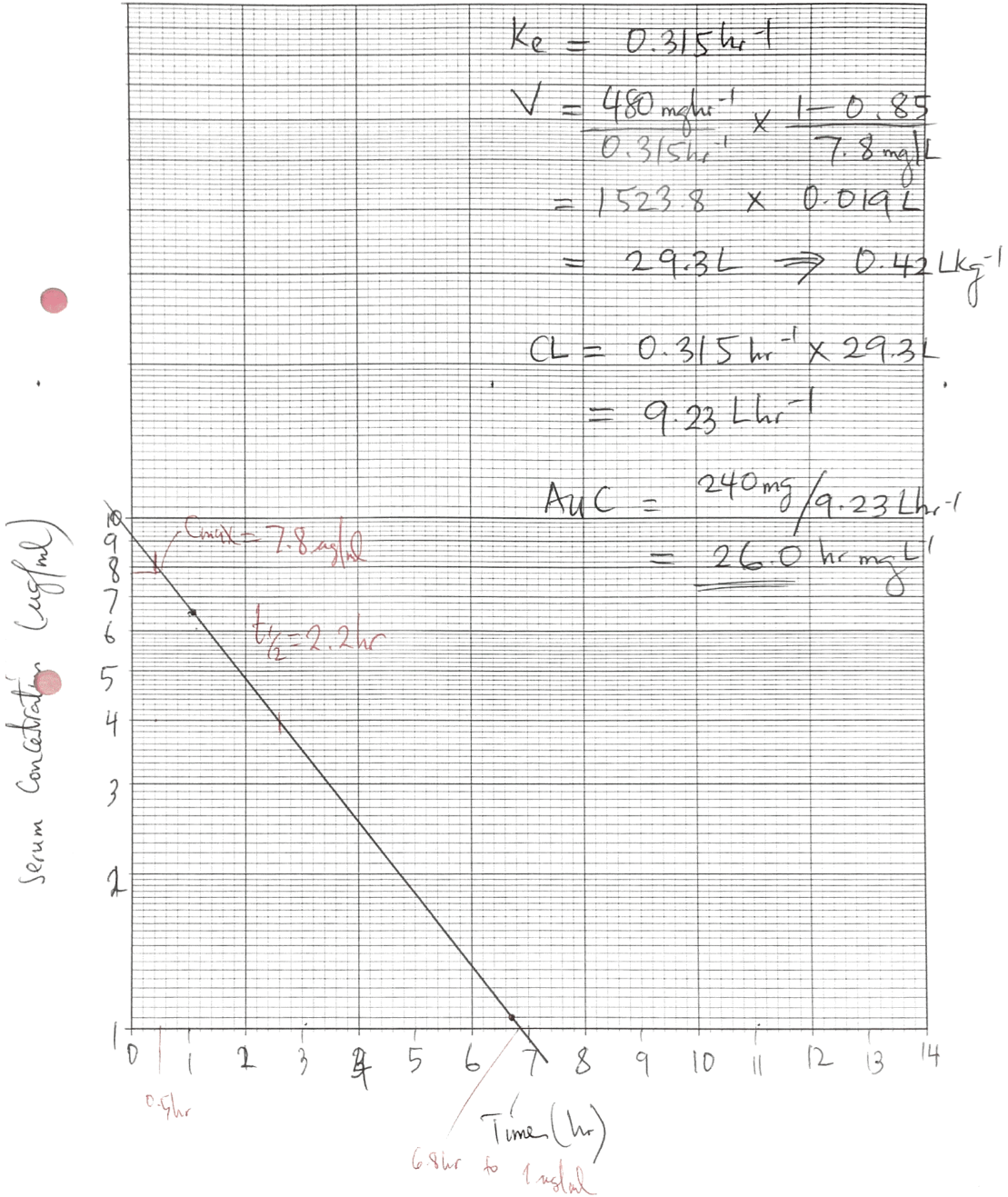
Patent 25

Two Cycle Semi-Log



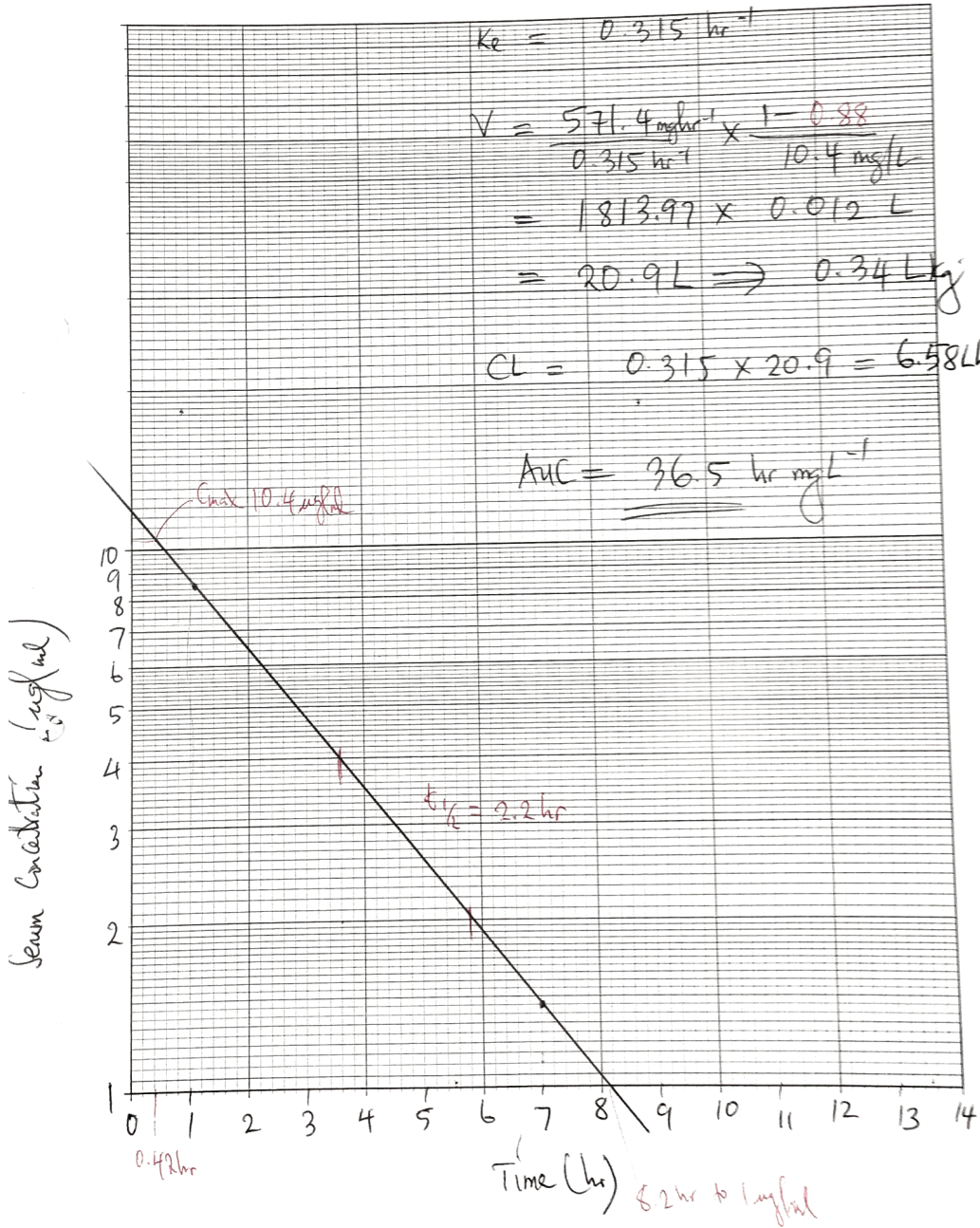
Patient 26

Two Cycle Semi-Log



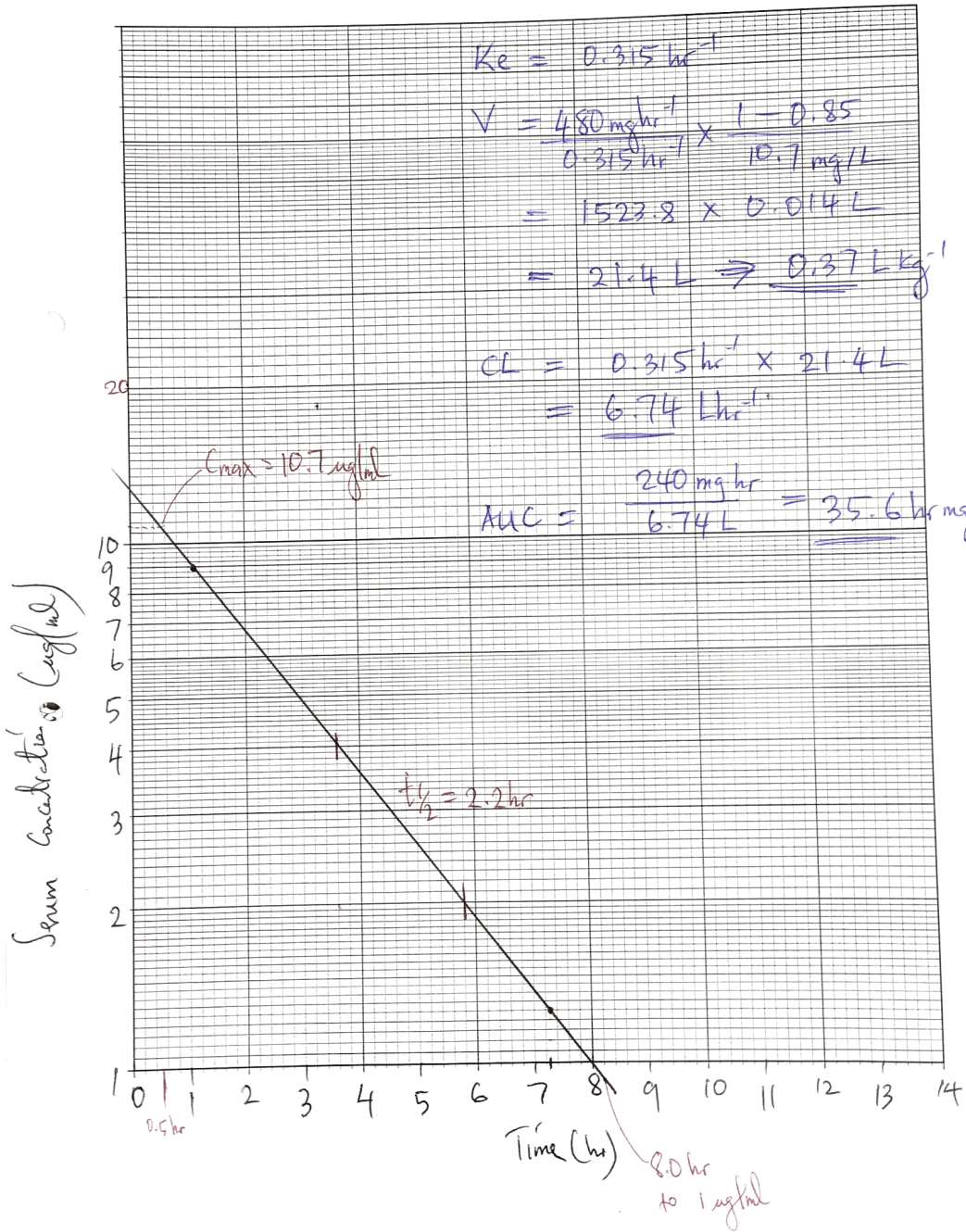
Patient 27

Two Cycle Semi-Log



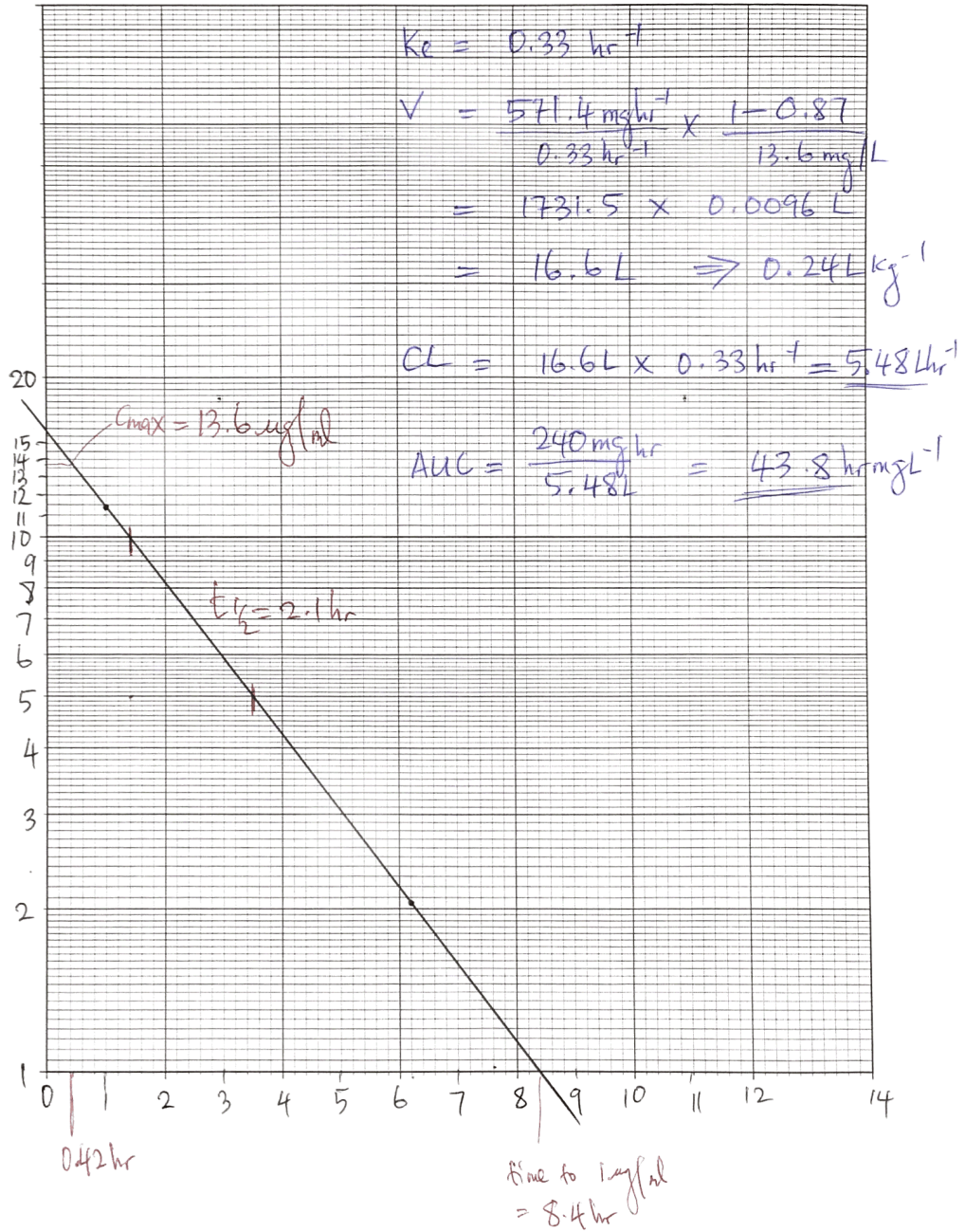
Patient 28

Two Cycle Semi-Log



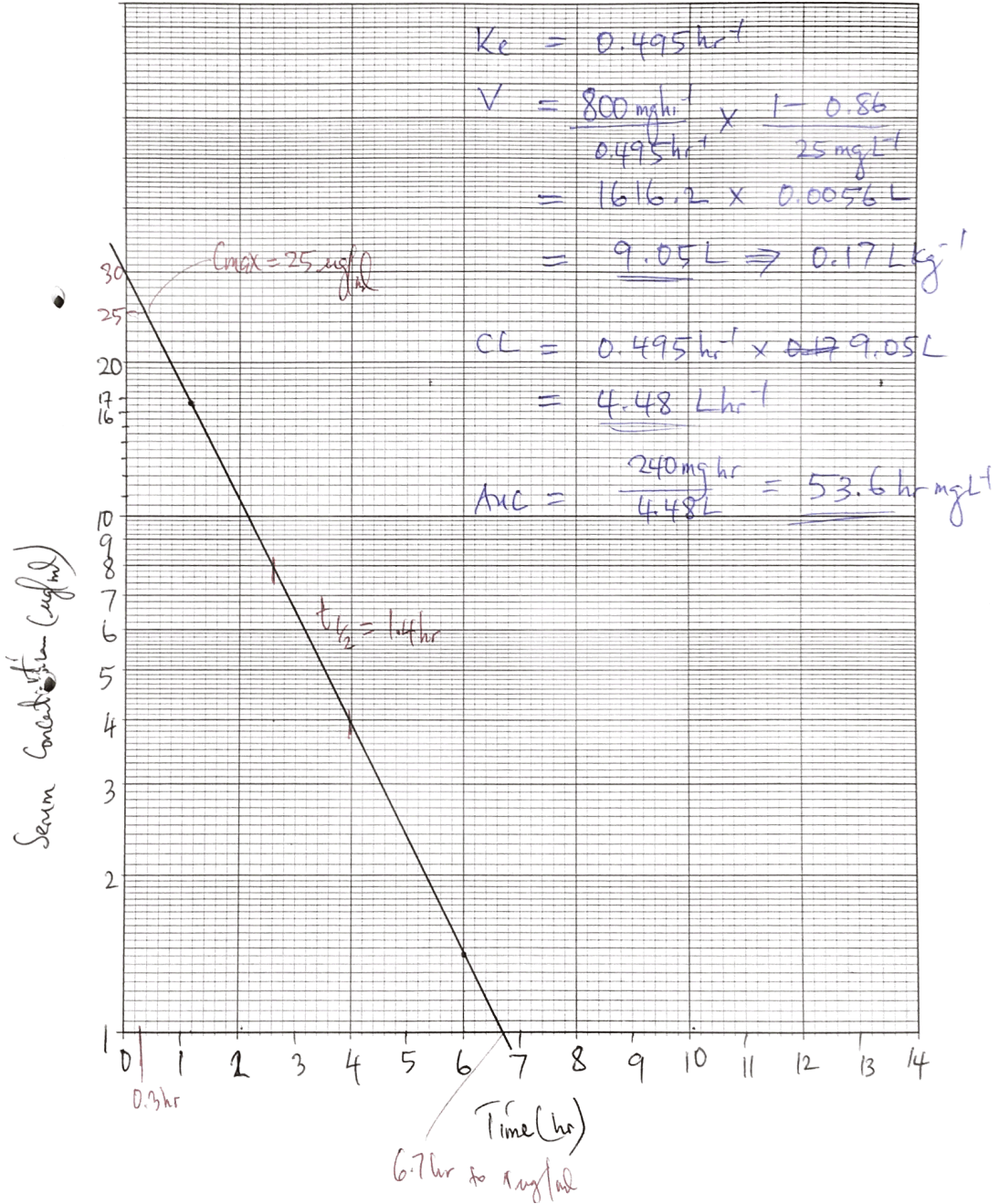
Patient 29

Two Cycle Semi-Log



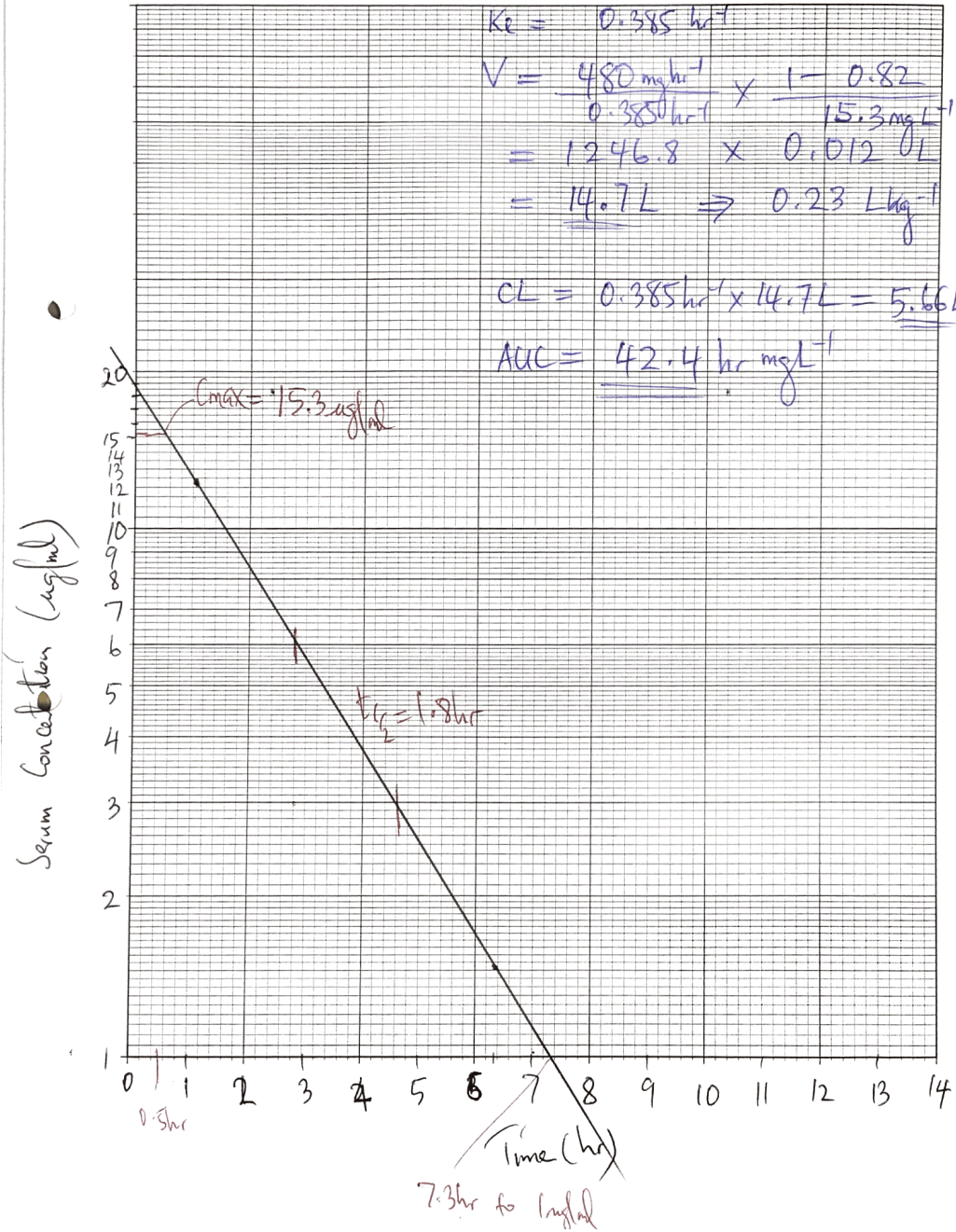
Patent 30

Two Cycle Semi-Log



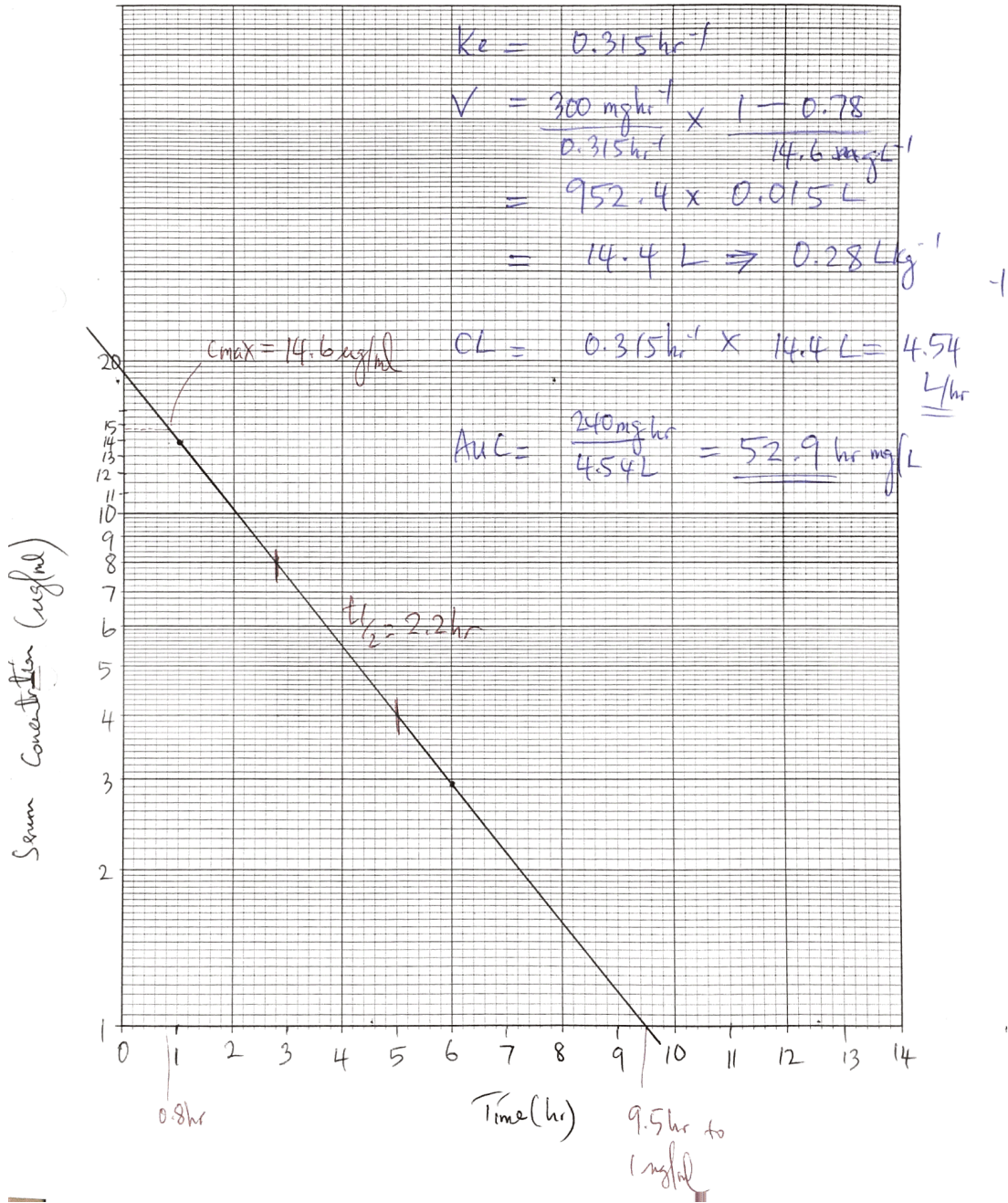
Patient 32

Two Cycle Semi-Log



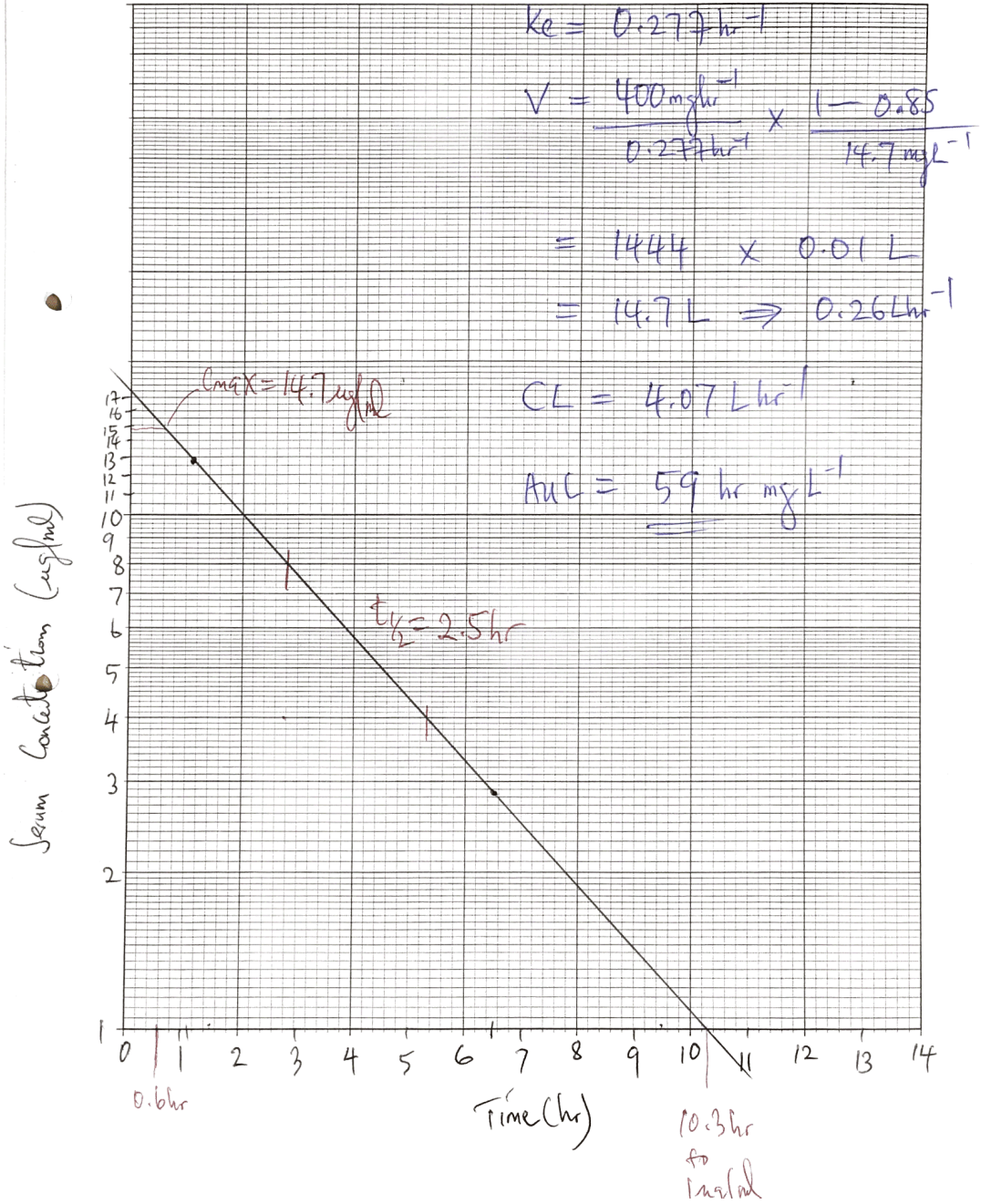
Patient 31

Two Cycle Semi-Log



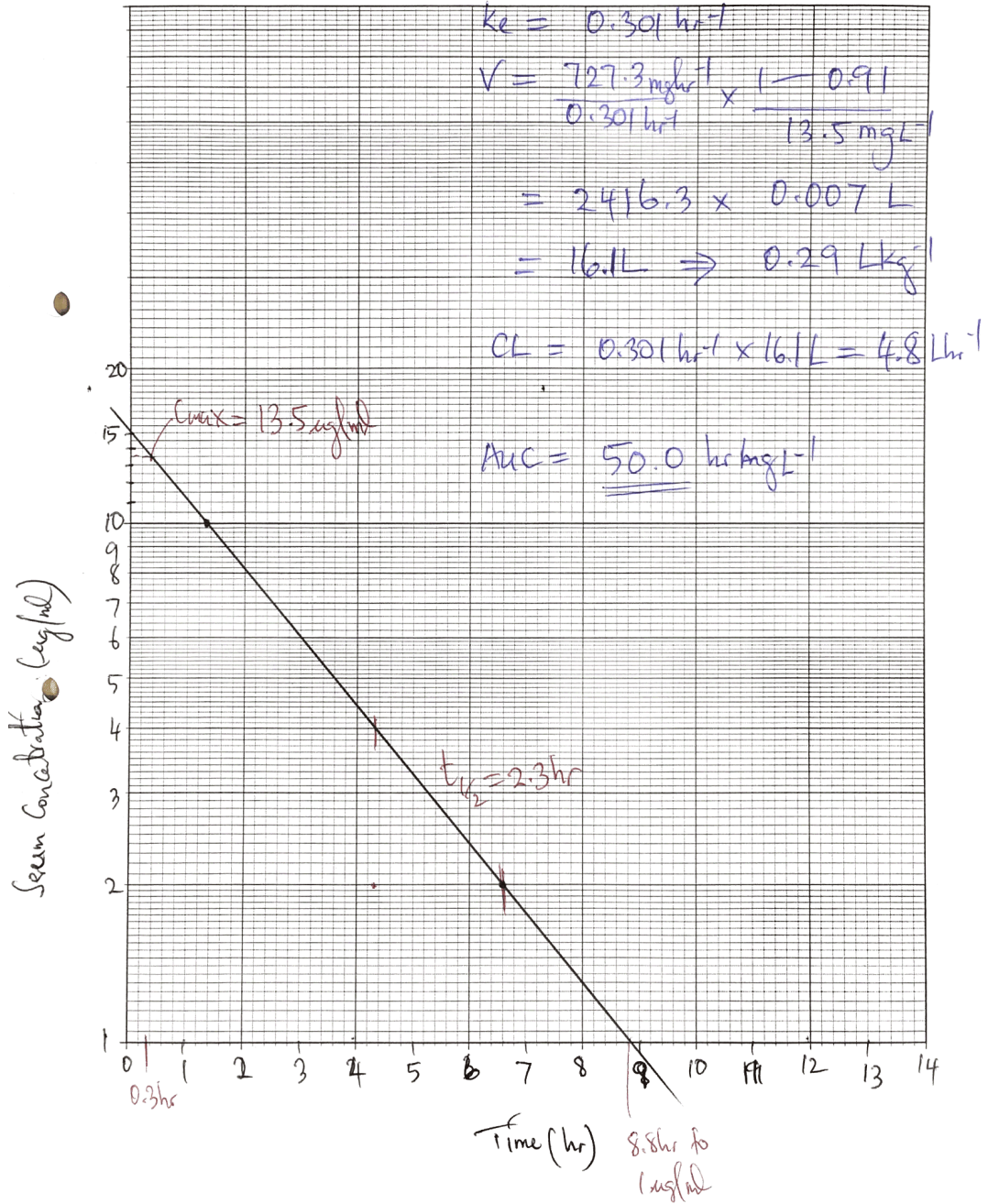
Patient 33

Two Cycle Semi-Log



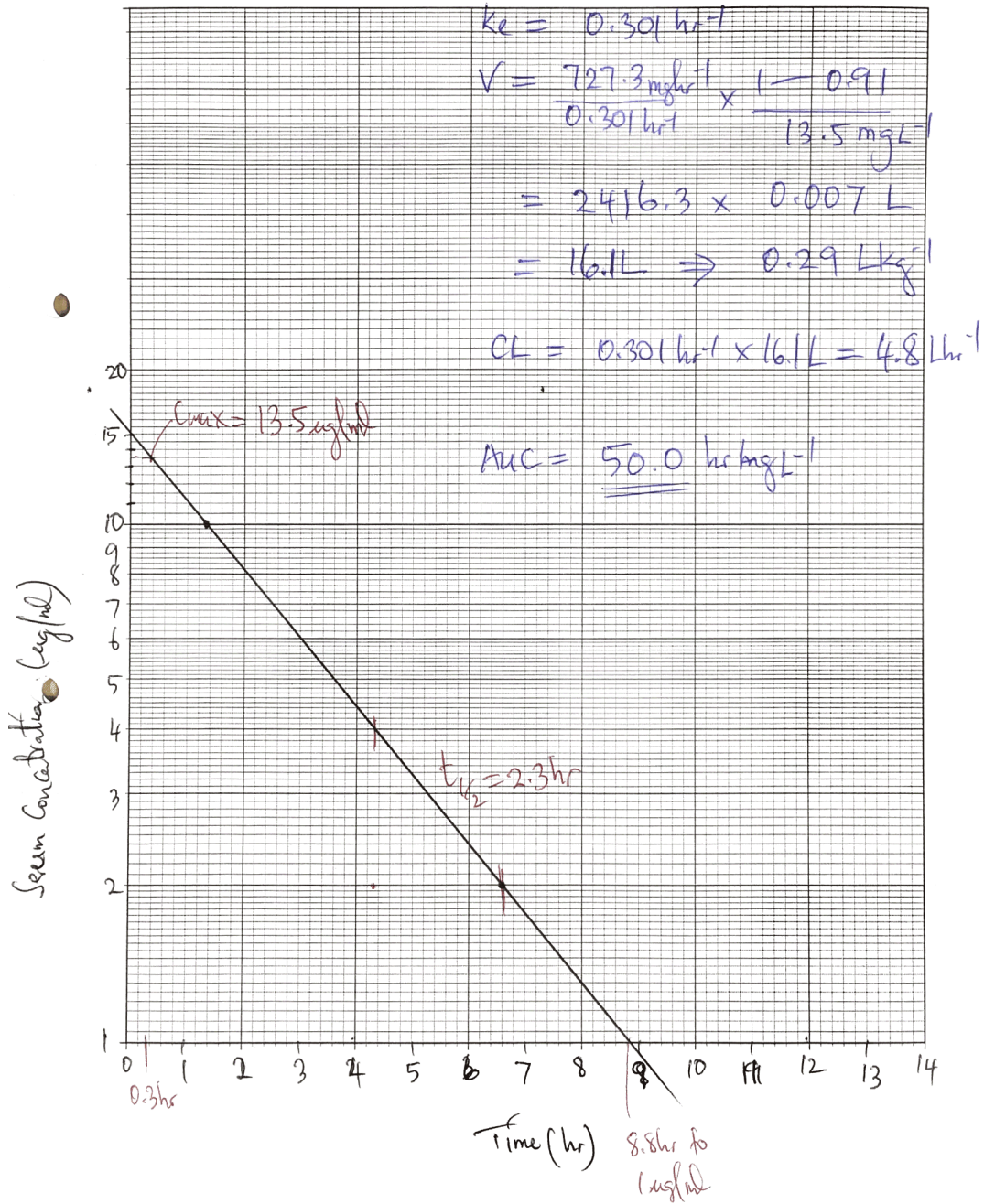
Patient 34

Two Cycle Semi-Log



Patient 34

Two Cycle Semi-Log



Patient 35

Two Cycle Semi-Log

