Development of Assessment Techniques for Methadone Dependent Patients

by

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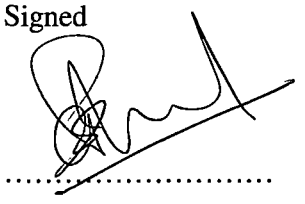

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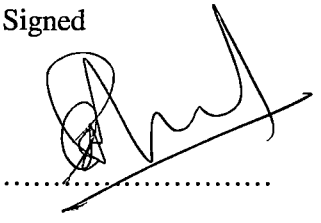

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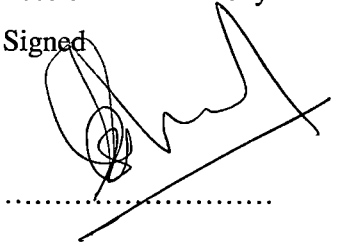

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Abstract

Background: During the past decades, use of urine testing, methadone plasma concentration (MPC), pupillometry and to some extent Reaction Time Test (RTT) has been use for assessment of opioid dependent patients (ODP). Urine and blood testing techniques have been traditionally used while others are still widely been investigated. Pupillometry and RTT techniques provide a direct measurement of the of the patients level of intoxication. However, pupillometry still requires a better understanding of the pupil characterization, eye safety standards, accommodation effects, and understanding consensual response of pupil, discretely. In addition, a systematically designed multifunctional pupillometer is required to gather all the above information and test its usefulness in clinical settings. RTT has been used in the past to some extent however its usability as an assessment tool is still under investigation.

Objectives: A novel multifunctional pupillometry goggles was developed and used for pupil characterization measurement. We also developed a Simple Visual RTT (SVRTT) for the assessment of methadone patients. In addition, blood testing was conducted to investigate its relationship with pupillometry and SVRTT. Lastly, an arousal index was developed, that can be potentially used by physicians in clinical settings.

Material and Methods: The pupillomety system consisted of a personal computer, goggles and a control unit. The system, capable of recoding pupil images at 10f/s for 140 seconds in one single test, was used for ipsi-lateral (IPL) and contra-lateral (COL) measurements. Pupillary characterization was studied using varied light intensities. Furthermore, SVRTT consisted of a personal computer and a mouse. The patient clicked the mouse button when visual stimulus was displayed on the computer monitor and RT was automatically recorded. Blood samples were collected for 6hrs (duration of the test) at an interval of one hour.

Results: High correlation was obtained between MPC-Pupillometry, MPC-SVRTT, and Pupillometry-SVRTT. Pupil characterization parameter (Constriction Amplitude, Constriction Velocity, Redilation Amplitude, Redilation Velocity, Relaxation Standard Deviation) were measured and high correlations were obtained when compared with MPC and SVRTT. No significant effect was obtained in accommodation response. Differences were obtained between IPL and COL results however the differences were constant. Pupil measurements were best

obtained at higher light intensity (100lux). Based on the data an Arousal Index (AI) was developed which correlated highly with MPC.

Discussion and Conclusion: This work is significant advancement in understanding pupillometry characteristics discretely. The results revealed that pupil characterization can be indicative of methadone metabolism and absorption and has the potential to be used in clinical settings. It is concluded that COL and IPL measurements showed significant differences, however the differences were approximately constant and pupil response was similar in both measurements. The safety of eye while conducting pupillomety test is important, especially when IR light or higher intensity visual light is used. SVRTT for the first time, has shown, it's potential to be used along with pupillometry in clinical settings in controlled environment. These findings, built on a sound experimental design and development, can be seen as a step towards establishing pupillometry and SVRTT as clinical assessment tool. An arousal index was developed based on the pupil measurements which can be used to predict MPC of patients during methadone absorption and metabolism.

Key Words: pupillometry, pupillometry characterization, methadone dependent patients, reaction time test, eye safety

This thesis is dedicated in the fond memory of my mother,
Late Smt Suman G. Patil

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Abbreviations and Nomenclature

HTC	Humanitas Trust Centre
RT	Reaction Time
RTT	Reaction Time Test
EDDP	2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
LED	Light Emitting Diode
CCD	Charged Coupled Device
IR	Infra Red
IRED's	Infra Red Light Emitting Diode's
IR LED's	Infra Red Light Emitting Diode's
PBC	Plasma Methadone Concentration
ODP	Opioid Dependent Patients
MDP	Methadone Dependent Patients
SVRTT	Simple Visual Reaction Time Test
IPL	Ipsi-Lateral
COL	Contra-Lateral
Ac	Accommodation
MDP	Methadone dependent patients
CTN	Clinical Trial Notification
MPC	Methadone Plasma Concentration
SRTT	Simple Reaction Time Test
RT	Reaction Time
CA	Constriction Amplitude
CV	Constriction Velocity
R-dA	Re-dilation Amplitude
R-dV	Re-dilation Velocity
Relaxation SD	Relaxation Standard Deviation
MDP	Methadone Dependent Patients
fl	foot-lamberts
B (λ)	blue light hazard function
cd	candela
mcd	milli candela
mA	milli Ampere
ms/msec	milliseconds
lm/W	lumens per watt
$d\phi$	radiant flux
dA	area
W/m^2	watt per square meter
CS	Consent Form
IS	Information Sheet
PCB	Printed Circuit Board

1. Thesis Structure

1.1 Motivation for the Study

The treatment options for opioid dependence has for many years been restricted by the availability of suitable assessment techniques. Assessment of opioid dependent patients (ODP) pre and post dosing has traditionally relied on conventional methods such as urine testing, blood testing, or face to face assessment by a physician. These techniques all have a number of drawbacks such as being highly expensive, time consuming, less convenient and the accuracy can be questioned, and the inability to be used remotely. This study centers on the development of new assessment techniques in the form of pupillometry and reaction time testing (RTT) that can overcome these limitations and be able to be employed in clinical settings.

Pupil diameter measurement using pupillometry procedure has been found effective in the assessment of ODP in the past since it is non invasive, time efficient and easy to use. However, its application to date in the area of addiction has been limited due to a number of drawbacks. These drawbacks include the effects of ambient light effecting the pupil diameter, and eye safety issues due to infra-red (IR) and visible light levels when used repeatedly during testing. Furthermore, a systematic study using ODP has not yet been documented providing information on the optimum light intensity under which pupillometry test can be conducted. The differences in results obtained when pupillometry is carried out using ipsi-lateral (IPL) and contra-lateral (COL) light stimulation of the pupil can affect the assessment as well. Other effects such as accommodation (Ac) in pupillometry procedures and the relationship between pupil responses during all phases of pupil activity post-light stimulation and methadone plasma concentration (MPC) can have a potential effect on patient assessment. In addition, there is a lack of systematic investigation of pupillary response characterization which can be useful for physicians in understanding pupillograms and their relationship with methadone plasma concentration (MPC) better. MPC is a technique traditionally used for ODP assessment. Lastly, considering the dearth of techniques used for assessment of ODP a new Simple Visual Reaction Time Test (SVRTT) is introduced as a potential for assessment tool.

1.2 Flow of the thesis

Opioid dependence is a problem of global concern, especially with dramatically increased rates of abuse and dependence of prescription opioids. The concerns are not limited to

the abuse of opioids alone but it can get worse as opioids effect the brain which is one of the most complex parts of human body. Methadone treatment is one of the most widely used methods to treat ODP. A review of opioids, opioid dependence and its effect on patients and the role of methadone in the treatment of opioid dependence are explained in section 2. While these processes are highly complex this section tries to explain them in terms that can be easily understood and explained to patients.

Assessment of MDP is a crucial step in understanding their rehabilitation progress. As mentioned earlier the assessment techniques are either subjective (face to face assessment with physician), or impractical (urine and blood testing), or expensive and the accuracy can be questioned (pupillometry). Therefore a novel technique in the form of SVRTT was introduced to investigate its potential as a new assessment tool for (MDP). A detailed description of the traditional and existing techniques for assessment leading towards their advantages and drawbacks followed by the potential use of SVRTT is presented in section 3. At the end of the section the aims of the study are given in a systematic manner (section 4).

Experiments were carried out after receiving an approval from the Tasmania Scientific Research Advisory Committee, the Tasmania Health and Medical Human Research Ethics Committee, and after notification of the Clinical Trial Notification (CTN) scheme, pursuant to Schedule 4 of Regulation 7.1 of the Thempeutic Goods (Medical Devices) Regulations 2000. Experimental procedure included the following: testing on an hourly basis for 6hrs; pupil image acquisition, SVRTT measurement, and blood sample collection. Urine sample were collected before the start of the test. Pupillometry system consisted of specially made goggles which were developed giving due consideration to the eye safety of the patient and the automation of the pupillometry technique. The goggles development included fixation of a web-camera to allow the collection of pictures of the pupil from one eye, while simultaneously stimulating both eyes alternately at variable light levels. Pupil diameter was measured using published pupil diameter measurement technique. The pupillometry data was further characterized to find constriction amplitude (CA), constriction velocity (CV), re-dilation amplitude (R-dA), re-dilation velocity (R-dV), and relaxation SD (standard deviation), during pupillary light reflex. SVRTT was also assessed along with pupillometry and the data was saved systematically for offline data analysis. The development of the experimental techniques and the procedure is given in detail in Section 5. A thorough eye safety assessment was carried out using British

Standards 2008 (BS EN 62471:2008) (Section 6). Once the goggles were termed safe they were considered for use with patients.

Pupillometry results are divided in two sections; section 7 and section 8, corresponding to IPL and COL results, respectively. Both section consisted to detailed analysis of pupil response to the number of test and visual stimulus. Systematic presentation of the result is followed by discussion and interpretation of the results. SVRTT results were described in detail in section 9. The section concludes with the discussion of the results.

After individually assessing each assessment technique comparisons were drawn between tests in section 10. It included correlation between MPC and Pupillometry, MPC and SVRTT, Pupil diameter with SVRTT, and other combinations such as SVRTT correlation with pupil diameter at different light intensities was presented. An arousal index (AI) was developed based on pupillometry parameters that can potentially be used in clinical settings. The section concludes with the discussion of the results.

All the result sections were further summarized in section 11 followed by conclusions and future work (section 12).

2. Introduction

This section introduces opioids and their actions in the human body. It further explains the factors affecting ODP in social settings and the related health problems. This section is intended to give the reader an idea of the severity of the problems associated with ODP.

2.1 What are Opioids?

Opioids are a class of drugs that include both natural and synthetic substances. The natural compounds are usually denoted "opiates" such as morphine and codeine, and then synthetic and semi synthetic compounds such as oxycodone, buprenorphine, fentanyl, heroin, methadone [1, 2, 3]. The primary medical use of these opioids is to reduce pain. However, synthetic opioids may cause dependency or addiction when consumed over time to reduce pain [4]. In addition, if these drugs are consumed repeatedly a tolerance is developed. Tolerance causes consumption of higher doses of drugs and generates craving feelings if the drugs are stopped. This causes drug dependency.

2.2 Mechanism of Action

Opioids act by attaching to specific proteins called opioid receptors in brain (Fig 1). As one becomes tolerant to opioid, they become less sensitive and require more opioids to produce the same effect. Whenever there is an insufficient amount of opioid receptors activated, the user feels discomfort. This happens in withdrawal. When full agonist drug (heroine, codeine) attach to the opioid receptors (Fig 1), they block the transmission of pain messages to the brain, which is why opioids are highly effective analgesics in relieving chronic and/or acute pain.

The strong opioid effect of full agonist opioid can cause euphoria and stop the withdrawal for a period of time (4-24 hours), after which the brain begins to crave opioids, and the cycle repeats and escalates. Synthetic opioid (methadone, Fig 1(c)) competes with the full agonist opioids for the receptor. Since methadone has a higher affinity it expels existing opioids and blocks others from attaching. As a partial agonist, the methadone has a limited opioid effect, enough to stop withdrawal but not enough to cause intense euphoria.

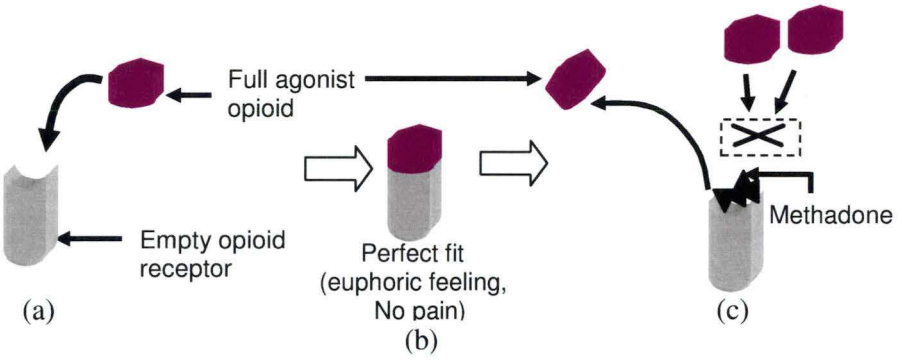

Figure 1. An illustration of the mechanism action of opioids with receptors in the brain. (a) An opioid occupies an empty receptor. (b) Due to a perfect fit, euphoric feelings are aroused. (c) However, on administration of methadone the patient gets similar feeling as opioid and restricts the opioids from acquiring any receptor. Over a period of 24hrs methadone dissipates and another dose needs to be intake or the patient starts craving for drugs.

2.3 Opioid Dependence

Opioid dependence is a complex condition involving communal, psychological and biological components [5]. Chronic administration of an opioid can be expected to cause physical dependence [3, 4, 5]. Excessive use of opioids can eventually cause physiological changes in the brain's function and chemistry, resulting in the chronic brain disease of opioid dependence. After long-term use, these changes persist even after the patient has stopped using the drug; it is for this reason that opioid dependence is properly characterized as a chronic, relapsing disease [6, 7, 8]. More specifically, opioid dependence is defined as the co-occurrence of several symptoms (e.g. drug tolerance, withdrawal symptoms, repeated unsuccessful attempts to decrease or discontinue use, significant time spent obtaining the substance or recovering from

its effects, drug use to avoid withdrawal and foregoing of important activities, or willingness to accept severe physical, social, or professional negative consequences, due to drug use) [3, 5].

2.4 Opioid Dependence Related Problems

Opioid dependence can have many negative effects, such as drug overdose, increased risk of contracting infections such as Human immunodeficiency virus (HIV) or hepatitis, family distress, disruption at work and possible involvement in criminal activities. It is difficult to stop using these drugs and remain abstinent due to a combination of craving, unpleasant withdrawal symptoms and the continued or worsening personal circumstances that led to illicit drug use in the first place. Even when a dependent opioid user manages to become abstinent, there is a high probability that he or she will return to using drugs within a short time. It is reported that approximately 185 million people worldwide aged 15 years and above were consuming drugs in the late 1990s [7].

2.5 Treatment of Opioid Dependence (Agonist Maintenance)

Agonist maintenance treatment usually consists of daily administration of an opioid agonist (methadone) or a partial agonist (buprenorphine). The resulting stable level of opioid effect is experienced by the dependent user as neither intoxication nor withdrawal, but more as "normal". The aims of agonist maintenance treatment include reduction of illicit opioids, reduction of injecting and associated risk of blood borne virus transmission, reduction of overdose risk, and improvement in psychological and physical health. In practice, most patients commencing opioid agonist treatment will cease heroin or use it infrequently, with only 20–30% reporting ongoing regular heroin use [9, 10]. However, relapse to heroin use following the termination of agonist maintenance treatment is common [11, 12, 13].

2.6 Role of Methadone in Treatment of ODP

Methadone is a synthetic opioid which was first reported as a maintenance treatment for opioid dependence by Dole and Nyswander (1965) [14]. Methadone first appeared in Europe in the late 1960's in response to an emerging use of heroine and was officially introduced into Britain in 1968 [14].

Methadone is a long-acting opioid medication. Unlike morphine, heroin, oxycodone, and other addictive opioids that remain in the brain and body for only a short time, methadone has effects that last for days. Methadone causes dependence, but because of its steadier influence on the opioid receptors it produces minimal tolerance and alleviates craving and compulsive drug use. In addition, methadone therapy tends to normalize many aspects of the hormonal disruptions found in addicted individuals [15, 16, 17]. Use of methadone produces symptoms similar to those observed for opioids.

The goals of the early induction of methadone patients in rehabilitation are to attenuate withdrawal symptoms, diminish opioid craving and arrive at a tolerance threshold, while preventing euphoria and sedation from overmedication [18, 19, 20]. Patients are generally started on smaller daily doses (20 mg to 30 mg), with increases of 5 mg to 10 mg until a dose of 60 mg to 100 mg per day is achieved. The higher doses produce full suppression of opioid craving and, consequently, opioid-free urine tests [21]. Periodic dosage increases are warranted in cases of patients who relapse or abuse other drugs. Patients generally stay on methadone for 6 months to 3 years, some much longer. While relapse is common among patients who discontinue methadone after only 2 years or less, many patients have benefited from lifelong methadone maintenance.

3. Assessment of MDP [34-40]

Assessment of MDP plays an important role in the patient's rehabilitation. During rehabilitation, it is considered essential to assess the patient before dose as well as after dose [22, 23, 24,]. Existing assessment techniques have been found to be either not convenient, time consuming, expensive or the accuracy can be questioned, which hampers patient assessment [23, 24]. The most traditional assessment techniques include subjective assessment by medical professionals, urine testing and blood testing [25, 26]. This section discusses the details of existing assessment techniques, followed by the aims of this study.

3.1 Urine Testing (Pre-Dose Assessment) [27-28]

Urine testing of patients immediately prior to methadone consumption is usually carried out and considered an important step, since dosing while intoxicated may be fatal. The usual procedure of assessing opioid users before dose at clinics involves urine analysis. Urinalysis drug screening is an important way of assessing drug misuse by patients undergoing methadone treatment. It is essential to know if a patient is taking all of their medication (correct time and amount) and to find out whether the patients is using extra opioid (obtained illicitly) for personal gain (in the case of patients on take away doses). Urinalysis only indicates whether or not a patient has taken some of the medication.

In general, urine testing of patients in methadone maintenance treatment programs is meant to verify compliance or to ascertain the adequacy of dosage by finding methadone or its demethylated metabolite in the urine and to detect the use of heroin (by finding morphine in the urine) or other illicit drugs. A positive test indicates that the patient has taken some illicit drugs and it's upon the physician's discretion whether to dose the patient or not. A negative test is usually an indicator that the patient can be dosed. However, urinalysis may not be valid unless it is certain, since patients can cheat by substituting a clean urine sample for an authentic one. In cases like these, one might as well save the cost and not bother conducting urine testing. On the other hand, factors such as fluid intake, rate of metabolism, glomerular clearance, and urine pH value affect urine concentration. Currently patient's given take-away doses are not assessed prior to consumption. Although urine analysis indicates past drug use, but fails to indicate when or how much drug was ingested. Therefore blood testing is used to detect the analytes found in the urine.

3.2 Methadone Plasma Concentration (MPC) (Post-Dose Assessment)

Blood is the primary biological sample for pharmacokinetic analyses, as both parent and metabolite concentrations can be quantified and samples cannot be adulterated by the donor [29]. Currently, MPC is considered useful test since it has shown good correlation with urine testing when compliance is good [29-31]. MPC can also be used to determine the metabolism of methadone in the patient over the period of testing until the next dose. Studies have also reported that there is a robust linear relationship between MPC and oral dose [31, 32]. This is important information in understanding the pharmacokinetics of the patient. Their relationship can be demonstrated over a wide range of dosages (3 to 100 mg), and the correlation has been reported at $r = 0.89$ [31]. It was demonstrated that MPC filled the gap and provided much needed evidence on compliance. Compliance in methadone maintenance patients has been measured using a pharmacological indicator to estimate MPC. The study showed that many patients took their medications incorrectly whereas others supplemented their dose with illicit methadone [31, 32].

The success or failure of patients in methadone treatment may be related to the determination of an appropriate daily oral dose [33], identifying patients who respond poorly to treatment [29], and ensuring compliance to the dosage regimen. Such tasks are difficult as clinicians are currently without an accurate, convenient and objective therapeutic tool for therapeutic drug monitoring. On the other hand it is impractical to do blood testing of patients after every single dose. Furthermore, the difficulty increases with the fact that the blood testing must be carried out over a period of 6hrs after methadone administration to understand the methadone metabolism in the patient. Blood testing technique gets further hampered since samples once collected need to be stored and then sent to pathology for analysis, which can be time consuming.

3.3 Pupillometry

Pupillometric methods are essentially used to measure the location of the pupil and the variations in the diameter of the pupillary aperture of the eye. Pupillometry is a procedure that measures the pupil size and pupil location with respect to the anatomical structure of the eye. Pupillometry has long been used in research on the autonomic nervous system [34, 35, 36, 37,

38], drug metabolism [39, 40, 41], pain responses [42, 43, 44], psychology [45, 46, 47], fatigue [48] and sleep disorders [49, 5, 51, 52].

3.3.1 Applications of Pupillometers

There is growing interest in pupillometry measures in various fields including cognitive ability [53] memory load [54], investigation of central autonomic pathways [55], alertness or anxiety [56] and in many optometry and ophthalmology applications. The information of pupil size and center is also important in keratorefractive procedures, such as laser in situ keratomileusis (LASIK) surgery, where it has a profound effect on the predicted outcome of the procedure [57, 58]. In addition pupillometry is also used as a substitute for urine testing in the drug related research. It has been shown in the past that opiates cause pupillary constriction [59-61].

3.3.2 Pupillometers

Pupil measurements are carried out in various ways including traditionally used rulers and gauges. One such gauge is Haab Pupil gauge which consist of a card with black circles ranging from size 2 mm to 10 mm in steps of 0.5 mm increments. When held of the temporal side of the eye the pupil coincides with one of the black circles and measurements can be taken with an accuracy of 2 mm [62]. However the disadvantages of such device are its accuracy and its usability in dark. Other gauges used for pupil diameter was measured to an accuracy of $\pm$1mm [62]. Another method used Polaroid close up cameras to take eye pictures before and after opiate administration and the pupil diameter was calculated using a vernier caliper and magnified scale that is concomitantly photographed [63, 64). The accuracy of this method is 0.1 mm. However the flash used in this measurement might possibly reduce the pupil size. The methods discussed above were not commercially viable as there performance to expense ratio can be questioned.

Commercial pupillometers range from specially designed rulers or gauges to highly sophisticated infrared video-based systems. Several recent studies of accuracy and reliability of popular pupillometers have been conducted [65, 66]. Infrared tube-based pupillometers are more accurate than the gauges. They often provide a digital read out of pupil size to $\pm$0.5mm. Most accurate systems are based on objective infrared video systems with accuracy of about

±0.1mm. Pupil parameters can also be estimated with a videokeratoscope, an instrument primarily used for measuring corneal elevation [25].

Advanced pupillometer technology has enabled its use in clinical research in many medical applications such as post-lasik treatment [67, 68, 69], neuro-ophthalmology [98, 99], and in estimating the visual field [70, 71, 72]. The critical care and emergency physicians employ pupillometry for quick and accurate measurement of pupillary responses in their patients [73, 74, 75]. In the recent years, computerized pupillometry has been regularly used since it has the ability to register and analyse numerically the parameters of the pupil. The pupil diameter and its dynamic change (pupil reactivity) measurement were made possible by the invention of computerised pupillometry. Due to such development the use of pupillogram became a common practice with physicians to understand the pattern of physiological process of patients. Pupil characterization parameters obtained by pupil light reflex is studied in a more discrete and detailed level.

Although modern pupillometry has been used in sophisticated areas, but its cost can be a major concern. In addition, these ccommercially available pupillographic devices which range in price from $3,000 to $30,000, differ remarkably because each instrument is designed for a special purpose. Furthermore, there are various effects (discussed in following sections) that can affect its accuracy.

3.3.3 Use of Pupillometry in Methadone Maintainance

In recent years pupillometry has been found to be effective in assessment of MDP however, its use has been scarce (Table 1). Pupillometry provides an objective measure of the intensity of opiate use in subjects [76]. Pupillometry has the advantage over urine testing and blood testing of being uncomplicated and quick, while still being accurate. Pupillometry has also shown good correlation with blood testing in the assessment of ODP (Table 1). The relationship between pupil size and subjective symptoms of opiate withdrawal during gradual opiate agonist detoxification has been studied in the past too [59, 60].

Usually MDP are given doses on daily basis depending on their level of tolerance. The amount of dose is decided by the physician. Post-dose assessment of methadone absorption and

metabolism is important indicator of patient's assessment. Pupil diameter is the most popular parameter of measure and has obvious "direct-effect" changes in reaction to MPC [76].

Constriction of the pupil is a common indicator of methadone intoxication. Pupil diameter is found significantly and consistently smaller in patients on methadone than the control subjects not on methadone [29]. Pupillary constriction is also highly correlated with MPC (Table 1). In fact, various studies have shown strong inverse relationships between MPC and pupil diameter [77, 78, 29]

Fewer useful studies have been conducted in the past to investigate pupil characterization in relation to metabolism of methadone in blood over the testing period. Pupillary characterization research on normal human subjects has been carried out however the focus was mainly on parameters such as measurement of pupil diameter after constriction, CV, and the dilation amplitude. It is understood that the components of the light reflex that were controlled by parasympathetic and sympathetic innervations of the smooth muscle control the pupil diameter. It was concluded that the parasympathetic nervous system must be intact to observe the light reflex and that the sympathetic nervous system influences the shape of the reflex. Further clarification was that in the absence of the sympathetic innervations, CV is increased and the dilation velocity is decreased.

The effects of opioids on these and other components of the light reflex were further studied. Some studies focused on external light stimulus during pupillometry procedures using MDP [76, 79, 80] (Table 1). However, their studies were limited to the pupil diameter and pupil constriction. Some of these studies have also reported great variability in the magnitudes of pupil constriction due to opioids. It maybe due to the use of static pupillometry technique, which does not take into account pupil dynamics. Some studies of parental opioids have reported pupillary changes of more than 2mm [81, 82] and other studies have reported miosis of less then 1mm [83].

Table 1. A summary of pupillmetry studies conducted on methadone patients with focus on pupil characterisation.

Authors, Year	Dose	Aim	Pupillometry type	Testing eye, Rate	Visual light stimulation	Parameter measured	Conclusion
Linda et al, 1993 [79]	Methadone	Quantitative characterization of effects of lightning intensities and exposure on opioid miosis.	Pupil photographs	Right eye, two photographs were taken at each light intensity, with left eye open and then with left eye close	Flood lamp at intensities of 4, 16, 40, 80, 160, 240 foot-lamberts (fl)	Pupil diameter	Peak methadone miosis was best detected at 4 and 16 fl at 90mins after dosing
Rosse et al, 1998 [76]	Methadone	Determine relationship between pupil size and intensity of opiate withdrawal scale symptom, Weak opiate withdrawal scale	RK-426 pupil/corneal reflection tracker (ISCAN, Cambridge, MA)	Right eye tested, other eye open	No stimulation	Pupil diameter, Subjective opiate withdrawal scale (SOWS), WOWS	Pupillometry provides an objective measure of intensity of opiate withdrawal
Murillo et al, 2004 [25]	Methadone		Video based pupillometer (FIT 2000)	Left eye tested, other eye always open, 60hz	Green light stimulation	Intial diameter , CA, saccadic velocity	Pupillometry can be used in verifying concomitant drug use in methadone maintained patients
Larson MD, 2008 [80]	Fentanyl	Testing the hypothesis that increased activity in pupillo-constrictor nucleus by addition of ambient light and fentanyl blocks pupil reflex dilation.	Portable IR pupillometer (Fairville Medica Opics, Inc)	Noxious	Light projected on left eye and right eye measured. Intensity not given.	Pupil reflex dilation (PRD) (in light and dark conditions)	No constriction due to fentanyl in darkness or light, however 40% decrease in PRD
Dyer et al 1999 [29]	Methadone	Comparing plasma methadone concentration with pupil response and investigating the pharmokinetic/pharmacodynamic factors influencing withdrawal severity	Video taped image of eye under constant illumination	Not given	Electric stimulation to ear lobe instead	Pupil diameter	Pupil diameter showed strong inverse correlation with plasma concentration

3.3.4 Pupil Diameter and Opioid Maintenance

A major goal of opioid maintenance is to reduce the fluctuation in opioid effect and opioid withdrawal associated with administration of a short-acting opioid such as heroin. Examination of opioid pharmacodynamics at steady state shows significant opioid effects (e.g. pupil constriction, reduced respiration rate) that are related to changes in methadone concentration in blood over the dosing interval [29, 78]. The relationship between pupil size and subjective symptoms of opiate withdrawal during gradual opiate agonist detoxification has been studied in the past [59, 60]. Another sign is the widening of the pupil, which had been constricted by previous intake of opiates. As the pupillary diameter decreases under the influence of opiates and increases during absorption and metabolism of the drug, indicates that the changes in size of the pupils can be used to determine the course of methadone level in the blood.

As advances in modern electronic technology have recently improved recording and evaluation techniques, it has become possible to measure pupillary diameter and reaction more objectively, economically and accurately. In addition, repeated measurements can be performed without discomfort to the patients [84, 85, 86]. Computer-assisted measuring of the pupillary diameter (static pupillometry) as well as the pupillary reaction to a light stimulus (light evoked dynamic pupillometry) has been carried out [84, 85]. The high reliability of the static variability of pupillometry has been confirmed [84-86].

3.3.5 Pupillary Reflex Characterization

Pupil characterization has been demonstrated as an important parameter for pupil examination (Table 1). However, the full potential of pupil characterization has not been achieved with methadone patients yet. A consistent observable effect of opioids in humans is pupil miosis (Table 1) [25, 76, 79, 80]. The clinical relevance of the miotic effect is well demonstrated and documented decades ago [87]. Some studies suggest that opioid induced pupillary changes can be demonstrated by directly stimulating the Edinger-Westphal complex of the oculomotor nerve [88, 89].

However, others proposed that opiates depress cortical centres that ordinarily inhibit the Edinger Westphal nucleus. Neuronal mechanisms responsible for opiate-induced changes have

recently been inferred from studies of dynamic pupillary measures such as the light reflex and pupillary fluctuations. Previous studies involving pupil light reflex with methadone patients have been listed in Table 1. However to our knowledge a systematic study showing relationship between pupil characteristics (pupil diameter, CA, CV, R-dA, R-dV, Relaxation SD) and blood plasma level, has not been documented. Such information can be helpful in improving and understating the outcomes of clinical studies. In addition it should be noted that the human pupil is never truly at rest and may even show large fluctuations or hippus in normal individuals under constant illumination and accommodation conditions [90, 91]. Large fluctuations in pupil size have also been reported to occur in the rabbit [92] and rat [93] after morphine administration.

In the past research, pupil illumination has been carried out either with lamps kept at a distance or with a LED fixed on the goggle. The light intensities used in the past have been in scotopic, mesopic or in photopic levels. The intensity used in these levels has varied with different studies. Furthermore, in most of the studies only one of these light levels have been used. In such cases, it would be difficult to indicate if the pupillary constriction is due to light intensity or methadone related constriction. So far, only [79] have used different levels of intensities to investigate pupillary constriction in MDP. In their study however, they used flood lamps kept at a distance from the patients. It is difficult to indicate the actual amount of light being directed towards the patient's eye. In addition, they used one single pupil photograph, which does not give full information of the pupil behaviour.

3.4 Variation in Pupillometry

Despite the use of pupillometry in various areas including methadone maintenance, its potential in methadone maintenance program can be questioned due the following reasons.

3.4.1 Accommodation (Dark Adaptation)

The human pupil size and its response ability are governed by the antagonistic actions of the dilator and sphincter muscles in the iris [94, 95]. Pupil size can be affected due to various factors such as retinal illuminance [96], accommodation state [97, 98], and various sensory and emotional conditions [99]. Most of the studies investigating pupil size of methadone patients

have done so with the eye in a dark-adapted state, or tested one eye while the other eye was left open to reflect the status of the pupil in its normal working conditions [100, 101]. Additionally, in the dark-adapted state, the pupil differs in its resting level of ocular accommodation (in the dark). Therefore the differences due to these factors may manifest themselves as differences in pupil size. There has been no systematic investigation of this effect as a function of luminance level using methadone patients. Also, in considering the luminance level it is essential that photopic experimental conditions be carefully controlled to eliminate accommodation and vergence effects.

3.4.2 Pupillometry Methods - Static and Dynamic

Pupillometric methods are classified as static and dynamic. Static methods require one second or more to measure pupil size and are suitable in situations where pupil size has come to a constant value or is varying very slowly. Most of the quantitative information available on the pupillary action of opiates has come from static methods using still photography [64] and direct measurement with callipers [102]. The data from these methods is limited to samples of pupil size taken over a few discrete time intervals and is often accompanied by a large variance due to experimental error and physiological fluctuation. A further problem associated with pupil measurements is that the pupil is never entirely at rest but undergoes small, continuous oscillations known as hippus [103]. A single "snapshot" estimate of pupil size cannot, therefore, be accepted as a reliable predictor of its true mean size. Instead, the pupil should be monitored continuously for a suitable period to enable a confident estimate of mean size and standard deviation to be obtained. With the advent of sophisticated pupillometers it has become easier to achieve discrete pupillary information. In MDP patient's discrete pupillary information is important to understand the methadone absorption and metabolism.

3.4.3 COL Pupil Response

It is generally accepted that on unilateral light stimulation the direct and consensual light reflexes are of equal magnitude provided there is no lesion in the reflex pathways. However, Lowenstein (1954) [104] described a syndrome, which she called 'alternating contraction anisocoria', in which the direct pupillary contraction of the illuminated eye in response to unilateral light stimulation exceeded the consensual contraction of the COL pupil. This light

induced anisocoria as said to be 'alternating' because it varied according to which eye was stimulated. It was said to indicate an abnormality in the reflex pathways because it was found in a third of 'unselected clinical cases' [105] but was rarely found in normal subjects. In their study evidence was presented to show that contraction anisocoria is common in normal subjects, although the extent to which the direct reaction exceeds the consensual is small. This can affect the pupil measurements in methadone patients, which may lead to wrong clinical results.

3.4.4 Pupil Measurement Techniques

Several studies of accuracy and reliability of popular pupillometers have been conducted [106-109]. Current pupillometers do not measure the location of the pupil center in reference to the corneal limbus [110]. As a consequence, measurement of the magnitude and direction of changes in the pupil center with light level changes cannot be directly performed. However, in other clinical applications the pupil parameters are often manually estimated from photographs and video images. Also, most commercial pupillometers assume that the outline of the pupil is circular, providing only an estimate of the pupil diameter.

Pupil and iris radius were calculated using Iskander's method [110, 111]. Their method utilizes several customized techniques. It first finds the initial centre of the eye [111]. Then the limbus outline algorithm is performed followed by the pupil outline algorithm. The detected candidate points of the limbus and pupil are then used to estimate the parameters of the pupil and limbus models. A circle and an ellipse are the most popular models. The limbus outline algorithm determines the area of an image that contains the transition from the limbus to the sclera. The pupil outline algorithm determines the pupil area. The whole procedure is iterated until the estimates of the pupil and limbus centers converge to stationary values. The method uses quadruple symmetry indicator to estimate the iris and pupil center of grey images.

3.5 Eye Safety

The potential hazard of Infrared (IR) and visual Light Emitting Diodes (LED) to eyes and skin has been well known [113, 114, 115]. Most of the pupillometry devices discussed here have used either visual or IR illumination to obtain good quality images of pupil. However, the safety standards related to potential hazards from IR and visual LED's are not stated. The

hazards associated with IR and visual LED should be considered as an important parameter before conducting tests with patients.

3.5.1 IR LED

Most IR LED's emit radiation in the 800nm to 960nm range. Radiation within these wavelengths causes thermal retina hazards and thermal injury risk to the cornea and possible delayed effects on the lens of the eye (cataractogenesis). Focused light is stronger in terms of irradiance than non-focused light. Hence, injury potential increases with focusing. For several years the safety standards for such LED's were classified under the laser safety standards [114]. However, recently the standards have changed and it becomes all the more important to do safety assessment with pupillometry development. In general the IEC 60825-1 is more restrictive in case of the thermal retinal hazard; the cornea/lens limits are only in IEC 62471:2008 [115] and in the European Directive 2006/25/EC. Most of the IR LED can be classified by the simplified method according IEC 60825-1 comparing the maximum intensity emitted under absolute maximum rating conditions. When the intensity is above that limit, the source size has to be taken into account. Most of the LED manufactures such as Vishay (Vishay Intertechnology, Inc.63Lancaster Avenue Malvern, PA 19355-2143), state in their technical document associated with products that none of the available (July/2008) Vishay IR LED's violate the class 1 limit (safety limits), and IEC 62471 and the European Directive 2006/25/EC have stated that all Vishay IR LED's are inside the exempt conditions. However, it is advisable when testing with patients, to conduct the safety testing of the pupillometry goggles.

3.5.2 Visual LED

Visual LED in the visible spectrum cover the wavelength range from 400nm to 780nm including wide band white LED's. LED's in the visible spectrum are used for lighting, signalling, or as indicators. Visual LED's were also classified under the risk assessment according to IEC 62471 and in the European Directive 2006/25/EC. However, since the change of safety standards [115], safety assessment of visual LED has become mandatory. In the updated standards, blue light hazard with the wavelength depending function B (λ) (blue light hazard function) is the limiting factor which is on the red side of the spectrum (for details refer fig 18). This limit has to be taken into account up to a wavelength of 700nm to ensure eye

safety. The intensity specification of visible LED's is carried out in terms of photometric units as Candela (cd). The complexity due to strong variation of the ratio to the radiometric units used for defining the limits is being recognised by Vishay. They advise nearly all LED's are far below the exempt limits. However, care should be taken on the short wavelength side of the spectrum. Therefore, a general statement for IRED's cannot be given. The risk assessment should be carried out based on the information provided by the manufacturers and the safety standards (BSI 62471).

3.6 Reaction Time Test (RTT)

Slower RT is a typical symptom of opioid intoxication, along with other typical symptoms of sedation including slurred speech, drowsiness, poor balance, retarded movement, unsteady gait, pin point pupils etc [5]. Several studies have been conducted on humans and animals in an attempt to understand long term opioid effects on sleep pattern, learning, mood locomotives and sexual activities (116-120). These studies have confirmed the cerebral side effects of long term opioid administration. While a lesser number of studies have reported use of reaction to a stimulus as assessment tools for opioid intoxication, the significance of RTT as a neuropsychological test is well documented [121-123]. In recent years, an increasing number of studies in cognitive functions in chronic non-malignant pain patients have appeared in the literature [121-124]. Most of these studies have focused on specific diagnosis of pain syndromes whereas others have investigated cognition in mixed pain population.

In the past, mixed results have been reported when RT was used as an assessment tool with patients. One study found that long-term opioid dependence showed no significant differences between the patients and control group [122]. They used the broad spectrum Halstead–Reitan neuropsychological test battery [125]. However in another study, cognitive and motor performance was found to be impaired in 15 patients receiving morphine [124]. The mixed responses of ODP in the above studies can either be due to the complexity of the topic itself (patient's reaction pathway, including mental processing and efferent and afferent paths) or the effects of opioid.

Remarkably, little research has been conducted to study its usefulness in the assessment of methadone rehabilitation patients. The literature concerned with cognitive functioning during

long-term opioid treatment so far has primarily dealt with drug addicts and cancer patients, or short-term administration of healthy volunteers. Although patients with non-malignant pain constitute a rather heterogeneous group, they are quite different from cancer patients in terms of pains, psychosocial circumstances, diseases, etc. To our knowledge, few or no studies have attempted to examine the cognitive functioning of methadone rehabilitation patients. One study focusing on methadone patients (ex Heroine addicts) was conducted in 1970 by Norman Gordon [128]. He used three different types of RT to investigate RT differences in 27 patients and some control subjects. His study also focussed on dose related RT. He concluded that RT difference between control group and patient group could be due to superior signal detection by control group. The apparatus used in that study was unlike the sophisticated programs and devices that exist these days. Therefore, use of modern day softwares with high accuracy can give more in-depth knowledge on the RT of patients and control groups.

It has been demonstrated in the past that RTT responses can vary with the type of RTT being used. The pioneer reaction time study was that of Donders (1868) [129]. He showed that a simple reaction time is shorter than a recognition reaction time, and that the choice reaction time is longest of all. The complexity further increases with the use of auditory RTT and visual RTT. Many researchers have confirmed that reaction to sound is faster than reaction to light, with mean auditory reaction times being 140-160ms and visual reaction times being 180-200ms [129-134]. Other factors that can affect RTT are the type of RTT used, the learning effect, the sample size, etc.

To understand the RT impairment in MDP and use of RTT as a potential assessment technique, above mentioned considerations must be made. Since the nature of RTT is such that variations are inherent in the test itself, therefore the use of a simple visual RTT (SVRTT) test is advisable.

3.7 Summary

During the last decade, use of urine testing, blood testing, pupillometry and to some extent RTT has been use for assessment of ODP. Commercial pupillometers became sophisticated with time and improvement in technology and gave valuable information. However, urine testing pre-dose and blood testing post-dose are still considered the way to go

since pupillometry techniques are still widely under investigation. RTT is relatively new and its usefulness with MDP is yet to be established. However, the potential of pupillometry and RTT techniques relies on it providing a direct measurement of the of the patients level of intoxication, non-invasiveness, easy to use, and strong potential of being used as a clinical tool in office settings.

As reviewed above, pupillometry requires a better understanding of the pupil characterization parameters, eye safety standards, accommodation effects, consensual response, and discrete pupillary information. Furthermore, pupillometers have a strong history and with the development of technology the cost of pupillometers make it impractical for daily usage. Therefore, a simple, but practical multifunctional pupillometer, which can be used for systematic study of the above mentioned pupillary parameters, can be useful in clinical settings. Furthermore, RTT, which can be simple in nature, can be used for better understanding on its usefulness in clinical settings. Accordingly, the present study was designed to develop a pupillometer which gives systematic information on the parameter described above and introduce a SVRTT which can be potentially used in clinical settings.

4. Objectives of the Study

The first objective of the study was to develop a multifunctional pupillometry goggles. The second objective was conducting a systematic investigation of the pupillary characteristics, eye safety, accommodation response and consensual response of pupil using methadone patients and control group. Based on the data obtained, the potential of the pupillometry system can be tested in clinical settings. Third objective was to investigate the potential of SVRTT as an assessment tool for methadone patients. The objectives can be summarized as follows:

- To develop a automatic multifunctional pupillometry system
- To do safety analysis of the pupillometry goggles (using standards: BSI 62471)
- To develop a SVRTT for the assessment of methadone patients
- To conduct urine testing before dose intake and blood testing before and after dose intake (0-6hrs, once every hour.).

Specific Objectives

Pupillometry

- To develop a multifunctional pupillometry system for pupil measurement
- To investigate the change of the pupil diameter induced by methadone over a period of (0-6hrs) at 0lux
- To investigate the effect of varied light intensity in detection of pupil constriction due to methadone
- To measure pupil characterization parameter (CA, CV, R-dA, R-dV, Relaxation SD)
- To investigate the correlation between pupil measurements versus methadone metabolism
- To investigate the effect of accommodation/dark adaptation on pupillometry results
- To conduct all above test on IPL eye and COL eye and investigate the differences
- To investigate the potential of the pupillometry system in clinical settings by developing an arousal index based on pupillometry results obtained from this study.

RTT

- To develop a SVRTT
- To measure RTT of patient and control group
- To investigate the potential of SVRTT as a clinical assessment tool used with MDP and ODP in general.

Inter-test relationship

- To investigate inter-correlation between MPC of methadone patients with pupil diameter, pupil characterisation parameters and SVRTT
- To investigate inter-correlation between MPC of methadone patients with pupil characterization parameter, and SVRTT
- To investigate the correlation between hysteresis (0-6hrs) in pupil diameter and SVRTT

Figure 4 shows the overall summary of the objectives of this thesis. It also represents how the objectives were achieved.

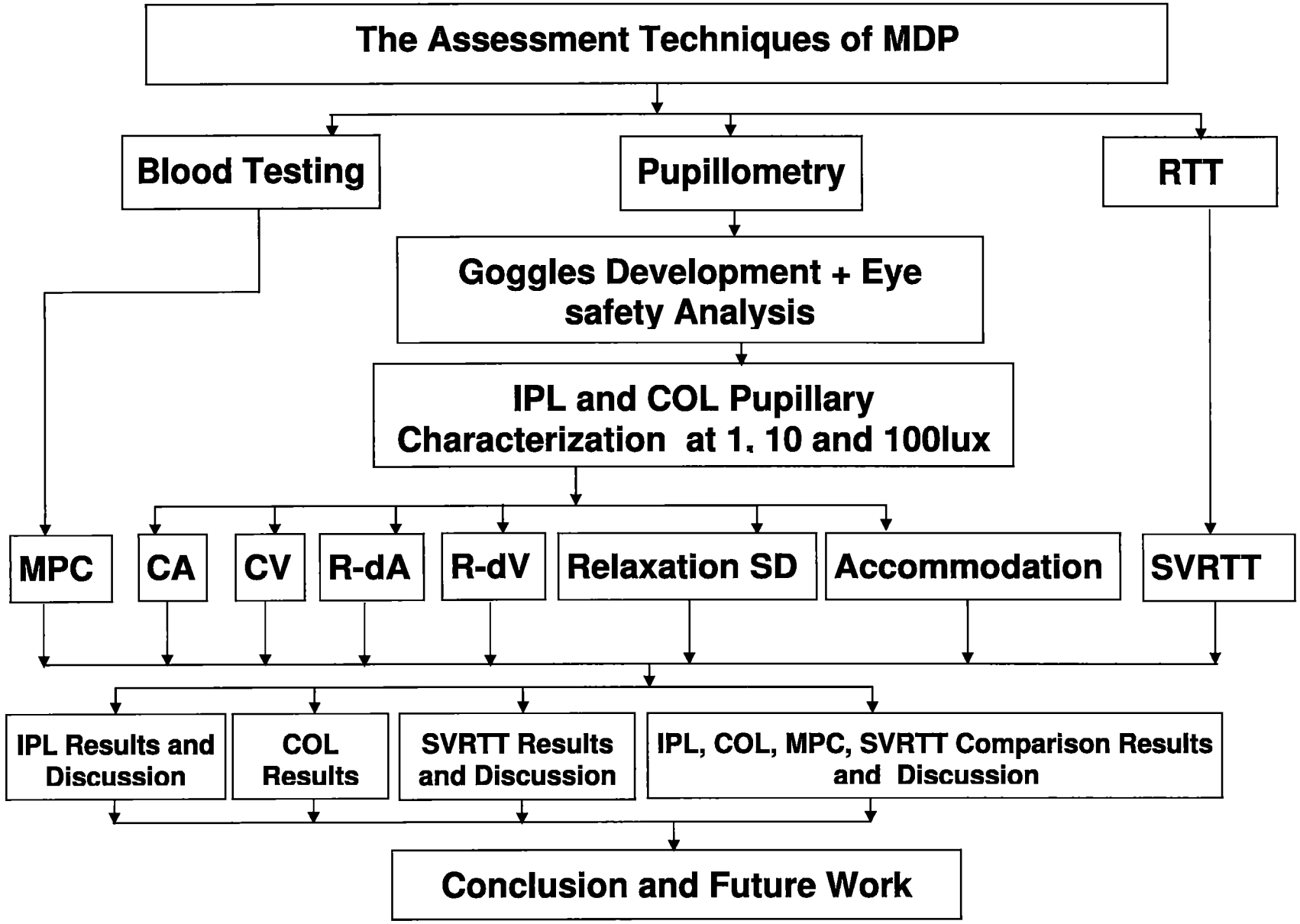

Figure 2. A Schematic representation of the contents of this thesis.

5. Material and Methods

5.1 Patients

Fifteen adult methadone patients and 2 control subjects, with mean age 22 years were recruited for the study. The patients were recruited from Humanitas Trust Centre (HTC) (primarily involved with drug dependency rehabilitation). The patients have been receiving treatment at HTC for several years and the patients and the physician (a member of this study) enjoyed a healthy trusting relationship. 13 of the 17 members used in the study were men and remaining 4 were women. Only those patients were selected for testing who were in rehabilitation program in HTC for at least one year. All subjects were free of any ocular or systemic disease and had no history of ocular surgery or significant trauma. None of the subjects wore contact lenses.

Several days before the study was conducted the patients were asked for their consent in volunteering to be a part of the research study. The potential risks and benefits involved in this study were also clearly explained during the physician and patient private meeting. After they indicated their willingness to participate in the study, they were given Information Sheet (IS) and a Consent Form (CF) to read and sign. The study was approved by the institutional review board and consent forms were obtained from each patient at the time of admission to the study.

5.2 Ethics Approval

Ethics approval for the study was obtained from The Tasmania University Human Research Ethics Committee and the Tasmanian State Government Ethics Body. The Ethics application consisted of the application form, the information sheet, and the consent form. The application form described the details of the procedure for the tests and the system used for testing the patients.

5.3 Experimental Procedure Overview

Figure 3 shows the schematic of the overview of the test procedure. After the patients arrived at the HTC they were familiarsied with the testing device and software. The patients reported the frequency of opioid, tobacco or any other illicit

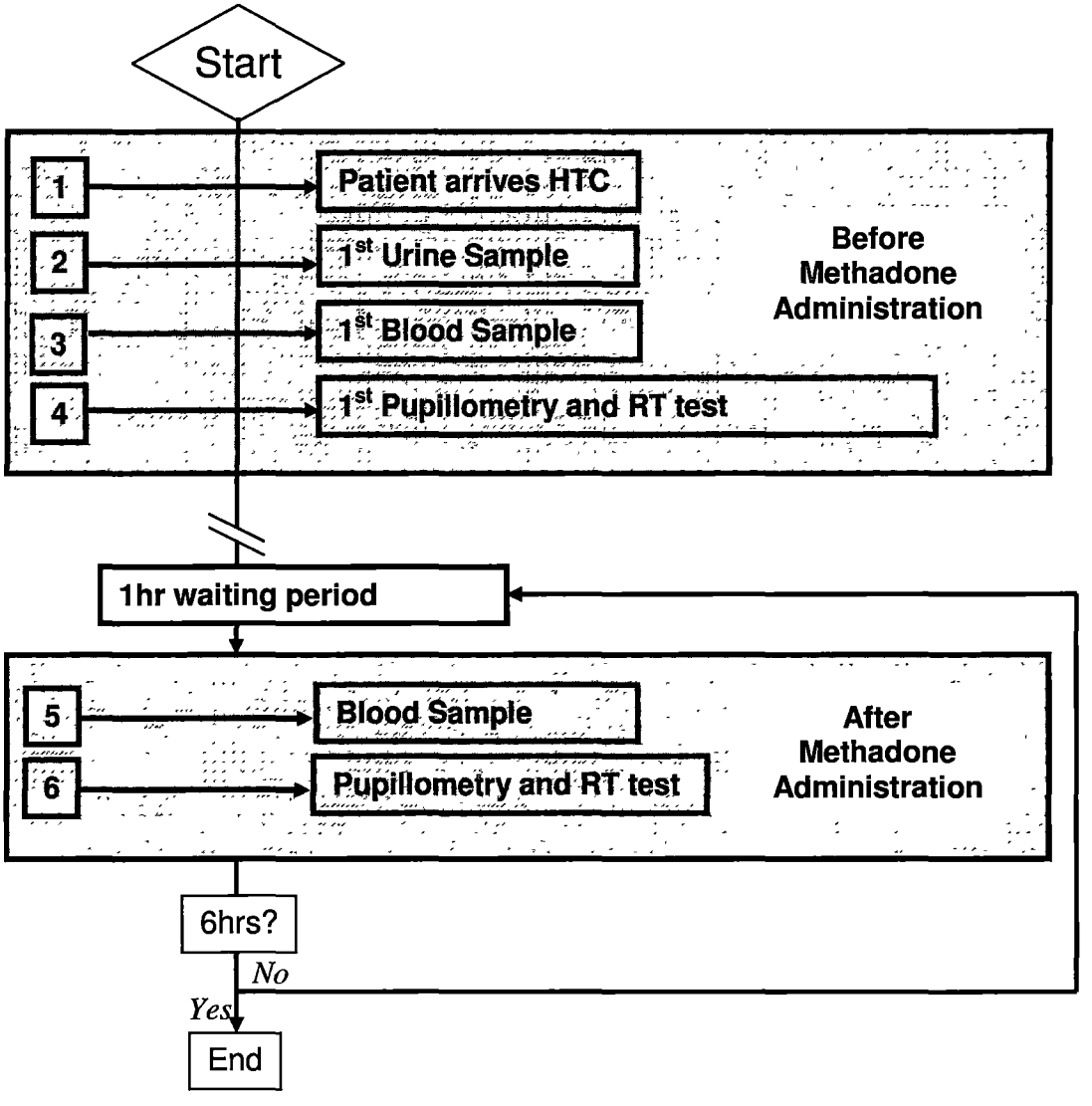

Figure 3. Schematic of the overview of the test procedure.

drug or alcohol use each day, starting with the testing day before admission and the day before testing. Of the 20 scheduled patients, 3 were excluded from testing because they were missing. None of the patients complained about the testing procedures. That was followed by the pre-

dose urine and blood sample collection under the supervision of a nurse, followed by their pre-dose patient testing with the assessment techniques. The assessment techniques pupillometry testing (IPL and COL measurements) followed by RTT. This data was collected and stored as pre-dose data of the patients. This denoted the completion of first post-dose tests. The patients were asked to rest for the next hour after which the tests were conducted again for 6hrs at interval of 1hr in the following sequence: blood test, pupillometry test and RTT.

5.4 Urine and Blood Testing

Urine samples were collected from each member of the patient group and the control group when they arrived HTC. Blood samples (2ml) were drawn too prior to the administration of the daily dose of methadone and every hour after dose for six hours. The blood samples were stored in a heparin tube. Both urine and blood samples were stored at 4°C until further analysis.

5.5 Simple Visual Reaction Time Test (SVRTT)

5.5.1 Apparatus

The apparatus used for the testing was used in our pervious study [135]. The apparatus was kept as simple as possible. A desktop computer with a mouse was used for the testing. Testing was conducted in a separate room with no external disturbance. An office desk and a comfortable chair were used. Patients were asked about their level of comfort prior to the test. The specifications of the essential parameters of the apparatus used are given in Table 2.

Table 2. Apparatus used for SVRTT

Name	Brand	Specifications
Personal Desktop Computer	Dell	Intel(R), Pentium 4 CPU 3 GHz, 1.99 GB RAM
Optical Mouse	Dell	Right Hand user (Left Click used)
RT Testing Software	Developed using Matlab	Matlab R2006b (Mathworks, USA)

5.5.2 Design of SVRTT

The SVRTT was developed in Matlab R2006b (Mathworks, USA). A stimulus was displayed on the background of a grey screen of a 15" SVGA monitor placed at a distance of 80 cm from the subject's eyes. Screen resolution was 1280 x 1024 pixels.

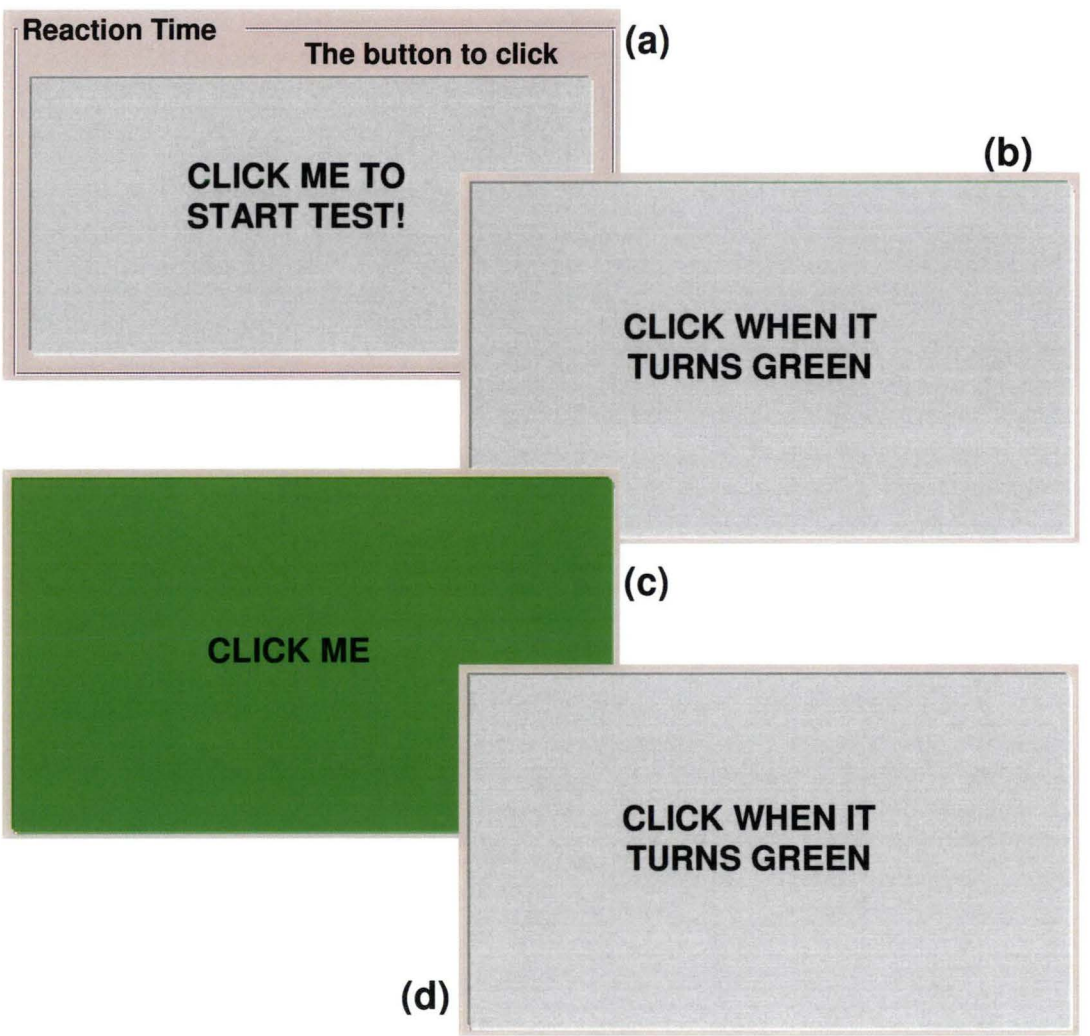

Figure 4. An illustration of SVRTT (a) The screen identifying the start of the test (b) The screen showing the grey button to be pressed to initiate the test (c) The screen showing the fluorescent green button (d) The screen showing the target turning back to grey and repeats stages (a)(b)(c).

A single target, a bright green fluorescent filled square covering the dimensions of the computer monitor was used as the visual stimulus (Fig 4). The target transformed from green color to grey color at random time intervals during the test. The time between the color change and the clicking of the left mouse button was recorded as the RT. The RT in milliseconds was saved in .txt format in the background. The test number was incremented following each response from the user. The software was designed to record 90 RT's in one test set.

5.5.3 SVRTT Procedure

The patients sat on a chair in a lit room and were instructed to look at the monitor and click the left mouse button as soon as the stimulus turned green. The mouse was kept at approximately the same height as that used by the subjects in their daily use. To more closely simulate normal conditions, fixation was not required; however, to ensure that the participants did not divert their gaze constantly, the button to click in the RT software was made of the same dimension of that of the computer monitor. The test was interrupted if the participant became slumbered, distracted or volunteered to stop. An interruption only occurred once and during this occasion the patient took his sight off the computer monitor and returned to the test after few minutes of rest.

Prior to the initiation of the test, patients were given a system demonstration. They were given the opportunity to perform a training session involving 3 trials, simulating the same conditions as that of the real test. Following the training sessions, the participants were asked to do the real test. One hour rest was given after each test of 90 responses, during which the subjects were allowed to occupy themselves otherwise. They returned for the test after every hour.

Figure 4 shows a step-wise visual illustration of a typical SVRTT. The patients were already briefed about the test therefore the initial display on computer monitor was kept very simple and with less text (Fig 4(a)). The stimulus button or the target to be clicked is clearly recognized by a rectangular shape box with "CLICK ME TO START TEST!" displayed on it. Once the user clicks the left mouse button the test commences. After clicking the target changes the message on it to "CLICK WHEN IT TURNS GREEN". Once the target turns color to green the user has to respond by clicking the left mouse button. The time taken from the target turning

green to the user clicking the mouse is considered the RT. The time is displayed in the text window besides the target in seconds.

5.6 Experimental Setup for Pupillometry

5.6.1 Design of Pupillometry System Development

Figure 5 shows a schematic of the architecture of the pupillometry system used for the testing. Figure 6 shows the developed pupillometry system used for testing.

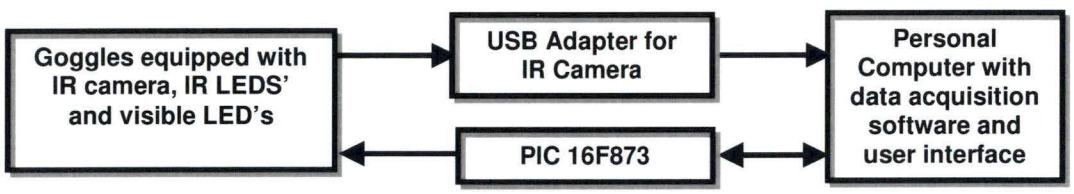

Figure 5. Schematic of the architecture of the pupillometry system.

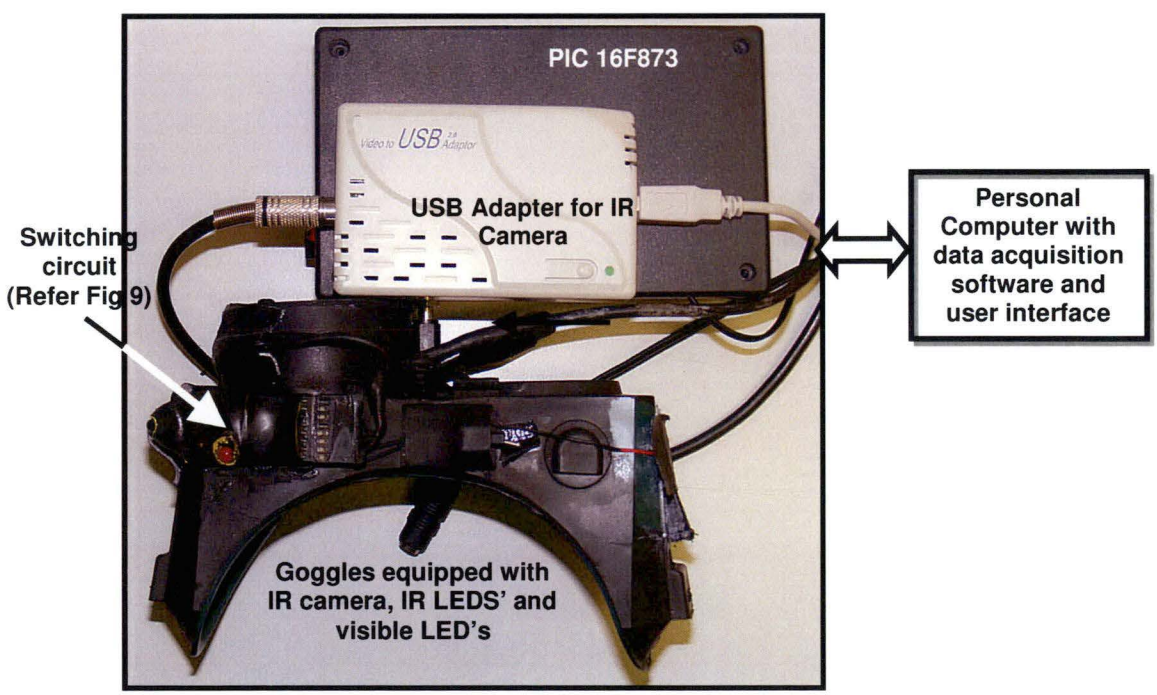

Figure 6. The pupillometry system.

A pair of welding goggles equipped with an IR Charged Coupled Device (CCD) camera and IR and visible LED are used for the testing (Fig 6). A USB adapter (Video Capture Box USB 2.0) was used to convert the video out from the camera to a USB input of a desktop

computer. A microprocessor (PIC16f873) was used to control and synchronies the frame capturing from the camera and turning the visible LED's On and OFF. The PIC18f873 board was connected to the computer using a USB connection. A software user interface was developed in LabView 6 (National Instruments) for the physician to get a visible feedback on the computer screen. A detailed description of each component in the pupillometry system is explained in the sections below.

5.6.2 The Pupillometry System Development

The goggles (Welding goggles, with eye protection removed, Protector) consisted of a head mounted device to which a IR CCD camera (Colour Domes-Standard Resolution Omni vision QC-3489, 1/4" Medium Resolution Colour Dome Camera, Panasonic Colour CCD Image Sensor, 3.6mm PCB Lens, 12V DC, 80mA Power Consumption) was attached on the left eye piece (Fig 7).

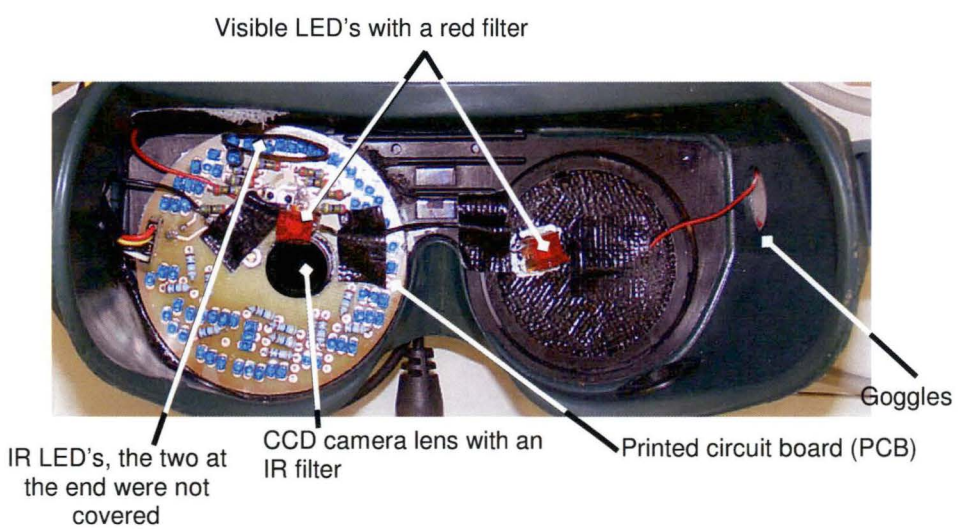

Figure 7. Figure shows the inner part of the goggles. The inner part of the goggles consists of a CCD camera for image capture, twelve IR LED's for eye illumination and one two whit LED's for stimulation.

Pupil images were captured at the rate of 10 f/s and saved in a systematic format for offline data analysis. An IR filter (broad band acrylic filter, filters wavelength below 780nm (Fig 7) was attached to the lens of the camera. The IR filter was used to stop IR light from entering the camera lens and distort the image. Visible light excitement was produced using

visible LED's. Two visible LED's, each on one eye piece of the goggles, were installed. The LED's were installed in such a way that the illumination was normal to the pupil (light falls directly in the eye). The distance between the visible LED, IR LED and the eye was approximately 35 mm (Fig 8).

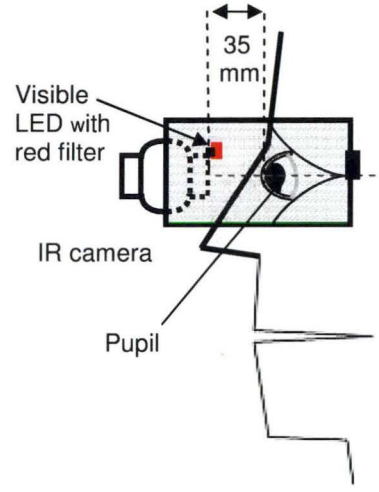

Figure 8. A schematic of the patient wearing the goggles. The distance between the eye and the camera lens was 35mm.

A red filter was placed on top of the visible LED's to protect the eyes from any potential danger due to prolonged exposure of the visible white light on the eyes. That is also one of the reasons the goggles were not designed to be head mounted (strapless). The patient held the goggles with their own hands during the test, so that they are in full control of the goggles, and immediately remove it in the case of emergency. The connections from the IR LED's and the visible LED's were fed to the PIC16F873 microcontroller via a small switching circuit (Fig 9(b)). The function of the switching circuit was to provide sufficient current to drive the visible and IR LED's. A (Pulse Width Modulation) PWM signal from PIC16F873 was used to control the illumination of the IR LED's. The connections from the microcontroller to the goggles were fed using a 4 pin connector (Fig 9 (d)). Two visible LED's installed on the microcontroller board were used for visual stimulus inside the goggles (Fig 9 (c)).

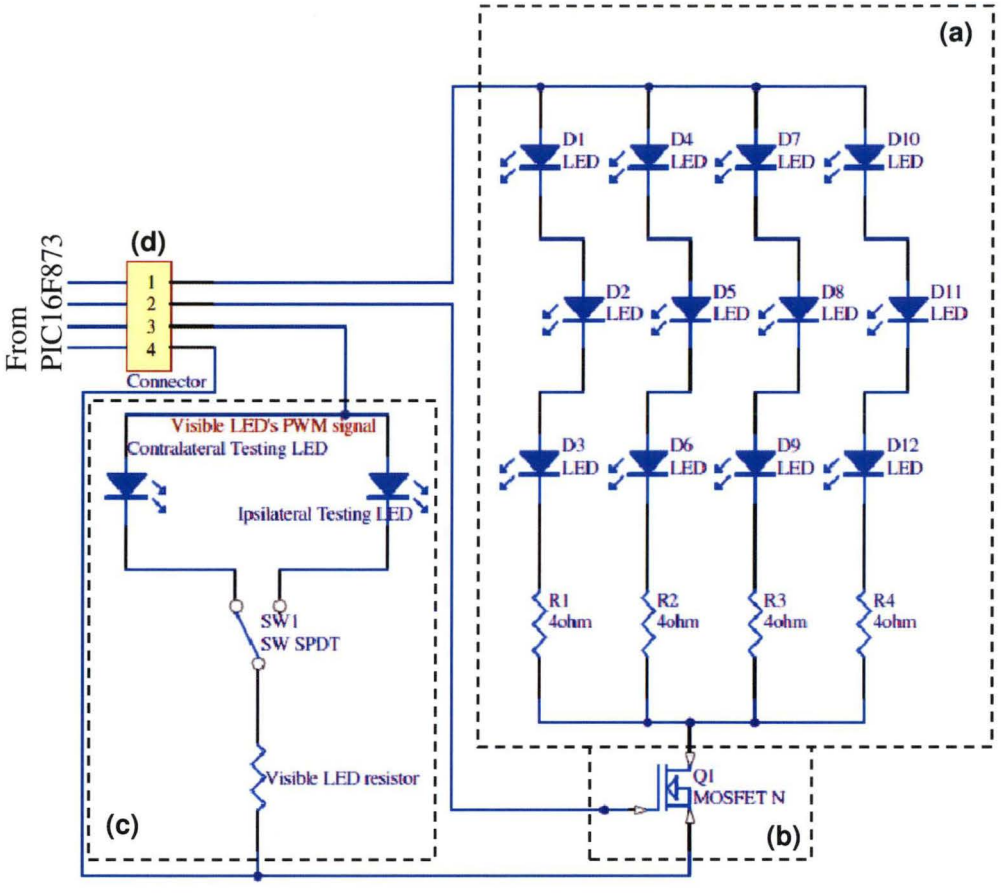

Figure 9. A schematic of the circuit on the PCB board installed on the goggles' eye piece. The PCB board was fastened in a polymer plastic box base to make it portable in nature. (a) Represents the 12 IR LED's used for IR illumination of the pupil. (b) Represents the switching circuit used to provide sufficient current to drive the visible and IR LED's (c) Represents the visible LED circuit. Both IPL and COL testing visible LED's were toggled using a switch (SW1). (d) shows that the signal to the goggles was sent by the microcontroller PIC16f873.

The camera lens was surrounded by I2 IR LED's mounted on a PCB board to illuminate the eye and acquire good quality images of pupil (Fig 7). The PCB formed the base of the camera lens. It consisted of 12 IR LED's in groups of fours (Fig 9 (a), Fig 7). The two IR LED at the ends (D1 and D12 in Fig 9 (a) were kept open while the others were fixed with a solid tube to direct the IR radiation away from the eyes, however providing sufficient illumination for good quality pupil images. This arrangement was made such so that only two IR LED's

reflection can be seen on the pupil images. The distance between the two LED's was used for correcting any potential errors in pupil diameter measurement caused due to the movement of the goggles during the testing process (refer section 5.6.8). Another reason was to avoid the IR radiations falling directly on the eye. The safety of the eyes from IR and visible LED is discussed in section 6.

The illumination of IR LED's and the visible LED's was controlled by a microcontroller (PIC16F873) controller (Fig 9(d) and Fig 5). Figure 10 shows the schematic and a picture of the microcontroller circuit. It consists of two sections. Part (a) represents the PIC16f873 pin configuration and the associated electronic on the microcontroller boards designed and developed for controlling the IR and visible LED's on the goggles. The microcontroller was programmed in MPLAB IC2.

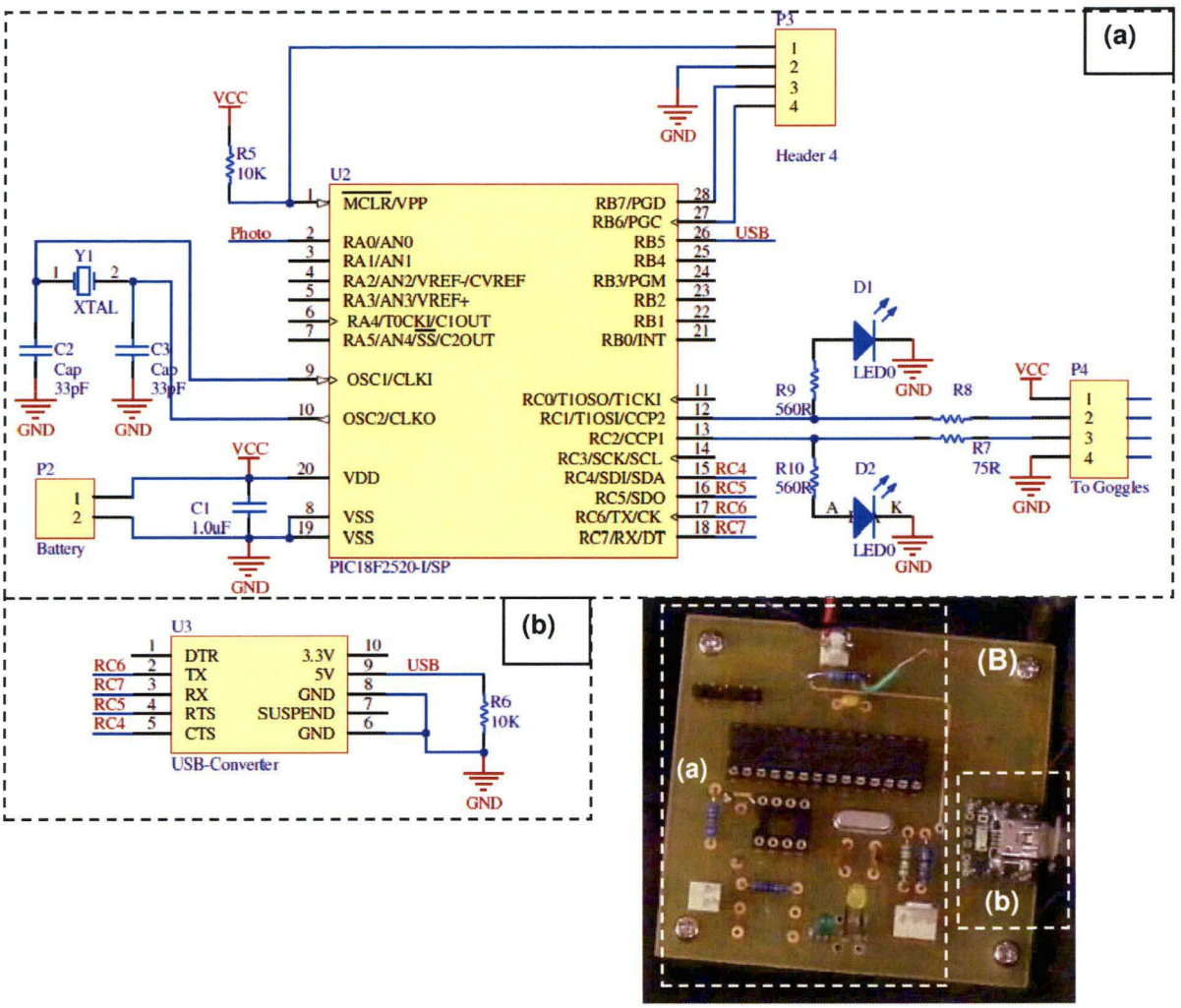

Figure 10. A schematic of the microcontroller circuit developed for automatic functioning of the goggles. (a) Represents the PIC16f873 pin diagram and associated electronics on the board. The connections from the microcontroller to the goggles were carried out using a 4 pin connector. (b) Represents the USB converter for signal transmission between PIC16f873 and the desktop computer with a visual feedback for the physician. (B) Inset is a picture of the PIC16f873 board (a) and the USB converter (b).

Figure 11 demonstrates the function of the visible LED as controlled by the PIC16f873. The LED has three levels of illumination; 1lux, 10lux and 100lux. In between these illuminating levels the LED is turned OFF for the pupil to dilate. At the 7th and 8th stage, the LED

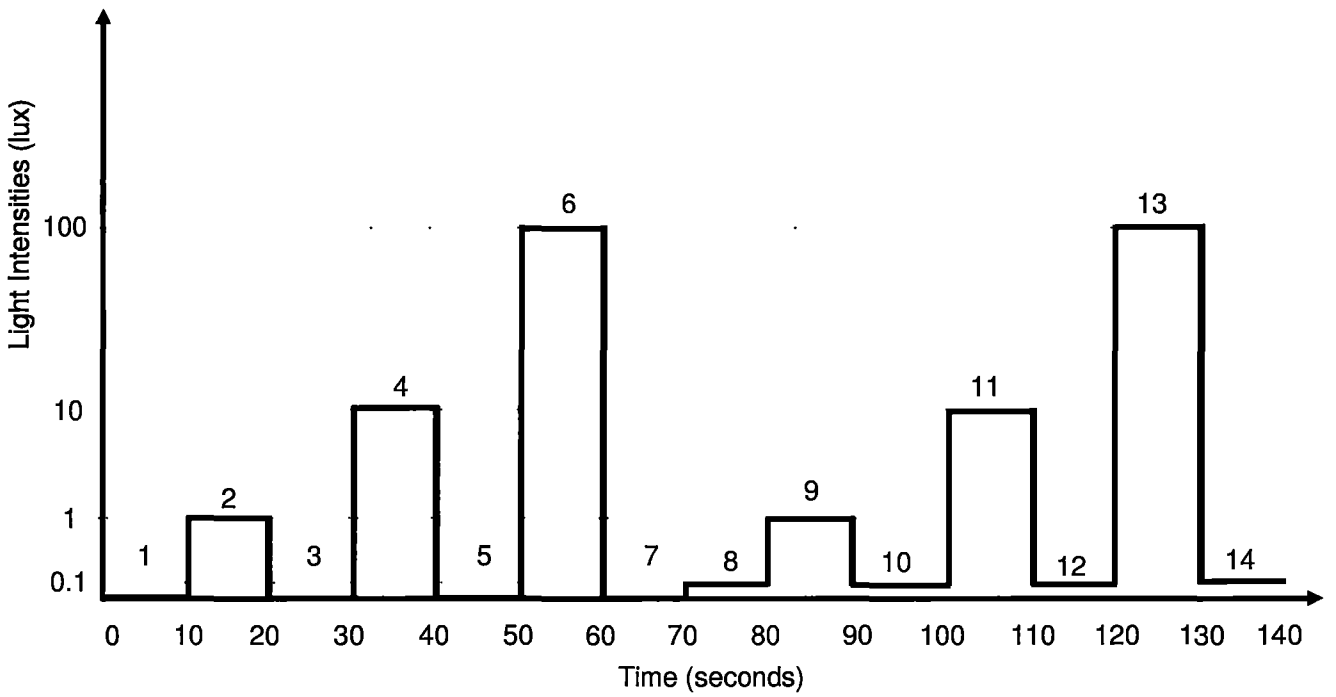

Figure 11. A schematic in the form of a plot to demonstrate function of the visible LED during IPL or COL test.

baseline illumination changes from 0lux to 0.1lux. This was designed to investigate if there are any accommodative effects on pupil response. The same procedure from stage 1 to 7 is repeated in stage 8-14, however replacing LED baseline intensity from 0lux to 0.1lux. Throughout this process the microcontroller synchronised the pulsating of the visible LED, the image capturing by LAbView, and the PWM signal to the IR LED to maintain its illumination.

5.6.3 Visual Feedback for Physician

Image capturing User interface: The patients were aware of the device and its usability as they were given training before the real test started. Figure 12 shows the user interface for the data acquisition procedure and Figure 13 demonstrates the pupillometry initialization process. The "Align" button was used to adjust the goggles such that the pupil was located in the center of the user interface window (Fig 12). When the Align button is pressed one visible LED turns ON (depending on COL or IPL measurement).

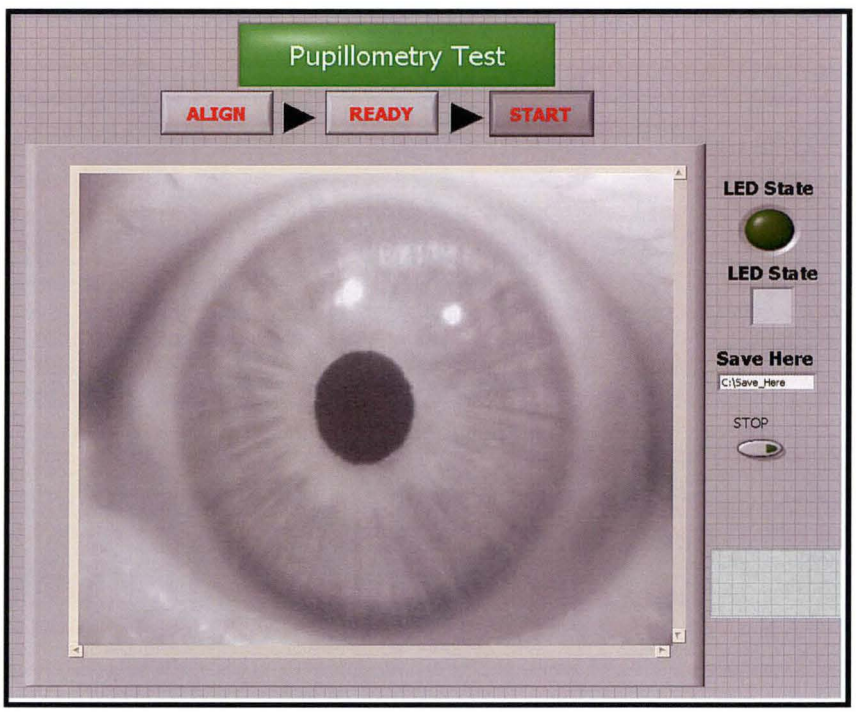

Figure 12. An illustration of the software developed for pupil image capture. The user interface also serves as a visual feedback for the physician to monitor the pupil during the test.

The visible LED's were positioned in such a way that the light falls directly on the pupil and when the patient focuses on the LED the pupil is automatically opposite the camera lens. After the pupil was centered the patients were asked about their comfort and permission to start the test. The patients were also not allowed to move throughout the test procedure, which lasted for 140sec. If the physician observed any movement that might affect the image quality the test was re-conducted immediately.

After centering the pupil, the "Ready" button was clicked indicating that the test is ready to start. Clicking the "Start" button started the test. A visual feedback of the state of visual LED was provided on the software user interface in the form "LED State" button. The green button turned fluorescent green when the visible LED intensity was 1lux, 10lux or 100lux and turned dark green when the visible LED intensity was 0lux or 0.1lux. "LED state" displayed the corresponding level light intensity of the visible LED. The "Save Here" option gave the physician the freedom to specify the location for saving the data in the computer. If this was not specified by the physician, then the data is stored in a default location corresponding to the patient Id and test number. The "stop" button was provided to allow the test to be stopped and

for the system to reset in case of emergency. In each test 1498 frames of pupil images were collected at 107f/10sec rate and stored in a systematic manner. For one patient the total number of images captured throughout the test (0-6hrs) was 20,972. This shows the discrete nature of pupillary information captured in this study. The images were stored in a systematic format corresponding to the different light levels of the visible LED's.

5.6.4 Procedure of Pupillometry Testing

As shown in Fig 13 the pupillometry testing was initiated when the patient wears the goggles and indicates about their comfort to the physician. Once the patient indicates his readiness the test was resumed. The physician instructed the patient to move the goggles such that the pupil is centred by checking the user interface window (Fig 13). During this time the patient looks at the LED, which has just enough intensity for the patient to focus on it. Once the patient and the physician are ready the physician clicks the ready button.

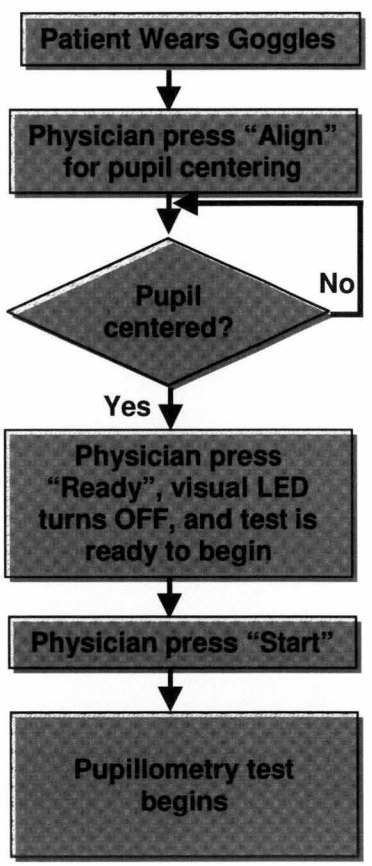

Figure 13. Flow chart demonstrating the pupillometry initialisation procedure

The LED turns OFF and after few seconds the physician starts the test by clicking the start button. During the period of the test the physician sat beside the patient and gives verbal feedback, based on visual feedback on the computer screen, in case the patient is closing their eyelids. Upon the discretion of the physician, they decide whether the test has been conducted successfully. If the test is successful, the patients is asked to leave the room and relax, otherwise the test is done again immediately.

5.6.5 Patient Profile

A software user interface was developed in LabView 6 for data acquisition and systematic storage (Fig 14). Patients were identified with a unique identity code such as SD0001.

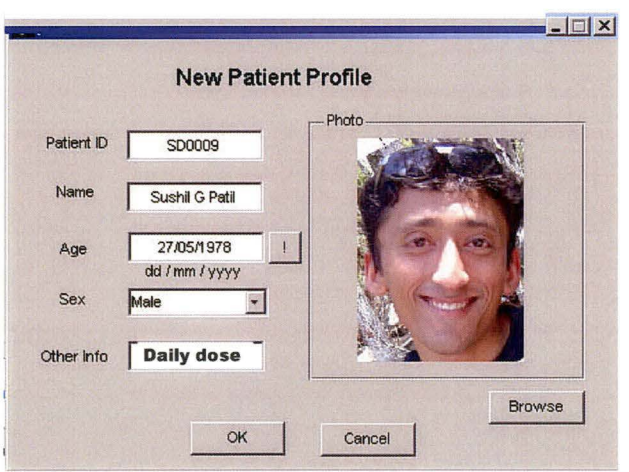

Figure 14. An example of a patient profile. The picture used in the patient profile does not represent any patient used in this study.

Every patient was given a new identity code by the physician before conducting the first test. The same code was used for the remaining tests. The details of the patient can be entered in a new window that assigns the unique identity code (Fig 5). The details include Name, Age, Sex, Patient Id. Patient photo, and other unique details specific to the patient.

Some additional functions were also developed but were not used in this study that includes selection of the patient Id from the drop down menu or entering of a new id. The test number is automatically incremented if a previous test has been carried out, or assigned number

1 by default. The software acquires the date and time for the test from the computer. The physician could also select a standard set of data from the user interface specific for the patient such as the type of medication and whether the patient is sedated or not (subjective assessment by the physician before the test). The details of the additional functions was published in our pervious study [136]

5.6.6 Procedure for Pupillometry Measurements

Figure 15 is an illustration of a typical pupil response during a single test. The pupillary response at different light intensities is shown in Figure 15. The white plot indicates the varying light intensity of the visual LED, and the blue plot indicates the corresponding pupillary response. The Y-axis represents the number of pixels and the X-axis the number of images captured during the period of the test. Both Y and X axes were converted to mm and time, respectively during data analysis.

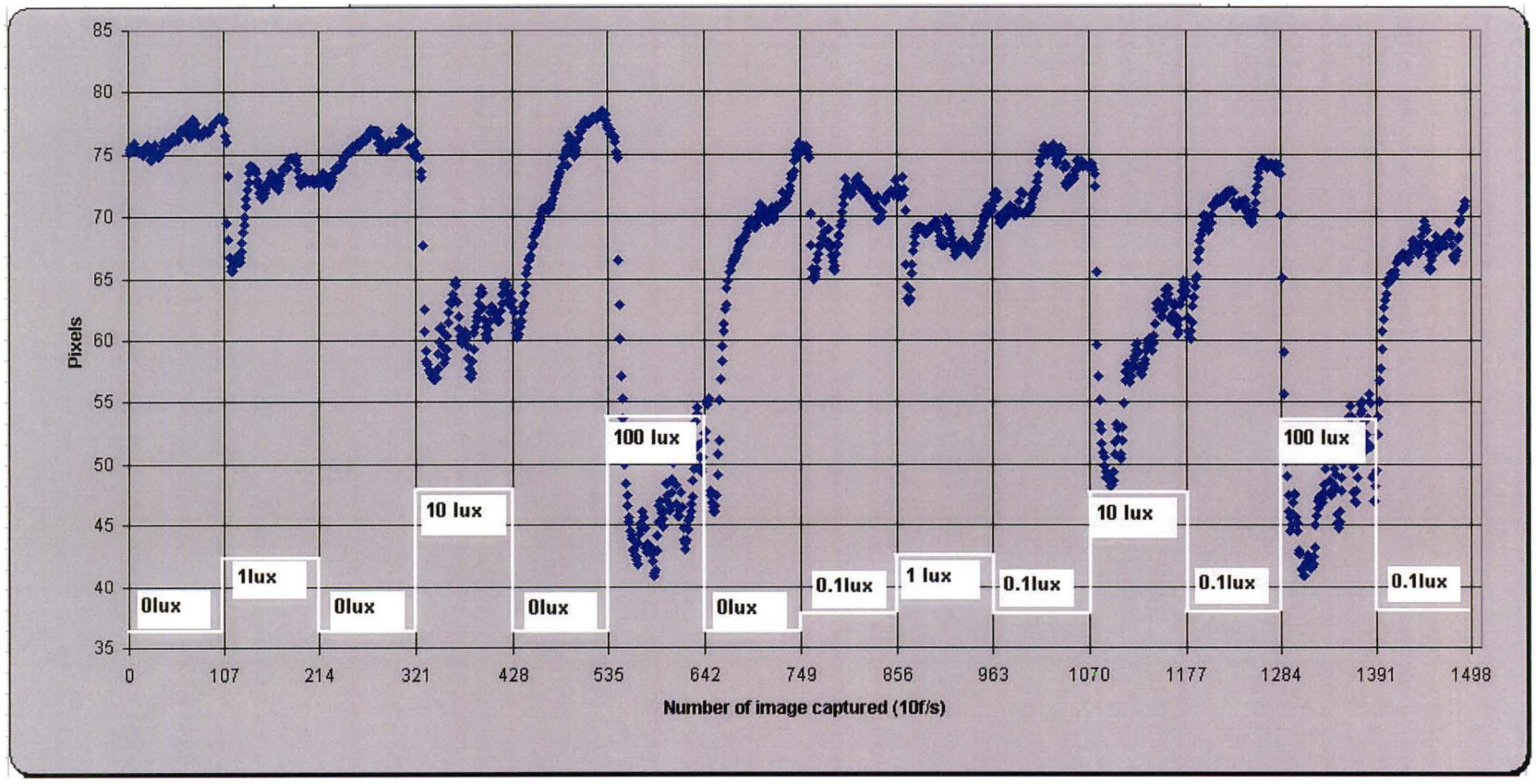

Figure 15. An illustration of a typical pupil response during a single test. The Y-axis represents the number of pixels and the X-axis the number of images captured during the period of the test. Both Y and X axes were converted to mm and time, respectively for data analysis. The light intensities are shown for ease in understanding the figure.

Figure 16 is a section of Figure 15. The figure represents the pupil response to light stimulus. It also shows the pupil response before and after the light stimulus when it's dark (baseline intensity of 0lux). The pupil response to light was divided into three sections.

Constriction: This parameter was used for measuring CA and CV. The time when the LED turns ON to the time the first maximum pupillary constriction was obtained was considered as the constriction phase (Fig 16). The corresponding amplitude was considered as CA. CV calculated using the ratio of CA to the corresponding time.

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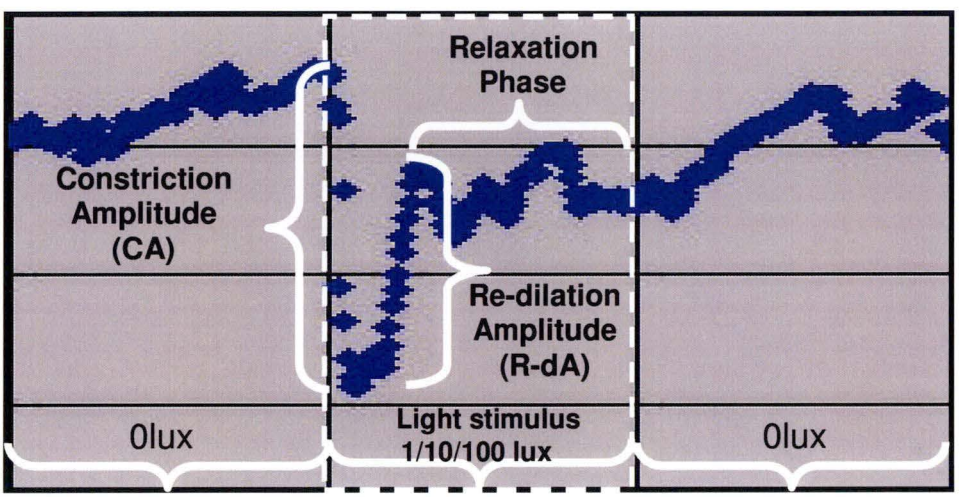

Figure 16. Pupil response before and after the light stimulus. A typical pupil shows three phases to a light stimulus; constriction, R-d and relaxation in this order.

Re-dilation (R-d): This parameter was used for measuring Re-dilation Amplitude (R-dA) and Re-dilation Velocity (R-dV). R-d phase represents the phase immediately after constriction to the point where the pupil reaches its first maximum peak (Fig 16). The images representing that phase were used for analysing the R-dA and R-dV. The absolute value of pupil diameter obtained in R-d phase was considered as the R-dA. R-dV was calculated using the ratio of R-dA and the corresponding time.

Relaxation: This phase represents the section after R-d to the end time the LED's switched to either of the baselines intensities (0lux or 0.1lux) (Fig 16)

5.6.7 Pupil Detection and Measurement

Pupil and iris radius are calculated using the method discussed in [110, 111]. The method first finds the initial centre of the eye. Then the limbus outline algorithm is performed followed by the pupil outline algorithm. The detected candidate points of the limbus and pupil are then used to estimate the parameters of the pupil and limbus models.

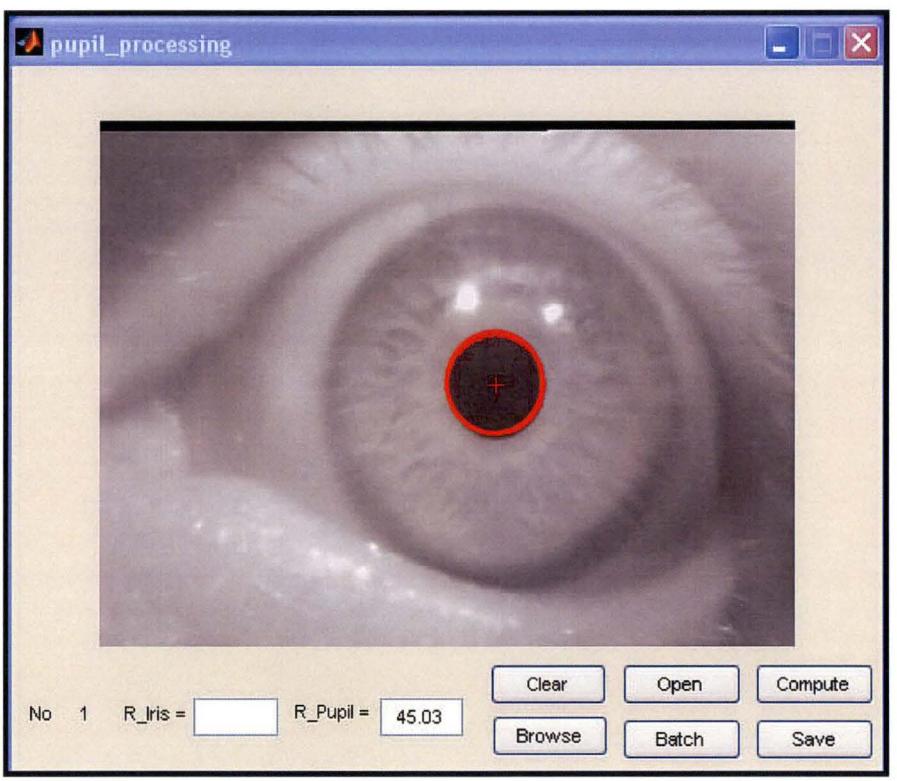

Figure 17. An illustration of pupil detection and diameter measurement in pixels.

The limbus outline algorithm determines the area of an image that contains the transition from the limbus to the sclera. The pupil outline algorithm determines the pupil area. The whole procedure is iterated until the estimates of the pupil and limbus centers converge to stationary values. The method uses quadruple symmetry indicator to estimate the iris and pupil center of grey images. A typical example of pupil detection and diameter calculation (in pixles) is shown in Figure 17.

5.6.8 Goggles Movement Drift Amendment

The distance between the pupil and the camera may vary with individual patients wearing the goggles and errors may be generated due to alterations in the size of the image projected by the camera. Therefore, to reduce such errors, the distance between the two illuminating visible LED's reflected on the pupil was measured to adjust for the errors generated due to the difference of the distance from the pupil and camera. As the distance between the pupil and the camera lengthens, the distance between the two illuminations reflected on the pupil shortens. Thus the distance of the reflected light was measured to adjust for the errors between individuals due to the distance of the pupil and the camera. The following assessments were performed to evaluate the accuracy of the pupil measurement. In the first experiment, to assess the accuracy of the actual pupil measurement once the camera had been calibrated, the distance between the pupil and the camera was fixed at 35 mm, and the size of 10 pupil images of 640 X 480 sizes were printed as 2mm by 9 mm in size was measured. Assuming that the camera was calibrated and proceeding with the 5 mm pupil images as the standard, all images showed a small range of error, within 0.1 mm. Second, the pupil size was fixed, and the effect of variation in distance from the camera to the pupil on the pupil measurement errors was assessed. The change of the image sizes formed on the camera was assessed while the distance between the camera and the pupil image was varied to 25±10 mm. There was a change of approximately 15 pixels when the distance from the eye to the camera was varied by ±5 mm, and the maximum error rate was 9% for the maximum change of 5 mm. The relationship between the error and the distance was linear. Based on these results, we believed that the necessity for the patient to wear goggles will alter the location of the camera according to individual head shape and eye position, and that adjustment is therefore required.

To adjust for the error generated by the distance between the camera and eye, the change of the location of the light source reflected on the pupil was utilized. As both the size of the pupil image on the camera and the relative distance of the location of a pair of LED images reflected on the corneal surface are affected by the distance from the camera to the eye, the obtained pupil size could be standardized. For this assessment, the location of the camera was moved while a subject was wearing the goggles with the visible LED's turned off, and the relationships between the camera adjustment, the change of the distance between the camera and the eye, and the change of the relative distance of the reflected light source were analysed.

From the result, although the pupil size did not change due to visible light, as the distance between the camera and eye changed, the pupil size on the camera and the relative distance of the reflected light source changed simultaneously. To adjust for this variation, the pupil size at a standard distance was calculated as the difference between the measured size of pupil and the product of relative distance of the reflection of IR LED's and α, where was α an experimentally determined constant. The results confirmed that the maximum error of the pupil size was 30% without adjustment, and maximally 1.2% with adjustment. As the error was approximately 0.01 mm, it was considered safe to ignore.

5.7. Data Analysis

The statistical tests used for analyzing the results are summarized as follows:

IPL/COL Measurements

- Pupil diameter was calculated by averaging the diameter obtained at the baseline intensities (0lux and 0.1lux)
- *Interclass correlation* – The Interclass correlation tool (SPSS v11.0.0, SPSS Inc.) was used to test the reproducibility of the IPL/COL pupillometry methods used in this study.
- *Analysis of Variance (ANOVA)* – used to investigate the difference between methadone conditions and time of measurement as well as a significant methadone by time interaction
- *T-test* – used for pair-wise comparison of data points from pre-methadone dose and post-methadone dose administration conditions.

SVRTT

- *Interclass correlation* – The Interclass correlation tool (SPSS v11.0.0, SPSS Inc.) was used to test the reproducibility of the SVRTT method used in this study.
- *Nonparametric statistical analyses* - were applied; mean values and standard deviations were derived
- *10th, 50th and 90th percentiles of the data were used for proper data representation*
- *The Mann-Whitney rank sum test* - was used for between group analyses (unpaired data).
- *The Wilcoxon signed rank test* - was used for within-group analysis (paired data).
- *Spearman's rank test* – was used for correlations analysis

6. Eye Safety

There are known retinal, corneal and blue light hazards associated with some LED's, especially ultra bright visible LED's and IRED's. The assessment of eye safety was important to conduct the experiments with the patients. The safety standard used to assess the LED's was IEC 62471 for incoherent sources [115]. The purpose for using this standard is to meet standardized requirements for reducing potential radiation hazards that may be associated with various lamps and lamp systems.

6.1 IR LED

In the case of IR LED's, the only limit which was relevant was the irradiance with IR in the longer wavelength range (780 nm to 3 μm). The measurement of safety standard according to IEC 62471 used 100 W/m^2 at a distance of 200 mm between source and target, which is considered as general irradiation. Under this condition any application is termed as "safe". The IR LED's in the goggles are at a distance of 28mm from the eye and at an angle of 25° from the normal to the eye. Applying IEC 62471 standards to the goggles with 12 IR LED's under the given maximum ratings (22 mW/sr/100 mA per LED) at full intensity, generated a maximum irradiance of 6.6 W/m^2 (Table3). However, the standard is based on general irradiation which is at a distance of 200mm. In the current study, the eyes are irradiated from a shorter distance. With a distance of 28 mm the irradiance would be considerably higher than the "current wave" (LED always on forever) limit.

Table 3. The table shows the values of lumens at different distances between source and target and the corresponding irradiance.

I [W/sr]	Distance (mm)	E=I/d^2	x12	Unit
0.022	20	55.0	660	W/m^2
0.022	25	35.2	422	W/m^2
0.022	30	24.4	293	W/m^2
0.022	35	18.0	216	W/m^2
0.022	40	13.8	165	W/m^2

The values given in Table 3 are calculated with maximum intensity @ 100 mA. For an 8 h working day this is too high. However the operating current used for the IR LED's in the goggles was much lower and therefore the exposure to the eye is much shorter.

There are other facts to be considered in addition to the amount of irradiance. In the IR wavelength range of 950 nm and above, the absorption in lens and cornea is quite low. The standard is related to equal IR absorption over the entire wavelength range. However, it's not true looking at the published absorption data of the human eye, especially lens and cornea of the eye [137]. This was one more reason to assume that the goggles were formalistically safe, provided that the exposure limit should not reach the full working day of 8 hrs per day. For a complete assessment of the safety of the goggles, the irradiation time is the most important factor to consider, because this value is valid for 8 hrs per day. Therefore the ON-time per day must be known.

IR Irradiation Hazards Exposure Limits for the Eye

To avoid thermal injury to the cornea and the possible delayed effects upon the lens of the eye (cataractogenisis), ocular exposure to infrared radiation (E_{IR}), over the wavelength range 780 nm to 3000 nm, for times less that 1000s, shall not exceed [115].

$$E_{IR} = \sum_{780}^{3000} E_{\lambda}.\Delta\lambda \le 18000.t^{-0\,75} \quad W.m^{-2} \qquad (t \le 1000s) \qquad \text{(eqn 1)}$$

The calculated limits are:

Table 4. Time Dependent Exposure Limits

EIR = 18000 x t^-0.75		
t [s]	E_{IR}	
	18000	W/m^2
1	18000	W/m^2
5	5383	W/m^2
10	3201	W/m^2
50	957	W/m^2
100	569	W/m^2
1000	101	W/m^2

Comment: Keep in mind that the given value of E_{IR} is already the integral

$$E_{IR} = \sum_{780}^{3000} E'_{\lambda}(\lambda) \times \Delta\lambda \qquad \text{(eqn 2)}$$

For times greater than 1000s the limit becomes

$$E_{IR} = \sum_{780}^{3000} E_{\lambda}.\Delta\lambda \le 100 \;\; W.m^{-2} \; (t \le 1000s) \qquad \text{(eqn 3)}$$

Where, E_λ is the spectral irradiance in $W.m^{-2}.nm^{-1}$, $\Delta\lambda$ is the bandwidth in nm, t is the exposure duration in seconds.

6.2 Visible LED

In case of white LED's or LED's with shorter wavelength the blue light risk may be more critical than the IR light especially when lens and cornea are irradiated (Fig 18). Similar effects as with IR may happen and the limits may be more critical. As shown in Figure 18 white and blue LED's pose higher risk of eye damage compared to longer wavelength in the visible spectrum.

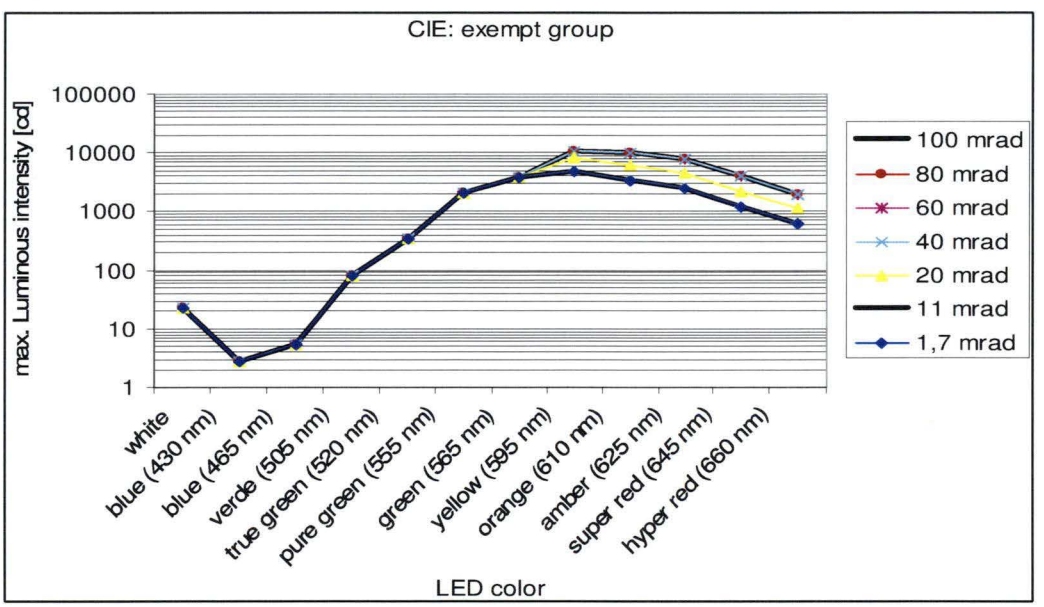

Figure 18. An illustration of the threshold of eye damage with respect to the different wavelengths in the visible spectrum [115].

6.2.1 Irradiation and Safety Calculations for White LED

The specification of visible LED's we used are given as below
Table 5 White LED specifications

Characteristics	Value
Type	White
Part Number	ASMT-SWBM-NU803
Min. I_v (mcd)	560.00
Typ. I_v (mcd)	1100.00

Max. I_v (mcd)	1400.00
Test Current (mA)	30
Dice Emitter chip material/technology	InGaN
Luminous Efficiency he (lm/W) (typ)	27
Total Flux / Luminous Intensity φv (lm) / Iv (cd) (typ)	2.7

Visible LED emission is specified in photometric values. However, for eye safety assessment, according to the standards, the radiometric values are to be applied. Given the measured visible spectrum (Fig 18) and the V(I) relationship the conversion coefficient between the radiometric and photometric values can be calculated. For the white LED's used in this study the conversion value is 281 lm/W. Based on this, the radiometric intensity of the white LED was calculated as follows.

Photometric Intensity (I_v) = 1400 mcd (Table 5)

Which is = 1400 mlm/sr (millilumen/steradian) will have a

Radiometric intensity (I_E) = 1400/281 = 4.98 mW/sr

Then we calculate the irradiance falling on the eye from the white LED. Irradiance is defined as quotient of the radiant flux ($d\phi$) incident on an element of a surface containing the point, by the area dA of the element

$$E = \frac{d\phi}{dA} \quad W/m^2 \qquad \text{(eqn 4)}$$

The distance between the LED and the pupil was 25mm on the IPL eye and 35mm on the COL eye. The irradiance (E_E) at a given distance (d) with given intensity (I_E) is given by,

$$E_{35} = \frac{I_{35}}{d^2}$$

Justification: *The inverse-square law generally applies when some force, energy, or other conserved quantity is radiated outward radially from a source. Because the surface area of a sphere (which is $4\pi r^2$) is proportional to the square of the radius, as the emitted radiation gets farther from the source, it must spread out over an area that is proportional to the square of the distance from the source. Hence, the radiation passing through any unit area is inversely proportional to the distance from the source.*

$$E_{35} = \frac{4.98\times10^{-3}}{35^2} = 4.07 \ W/m^2$$

Now, for eye safety assessment E_{35} and E_{25} is to be multiplied by the spectral efficiency factors according the calculated values of B_{eff}, S_{eff} for calculating the values to be compared with the exempt values of the Table 6

Table 6 Summary of the Exposure Limits (EL's) for the surface of the skin or cornea (irradiance based) [115].

Hazard Name	Relevant equation	Wavelength range	Exposure duration	Limiting aperture	EL in terms of constant irradiance
Units		(nm)	sec	rad (deg)	W/msquare
Actinic UV skin & eye	$E_S = \sum (E_\lambda \cdot S(\lambda) \cdot \Delta\lambda$	200 – 400	< 30000	1,4 (80)	$30/t$
Eye UV-A	$E_{UVA} = \sum (E_\lambda \cdot \Delta\lambda)$	315 – 400	≤1000 >1000	1,4 (80)	$10000/t$ 10
Blue-light small source	$E_B = \sum (E_\lambda \cdot B(\lambda) \cdot \Delta\lambda$	300 – 700	≤100 >100	< 0,011	$100/t$ 1,0

6.2.2 Calculations of B_{eff}, S_{eff} and R_{eff}

To calculate the B_{eff}, S_{eff} and R_{eff} values, the E_λ values were obtained from the manufacturers of the white LED's and were normalized to the peak of the spectrum (Fig 19).

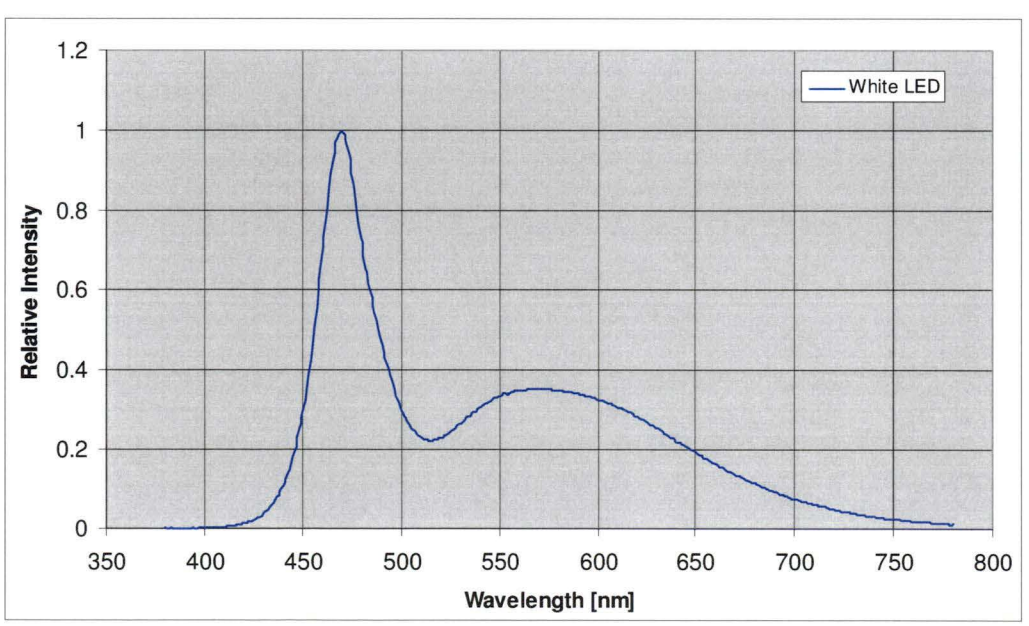

Figure 19. An illustration of relative intensity vs. wavelength of white LED's.

The normalized summation value of E_λ obtained over the whole visible spectrum (380-780 nm) was 88.39. The extrapolated values for the spectral action functions S, B, R are shown below in Tables 6.1 and 6.2 The original values for these spectral action functions were obtained from Tables 3 and 4 [115]. The values given in the reference were at interval of 5 nm, which were converted to an interval of 1nm to get a good resolution over the range of the visible spectrum.

Table 6.1 Spectral Action Function $S_{UV}(\lambda)$. Values of $S_{UV}(\lambda)$ were extrapolated for the given data over a range from 380 nm to 780 nm [115]

Wavelength[1] λ, nm	UV hazard function $S_{UV}(\lambda)$	Wavelength λ, nm	UV hazard function $S_{UV}(\lambda)$
200	0,030	313*	0,006
205	0,051	315	0,003
210	0,075	316	0,0024
215	0,095	317	0,0020
220	0,120	318	0,0016
225	0,150	319	0,0012
230	0,190	320	0,0010
235	0,240	322	0,00067
240	0,300	323	0,00054
245	0,360	325	0,00050
250	0,430	328	0,00044
254*	0,500	330	0,00041
255	0,520	333*	0,00037
260	0,650	335	0,00034
265	0,810	340	0,00028
270	1,000	345	0,00024
275	0,960	350	0,00020
280*	0,880	355	0,00016
285	0,770	360	0,00013
290	0,640	365*	0,00011
295	0,540	370	0,000093
297*	0,460	375	0,000077
300	0,300	380	0,000064
303*	0,120	385	0,000053
305	0,060	390	0,000044
308	0,026	395	0,000036
310	0,015	400	0,000030

[1] Wavelengths chosen are representative: other values should be obtained by logarithmic interpolation at intermediate wavelengths.

* Emission lines of a mercury discharge spectrum.

Table 6.2. Spectral Action Functions B(λ) and R(λ). Values were of B(λ) and R(λ) extrapolated for the given data over a range from 380 nm to 780 nm [115]

Wavelength nm	Blue-light hazard function *B(λ)*	Burn hazard function *R(λ)*
300	0,01	
305	0,01	
310	0,01	
315	0,01	
320	0,01	
325	0,01	
330	0,01	
335	0,01	
340	0,01	
345	0,01	
350	0,01	
355	0,01	
360	0,01	
365	0,01	
370	0,01	
375	0,01	
380	0,01	0,1
385	0,013	0,13
390	0,025	0,25
395	0,05	0,5
400	0,10	1,0
405	0,20	2,0
410	0,40	4,0
415	0,80	8,0
420	0,90	9,0
425	0,95	9,5
430	0,98	9,8
435	1,00	10,0
440	1,00	10,0
445	0,97	9,7
450	0,94	9,4
455	0,90	9,0
460	0,80	8,0
465	0,70	7,0
470	0,62	6,2
475	0,55	5,5
480	0,45	4,5
485	0,40	4,0
490	0,22	2,2
495	0,16	1,6
500-600	$10^{[(450-\lambda)/50]}$	1,0
600-700	0,001	1,0
700-1050		$10^{[(700-\lambda)/500]}$
1050-1150		0,2
1150-1200		$0{,}2 \cdot 10^{0{,}02(1150-\lambda)}$
1200-1400		0,02

Based on the values obtained/ interpreted from Table 6.1 and 6.2, and data obtained from white LED manufacturers and the formulae's given below, we derived the irradiance safety limits for the white LED's,

$$S_{eff} = \frac{\int E_e(\lambda) \bullet S(\lambda) \bullet d\lambda}{\int E_e(\lambda) \bullet d\lambda} \quad \text{(eqn 5)}$$

$$B_{eff} = \frac{\int E_e(\lambda) \bullet B(\lambda) \bullet d\lambda}{\int E_e(\lambda) \bullet d\lambda} \quad \text{(eqn 6)}$$

$$R_{eff} = \frac{\int E_e(\lambda) \bullet R(\lambda) \bullet d\lambda}{\int E_e(\lambda) \bullet d\lambda} \quad \text{(eqn 7)}$$

Integral (Irradiance) of the peak normalized function ($\sum_{380}^{780} E_\lambda$) = 88.39

$$S_{eff} = \frac{\sum_{380}^{780} S_{UV} * E_\lambda}{\sum_{380}^{780} E_\lambda} = \textbf{\textit{1.62161E-08}} \quad \text{(eqn 8)}$$

$$B_{eff} = \frac{\sum_{380}^{780} B_\lambda * E_\lambda}{\sum_{380}^{780} E_\lambda} = \textbf{\textit{0.24}} \quad \text{(eqn 9)}$$

$$R_{eff} = \frac{\sum_{380}^{780} R_\lambda * E_\lambda}{\sum_{380}^{780} E_\lambda} = \textbf{\textit{2.96}} \quad \text{(eqn 3.10)}$$

Comparing the values obtained above to EL values of Table 6, the goggles were considered safe for testing.

6.2.3 Red Pass Filter

During the development of the goggles it was observed that the light from the white LED's was uncomfortable. Therefore for the convenience to the eyes a red pass filter was placed on top of the white LED's. It was found that significant level of comfort was achieved due the red filter. The red filter was tested using a spectrophotometer (Metertech SP8001 UV/VIS Spectrophotometer, Taipei, Taiwan) to investigate the transmitting wavelength (Fig 20).

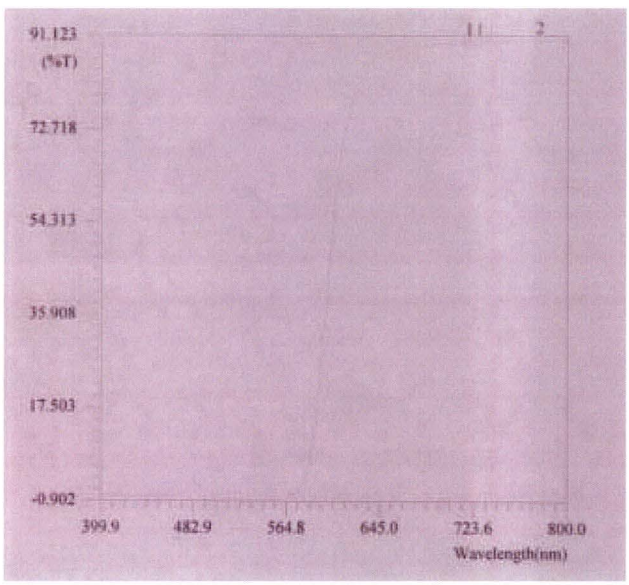

Figure 20. Results obtained from a spectrophotometer for the red filter.

The results indicated that the radiation with wavelengths consisting of blue and green light and posing hazard to eye was eliminated, while the rest of the visible spectrum (564.8 nm to 780 nm approximately) passed the filter. The maximum transmittance was obtained at 645 nm and stays at that level till 780 nm approximately.

Placing the red filter on the white LED's also meant that the values for E_λ would be less then the wavelength filtered by the filter. So the new values obtained for B_{eff}, S_{eff} and R_{eff} were 0, 0.000985386 and 0.987771128 respectively. These values were well below the exempt level demonstrating that the LED's were not only safe but also convenient for patients to wear during the period of the test.

7. Results

7.1 Pupil Measurements Validation

The pupil diameters of various artificial and real eye images were measured using the pupil diameter measurement technique [110]. The pupil diameters were also measured by finding the maximum horizontal distance between two pixels in a pupil image. This was done by applying the basic matlab function "imdistline" to each image.

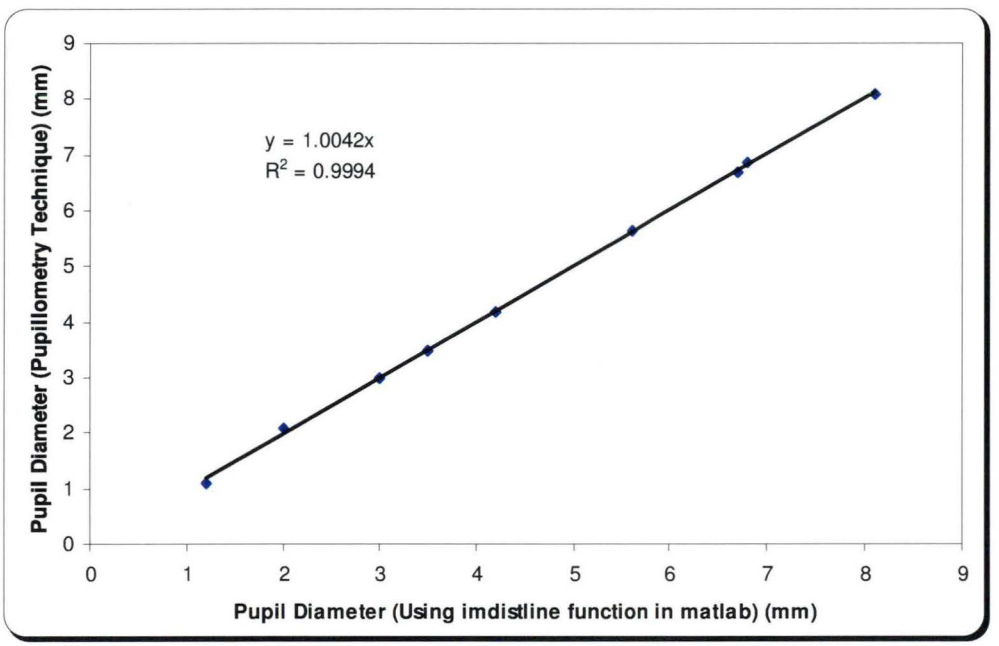

Figure 21. The pupil diameter measurement using the pupil diameter measurement technique [110] correlated highly with the "imdistline" function technique from matlab.

The results obtained from basic pixel distance measurement correlated highly with the results obtained by the pupil diameter measurement technique (Fig 21, $p < 0.001$, $R^2 \approx 1$, $n = 9$). The validation results demonstrated that the pupil diameter measurement technique was very reliable for the measurement of pupil diameter.

Reproducibility: To test the reproducibility of the pupillometry technique, the pupil diameter of 5 volunteers from the school were recorded and measured for 5 days at the same time everyday. The measurement data from each volunteer were compared using Interclass correlation. The reproducibility test showed that the pupil diameter measured for 5 days for each volunteer agreed well with a mean percentage SD of 2.2%. The Interclass Correlation

coefficient for a 95% confidence level was r = 0.9721 ($p < 0.001$), which showed high reproducibility.

7.2 IPL Eye Measurements for the Control Group

Table 7 summarizes the result of all control subjects in the form of Mean and SD versus different light intensities. Figure 22 is a visual representation of Table 7. Throughout this chapter "Ac" is used to denote accommodation response in Figures and Tables.

Table 7. Summary of the mean IPL pupil diameter result obtained for control group in the form of Mean and SD against different light levels during the period of the test (0hr-6hr).

Hours		Pupil diameter of IPL eye of control group (CG) [Mean±SD] (mm)													
	lux	0	1	0	10	0	100	0	Ac0.1	Ac1	Ac0.1	Ac10	Ac0.1	Ac100	Ac0.1
0hr	Mean	7.70	7.22	7.49	6.29	7.20	5.31	6.99	7.21	7.05	7.28	6.09	6.88	4.83	6.51
	SD	0.55	0.79	0.71	0.95	0.69	1.23	0.59	0.59	0.44	0.61	0.60	0.11	0.53	0.04
1hr	Mean	7.79	7.18	7.46	6.17	7.14	4.97	6.48	7.28	7.28	7.09	6.59	7.20	5.15	6.99
	SD	0.68	0.74	0.65	0.79	0.61	0.75	0.12	0.68	0.77	0.35	1.31	0.35	0.99	0.73
2hr	Mean	7.34	7.05	7.39	6.45	7.31	5.29	6.82	7.13	7.16	7.08	6.11	6.65	4.93	6.75
	SD	0.16	0.31	0.16	0.61	0.15	0.67	0.24	0.17	0.15	0.39	0.67	0.64	0.54	0.06
3hr	Mean	7.59	6.49	7.14	5.66	7.21	4.88	6.55	7.16	7.01	6.97	6.30	6.88	5.18	7.03
	SD	0.45	0.81	0.92	0.82	0.58	0.53	0.04	0.83	0.87	0.20	1.05	0.07	0.74	0.36
4hr	Mean	7.90	6.77	7.65	6.12	7.43	5.23	6.78	7.08	6.63	7.10	6.10	6.25	5.47	7.13
	SD	0.08	0.23	0.67	0.22	0.15	0.52	0.21	0.17	0.01	0.60	0.62	0.37	0.89	0.25
5hr	Mean	7.74	6.62	7.07	6.04	7.14	5.62	6.59	6.77	6.50	6.25	5.77	6.25	5.55	6.77
	SD	0.87	0.23	0.23	0.24	0.47	0.59	0.38	1.06	1.04	0.01	0.68	0.23	0.52	0.52
6hr	Mean	7.33	6.91	7.33	6.65	7.32	5.12	6.51	7.13	6.91	6.74	6.04	6.33	4.73	6.51
	SD	0.13	0.22	0.22	0.39	0.31	0.80	0.08	0.57	0.56	0.13	0.75	0.12	0.70	0.37

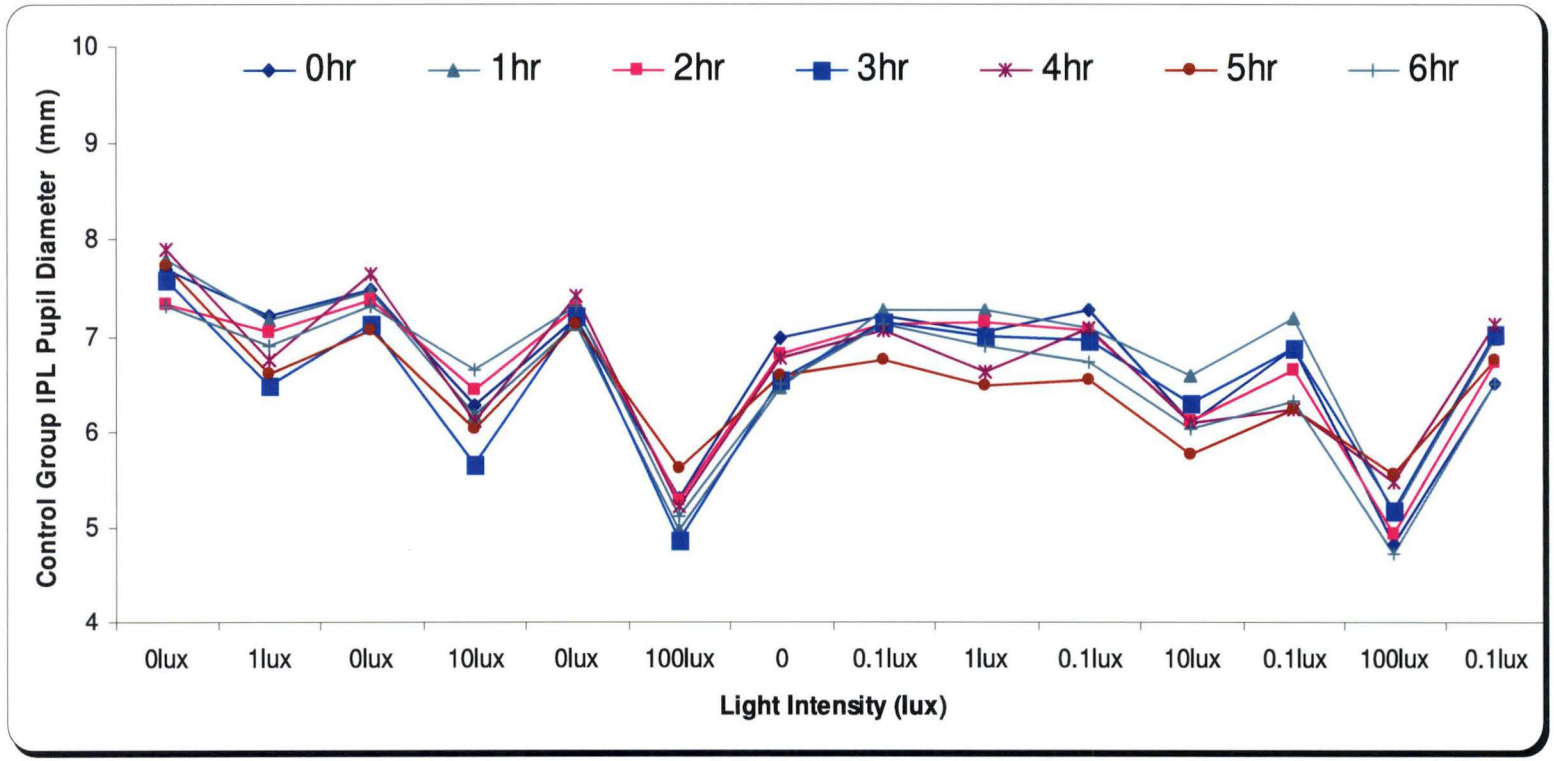

Figure 22. A plot of light intensities versus mean pupil diameter of control subjects during the period of the test (0hr-6hr). Each horizontal line represents the test performed at every hour for 6hrs. The SD was not included for better representation of the data. The values are given in Table 7.

Figure 23 demonstrates the mean pupil diameter of control subjects at 0 and 0.1lux with and without accommodation response. No statistical significance *($p < 0.05$. t-test, paired two samples for means)* was observed in pupil diameters measured at hourly intervals. The errors bars were not shown in Figure 23 because the number of sample size (n=2) was too small to demonstrate variance in population.

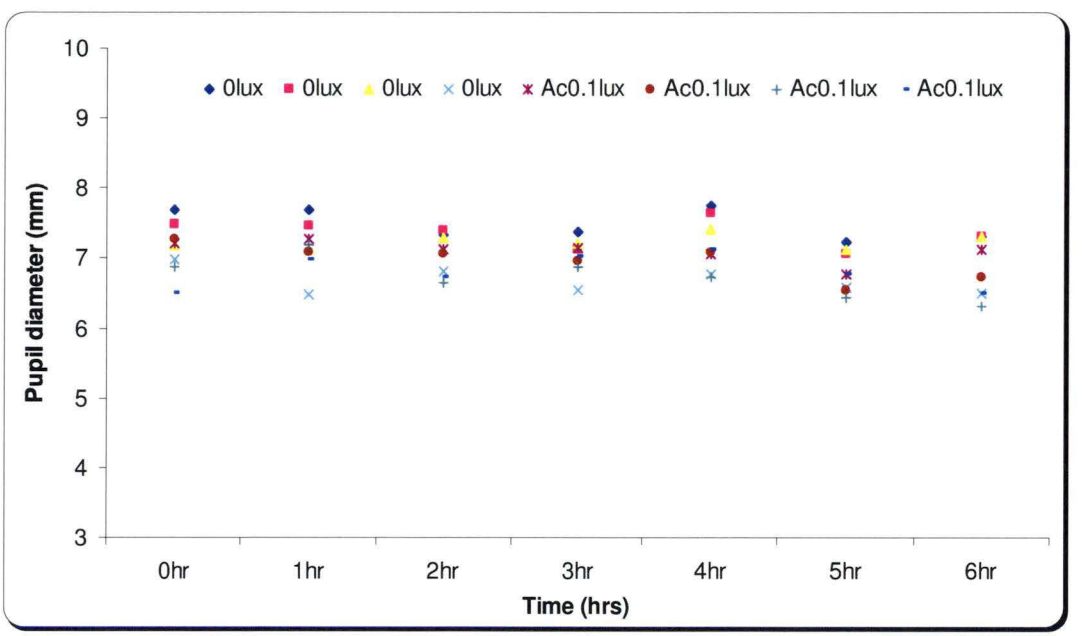

Figure 23. The mean pupil diameters of the control patients measured in the IPL eye throughout the test period (0-6hrs) for 0 and Ac0.1lux.

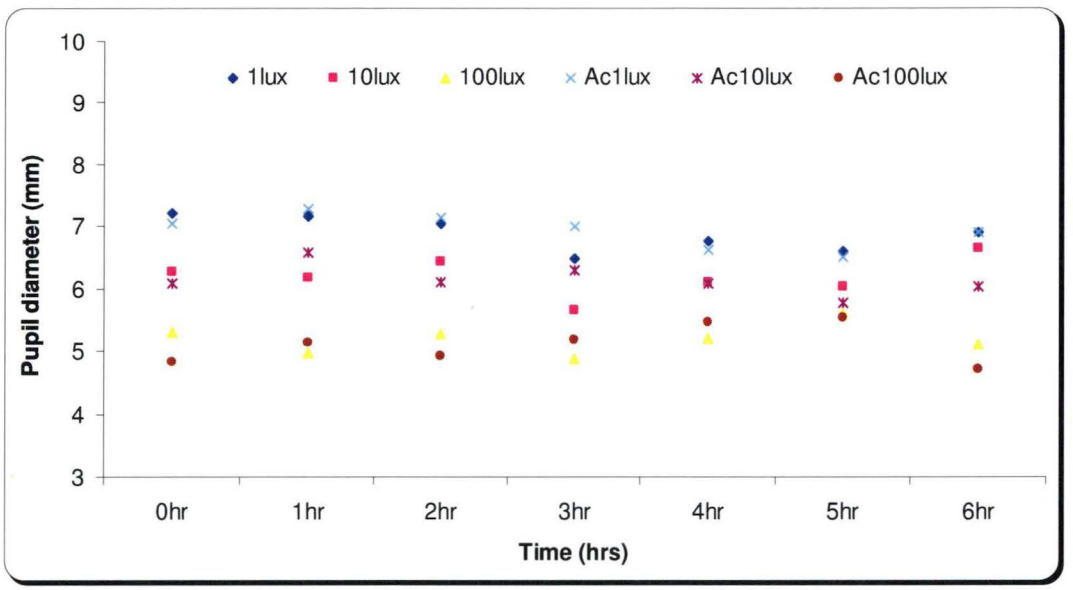

Figure 24. The mean pupil diameters of control patients measured in the IPL throughout the test period (0-6hrs) at light intensities of 1lux, 10lux and 100 lux.

Figure 24 shows the mean pupil diameter of control subjects at light intensities 1, 10lux and 100lux. Significant difference *($p < 0.01$, t-test, paired two samples for means)* was obtained for the pupil diameters measured at the 3 light intensities. Statistical significance was also observed at the same light intensities after the accommodation response, however of a lesser effect *($p < 0.05$).* Figure 25 shows a plot of mean pupil diameter versus various baseline light intensities (0lux and Ac0.1lux) in the order used in the tests. Figure 25 is a better visual representation of Figure 23 in understanding the pupil response to the baseline light intensities. It was observed that the pupil diameter decreased progressively from the first 0lux intensity to the last Ac0.1lux. In addition a consistent dip at 0lux and Ac0.1lux intensity after 100lux was observed at different hours. This may have caused because of the memory of 100lux light in the pupil which carried its big constriction effect on the 0lux and Ac0.1lux light intensity.

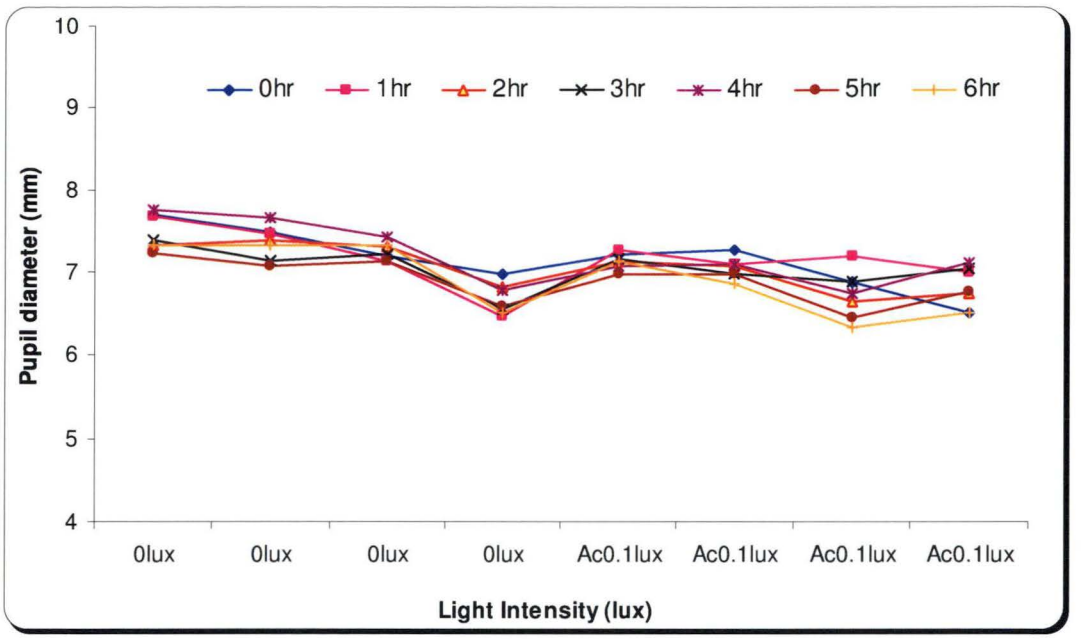

Figure 25. The mean pupil diameter of control group at intensities 0lux and Ac0.1lux. Each horizontal line represents the test performed at every hour for 6hrs. Figure 25 is a better visual representation of Figure 24 in understanding the pupil response light intensities 0lux and Ac0.1lux.

7.3 IPL Eye Measurements for the Patient Group

Table 8 summarizes the result of the patients group in the form of Mean and SD versus different light intensities including the baseline intensities (0lux and Ac0.1lux). Figure 26 is a visual representation of Table 8. The plot demonstrates the pupil diameter change in methadone patients with different light intensities as a function of time.

Table 8. Summary of the IPL results obtained for patient group in the form of Mean and SD versus different light levels during the period of the test (0-6hrs).

Hours	Pupil diameter of IPL eye of patient group [Mean±SD] (mm)														
	lux	0	1	0	10	0	100	0	Ac0.1	Ac1	Ac0.1	Ac10	Ac0.1	Ac100	Ac0.1
Before Dose	Mean	7.07	6.97	7.08	6.64	6.92	5.38	6.49	6.75	6.68	6.84	6.39	6.83	5.26	6.46
	SD	0.74	0.82	0.89	0.81	0.79	0.62	0.60	0.71	0.71	0.69	0.78	0.84	0.68	0.64
1hr	Mean	5.26	5.19	5.18	5.01	5.17	4.16	4.91	5.16	5.05	5.14	4.77	5.00	4.04	4.91
	SD	0.65	0.85	0.94	0.92	0.85	0.68	0.64	0.85	0.94	0.98	0.84	0.84	0.80	0.90
2hr	Mean	4.55	4.21	4.51	4.12	4.39	3.60	4.38	4.43	4.31	4.35	4.08	4.30	3.44	3.96
	SD	0.49	0.60	0.82	0.65	0.60	0.38	0.84	0.93	0.87	0.73	0.59	0.88	0.52	0.72
3hr	Mean	4.67	4.46	4.47	4.18	4.38	3.62	4.11	4.17	4.16	4.31	3.89	4.11	3.45	4.19
	SD	0.53	0.64	0.62	0.67	0.68	0.58	0.63	0.71	0.66	0.63	0.55	0.68	0.59	0.51
4hr	Mean	5.58	5.28	5.20	4.85	5.08	4.02	4.82	4.94	4.72	4.84	4.58	4.86	3.83	4.54
	SD	1.03	0.74	0.78	0.74	0.74	0.51	0.48	0.78	0.81	0.99	0.72	0.67	0.41	0.48
5hr	Mean	5.69	5.56	5.69	5.30	5.50	4.35	5.08	5.40	5.27	5.54	4.95	5.29	4.16	5.05
	SD	0.80	0.72	0.73	0.80	0.96	0.46	0.70	0.81	0.74	0.79	0.83	0.94	0.59	0.84
6hr	Mean	6.39	6.26	6.39	5.85	6.18	4.71	5.99	6.11	6.11	6.13	5.49	5.88	4.59	5.73
	SD	1.00	0.97	1.04	0.93	0.73	0.39	0.86	0.91	0.95	1.07	0.78	0.94	0.59	0.61

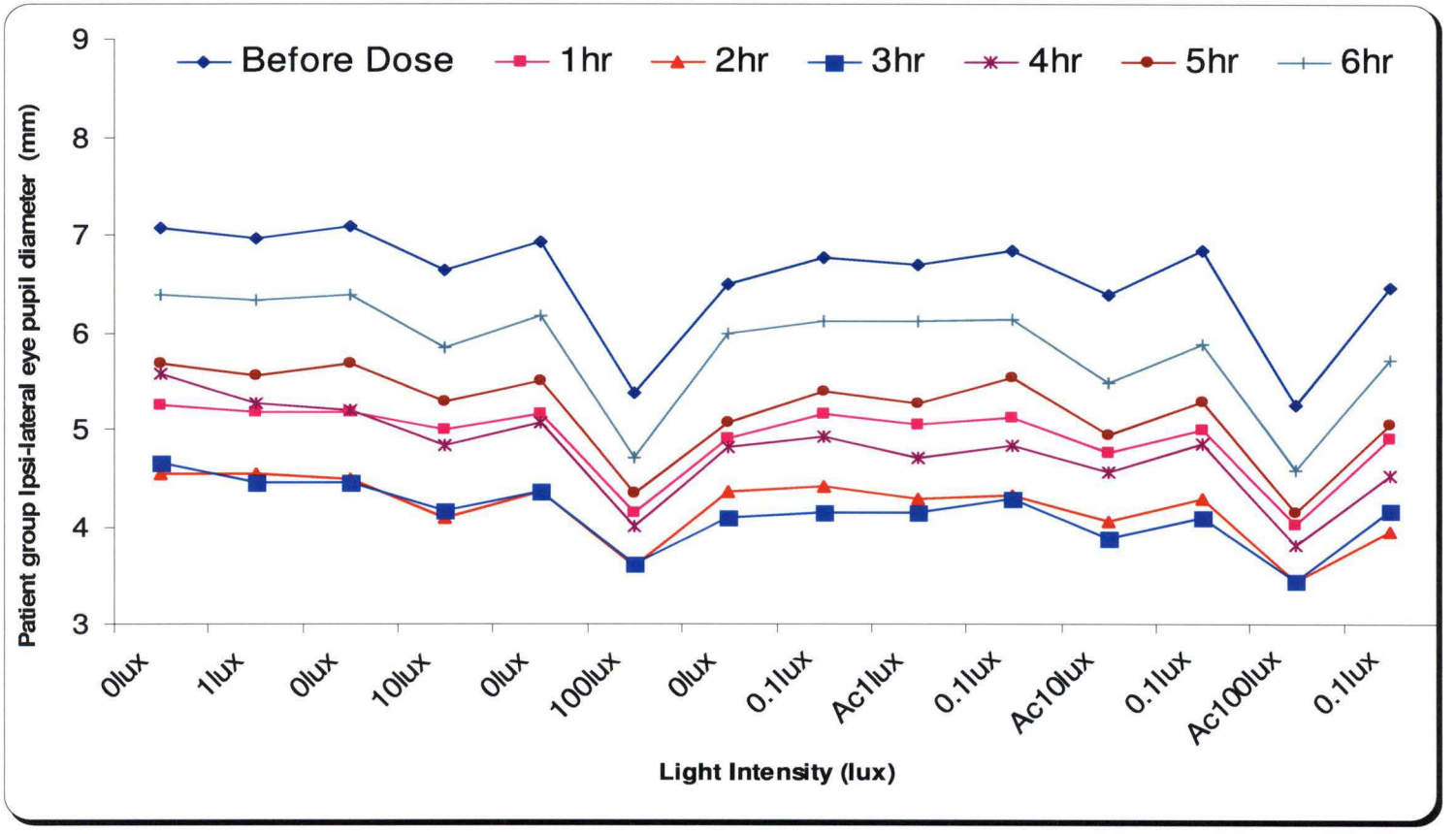

Figure 26. A plot of light intensities versus pupil diameter (IPL eye) of methadone patient group during the period of the test (0-6hrs). The values are given in Table 8. Each horizontal line represents the test performed at every hour for 6hrs. It can be observed that the pupil diameter decreased and recovered over the period of 6hrs.

Figure 27 demonstrates the pupil diameter of patients at 0lux and 0.1lux light levels throughout the test (0-6hrs). Pupil diameters were decreased significantly by methadone. The differences were significant *($p > 0.01$. t-test, paired two samples for means)* when pupil diameter measured before dose was compared with the pupil diameter after methadone administration. The results obtained for pupil diameter agreed with several past studies (Table 1). Peak miosis occurred approximately 2hrs after methadone administration by patients.

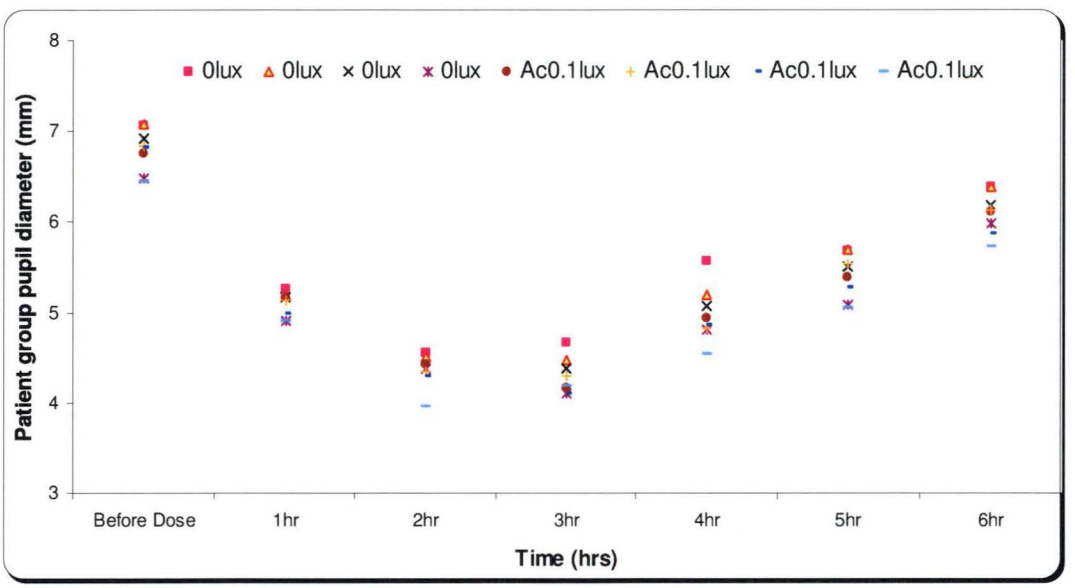

Figure 27. The mean pupil diameters of patient group measured in the IPL eye throughout the test period (0-6hrs) when the light intensity was at 0lux and Ac0.1lux.

Figure 28 is a better visual representation of Figure 27 to understand the pupil response to the baseline light intensities in the order used in the tests. Each horizontal line represents the test performed at every hour for 6hrs It was observed that the pupil diameter decreased progressively from the first 0lux intensity to the last 0.1lux. However, this progressive decrease was not as prominent as observed in the control subjects (Fig 25).

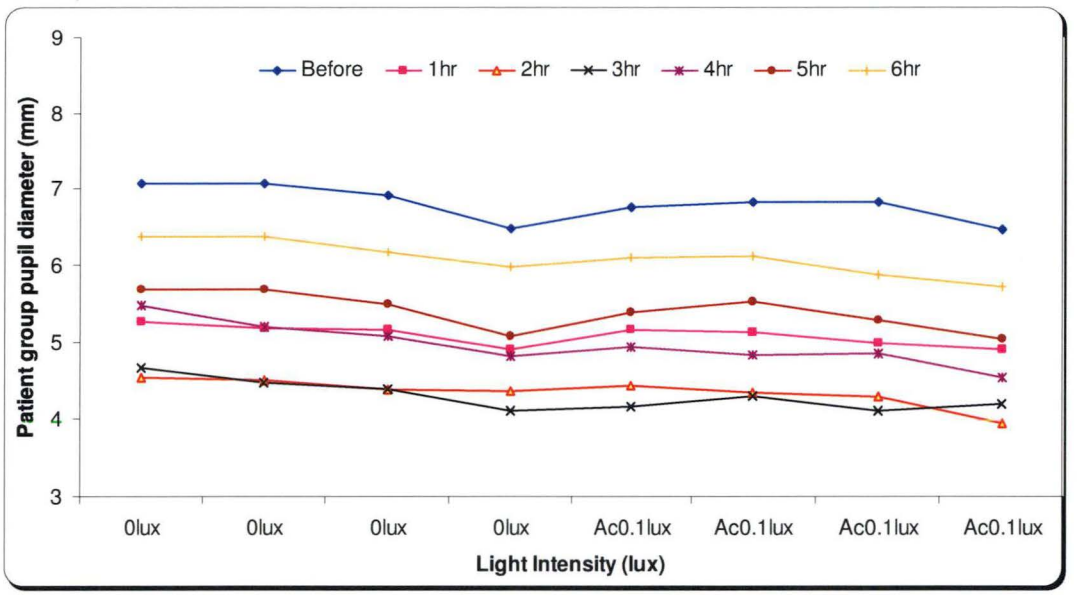

Figure 28. The plot represents the mean IPL pupil diameter response of methadone patients group at various baseline intensities. Each horizontal line represents the test performed at every hour for 6hrs

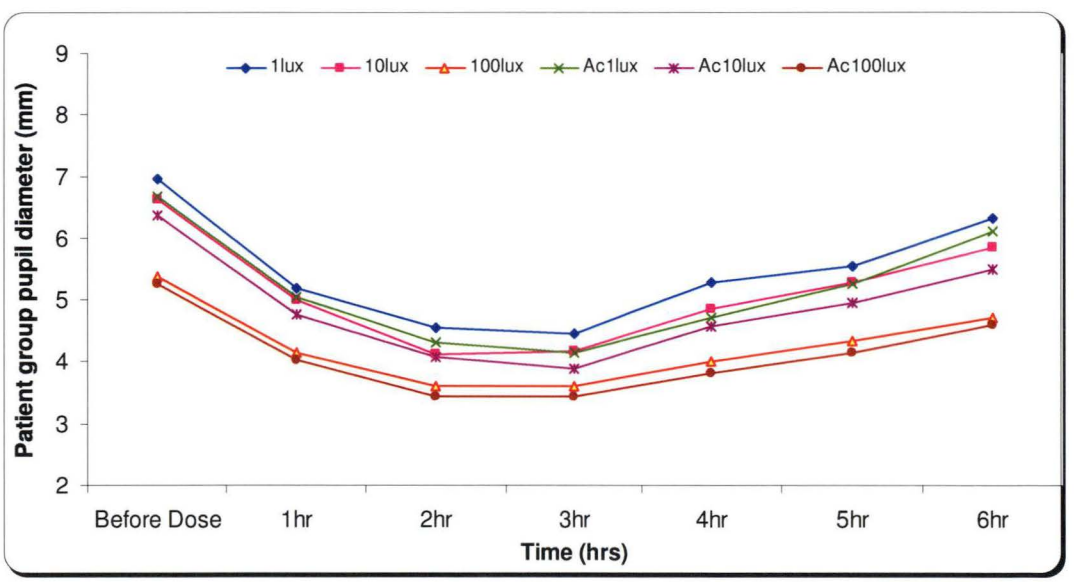

Figure 29. The average pupil diameters of the patients measured in the IPL eye throughout the test period (0-6hrs) at light intensities of 1lux, 10lux and 100lux. Each horizontal line represents the test performed at light intensities 1lux, 10lux and 100lux.

Figure 29 shows the pupil diameter of patient group at the three light intensities (1lux, 10lux and 100lux) (Table 8). Significant difference *($p > 0.01$ t-test, paired two samples for*

means) was obtained in the pupil diameters measured at the 3 intensities. Similar statistical significance was observed at the same intensities in the accommodation response of the pupil. Figure 30 shows the plot of pupil diameter response of the patient group at various light intensities. Each horizontal line represents the test performed at every hour for 6hrs. At maximum intensity (100lux) the pupil response was the strongest. Similar response was obtained at Ac100lux in accommodation response.

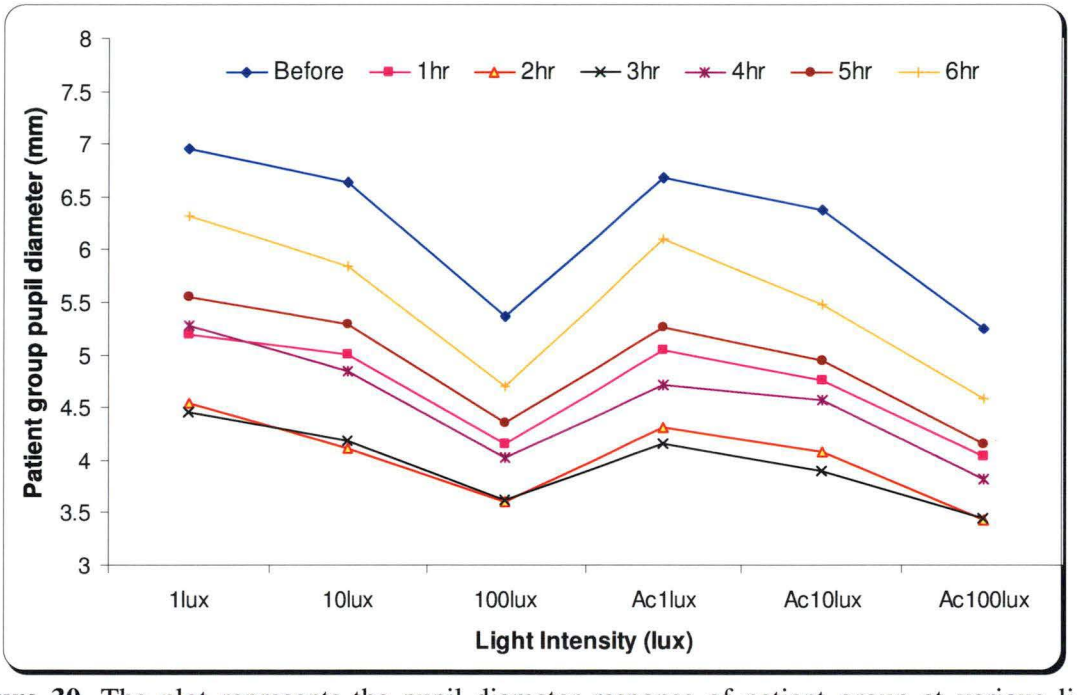

Figure 30. The plot represents the pupil diameter response of patient group at various light intensities. Each horizontal line represents the test performed at every hour for 6hrs

7.4 Effect of Accommodation Response

To understand the effect of accommodation response on pupil diameter the baseline intensity was increased from 0lux to 0.1lux. Figure 31 shows the pupil diameter as a function of accommodation response for control as well as patient group throughout the time course of the test (0-6hrs). No significant accommodation effect was observed in control as well as patient group.

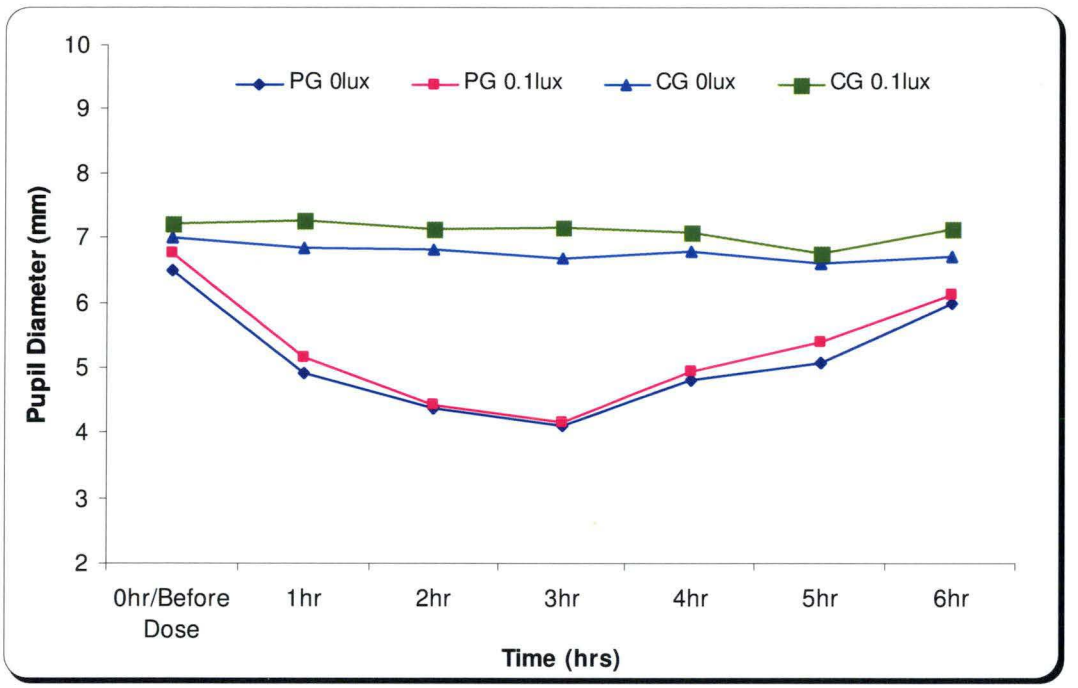

Figure 31. Effects of accommodation on mean pupil diameter when light intensity was increased from 0lux to 0.1lux. The plot consists of combined results from control group (CG) and patients group (PG).

The maximum percentage difference of accommodation response in pupil diameter for the patient group was 0.06% and for the control group was 0.07%. The pupil diameter in the control as well as the patient group was found to be higher in accommodation response. In both accommodation and non-accommodation responses of pupil diameter it was indicated that the slight difference in pupil diameter measures were accounted by the differences in light intensity.

The detection of methadone miosis was affected by lighting intensity and at zero intensity at different levels of statistical significance (Figure 31). The best level of significance ($p < 0.001$) was obtained at maximum intensity of 100lux (Fig 26). However, methadone miosis was mostly readily detected irrespective of visible light excitement (at baseline intensities). Miosis was statistically significant at different levels under these lighting conditions as well as no light condition according to the paired T-test.

7.5 Constriction Amplitude (CA)

Figure 32 shows a plot of change in CA at various light intensities. The plot demonstrates that the CA increased with increasing light intensities for the control group as well as the patient group. The results of CA obtained from the control group were averaged over the period of the test and plotted in Figure 32 to indicate the distinction in CA of the control group and the PG pre and post methadone administration. The error bar represents the SD obtained from control group. Pupil response to changes in light levels were non linear for both the patients and control group. This was expected as the light levels were logarithmically increasing. In both groups the maximum constriction was observed at 100lux. Differences in constriction was observed at the same light levels in accommodation responses, however they were not significant. This difference can be speculated as the memory response of the pupil or lack to time for the pupil to reach equilibrium in dilation periods, which were 10 seconds.

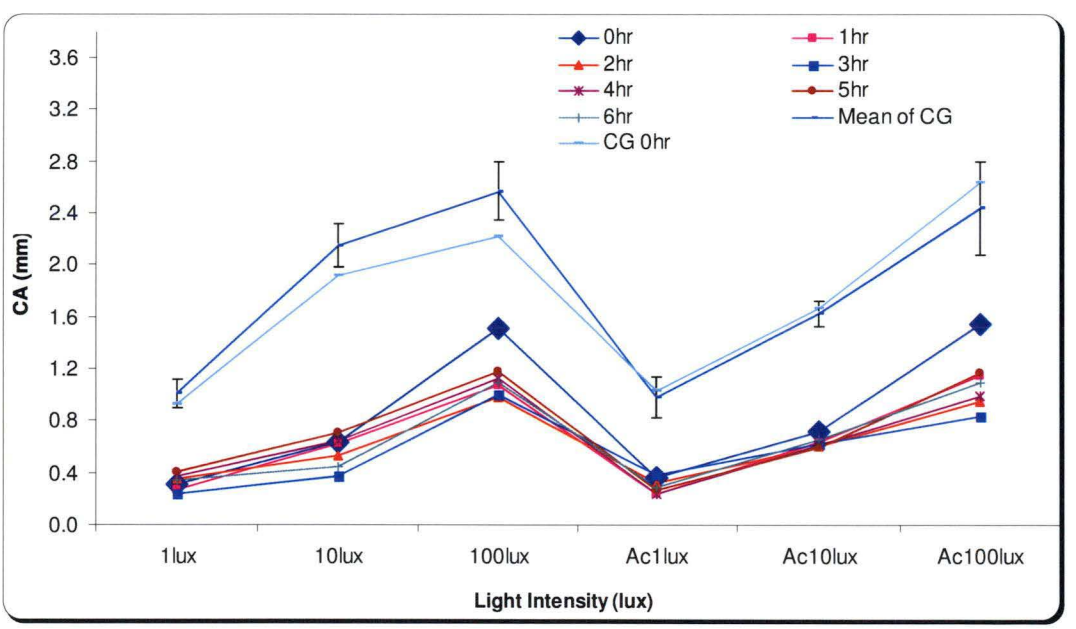

Figure 32. The plot represents the change in mean CA at different light intensities as a function of time.

The CA of the light reflex differed significantly among the patient group. A two-way ANOVA indicated significant differences ($p < 0.01$) among methadone conditions and time of measurement as well as a significant methadone by time interaction. As shown in Figure 32, CA

significantly decreased as a function of time. The magnitudes of the effects were small at light intensities 1lux and 10lux and maximal changes occurred at the time of the maximal change in pupillary size (100lux).

7.6 Constriction Velocity (CV)

Figure 33 shows a plot of pupil CV at various light intensities. The plot demonstrates that the pupil constriction increased with increasing light intensities for control group as well as patient group.

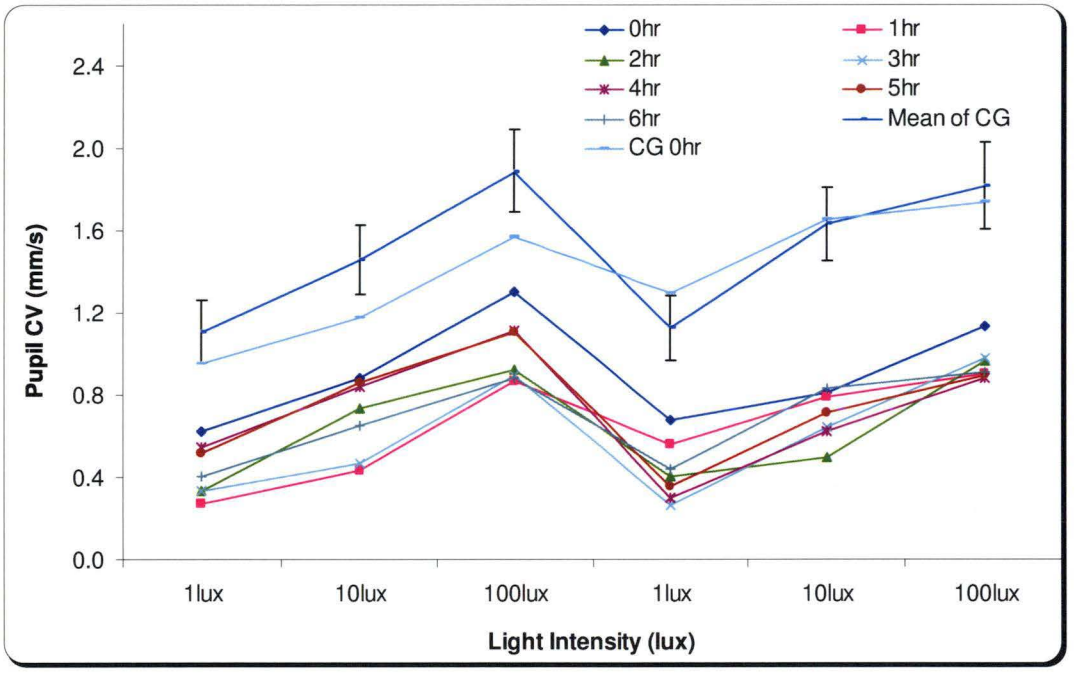

Figure 33. The plot represents the change in CV at different light intensities as a function of time.

The results of pupil constriction obtained from the control group were averaged over the period of the tests and plotted in Figure 33 to indicate the distinction in pupil CV of control group and patients group pre and post methadone administration. The error bar represents the SD obtained from the control group. Pupil CV response to changes in light levels were non linear for both the patients and control group. This was expected as the light levels were logarithmically increasing. In both groups the maximum CV was observed at 100lux.

Differences in constriction were observed at the same light levels in accommodation response however they were not statistically significant. This difference can be speculated as the memory response of the pupil or lack to time for the pupil to reach equilibrium in dilation periods.

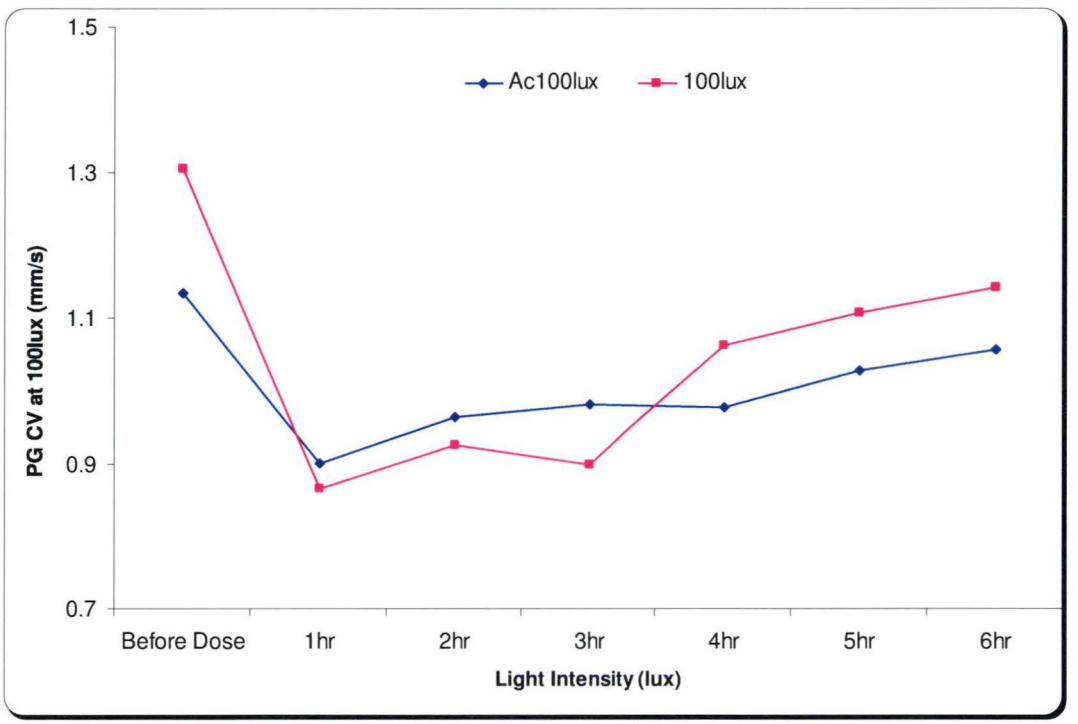

Figure 34. The plot represents the change in CV during the course of the test at 100lux (0-6hrs).

Statistically significant and near significant changes were observed in pupil velocity as a function of light intensity in the control group, the patient group before dose ($p < 0.05$) and the methadone group after dose ($p < 0.01$), respectively. The velocity of pupillary constriction changed significantly as a function of the drug treatment (Figure 33). A two-way ANOVA indicated significant differences among methadone conditions and the time of measurement, as well as a significant ($p < 0.05$) methadone by time interaction.

The CV at various light intensities over the period of 6hrs was measured. A significant ($p<0.05$) difference in pupil CV before and after methadone administration was observed at 100lux (with and without accommodation) (Fig 34). No significant differences were observed at lower light intensities ((with and without accommodation).

7.7 Re-dilation Amplitude (R-dA)

The R-dA change in the patient group without accommodation response changed from a maximum of 0.47 mm before dose to 0.31 mm at the 6hr. It achieved the lowest R-dA change at the 2hr period (0.15mm).

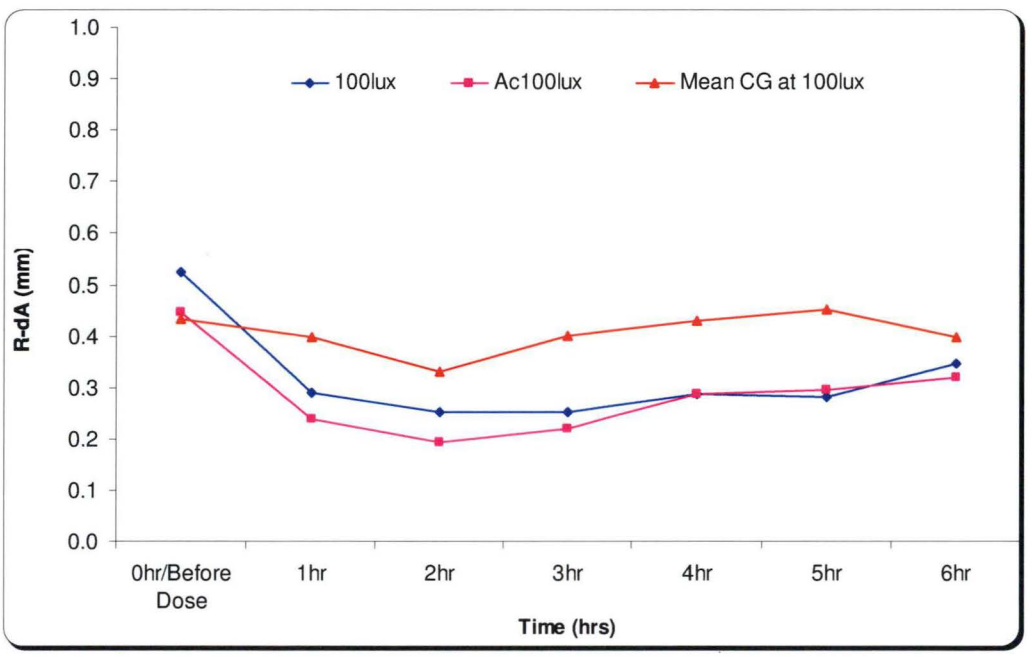

Figure 35. The plot of R-dA change in the patient group and control group. It was demonstrated that the magnitude of R-dA at 100lux progressively dampened and then recovered after intake of methadone dose. The control group showed no trend as expected unlike the control group.

The R-dA change in the patient group with accommodation response changed from a maximum of 0.38 mm before dose to 0.27 mm at the 6hr. It achieved the lowest R-dA change at the 2hr and 3hr period (0.16mm). It was observed that the magnitude of R-dA progressively dampened and then recovered after methadone administration at 100lux, with and without accommodation response (Figure 35). A similar trend was observed at 1lux and 10lux however the response was not obvious. The changes in R-dA after methadone dose over a period of the test were compared with the pupil response of control group. The R-dA change in CG at 0hr was 0.43mm/s and at 6hr was 0.40mm/s. Figure 35 shows an obvious difference between the patient group and the control group from the period between 2hr to 5hr. The differences observed during this period were significant ($p < 0.01$). The response crosses over at the

0hr/Before dose and 6hr. This could be attributed to the fact that the pupil diameter of the control group was inherently smaller than then patient group. In addition, pupil diameters have been known to decrease from morning to evening [94], which was observed in this study (Figure 22, Control group), and that maybe one of the reasons behind the response obtained at 6hr.

7.8 Re-dilation Velocity (R-dV)

The R-dV in the patient group with accommodation changed from a maximum of 0.32 mm/s before dose to 0.26 mm/s at the 6hr. It achieved the lowest R-dV at 2hr (0.13mm/s). The maximum R-dV at Ac100lux was obtained at the 0hr/before dose (0.26mm/s) with a minimum at 3hr (0.14mm/s). At 6hr the velocity was 0.25mm/s. It was observed that the magnitude of R-dV progressively dampened and then recovered after methadone administration at 100lux, with and without accommodation response (Figure 36). The factor that differentiates R-dA from R-dV is the time. It is expected that the pupillary responses low down upon methadone intoxication and it increases as it reaches its peak. The pupil regains its normal functions as the peak gradually drops.

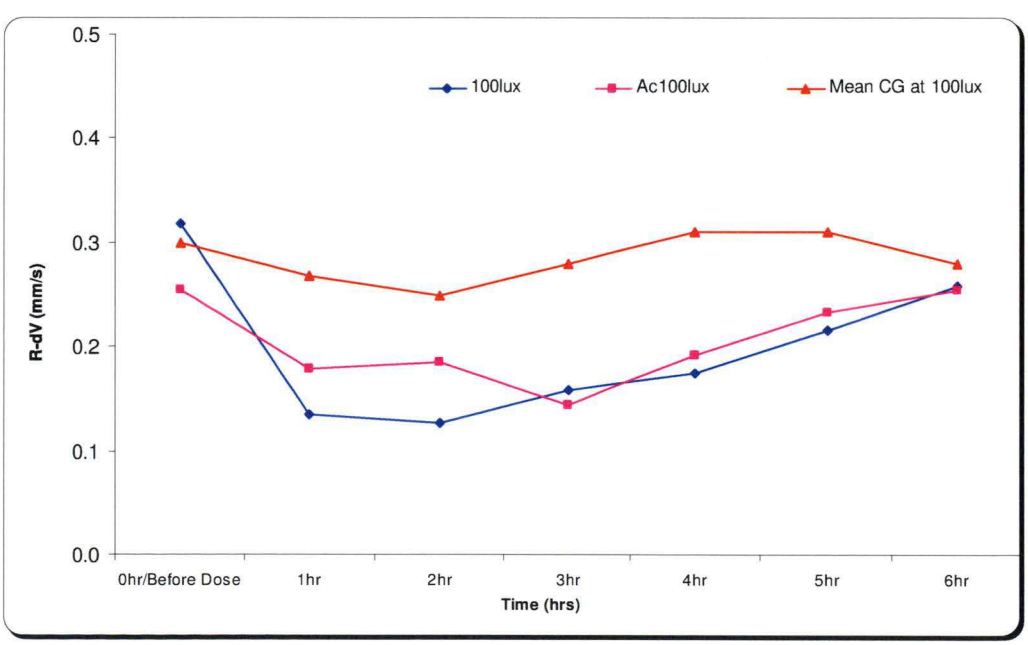

Figure 36. The plot of R-dV in patient group and control group. It was demonstrated that the R-dV at 100lux progressively dampened and then recovered after intake of methadone dose. The control group showed no trend as expected unlike the control group.

Similar, trend was observed at 1lux and 10lux however the response was not obvious. The changes in R-dV after methadone dose over a period of the test were compared with the pupil response of control group. The R-dV at 0hr was 0.30mm/s and at 6hr was 0.28mm/s. Figure 36 shows an obvious difference between the patient group and the control group throughout the test. The differences observed during this period were significantly ($p < 0.01$). The response nearly come together or cross over at the 0hr/Before dose and 6hr indicating that during the period between 0hr to 6hr the pupil responded to methadone metabolism(Fig 36).

7.9 Relaxation SD

Considerable amount of fluctuations were observed in the relaxation phase of the pupil response under light. The fluctuations were higher with increasing light intensity. It was difficult to find the amplitude and velocity of the relaxation phase. Therefore the SD of relaxation phase was compared over the period of the test (0-6hrs). The relaxation SD of the light reflex changed significantly ($p<0.05$) among

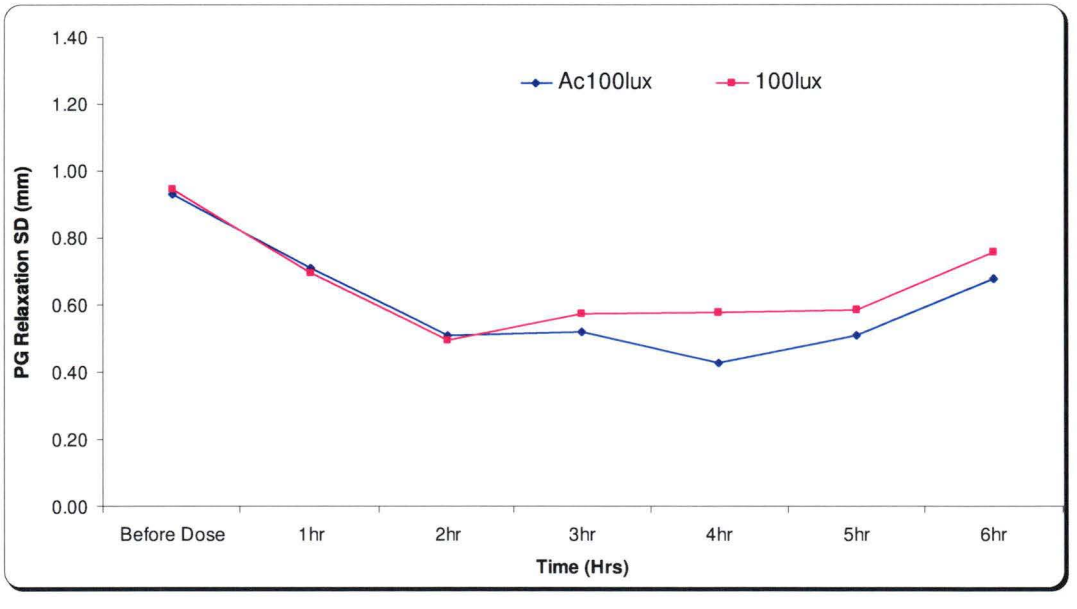

Figure 37. The plot shows the Relaxation SD in patient group with and without accommodation response. Both responses showed high degree of similarity.

the pre-dose and post-dose measurements. The highest level of significance ($p<0.01$) was obtained at the 2hrs interval in both accommodation and non-accommodation response, although a lower value at 4hr was obtained in accommodation response. The trend observed in the Ac response showed high degree of similarity to the response obtained before Ac response (Fig 37). The difference between the two trends was 0.1mm.

7.10 Discussion

The main purpose of the IPL testing was to examine the methadone effect on the pupil and to investigate the effect of illumination and exposure conditions on the detection of miosis. Furthermore, the aim was to characterize pupillometric parameters and investigate their response to methadone.

No light intensity (0lux)

The pupil diameter at 0hrs was 7.70±0.55 (Mean±SD) and at 6hrs was 7.33±0.13 for the control group. The pupil diameter was 7.07 ±0.74 pre methadone administration and 7.39 ± 0.99 at 6hrs post methadone administration for the patient group. The pupil diameters in the control group although not significantly different from patient groups, were bigger throughout the test. This can be due to two reasons. (1) The control group patients had bigger pupils (2) the patient group had smaller pupil diameters due to the methadone dose from the previous day.

Pupil diameter change over the period of testing

The pupil diameter changed as expected during the period of absorption and metabolism after methadone administration and reached a mean peak constriction of 2.4mm at 2hrs. The mean pupil diameter at peak constriction was significantly different when compared with pre-dose pupil diameter. After 2hrs the pupil dilated progressively and reached a maximum dilation at 6hrs, which was 0.96mm less than pre-dose pupil diameter measured at 0hrs. The result thus illustrates that rising methadone levels produces increasing pupillary miosis in human subjects [87] (Table 1), while decreasing methadone levels cause increasing pupillary dilation.

The dilation phase that follows pupil constriction can differ with the use of different opiates. In this study, methadone metabolism corresponded strongly with pupil dilation. The dilation phase following the peak constriction of the pupil, took place progressively as expected.

It can be due to the increase in the central nonradrenergic activity which is commonly known to be responsible for increase in pupil size (pupil dilation) [76]. It was also suggested that the pupil dilatation may be due to the removal of the dopaminergic excitation of the Edinger–Westphal nucleus (via a putative meso-pupillomotor pathway) [138].

Pupil Characterization Parameters

The pupil goes through a few stages of constriction and relaxation if light is directed on it. In a broader sense, the pupil is characterised into two phases; constriction and dilation. During data analysis it was observed that the pupil can be characterised in more than two stages. The aim of the current study was to characterize pupil in three stages objectively and investigate their potential in assessment of methadone patients. The stages were constriction, re-dilation and relaxation (Fig 16). The intensities used in this study were logarithmic (1lux, 10lux, and 100lux). The selection of these light intensities was to record pupillary characteristics at low, medium and high level of visual stimulation and study pupil response before and after methadone administration.

It was observed that all intensities of visual light affected the pupillary constriction significantly. Although pupil constriction was detectable at 1lux and 10lux, most significant changes were detectable at 100lux. The peak constriction at 100lux occurred 2hrs after dosing. Similar time course was documented in previous studies to achieve peak pupillary constriction in methadone maintenance patients [139].

With methadone patients it is expected that the pupil reaches its maximum constriction at peak methadone levels in blood. Further constriction of the pupil observed in our results may be due to the fact that patients were on different doses of methadone. It can be speculated that patients with higher doses may have reached peak pupillary constriction, and visual light stimulation would not constrict it more. However, since the data in this study is based on averages of all the patients, this cannot be claimed strongly. Due to time constraints and large volume of information gathered, it was not possible to present individual patient data in this thesis.

The results obtained at 100lux also demonstrated that at 100lux, the pupil can be constricted further after being constricted due to methadone. This confirms form studies in the past on humans by [80], and on cats by [140]. The result confirms with some previous studies

where patients were anesthetized while being on opiates and stimulated with visual light [80]. This study further goes to show that pupillary characterization parameters were also affected due to the influence of visual light at 100lux, even at peak constriction. The parameters were inhibited for the first couple of hours after methadone administration; however the activity of the parameters increased as methadone metabolism progressed.

An obvious trend that included a peak at 2-3hrs and then recovery phase was obtained at CA, CV, R-dA, R-dV and relaxation SD. This major finding shows that pupil constriction is not the only parameter that can indicate methadone metabolism in patients. Mixed results were obtained in the past when studies were conducted on CA and R-d. It may be either due to use of different drugs, or the measurements used less data (f/s) collected over a short period of time to find discrete information. The results obtained in this study at high resolution pupil information (10f/s for 140secs gives 1498 images of pupil) demonstrate that CA and R-d can be used as potential tools for indentifying methadone metabolism. Other pupillary characteristics such as CV, R-dV and relaxation SD also demonstrated obvious trends and significant relationship over the period of the test (0-6hrs) using MDP. This is a major finding considering pupil diameter was the only parameter used in the past for assessing MDP.

In addition, at 1lux and 10lux, the pupil diameters showed significant difference when compared pre and post dose. However, pupillary characterisation parameters did not show significant changes; however trends were observed (inhibition and then recovery) over the period of the test that could be comparable to methadone metabolism. These findings are compatible with the general hypothesis that opioid depresses pupillary light reflex by increasing the activity in the Edinger-Westphal nucleus (pupilloconstrictor nucleus) [142]. It can be concluded from the investigation that 100lux is a better intensity compared to 1lux and 10lux to measure the pupils of MDP. However, it is advisable to use the results obtained from lower intensities to study the pupillogram, where effects of multiple lights as a function of time and drug can be studies in detail by physicians.

The response of various pupillometric parameters over the period 0-6hrs was inhibited by methadone. It was observed that the inhibition also occurred at the time of peak pupillary constriction. Similar methadone inhibitions were obtained for CA, CV, R-dA, R-dV, and Relaxation SD at various levels of statistical significance. Although not many studies in the past

have studied all these parameters in one single study, the results in study of pupillary diameter and pupillary R-dA agreed with [63]. They used buprenorhine in their study.

In previous studies opioid induced mydriasis was observed in cats while in humans this effect was not parallel. The results of pupillary constriction obtained in this study agree with these results. It was demonstrated in this study that as the intensity of light increased from 1 to 100lux the sensitivity of the pupil characterisation parameters increased even at the peak methadone metabolism and absorption. When the metabolism reached its peak, the pupillary characteristic parameters also reached their reduction peak and then regained its sensitivity as metabolism progressively slowed down until 6hrs.

Pupillogram Trend

Figures 15, 22 and 26 represent a typical pupillogram, IPL and COL responses of pupil during one test of 140secs. In all the responses, it was observed that the pupil does not reach its original size at the end of 140secs. The difference was not significant however the trend was obvious. This can happen due to two reasons (1) either the goggles move discretely during the testing procedures or (2) the effect of visual light stimulus for a longer time might have a memory effect on the pupil. The goggles drift amendment was carried out by us as shown in section (5.6.8). Therefore it was assumed that this slope was due to the light stimulation memory in the eye.

Accommodation Effects

The results demonstrated that pupil response to light has no effect when the pupil was in total darkness and when exposed to a minimum level of light, just for the eye to focus. This result agreed with various studies conducted in the past with control groups or patients not on methadone rehabilitation. We conducted this study to clarify if this parameter should be considered while developing pupillometry goggles to use with methadone dependent patients. From the results it can be suggested that the small but not significant changes in pupil diameter due to accommodation can be taken into account before development of pupillometry goggles.

In summary, it can be concluded that opioid constriction is best detected under the high light intensity of 100lux. Pupil characterization parameters including CA, CV, R-dA. R-dV and Relaxation SD are all capable of indicating methadone metabolism in the patient, and show

potential to be used in clinical settings along with pupil diameter measurement. These parameters at lower intensities can be used to study the pupillogram. A pupillogram based on three light intensities, discrete pupillary information, and effect of methadone can be used to investigate patterns in pupil behaviour. If these patterns show some correlation with pupil physiology, this can be potentially used as another clinical tool. It is also suggested that accommodation, dark adaptation, or pupil dark focus does not affect pupil measurements significantly.

8. COL Results

This section includes the results obtained from COL measurements. For easiness of understanding this section follows the same pattern as IPL measurements (section 7). The results obtained from IPL measurements and COL measurements, were significantly different in some areas however the difference was constant (for details refer section 9). Therefore, this section only consists of COL results. The discussion from the IPL measurements (7.10) can be applied to these results. Though, results were discussed wherever need be throughout this section. Due to time constraints and large volume of information gathered, it was not possible to do complex data analysis and in-depth comparisons of results obtained from IPL and COL measurements.

8.1 COL Eye Measurements for the Control Group

Table 9 summarizes the result of all control subjects in the form of Mean and SD versus different light intensities. Figure 38 is a visual representation of Table 9. Throughout this section "Ac" is used to denote accommodation response in Figures and Tables.

Table 9. Summary of the mean COL pupil diameter result obtained for control group in the form of Mean and SD against different light levels during the period of the test (0hr-6hr).

Hours	Pupil diameter of COL eye of Control Group (CG) [Mean±SD] (mm)														
	lux	0	1	0	10	0	100	0	Ac0.1	Ac1	Ac0.1	Ac10	Ac0.1	Ac100	Ac0.1
0hr	Mean	7.46	7.07	7.68	6.26	7.40	5.25	7.08	7.39	6.63	7.25	5.76	6.88	4.87	6.49
	SD	1.25	1.33	1.39	1.32	1.26	1.45	1.56	1.55	1.13	1.04	0.97	0.83	0.77	0.88
1hr	Mean	7.89	7.10	7.62	6.02	7.36	5.41	6.72	7.03	6.70	7.00	5.88	6.81	4.86	6.29
	SD	1.01	1.71	1.44	1.38	1.19	1.17	0.80	1.18	1.42	1.36	1.18	1.45	1.45	1.37
2hr	Mean	7.63	7.02	7.41	6.71	7.32	5.92	7.18	7.67	7.37	7.09	6.10	7.03	5.35	6.91
	SD	0.88	1.59	0.88	1.13	0.64	1.15	0.92	0.82	0.71	1.12	0.63	0.89	1.18	0.98
3hr	Mean	7.81	6.99	7.30	5.71	7.23	5.23	7.09	7.21	6.87	7.27	5.82	6.98	4.98	6.70
	SD	1.12	1.89	1.51	1.26	0.97	1.39	1.10	1.26	1.03	0.99	0.72	0.44	0.81	0.26
4hr	Mean	7.63	6.39	7.53	5.82	7.14	5.17	6.64	7.14	6.63	7.27	6.25	7.09	5.28	7.02
	SD	0.64	1.42	1.23	1.37	1.04	1.25	1.27	1.55	1.73	1.31	1.40	1.01	1.04	0.62
5hr	Mean	7.41	6.49	7.00	5.60	6.74	4.77	6.39	7.21	6.41	7.02	5.31	6.62	4.96	6.32
	SD	0.59	0.84	1.02	0.99	0.10	1.42	0.64	1.33	1.33	1.24	1.12	0.32	1.19	0.35
6hr	Mean	7.78	6.97	7.65	6.15	7.21	5.26	6.88	6.83	6.80	7.25	6.16	6.79	4.99	6.69
	SD	0.43	1.13	0.49	1.15	0.62	1.29	0.75	1.14	0.80	0.65	0.54	0.01	0.30	0.21

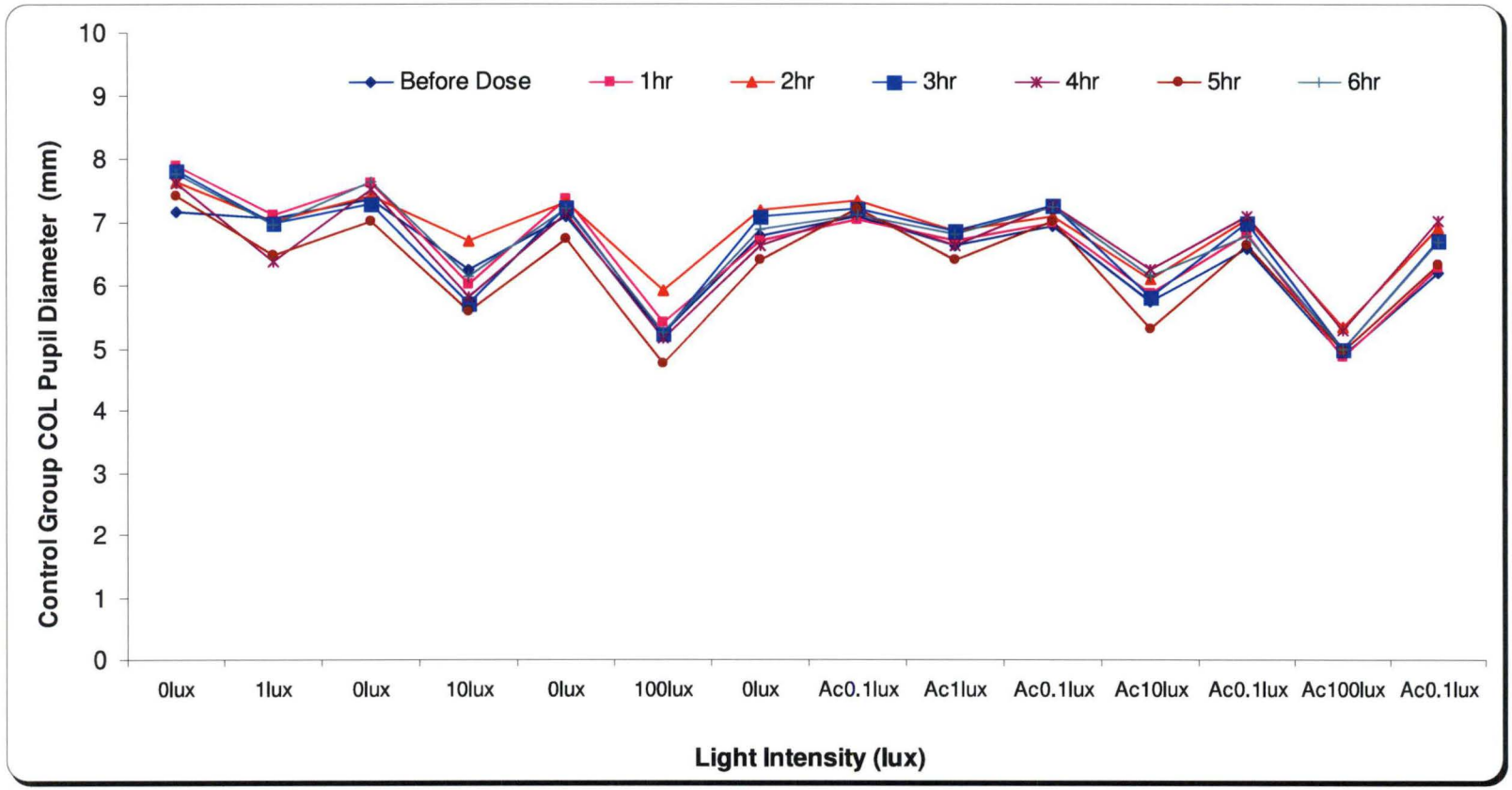

Figure 38. A plot of light intensities versus mean pupil diameter of control subjects during the period of the test (0hr-6hr). Each horizontal line represents the test performed at every hour for 6hrs. The SD was not included for better representation of the data. The values are given in Table 9.

Figure 39 demonstrates the mean pupil diameter of control subjects at 0 and 0.1lux with and without accommodation response. No statistical significance *($p < 0.05$. t-test, paired two samples for means)* was observed in pupil diameters measured at hourly intervals. The errors bars were not shown in Figure 23 because the number of sample size (n=2) was too small to demonstrate variance in population.

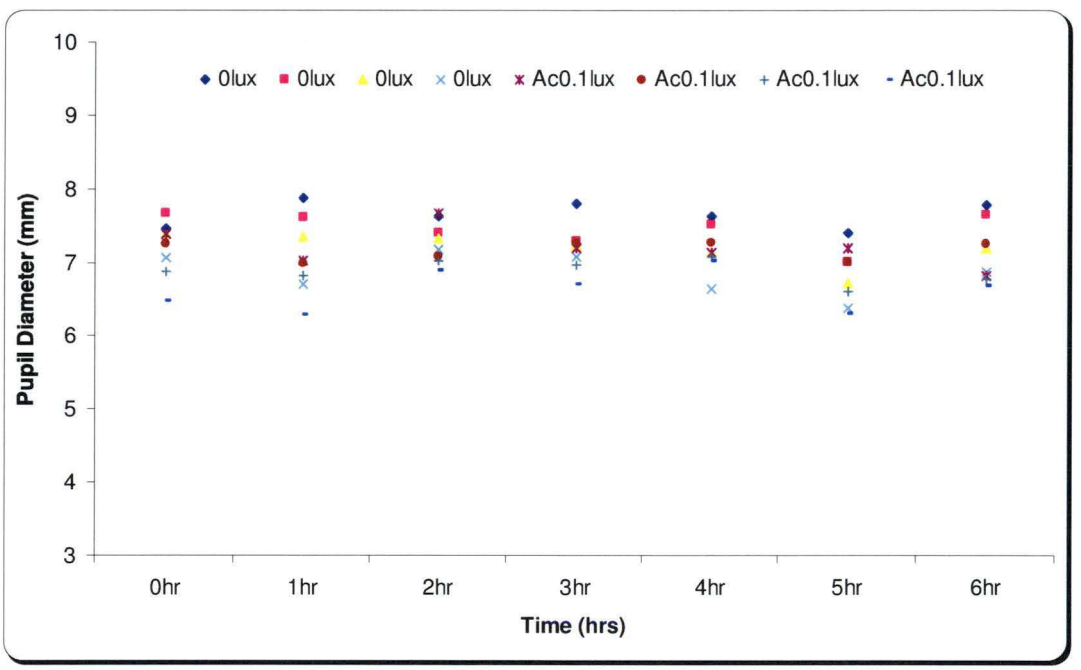

Figure 39. The mean pupil diameters of the control patients measured in the COL eye throughout the test period (0-6hrs) for 0 and Ac0.1lux.

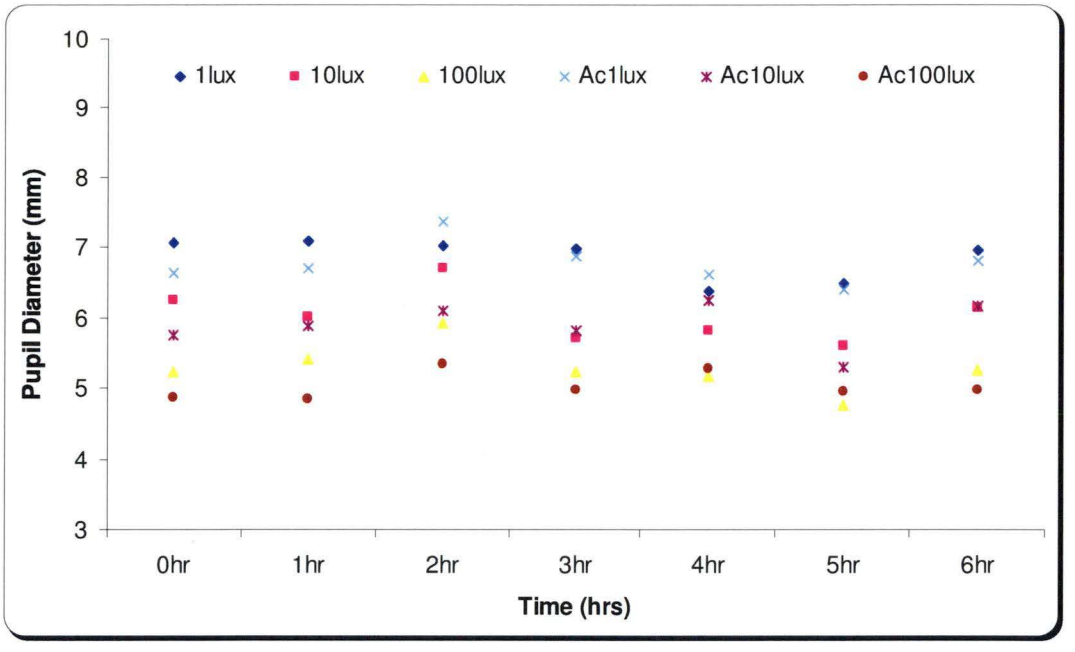

Figure 40. The mean pupil diameters of control patients measured in the COL throughout the test period (0-6hrs) at light intensities of 1, 10 and 100lux.

Figure 40 shows the mean pupil diameter of control subjects at light intensities 1, 10 and 100lux. Significant difference *($p < 0.01$, t-test, paired two samples for means)* was obtained for the pupil diameters measured at the 3 light intensities. Statistical significance was also observed at the same light intensities after the accommodation response, however of a lesser effect *($p < 0.05$)*. Figure 41 shows a plot of mean pupil diameter versus various baseline light intensities (0 and Ac0.1lux) in the order used in the tests. Figure 41 is a better visual representation of Figure 40 in understanding the pupil response to the baseline light intensities. It was observed that the pupil diameter decreased progressively from the first 0lux intensity to the last Ac0.1lux. In addition a consistent dip at 0lux and Ac0.1lux intensity after 100lux was observed at different hours. This may have caused because of the memory of 100lux light in the pupil which carried its big constriction effect on the 0lux and Ac0.1lux light intensity.

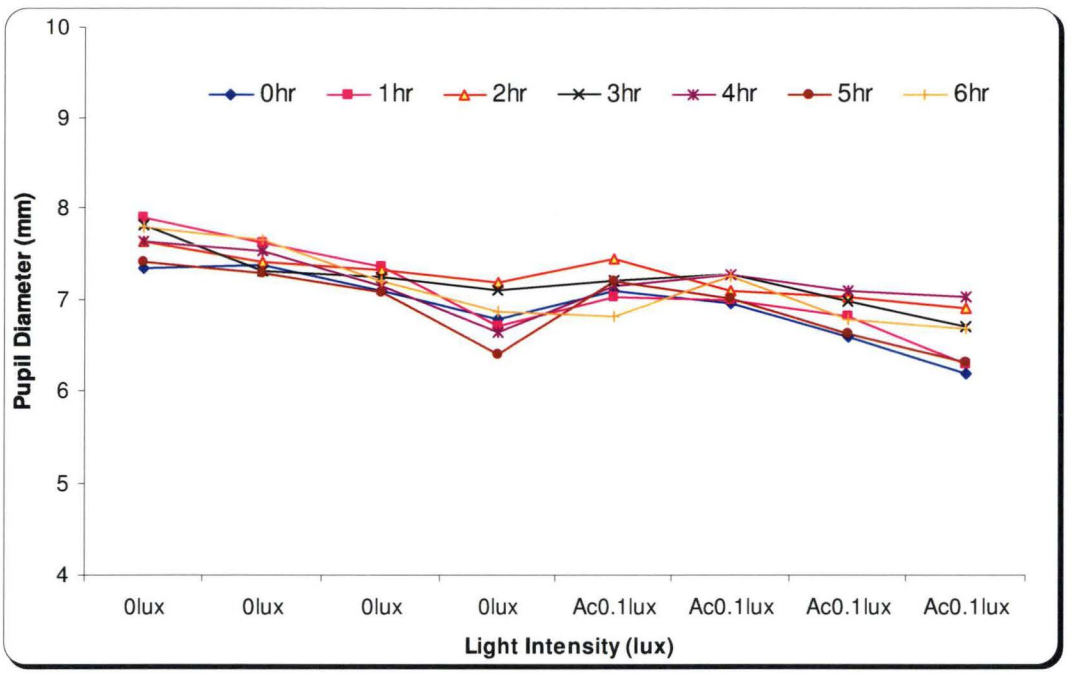

Figure 41. The mean pupil diameter of control group at intensities 0lux and Ac0.1lux. Each horizontal line represents the test performed at every hour for 6hrs. Figure 41 is a better visual representation of Figure 40 in understanding the pupil response light intensities 0lux and Ac0.1lux.

8.2 COL Eye Measurements for the Patient Group

Table 10 summarizes the result of the patients group in the form of Mean and SD versus different light intensities including the baseline intensities (0lux and Ac0.1lux). Figure 42 is a visual representation of Table 10. The plot demonstrates the pupil diameter change in methadone patients with different light intensities as a function of time.

Table 10. Summary of the COL results obtained for patient group in the form of Mean and SD versus different light levels during the period of the test (0-6hrs).

Hours		Pupil diameter of COL eye of patient group [Mean±SD] (mm)													
	lux	0	1	0	10	0	100	0	Ac0.1	Ac1	Ac0.1	Ac10	Ac0.1	Ac100	Ac0.1
Before Dose	Mean	7.39	7.31	7.31	7.12	7.12	6.14	6.92	7.13	7.13	7.08	7.08	7.25	6.09	6.74
	SD	0.53	0.53	0.65	0.82	0.79	0.69	0.80	0.95	0.85	0.75	0.86	0.75	0.86	0.83
1hr	Mean	5.80	5.87	5.87	5.69	5.69	4.72	5.59	5.75	5.75	5.25	5.25	5.44	4.51	5.29
	SD	0.37	0.44	0.71	0.40	0.37	0.69	0.59	0.71	0.74	0.75	0.64	0.84	0.66	0.74
2hr	Mean	5.03	4.82	4.82	4.46	4.46	3.99	4.65	4.33	4.33	4.33	4.33	4.55	3.87	4.50
	SD	0.55	0.77	0.61	0.78	0.78	0.79	0.77	0.84	0.93	0.90	1.00	0.96	0.88	0.81
3hr	Mean	5.15	5.25	5.25	4.93	4.93	4.33	4.83	4.84	4.84	4.62	4.62	4.81	3.96	4.60
	SD	0.57	0.58	0.64	0.69	0.70	0.69	0.66	0.78	0.82	0.78	0.84	0.84	0.70	0.75
4hr	Mean	6.05	5.91	5.91	5.42	5.42	4.75	5.42	5.57	5.57	5.17	5.17	5.64	4.45	5.26
	SD	0.86	0.80	0.75	0.73	0.81	0.73	0.69	0.74	0.83	0.84	0.88	0.87	0.82	0.81
5hr	Mean	6.20	6.14	6.02	5.81	5.91	5.17	5.89	6.13	6.13	5.70	5.70	6.05	5.02	5.67
	SD	0.63	0.74	0.91	0.87	0.81	0.72	0.72	0.65	0.72	0.98	0.83	0.72	0.89	0.92
6hr	Mean	6.43	6.33	6.47	6.15	6.35	5.39	6.06	6.17	6.17	5.75	5.75	6.18	5.21	6.02
	SD	0.58	0.57	0.49	0.42	0.42	0.47	0.36	0.38	0.53	0.47	0.47	0.59	0.57	0.51

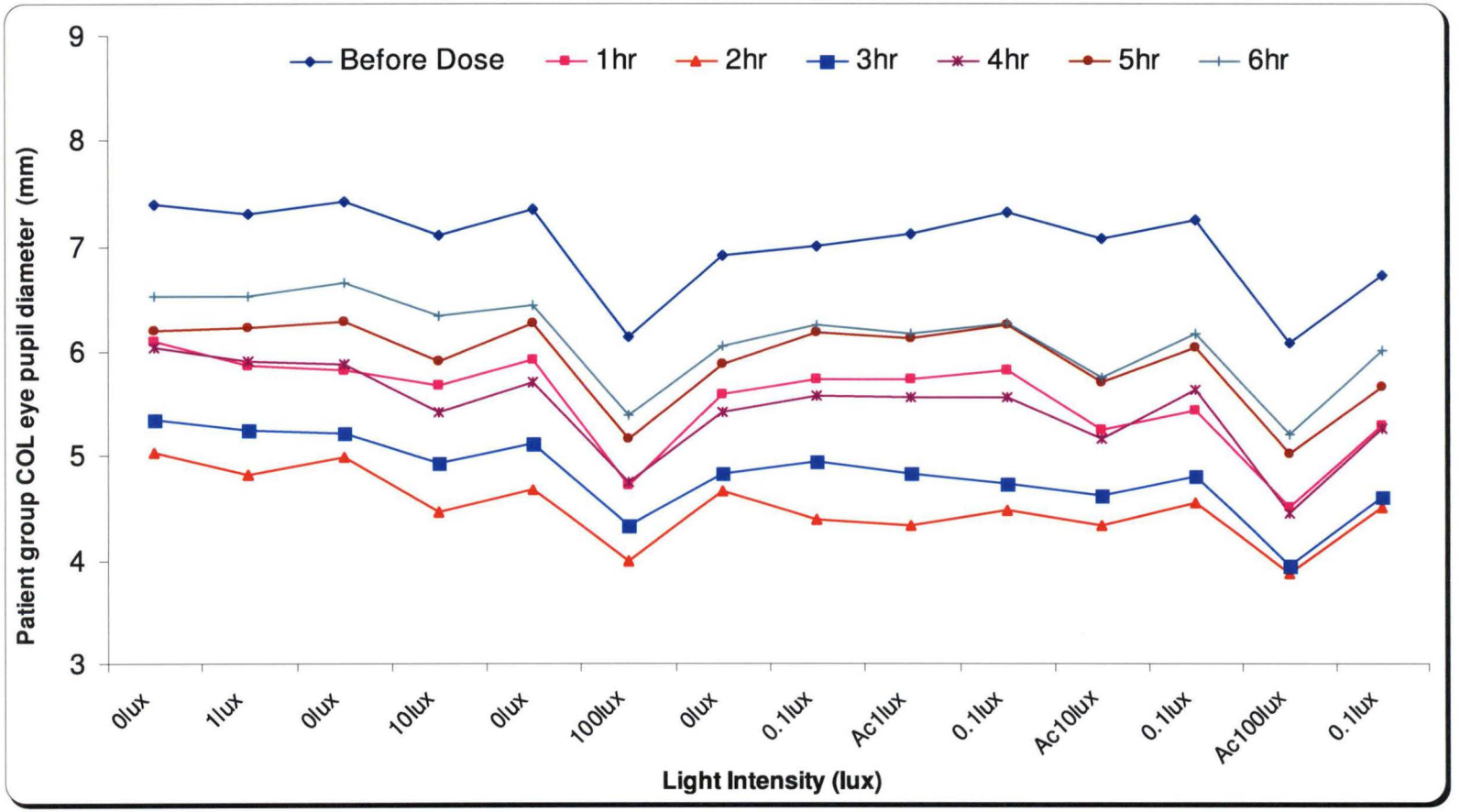

Figure 42. A plot of light intensities versus pupil diameter (COL eye) of methadone patient group during the period of the test (0-6hrs). The values are given in Table 10. Each horizontal line represents the test performed at every hour for 6hrs. It can be observed that the pupil diameter decreased and recovered over the period of 6hrs.

Figure 43 demonstrates the pupil diameter of patients at 0lux and 0.1lux light levels throughout the test (0-6hrs). Pupil diameters were decreased significantly by methadone. The differences were significant *($p > 0.01$. t-test, paired two samples for means)* when pupil diameter measured before dose was compared with the pupil diameter after methadone administration. Peak miosis occurred approximately 2hrs after methadone administration by patients.

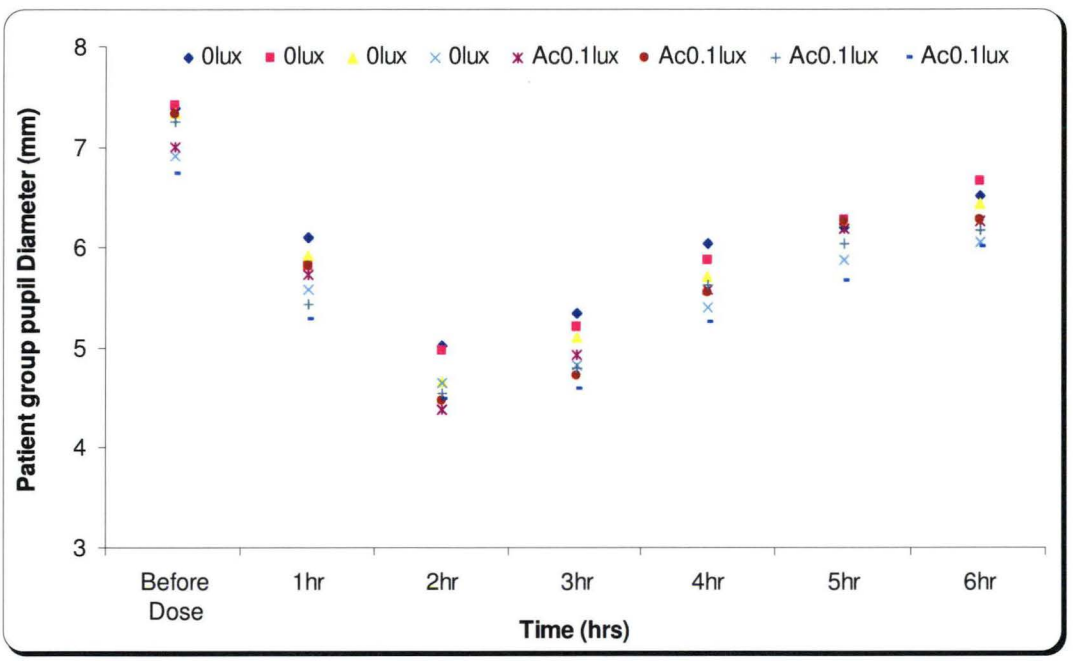

Figure 43. The mean pupil diameters of patient group measured in the COL eye throughout the test period (0-6hrs) when the light intensity was at 0lux and Ac0.1lux.

Figure 44 is a better visual representation of Figure 43 to understand the pupil response to the baseline light intensities in the order used in the tests. Each horizontal line represents the test performed at every hour for 6hrs It was observed that the pupil diameter decreased progressively from the first 0lux intensity to the last 0.1lux. However, this progressive decrease was not as prominent as observed in the control subjects (Fig 41).

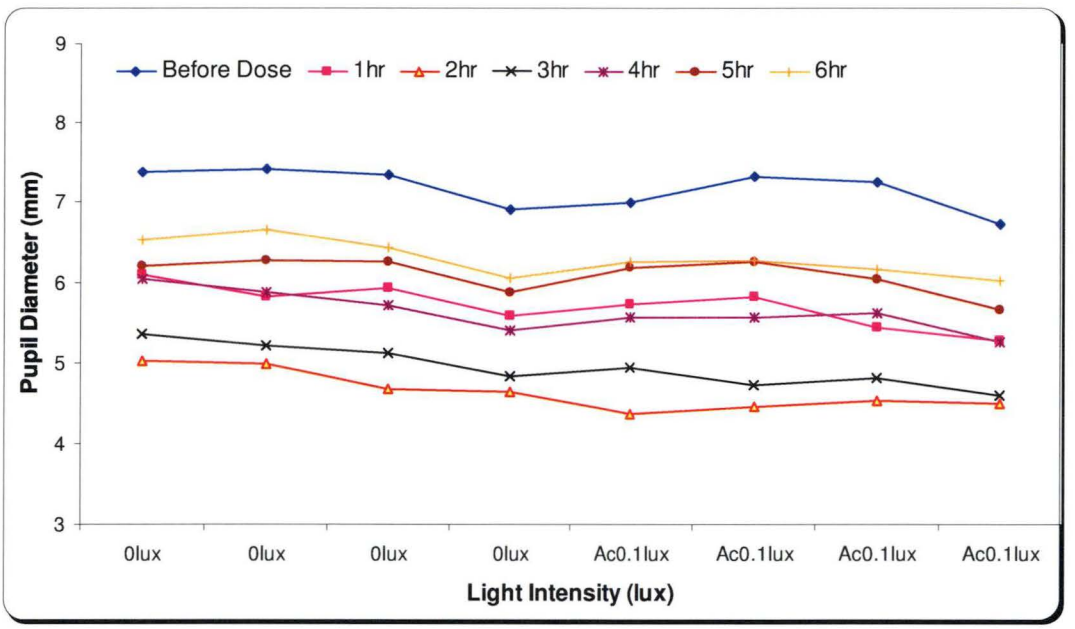

Figure 44. The plot represents the mean COL pupil diameter response of methadone patients group at various baseline intensities. Each horizontal line represents the test performed at every hour for 6hrs

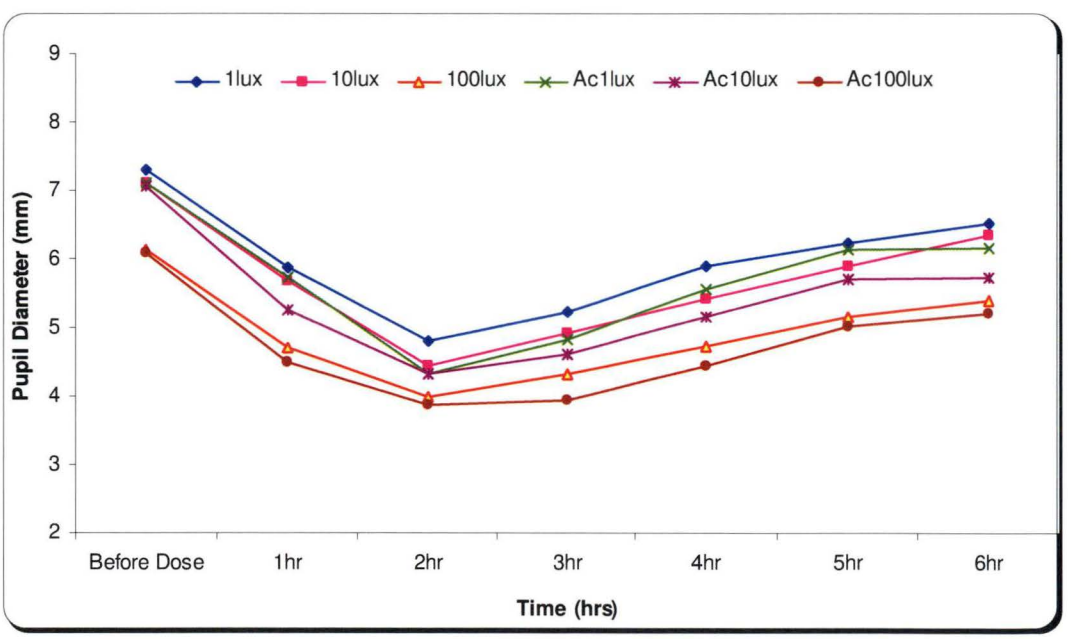

Figure 45. The average pupil diameters of the patients measured in the COL eye throughout the test period (0-6hrs) at light intensities of 1lux, 10lux and 100lux. Each horizontal line represents the test performed at light intensities 1lux, 10lux and 100lux.

Figure 45 shows the pupil diameter of patient group at the three light intensities (1lux, 10lux and 100lux) (Table 10). Significant difference *($p > 0.01$ t-test, paired two samples for means)* was obtained in the pupil diameters measured at the 3 intensities. Similar statistical significance was observed at the same intensities in the accommodation response of the pupil. Figure 46 shows the plot of pupil diameter response of the patient group at various light intensities. Each horizontal line represents the test performed at every hour for 6hrs. At maximum intensity (100lux) the pupil response was the strongest. Similar response was obtained at Ac100lux in accommodation response.

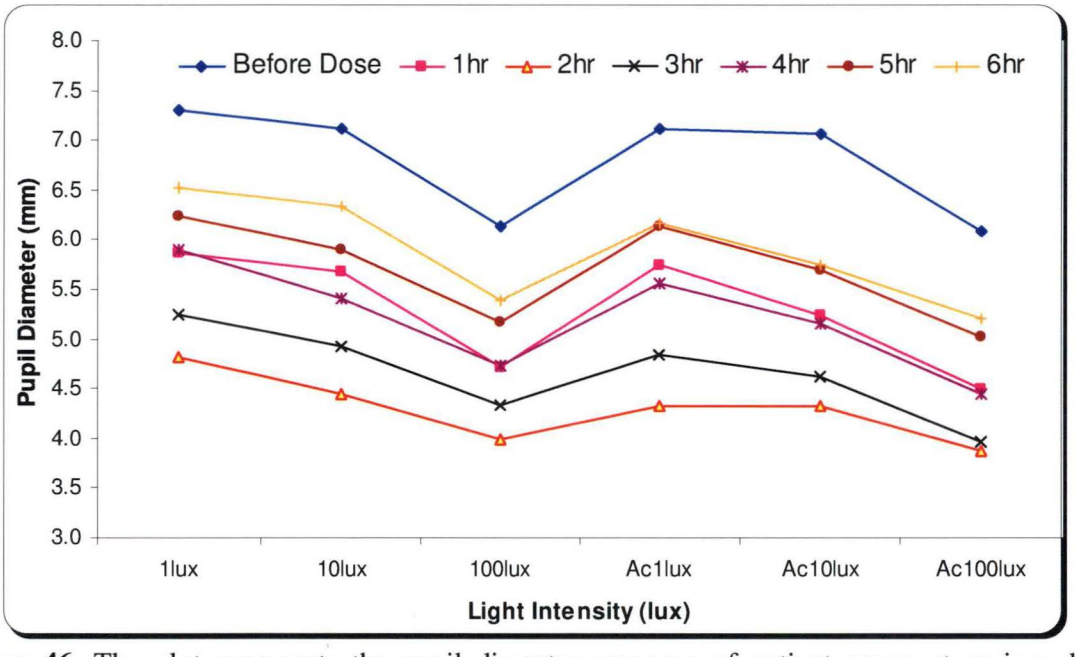

Figure 46. The plot represents the pupil diameter response of patient group at various light intensities. Each horizontal line represents the test performed at every hour for 6hrs

8.3 Effect of Accommodation Response

To understand the effect of accommodation response on pupil diameter the baseline intensity was increased from 0lux to 0.1lux. Figure 47 shows the pupil diameter as a function of accommodation response for control as well as patient group throughout the time course of the test (0-6hrs). No significant accommodation effect was observed in control as well as patient group.

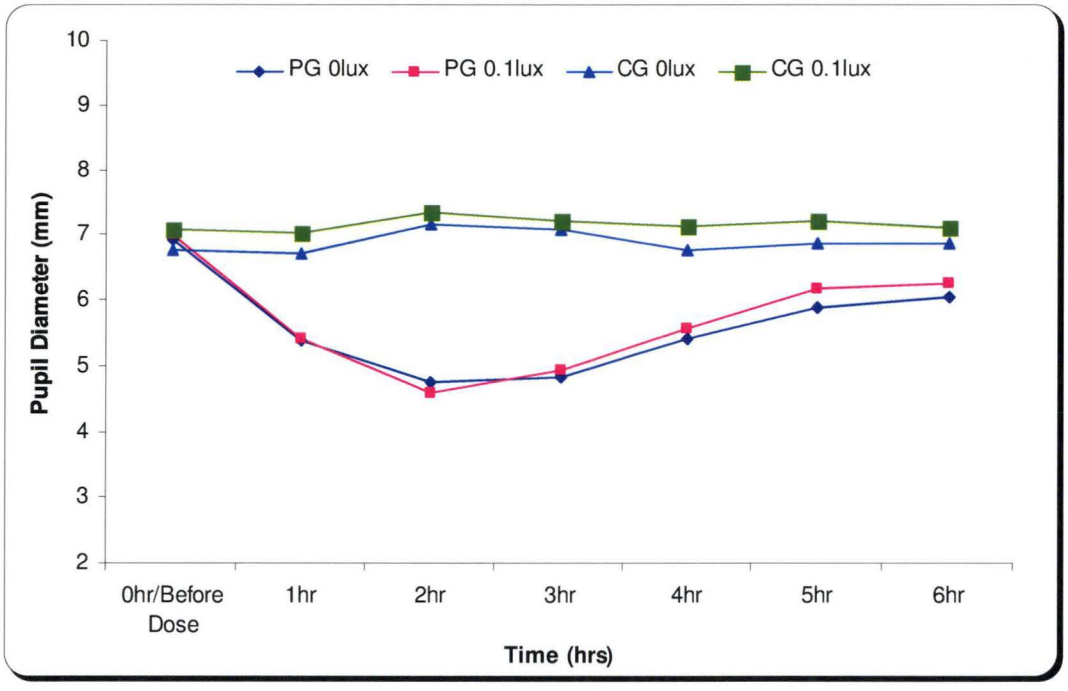

Figure 47. Effects of accommodation on mean pupil diameter when light intensity was increased from 0lux to 0.1lux. The plot consists of combined results from control group (CG) and patients group (PG).

The maximum percentage difference in pupil diameter for both patient group and control group was 0.05%. The pupil diameter in control as was a patient group was found higher in accommodation response. In both accommodation and non-accommodation responses of pupil diameter it was indicated that the slight difference in pupil diameter measures were accounted by the differences in lightning intensity.

The detection of methadone miosis was affected by lighting intensity and at zero intensity at different levels of statistical significance (Figure 47). The best level of significance ($p < 0.001$) was obtained at maximum intensity of 100lux (Fig 42). However, methadone miosis was mostly readily detected irrespective of visible light excitement (at baseline intensities). Miosis was statistically significant at different levels under these lighting conditions as well as no light condition according to the paired T-test.

8.4 Constriction Amplitude (CA)

Figure 48 shows a plot of change in CA at various light intensities. The plot demonstrates that the CA increased with increasing light intensities for the control group as well as the patient

group. The results of CA obtained from the control group were averaged over the period of the test and plotted in Figure 48 to indicate the distinction in CA of the control group and the PG pre and post methadone administration. The error bar represents the SD obtained from control group. Pupil response to changes in light levels were non linear for both the patients and control group. This was expected as the light levels were logarithmically increasing. In both groups the maximum constriction was observed at 100lux. Differences in constriction was observed at the same light levels in accommodation responses, however they were not significant. This difference can be speculated as the memory response of the pupil or lack to time for the pupil to reach equilibrium in dilation periods, which were 10 seconds.

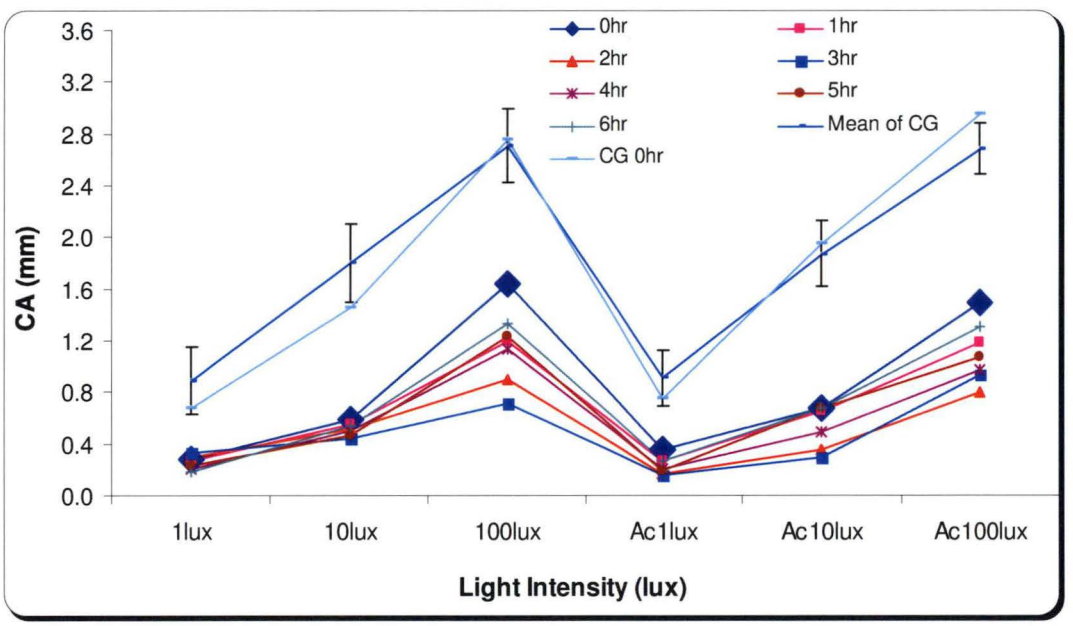

Figure 48. The plot represents the change in mean CA at different light intensities as a function of time.

The CA of the light reflex differed significantly among the patient group. A two-way ANOVA indicated significant differences ($p < 0.01$) among methadone conditions and time of measurement as well as a significant methadone by time interaction. As shown in Figure 48, CA significantly decreased as a function of time. The magnitudes of the effects were small at light intensities 1lux and 10lux and maximal changes occurred at the time of the maximal change in pupillary size (100lux).

8.5 Constriction Velocity (CV)

Figure 13 shows a plot of pupil CV at various light intensities. The plot demonstrates that the pupil constriction increased with increasing light intensities for control group as well as patient group.

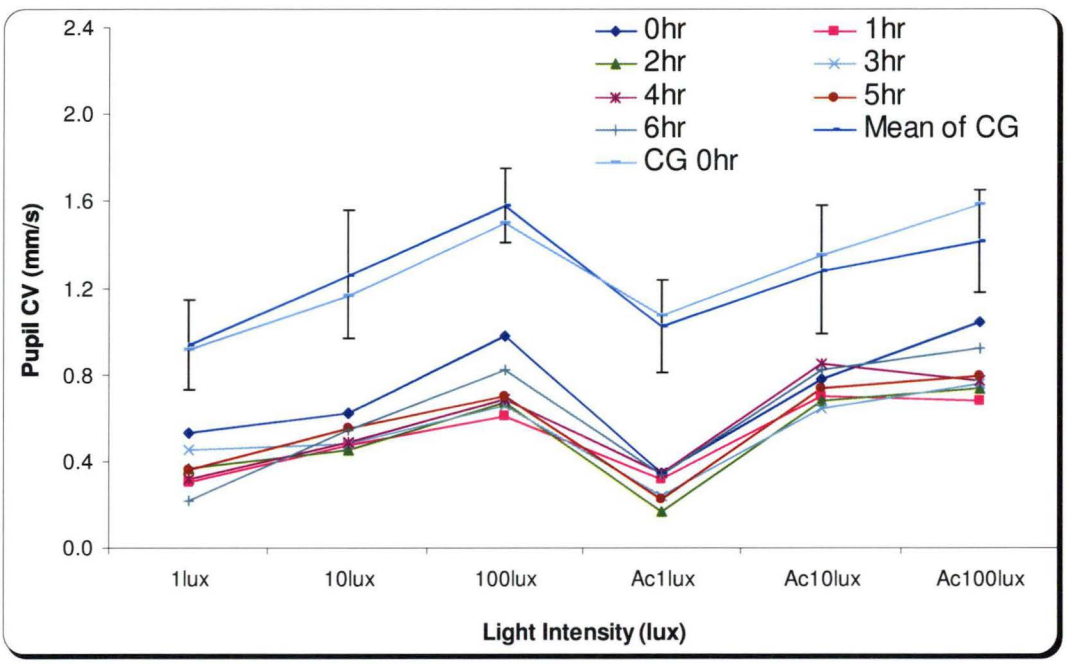

Figure 49. The plot represents the change in CV at different light intensities as a function of time.

The results of pupil constriction obtained from the control subjects were averaged over the 7hrs and plotted in Fig 13 to indicate the distinction in pupil CV of control group and patients group pre and post methadone administration. The error bar represents the SD obtained from control group. Pupil CV response to changes in light levels were non linear for both patients and control group. This was expected as the light levels were logarithmically increasing. In both groups the maximum CV was observed at 100lux. A difference in constriction was observed at same light levels in accommodation response however they were not statistically significant. This difference can be speculated as the memory response of the pupil or lack to time for the pupil to reach equilibrium in dilation periods.

Statistically significant and near significant changes were observed in pupil velocity as a function of light intensity in the control group, the patient group before dose ($p < 0.05$) and the methadone group after dose ($p < 0.1$), respectively. The velocity of pupillary constriction changed significantly as a function of the drug treatment (Figure 49). A two-way ANOVA indicated significant differences among methadone conditions and the time of measurement, as well as a significant ($p < 0.05$) methadone by time interaction.

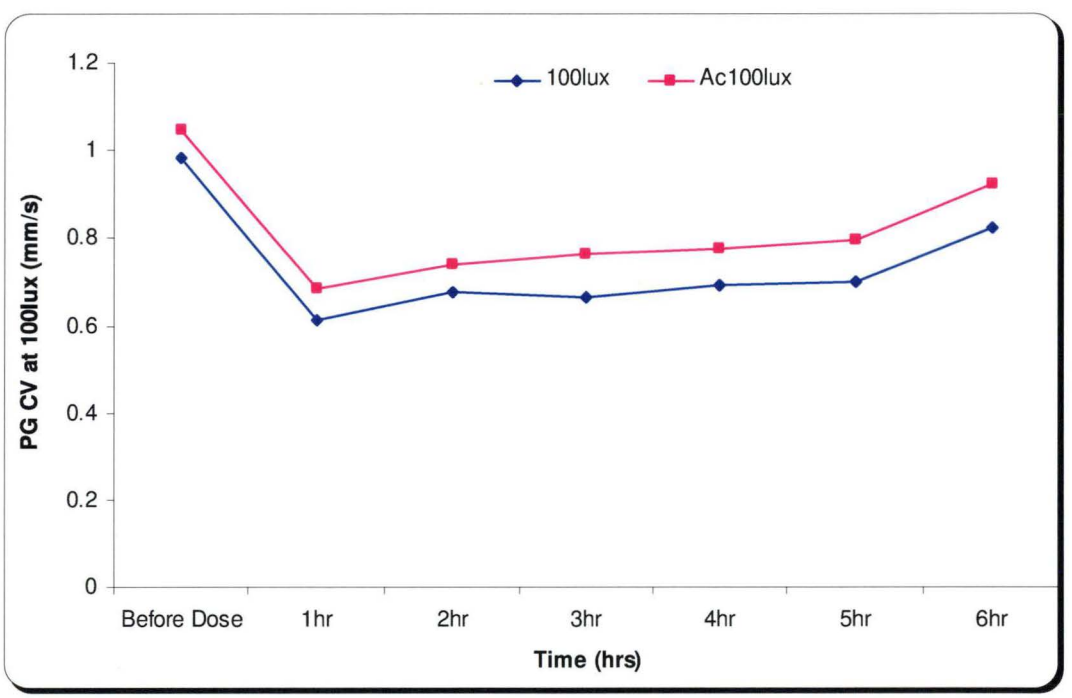

Figure 50. The plot represents the change in CV during the course of the test at 100lux (0-6hrs).

The CV at various light intensities over the period of 6hrs was measured. A significant ($p<0.05$) difference in pupil CV before and after methadone administration was observed at 100lux (with and without accommodation) (Fig 50) . No significant differences were observed at lower light intensities ((with and without accommodation).

8.6 Re-dilation Amplitude (R-dA)

The R-dA change in the patient group without accommodation response changed from a maximum of 0.52 mm before dose to 0.35 mm at the 6hr. It achieved the lowest R-dA change at the 2hr period (0.25mm). The R-dA change in the patient group with accommodation response changed from a maximum of 0.45 mm before dose to 0.32 mm at the 6hr. It achieved the lowest R-dA change at the 2hr period (0.20mm). It was observed that the magnitude of R-dA

progressively dampened and then recovered after methadone administration at 100lux, with and without accommodation response (Figure 51).

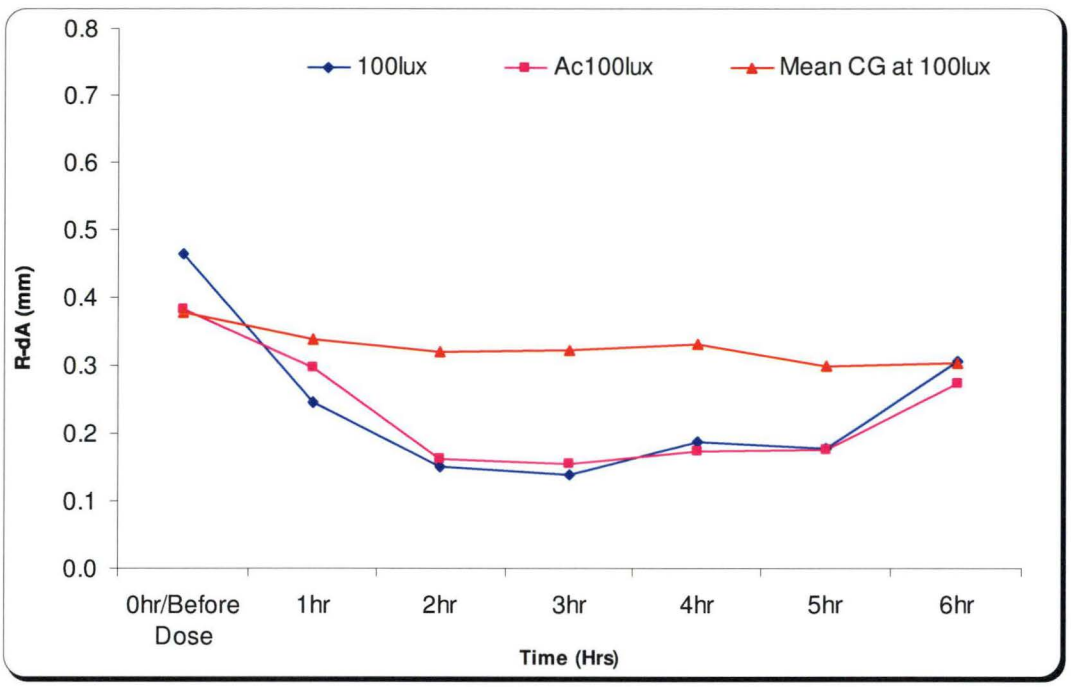

Figure 51. The plot of R-dA change in the patient group and control group. It was demonstrated that the magnitude of R-dA at 100lux progressively dampened and then recovered after intake of methadone dose. The control group showed no trend as expected unlike the control group.

A similar trend was observed at 1lux and 10lux however the response was not obvious. The changes in R-dA after methadone dose over a period of the test were compared with the pupil response of control group. The R-dA change in CG at 0hr was 0.43mm/s and at 6hr was 0.40mm/s. Figure 51 shows an obvious difference between the patient group and the control group from the period between 2hr to 5hr. The differences observed during this period were significant ($p < 0.01$). The response crosses over at the 0hr/Before dose and 6hr. This could be attributed to the fact that the pupil diameter of the control group was inherently smaller than then patient group. In addition, pupil diameters have been known to decrease from morning to evening [94], which was observed in our study (Figure 38, Control group), and that maybe one of the reasons behind the response obtained at 6hr.

8.7 Re-dilation Velocity (R-dV)

The R-dV in the patient group with accommodation changed from a maximum of 0.32 mm/s before dose to 0.25 mm/s at the 6hr.

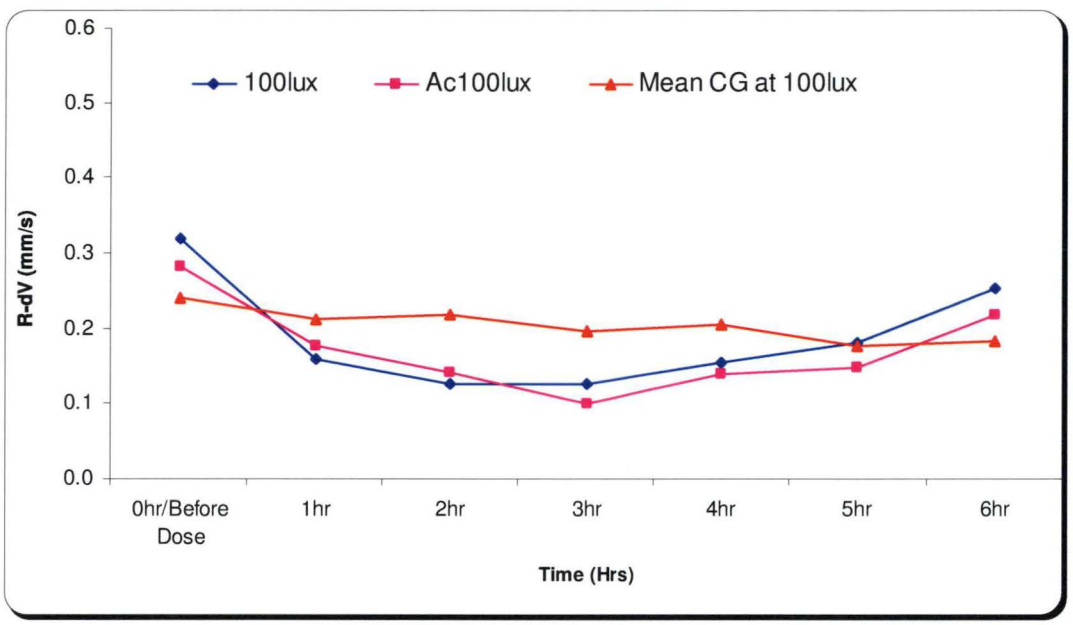

Figure 52. The plot shows the R-dV in patient group and control group.

It was demonstrated that the R-dV at 100lux progressively dampened and then recovered It achieved the lowest R-dV at 2hr and 3hr (0.13mm/s). The maximum R-dV at Ac100lux was obtained at the 0hr/before dose (0.28mm/s) with a minimum at 3hr (0.10mm/s). At 6hr the velocity was 0.22mm/s. It was observed that the magnitude of R-dV progressively dampened and then recovered after methadone administration at 100lux, with and without accommodation response (Figure 52). The factor that differentiates R-dA from R-dV is the time. It is expected that the pupillary responses low down upon methadone intoxication and it increases as it reaches its peak. The pupil regains its normal functions as the peak gradually drops after methadone administration. The control group showed no trend as expected unlike the control group.

Similar, trend was observed at 1lux and 10lux however the response was not obvious. The changes in R-dV after methadone dose over a period of the test were compared with the pupil response of control group. The R-dV at 0hr was 0.24mm/s and at 6hr was 0.18mm/s. Figure 36 shows an obvious difference between the patient group and the control group throughout the test. The differences observed during this period were significantly ($p < 0.01$). The response nearly

come together or cross over at the 0hr/Before dose and 6hr indicating that during the period between 0hr to 6hr the pupil responded to methadone metabolism (Fig 52).

8.8 Relaxation SD

Considerable amount of fluctuations were observed in the relaxation phase of the pupil response under light. The fluctuations were higher with increasing light intensity. It was difficult to find the amplitude and velocity of the relaxation phase. Therefore the SD of relaxation phase was compared over the period of the test (0-6hrs). The relaxation SD of the light reflex changed significantly ($p<0.05$) among the pre-dose and post-dose measurements. The highest level of significance ($p<0.01$) was obtained at the 2hrs interval. The trend observed in the Ac response showed high degree of similarity to the response obtained before Ac response (Fig 16). The difference between the two trends was 0.22mm.

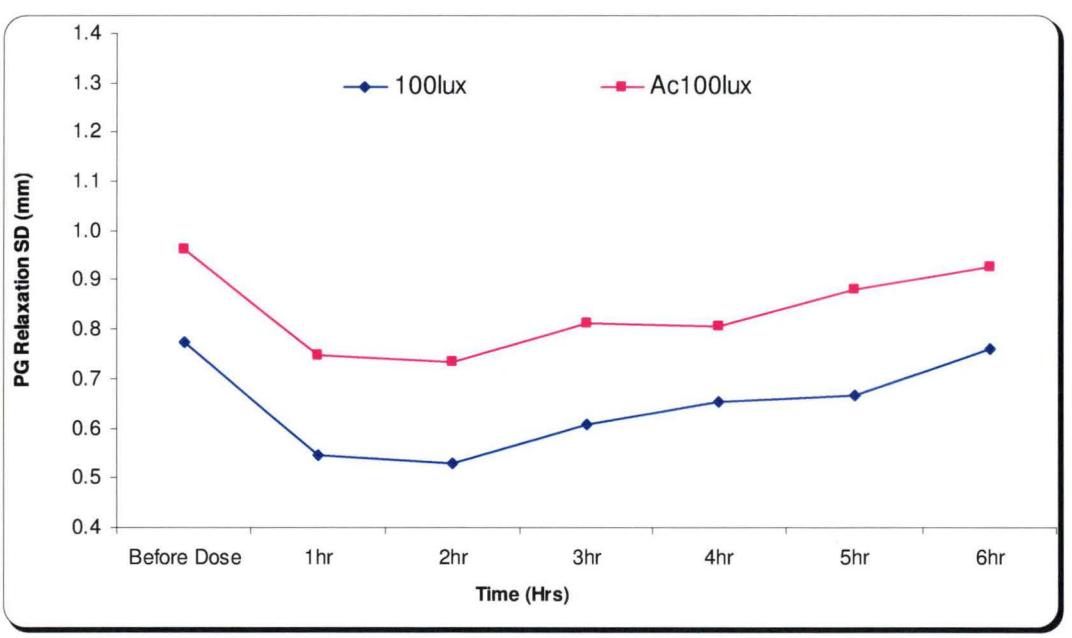

Figure 53. The plot shows the Relaxation SD in patient group with and without accommodation response. Both responses showed high degree of similarity.

8.9 Discussion

Under normal conditions, the pupils of both eyes respond identically, regardless of which eye is stimulated; that is, light in one eye produces constriction of both the stimulated eye (the

direct response) and the unstimulated eye (COL response). The results obtained in COL measurements were similar to the one obtained in IPL measurements. Therefore the discussion from IPL can be used for COL measurements. For further discussion on IPL-COL comparison please refer section 10.

9. SVRTT Results

9.1 SVRTT Validation

Before doing the testing on patients, the RTT was tested for its reliability and reproducibility. 15 people volunteered from the school for the validation test. These subjects were considered normal test subjects. Repetitive testing was done on these subjects in two phases. Phase 1 consisted of testing at an interval of every half hour and Phase 2 at an interval of every 24hrs. The Mean $\pm$ SD obtained from Phase 1 was 0.30 ± 0.09, from Phase 2 was 0.30 ± 0.08 and combined was 0.30 ± 0.09 (Fig 54). There was no significant difference among the mean reaction times of the 5 subjects in Phase 1 and the 10 subjects in Phase 2, indicating that the test was reliable (Fig 54).

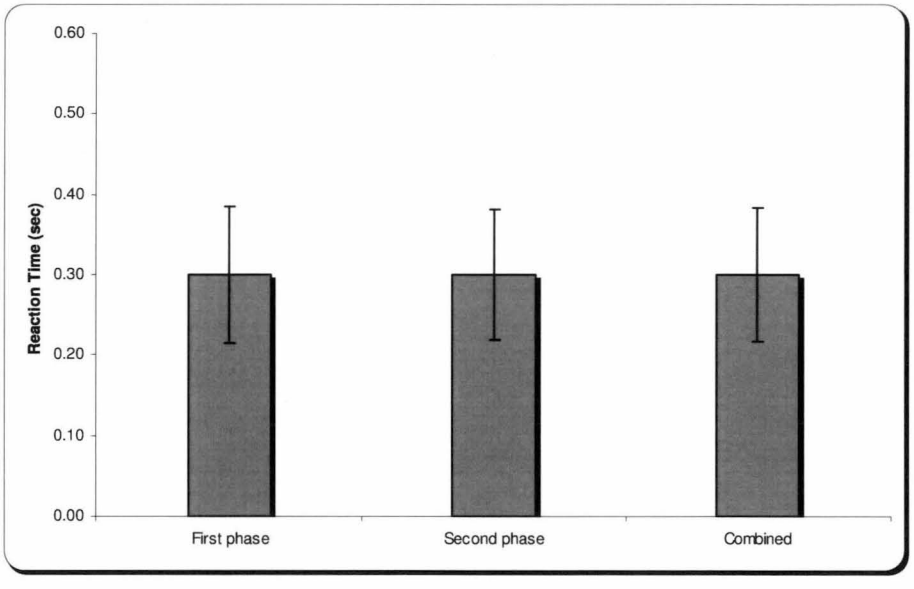

Figure 54. Comparison of the means of the RT's during the two different testing phases and the combination of the means of the two phases. The maximum difference in the mean was obtained between the combined results and the mean obtained from the first phase.

To validate the reproducibility of the SVRTT, the results for each subject from the 5 days were compared. The reproducibility test showed that the SVRTT measured for each subject during the first phase of the study agreed well with a mean percentage SD of 9.2%. The intraclass

correlation coefficient (correlation of the 5 test sets) for 95% confidence level was r = 0.922 *($p<0.001$)*, which showed a high reproducibility.

9.2 SVRT

In the SVRTT tests, a total of 15 methadone patients and two control subjects were tested. The control subjects followed the same procedure as the patients except methadone administration. Figure 55 shows a typical response of RT variation conducted on one control subject from test 1 to test 7. The plot contains all the data points measured from test 1 to test 7 (0hr to 6hr). Each test consisted of a test set of 90 RT.

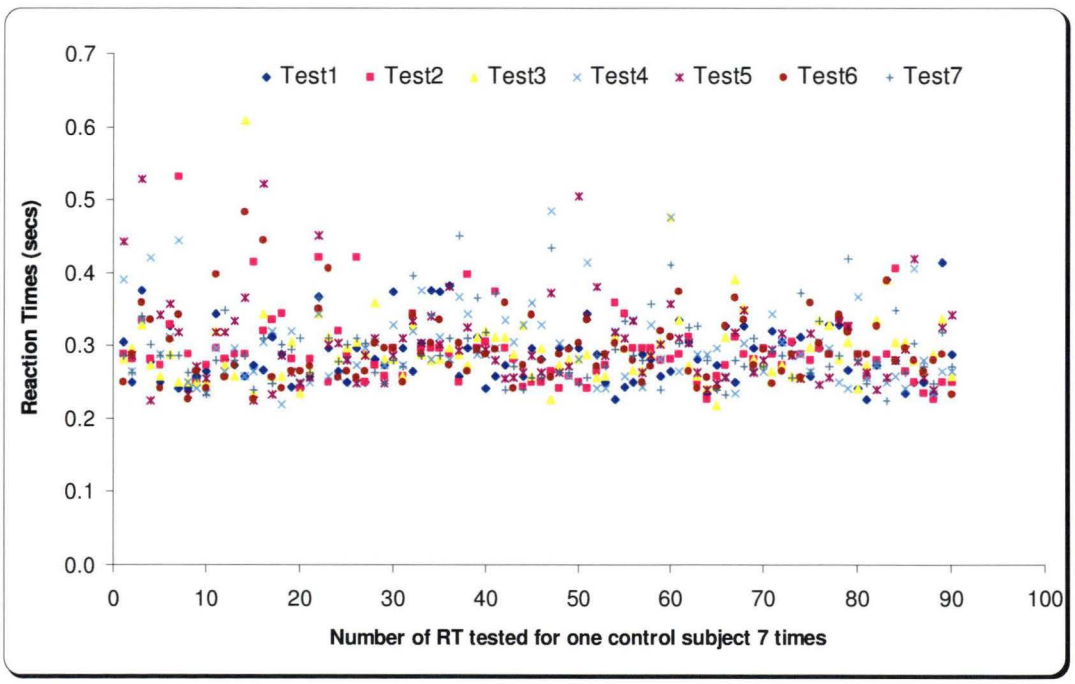

Figure 55. The graph indicates a typical response of RT's versus the number of times tested for one control subject.

The plot indicates the variance of the data from mean. The variance was also similar for the other control subject. RT's were measured at an accuracy of $0.1s$ Valid RT responses were defined as scores between $1.2s$ and $1.5s$ (responses faster than $1.2s$ were considered anticipatory and responses slower than $1.5s$ were considered late.) The range of variance between the RT's

observed in Figure 55, matched with values obtained by [141]. The range of RT values found in that study was from 0.42 s to 0.63 s.

Table 11. The mean RT of the control group and the patient group measured at hourly interval during the course of the test.

Hours	Reaction Times (seconds)			
	Patient Group		Control Group	
	Mean	SD	Mean	SD
0hr/Before Dose	*0.379*	*0.045*	*0.287*	*0.041*
1	0.501	0.042	*0.294*	*0.048*
2	0.528	0.065	*0.294*	*0.051*
3	0.530	0.065	*0.298*	*0.052*
4	0.509	0.058	*0.304*	*0.060*
5	0.512	0.063	*0.295*	*0.047*
6	0.510	0.068	*0.294*	*0.046*
Mean Control	*0.379*	*0.045*	*0.0296*	*0.068*

Table 11 presents the results of the SVRTT of patient and control group in the form of Mean and SD from 0hr to the 6hr. The RT of test 1 of the control group and the patient group was 0.29±0.06s and 0.38±0.18s, respectively. The patient group was observed to be 24.8% higher that the control group. This difference was not statistically significant.

Figure 56 and Table 11 illustrates the Mean±SD plot of the control group. No significant statistical difference was observed in the results obtained during the 6hrs. The Mean±SD of the control group was 0.28±0.04s. No significant trend of RT was observed indicating no obvious learning effects of SVRTT. Infect, a slight, but not significant, trend of RT was observed from 0hr to 4hr after which a small decrease was observed. In our pilot study [135] the tests were carried out at an interval of 15 to 30 mins apart and the number of RT tested in each test were 300. In the current test the RT test set of 90 was used considering the comfort of the patients and the instability that might set in after dose if large number is used. In addition, the control subject's were asked to do the exact test the patients did. The minimum RT obtained in the first phase of testing was 0.17s and the maximum was 0.78s.

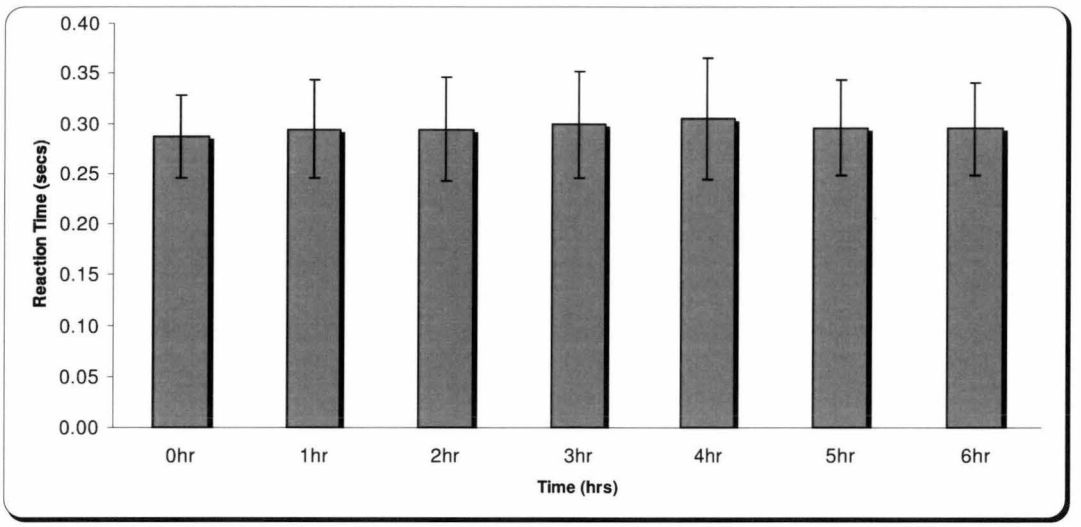

Figure 56. The RT of the control subjects tested every hour for 6 hrs. The presented data were the average of the 2 control subjects and the error bars indicated the standard deviations.

RT's are not normally distributed and therefore the RT from patient group was summarized using 10th, 50th and 90th percentiles. Figure 57 shows a plot comparing the tests in the control group. No differences in all RT percentiles were found. No statistical significance was observed when RT values at 10th, 50th and 90th percentile were compared between the 7 tests (0hr to 6hr) of the control group.

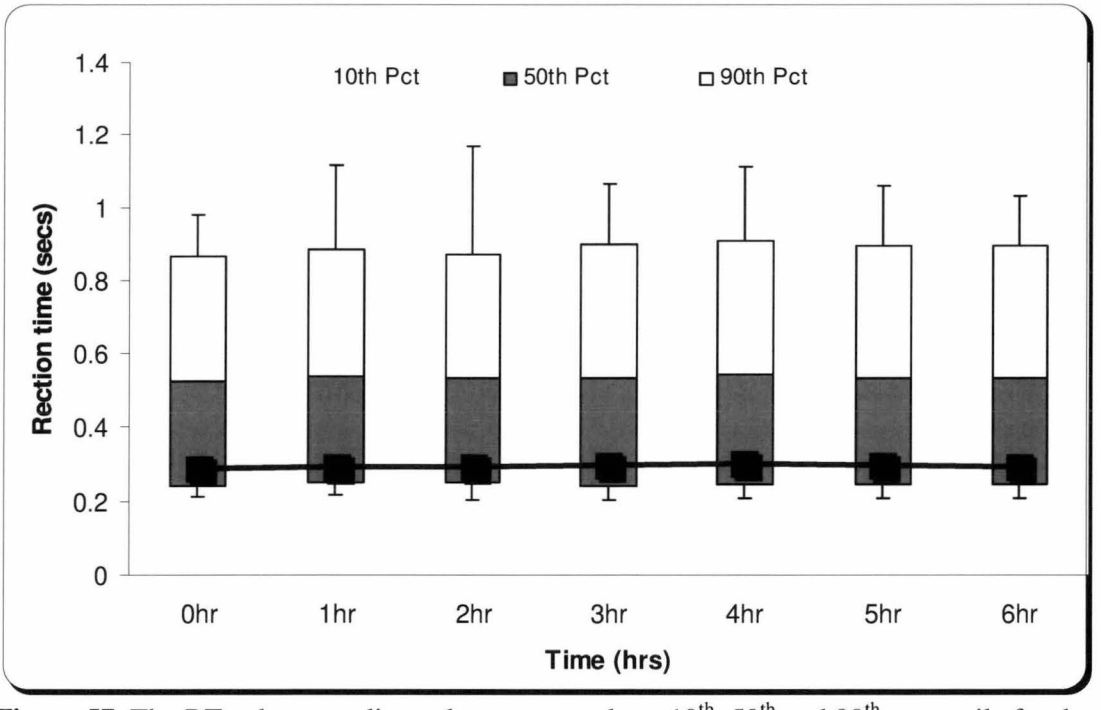

Figure 57. The RT values, median values, mean values, 10th, 50th and 90th percentile for the control group.

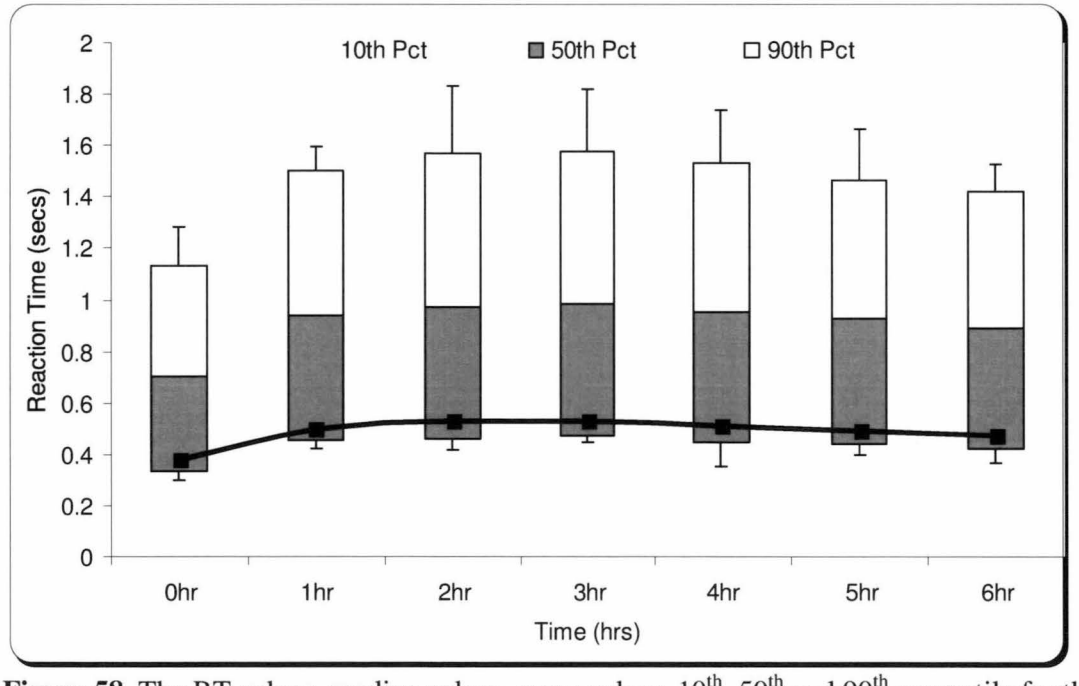

Figure 58. The RT values, median values, mean values, 10th, 50th and 90th percentile for the patient group.

Differences in all RT percentiles were found in the patients when compared before and after dose. The values recorded after the methadone administration was significantly higher than the RT obtained before the dose (Fig. 58). No significant difference was obtained between RT's measured after methadone administration.

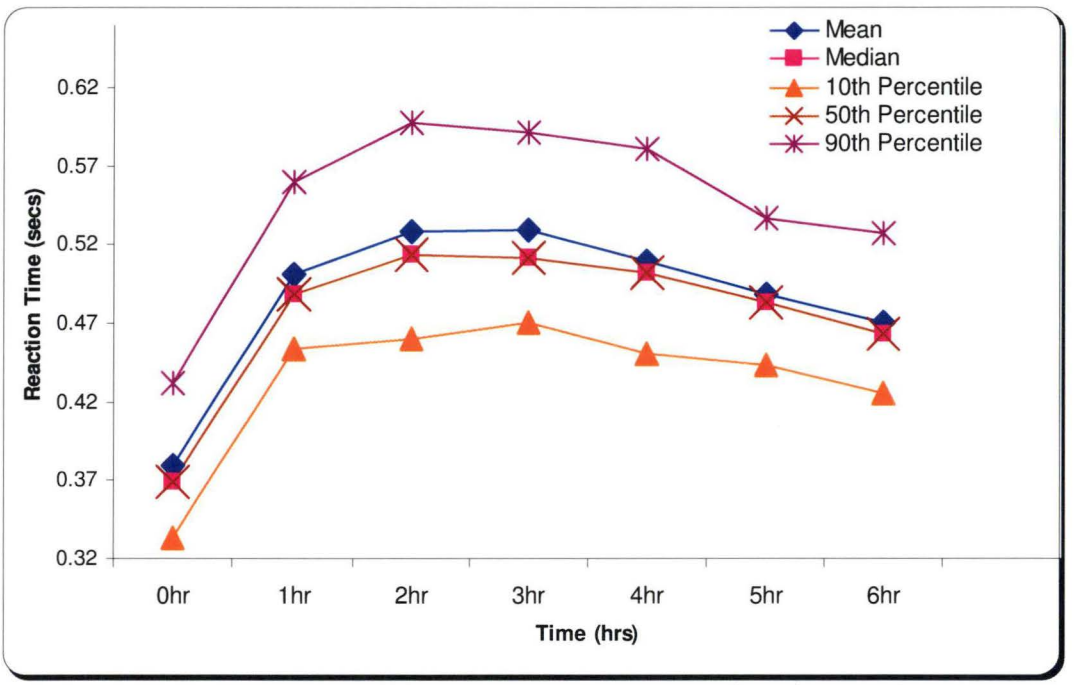

Figure 59. The RT mean values, median values, mean values, 10th, 50th and 90th percentile for the patient group.

Therefore further investigation was carried out to investigate statistical differences at different percentiles. The RT values at 10th, 50th, and 90th% percentiles, mean and median are summarised in the plot (Fig 59). The 90th% percentile showed statistically significantly slower values in the patient group ($p < 0.05$) after dose. In addition, a slowing RT trend can be observed until 2hr after which RT stabilises and then decreases, however still remains significantly slower ($p < 0.05$) than the RT obtained pre methadone administration.

9.3 Sex Differences in RT

Out of the 11 patients 3 were female. The sex differences on RT were demonstrated in this study. Figure 60 shows the plot of RT (Mean±SD). The plot demonstrates that males were slower than females specifically post methadone administration. However, pre methadone dose the response was opposite post dose.

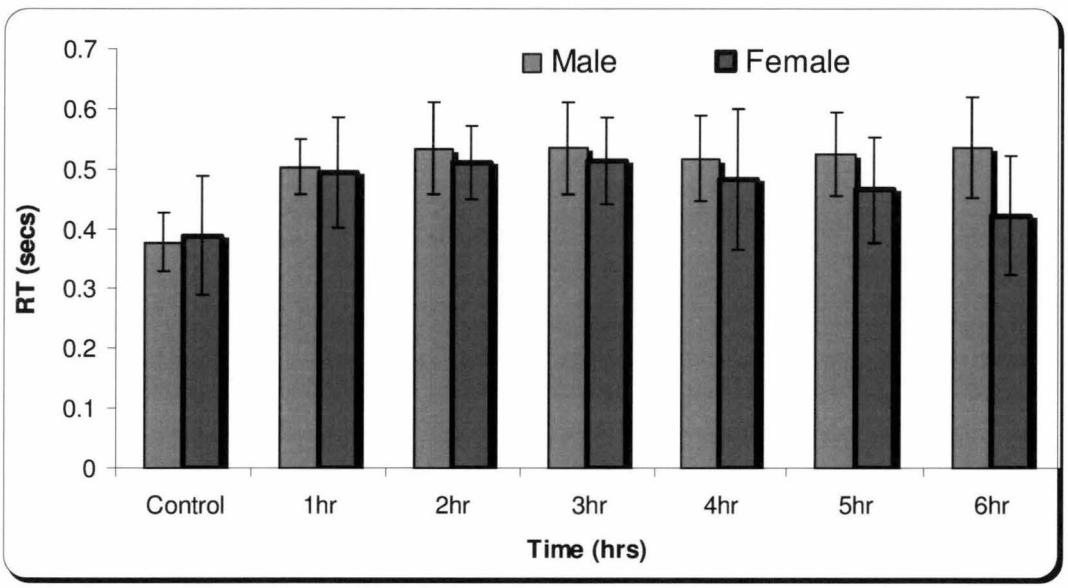

Figure 60. The mean RT values of male and female patients. The error bars represents the standard deviation.

However, these differences were not statistically significant. Pre-methadone dose RT for female was 2.9% higher than the males. The maximum percentage difference was obtained in the 6th hour at 23.6% and the minimum difference was obtained immediately after the dose intake at 1.82%. In both sexes, RT increased after the intake of methadone. Two-way Analysis of Variance was performed on the RT with test numbers after dose as in between factor and condition (not-dosed and dosed) as the within factor (Two way ANOVA, $p>0.05$). There was no significant main effect of group, indicating no difference in the RT between tests after methadone dose intake.

9.4 Age Differences in RT

When analyzing for correlation between RT and the age, the 90th percentile of the RT was used to plot against the age. Fig 9 shows the plot of age v/s 90th percentile RT. It was observed that the 90th percentile proved to be positively correlated to age: 90th percentile age (R = 0.49, *p=0.04*, Fig 61).

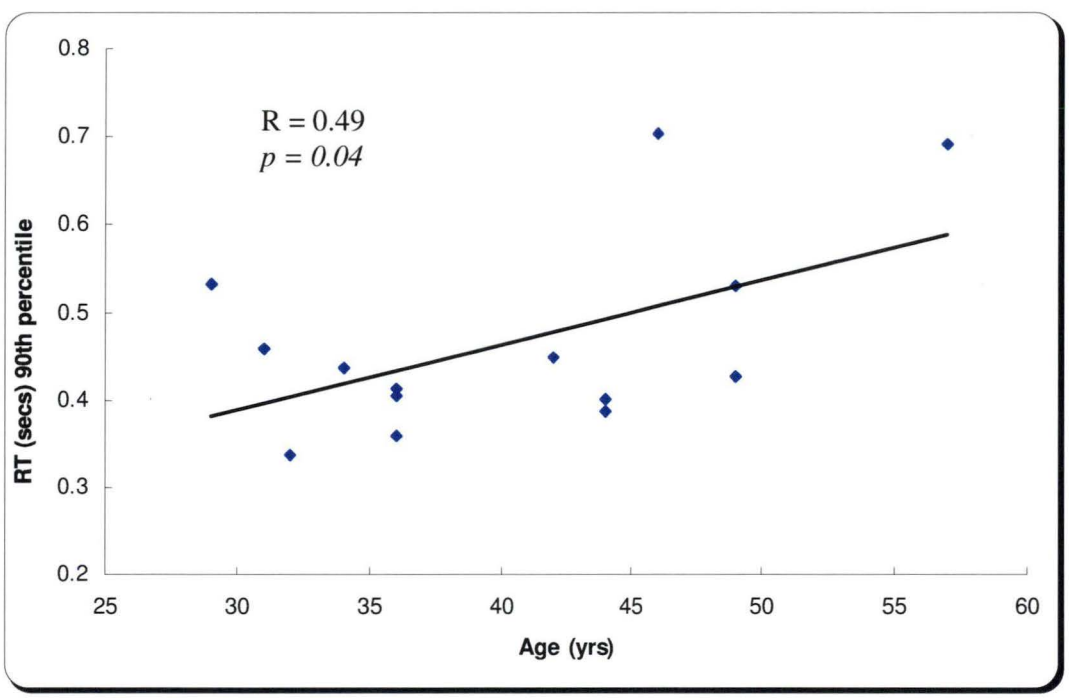

Figure 61. The 90 percentile RT values of male and female patients together plotted against age.

9.5 Discussion

The RT test used in the present study was validated using subjects from the school of engineering (Fig 54). A SVRTT was selected because it is considered to represent cognitive functioning of the most crucial to general adaptive and information processing aspects of human behaviour [120] at the same time its simple nature makes it a good test for testing its potential with MDP.

The results of the present study indicate that methadone treated subjects show evidence of impaired RT when compared their RT before methadone administration and also when compared to the control group. Methadone patients appear to have inferior RT (Table 11). It also appears

that the female methadone subjects have slightly faster RT than men post methadone, however similar results were not observed in the RT results obtained from pre-methadone dosing. This is unlike some past results that suggest otherwise. It could be speculated that the females in the patient group in general may consist of individuals who through some natural selection process, are more reactive than the male patients. It is also possible that prolonged use of narcotic drugs may have lead to increased levels of arousal in females. This could be further studied with equal number of male and female subject.

A curving trend of RT versus time was observed in the 90th percentile (Fig 58 and Fig 59). It might be speculated that the response can be due to the learning effect of RT. However, the effects of methadone will have a strong effect on the RT responses compared to learning tendency of patients. This can be further confirmed by some results in the previous studies. In addition, the learning effect in our study was not observed even with the control group, however further investigation needs to be carried out as the number of samples in the control group were less than the patient group. Previous studies have demonstrated that performance of the methadone patients could be ascribed to a desire to perform well on a test that patients might have presumed had important implications for the future of methadone treatment. They further suggested the need to investigate the nature of responses at different times after daily dose administration, while controlling for time of day effects. The results demonstrate systematically the effect of methadone dose over the RT of the patient throughout the methadone metabolism period.

The neuropsychological effect of long-term methadone administration patients is a matter of interest. While some studies suggest that opioids cause cognitive dysfunctioning, while others claim that opioids have beneficial effects on cognition. Responding to a stimulus involved activation of the receptor organ, afferent pathway and cerebral processing by cortical as well as sub cortical structures. The 90th, 50th, 10th percentile values and then mean and median values of RT found in this study indicate the level of cerebral activation at different times. Few studies have been performed investigating the cerebral effects of long term opioids administration. In those studies it was concluded that patients in pain may have arousal effect which might affect neuropsychological performance [144]. Other studies conducted SVRTT on methadone addicts and non addicts and concluded that methadone addicts have faster RT. [144] found decrease in euphoric behaviour when drug addicts after single dose methadone, methadone after long term

treatment were tested. Thus, the sparse literature concerning RT with methadone dependent patients (MDP) is conflicting.

The present study was performed in order to test SVRTT as a potential tool for assessment of methadone patients. The simple nature of the test and the simplicity of the testing procedure make SVRTT a good experimental assessment tool. The time dependent differences were greatest when considering the 90th percentile results (Fig 7). Similar trend was observed when the means, medians and the 10th and 50th percentile results were plotted against time. Furthermore, the SD remained similar for the patient group before and after dose and the control group. This result did not agree with the results obtained by [124, 127]. The reason behind this difference may be the unequal number of sample size in both groups and even smaller sample size in the control group.

It was also observed that stronger variation of correlation was observed inter-patient data compared to intra-patient data. As there is great overlapping between the results of the patient group it is impossible to setup meaningful conclusion on the variation. Though, from the available data it seems reasonable to consider that the SVRTT can be used as a potential assessment tool for MDP. Whether this test can be used with pain and other such patients using opioid as analgesics still remains a question, since they are quite different from long term methadone rehabilitation patients in terms of pain, psychosocial circumstances disease etc. Many aspects of the cerebral influence of long term opioid administration still remain to be studied.

10. Comparison of Results and Discussion

In this section IPL, COL, MPC and SVRTT results are compared. Possible correlations between techniques are investigated. The potential uses of such correlations in clinical applications are discussed. The discussion is merged with the results to make the section more presentable and meaningful.

In pupillometry results shown in the pervious sections, the pupil was significantly affected at all light intensities. However at 100lux, the level of significance was at its highest. The pupillary constriction was readily detected at 0lux intensity too. Having identified light level 100lux as optimal level for light reflex and 0lux as optimal level at no light, this section uses pupil characterization parameters obtained at these two light levels only. Peak pupillary constriction was achieved 2hrs after methadone administration which could to be related to peak MPC, also achieved at the same time. The peak pupillary constriction was significantly different ($p<.001$) to the pupil diameter pre-dose in IPL as well as COL measurements. All patients who participated in the study were found negative when test for urinalysis.

10.1 Comparison of IPL-COL Pupil Diameter Response

The percentage difference in the pupil diameter obtained from IPL and COL results from CG and PG is shown in Table 1. In general the average pupil size was smaller in IPL compared to COL in both CG and PG. This is expected as the visual light stimulus and the eye capture was on the same eye in the case of IPL. This is also the reason behind a higher SD in IPL compared to COL. The percentage difference between IPL and COL under all light levels was 0.4% (0.03mm) in the case of the CG. For the CG, the highest percentage difference (5.95%) was obtained at the 6th hour of testing, while the lowest percentage difference (1.27%) was obtained at one hour.. There were no significant differences observed in the CG between IPL and COL measurements of pupil diameter.

In the PG, the difference here was higher compared to the CG throughout the whole test (0-6hrs). The highest difference between IPL and COL was 10.02% which was obtained at the peak constriction of the pupil. The differences decreased as we move away for the peak constriction on either side. Therefore it can be assumed that the differences are due to the effect of methadone.

Table 12. Mean pupil diameters of CG and PG obtained from IPL and COL measurements at 0lux over the full period of the tests (0-6hrs)

	Control Group			Patient Group		
Hrs	Mean pupil diameter at 0lux		$\%diff = \frac{IPL-COL}{(IPL+COL)/2} \times 100$	Mean pupil diameter at 0lux		$\%diff = \frac{IPL-COL}{(IPL+COL)/2} \times 100$
	IPL	**COL**		**IPL**	**COL**	
0hr	7.70	7.46	3.16	7.07	7.39	4.43
1hr	7.79	7.89	1.27	5.26	5.8	9.76
2hr	7.34	7.63	3.87	4.55	5.03	10.02
3hr	7.59	7.81	2.85	4.67	5.15	9.78
4hr	7.90	7.63	3.47	5.58	6.05	8.08
5hr	7.74	7.41	4.35	5.69	6.20	8.58
6hr	7.33	7.78	5.95	6.39	6.43	0.62
Mean	7.62	7.65	0.40			

10.2 MPC

The mean MPC obtained at different hours for 6hrs is shown in Table 13. Figure 62 shows the MPC obtained from all patients as a function of time (0-6hrs). The MPC increased significantly *($p<0.001$)* when compared to measurements before dose and at the peak MPC (obtained 2hrs after methadone intake). It was observed that MPC increased almost linearly for the first two hours, before a decline was observed. The decline was slow and gradual indicating the detoxification phase. The MPC value at the end of 6hrs was 343$\pm$121 (Mean$\pm$SD).

Table 13. MPC of methadone patients over the period of the tests (0-6hrs)

Methadone Plasma Concentration (MPC)		
	Average	**SD**
Before Dose	242	93
1hr	376	114
2hr	410	119
3hr	400	119
4hr	392	117
5hr	379	127
6hr	343	121

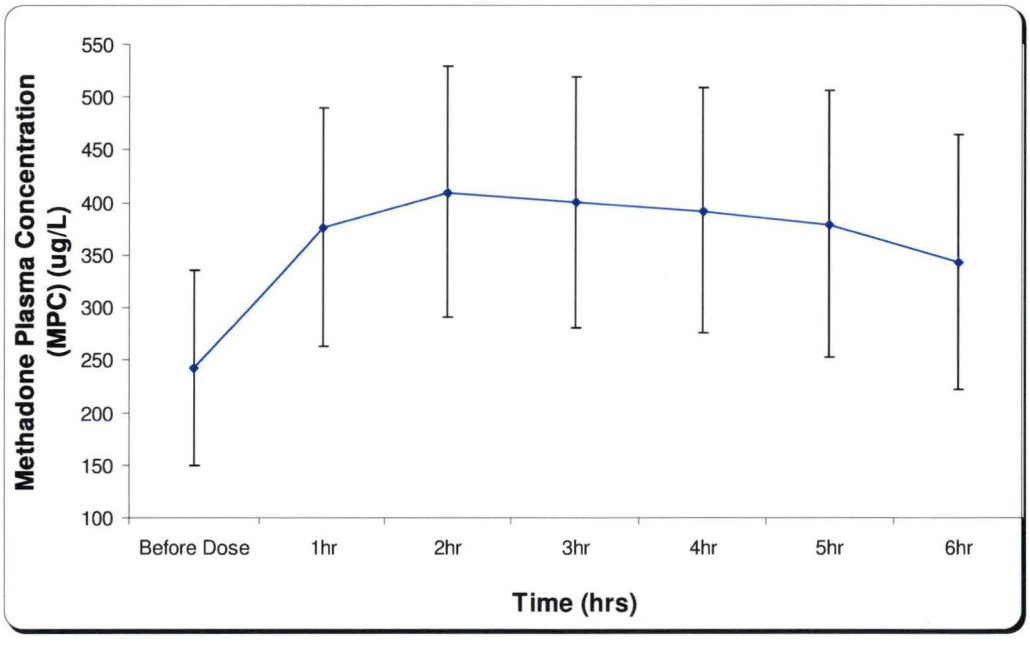

Figure 62. The change in MPC over the period of 6hrs after methadone administration by the patients. The error bars indicate the SD.

10.3 Pupil Diameter, MPC, 90th Percentile SVRTT v/s Time

Figure 63 shows a plot comparing all of the assessment techniques used in this study. The plot indicates that the trend of pupillary response for IPL and COL was similar. The two trends although significantly different *($p<0.01$)* there was a constant difference throughout the tests between the two measures. This constant difference only decreased at the 5hr, while this constant difference remained the same for other hourly interval measurements. This indicates that either of

the two tests (IPL or COL) can be used while the other can be predicted using a compensatory common factor. The MPC and SVRT trend showed strong similarity too. A visual comparison of IPL, COL, SVRT, and MPC indicates that in all measurements the maximal changes after methadone intake occurred at the same time (2hr). It was also observed that all the responses showed in Fig 63 showed signs of saturation at around 6hrs after the medication intake. Fig 63 encourages investigating the correlations between MPC and other assessment techniques.

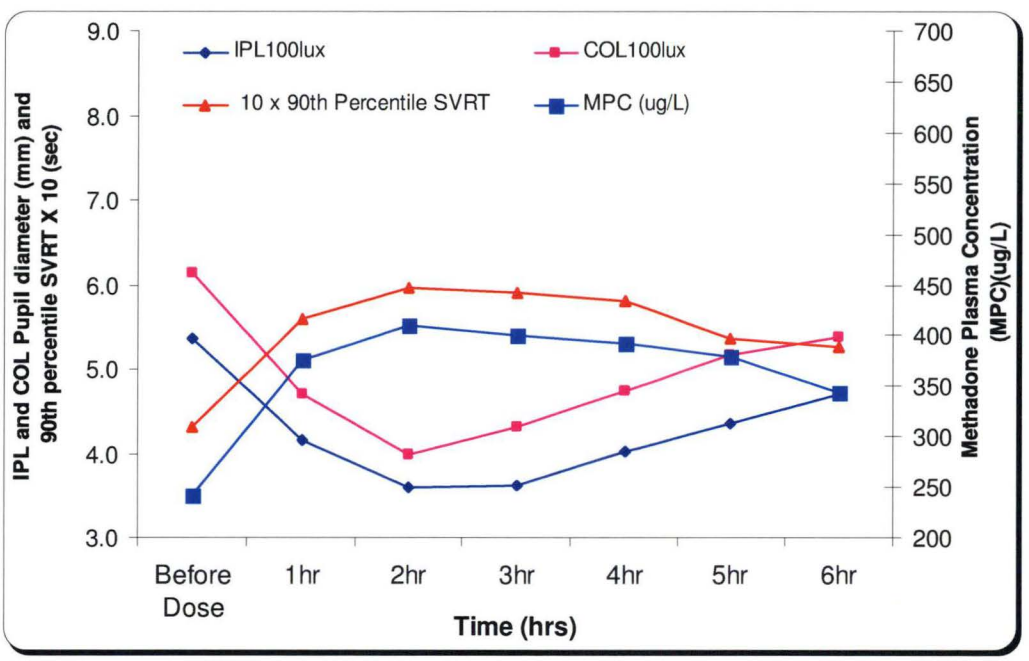

Figure 63. Plot of IPL, COL, MPC, and SVRT versus Time over the period of the whole test (0-6hrs).

Therefore Correlation analysis was performed to determine if SVRT changed as a function of MPC.

10.4 MPC versus SVRT at 90th Percentile

The correlation between MPC and SVRT at 90th percentile showed strong correlation (R^2 = 0.95) (Fig 64). A linear relationship obtained from the plot showed SVRT increases with the increase in the methadone concentration in the blood. It might be too early to make a claim that the sedation caused due to methadone is pretty evident from the result as the numbers of patients used in the test are small in numbers. However, the high correlation factor in Figure 64 indicates strongly the sedation effect of methadone on patients.

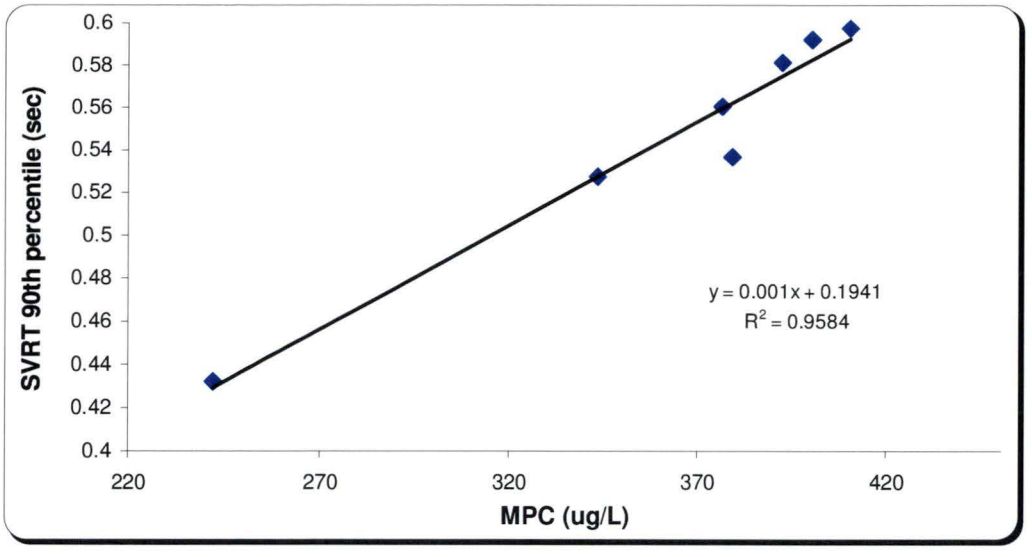

Figure 64. The plot represents the correlation between MPC and SVRT.

10.5 SVRT at 90th Percentile versus Pupil Diameter at 100lux

The relationship between pupil diameters at 100lux was compared with SVRT at 90th percentile (Fig 65). For this comparison, pupil diameter from IPL and COL were used and showed separately (Fig 65). In both comparisons high inverse correlation was obtained between pupil diameter and SVRT. In case of IPL the correlation was $R^2 = 0.91$, where as for COL the correlation was $R^2 = 0.94$.

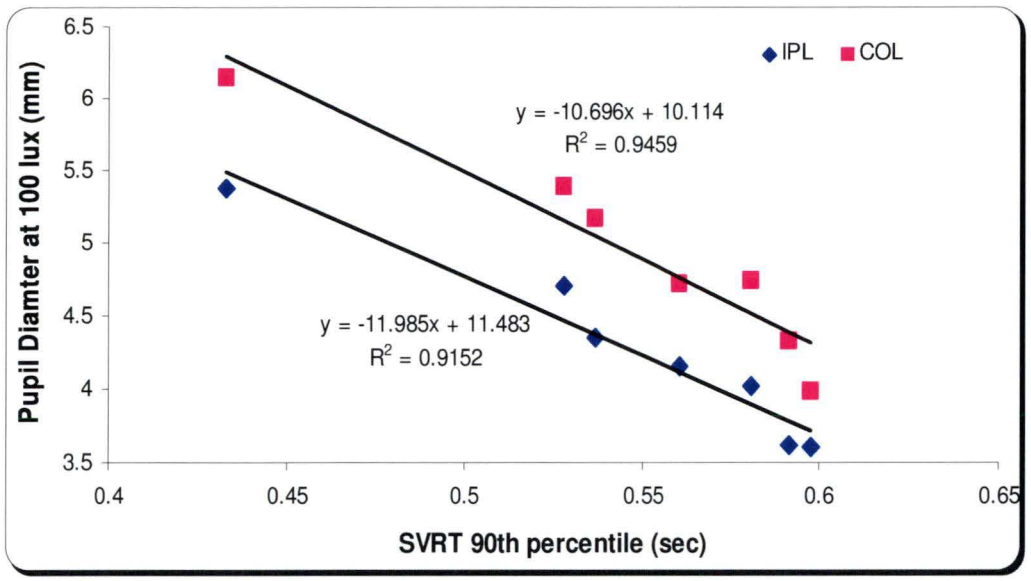

Figure 65. The plot represents the correlation between pupil diameter at 100lux (IPL and COL) against SVRT at 90th percentile.

The plot in Fig 65 again shows that IPL and COL maintain a constant difference. It is encouraging to see these results since clinicians can use either IPL or COL measurements and predict the other using a common constant.

10.6 Hysteresis

The peak reduction in pupil diameter, the peak obtained in MPC and SVRT occurred at the same time interval, at 2hrs after methadone admission. When the reduction in pupil size and the response of SVRT was plotted against MPC, a hysteresis curve was obtained (Fig 66). The pupil diameter is not affected by the methadone absorption and metabolism in the blood, but it is understood that the dilator muscle of the pupil is innervated by noradrenergic nerve fibers that use norepinephrine as a transmitter, whereas the constrictor muscle uses acetylcholine (Ach) as a neurotransmitter. Increased dosing results in a smaller pupil diameter, presumably related to methadone to diminish central noradrenergic activity [11]. This hysteresis response indicates and further confirms that the peak methadone concentration in the blood, the pupillary miosis and the SVRT reaches the peak at the same time.

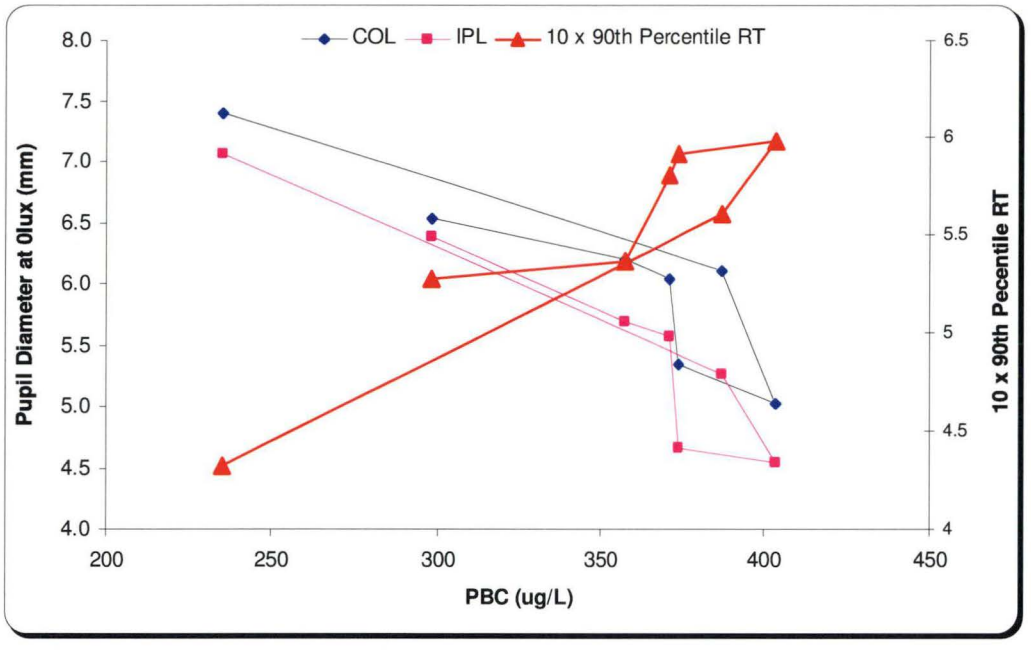

Figure 66. The plot represents the hysteresis response of pupil diameter at 0lux (IPL and COL) and SVRT at 90th percentile against MPC.

10.7 Pupil Characterization versus MPC and SVRT

The relationship between pupil characteristic parameters of IPL and COL at 100lux was compared with MPC and SVRT at 90th percentile (Table 14). High correlation was obtained between most of the parameters when correlated with MPC and SVRT, except for CV (IPL and COL) and relaxation SD (COL).

Table 14. Pupil Characterization versus MPC and SVRT

		MPC	SVRT (90th Percentile)
	CA	y = -301.68x + 707.57 R^2 = 0.8616	y = -0.304x + 0.8937 R^2 = 0.889
IPL at 100lux	**CV**	y = -237.01x + 603.96 R^2 = 0.4605	y = -0.246x + 0.7966 R^2 = 0.5042
	R-dA	y = -599.09x + 555.63 R^2= 0.9888	y = -0.5751x + 0.7315 R^2= 0.9261
	R-dV	y = -0.7513x + 0.6962 R^2 = 0.8504	y = -738.58x + 510.18 R^2 = 0.8086
	Relaxation SD	y = -362.11x + 603.15 R^2 = 0.9249	y = -0.3468x + 0.7766 R^2 = 0.8623
	0lux	y = -83.978x + 723.31 R^2 = 0.9197	y = -0.0846x + 0.9096 R^2 = 0.9489
COL at 100lux	**CA**	y = -0.1747x + 0.7513 R^2 = 0.8235	y = -168.18x + 560.14 R^2 = 0.7511
	CV	y = -388.57x + 642.89 R^2 = 0.5485	y = -0.3566x + 0.8034 R^2 = 0.4694
	R-dA	y = -486.27x + 480.13 R^2 = 0.9532	y = -0.4653x + 0.6586 R^2 = 0.8872
	R-dV	y = -766.9x + 508.69 R^2 = 0.9144	y = -0.7625x + 0.6914 R^2= 0.9188
	Relaxation SD	y = -460.07x + 661.93 R^2 = 0.5903	y = -0.4671x + 0.85 R^2 = 0.6184
	0lux	y = -75.235x + 734.09 R^2 = 0.8654	y = -0.0775x + 0.9289 R^2 = 0.9341

10.8 Arousal Index (AI)

Based on the results obtained from Table 14, the Arousal Index (AI) was calculated for every hour over the period of 0-6hrs. The parameters considered to calculate AI are

- Pupil diameter at 0lux – reflects the steady state pupil diameter which probably reflects the relative balance between sympathetic and parasympathetic activity on the pupil
- Pupil diameter at 100lux – represents the sensitivity of the pupillary constrictor circuits to light. Methadone reduces the sensitivity of pupillary response to light stimulation
- Relaxation SD at 100lux - Relaxation SD at 100lux represents the transients as the pupil settles into its new state, which represents the level of dampening of the reflex arc.
- Ratio of Change in Pupil diameter at 100lux to 1lux - this represents the degree to which the pupil can change its diameter. The smaller the diameter change the closer the pupil is to its level of maximal constriction

- Ratio of Absolute Pupil diameter at 100lux to 1lux – this represents the degree of absolute change in the pupil diameter. The smaller the absolute level, the closer the pupil is to its level of maximal constriction
- R-dA - represents the degree of initial correction of overshoot and hence it gets smaller as the degree of dampening by the methadone increases.

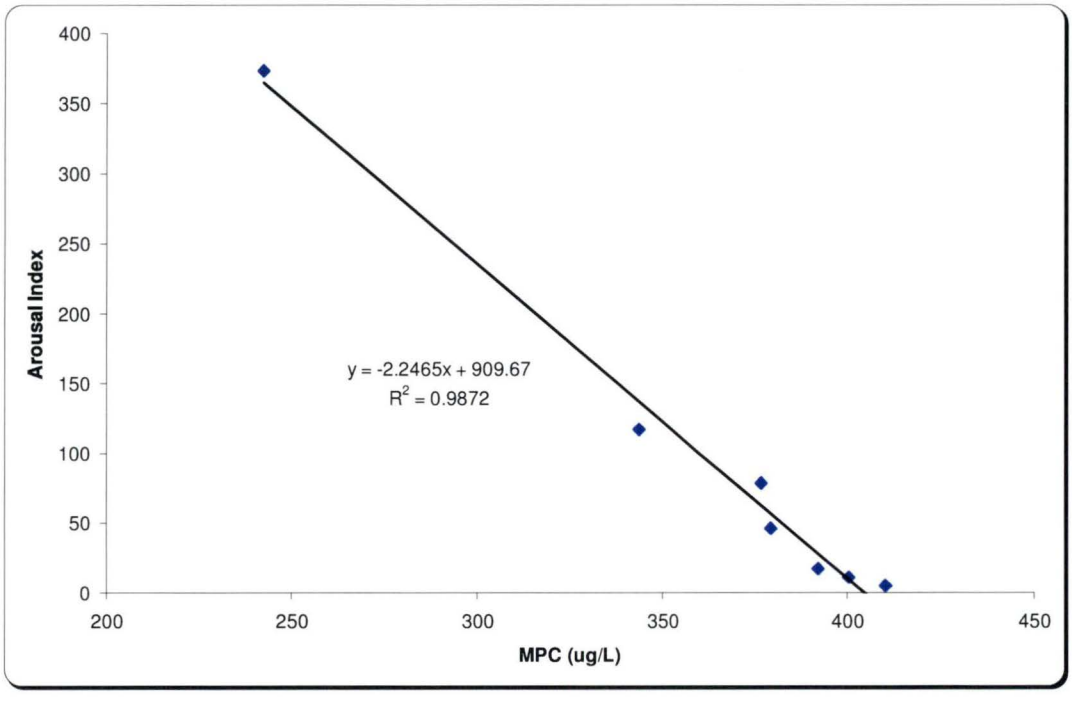

Figure 67. The plot represents the correlation between AI (IPL) versus MPC.

These 5 aspects of pupillary physiology found in the pupillogram that changes relative to MPC were multiplied to obtain an "arousal index". This is to see if a better predictor of MPC can be achieved from pupillometry parameters. When this is done and plotted against MPC with the averaged results it suggest there is a very high degree of correlation ($R^2 = 0.98$) (Fig 76) between this combined index (AI) and MPC. From the response obtained in the Figure 67 it can be predicted that the higher the methadone level the smaller the change in pupil size to a given level of light stimulation. This index also indicates that pupillary characterization parameter can be used as an indicator of methadone metabolism in clinical settings based on a pupillogram.

11. Conclusion, Discussion and Future Work

It was concluded that the pupillometry system introduced in the present study was reliable and reproducible for pupillary measurement. It was also demonstrated that the measurement of pupillary characteristic parameters can be used to assess MDP in addition to the pupil diameter. The details are summarized as follows.

- The pupil diameter results at 0lux showed that the pupil constricted significantly ($p < 0.001$) with time and MPC in both IPL and COL measurements. The maximum pupillary constriction was obtained at 2hrs after methadone administration. The pupil diameter obtained at 0lux matched and agreed with previous studies.
- The pupil diameter results at various light levels including 1lux, 10lux, and 100lux showed significant pupillary constriction. However, the maximum level of significance was obtained at 100lux. Therefore, it is concluded that the pupil constriction is better detected at 100lux.
- There was no obvious changes obtained in the results for CA, CV, Rd-A, Rd-V, and relaxation SD at 1lux and 10lux over the period of the test from 0-6hrs. Therefore it is concluded that those intensities are not suitable to measure pupil characterization parameters.
- The pupil CA results at 100lux obtained in PG and CG were significantly ($p < 0.01$) different. Significant differences were also obtained among methadone levels and time of measurement as well as significant methadone by time interaction.
- The pupil CV results at 100lux obtained in PG and CG were significantly ($p < 0.01$) different. Significant differences were also obtained among methadone levels and time of measurement as well as significant methadone by time interaction.
- The pupil R-dA results at 100lux obtained in PG and CG were significantly ($p < 0.01$) different. The pupil R-dA in patient group at 0hr was significantly ($p < 0.01$) different compared to R-DA measured at maximum constriction at 2hrs after methadone administration. The pupil R-dA trend obtained at 100lux over the period of the test (0-6hrs) was highly comparable to methadone metabolism in the blood which was indicated by MPC. In both trends the peak constriction (R-dA) and peak MPC was obtained at 2hrs after methadone administration.
- The pupil R-dV results at 100lux obtained in PG and CG were significantly ($p < 0.01$) different. The pupil R-dV in patient group at 0hr was significantly ($p < 0.01$) different compared to R-dA measured at maximum constriction at 2hrs after methadone administration. The pupil R-

dV trend obtained at 100lux over the period of the test (0-6hrs) was highly comparable to methadone metabolism in the blood which was indicated by MPC. In both trends the peak constriction (R-dV) and peak MPC was obtained at 2hrs after methadone administration.

- The pupil relaxation SD in patient group at 0hr was significantly ($p < 0.01$) different compared to R-dA measured at maximum constriction at 2hrs after methadone administration.
- It is concluded that accommodation response does not effect the pupil measurement significantly
- It is concluded that IPL and COL measurements can be used for pupillary measurements with MDP. However, it is recommended that while measuring pupillary reflex parameters COL measurements should be carried out so that the visual light falling on the eye does not affect the pupil image and in turn pupil detection and measurement.
- It is also concluded that the results obtained from IPL and COL measurements were significantly different; however the difference between the two was similar throughout the testing period (0-6hrs). Therefore, a factor that indicates the difference can be calculated and used to predict IPL and COL values if only one of the two measurement techniques are used for testing the patients.
- SVRTT showed significant ($p < 0.01$) difference between the PG and the CG.
- The trend obtained from SVRTT at 90th percentile over the period of the test (0-6hrs) was highly comparable to methadone metabolism in the blood which was indicated by MPC.
- It can be concluded that SVRTT measured in control settings can be used to predict the MPC values of patient's.
- The correlation between MPC and SVRT at 90th percentile showed strong correlation ($R^2 = 0.95$).
- The correlation between pupil diameter at 100lux and SVRT at 90th percentile showed strong correlation ($R^2 = 0.91$, IPL measurement) and ($R^2 = 0.94$, COL measurement).
- All pupil characterization parameters, except CV (both IPL and COL) and relaxation SD (both IPL and COL) showed high correlation with MPC and SVRTT.
- A high correlation ($R^2 = 0.98$) was obtained between AI and MPC indicating the combination of pupil characterization parameters can be used in addition to pupil diameter as a tool for assessment of MDP.

Discussion and Future Work

This section focuses on summarizing the results obtained in this study and describes their potential use in the area of assessment of MDP. Before beginning the summary, a brief discussion is provided on the reasons behind conducting this study.

The present study aimed to develop multifunctional goggles along with a pupillometry system and a novel SVRTT. The development involved systematic investigation of pupillometry and SVRTT techniques associated with MDP assessment and investigating their potential in clinical settings. An AI was calculated based on the parameters measured using pupillometry in this study.

From the values reported in the literature it is observed that the pupil measurements are usually performed under no light or varying light conditions, with the assumption of constant light levels. The other assumptions in the literature were that IR and visual light are safe without conducting proper safety procedures before doing pupillometry measurements. In addition, the importance of pupil size variation due to accommodation is known, with control subjects. COL response of the eye has also shown mixed results. The changes in the pupillometry due to the variation in parameters mentioned above can play an important role in understanding assessment of MDP. Furthermore, pupil's CA, CV, R-dA, Rd-V, Relaxation SD are parameters that are not widely investigated and documented yet in assessment of ODP, however their importance is well documented in other areas of pupillometry measurements. SVRTT has also been used in the past; however mixed results have been obtained regarding its usability.

This study provided an investigation of pupil measurements of methadone dependent patients, while also studying the effects of different light levels, accommodation, CA, CV, R-dA, Rd-V, Relaxation SD, eye safety and COL measurements. The SVRTT technique was developed and tested. MPC was also measured and tested and results were compared with the pupillometry and SVRTT techniques. An AI was calculated using the parameters found from pupillometry and SVRTT and a relationship between AI and MPC was formed.

In the current study multifunctional goggles was successfully developed and used with MDP. The goggles development was based on two considerations (1) the eye safety and (2) the automation, so that the patients use the goggles with ease. Eye safety procedures were followed using the correct standards and goggles were used only when they were termed safe. The goggles used were automated using a microcontroller. The microcontroller synchronized the visual LED

intensities with the image capturing, while keeping the IR LED illumination at a constant level. Images were captured at 10f/s. 2996 images were captured in IPL and COL measurements combined in one test. For one patient the total number of images captured throughout the test (0-6hrs) was 20,972. This shows the discrete nature of pupillary information captured in this study.

The goggles were less expensive in comparison to the commercial pupillometers available in the market. The economic analysis was not conducted in this study sine the focus was on the results obtained and assessing the potential of the techniques in clinical settings. However, the material used for the development of the goggles itself was simple and an easy assumption of the goggles being cheap can be made. Successful use of goggles in HTC confirms the usefulness of the pupillometry system in clinical settings. This pupillometry system can also facilitate in producing simple, but informative pupillogram. Physicians are known to make decisions about patient's physiology looking at their physiology pattern over time. Pupillograms obtained from this Pupillometry system has the potential to offer large information on a single plot. One example can be the AI found in this study.

IPL and COL measurements showed significant differences in pupil diameter results and some pupil characterization parameters. However, it is interesting to notice that the difference between IPL and COL measurements were nearly constant indicating that one test can be used to predict the outcome of the other. Although in this study the focus was limited to measuring the parameters and comparing on a superficial basis, but a in-depth comparison using more patients can be useful in supporting the claim made.

It was observed that accommodation response can ignored while testing MDP. Either the effect is too small or negligible. Most of the pupil characteristic parameters showed high degree of correlation with MPC and SVRTT. It shows that pupil diameter along with these parameters can be used as the indicator of methadone intoxication. Measuring absolute pupil diameter is still a matter of investigation, since pupil is not a perfect circle, pupil characterization parameters can be used as an alternative. The parameters are either ratio (velocity) or difference (amplitude). So a relative change can be potentially used based on the parameters obtained. SVRTT was successfully developed, tested and results show its usefulness in the area of assessment of MDP. Since many factors affect RTT, it is suggested that it should be further tested highly controlled settings.

This study was focussed on one hour before methadone administration and 6hrs after. Figure 68 is a predictive plot based on the data achieved from this study. This plot uses data from Figure 67, and the 24th hour AI value was the same as the 0th hour.

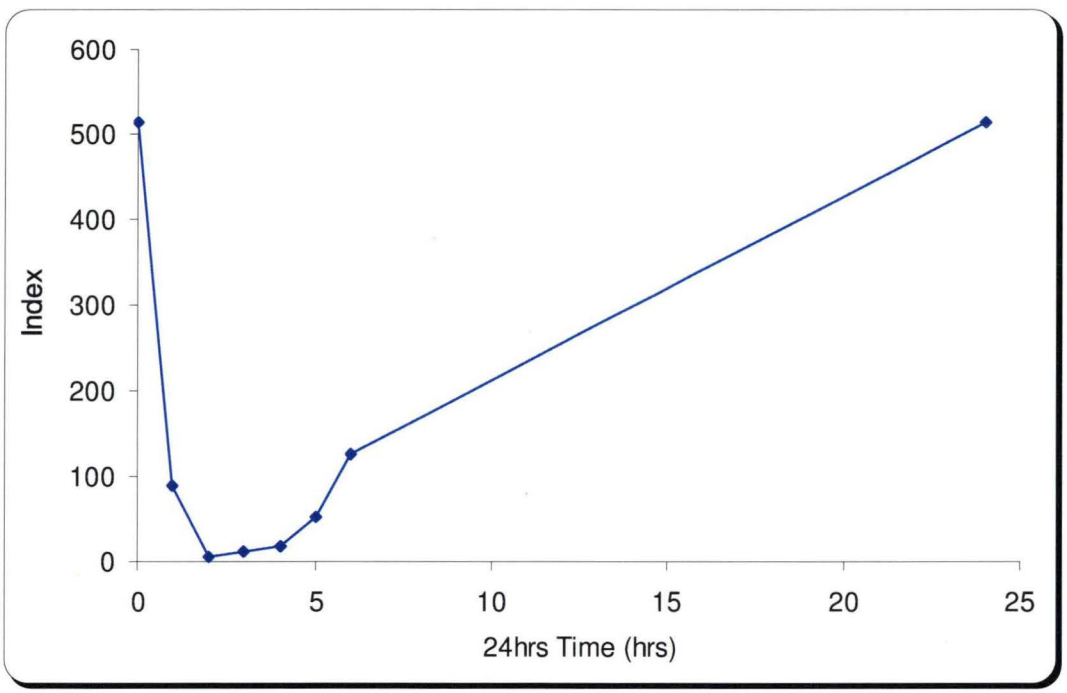

Figure 68. The plot indicates and predictive model of the AI versus a 24 hour period. The value of AI at the 24th hour is the same as the 0th hour.

Usually MDP are on daily dose. Depending on the amount of dose and their tolerance they feel the effect of the does. When the effect disappears patients suffer withdrawal symptoms. Figure 67 adds in the rest of the 18 hours along with this study and plots a graph of index versus time. It would be interesting if studies are conducted in the past to test the patients during those 18hrs. This can show the potential of pupil characteristic parameters is assessing MDP during pre and post withdrawal phase. At the same time a dose dependent test can be carried out. A combination of all the data achieved can be used to identifying patient's status after dose till the next dose.

The assessment techniques used in this study should also be investigated for its potential as a remote assessment tool. The simple nature of SVRTT used in this study makes it a good potential to be used as a remote assessment technique for MDP. MDP patients are sometimes on take away doses. Therefore, assessing them after every does becomes difficult.

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