THE FORMULATION OF STABLE ADRENALINE EYE DROPS FOR USE IN THE MANAGEMENT OF GLAUCOMA

BY

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"A Thesis submitted in part fulfilment for the Degree of Master of Science (Pharmacy), University of Nairobi."

SEPTEMBER 1981

DECLARATION

"This thesis is my original work and has not been presented for a degree in any other University".

SIGNED .....

G.O. KOKWARO

"This thesis has been submitted for examination with our approval as University Supervisors".

SIGNED..... and .....

DR. W.M. WATKINS DR. D. SIXSMITH

A C K N O W L E D G E M E N T S

I wish to thank all those individuals who have helped me during the course of this study.

My sincere thanks go to Professor G.D.H. Leach of the University of Bradford in securing some of the Published material relevant to my project.

I also wish to thank Drs. V. Klauss and H. Adala and the Post graduate students in the Department of Ophthalmology, Kenyatta National Hospital for their assistance with the studies on glaucoma patients. Their enthusiasm was always a source of encouragement.

I am above all indebted to my Supervisors, Dr. W.M. Watkins and Dr. Sixsmith for their constructive criticism which I found most invaluable.

I would also like to take this opportunity to thank Mrs. Chaggar for working hard to ensure that the typing was completed on time.

Finally, my gratitude goes to the University of Nairobi and the Government of the Federal Republic of Germany, through DAAD, for the financial assistance to enable me to carry out the present research.

C O N T E N T S

L I S T O F T A B L E S

KEY: F = Ferrous Sulphite method.

T = Thiosemicarbazide method.

P-C-B = Phosphate-Citrate-Borate Buffer

IOP = Intraocular Pressure.

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CHAPTER 1

INTRODUCTION

At the present time most of the routinely prescribed Ophthalmic Medications are prepared by Pharmaceutical Manufacturers. Stability, uniformity, and sterility characterise these products. Nonetheless, the prohibitive cost of obtaining such commercial products for a large hospital sometimes necessitates the production of some of these products within the hospital itself. Then the stability, uniformity and sterility of such products must be closely monitored to ensure that the patients derives the same therapeutic benefits as would be obtained by using a similar commercial product.

The eye is a very sensitive organ to physical and chemical injury and it is a well known fact that ocular drug bioavailability from traditional Ophthalmic drug delivery systems is generally poor (CONRAD, et al, 1978). This is due, in part, to the tremendous Physiological constraints imposed by the eye through such factors as fluid turnover (HAVERER, 1974; CHRAI, et al, 1974; PATTON and ROBINSON, 1975, 1976) instilled fluid drainage (CHRAI et al, 1973), drug-protein interactions (MIKKELSON et al, 1973), the relative impermeability of the cornea (ASSEF, et al, 1973; CHRAI and ROBINSON, 1974; SIEG and ROBINSON, 1976, and MAKOID et al, 1976), the substential surface area of the conjunctiva as compared to the cornea (MISHIMA et al, 1966), aqueous humor turnover (CONRAD and ROBINSON, 1977), and lacrimation.

Equally important is the type of vehicle employed, and associated with this is the question of patient comfort to ocular products, which in turn is again related to lacrimation

Thus, apart from the wide spectrum of ocular tissue reaction to the physical and chemical nature of the ophthalmic medication, the question of the stability of the drug itself must also be considered.

1.1 THE USES OF ADRENALINE IN OPHTHALMOLOGY

Adrenaline (Epinephrine) is a catecholamine having both alpha (α) and beta (β) adrenergic agonist properties. The α-receptors are excitatory and mediate the vasoconstrictor effects of adrenaline, while the β-receptors are inhibitory and mediate the vasodilator effects.

In Ophthalmology, adrenaline is useful because of its vasoconstrictor and ocular hypotensive effects, hence its use in three main conditions, namely:-

(a) To decrease conjunctival congestion

(b) To control heamorrhage

(c) To decrease intraocular pressure (10 P) in simple glaucoma.

To decrease conjunctival congestion preparation such as 1% and 2% Epinephrine solutions USP are used. For the control of heamorrhage weaker solution such as 0.002% Epinephrine solution USP (freshly prepared from the official 0.01% Epinephrine solution USP) are employed. The use of Adrenaline to decrease raised intraocular pressure in simple glaucoma will be discussed in this Section.

The intraocular pressue of the non-glaucomatous population approximates a normal (Gaussian) distribution and may be described in statistical terms (KOLKER and HETHERINGTON, 1976). By Applanation Tonometry (the principles of which are explained

in the experimental section), mean values of 15.4 (± 2.5) MM Hg (sitting) and 16.5 (± 2.6) MM Hg (reclining) were obtained by the above mentioned workers. These values are only approximate, and the actual frequency distribution of intraocular pressures in the population is skewed towards the higher levels. This skewness is the result of several statistically different subpopulations (glaucoma relatives, people of different age groups, etc) that comprise the general population.

KOLKER and HETHERINGTON (1976) also reported that Mean intraocular pressure increases with age and is slightly higher in women than in men over the age of 40. They also reported that pressures measured in the morning are usually higher than those measured in the afternoon.

Glaucoma may be defined for the individual eye as that intraoculer pressure which produces damage to the optic nerve (PORTNEY, 1977, KOLKER and HETHERINGTON, 1976). The latter two workers also found that, statistically, an intraocular pressure over 21 MM Hg (mean ± 2) should occur in less than 2.5% of the population, and a pressure over 24 MM Hg in less than 0.15% of the normal population.

Glaucoma can be divided into three major categories (PORTNEY, 1977).

(a) Open-angle glaucoma.

(b) Angle-closure glaucoma

(c) Congenital glaucoma

The flow of aqueous humor (or 'aqueous') in the normal eye is depicted in diagram A.I.I. (Appendix I, page 162.). Aqueous is secreted by the epitheliel cells of the ciliary process into the posterior chamber. It then passes through the pupil into the anterior chamber and leaves through the trabecular meshwork into the schlemm's canal and the venous system.

Primary open-angle glaucoma (chronic simple glaucoma) usually results from an interferance with the outflow of aqueous due primarily to a degenerative change in the trabecular meshwork and the inner wall of the Schlemm's canal resulting in decreased trabecular porosity. It is called open-angle glaucoma because physical obstruction to the flow at the angle formed by the cornea and the iris is not evident on examination and peripheral anterior synechiae (iris adhesions') to the lens structures are absent or insignificant in extent. A schematic depiction of aqueous dynamics in open-angle glaucoma is given in Fig. A.1.2 (page 163.)

Angle closure glaucoma comprises those that have an actual physically identifiable block posterior or adjacent to the innermost trabeacular sheet that prevents the egress of aqueous. The aqueous dynamics in angle-closure glaucoma is given in Fig. A.1.3 (page 164.)

In the human eye, α-adrenergic stimulation results in

decreased resistance to aqueous out flow, while β -adrenergic stimulation results in a decreased rate of aqueous formation. Thus adrenaline, having both α- and β - adrenergic agonistic effects reduces the intraocular pressure by both mechanisms. This has been reported by several workers. (SEARS and BA'RANY, 1960, BECKER et al, 1961; EAKINS, 1963; LANGHAM, 1965; KRONFELD, 1967; ROSS and DRANCE, 1970; MARCI, 1972; ROTH, 1973, and GAASTERLAND et al; 1973).

The improvement of the facility of aqueous out flow is not an immediate effect, but is observed after several months of treatment with adrenaline (BECKER et al; 1961). This action of adrenaline appears to result from its action on the alpha-adrenoceptors located in the region of the trabecular meshwork (SEARS and SHERK, 1964). The occupation of beta-adrenoceptors in the region of the ciliary body results in a decrease in aqeuos secretion. HARIS et al (1971) believe that topically applied adrenaline has primarily a beta effect at low concentrations (0.06% - 0.5%), whereas at higher concentrations (1.0% -2%) alpha effects are predominant.

When adrenaline is instilled into the eye a brief mydriasis and vaso constriction is produced followed by a more prolonged lowering of the intraocular pressure lasting 24 - 36 hours. Due to this mydriatic effect, adrenaline is contraindicted in Patients with angle-closure glaucoma (YANCHICK, 1978). This can be explained as follows:

Adrenaline causes pupillary dilatation leading to folding of the iris against the trabecular meshwork. This isolates the meshwork from the anterior chamber, thereby preventing normal removal of aqueous.

The use of adrenaline in the treatment of glaucoma is not new but has only been widespread in the last 20 years (JONES, 1977). Earlier uses had been reported (GREEN, 1934; POST, 1934; HOWELL, 1934, 1936). Depending on the severity of the glaucoma, several strengths of adrenaline eye drops have been suggested for the management of open angle glaucoma (PORTENEY, 1977). Because of the wide dose range possible, several formulations of adrenaline eye drops have been suggested, some of which are not official preparations. The USP mentions Adrenaline (Epinephrine) Ophthalmic solution, but is not specific on strengths or how it is to be prepared (USP XIX, 1975 (a)). Martindale lists several ophthalmic preprations containing adrenaline, but these are not official preparations (Martindale 1977 (a)).

Thus one often finds that preparations for adrenaline eye drops are not based upon one or even a few universally accepted formulae, but can be varied almost at will. This point will be highlighted further in the section dealing with literature survey.

2 THE STABILITY OF ADRENALINE IN AQUEOUS SOLUTION

The stability of adrenaline in solution is an important

factor which must be taken into account whenever the formulation of adrenaline eye drops is considered. Some of the factors affecting the stability of adrenaline in solution will now be discussed.

1.2.1 THE EFFECT OF MOLECULAR OXYGEN

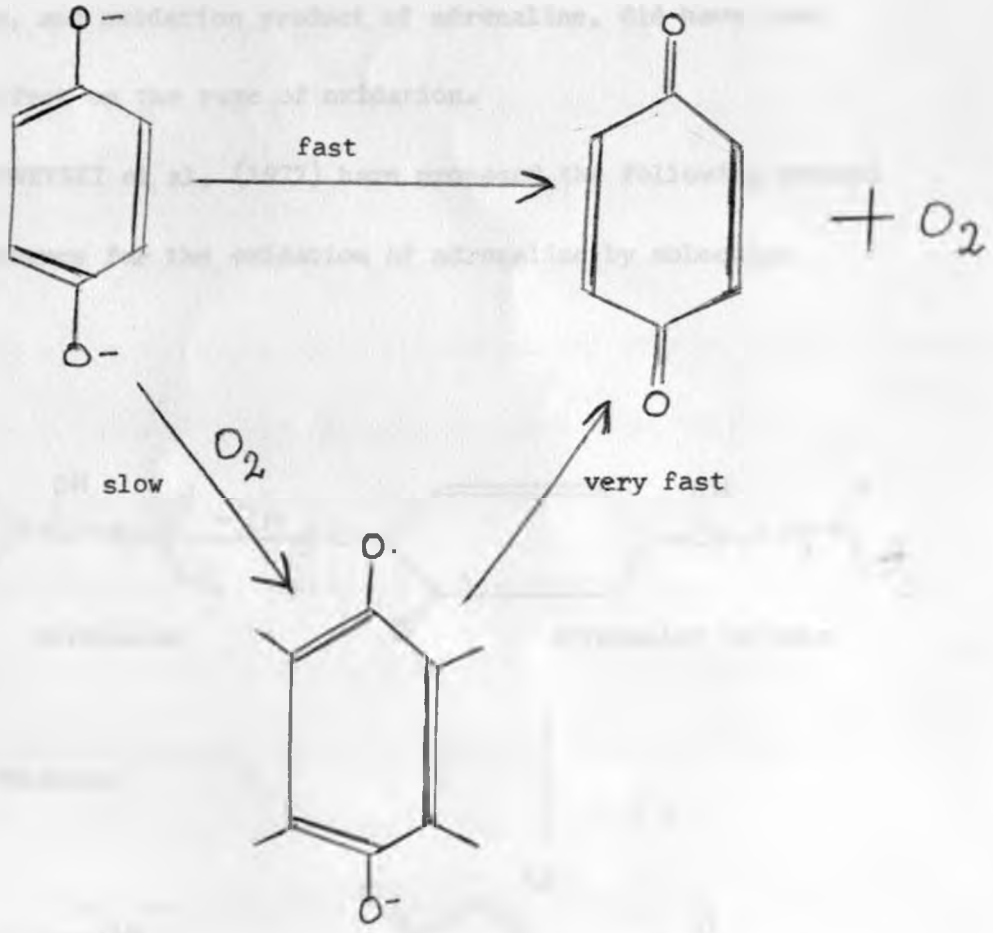
The mechanisms and Kinetics of the reaction between molecular oxygen and adrenaline have provided a challenge to pharmaceutical workers ever since the isolation of the hormone in 1901 by TAKAMINE (SOKOLOSKI and HIGUCHI, 1962). This has been so because of lack of a suitable analytical procedure for the intact catecholamine, and the complexity of the oxidative processes involved.

The first investigation on the rate dependency of oxidation on the concentration of oxygen and adrenaline in solution was carried out by SOKOLOSKI and HIGUCHI (1962). They found that the dependence of the reaction rate with respect to the drug concentration varied with the oxygen tension present, and ranged from a simple proportionality at low oxygen tensions to a half-order at higher oxygen-tensions.

They further showed that the degradation proceeds with no apparent lag phase. This would suggest that free radical participation in the process was unlikely because, as they pointed out, reactions which follow a free radical path are often characterized by an initial lag time during which intermediates accumulate. However,

the appearance of fractional orders strongly suggested that the degradation might be free radical mediated. This would be in agreement with similar findings by JAMES and WEISBERGER (1938) who had proposed the following autoxidation of durohydroquinone (2,3,5,6-tetramethy (hydroquinone).

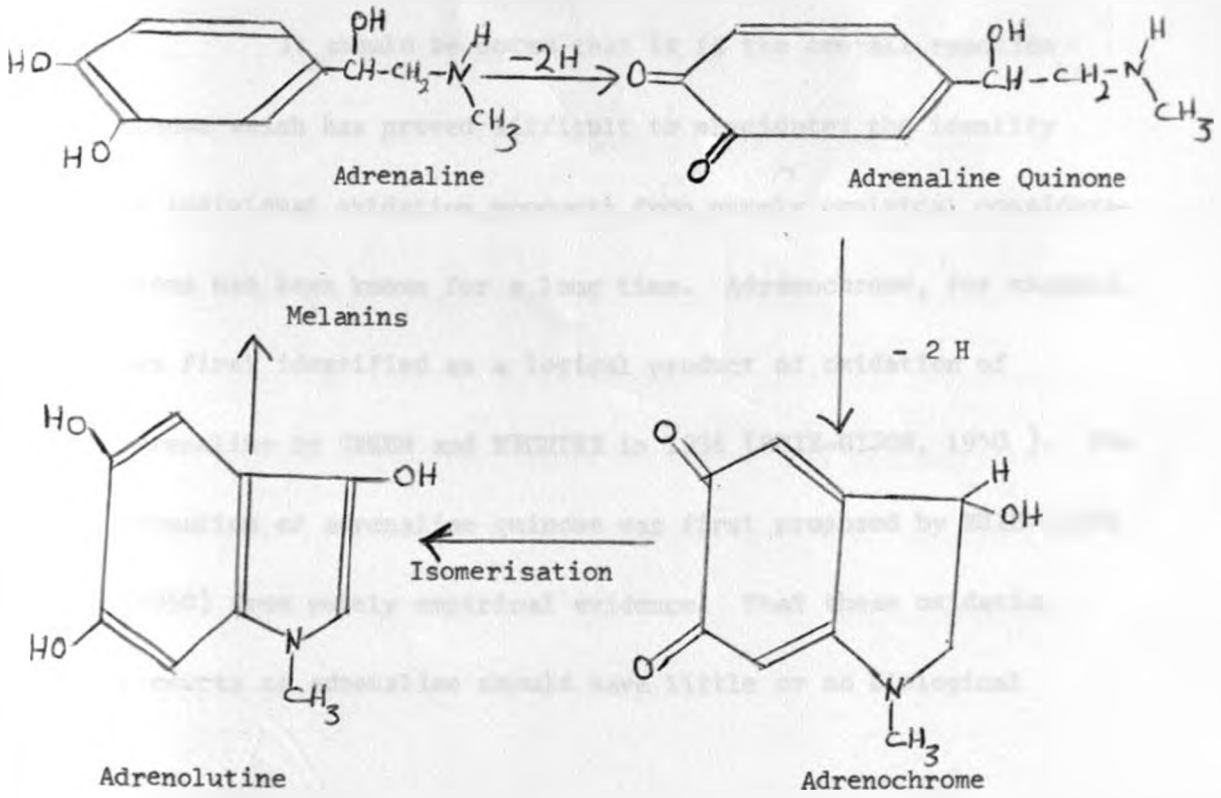
FIG. I



Such a reaction scheme would rationalise the one-half ($\frac{1}{2}$) power dependency of adrenaline at higher concentrations but, in turn, requires some sort of lag phase for the build up of the quinoid species and a catalytic activity on the part of the oxidative products of the catecholamine. SOKOLOSKI and HIGUCHI (1962) did not find any evidence of a lag phase nor data to suggest that the reaction was autocatalytic. It is worth noting, however, that in an earlier work, TRAUTNER and BRADLEY, (1951) had reported that adrenochrome, and oxidation product of adrenaline, did have some catalytic effect on the rate of oxidation.

BONEVSKI et al, (1977) have proposed the following overall reaction sequence for the oxidation of adrenaline by molecular oxygen.

FIG 2.



The above reaction scheme (Fig. 2) has also been proposed by MARTIN (1969) who demonstrated that the formation of adrenolutin was the intermediate stage preceeding the formation of melanins.

HARRISON et al., (1968) have demonstrated the formation of an aminochrome (adrenochrome) by performing experiments using ring-labelled adrenaline. They found that oxidation of adrenaline at pH 7.0 involved the reduction of 4 equivalents of ferricyanide (transfer of 4 electrons) and the release of 5 protons. The stochiometry indicated that the release of 4H+ equivalents resulted from the oxidation; the additional H+ equivalent release reflects the protonation of the side-chain amino N-atom which most probably is converted in the cyclization step into an imino nitrogen, which has negligible affinity for protons at pH 7.0.

It should be noted that it is the overall reaction scheme which has proved difficult to elucidate; the identity of individual oxidation products from purely empirical considerations has been known for a long time. Adrenochrome, for example, was first identified as a logical product of oxidation of adrenaline by GREEN and RICHTER in 1936 (RUIZ-GIJON, 1950). The formation of adrenaline quinone was first proposed by RUIZ-GIJON (1950) from purely empirical evidence. That these oxidation products of adrenaline should have little or no biological

activity similar to adrenaline should be clear when it is recalled that the activity of adrenaline can be explained by a three-point attachment at the receptor, requiring the presence of the phenolic hydroxy, alcoholic and amino groups for binding (GEARIN, 1975)

1.2.2 THE EFFECT OF pH

A consideration of pH of ophthalmic formulations lays emphasis on two aspects: (i) the effect of pH on the bioavailability of the drug and, (ii) the effect of pH on the stability of the drug.

Alkaloids and other weak bases are much more stable at pH 5 than at pH 7. This is related to the proportion of the drug that exists in the less stable indissociated form at a given pH. With decreasing pH, the dissociation of the drug increases, and therefore stability increases (ELLIS, 1977).

Adrenaline is a weak base with three PKa values 8.7, 10.2, and 12, at 20° C (Martindale 1977 (b)), The earliest recorded work on the correlation between pH and the physiological activity of adrenaline is that of WEST (1950), who showed that the pH of maximum stability (corresponding to greatest physiological activity) was at pH 3.6. Above and below this pH value, he demonstrated a decrease in physiological activity. However, the pH range used by WEST was very limited and did not include pH values above 4.2.

graphical scheme ?

Degradation of adrenaline below pH3 appears to be predominantly via racemization, as has been reported by several workers (BERY and WEST, 1944; HELLBERG, 1955; and SCHROETER and HIGUCHI, 1958). KISBYE and SCHOU (1951) showed that racemization of adrenaline ~~of xxxxxxxxxx~~ is a monomolecular process, whose velocity constant increases rapidly when the pH drops below 2.

In the formulation of ophthalmic drugs, stability is not the only factor when the effect of pH is considered. There is also the question of bioavailability of the drug. According to the pH partition hypothesis, more drug molecules will pass across a lipid barrier at pH values which result in a greater proportion of the drug being in the non-ionised form. For adrenaline, this means alkaline pH values.

However, bioavailability is also influenced by the effect of pH on the degree of corneal epithelial permeability to drug molecules (CONRAD et al, 1978). They concluded that the greater bioavailability of basic drugs at higher pH values is also due to the cornea. Their explanation is supported by scanning electron microscopy which shows substantial changes in surface layer cells of the cornea when exposed to mildly alkaline solutions (MATSUDA and SMELSER, 1973; PFISTER and BURSTEIN, 1976).

But it should also be noted that the eye, like most body tissues, is more comfortable with an acid than an alkaline pH (~~CONARD~~ CONRAD,

1973). Thus in considering pH of an ophthalmic formulation, the stability of the drug, the comfort of the patient and the bio-availability of the drug are important considerations.

1.2.3 THE EFFECT OF HEAVY METALS

Heavy metal ions, particularly copper, manganese, iron and nickel have been known to initiate oxidation of adrenaline in solution, possibly by forming autoxidisable adrenaline-metal chelates (BOUVET, 1949; TIMBERLAKE, 1957; NASANEM, 1958). RIEGELMAN and FISCHER (1959), however, have suggested that heavy metal ions increase the rate of oxidation of adrenaline in solution indirectly by increasing the rate of oxidation of the sulphite antioxidants present.

Thus steps are always taken to minimise the amount of heavy metal ions in solutions of adrenaline. These include the use of distilled water for formulation and the inclusion of a chelating agent.

1.2.4 THE EFFECT OF LIGHT

It is not exactly known how light accelerates the degradation of adrenaline in solution. For aqueous solutions containing sulphite and exposed the light RIEGELMAN and FISCHER (1962) have proposed an initial oxidation step of the sulphite ions to sulphate ions. At a critical sulphite concentration adrenochrome starts to form and the residual sulphite ions react with adrenaline

to give a colourless sulphonate until all the sulphite is consumed. This is followed by deterioration of the solution with visible colour change.

This may be just one of the several possible explanations of the effect of light on solutions of adrenaline, since it does not explain the deterioration of such solutions exposed to light, but containing no sulphite ions.

1.2.5. THE EFFECT OF TEMPERATURE

Decomposition of ophthalmic preparations occurs much more rapidly at elevated temperatures encountered in autoclaving than at room temperature. The rate of decomposition with autoclaving is much less at lower pH values (around pH 5). Therefore it is desirable to buffer the solutions around this pH (ELLIS, 1977). However, it is also a well known fact that oxidative processes are often slower at higher temperatures because of the reduced oxygen solubility (LACHMAN et al, 1970). The solubility of oxygen in water at atmospheric pressure is virtually zer 100°c (STEPHEN and STEPHEN, 1891; WINKLER, 1899).

Thus oxidation reactions are affected not only by direct temperature effects but also by the effect of temperature on the concentration of oxygen in solution (AKERS, 1979). Moderately high temperatures during storage may do more damage to formulations of adrenaline eye drops than the high but short-lasting autoclaving

temperatures.

1.2.6 OTHER FACTORS AFFECTING THE STABILITY OF ADRENALINE IN SOLUTION

1.2.6.1 THE EFFECT OF SULPHITE IONS

SCHROETER et al, (1958) showed that the degradation of adrenaline in solutions stored in an oxygen-free atmosphere in the presence of as little as 0.1.% $^{w}/_{v}$ bisulphite occured at a faster rate than in the complete absence of bisulphite. RIEGELMAN and FISCHER (1962) also implicated sulphite ions in the degradation of adrenaline in solution. Finally, HAJRATWALA (1975) has reported that sulphite-induced anaerobic degradation of adrenaline is more pronounced with sodium metabisulphite, and is pH dependent, increasing with increase in pH.

The mechanism of sulphite-induced degradation of adrenaline is not clear, but BONEVSKI et al (1977) have suggested that, at least in alkaline media, sulphate ions formed by the oxidation of sulphite ions, might be responsible for the initial oxidation of adrenaline to adrenaline quinone.

1.2.6.2 THE EFFECT OF ASCORBIC ACID

Under certain conditions, ascorbic acid can accelerate the rate of oxidative degradation of adrenaline. It has been suggested that conditions which lead to cleavage between carbon 2 and carbon 3 of ascorbic acid permit the antioxidant effects to be observed

whereas conditions leading to decarboxylation or to a dismutative side chain reaction favour pro-oxidative effects (OESTERLING, 1957).

The effect has been observed in acetate buffer (pH 4-6) and the mechanism of the destructive effect is thought to depend on the generation of semiquinone-like free radicals by the removal of one electron from the ene-diolate group of ascorbic acid.

1.2.7 STEPS THAT HAVE BEEN TAKEN TO IMPROVE THE STABILITY OF ADRENALINE IN SOLUTION

1.2.7.1. THE USE OF ANTIOXIDANTS

Antioxidants are compounds which have the capability of functioning chemically as reducing agents. They are used in pharmaceutical preparations containg easily oxidised substances. They seem to act in two ways, both achieving the same results (NASH, 1958):

(i) either the antioxidant is oxidised in place of the active component or,

(ii) if the active component is oxidised, the antioxidant reduces it back to its normal oxidation state.

According to AKERS (1979) the formulator of a solution dosage form of an oxygen-sensitive drug must consider the following questions: Given the drug concentration, its solubility pH stability, and desired shelf-life, what antioxidant choices are there, what are the concentration limits, and how effective is the

is the antioxidant system in protecting the drug from being oxidised?

Selection of an antioxidant is not an easy task for a formulator (WHNG and KOWAL, 1980). Not only is the preformulation screening of antioxidant efficacy misleading in certain cases, (AKERS, 1979), but other factors such as interaction with the closure, effectiveness of nitrogen purge (where applicable), and the stability of the antioxidant itself could complicate the entire picture.

In general, for a compound to be used as an antioxidant in eye preparations it must satisfy the following condition:-

(i) it must be physiologically inert., (ii) it must not react chemically with the other ingredients in the eye drop., (iii) it must be effective at low concentrations to avoid adverse systemic effects after absorption from the eye., (iv) it must remain chemically unaltered during the preparation of the eye drops.

The following compounds have been used as antioxidants in solutions of adrenaline (AKERS, 1979):

(i) the sulphite, thiosulphate and metabisulphite salts of sodium.,

(ii) thiourea;

(iii) ascorbic acid.,

(iv) acetyl cysteine.

Bisulphite salts of sodium have been used for many years (GIRARD and KERNEY, 1950) and are still used despite some of their drawbacks that have already been discussed.

Ascorbic acid has also been known for several years now to exhibit antioxidant activity in solutions containing adrenaline (CLARKE and GEISMANN, 1949). MANN (1953) was able to demonstrate that it was the endogenous ascorbic acid that was responsible for the stability of adrenaline and noradrenaline in human urine. SZEPESY (1962) recommended the use of ascorbic acid and sodium metabisulphite in solutions of adrenaline, and the same combination has been recommended by the Moorfields Eye Hospital for use in preparations of 1% adrenaline eye drops (Martindale 1979 (a).

AKERS (1979) reported that epinephryl (adrenaline) - borate solutions containing thiourea with either acetylcysteine or ascorbic acid were more stable than similar solutions containing ascorbic acid and acetylcysteine. Earlier worksby DOLDER (1952) and FENECH (1958) has shown that thiourea is an effective stabilizer of ascorbic acid.

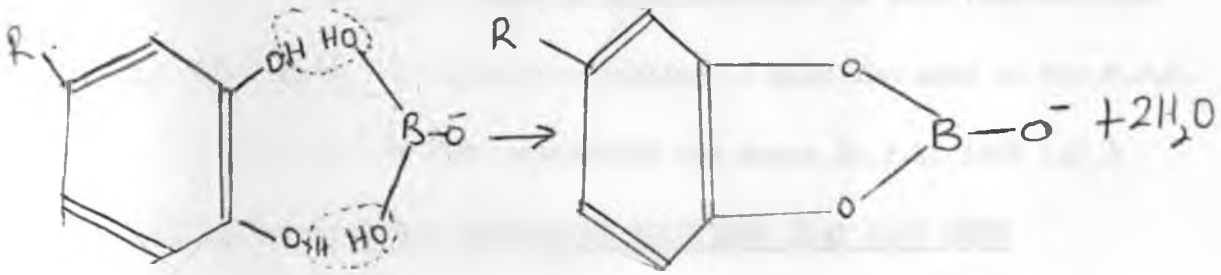
1.2.7.2. THE USE OF BUFFERS

The ideal buffer for ophthalmic preparations is one with a low buffer capacity, i.e. one whose pH can be easily shifted to tne tnat of the lachrymal secretions when a drop of this buffer

is instilled into the eye. As is illustrated in Appendix ..5... (page.199...), borate buffer has a very low buffer capacity, and is ideal for such preparation. It has been recommended as buffer for adrenaline eye drops by several workers (RIEGELMAN, 1962; RIEGELMAN and FISCHER, 1962: SZEPESY, 1962; MOERCH and MOERCH, 1962).

Borate also has the advantage that it protects adrenaline from oxidation by a process of chelation (TRAUTNER and MESSER, 1952) and from attack by sulphite ions (RIEGELMAN and FISCHER, 1962), by the same process. A 1:1 chelate, as illustrated in Figure 3 is formed between adrenaline and Borate (ANTIKAINEN and TEVANEN, 1966).

FIG 3



R = Adrenaline side chain.

1.2.7.3 REPLACEMENT OF OXYGEN WITH AN INERT GAS

Using an inert gas such as nitrogen or carbon-dioxide to replace oxygen in containers of adrenaline solutions has also been recommended by several workers as a means of retarding the rate of oxidative degradation; (GIRARD and KERNEY, 1950; MEINHARD, 1958; LUNDGREN and STORM, 1966; HARMNETT, 1975).

1.2.7.4 USE OF CHELATING AGENTS

Chelating agents have been used to remove trace heavy metal ions that may be present in solutions of adrenaline, especially following leaching from glass containers.

Disodium edetate (EDTA) is included as a chelating agent in the Moorfields Eye Hospital formula for 1% neutral adrenaline eye drops, (MARTINDALE, 1979 (a)). HAMNETT (1975) has recommended the use of 8 -Hydroxyquinoline as a chelating agent and as a synergist for sodium metabisulphite in solutions containing adrenaline. 8 -hydroxyquinoline is also included in the B.P.C. preparation for neutral adrenaline eye drops (B.P.C; 1973 (a))

1.2.8 SOME FORMULATIONS OF ADRENALINE SOLUTIONS THAT HAVE BEEN PROPOSED

The formulation of stable adrenaline solutions (including eye drops) has proved to be a problem for formulators for many years now.

MEINHARD (1958) gave a summary of the various official

requirements for the preservation and stabilization of solutions of adrenaline. These stated, amongst other things, that the solution was not to be heated, was to be protected from light, to be packaged in alkali-free glass under nitrogen or carbon dioxide and to contain either sodium sulphite or sodium metabisulphite as stabiliser.

HOSSEIN et al (1960) reported that the most stable solution of adrenaline contained 1% Nicotinic acid, 0.5% chloretone, 0.05% sodium metabsulphite and packed under carbon-dioxide. The solution was reported to have been stable for 420 days.

MOERCH and MOERCH (1965) proposed the formulation of stable adrenaline eye drops by dissolving adrenaline bitartrate in distilled water with 0.05% sodium metabisulphite as the anti-oxidant, and the addition of boric acid for isotonicity.

FUETIG and METHKE (1965) proposed a formula for stable adrenaline eye drops suitable for use in glaucoma therepy as consisting a 2% solution of adrenaline base with 0.05% sodium metabisulphite as stabilizer.

HAMNETT (1975) proposed a formula for 1% neutral adrenaline eye drops that included 8 - Hydroxyquinoline, sodium metabisulphite, phenylmercuric nitrate, and adjusted to pH 7.4. This solution was claimed to be stable for upto 5 months. The USP of the same year (U.S.P. XIX, 1975 (a)) gave a general description

of adrenaline ophthalmic solutions, without being specific on the composition or method of stabilization.

The latest issue of the B.P.C. (1979 (a)) gives two formulas: for neutral adrenaline solution (pH 7.4.), in borate buffer and containing a suitable stabilizing agent; and a formula for neutral viscous adrenaline eye drops, pH 6.5, and containing suitable stabilizing agents.

It is clear from this brief review that a universally accepted formula for adrenaline eye drops is still to be found, and the present work is a continuation of previous efforts to find a simple and stable formulation.

1.2.9 THE SCOPE OF THE WORK

The present work is aimed at studying the stability of adrenaline in an eye drop formulation, under various conditions of vehicle composition and pH.

To achieve this a preformulation study is to be undertaken to select excipients to be included in the final formulation. This will involve a preliminary screening of the various antioxident compounds suitable for use in preparations of adrenaline eye drops; an investigation into the effect of various sterilization methods on the stability of adrenaline in a well tried formulation, both during sterilization and on long-term storage; and an investigation, by accelerated stability tests,

of the effect of changing the pH and the buffer system, on the stability of adrenaline in solution.

The final aim will be to arrive at a formulation of adequate stability, minimal ocular side effects and adequate therapeutic effectiveness, which can be used in the management of raised intraocular pressure in glaucoma patients. These properties of the final formulation will be assesed by performing clinical tests on hospitalised patients with glaucoma.

CHAPTER 2

EXPERIMENTAL

2.1. INTRODUCTION

A brief description of the principles underlying the experimental methods will be given in the sections which follow:

2.1.1. PREFORMULATION SCREENING OF ANTIOXIDANTS

The purpose was to select a suitable antioxidant or combination of antioxidants to be used in the final formulation of adrenaline eye drops.

Each of the antioxidants used in pharmaceutical preparations has certain limitations with regard to their pH stability (most are stable in acidic pH), their maximum allowable concentration (range of 0.05 to 1.0%), and their ability to protect oxygen sensitive drugs for long storage periods (AKERS, 1979). Preformulation screening involves the consideration of these limitations.

The most common method for screening as well as stability testing of antioxidant efficiency is the assay of the ingredient and the antioxidant in solution with time under demanding conditions. This method is essential once the final formulation (s) have been selected and long term stability studies initiated. However, in the preliminary phase, this method is too time consuming to allow a comprehensive study of all the potential antioxidants and combinations of antioxidants.

Although the selection of antioxidants can be made on

sound theoretical grounds (based on the difference in redox potentials between the drug and the antioxidant) it is a more intricate problem to predict the efficiency of antioxidants in complex pharmaceutical systems (LACHMAN, 1968). In none of the recently published papers except one by AKERS (1979), were antioxidants screened for effectiveness prior to testing in presence of the oxygen sensitive drug (DAVIES, 1970; MOORE, 1976; and ENEVER et al, 1977).

In the present work a very simple method for antioxidant screening prior to testing in the presence of the drug was adopted. Ceric Ammonium Sulphate standard solution was used to titrate solutions containing reduced substances (antioxidants), and the oxidation process was measured potentiometrically, using a pH meter. The electrodes were connected to the potentiometer in such a way that the reaction proceeded according to the following half equation.

Reduced form $\rightleftharpoons$ oxidised form + n Electrons.

The standard oxidation potential., E^{o}, of the anti-oxidant was determined from the Nernst equation as follows:

$$E = E^{o} + \frac{RT}{nF} \ln \frac{[OX]}{[RED]}$$

where E is the oxidation-reduction potential, E^{o} is the standard oxidation-reduction potential, R is the gas constant, T is the

absolute temperature, n is the number of electrons involved in the oxidation process, F is the Faraday constant, [OX] is the concentration of the oxidised species, and [Red] is the concentration of the reduced species in solution .

By plotting E versus log $\frac{[ox]}{[Red]}$, E^o was determined from the intercept of the resultant straight line, after fitting the data by least square regression analysis. E^o was determined at various pH values.

2.1.2. ACCELERATED STABILITY TESTS

Accelerated stability tests were carried out on adrenaline solutions to determine the extrapolated reaction rate constants of degradation at room temperature (25°c) under different conditions of formulation.

Accelerated stability tests are generally used to predict the shelf-life of pharmaceutical products, by storing such products at two or more elevated temperatures and sampling at suitable time intervals to determine the potency (DAVIES and BUDGETT, 1980).

For many drugs the deterioration is first order, that is,

$$\log Y = \log Yo - \frac{KT}{2.303}$$

where Y is the potency at time t, Yo is the initial potency and K the rate of deterioration at temperature T.

An ampule stored at an elevated temperature is usually assayed alongside an ampule stored at a low temperature where no appreciable deterioration occurs. The latter is regarded as a standard, and the potency of the sample is expressed as a percent or proportion of the standard. By fitting the data by least square regression analysis as will be illustrated in Appendix 2 (page 178.) It is possible to determine the reaction rate constant of degradation at any temperature.

2.1.3. APPLANATION TONOMETRY

The final section on experimental involved the measurement of intraocular pressure in hospitalised patients using the method of Applanation Tonometry. The principle and procedure involved are discussed briefly below.

Intraocular pressure (P_o) is measured accurately if one measures the force required to flatten (applanate) a small corneal surface of constant area, and uses the formula: Pressure = Force ÷ area. The minimal area involved (usually corresponding to a diameter of 3.06 mm) results in a minimum volume of fluid displacement due to the applanation of the cornea, and eliminates almost completely any rise in the P_o value. For this purpose, the Goldmann Applanation Tonometer is used.

The measurement procedure is performed at a slit-lamp

where a dye such as fluorescein is placed into the patient's eye to make the tear film more apparent, and a local anaesthetic such as decicaine is used to reduce corneal sensation.

The Tonometer has a conoid, flat-tipped instrument with a transpararent centre which is pressed against the cornea, while the observer sights through tne double-prism in the shaft of the slit-lamp. There, two semi-circles of fluorescent tear films are seen which have to be moved so that they just interlock. This is done by adjusting the pressure with a micrometer on the Tonometer. The diagram A.I.4 in Appendix 1 (page.165..) illustrates the appearance of the semi circles.

2.2 MATERIALS AND APPARATUS

M A T E R I A L S

The following materials and reagents were used in the present work. A key to the abbrevations used is given at the end of this section.

MATERIAL/REAGENT/MEDIUM	GRADE	MANUFACTURER/SUPPLIER
Acetic acid (glacial)	Anal reagent	M & B, England
Acetic anhydride	Anal. reagent	Merk, Germany
Adrenaline bitartrate		BDH Chemicals
Ammonium Ceric Sulphate	Lab. reagent	BDH Chemicals
Ammonia '0.91' Solution	Lab. chemical	Kobian (K) Ltd.
Ascorbic acid	Anal. reagent	BDH Chemicals
Boric Acid	Lab. chemical	Kobian (K) Ltd.
Borate Powder	Lab. chemicals	H & MaG.
Buffer Tablets		BDH Chemicals
Citric acid	Gen. purpose reagent	Hopkins & Williams
L (-) Cysteine hydrochloride	Lab. reagent	S.D's Industry
Decicaine (1%) drops		S.P.U.
Disodium hydrogen phosphate	Lab. chemicals	H & McG. Ltd.
Disodium Tetroborate	Lab. chemicals	H & McG. Ltd.
EDTA	Anal. reagent	BDH Chemicals
Ferrous sulphate	Anal. reagent	BDH Chemicals

MATERAL/REAGENT/MEDIUM	GRADE	MANUFACTURER/SUPPLIER
Fluorescein (1%) drops		S.P.U.
Fluorescein Strips		AG, Germany
Glaucothil (1% Dipivefrin) drops		Dr. Thilo, Germany
Glycine crystals	Lab. chemicals	M & B, England
Hydrochloric acid (conc.)		E.T. Monks
(8-) Hydroxyquinoline	Lab. chemicals	M & B, England
Lecithin Egg (95%/100%)		BDH Chemicals
Millipore Filters		Millipore Corporation
Pilocarpine drops (2%, 4%)		S.P.U.
Pilocarpine drops (2%)		Dr. Thilo, Germany
Potassium chloride powder	Lab. chemicals	M & B, England
Potassium bicarbonate	Lab. reagent	BDH Chemicals
Potassium hydrogen Phthalate	Lab. chemicals	M & B, England
Sabouraud Liquid Medium		Oxoid Ltd.
Sodium acetate (anhydrous)	Lab. reagent	BDH chemicals
Sodium bicarbonate powder	Lab. chemicals	BDH Chemicals
Sodium Carbonate	Lab. chemicals	E.T. Monks
Sodium Citrate	Lab. chemicals	H & MaG.
Sodium hydroxide pellets	Lab. chemicals	E.T. Monks
Sodium metabisulphite	Lab. chemicals	H & MaG.
Sodium nitrate	Lab. chemicals	H & MaG.

MATERIAL/REAGENT/MEDIUM	GRADE	MANUFACTURER/SUPPLIER
Sodium sulphite	Lab. reagent	BDH Chemicals
Sterile Eye swabs		CSSD
Thioglycollate medium	U.S.P.	Oxoid Ltd
Tween 80		Mark, Germany

KEY

M & B = May and Baker

H & McG = Howse and McGeorge

S.P.U. = Sterile preparation unit

A P P A R A T U S

APPARATUS	MANUFACTURER/SUPPLIER
Bench Autoclave	Express Equipment
Calomel Electrodes	EIL, Surrey, England
Electric heaters (TE-7)	Techne, Cambridge
Eye drop bottles	Beetson clarke, England
Goldmann Applanation Tonometer	Hagg-streit, Germany
Griffin Pipetle Filler	Griffin & George Ltd., England
Memmert drying oven	Schwabach, Germany
Pye Unicom SP 8000 spectrophotmeter	Phillips, Holland
Pye Unicom PH meter	Phillips, Holland
Platinum Electrode (IM)	EIL, Surrey, England
Sartorius Microanalytical Balance	Archelis (K) Ltd.
Tecam Magnetic stirrer	Techne, Cambridge
Toughend PH Electrode	EIL, Surrey, England
Vacuum pump/compressor	Edwards, England

2.2.3. ORGANISMS:

Anaerobic bacillus (isolated from the soil)

Candida albicans ( ) } from
Staphylococcus aureus (Oxford strain) } Kenyatta Hospital

2.3. PREFORMULATION SCREENING OF ANTIOXIDANTS

2.3.1. PREPARATION OF REAGENTS

2.3.1.1. 0.1N CERIC AMMONIUM SULPHATE SOLUTION

The solution was prepared and standardized with ferrous sulphate according to the method described in the B.P., 1973 (a). The calculated normality of the solution was 0.157N.

2.3.1.2. CITRATE PHOSPHATE BUFFERS

Buffer solutions of pH 3.8, 4.4, 4.0, 6.0, and 7.0 were prepared according to the method given in the Pharmaceutical Handbook (1975 (a)). The exact pH of the solutions was adjusted by gradual addition of 1N Hydrochloric acid or 1N sodium hydroxide solution and monitoring the pH with a pH meter. The pH meter had been previously calibrated using standard buffer solutions (pH 4 and 9) prepared from standard buffer Tablets.

2.3.1.3. 0.1N ACETOUS PERCHLORIC ACID

Acetous perchloric acid was prepared and standardized with potassium hydrogen phthalate according to the method described in the B.P., 1973(b). The normality of the acetous perchloric acid was determined to be 0.096 7 N.

2.3.1.4 STANDARDIZATION OF ADRENALINE BITARTRATE

It was necessary to determine the quantity of adrenaline base in a sample from a batch of adrenaline bitartrate powder to be used in subsequent experiments. This was done by titrating solutions

of adrenaline bitartrate with standard acetous perchloric acid, according to the procedure described in the B.P., 1973 (c).

The average amount of adrenaline base found was 0.5416g/1g of powder, giving a percentage purity of 98.78 (±0.48) %.

2.3.2. DETERMINATION OF STANDARD OXIDATION-REDUCTION POTENTIALS

2.3.2.1. ADRENALINE ALONE

The method used has been described before, although actual experimental details were not given in that particular report, (AKERS, 1979). The description given below for adrenaline also applied to the other compounds whose standard oxidation-reduction potentials were determined.

Ceric ammonium sulphate solution was used as the oxidising agent and the oxidation process was followed using a previously calibrated pH meter. A known amount of adrenaline bitartrate powder was accurately weighed into a beaker and dissolved in a previously prepared phosphate-citrate buffer solution of known pH. The solution was adjusted to 50 mls. with more buffer, and a magnet was placed into the solution for continous stirring as the ceric ammonium sulphate solution was added from a burette.

A platinum and a calomel electrode were dipping into the adrenaline solution as the ceric ammonium sulphate solution was being added. The electrodes were connected to a pH meter and the potential difference (MV) was noted after each addition of a known volume of the oxidising agent. The results given in Table 1 (page..75..) and Figure 4 (page..77..) are an average of two such determinations. Regression analysis of a sample set of such results is given in Appendix 2 (page.172.). The oxidation

of adrenaline by ceric ions is illustrated in scheme 1 (page.42..).

2.3.2.2. ANTIOXIDANTS ALONE

The standard oxidation-reduction potentials of the following antioxidants were determined; Ascorbic acid, L-Cysteine, sodium sulphite and sodium metabisulphite. The procedure followed was the same as has been described for adrenaline, except that in the case of ascorbic acid, a slight modification was necessary. According to MARTIN et al (1973) because the oxidation of ascorbic acid in aqueous solution proceeds sluggishly, a potential mediator, such as methylene blue should be added to the solution of ascorbic acid, in a small amount (0.001M) before addition of the oxidising agent. Thus ascorbic acid was dissolved in buffer solution containing 0.001M Methylene blue before the addition of ceric ammonium sulphate solution.

The results are summarised in Table 1 (page..75..) and figures (5 - 7) (pages..78-80). The method of calculation of the standard oxidation-reduction potentials, the coefficients of correlation, the regression equations together with a sample set of results is given in Appendix 2 (p..172). The oxidation process of ascorbic acid, L-Cysteine, sodium sulphite and sodium metabisulphite are summarised in schemes (II - VI) pages ..43..to ..47..)

2.3.2.3 8 - HYDROXYQUINOLINE

This compound is not an antioxidant, but its standard

oxidation reduction potential was also determined for comparision purposes. The results are summarised in Table 1 (Page..75..) and Figure (8) (page..81..). The oxidation process is illustrated in scheme (V) (page..46..)

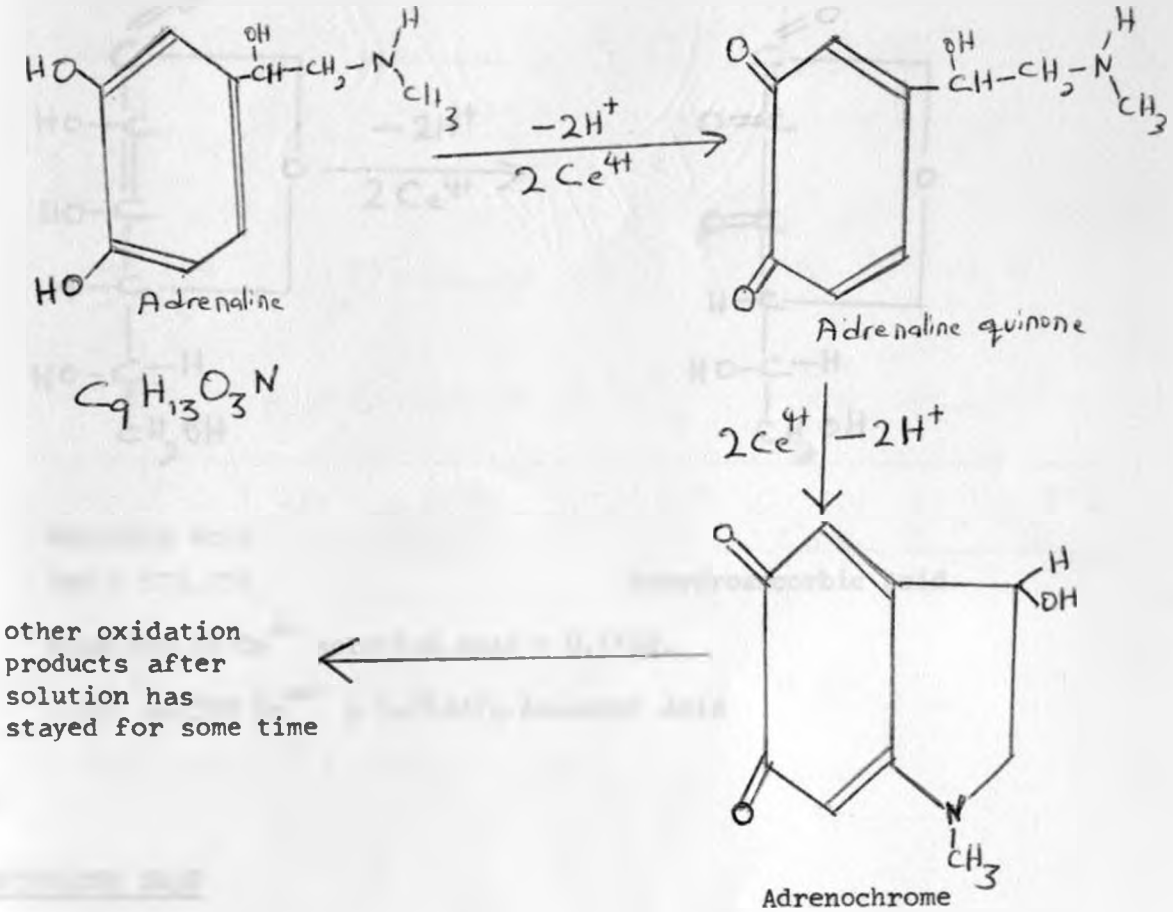
For each of the compounds whose standard redox potentials at various pH values were determined, a plot of the standard redox values versus the pH was made after analysing the data by least square regression. Table (2) (page..82..) and figure (9) (p..83..) give a summary the regression equations for all the compounds and the corresponding straight line plots respectively. The generation of the regression equations is illustrated in Appendix 2 (page..172..) using the data for sodium metabisulphite as an example.

2.4. REACTION SCHEMES

All the reaction schemes mentioned in the present work are given in this section. It should be noted that the last reaction (scheme VII) is an enzymatic hydrolytic reaction which will be referred to again in section 8.5 which deals with general discussion. Where the source of the reaction pathway is not mentioned, this was worked out from first principles.

S C H E M E 1

2.4.1. **OXIDATION OF ADRENALINE BY CERIC IONS**



183.2g $C_9H_{13}O_3N \equiv 4\,Ce^{4+} \equiv 4{,}000\,NCe^{4+}$

1ML 0·1 $Ce^{4+} \equiv 0.00458g\ C_9H_{13}O_3N$.

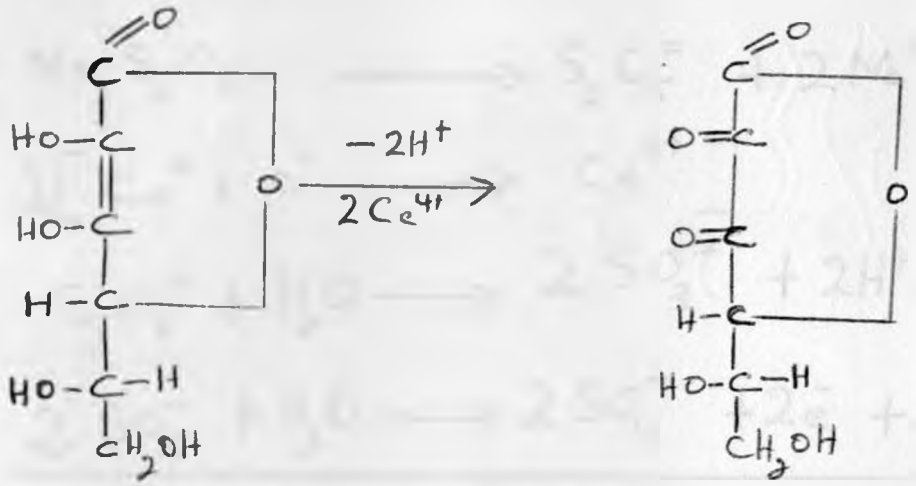
1ML 0·157N $Ce^{4+} \equiv 0.007g\ C_9H_{13}O_3N$

(Oxidative pathways from : BONESKI et at, 1977)

S C H E M E II

2.4.2.1(a)

OXIDATION OF ASCORBIC ACID BY CERIC IONS



Ascorbic Acid
(MW = 176.13)

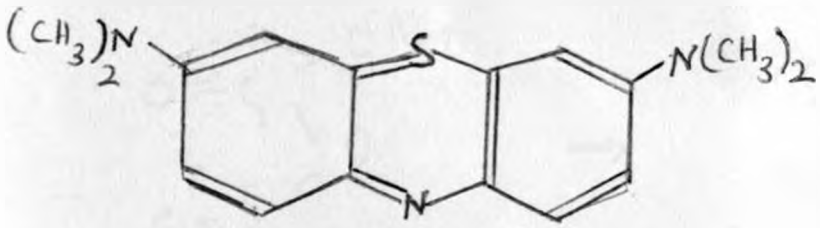
Dehydroascorbic Acid.

Nomality of Ce^{4+} solution used = 0.153N.

I ml. 0.153N Ce^{4+} ≡ 0.01347g Ascorbic Acid

2.4.2.2.(b)

METHYLENE BLUE



$C_{16}H_{18}CNS$

Mwt = 284.4

In 50 mls, a 0.001M solution would contain 0.01422 g of Methylene Blue.

S C H E M E III

2.4.3 **OXIDATION OF METABISULPHITE IONS BY CERIC IONS**

no sum Formula

$$Na_2S_2O_5 \longrightarrow S_2O_5^{=} + 2Na^{+}$$

$$2[Ce^{4+} + e^{-} \longrightarrow Ce^{3+}]$$

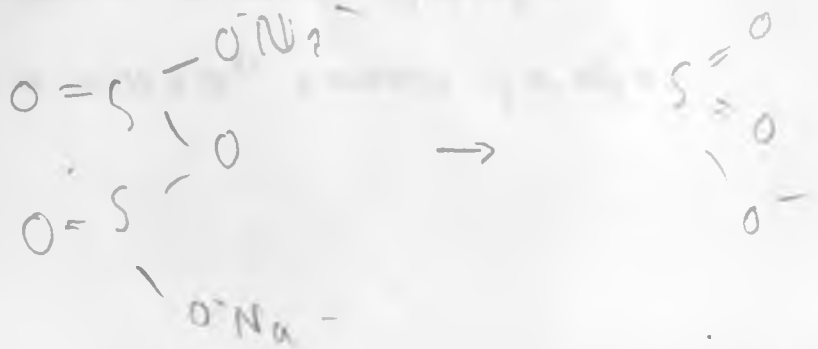
$$S_2O_5^{=} + H_2O \longrightarrow 2SO_3^{-} + 2H^{+} + e^{-}$$

$$2SO_3^{-} + H_2O \longrightarrow 2SO_4^{=} + 2e^{-} + 2H^{+}$$

$$2Ce^{4+} + 2SO_3^{-} + H_2O \rightarrow 2SO_4^{=} + 2H^{+} + 2Ce^{3+}$$

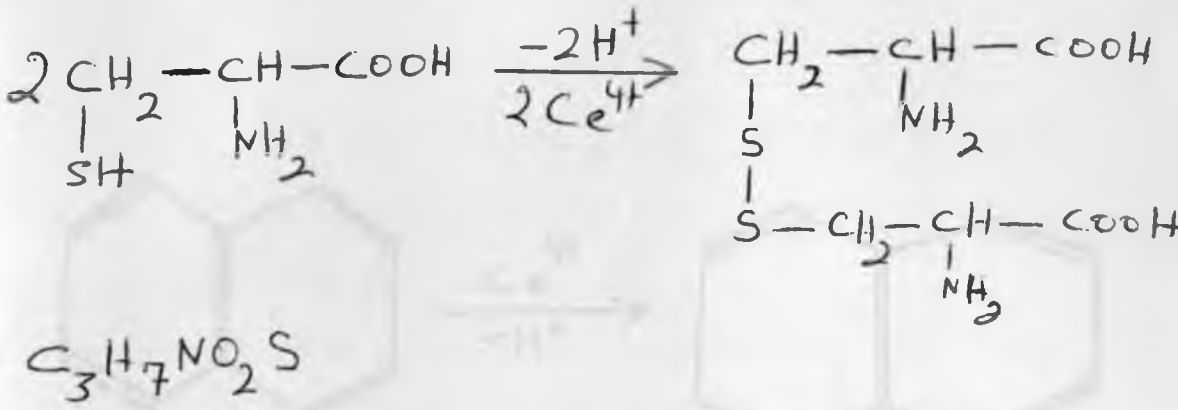
190.1 g $Na_2S_2O_5$ ≡ 2,000 mls N Ce^{4+}

∴ 1 ml 0.153N Ce^{4+} ≡ 0.0145g $Na_2S_2O_5$



S C H E M E IV

2.4.4. OXIDATION OF L-CYSTEINE BY CERIC IONS:



$C_3H_7NO_2S$

$N Ce^{4+} \equiv$ g $C_3H_7NO_2S$

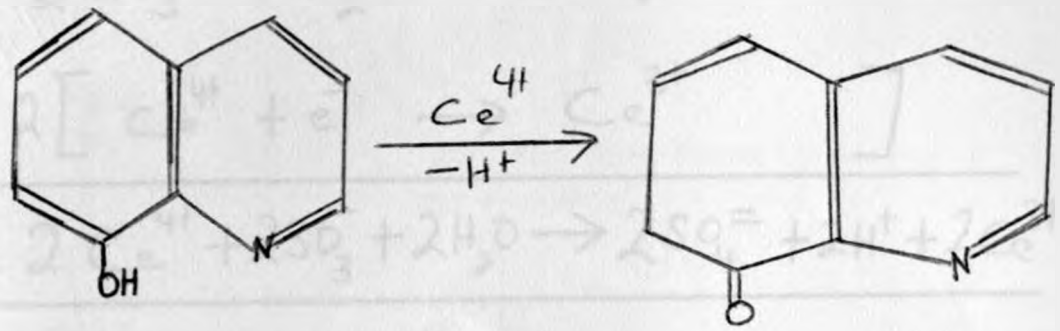
$\therefore$ $N Ce^{4+} \equiv$ g $C_3H_7NO_2S$

1000 Mls N Ce^{4+} ≡ 121.14g $C_3H_7NO_2S$

∴ 1 ml. 0.153 N Ce^{4+} ≡ 0.01853g $C_3H_7NO_2S$

S C H E M E V

2.4.5. THE OXIDATION OF 8-HYDROXYQUINOLINE BY CERIC IONS



8-Hydroxyquinoline

C_9H_7ON

(Mol. mwt = 145.15g)

.: 1000 mls N Ce^{4+} ≡ 145.15g C_9H_7 ON

.: 1 Ml 0.1N Ce^{4+} ≡ 0.014515 g C_9H_7 ON

1 Ml 0.153 N Ce^{4+} ≡ 0.0222g C_9H_7 ON

8-Quinolone

S C H E M E VI

THE OXIDATION OF SODIUM SULPHITE BY CERIC IONS

$$2SO_3^- + H_2O \longrightarrow 2SO_4^{=} + 2e^- + 2H^+$$

$$2\left[\, Ce^{4+} + e^- \longrightarrow Ce^{3+} \,\right]$$

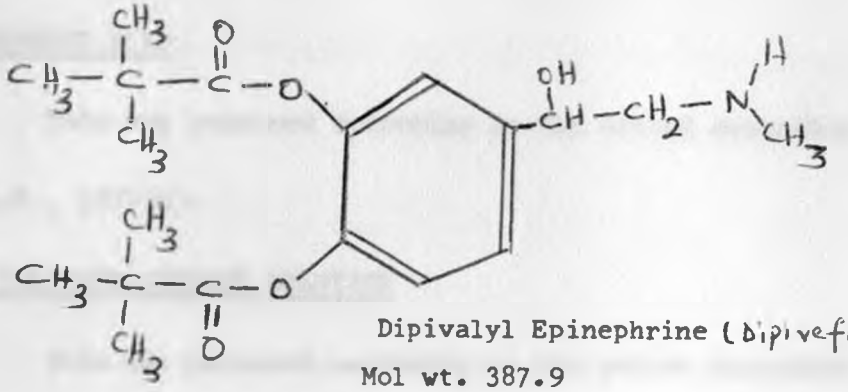
$$2\,Ce^{4+} + 2SO_3^- + 2H_2O \longrightarrow 2SO_4^{=} + 2H^+ + 2Ce^{3+}$$

126.04 g Na_2SO_3 ≡ 1000 mls. N Ce^{4+}

∴ 0.147NCe^{3+} ≡ 0.01852g Na_2SO_3

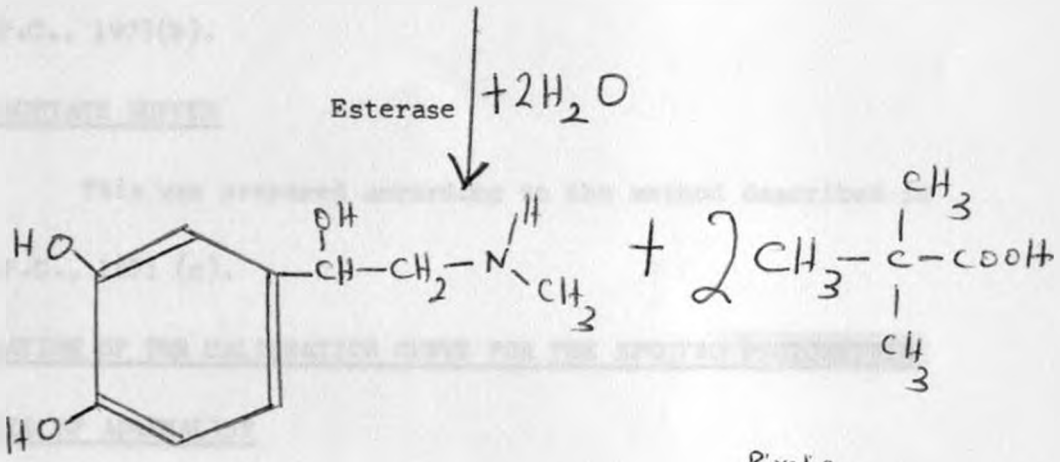
S C H E M E VII

2.4.6. ENZYMATIC CONVERSION OF DIPIVEFRIN TO ADRENALINE BY HYDROLYSIS OF ESTER LINKAGES



Dipivalyl Epinephrine (Dipivefrin)

Mol wt. 387.9



Adrenaline

Mol. wt. 183.2

Pivalic Acid

(From ABRAMOVSKY and MINDEL, 1979).

2.5 INVESTIGATION OF THE EFFECT OF VARIOUS STERILIZATION METHODS ON THE STABILITY OF ADRENALINE EYE DROPS

2.5.1. PREPARATION OF REAGENTS

2.5.1.1. IN HYDROCHORIC ACID

This was prepared according to the method described in the B.P., 1973(d).

2.5.1.2 FERROUS SULPHATE-CITRATE SOLUTION

This was prepared according to the method described in the B.P.C., 1973(b).

2.5.1.3 AMINO ACETATE BUFFER

This was prepared according to the method described in the B.P.C., 1973 (c).

2.5.2. PREPARATION OF THE CALIBRATION CURVE FOR THE SPECTRO PHOTOMETRIC ANALYSIS OF ADRENALINE

It was necessary to construct a calibration curve to be used in the subsequent spectro photometric analysis of adrenaline.

From a stock solution of adrenaline bitartrate containing 5.416 mg/ml adrenaline, and 0.3 g. of sodium metabisulphite the following quantities were pipetted into 100-ml. volumetric flasks: 0.50ml, 0.80ml, 1.0ml, 1.2mls, 1.50mls, 1.80mls, and 2.0mls. The samples were then used to determine the absorbance at 540nm according to the method described by HANMETT (1975). Replicate determinations of the absorbance were performed and

the average of the two absorbance readings was noted.

The data for absorbance and concentration were then analysed by regression and the regression equation generated was then used to construct the calibration curve. The results are summarised in Table 3 (page..85..) and figure 10 (page..86..).

2.5.3 THE EFFECT OF HEAT STERILIZATION ON THE STABILITY OF ADRENALINE SOLUTIONS.

The effect of heat sterilization of 98°c, 115°c and 121°c on the stability of solutions of adrenaline bitartrate was investigated. For comparision, samples of unheated solutions of adrenaline bitartrate were included in the study.

The adrenaline bitartrate solutions were prepared according to the formula for 1% neutral adrenaline solution given in Martindale (1977(a)), which has the following composition:

Adrenaline base	= 1.2% w/v
Boric Acid	= 1.0% w/v
Borate	= 0.6% w/v
Sodium Metabisulphite	= 0.3% w/v
Ascorbic acid	= 0.2% w/v
EDTA	= 0.1% w/v

Water for injection to 100mls.

The pH of the solution was adjusted to 7.4 with 1N sodium hydroxide solution, using a previously calibrated pH meter. The solution was then distributed into seven amber coloured eye-drop bottles which were then treated as follows:-

(i) Heated at 98°c for 30 minutes (2 bottles).

(ii) Autoclaved at 115°c (12.4 p.s.i.) for 30 minutes (2 bottles).

(iii) Autoclaved at 121°c (17.4 p.s.i.) for 15 minutes (2 bottles).

(iv) One bottle with the adrenaline solution was stored at room temperature throughout. The average room temperature was taken to be 25°c.

The amount of adrenaline remaining after each heat treatment was then determined in duplicate by the colorimetric method described by HAMNETT (1975). A statistical analysis of the data obtained was then carried out to test for the "Null-hypothesis", according to the method given in Appendix 2 (page.181..). The results are summerised in Table 4a (page..87..)

All the bottles containing the adrenaline solutions were then stored at room temperature for a period of four weeks. At weekly intervals, the amount of adrenaline remaining was determined (in duplicate) and expressed as a percentage of the amount of adrenaline present at time zero, i.e. the amount present prior to storage.

The data obtained was then analysed by regression to determine the rate of degradation at room temperature. The results are summarised in Table 5. (page..89.).

2.6. INVESTIGATION OF THE VARIOUS FACTORS AFFECTING THE STABILITY OF ADRENALINE SOLUTIONS

2.6.1. EFFECT OF THE NATURE OF THE BUFFER ON THE STABILITY OF ADRENALINE, AT BpH 7.4

The effect of changing the buffer on the stability of adrenaline solutions at pH 7.4 was investigated by performing accelerated stability tests on such solutions.

The following buffers (all with approximate buffer concentration of 0.177.5 M) were used:

(a) Phosphate - Citrate buffer. (0·1774M)

(b) Borax - Borate buffer (0·1775 M)

(c) Giffords buffer (0·1775M)

(d) Phosphate - Citrate - Borate buffer (0·1743M)

Since it is known that Borate retards the degradation of adrenaline by a chelation process (TRAUTNER and MESSER, 1952; RIEGELMAN and FISCHER, 1962) it was decided to investigate the degradation rates of adrenaline in buffers containing Borate (buffers b,c, and d, above) and compare these with the degradation rates in a buffer containing no Borate (buffer 'a' above). Intact adrenaline was analysed by the method described by SALAMA et al (1974)

2.6.1.1. PREPARATION OF REAGENTS

2.6.1.1.1.

PHOSPHATE - CITRATE BUFFER, pH 7.4

This was prepared according to the procedure described in the Pharmaceutical Handbook, 1975 (a), with the following modification: 0.442g of citric acid and 6.00 6g of disodium hydrogen phosphate were dissolved in 245mls of distilled water in a 250-ml volumetric flask. The pH of the solution was adjusted to 7.4 by the addition of small amounts of INsodium hydroxide solution and IN Hydrochloric acid, and monitoring the pH with a previously calibrated pH meter. The volume of the solution was finally adjusted to 250mls by further addition of distilled water.

2.6.1.1.2.

GIFFORDS BUFFER, pH 7.4.

This was prepared according to the method described in "Rogers Inorganic Pharmaceutical Chemistry" (1967), with the following modification: 47.2 mls of Giffords acid buffer were mixed with 2.8 mls of Giffords alkaline buffer in a 50 ml volumetric flask. The pH was adjusted to 7.4 with 1N sodium hydroxide, and the volume of the solution was finally adjusted to 50 mls by further addition of distilled water.

2.6.1.1.3 BORAX - BORATE BUFFER, pH 7.4

This was prepared using the quantities of Borax and Boric acid given in Martindale, 1977 (a). The pH of the solution was adjusted to 7.4 using 1N sodium hydroxide.

2.6.1.1.4. PHOSPHATE - CITRATE - BORATE BUFFER

0.442 g of citric acid, 6.006g of dissodium hydrogen Phosphate and 2.5 g of boric acid were dissolved in 245 mls of distilled water in a 250 ml volumetric flask. The pH was adjusted to 7.4, and the final volume of the solution was adjusted to 250mls by further addition of distilled water.

125.9 mls of this solution was then pipetted into a second 250 ml volumetric flask, and diluted to 250 mls with distilled water.

2.6.1.2. CALIBRATION CURVE FOR THIOSEMI CARBAZIDE ANALYSIS

From a stock solution containing 0.1667 mg/ml adrenaline base the following quantities were accurately pipetted into 25 ml volumetric flasks:-

0.5 ml, 1.0 ml, 1.2 ml, 1.5 ml, and 2.0 mls.

The aliquots were then analysed in duplicate for intact adrenaline by the method described by SALAMA et al (1974). Regression analysis of the absorbance and concentration data was then performed and the resulting equation for the straight line was used to construct the calibration graph shown in Figure (11)

(page..92..). Table 6 (page..91..) summarises the data used for the regression analysis.

2.6.1.3. ACCELERATED STABILITY TESTS ON ADRENALINE SOLUTIONS PREPARED IN VARIOUS BUFFERS (pH 7.4)

Accelerated stability tests were performed in solutions of adrenaline bitartrate prepared in the four different buffers at pH 7.4. The solutions were prepared according to the formula for 1% adrenaline eye drops given previously (page..51.).

A preliminary experiment to compare the two methods of analysis for intact adrenaline (method of SALAMA et al, 1974; and method of HAMNETT, 1975) was performed in phosphate - citrate buffer. The description for the accelerated stability tests which follows will serve for all such tests which were performed.

Five water baths were set at temperatures of 60°c, 66°c, 70°c, 73°c, and 76°c, respectively, by fitting each bath with an adjustible electric heater set at the required temperature. The water in the baths was allowed to attain the temperature before the experiments were started.

Solutions of the eye drops in the particular buffer were prepared according to the formula for 1% Neutral adrenaline given earlier (page..51.). A 2 ml-sample was taken from the eye drop solution and transferred into an amber-coloured eye drop bottle, which was then closed and stored at - 4°c in the refri-

gerator. The amount of adrenaline in this sample would represent the amount at zero time.

The rest of the adrenaline solution was quickly distributed into five 50 ml amber coloured miltidose injection containers (vials) which were then sealed with rubber stoppers and aluminium seals using a capsolut sealer.

Each vial contained exactly 18 mls of the adrenaline solution. The sealed vials were then heated in individual water baths.

A second vial containing 18 mls of distilled water was also placed into each water bath at the same time as the vial containing the adrenaline solution. The second vial was not sealed and was used to monitor the rise in temperature of the adrenaline solution in the sealed vial. Timing was started when the temperature of the water in the unsealed vial reached the set temperature of the water bath.

At appropriate time intervals, 2 ml samples were with drawn from the adrenaline solutions using a 5-ml plastic syringe, by inserting the syringe needle through the rubber seal into the solution. 2 mls of air was injected into the vial before withdrawing the 2 ml sample solution, to maintain a constant pressure inside the vial.

The withdrawn samples were transferred into [illegible] 10-ml

amber coloured eye drop bottles, wich were then closed and stored at - 4°c until analysis. At the end of the heating period, all the samples were analysed spectro photometrically (1) in duplicate for the intact adrenaline remaining. These amounts were expressed as percentages of the amount of adrenaline present at zero-time.

The results were subjected to regression analysis, from which the correlation coefficient was determined. The regression equation was then used to determine the theoretical reaction rate constant at room temperature (25°c) as illustrated in Table 15 (Appendix 2, page.175.). The experiments were carried out in duplicate or triplicate.

Table 8 (page..94..) gives a summary of all the extrapolated values of the reaction rate constants determined in various buffers at pH 7.4.

(1) FOOTNOTE

Several spectrophotometric fluorometric methods based on the oxidation of epinephrine to 'adrenochrome' have been developed. These methods show high sensitivity, but the presence of certain antioxidants interfers with the development of fluorescence (SALAMA and KHALIL, 1974)

2.6.2 EFFECT OF CHANGING THE PH ON THE STABILITY OF ADRENALINE SOLUTIONS

2.6.2.1. ACCELERATED STABILITY TESTS AT PH's 6,5.4,3, AND 2.

Accelerated stability tests were also used to investigate the effect of changing the pH on the stability of adrenaline solutions. The experiment were carried out in phosphate-citrate-Borate buffer using the same formula for adrenaline solution given earlier (page.57.).

The accelerated stability tests were carried out following the same procedure as has been described in section 2.6.1., with one modification on the heating duration, which was extended to 30 hours. This was necessary to give more date points especially at the lower pH values where it was anticipated that the stability of adrenaline would increase. A longer heating period was not attempted as it was felt this might have some significant effect on the stablizers included in the solutions, and hence complicate the interpretation of the results.

At each pH value the extrapolated theoretical value of reaction rate constant at 25°c was determined by regression analysis. The results are summarised in Table 10 (page..98..).

A sample set of results for one of the experiments performed at pH 6.0 at a temperature of 66°c is given in Appendix 2 (page..178.) and illustrates the use of regression analysis to calculate the correlation coefficient and the reaction rate constant

of degradation (K) of adrenaline heated for a total of 30 hours.

A plot of the log of K versus the pH is given in Figure 12 (page..188.). The extrapolated portions of the curve in Figure 12 intersected at a point corresponding to pH 3.7. This point was taken to represent the pH at which adrenaline had maximum stability.

2.6.2.2. ACCELERATED STABILITY TESTS AT pH 3.7

Accelerated stability tests were carried out on adrenaline solutions prepared in Phosphate-Citrate-Borate buffer to determine the reaction rate constant of the degradation of adrenaline at pH 3.7. These tests were also used to make a comparision of the effectiveness of sodium sulphite and a combination of sodium metabisulphite and ascorbic acid in preventing the oxidation of adrenaline in solution. The following two formulae of adrenaline solutions were used.

FORMULA 1

Adrenaline base	= 1.2 g.
Sodium Metabisulphite	= 0.3 g.
Ascorbic acid	= 0.2 g.
EDTA	= 0.1 g.

Buffer solution to 100 mls.

FORMULA 2

Adrenaline base	= 12 g.
Sodium sulphite	= 0.1. g.

EDTA = 0.1 g.

Buffer solution to 100 mls.

The results of the accelerated stability tests are given in Tables 9 and 10 (pages ..96.. and ..98..).

2.7.1.2.7. FORMULATION OF ADRENALINE EYE DROPS FOR CLINICAL TESTING

INTRODUCTION

THE FORMULATION

Using the information gained from the preceding experiments, a formula for 1% Adrenaline eye drops suitable for use in glaucoma therapy was chosen. The formula had the following composition:

Adrenaline = 1.2 g

Sodium Sulphite = 0.1 g.

Disodium Edetate = 0.1 g.

Boric Acid = 1.5 g.

Benzalkonium Chloride = 0.01% w/v

Distilled water to 100 mls.

The amounts of adrenaline base and Disodium Edetate were kept the same as they were in the original Moorfield's Eye hospital formula for 1% adrenaline eye drops, which was used for the accelerated stability tests. From Table 10 (page..98..) it can be seen that 0.1% w/v sodium sulphite was a better antioxidant than the combination of 0.3% w/v sodium metabisulphite and 0.2% w/v ascorbic acid. Therefore 0.1% w/v sodium sulphite was chosen as the antioxidant, although its superiority over the combination of sodium metabisulphite and ascorbic acid was demonstrated only at a single pH value (3.7). The choice of the

antioxidant is discussed further in the discussion section (page..107.).

The choice of the borate buffer was due to two advantages of borate. First, as was mentioned in the introduction (page..20.) borate protects adrenaline against degradation by forming a 1:1 chelate complex with adrenaline. Second, borate buffer has a low buffer capacity, and therefore a solution of an eye drop prepared in borate buffer is quickly neutralised by the lacrymal secretions when it is instilled into the eyes. This point is analysed in more detail in the discussion section (page..122.). How the figure of 1.5% w/v boric acid was arrived at is illustrated by the calculation given in Appendix 4 (page.197). Benzalkonium chloride was chosen as the preservative because it is more effective in acidic conditions, with an optimal pH range of 4 - 5 ("The Quantity Control of Medicines" 1976).

It has been shown that the pH of maximum stability for adrenaline solutions is around pH 3.6 (WEST, 1950). However, eye drops of adrenaline salts, especially the bitartrate salts, have been shown to be irritating to the eyes when formulated at low pH values (PORTNEY, 1977). Therefore it was necessary to choose a pH for formulating the eye drops, which was high enough to eliminate the irritant effects of adrenaline bitartrate, yet still low enough for the stability of adrenaline. It was also found that, due to the presence of benzalkonium chloride, solutions of eye drops prepared according to the proposed formula were turbid below pH 5.7. There-

fore it was necessary to prepare the eye drops at a pH above 5.7 to eliminate the turbidity.

The most common and dangerous pathogenic bacterium which contaminates eye preparations is Pseudomonas aeroginosa (KALLINGS, et al, 1966). EDTA increases the permeability of the cell wall of certain bacteria, including Pseudomonas aeroginosa, to several substances including benzalkonium chloride, apparently by removing calicum and/or magnesium ions from the cell membrane (BROWN and RICHARDS, 1965). However, the antimicrobial effect of benzalkonium chloride is reported to diminish below pH 5(CARTER, 1975). Since the proposed formula for adrenaline eye drops included both EDTA and benzalkonium chloride it was necessary to formulate the eye drops at a pH above 5 in order to take advantage of the enhanced antimocrobial activity of benzalkonium chloride by EDTA above this pH.

Formulation of the eye drops at a pH of 5.8 was therefore considered as a reasonable compromise after consideration of the facts mentioned above on stability, physiological activity, irritation and preservation of the eye drops.

2.7.1.2. ACCELERATED STABILITY TESTS.

It was necessary to perform accelerated stability tests on the final proposed formulation since its composition and the buffer used were different from those of the solutions used for

earlier accelerated stability test experiments. The procedure was the same as was described earlier (page..56..) and the results are summarised in Table 11(a)(page..99..).

2.7.1.3. STERILITY REQUIREMENTS FOR OPHTHALMIC PREPARATIONS

It was necessary to test the new formulation for sterility before testing for its clinical effectiveness. Such a test is a mandatory, requirement in both the U.S.P. and the European Pharmacopoeias.

2.7.2 EXPERIMENTAL

2.7.2.1. FORMULATION OF ADRENALINE EYE DROPS

The formula used has been given in section 2.7.1.1. 0.04 mls of 50% w/v Benzalkonium Chloride solution was diluted to 100 mls. with distilled water to give a 0.02% w/v solution. 50 mls. of this diluted solution were used to dissolve the other ingredients in the formula. The solution was then made upto 95 mls. with distilled water, and the pH was then adjusted to 5.8 by the addition of small quantities of IN Sodium hydroxide solution, and monitoring the pH using a previously calibrated pH meter. Finally, the solution was made up to 100 mls. with distilled water.

The solution was then transferred into a 'Swinex - 25' filter fitted with a membrane filter (pore size 0.45μm). The filter was connected to a vaccum pump and the solution was filtered and then distributed into 10 - ml amber coloured dropper bottles. These were then sealed and sterilized by heating in water at 98^{o}c for 30 minutes. This method of sterilization was chosen since the results from earlier experiments (page..51..) had shown there was no loss of adrenaline base immediately following sterilization by this method.

2.7.2.2 THE STERILITY TESTING

This was performed according to the method described in the European Pharmacopoeia (1972), with the following modifications :

(a) The Benzalkonium Chloride in the eye drops was inactivated by the inclusion of 0.05% commercial Egg Lecithin solubulized in 3% Tween 80 in Thioglycollate test medium.

(b) No authentic culture of Plectridium Sphenoides was available as an anaerobic control organism. An anaerobic spore-forming bacillus was obtained from a local deep-sample of soil. An aqueous suspension was boiled for 10 minutes to kill vegetative bacteria, and a loopful of the heated sample used to inoculate 10ml. liquid thioglycollate medium, which was then incubated at 37°c for 24 hours. This culture was then diluted as described in the European Pharmacopoeia for anaerobic test and control.

(c) Since the Thioglycollate medium was slightly cloudy at the beginning of the experiment due to the presence of solubulized lecithin, the test series was subcultured into fresh Thioglycollate medium and incubated for a further one week. This was to check for any growth which might have been masked by the cloudiness of the first test medium.

The results of the sterility test are summarised in Table 11b (page..102..).

2.7.2.3. ACCELERATED STABILITY TESTS ON THE FINAL FORMULATION

Accelerated stability tests were also performed on the final formulation, and the extrapolated value of the reaction rate constant at 25°c was determined as has been described in section 2.6.1.3. The results are summarised in Table 9 (page..96..), and Table 11a (page..99..).

2.7.2.4. EFFECT OF HEAT STERILIZATION ON THE NEW FORMULATION

The sterilization and subsequent storage of the solutions was performed in exactly the same way as was described in section 2.5.3. (page..54..) for solutions of adrenaline prepared at pH 7.4. The analysis for adrenaline was, however, carried out according to the method described by SALAMA et al (1977). The results are summarised in Tables 4b and 5 (pages..88... and ...89.).

2.8. INVESTIGATION OF THE CLINICAL EFFECTIVENESS OF THE NEW FORMULATION OF ADRENALINE EYE DROPS

2.8.1. INTRODUCTION

It was necessary to test the new formulation of adrenaline eye drops on hospitalised patients in order to assessthe effectiveness of the drops in controlling glaucoma. All the patients tested were advanced cases of glaucoma simplex that were admitted for surgery as they could not be controlled medically as out patients. It is possible that most of these patients failed to take their medications as regularly as adviced, leading to the uncontrolled glaucoma.

It was not possible to test adrenaline eye drops alone because these patients were high risk patients who needed to be placed on a treatment regime immediately. The time interval during the 'control' days when the patients were not receiving adrenaline could be critical if the pressures rose to dangerously high levels unless the patients were receiving other medications for glaucoma.

2.8.2 EXPERIMENTAL PROCEDURE

2.8.2.1. TREATMENT REGIME

All the patients tested were receiving Pilocarpine eye drops concurrently. Two of these patients were in addition receiving Diamox Tablets. The times for giving diamox tablets will be given under the sections for the individual patients.

On two patients, Depivefrin, a pro-drug of adrenaline, was also tested. Under the sections dealing with the individual patients it will be indicated whether the patient received commercial Pilocarpine or Pilocarpine prepared in S.P.U. at Kenyatta hospital.

In all the cases, Pilocarpine drops and adrenaline or Dipivefrin drops were given at the following times:

(a) Pilocarpine drops : - Given four times a day (QID) at 6 a.m., 11 a.m., 4 p.m., and 10 p.m.

(b) Adrenaline/Dipivefrin - Given twice daily (BD) at 7 a.m. and 5 p.m.

2.8.2.2. PREPARATION OF PATIENT FOR TONOMETRY

The intraocular pressure was determined four times daily at 7 a.m., 10 a.m., 2 p.m., and 7 p.m. Before each measurement of the pressure, the patient was prepared as described below.

The patient was seated in a chair positioned before the slit-lamp assembly and asked to tilt the head slightly backwards. A drop of topical anaesthetic (1% decicaine) was instilled into the eye, and the patient was asked to close the eye slowly to avoid squeezing the drops out. The patient was then asked to open the eyes when all the st: ing had gone. The instillation of the anaesthetic was then repeated to ensure complete anaesthesia.

Either of the two methods was used to stain the cornea

to enable to tear films to be seen at the slit lamp. One method involved using Fluorescein paper, and the other method involved using Fluorescein drops. In the first method, the patient was asked to gaze upwards, the lower lid of the eye was then retracted and the strip was inserted into the lateral aspect of the exposed conjunctival sac area. The lower lid was then slowly permitted to regain its normal position, by gradual release while the patient continued to gaze upwards. After about 20 seconds the strip was removed.

In the second method, Fluorescein drops were instilled into the eye with the lids retracted as described above and the patient positioned as described above. In either case, excess stain was wiped off before measurement of the intraocular pressure. Details of the measurements of the intraocular pressure were given on page (..39.).

2.8.2.3. INTERPRETATION OF THE PRESSURE READINGS

The first readings of the intraocular pressure were taken at 7 a.m. These readings were taken as the initial readings and all the changes in subsequent readings were expressed as percentages of the initial readings. Readings taken while the patient was on the prescribed medication were taken as the 'control' readings. Readings taken when the patient was receiving adrenaline drops in addition to the prescribed medication were

taken as the 'test' readings.

Graphs of the intraocular pressure readings (percentage values) versus time of day were then plotted for both control and test treatments. Table 12 (page..105.) and Figure 13 (page.....105(b).) summarise the results for all the five patients tested. A summary of the treatment for each patient data for each patient, and the figures for individual diurnal pressure changes are given in Appendix 3 (page..183.)

CHAPTER 3

RESULTS

(3.1.) RESULTS FOR THE DETERMINATION OF THE OXIDATION-REDUCTION POTENTIALS

TABLE 1

STANDARD REDOX POTENTIALS (E^{o}) OF VARIOUS COMPOUNDS AT VARIOUS PH VALUES

PH OF DETERMINATION	2.2	3.0	3.8	4.0	4.4
ADRENALINE					
E^{o} =	0.746	0.668	0.598	-	0.566
r =	0.985	0.996	0.862	-	0.970
y =	746+78.97x	668.5+67.16x	598+73.61x	-	556+85.41x
ASCORBIC ACID					
E^{o} =	0.344	-	-	0.269	-
r =	0.94			0.878	
y =	344.47+15.46x			269.15+57.19x	
SODIUM METABISULPHITE					
E^{o} =	-	-	-	0.317	-
r =				0.973	
y =				317.34+17x	
L(-)CYSTEINE HYDROCHLORIDE					
E^{o} =	-	-	-	0.510	-
r =				0.956	
y =				509.97+69.9x	
8-HYDROXY QUINOLINE					
E^{o} =	-	-	-	0.687	-
r =				0.981	
y =				686.9+66x	
SODIUM SULPHITE					
E^{o} =	-	0.345	-	0.300	-
r =		0.937		1.1	
y =		344.8+86.3x		300.12+80.9x	

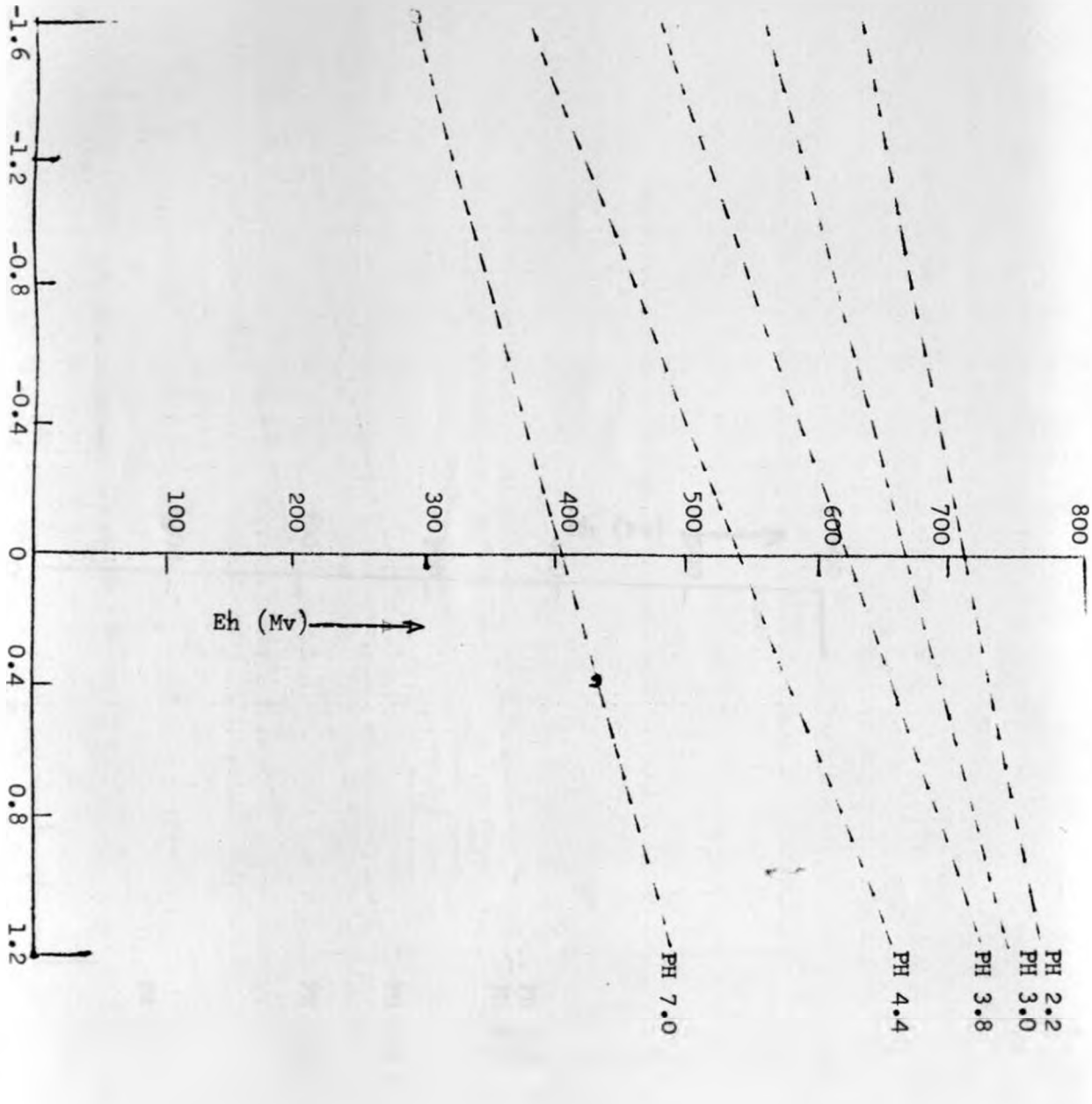
cont....

5.0	6.0	7.0
0.5379 0.980 537.86+92.96x	0.407 0.937 407.5+73.6x	0.381 0.920 381.1+85.89x
0.20 0.867 199.55+54.28x	0.144 0.92 143.83+49x	0.047 0.94 46.89+9.8x
0.303 0.969 302.62+54.7x	0.236 0.921 236.2+47.8x	0.178 0.897 178+14.9x
0.378 0.976 378+13.1x	0.339 0.919 339.4+52.9x	0.276 0.940 275.78+107x
0.595 0.969 595.1+115x	-	0.459 0.943 458.49+159.7x
0.296 0.989 296.45+102.8x	0.285 0.96 285.06+99.12x	0.272 0.877 272.39+95.64x

KEY : r = Correlation Coefficient, E^{o} = Standard Redox Potential (Volts),
y = a+bx = regression equation.

FIGURE (4)

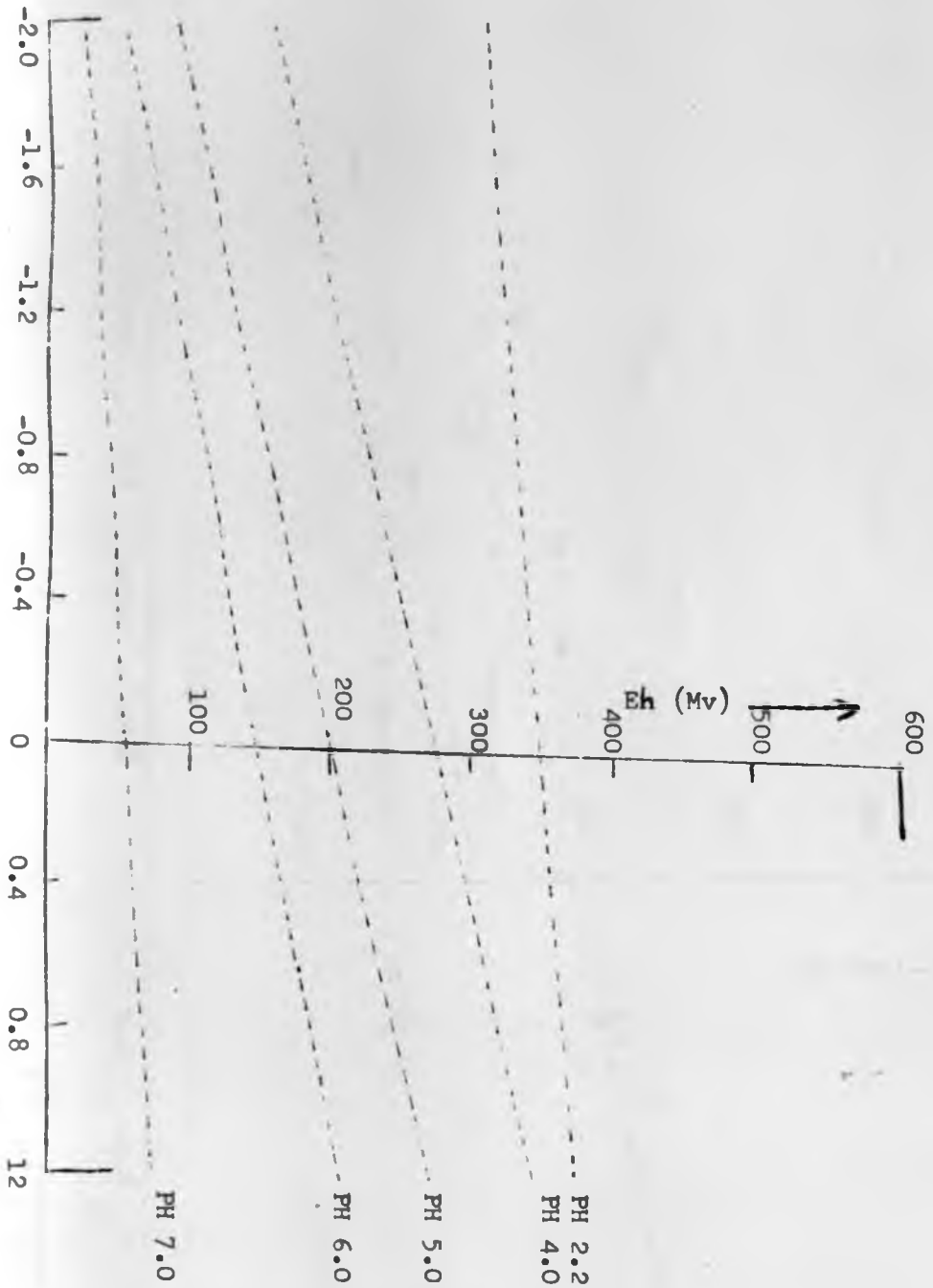
REGRESSION LINES FOR THE OXIDATION OF ADRENALINE DRAWN USING THE EQUATIONS IN TABLE (1)



3.1.2.

FIGURE (5)

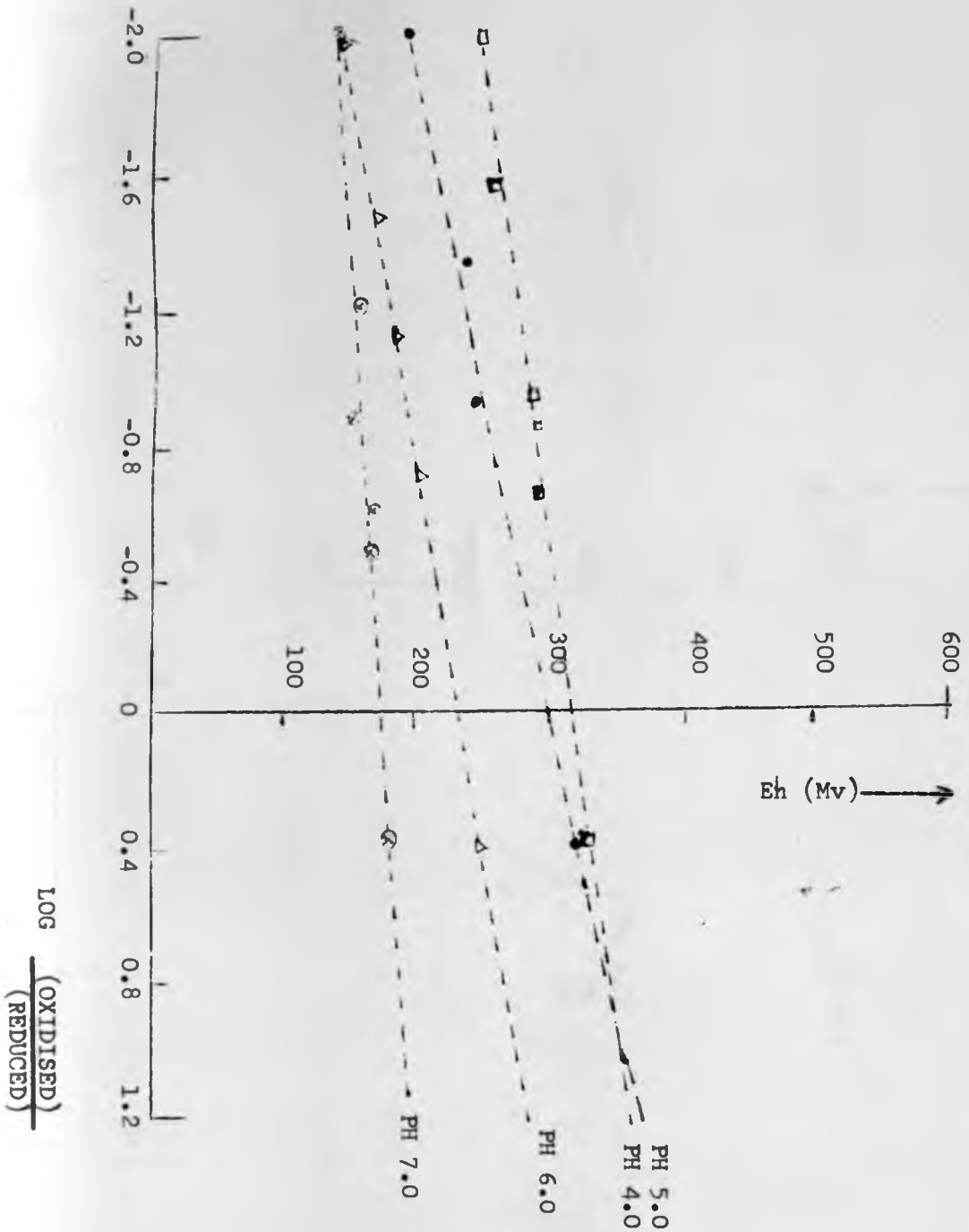
REGRESSION LINES FOR THE OXIDATION OF ASCORBIC ACID DRAWN USING THE REGRESSION EQUATIONS IN TABLE (1)



3.1.3.

FIGURE (6)

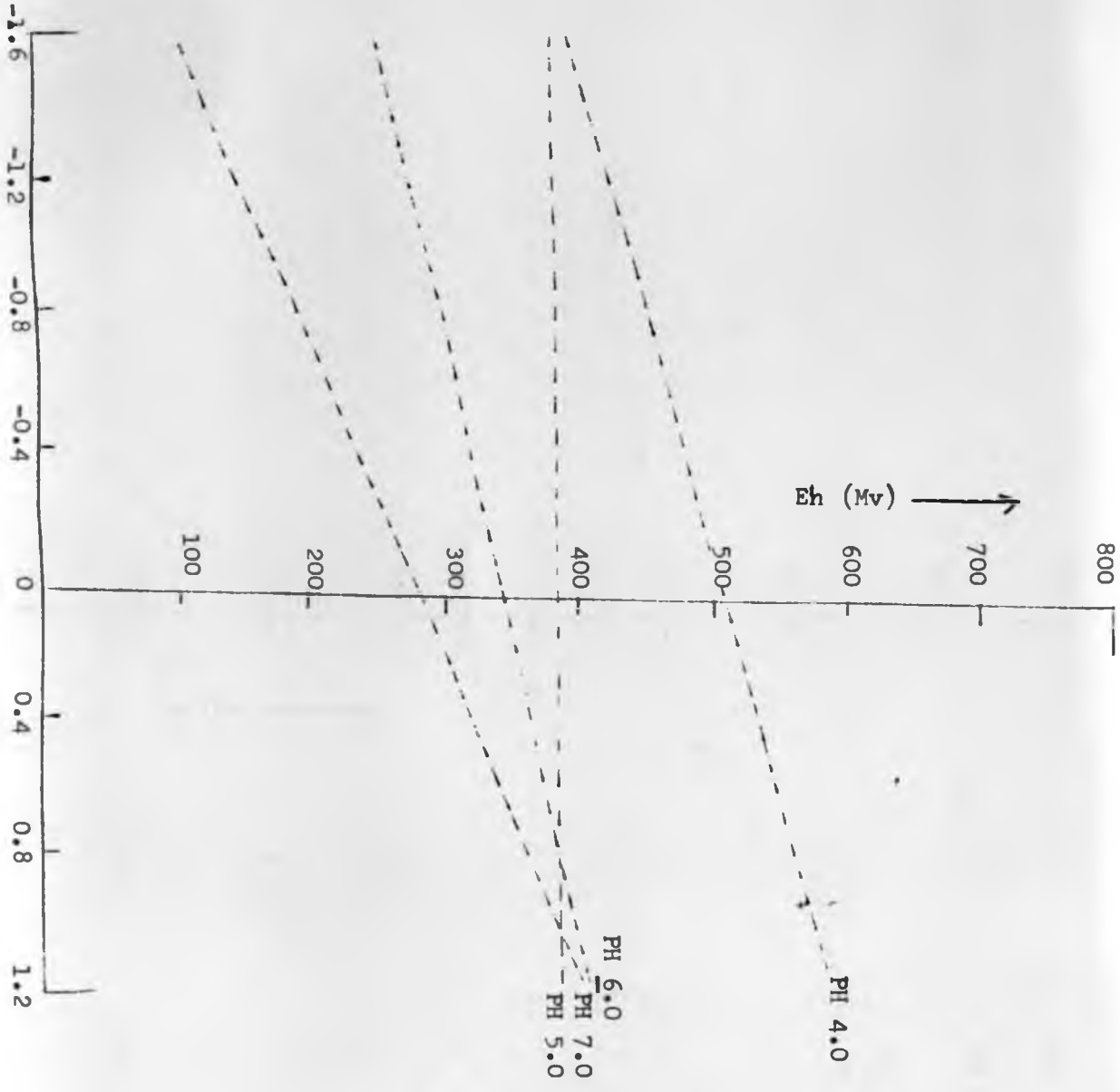
REGRESSION LINES FOR THE OXIDATION OF SODIUM METABISULPHITE DRAWN USING THE REGRESSION EQUATIONS IN TABLE (1)



7.1.4

FIGURE (7)

REGRESSION LINES FOR THE OXIDATION OF L-CYSTEINE DRAWN USING THE REGRESSION EQUATIONS IN TABLE (1)



7.1.5

FIGURE (8)

REGRESSION LINES FOR THE OXIDATION OF 8-HYDRO QUINALINE

DRAWN USING THE REGRESSION EQUATIONS IN TABLE (1)

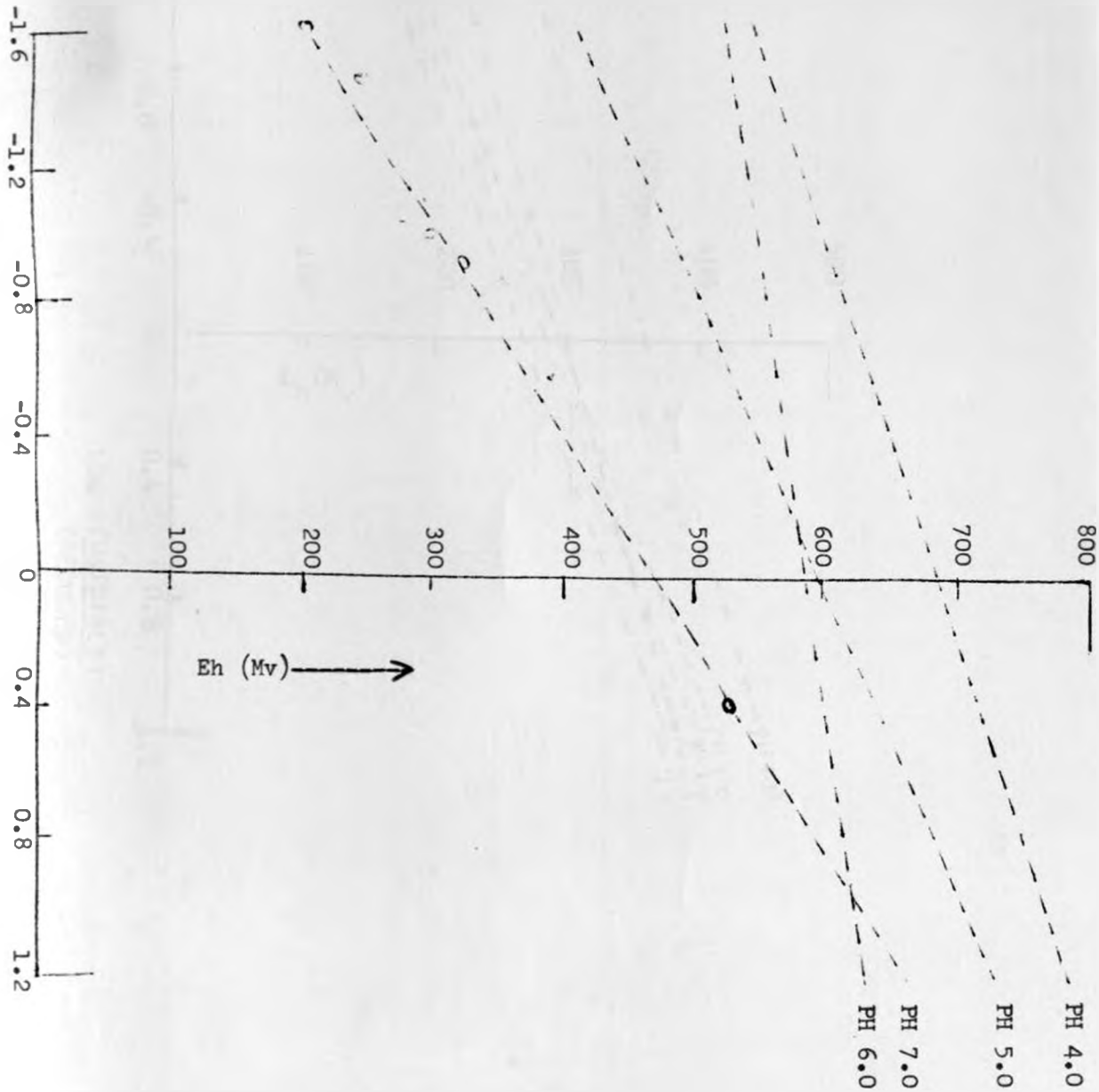
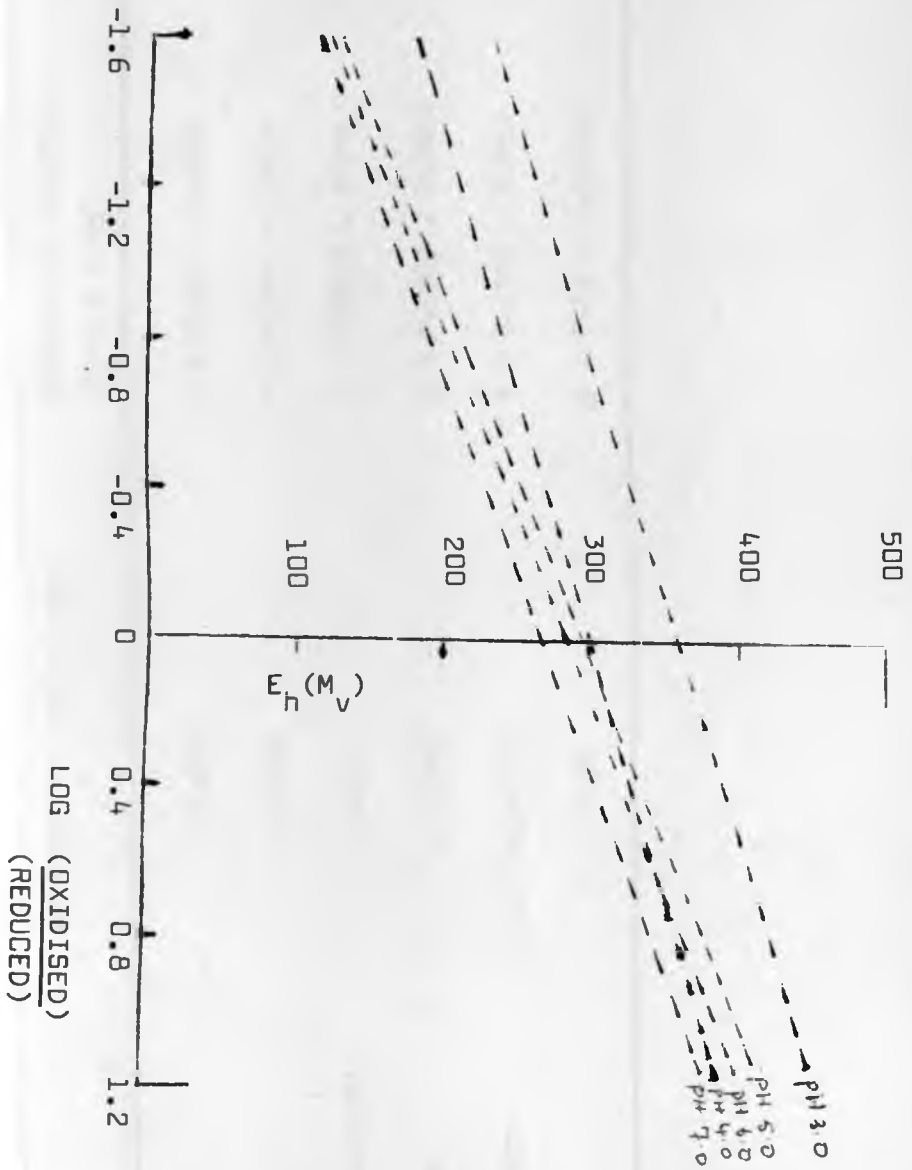


Fig 8(a)

REGRESSION LINES FOR THE OXIDATION OF SODIUM SULPHITE
DRAWN USING THE REGRESSION EQUATIONS IN TABLE 1



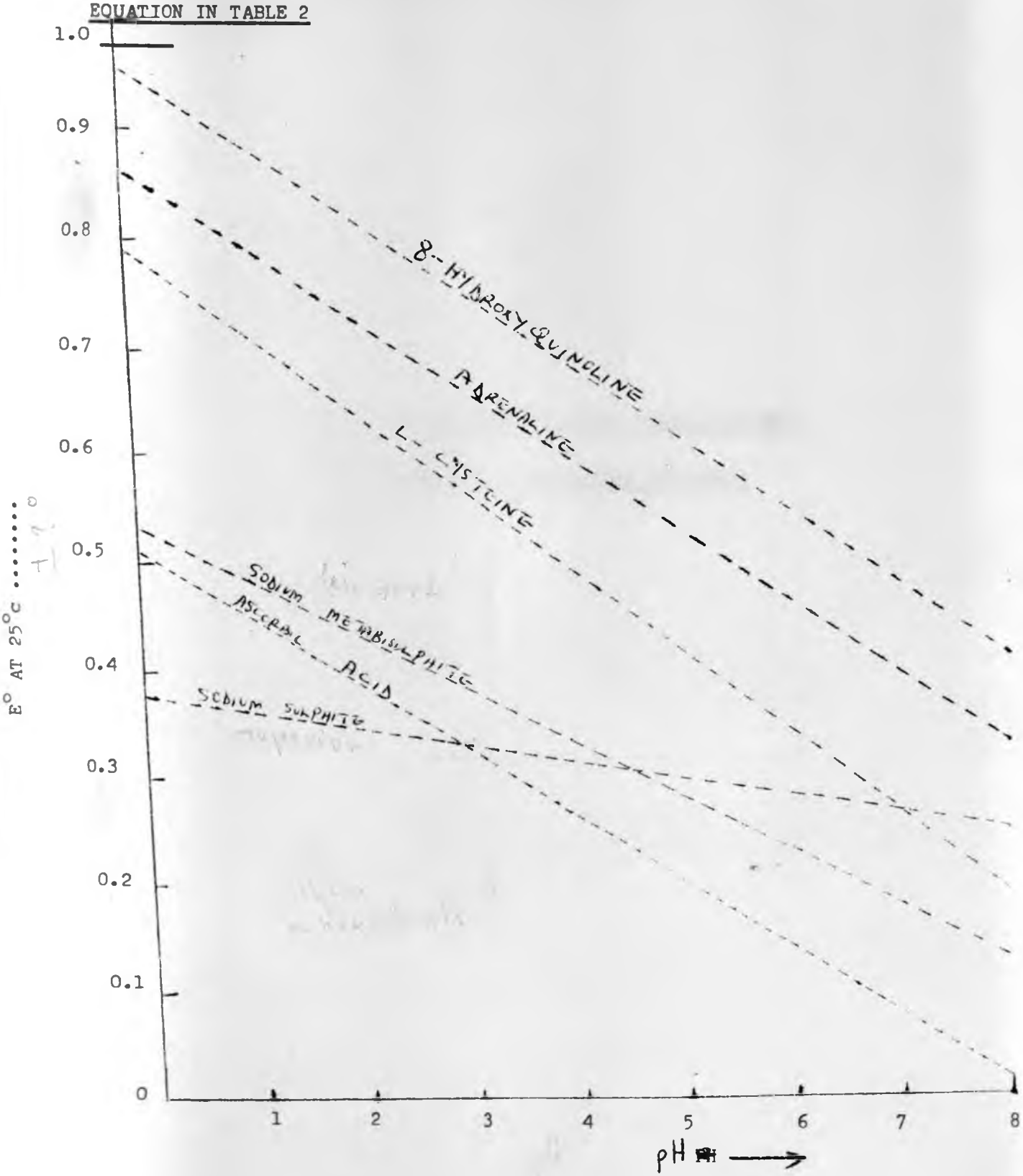
T A B L E 2 REGRESSION EQUATIONS FOR THE STRA' IGHT LINE PLOTS SHOWN IN FIGURE 9

COMPOUND	CORRELATION COEFFICIENT	REGRESSION EQUATION. (y = a + bx)
1. ADRENALINE	- 0.968	y = 0.867 - 0.068x
2. ASCORBIC ACID	- 0.933	y = 0.509 - 0.063x
3. L-CYSTEINE HYDROCHLORIDE	- 0.91	y = 0.7858 - 0745x
4. 8-HYDROXYQUINOLINE	- 0.892	y = 0.9545 - 0.069x
5. SODIUM METABISULPHITE	- 0.938	y = 0.5325 - 0.05x
6. SODIUM SULPHITE	- 0.88	y = 0.38 - 0.0161x

7.1.6 (b)

FIGURE (9)

PLOTS OF STANDARD REDOX POTENTIALS VERSUS PH USING THE REGRESSION EQUATION IN TABLE 2



3.2. RESULTS OF THE EFFECT OF HEAT STERILIZATION ON THE STABILITY OF ADRENALINE EYE DROPS

TABLE 3

DATA FOR THE CALIBRATION GRAPH FOR ADRENALINE:- ANALYSIS BY THE FERROUS SULPHATE METHOD

CONCENTRATION OF ADRENALINE BASE (MG/100 MLS) (x)	AVERAGE OF DUPLICATE ABSORBANCE VALUES AT 535 NM (y)
2.7080	0.23
4.3330	0.38
5.4160	0.49
6.4990	0.59
8.1240	0.74
9.7490	0.86
10.8380	0.92
REGRESSION EQUATION : $y = 0.0134 + 0.08625x$	

3.2.1.(b)

FIGURE (10)

CALIBRATION CURVE FOR THE ANALYSIS OF ADRENALINE BY THE FERROUS SULPHATE METHOD. REGRESSION LINE DRAWN USING THE EQUATION GIVEN IN TABLE 3

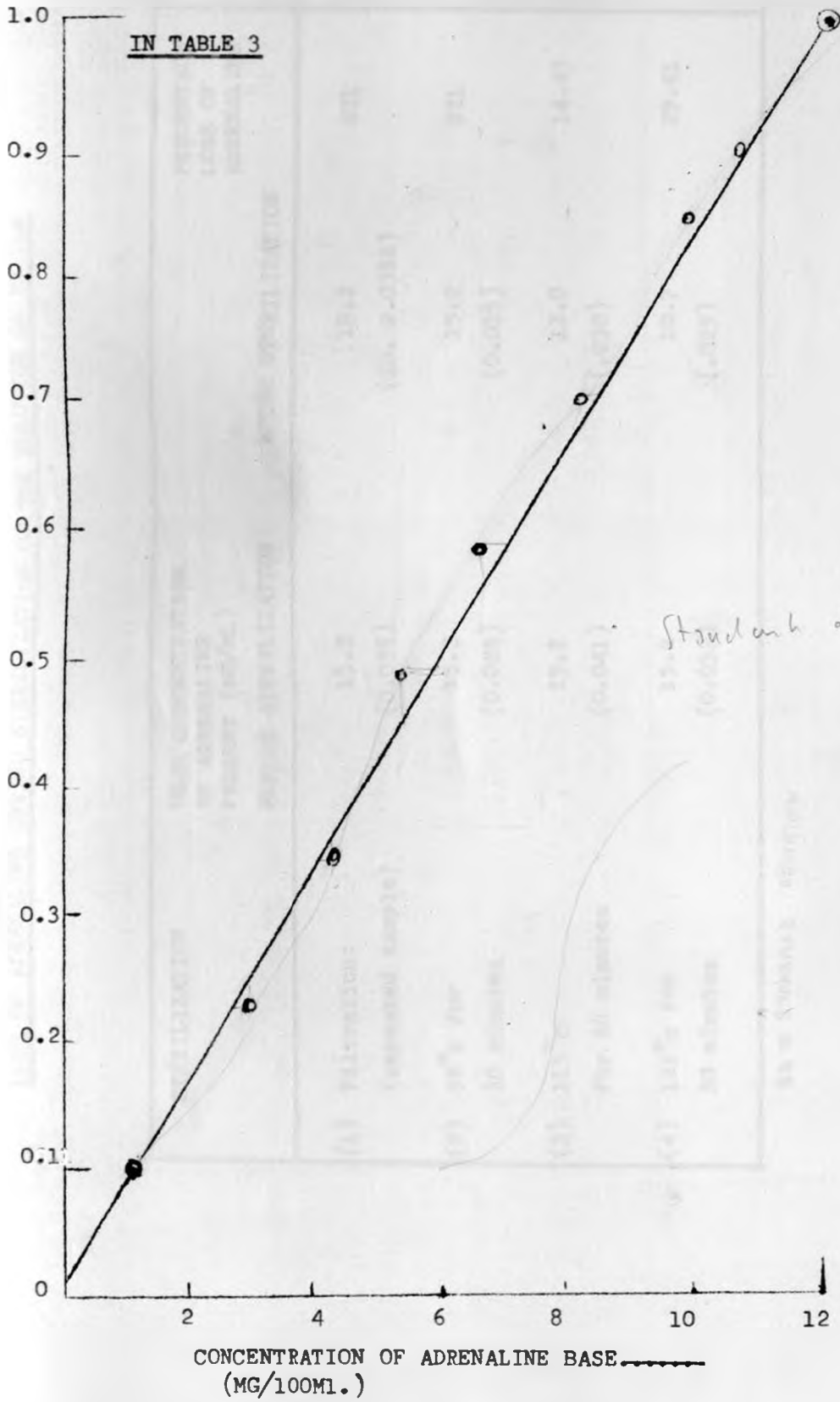


TABLE 4 (a)

LOSS OF ADRENALINE DURING STERILIZATION OF THE SOLUTION OF PH 7.4

STERILIZATION	MEAN CONCENTRATION OF ADRENALINE PRESENT (MG/ML)		PERCENTAGE LOSS OF ADRENALINE
	BEFORE STERILIZATION	AFTER STERILIZATION	
(1) Filtration: (unheated sample)	15.2 (0.038)	15.2 (SD. 0.0388)	NIL
(2) 98°c for 30 minutes	15.2 (0.024)	15.2 (0.035)	NIL
(3) 115°c for 30 minutes	15.2 (0.041)	13.0 (.030)	14.47
(4) 121°c for 30 minutes	15.2 (0.032)	10.7 (.029)	29.61

SD = STANDARD DEVIATION

TABLE 4 (b)

LOSS OF ADRENALINE DURING STERILIZATION OF THE SOLUTION OF PH 5.8

STERILIZATION METHOD	MEAN CONCENTRATION OF ADRENALINE(MG/ML)		PERCENTAGE LOSS OF ADRENALINE
	BEFORE STERILIZATION	AFTER STERILIZATION	
(1) Filtration (unheated sample)	14.2 (SD=0.012)	14.2 (0.0176)	NIL
(2) 98°c for 30 minutes	14.2 (SD=0.032)	14.2 (SD=0.0167)	NIL
(3) 115°c for 30 minutes	14.2 (SD=0.019)	13.8 (SD=0.0143)	2.82
(4) 121°c for 15 minutes	14.2 (0.024)	13.8 (SD=0.0197)	2.82

SD = STANDARD DEVIATION

TABLE 5

LOSS OF ADRENALINE ON STORAGE AT ROOM TEMPERATURE

DURATION OF STORAGE (WEEKS)	PH 5.8				PH 7.4			
	UNHEATED SAMPLE	98°c/30 MINUTES	115°/30 MINUTES	121°c/15 MINUTES	UNHEATED SAMPLE	98°c/30 MINUTES	115°c/30 MINUTES	121°/15 MINUTES
0	14.2	14.2	13.8	13.8	15.2	15.2	13.0	10.7
1	14.2	14.2	13.6	13.2	15.0	14.8	10.7	10.0
2	14.2	13.7	12.7	11.9	14.8	12.9	9.8	9.7
3	12.2	12.2	11.0	10.7	11.6	10.7	8.8	8.8
4	12.0	11.5	10.1	10.0	10.9	10.12	8.3	7.8
r =	-0.88	-0.95	-0.96	-0.995	-0.98	-0.973	-0.98	-0.98
y =	2-0.0212x	2-0.025x	2-0.036x	2-0.037x	2-0.04x	2-0.049x	2-0.0475x	2.0.03x
k =	$2.906 \times 10^{-4} HR^{-1}$	$3.416 \times 10^{-4} HR^{-1}$	$4.979 \times 10^{-4} HR^{-1}$	$5.085 \times 10^{-4} HR^{-1}$	$5.49 \times 10^{-4} HR^{-1}$	$6.77 \times 10^{-4} HR^{-1}$	$6.77 \times 10^{-4} HR^{-1}$	$4.525 \times 10^{-4} HR^{-1}$
Total % Loss	15.49%	19%	28.87%	29.58%	28.29%	33.4%	45.39%	48.68%

3.3: RESULTS OF THE EFFECT OF THE NATURE OF THE BUFFER ON THE STABILITY OF ADRENALINE AT PH 7.4

3.3.1(a)

TABLE 6

DATA FOR THE CALIBRATION CURVE FOR ADRENALINE ANALYSIS BY THE THOSEMICARBAZISE METHOD

CONCENTRATION OF ADRENALINE BASE (mg/ml) (x)	AVERAGE OF DUPLICATE ABSORBANCE READINGS AT 380 nm (y)
3.334	0.180
6.668	0.380
8.000	0.510
10.000	0.620
13.336	0.845
CORRELATION COEFICIENT (r)	= 0.998
REGRESSION EQUATION: (y)	= 0.0462+0.067x

3.3.1.(b)

FIGURE (11)

CALIBRATION CURVE FOR THE ANALYSIS OF ADRENALINE BY THE THOSEMICARBAZIDE METHOD.

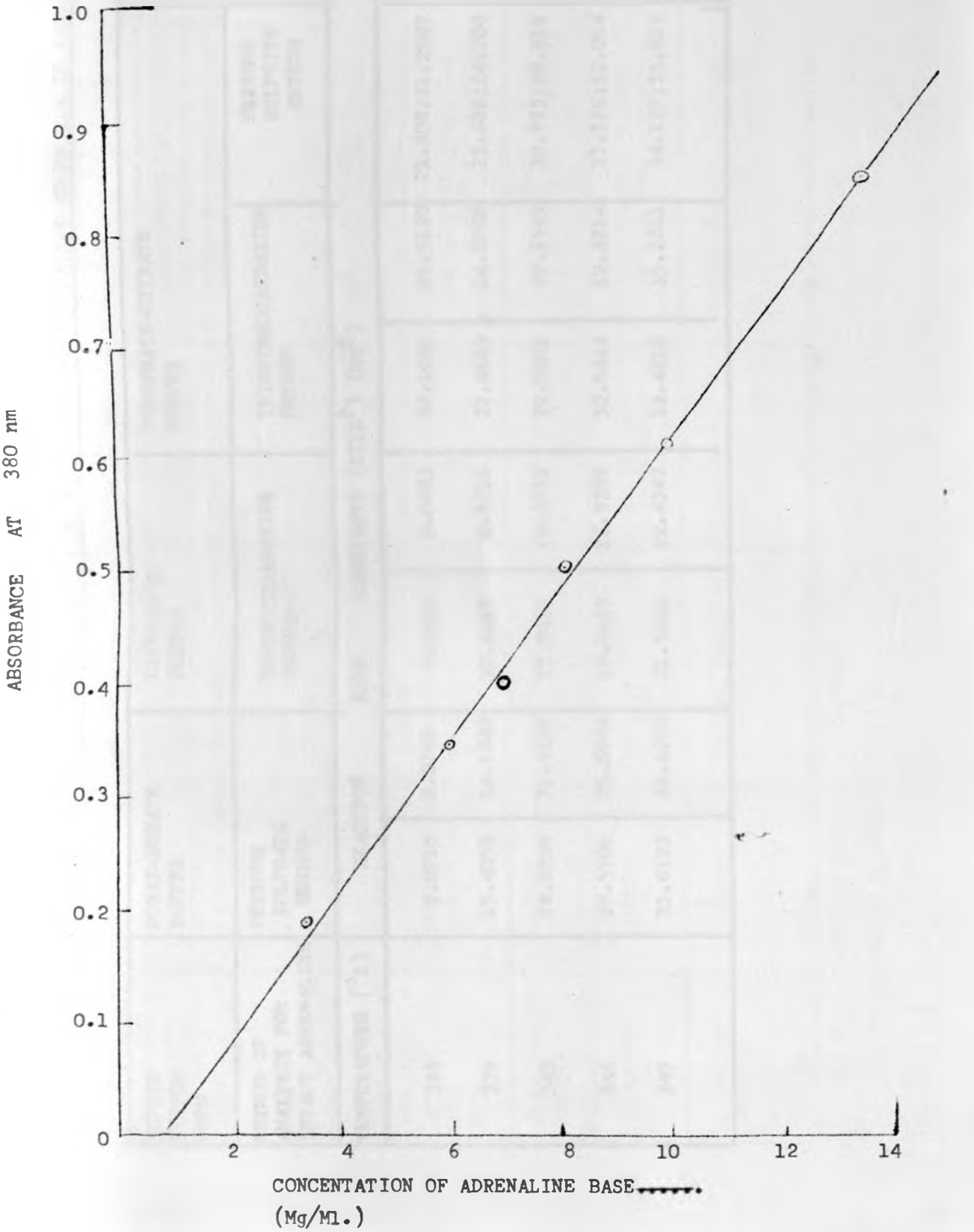


TABLE 7

SUMMARY OF ALL THE VALUES OF THE REACTION RATE CONSTANT DETERMINED AT PH 7.4 IN VARIOUS BUFFERS

BUFFER SYSTEM USED	BORAX-BORATE BUFFER		GIFFORD'S BUFFER		PHOSPHATE-CITRATE BUFFER			PHOSPHATE-CITRATE-BORATE BUFFER	
METHOD OF ANALYSIS FOR INTACT ADRENALINE	FERROUS SULPHATE METHOD		THOSEMICARBAZIDE METHOD		THIOSTEMICARBAZIDE METHOD		FERROUS SULPHITE METHOD	THIOSEMICARBAZIDE METHOD	
TEMPERATURE (^{o}K)	REACTION		RATE	CONSTANTS ($KX10^{3}$) (HR^{-1})					
333	9.0715	8.0782	9.8863	9.0683	22.1920	21.5120	21.808;21.2507	9.2640	9.4978
339	15.4055	14.1342	10.6412	9.8838	22.4689	24.8908	23.686;24.208	9.5879	10.1340
343	24.3934	21.4156	13.8417	14.4522	29.5601	27.6549	30.935;28.616	15.4264	13.7636
346	28.5706	25.6764	19.8417	16.9389	32.8444	32.4214	33.526;32.054	21.7705	20.6134
349	33.6123	33.4000	21.5660	22.4347	33.4810	35.2801	34.127;33.672	22.2868	23.7105

TABLE (8)

SUMMARY OF ALL THE EXPRAPOLATED VALUES OF THE REACTION RATE CONSTANTS DETERMINED AT PH 7.4 IN VARIOUS BUFFERS

BUFFER SYSTEM USED	AVERAGE EXTRAPOLATED REACTION RATE CONSTANT AT $25^{o}c$ $(Kx10^{3})$ HR^{-1}	VALUES FROM WHICH THE AVERAGE REACTION RATE CONSTANTS WERE CALCULATED $(Kx10^{3})$ HR^{-1}	CORRELATION COEFFICIENT (r)	REGRESSION EQUATIONS USED TO CALCULATE THE EXTRAPOLATED VALUES OF THE REACTION RATE CONSTANTS.
PHOSPHATE -CITRATE BUFFER	5.8728	6.0610 (TA) 5.6845	-0.940 -0.994	y = 5.917 - 1.53x y = 6.1356 - 1.6035x
	5.9727	5.6864 (FA) 6.2559	-0.964 -0.989	y =6.1863 - 1.6186x y = 5.7897 - 1.488x
BORAX-BORATE BUFFER	o.2387	0.2702 (FA) 0.2066	-0.952 -1.002	y = 13.9962 - 4.34x y = 14.4915 - 4.5226x
GILFORD'S BUFFER	0.8526	0.9706 (TA) 0.7346	-0.944 -0.9626	y = 9.094 - 2.714x y = 9.7696 - 2.9513x
PHOSPHATE CITRATE-BORATE BUFFER	0.6242	0.5820 (TA) 0.6663	-0.940 -0.9316	y = 10.665 - 3.249x y = 10.246 - 3.106x

KEY TA = THIOSEMICARBAZIDE ANALYSIS

FA = FERROUS SULPHATE ANALYSIS

3.4 RESULTS OF THE EFFECT OF CHANGING THE PH ON THE STABILITY OF ADRENALINE SOLUTIONS

3.4.1.

TABLE 9

SUMMARY OF ALL THE REACTION RATE CONSTANTS DEVERMINED IN PHOSPHATE-CITRATE-BORATE BUFFER, AT VARIOUSPH VALUES

TEMP (^{o}K)	REACTION RATE CONSTANTS ($K \times 10^{3}$) (HR^{-1})															
PH	6.0		AVERAGE	5.0		AVERAGE	4.0		AVERAGE	3.7	(SS)	AVERAGE	3.7 (SM=AA)			AVERAGE
333	4.4228	3.9109	4.1669	3.0559	3.8097	3.4328	2.6014	2.4731	2.5373	2.1440	2.0473	2.0957	2.8545	2.5529	2.2204	2.5426
339	5.4990	5.6643	5.6643	3.7482	4.5385	4.1434	3.2283	3.1458	3.1871	3.3454	3.1271	3.2363	3.5889	2.6276	3.0792	3.0986
343	7.4134	8.4647	8.4647	5.4975	5.5800	5.5388	4.3978	4.4107	4.4043	5.0189	4.2136	4.6163	5.8430	3.6456	3.2968	4.2618
346	10.0017	8.8277	9.4147	7.1394	7.9045	7.5220	5.0912	4.9977	5.0445	5.9076	5.9773	5.9425	6.5427	5.1086	4.0188	5.2234
349	11.7455	9.7712	10.7584	9.3467	10.6465	9.9966	7.6269	7.3728	7.4999	8.3922	7.1593	7.9758	8.2288	8.3953	7.9832	8.2024

cont...

TABLE 9

TEMP. (^{O}K)	PH	REACTION RATE CONSTANTS ($Kx\ 10^{3}$) (HR^{-1})					
		3.0		AVERAGE	2.0		AVERAGE
333		2.6519	2.6443	2.6481	4.3706	4.3412	4.3559
339		3.3400	3.8764	3.6082	4.8229	4.6867	4.7548
343		4.9703	4.7763	4.8733	6.4212	5.6198	6.0205
346		6.0807	5.9961	6.0384	7.1821	5.3931	6.2876
349		7.379	7.1569	7.1474	10.0193	10.3566	10.880

KEY: PH 3.7 SS = Sodium Sulphite as the antioxidant

SM + AA = Sodium Metabisulphite + Ascorbic acid as the antioxidants.

TABLE 10

3.4.2 SUMMARY OF ALL THE VALUES OF THE EXTRAPOLATED REACTION RATE CONSTANTS AT 25°c DETERMINED IN PHOSPHATE-CITRATE-BORATE BUFFER AT VARIOUS PH VALUES

PH OF DETERMINATION	EXPERIMENT NO.	REACTION RATE CONSTANT AT 25°c ($K \times 10^{-3}$) (HR^{-1})	(AVERAGE)	COEFFICIENT OF CORRELATION (r)	REGRESSION EQUATION FOR THE STRAIGHT LINE (y = a+bx)
6	1	0.2.775	0.2808	- 1.004	y = 10.5148 - 3.2993x
	2	0.2841		- 1.046	y = 10.2980 - 3.2317x
5	1	0.1422	0.1627	- 0.984	y = 11.39 - 3.647x
	2	0.1832		- 1.049	y = 11.09 - 3.5249x
4	1	0.1585	0.1506	- 0.9809	y = 10.3453 - 3.3214x
	2	0.1427		- 0.993	y = 10.5681 - 3.4013x
3	1	0.1613	0.1615	- 1.004	y = 10.4874 - 3.3614x
	2	0.1617	0.1615	- 0.9864	y = 10.6432 - 3.3467x
2	1	0.4685	0.4390	-0.9745	y = 8.4458 - 2.6150x
	2	0.4095		-0.9434	y = 8.7705 - 2.7292x
3.7	1) SS	0.0614	0.0652	- 1.01	y = 13.2858 - 4.320x
	2)	0.0689		- 1.01	y = 12.6535 - 4.117x
3.7	1) SM	0.1484	0.1217	- 0.9984	y = 11.0862 - 3.5506x
	2) +	0.1044		-0.9230	y = 11.3465 - 3.6737x
	3) AA	0.1122		- 0.916	y = 10.8998 - 3.5313x

KEY : (for PH 3.7)

SS = Sodium sulphite as the antioxidant

(SM +AA) = Sodium Metabisulphite and Ascorbic acid as the antioxidants.

T A B L E 11(a)

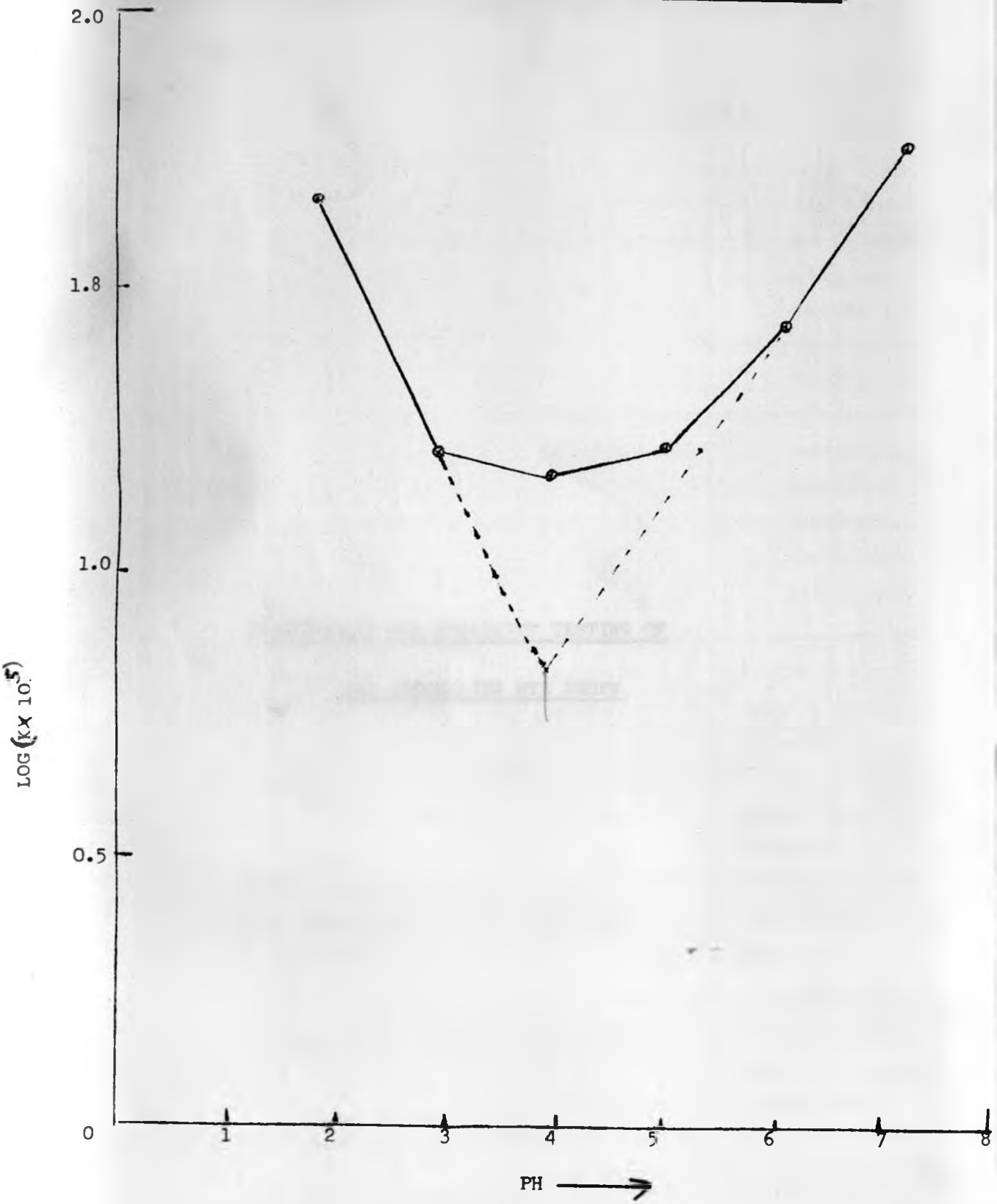
SUMMARY OF THE REACTION RATE CONSTANTS DETERMINED AT PH 5.8. ON THE FINAL FORMULATION

TEMP. (°K)	REACTION RATE CONSTANTS ($K \times 10^3$) (HR^{-1})		
	Experiment (1)	Experiment (2)	Average
298 (Extrapolated)	0.240	0.107	0.174
333	3.415	2.9492	3.182
339	4.6889	3.9646	4.3268
343	5.9319	5.3467	5.6393
346	7.896	8.707	8.302
349	9.145	9.434	9.290
	r = - 0.995	- 0.980	For the extrapolated reaction rate constants.
	y = 10.167 - 3.215x	12.357 - 3.972x	

3.4.3

FIGURE 12

PLOT OF LOG. REACTION RATE CONSTANT VERSUS PH (DATE FROM TABLE 10)



3. 5 RESULTS FOR THE STERILITY TESTING OF THE ADRENALINE EYE DROPS

T A B L E 11(b)

RESULTS FOR THE STERILITY TEST ON THE FINAL FORMULATION OF ADRENALINE EYE DROPS

SERIES	MEDIA BOTTLE NO.	CONTENTS	OBSERVATIONS	REMARKS
S T E R I L	1	Thioglycollate + 2 mls Eye drops + Staph. aureus	Growth present	(i) Medium supported growth of aerobes. (ii) Bactericide inactivated
C O N T R O	2	Thioglycollate + 2 mls. Eye drops + local isolate of anaerobic bacterium from the soil.	Growth present	(i) Medium supported growth of anaerobes. (ii) Bactericide inactivated.
	3	Sabouraud + 2 mls. Eye drops + Candida albicans	No growth present	Medium did not support growth of yeast.
	4	Thioglycollate alone	No growth	Medium was sterile
	5	Sabouraud alone	No growth	Medium was sterile.
	6	Thioglycollate + 2mls. Eye drops	No growth	No aerobic or anaerobic bacteria in Eye drops.

cont.....

cont..

T A B L E 11(b)

SERIES	MEDIA BOTTLE NO.	CONTENTS	OBSERVATIONS	REMARKS
TESTS	7	Sabouraud + 2mls. of the Eye drops.	No growth	Not possible to infer since (3) was negative.
SUB-CULTURED	8	Thioglycollate containing no lecithin + 2 mls. of the Eye drops.	No growth	Findings in (6) confirmed, i.e. the original cloudiness in (6) did not mask any growth that might have occured

3.6 RESULTS OF THE CLINICAL TESTING OF THE EFFECTIVENESS OF THE ADRENALINE DROPS IN REDUCING RAISED INTRAOCULAR PRESSURE

TABLE 12

3.6.1.(a) SUMMARY OF THE DIURNAL IOP READINGS FOR ALL PATIENTS TESTED

PATIENT NO.	EYE TESTED	MEAN DIURNAL CHANGE IN IOP AS A PERCENTAGE OF INITIAL IOP					
		DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
(1) IP.NO.423565	LE	- 1.83 (CONTROL)	- 4.76 (TEST)	+ 28.57 (TEST)	x	x	x
(2) IP.NO.3613-43	LE	- 3.55 (CONTROL)	- 7.24 (TEST)	- 42.24 (TEST, DEPIVEFRINE)	- 14.29 (CONTROL)	- 7.87 (CONTROL)	+ 9.38 (TEST)
(3) IP.NO.41805	LE	+ 1.82 (CONTROL)	- 6.82 (TEST)	- 16.25 (TEST)	x	x	x
(4) IP.NO.273089	RE	- 13.85 (CONTROL)	- 19.44 (TEST)	x	x	x	x
(5) IP.NO.1696-92	RE	-1.45 (CONTROL)	- 10.37 (TEST)	- 1.48 (CONTROL)	- 5.55 (TEST, DIPIVEFRINE)	- 9.85 (TEST)	x

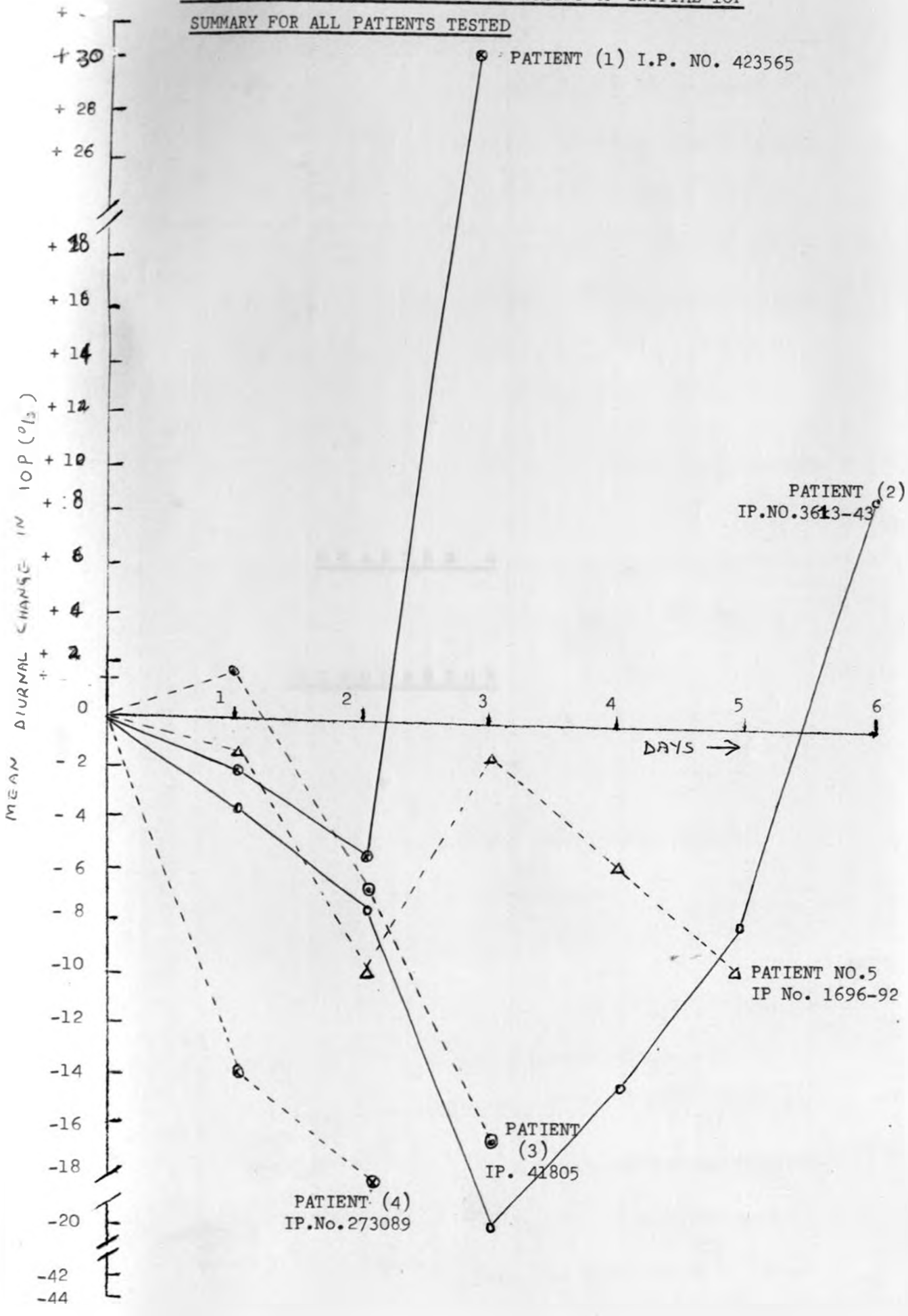
KEY: LE = Left Eye, RE = Right Eye, x = No experiment done on these days, IOP = Intraocular Pressure, IP.NO. = In-Patient Number, (-) = Values showing mean pressure reduction (as % of Initial Pressure) (+) = Values showing mean pressure increase (as % of Initial pressure) TEST = Test with 1% adrenaline, unless otherwise stated.

3.6.1.(b)

FIGURE 13

MEAN DIURNAL CHANGE IN IOP AS A PERCENT OF INITIAL IOP

SUMMARY FOR ALL PATIENTS TESTED



CHAPTER 4

DISCUSSION

4.1. PREFORMULATION SCREENING OF ANTIOXIDANTS

From Figure 9 (page..83..) the values of the standard oxidation-reduction potentials indicate (theoretically) the following order of (decreasing) effectiveness of the antioxidants.

(a) Below pH 3.0:

Sodium Sulphite > Ascorbic acid > sodium metabisulphite > L-cysteine.

(b) Between pH 3.0 - pH 5.2

Ascorbic acid > Sodium Sulphite > Sodium metabisulphite > L-cysteine.

At pH 3.0, sodium sulphite and Ascorbic Acid appear to have equal effectiveness as antioxidants.

(c) Between pH 5.2 - pH 7.0

Ascorbic acid > Sodium metabisulphite > Sodium Sulphite > L-cysteine.

At pH 5.3, sodium sulphite and sodium metabisulphite appear to have equal effectivness as antioxidants.

(d) Between pH 7.0 - pH 7.4

The order is the same as in (c) except that at pH 7.4, sodium sulphite and L-cysteine appear to have equal effectiveness as antioxidants.

AKERS (1979) found a similar trend in antioxidant effectiveness based on standard oxidation potentials (E^{o}) of Ascorbic acid, sodium metabisulphite and Acetyleysteine. He found that both sodium metabisulphite and sodium sulphite were more effective antioxidants

than Acetylcysteine but less effective than Ascorbic Acid. However, based on the change in Ceric Sulphate Equivalence volume as a function of time, he found that Acetylcysteine was next to Ascorbic acid in effectiveness, replacing the sulphite salts in the position as predicted from standard oxidation potential values. He pointed out that while a general range of E^{o} values may be acceptable as a starting point for selecting antioxidants for further testing, such E^{o} values do not relate in absolute sense to antioxidant activity over a length of storage time.

Antioxidant efficiency is dependent upon the ability of the antioxidant preferentially to be oxidised by serving as an inhibitor of the propagation of the free radical process, where such a process occurs during oxidation (OESTENDOP, 1965). However, oxidation potentials and stability studies of antioxidants in solutions without oxygenic drugs are sometimes poor prognosticators of the potential antioxidant efficiency of these substances when the complete drug product is stability-tested (AKERS, 1979).

Although the present work was designed to evalute E^{o} values of antioxidants in the absence of the oxygenic drug (adrenaline) the results obtained were taken to reflect the situation in the presence of adrenaline since they correlated well with the results obtained by AKERS who performed similar experiments in the presence of adrenaline, and over a period of time (3 weeks).

The present work showed that between pH 3.0 and 5.2, ascorbic acid was more effective than sodium sulphite as on anti-oxidant. However, accelerated stability tests at pH 3.7 showed that sodium sulphite was a more effective antioxidant than the combination of ascorbic acid and sodium Metabisulphite. This was probably due to the effect of heat during the accelerated stability tests, which resulted in the degradation of ascorbic acid. The degree of degradation of ascorbic acid was not measured, but was noted due to the colour change of the solution after prolonged heating. Therefore, in the solutions containing both ascorbic acid and sodium Metabisulphite as antioxidants, it is possible that after sometime sodium metabisulphite would play the major role of antioxidant, as the concentration of ascorbic acid would diminish faster with time. Since sodium sulphite was shown to be more effective than sodium Metabisulphite between pH 3.0 and pH 5.2, the results of the accelerated stability tests at pH 3.7 fitted the above explanation.

4.2 THE EFFECT OF HEAT STERILIZATION ON THE DEGRADATION OF ADRENALINE

4.2.1. DEGRADATION DURING STERILIZATION

Tables 4a and 4b (page..87..and ..88...) give summaries of the mean concentrations of adrenaline determined immediately following heat sterilization at pHs 7.4 and 5.8 respectively.

In both cases, sterilization by heating at 98°c for 30 minutes did not result in any loss of adrenaline. From similar experiments performed on a modified formulation of adrenaline eye drops based on the Moorfield Eye hospital formula, HAMNETT (1975) showed that there was no difference in the amount of adrenaline present in solutions heated at 98°c for 30 minutes and solutions which had been sterilized by filtration.

Earlier work on the Moorfields Hospital adrenaline eye drops, cited by HAMNETT, had reported a loss of upto 20% on heating at 98°c for 30 minutes. This reported loss probably explains why the Moorfields formula as given in Martindale (1979 (a)) includes an excess of 20% for a 1% solution of adrenaline eye drops.

Autoclaving the solutions at 115°c for 30 minutes resulted in a significant loss of adrenaline at both pHs 5.8 and 7.4 (Appendix A.2.11, page..181..). A greater loss (14.47%) occured at pH 7.4 compared to the loss at pH 5.8 (2.27%). This was explained by the fact that at the higher pH value adrenaline, like other alkaloids, becomes more unstable (ELLIS, 1977).

Autoclaving the solutions at 121°c for 15 minutes resulted in a loss of 29.6% for solutions at pH 7.4, but the same percentage loss (2.27%) was obtained for solutions at pH 5.8 as was obtained for similar solutions heated at 115°c.

The results of this work showed that autoclaving as a means of sterilization is more detrimental to solutions of adrenaline eye drops prepared at pH values near neutrality than it is to similar solutions at lower pH values. Sterilization by filtration or steaming at 98°c for 30 minutes appear to be safe over the entire pH range where most such eye drops are prepared. The present work also showed that it was not necessary to include an excess of upto 20% adrenaline base as is the case in the original Moorfield formula, if filtration or steaming were to be used as methods of sterilization. However, such an excess would be advisable if sterilization by autoclaving were contemplated.

In the final proposed formula it was decided to retain the proportion of adrenaline as had been suggested in the original Moorfields Eye Hospital formula for 1% adrenaline eye drops. This was because storage of the eye drops prepared at pH 5.8 and containing a similar proportion of adrenaline had shown that for eye drops sterilized by either filtration or heating at 98°c/30 minutes, there was a total loss of adrenaline of between 15% and 20% after one month's storage at room temperature. Since most patients

receiving such eye drops in this country are unlikely to have proper storage facilities it was considered appropriate to retain the original excess amount of adrenaline in the formula.

4.2.2. DEGRADATION DURING STORAGE AT ROOM TEMPERATURE

Table 5 (page...89.) gives a summary of the degradation rate constants of solutions of adrenaline on storage, after initial heat treatment (98°c, 30 minutes; 115°c, 30 minutes; and 121°c 15 minutes) and for solutions not initially subjected to heat treatment. The average room temperature during the entire storage period (4 weeks) was taken to be 25°c.

Tables 4a and 4b (pages..87. and ..88..) give a summary of the loss of adrenaline during the initial heat sterilization period. The results showed that degradation occured at 115°c and 121°c at both pH 5.8 and 7.4. Degradation rate were higher at the latter pH. It was also found that degradation rates were higher at 121°c than at 115°c, at both pH values.

During storage it was found that the rate of degradation of adrenaline was faster at pH 7.4 than at pH 5.8. It was found that at pH 7.4, in Borax-Borate buffer the subsequent rate of degradation on storage appeared to be unaffected by the initial method of sterilization. Table 8 (page..94.) gives a summary of the extrapolated values of the reaction rate constants from accelerated stability tests performed on adrenaline ~~solutions~~

solutions prepared in Borax-Borate buffer at pH 7.4. The reaction rate constants at 25°c from accelerated stability tests were about half the value found from the long-term storage experiment. This was not considered to be a significant difference when it is recalled that for the storage experiment it was assumed that room temperature remained constant at 25°c throughout the 4-week period, which was not the case.

The storage experiments performed on solutions of adrenaline prepared in Borate buffer at pH 5.8 showed that there was a general increase in the degradation rate the higher the initial sterilization temperature. One explanation would be a rise in pH as a result of heating, but this was ruled out since a check on the pH immediately following heat sterilization, and at the end of the 4-week storage period had shown that the pH had not changed. Another explanation would be the degradation of sodium sulphite with increasing temperature, reducing the amount available to protect adrenaline during the subsequent storage. However, it was not possible to confirm this since no experiments were performed using varying concentrations of sodium sulphite.

It had been noted earlier that the standard redox-potential versus pH plot for sodium sulphite was 'flatter' than the other plots (Figure 9, page..83.), indicating that the antioxidant effectiveness of sodium sulphite diminished more rapidly with

increase in pH than those for the other antioxidants tested. Visual examination of the adrenaline solutions prepared at pH 5.8, and stored showed that for the non-heated solutions and those heated at 98°c for 30 minutes, there was a slight discolouration after 3 weeks storage. For those solutions heated at higher temperatures, discolouration occured within a few days on storage.

Considering the effect of temperature on the subsequent rate of degradation on storage, and the fact that sodium sulphite was less effective at higher pH values, it was concluded that a formulator who wishes to prepare adrenaline eye drops at pH 5.8 would have to increase the amount of sodium sulphite slightly above the 0.1% w/v proposed in the present work. It was not possible to propose an exact amount of sodium sulphite that would suffice since time did not allow for further storage experiments which would have made this possible. However, a sodium sulphite concentrate within the range of 0.2 - 0.3% w/v was not thought to be excessive.

4.3 ACCELERATED STABILITY TESTS

It is a fairly well known fact that oxidation processes are often slower at higher temperatures because of reduced oxygen solubility (LACHMAN et al, 1970). Thus, oxidation reactions are affected not only by direct temperature effects but also by the effect of temperature on the concentration of oxygen in the solution. Therefore the predictive advantage of accelerated kinetic studies done in preformulation of oxidisable products should always be treated with caution, especially when such predictions are extrapolated to ambient temperatures and used to estimate the shelf-life of such products (AKERS, 1979).

AKERS performed accelerated stability tests on adrenaline-borate solutions at 45°c, 55°c, and 65°c over a period of several weeks and found that the resultant logarithmic plots of the rate constants were not linear. He concluded that the predicted rate constants at 25°c(average room temperature) would be accompanied by a rather sizable confidence limits . In the present study,a preliminary investigation showed that linear logarithmic plots were obtained by performing accelerated stability experiments at 60°c, 66°c, 70°c, 73°c, and 76°c.

It is possible that AKER'S results did not yield linear plots because he used too few temperature values (three) than were used in the present work (five values). The problem

of diminished oxygen solubility at higher temperatures was also not experienced in tne present work. It is possible that free radicals play a part in the oxidation of adrenaline in solution. In such a case, the solubility of oxygen would not play a significant role in the subsequent oxidation process once the reaction has been initiated.

The heating period in the present work was 30 hours, except for the few preliminary experiments done at pH 7.4, where heating was for 8 hours.

4.3.1. ACCELERATED STABILITY TESTS AT pH 7.4

A summary of the results of accelerated stability tests carried out in various buffers at pH 7.4 is given in Table 8 (page..94.). At this pH it was found that the rate of degradation of adrenaline was fastest in Phosphate-Citrate buffer and slowest in Borax-Borate buffer. When a Phosphate-Citrate-Borate buffer was used, the reaction rate was considerably slowed, though still higher than the reaction rate obtained in Borax-Borate buffer. The use of Gifford's buffer, which also contained boric acid, yielded a reaction rate value which was much lower than that obtained in Phosphate-Citrate buffer.

The above results confirmed the protective role of boric acid on the oxidation of adrenaline as has been suggested by TRAUTNER and MESSER, (1952), and by RIEGELMAN and FISCHER,(1962).

The latter workers had concluded that this protective action of boric acid was due to a chelation process with the catechol nucleus of adrenaline to form a stable 1:1 complex. It also appeared that boric acid protected ascorbic acid from degradation, as it was observed that solutions containing ascorbic acid but no boric acid turned yellow (indicating ascorbic acid degradation) more rapidly than solutions containing both asborbic acid and boric acid. Similar observations have been reported before (HAMNETT, 1975). However, this protective action of boric acid on ascorbic acid was not investigated further in the present work.

From the results of the present work, it was hypothised that boric acid slowed the rate of degradation of adrenaline in solution by two mechanisms:

(a) Directly, by chelation of the catechol nucleus, thus protecting adrenaline from degradation by molecular oxygen, and bisulphite ions, as has been suggested by RIEGELMAN and FISCHER, (1962).

(b) Indirectly, by protecting the ascorbic acid from degradation. Further experiments are needed to confirm the second hypothesis.

For all the subsequent experiments at other pH values,

it was decided to use a Phosphate-Citrate-Borate buffer for the accelerated stability tests. This was because this buffer enabled experiments to be performed over a wider pH range, and the presence of borate would retard the rate of degradation of the antioxidant ascorbic acid.

4.3.2. ACCELERATED STABILITY TESTS AT OTHER pH VALUES

Table 10 (page..98.) gives a summary of all the average values of the extrapolated reaction rate constants at room temperature ($25^{\circ}c$) determined at other pH values, in phosphate-Citrate-Borate buffer. A plot of the log. values of the average reaction rate constants versus the pH is given in Figure 12 (page..100..). The plot showed a point of inflexion between pH 3.0 and pH 4.0. The extrapolated portions of the first straight sections of the curve on either side of this pH range intersected at a point corresponding to pH 3.7.

A solution of adrenaline formulated of pH 3.7 was then subjected to accelerated stability tests, and the results showed that the reaction rate constant at this pH was lower than values obtained at pH 3 and 4 respectively. A point of maximum stability was therefore assumed to exist at approximately pH 3.7. It would have required the performance of several accelerated stability tests around pH 3.7 to determine the exact pH of maximum stability.

The existance of a pH of maximum stability around pH 3.7 was first shown indirectly by WEST (1950), who used a physiological method to determine the amount of adrenaline remaining. For adrenaline solutions of the same strength and prepared in buffers of different pH values, the immediate physiological activity should be greater in the solution at the higher pH because more adrenaline molecules can pass through the cell membrane at this pH. However, on storage over along period of time, the concentration of adrenaline will be less in the solution stored at the higher pH than that at the lower pH because adrenaline is more stable in the latter solution. Therefore if the physiological activity is monitored over a long period of time, the higher activity will be obtained with the solution stored at the lower pH value.

The explanation given above was the principle behind WEST'S work. He prepared adrenaline solutions in tartaric acid and adjusted the pHs to 3.0, 3.6 and 4.6. Some samples were not subjected to any heat treatment while others were heated at 115^{o}c for 30 minutes, 3 hours and 6 hours respectively, prior to storage. He then determined the physiological activity during storage, by utilizing the contraction produced on the nictating membrane of the cat. He found that samples stored at pH 3.6 showed the greatest activity.

The accelerated stability tests performed at pH 3.7

were also used to compare the effectiveness of sodium sulphite and the combination of sodium metabisulphite and ascorbic acid as antioxidants. The results (Table 10, page..98..) showed that sodium sulphite was more effective than the latter combination. The correlation of these results with the results for the standard redox potentials was discussed in section 4.1. (page..187..).

4.3.3 LIMITATIONS OF ACCELERATED STABILITY TESTS

Accelerated stability tests have certain limitations which will be discussed briefly in this section.

When the decomposition process is complex, involving a series of simulteneous and/or consecutive reactions each having a characteristic energy of activation, accelerated stability tests may produce a change in the relative contributions of the component reactions. This could result in errors in the predicted shelf-life. As was stated elsewhere (page..8..) the oxidation of adrenaline in solution appears to be a complex reaction, and this could be a major source of error in the extrapolated reaction rate constants.

Secondly, the average room temperature was assumed in the present work to be 25°c. The average room temperature in this country may be quite different from this value. A more realistic approach would have been to use simulated cycling ovens which repeatedly cycle the product through a wide range of temperatures.

This was not feasable in the present study.

Because of the above limitations, no attempt was made in the present work to predict the shelf-life of adrenaline preparations that one would expect in this country.

4.4 FORMULATION AND CLINICAL TESTING OF THE ADRENALINE EYE DROPS

4.4.1. FORMULATION:

The details of the ingredients in the formulation were given in section 2.7.1. (page..62..). It was mentioned briefly in section 2.7.1. that borate buffer was used because one of the advantages was the low buffer capacity. The calculation given in Appendix 5 (page..201..) shows that at pH 5.8, borate buffer has a buffer capacity of only 0.145% of the maximum buffer capacity which is a very low value and would ensure rapid neutralisation when a drop of such a buffer is instilled into the eye.

A solution of similar composition to the final formulation was also subjected to accelerated stability tests. The results are shown in Table 9 (page..96..). The extrapolated reaction rate constants are given in Table 11 (page..99..). The average extrapolated reaction rate constent at pH 5.8 in borate buffer was found to be intermediate between the values obtained at pHs 5 and 6 in Phosphate-Citrate-Borate buffer. At the latter two pH values, a combination of sodium metabisulphite and ascorbic acid was used as the antioxidant, whereas at pH 5.8 sodium sulphite was used as the antioxidant. From the values of the standard redox potentials in Figure 9 (page..83..) it can be seen that both sodium metabisulphite and ascorbic acid were

shown to be more effective antioxidants than sodium sulphite at pH 5.8. However, the results of the accelerated stability tests at pH 5.8 showed that using sodium sulphite alone as the antioxidant was reasonable since a reaction rate within the range obtained using sodium sulphite alone would have been expected if the combination of sodium metabisulphite and ascorbic acid had been used instead.

4.4.2 STERILITY TESTING

After sterilization of the eye drops by heating at 98°c for 30 minutes, a sterility test was performed. The results are summarised in Table 11(b)(page.192.).

No anaerobic or aerobic bacteria were detected in the eye drops. The growth media used were also shown to be sterile and capable of supporting bacterial growth. The presence of solibilised lecithin in the bacterial growth media resulted in a cloudy appearance before the start of the testing. It was therefore necessary to subculture the incubated media in a similar media, but having no activator. This was to make sure that no growth had escaped notice due to the slight cloudiness of the media containing the activator. Inspection after incubation for a further one week revealed no growth.

There was no growth in all the fungal tests and the controls. It is possible that the medium used lacked growth promoting properties, but this could not be confirmed as the experiment was not repeated. It was, however, decided to proceed with the clinical tests as it was felt that since the eye drops were free of bacterial contamination the chances of fungal contamination were remote.

4.4.3. CLINICAL TESTING OF ADRENALINE EYE DROPS

The new formulation was tested on a total of five patients with glaucoma. All the patients tested were advanced cases of glaucoma simplex that were admitted for surgery as they could not be controlled medically as out-patients.

These patients were high risk patients who needed to be placed on a treatment regime immediately while awaiting surgery. Therefore the patients could not be tested on adrenaline alone. The time interval during the 'control' days when the patients were not receiving adrenaline could be critical if the pressure rose to dangerously high levels. This could easily result in total loss of vision(KLAUSS, 1981).

For the purposes of the experiment, therefore, it was decided to take the diurnal variations in the intraocular pressure when patients were receiving the prescribed medication (usually pilocarpine drops) as the 'controls'. These were then compared with the variations in the intraocular pressures obtained when the patients received adrenaline eye drops in addition to the prescribed medication.

Since the duration for the clinical testing varied from patient to patient and was less than one week for all the patients, it was not possible to make a comparision of the long term prognosis of all the five cases. Also as can be seen from

the summary of the treatment schedule given in Table 17 (Appendix 3.1.a, page..183.) the treatment was not uniform for all the five patients tested.

Considering the first 'test' day (day 2) following the first 'control' day (day 1) which was common to all the five patients tested (Table 12, page..105.) it was noted that there was a greater drop in the mean intraocular pressure on day 2 when adrenaline was included in the treatment schedule than on day 1 when no adrenaline was given. In all the five cases, the mean percentage drop in the intraocular pressure on day 2 was more than 50% of the pressure drop obtained on day 1. This could not be explained on the basis of chance alone. It was therefore concluded that the new formulation of adrenaline eye drops was effective in reducing raised intraocular pressure in glaucoma.

The pressure variations on subsequent test days with adrenaline showed the same general trend of a more pronounced pressure reduction on the test day as compared to the control day. There was, however, one exception (patient no.2, Table 12) who showed an increase on the mean diurnal intraocular pressure on a test day (day 6) as compared to control days (days 4 and 5). However, this particular patient had presented a long history of poor control of pressure by medication. The gradual rise in the mean diurnal intraocular pressure from day 4 to day 6 was

therefore not considered to be unusual.

Adrenaline is rarely given alone for the management of glaucoma, except in such cases as in Neovascularisation glaucoma (KLAUSS, ., 1981). It was therefore concluded that the new formulation for adrenaline eye drops would be an effective additional therapy in glaucoma when used together with other medications for glaucoma management.

In the present work, two of the patients received Dipivefrin on certain test days, and adrenaline on other test days. In one case, the reduction in intraocular pressure was greater on the test day when Dipivefrin was given, while in the other patient the reduction was greater on the test day when adrenaline was given as compared to the day when Dipivefrin was given. It was impossible to make a proper comparision with the results from two patients only. However, the experience in this hospital has been that patients given Dipivefrin show no better prognosis than those given adrenaline drops (KLAUSS, 1981).

Dipivefrin is a prodrug of adrenaline which has been produced as an alternative to adrenaline, with fewer side effects. These side effects include allergic blephero conjuntivitis (GARNER et al, 1959; BECKER et al, 1961; BECKER and MORTON, 1966), deposits of adrenochrome in the corneal and conjunctival epithelium

(CORWIN and SPENCER, 1963; REINECKE and KUWAMARA, 1963; FERRY and ZIMMERMAN, 1964) and occasional blurred vision and corneal edema (BECKER, 1967). The aphakic eye is suceeptible to the development of macular edemas (KALKER and BECKER, 1968). Cardiac arrhythmias, with extrasystoles, and elevation of systemic blood pressure require cessation of therapy in a number of patients (BALLIN et al, 1968).

Dipivefrin has been claimed to have fewer side effects because it is more lipophilic than the parent compound and therefore can be used at a much lower concentration. It has been shown to be ten times more lipophilic than adrenaline (WEI et al, 1978), and is converted to its pharmacologically active form, adrenaline, by hydrolysis by estearase enzyms witnin the eye (ABRAMOVSKY and MINDEL, 1979).

KABACK et al (1976) noted that 0.1% Depivefrin instilled twice daily for one month showed no side effects, but that the response was dramatic in some patients, while in others it had minimal effect. It was not possible confirm these findings in the present work.

From the points raised in this section it would appear that a number of points still need to be investigated further as far as formulations of adrenaline eye drops are concerned. These include the following:

(a) Investigation of the protective action of boric acid on solutions containing ascorbic acid, at various pH values and in various buffers. This would reveal whether there are particular formulating conditions where ascorbic acid could be used as the sole antioxidant in place of sodium sulphite or a combination of sodium Metabisulphite and ascorbic acid.

(b) Repetition of the work done by WEST (1950) but using a wider pH range and different buffers. This would reveal whether the same exhibition of a point of maximum stability shown in the present work is shown from physiological assays, and with different buffers.

(c) Not much clinical investigation has been done to compare the extent of side effects produced when using Dipivefrin and adrenaline on patients over a long period of time. It would also be worthwhile to investigate further the response when using 0.1% Dipivefrin and that obtained when using 1% adrenaline. Theoretically, there should be no difference, but at present there is no sufficient clinical data to support this claim.

S U M M A R Y

The different factors affecting the stability of adrenaline in solution have been examined with a view to producing a pharmaceutically active eye drop preparation of adrenaline. It was important that such a formulation should be simple enough to enable preperation using the available facilities in this country.

A preformulation screening of antioxidants in the absence of adrenaline showed that at low pH values (around pH 3.0) sodium sulphite was superior to either sodium metabisulphite or ascorbic acid. Accelerated stability studies showed that the pH of maximum stability for aqueous solutions of adrenaline was approximately pH 3.7. Accelerated stability tests at this pH confirmed the superiority of sodium sulphite over a combination of sodium metabisulphite and ascorbic acid as antioxidants.

Accelerated stability studies also confirmed the important role of boric acid in enhancing the stability of adrenaline in aqueous solutions.

An investigation of four sterilization procedures showed that the immediate loss of adrenaline was negligible after either sterilization by filtration or by heating at $98^{o}c$ for 30 minutes. Higher sterilization temperatures caused substantial loss of adrenaline and discolouration of the solutions.

For reasons of comfort to the patient on instillation

into the eye and for clarity of the solution in presence of the preservative used (Benzalkonium Chloride), a final formulation of adrenaline eye drops was prepared in borate buffer at pH 5.8, with sodium sulphite as the antioxidant. Accelerated stability studies and long term storage studies at ambient temperatures showed that the final preparation was reasonably stable. Clinical testing of the preparation on hospitalized glaucoma patients showed that the preparation compared favourably with commercial and other preparations used in the management of raised intraocular pressures.

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Elsevier Scientific Publi. Co.

New York.

(9) European Pharmacopoeia (vol. 2) 1971

APPENDIX

A P P E N D I X 1

1. SCHEMATIC REPRESENTATION OF AQUEOUS HUMOR DYNAMICS IN THE NORMAL EYE.

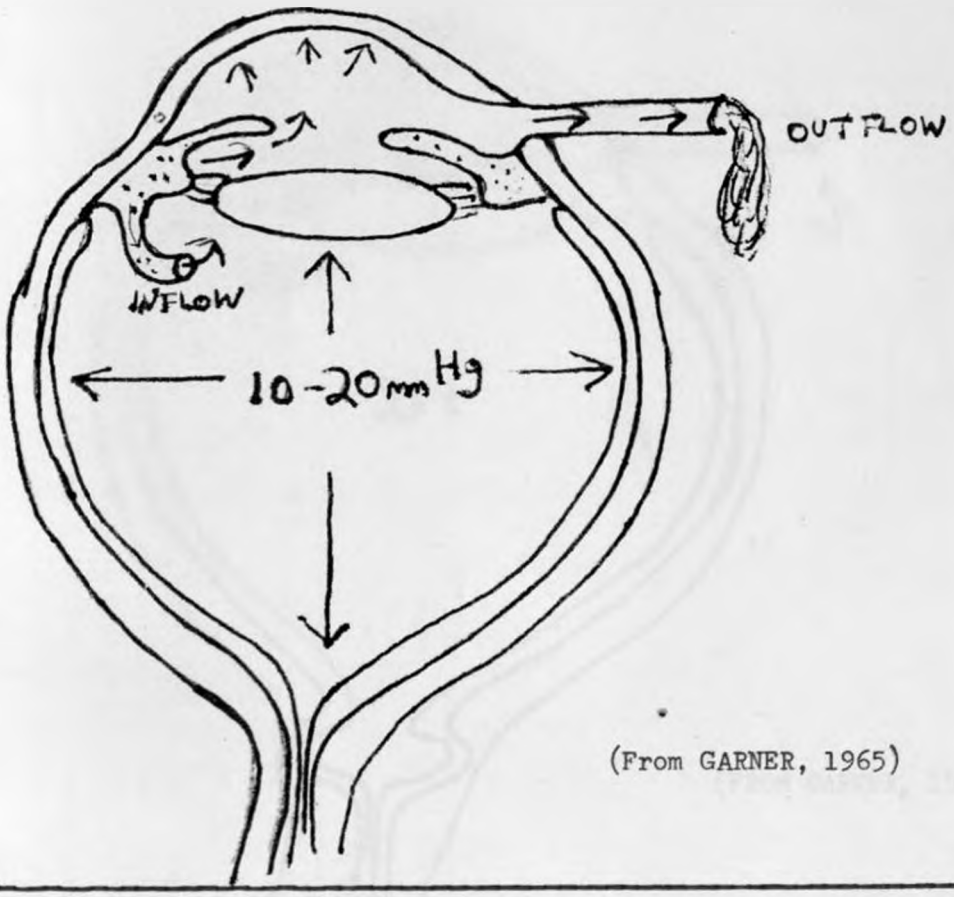
2. SCHEMATIC REPRESENTATION OF AQUEOUS HUMOR DYNAMICS IN OPEN-ANGLE GLAUCOMA.

3. SCHEMATIC REPRESENTATION OF AQUEOUS HUMOR DYNAMICS IN ANGLE-CLOSURE GLAUCOMA.

4. SCHEMATIC REPRESENTATION OF THE FLUORESCEIN-STAINED TEAR FILM RINGS AS SEEN DURING APPLANATION TONOMETRY.

A.1.1.

THE NORMAL EYE

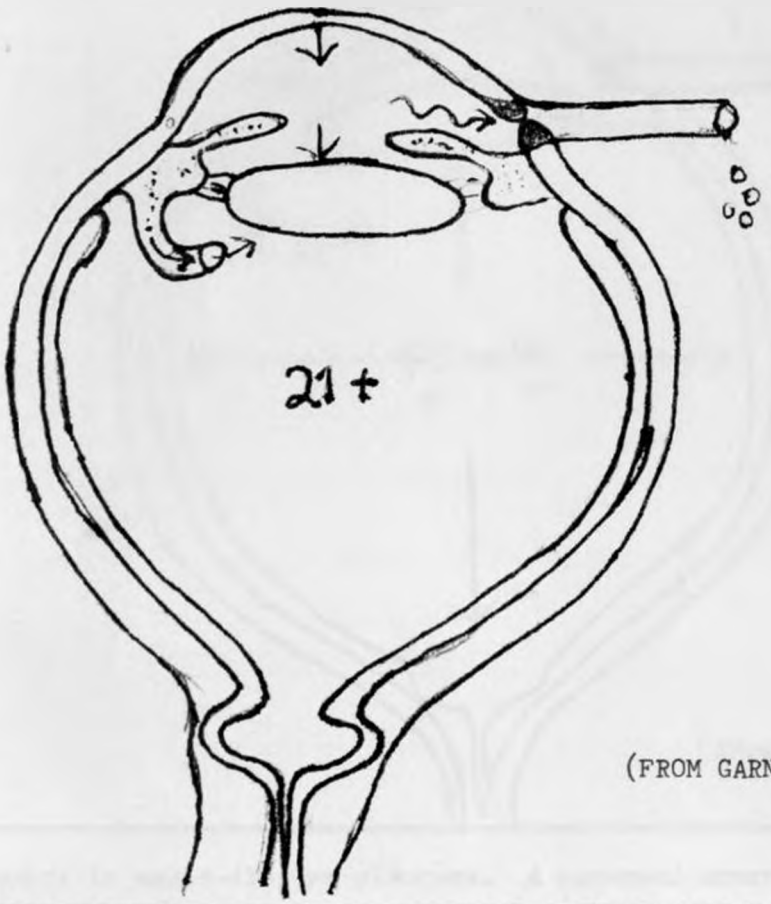


(From GARNER, 1965)

The normal intraocular pressure dynamics. The normal IOP dynamics can be visualized in the diagram above and it represents the steady-state that exists under normal conditions. Normal IOP is maintained by a steady-state of aqueous inflow due to secretion of aqueous from the ciliary body and a steady outflow via the chamber angle mechanisms.

A.1.2

OPEN-ANGLE GLAUCOMA

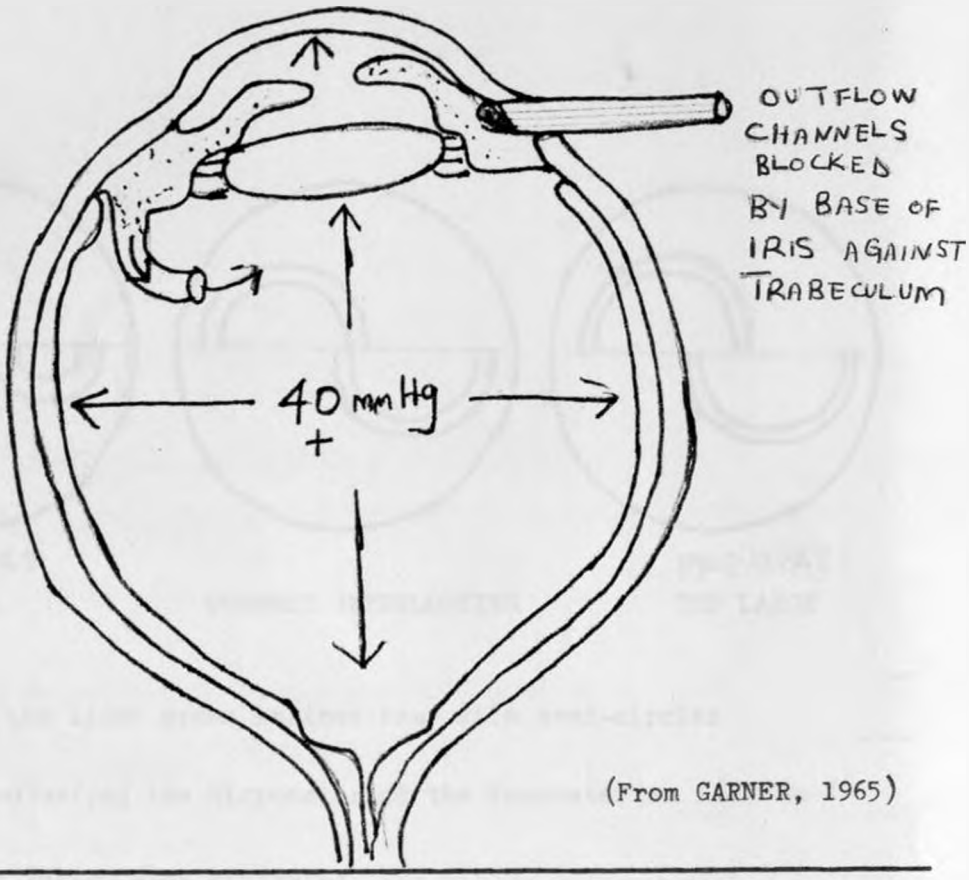


(FROM GARNER, 1965)

Aqueous humor dynamics in open-angle glaucoma. The anterior chamber remains deep with chamber angles easily visualized. The flow of aqueous is produced from the posterior chamber and meets with obstruction to the egress of aqueous from the anterior chamber. The obstruction cannot be visualized anatomically but may be illustrated diagrammatically since the outflow facility impediment takes place somewhere in the exit channel system and is depicted as a narrowed outlet in this schematic drawing. As a result, the aqueous fluid leaves the eye slower than the aqueous secreted into the anterior chamber, producing a gradual rise in IOP. Given sufficient time, this pressure will compress the optic nerve fibres against

A.1.3

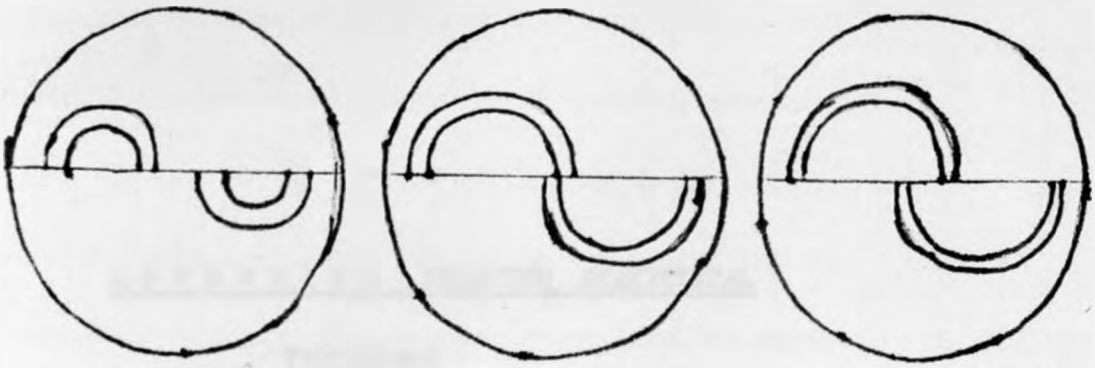
ANGLE-CLOSURE GLAUCOMA



Aqueous humor dynamics in angle-closure glaucoma. A narrowed anterior chamber is usually easily demostrable with chamber angle poorly visualized. The flow of aqueous through exit channels, beginning in the trabecular meshwork, may be obstructed by any condition causing the base of the iris to impinge against the trabecular area sealing off the out flow site. Dilatation of the pupil in such an individual is capable of setting off the acute picture of angle-closure. Outflow is suddenly reduced to almost zero, and the IOP rises rapidly to height well over 40 MM Hg. If permitted to continue indefinitely, nerve head damage will ensue with permanent loss of vision and eventual cupping of the nerve head.

A.1.4.

APPLANATION TONOMETRY



Pattern of the light green stained tear film semi-circles seen when adjusting the Micrometer of the Tonometer to measure the intraocular pressure.

A P P E N D I X 2 SELECTED STATISTICAL PROCEDURES

(a) Derivation and Use of Regression Equations in sample calculations.

(b) The Student's t-Test.

REGREGRESSION ANALYSIS

A.a.1. INTRODUCTION

A brief outline of the principles underlying the derivation and use of regression equations will be given here. However, the derivations of the formulae used will not be given as these can be found in any standard Textbook of statistics. The sample calculations which follow this introduction will illustrate the procedures followed in determining some of the data given earlier in the results section where regression equations for calculating the individual parameters in the main regression equation will be given once only, to serve as basis for reference.

A.2.1.1. LINEAR REGRESSION

Broadly defined, regression analysis is "the analysis of relationships among variables" (CHATTERJIE and PRICE, 1977). It tries to answer the question, "how does variable Y depend on variable X." (KERLINGER, 1973).

The format for data to be analysed with regression analysis consists of multiple observations on a dependant variable Y and an independant variable X. The relationship can be expressed as a linear Model thus:

$$Y = a + bX \quad \ldots\ldots\ldots\ldots\ldots (1)$$

where a and b are constants in the regression equation (NELSON,

1981). The analysis assumes that, in the range of observations studied, the linear equation provides a reasonable approximation to the true relationship between X and Y.

A.2.1.2. ESTIMATION OF THE REGRESSION CONSTANTS

The constants a and b are estimated with sample data from the linear function given in equation(1), where 'a' is called the Y intercept and 'b' is called the slope. They are determined using the following formulae:

$$b = \frac{\Sigma(x-\bar{x})(y-\bar{y})}{\Sigma(x-\bar{x})^2} = \frac{\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}} \quad \cdots (2)$$

where n is the number of observations.

$$a = \bar{Y} - b\bar{X} \quad \ldots\ldots\ldots\ldots (3)$$

where $\bar{X}$ and $\bar{Y}$ are the means of the X and the Y observations respectively.

A.2.1.3. INDEX OF FIT.

The index of assesing the goodness of fit of the regression model to the observed data is the Pearson Product Moment Correlation Coefficient (r). Generally, correlation coefficients such as r show if high measures of one variable tend to be associated with high measures on the other variable, or low values or none (JACKSON, 1980).

Correlation coefficients usually vary from - 1 to + 1 and give information on two aspects of the relationship:

1. the direction of relationship, and 2. the strength of the relationship.

If the correlation coefficient is positive, this means that as one variable increases, so does the other. If the correlation coefficient is negative, if indicates that as one variable increases, the other decreases.

A correlation coefficient (r) of + 1 indicates a perfect positive relationship, - 1 indicates a perfect negative relationship, and 0, no relationship. The square of the correlation coefficient, r^2, is another useful index of fit (NELSON, 1981). It is called the coefficient of determination and may be interpreteted as the proportion of total variation in the dependent variable Y explained by the independent variable X.

Sample calculations from the redox determinations and the accelerated stability tests will now be given to illustrate the use of regression equations. Coefficient of correlation (r) will be calculated using the following equation:

$$r = \frac{\Sigma(x-\bar{x})(y-\bar{y})}{\sqrt{\Sigma(x-\bar{x})^2\Sigma(y-\bar{y})^2}} = \frac{\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n}}{\sqrt{\left(\Sigma x^2 - \frac{(\Sigma x)^2}{n}\right)\left(\Sigma y^2 - \frac{(\Sigma y)^2}{n}\right)}} \quad \cdots (4)$$

A.2.2.(a)

TABLE 13

STANDARD OXIDATION-REDUCTION POTENTIAL OF ADRENALINE DETERMINED IN PHOSPHATE-CITRATE BUFFER. PH 2.2

VOLUME OF Ce^{4+} SOLUTION USED MLS.	AVERAGE VOLTAGE ACROSS HALF-CELL MV	REPLICATE VALUES OF VOLTAGE MV	REDOX POTENTIAL (Mv) (Y)	CONC. OF OXIDISED ADRENALINE (OX) (g/50MLS)	CONC. OF REDUCED ADRENALINE (RED) (g/50MLS).	LOG. (OX)/(RED) (X)	(X^2)	(XY)
0.50	380	380,380	662	0.0035	0.1303	-1.570	2.47	-976.54
1.00	400	390,410	642	0.0070	0.1268	-1.258	1.58	-807.64
1.50	420	415,425	662	0.0105	0.1233	-1.070	1.14	-708.34
2.00	430	430,430	672	0.0140	0.1198	0.930	0.87	-624.96
2.50	440	440,440	682	0.0175	0.1163	0.820	0.68	-559.24
3.00	445	440,450	687	0.0210	0.1128	0.730	0.53	-501.51

WEIGHT OF ADRENALINE POWDER USED=0.1338g ADRENALINE BASE.

1 ML 0.157N Ce^{4t} ≡ 0.007g ADRENABLNE BASE

REGRESSION EQUATION: Y = 745.61 + 78.97x

A.2.2.(b) CALCULATION OF THE STANDARD OXIDATION-REDUCTION POTENTIAL OF ADRENALINE AT PH 2.2 DATA FROM TABLE

$\Sigma x = -6.378$ $\quad\quad$ $\Sigma y = 3967$

$(\Sigma x)^2 = 40.679$ $\quad\quad$ $(\Sigma y)^2 = 15737089$

$\bar{X} = -1.063$ $\quad\quad$ $\bar{Y} = 661.1667$

$\Sigma x^2 = 7.27$ $\quad\quad$ $\Sigma y^2 = 2625969$

$(\Sigma x)^2/n = 6.78$ $\quad\quad$ $(\Sigma y)^2/n = 262284.1$

$\Sigma x^2 - (\Sigma x)^2/n$ $\quad\quad$ $\Sigma y^2 - (\Sigma y)^2/n = 3121$

$$\Sigma xy = -41.78.226$$

$$\frac{(\Sigma x)(\Sigma y)}{n} = -4216.92$$

$$\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n} = 38.694 \ldots\ldots(1)$$

$$\left(\Sigma x^2 - \frac{(\Sigma x^2)}{n}\right)^n (\Sigma y^2 - (\Sigma y)^2/n) = 1592.29$$

$$\sqrt{1592.29} = 39.106\ldots..(2).$$

Dividing 1 by 2, we have,

$r = 0.989$

Also $b = 78.97$

$a = 745.61$

$Y = 745.61 + 78.97\ X.$

This sample calculation for adrenaline will serve as model for the rest of compounds whose redox data are summarised in the Tables which follow.

A.2.7.(a)

TABLE 14

DATA FOR THE STANDARD REDOX POTENTIALS FOR SODIUM METABISULPHITE AT VARIOUS PH VALUES USED TO ILLUSTRATE THE DERIVATION OF THE REGRESSION EQUATIONS FOR THE STRAIGHT LINE PLOTS SHOWN IN FIGURE 9 (PAGE 83)

PH AT WHICH E° WAS DETERMINED (x)	(x^2)	STANDARD REDOX POTENTIAL (VOLTS) AT 25°c (y)	(xy)	(y^2)
4	16	0.320	1.280	0.1024
5	25	0.300	1.500	0.0900
6	36	0.235	1.410	0.0550
7	49	0.175	1.225	0.0306

COEFFICIENT OF CORRELATION (r) = - 0.980

REGRESSION EQUATION : y = 0.5325 - 0.05x

A.2.7. (b) USING DATA SHOWN IN TABLE 14 TO ILLUSTRATE THE GENERATION OF THE REGRESSION EQUATIONS UNED FOR THE STRAIGHT LINE PLOTS SHOWN IN FIGURE 9 (PAGE 83)

$n = 4$

$\Sigma x = 22$ $\qquad$ $\Sigma y = 1.03$

$\bar{X} = 5.5$ $\qquad$ $\bar{Y} = 0.2575$

$(\Sigma x)^2 = 484$ $\qquad$ $(\Sigma y)^2 = 1.0691$

$\Sigma \bar{X}^2 = 126$ $\qquad$ $\Sigma Y^2 = 0.27825$

$(\Sigma x)^2/n = 121$ $\qquad$ $(\Sigma y)^2/n = 0.2652$

$\Sigma x^2 - (\Sigma x)^2/n = 5$ $\qquad$ $\Sigma y^2 - (\Sigma y)^2/n = 0.01302$

$$\Sigma xy = 5.415$$

$$\frac{(\Sigma x)(\Sigma y)}{n} = 5.665$$

$$\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n} = -0.25 \quad \ldots\ldots\ldots(1)$$

$$(\Sigma x^2 - (\Sigma x)^2/n)(\Sigma y^2 - (\Sigma y)^2/n) = 0.0651$$

$$\sqrt{0.0651} = 0.2551 \quad \ldots\ldots\ldots(2)$$

Dividing (1) by (2), we have;

$$r = -0.980$$

Also $\qquad b = -\frac{0.25}{5} = -0.05$

$$a = \bar{y} - 5\bar{x} = 0.5325$$

$\therefore \qquad y = 0.5325 - 0.05x.$

TABLE 15

A.2.8 (a)

SAMPLE SET OF RESULTS: ACCELERATED STABILITY TESTS CARRIED OUT IN GIFFORDS BUFFER, PH 7.4

SUMMARY OF THE REACTION RATE CONSTANTS

TEMPERATURE OF DETERMINATION (T) (^{o}K)	$(I/T) \times 10^3$ (X)	(X^2)	REACTION RATE CONSTANT $(K \times 10^3)$ (HR^{-1})	LOG. $(K \times 10^3)$ (Y)	(XY)	(Y^2)
333	3.0030	9.0180	9.8863	0.9903	2.9881	0.9901
339	2.9499	8.7019	10.6412	1.0270	3.0295	1.0547
343	2.1955	8.5000	13.4965	1.1302	3.2952	1.2774
346	2.8902	8.3533	19.8417	1.2976	3.7503	1.6837
349	2.8650	8.2032	21.56660	1.3338	3.8213	1.7789

COEFFICIENT OF CORRELATION (r) = - 0.9436

REGRESSION EQUATION: y = 9.0943 - 2.7140x

EXTRAPOLATED VALUE OF $(K \times 10^3)$ AT $25^{o}c$ = 0.9706 HR^{-1}

A.2.8. (b) USING DATA GIVEN IN TABLE 15 TO ILLUSTRATE THE DETERMINATION OF THE REACTION RATE CONSTANT (K) AT ROOM TEMPERATURE ($25^{o}c$)

$Ex = 14.6236$ $(n = 5)$ $Ey = 5.7836$

$\overline{X} = 2.9247$ $\overline{Y} = 1.1567$

$(Ex)^2 = 213.84967$ $(Ey)^2 = 33.4450$

$Ex^2 = 42.7814$ $Ey^2 = 6.78485$

$Ex^2 - (Ex)^2/n = 0.011466$ $Ey^2 - (Ey)^2/n = 0.09485$

$Exy = 16.88428$

$$Exy - \frac{(Ex)(Ey)}{n} = -0.0311 \quad \ldots\ldots\ldots(1)$$

$$(Ex^2 - (Ex)^2/n)(Ey^2 - (Ey)^2/n) = 1.0876 \times 10^{-3}$$

$$\sqrt{1.0876 \times 10^{-3}} = 0.03298 \quad \ldots\ldots\ldots(2)$$

Dividing (1) by (2) we have,

$r = -0.9436$

Also, $b = -2.714$

$a = 9.0944$

$\therefore Y = 9.0944 - 2.714\,x$

$(K \times 10^3)$ at $25^{o}c = 0.9706\ HR^{-1}$

A.2.9. (a)

TABLE 16

SAMPLE SET OF RESULTS: ACCELERATED STABILITY TEST DONE AT 66°c IN PHOSPHATE-CITRATE-BORATE BUFFER (PH 6.0):

(DILUTION FACTOR: 1000)

DURATION OF HEATING (HOURS) (x)	(x^2)	AVERAGE ABSORBANCE AT 380nm	CONCENTRATION OF ADRENALINE REMAINING (Mg/ML.)	LOG. PERCENT ADRENALINE REMAINING (y)	(xy)	(y^2)
0	0	0.69	11.05	2.0000	0.0000	4.0000
1	1	0.67	10.80	1.9901	1.9901	3.9603
3	9	0.66	10.60	1.9819	5.9458	3.9281
5	25	0.66	10.60	1.9819	9.9097	3.9281
8	64	0.65	10.50	1.9778	15.8226	3.9118
10	100	0.64	10.30	1.9695	19.6948	3.8788
15	225	0.63	10.10	1.9610	29.4144	3.8454
20	400	0.61	9.85	1.9501	39.0015	3.8028
25	625	0.59	9.55	1.9366	48.4160	3.7506
30	900	0.57	9.10	1.9157	57.4704	3.6698

COEFFICIENT OF CORRELATION (r) = - 0.9874

REGRESSION EQUATION: Y = 1.9952 - 0.00246x

REACTION RATE CONSTANT(K) = 5.664×10^{-3} HR^{-1}

A.2.9.(b) REGRESSION ANALYSIS OF DATA GIVEN IN TABLE 16 TO ILLUSTRATE THE DETERMINATION OF THE REACTION RATE CONSTANT AT A GIVEN TEMPERATURE

$\Sigma x = 117 \qquad n = 10 \qquad \Sigma y = 19.6646$

$\bar{X} = 11.7 \qquad \bar{Y} = 1.96646$

$(\Sigma x)^2 = 13689 \qquad (\Sigma y)^2 = 386.6965$

$\Sigma x^2 = 2349 \qquad \Sigma y^2 = 38.6757$

$(\Sigma x)^2/n = 136.89 \qquad (\Sigma y)^2/n = 38.6696$

$\Sigma x^2 - (\Sigma x)^2/n = 980.1 \qquad \Sigma y^2 - (\Sigma y)^2/n = 0.0061$

$\Sigma xy = 227.6652$

$$\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n} = -2.41058$$

$$(\Sigma x^2 - (\Sigma x)^2/n\ (\Sigma y^2) - (\Sigma y)^2/n) = 5.9786$$

$$\sqrt{5.9786} = 2.4451 \ldots\ldots\ldots (2)$$

Dividing (1) by (2) we have,

$$r = -0.9874$$

Also $b = -0.0024595$

$a = 1.9950$

$\therefore \quad y = 1,9952 - 0.0024\ 6x$

Reaction rate constant (k) = 5.664×10^{-3} HR^{-1}

A.2.10 THE STUDENT'S TEST

INTRODUCTION

Basically, t-tests are statistical procedures used to test the null hypothesis of no difference between two means (JACKSON, 1980). The means that are tested are the population means from which the samples have been selected.

The procedure for performing a t-test involves first calculating the means for both groups of measures. The t-test indicates whether the difference between the means is a "true" difference or whether it may have come about by chance alone. The null hypothesis is tested by calculating a value for t using a formula that will be given here.

After calculating t, the investigator refers to a statistical table containing a distribution of t probability, where a 'tabled' value for t is obtained. Since the null hypothesis is being tested, only that portion of the table labelled 2p is used. The tabled value is found at the intersection of the row associated with the degrees of freedom (df) for the experiment and the column associated with the alpha level for the testing of the null hypothesis.

Degrees of freedom is equal to $N_1 + N_2 - 2$, where N_1 and N_2 are the number of subjects in each of the two samples of the study. The tabled value of t is compared with the

calculated value of t. If the calculated value (absolute figures) does not exceed the tabled value, the null hypothesis is not rejected. If the calculated value exceeds the tabled value, the null hypothesis is rejected and it may be concluded that there is a significant difference in the means, or the difference is "significant."

Tables showing the t-distribution can be obtained from any standard statistics Textbook. The formula given below will be used in one sample calculation to illustrate the determination of t.

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{SD_1^2}{N_1} + \frac{SD_2^2}{N_2}}}$$

Where $\bar{X}$, SD and N represent the means, the standard deviation and the number of subjects in each group, respectively.

A.2.11 SAMPLE CALCULATION : COMPARISION OF SOLUTIONS LEFT AT ROOM TEMPERATURE AND THOSE HEATED AT 115°c FOR 30 MINUTES (pH 7.4).

For all the solutions, the mean concentration of adrenaline was determined by analysing five samples (n = 5).

For the unheated samples:

$\bar{X}_1 = 7.6, \qquad SD = 0.0189.$

For the samples autoclaved at 115°c

$\bar{X}_2 = 6.5., \qquad SD_2 = 0.0152.$

$$\therefore t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{SD_1^2}{n_1} + \frac{SD_2^2}{n_2}}}$$

$$= 101$$

From a table of t (from any standard statistics Textbook) it can be seen that for 8 degree of freedom, the Null hypothesis does not hold.

For similarly treated solutions at pH 5.8, t was calculated to be 31.9, i.e. the Null hypothesis is again rejected. The same applied for samples heated at 121°c at the two pH values when compared to the unheated samples.

A.2.11 SAMPLE CALCULATION : COMPARISION OF SOLUTIONS LEFT AT ROOM TEMPERATURE AND THOSE HEATED AT 115°c FOR 30 MINUTES (pH 7.4).

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$\bar{X}_2 = 6.5., \qquad SD_2 = 0.0152.$

$$\therefore t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{SD_1^2}{n_1} + \frac{SD_2^2}{n_2}}}$$

$$= 101$$

From a table of t (from any standard statistics Textbook) it can be seen that for 8 degree of freedom, the Null hypothesis does not hold.

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APPENDIX 3

TREATMENT SUMMARY AND THE DETERMINATION OF DIURNAL VARIATIONS IN THE INTRAOCULAR PRESSURE FOR INDIVIDUAL PATIENTS

A.3.1.(a)

TABLE 17

SUMMARY OF THE TREATMENT SCHEDULE FOR ALL THE PATIENTS USED FOR THE CLINICAL TEST

PATIENT NO: AGE & SEX	IP NO. 423565 MALE 28 YEARS	IP NO. 3613-43 MALE + 70 YEARS	IP NO.41805 FEMALE + 50 YEARS	IP NO.273089 MALE + 80 YEARS	IP NO.1696-92 MALE 33 YEARS
DIAGNOSIS	Bilateral chronic simple glaucoma. Intraocular pressures on admission were as follows: RE 48 | LE 46	Bilateral glaucoma with bilateral mature cataracts. Had been admitted for operation on the Right eye.	Bilateral glaucoma simplex with bilateral mature cateracts. Had been admitted for operation on the Right eye.	Absolute glaucoma in Right eye(RE). Eye completely blind.	Chronic glaucoma simplex in the Right eye (RE)
TREATMENT SCHEDULE	Placed on 2% Pilocarpine drops (QID) and Diamox Tablets, 250 mg, TID. Adrenaline was tested on the Left eye (LE) only. Experiment was started three days after admission into the ward. Results given in Table 18 and Fig. 14.	Patient was placed on 2% Pilocarpine drops, QID (LE). For experiment, adrenaline (1%) and Dipivefrin (0.1%) were instilled into the Left eye, BD. The results are summarised in Table 19 and Fig. 15.	Placed on 2% Pilocorpine drops, QID (LE). For experiment, Adrenaline drops (1%) were instilled into Left eye BD. The results are summarised in Table 20 and Figure 16.	Placed on 2% pilocarpine drops, QID, into RE. For experiment, 1% Adrenaline instilled BD into RE. Results are given in Table 21 and Figure 17.	4% Pilocorpine into RE, QID. Diamox Tablets, 250mg, QID. For experiment, Adrenaline (1%) and Dipivefrin (0.1.) were instilled, BD, into RE. Results given in Table 22 and Figure 18.

TABLE 18

A.3.1(b) DIURNAL VARIATION IN INTRAOCULAR PRESSURE FOR PATIENT NO. 423565.

DAY AND TREATMENT RECEIVED	TIME OF DAY	INTRAOCULAR PRESSURE (IO) (MM Hg)		CHANGE IN IOP AS PERCENTAGE OF INITIAL IOP	
		RE	LE	RE	LE
(1) Pilocarpine	7 a.m.	16	18	0 (initial)	0 (initial)
(2%) QID (BE)	10 a.m.	18	17	+ 12.5	- 5.5
- Diamox 250 mg.	2 p.m.	17	18	+ 6.25	0
tid	7 p.m.	17	18	+ 6.25	0
(2) Pilocarpine	7 a.m.	16	14	0 (initial)	0 (initial)
(2%) QUID (BE)	10 a.m.	16	12	0	- 14.29
Adrenaline (1%)	2 p.m.	14	14	-12.5	0
BD 'LE)	7p.m.	14	14	-12.5	0
Diamox 250 mg.					
tid					
(3) Pilocarpine	7 a.m.	18	14	0 (initial)	0 (initial)
(2%) QID (BE)	10 a.m.	22	16	+ 22.2	+ 14.29
Adrenaline (1%)	2 p.m.	21	17	+ 16.67	+ 21.43
BE, (LE)	7 p.m.	24	21	+ 33.33	+ 50
No Diamox					

(c)

FIGURE (14)

(IP NO. 423565)

CHANGES IN OCULAR TENSION EXPRESSED AS PERCENTAGE OF INITIAL PRESSURE, FOR BOTH EYES (BE)

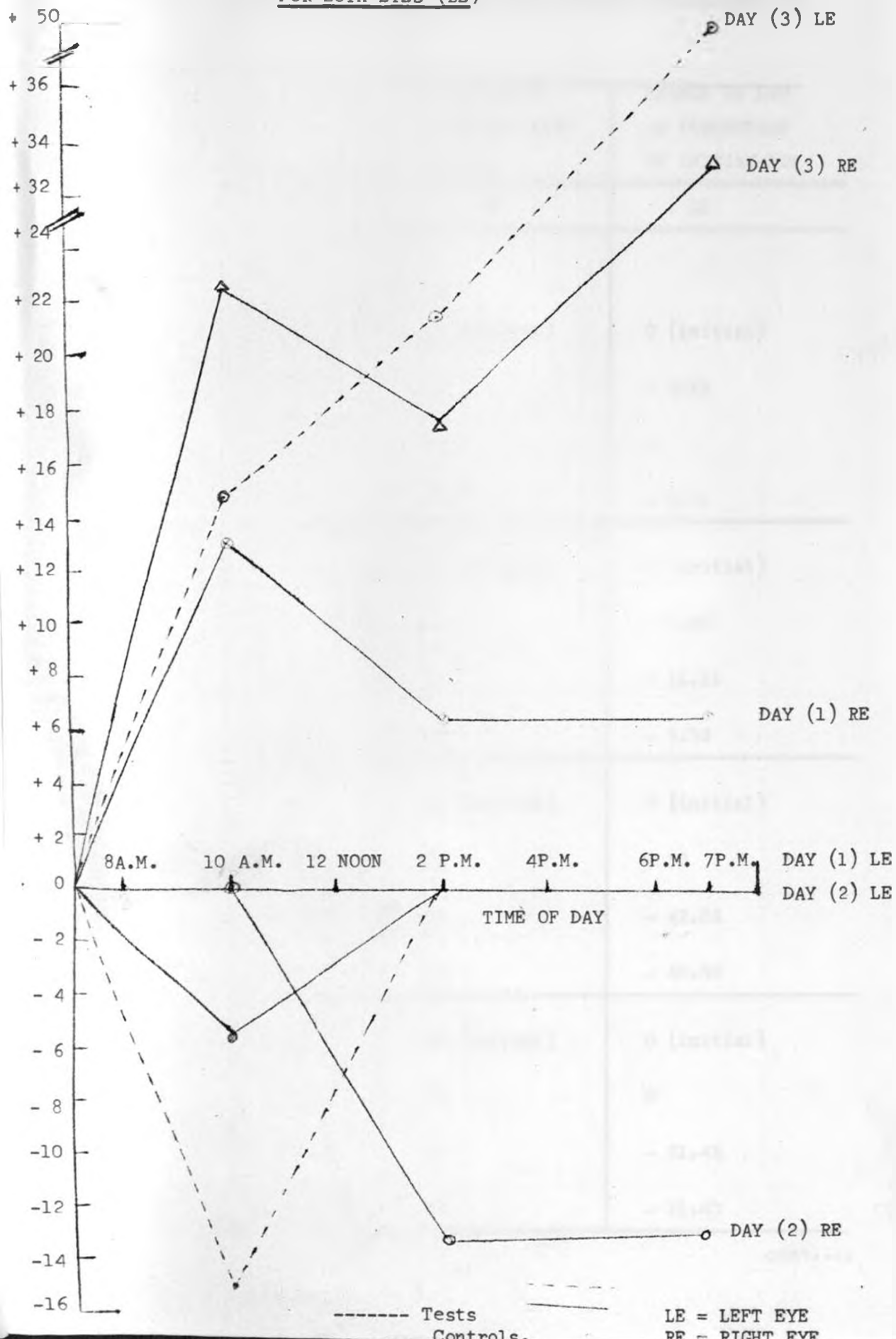


TABLE 19

A.3.2(b) DIURNAL VARIATIONS IN INTRAOCULAR PRESSURE FOR PATIENT NO. 3613-43

DAY AND TREATMENT RECEIVED	TIME OF DAY	INTRAOCULAR PRESSURE (IOP) (MM Hg)	CHANGE IN IOP AS PERCENTAGE OF INITIAL IOP
		LE	LE
(1) Pilocarpine (2%) drops, QID (LE)	7 a.m.	47 (initial)	0 (initial)
	10 a.m.	46	- 2.13
	2 p.m.	47	0
	7 p.m.	43	- 8.51
(2)Pilocarpine (2%) QID (LE) and Adrenaline (1%) BD (LE)	7 a.m.	36 (initial)	0 (initial)
	10 a.m.	34	- 5.56
	2 p.m.	32	- 11.11
	7 p.m.	34	- 5.56
(3) Pilocarpine (2%) QID and Dipivefrin (0.1%) BD (LE)	7 a.m.	42 (initial)	0 (initial)
	10 a.m.	21	- 50
	2 p.m.	24	- 42.86
	7 p.m.	24	- 42.86
(4) Pilocarpine (2%) QID (LE)	7 a.m.	28 (initial)	0 (initial)
	10 a.m.	28	0
	2 p.m.	22	- 21.43
	7 p.m.	22	- 21.43

cont....

TABLE 19

DAY AND TREATMENT RECEIVED	TIME OF DAY	INTRAOCULAR PRESSURE (IOP) (MM Hg)	CHANGE IN IOP AS PERCENTAGE OF INITIAL IOP
		LE	LE
(5) Pilocarpine (2%)	7 a.m.	40	0 (initial)
(2%) QID	10 a.m.	35	- 11.11
(LE)	2 p.m.	42	+ 5.0
	7 p.m.	31	- 17.5
(6) Pilocarpine	7 a.m.	32	0
(2%) QID(LE) and	10 a.m.	32	0
Adrenaline (1%)	2 p.m.	37	+ 15.63
BD (LE)	7 p.m.	36	+ 12.5

A.3.2 (c) FIGURE (15)

CHANGE IN OCULAR TENSION EXPRESSED AS PERCENTAGE OF INITIAL PRESSURE.

DATA FOR LEFT EYE (LE)

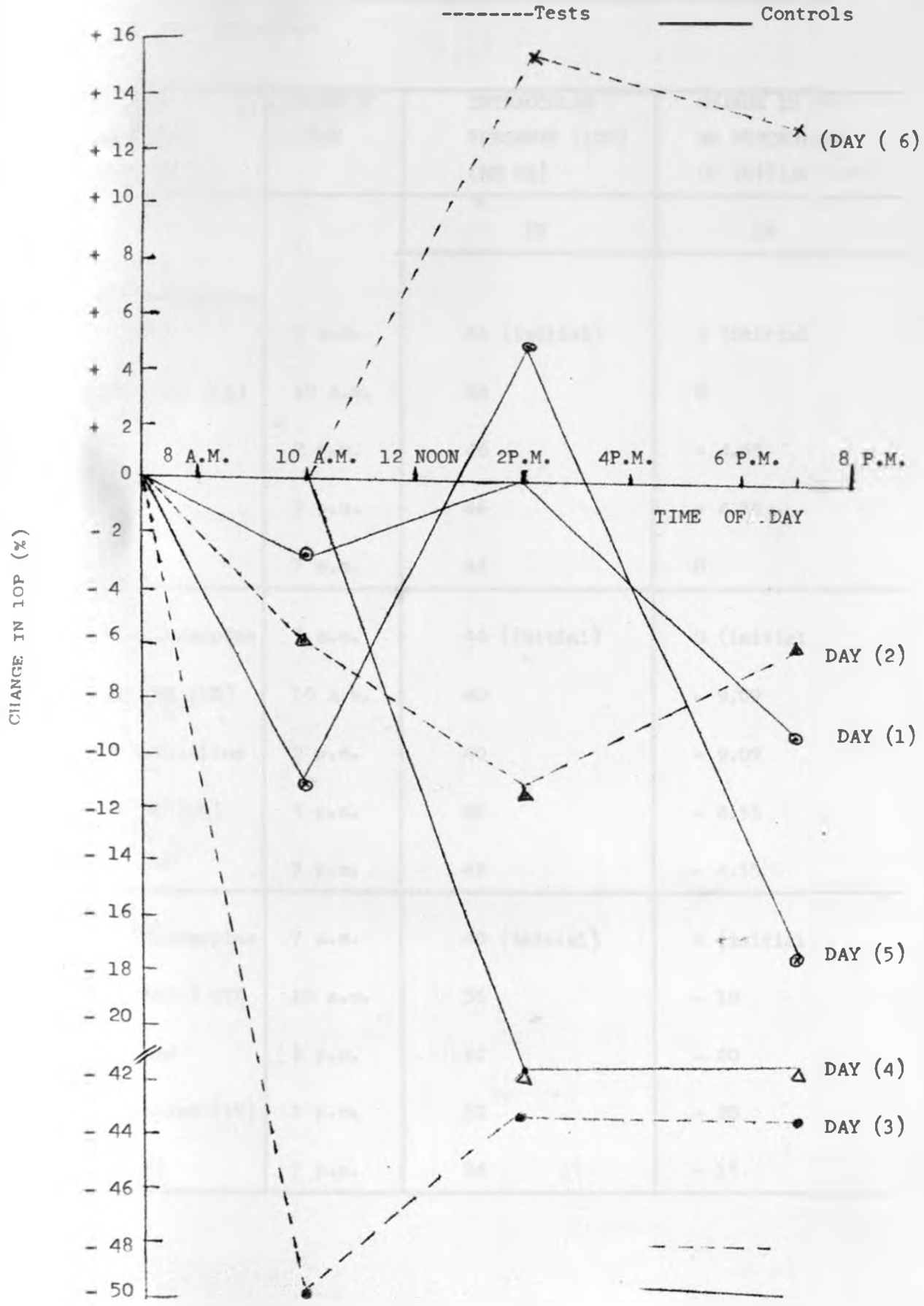


TABLE 20

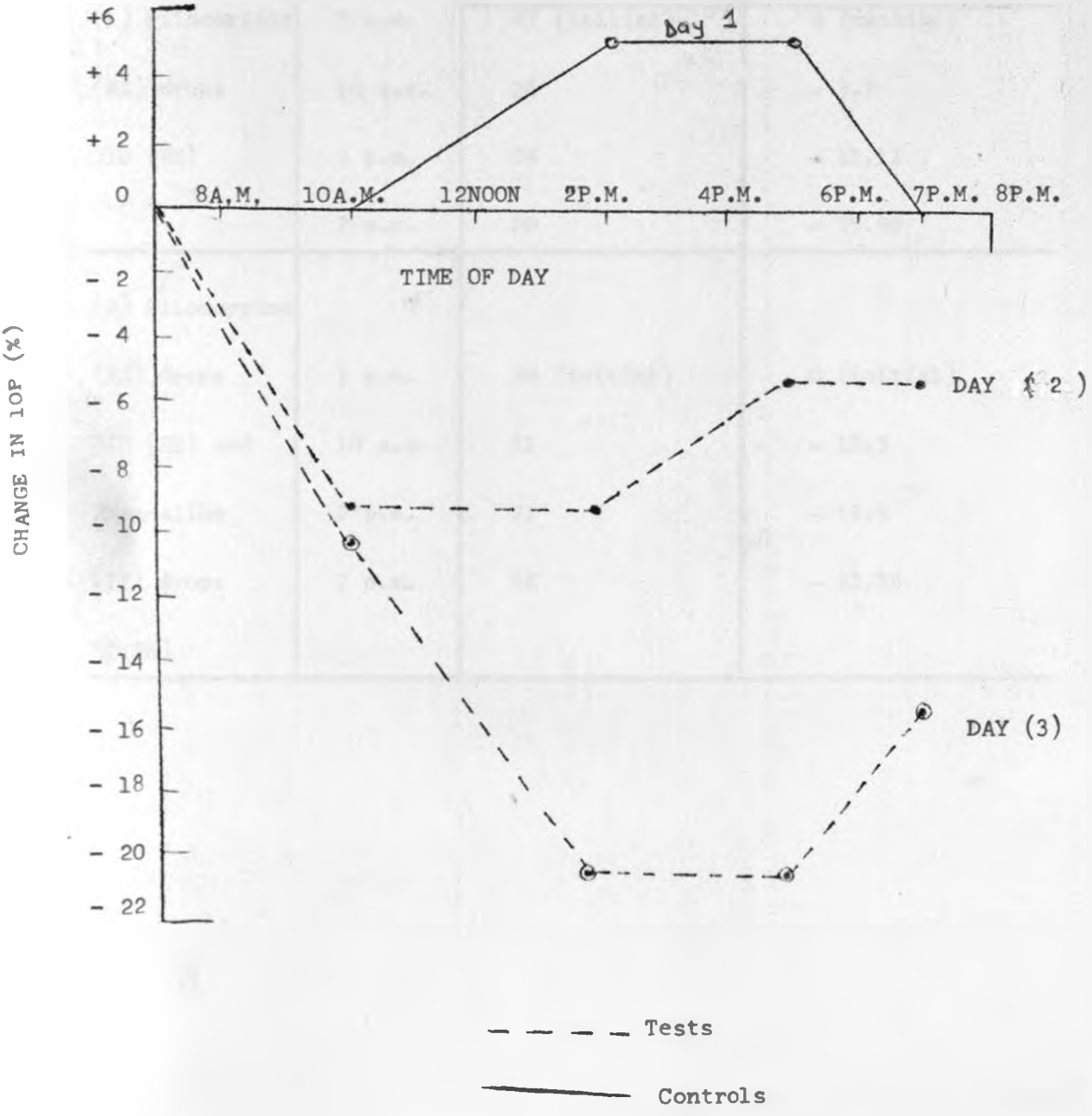
A.3.3.(b) DIURNAL VARIATIONS IN INTRAOCULAR PRESSURE FOR PATIENT NO. 41805

DAY AND TREATMENT RECEIVED	TIME OF DAY	INTRAOCULAR PRESSURE (IOP) (MM Hg)	CHANGE IN IOP AS PERCENTAGE OF INITIAL IOP
		LE	LE
(1) Pilocarpine (2%) drops QID (LE)	7 a.m.	44 (initial)	0 (initial)
	10 a.m.	44	0
	2 p.m.	46	+ 4.55
	5 p.m.	46	+ 4.55
	7 p.m.	44	0
(2) Pilocarpine (2%) QID (LE) and Adrenaline (1%) BD (LE)	7 a.m.	44 (initial)	0 (initial)
	10 a.m.	40	- 9.09
	2 p.m.	40	- 9.09
	5 p.m.	42	- 4.55
	7 p.m.	42	- 4.55
(3) Pilocarpine (2%, Thilo) QID (LE) and Adrenaline (1%) BD (LE)	7 a.m.	40 (initial)	0 (initial)
	10 a.m.	36	- 10
	2 p.m.	32	- 20
	5 p.m.	32	- 20
	7 p.m.	34	- 15

A.3.3.(c)

FIGURE (16) IP NO. 41805

CHANGE IN OCULAR TENSION EXPRESSED AS PERCENTAGE OF INITIAL PRESSURE (FOR LE)



A.3.4.(b) TABLE 21

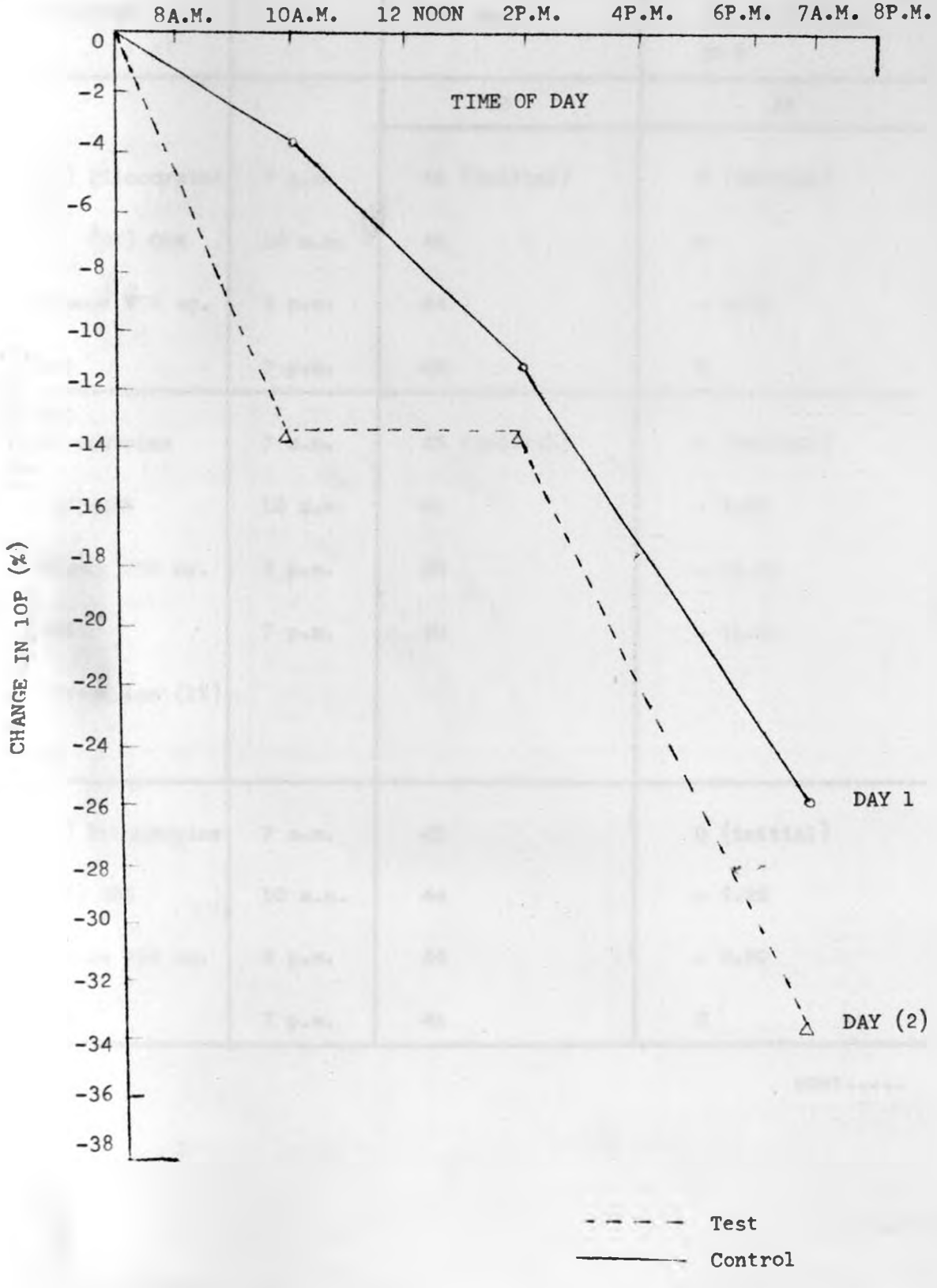
DIURNAL VARIATION IN THE INTRAOCULAR PRESSURE FOR PATIENT NO. 273089

DAY AND TREATMENT RECEIVED	TIME OF DAY	INTRAOCULAR PRESSURE (IOP) (MM Hg)	CHANGE IN IOP AS PERCENTAGE OF INITIAL IOP
		RE	RE
(1) Pilocarpine	7 a.m.	27 (initial)	0 (initial)
(2%) drops	10 a.m.	26	- 3.7
QID (RE)	2 p.m.	24	- 11.11
	7 p.m.	20	- 25.93
(2) Pilocarpine			
(2%) drops	7 a.m.	24 (initial)	0 (initial)
QID (RE) and	10 a.m.	21	- 12.5
Adrenaline	2 p.m.	21	- 12.5
(1%) drops	7 p.m.	16	- 33.33
BD(RE)			

A.3.4 (c) FIGURE (17) IP. NO. 273089

CHANGE IN OCULAR TENSION EXPRESSED AS PERCENTAGE OF INITIAL PRESSURE

DATA FOR RIGHT EYE (RE)



A.3.5.(b)

TABLE 22

Diurnal variation in the intraocular pressure for patient No. 1696 - 92.

DAY AND TREATMENT RECEIVED	TIME OF DAY	INTRAOCULAR PRESSURE (IOP) (MM Hg.)	CHANGE IN IOP AS PERCENTAGE OF INITIAL IO P
		RE	RE
(1) Pilocarpine	7 a.m.	46 (initial)	0 (initial)
(4%) QDS	10 a.m.	46	0
Diamox 250 mg.	2 p.m.	44	- 4.35
QDS	7 p.m.	46	0
(2) Pilocarpine	7 a.m.	45 (initial)	0 (initial)
(4%) QDS	10 a.m.	41	- 8.89
Diamox 250 mg.	2 p.m.	40	- 11.11
QDS	7 p.m.	40	- 11.11
Adrenaline (1%) BD			
(3) Pilocarpine	7 a.m.	45	0 (initial)
(4%) QDS	10 a.m.	44	- 2.22
Diamox 250 mg.	2 p.m.	44	- 2.22
QDS	7 p.m.	45	0

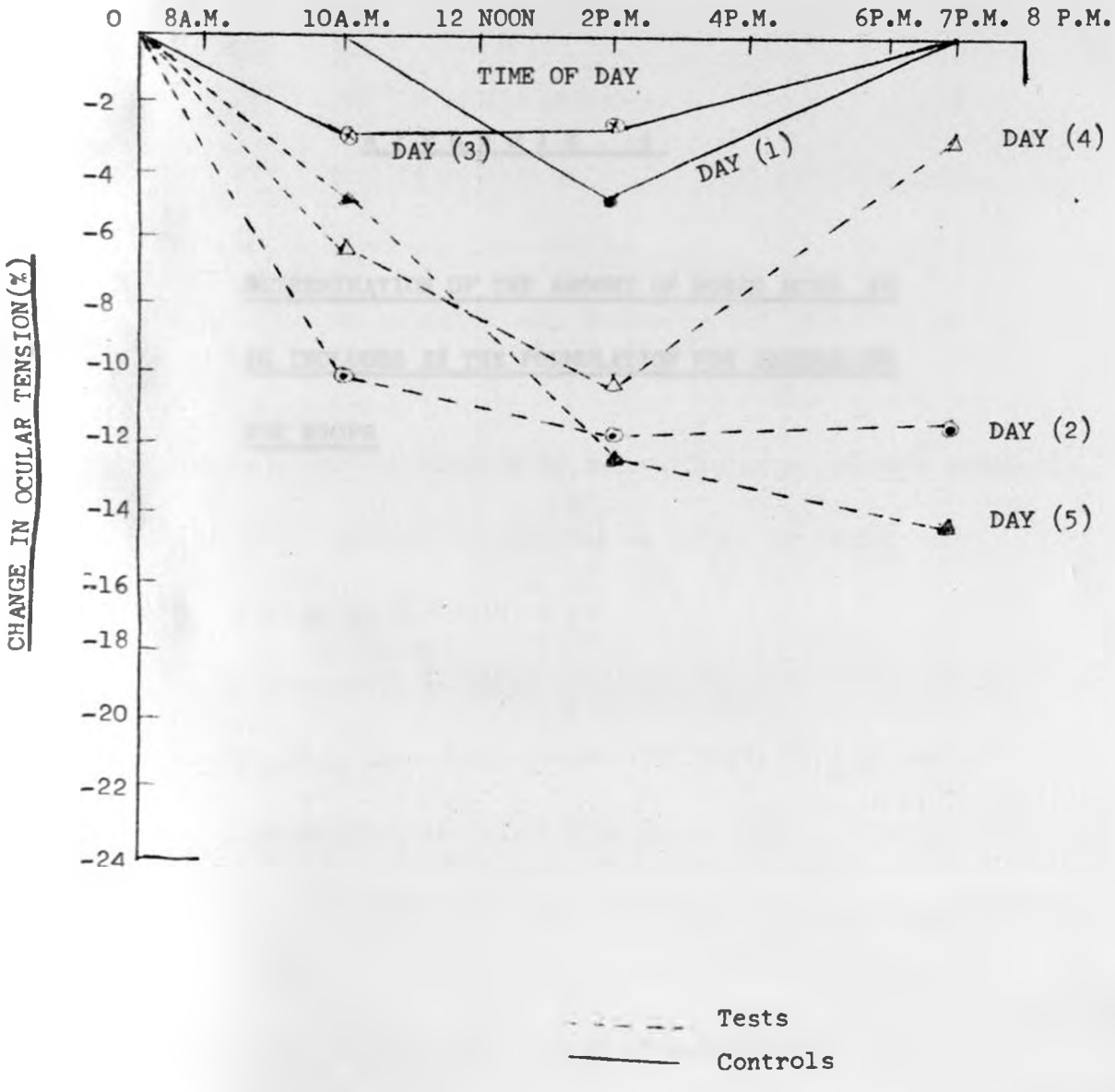
cont.....

TABLE 22

DAY AND TREATMENT RECEIVED	TIME OF DAY	INTRAOCULAR PRESSURE(IOP) (MM Hg.)	CHANGE IN IOP AS PERCENTAGE OF INITIAL IOP
(4) Pilocarpine	7 a.m.	42 (initial)	0 (initial)
(4%) QDS;	10 a.m.	40	- 4.76
Diamox 250 mg.	2 p.m.	38	- 9.52
QDS	7 p.m.	41	- 2.38
Dip. Adrenaline			
(0.1%) BD			
(5) Pilocarpine	7 a.m.	44 (initial)	0 (initial)
(4%) QDS	10 a.m.	42	- 4.55
Adrenaline	2 p.m.	39	- 11.36
(1%) BD	7 a.m.	38	- 13.64
No Diamox			

A.3.5(c) FIGURE (18) IP. NO. 1696-92

CHANGE OF OCULAR TENSION EXPRESSED AS PERCENTAGE OF INITIAL PRESSURE. DATA FOR RIGHT EYE (RE)



A P P E N D I X 4

DETERMINATION OF THE AMOUNT OF BORIC ACID TO BE INCLUDED IN THE FORMULATION FOR ADRENALINE EYE DROPS

4.1. AMOUNT OF BORIC ACID REQUIRED TO MAKE 2% ADRENALINE ACID TARTRATE EYE DROPS ISOTONIC WITH TEARS:

A 2% $^{w}/_{v}$ solution of adrenaline bitartrate contains approximately 1% Adrenaline base. This solution has a sodium chloride equivalent of 0.17 (Pharmaceutical Handbook, 1975 (b)).

Therefore, percentage of sodium chloride required to make this solution isotonic.

= 0.9 - (% strength of solu. X Sod. chloride Equivalent)

= 0.9 - (2 X 0.17)

= 0.56 $^{w}/_{v}$ sodium chloride.

But 1g of Boric acid is equivalent to 0.5 g of sodium chloride (Pharmaceutical Handbook, 1975 (b)).

∴ 0.56 g of sodium chloride will be equivalent to 1.12 g of Boric acid.

∴ To render a 2% $^{w}/_{v}$ solution of adrenaline acid tartrate isotonic with tears will require the addition of 1.12 g of boric acid.

Mol. wt. of Boric acid = 61.8

Mol. wt. of Adrenaline = 183.2

1 gm of Adrenaline base would require (61.8/183.2) g of boric acid for complexation, or 0.3378 g of boric acid.

∴ Amount of uncompleted boric acid in solution = (1.12 - 0.3373)g

= 0.7827 g.

Therefore, to make up for the amount of boric acid used to complex

with adrenaline, an additional 0.3373 g of boric acid would have to be added to the amount required to make 2% $^{w}/_{v}$ adrenaline bitartrate solution isotonic, i.e. 1.12 g of boric acid. This gives a total boric acid concentration of 1.4573% $^{w}/_{v}$. The amount 1.5% $^{w}/_{v}$ was used in the present work.

A P P E N D I X 5

DETERMINATION OF THE BUFFER CAPACITY OF BORATE BUFFER AT PH 5.8

A.5.1. DETERMINATION OF MAXIMUM BUFFER CAPACITY

Maximum buffer capacity occurs at the point where the pH is equal to the pKa. At this point, the following equation can be used to calculate the maximum buffer capacity (FLYNN, 1980).

$$B_{max} = 2.303\ C_t \frac{[H^+]^2}{-(2[H^+])^2} \quad \ldots\ldots\ldots (1)$$

$$= 0.576\ C_t \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where B_{max} is the maximum buffer capacity, and C_t is the total buffer concentration.

Boric acid has a moleculer weight of 61.84 and a PKa of 9.24 (Pharmaceutical Handbook, p. 216). The concentration of boric acid used in the final formulation of adrenaline drops was 1.5 g in 100 mls or 15 g in a litre, which gives a buffer concentration of 0.2426 M.

Using equation (2), the maximum buffer capacity for boric acid is given by,

$$B_{max} = 0.576\ C_t = (0576 \times 0.2426)$$

$$= 0.1397.$$

A.5.2. TO CALCULATE H^+ FOR A SOLUTION OF BORIC ACID, AND THE DISSOCIATION CONSTANT (Ka)

5.8 is equivalent to - log H^+ at PH 5.8

∴ H^+ at pH 5.8 = 1.585×10^{-6} moles/litre.

Boric acid has a pKa of 9.24, which is equal to - log. Ka.

∴ Ka = 5.75×10^{-10}

A.5.3. BUFFER CAPACITY OF BORIC ACID AT pH 5.8

According to FLYNN (1980), buffer capacity can be calculated using the following equation:

$$B = 2.303\, C_t \frac{Ka \cdot H^+}{(Ka + H^+)^2} \quad \ldots\ldots\ldots\ldots (3)$$

Substituting the values of Ka and H^+ calculated above, and ignoring the value of Ka in the denominator since it is very small compared to H^+ J, the value of B is found to be 2.027×10^{-4}. In sect. A.5.1, B_{max} was calculated to be 0.1397. Therefore the buffer capacity of boric acid at pH 5.8 is only 0.145 % of the maximum buffer capacity.