CHAPTER III

EXPERIMENTALS

3.1 Equipments

3.1.1 Co^{60} Source

Throughout the experimental work, the samples were irradiated by using the Co^{60} source (Gamma Beam 160, Atomic Energy of Canada Ltd., Commercial Products, Ottawa, Canada) at the Office of the Atomic Energy for Peace, Bang-Khen. The half life ($t_{½}$) of Co^{60} was 5.24 years. The decay factor was 0.011023 per month (recorded from the reactor office).

The radiation source consisted of 12 rods in which the Co^{60} pellets were blown up to radiate gamma rays during the irradiation process. The pitch diameter was adjusted to the largest size i.e. 32.5 inches. The dose rate at the centre of the largest pitch diameter was about 2 k rad $min.^{-1}$

3.1.2 Spectrophotometer

The spectrophotometer used in this work was the Perkin-Elmer 124, Double Beam Spectrophotometer with 1 centimetre quartz cells. The absorbance could be read to $\pm$ 0.001.

3.2 Chemicals

Chemicals used for dosimetry and competition kinetics studies were BDH, Merck and Carlo Erba of reagent grade. Chemicals used in the kinetics studies were:

glycine NH_2-CH_2-COOH

DL-Alanine $CH_3-\underset{\displaystyle NH_2}{\underset{|}{CH}}-COOH$

Sodium nitrate $NaNO_3$

Sodium formate $HCOONa$

3.3 Water Preparation and Purity Check

In the preparation of solutions for irradiation it was necessary to remove trace organic materials from the water. Water used in all preparations was triply distilled. The distillation system is shown in Figure 3.1

The first flask contained a dilute solution of potassium dichromate acidified with sulphuric acid (1 ml conc.H_2SO_4/litre). Doubly distilled water was used initially. The water was distilled to the second flask which contained an alkaline solution of potassium permanganate (1 gm $KMnO_4$ + 1 gm KOH). A final distillation was carried out in the third flask which contained no additives. Nitrogen gas, filtered through glass wool and scrubbed in KOH solution to eliminate CO_2, was passed through the system to equalize

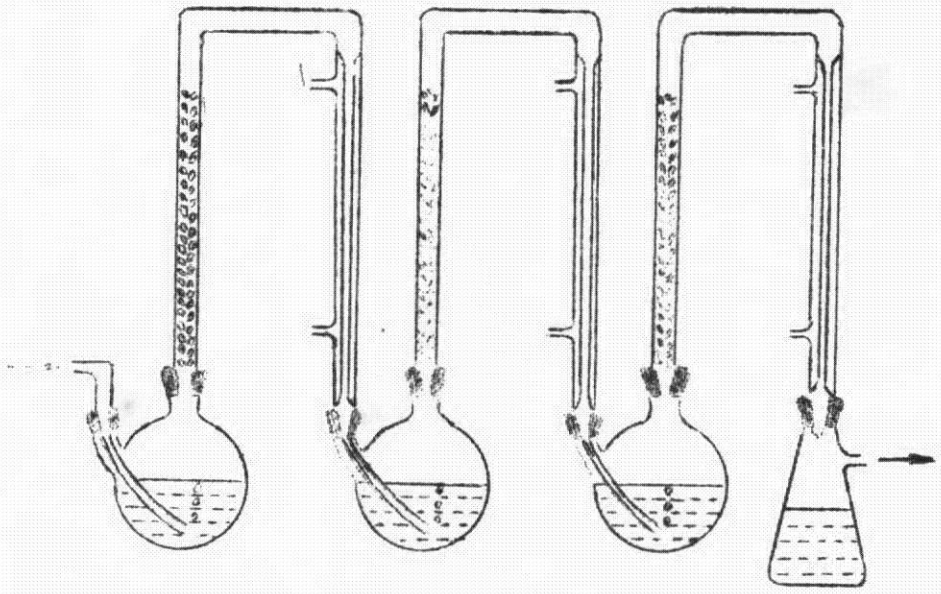


Figure 3.1 Purification of water for radiation-chemical experiments.

the pressure and temperature during distillation in all three stills.

The purity of the water was checked by a standard method. This radiation chemical yield of hydrogen peroxide $G(H_2O_2)$ was

measured in air-saturated water containing 1 X 10^{-4}M KBr. Water samples were placed in the Co^{60} source and irradiated for various lengths of time. The pitch diameter was fixed at 32.5 inches throughout the irradiation. The irradiated samples were analysed spectrophotometrically using a standard method for the determination of H_2O_2(10). Solution A containing 1 gram of NaOH, 33 grams of KI, 0.1 gram of $(NH_4)_6Mo_7O_{24}.4H_2O$ in 500 ml of distilled water, was mixed with an equal volume of solution B containing 10 grams of potassium acid phthalate in 500 ml distilled water. 1 ml of 1 X 10^{-6}M standard H_2O_2 solution was pipetted into a 25 ml volumetric flask, together with 5 ml of the irradiated KBr solution. 12.5 ml of mixed solution A and B was added and the total volume made up. The optical densities were determined at 350 nm, the wavelenght of maximum absorption for the triiodide ion. The molar extinction coefficient obtained from standard H_2O_2 determination was 25050 $cm^{-1}M^{-1}$. The optical density was plotted against time (Figure 3.2). The radiation chemical yields of peroxide, $G(H_2O_2)$, were calculated at various absorbed doses. The $G(H_2O_2)$value was replotted against irradiation time and $G_0(H_2O_2)$ was obtained by extrapolation to zero time (Figure 3.3). The value of $G_0(H_2O_2)$ was found to be 0.90 which was in good agreement with the expected value, 0.85 $\pm$ 0.04.

Fig. 3.2 Plot of Absorbance of Triiodide Ions (350 nm) against Irradiation Time for $G(H_2O_2)$ Determination

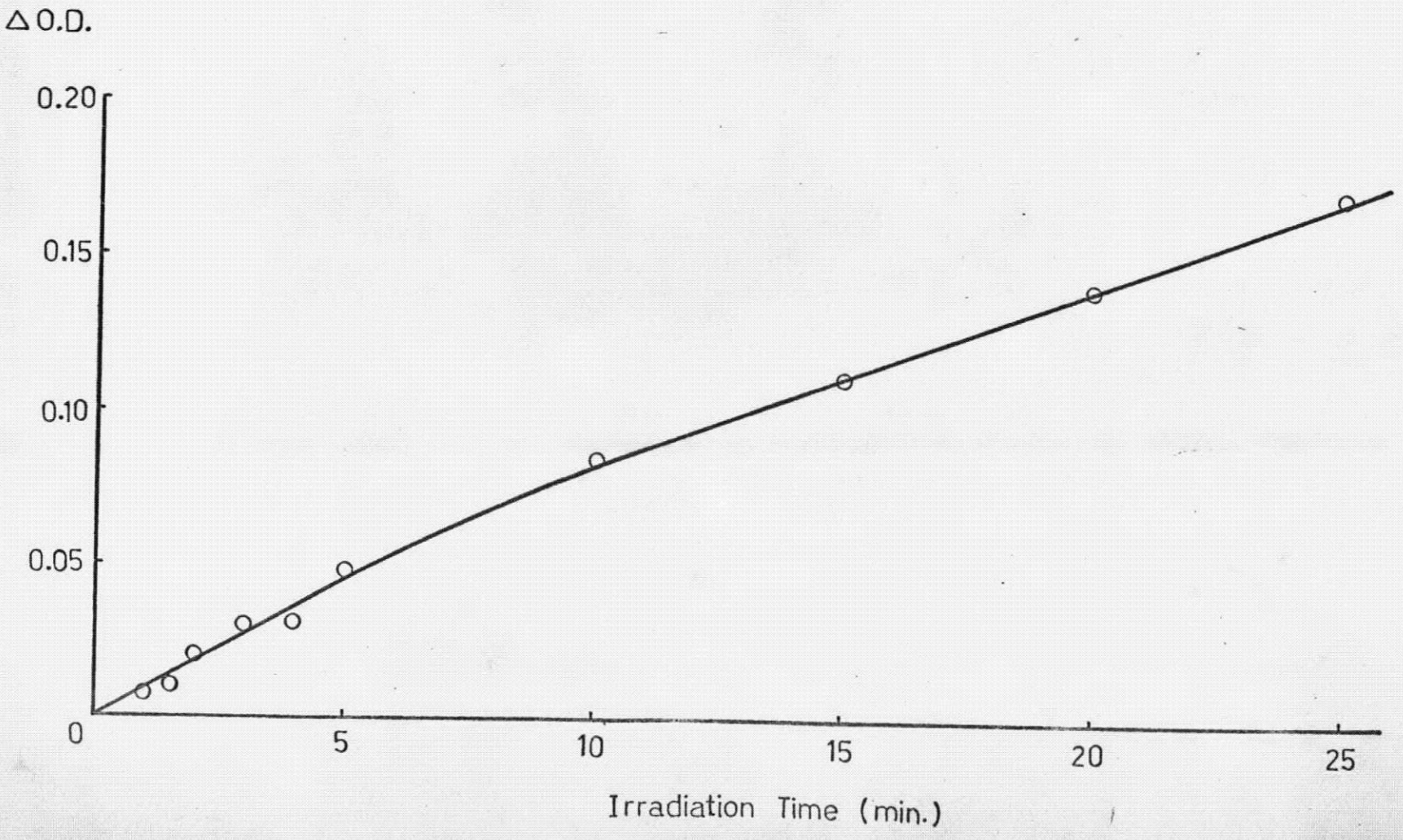
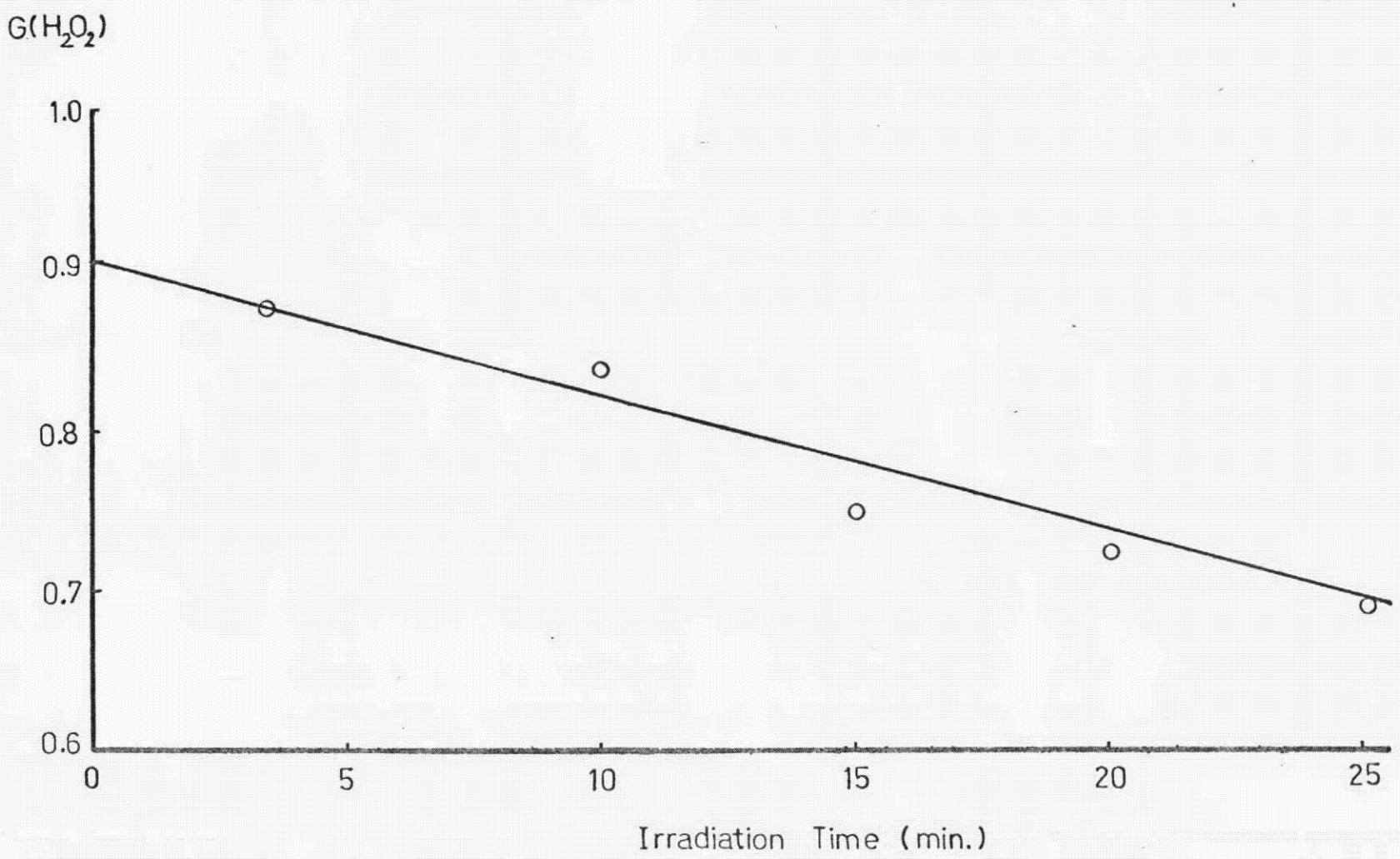


Fig. 3.3 Plot for Determination of $G_o(H_2O_2)$



3.4 Dosimetry Techniques and Calculation of Dose

3.4.1 Radiation Dose Measurements

The absorbed dose obtained from this specific Co^{60} source was determined by chemical dosimetry. The dosimetry was carried out by chemical means using a ferrous sulphate system (Fricke dosimeter). This system has been adopted as a standard method of chemical dosimetry (11). The dosimeter solution was made of 1×10^{-3} M $FeSO_4$, 1×10^{-3} M NaCl in air-saturated 0.4 M H_2SO_4. Triply distilled water was used in solution preparations. Cylindrical ampoules with B 14 joints were used for irradiation of the ferrous sulphate solutions by Co^{60} source for various leng s of time. The concentrations of ferric ions produced by irradiation were measured spectrophotometrically at 302 nm. The molar extinction coefficient (ε) for ferric ion at 302 nm was checked and compared favorably with the accepted value of ε for Fe^{3+} at 302 nm. This accepted value of 2197 $M^{-1}cm^{-1}$ at $25^{o}c$ was used for the dose calculations.

3.4.2 Calculation of Absorbed Dose

Absorbed dose, D (in rad), was calculated by the following formula (1):

$$D = \frac{N \times \Delta O.D. \times 100}{\varepsilon \times 10^3 \times G(Fe^{3+}) \times f \rho \, l} \quad \text{rad} \text{ --------- (1)}$$

where N = Avogadro's number, 6.023×10^{23} molecules per mole

$\Delta O.D.$ = the difference between the optical densities of irradiated and control samples

ε = the molar extinction coefficient, 2197 $M^{-1} cm^{-1}$ at 25°c

f = the conversion factor for transition from electron volts per millilitre units into rad, 6.245×10^{13}

ρ = the density of the dosimeter solution, 1.024 for 0.4 M H_2SO_4

l = the optical path length, centimetres

$G(Fe^{3+})$ = the radiation-chemical reaction yield under given conditions

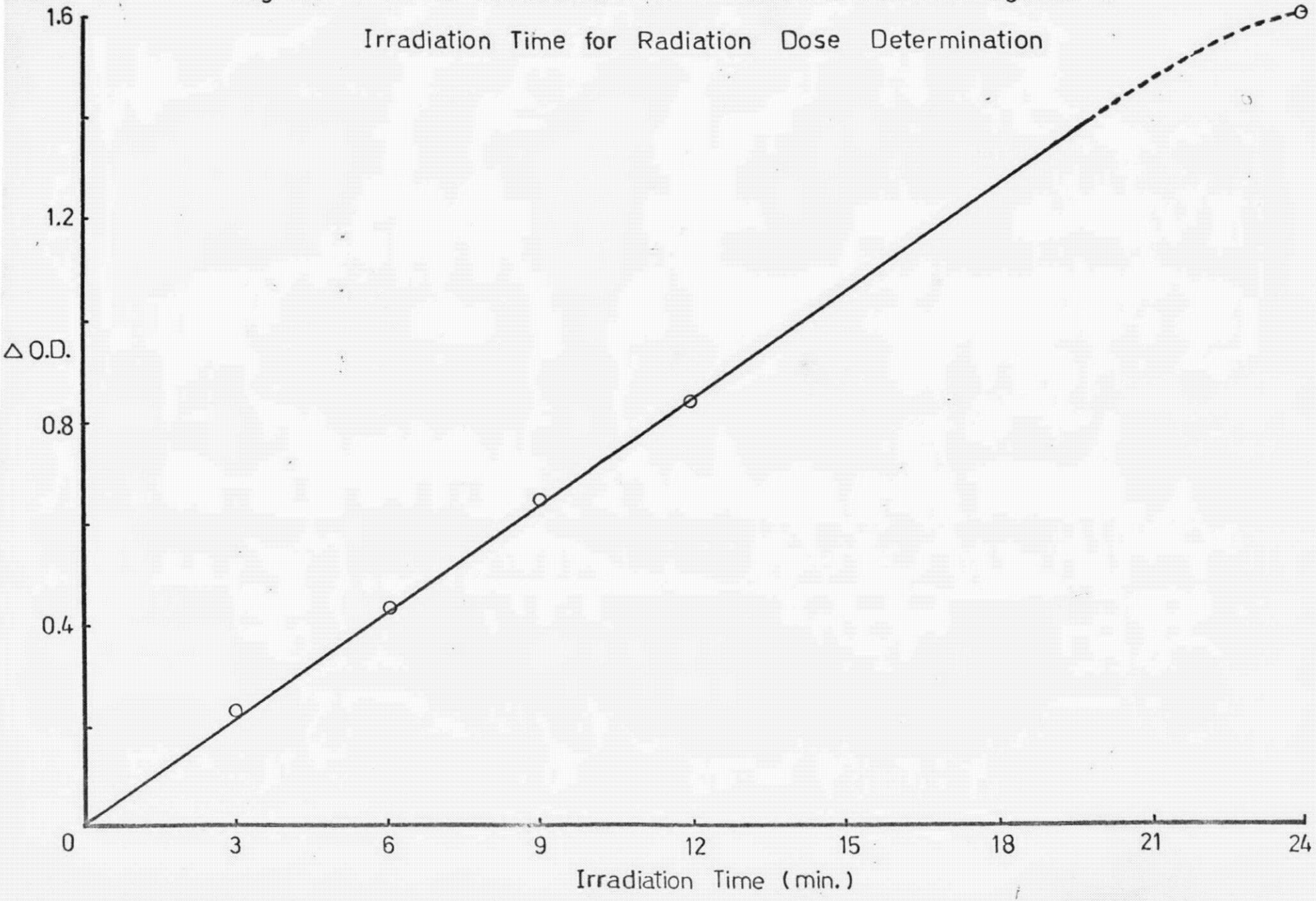
For 0.4 M H_2SO_4, 1 cm absorption cell, and $G(Fe^{3+}) = 15.6$ this equation reduced to

$$D = 2.75 \times 10^4 \times \Delta O.D. \quad \text{rad} \text{ --------- (2)}$$

Plotting of Δ O.D. against irradiation time produced a linear graph up to 21 minutes (see Figure 3.4). The dose range varied from 1×10^3 to about 4.09×10^4 rad in this time interval.

From the dosimetry curve the dose rate calculated by equation (2) was 1.92 k rad min^{-1} with 1.6% error (the actual dose rate, calculated from the curve done by the reactor office, was 1.89 k rad min^{-1}). The agreement between the two values was acceptable.

Fig. 3.4 Plot of Absorbance of Ferric Ions (302 nm) against Irradiation Time for Radiation Dose Determination



3.5 Kinetics Experiments and NO_2^- Determinations

3.5.1 Solution Preparations

The solutions for the kinetic studies consisted of amino acid (glycine or alanine) dissolved together with various concentrations of $NaNO_3$ and 0.1 M HCOONa in aqueous solution. Triply distilled water was used throughout. Concentrations of nitrate and formate were fixed throughout and the concentration of amino acid was varied, giving a range of ratios of concentrations of amino acid to nitrate ion.

Six sets of solutions were used altogether; three sets for glycine/nitrate ion system and three for alanine/nitrate ion system.

3.5.2 Degassing of Samples

For the kinetic experiments, it was necessary to degas the sample solutions before irradiating. The degassing vessel used is shown in Figure 3.5. It was capable of filling five ampoules at once. The vacuum pump used had a capability of 10^{-4} - 10^{-5} mm Hg. Pyrex cylindrical ampoules were fitted with ground joint cones which were of standard size and identical to the sockets on the degassing vessel. The cells had a capacity of 20 ml and were fitted with a narrow neck 50 mm long and only 6 mm in diameter

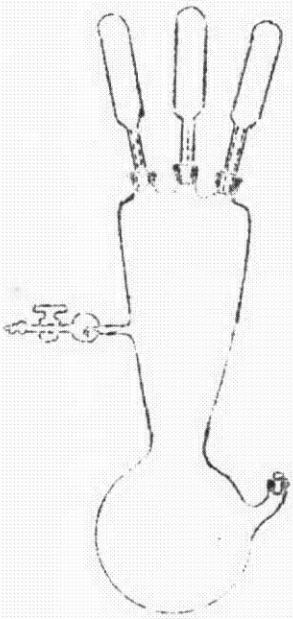


Figure 3.5 A vessel for solution degassing and simultaneous preparation of many samples.

(B 17 joints were used). The narrow neck prevented rediffusion of air from the atmosphere during transfer. The cells were cleaned first by immersion in a boiling nitric acid-sulphuric acid mixture followed by thorough rinsing with triply distilled water.

For the kinetic measurements, the ampoules were filled with solutions containing amino acid, sodium nitrate and sodium formate as mentioned in 3.5.1. A single solution was placed in the degassing flask and five ampoules were fitted on top. The vacuum line was connected as indicated through a trap to the high-vacuum pump. By alternatively shaking and pumping, the air was removed from the solution. By turning the vessel, the ampoules

were filled with degassed solution up to the top, so that no gas phase remained, and then closed with stoppers full of solution.

3.5.3. NO_2^- Determination

After irradiation of the kinetic solutions the yields of nitrite ion were determined. The Rider-Mellon method (12) was used with modifications. Ten millilitre of the irradiated sample was pipetted into 25 ml volumetric flask, followed by 0.5 ml of 0.6% sulphanilic acid in 20% HCl. After standing for 3-10 mins., 0.5 ml of 0.4% α-naphthylamine in 1.3% HCl and 0.5 ml of 2 M CH_3COONa was added respectively. The red colour developed in the solution was due to the diazotization reaction followed by coupling with the amine to form a dye. The reaction mixtures were allowed to stand for half an hour at 25^0 C and the optical densities were measured at 518 nm.

The yield of nitrite $G(NO_2^-)$ were calculated by the following formula:

$$G = \frac{C}{D} \times 100 \qquad \text{.....(3)}$$

where C = concentration in molecule ml^{-1}

D = absorbed dose in electron volt ml^{-1}

The concentration in molar was converted into molecule ml^{-1} by using the following

$$C = \frac{\triangle O.D. \times 6.02 \times 10^{23}}{\epsilon \times b \times 10^{3}} \times \text{dilution factor} \quad \ldots (4)$$

$\triangle$ O.D. = measured optical density

6.02×10^{23} = Avogadro's number, molecules per mole

ϵ = the extinction coefficient of the solution measured

b = the optical path length in cm = 1

dilution factor = $\frac{25}{10}$ for this case

The dose in rad was transformed into eV ml^{-1} using:

$$D = \text{absorbed dose in rad} \times 6.245 \times 10^{13} \quad \ldots (5)$$

Practically, absorbed doses in rad could be obtained from the dosimetry curve done at the beginning of the experiment. Unfortunately during the work the Co^{60} source has been under repair and the doses could not be determined accurately by using the irradiation time. Therefore Fricke solutions were used each time throughout the experimental work to obtain the dose measurement. The absorbed dose in rad was equal to $\triangle$ O.D. $\times 2.75 \times 10^{4}$ (see 3.4.2). Thus the competition plots for $\frac{1}{G(NO_2^-)}$ and $\frac{[\text{amino acid}]}{[NO_3^-]}$ were obtained.

3.6 The Molar Extinction Coefficient and the Influence of Amino Acid Concentration

The molar extinction coefficient (ϵ) was determined by using the standard nitrite solution with nitrite nitrogen

Fig. 3.6 Variation of Molar Absorptivity in Nitrite Analysis with Glycine Concentration

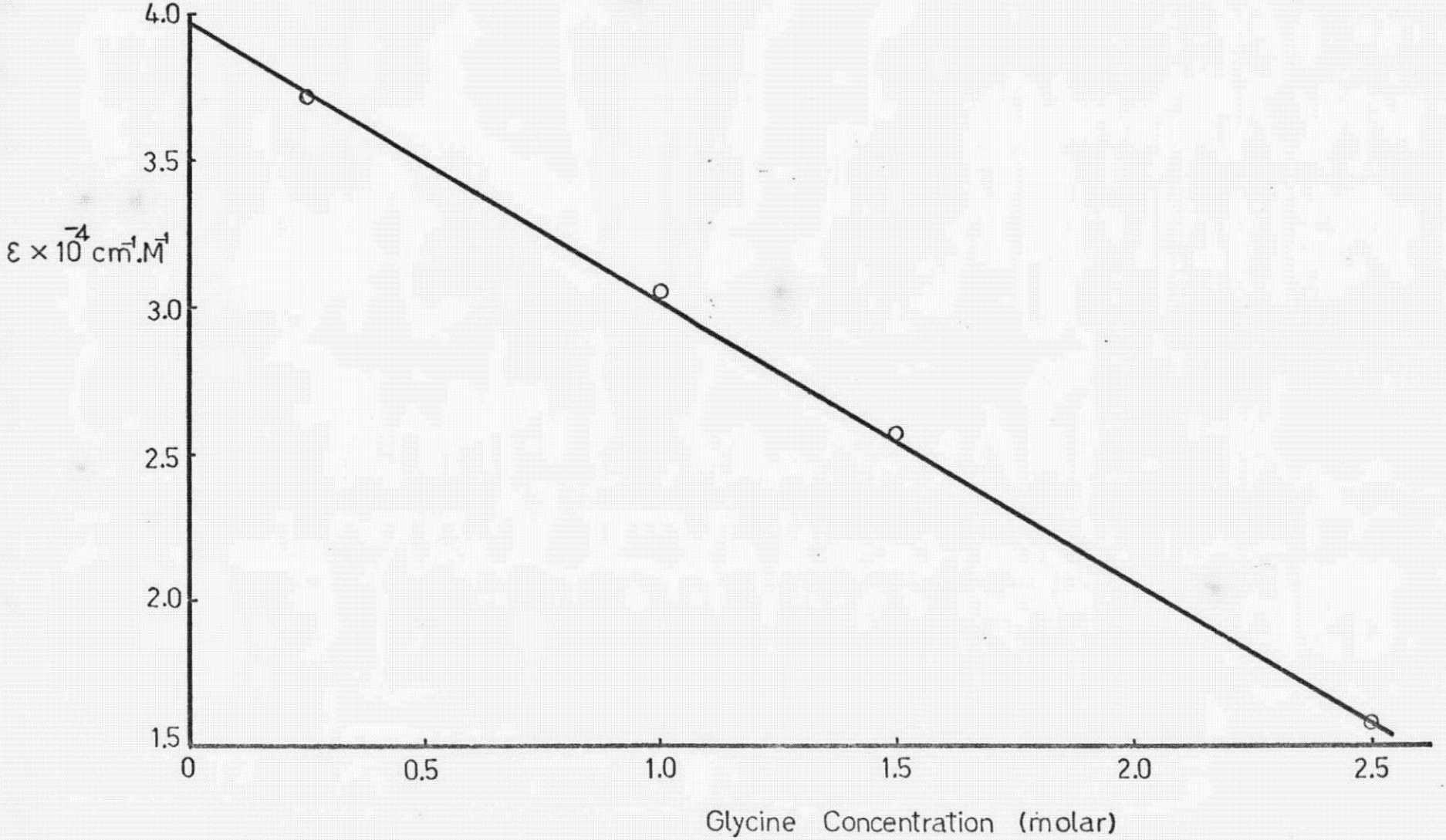
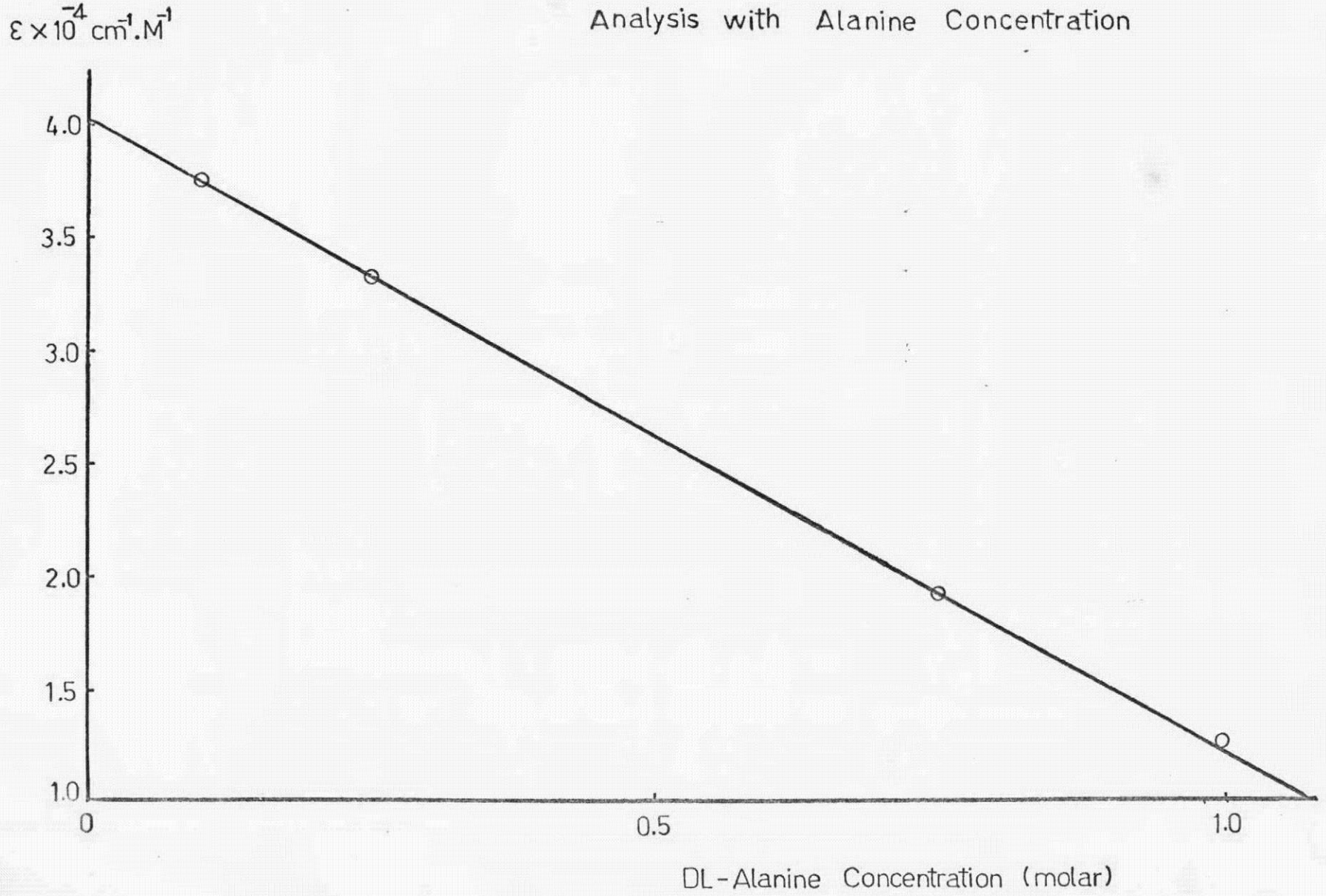


Fig. 3.7 Variation of Molar Absorptivity in Nitrite Analysis with Alanine Concentration



concentration ranging from zero to 101.3 μg/litre. It was found that varying the concentration of amino acids produced a corresponding change in the value of ϵ . Therefore it was necessary to use correction graphs for molar extinction coefficient and at various concentrations of glycine and alanine. The correction graphs were found to be linear (Figure 3.6 and Figure 3.7)

This phenomenon was attributed to a shift in the equilibrium of the dye complex formation. pH effects were investigated but no simple correlation was observed.

3.7 The Molar Extinction coefficient and the Influence of Temperature

The effect of temperature on $\epsilon_{NO_2^-}$ was investigated. It was found that as the temperature increased from 21°C to 31°C, absorbances were found not to vary significantly in this temperature range for lower concentrations of amino acid. However, at higher concentrations of amino acid, the absorbances increased as the temperature decreased. All the optical density measurements were performed at 25 $\pm$ 1°C throughout the experimental work.