CHAPTER II

MATERIALS AND METHODS

1. Materials

1.1 Sulfanilamide B.P.C.

1.2 Sulfacetamide sodium B.P.1973 (L.S. Raw Materials Ltd.)

1.3 Sodium Metabisulphite B.P. (E. Merck)

1.4 Sodium Thiosulfate B.P.

1.5 Disodium ethylenediamine tetraacetate (May & Baker)

1.6 Sodium acid phosphate B.P.1973 (Albright Wilson)

1.7 Sodium phosphate B.P.1973

1.8 Sodium Hydroxide AR (BDH Chemical Ltd.)

1.9 Hydrochloric acid AR (Peking Chemical Works)

1.10 Hydroxylamine Hydrochloride AR (May & Baker)

1.11 Ferric Chloride AR

1.12 Instant caramel color powder, E.150 Type 600 No.60384 (B.V. Wederlandse Klenrstefindustrial Holland)

1.13 Freshly boiled distilled water

1.14 Clear ampule type 1, 5 c.c. (APA)

1.15 Amber ampule type 1, 5 c.c. (APA)

2. Equipments

2.1 Spectrophotometer and recorder, Pye Unicam, Model SP 1800

2.2 Electrical Balance, Sartorius, Model 2442

2.3 pH meter, Pye Unicam Model 292

2.4 Ampule filling instrument, AupeHe, Clay Adams Inc.

2.5 Water bath, heater thermostat, Tecam Model TE 7 Tempette

2.6 Fluorescent lamp "Toshiba" 40 W.

2.7 Hot Plate

3. Methodology

3.1 Experiment to find the most suitable method to evaluate the decomposition and the color formation of sulfacetamide sodium in aqueous solution.

3.1.1 Preparation of samples

The following solutions were prepared in distilled water:

1. Sulfanilamide, 0.5% in water
2. Disodium EDTA, 0.05% in water
3. Sodium metabisulfite, 0.10% in water
4. Sodium thiosulfate, 0.10% in water
5. Sulfacetamide sodium, 0.5, 1.0, and 10% in water
6. Sulfacetamide sodium 0.5%, containing sodium metabisulfite, 0.1, 0.3 and 0.5%
7. Sulfacetamide sodium 0.5%, containing sodium thiosulfate, 0.1, 0.3 and 0.5%
8. Solutions as in 6 and 7 containing disodium EDTA, 0.5%
9. Sulfacetamide sodium, 10% solution buffered at pH 7, 7.4, 8 by using opthalmic phosphate buffer (30) and unbuffered.

All solutions were filled in 5 milliters clear ampules and sealed.

3.1.2 Samples were exposed to artificial daylight lamp, fluorescent, 40 W. at a distance of 5 centimeters from the lamp. The empules were removed after 1, 2, 3, 4, 5, 6 and 7 days of exposure. After removal, all samples were refrigerated until analysed or examined.

3.1.3 Analysis and examination of samples were done as follows:

1. The percentages of sulfacetamide sodium solutions were analysed before and after exposure to light by using Schleider, Feldman and Galinsky method (31).
2. The color formation of the solutions before and after exposure to light was compared with standard caramel solutions prepared at concentrations according to table 1.
3. The absorption spectra of solutions No. 1, 2, 3, 4 and 5 were recorded in the wavelength range of 200-600 nm, before and after exposure to light for a period of time.
4. The visible absorption spectra of solutions No. 5, 6, 7 and 8 were recorded in the wavelength range of 300-600 nm after exposure to light for a period of time.

3.1.4 The results obtained were evaluated to find the most suitable method to be used in further experiments.

3.2 Experiment to find the most suitable antioxidants to be used in sulfacetamide solution eye drop.

3.2.1 Preparation of samples

Samples were formulated and prepared as follows:

1. Samples of 0.5% sulfacetamide sodium solutions were prepared by using sodium thiosulfate or sodium metabisulfite, 0.1, 0.3, 0.5% as antioxidant. Solutions were prepared with and without disodium EDTA as chelating agent, in water. Another set of solutions were also prepared in phosphate buffer, pH 7.4.
2. Samples of 10% sulfacetamide sodium solutions were prepared by using antioxidants of concentrations 0.1, 0.2, 0.3% and chelating agent (disodium EDTA) of concentrations 0.01, 0.05 and 0.1% according to 1, in water and in phosphate buffer, pH 7.4.

All samples were filled in 5 milliliters clear ampules and sealed. Some solutions of some particular formula were also filled in amber ampules.

3.2.2 Samples were exposed to light and treated according to 3.1.3.

3.2.3 The results were evaluated to find the most suitable antioxidant and its proper concentration to be used in sulfacetamide sodium eye drop in order to make the most stable formulation.

3.3 Analytical procedure in analysing sulfacetmaide sodium concentration (31).

The potency of sulfacetamide sodium solutions was determined as follows:

3.3.1 Preparation of standard curve.

1. Standard sulfacetamide sodium solution equivalent to 3, 4, 5, 6 mg of sulfacetamide sodium were prepared in distilled water.
2. Two millititers of sodium hydroxide solution (3.5 M) and 2 ml of hydroxylamine hydrochloride solution, (2.0 M) were added concurrently.
3. Mixtures were put into water bath at 80 $\pm$ 0.5°C for 45 minutes.
4. Three millititers of hydrochloric acid (3.5 M) was added to make the pH to 1.2 and then 2 ml of ferric chloride (0.37 M in 0.1 M hydrochloric acid) was added.
5. The solution was made up to 25 ml with water.
6. The absorbance of the solution was measure at 540 nm.

The standard curve was prepared by plotting the concentration of sulfacetamide sodium V.S. absorbance as illustrate in fig. 1 which shows a straight-line.

3.3.2 The samples were pipetted in the amount equivalent to about 4 mg of sulfacetamide sodium. The same procedures as in preparing the standard curve were followed. Then the amount of sulfacetamide sodium was calculated from the standard curve.

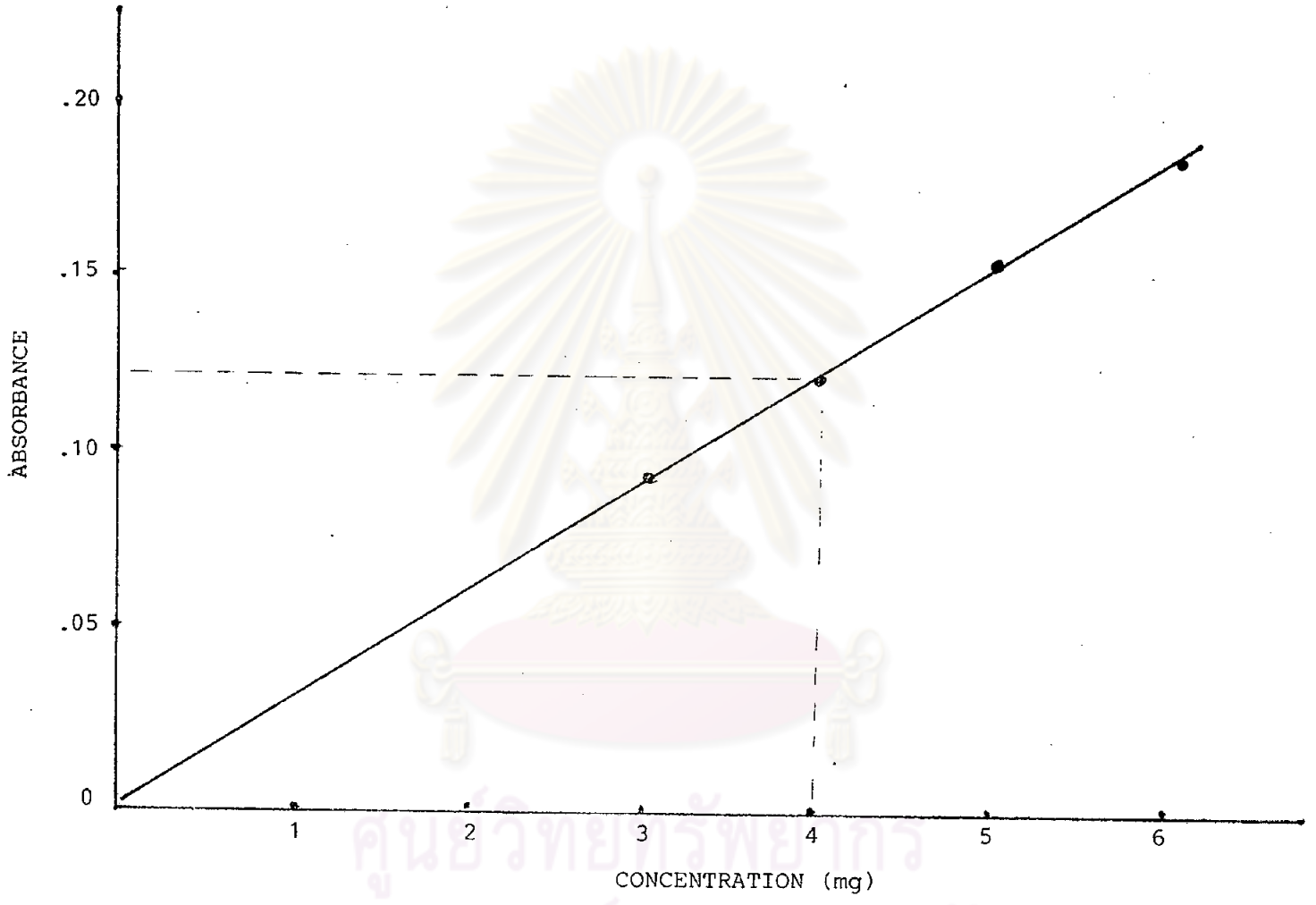

Fig. 1 Standard curve plotting the concentration of sulfacetamide sodium versus absorbance at 540 nm, slope=0.031 A/mg.