CHAPTER II

MATERIALS AND METHODS

Materials

Vitamin A, D_3, C, thiamine hydrochloride, riboflavin 5' phosphate sodium were obtained from F. Hoffmann-La Roche Co. Pyridoxine hydrochloride was purchased from Pliva. Cyanocobalamine was purchased from LPC Chemicals and Dyes Ltd. Nicotinamide was obtained from Yuki Gosei Kogyo Co Ltd. All other chemicals used in formulation of multivitamin syrup were pharmaceutical grade. All reagents used in the analysis were analytical grade.

A pH-meter used was model PHM 62 standard (Radiometer)

Methods

1. Preparation of experimental formulation

The core formula which contains all active ingredients has been selected from commercial products available in the market. The following multivitamin combination was shown to be unstable under normal storage conditions (1). It was selected for product development in this study.

Core formula :

Each 5 ml. contains :-

Vitamin A	5000 IU.
Vitamin D	1000 IU.
Thiamine hydrochloride	2 mg
Riboflavin 5' phosphate sodium	2 mg
Pyridoxine hydrochloride	2 mg
Vitamin B_{12}	3 µg
Vitamin C	75 mg
Nicotinamide	20 mg
Saccharin sodium	0.1 %

The formulations were prepared by mixing all active ingredients as in the core formula with various combination of pharmaceutical additives such as vehicle (Table 2), antioxidants and chelating agents (Table 3), suspending agents, preservative and flavoring agents (Table 4), buffer and pH (Table 5), and other formulations (Table 6)

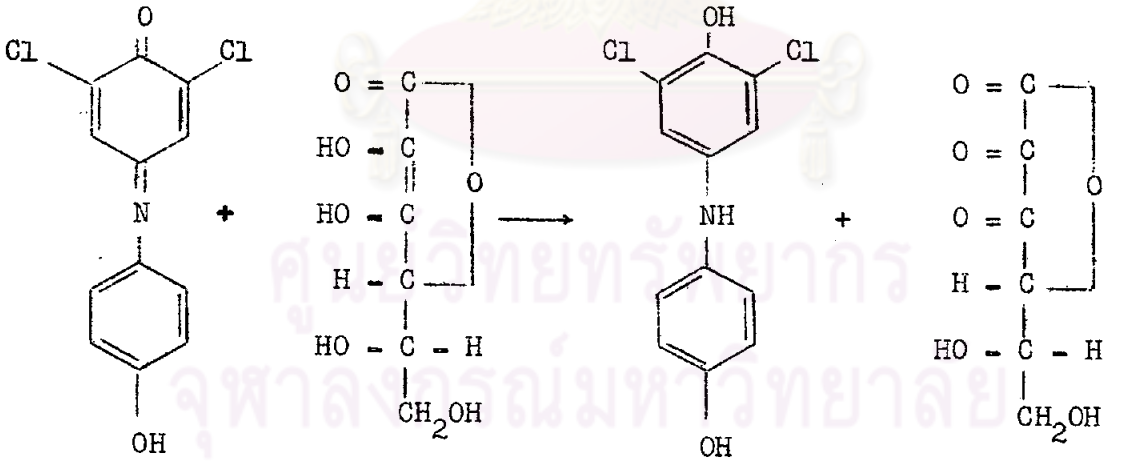
One hundred milliliters of preparation was prepared for evaluation. It was divided into about 6 ml-portion and filled in 10 ml-amber glass vials with rubber closures and aluminum seals, so that the error of head space and sample volume could be avoided. The vials were kept in an incubator at $60^{\circ} \pm 0.5^{\circ}C$ and analyzed for vitamin C content at suitable time intervals.

2. Analysis of vitamin C

The sample bottle of each formulation at each temperature was periodically removed from the incubator at the appropriate time intervals and immediately equilibrate to room temperature. The remaining vitamin C was analyzed by using 2,6-dichlorophenolindophenol titration

The 2,6-dichlorophenol indophenol method (48, 49) is based on the oxidation of vitamin C in an acid solution. The titrant is a self-indicator which has blue color in alkali solution, pink color in acid solution and colorless when reduced by vitamin C.

The reaction proceeds as follows :



Dye (pink) Ascorbic acid Dye (colourless) Dehydroascorbic acid

Procedure

The specific gravity of each preparation was determined by using a pycnometer. Sample containing about 50 mg of vitamin C was transfered into 50 ml volumetric flask and accurately weighed. It was then diluted with 8 % acetic acid in 0.3 mol/l sulfuric acid solvent (48) to obtained about 1 mg/ml solution, two millilitres of the diluted sample was titrated rapidly with 2,6-dichlorophenol indophenol standard solution until a rose-pink color end point that persist for at least 5 seconds was reached. Blank titration was also carried out in the same manner, but contained no sample.

The amount of vitamin C was calculated from :

$$\text{vitamin C (mg)} = \frac{(U - B) \times C \times S}{(A - B) \times W}$$

where U is the average volume for sample titration

A is the average volume for standard titration

B is the average volume for blank titration

C is the weight of standard, mg

S is the specific gravity of the sample

W is the weight of the sample

The first sample was considered to be 100 % of the remaining concentration of vitamin C and the subsequent samples were calculated as a relative percentage amount of the original vitamin C.

3. Obervations on physical changes

Physical appearance such as color, viscosity, clarity were inspected visually. It is a relative physical changes of a group of formulations tested at the same period.

4. Evaluation of the effects of additives on vitamin C stability

The total effects of additives on chemical stability of vitamin C were compared by the method of Connors, et al (11). The summation of the rate constants (k) effected by each additive in various formulations was averaged and compared to its counterparts as shown in each Table. Higher k values would result in more rapid degradation. This method has been applied where possible.

5. Kinetic study on the stability of vitamin C

Preparations of multivitamin syrup were made according to formulas (Table 7). Each formula was divided and filled in vials. The prepared vials were kept in constant temperature incubator at 70°, 60°, 50°, 40°, 20°C and room temperature. The samples taken from each temperature were analyzed at suitable time intervals.