THE BIOPHARMACEUTICAL PROPERTIES OF SOLID DOSAGE FORMS

The Stability of p-Aminosalicylic Acid
and
Sodium p-Aminosalicylate in Tablets

by

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ABSTRACT

A product must comply with pharmacopeial specifications at all times. If the drug in the tablet degrades to a therapeutically inactive (and sometimes toxic) substance, the patient will not receive the correct amount of drug and, more important, may be adversely affected by the degradation product. The stability characteristics of four products containing either p-aminosalicylic acid or sodium aminosalicylate were, therefore, determined in this laboratory. The products were selected at random and are currently being sold to pharmacies and hospitals in Canada.

The products were analyzed and their disintegration times determined. All products complied with existing specifications.

The products were then stored in a Vapor-Temp Controlled Humidity Chamber at various temperatures ($30^{\circ}C$. to $60^{\circ}C$.) and at 65 % and 90 % relative humidity for varying periods of time. Tablets were withdrawn from the chamber at various times and analyzed. Using the data so obtained, rate constants were calculated for each product stored at the two basic conditions, that is, at 65 % and 90 % relative humidity.

Products 1 and 4 were not affected by the environmental conditions in the humidity chamber. However, the drug in Products 5 and 7 degraded quickly to m-aminophenol when the tablets were exposed to temperatures in excess of $40^{\circ}C$. and a relative humidity of 90 %. Product 7, in particular, was very susceptible to both heat and moisture. The product would, therefore, be unacceptable to the profession. It contains buffers and it is assumed that these substances are responsible for the product's instability.

This then is a good example of poor product development.

The data accumulated during this investigation was analyzed mathematically and it was concluded that the pseudo first-order reaction equation may be used to explain the degradation process.

Arrhenius plots (that is, plots of the logarithm of the rate constant versus the reciprocal of the absolute temperature) were prepared for Products 5 and 7. On the basis of these plots, product stability at various temperatures and a relative humidity of 90 % was determined. As an example, Product 7 will contain only 90 % of the drug claimed on the label if it is stored at a temperature of 25°C. and a relative humidity of 90 % for 70 days. Values at other temperatures and for Product 5 are given in this thesis.

The data obtained during this investigation suggests that the following stability specification may be used to quickly evaluate a product containing p-Aminosalicylic acid or its sodium salt.

> Place 20 tablets in a petrie dish and transfer
> to a humidity chamber adjusted to 40°C. and a
> relative humidity of 90 %. Store in the chamber
> for ten days. Remove and assay the tablets.
> The mean potency of the 20 tablets must be
> not less than 90 % of the amount claimed on
> the label.

The drug in products which do not meet this specification would degrade to m-aminophenol even if it is stored at normal temperatures for relatively short periods of time. Product 7 falls into this category.

This study shows that humidity must be taken into consideration in any investigation on product stability. Moreover, it is not enough to determine the stability of the drug as such. The excipients (and other drugs combined with the anti-tubercular in the same dosage form) can influence the degradation process in the tablet.

This abstract represents the true contents of the thesis submitted.

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Supervisor

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I. INTRODUCTION

Aminosalicylic Acid Tablets U.S.P. must contain not less than 95% and not more than 105% of the amount of drug claimed on the label (1). The pharmacopeia permits such deviations from label claim for three basic reasons.

(a) The 5% deviation from label claim allows for errors in the manufacturing process.

(b) The deviation allows for errors in the assay procedure given in the monograph.

(c) The deviation allows for some drug degradation. In the case of Aminosalicylic Acid Tablets U.S.P., the pharmacopeia permits a maximum of 1% m-aminophenol (MAP), the principle degradation product of p-aminosalicylic acid (PAS).

Each of the above factors should be taken into consideration in the manufacture and control of any drug product. In the case of 'new' drug products, the law requires that the errors in manufacturing and assay be clearly defined and that the stability of the drug product should be such that normal storage conditions do not adversely affect the drug. If the stability of the drug in the dosage form is poor, an expiration date must be given on the label. Such a date is now seen on antibiotic and vitamin preparations and on many parenterals which degrade quickly in solution. Unfortunately, 'old' drugs need not comply with these requirements and many manufacturers do not check product stability prior to marketing the drug product they have created.

Physical changes in a pharmaceutical are readily discern-able. The tablet may crumble when exposed to moisture; molds may grow in a liquid medium; changes in the pH of the pharmaceutical may produce a precipitate; and the color of the tablet coating may fade with time. Such changes concern the pharmacist because they are easily detected by the patient. However, chemical changes in a product are not always apparent to either the pharmacist or the patient. These changes can, at times, have a much greater bearing on the health of the patient than those changes previously mentioned.

Chemical changes in a product are induced by many internal and external factors. However, the factors which affect product stability may not be the same as those that affect the stability of the chemical substance. For example, PAS is affected by heat and moisture. However, the excipients within the tablet can have a pronounced effect on the rate of degradation. This implies, therefore, that stability is a characteristic of the product and not of the chemical substance incorporated into it.

Tuberculosis is still a major disease in many parts of the world. PAS and other anti-tubercular drugs are, therefore, used in large quantities in countries with temperate and tropical climates. The World Health Organization (2) has expressed con-cern over the stability of these drugs under various temperature and humidity conditions. It is known, for example, that the drug will break down under normal storage environments but there is no general test for measuring stability under accelerated conditions. Four brands were selected for this study and were

subjected to two levels of humidity (65% and 95%) and to three or more temperatures. One of these temperatures (30°C) is common to most parts of the world and another (40°C) is reached, at times, in some parts of the world. Products were also subjected to higher temperatures in order to yield the data necessary for the calculation of degradation rates.

Rates of decomposition and velocity constants at various temperatures and relative humidities can be determined in the laboratory. The data may be plotted and the plots linearized depending on the order of the reaction. Velocity constants at room or other temperatures can then be determined from a logarithm of the velocity constant versus the reciprocal of the absolute temperature plot. Knowing these values, the half life and the stability of the product at room temperature can be established quickly and easily.

The main objective of this research project is, therefore, to determine the stability characteristics of four brands of PAS and Sodium PAS tablets marketed in Canada. Secondary to this objective, a test for measuring stability of PAS (or Sodium PAS) will be proposed.

II. LITERATURE SURVEY

Salicylates increase the oxygen uptake of pathogenic strains of mycobacteria tuberculosis. Because of this, more than fifty derivatives of benzoic acid have been tested for their effectiveness in the treatment of tuberculosis. The most active compound in this series is 2-hydroxy-4-amino-benzoic acid, better known as p-aminosalicylic acid or PAS. It was first used to treat tuberculosis in animals and man in 1946 (3).

p-Aminosalicylic acid is relatively nontoxic. Doses as high as 20 grams per day may be taken without risk. The chief manifestations of toxicity are gastrointestinal irritation, nausea, anorexia, vomiting, and diarrhea. These may be due, in part, to the decarboxylation product of PAS, m-aminophenol. Occasionally, more serious side effects such as hypokalemia, goitrogenic effect, allergic reactions, and liver damage have been observed (4).

PAS may be administered at a 5% level in food to rats over a one to two month period without serious side effects arising in the animals. The blood concentration in such studies was of the order of three to seven mg. of free PAS per 100 ml. of blood. Guinea pigs are more sensitive to the drug and show a marked decrease in appetite and growth. No changes in blood cells or hemoglobin content have been observed when rats and guinea pigs have been fed the drug.

PAS can be administered orally, subcutaneously, intramuscularly, or intravenously. A 10% solution of PAS has healed

tuberculosis abscesses after thoracoplasty in humans. Doses of up to 15 grams per day have been administered to patients and these have produced a prompt fall in temperature, a gain in weight, an increase in appetite, and a greater production of red cells and hemoglobin.

PAS is seldom used alone in the treatment of tuberculosis in man. It is usually administered with other antimicrobial agents such as isoniazid, streptomycin, or other antibiotics. Studies have been carried out that show that streptomycin-resistant tubercle bacilli are sensitive to PAS. For this reason, it is often combined with this antibiotic. The over-all therapeutic effect of the two drugs is more pronounced and there is some reduction in the production of streptomycin-resistant tubercle bacilli.

Other drug combinations have been used to treat tuberculosis (4). In hospitals, mixtures of streptomycin, isoniazid, and PAS are frequently used. However, after discharge, the most common combination used is PAS and isoniazid. The drug combination may be administered for as long as two years after discharge if the patient still shows signs of the disease.

According to Way, Smith, Howie, Weiss and Swanson (5), PAS is rapidly and completely absorbed from the gastrointestinal tract. Peak plasma levels occur within one to two hours after the drug has been administered. Within seven hours, 85% of the substance can be accounted for in the urine as free and conjugated amines. Because the drug is rapidly excreted, tablets must be administered every four hours if an adequate blood level is to be maintained.

The drug is usually administered to the patient in tablet form. Chapman, Crisafio and Campbell (6) carried out a study on the in vitro and in vivo characteristics of a number of different products containing PAS and concluded that tablets which disintegrate in more than sixty seven minutes were not completely available. These researchers measured the urinary excretion of the drug and found that an eight hour excretion period gave the same general indication of availability as did a twenty-four hour excretion period. Their data suggested that the maximum allowable time for the in vitro disintegration of tablets should be sixty minutes.

In view of the absorption and excretion characteristics of PAS and the long term therapy required to produce desired results, high concentrations of PAS in the blood and the maintenance of such levels is an important factor in the treatment of tuberculosis. Patients may not recover from the disease or may develop resistant strains of bacteria if the dose is inadequate. In order to guarantee that the dosage form contains the labelled amount of drug and that it is available to the patient, PAS research has centred around the in vivo availability of the drug and on the stability of the PAS in solution and as a chemical substance.

Stability of p-Aminosalicylic Acid.

When powdered p-aminosalicylic acid is stored under dry atmospheric conditions, it undergoes a slow change to m-aminophenol. This change is readily detectable by the darkening of the powder with time. On the other hand, if p-aminosalicylic acid

is dissolved in water and held at temperatures in excess of 80°C., (7) the solution quickly turns brown. Under such conditions, decarboxylation occurs quickly and the solution consists mainly of m-aminophenol.

Solutions of sodium p-aminosalicylate are more stable even if they are exposed to elevated temperatures. Such solutions have been studied in depth and decarboxylation, oxidation, and polymerization of both the drug and its degradation product have been observed. The toxicity of the degraded solution appears to be due to the oxidation product and to the colorless polymers of p-aminosalicylic acid (8).

Tanaka and Nakagaki (9) studied the decomposition of p-aminosalicylic acid and its salts in aqueous solution. Their research emphasized the effect of pH and the addition of propylene glycol, polyvinyl pyrrolidone, and certain surface active agents on the decarboxylation of p-aminosalicylic acid in solution. Solutions appeared to be most stable in the 9.2 to 9.5 pH range. Solutions of p-aminosalicylic acid were prepared, and the pH was adjusted to 1.9, 2.9, or 3.9, either propylene glycol, polyvinyl pyrrolidone, or a surface active agent polyoxyethylene monolauryl ether was added to each of the above solutions. The authors found that the solutions containing additives with high HLB value were more stable.

Kornblum and Sciarone (10) studied the decarboxylation of p-aminosalicylic acid in the solid state. They found that this decarboxylation depended on temperature, moisture, pressure, and particle size. If decomposition occurs in the solid state

without partial melting, it may be assumed that the formation of product molecules induces a strain in the crystal. The crystal cracks and thus exposes a greater surface area to the atmosphere. If the p-aminosalicylic acid is finely powdered, this cracking process does not occur. The authors concluded that the fracturing of large crystals of p-aminosalicylic acid was due to either uneven heating of the crystals or to the formation of carbon dioxide during the decarboxylation reaction.

Kornblum and Sciarone noted that a typical percent decomposition versus time curve consisted of three parts. They described these parts as the induction period, the acceleration period, and the decay period. At a given temperature, the induction period was found to occur only once in the thermal decarboxylation reaction. The acceleration and decay periods best fit an apparent zero-order reaction equation which indicates an independence of the rate of reaction on the fraction of p-aminosalicylic acid remaining. These studies were conducted on powdered p-aminosalicylic acid at $80^{\circ}C$.

The decarboxylation of p-aminosalicylic acid in the presence of heat and moisture was also investigated by these authors. If the temperature is constant and the vapor pressure of the water is doubled, the velocity constant is doubled. Zero order solution kinetics seem to be indicative of this relationship since the solubility of the p-aminosalicylic acid in the adsorbed moisture appears to be a function of the aqueous tension over the sample. However, when attempts were made to determine the rate constant (k_1) of the first order reaction and the rate constant (k_0) of the zero order reaction for a

saturated solution on the surface of the drug, a reasonable correlation could not be made unless it was assumed that 80% of the solid was in solution. If this assumption was made, the following relationship exists between the constants and the amount of drug which has degraded.

$$\frac{d(PAS)}{dt} = K_1 (PAS) = K_o$$

The rate of dissolution and diffusion in a stagnant system is very slow. Therefore, pure solution kinetics cannot be assumed. Kornblum and Sciarone concluded that their data was not sufficiently quantitative to attempt an analysis based on this type of kinetics. They observed that the decomposition product (m-aminophenol) was removed from the sample and that this removal was aided by the moisture surrounding the drug. Scheinker and Persiyanova (11) claimed that water functions as a catalyst. This contradicts the findings of Kornblum and Sciarone (10). In a similar study (12) it was reported that m-aminophenol, when physically incorporated into p-aminosalicylic acid, acted as a catalyst.

Stability Testing of Pharmaceuticals

There is both a legal and a moral obligation on the part of the pharmaceutical industry to market products that comply with label claim and that maintain their elegance from the time they are sold to the time that they are consumed by the patient. The pharmaceutical industry is, therefore, vitally interested in the shelf life of the products that they market.

The shelf life of a pharmaceutical can be predicted by calculating rates of degradation at various temperatures, concentrations, times, humidity conditions, and pH values. The relationships between two or more of the parameters can be determined by utilizing the mathematical principles of classical physical chemistry (13). Carefully designed experiments can yield results which may be used to evaluate shelf life quickly and with a reasonable degree of accuracy.

Although drug stability has always been important to the pharmaceutical chemist, it is only during the past 20 years that stability predictions have been made on the basis of quantitative relationships between the factors involved. Several examples will be given herein in order to illustrate the mathematics and methodology used to predict shelf life.

Garrett and Carper (14) investigated the stability of the color in a liquid sulfonamide preparation. The Arrhenius relationship should apply to the degradation of the color in the solution. The rate of change of some property can be evaluated as zero, first, or second order by normal, reciprocal or logarithm plots of a concentration function against time. The slopes of the lines can be used to estimate the specific rates within specified concentration ranges. The values at various temperatures can then be plotted versus the reciprocal of the absolute temperature and an estimate of the stability at some other temperature can then be made. Garrett and Carper found that the decrease in color in the liquid sulfonamide preparation was proportional to time (that is, it was zero order) at all the temperatures studied. The logarithm of the slopes of such plots versus the

reciprocal of the absolute temperature was linear. On the basis of this type of plot, these authors predicted the stability of preparation at room temperature.

The thermal degradation of vitamins in liquid multi-vitamin preparations was investigated by Garrett (15). He found that these preparations followed either a zero or a first order plot. Tardiff (16) showed that the mathematical principles outlined by Garrett for liquids were applicable to solid dosage forms containing vitamins.

Moore (17) investigated the stability of ergonovine maleate in tablets by using accelerated storage tests. Garrett (18) attempted to predict the stability of streptozotocin not only in vitro but also in vivo. In this study, Garrett derived equations that characterized the degradation not only as a function of temperature but also of pH.

Uprety and Brevis (19) showed by studies at elevated temperatures that the stability of ascorbic acid depended on pH, temperature, type of vehicle used, and the amount of head space in the container.

All researchers use similar methods to determine the stability of the pharmaceutical at room temperature. The pharmaceutical is subjected to three or more elevated temperatures and the results obtained under these conditions are plots in the appropriate way to determine the stability at room temperature. Degradation in a pharmaceutical is complicated by the presence of other 'inactive' components. It is not possible, therefore, to generalize and each product and variable must be investigated separately in order to determine the shelf life of that preparation.

The Analysis of p-Aminosalicylic Acid and of Sodium Aminosalicylate

The U.S.P. XVII method for the analysis of sodium amino-
salicylate and p-aminosalicylic acid is based on a diazotization
(20,21) followed by titration with sodium nitrite (22). The
endpoint may be detected externally with starch iodide paper or
potentiometrically. However, m-aminophenol is also diazotable
and it is not possible, therefore, to determine either p-amino-
salicylic acid or sodium aminosalicylate in the presence of this
degradation product by this method of analysis. The U.S.P. does
specify a method of analysis for m-aminophenol. The analysis
is carried out spectrophotometrically and is specific for the
degradation product.

A colorimetric method of analysis for sodium aminosalicy-
late has been reported by Baiulescu (23). This method is based on
the reaction of uranyl nitrate hexahydrate with sodium amino-
salicylate to form a characteristic orange color. Under given
conditions, m-aminophenol does not interfere with the analysis of
sodium p-aminosalicylate.

Many methods of analysis have been reported in the literature
for the determination of the drug as such and the drug in various
dosage forms (24,25,26,27,28,29,30,31,32). The nonaqueous tit-
rimetric methods of analysis reported by Chatten (33) are more
specific than the official method of analysis. The method can be
used to determine p-aminosalicyclic acid or sodium aminosalicylate
in the presence of its degradation product. This method of
analysis was used to determine the drug content in the four products
subjected to this stability study.

III. EXPERIMENTAL

Apparatus

(1) Blue M Vapor-Temp Controlled Relative Humidity Chamber.

(2) Microburette, graduated to 0.01 ml.

(3) 25.0 ml. pipettes.

(4) 250.0 ml. volumetric flasks.

(5) Fisher pH Meter.

(6) Thin-Layer Chromatographic Apparatus Model C-200

(Research Specialties Co.)

(7) Iodine Chamber.

(8) Erweka Tablet Disintegration Apparatus (Type ZT2).

Reagents and Solutions

(1) Anhydrous acetone, reagent grade.

(2) Anhydrous methanol, reagent grade.

(3) Dioxane, Eastman white label.

(4) 0.1 N Perchloric Acid in Dioxane.

(5) 0.1 N Potassium Hydroxide in Methanol.

(6) Thymol Blue Indicator Solution, 0.5 % w/v in methanol.

(7) Crystal Violet Indicator Solution, 0.5 % w/v in glacial

acetic acid.

(8) Phenolphthalein Indicator Solution, U.S.P.

(9) Potassium acid phthalate, U.S.P.

(10) Glacial acetic acid, U.S.P.

Standardization of Potassium Hydroxide in Methanol

Weigh accurately 200 mg. of potassium acid phthalate and dissolve in 50 ml. of methanol. Add one drop of phenolphthalein indicator solution and titrate to a pink color with the titrant. Calculate the normality.

Standardization of Perchloric Acid in Dioxane

Weigh accurately 200 mg. of potassium acid phthalate and dissolve in 50 ml. of glacial acetic acid. Add one drop of crystal violet indicator solution and titrate to a blue color with the titrant. Calculate the normality.

The Analysis of p-Aminosalicylic Acid Tablets

Select, at random four p-aminosalicylic acid tablets for the analysis. Using a glass mortar and pestle, reduce the tablets to a fine powder. Add acetone and stir. Transfer the acetone and residue to a 250.0 ml. volumetric flask and make to volume with acetone. Shake for ten minutes and then allow the residue to settle to the bottom of the flask. Transfer 25.0 ml. of the supernatant liquid to an erlenmeyer flask, add four drops of thymol blue indicator solution, and titrate the solution with potassium hydroxide in methanol to a blue end point. Calculate the number of mg. of p-aminosalicylic acid in one tablet.

$$\text{Mg. PAS} = V \times N \times \text{eq.} \times \frac{250}{25} \times \frac{1}{4}$$

where V is the number of ml. of potassium hydroxide in
methanol used in the determination,

N is the normality of the titrant, and

eq. is the equivalent weight of PAS (153.14).

Products No. 5 and 7 contained p-aminosalicylic acid and were analyzed in the manner described above. The method of analysis is specific for p-aminosalicylic acid in the presence of m-aminophenol (33,34).

The Analysis of Sodium Aminosalicylate Tablets

Select, at random four sodium aminosalicylate tablets for the analysis. Using a glass mortar and pestle, reduce the tablets to a fine powder. Add methanol and stir. Transfer the methanol and residue to a 250.0 ml. volumetric flask and make to volume with methanol. Shake for ten minutes and then allow the residue to settle to the bottom of the flask. Transfer 25.0 ml. of the supernatant liquid to an erlenmeyer flask, add one drop of thymol blue indicator solution, and titrate the solution with perchloric acid in dioxane to a peach end point. Calculate the number of mg. of sodium aminosalicylate in one tablet.

$$\text{Mg. Sodium PAS} = V \times N \times eq. \times \frac{250}{25} \times \frac{1}{4}$$

where V is the number of ml. of perchloric acid in dioxane
        used in the determination,

N is the normality of the titrant, and

eq. is the equivalent weight of Sodium PAS (211.15).

Products No. 1 and 4 contained sodium aminosalicylate and were analyzed in the manner described above. The method of analysis is specific for sodium aminosalicylate in the presence of m-aminophenol. Both this and the previous end point can be detected potentiometrically by using a Fisher pH meter.

Stability Studies

Four brands of p-aminosalicylic acid or sodium aminosalicy-
late tablets were selected at random and subjected to the follow-
ing temperature - humidity conditions.

(a) 90 % relative humidity, 50°C.

(b) 90 % relative humidity, 40°C.

(c) 90 % relative humidity, 30°C.

(d) 65 % relative humidity, 60°C.

(e) 65 % relative humidity, 55°C.

(f) 65 % relative humidity, 50°C.

(g) 65 % relative humidity, 30°C.

Products No. 1 and 4 were not studied at 65% relative humidity,
55°C. The following general procedure was used in this stab-
ility study.

Set the Blue M Vapor-Temp Controlled Relative Humidity

Chamber to the desired condition. Place the tablets in

petrie dishes and transfer to the chamber. Depending

on the rate of degradation, store the tablets contin-

uously in the chamber from four to fourteen days. Re-

move four tablets from each petrie dish at suitable

intervals and assay immediately. If this is not possi-

ble, store the tablets in a refrigerator for not more

than 24 hours and then assay. Samples were taken at 24

hour intervals or multiples thereof depending on the

rate of degradation. Calculate the number of mg. of p-

aminosalicylic acid or sodium aminosalicylate in each

tablet.

Stability data for the four products is shown in Tables I to VIII. Figures 1, 2, 3, 4 and 5 show psuedo first-order plots for Products 4, 5 and 7. The calculated slopes for the lines shown in these figures are reported in Tables IX and X. These slopes are calculated by the method of least squares (35) and were used to prepare the Arrhenius plots for Products 5 and 7 shown in Figures 6 and 7.

Determination of the Disintegration Time of the Tablets

The disintegration times of the tablets were determined by using an Erweka Tablet Disintegration Apparatus. The method is described in detail in a document published by the Food and Drug Directorate (36). All tablets disintegrated in less than 60 minutes and, therefore, comply with the disintegration regulation appended to the Food and Drug Act. Mean disintegration times for the four products are shown in Table XI.

Identification of the Degradation Product

The acetone or methanolic solutions of p-aminosalicylic acid or sodium aminosalicylate were checked for the presence of m-aminophenol by chromatographing aliquots on Silica Gel G. (37). The following procedure may be used to determine the presence of m-aminophenol in the degraded product.

Coat 8" x 8" glass plates with a 100 mu layer of Silica Gel G. Activate the plates by heating in an oven set at 105°C. for half an hour. Reserve a 30 ml. portion of the solutions prepared for the assay, reduce volume to 5 ml by allowing solvent to evaporate spontaneously, and spot 5 lambda aliquots on the starting line. Using a 10:30:5 v/v mixture

TABLE I

Stability Data for p-Aminosalicylic Acid Tablets* - Product
No. 7 at 90% Relative Humidity

Time in days	50°C	40°C	30°C
	mg. per tablet	mg. per tablet	mg. per tablet
0	498.5	477.0	477.0
1	403.1	475.0	-
2	346.9	-	464.7
3	301.7	-	-
4	240.0	448.1	456.9
5	-	-	-
6	-	412.8	459.4
7	-	-	-
8	-	393.9	451.5
9	-	-	-
10	-	369.7	-
11	-	-	-
12	-	-	-

*Compressed Tablets

TABLE II.

Stability Data for p-Aminosalicylic Acid Tablets* - Product
No. 7 at 65% Relative Humitity

Time in days	60°C mg. per tablet	55°C mg. per tablet	50°C mg. per tablet	30°C mg. per tablet
0	477.0	492.8	476.9	492.0
1	-	-	-	-
2	439.1	481.7	471.8	-
3	-	-	-	483.5
4	413.6	469.0	471.6	-
5	-	-	-	-
6	375.95	453.2	467.9	485.0
7	-	-	-	-
8	-	432.8	-	-
9	332.9	-	462.8	494.5
10	-	408.5	-	-
11	-	-	-	-
12	-	-	469.7	482.7

*Compressed tablets

TABLE III

Stability Data for p-Aminosalicylic Acid Tablets* - Product
No. 5 at 90% Relative Humidity

Time in days	50^0C	40^0C	30^0C
	mg. per tablet	mg. per tablet	mg. per tablet
0	485.9	478.7	478.7
1	480.1	481.3	-
2	-	-	474.7
3	465.7	-	-
4	-	480.0	474.7
5	456.0	-	-
6	439.0	479.7	474.8
7	435.0	-	-
8	-	476.6	476.6
9	-	-	-
10	-	471.6	-
11	391.6	-	-
12	-	-	-
13	-	-	-
14	-	-	463.1

*Compressed Tablets

TABLE IV

Stability Data for p-Aminosalicylic Acid Tablets* - Product
No. 5 at 65% Relative Humidity

Time in days	60°C mg. per tablet	55°C mg. per tablet	50°C mg. per tablet	30°C mg. per tablet
0	478.7	492.5	477.5	488.5
1	-	-	-	-
2	460.7	483.5	470.5	-
3	441.3	-	-	484.4
4	-	475.5	477.0	-
5	406.3	-	-	-
6	-	466.0	476.5	501.10
7	361.6	-	-	-
8	-	445.2	-	-
9	-	-	470.3	488.75
10	-	427.1	-	-
11	-	-	-	-
12	-	-	466.7	489.50
13	-	-	-	-
14	-	-	-	-

*Compressed Tablets

TABLE No.V

Stability Data for Sodium p-Aminosalicylate Tablets* - Product No. 4 at 90% Relative Humidity

Time in days	50°C mg. per tablet	40°C mg. per tablet	30°C mg. per tablet
0	500.0	509.4	497.0
1	492.8	499.0	-
2	-	-	496.0
3	481.3	-	-
4	-	498.4	-
5	480.9	-	-
6	465.3	495.3	497.5
7	-	-	-
8	459.0	498.8	494.1
9	-	-	-
10	-	500.0	-
11	-	-	-
12	-	-	-
13	-	-	-
14	-	-	493.5

*Compressed Tablets

TABLET NO. VI

Stability Data for Sodium p-Aminosalicylate Tablets*- Product
No. 4 at 65% Relative Humidity

Time in days	60°C	50°C	30°C
	mg. per tablet	mg. per tablet	mg per tablet
0	497.0	509.4	496.0
1	-	-	-
2	500.0	506.1	-
3	-	-	499.7
4	502.2	500.1	-
5	-	-	-
6	499.4	500.8	492.2
7	-	-	-
8	499.7	-	-
9	-	500.6	494.5
10	-	-	-
11	-	-	-
12	-	497.0	494.8

*Compressed Tablets

TABLE VII

Stability Data for Sodium p-Aminosalicylate Tablets* - Product
No. 1 at 90% Relative Humidity

Time in days	50°C	40°C	30°C
	mg. per tablet	mg. per tablet	mg. per tablet
0	499.5	500.9	500.9
1	-	499.9	-
2	504.7	-	500.4
3	-	-	-
4	478.2	504.0	502.0
5	-	-	-
6	465.2	504.0	498.7
7	-	-	-
8	-	502.7	499.1
9	-	-	-
10	-	502.7	-
11	-	-	-
12	-	-	-
13	-	-	-
14	-	-	498.7

*Sugar Coated Tablets

TABLE VIII

Stability Data for Sodium p-Aminosalicylate Tablets* - Product
No. 1 at 65% Relative Humidity

Time in days	60°C mg. per tablet	50°C mg. per tablet	30°C mg. per tablet
0	500.9	503.4	506.5
1	-	-	-
2	499.8	503.4	-
3	-	-	498.3
4	499.1	500.4	-
5	-	-	-
6	497.5	502.8	503.0
7	-	-	-
8	496.9	-	-
9	-	504.7	492.2
10	-	-	-
11	-	-	-
12	-	-	498.2

*Sugar Coated Tablets

TABLE IX

Slopes of the Pseudo-First Order Plots of the Thermodegradation of Product No. 7

Relative Humidity	Temperature C°	Reciprocal of Absolute Temp. $1/T$	Slopes
90%	50°C	3.10×10^{-3}	-0.07606
90%	40°C	3.20×10^{-3}	-0.01144
90%	30°C	3.30×10^{-3}	-0.00130
90%	25°C	3.35×10^{-3}	-0.00045*
65%	60°C	3.00×10^{-3}	-0.01710
65%	55°C	3.05×10^{-3}	-0.00870
65%	50°C	3.10×10^{-3}	-0.00085
65%	30°C	3.30×10^{-3}	≈ 0.00000

* By Extropolation - (See Figure 6)

TABLE X

Slopes of the Pseudo-First Order Plots of the Thermodegradation of Product No. 5

Relative Humidity	Temperature C^{o}	Reciprocal of Absolute Temp. $1/_{T}$	Slopes
90%	50°C	3.10×10^{-3}	-0.00790
90%	40°C	3.20×10^{-3}	-0.00060
90%	30°C	3.30×10^{-3}	-0.00015
65%	60°C	3.00×10^{-3}	-0.02100
65%	55°C	3.05×10^{-3}	-0.00680
65%	50°C	3.10×10^{-3}	-0.00110
65%	30°C	3.30×10^{-3}	≈ 0.00000

TABLE XI

Disintegration Times for Four Brands of p-Aminosalicylic
Acid and Sodium p-Aminosalicylate Tablets

Tablet No.	Disintegration Time			
	Product No. 1*	Product No.4	Product No.5	Product No.7
1	37 min.	17 min.	less than 1 min.	less than 1 min.
2	34 min.	17 min.	"	"
3	40 min.	17 min.	"	"
4	37 min.	14 min.	"	"
5	42 min.	20 min.	"	"
6	39 min.	15 min.	"	"
AVERAGE	38 min.	17 min.	less than 1 min.	less than 1 min.

*Enteric Coated Tablets

Figure 1. Psuedo First-order plots of the thermo-
degradation of Product No. 7 at 90%
Relative Humidity.

Figure 2.   Pseudo First-order plots of the thermo-
            degradation of Product No. 7 at 65%
            Relative Humidity
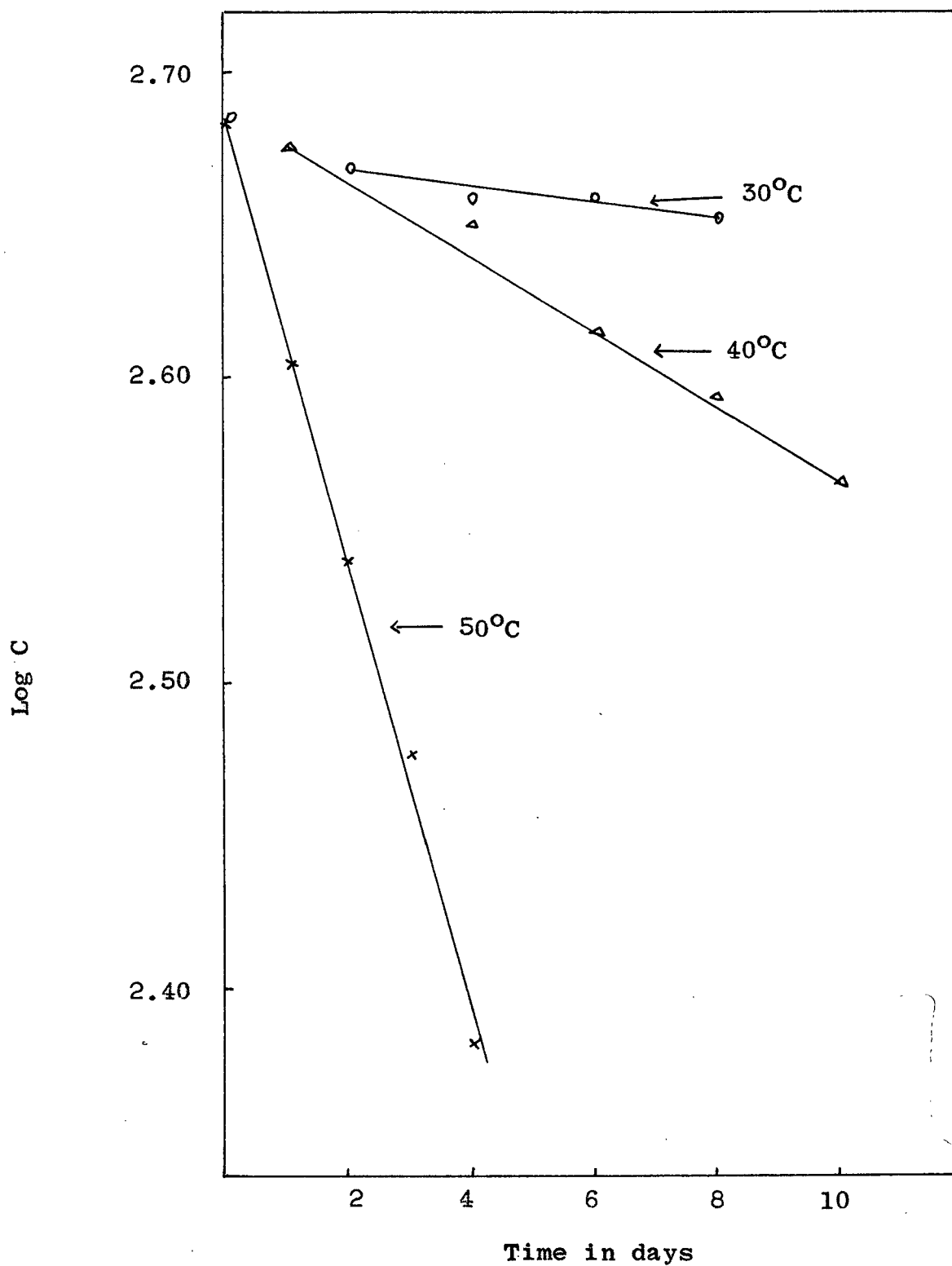
Figure 3.   Pseudo first-order plots of the thermo-
            degradation of Product No. 5 at 90%
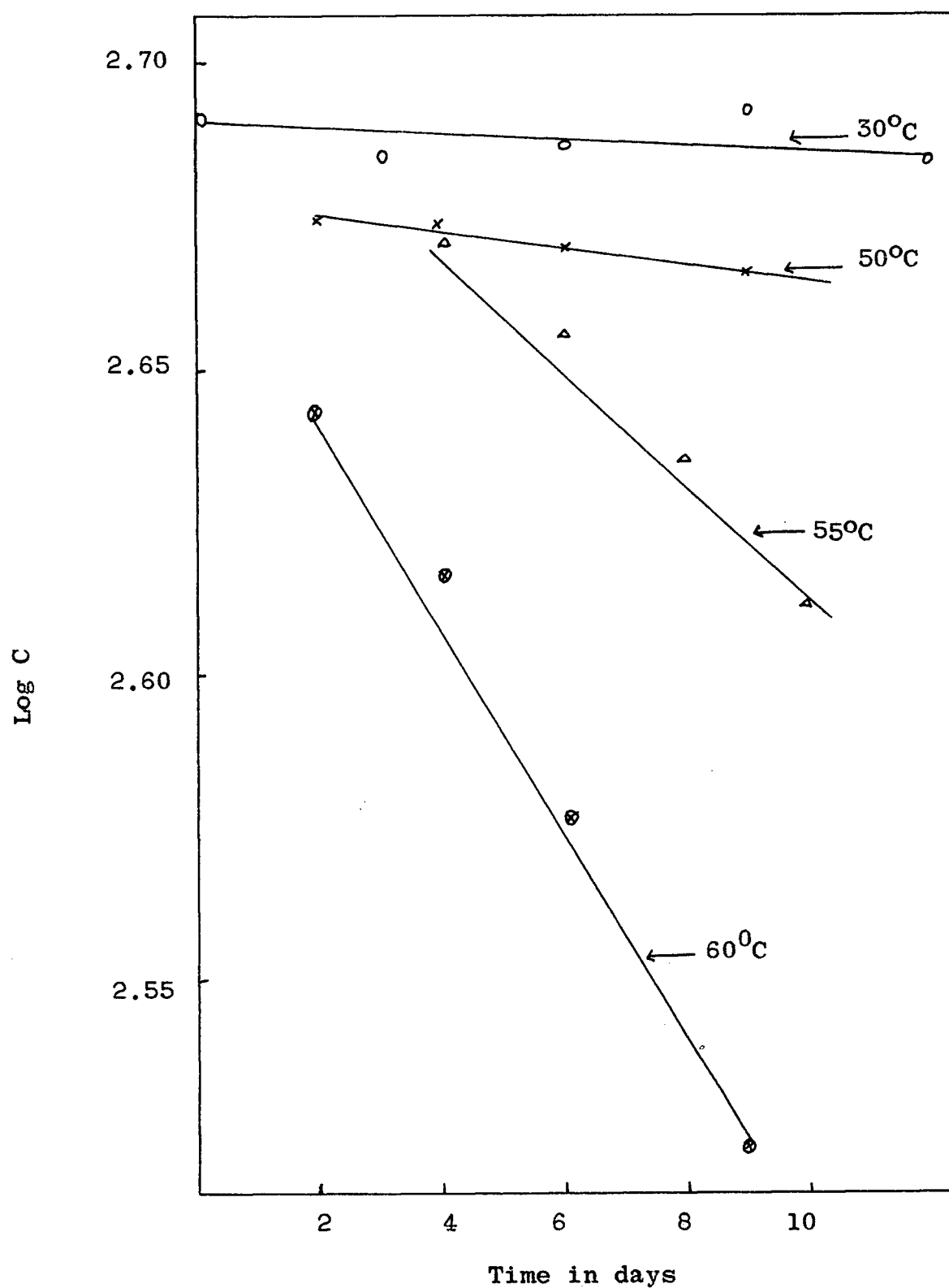   Relative Humidity

Figure 4. Pseudo first-order plots of the thermo-
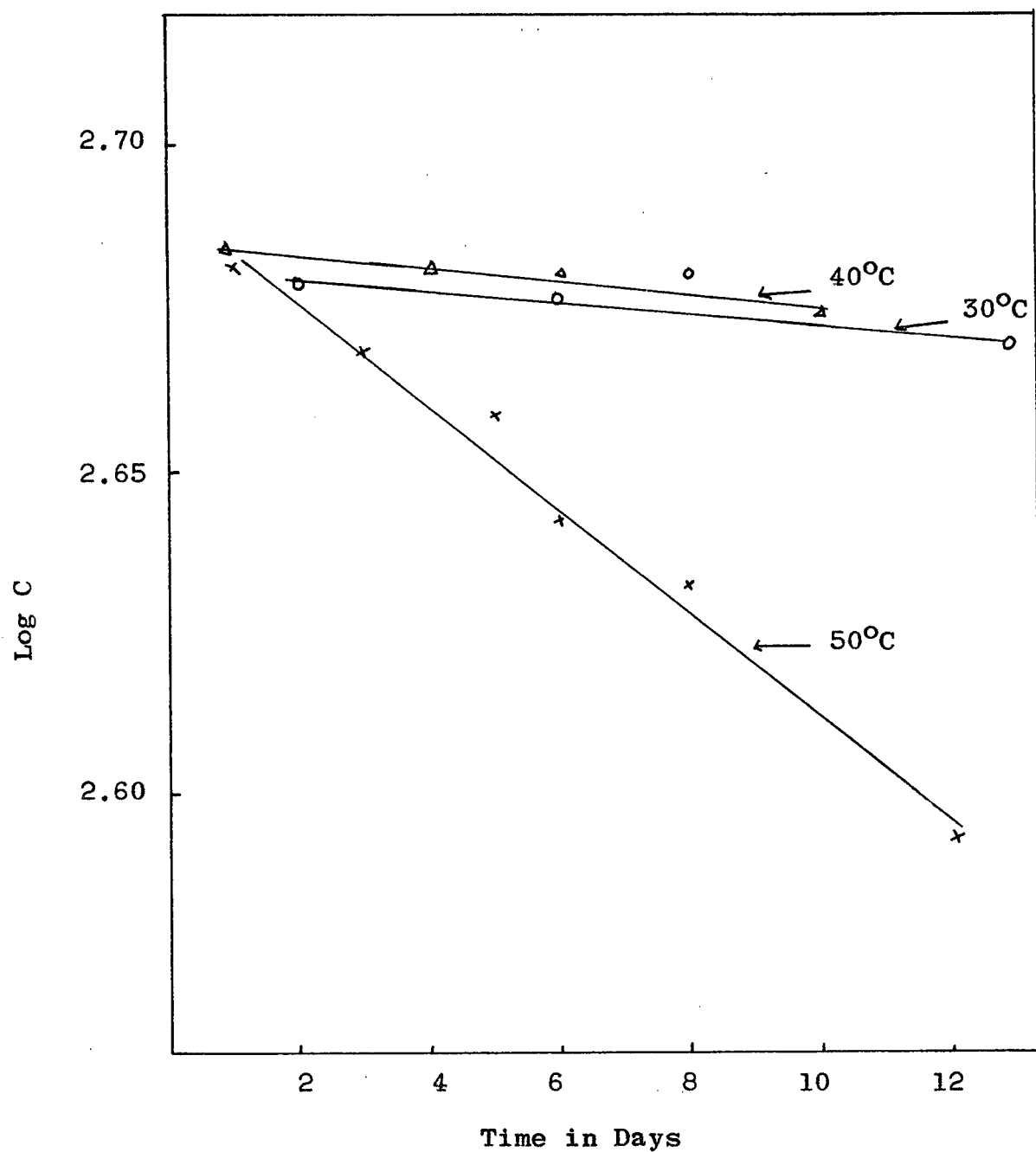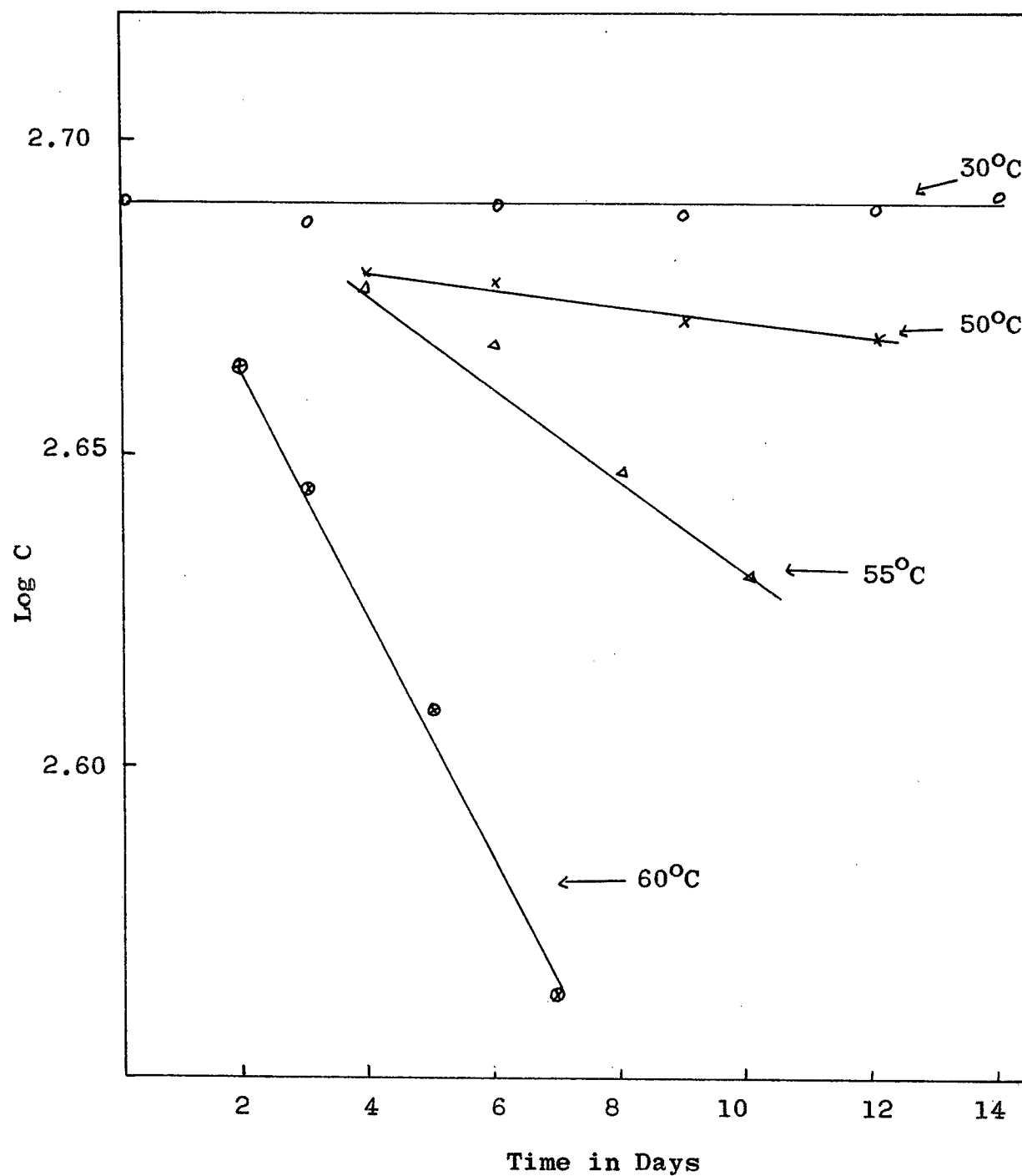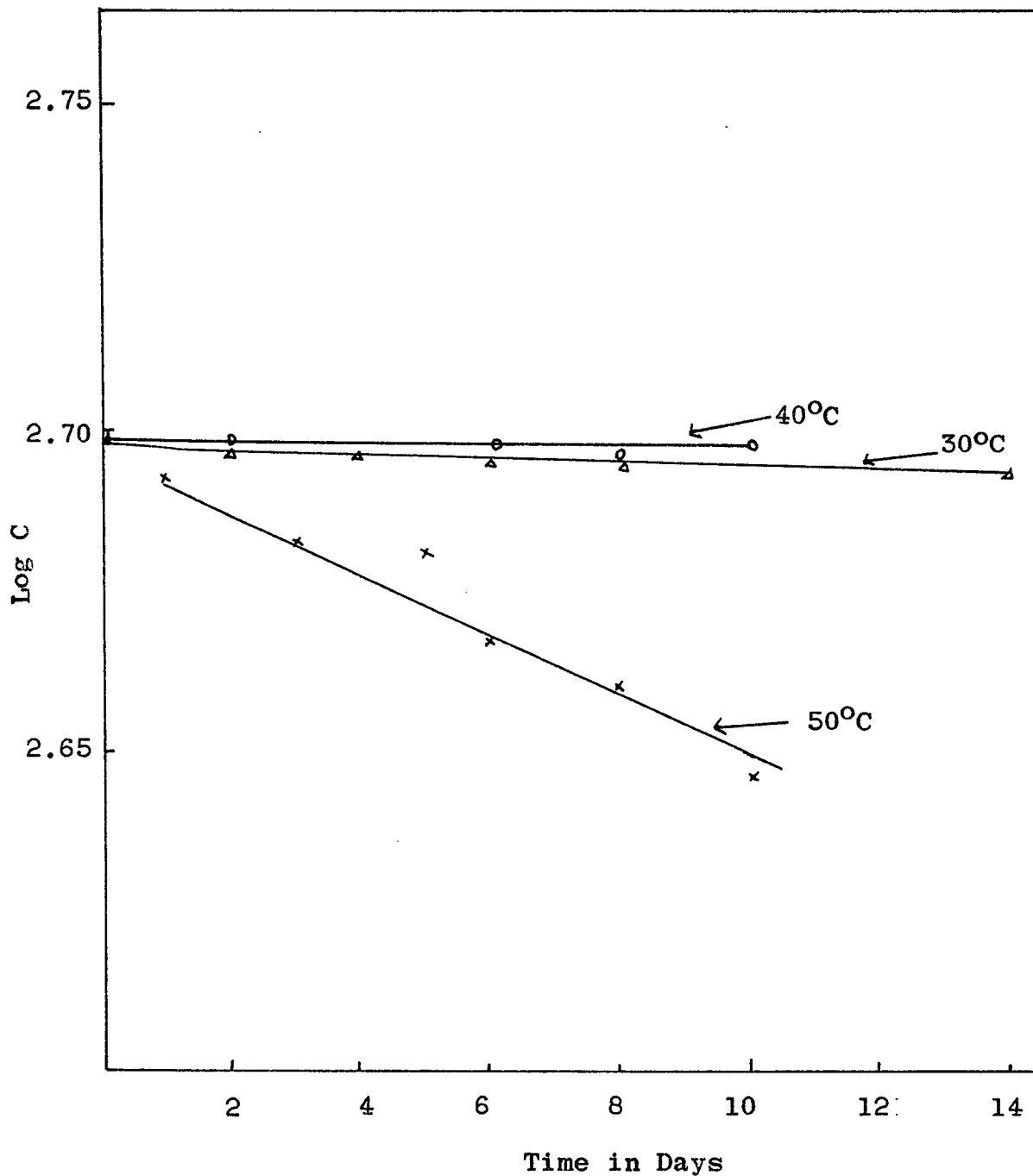degradation of Product No. 5 at 65% Relative
Humidity

Figure 5.   Pseudo first-order plots of the thermodegradation
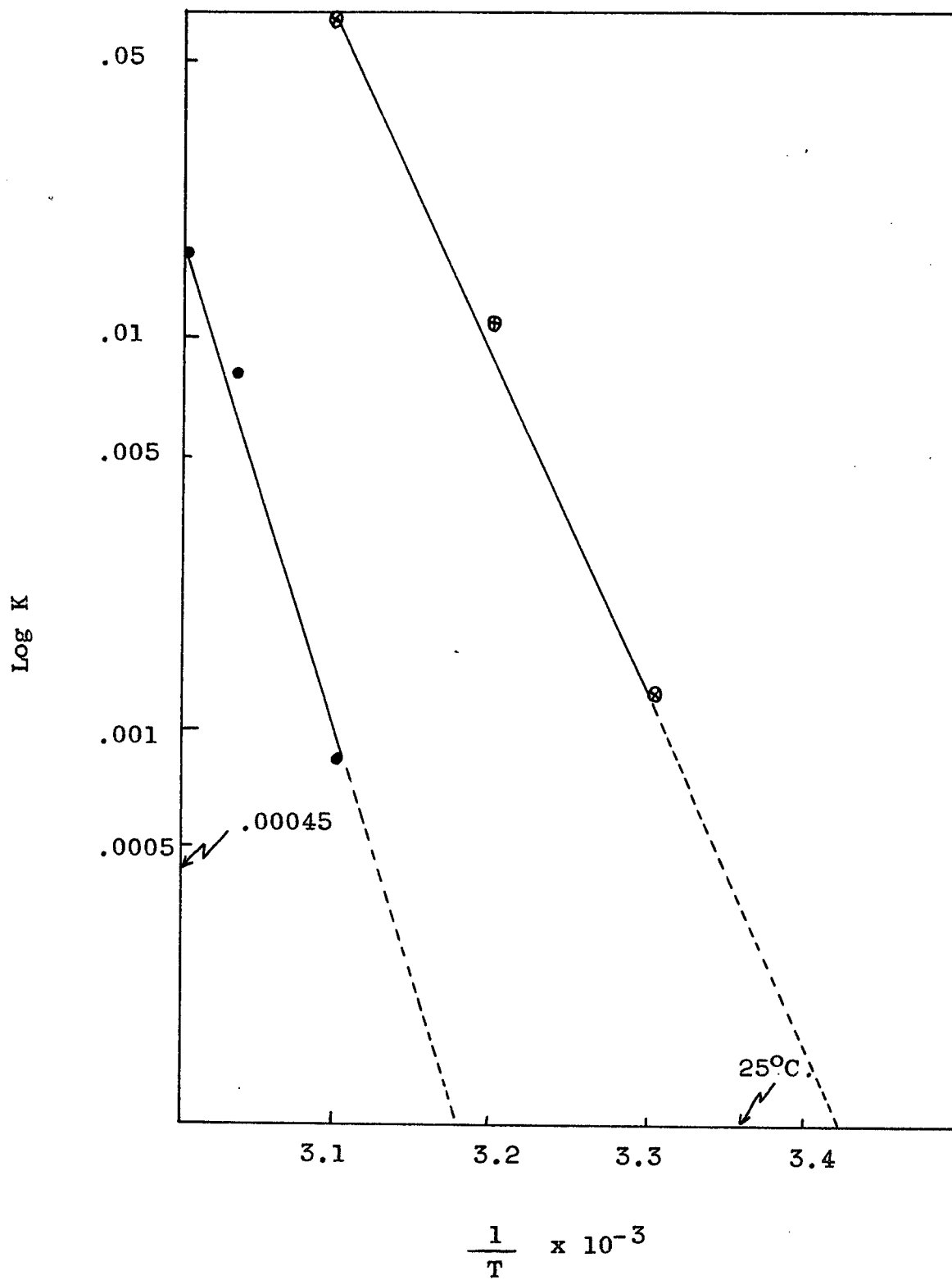   of Product No. 4 at 90% Relative Humidity.
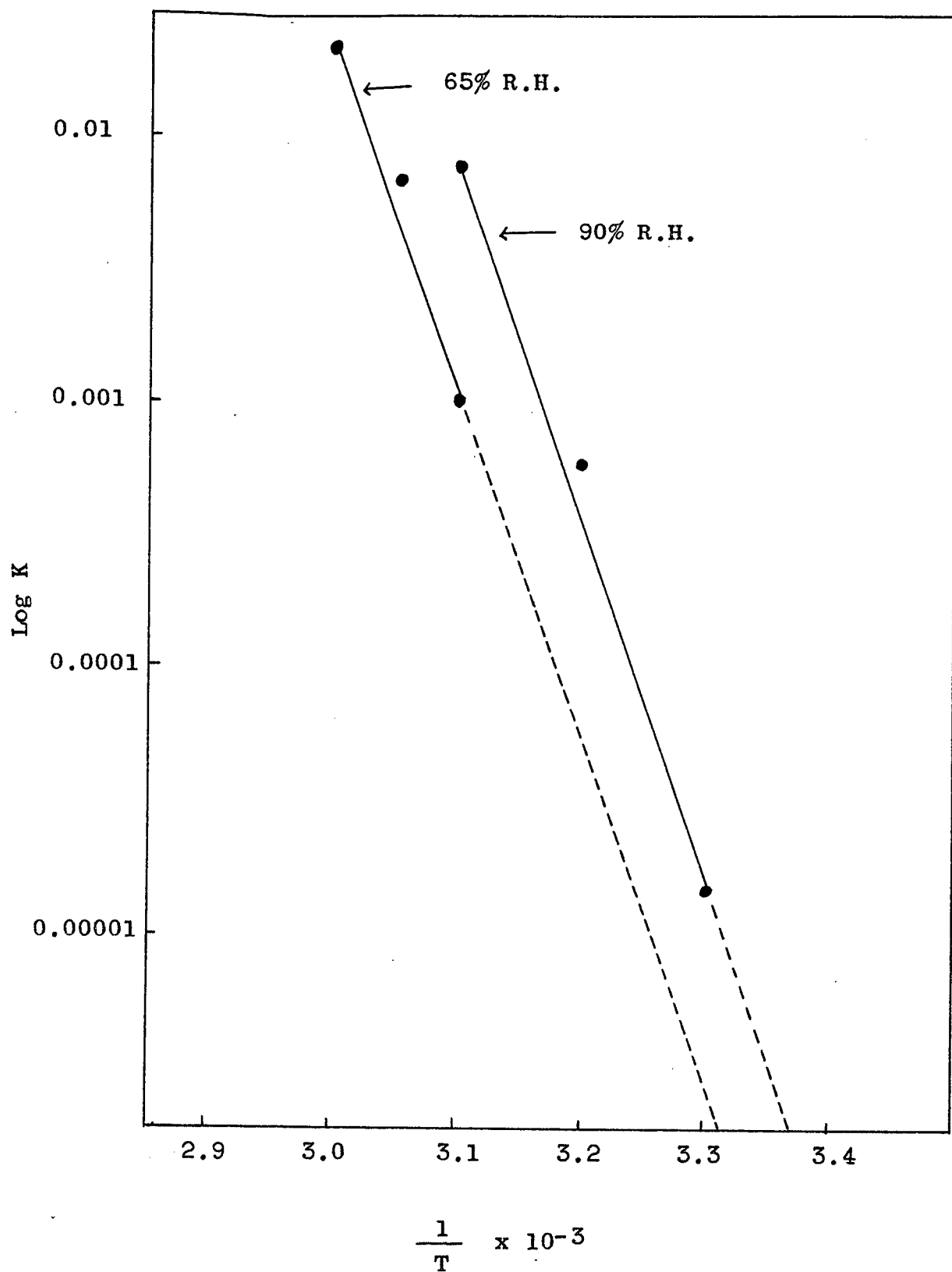
Figure 6. Arrhenius plots for Product No. 7

Figure 7. Arrhenius plots for Product No. 5

of 28 % ammonia, isopropanol and water, develop the chromatogram for three hours. Remove the plates from the developing chamber, mark the solvent front, dry the plates at room temperature, and place in an iodine chamber to develop the spots. Calculate R_f values

$$R_f = \frac{\text{The distance the individual spot has moved}}{\text{The distance the solvent front has moved}}$$

Preliminary studies with p-aminosalicylic acid, m-aminophenol, and a mixture of the two showed that the R_f value for m-aminophenol was 0.875 and for p-aminosalicylic acid was 0.687.

A typical chromatogram is shown in Figure 8. The products specified on this chromatogram had been exposed to 65 % relative humidity and a temperature of 60°C. for 48 hours. Products 1 and 4 contained only p-aminosalicylic acid. However, Products 5 and 7 contained m-aminophenol. Chromatograms were prepared for products subjected to other temperature and humidity conditions. Results varied with storage conditions but only the drug and m-aminophenol could be detected on the chromatograms. It would appear, therefore, that the only degradation product formed in the products under the conditions specified is m-aminophenol.
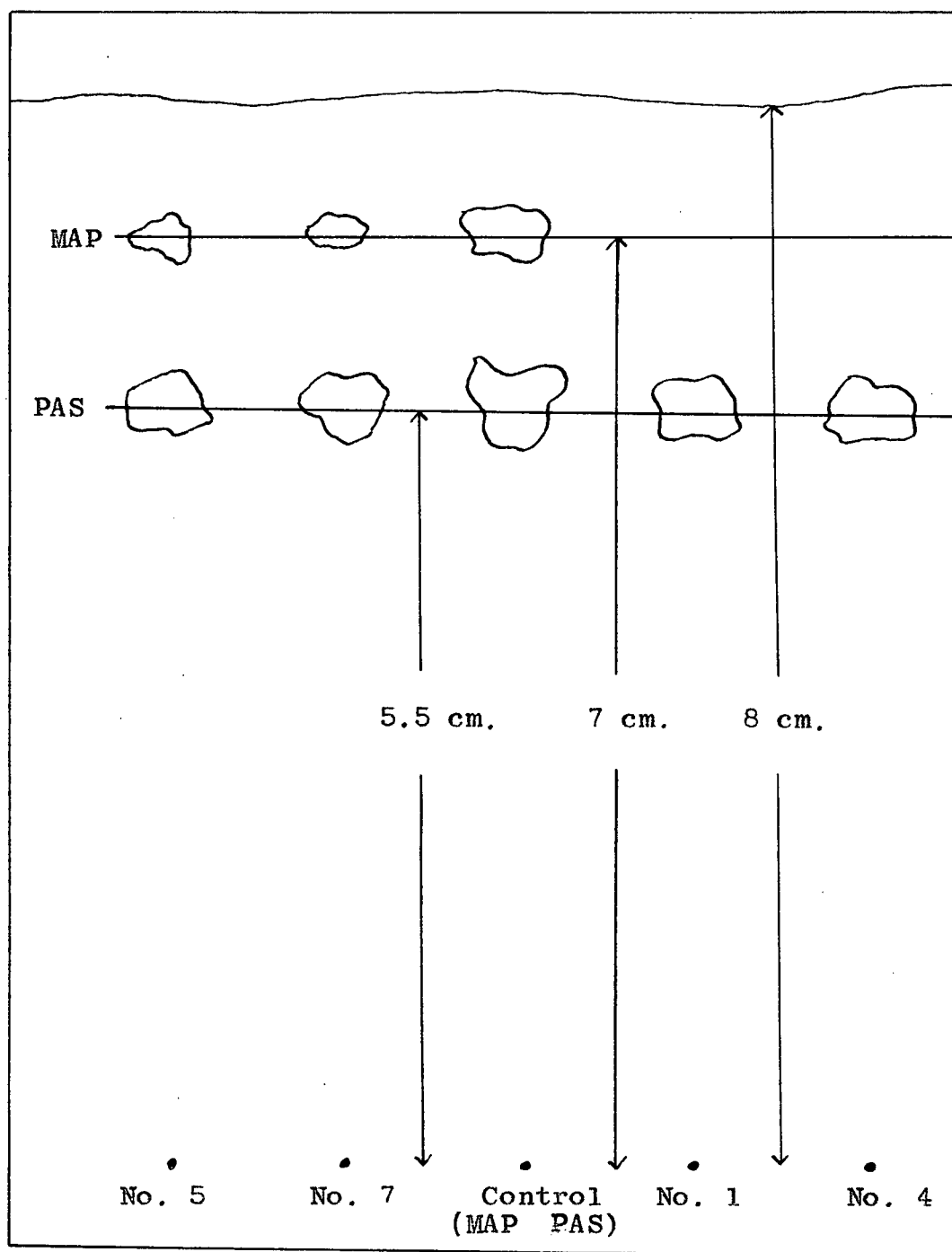
Figure 8.   Thin Layer Chromatograms for Four Products
             after 48 hours at 60°C. and 65 % Relative
             Humidity.

IV. THEORY

The Order of a Reaction

A first-order reaction is defined as one for which, at a given temperature, the rate of the reaction depends only on the first power of the concentration of a single reacting species. If the concentration of this species is represented as c and if the volume of the system remains essentially constant during the course of the reaction, the first-order equation may be written in the following way (38).

$$- \frac{dc}{dt} = kc$$

The rate constant (k) is thus a positive quantity and the unit for this constant is reciprocal time.

The experimental results obtained in a study of the rate of a reaction are usually values of c, or some quantity related to c, at various times. Such data is best evaluated by using the integrated form of the first-order reaction equation. If the initial concentration at time zero is c_0 and the concentration at some later time t has fallen to c, the integrated form of the equation is:

$$- \int_{c_0}^{c} \frac{dc}{c} = k \int_{0}^{t} dt$$

and

$$- \ln \frac{c}{c_0} = \ln \frac{c_0}{c} = kt$$

or

$$\text{Log} \frac{c}{c_0} = \frac{k}{2.303} t$$

The final form of this equation is usually that given below

$$\text{Log } c = \frac{kt}{2.303} + \text{Log } c_o$$

A reaction is, therefore, considered to be a first-order reaction
if a plot of Log c versus t results in a straight line. The
slope of this line can be calculated and the rate constant for
the reaction determined.

The above equations may be used to evaluate experimental
data but they do not always define the mechanism of the reaction.
For example, in this particular stability study, the water con-
tent of the atmosphere in the humidity chamber is constant for
any set of conditions. Even if the water molecules are involved
in the rate determining step, the water concentration would not
be defined in the basic equation but would be a part of the rate
constant term. If the water in the atmosphere (or, for that
matter, any of the other ingredients in the tablet) contributes
to the constant in the above equations, the reaction is consider-
ed to be psuedo-first order.

Reaction Rate and Temperature

The rate equation and the rate constant for a reaction are
determined from kinetic data at a fixed temperature. If ex-
periments are performed at several different temperatures, it
is generally found that the concentration changes do not affect
the rate equation. However, the rate constant value is much
greater at the higher temperatures.

In 1889, Arrhenius showed that the rate constant increases
in an exponential manner as the temperature increases. This

implies that a plot of Log k (the rate constant) versus 1/T
(where T is the absolute temperature) is a straight line. This
may be stated mathematically in the following way (39).

$$\ln\ k\ \propto\ 1/T$$

or

$$k\ =\ K'e^{1/T}$$

where K' is a constant

This relationship is now defined more accurately by including
certain other factors which are involved in the relationship be-
tween k and T (40).

$$k\ =\ A\ e^{-Ea/RT}$$

A, in this equation, is called the pre-exponential factor, Ea is
the activation energy, and R is the gas constant. The logarithmic
form of this equation is given below.

$$\ln\ K\ =\ -\ \frac{Ea}{RT}\ +\ A$$

$$Log\ K\ =\ -\ \frac{Ea}{2.303R}\ \times\ \frac{1}{T}\ +\ \frac{Log\ A}{2.303}$$

This implies that a plot of Log k versus 1/T is a straight line.
The slope of this line is equal to $-$ Ea/2.303 R and the intercept
value is equal to Log A / 2.303. It is, therefore, possible to
calculate the slope of the line from rate constant determined at
three or more temperatures. It is then possible to determine
mathematically the rate of the reaction at any other temperature.
These, then, are the mathematical principles used by most research-
ers (14, 18, 19) to determine product stability at room temperature
by studying degradation at elevated temperatures.

V. DISCUSSION

Method of Analysis

Many methods of analysis are available for the determination of p-aminosalicylic acid or its sodium salt (24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 23). However, only several of these methods are suitable for the determination of the drug in the presence of m-aminophenol. The simplest of these procedures is that described by Chatten (33). This method is based on the reaction between the acidic substance or its sodium salt and either a basic or acidic titrant. The determinations are carried out in nonaqueous media. The method has been collaboratively evaluated (34) and proven to be both accurate and reliable. Because of its simplicity, the method is particularly suited to stability studies because such studies require a large number of determinations of the p-aminosalicylic acid content of tablets over a period of time. Unlike the method of analysis described in the U.S.P. (20), this method is specific for the drug and it is, therefore, not necessary to compensate for the presence of the degradation product.

The authors of the specified papers (33,34) recommended that the end point of the titration be detected by using thymol blue indicator solution. However, the amount of m-aminophenol in the product they assayed was small. In this study, the concentration of m-aminophenol in the product was, in certain instances, sufficiently high to produce a dark brown solution. Visual detection of the endpoint was difficult and, for this reason, the endpoint

was detected potentiometrically using a Fisher pH meter whenever the solutions darkened excessively. Chatten (33) reported in his paper that the visual and potentiometric end points coincide.

Product Potency

The U. S. P. (1) states that p-aminosalicylic acid tablets should contain not less than 95 % and not more than 105 % of the amount of drug claimed on the label. All four products complied with this specification.

Stability studies on tablets are complicated by between tablet variability. The analyst does not know, at least initially, if a change in potency is due to this type of variability or to the degradation of the p-aminosalicylic acid in the tablet. Initial assays for the four products subjected to the stability study showed that there was a maximum range of 15.9 mg. in potency for Product 7. The range values for Products 5, 4 and 1 were 13.0 mg, 13.4 mg and 7.0 mg respectively. This in iteself is not serious but, in an attempt to further minimize this problem, four tablets were drawn from the humidity chamber during the stability study and analyzed collectively. The potency values given in Tables I to VIII are, therefore, mean values for the four tablets. The answers obtained in this way are probably closer to the true potency of the product at any particular time than are the answers obtained by analyzing only one tablet.

Tablet Disintegration

Product 1 is an enteric coated tablet. It resisted gastric juice for one hour and disintegrated in 38 minutes in intestinal

juice. Products 4, 5, and 7 are compressed tablets and disintegrated quickly. All products comply, therefore, with the disintegration regulations appended to the Food and Drug Act.

The physiological availability of sodium p-aminosalicylate in tablets has been thoroughly investigated by Champman, Crisafio and Campbell (6). These authors concluded that the drug in tablets which disintegrated in less than 60 minutes was physiologically available. Since all the products studied disintegrated quickly, it must be assumed that the drug in the tablets would be available to the patient.

The Effect of Moisture and Temperature on the Physical Characteristics of the Products.

Products 1, 4, 5 and 7 were placed in the Vapor-Temp Controlled Humidity Chamber and subjected to a temperature of $50^{\circ}C$. and a relative humidity of 90 % for 24 hours. The tablets were removed and examined visually for changes in physical appearance.

Product 1. The tablets are coated and bright yellow in color. The coating started to melt after 24 hours in the humidity chamber but tablet remained intact.

Product 4. The tablets are white in color. After 24 hours in the humidity chamber, the color of the Product changed from white to light brown.

Product 5. The tablets are beige in color. The tablets were not affected by environmental conditions in the humidity chamber.

Product 7. The tablets are white in color. After 24 hours in the humidity chamber, the tablets began to swell, the color changed to brown, and isolated black spots were observed on the surface of the tablet.

Three of the four products are not significantly affected by the conditions within the humidity chamber. Product 7 was significantly affected by a temperature of 50°C and a relative humidity of 90 %. Furthermore, the appearance of this product changed in much the same way (but at a slower rate) when the temperature in the chamber was decreased. It would appear, therefore, that Product 7 is highly susceptible to the moisture in the atmosphere.

The Effect of Moisture and Temperature on the Chemical Characteristics of the Products

Stability data for Products 1, 4, 5, and 7 is shown in Tables I to VIII.

Product 1 maintains its potency under all experimental conditions. The physical appearance of the product did change when the temperature and humidity in the chamber were high. However, it would appear that only the outer sugar-coating is affected. Since the tablets are enteric coated, the coating probably acts as a barrier to moisture. Temperature did not appear to affect the potency of the product. The changes in potency shown in Table IV may represent some degradation but, more than likely, reflect the normal variations between groups of tablets or errors in the method of analysis.

The potency of Product 4 was affected by only high temperatures and humidities. However, the product contains sodium aminosalicylate and it is known that the salt is more stable than the acidic substance (7). No m-aminophenol was formed except when the product was subjected to a temperature of 50°C and a humidity of 90 %. Even when the product was held in the humidity chamber

set to these conditions, changes in potency were minimal. Potency changes for this product are shown graphically in Figure 5.

Product 5 contains p-aminosalicylic acid. Its initial color was a light brown and this suggested the presence of m-aminophenol. However, analysis showed that the product met U. S. P. specifications. When the product was subjected to the various experimental conditions, a certain amount of degradation occurred. Data for this product is given in Table III and is illustrated graphically in Figures 3 and 4. Because a plot of the logarithm of the concentration versus time is a straight line, the reaction is probably first order. Initial assay values were disregarded in calculating slope values because Scott and Lachman (40,41) reported that when heating and cooling lags occur during a stability study the initial assay value (at zero time) does not necessarily fall on the straight line. The physical appearance of this product did not change much during the studies but, on the basis of the determinations carried out, the potency of the preparation is affected by both temperature and humidity.

If the rate constants for Product 5 are compared (that is, the slopes of the lines illustrated in Figures 3 and 4), it is obvious that moisture plays a more important role in the degradation process than does temperature. For example, the slope of the line for the 90 % relative humidity - 50°C. condition is approximately eight times that found for the 65 % relative humidity - 50°C. condition. If the humidity is kept constant, an increase in temperature will increase the rate of degradation.

The data obtained for Product 5 at the higher temperatures (e.g. 60°C.) and higher humidities (e.g. 90 %) may be illustrated graphically by plotting the logarithm of the concentration versus time. This implies that the reaction is first order. However, at lower humidities, the plots imply either a zero order or a first order reaction. This data must, therefore, be judged on the basis of the observations made by Scott and Lachman (40) and by Kornblum and Sciarone (10). The former researchers claim that initial assay values must be omitted and slopes calculated only on the basis of data obtained after time zero. The latter researchers pointed out that the rate of degradation can change during the stability study. It would appear, therefore, that the changes in Product 5 are pseudo first order. The data suggests two different rates of degradation.

The initial rate of degradation is slow and depends probably on the temperature in the chamber. As moisture builds up in and around the tablet, the rate of degradation increases. The latter rate appears to be the more important and was used, therefore, to predict the shelf life of this product.

Product 5 contains little more than p-aminosalicylic acid. The mean weight of the tablets is 503 mg. and the mean assay value is 482.9 mg. This means that the product contains little binder, disintegrant, and lubricant. This may be further confirmed by dissolving the tablet in acetone. A clear solution results which contains little insoluble matter. This implies that substances added to the product do not significantly affect product stability. The stability of Product 5 should, therefore, be similar to the stability of p-aminosalicylic acid compressed as such into a tablet.

The mean assay value for Product 7 was found to be 484.6 mg. The mean tablet weight for this product is approximately 650 mg. It is claimed on the label that the product is buffered and contains, in addition to the usual binders, lubricants, and disintegrants, Dihydroxyaluminum Aminoacetate N.F. and Calcium Carbonate, U.S.P. When this product was stored in the Vapor-Temp Controlled Humidity Chamber adjusted to a temperature of $50^{\circ}C$. and a relative humidity of 90 %, its potency decreased to approximately 50 % of that claimed on the label in four days. This change in potency parallels the change in the physical appearance of the product. The rate of degradation at lower temperatures but at the same humidity is less than that given above but is still much more (approximately ten times more) than that found for Product 5. The results for this product are shown in Table I and may be compared with those given in Table III for Product 5. If the relative humidity in the chamber is lowered to 65 %, potency losses are still significant but not as great as those found when the tablets were stored at higher humidities.

Product 7 is, therefore, an example of a highly instable pharmaceutical and must be considered as unsatisfactory even though it complies with existing specifications. On the basis of the data obtained, it must be assumed that the manufacturer of this product did not check its stability either before or after it had been placed on the market. If he had done so, the product would not have been marketed. This then is a good example of poor product development.

Product 7 is affected by both heat and moisture. On the

basis of the data in Tables I and II, it is obvious that moisture plays a much more important role in the degradation process than does temperature. However, neither temperature nor moisture can fully account for the potency changes in this product. The excipients and/or buffers in this preparation appear to play an important role in the degradation process. Of the two, the buffers are probably responsible for the poor stability of the product. In the presence of moisture, these buffers produce an unfavorable pH situation which leads to the rapid degradation of p-aminosalicylic acid. The addition of these buffers to the tablet was not based, therefore, on the principles associated with good quality control. If the manufacturer failed to do this, it is doubtful if he assessed the product's ability to alleviate the gastrointestinal disturbances usually associated with the ingestion of large amounts of acid substances, that is, with the ingestion of p-aminosalicylic acid.

Some of the solutions of p-aminosalicylic acid (and its degradation product) prepared for the analysis were chromatographed in the manner specified in a previous section. The only detectable degradation product in all instances was m-aminophenol. The drug in Product 7, therefore, degrades rapidly to m-aminophenol and should not be administered to a patient unless it is known that the product has been recently manufactured or has been carefully stored in a dry cool place.

The data in Tables I and II is illustrated graphically in Figure 1 and 2. These plots indicate the the reaction is pseudo-first order.

Prediction of Product Stability

The stability characteristics of the four products at various temperatures and at 65 % and 90 % relative humidity have been determined. The two basic conditions are, therefore, the two humidity values. This means that the rate of degradation for either of the two conditions can be calculated by subjecting the data to the method of least squares (35). The slope of the line is then equal to the rate constant (k).

The logarithm of rate constants for the three temperatures (in general, 30°C., 40°C., and 50°C.) can then be plotted versus the reciprocal of the absolute temperature. This Arrhenius plot can then be used to determine the reaction constant (either mathematically or graphically) at any desired temperature. When the rate constant for this temperature has been determined, it is possible to establish a half life for that product at that temperature.

As an example, Arrhenius plots for Product 7 are given in Figure 6. Predictions may be made on the basis of either the 65 % or the 90 % relative humidity plots. In the example below, the prediction is based on 90 % relative humidity plot. By extrapolating the curve to 25°C. (that is, to room temperature), the rate constant at this temperature is found to be 0.00045. The half life for the product may then be calculated.

$$t_{\frac{1}{2}} = \frac{\ln 2}{k}$$

$$= \frac{0.693}{0.00045} = 1540 \text{ days}$$

This means that the product would lose 50 % of its potency in 1540 days if stored at a temperature of 25°C and a relative humidity of 90 %. Similar calculations may be made for other conditions and products.

Most manufacturers study product stability at both accelerated temperatures and at normal temperatures. However, the studies at room temperature require time and, in most instances, the product is released for sale before the accuracy of the prediction based on the accelerated studies is known. In this study, it was not possible to substantiate the predictions. However, on the basis of literature observations on stability testing, the predictions made herein are probably reasonably accurate.

The accuracy of the prediction depends entirely on the accuracy with which the rate constants have been determined. In the first instances, the analytical errors in the method of analysis must be minimal. This usually presents few problems. However, it is known that degradation rates can change even if the product is stored at the same temperature over the entire stability investigation. Moreover, the basic assumption made in all stability studies is that the Arrhenius plot is linear for the drug in the dosage over the entire temperature range. The assumption here is that mechanism of the reaction is the same at all temperatures. There is some indication that this is true for at least one of the products studied. This was previously presented in the discussion on the stability of Product 5. It must be assumed, therefore, that the results of this investigation are qualitative rather than quantitative. However, this basic approach (that is, utilizing the Arrhenius plot) to the prediction of stability

yields reasonably accurate results and cannot be replaced until the pharmaceutical chemist knows more about the interactions between the drug, the excipients in the tablet, and the conditions existing in the environment surrounding the tablet.

Comparison of Products

The purpose of this investigation was to compare the stability of four brands of p-aminosalicylic or sodium aminosalicylate tablets. Secondary to this, it was hoped that the results of the investigations would be sufficiently meaningful to enable the researcher to propose a stability specification that would be acceptable to the World Health Organization and to the pharmacopoeia. The four products are compared in this section. The proposed specification is given in the next section.

Product elegance does not necessarily imply product stability. Products 1 and 4 (a yellow enteric coated tablet and a white compressed tablet) were well made and complied with existing specifications. Product 5 was light beige in color. It would appear that this color in the product is not due to m-aminophenol but it does stain the cotton packing in the original bottle. Product 7, a white compressed tablet, was well made and, on the basis of initial assays and disintegration times, would be acceptable to a regulatory agency.

Products 1 and 4 are stable under all conditions. Product 5 is reasonably stable but the p-aminosalicylic acid in Product 7 breakds down to m-aminophenol at all temperatures and humidities. This means that one of the four products would not be acceptable to the pharmaceutical chemist.

A product is considered to be unsatisfactory if it assays at less than 90 % of label claim or contains more than 1 % m-aminophenol. This means that the product should meet these specifications at all times assuming that it is stored under suitable conditions.

It is obvious that products would not be normally exposed to temperatures of 50°C. and relative humidities of 90 %. However, a temperature of 40°C. and a relative humidity of 90 % is not an unreasonable environmental condition in many parts of the world. Moreover, this condition is reached, at times, in many parts of Canada during the summer months. The drug in the product should not, therefore, degrade when it is exposed to such conditions.

It is possible to calculate the half life of the drug in each of the products stored at 40°C. and 90 % relative humidity. However, this value has little meaning to the pharmaceutical analyst. He is interested in the number of days it takes for the potency of the preparation to drop to the minimal amount allowed by the pharmacopeia. Such a calculation can be made but it would make no allowance for normal variations from label claim. For example, a product, when manufactured, might contain 95 % of the drug claimed on the label. If the product degraded by 0.5 %, it would automatically be rejected by the analyst. This is obviously not fair to the manufacturer and, for this reason, a minimal value of 90 % p-aminosalicylic acid (or its sodium salt) was chosen for the purpose of product evaluation.

It would be theoretically possible to calculate the time required for Products 1 and 4 to reach 90 % of label claim but the value so obtained would be meaningless because the products are

stable under all conditions. However, such calculations can be
made for Products 5 and 7. These calculations are made in the
manner given in a previous section. The only difference between
this calculation and that for the half life is that this value
represents a minimal value of 90 % of label claim. The results
at four temperatures for the two products are given in Table
XII.

The results in Table XII show that, although Product 5 is
not as stable as Products 1 and 4, it cannot be classified as
an unsatisfactory product. This conclusion is reached on the
basis of product packaging. If a bottle contains 500 tablets,
the tablets in that bottle would be consumed by the patient in a
relatively short period of time. (The usual dose of p-aminosal-
icylic acid is twenty four tablets per day. This means the
patient would consume the contents of the bottle in twenty days.)
Even at 40°C., the product would still assay at more than 90 %
at the end of this time period. This situation does not apply to
Product 7. It would fall to 90 % of label potency in 4 days.
After this time, it would have to be considered as unacceptable.
Even at 30°C., its shelf life would be only 25 days.

A Stability Specification for p-Aminosalicylic Acid
and Sodium Aminosalicylate Tablets

Product 5 is a 'good' product in the sense that it maintains
its potency reasonably well at all the temperatures given in
Table XII. An 'unacceptable' product would be one whose stability
is better than that shown for Product 7 but somewhat less than
that of Product 5. This means that the time required for the pro-
duct to fall to 90% of label claim must fall between the values

TABLE XII

Time Required for Products No. 7 and No. 5 to Drop to 90% of Label Claim when Exposed to 90% Relative Humidity.*

Temperature	Product No. 7	Product No. 5
25°C	70 days	765 days
30°C	25 days	204 days
40°C	3 days	51 days
50°C	1/2 day	4 days

* Label Claim = 500 mg of p-Aminosalicylic Acid per Tablet. (All calculations were based on the average initial potency of the product - 484.6 mg for Product 7 and 482.9 mg for Product 5).

given for the two products at all temperatures.

To be practical, a stability study must be carried out quickly. The obvious temperature choice is then 40°C. or higher. Although products will degrade quickly at elevated temperatures, it is better to approximate a 'normal' condition for the stability specification. For this reason, a temperature of 40°C. was selected.

Rather arbitrarily, therefore, a temperature of 40°C., a relative humidity of 90 %, and a time of ten days were chosen for the stability specification. The specification is given below:

Place 20 tablets in a petrie dish and transfer to a humidity chamber adjusted to 40°C and a relative humidity of 90 %. Store in the chamber for ten days. Remove and assay the tablets. The mean potency of the 20 tablets must be not less than 90 % of the amount claimed on the label.

Product 7 would not comply with this specification. The test is not severe and would quickly establish the relative stability of a preparation. No pharmacopeia, at present, requires a test such as this for any product. However, on the basis of the results obtained in this investigation, such a test is required and provides a way to better evaluate the products that are available to the patient.

Comments on Methodology

All researchers predict product stability by studying degradation at several elevated temperatures, calculating rates,

and plotting these values versus the reciprocal of the absolute temperature. The studies are usually carried out in stability ovens and no attempt is made to evaluate the effect of both temperature and humidity on the products. In this study, both factors (that is, temperature and humidity) were evaluated. For a particular series of experiments, the humidity value is a constant. However, like the temperature, the humidity in the chamber will vary when samples are withdrawn for analysis. This means that the samples in the chamber are cooled and exposed to drier atmospheric conditions for short periods of time throughout the stability study. Other authors have pointed out that this may lead to erroneous results (41) but there is no evidence in this study to support this hypothesis. However, it must be taken into consideration in any assessment of the accuracy of the predictions made in Table XII.

The magnitude of the error introduced by this disturbance of equilibrium is related to the rate constant, the length of the stability test, the storage temperature, and the activation energy of the breakdown reaction (42). A fifth factor, the humidity, must be added to this list. Because these factors may affect the accuracy of the prediction, the stability specification given in the previous section was based on a definite period of time (that is, ten days). This means that the sample is exposed continuously to the same conditions for the entire time period. Moreover, in carrying out stability tests, samples should not be taken from the chamber unless an analytical value on that particular day is essential. In this way, equilibrium conditions are maintained over most of the stability test period.

Despite the shortcomings in methodology, the stability characteristics of the four products may be compared directly. The four products were placed in the chamber at the same time, stored for the same time, and samples withdrawn for analysis at the same time. Consequently, reaction rates may be compared directly. Other researchers have made similar claims and have pointed out that predictions made on the basis of the experimental data accumulated under such conditions are reasonably accurate (42).

Ideally, the stability of the products at a third humidity value should have been investigated. It would then have been possible to estimate the effect of humidity on product stability. Although this was not done, sufficient data is presented in this thesis to show that humidity does have an effect on product stability. This means that future stability studies should be carried out by exposing products to an environment in which both the temperature and humidity are controlled. Drug products are rarely exposed to the idealized conditions described in the literature. Bottles are opened, drums of tablets are left open, sometimes for two or three days, and are, therefore, exposed to higher humidities than those found in standard stability ovens. The humidity factor cannot, therefore, be disregarded.

Although no quantitative relationship can be given for the humidity - potency factors, an examination of Figures 6 and 7 (Arrhenius plots for Products 5 and 7) shows that the slopes of the two lines are similar. This suggests that, at two different humidities, the mechanisms of degradation are similar. Again

this statement must be tempered by the observation that little

is known about mechanisms of degradation in solid dosage forms.

It is admitted that the stability specification proposed

herein is arbritrary. However, it is possible to make stability

predictions for products stored at other humidity values. For

example, Product 7 will fall to 90 % of label claim in 70 days if

it is stored at a temperature of 25°C. and a humidity of 90 %.

If a line is drawn through the 25°C. point parallel to the base

line (see Figure 6), it intercepts the 65% plot at 45°C. This

means that the rate constant is the same for this product at both

temperatures. The product should, therefore, fall to 90 % of

label claim if it is stored in the chamber at a temperature of

45°C. and a humidity of 65 % for 70 days. It should be obvious,

therefore, that many values may be derived from the data in this

thesis. However, these values have meaning only during the pro-

duct development process. The pharmaceutical chemist cannot sub-

ject a product to a wide variety of conditions during the normal

process of product evaluation. He must rely on a specification

that takes into consideration all factors. The specification

given in the previous section may be criticized, therefore, in a

number of ways. However, it is a first attempt at stability

assessment of products by utilizing data obtained from a comparative

study.

VI. SUMMARY AND CONCLUSIONS

(1) Four p-aminosalicylate acid or sodium p-aminosalicylate tablets were selected at random for this investigation. These products were stored in a Vapor-Temp Controlled Humidity Chamber at various temperatures (30°C. to 60°C.) and at 90 % and 65 % relative humidity for varying periods of time. The environmental conditions in the chamber did not affect the stability of the drug in Products 1 and 4. Products 5 and 7 were affected by the conditions in the chamber. However, potency losses for Product 5 were less than those for Product 7.

(2) An increase in temperature increases the rate of degradation. Moreover, humidity plays an important role in the degradation process. At 90 % relative humidity, the drug in Product 7 degrades rapidly to m-aminophenol. The physical appearance of Product 1 (a yellow enteric coated tablet) is altered drastically when it is exposed to a temperature of 50°C. and a relative humidity of 90 %. However, no potency losses were detected. The environmental conditions appear to affect only the outer coating.

(3) Sodium aminosalicylate tablets appear to be more stable than p-aminosalicylic acid tablets. This confirms the observations in the literature that the salt is more stable than the acidic substance.

(4) The data accumulated in this investigation is best illustrated by a pseudo-first order plot. Only the plots for Products

5 and 7 are significant. The slopes of these lines (that is, the rate constants) are presented in Tables IX and X.

(5) Arrhenius plots for Products 5 and 7 are presented in Figures 6 and 7. These plots were used to predict stability of the Products at room temperature. Results are shown in Table XII. The results in this table show that Product 7 will fall to 90 % of label potency in 70 days if it is stored at a temperature of 25°C. and a relative humidity of 90 %.

(6) All four brands complied with the specifications given in the pharmacopeia for this type of product. However, on the basis of the data accumulated in this investigation, Product 7 was considered to be unsatisfactory.

(7) The only detectable degradation product in Products 5 and 7 was m-aminophenol. This was confirmed by thin layer chromatography.

(8) The following stability specification is proposed:

Place 20 tablets in a petrie dish and transfer to a humidity chamber adjusted to 40°C. and a relative humidity of 90 %. Store in the chamber for ten days. Remove and assay the tablets. The mean potency of the 20 tablets must be not less than 90 % of the amount claimed on the label.

Product 7 would not comply with this specification. This specification provides the analyst with a way to quickly evaluate the stability characteristics of products containing either p-aminosalicylic acid or sodium aminosalicylate.

(9) The data accumulated during this investigation shows that humidity plays an important role in the degradation process.

Future stability studies should, therefore, be so designed that products are stored in environments in which both the temperature and humidity are controlled.

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