ESTIMATION OF SALICYLIC ACID IN ASPIRIN (PEDIATRIC) MIXTURES BY COLORIMETRIC METHOD

BY

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A DISSERTATION PAPER SUBMITTED IN PARTIAL FULFILMENT FOR THE AWARD OF THE BACHELOR OF PHARMACY DEGREE

DEPARTMENT OF PHARMACY
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NAIROBI, KENYA.
I wish to say my special thanks to:

- Mr. Ogeto, my project supervisor, who patiently and tirelessly helped and guided me through this project.

- My lecturers, other members of staffs and students who gave me a peaceful atmosphere necessary for the success of such tedious work.

- Mr. Mureithi and Mr. Thuranira, Laboratory Technicians in the Pharmaceutical Chemistry section for being most helpful especially in the operation of the analytical instruments.

- My Relatives living in Nairobi for their long endurance with my demands and for giving me constant moral support even at times when I had reached the end of the tether.

- My auntie Miss E.B. Muthoni for her encouragements and above all for typing this script.
DEDICATION

This work is dedicated to all its worth:—
To my dear parents Mr. & Mrs. James Kiama
To my brothers and sisters and especially
to my beloved son Jim, for providing me with
the most needed factor;

A constant inspiration.
The relative frequency of salicylate poisoning has led to the development of many methods of estimation of salicylates in plasma and urine (1). Numerous methods have also been described for the additional determination of aspirin and salicylic acid in pharmaceutical and biological media.

Among the techniques employed is a group of U.V. assays. By this technique either salicylic acid alone or salicylic acid and aspirin may be determined, on the basis of the beer-lambert laws. Salicylic acid has been estimated in combination with aspirin or individually following a physical separation step. Both salicylates have been determined simultaneously by use of a pH - dependent shift in their individual absorption spectra. These hyposochromic shifts technique results in some overlap of absorption spectra for salicylic acid and aspirin and correction may be required for the spectra interferences (2) Routh, shame et al (1967) (3) used this method for the estimation of aspirin and salicylates in plasma and urine.

Salicylates have also been assayed flourimetrically. Here aspirin may be determined directly or more commonly as salicylic acid. This has been accomplished by direct hydrolysis. Flourimetric assays generally require a hydrolytic procedure and a dual determination to estimate aspirin (4).
The most quantitative methods employed to date are chromatographic estimations. Thin Layer Chromatography (5,6) and Liquid Chromatography (7) have been used, but the most quantitative chromatographic procedures for salicylates involve Gas Liquid Chromatography. This is currently being used for the determination of the comparative bio-availability of the various dosage forms of aspirins in arthritic patients. Rowland and Aregelman (1967) described the procedure as the most specific and sensitive for the assay of salicylates (8).

Among the oldest and most widely used methods for the assay of salicylic acid in aspirin dosage forms is the colorimetric method. Its ease of operation and sensitivity of the method allows the determination to be carried out very easily from the dosage forms being used. It was first used by Blodie (1946) and then by Trinder (1954) to assay salicylates in urine (9). A large group of these colorimetric assays involve diazotization with para-introaniline and Nitron acid (10), use of Fällin and Coucalteaphend reagent (11), complexation with cupric ion, in Nitron acid(12), estimation of the intro derivative (13) or complexation with the ferric ions. The last of this group, the ferric ion complexation, is the most popular method, because of its ease of operation, for the assay of salicylic and present in dosage forms. The colour produced when a phenol is treated with ferric ions is characteristic of the class of compound and can be used for
quantitative analysis (13). By using the last method, Trinder (1954) made measurements of urinary salicylates in order to study the bioavailability of salicylates from their dosage forms, thus the birth of Trinder’s reagent for colorimetric assay of salicylates. Levy (1972) modified this method; he was aware of the fact that Trinder’s method did not react with the glucuronide metabolites of salicylic acid and underreacts with salicyluric acid a major metabolite so he replaced the 5ml of Trinder’s reagent with 1ml of urine sample used by Trinder to get the absorbance to 3ml of the urine sample and 3ml of concentrated hydrochloric acid are heated to 100°C for 16 hours in order to hydrolyse the metabolites to salicylic acid. An aliquot of the hydrolysed sample is then accurately removed acidified and extracted with organic solvents and the salicylic acid from an aliquot of this solvent was assayed calorimetrically after complexing with ferric ion in an acidic medium. In this method the total salicylate, which includes aspirin, salicylic acid and other metabolites is analyzed (14). Therefore this was a simpler and yet sensitive procedure for assay of total salicylates in urine; it is Levy’s modification of the method of Smith et al (15).

Handy (1978) used the colorimetric method for stability tests on aspirin tablets and mixtures and obtained very reliable results (16). Kutsum (1979) used ferric ammonium sulphate for the metal determination in streptomycin aqueous solutions, this was found to give better results than other ferric salts in aqueous solutions phase because compared to ferric chloride which is coloured or other ferric salts it gives the most lightly
coloured solution especially in an acidic media, its very pale yellow almost colourless which is preferred for good comparison in colorimetry, since colour formation may interfere with the purple colour formed due to complexation and alter the absorption. With salicylates it gives a clear purple colour (17).
Until very recently aspirin was almost automatically prescribed for any patient with pain of moderate severity since its isolation as a drug in 1853 and its introduction as a drug in 1899 by Dreser under the trade name of "aspirin" (an abbreviation made by Dreser from the German word acetylirten - acetyl salicylic acid). Acetyl salicylic acid has been widely accepted as an effective and safe analgesic preparation but in recent years the occurrence of severe gastric hemorrhage in patients taking it has caused concern in the profession.

Acetyl salicylic acid was first produced by the German Bayer Company from sodium salicylate which until that time was the commonly used salicylate analgesic. Sodium salicylate is hydrolysed into salicylic acid by the presence of hydrochloric acid in the stomach. Salicylic acid is a corrosive agent, in fact it is used today to erode coral due to this erosion effect it irritates the stomach when it is released from medicaments. In the same way acetyl salicylic acid (where the phenolic OH is replaced by an acetyl group) is hydrolysed by water to salicylic acid thus freeing the phenolic hydroxyl group the gastric irritant, although pure aspirin is relatively free of gastric irritant effects, showing that irritation is associated with the free phenolic hydroxyl group of the compound.
This reaction is acid catalysed.

Aspirin has been formulated into various dosage forms:—

1. Suppositories
2. Ointments and lotions
3. Tablets
4. Mixtures

The first 2 preparations are free from the above mentioned salicylic acid undesirable side effects because they are free from hydrochloric acid and aqueous environment which are responsible for the hydrolysis. The last 2 dosage forms, for oral administration the problem is relevant.

Aspirin tablets are the most common dosage form available. They disentigrate in presence of water, moisture and acid to release salicylic acid, but if they are stored in well closed containers for use for a long time; particularly when they are sugar coated. When tablets prove to be too cumbersome for effective administration then the mixture is prescribed particularly in the case of Paediatrics and geriatrics.

The mixture is a suspension of aspirin in water, therefore in this form aspirin is more prone to water hydrolysis.

The aspirin mixtures used in Kenya are compounded in Kenyatta National Hospital manufacturing unit and in the Provincial Hospital Pharmacies. Here the mixture is stored under very poor conditions usually in plastic jars of around 20 litres at room temperature, they are then distributed to the sub units of the hospitals or transported to smaller
hospitals, dispensaries and health centres for use, here again they are not used for a long time and due to the variety of climatic conditions from place to place; room temperature differ from the hot humid 27°C day temperature in Coast General Hospital to the 20°C day temperature of a dispensary in the Aberdares region or Nyeri District added to this is the fact that once dispensed the product has to be used for about 5 days and this further increases the time interval between manufacture at one place and use at some other place; for example from Nakuru Hospital for use in the Turkana region. All this tells that the mixture, (a) takes long before it is used, i.e. (there is a long shelf life) (b) is stored in poor conditions at various places depending on the facilities available in the hospitals and homes of patients (c) as seen above it stored at different temperatures depending on climatic variations; from place to place.

Therefore it is here that the problem of dispensing a degraded product with containing toxic salicylic acid is most acute. All the above affect the rate of hydrolysis of the aspirin mixtures, and to be able to tell the extent of the degradation, rate tests have to be carried out. Their findings are important in that:

1) They will convey to the pharmacist the amount of salicylic acid present in the mixture at any given time; based on this the pharmacist should be able to decide which mixture doesn't comply with the B.P. requirements.
2) Also based on this the pharmacist should be able to predict the shelf life of the mixture and give advise to patients to discard the mixture after a given number of days.

3) The findings could also aim at establishing which temperatures are fit for the storage of the mixture to reduce rate of hydrolysis and increase shelf life.

4) They may correlate stability with formulation showing whether stabilizers claimed by official compendias could be used to reduce rate of hydrolysis and thus lengthen shelf life and possibly show expiring dates.

5) Once established the findings could reflect a way of quality control of the preparation received into dispensaries and avoid expired preparations from being accepted.
OBJECTIVE

Most of the aspirin (Paediatric) suspensions used in Kenya take long before they are supplied to patients for use since they are only manufactured in very few centres and have to be used almost in every dispensary in the country. (see introduction) But it is known that aspirin in solution hydrolysis in time to salicylic acid. Thus leaving the patient to take variable doses of salicylic acid which is toxic to the gastric mucosa and has largely been responsible for such stomach lesions (see review).

For this reason a quick and yet sensitive method of assay should be available to the dispenser of the mixture of aspirin to provide him with a rough guide of giving out only those preparations that are within the limits of salicylic acid specified in the official compendias as safe for administration, and possibly advise the patient on storage conditions and safe use of the mixture when at home. The method chosen was colorimetric.

It is considered that a colorimetric method using ferric ammonium sulphate reagent and a simple spectrophotometer, would be a quick method of estimating the quantities of salicylic acid in the mixture at any given time. This is the objective of this investigation.
**INSTRUMENT AND APPARATUS**

The following instruments were used in the course of the experiments:

<table>
<thead>
<tr>
<th>Name</th>
<th>Maker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectronic 20 spectrophotometer</td>
<td>Banach &amp; Lamb</td>
</tr>
<tr>
<td>Ultra violet Sp 8000 spectrophotometer</td>
<td>Pye unicam</td>
</tr>
<tr>
<td>Thermostatic water bath</td>
<td>Techne</td>
</tr>
<tr>
<td>Weighing balance</td>
<td>Scotorius 2354</td>
</tr>
<tr>
<td>Refrigerator</td>
<td></td>
</tr>
</tbody>
</table>

The following apparatus were used in the course of the experiment:

- Pipettes 1ml, 2ml, 3ml, 10ml, 25ml
- Volumetric flasks 50mls, 100mls, 500mls, 1000ml
- Flasks
- Beakers

The following materials were used in the course of the experiment:

- Aspirin mixture (Paediatric) - supplied by Kenyatta National Hospital
- BPC (1963)

The following chemicals were used in the course of the experiment:

<table>
<thead>
<tr>
<th>Name</th>
<th>Grade</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferric ammonium sulphate</td>
<td>Aniralar</td>
<td>Merck</td>
</tr>
<tr>
<td>$\text{NH}_4\text{Fe(504)}_2\text{LH}_2\text{O}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>concentrated sulphuric acid</td>
<td>Laboratory Grade</td>
<td>Howse &amp; McGeorge Ltd.</td>
</tr>
<tr>
<td>Calcium Carbonate</td>
<td>Analytical Reagents</td>
<td>BD. H Chemical Ltd.</td>
</tr>
</tbody>
</table>
PREPARATION OF REAGENTS

1% Sulphuric Acid:

About 24.52g of concentrated sulphuric acid was weighed and added to a 500ml volumetric flask containing about 300mls of distilled water. The flask was kept cool by immersing it in a trough containing some cold water because a lot of heat was evolved during the process. The solution was then made up to 500mls with distilled water.

1% Ferric ammonium sulphate in IN sulphuric acid:-

About 1gm of ferric ammonium sulphate was dissolved in about 50mls of IN sulphuric acid contained in a 100ml volumetric flask and the solution was made up to 100mls with distilled water. The resulting solution was pale yellow in colour (very lightly coloured).
EXPERIMENTAL

The experimental work was divided into the following sections:

i) Construction of the Calibration Curve.

ii) The effect of temperature on the rate of hydrolysis of aspirin at fixed concentration.

iii) The effect of concentration on the rate of hydrolysis of aspirin at fixed temperature.

iv) Effect of time on the hydrolysis of aspirin at fixed concentration and temperature.

(i) Construction of the calibration curve

The calibration curve was constructed using sodium salicylate because salicylic acid is poorly soluble in water. It was believed therefore that while in water sodium salicylate will exist as salicylic acid and sodium hydroxide ions and the presence of sodium hydroxide has no effect on the colour formed because the colour depends on the phenolic group.

Solubility of salicylic acid in water is only

Procedure:

Preparation of the standard solutions:

A quantity of sodium salicylate powder equivalent to 10.00gms was accurately weighed and placed in a 100ml volumetric flask, distilled water was added and the salt dissolved with shaking, the resulting solution is clear
and colourless, this was made up to volume with distilled water with thorough shaking. 10mls of the resulting solution was pipetted into another 100ml volumetric flask and made up to volume with water. This serial dilution was repeated to give nine standard solutions of the following concentrations:

\[ 10^{-1}\text{gm/ml} \quad 10^{-2}\text{gm/ml} \quad 10^{-3}\text{gm/ml} \quad 10^{-4}\text{gm/ml} \quad \ldots \quad 10^{-9}\text{gm/ml}. \]

3.0mls of each of the standard solutions prepared above was pipetted out into a cuvette and to each 3mls of the 1% Ferric ammonium sulphate in 1N sulphuric acid was added using a pipette and then shaken thoroughly to mix. The solution was allowed to stand for 3 minutes before reading the absorbance at 540nm on the spectronic 20. The procedure was repeated without the sodium salicylate solution but using distilled water and the difference between the two readings was taken to represent the colour due to salicylic acid in solution. The results obtained are shown in table I and the calibration curve constructed is shown in figure I.
(11) The effect of temperature on the hydrolysis of aspirin at fixed concentration: - \(10^{-4}\text{mg/ml}\) i.e. 0.1mg/ml

About 500mls of freshly prepared aspirin suspension was collected from the manufacturing unit of Kenyatta National Hospital. The label claim of the mixture was that 300ml of aspirin was contained in every 5ml of the suspension (aspirin pro infants RFC 1963). Therefore a 1:600 dilution would give the required concentration.

10mls of the mixture was filtered under vacuum and the precipitate discarded. A 1:600 dilution was done as follows:

1ml of the aspirin filtrate was pipetted into a one litre flask followed by addition of 500ml of distilled water from a 500ml volumetric flask and then 99mls of distilled water from a burette.

The above (0.1mg/ml) of aspirin solution was divided into 4 aliquots each of about 150mls and placed in 4 (250ml) volumetric flasks. At zero time and room temperature 3mls of the aspirin solution 0.1mg/ml was pipetted out from each of the 4 flasks above and placed in 4 different test tubes then 3mls of ferric ammonium sulphate reagent was pipetted into each one of the four, the solutions were shaken well to mix and left to stand for 3 minutes. Absorbance from each solution was taken at 540nm after the reading the four flasks were labelled accordingly and placed at 4 different temperatures as follows:-
Flask 1 - placed at deep-freeze temperature between 4°C - 0°C

Flask 2 - placed in the refrigerator average temperature between 1°C-10°C

Flask 3 - placed at room temperatures between 21°C - 25°C

Flask 4 - placed in a water bath temperature 30°C

The four flasks were left at the above temperatures and 3ml aliquots were taken from each flask. These aliquots were examined for the amount of salicylic acid as described above see page 14. The results obtained from the above investigation after 7 days are shown in Table 2 and the graph obtained from the tables is shown in Figure 2 page.
The effects of concentration on the rate of hydrolysis of aspirin at fixed temperature

20mls of freshly prepared aspirin suspension of concentration 300mg/5ml was shaken thoroughly and filtered under vacuum. 10mls of the filtrate was pipetted using a 10ml bulb pipette and transferred into a 100ml volumetric flask. 50mls of distilled water was added from the burette to make a total volume of 60mls containing 600mgs of the aspirin. (i.e. 10mg/ml). Then 10ml of this solution was pipetted out and made 100mls with distilled water in a 100ml volumetric flask to give a concentration of 1ml/ml. The above procedure was repeated to obtain the following concentrations 0.1mg/ml, and 0.01 mg/ml.

At zero time, 3mls from each of the above concentrations was investigated for the amount of salicylic acid as described in page 14 after this:- The solutions above were kept in room temperature 23-25°C and a daily absorbance taken as described above. The results obtained for four days are shown in table 3 and the resulting graph is shown in figure 3.
(iv) **Effect of concentration and temperature on the rate of hydrolysis of aspirin mixture at varied time:**

About 200mls of freshly prepared aspirin suspension was obtained from the hospital manufacturing unit and stored at room temperature 20 - 25°C. 20mls of the suspension was shaken thoroughly and filtered under vacuum. 10mls of the filtrate was pipetted out using a 1 ml. bulb pipette and transferred to 50ml. volumetric flask and made upto volume with distilled water. To give a concentration of 1ml. of aspirin in 50mls of water. A few ml's of this solution was placed in a covette and its absorbance read in UV 8000 against a water blank. Results in Table 4 and peaks on Fig.5. Then 1ml. of the remaining 1:50 solution of aspirin was pipetted out and placed in a 5ml. volumetric flask and made upto volume with distilled water. Then this was again placed in a covette and the UV absorbance read in UV 8000 against a water blank on a different sheet of paper; (this was the reading for A, concentration of 1ml of aspirin in 250mls of water). From the above 1/50 solution of aspirin, a further 5ml. of the solution is pipetted out using a 5ml. bulb pipette and placed in a 1000ml volumetric flask and made upto volume with water then 3ml. of the resulting dilute mixture (dilution factor 1:10,000) was investigated for the amount of salicyclic acid using the spec-20 as described in Page 14.
The above procedure, for the estimate of salicylic acid present in the stored aspirin suspension was repeated for 10 days, and the results obtained from the UV. were recorded on the same sheet of paper everyday for each of the 1:50 dilution mixture and 1:250 dilution mixture and the resulting peaks are shown in figure 5 and 6 respectively, while the readings for spec. 20 and the U.V. data got from the peaks are shown in Table 4; the graphs for both is in figure 4.

(v) The effect of calcium carbonate on the stability of aspirin:

100ml. of aspirin paediatric mixture was dispensed with 1gm. of calcium carbonate B.P. added in the formula; and the mixture was diluted to a 1:50 dilution as described above in page 17 and the whole procedure repeated for 3 days; using U.V. 8000 in order to estimate the amount of salicylic acid released. The results can be seen in figure 7.
# RESULTS

## TABLE 1 - CALIBRATION CURVE

<table>
<thead>
<tr>
<th>Conc of salicylic Acid in solution in μg/ml</th>
<th>Conc of Salicylic Acid in Solution in ng/ml</th>
<th>Absorbance at 540 nm (O.D.)</th>
<th>Absorbance at 540nm O.D.</th>
<th>Absorbance at 540 mm O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-1}$ μg/ml</td>
<td>100 mg/ml</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>10</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>1</td>
<td>0.5000</td>
<td>0.4800</td>
<td>0.4900</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>0.1</td>
<td>0.3300</td>
<td>0.3400</td>
<td>0.3350</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>0.01</td>
<td>0.2250</td>
<td>0.2800</td>
<td>0.2500</td>
</tr>
<tr>
<td>$10^{-6}$</td>
<td>0.001</td>
<td>0.1600</td>
<td>0.1200</td>
<td>0.1400</td>
</tr>
<tr>
<td>$10^{-7}$</td>
<td>0.0001</td>
<td>0.3600</td>
<td>0.0145</td>
<td>0.0250</td>
</tr>
<tr>
<td>$10^{-8}$</td>
<td>0.00001</td>
<td>0.0090</td>
<td>0.0070</td>
<td>0.0080</td>
</tr>
<tr>
<td>$10^{-9}$</td>
<td>0.000001</td>
<td>0.0010</td>
<td>0.0010</td>
<td>0.0010</td>
</tr>
</tbody>
</table>

Graph in Page Figure 2
### Table 2

Results for the experiment (ii) - effect of Temperature on the rate of hydrolysis of aspirin mixture at fixed concentration (conc. 0.1mg/ml), graph in figure 2.

1. Incubation temperature 0°C

<table>
<thead>
<tr>
<th>Time in days</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance at 540 nm</td>
<td>0.010</td>
<td>0.010</td>
<td>0.030</td>
<td>0.140</td>
<td>0.165</td>
<td>0.170</td>
<td>0.200</td>
</tr>
<tr>
<td>Conc. of Salicylic Acid in mg/ml</td>
<td>0.00015</td>
<td>0.000150</td>
<td>0.00025</td>
<td>0.0019</td>
<td>0.0015</td>
<td>0.0014</td>
<td>0.0016</td>
</tr>
</tbody>
</table>

2. Incubation temperature 0°-10°C

<table>
<thead>
<tr>
<th>Time in days</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance at 540 nm</td>
<td>0.075</td>
<td>0.1250</td>
<td>0.220</td>
<td>0.280</td>
<td>0.390</td>
<td>0.285</td>
<td>0.280</td>
</tr>
<tr>
<td>Conc. of Salicylic Acid in mg/ml</td>
<td>0.0006</td>
<td>0.00010</td>
<td>0.00075</td>
<td>0.025</td>
<td>0.025</td>
<td>0.03</td>
<td>0.025</td>
</tr>
</tbody>
</table>

3. Room Temperature

<table>
<thead>
<tr>
<th>Time in days</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance at 540 nm</td>
<td>0.08</td>
<td>0.16</td>
<td>0.280</td>
<td>0.340</td>
<td>0.485</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Conc. of Salicylic Acid in mg/ml</td>
<td>0.00075</td>
<td>0.0030</td>
<td>0.025</td>
<td>0.075</td>
<td>0.80</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

4. Temperature 30°C

<table>
<thead>
<tr>
<th>Time in Days</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance at 540 nm</td>
<td>0.185</td>
<td>0.31</td>
<td>0.41</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Conc. of Salicylic Acid in mg/ml</td>
<td>0.005</td>
<td>0.05</td>
<td>0.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
TABLE 3

Results of the Experiment (iii) - Effect of concentration on the rate of hydrolysis of Aspirin mixture at fixed temperature (Temp = Room Temperature) - Reading on alternate days

1. Conc. 0.01 mg/ml

<table>
<thead>
<tr>
<th>Time in days</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance at 540 nm</td>
<td>0.010</td>
<td>0.015</td>
<td>0.025</td>
<td>0.030</td>
<td>0.030</td>
</tr>
<tr>
<td>Conc. of Salicylic acid in mg/ml</td>
<td>0.00011</td>
<td>0.00015</td>
<td>0.0002</td>
<td>0.00025</td>
<td>0.00025</td>
</tr>
</tbody>
</table>

2. Conc. 0.1mg/ml

<table>
<thead>
<tr>
<th>Time in days</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance at 540 nm</td>
<td>0.025</td>
<td>0.025</td>
<td>0.0350</td>
<td>0.050</td>
<td>0.060</td>
</tr>
<tr>
<td>Conc. of salicylic Acid in mg/ml</td>
<td>0.0002</td>
<td>0.0002</td>
<td>0.00035</td>
<td>0.00040</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

3. Conc. 1mg/ml

<table>
<thead>
<tr>
<th>Time in days</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance at 540 nm</td>
<td>0.140</td>
<td>0.155</td>
<td>0.165</td>
<td>0.180</td>
<td>0.175</td>
</tr>
<tr>
<td>Conc. of salicylic Acid in mg/ml</td>
<td>0.0015</td>
<td>0.0025</td>
<td>0.0035</td>
<td>0.0045</td>
<td>0.0040</td>
</tr>
</tbody>
</table>

4. Conc. 10mg/ml

<table>
<thead>
<tr>
<th>Time in days</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance at 540 nm</td>
<td>0.205</td>
<td>0.250</td>
<td>0.300</td>
<td>0.375</td>
<td>0.495</td>
</tr>
<tr>
<td>Conc. of salicylic Acid in mg/ml</td>
<td>0.0065</td>
<td>0.010</td>
<td>0.040</td>
<td>0.10</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Graph shown in Figure 3
**TABLE 4**

**Effect of Concentration and Temperature on the rate of Hydrolysis of the Aspirin Mixture at varied time:**

<table>
<thead>
<tr>
<th>Time in days</th>
<th>Concentration of salicylic acid in the ASA Sample</th>
<th>Spec. 20 Absorbance of ASA sample at 540nm (o.b.)</th>
<th>Conc. of salic. acid in sample of ASA in mg/ml</th>
<th>U.V. Absorbance of ASA sample (1:250) in U.V. 8000 (o.d.)</th>
<th>Conc. of Salic acid in sample of ASA IN mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.025</td>
<td>0.00020</td>
<td>0.040</td>
<td>0.00035</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.055</td>
<td>0.00045</td>
<td>0.1400</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.220</td>
<td>0.0075</td>
<td>0.2000</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.450</td>
<td>0.65</td>
<td>0.640</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.585</td>
<td></td>
<td>0.745</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.620</td>
<td></td>
<td>0.300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.825</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key:
1) - ; These concentrations cannot be mapped on the calibration curve.
2) A B C D E F - Peaks on the record sheet figure 5 which correspond to those of figure 6. The readings were taken on alternate days.
Figure 21: EFFECT OF TEMPERATURE ON THE HYDROLYSIS RATE OF ASPIRIN (0.1 M/L)

Concentration (M) vs Time (Days)

- 50°C
- Room Temperature
- 0-10°C
- 0°C
FIG. 3: EFFECT OF CONCENTRATION ON THE PROGRESS OF RATE OF HYDROLYSIS OF ASPIRIN MIXTURE AT ROOM TEMPERATURE (SALICYLIC ACID CONC V. VS. TIME)

Key
- Aspirin:
  - O --- O --- Concentration 10 mg/mL
  - A --- A --- Concentration 4 mg/mL
  - C --- C --- Concentration 0.1 mg/mL
  - X --- X --- Concentration 0.04 mg/mL

Time in Days
Comparison of the data obtained for the highways of cement and non-cement mixtures.
Figure 5: Salicylic acid peaks A, B, C, D, E, F taken on alternating days, at 1 ns channel.
Absorbance Figure 6: Hydolytic Uptake of Aspargine at Room Temperature Comparison

Transmittance 0-1A Range

A - 0 day
B - 1 day
C - 2 days
D - 7 days
E - 14 days
F - 21 days

Aspargine peaks

Sodicite acid peaks

Concentration: Gomphcal (1:250mL)
Scan Speed: Fast
Atmospheric Error: ±0.25
Figure 8.7: Stability of Aspirin Mixture in Calcium Carbonate
Transmittance 0-1A Range

Absorbance

Concentration

Scan Speed

Absorbance Range
0-0.2A
0-0.1A

Operator

Date
08-08-85

Sample and Formula

C6H5O7

Reference
Distilled Water

Path Length
10 cm

1st day = A
2nd day = B
3rd day = C
DISCUSSION

One of the objectives of the present investigation was to obtain a quick, sensitive and reliable method which could be used by the dispenser to determine the shelf life of aspirin mixture made in Kenyan hospitals.

To this the results obtained show that the rate of hydrolysis of aspirin to salicylic acid is dependent on concentration and temperature of storage. This is in agreement with previous workers; (Ochieng (1978)). While working on accelerated stability testing on pharmaceuticals showed that hydrolysis/temperature and concentration dependent (19).

Working with sodium salicylate while constructing a calibration curve it was observed that the reaction between ferric ammonium sulphate and the phenolic OH on the salicylic acid produced a coloured complex which obeyed Beer and Lambert Laws. It was then possible to use this straight line curve to indicate the amount of salicylic acid produced from the absorbance obtained.

Table 2 shows the results obtained when investigating the effect of temperature on the rate of hydrolysis of aspirin. The concentration used was 0.1mg/ml, the mid-point in the calibration curve. The temperature investigated was 0°C, 10°C, room temperature and 30°C.
The last two temperatures were supposed to indicate the possibility of storing, the mixture in reference, particularly in the hospitals (manufacturing) health centres and home where this facility is available.

The room temperature of Nairobi is midway between the range of temperatures normally regarded in Kenyan Hospitals and homes. The 30°C represents the upper extreme of the room temperatures normally recorded in some parts of Kenya e.g. Mombasa, Kisumu, Mandera etc.

Observations indicated that at 0°C hydrolysis is minimal at least for the last 3 days. This is followed by a fast increase between the 3-4th day to a maximum of 0.15x10^-2mg/ml of salicylic acid and this stabilizes at this temperature for more than 7 days. This concentration of salicylic acid is below the minimum BP(1973) requirements for internal preparations containing salicylic acid which is 0.1mg/ml.

At 0-10°C, observations from the graph indicate that there is fast hydrolysis (curve gradient 1) for the first 4 days until a maximum of 0.125x10^-1mg/ml of salicylic acid and it stabilizes at this concentration for more than 7 days. This concentration of salicylic acid is below the minimum B.P. requirements for internal preparation.
At room temperature observations show that the rate of hydrolysis is very fast from the 1st day but it never reaches a maximum in 7 days. However in 4 days time the concentration of salicylic acid is equal to the minimum B.P. (1973) requirements for internal preparations containing salicylic acid, so that after 4 days the concentration is above this limit (i.e. above 0.1mg/ml).

At 30°C observations indicate that the rate of hydrolysis is very very fast and within 2-3 days it reaches the 0.1mg/ml which is the minimum concentration of salicylic acid required by the B.P. 1973 for internal preparations containing salicylic acid and continues increasing so that it does not reach a maximum in 7 days.

Table 3 shows the results obtained when investigating the effect of concentration on the hydrolysis of aspirin.

The temperature for the storage was room temperature, this is the most common method of storing the mixtures. The concentration investigated were 0.01mg/ml, 0.1mg/ml, 1.00mg/ml and 10mg/ml. The 1st 3 were taken to indicate the concentrations under which the investigations were carried out while the last concentration indicates the range of concentration of the mixtures prepared and stored in the manufacturing units of Kenyatta National Hospital 60mg is prepared and 30mg/ml is dispensed.

Observations indicate that at 0.01mg/ml concentration of the mixture very little hydrolysis takes place for all the 8 days. The concentration of salicylic acid at the last day reaches 0.25x10^{-3}mg/ml and this is far below the B.P. (1973)
limit for the preparations containing salicylic acid.

At 0.1mg/ml again there is very little hydrolysis, and the line is parallel to the 0.01mg/ml concentration curve, and at the end of 8 days the concentration of salicylic acid attained is $0.5 \times 10^{-3}$ which is still below the limit of the R.P. for the preparations containing aspirin.

At 1 mg/ml the hydrolysis is still at a slow rate and the highest level of salicylic acid achieved is $0.26 \times 10^{-2}$ from the original $0.2 \times 10^{-2}$ in 3 days. This is below the R.P. (1973) requirements mentioned above.

At 10mg/ml observations show that the rate of hydrolysis is high and in 3 days it changes from $0.5 \times 10^{-2}$ to 1mg/ml; after 6 days the amount of acid released is 0.1mg/ml which is the R.P. (1973) limit of salicylic acid present in internal preparations where after 6 days the mixtures salicylic levels exceed the stated requirements.

In order to limit hydrolysis to a minimum as seen above it can be deduced that the storage temperatures have to be adjusted to below 10°C. Since this is the range of temperatures where toxic levels of salicylic acid are not reached for over 7 days. However aspirin stored at room temperature and 30°C becomes toxic to live only after a few days. Reduction of temperatures to 10°C in this country means storage in a refrigerator. This equipment is expensive and can only be afforded by large hospitals for example Kenyatta National Hospital and provincial hospitals, while some health
centres and dispensaries would find it a luxury for example the remote rural areas for example Mandera, very few homes would have them. Meaning that at these places where it is available, the refrigerator can be used to store aspirin mixtures in order to lengthen their shelf life, but this is only applicable in the places where these facilities are found. However it should be emphasized that it should be preferred from room temperature wherever possible especially in the manufacturing units while it awaits distribution and dispensing; and this could be aided by the attachment of a label to indicate this i.e. 'store in a cool place' so this will only be a solution to a very small extent and patients will still store it at room temperature wherever they are where they cannot afford a cool place. So some other measure will have to be employed to limit hydrolysis.

Adjustment of concentration as shown above, may be another measure since hydrolysis can be limited by decreasing the concentration of the aspirin mixture, as it has been shown that dilute mixtures have a longer shelf life, than concentrated mixtures. It can be deduced that the best concentration would be about 1mg/ml mixtures or even less. But the lower the concentration the lower potency, therefore dispensing dilute mixtures will interfere with the intended convenience of administering the usual 60mg/ml strength meaning 150mg/ml, which is well measured with a teaspoonful and the usual dose is 150mg to 300mg of aspirin in divided doses for children.
So if the concentration is decreased to 1mg/ml it will mean that one has to give 50mls of aspirin dilute mixture to achieve the needed therapeutic dose which means taking 10 teaspoons of which is not only difficult but also has very little chances of patience compliance. Furthermore it means that a patient will have to be supplied with gallons of aspirin for a single treatment which is again unpracticable. Therefore the usual concentration of 60mg/ml has to be made and another measure of minimizing the hydrolysis employed.

From the above deductions it has been seen that since little can be done on the storage temperatures and concentration other measures have to be employed; this will include the critical check on the duration of storage. The shelf-life must be shortened by taking precautions like preparing the aspirin at shorter intervals, this meaning that it should be made on demand but as seen above in the introduction, supplies are from distant places and so the time lag between manufacture and use is always there. It can be suggested that the system be changed and the aspirin be compounded at the place of use, by the staff of lets say the dispensaries and health centres, in accordance with their needs making sure that what is fetched from the large hospitals are the dry ingredients which will not be subject to hydrolysis. This can be effected by equipping all the dispensaries etc with manuals directing the staff on how to make up the mixtures for dispensing and since its not a tedious process it can be carried out by anybody working in such a premises, who has been prepared for such work.
This will mean that fresh samples are supplied to patients.

In order to make sure that hydrolysis which occur at the time of use does not result to intake of salicylic acid the patient can be advised to discard the mixture after 4 days and if need be go back to fetch a new supply for the dispensary.

On the other hand the formulation of aspirin mixture should be adjusted to lengthen the shelf life of the preparation. It has been suggested that aspirin mixtures are stable when mixed with anti-acids indicated by the amount of salicylic acid present in a mixture stored at room temperature in screw capped amber bottles were as follows; dihydro oxy aluminium, amino acetate 0.65%, calcium gluconate 0.8%, dried aluminium hydroxide gel 3.9%, calcium carbonate 4.4% magnesium carbonate 11% (20). This shows that anti-acids may be used to minimise hydrolysis and with this in mind calcium carbonate was formulated with aspirin and stored for a week but results reviewed that there was more decomposition than when aspirin was stored without the anti-acid thus it disagreed with the suggestion above; see figure 7

Crystalline sorbitol also has a stabilising effect on aspirin mixtures and it is a better stabilizer than glycemol macrogol calcium gluconate or povidone. The martindale further suggests that stability could be further allowed by change of the vehicle from water to alcohol a 12.5% solution of aspirin in dehydrated alcohol is stable for at least 2 years (21)
however alcohol is very expensive compared to water which is freely available and therefore would not be a good choice for improvement of the formulation because aspirin is a very commonly used drug and should be easily available therefore should made as cheaply as possible.

As to the purpose of these findings the above considerations have been satisfactory and the method of choice to review the needed pharmaceutical perspective is colorimetry.

It was chosen for its

Simplicity of operation:-

The instrument – the calorimeter is comparatively easy to operate than any other analytical machine with comparable accuracy; meaning that a person without much training can operate it. It is easy to move it from place to place because of its small size; and is not sensitive to vibrations unlike other analytical instruments.

The procedure is simple and short and it offers conclusion at the spot needed in a dispensary.

Convenience and cost:-

The reagents used are few and cheap and they are required in small quantities for a single determination therefore little money is needed. The work involved is also little and therefore its economical sensitivity:-

The calorimetric results compare well with the results obtained using a UV machine (ultraviolet sp 8000) of Bye Unicham as shown by the graph in figure 4 which shows the day
to day increase of salicylic acid in the mixtures.

Both methods review the same picture that the rate of hydrolysis of aspirin in room temperature is high and reaches to about 1mg/ml in 7 days. Showing that from such a sample 1mg of salicylic acid is contained in every 1ml of aspirin taken; toxic levels of salicylic acid would be about 0.1mg/ml depending on individuals. The level is significant since aspirin is taken in a period of one week and as seen above depending on temperatures, rate varies from place to place. So these results should allow us to advise patients accordingly. For example should always say 'discard after a certain number of days,' or store in a cool place etc.
The objective of the present work was to find a quick sensitive way of determining the shelf life of aspirin mixture. To this the author's investigations have revealed that the best storage temperatures is at 10°C and a concentration of 1mg/ml. Suggestions will have to be made to the manufacturer and the patients as regards storage and shelf life at various hospitals since Kenya has varying temperatures.

To date, the work does not solve the problem but it goes a long way to suggest a quick method of determining the shelf-life of the mixture.

For further work it could be suggested that investigations be made so that the mixture is dispensed as a powder for reconstitution by adding a specified amount of water. This could to the author's opinion solve the hydrolysis problem and make available fresh preparations when and where they are used.
REFERENCES

18. Ochieng M.B. accelerated stability testing of Pharmaceutical...
Page 5-8 June, 1978.