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Project Title: "Influence of Storage under Tropical Conditions on the In-Vitro Availability of Certain Drugs from Tablets"

Principal Investigator: Dr. Ahmed F. Asker
Sponsoring Institution: Florida A&M University
Collaborating institutions and scientists:

Specific significant new developments that have resulted from the research conducted:

The results of this investigation have indicated that storage of tablets of ascorbic acid and of neomycin sulfate manufactured and marketed in Egypt where tropical climate predominates could greatly affect chemical stability of the contained drugs as well as certain physical parameters of the tablets such as hardness, disintegration time and dissolution rate. Dissolution rate of drugs from tablets can significantly affect physiological availability of the drug.

Appropriate storage conditions of tablets marketed in tropical countries should be adhered to in order to ensure that the patient would not be receiving subpotent medication or less biologically available drugs from tablet dosage forms.

NOTE: Please attach copies of manuscripts/publications/abstracts/reports that provide documentation of the scientific results.

Return this form and any attachments to: Floyd O'Quinn, Project Manager, HBCU Research Program, U.S. AID, Bureau for Science and Technology, Office of RUR, Room 309, SA-18, Washington, DC 20023.
INFLUENCE OF STORAGE UNDER TROPICAL CONDITIONS ON THE STABILITY AND DISSOLUTION OF ASCORBIC ACID TABLETS

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ABSTRACT

Ascorbic acid tablets stored at 40°C and 90% R.H. demonstrated substantial changes in chemical as well as physical stability including color, disintegration time, hardness and dissolution rate. Tablets stored at 40°C and 35% R.H. showed virtually no change in chemical stability and hardness but measurable changes in disintegration time and dissolution rate. Moisture appeared to be a significant contributing factor to the enhancement of the chemical and physical instability of ascorbic acid tablets.

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▲ To whom inquiries should be directed.
INTRODUCTION

Tablet aging can affect not only the chemical stability of the contained drug, but also certain physical parameters such as color, hardness, disintegration time and dissolution rate. Storage of tablets under tropical conditions is expected to enhance chemical instability of the drug as well as physical instability of the tablet dosage form.

Aged tetracycline tablets have been found to demonstrate decreased chemical stability (1) and increased dissolution time (2) during storage. Retardation of dissolution rate due to aging has been reported for tablets of sodium salicylate (3), acetaminophen (4), phenylbutazone (5), prednisone (6), hydrochlorothiazide (7) and aspirin (8).

The problem of tablet aging with accompanying changes in stability, disintegration time and dissolution rate has received increasing attention (9,10).

The purpose of this study therefore, was to investigate the influence of storage under tropical conditions on the stability and in-vitro availability of ascorbic acid from commercial tablets produced and marketed in one of the African countries where tropical climate predominates.

EXPERIMENTAL

Materials: Ascorbic acid, glacial acetic acid, metaphosphoric acid, 2,6 dichlorophenol-indophenol sodium, sodium bicarbonate were obtained from commercial sources in pharma-
ceutical or reagent grade and were used without further purification. Ascorbic acid tablets, freshly made and having the same batch number were obtained from a pharmaceutical company in an African country and used in this investigation.

Equipment: The following equipment were used: Incubators with controlled temperature and humidity; USP tablet disintegration apparatus; USP tablet dissolution apparatus; Erweka tablet hardness tester.

Procedure:

Tablet Storage: The freshly made tablets were placed in loosely capped plastic vials and placed in dessiccatos containing saturated solutions of either monobasic ammonium phosphate or magnesium chloride hexahydrate. The dessiccators were then placed in incubators maintained at 40°C + 3°C. These solutions produced relative humidity of 90% and 35% respectively at 40°C. Tablets were withdrawn periodically and evaluated for appearance, drug content, hardness, disintegration time and dissolution rate.

Assay of Ascorbic Acid: Analysis of ascorbic acid in the tablets was carried out on duplicate samples at different time intervals using the official dichlorophenol-indophenol method (11).

Content Uniformity: The USPXXI-NFXVI (12) general procedure was followed. Ascorbic acid content was determined by the dichlorophenol-indophenol method (11).
**Disintegration:** Disintegration time was determined by the USPXXI-NFXVI (13) method using distilled water as the disintegration medium. Six tablets were used for each determination.

**Dissolution:** Dissolution profiles of ascorbic acid tablets were determined by the USPXXI-NFXVI (13) rotating-basket method at 100rpm. A volume of exactly 900 ml of distilled water maintained at 37 ± 0.5°C was used as the dissolution medium. A 4-ml sample from the dissolution vessel was withdrawn at various time intervals by means of a filtering pipet into a 50-ml volumetric flask containing 6 ml of metaphosphoric -acetic acid mixture and titrated against 2,6 dichlorophenol-indophenol using appropriate blank. Four milliliters of distilled water was introduced to the dissolution vessel to replace the sample withdrawn. A cumulative correction was made for the previously remove samples in determining the total amount of drug released according to the equation used by Sciarra and Patel (14). Six tablets were used for each determination.

**Hardness:** Tablet hardness was determined on 10 tablet using the Erweka tablet hardness tester.

**DISCUSSION OF RESULTS**

**Content uniformity of Fresh Tablets:**

The tablets were found to comply with the official requirements for content uniformity since average ascorbic acid content was found to be 100.01 ± 4.3%.
**Chemical Stability:**

It is evident from Table 1 that the chemical instability of ascorbic acid was enhanced during storage of the tablets at 40°C and 90% R.H. On the other hand, tablets stored at 40°C and 35% R.H., were found to be chemically stable. It appears therefore, that the moisture was a significant contributing factor to the enhancement of the chemical instability of ascorbic acid tablets.

**Physical Stability:**

Tablets stored at 40°C and 90% R.H. were found to discolor progressively from white to yellow, tan, dark brown and then almost black. However, tablets stored at 40°C and 35% R.H., demonstrated only a very slight change in color at the end of the storage period when they assumed only a light buff color.

Tablets stored at 40°C and 90% R.H. demonstrated decreasing hardness on storage, whereas the hardness of the tablets stored at 40°C and 35% R.H. remained virtually unchanged as evidenced from Table 2.

Disintegration time was found to increase substantially during storage under either dry or wet conditions as can be seen from Table 2. This may be attributed to thermal gelation of the cellulose ester coat applied to the ascorbic acid particles used in the preparation of the tablets.

Figures 1 and 2 illustrate the influence of storage conditions on the release of ascorbic acid from the tablets. It is evident that the decrease in dissolution rate was more pronounced for tablets stored at 40°C and 90% R.H. This again can
### TABLE 1. Ascorbic Acid Content of Tablets
Stored under Tropical Conditions

<table>
<thead>
<tr>
<th>Storage Time (Days)</th>
<th>% Ascorbic Acid in Tablets Stored at:</th>
<th>Avg. (\pm) S.D.</th>
<th>Avg. (\pm) S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40°C &amp; 90% R.H. 40°C &amp; 35% R.H.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>100.01 4.3</td>
<td>100.01 4.3</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>100.74 2.9</td>
<td>99.3 3.2</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>94.0 3.5</td>
<td>100.2 2.2</td>
<td></td>
</tr>
<tr>
<td>105</td>
<td>76.7 7.4</td>
<td>101.7 7.6</td>
<td></td>
</tr>
<tr>
<td>164</td>
<td>62.4 1.8</td>
<td>101.7 4.2</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 2. Hardness and Disintegration of Ascorbic Acid Tablets Stored under Tropical Conditions

<table>
<thead>
<tr>
<th>Storage Time (Days)</th>
<th>40°C &amp; 90% R.H.</th>
<th>40°C &amp; 35% R.H.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hardness Kg. Avg. (\pm) S.D.</td>
<td>Disintegration (Min.) Avg. (\pm) S.D.</td>
</tr>
<tr>
<td>0</td>
<td>6.53 0.85 145 36</td>
<td>6.53 0.85 145 36</td>
</tr>
<tr>
<td>20</td>
<td>1.78 0.43 201 50</td>
<td>5.6 0.92 247 34</td>
</tr>
<tr>
<td>48</td>
<td>Too soft (&gt;) 240</td>
<td>6.2 0.76 (&gt;) 240</td>
</tr>
<tr>
<td>105</td>
<td>Too soft (&gt;) 240</td>
<td>6.2 0.35 (&gt;) 240</td>
</tr>
<tr>
<td>164</td>
<td>Too soft (&gt;) 240</td>
<td>5.4 0.43 (&gt;) 240</td>
</tr>
</tbody>
</table>
FIGURE 1. Dissolution Profiles of Ascorbic Acid from Tablets Stored at 40 °C and 90% R.H.

- Fresh tablets
- Tablets stored for 20 days
- Tablets stored for 48 days
- Tablets stored for 125 days
FIGURE 2. Dissolution Profiles of Ascorbic Acid from Tablets Stored at 40°C and 35% R.H.

- Fresh tablets
- Tablets stored for 20 days
- Tablets stored for 48 days
- Tablets stored for 133 days
be attributed to thermal gelation of the cellulose ester coat of the ascorbic acid particles.

CONCLUSIONS

The study has shown that storage under tropical conditions can substantially affect chemical as well as physical stability of ascorbic acid tablets. Moisture appeared to be a significant contributing factor to the enhancement of tablet instability.

REFERENCES

1. A. Asker, M. El-Nakeeb, M. Motawi and N. El-Gindy, Pharmazie, 27, 600 (1972).
5. D. Barret and J.T. Fell, ibid., 64, 335 (1975).
12. Ibid., p. 1277.

13. Ibid., p. 1242, 1243.

INFLUENCE OF STORAGE UNDER TROPICAL CONDITIONS ON THE STABILITY AND DISSOLUTION OF NEOMYCIN SULFATE TABLETS

Ahmed F. Asker* and Cheryl Harris

ABSTRACT

A study was made of the effects of storage of neomycin sulfate tablets under tropical climate on the stability and release of the antibiotic. Changes in drug content, appearance, disintegration time and release patterns were observed as a result of storing the tablets at 40°C and 90% R.H., and at 40°C and 35% R.H. These storage conditions represent hot/humid and hot/dry tropical climate respectively. Higher humidity appeared to be an important factor contributing to the physical and chemical instability of neomycin sulfate tablets.

* Supported by a grant from The Agency for International Development, Washington, D.C.
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INTRODUCTION

The problem of tablet aging with accompanying changes in stability, disintegration time and dissolution rate has received increasing attention. Although the chemical stability of drugs in tablet dosage forms has been extensively studied, the problem of change of dissolution patterns of aged tablets has been given less consideration.

Aged tetracycline tablets have been found to demonstrate increased dissolution time which was reflected by decreased bioavailability (1). Retardation of dissolution rate due to aging has been reported for tablets of sodium salicylate (2), acetaminophen (3), phenylbutazone (4) and prednisone (5).

There is a particular concern about the effects of storage under tropical climate that prevails in African and Middle Easter countries, on drug stability and release from various solid dosage forms.

The purpose of this study therefore, was to investigate the influence of storage under tropical conditions on the stability and release of neomycin sulfate from tablets.

EXPERIMENTAL

Materials: Neomycin sulfate, amaranth, potassium hydrogen phthalate, magnesium chloride hexahydrate and monobasic ammonium phosphate were obtained from commercial sources in pharmaceutical or reagent grade and were used without further purification. Tablets having the same batch number with a label claim of 500mg of neomycin sulfate, and marketed in Egypt were used in this study.
**Equipment:** The following equipment were used: A Spectronic 20 spectrophotometer; Orion digital pH meter; USP tablet disintegration apparatus; USP tablet dissolution apparatus; Incubators with controlled temperature and humidity.

**Procedure:**

**Tablet Storage:** Neomycin sulfate tablets of the same batch and in their original aluminum/plastic blister packs were used. The tablets were placed in dessicators containing saturated solutions of either monobasic ammonium phosphate or magnesium chloride hexahydrate at 40°C. These solutions produced relative humidities of 90% and 35% respectively at 40°C. The dessicators were placed in ovens maintained at a temperature of 40 ± 0.5°C. Tablets were withdrawn periodically and evaluated for appearance, drug content, disintegration time and dissolution rate.

**Assay of Neomycin Sulfate:** The colorimetric method used by McGinity and Hill (6) and by Hodges and Singh (7) was followed in this study. Excellent agreement was reported between the results of the colorimetric assay and those obtained by an assay of microbiological activity (6,7).

The assay is based on a colorimetric reaction between amaranth and neomycin sulfate buffered to pH 4.0 with potassium hydrogen phthalate. The absorbance of the uncomplexed dye was read spectrophotometrically at 520 nm. A linear relationship existed between the absorbance of the dye and the concentration of antibiotic; the amount of free amaranth was inversely related to the concentration of the neomycin sulfate present as shown in Fig. 1. The procedure for the preparation of the calibration curve was as follows: Several dilutions of neomycin
Concentration of Neomycin Sulfate (mg/ml)

FIGURE 1: Calibration Curve for the Assay of Neomycin Sulfate
sulfate in distilled water in the range of 0.2 - 2 mg/ml were prepared. A 1-ml sample of each dilution was added to 3 ml of a 5% solution of potassium hydrogen phthalate (pH = 4.0) in a culture tube and mixed. Two milliliters of 0.2% amaranth solution was added followed by 5 ml of distilled water and the solution was mixed on a vortex mixer for 1 minute. The mixture was allowed to stand for 15 minutes and then filtered through Whatman No. 50 filter paper. A 1-ml sample of the filtrate was introduced into a 25-ml volumetric flask, diluted to volume with distilled water and mixed. The absorbance of this solution was measured at 520 nm on the Spectronic 20 spectrophotometer using an appropriate blank.

**Drug Content:** One tablet was introduced into a small beaker and 25 ml of distilled water was added. The tablet was allowed to soak until completely disintegrated and reduced to a fine suspension with the aid of a stirring rod. The mixture was filtered into a 50-ml volumetric flask. The beaker was rinsed with 4 portions each of 5 ml of distilled water. The rinsings were poured onto the filter paper and the solution in the flask was brought to volume with distilled water. Fifteen milliliters of the filtrate were placed into a 100-ml volumetric flask and the solution was brought to volume with distilled water. A 1-ml sample of this solution was assayed for drug content as previously described under assay of neomycin sulfate. The amount of neomycin sulfate was calculated by utilizing the values of the slope and the intercept of the calibration
The USP XXI (7) general procedure for content uniformity was followed in this study. Thirty tablets were used to determine content uniformity of fresh tablets. In case of tablets stored under tropical conditions, 6 tablets were used for each determination.

Disintegration: Disintegration time was determined by the USP XXI method using distilled water as the disintegration medium. Six tablets were used for each determination.

Dissolution: Dissolution rates of neomycin sulfate were determined by the USP XXI rotating-basket method at 100 rpm. A volume of exactly 900 ml of distilled water maintained at 37 ± 0.5°C was used as the dissolution medium. A 3-ml sample from the dissolution vessel was withdrawn at various time intervals and assayed for neomycin sulfate content. Three milliliters of distilled water was introduced into the dissolution vessel to replace the sample withdrawn. Six tablets were used for each determination.

DISCUSSION OF RESULTS

Fresh Tablets:

It appears from Fig. 2 that fresh neomycin sulfate tablets of the same batch failed the USP XXI test for uniformity of dosage units by a narrow margin. Moreover, none of the tablets complied with the USP XXI requirement for disintegration. The USP XXI (9) specifies 60 minutes as a disintegration time for neomycin sulfate tablets. Fig. 3 shows the variability in disintegration time for the 6 tablets evaluated. The disintegration time ranged from 70 to 124 minutes.

Aged Tablets:

It can be seen from Table 1 that storage of neomycin
FIGURE 2: Inter-Tablet Variability in Content Uniformity of Neomycin Sulfate Tablets

x = Tablets outside the Acceptable Range
FIGURE 3. Inter-Tablet Variability in Disintegration Time of Neomycin Sulfate Tablets
**TABLE 1. Effect of Storage Conditions on the Physical and Chemical Stability of Neomycin Sulfate Tablets**

<table>
<thead>
<tr>
<th>Appearance</th>
<th>Disintegration Time (Minutes)</th>
<th>% Drug Released in 90 Minutes</th>
<th>Drug Content %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Tablets</td>
<td>White</td>
<td>103</td>
<td>87.03</td>
</tr>
<tr>
<td>Tablets stored at 40°C &amp; 90% R.H. for 30 days</td>
<td>Discolored (Tan)</td>
<td>120</td>
<td>82.40</td>
</tr>
<tr>
<td>Tablets stored at 40°C &amp; 35% R.H. for 103 days</td>
<td>White</td>
<td>116</td>
<td>80.76</td>
</tr>
</tbody>
</table>
sulfate tablets under tropical conditions affected the physical as well as the chemical stability of the tablets. It appeared also that higher humidity was an important factor contributing to the discoloration of neomycin sulfate tablets, since tablets stored at 40°C and 35% R.H. demonstrated no discoloration, whereas tablets stored at 40°C and 90% R.H. assumed a tan color.

Tablets stored at 40°C and 90% R.H. were less chemically stable than those stored at 40°C and 35% R.H. in spite of the fact that the latter tablets were stored for a much longer time. High humidity, therefore appeared to be a significant factor contributing to the chemical instability of neomycin sulfate tablets.

There was a change in the average disintegration time of the tablets as a result of aging under the two storage conditions. The disintegration time after a month of storage at 40°C and 90% R.H. was slightly longer than that for the fresh tablets. However, the disintegration time then began to decrease with storage. This may be attributed to the high humidity which rendered the tablets soft in texture. On the other hand, the disintegration time of tablets stored at 40°C and 35% R.H. remained practically unchanged after a month of storage as can be seen from Fig. 4.

Dissolution of neomycin sulfate tablets appeared to be retarded by storage under tropical conditions. Tablets stored at 40°C and 90% R.H. showed less retardation in dissolution rate than tablets stored at 40°C and 35% R.H., possibly due to the fact that tablets stored at the higher humidity became soft in texture and were stored for a shorter period of time.
FIGURE 4: Effect of Storage on Disintegration Time of Neomycin Sulfate Tablets

○ Tablets stored at 40°C & 90% R.H.
△ Tablets stored at 40°C & 35% R.H.
FIGURE 5: Dissolution Profiles of Neomycin Sulfate Tablets

- Fresh tablets
- Tablets stored at 40°C & 90% R.H. for 30 days
- Tablets stored at 40°C & 35% R.H. for 103 days
Based on the results of this study, a cool and dry place for the storage of neomycin sulfate tablets would enhance their physical and chemical stability.

REFERENCES


(4) D. Barret and J. T. Fell, ibid., 64, 335 (1975).


(9) Ibid., p. 713.