STABILITY STUDIES ON FLUCLOXACILLIN SODIUM IN RECONSTITUTED ORAL SUSPENSIONS

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ABSTRACT

Objective: Stability studies on flucloxacillin sodium in reconstituted oral suspensions were carried out. The experiment sought to investigate the effects that the different types of water for reconstitution and different storage conditions have on the stability of flucloxacillin sodium in the reconstituted suspensions.

Methods: Suspensions of flucloxacillin sodium were reconstituted with tap water, commercial bottled water (Voltic brand was used), commercial sachet water (Everpure brand was used) treated tap water and distilled water and stored under refrigeration (RF) (4-6 °C), at room temperature (RT) (31-33 °C) and in a bowl of water (BW) (26-27 °C). Assay of flucloxacillin sodium was by iodimetry at predetermined time intervals for 8 d.

Results: The amount of flucloxacillin sodium in all the suspensions stored under the various storage conditions reduced with time and at different rates. The percentage breakdown, a parameter of stability, was calculated for each reconstituted suspension stored at the different conditions investigated and they were as follows: commercial bottled water (RT-22.40 %, RF-9.90 % and BW-15.90 %), distilled water (RT-29.14 %, RF-18.0 %, BW-28.80 %), tap water (RT-25.0%, RF-14.60 % and BW-25.10 %) and commercial sachet water (RT-25.0 %, RF-10.17 % and BW-22.50 %).

Conclusion: At the end of the study, it was found that those suspensions reconstituted with the commercial bottled water were the most stable and had the smallest breakdown of flucloxacillin sodium whereas those reconstituted with distilled water were the least stable and had the largest breakdown of flucloxacillin sodium. Commercial sachet water reconstituted more stable suspensions than tap water. Also, the suspensions stored under refrigeration were the most stable followed by those stored in a bowl of water. The formulations kept at room temperature were the least stable and thus, had the largest breakdown of flucloxacillin sodium.

Keywords: Flucloxacillin sodium, Iodimetry, Storage condition, Suspensions, Water

INTRODUCTION

Antibiotics are compounds of natural, semi-synthetic, or synthetic origin which kill or inhibit the growth of microorganisms without any significant toxicity to the host. In September 1928, Sir Alexander Fleming discovered penicillin, the first true antibiotic, while working on Petri dishes containing colonies of Staphylococcus [1].

A beta-lactam antibiotic is an agent that has the beta-lactam ring in its molecular structure. Most beta-lactam antibiotics are bactericidal and they work by inhibiting bacterial cell wall synthesis thereby causing the death of bacteria [2, 3]. They are said to be the safest and most common antibacterial agents because they have a short elimination half-life, they are eliminated via the renal route, they have a good distribution in the body and have a low toxicity [4]. There are four classes of beta-lactam antibiotics namely; penicillins, cephalosporins, monobactams and carbapenems [5].

Flucloxacillin which is usually marketed as the sodium salt is a narrow spectrum beta-lactam antibiotic of the penicillin class. It has activity against bacteria like Staphylococcus aureus, beta-hemolytic Streptococci (Streptococcus pyogenes) and Streptococcus pneumoniae which produce the beta-lactamase enzyme (penicillinase), an enzyme that destroys beta-lactam rings. It is resistant to the beta-lactamase enzyme because of its bulky isoxazolyl side chain [4, 6]. It is however, contraindicated in persons that are allergic to penicillins [7, 8].

Flucloxacillin sodium is available as the injection, suspension, capsules and oral solution [9]. In all of these forms, it is unstable and easily broken down by moisture, acids, bases and heavy metal ions [10, 11].

The recommended storage condition for reconstituted flucloxacillin sodium suspension is refrigeration, but in Ghana and some parts of Africa, not all persons are able to provide this means of storage thereby raising stability concerns of the drug. Also, different people based on their economic status reconstitute suspensions with different types of water and this could also affect the stability of the drug during its period of usage. This research, therefore, seeks to investigate the effects storage conditions and the type of water for reconstitution have on the stability of flucloxacillin sodium in reconstituted suspensions with a view to recommending the best type of water for reconstituting flucloxacillin sodium suspensions to minimize breakdown of the drug.

MATERIALS AND METHODS

Materials/reagents

Distilled water, Voltic brand of commercial bottled mineral water (bottled water), Everpure brand of commercial sachet water (sachet water), treated tap water, bottles of flucloxacillin suspension of the same batch obtained from a local manufacturer (Ernest Chemist Ltd, Ghana), sulphuric acid, hydrochloric acid, glacial acetic acid and sodium hydroxide (all obtained from Daejung Chemicals and Metals Company Limited); iodine, potassium iodide and potassium iodate (all obtained from Fisher Scientific, UK); sodium acetate (Gatt-Koller) and starch mucilage powder (Qualimaks Fine Chemicals, PVT Limited). All the chemicals used were of analytical grade.

Qualitative tests on water types used

The types of water used; tap water, the commercially bottled mineral water, commercial sachet water, treated tap water and distilled water were tested for pH and the presence of copper (Cu), zinc (Zn), mercury (Hg) and lead (Pb) ions that are known to breakdown penicillins [10, 12].
Method of assay

Each type of water (tap water, bottled water, sachet water and distilled water) was used in reconstituting three bottles of flucloxacillin suspension and a bottle stored in a bowl of water (26-27 °C) such that half the height of the bottle was submerged, at room temperature (31-33 °C) and in a refrigerator/fridge (4-6 °C). The samples were assayed in triplicates at predetermined time intervals for 8 d by iodimetry because the method is cheap, accurate and can be done routinely [13]. The percentage of flucloxacillin in each case was expressed as the mean with the standard deviation (SD).

Percentage breakdown determination

\[ \text{Percentage breakdown} = \frac{C_t - C_0}{C_0} \times 100% \]

C<sub>t</sub> is the concentration of flucloxacillin sodium in suspension at 0 d
C<sub>0</sub> is the concentration of flucloxacillin sodium in suspension at 8 d

Reaction rate equations

**Zero-order reaction equation**

\[ C = C_0 - kt \]

For a zero-order graph, the final concentration, C<sub>t</sub>, was plotted against the time of assay, t, and the slope, k, obtained is the zero order reaction rate constant. Co is the initial concentration.

**First order reaction equation**

\[ \ln C = \ln C_0 - k't \]

For a first order graph, ln C was plotted against the time of assay, t, and the slope, k, obtained is the first order reaction rate constant. Co is the initial concentration and C is the final concentration after a certain time, t.

**Second order reaction equation**

\[ \frac{1}{C} = \frac{1}{C_0} + k't \]

For a second-order graph, 1/C was plotted against the time of assay, t, and the slope, k, obtained is the second order reaction rate constant. Co is the initial concentration and C is the final concentration after a certain time, t.

**Determination of reaction order of breakdown**

The percentage content of flucloxacillin sodium in each sample was converted to the appropriate concentration terms needed to plot line graphs for zero, first and second orders. The line graphs were obtained by plotting the concentration terms against time [15].

**Statistical data**

All line graphs and the bar chart were drawn with Microsoft Excel 2013. The coefficients of correlation (R<sup>2</sup>) and the slopes were all obtained from the linear equations generated with Microsoft Excel 2013.

**RESULTS AND DISCUSSION**

The pH ranges of the types of water used were within acceptable pH ranges of 6.5-8.5 according to the World Health Organization for drinking water, and they did not contain Cu<sup>2+</sup>, Zn<sup>2+</sup>, Hg<sup>2+</sup> and Pb<sup>2+</sup>ions that are known to breakdown penicillins (table 1) [10,16].

### Table 1: Results of qualitative tests done on the water types

| Water type       | Qualitative tests | pH range   |
|------------------|-------------------|------------|
|                  | Test for ions     |            |
|                  | Zn<sup>2+</sup>   | Cu<sup>2+</sup> | Hg<sup>2+</sup> | Pb<sup>2+</sup> |
| Bottled water    | -ve               | -ve        | -ve            | -ve            | 7.29-7.30 |
| Sachet water     | -ve               | -ve        | -ve            | -ve            | 8.19-8.20 |
| Distilled water  | -ve               | -ve        | -ve            | -ve            | 7.73-7.79 |
| Tap water        | -ve               | -ve        | -ve            | -ve            | 7.30-7.37 |

-ve means ions were absent

The concentrations of flucloxacillin sodium in all the suspensions reconstituted with all the various types of water under all the storage conditions under investigation reduced with time (tables 2, 3, 4 and 5) [17].

### Table 2: Assay results for flucloxacillin sodium in suspensions reconstituted with bottled water under the various storage conditions.

| Time of assay (d) | Concentration (% w/w) | Room temperature | Bowl of water | Refrigerator |
|-------------------|------------------------|------------------|--------------|-------------|
|                   |                        |                  |              |             |
| 0                 | 204.76±1.380           | 185.08±1.570     | 201.47±1.380 |             |
| 2                 | 191.16±2.710           | 172.83±3.210     | 195.68±0.540 |             |
| 4                 | 184.34±0.530           | 170.51±0.960     | 192.58±0.560 |             |
| 6                 | 172.66±1.990           | 165.85±2.540     | 188.49±0.930 |             |
| 8                 | 158.81±0.540           | 155.74±0.940     | 181.48±1.110 |             |

Concentration of flucloxacillin sodium given as mean (n=3)±SD

### Table 3: Assay results for flucloxacillin sodium in suspensions reconstituted with distilled water under the various storage conditions.

| Time of assay (d) | Concentration (% w/w) | Room temperature | Bowl of water | Refrigerator |
|-------------------|------------------------|------------------|--------------|-------------|
|                   |                        |                  |              |             |
| 0                 | 217.33±2.867           | 196.67±1.670     | 220.67±0.943 |             |
| 2                 | 199.67±2.055           | 185.33±4.784     | 202.33±1.700 |             |
| 4                 | 173.67±1.289           | 175.00±4.144     | 194.67±1.866 |             |
| 6                 | 159.67±2.357           | 153.33±2.055     | 190.33±0.949 |             |
| 8                 | 154.00±1.414           | 140.67±4.497     | 181.67±0.471 |             |

Concentration of flucloxacillin sodium given as mean (n=3)±SD
Table 4: Assay results for flucloxacillin sodium in suspensions reconstituted with sachet water under the various storage conditions

| Time of assay (d) | Concentration (% w/w) | Room temperature | Refrigerator | Bowl of water |
|-------------------|------------------------|------------------|--------------|---------------|
| 0                 | 205.15±1.380           | 212.15±0.900     | 204.79±4.970 |
| 2                 | 179.32±1.020           | 208.89±1.060     | 190.01±1.400 |
| 4                 | 177.17±2.670           | 199.61±1.100     | 184.58±0.950 |
| 6                 | 158.40±1.110           | 195.77±0.540     | 165.51±0.940 |
| 8                 | 153.82±0.540           | 190.58±1.030     | 158.67±0.550 |

Concentration of flucloxacillin sodium given as mean (n=3)±SD

Table 5: Assay results for flucloxacillin sodium in suspensions reconstituted with tap water under the various storage conditions

| Time of assay (d) | Concentration (% w/w) | Room temperature | Refrigerator | Bowl of water |
|-------------------|------------------------|------------------|--------------|---------------|
| 0                 | 226.15±1.040           | 224.31±0.900     | 228.73±0.900 |
| 2                 | 212.85±3.760           | 221.74±0.530     | 216.65±1.930 |
| 4                 | 191.14±2.330           | 205.06±0.960     | 207.63±2.210 |
| 6                 | 181.21±3.110           | 195.78±0.550     | 190.79±0.940 |
| 8                 | 169.72±1.950           | 191.45±0.950     | 171.21±0.940 |

The concentration of flucloxacillin sodium given as mean (n=3)±SD

The reduction of flucloxacillin sodium in all the suspensions occurred at different rates with time (fig. 1, 2, 3 and 4).

![Fig. 1: Rates of degradation of flucloxacillin sodium (FS) in suspensions reconstituted with bottled water under the various storage conditions](image1)

![Fig. 2: Rates of degradation of flucloxacillin sodium (FS) in suspensions reconstituted with distilled water under the various storage conditions](image2)
It was generally observed that, for all the suspensions reconstituted with the various types of water under study, those stored under refrigeration experienced the least breakdown and were the most stable, followed by those stored in a bowl of water while the suspensions kept at room temperature were the least stable (fig. 5)[18]. Comparatively, suspensions made with bottled water generally had the smallest breakdown while those made with distilled water had the largest breakdown per storage condition. Suspensions made with sachet water gave a lower percentage breakdown than those made with tap water (fig. 5).

To study the kinetics of the breakdown of flucloxacillin sodium in the samples used, the concentrations of flucloxacillin sodium in the suspensions at the various times were changed to concentration terms that satisfied a zero order, first order and second order rates of reaction (Tables 6, 7, 8 and 9).
Fig. 5: Percentage breakdown for flucloxacillin sodium in suspensions reconstituted with the various types of water under the different storage conditions studied

Table 6: Rates of reaction values for flucloxacillin sodium in suspensions reconstituted with bottled water under the various storage conditions

| Time of assay (d) | Storage condition | Room temp | Fridge | Bowl of water |
|------------------|------------------|-----------|--------|--------------|
|                  | Zero order C (%w/w) | First order ln c (% w/w) | Second order 1/c (%w/w-1) | Zero order C (%w/w) | First order ln c (% w/w) | Second order 1/c (%w/w-1) | Zero order C (%w/w) | First order ln c (% w/w) | Second order 1/c (%w/w-1) |
| 0                | 204.78 5.32       | 0.0049    | 201.47 5.31 | 0.0050 185.08 5.22 | 0.0054                  |
| 2                | 191.16 5.25       | 0.0058    | 195.68 5.28 | 0.0051 172.83 5.15 | 0.0058                  |
| 4                | 184.34 5.22       | 0.0054    | 192.59 5.26 | 0.0052 170.51 5.14 | 0.0059                  |
| 6                | 172.66 5.15       | 0.0058    | 188.49 5.24 | 0.0053 165.85 5.11 | 0.0060                  |
| 8                | 158.81 5.07       | 0.0063    | 181.48 5.02 | 0.0055 155.74 5.05 | 0.0064                  |

Table 7: Rates of reaction values for flucloxacillin sodium in suspensions reconstituted with distilled water under the various storage conditions

| Time of assay (d) | Storage condition | Room temp | Fridge | Bowl of water |
|------------------|------------------|-----------|--------|--------------|
|                  | Zero order C (%w/w) | First order ln c (% w/w) | Second order 1/c (%w/w-1) | Zero order C (%w/w) | First order ln c (% w/w) | Second order 1/c (%w/w-1) | Zero order C (%w/w) | First order ln c (% w/w) | Second order 1/c (%w/w-1) |
| 0                | 217.33 5.38       | 0.0046    | 196.67 5.28 | 0.0051 220.67 5.40 | 0.0045                  |
| 2                | 199.67 5.30       | 0.0050    | 185.33 5.22 | 0.0054 202.33 5.31 | 0.0049                  |
| 4                | 173.67 5.16       | 0.0058    | 175.00 5.16 | 0.0057 194.67 5.27 | 0.0051                  |
| 6                | 159.67 5.07       | 0.0063    | 133.33 4.09 | 0.0075 190.33 5.25 | 0.0053                  |
| 8                | 154.00 5.04       | 0.0065    | 140.67 4.95 | 0.0071 181.67 5.20 | 0.0055                  |

Table 8: Rates of reaction values for flucloxacillin sodium in suspensions reconstituted with sachet water under the various storage conditions

| Time of assay (d) | Storage condition | Room temp | Fridge | Bowl of water |
|------------------|------------------|-----------|--------|--------------|
|                  | Zero order C (%w/w) | First order ln c (% w/w) | Second order 1/c (%w/w-1) | Zero order C (%w/w) | First order ln c (% w/w) | Second order 1/c (%w/w-1) | Zero order C (%w/w) | First order ln c (% w/w) | Second order 1/c (%w/w-1) |
| 0                | 205.15 5.32       | 0.0049    | 212.15 5.36 | 0.0047 204.79 5.32 | 0.0049                  |
| 2                | 179.32 5.19       | 0.0055    | 208.89 5.34 | 0.0048 190.01 5.24 | 0.0053                  |
| 4                | 177.17 5.17       | 0.0056    | 199.61 5.30 | 0.0050 184.58 5.22 | 0.0054                  |
| 6                | 158.40 5.07       | 0.0063    | 195.77 5.28 | 0.0051 165.51 5.11 | 0.0060                  |
| 8                | 153.82 5.04       | 0.0065    | 190.58 5.25 | 0.0052 158.67 5.07 | 0.0063                  |

Table 9: Rates of reaction values for flucloxacillin sodium in suspensions reconstituted with tap water under the various storage conditions

| Time of assay (d) | Storage condition | Room temp | Fridge | Bowl of water |
|------------------|------------------|-----------|--------|--------------|
|                  | Zero order C (%w/w) | First order ln c (% w/w) | Second order 1/c (%w/w-1) | Zero order C (%w/w) | First order ln c (% w/w) | Second order 1/c (%w/w-1) | Zero order C (%w/w) | First order ln c (% w/w) | Second order 1/c (%w/w-1) |
| 0                | 226.15 5.42       | 0.0044    | 224.31 5.41 | 0.0045 228.73 5.43 | 0.0044                  |
| 2                | 212.85 5.36       | 0.0047    | 221.74 5.40 | 0.0045 216.65 5.38 | 0.0046                  |
| 4                | 191.14 5.25       | 0.0052    | 205.06 5.32 | 0.0049 207.63 5.34 | 0.0048                  |
| 6                | 181.21 5.20       | 0.0055    | 195.79 5.28 | 0.0051 190.79 5.25 | 0.0052                  |
| 8                | 169.72 5.13       | 0.0059    | 191.45 5.25 | 0.0052 171.21 5.14 | 0.0058                  |

For a particular order of reaction, the concentration terms were plotted against the corresponding times of assay to generate a line graph using Microsoft Excel 2013. This was done for zero, first and second orders for all samples reconstituted with the various types of water under all three storage conditions. Examples of the line graphs for each order of reaction have been given in figs. 6, 7 and 8 below: For every line graph plotted for all the orders of reaction, the correlation coefficient, $R^2$, and the slope, $k$, which is the reaction rate constant, were obtained (Tables 10, 11, 12 and 13). The $R^2$ measures the strength of the correlation between the values on the y-axis and the values on the x-axis. The higher the $R^2$, the greater the correlation and the more linear the graph. Conversely, the smaller the $R^2$, the smaller the correlation and the less linear the graph [19, 20].
Fig. 6: Zero order graph for flucloxacillin sodium in suspension reconstituted with sachet water at room temperature

Fig. 7: First order graph for flucloxacillin sodium in suspension reconstituted with bottled water at room temperature

Fig. 8: Second order graph for flucloxacillin sodium in suspension reconstituted with distilled water at room temperature

Table 10: Data obtained from reaction order graphs for flucloxacillin sodium suspension reconstituted with bottled water under the various storage conditions

| Storage condition | Reaction order |   |   |   |
|-------------------|----------------|---|---|---|
|                   | Zero order     | First order | Second order |
|                   | $R^2$          | $k$          | $R^2$ | $k$ | $R^2$ | $k$ |
| Room temp         | 0.9894         | 5.5220       | 0.9615 | 0.030 | 0.7179 | 0.0001 |
| Fridge            | 0.9927         | 2.3538       | 0.7161 | 0.031 | 0.9730 | 0.0001 |
| Bowl of water     | 0.9449         | 3.2830       | 0.9426 | 0.019 | 0.9508 | 0.0001 |
For every reaction order graph, a plot of the right concentration term against time produced a straight line. The reaction order graphs for a particular water type for all the storage conditions that gave the highest values for R$^2$ (i.e. the most linear graphs) were chosen to give the order of breakdown of flucloxacillin sodium in the suspension reconstituted with that water type [15].

Degradation of flucloxacillin sodium in suspensions reconstituted with bottled water generally followed a zero-order pathway because the highest R$^2$ values were seen for zero order (table 10).

Hence comparing the zero order rate constant ($k$) values for the three storage conditions in table 10, it was observed that, the value for storage under refrigeration (fridge) was the least, followed by that for bowl of water while storage at room temperature gave the highest value. The higher the value of $k$, the faster the breakdown and the least stable the product whereas a small value of $k$ means a slow rate of breakdown hence a stable product [14]. This observation agrees with the breakdown trend in fig. 5.

Breakdown of flucloxacillin sodium in suspensions reconstituted with distilled water and commercial sachet water was via a second order pathway because it gave the highest R$^2$ values. Comparing the second order rate constant values shows that, storage under refrigeration was the best with the values for a bowl of water being very close due to approximations (Tables 11 and 12). Though data from fig. 5 also reveal this closeness of $k'$ (second-order rate constant) for a bowl of water and room temperature, it showed that storage in a bowl of water was superior.

Flucloxacillin sodium breakdown in suspensions reconstituted with tap water gave a first order breakdown pattern since it generally gave the highest R$^2$ for first order. Again, the $k'$ values reveal that the suspensions kept under refrigeration were the most stable whereas the suspensions stored at room temperature were the least stable (table 13).

CONCLUSION
The results of the analysis showed that flucloxacillin sodium broke down with time and at different rates in all the suspensions and under the various conditions of storage. It was seen that the suspensions made with bottled water experienced the least breakdown of flucloxacillin sodium and were the most stable whereas those reconstituted with distilled water had the largest breakdown of flucloxacillin sodium and were the least stable. Sachet water reconstituted more stable suspensions than tap water. Hence, the preferred type of water for reconstitution is bottled water. Sachet water may be used as an alternative. Distilled water should be avoided in the reconstitution of suspensions to minimize the breakdown of flucloxacillin sodium.

The rate of breakdown of flucloxacillin sodium in suspensions stored under refrigeration was the smallest, and those suspensions were the most stable. The suspensions stored at room temperature experienced the largest breakdown of flucloxacillin sodium and were the least stable. The suspensions stored in a bowl of water were more stable than those stored at room temperature since the rate of breakdown of flucloxacillin sodium was higher in the latter than in the former. As such, in the absence of refrigeration, suspensions may be stored in a bowl of water to reduce degradation.

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AUTHORS CONTRIBUTIONS
The corresponding author, Michael W. Klu, was the lead investigator and supervised the work. Elikem Katsekpor carried out the laboratory work. John A. Apenteng and Bright S. Addy drafted the manuscript while David Mintah did the critical review and editing of the manuscript.

CONFLICT OF INTERESTS
The authors declare that they have no competing interest

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