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Stability of Chloroquine Phosphate Tablets Inoculated with Bacterial Species

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Five popular brands of chloroquine tablets available to the average Nigerian consumers were examined for the effects of *Staphylococcus aureus* and *Bacillus cereus*, on the dissolution, disintegration and hardness after six weeks of incubation. The maximum percent dissolution was 98.34 % with *Bacillus subtilis* while the minimum was 19.12% with *Staphylococcus aureus*. The disintegration results showed a maximum of 69 min. 19 sec with *Staphylococcus aureus* while the least was 56 sec with *Bacillus subtilis*. The maximum hardness obtained was 12.75 kg and the least was 1.25kg also with *Staphylococcus aureus*. The dissolution, disintegration and hardness also varied with the control. The metabolic activities of the bacterial species were believed to have caused the variations in the physical properties of the chloroquine phosphate tablets. The results from this investigation strongly advises adequate storage of chloroquine phosphate tablets, especially when it is the drug of choice for the treatment of malaria in most endemic countries of sub-Saharan Africa.

Key words: Stability, Chloroquine phosphate, Tablets, *Staphylococcus aureus*, *Bacillus subtilis*

Introduction

Bacteria and fungi are commonly found in any environment. They constitute a diverse group of microorganism that is freely abundant in nature. One major problem commonly, encountered in the production of pharmaceuticals is caused by microbial spoilage and contamination.

This spoilage of drugs could result from improper storage of the drugs in pharmacies, during use at home or in the hospital. Poor storage of raw materials can also lead to a poor finished product. Furthermore, tropical regions like Nigeria with hot and humid climate provide favourable conditions for the growth of contaminating microorganisms. Obuekwe *et al* (1996), studied the effects of some bacterial species on the disintegration of acetylsalicylic acid (aspirin) tablets and found that the presence of bacteria on the tablets had an effect on their physical properties.

Contaminating organisms may also alter some tablet properties such as, dissolution of the active drug from the tablet by either increasing or decreasing the amount dissolved in a suitable solvent after a given time, (Esezobo 1985). Chloroquine phosphate is available as a white or almost white odourless tablet and is arguably the most prescribed anti-malarial. It is orally absorbed and the rate of absorption is determined by many factors, particularly the dissolution and disintegration rates of the tablets. Obuekwe and Sadoh (2000) studied the effects of some fungi on the physical properties of paracetamol tablets. They observed that the presence of these organisms actually had some effects on the disintegration and hardness of the tablets.

This study ascertains the effects of *Staphylococcus aureus* and *Bacillus subtilis* on the stability of chloroquine phosphate tablets and questions its use as a cure in malarial therapy, especially in the tropical regions of the world.

Materials and Methods

Five popular brands of chloroquine phosphate tablets from retail pharmacies in Benin City, Nigeria were used for this study. Overnight cultures of *Staphylococcus aureus* and *Bacillus subtilis* were inoculated onto the surfaces of 42 tablets of each brand of the chloroquine phosphate tablets by means of a sterile pipette. About 0.2 milliliters (approximately, 1.0×10^2 colony forming units per milliliter) of the culture was used to inoculate each of the tablets.

Dissolution test. At two week- intervals for six weeks, the dissolution and disintegration rates as well as hardness for each brand of the chloroquine phosphate tablets were determined and results were recorded. For dissolution, the G. B. Dissolution Test Unit, Model ST7 (G. B. Caleva Ltd. Dorset,

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England) was used. The unit was set at 100 revolutions per minute and the medium, which was 0.1N HCl was kept at 37°C ± 0.5°C. One tablet from each brand of the chloroquine tablets was tested. The dissolution machine was run for 45 min after which 2.0 milliliters of the solution was pipetted out and made up to 100 ml. with 0.1N HCl (that is 1mg %). Quadruplicate determinations were carried out and the mean value was taken. The absorbance readings were taken and recorded from the UV-Visible Spectrophotometer (SP 1800, Pye Unicam, Cambridge, UK)

Disintegration test. For disintegration, the B. P. Disintegration Test Unit (MK 4, Manesty Machine Ltd. Liverpool, England) was used. The unit was set at 30 oscillations per minute and the disintegration medium was water kept at 37°C ± 0.5°C. Six tablets from each brand were tested and duplicate determinations carried out and the mean disintegration times were recorded. The disintegration times of the control tablets were also noted.

Hardness test. The Monsanto tablet hardness tester was used to measure the hardness of both the inoculated and control tablets. The tablet was placed between the anvil and applied the pressure of the hardness tester. Pressure was applied to the tablet until it fractured, whereupon the hardness was read from the graduated scale. This determination was carried out on ten tablets for the brand of the chloroquine phosphate tablets and the mean values recorded.

Results and Discussion

Most brands of the chloroquine phosphate tablets tested, retained their original colour and consistency, except in some tablets inoculated with *Staphylococcus aureus* where dark brownish spots were observed.

Figure 1 showed the effect of *Staphylococcus aureus* on the dissolution rates of different brands of chloroquine phosphate tablets. The maximum amount dissolved (%) was 92.64 for brand 5 in week 2, while the least was 19.12 for brand 1 in week 6. Figure 2 also showed a maximum amount dissolved (%) of 98.34 for brand 5 in week 6 and a minimum of 30.88 for brand 3 in week 6 when *Bacillus subtilis* was inoculated into the five brands of chloroquine phosphate tablets. All graphs in figure 1 showed similar patterns. The peak amounts dissolved were mainly at week 2. The reverse was observed in figure 2 which also showed similar patterns of decline in the graphs at week while the maximum amount dissolved was mainly at week 4.

Figure 3 showed the effect of *Staphylococcus aureus* on the disintegration time of the different brands of chloroquine phosphate tablets. The maximum disintegration time was 69.3 min for brand 1 and the least was 2 min for brand 4. Figure 4 also showed the effect of *Bacillus subtilis* on the disintegration properties of the different brands of chloroquine phosphate tablets examined. The maximum disintegration time was 41 min for brand 3 with a minimum of 56 sec for brand 4.

The effects of *Staphylococcus aureus* and *Bacillus subtilis* on the hardness of the five brands of chloroquine phosphate tablets are shown in Tables 1 and 2. For tablets inoculated with *Staphylococcus aureus*, the maximum hardness observed was 12.75kg in brand 1 while the least was 1.25 kg in brand 4 (Table 1). With *Bacillus subtilis*, the maximum hardness was 11.0 kg for brand 3 and 1.6 kg for brand 4. With both organisms, there was similar pattern of change in hardness reaching a maximum at week 4 before a decline in week 6.

The results obtained from the dissolution test showed that there were great variations in the amount dissolved (%) of the inoculated chloroquine phosphate tablets.It was an indication that the bacteria affected the dissolution rates of the tablets.

Khattab *et al* (1993), studied the mode of incorporation of disintegration on the characteristics of fluid-bed granulated tablets. They found that the combined mode resulted in significantly, faster dissolution rates than extra-granular mode, which in turn was superior to the intra-granular mode of inclusion. In the present study, there was a very rapid initial increase in the dissolution rates before a decline (Fig 1). The effects of humidity and temperature on *in vitro* dissolution of carbamazepine (CBZ) tablets have been evaluated (Wang *et al* 1993). The effects of different humidity and temperatures on the dissolution property of CBZ tablets were compared to fresh and unstressed tablets. The results showed that expo-

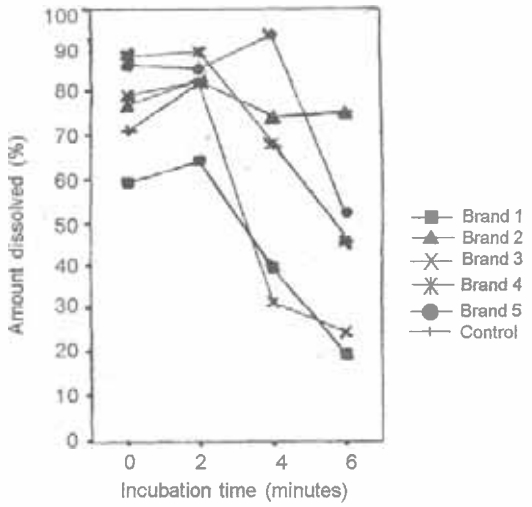

Fig 1. Effect of *Staphylococcus aureus* on the dissolution properties of chloroquine phosphate tablets.

Table 1
Effect of *Staphylococcus aureus* on the hardness of five brands of chloroquine phosphate tablets

Brand	Wk 0		Wk 2		Wk 4		WK 6	
	Control	Sa.	Control	Sa.	Control	Sa.	Control	Sa.
Brand 1	8.00	7.20	8.90	6.50	9.00	12.75	11.00	8.50
Brand 2	3.90	3.50	5.00	3.10	8.00	3.75	6.00	2.75
Brand 3	6.50	5.00	7.00	9.00	6.00	11.00	6.00	10.75
Brand 4	1.90	2.50	2.00	1.50	3.00	2.50	4.00	1.25
Brand 5	2.90	2.50	3.00	3.50	5.00	5.75	4.00	3.50

Wk, week; Sa, *Staphylococcus aureus.*

sure of CBZ tablets to high humidity and temperature could have a profound effect on tablet disintegration and dissolution. Furthermore, the dissolution rates of some batches of CBZ products exposed to very high humidity and temperature were drastically reduced in only 6-7 days.

Also the effects of treating cassava starch with sodium lauryl sulfate (SLP) and polysorbate 80 (both preservatives) have been studied by Nasipuri and Omotosho (1985). Dissolution and disintegration rates were found to be faster with starch in which surfactant was incorporated in dry state. A direct correlation was observed between the hardness and friability index. They finally concluded that polysorbate 80 treated-starch exhibited a better dissolution profile than SLS-treated starch. Gebre-Mariam and Nikolayer (1993) studied the binding and disintegrating properties of starch obtained from Ensete starch. The effects of the starch on the physical properties such as, crushing strength, friability and disintegration of tablets of chloroquine phosphate, dipyrone and paracetamol were compared with potato starch. They found that Ensete starch has a better binding ability and less disintegration power than potato starch. In the present study, the differences in the disintegration times observed with the different brands of chloroquine phosphate tablets as well as with the control tablets. It could be, as a result of the different disintegrants used by different manufacturers.

Fassihi and Parker (1987) studied the effects of incorporating cells of *Staphylococcus aureus* and *Bacillus subtilis* in tablets prepared from compaction at various pressures and compression speeds. They found that the compression behavior of powders on the speed of compaction and the degree of densification of the compacted tablets was responsible for the extent of inactivation of these organisms. In the present study, cells of *Staphylococcus aureus* and *Bacillus subtilis* were inoculated onto surfaces of tablets chloroquine phosphate tablets and the results obtained showed that there were changes in the dissolution and disintegration rates of the tablets as well as the hardness.

Table 2
Effect of *Bacillus subtilis* on the hardness of some brands of chloroquine phosphate tablets

Brands	Wk 0		Wk 2		Wk 4		Wk 6	
	Control	Bs.	Control	Bs.	Control	Bs.	Control	Bs.
Brand 1	8.00	7.20	8.90	7.50	9.00	9.25	11.00	6.50
Brand 2	3.90	3.50	5.00	3.00	8.00	3.75	6.00	3.00
Brand 3	6.50	5.00	7.00	9.50	6.00	11.00	6.00	7.00
Brand 4	1.90	2.50	2.00	1.90	3.00	2.50	4.00	1.60
Brand 5	2.90	2.50	3.00	3.20	5.00	3.75	4.00	1.60

Bs, *Bacillus subtilis.*

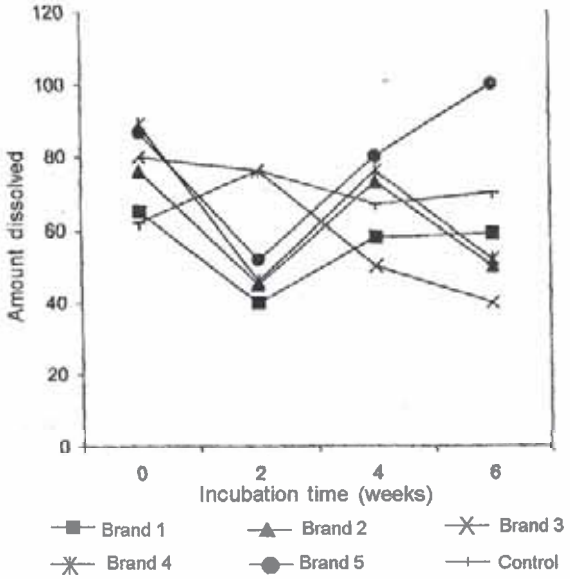

Fig 2. Effect of *Bacillus subtilis* on the dissolution of chloroquine phosphate tablets.

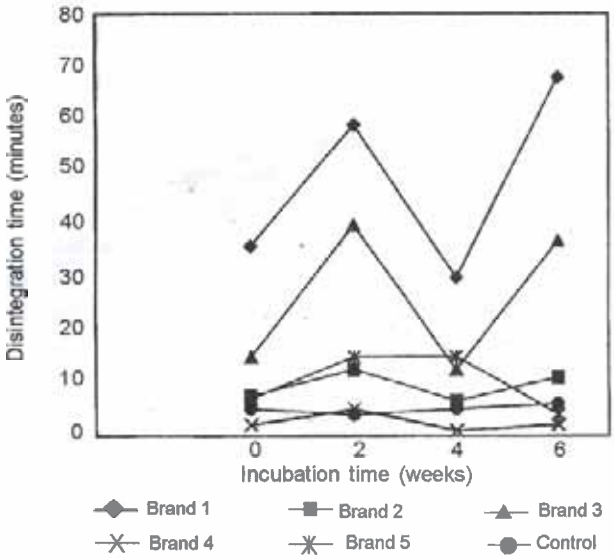

Fig 3. Effect of *Staphylococcus aureus* on the disintegration properties of chloroquine phosphate tablets.

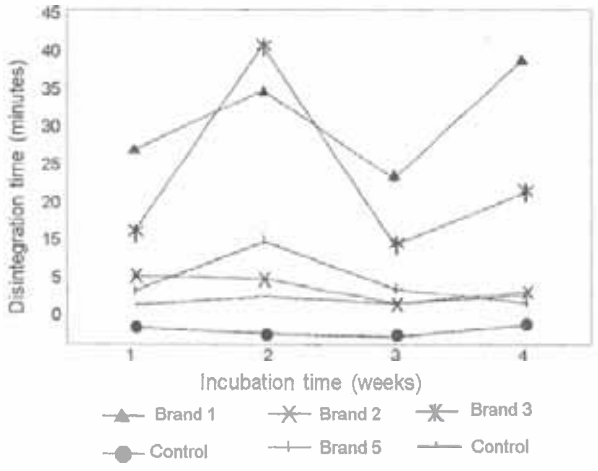

Fig 4. Effect of *Bacillus subtilis* on the disintegration properties of chloroquine phosphate tablets.

The hardness values of the inoculated chloroquine phosphate tablets were observed to be gradually increasing, for most brands reaching at maximum in week 4 before a decline (Tables 1 and 2). The increase in hardness could be due to an increase in the tensile strength of the tablets, that resulted because of increased water activity caused by the high humid conditions of the environment. Results obtained from this study correlated with similar work carried out by Obuekwe and Sadoh (2000) where fungal cells were used in place of bacteria. The graphs obtained in this study showed similar patterns in the disintegration rates of the tablets where maximum peaks were obtained before a decline.

This study has revealed that there was a correlation among the amount dissolved (%), the disintegration times as well as the hardness of chloroquine phosphate tablets in the presence of bacterial species. These must be viewed with great concern, especially when such organisms had already been found as contaminants in drugs (Obuekwe and Ogbimi 1998). This could lead to a reduction in the pharmacological activity of the drug as there was delayed release of active ingredients from the tablets, which subsequently could lead to delay and eventual loss of therapeutic efficacy, especially when the drug is used for the treatment of malaria. It is known that thousands of people die of malaria every year in countries where the disease is endemic. To enhance good pharmaceutical products at retail outlets, manufacturers should ensure that raw materials are adequately checked before use by their Quality Control (QC) laboratories. Proper handling of drugs and storage by consumers as well as good manufacturing practice (GMP) in drug production are highly recommended for the continued safety and efficacy of drugs.

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