THE PRESENCE OF MICROORGANISMS IN SOME COMMON EXCIPIENTS USED IN TABLET FORMULATION

IFEYINWA F. OBUEKWE* and FLORENCE EICHIE

1 Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria
2 Department of Pharmaceuticals and Pharmaceutical Technology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Abstract: Strict measure on the need to control microbial contamination in the formulation of solid dosage forms such as tablets and capsules are not usually in place as is done in other pharmaceutical preparations. The presence of microorganisms in some common excipients such as starch and lactose powders, and distilled water used in tablet formulation was investigated in this study. Organisms isolated were Geotrichum and Aspergillus spp as well as two strains of Staphylococcus aureus. Lactose tablets were further formulated using 5% starch as binder. Some of the isolated organisms were, in turn, inoculated into these tablets and stored over a period of several weeks. The physical properties of these tablets were determined at various time intervals. Marked increases in the disintegration times of these tablets were observed, while there was a decrease in the hardness property for both the fungi and bacteria. These observed effects have serious implications in the overall bioavailability of drugs such as tablets formulated from contaminated excipients.

Keywords: microorganisms, excipients, tablet formulation

The major advantage of tablets as a dosage form is that they provide an accurate dosage of medication. Each tablet must contain a known amount of drug and must be uniform in diameter, appearance and weight. Tablets for oral use once swallowed whole, should readily disintegrate in the stomach. This property represents a great paradox in formulation, hence tablets should be produced with sufficient strength to withstand the rigors of processing coating and packaging, yet be capable of order to release the drug rapidly (1). This disintegration involves the bursting apart of the compact by aqueous fluids penetrating the time residual pore structured the tablet.

The active ingredient must be pharmacologically available and since drugs cannot be absorbed into the blood stream from the solid state, the active ingredients must dissolve in the gastric or intestinal fluids before absorption can take place.

One of the major setbacks commonly encountered is that carried by the storage due to microorganisms (2). A lot of factors contribute to microbial load carried by a pharmaceutical preparation at every stage. These include: raw materials used, manufacturing processes or personnel, conditions of storage in the industry, at home, hospitals or from packaging materials (3). Another source of microbial contamination is the preservative intended to protect the formulation against microorganisms. They can be used as a ready source of microbial nutrition, particularly if their levels become depleted and if they are aromatic in nature (4).

A spoilage of pharmaceutical products could occur over a temperature range from about –20°C to 60°C, although it is generally poor at the extremes. The effect of transportation and storage of products at ambient temperatures in the tropics or subtropics should be considered in this respect. It is known that some microorganisms make use of some tablet excipients such as starches used as binders and disintegrants, as substrates for their growth (5-10).

This study, therefore, investigates the sources and levels of such microbial contamination on raw materials used for tablet formulation and the resulting consequences on the physical properties of tablets such as hardness and disintegration.

MATERIALS AND METHODS

Enumeration of microorganisms from the raw materials used for tablet formulation

Raw materials used in this study were starch powder, lactose powder and distilled water. Ten
grams of starch and lactose powders were weighed separately and dissolved in two flasks containing 90 mL of sterile distilled water to make a 1:10 dilution (stock solution). Also 10 mL of distilled water was added to 90 mL of sterile distilled water to make a 1:10 dilution. Serial dilutions were made up to 10^4 from the different stock solutions (containing the raw materials – starch and lactose powders and distilled water, respectively). Using 0.9% normal saline as diluent subsequently, 1 mL of the dilutions (10^5 to 10^4) from the three different stock solution were then pipetted into 19 mL of molten nutrient Agar, sabouroid and agar media, respectively. These were individually thoroughly mixed and poured into sterile Petri dishes (pour plate method). All plates were then incubated for 24 h at 37°C for bacterial growth and at room temperature (±28°C) for 5-7 days for fungal growth. Bacteria were identified by Gram stain and biochemical tests whereas the fungi were identified using a lactophenol stain preparation of lactose tablets.

Lactose powder (400 g) was wet mixed with 5% starch mucilage in a mortar. The wet mass was then passed through a 1.40 mm mesh screen and dried at 60°C for 1 h. The granules were then dry ground to a uniform size. The dried granules were passed through a 710 mm mesh screen and finally dried at 60°C for 3 h to obtain uniform size granules. The dried granules were mixed with 1% magnesium stearate (lubricant) and 5% maize starch (disintegrant). The final tablet mixture was compressed at a compression pressure of 25 mmHg to produce the lactose tablets. The tablets were stored overnight in a dessicator before evaluations.

Inoculation of tablets
A suspension of pure cultures of Geotrichium and Aspergillus spp isolated from the raw materials (i.e. starch and lactose powders, and distilled water) was made by scooping layers of the mycelia using a sterile inoculation needle into 20 mL of normal saline (0.9% sodium chloride) and shaken thoroughly. From this suspension, 0.1 mL (10^4 conidia/mL) was inoculated on the tablets and incubated at room temperature (±28°C) for 7 weeks. The uninoculated tablets served as control.

A suspension of isolated bacteria spp. (Staphylococcus aureus) was inoculated into 20 mL of normal saline and incubating at 37°C for 24 h. From this suspension, 0.1 mL (1 x 10^2 cfu/mL) was inoculated on the lactose tablets and incubated at 37°C for 7 weeks. The uninoculated tablets also served as control.

At weekly intervals for seven weeks, the disintegration and hardness tests for the lactose tablets were carried out and results recorded.

Disintegration test
A BP tablet disintegration unit apparatus (type MK4 Manesty Machine Ltd., Liverpool, England) was used. The disintegration medium was distilled water maintained at 37±1°C. Six tablets from each batch were placed in each tube and allowed to oscillate. The time taken for the primary particles to pass through the mesh was noted. The mean values of six determinations are reported.

Hardness test
A Mosanto tablet hardness tester was used to measure the hardness properties of the tablets before and after inoculation at various time intervals. The tablet was placed between the anvil and the pressure plunger of the hardness tester; pressure was applied to the tablet until it fractured diametrically. The load causing the diametrical fracture was read from the graduated scale. The determination was carried out in five replicates and the mean values are reported.

<table>
<thead>
<tr>
<th>Excipient</th>
<th>Bacteria*</th>
<th>Fungi*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose</td>
<td>S. aureus A+++</td>
<td>Aspergillus ++</td>
</tr>
<tr>
<td></td>
<td>S. aureus A++</td>
<td>Penicillium sp. ++</td>
</tr>
<tr>
<td></td>
<td>S. aureus B ++</td>
<td>Aspergillus sp. +</td>
</tr>
<tr>
<td>Starch</td>
<td>S. aureus A++</td>
<td>Saccharomyces sp.+</td>
</tr>
<tr>
<td></td>
<td>S. aureus B ++</td>
<td>Rhodotorula ++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geotrichium sp. ++</td>
</tr>
<tr>
<td>Distilled water</td>
<td>S. aureus A ++</td>
<td>Penicillium ++</td>
</tr>
</tbody>
</table>

* low (+), medium (++), high (+++) level of growth
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Figure 1. Changes in the disintegration time of lactose tablets inoculated with: (♦) control Geotrichium (▲) and (■) Aspergillus species.

Figure 2. Changes in the disintegration time of lactose tablets inoculated with: (♦) control (▲) Staphylococcus aureus strain A and (■) Staphylococcus aureus strain B species.

Figure 3. Changes on the hardness of lactose tablets inoculated with: (♦) control Geotrichium (■) and (▲) Aspergillus species.

Figure 4. Changes on the hardness of lactose tablets inoculated with: (♦) control, (■) Staphylococcus aureus (A) and (▲) Staphylococcus aureus (B) species.
Apart from the disintegration and hardness properties of the tablets, other observations made included color changes, size and texture variations.

RESULTS AND DISCUSSION

The microorganisms isolated form the raw materials (starch, lactose powder and distilled water) are shown in Table 1. Generally, the major raw materials employed in this study contained both bacterial and fungi. The organisms identified included *Staphylococcus aureus* strains A and B, *Geotrichium* and *Aspergillus* spp. Others included *Penicillium*, *Rhodotolila* and *Saccharomyces* spp. The spores of these fungi could persist for a long time in the raw material. *Staphylococcus aureus* was found to be widely distributed in the entire raw materials examined. The lactose tablets were originally white in color and most of the tablets turned brown after one week of incubation except for the control. Other changes observed included cracks at the sides and rough surfaces. There observed color changes are physical manifestation of chemical breakdown of the tablet component, such as starch, by the microorganisms. This lead to a loss of binding properties and manifested as cracks. The tablets also became softer after three weeks of inoculation as evidenced by the low tensile strength values except for the control. This may have been due to the breakdown of starch used as a binder as the organisms must have derived nutrients for growth from it.

The disintegration time increased more markedly with time after inoculation with the organisms on storage. This effect was more marked with *Aspergillus* than with *Geotrichium* species (Figure 1). The disintegration time increased drastically from 1 min (control) to 35 min after inoculation with *Aspergillus* and *Geotrichium* species for 3 weeks. This observed effect might be attributed to failure of the compact to disintegrate as the organisms might have utilized the tablet excipients as nutrients for growth. However, the control tablets did not show marked changes over the period of storage. This finding correlated with the previous reports by earlier researchers in this field (2, 6-9).

The results of the disintegration times of lactose tablets inoculated with two strains of *Staphylococcus aureus* are shown in Figure 2. There were no marked changes in the disintegration times of the control tablets but the inoculated tablets were markedly affected. Fibrils were seen after the disintegration of the tablets except the control after two weeks of incubation. Formation of cotton wool like fibrils had been found interwoven with the tablet matrix in our earlier study (1). The initial increase observed in the disintegration times could also be attributed to an increase in biosynthetic activity and cell division of the microorganisms upon arrival on a suitable substrate, this time – the lactose tablets.

The prolonged disintegration observed after inoculation and storage with the various organisms has serious biopharmaceutic implications in terms of bioavailability of drugs from such tablets. A drug is supposed to disintegrate before the dissolution and absorption. However, in the absence of complete disintegration of a tablet, there will be a loss of therapeutic action.

The results of the changes in the hardness of tablets inoculated with both *Geotrichium* and *Aspergillus* spp, as well as the two strains of *Staphylococcus aureus* are shown in Figures 3 and 4. There were progressive decreases in the hardness throughout the periods of incubation but the controls remained unchanged. Microorganisms require ready access to water for growth. Condensed water films can accumulate on the surface of the otherwise ‘dry’ products, such as tablets, resulting in sufficiently high localized water activity (Aw) to initiate fungal growth (3). In this study, water came form the inoculated drops, leading to an increase in microbial growth.

The hardness of a tablet is tied in with the strength of the bond resulting from binders, which may form bridges between particles. When tablets absorb moisture, there is softening of the binder bridge, therefore resulting in soft tablets. In addition to the above, the decrease in the hardness during incubation could be due to the loss of the binding properties of the starch as the organism might have utilized it as a nutrient for growth leading to the soft crumbling tablets.

The microbial deterioration of tablets has serious pharmaceutical implication as it affects therapeutic efficacy of drugs. During storage, transportation or handling, the soft tablets may break up since they are not able to withstand minimal shocks; therefore the amount of active drug is reduced. The process of good manufacturing practice must be observed; raw materials should be properly sterilized before use and checked by quality control laboratories. Production processes should be carried out with regular microbial monitoring and drugs should be properly stored.

REFERENCES

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