

MODERN ASPECTS REGARDING THE MICROBIAL SPOILAGE OF PHARMACEUTICAL PRODUCTS

ODAY HUSHAM KAMIL^{1*}, DUMITRU LUPULIASA²

¹*University of Baghdad, Faculty of Pharmacy, Iraq*

²*University of Medicine and Pharmacy "Carol Davila" Bucharest, Faculty of Pharmacy, Department of Pharmaceutical Technology*

**corresponding author: pharma_oday@yahoo.com*

Abstract

The aim of this article is to review some of the scientific data dealing with the effect of some environmental factors (water activity, pH and temperature) on microbial spoilage. Contamination of pharmaceutical products with microorganisms could make changes in physico-chemical characteristics as well as the toxicity of pharmaceutical preparations. All the contents of the dosage forms (active ingredients and excipients) are susceptible to microbial contamination and spoilage. Strict measures are required to control microbial contamination in the formulation of pharmaceutical preparations.

Rezumat

Scopul acestui articol este de a analiza cele mai recente date din literatura de specialitate referitor la influența unor factori de mediu (umiditate, pH, temperatură) asupra contaminării microbiene a produselor farmaceutice. Toate componentele medicamentelor (substanțe active și excipienți) sunt puternic afectate de prezența microorganismelor, prin urmare sunt necesare măsuri exigente pentru controlul contaminării microbiene al tuturor produselor farmaceutice.

Keywords: Microbial spoilage, environmental factors.

Introduction

Non-sterile pharmaceutical products with a high degree of water content may be contaminated with microorganisms. The contaminating microorganisms may cause spoilage of the product with loss of its therapeutic properties and, if they are pathogenic, serious infections can arise [1].

Modern research identified different types of microorganisms from the raw materials used during pharmaceutical productions; these organisms include *Aspergillus spp.* *Penicillium spp.* [2].

A study carried by Mugoyela and Mwambete [3] involved structured selection of representative tablets, syrups, and capsules from the hospital's outpatient pharmacy in Tanzania. They found that the majority of

microbial contaminants in non-sterile pharmaceuticals are *Aspergillus spp.*, *Bacillus spp.*, *Candida albicans* and *Klebsiella spp.*

Apart from health problems of microbial contamination of pharmaceuticals [4, 5], the deteriorating effects on the products are various, ranging from introduction of toxic metabolites and cell fractions to chemical and physical modifications [6, 7].

Several reports have been published describing clinical hazards that are attributable to microbiologically contaminated pharmaceuticals [8]. In 1966, more than 200 people in Sweden became ill after taking thyroid tablets heavily contaminated with *Salmonella muenchen* [9, 10]. In Sweden in 1964 eight cases of infection of the eye after removal of foreign bodies occurred at two different centers. The infections were caused by *Pseudomonas aeruginosa* [9]. Reports from India, Japan and Indonesia showed mycoflora and mycotoxins contamination of herbal drugs [11, 12].

Microbial contamination of pharmaceuticals

A pharmaceutical raw material is an active or inactive substance used in the manufacture of a pharmaceutical dosage form. It includes materials manufactured by processes such as, chemical synthesis, fermentation, recombinant DNA or other biotechnology methods, isolation/recovery from natural sources, or any combination of these processes [13]. Microbial contamination of pharmaceutical products may originate from the raw materials, as microorganisms from the raw materials will invariably be transferred to the product [14].

In addition, 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 or from packaging materials [15].

Most raw materials for pharmaceutical products support some forms of microbial growth, depending on the nutritive properties and moisture contents. Hence, dry powder or tablets are capable of undergoing some form of microbial spoilage or degradation. The more serious problem of microbial contamination of tablets is where there are no obvious signs of spoilage; hence, it is usually advisable to have knowledge of the microbial content of all drugs and medicines, whether they are required to be sterile or non-sterile. 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 [16].

Microbial contamination of tablets

The major advantage of tablets as a dosage form is that they provide an accurate dosage of the active substance. Each tablet must contain a known amount of drug and excipients.

Lactose is one of the most used excipient in tablets. It is originally white in color and most of the tablets turn brown after one week of incubation when contaminated with microorganisms.

The literature also reports other changes after microbial contamination including cracks at the sides and rough surfaces. Color changes are physical manifestation of chemical alteration of the tablet component, such as starch, by the microorganisms. This leads 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. This may have been due to the breakdown of starch used as a binder as the microorganisms transformed it into nutrients for growth from it. The disintegration time increased significantly with time after inoculation with the organisms on storage. The literature reports that this effect is important for *Aspergillus spp.* [2].

Environmental factors affecting microbial growth [15]

Nutritional factors

The simple nutritional requirements and metabolic adaptability of many common spoilage microorganisms enable them to utilize many formulation components as substrates for biosynthesis and growth. A formulation containing crude vegetable or animal products provide additional nutritious environment. Even demineralized water prepared by ion exchange method normally contains sufficient nutrients to allow growth of some *Pseudomonas spp.* Acute pathogens require specific growth factors which are often absent in pharmaceutical formulations so they do not multiply but remain viable and infective for an appreciable time.

Moisture contents (water activity - a_w)

Microorganisms require readily accessible water in appreciable quantities for growth. Water activity of aqueous formulations can be reduced by the addition of high concentration of sugars or PEG or by drying. Condensed water films can accumulate on the surface of dry products such as tablets or bulk oils due to storage in damp atmospheres resulting in fungal growth.

Effect of water activity (a_w) on microbial growth and toxins production

Gervais, Bensoussan, and Grajek [25] compared the effects of water content and water activity (a_w) values of cellulose substrate on the growth of a filamentous fungus *Penicillium roqueforti*. Four water activity values (0.94, 0.96, 0.97, 0.99) and four water content values: 0.4, 0.6, 0.8, 1 (g/g dry matter) have been tested in a cross experiment. The effect of water activity has been shown to be highly significant while the water content level did not significantly modify the development of the fungus. The effect of water activity was highly significant (0.1% level) both on estimated growth as well as on growth rate, as shown by diameter measurement; water content, however, had no significant effect. Thus it appeared clear that a decrease of a_w drastically decreased the development of the fungus, whatever the water content level.

The effect of butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), trihydroxybutyrophenone (THB) and propyl paraben (PP) antioxidants (at concentrations of 1, 10 and 20 mmol/L) at different water activities and pH values on germination, growth and aflatoxin B₁ production by *Aspergillus flavus* was evaluated by Nesci *et al.* [26]. Their studies on the percentage of spore germination, elongation rate, growth rate and aflatoxin B₁ production were carried out *in vitro* in relation to water activity (a_w) at 0.982, 0.937, 0.809 and 0.747 values. At 0.809 and 0.747 (a_w) values, none of the isolates was able to germinate. Overall, propyl paraben and butylated hydroxyanisole were the antioxidants most effective at inhibiting germination of both species. In the presence of the lowest concentration of butylated hydroxyanisole and propyl paraben (1mmol/L) the conidial germination percentage ranged from 2 to 19% after 15 hours of incubation at the highest water activity tested. Butylated hydroxyanisole and propyl paraben at 10-20 mmol/L completely inhibited conidial germination. The antioxidants more efficient in controlling *Aspergillus* elongation rate were propyl paraben, butylated hydroxyanisole, butylated hydroxytoluene. All strains were much more sensitive to all antioxidants tested on the percentage of spore germination and growth rate at 0.937 (a_w). The antioxidants propyl paraben and butylated hydroxyanisole completely inhibited aflatoxin B₁ production by all strains when added at 1mmol/L.

Decreased aflatoxin B₁ levels, were observed with butylated hydroxyanisole at 1, 10 and 20 mmol/L with the strain T20 at 0.982 (a_w). In contrast, stimulation was observed with the antioxidant trihydroxybutyrophenone at 10 and 20 mmol/L at 0.937 (a_w) with the strains T20 and

T23. The effect of butylated hydroxyanisole and propyl paraben at 1mmol/L on lag phase and growth rate was maintained in the pH range between 6 and 8. At all pH values, the inhibitory effect of butylated hydroxyanisole was higher than propyl paraben. No aflatoxin B₁ was detected at all pH values. Their conclusion was butylated hydroxyanisole and propyl paraben could be considered as effective fungitoxicants for *Aspergillus flavus* and *Aspergillus parasiticus* [26].

Redox potential

Microbial growth in an environment is influenced by its oxidation-reduction balance as they require compatible terminal electron acceptor for their respiratory pathways to function. The redox potential in viscous emulsions may be high due to the appreciable content of oxygen in fats and oils.

Storage temperature

Spoilage of pharmaceuticals may occur potentially over the range of 20°C to 60°C. Reconstituted syrup and multidose eye drop packs are instructed to store at 8-12°C, to reduce the risk of growth inadvertently introduced during use. Water for injection should be held at 80°C after distillation and before packing and sterilization to prevent possible growth of gram negative bacteria.

Effect of temperature on microbial growth and toxins production

Spoilage of pharmaceutical products could occur over a temperature ranging from 20 to 60°C, although it is generally low 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 [19, 20].

Schindler *et al.* [21] studied the production of two aflatoxins isolated from *Aspergillus flavus* grown for 5 days on work media at 2, 7, 13, 18, 24, 29, 35, 41, 46, and 52°C. They found that maximal growth for the two isolates occurred at 29 and 35°C and the optimal temperature for growth of both *Aspergillus flavus* isolates was higher than optimal temperatures for aflatoxin production. The ratio of the production of aflatoxin B₁ to aflatoxin G₁ varied with temperature. Aflatoxin production was not related to growth rate of *Aspergillus flavus* aflatoxin. B₁ was produced at 35°C. They also found that aflatoxin G₁ is produced at 18°C; production starts between 7 and 13°C, and is maximal at 24°C. Most of these differences in results can probably be explained by the variability of isolates.

Sorenson *et al.* [22] determined the effect of temperature on aflatoxin production by *Aspergillus flavus* when grown on a cereal grain substrate. They found that the optimum temperature for production of both aflatoxins B₁ and G₁ under the conditions employed was 28°C. Comparable yields of aflatoxin B₁ were obtained at 32°C, but considerably less G₁ was produced at this temperature. Both B₁ and G₁ were found in lesser amounts at temperatures above 32°C, and the aflatoxin content of rice incubated at 37°C was low (300-700 ppb) even though growth was good. Reducing the temperature from 28 to 15°C resulted in progressively less aflatoxin, but 100 ppb of B₁ was detected in cultures incubated 3 weeks at 11°C. No aflatoxin was produced at 8°C. The ratio of the four aflatoxins was affected by temperature. At the lower temperatures, essentially equal amounts of aflatoxin B₁ and G₁ were produced, whereas at 28°C, approximately four times as much B₁ was detected as G₁. At the higher temperatures, relatively less G₁ was formed, until at 37°C, less than 10 ppb was detected.

The time required for devitalization of *Penicillium glabrum* and *Aspergillus niger* germs (as an example of *Aspergillus* and *Penicillium spp.*) and other microorganisms at different temperatures was studied by Laciaková *et al.* [23] at temperatures of 22, 37, 60°C. All of the tested strains withstood the temperature 22°C during the period of 42 days in Sabouraud agar. At the temperature of 37°C the devitalization of *Penicillium glabrum* occurred after 21 days and *Aspergillus niger* devitalization occurred after 35 days. Temperature of 60°C devitalized all tested strains of microorganisms within 5 hours.

Rabie and Smalley [24] also demonstrated that the optimal temperatures for growth of *Aspergillus flavus* did not coincide with those for aflatoxins production, and that maximal production of aflatoxin B₁ occurred at 24°C, however, they found that maximal growth occurred at 18 °C and optimal temperature for growth was lower than optimal temperature for aflatoxin production. No aflatoxin B₁ was produced at 36°C. They also found that aflatoxin G₁ is not produced at 18°C; production starts between 18 and 24°C and is at a maximum at 30°C.

Effect of temperature and water activity (a_w)

The water relations of three isolates representative of each of the closely related species *Aspergillus flavus*, *Aspergillus nomius*, *Aspergillus oryzae* and *Aspergillus parasiticus* were examined by Pitt and Miscamble [29] at three temperatures, 25°C, 30°C and 37°C. Media were prepared over a wide range of water activity (a_w) from 0.996 to 0.75, controlled by a mixture of glucose and fructose. Water relations of *Aspergillus flavus*,

Aspergillus oryzae and *Aspergillus parasiticus* were very similar. The minimum (a_w) for germination and growth of each of these three species was 0.82 at 25°C, 0.81 at 30°C and 0.80 at 37°C. *Aspergillus nomius* was slightly less xerophilic, with minimum (a_w) values for germination and growth of 0.83 at 25 and 30°C, and 0.81 at 37°C. Reported differences in water relations between *Aspergillus flavus* and *Aspergillus parasiticus* were not substantiated. The "domestication" of *Aspergillus oryzae* has not affected its water relations.

Marin *et al.* [18] compared the effect of temperature (5–45°C), water availability (water activity values 0.95–0.75) and their interactions on the temporal rates of germination and mycelial growth of mycotoxigenic strains of *Aspergillus ochraceus*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium aurantiogriseum* and *Penicillium hordei*, *in vitro* on a maize extract medium. Germination was very rapid at >0.90 (a_w) with an almost linear increase with time for all species. However, at <0.90 (a_w), the germination rates of *Aspergillus flavus* and *Penicillium hordei* were slower. The minimal (a_w) for germination were usually lower than for growth and varied with temperature. The effect of water activity and temperature interactions on the lag phases (h), prior to germination, and on the germination rates (h^{-1}), were predicted for the first time for these fungi using the Gompertz model modified by Zwietering. This showed that *Aspergillus flavus*, *Aspergillus niger* and the two *Penicillium* strains had very short lag times between 0.995–0.95 (a_w) over a wide temperature range. At marginal temperatures, these were significantly higher, especially at $<10^\circ\text{C}$ for *Aspergillus spp.* and $>30^\circ\text{C}$ for *Penicillium spp.* There were also statistically significant differences between lag phases and germination rates for three different isolates of *Aspergillus ochraceus*. The *Aspergillus spp.* also germinated faster than the *Penicillium spp.* The temperature water activity profiles for mycelial growth varied considerably between species, both in terms of rates (mm/d) and tolerances.

The influence of temperature (T) and water activity (a_w) on the growth rate of seven moulds (*Aspergillus flavus*, *Penicillium chrysogenum*, *Alternaria alternata*, *Cladosporium cladosporioides*, *Mucor racemosus*, *Rhizopus oryzae* and *Trichoderma harsianum*) was assessed by Sautour *et al.* [31] in suboptimal conditions, confirming that water activity has a greater influence than temperature on fungal development. At 0.99 (a_w), it appeared that the optimal growth rate for *Aspergillus flavus* but *Penicillium chrysogenum* growth remained low, ranging from 3 to 6 mm/day. The differences in the optimal growth rate observed between the moulds can be attributed to the nature of the microorganism; the optimum temperature for

growth was about 25°C with the exception of *Aspergillus flavus* and *Rhizopus oryzae*, which exhibited values of 31 and 35°C, respectively. The genus *Aspergillus* is typical, growing readily at temperatures between 15 and 40°C [30]. By decreasing (a_w) from 0.99 to 0.97 at 31°C, the optimum growth rate for *Aspergillus flavus* was increased from 5.7 to 9.7 mm/day. *Penicillium chrysogenum* grew faster at 0.985 (a_w) than at 0.99, suggesting that these moulds required less humidity to grow at the optimum rate.

Abellana, Sanchis and Ramos [32] compared the effect of temperature and water activity and their interactions on the rate of mycelial growth of *Penicillium aurantiogriseum*, *Penicillium chrysogenum*, *Penicillium corylophilum* and *Aspergillus flavus* on a sponge cake analogue. As expected, growth rates showed dependence on (a_w), and temperature. However, no significant differences were observed in the growth rates of different isolates. The minimum (a_w) values for growth of the *Penicillium spp.* was 0.85-0.90. *Aspergillus flavus* was able to grow at 0.90 (a_w) when the temperature was above 15°C.

Plaza *et al.* [33] compared the effect of temperature (4–37°C) and water activity (0.99–0.87) and their interactions on the germination rates, lag times prior to germination and mycelial growth (*in vitro*) of *Penicillium digitatum*, *Penicillium italicum* and *Geotrichum candidum*. Germination and growth were markedly influenced by temperature and (a_w). Generally, lag times were longer and germination and growth rates were slower when conditions of temperature and (a_w) were far from optimum. All the studied species were able to germinate over a range of 4–30°C at 0.995 (a_w), although in non-optimal conditions *Penicillium digitatum* only reached 40–60% of germinated conidia. At low temperatures, *Penicillium italicum* germinated and grew faster than *Penicillium digitatum* and *Geotrichum candidum*, particularly at 0.95 (a_w). *Penicillium italicum* was also able to germinate and grow in the driest studied conditions (0.87 (a_w) value).

Lahlali *et al.* [34] evaluated the effect of water activity (a_w) 0.98–0.89, adjusted with glycerol, sorbitol, glucose, or NaCl and temperature (5–25°C) on the lag phase and radial growth rate (mm/day) of the important citrus spoilage fungi, such as *Penicillium italicum* and *Penicillium digitatum* grown in potato dextrose agar (PDA) medium. Highly significant effect of (a_w), temperature, solutes and their interactions on radial growth rate. Radial growth rate was inhibited and the lag phase (i.e. the time required for growth) lengthened as the water activity of the medium decreased. NaCl appeared to cause the greatest stress on growth when compared with other non-ionic solutes. *Penicillium italicum* stopped growing at 0.96 (a_w) and *Penicillium digitatum* at 0.93 (a_w). Under the dry conditions where growth

was observed, *Penicillium italicum* grew faster than *Penicillium digitatum* at low temperature and *Penicillium digitatum* remained more active at ambient temperature.

Rashmi *et al.* [35] studied the growth of *Aspergillus flavus* was observed on selected ten medicinal herbs with water activity (a_w) above 0.81 when stored at $25 \pm 2^\circ\text{C}$, $30 \pm 2^\circ\text{C}$ and $40 \pm 2^\circ\text{C}$ except for *Picrorhiza kurrooa* and *Alpinia galanga* which were found to have anti-fungal properties. *Aspergillus flavus* did not grow in any samples of medicinal herbs with water activity (a_w) below 0.81 at temperatures of $25 \pm 2^\circ\text{C}$, $30 \pm 2^\circ\text{C}$ and $40 \pm 2^\circ\text{C}$. Also *Aspergillus flavus* did not grow in any samples of medicinal herbs with water activity (a_w) above 0.81 when stored below $10 \pm 2^\circ\text{C}$. Therefore it can be concluded that the contamination of medicinal herbs with aflatoxins can be minimized by controlling water activity and storage temperature. Sorption isotherms (desorption) can be interpreted to determine the optimum drying which can lower the water activity to the level required for preventing growth of *Aspergillus flavus* and also for ensuring quality of medicinal herbs which may get destroyed upon over drying. Furthermore, it also saves incremental cost in prolonged drying over the optimum drying.

Nceba *et al.* [36] studied the effects of and interactions among temperature, water activity (a_w), incubation period, and substrate on coproduction of aflatoxins and cyclopiazonic acid by an isolate of *Aspergillus flavus*. Analysis of variance showed that there was a complex interaction among all of these factors and that this influenced the relative concentrations of the mycotoxins produced. The optimum temperatures for the production of aflatoxins and cyclopiazonic acid were 30°C and 25°C , respectively. Both mycotoxins were maximally produced (0.306 to 0.330 μg of aflatoxins / mL of medium, 4.040 to 6.256 μg of cyclopiazonic acid / mL of medium) at (a_w) of 0.996 and after 15 days of incubation. No aflatoxin were produced in either yeast extract agar or Czapek yeast autolysate agar medium at a water activity of 0.90 at 20 or 37°C after 15 days (minimum conditions), while 0.077 to 0.439 μg of cyclopiazonic acid / mL of medium was produced under the same conditions. Yeast extract agar favored maximum aflatoxin production, and Czapek yeast autolysate agar favored maximum cyclopiazonic acid production.

pH

Extremes of pH prevent microbial attack. At pH values above 8 (e.g. soap based emulsions) spoilage is rare. In products with low pH levels, e.g. fruit juice flavored syrups with a pH value between 3-4, mould or yeast

attack is more likely. Yeast can metabolize organic acids and raise the pH, and secondary bacterial growth can occur.

Effect of pH on microbial growth and toxins production

Kathryn *et al.* [27] determined the effect of pH on the growth rates of 61 isolates belonging to 13 important toxigenic fungi derived from *Aspergillus spp.*, *Fusarium spp.*, *Penicillium spp.*, over the pH range 2 to 11 at 25, 30 and 37°C. Nearly all species studied were able to grow over the entire range examined on a laboratory agar medium. However, in general, *Aspergillus spp.* was more tolerant on alkaline pH while *Penicillium spp.* appeared to be more tolerant on acidic pH.

Joffe and Lisker [28] determined the effect of pH and light on aflatoxins production of *Aspergillus flavus*. Results clearly showed that Czapek's medium produced 26 to 83 times more of the toxin when initially adjusted to a pH value of 4, than at the initial pH of 7.4. The effect of light however, was most striking. The data showed that light was deleterious for the aflatoxin formation, since in the complete absence of light increased fivefold the production of toxin (from 35,000 to 178,000 pg/g).

Combined effects of water activity (a_w), pH and antimicrobial agent on microbial growth

The combined effects of water activity (0.99 or 0.95), pH (4.5 or 3.5) and antimicrobial agent (potassium sorbate, sodium benzoate, sodium bisulfate, carvacrol, citral, eugenol, thymol, or vanillin) concentration (0, 100, 200 up to 1800 ppm) on the growth of *Aspergillus flavus* were evaluated by López-Malo *et al.* [37] in potato dextrose agar (PDA). Mold spore germination time and radial growth rates (RGR) were significantly ($p < 0.05$) affected by the variables. For equal antimicrobial concentration, reduction in pH or (a_w) had important effects, lowering RGR and delaying germination time. Depending on (a_w) and pH, an increase in antimicrobial concentration slightly reduced RGR until a critical concentration where RGR was drastically reduced or mold growth was inhibited. Germination time increased as antimicrobial agent concentration increased and when (a_w) and pH decreased. Important antimicrobial differences were observed, being, in general, the natural antimicrobials less pH-dependent than chemical preservatives. *Aspergillus flavus* exhibited higher sensitivity to thymol, eugenol, carvacrol, potassium sorbate, sodium bisulfate, and sodium benzoate (at pH 3.5) than to vanillin or citral.

Conidial germination of *Penicillium chrysogenum* was carried out by Sautour *et al.* [38] under operating conditions compatible with a pastries manufacturing process. A range, limited by two experimental values, was

defined for each environmental factor tested: temperature (15 or 25°C), water activity (0.75 or 0.85) and pH (3.5 or 5.5). A closed device was made, which maintained equilibrium between water activity of the culture medium and atmospheric relative humidity during 25 days, to follow spore germination. The combined effects of temperature, water activity and pH on spore germination were studied by applying factorial design methodology.

Higher rates of spore germination were associated with a high level of water activity (a_w). The incubation temperature also had a positive effect while pH did not have a significant effect on conidial germination. They said that at low temperature (15°C), when the water activity increased (from 0.75 to 0.85), the rate of germination increases 10-fold. This observation was confirmed at the higher temperature (25°C). Likewise, the effect of temperature on spore germination was more pronounced at the higher (a_w) value (0.85) than at 0.75. Under these particular experimental conditions, pH showed no significant effect on conidial germination after 25 days [38].

Holmquist, Walker and Stahr [39] studied the effect of temperature, pH, water activity, and nine antifungal agents on growth of *Aspergillus flavus* and *Aspergillus parasiticus*, determined on Sabouraud Dextrose Agar and on corn. Maximal growth of the two molds occurred at 33°C, the highest temperature used, pH of 5.0 and (a_w) of 0.99. At 15°C, growth was observed at (a_w) of 0.95 but not 0.90. Slight growth was observed at (a_w), of 0.85 at 27°C and 33°C. Nine antifungal agents (Botran, Orthocide, Poly-ram 80, Topsin-M, Thiram, Imazalil, sodium propionate, sodium sulfite and DDVP - 2,2dichlorovinyl dimethyl phosphate) were tested for inhibition of growth. Activity of the antifungal increased as (a_w) was decreased. All antifungal showed inhibitory activity, but Imazalil and DDVP were the most effective agents at the lowest concentrations.

Packaging design

Packaging can have a major influence on microbial stability of some formulations in controlling the entry of contaminants during both storage and use. Self-sealing rubber wads must be used to prevent microbial entry into multidose injection containers. Wide mouthed cream jars have now been replaced by narrow nozzles and flexible screw capped tubes.

Survival of microorganisms within pharmaceutical products

The survival of microorganism in particular environments is sometimes influenced by the presence of relatively inert materials. Thus, microbes can be more resistant to heat or desiccation in the presence of starch, acacia or gelatin.

Effect of the compaction process

Raw materials used for tablet production were assessed by Ibrahim and Olurinola [40] for their microbiological quality. Tablets were produced by wet granulation and direct compression methods and assessed for compliance with the British Pharmacopoeia specifications. The effect of the microbial levels of the raw materials and the formulation technology on the microbial levels of the produced tablets were also investigated. Results indicated that the microbial levels of the tablets were influenced by the microbial quality of the starting raw materials, the production environment and the method of production. Generally, tablets produced by direct compression method gave lower microbial levels than those of the wet granulation method. The compaction process exerts lethal effect on the survival of microorganisms.

The lethal effect of the compaction process on *Aspergillus niger* present in direct compression materials has been also studied by Plumpton *et al.* [41]. Results showed that low pressures have no such effect on *Aspergillus niger*. Higher pressures caused destroying of both microorganisms, the extent of killing being determined by the relative size of the excipient and the organism. The results indicate that the lethal effect of compression is due to shearing forces caused by inter-particulate movement.

Conclusions

Each pharmaceutical product should be submitted to predictive microbiology studies on the behavior of microorganisms under different physical, physicochemical or chemical conditions such as temperature, water activity, pH, or antimicrobial compounds. This can help the identification of critical points of the production and distribution process, and the optimization of production and distribution chains [17], also studying their interactions on lag times to germination, germination rates and mycelial growth is important in the development of hurdle technology approaches to predicting fungal spoilage in agricultural and food products [18].

References

1. Danyer S. and Baird R., Guide to Microbiological Control in Pharmaceuticals, Chichester, Ellis Horwood. 1990, 29–52.
2. Ifeyinwa F. Obuekwe and Florence Eichie, -The presence of microorganisms in some common excipients used in tablet formulation, *Acta Poloniae Pharmaceutica, Drug Research*, Vol. 63. 2006, 2, 124.

3. Mugoyela Veronica and Mwambete D. Kennedy -Microbial contamination of nonsterile pharmaceuticals in public hospital settings, *Therapeutics and Clinical Risk Management, Dove Press Journal*, 2010, 6, 447.
4. Veber B., Gachot B., Bedos J., Wolff M. -Severe sepsis after intravenous injection of contaminated propofol, *Anesthesiology*, 1994, 80, 712.
5. Bennett SN., McNeil MM., Bland LA., Arduino MJ., Villarino ME., Perrotta DM., Burwen DR., Welbel SF., Pegues DA., Stroud L., Zeitz P., Jarvis W., Postoperative infections traced to contamination of an intravenous anesthetic, propofol, *N. Eng. J. Med.* 1995, 333, 147–154.
6. Hugo W.B., Russell A.D., *Pharmaceutical Microbiology*, Edition 5, London, Blackwell Scientific. 1992, 369–390.
7. Obuekwe I.F., Ogbimi A.O., Obuekwe C.O., Effect of some bacterial species on the disintegration properties of four commercial brands of acetyl salicylic acid tablets, *Biomed Lett*, 1996, 54, 199–205.
8. Mwambete K.D., Justin-Temu M., Fazleabbas S.F., Microbiological assessment of commercially available quinine syrups and water for injections in Dar es Salaam, Tanzania, *Tropical J. Pharm. Res.* 2009, 8, 5, 441–447.
9. Kallings L.O., Ringertz O., Silverstolpe L., Ernerfeldt F., Microbiological contamination of medical preparations, *Acta Pharm. Suecica*. 1966, 3, 219–228.
10. Lennington K.R., Salmonella in drugs and dietary supplements, *Drug Cosmetic Ind.* 1967, 100, 42–43.
11. Roy A.K., Sinha K.K., Chourasia H.K., Aflatoxins contamination of some drug plants, *Appl. Environ. Microbiol.* 1988, 54, 842–843.
12. Tanaka T., Hasegawa A., Yamamoto S., Aoki N., Toyazaki N., Matsuda Y., Udagawa S., Sekita S., Narita N., Suzuki M., Harada M., Natural occurrence of aflatoxins in traditional herbal drugs from Indonesia, *Proc. Jpn. Assoc. Mycotoxicol.* 1988, 28, 33–35.
13. Food and Drug Administration, Guidance for industry: Manufacture, processing or holding of Active Pharmaceutical Ingredients, 1996.
14. Denyer S., Baird R., Guide to Microbiological Control in Pharmaceuticals, 1990, 53–56.
15. Hugo W.B., Russell A.D., *Pharmaceutical Microbiology*, Edition 6. London, Blackwell Scientific. 1998, 355–376.
16. Aulton M.E. -Pharmaceutics: The science of dosage from design, Churchill Livingstone. 2000, 479–508.
17. Zwietering M.H., Jongenburger I., Rombouts F.M., Riet K. -Modeling of the bacterial growth curve, *Appl. Environ. Microbiol.* 1990, 56, 1875–1881.
18. Marin S., Sanchis V., Saenz R., Ramos A.J., Vinas I. and Magan N. -Ecological determinants for germination and growth of some *Aspergillus* and *Penicillium spp.* from maize grain, *Journal of Applied Microbiology*. 1998. 84, 25–36.
19. Bhagavan N.H., Wolkoff B.I., Correlation between the disintegration time and the bioavailability of vitamin C tablets. *Pharm Res*, 1993, 10, 239–242.
20. Esezobo S., The effect of some excipients on the physical properties of a paracetamol tablet formulation, *J Pharm Pharmacol*, 1985 37(3), 193–5.
21. Schindler A.F., John G. Palmer, Eisenberg W.V., Aflatoxin production by *Aspergillus flavus* as related to various temperatures, *Applied Microbiology*. Vol. 15. 1967, 5, 1006–1009.
22. Sorenson W.G., Hesseltine C.W., Odett Shotwell, Effect of temperature on production of aflatoxin on rice by *Aspergillus flavus*, Northern Regional Research Laboratory, Peoria, Illinois 6160. 1966, 20, 49–55.
23. Laciaková A., Kocisová A., Kukucka D., Survival of microscopic filamentous fungi under various temperature conditions, *Vet. Med. (Praha)*. 1995, 40, 7, 233–235.
24. Rabie C.J., Smalley E.B., Influence of temperature on the production of aflatoxin, Symposium on mycotoxins in foodstuffs, Agricultural Aspects. Pretoria, *Dept. Agric. Tech. Serv.* 1965, 2, 18–29.

25. Gervais P., Bensoussan M., Grajek W., Water activity and water content: comparative effects on the growth of *Penicillium roqueforti* on solid substrate, *Applied Microbiology Biotechnology*. 1988, 27, 389-392.
26. Nesci A., Rodriguez M., Etcheverry M., Control of *Aspergillus* growth and aflatoxin production using antioxidants at different conditions of water activity and pH, *Journal of Applied Microbiology*, 2003, 95, 279-287.
27. Kathryn A. Wheeler, Beverly F. Hurdman, Pitt J.I., Influence of pH on the growth of some toxigenic species of *Aspergillus*, *Penicillium* and *Fusarium*, *International Journal of Food Microbiology*, Volume 12. 1991, 2-3, 141-149.
28. Joffe A.Z., Lisker N., Effects of Light, Temperature, and pH Value on Aflatoxin Production *in vitro*, *Applied Microbiology*, Vol. 18. 1969, 3, 517-518.
29. Pitt John I. Miscamble Beverly F. Water Relations of *Aspergillus flavus* and Closely Related Specie, *Journal of Food Protection*, Volume 58. 1995, 1, 5, 86-90.
30. Smith J.E., Physiology of *Aspergillus*. In: *Aspergillus*. Plenum, New York. 1994, 23- 39.
31. Sautour M., Soares C. Mansur, Divies C., Bensoussan M. and Dantigny P., Comparison of the effects of temperature and water activity on growth rate of food spoilage moulds- *Journal of Industrial Microbiology and Biotechnology*. 2008, 28, 311-315.
32. Abellana M., Sanchis V., Ramos A.J., Effect of water activity and temperature on growth of three *Penicillium* species and *Aspergillus flavus* on a sponge cake analogue, *International Journal of Food Microbiology*, Volume 71. 2001, 2-3, 151-157, 30.
33. Plaza P., Usall J., Teixido N. and Vinas I., Effect of water activity and temperature on germination and growth of *Penicillium digitatum*, *Penicillium italicum* and *Geotrichum candidum*, *Journal of Applied Microbiology*, 2003, 94, 549-554.
34. Lahlali R., Serrhini M.N., Friel D. Jijakli M.H., *In vitro* effects of water activity, temperature and solutes on the growth rate of *Penicillium italicum* Wehmer and *Penicillium digitatum* Sacc., *J. Appl. Microbiol*, 2006, 101, 3, 628-36.
35. Kulshrestha R., Gupta C.P., Shukla G., Kundu M.G., Bhatnagar P.S., Katiyar C.K., The effect of water activity and storage temperature on the growth of *Aspergillus flavus* in medicinal herbs, *Planta Med*. 2008, 74, 10, 1308-1315.
36. Gqaleni N., Smith J.E., Lacey J., Gettinby G. Effects of temperature, water activity, and incubation time on production of aflatoxins and cyclopiazonic acid by an isolate of *Aspergillus flavus* in surface agar culture, *Applied and Environmental Microbiology*. Vol. 63. 1997, 3, 1048-1053.
37. López-Malo A., Maris Alzamora S., Palou E., *Aspergillus flavus* growth in the presence of chemical preservatives and naturally occurring antimicrobial compounds, *Int. J. Food Microbial*. 2005, 99, 2, 119-128.
38. Sautour M., Rouget A., Dantigny P., Divies C., Bensoussan M., Prediction of conidial germination of *Penicillium chrysogenum* as influenced by temperature, water activity and pH, *Letters in Applied Microbiology*. 2001, 32, 131-134.
39. Holmquist G.U., Walker H.W., Stahr H.M., Influence of temperature, pH, water Activity and antifungal agents on growth of *Aspergillus flavus* and *Aspergillus parasiticus*, *Journal of Food Science*, Volume 48. 1983, 3, 778-782.
40. Ibrahim Y.K., Olurinola P.F., Comparative microbial contamination levels in wet granulation and direct compression methods of tablet production, *Pharm Acta Helv*. 1991, 66, 11, 298-301.
41. Plumpton J.E., Gilbert P., Fe J.T., The survival of microorganisms during tableting, *International Journal of Pharmaceutics* Volume 30. 1986, 2-3, 241-246.