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Microbiological stability of tablets stored under tropical conditions

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Summary

The effect of storage under tropical conditions on the behaviour of microbial contamination of tablets was studied. The investigation of the microbiological quality of the starting materials showed that rice and tapioca starch had a higher level of natural contamination than potato starch. Lactose/potato starch tablets without preservatives, inoculated with *Aspergillus niger* spores, spoiled due to mould growth when stored under tropical conditions (31° C and 95% RH). Under these conditions tablets prepared with lactose and rice or tapioca starch spoiled due to growth of natural contaminants. No growth of bacterial cells (*Bacillus brevis*) was observed during storage under these conditions. When the tablets were stored under more moderate conditions (31° C, 75% RH) they were not at risk of microbiological spoilage. Sodium methylhydroxybenzoate and potassium sorbate were evaluated for their efficacy against microbiological spoilage of tablets. A concentration of 1% w/w of either preservative prevented growth of *Aspergillus niger* on lactose/potato starch tablets stored at 31° and 95% relative humidity. A 0.1% w/w concentration level of preservative was not as effective. Adding a preservative to lactose/potato starch tablets contaminated with *Bacillus brevis* spores did not affect the viability of these bacterial spores. The addition of preservatives to tablets prepared with lactose and rice or tapioca starch and stored under tropical conditions prevented microbiological spoilage caused by growth of natural contaminants.

Introduction

The microbiological quality at the moment of administration of non-sterile pharmaceutical preparations such as tablets is largely determined by 3 factors:
(i) the microbial contamination of the raw materials;
(ii) the effect of the manufacturing process on the microorganisms; and
(iii) the fate of the contaminating micro-organisms during storage.

The predominant factor is probably the bioburden of the raw materials used, both active drug and excipient (Devleeschouwer and Dony 1979; Khante et al., 1979; Somerville, 1981). Several infection outbreaks which could be traced back to the use of heavily contaminated raw materials of natural origin have been reported (Kallings et al., 1966; Komarmy et al., 1967). During the manufacturing of tablets the viability of microbial cells can be significantly affected by the drying process of granulates (Parker, 1984) and by the actual compaction (Fassihi and Parker, 1987; Plumpton et al., 1986).

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Despite the fact that suitable substrates such as starch and lactose are abundantly present in tablets, microbial growth has rarely been observed. The availability of water probably plays an important role. As long as tablets are stored under dry conditions, spoilage due to growth of micro-organisms is unlikely to occur (Blair et al., 1988). However, in tropical regions with a hot and humid climate, growth of contaminating micro-organisms cannot be excluded. In such regions the average kinetic temperature is 31 °C and the average relative humidity (RH) is 75%, with maxima up to 100% during the rainy season (Grimm, 1986). Moreover, in these countries pharmaceutical preparations are frequently stored under uncontrolled conditions and may be dispended in non-protective packaging or even without any packaging at all.

Few studies that investigate the effect of storage on the microbiological stability of tablets have been published (Blair et al., 1987); Fassihi and Parker, 1977; Waterman et al., 1973). In none of these studies are the effects of tropical storage conditions (high temperature and high relative humidity) investigated nor are the storage conditions controlled for relative humidity (Fassihi et al., 1978). Parker (1984) suggests that the addition of preservatives to tablets could be effective, but he does not give any experimental evidence.

The aim of the study described in this paper was to investigate the effect of storage under carefully controlled conditions of temperature and relative humidity on the growth of contaminating micro-organisms in tablets. Also the rationale of the addition of a preservative to tablets was investigated.

The effectiveness of sodium methylhydroxybenzoate (Na-MOB) and potassium sorbate (K-sorbate) in lactose/potato starch tablets was assessed by microbial challenge tests, using a mould (*Aspergillus niger*) and a spore-forming bacterium (*Bacillus brevis*), both isolated from starch. The inoculated tablets were stored at 31 °C and 75% RH or at 31 °C and 95% RH. The total viable count of the tablets after storage was compared with the count directly after inoculation. Finally the effectiveness of preservatives in tablets prepared with lactose and rice or tapioca starch was assessed.

Materials and Methods

Materials

The tablet ingredients used were: lactose monohydrate 200 mesh (D.M.V., Veghel, The Netherlands), potato starch Ph. Eur. grade (Avebe, Foxhol, The Netherlands), rice starch Ph. Eur. grade (Chemiefarma, Maarssen, The Netherlands) and tapioca starch (P.T. Delta Mulya Chemical Industries, Cimindi, Indonesia). Magnesium stearate (Centrachemie, Etten-Leur, The Netherlands) was used as a lubricant. Prior to use, magnesium stearate was sieved through a 150 μm sieve.

The used preservatives were sodium methylhydroxybenzoate (Na-MOB) B.P. grade (Chemiefarma, Maarssen, The Netherlands) and potassium sorbate (K-sorbate) N.F. grade (O.P.G., Utrecht, The Netherlands).

All the other materials used were of analytical quality.

The micro-organisms used were *Aspergillus niger* van Tieghem (a spoilage isolate from rice starch) and *Bacillus brevis* (a spoilage isolate from tapioca starch).

Preparation of spore suspension

Aspergillus niger (A. niger) was grown on Trypton Soya Agar (Oxoid, Basingstoke, U.K.) at 30 °C. After 5 days the spores were harvested by washing the plates with a 0.1% polysorbate 80 solution. The spore suspension was diluted to contain: 5×10^7 spores/ml. *Bacillus brevis (B. brevis)* was grown on Lab Lemco Agar (Oxoid, Basingstoke, U.K.), containing 0.88 mg/l manganese chloride, at 30 °C. After 7 days the spores were harvested by washing the plates with a 0.1% polysorbate 80 solution. The spore suspension was diluted to contain: 5×10^7 spores/ml.

Tablet preparation

Tablets with 0.1% and 1% w/w preservative and control tablets without preservative were prepared. The composition of the tablets is given in

TABLE 1

Tablet composition

	Control tablets (mg)	0.1% w/w preservative (mg)	1% w/w preservative (mg)
Lactose monohydrate	245	245	242.5
Starch [a]	245	245	242.5
Preservative [b]	–	0.5	5
5% starch [a] paste	163	163	163
Sterilized water	q.s.	q.s.	q.s.
Magnesium stearate	2.5	2.5	2.5

[a] Potato starch, rice starch or tapioca starch.
[b] Sodium methylhydroxybenzoate or potassium sorbate.

Table 1. Lactose and starch were mixed in a planetary mixer (Kenwood model A710A, Hants, U.K.). The preservative was dissolved in a freshly prepared starch paste and slowly added to the lactose/starch mixture. Sufficient water was added to granulate and the mixing was continued for 15 min. The wet mass was passed through a 1000 μm screen. The granules were dried overnight at room temperature on a laminar flow bench. After drying, the granules were passed through a 1000 μm screen. Magnesium stearate was added and mixed for 2 min in a Turbula mixer (90 rpm, W.A. Bachofen, Basel, Switzerland). Tablets (500 mg) were prepared on a single-punch tabletting machine (HOKO, Rijswijk, The Netherlands), using 13 mm flat punches (compression load: 75 MPa). During preparation, care was taken not to contaminate the product. Prior to use all utensils were sterilized or disinfected.

Determination of microbiological quality of tablet ingredients

The used tablet ingredients were tested for microbial contamination. The total viable aerobic count (plate count method) and the total viable count for moulds (plate count method) as well as the absence of *Escherichia coli* and *Staphylococcus aureus* were assessed as described in the European Pharmacopoeia Ed. II. The number of *Enterobacteriaceae* was assessed as described by van Doorne and Claushuis (1979). The selective enumeration of *Bacillus cereus* was performed on Bacillus cereus Selective Agar (Oxoid, Basingstoke, U.K.) as described by Mossel et al. (1967).

Microbial challenge to tablets

From each batch, tablets were placed in Petri dishes (5 tablets/dish). Each tablet was inoculated with 5 μl of spore suspension, of either *A. niger* or *B. brevis*, on the tablet surface.

Storage of tablets

From each batch, two dishes with tablets were stored at 31°C and 75% RH and two at 31°C and 95% RH in climate chambers (Heraus Vötsch, Balingen, F.R.G.). Lactose/potato starch tablets inoculated with *A. niger* were also stored at 31°C and 45% RH.

Estimation of total viable count of the tablets

After 1 and 4 weeks of storage, the tablets inoculated with *A. niger* were examined for visible mould growth. Before and directly after inoculation with *A. niger* or with *B. brevis* and after 1 and 4 weeks of storage the total viable count of each batch of tablets was estimated in 5-fold. Of the tablets prepared with lactose and rice or tapioca starch, the total viable count was estimated directly after tabletting and after storage for 1 and 4 weeks. The tablets were suspended in 9.5 ml of universal neutralization liquid (U.N.L., Table 2) and suitable serial dilutions in U.N.L. were made. 1 ml samples of each dilution were plated in duplicate in Trypton Soya Agar (Oxoid, Basingstoke, U.K.). Plates were incubated at 30°C for 40 h and the colonies were counted. The results were expressed as log N/N_o, where N and

TABLE 2

Composition of universal neutralization liquid

Soya lecithin	3.0 g
Polysorbate 80	30.0 g
Sodium thiosulfate pentahydrate	5.0 g
L-Histidine	1.0 g
Peptone	1.0 g
Sodium chloride	8.5 g
Disodium hydrogen phosphate dihydrate	4.5 g
Potassium dihydrogen phosphate	1.5 g
Purified water	to 1000 ml

N_o (colony forming units [c.f.u.]/tablet) are the total viable counts respectively after storage and directly after inoculation.

Adsorption of preservative onto granulate

Ten g of lactose/potato starch granulate was mixed with 100 ml preservative solution (0.4 mg/ml) and shaken for 72 h. The decrease in preservative concentration was measured spectrophotometrically, after filtration through a 0.45 μm filter. The absorption measurements were carried out on a Philips spectrophotometer (model PU 8720, Philips, Eindhoven, The Netherlands). The absorbance was measured at 258 nm, 254 nm and 259 nm for respectively benzalkonium chloride ($E_{1\%} = 8.43$), K-sorbate ($E_{1\%} = 1554$) and Na-MOB ($E_{1\%} = 670$).

Results and Discussion

Microbiological quality of the tablet ingredients

The results of the microbiological examinations are shown in Table 3. The investigated batches of rice and tapioca starch barely met the requirements of the European Pharmacopoeia Ed. I for preparations for oral use, which are: total viable aerobic count $\leq$ 1000–10,000 c.f.u./g and total viable count for fungi $\leq$ 100 c.f.u./g. The investigated batches of lactose monohydrate, potato starch and magnesium stearate met these requirements for preparations for oral use. None of the investigated materials were contaminated with the specific organisms as mentioned under Materials and Methods.

TABLE 4

Microbiological quality of tablets directly after compaction

Batch	c.f.u./tablet [a]	
	Expected	Measured
Potato starch/lactose	1.8×10^1	$< 10^1$
Rice starch/lactose	2.5×10^2	5.1×10^1
Tapioca starch/lactose	3.2×10^3	5.3×10^2

[a] Colony forming units/tablet.

Because of their satisfactory microbiological quality, potato starch and lactose were used without further treatment in the microbial challenge tests. Rice and tapioca starch were used to study the effect of storage on the viability of natural contamination. Earlier investigations had proven that these starches are suitable for use as excipient in tablets (Bos et al., 1987).

Microbiological quality of unpreserved tablets after compaction and storage

The total viable count of the control tablets, without preservatives, was lower than expected from the microbiological examinations of the raw materials (Table 4). This decrease in contamination level can be attributed to the drying process of the granules and to the compaction of the tablets. This is in accordance with the results found by Fassihi and Parker (1987), Parker (1984) and Plumpton et al. (1986).

The total viable counts of the uninoculated lactose/potato starch tablets, prepared without preservatives, measured directly after preparation and after 4 weeks of storage at 31° C and 75% RH or 95% RH were $< 10^1$ c.f.u./tablet.

TABLE 3

Microbiological quality of tablet ingredients

	Mg-stearate	Lactose	Potato starch	Rice starch	Tapioca starch
Total viable aerobic count [a]	$< 10^1$	$< 10^1$	3.5×10^1	5.0×10^2	6.3×10^3
Total viable count for fungi [a]	$< 10^1$	$< 10^1$	$< 10^1$	1.0×10^2	5.6×10^1
Enterobacteriaceae [a]	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$
Escherichia coli [a]	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$
Staphylococcus aureus	neg.	neg.	neg.	neg.	neg.
Bacillus cereus	neg.	neg.	neg.	neg.	neg.

[a] Colony forming units/g.

TABLE 5

Microbiological quality of unpreserved tablets after storage

Batch		time (weeks)	Storage condition		
			31°C 45% RH	31°C 75% RH	31°C 95% RH
Lactose/potato starch tablets inoculated with *A. niger*	N_o [a]	0	1.5×10^5	1.5×10^5	2.7×10^5
	log N/N_o [a] after storage	1	−0.8	−0.4	1.4
		4	−1.6	−0.7	2.8
Lactose/potato starch tablets inoculated with *B. brevis*	N_o [a]	0	n.d. [c]	2.5×10^5	2.2×10^5
	log N/N_o [a] after storage	1	n.d.	−0.2	−0.2
		4	n.d.	−0.1	−0.1
Lactose/tapioca starch tablets (uninoculated)	N_o [a]	0	n.d.	5.3×10^2	5.3×10^2
	log N/N_o [b] after storage	1	n.d.	−0.1	−0.1
		4	n.d.	−0.2	4.9
Lactose/rice starch tablets (uninoculated)	N_o [b]	0	n.d.	5.1×10^1	5.1×10^1
	log N/N_o [b] after storage	1	n.d.	0.0	−0.1
		4	n.d.	−0.1	5.9

[a] N_o = total viable count directly after inoculation; N = total viable count after storage.
[b] N_o = total viable count directly after tablet preparation; N = total viable count after storage.
[c] n.d. = not determined.

The behaviour of *A. niger* spores, inoculated onto potato starch/lactose tablets and after storage for 4 weeks at different relative humidities is shown in Table 5. When stored at relative humidity values up to 75% there is a slight decrease of viability of the spores and at high RH (95%) a significant increase in total viable count was observed. After 4 weeks of storage, visible growth of *A. niger* was seen and the total viable count had increased with almost 3 log cycles. These results were in good agreement with those obtained with uninoculated tablets prepared with lactose and either rice or tapioca starch (Table 5). When stored at 75% RH no change in the number of microorganisms was observed. However, when stored at 95% RH the total viable counts increased dramatically after 4 weeks of storage.

In order to test the possibility for bacterial growth under tropical conditions potato starch/lactose tablets were inoculated with spores of *B. brevis*. The results (Table 5) show that even when stored at the highest relative humidity there is no increase in the number of bacterial cells.

TABLE 6

Adsorption of preservative onto lactose/potato starch granulates

Preservative	mg preservative adsorbed onto 10 g granulate
Benzalkonium chloride	30
Na-MOB	0.9
K-sorbate	3.5

Microbiological quality of preserved tablets after storage

As the results so far had demonstrated that, after storage under tropical conditions, a dramatic loss of microbiological quality may occur within a relatively short period of time (4 weeks), the effect of the addition of preservatives was studied.

Preservatives used in pharmaceutical oral dosage forms are: alcohol, chlorhexidine, chloroform, organic acids (benzoic acid and sorbic acid), parabens and quaternary ammonium salts (benzalkonium chloride) (Parker, 1984). In this study the effectiveness of sodium methylhydroxybenzoate, a paraben, and potassium sorbate was investigated. Both preservatives are permitted world-wide as an additive in food. They also have

acquired the American Food and Drug Administration (F.D.A.)-status: 'Generally Regarded As Safe' (G.R.A.S.). Due to its volatility, alcohol and chloroform are not suitable for use in tablets; besides, chloroform has been proven to be nephro- and hepatotoxic. Benzoic acid was not included in this study because its spectrum of activity is almost identical to that of sorbic acid. Chlorhexidine is incompatible with starch and magnesium salts (McCarthy, 1969). It was seen that benzalkonium chloride was adsorbed substantially onto the lactose/potato starch granulation, whereas a slight adsorption occurred with Na-MOB and K-sorbate (Table 6).

Na-MOB and K-sorbate were incorporated into potato starch/lactose tablets at two concentration levels: 0.1% and 1.0% w/w. After inoculation with *A. niger* or *B. brevis* the tablets were stored at 31° C and 75% RH and at 31° C and 95% RH. Tablets with lactose and rice or tapioca starch were prepared with 1.0% w/w Na-MOB or K-sorbate. These tablets were also stored at 31° C and 75% RH and at 31° C and 95% RH.

Challenge test with Aspergillus niger

When stored at 31° C and 95% RH, tablets preserved with 0.1% Na-MOB or 0.1% K-sorbate showed no visible mould growth after 1 week of storage; but after 4 weeks slight visible mould growth was seen on both batches, whereas the control tablets showed strong visible growth even after 1 week of storage. Similar results were seen with the total viable counts: where mould growth was seen log $N/N_0 > 0$. No visible growth corresponded with log $N/N_0 < 0$ (Fig. 1). When stored at 75% RH and 31° C both the control tablets and the tablets prepared with 0.1% preservative showed no visible growth at all. The total viable counts after 1 and 4 weeks of these batches all showed a slight decrease: log $N/N_0 \leq 0$.

The results of the experiments with tablets prepared with 1% w/w preservative and stored at 31° C and 75% RH did not differ much as compared to tablets prepared with 0.1% preservative (Fig. 2). However, when these tablets were stored at 95% RH and 31° C for 1 week, the total viable count of the preserved tablets decreased to $< 10^1$

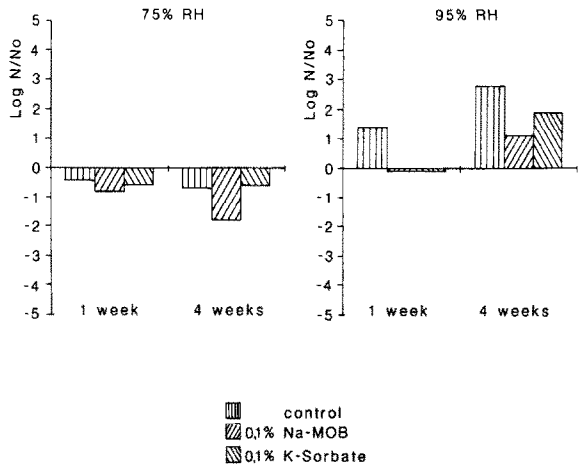

Fig. 1. Growth of *Aspergillus niger* on potato starch/ lactose tablets prepared with 0.1% w/w preservative, stored at 31° C and 75% RH or 95% RH.

c.f.u./tablet, whereas the control tablets showed visible growth: log $N/N_0 = 1.4$ (Fig. 2).

Under the circumstances of this study, the addition of preservatives to tablets that are stored at $\leq$ 75% RH and 31° C has no effect on the viability of mould spores. However, when the tablets are stored at high relative humidity (95%), the addition of preservatives does have a positive effect on the microbial quality of the product. Especially with the high preservative concentrations, the ef-

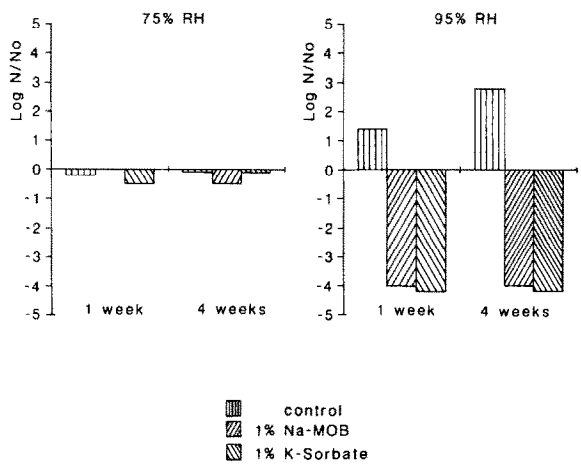

Fig. 2. Growth of *Aspergillus niger* on potato starch/lactose tablets prepared with 1.0% w/w preservative, stored at 31° C and 75% RH or 95% RH.

TABLE 7

Challenge test with Bacillus brevis

Batch	Storage conditions			
	75% RH, 31° C		95% RH, 31° C	
	1 week	4 weeks	1 week	4 weeks
	log N/N_0 [a]	log N/N_0 [a]	log N/N_0 [a]	log N/N_0 [a]
control	−0.2	−0.2	−0.1	−0.1
0.1% Na-MOB	0.0	0.0	−0.1	0.0
0.1% K-sorbate	−0.1	0.0	−0.2	−0.1
1% Na-MOB	−0.1	−0.1	0.0	−0.1
1% K-sorbate	0.0	−0.2	0.0	−0.1

[a] N = total viable count after storage; N_0 = total viable count directly after inoculation.

fect is dramatic: $< 10^1$ c.f.u./tablet after 1 week of storage under humid conditions.

Challenge test with Bacillus brevis

Preservation of tablets did not have any effect on the viability of *B. brevis* spores. The log N/N_0 for all batches varied between 0.0 and −0.2 (Table 7). Under the conditions of this study, the addition of a preservative has no effect on the viability of bacterial spores.

Microbiological stability of tablets containing rice or tapioca starch

When stored at 75% RH and 31° C for 4 weeks, preservation of tablets prepared with lactose and rice or tapioca starch had no effect on the micro-

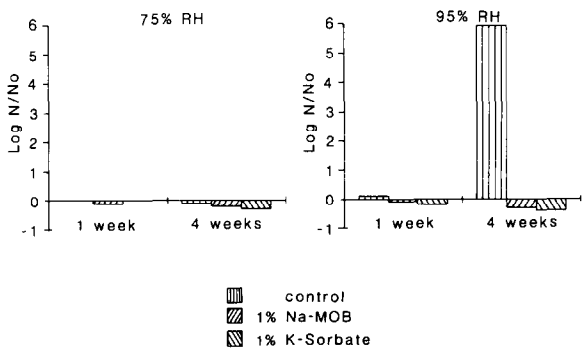

Fig. 3. Microbiological quality of rice starch/lactose tablets prepared with 1.0% w/w preservative, stored at 31° C and 75% RH or 95% RH.

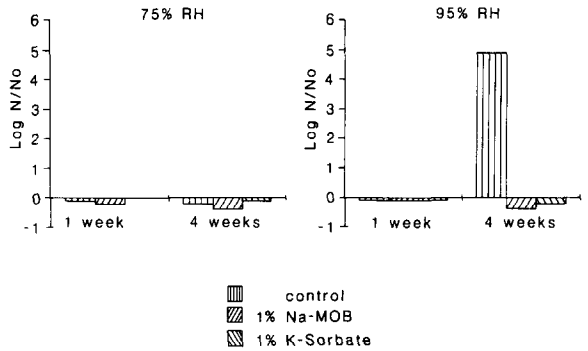

Fig. 4. Microbiological quality of tapioca starch/lactose tablets prepared with 1.0% w/w preservative, stored at 31° C and 75% RH or 95% RH.

biological quality (Figs. 3, 4). When stored at 31° C and 95% RH both batches of control tablets showed visible mould growth (log $N/N_0 \gg 0$), whereas the tablets prepared with a preservative showed no such mould growth: log $N/N_0 \leq 0$ (Figs. 3, 4).

The water content of solid oral dosage forms depends on the water content at production, the physical properties and the storage conditions (relative humidity and temperature). The importance of water to contaminating micro-organisms depends on its availability to the contaminant. Water can be bound by chemical adsorption, e.g. water of crystallization, and by physical adsorption. This latter type of water is less tightly bound and is therefore more readily available to the contaminating micro-organisms. In this water dissolution of soluble tablet ingredients, e.g. preservatives, is thought to occur (Carstensen et al., 1969). Tablets containing hygroscopic materials, either active drug or excipients, are likely to physically adsorb substantial amounts of water.

The water requirements for micro-organisms vary depending on the organism (Blair et al., 1988). For different mould spores the minimum RH required for germination varies from 70% to 98%. The optimum temperatures for growth of moulds vary from 23 to 40° C. Mould spores are capable of surviving for considerable periods of time at much lower RHs and temperatures. The minimum water requirements for bacteria are in the range of 91–99% RH, depending on the organism. Bacterial spores are capable of surviv-

ing for considerable periods of time at much lower RHs and temperatures. The water requirements of yeasts are somewhat higher than those of moulds.

In accordance with these water requirements, tablets without preservatives, inoculated with *A. niger* spores or prepared with rice or tapioca starch, spoiled due to mould growth when stored under tropical conditions (31°C and 95% RH). No growth of bacterial cells was observed during storage under these conditions. When stored under more moderate conditions (31°C and 75% RH) the tablets were not at risk to microbiological spoilage. Both an optimum relative humidity and an optimum temperature are required before tablets are at risk of microbiological spoilage. Fassihi and Parker (1977) found that tablets stored at a lower temperature (25°C) and at 96% RH did not spoil due to mould growth (*Aspergillus niger* and *Penicillium sp.*). When stored under these conditions, the viability of the mould spores decreased slightly.

The higher concentration of preservative (1% w/w) investigated was effective in protecting the tablets against spoilage arising from the growth of mould spores, both of *A. niger* and of natural contaminants in rice or tapioca starch. Further investigation is needed to determine the minimal effective concentration of preservative.

The results showed that 0.1% w/w preservative, either of sodium methylhydroxybenzoate or potassium sorbate, in tablets was not adequate to have a lasting positive effect on the microbial tablet quality. The viability of the mould spores was suppressed for some time, but finally the spores were able to multiply. This is not in accordance with the results found by Fassihi et al. (1978), who found that 0.1% Na-MOB in tablets prevented spores of *A. niger* from growing. This difference in results can be explained by the storage conditions used by Fassihi et al.: 25°C and no controlled relative humidity.

After storage under tropical conditions, adsorption of water into tablets is inevitable since in most cases tablets have to contain disintegrants, which are highly hygroscopic materials. Under these conditions spoilage due to growth of natural contaminants is hardly avoidable, although tablets initially meet the pharmacopoeial requirements for microbiological quality of solid oral dosage forms. Therefore the addition of a preservative to tablets that are to be stored under tropical conditions is necessary in order to guarantee sufficient microbiological quality. The choice of the preservative depends on the tablets formulation. Attention must be paid to incompatibilities with other ingredients.

Conclusions

Of the investigated batches of tablet excipients, the highest level of natural contamination was found for rice and tapioca starch. Lactose/starch tablets which are stored under tropical conditions with high relative humidities (31°C, 95% RH), even for a relatively short period of time (4 weeks), are at risk to microbial spoilage due to mould growth. The addition of a preservative to these tablets can prolong their microbiological shelf-life. Both sodium methylhydroxybenzoate and potassium sorbate are effective, at a concentration level of 1.0% w/w. The choice of preservatives depends on other formulation factors, such as incompatibility. When tablets are stored under conditions with RH $\leq$ 75%, the addition of preservatives is not necessary to guarantee their microbiological quality.

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