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On the mechanism of kill of microbial contaminants during tablet compression

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Summary

Separate batches of three tabletting excipients, lactose monohydrate, maize starch and Avicel PH-101, were contaminated with vegetative bacteria, *Staphylococcus aureus* or *Enterobacter cloacae*. The bacteria were allowed to grow in the excipient, such that they were adhered to the surface of the powder. The contaminated powders were tabletted at various compression forces. The extent of kill was assessed for each organism and compression/kill curves were produced. The extent of kill on tablet compression was greatest for the larger organism, *E. cloacae*, indicating that size is important and that cell rupture by shear, rather than heat, is the mechanism of kill. The type of consolidation during compaction (plastic flow or brittle fracture) influenced survival, plastic flow causing the greatest kill at low compression forces. The results are related to published data for the kill of spores during compaction.

Introduction

The low water content of compressed tablets leads to the assumption that these dosage forms are not at risk from microbial spoilage. There are, however, numerous anecdotal and published reports (e.g. Kallings et al., 1966a,b; Fischer et al., 1968; Jain and Chauhan, 1978; Somerville, 1981; Bos et al., 1989) which show that tablet contamination can be a problem, and may even result in clinical infection.

In this study, one aspect of tablet production, the compression stage, was investigated to determine the mechanism(s) of kill of microorganisms (especially vegetative bacteria) during this process.

The increased use of direct compression as a means of tablet manufacture means that contaminated drugs or excipients avoid the lethal drying stage of wet granulation (see Fry and Greaves, 1951; Chesworth et al., 1977; Fassihi and

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Parker, 1977a and Wallhauser, 1977 for detail of the effects of wet granulation), resulting in a potentially much higher bio-burden prior to compression. Therefore, it is vital to understand how microbes are killed during compression. The factors that must be considered are the compression force that is applied, the properties of the micro-organisms and the properties of the formulation.

There has been debate on the mechanism of kill of micro-organisms during the compaction process. Chesworth et al. (1977) reported that compaction resulted in a drop of about 70% in the viable count compared to the original natural raw materials. Following compaction, the tablets had a final temperature of 40–50°C, and thus the killing effect was partially attributed to heat. Fassihi and Parker (1977a) proposed that at low compaction pressures kill was due to mechanical disruption, but at higher pressures it was due to high local heat. Yanagita et al. (1978) acknowledged the possibility of temperature affecting microbial kill on tablet production, but also noted that larger organisms were proportionally more likely to be killed than smaller ones, implying that shearing forces are significant in the killing process. Plumpton et al. (1982, 1986a) argued that shearing, and not temperature, effects were important in the killing of micro-organisms (yeasts) during compression.

Previous workers (e.g. Fassihi and Parker, 1977a,b, 1987; Plumpton et al., 1986a,b) have added spores as contaminants. Generally these were added dry to compression mixes just prior to tabletting. In this work, non spore forming bacteria were used, which were introduced to the formulation by a method similar to that of Ishag (1973), in which the bacteria are cultured within a suspension of the powder to be tested. This approach produced much higher levels of survival after drying, demonstrating that bacteria that are grown in situ are more resistant (to drying) than those which are grown separately in nutrient media and inoculated into the test system. The aims of this work were: (1) to investigate the killing effects of compression on two different vegetative organisms which were grown in the powder, one of which was a spoilage isolate from a pharmaceutical product; (2) to investigate the effect of different excipients, which compact in different ways, in order to investigate the mechanism of microbial kill during tablet compression.

Materials and Methods

Materials

Maize starch BP special (Roquette (U.K.) Ltd), lactose 170 mesh (Dairy Crest) (both gifts of Glaxo Group Research), Avicel PH-101 (FMC Corp. Ltd) and magnesium stearate BP (Durham Chemical Distributors Ltd) were used to prepare the tablets.

Methods

Contamination of powders

Two organisms were investigated, *Staphylococcus aureus* (NCTC strain, 10788) and *Enterobacter cloacae* (spoilage isolate). A 37°C, 18 h tryptone soya Roux slope was harvested, with a peptone buffer (Buhlmann, 1968), and adjusted to approx. 1.6×10^6 colony forming units (cfu) per ml. Sterilised excipient (120 g, lactose, Avicel PH-101 or maize starch) was mixed with 180 ml of the suspension. These suspensions were incubated at 37°C for 48 h, then filtered through a 0.45 μm filter (Millipore). The damp powders were screened (10 mesh) and spread onto sterile pyrex trays in a laminar flow cabinet, and allowed to dry at room temperature. The water content of the powders was measured at 25°C using a thermostated humidity sensor (Rotronic BT Hygroskop) and drying was terminated at 75% RH. The 'dry' powder was then re-screened (40 mesh) and mixed with 1% w/w sterile magnesium stearate before tabletting. Viable counts were approx. 10^8 cfu/g.

Tablet production

Tablets were prepared from maize starch or lactose contaminated with *E. cloacae* and maize starch, lactose or Avicel PH-101, contaminated with *S. aureus*. The 10.5 mm flat-faced punch and die assembly of a Manesty F3 tabletting machine was disinfected with 70% alcohol, and the feed shoe was heat sterilised, before use. Tablets of 330 mg were produced at a constant (slow) rate of 42

per min. The lower punch was instrumented (quartz ring, 202A, PCB Piezotronics Inc.), to allow measurement of the compression. The compression pressure used to prepare the tablets was varied up to about 323 MPa.

Viable counts were performed on weighed samples consisting of three whole tablets and were compared with viable counts for the uncompressed material.

Viable counting

Approx. 1 g of powder (or tablets) was accurately weighed into 9 ml of peptone buffer. The solids were disintegrated or dissolved, and the resultant suspensions were serially diluted in peptone buffer and dispensed immediately onto over dried tryptone soya agar plates. Where possible, the method of Miles and Misra (1938) was used, otherwise 0.5 ml or 1 ml surface spread plates were produced.

Results and Discussion

The results are presented as a graph of compression force as a function of log survivors for *E. cloacae* (Fig. 1) and *S. aureus* (Fig. 2).

The results presented here for vegetative cells are in agreement with previously published data for spores (Chesworth et al., 1977; Fassihi and Parker, 1977a; Plumpton et al., 1982, 1986a,b)

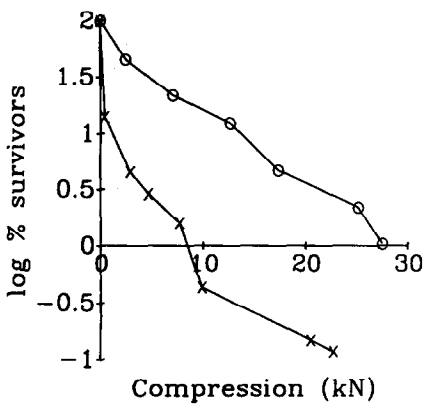

Fig. 1. Compression/survival graph for *E. cloacae* encorporated in lactose (○) and maize starch (×).

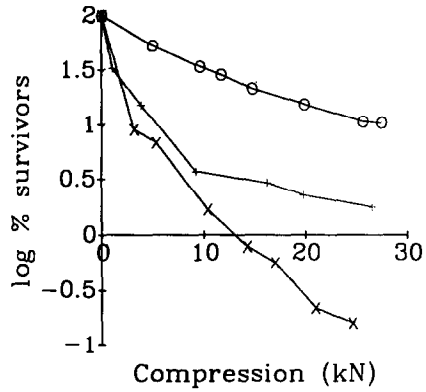

Fig. 2. Compression/survival graph for *S. aureus* encorporated in lactose (○), maize starch (×) and Avicel PH-101 (+).

indicating that compaction pressure produces a substantial loss of viability. The extent of kill was, however, related to the excipient used and the contaminating organism, as well as the compression force. With maize starch tablets it was possible to achieve up to a three log unit reduction in viability (i.e. 99.9% kill) by the application of a compression force. Previous publications, using alternative means of contamination, have not reported such extensive kill.

The smaller coccoid cells of *S. aureus* were more resistant to the effects of compression than the larger rod-shaped bacteria (*E. cloacae*). This relationship between cell size and kill is in agreement with the work reported by Plumpton et al. (1982, 1986a,b), and provides further evidence to suggest that kill is predominantly due to shear rather than heat.

Overall, the survival of bacteria during tabletting was inversely related to the compaction pressure applied (Figs 1 and 2). For lactose, this relationship appears to be a direct inverse proportionality, with a correlation coefficient for linearity of −0.99 for both organisms. For maize starch and Avicel PH-101 however, the slope of the pressure/survival plots was greatest at low compaction pressures.

The three excipients have different mechanisms of consolidation on compaction. Lactose monohydrate is a brittle crystalline material, and tends to consolidate by fracture initiated at the early stages of compression (Hersey et al., 1973). As the ap-

plied force is increased, more of the material fractures and rearranges to fill available void spaces. In contaminated lactose granules, the inverse proportionality of bacterial survival to applied pressure probably results from increasing the area of interparticulate contact on fracturing; the microorganisms may then be inactivated by shearing forces, as described by Plumpton et al. (1986a).

Maize starch is particularly prone to plastic flow, and less prone to plastic recovery than other starches (Paronen and Juslin, 1983). According to Hiestand et al. (1977), plastic deformation results in localised shear flow, which under a compression load would lead to a greater interparticulate contact, and enhance mechanical disruption of microbial contaminants. In the early stages of compression, maize starch deforms plastically, accompanied by particle rearrangement (Paronen and Juslin, 1983). At low compaction pressures, more extensive kill was observed in starch than lactose. As the compaction pressure is increased, densification of the starch compact hinders particle rearrangement, and this explained the relatively smaller rise in the extent of bacterial kill in starch at higher compaction pressures.

The pressure/survival plot obtained from compressions of Avicel PH-101 contaminated with *S. aureus* initially follows the same pattern as for maize starch. However, at compression forces exceeding 8 kN (corresponding to 92 MPa for the punches used), a linear relationship developed, with a correlation coefficient of -0.99, and a slope similar to that obtained for *S. aureus* tabletted in lactose. The plastically deforming nature of Avicel PH-101 has been confirmed by David and Augsberger (1977), but it was reported by Sixsmith (1982) that Avicel PH-101 exceeds its elastic limit after about 80 MPa, and at higher pressures consolidates chiefly by brittle fracture. This explains the change in the extent of bacterial kill in Avicel PH-101 tablets as the compression forces were increased, and provides strong evidence that shear flow is the chief mechanism of kill, since inactivation was substantially reduced once the elastic limit was exceeded.

The results indicate that compression of plastically deforming materials causes more bacterial inactivation than fracturing materials, especially at low pressures. A consideration of these results, along with previously published data reveals uncertainty about the mechanisms which determine the shape of the compression/kill curves for contaminated tablets. Fassihi and Parker (1977a) reported that log % survivors decreased linearly with applied compression force, whilst Yanagita et al. (1978) described a non-linear relationship between pressure and survival. Results presented in this study demonstrate both trends; when lactose is used as the excipient a linear response is observed, but for starch and Avicel PH-101, the pressure/kill profile exhibits a more complicated (non-linear) relationship (Figs 1 and 2). Plumpton et al. (1986b) argued that (for any one organism in any one excipient) the compression/kill relationship was linked to the spatial distribution of the organism. Their results indicated that using samples contaminated with spores via the granulating fluid, in which the organisms were present within the granules, a non-linear compression/kill relationship was obtained: when spores were added as a dry mix to previously prepared granules or direct compression material, where contaminants were present only at the surface of the granule, then a linear relationship was obtained. By contrast, the results in this study indicate that both linear and non-linear compression/kill curves may be obtained with powders in which the organisms were present only inside the granules (i.e. inoculated via the granulating fluid).

From their results, Plumpton et al. (1986b) also concluded that for organisms (spores) present only at the surface of the granules, compaction by brittle fracture produces the greatest inactivation, but for organisms present inside the granule (i.e. not just at the surface), inactivation is maximised where compaction was achieved by plastic deformation. Results of the present study confirm that where organisms are present inside the granule, inactivation is greater for materials such as maize starch or Avicel at low compaction pressures, which compact by plastic deformation, than for materials such as lactose, or Avicel at high compaction pressures, where compaction occurs by fracturing.

In view of the results presented here, we suggest that the interpretation of Plumpton et al

(1986b) should be modified thus: microorganisms that are bonded to or in powders/granules which fracture on compression, may be protected to some extent, and only damaged when directly fractured. Materials which compact by plastic flow, will exhibit greater surface disruption and thus more kill of organisms which are bonded to or in powders/granules than for brittle fracture samples. When the contamination is present in void spaces between powders (Plumpton et al., 1986b) then fracturing materials may well result in greater kill than those which exhibit plastic flow.

A further variable between published studies is that of the use of lubricants. Fassihi and Parker (1987) did not include a lubricant, and thus friction and kill may be expected to be greater than in studies where such an excipient was included (e.g. this work and that of Plumpton et al., 1986a,b).

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