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The survival of microorganisms during tabletting

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Summary

The lethal effect of the compaction process on *S. cerevisiae* and *A. niger* present in direct compression materials has been studied. For *S. cerevisiae*, compaction at low pressures leads to an increase in colony forming ability, presumably due to an activation process. Low pressures have no such effect on *A. niger*. Higher pressures cause killing of both organisms, 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.

Introduction

Microbiologically contaminated tablets may cause disease and under humid storage conditions can visibly cause biodeterioration. Granulation steps prior to compression into tablets often employ temperatures in excess of 50°C and are themselves detrimental to microbial survival (Fassihi and Parker, 1977). Increasing use of direct compression vehicles has enhanced the likelihood of organisms surviving the initial stages of tablet manufacture. The effects of compaction per se upon microbial survival in tablets is therefore of increased significance. Morris (1981), Chesworth et al. (1977) and Schilder et al. (1968) have all shown that the compression of naturally contaminated formulations led to significant reductions in the number of viable organisms. To obtain more quantitative estimates of survival during compression, Fassihi and Parker (1977) and Fassihi et al. (1977) deliberately contaminated a formulation and demonstrated a linear relationship between log survival and applied pressure. In a second study, again using a deliberately contaminated formulation (Yanagita et al., 1978), such linearity was not found, rather a relationship between cell size and survival was observed, with larger cells being more sensitive to compaction pressure than smaller ones.

Two mechanisms have been proposed for the lethal effect of compression, namely local heating and shearing (Chesworth et al., 1977). It is likely therefore, that properties of the formulation such as particle size and mode of compaction (plastic flow or fragmentation) will influence the degree of killing brought about. These factors have not been previously taken into account and are examined in this paper.

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Materials and Methods

Materials

Four direct compression materials were used, Edmex (K.&K. Greeff Chemicals, Croydon, U.K.), Sta-Rx 1500 (Colorcon, Orpington, Kent, U.K.), spray-dried lactose (Unigate Foods, London, U.K.), and potassium chloride (BDH, London, U.K.). Where necessary these were fractionated with respect to particle size using stacked 295, 251, 178 and 104 μm sieves, on a mechanical shaker. Sodium starch glycollate (Explotab) and magnesium lauryl sulphate were kindly donated by Thomas Kerfoot Ltd (Ashton-under-Lyme, U.K.). Polysorbate 80 was obtained from Sigma Chemicals (London, U.K.).

Preparation of tablets

The direct compression excipients were used in conjunction with magnesium lauryl sulphate (1% w/w) as a lubricant and sodium starch glycollate (1% w/w) as a tablet disintegrant.

Ingredients, including the dried inocula, were weighed and lightly mixed in a glass mortar by the method of increasing quantities. Preliminary experiments had established that this gave an even dispersion of the contaminating microorganisms throughout the mixture.

Quantities, each of 500 mg were accurately weighed and poured into a 12.6 mm diameter die and compressed between flat-faced punches, maintaining the die and lower punch at a fixed position, using a 10 ton hydraulic press. Compacts were brought as rapidly as possible to the required pressure (0–271 $MN \cdot m^{-2}$) and were maintained at this value for 30 s before release. The tablets so produced disintegrated upon shaking in distilled water within 10 min at all compaction pressures employed.

Preparation of inocula

Saccharomyces cerevisiae, and *Aspergillus niger* ATCC 16404 spores were incorporated into the direct compression vehicles by dry mixing. It was necessary to prepare suitable dried samples of these microorganisms. *S. cerevisiae* was obtained as a commercially available granulated dried yeast (Be-Ro dried yeast, J.A. Sharwood, London, U.K.). The particle size of the granules was reduced using a fluid energy mill and the resultant powder was passed through a 251 μm sieve. Where necessary yeast powders were further fractionated according to size using 251, 178 and 104 μm sieves. The powders were incorporated directly into tablet formulations (2% w/w). Cultures of *A. niger* were grown, for 48 h at 35°C on Sabourand dextrose agar plates (Oxoid, CM41) and subsequently left at room temperature for 48 h. Spores were harvested from the plates in 3 ml volumes of sterile water containing polysorbate 80 (0.1% w/w) as a surfactant. Resultant suspensions were centrifuged at 30°C ($10{,}000 \times g$, 10 min) washed and resuspended in distilled water. These were air-dried in a sterile mortar at 30°C for 5 days, collected and incorporated directly within the tablet formulations (0.05% w/w) by dry mixing.

Determination of viability

Inactivation of microorganisms during compression required assessments of viability to be made directly upon the tabletted product. Tablets were disintegrated in distilled water (10 ml) using a Griffin flask shaker (1800 oscillations $\cdot$ min^{-1} for 10 min). Serial dilutions were made and viability assessed using a surface spread method and predried agar plates. Tryptone soya agar (Oxoid CMB1) plates were incubated at 30°C for 16 h for the bacteria and Sabaraud dextrose agar plates at 30°C for 48 h for the yeast and fungi. Survival, estimated as the mean of triplicate determinations, made upon each of three tablets for each compaction pressure, were expressed as percentages relative to uncompressed control samples of the contaminated formulations.

Pore size distribution of tablets

This was assessed using a mercury porosimeter (model 820, Carla Erba, Strumentazione, Italy) and the methods described by Carli et al. (1981) and Plumpton (1982).

Results

Fig. 1 shows the relation between compaction pressure and log% survival for different particle

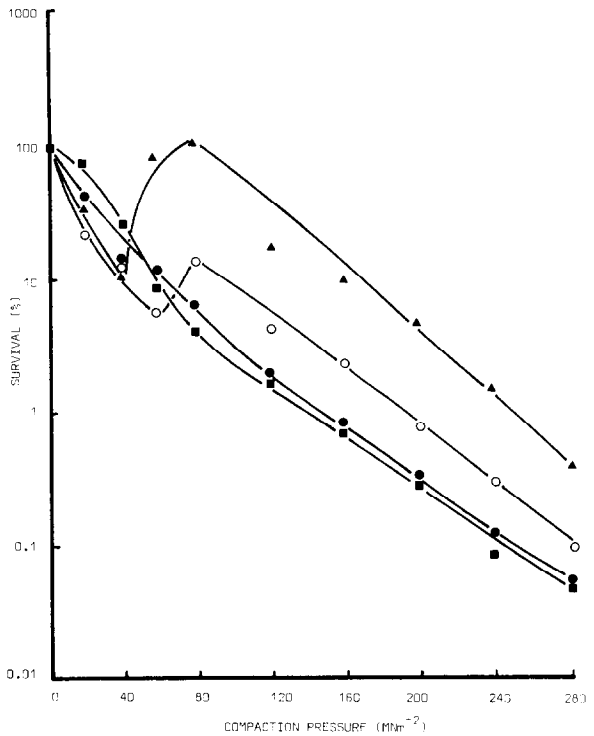

Fig. 1. Survival of dried yeast of 251–178 μm (■), 178–104 μm (●), 104–74 μm (○), and < 74 μm (▲) during compaction in potassium chloride.

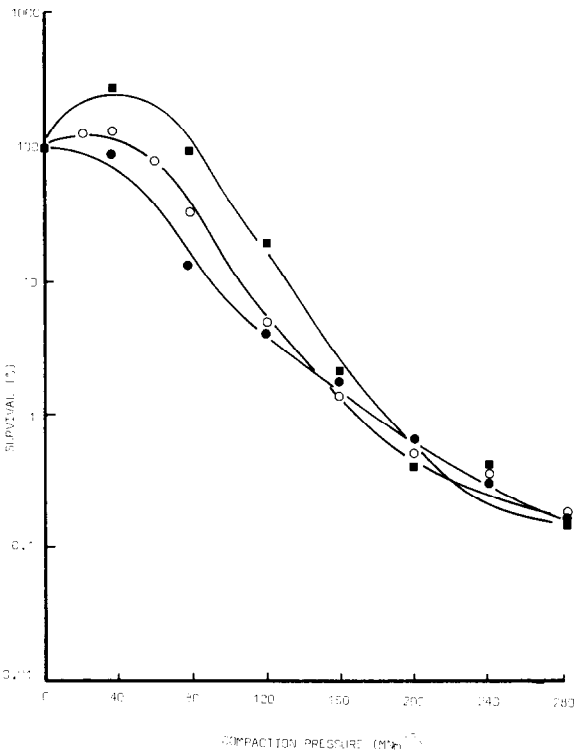

Fig. 3. The influence of compaction pressure upon the survival of dried yeast (< 74 μm) in lactose (●), Emdex (■) and Sta-Rx (○).

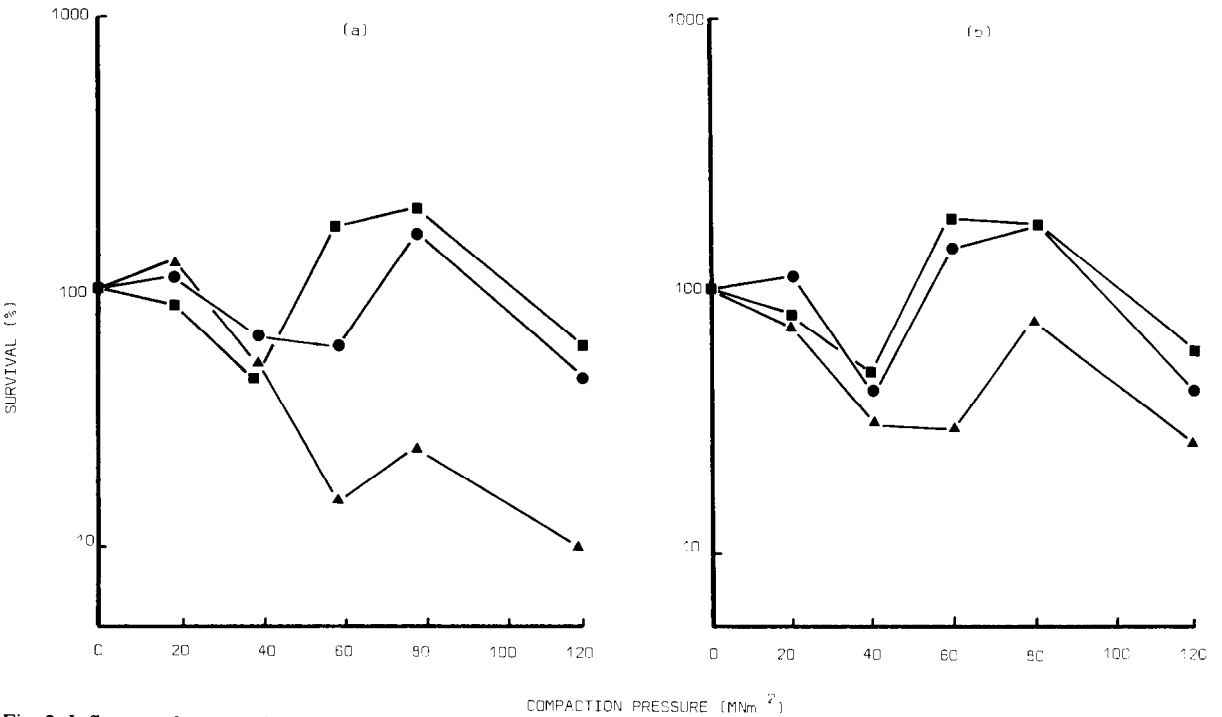

Fig. 2. Influence of compaction pressure upon the survival of dried yeast of: (a) 90–74 μm and (b) 74–53 μm during compaction in potassium chloride of 295–251 μm (■), 251–178 μm (●) and 178–104 μm (▲).

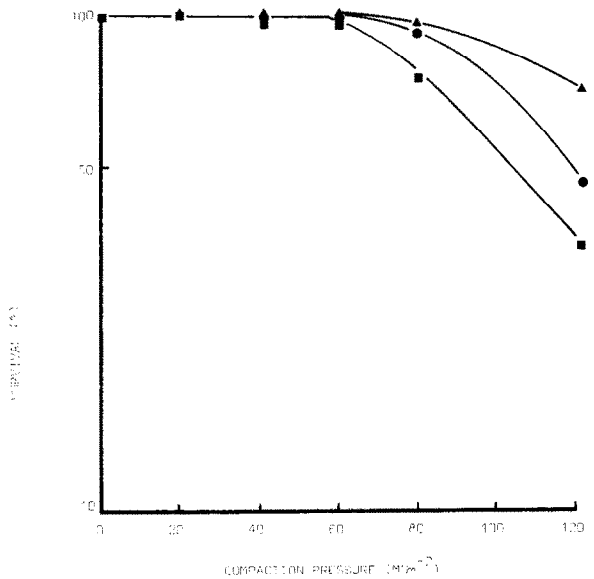

Fig. 4. Survival of *A. niger* spores during compaction in potassium chloride of particle size 295–251 μm (■), 251–178 μm (●) and 178–104 μm (▲).

size fractions of milled yeast incorporated into unfractionated potassium chloride. The results show a decreasing level of survival with increasing compaction pressure for the larger sized yeast particles, whereas for the smaller yeast particles, an increase in colony-forming ability occurred between 39 and 77 MN · m^{-2}. These data show clear differences in survival for the various yeast size fractions and suggest that the relative particle size between the contaminating organism and the excipient is important in determining patterns of survival. This was tested directly utilizing various particle size fractions of potassium chloride with the two smaller sizes of yeast particles (Fig. 2). Pressures greater than 116 MN · m^{-2} were not used as little significant difference in survival was present above this pressure. Colony-forming ability increased between 39 and 77 MN · m^{-2} and was most marked for the largest potassium chloride size fraction and the smallest yeast particles. Incorporation of the smallest sized yeast particles

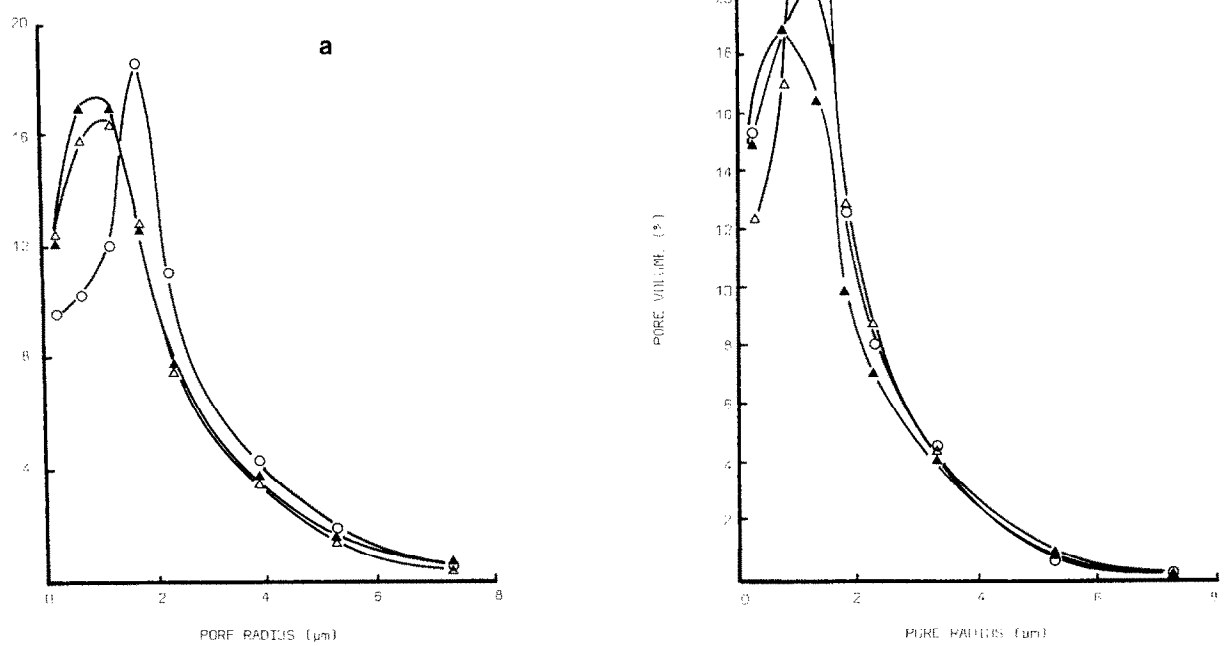

Fig. 5. Pore size distributions for tablets of potassium chloride prepared from 295–251 μm (▲), 251–178 μm (△) and 178–104 μm (○) size fractions. (a) Compaction pressure 96 MN · m^{-2}; (b) compaction pressure 116 MN · m^{-2}.

(< 74 μm) into the other tablet excipients was carried out to test whether compaction mechanism or particle size was important in giving rise to the increase in colony-forming ability (Fig. 3). Formulations based on Emdex and Sta-Rx exhibited initial increases in colony-forming ability. These materials compact by different mechanisms, Emdex fracturing on compaction, and Sta-Rx consolidating by plastic flow.

The survival of *A. niger* spores compacted in various size fractions of potassium chloride is illustrated in Fig. 4. Little inactivation occurs up to a pressure of 58 MN $\cdot$ m^{-2} whilst higher pressures decrease colony-forming ability with the greatest effect occurring with the largest size fraction of potassium chloride.

The lethal effect of compaction may be related to the pore size distribution achieved at any given pressure. Pores above 15 μm are outside the range of the mercury porosimeter but their total volume can be estimated. After compaction at 58 MN $\cdot$ m^{-2} approximately 55% of the pore volume for tablets of 295–251 μm and 251–178 μm potassium chloride and 36% for 178–104 μm potassium chloride were for pores of greater than 15 μm. Fig. 5 illustrates the pore size distributions for tablets of size fractions of potassium chloride prepared at 96 and 116 MN $\cdot$ m^{-2}. The pore size distributions for the three size fractions are similar with the larger fraction exhibiting a predominantly smaller radius, having thus undergone a greater degree of compaction.

Discussion

The initial stages of the compaction of powders consists of particle movement into void spaces within the powder bed. During this process, no decrease in survival was detected. This indicates that frictional or shearing forces occurring at the time were insufficient to damage microorganisms. For *S. cerevisiae*, application of low pressures caused increases in colony-forming ability relative to the initial inocula. This may have been due to cleavage of budded cells or their activation. Examination of reconstituted yeast inocula under phase-contrast microscopy showed insufficient budded cells to account for the increase obtained. It is likely, therefore, that activation of dormant cells, probably due to a combination of heat generation and mild mechanical abrasion, accounts for the increased colony-forming ability.

Further increases in pressure on a powder bed cause consolidation by mechanisms such as fragmentation or plastic flow. Decreases in survival for both microorganisms were observed in this range of pressure. Suggested mechanisms for the lethal effects of compaction are localized "hot spots", shearing forces or pressure itself (Chesworth et al., 1977; Fassihi et al., 1977). Since different survival patterns were observed for the various particle size of yeast (Fig. 1), pressure alone could not have been responsible for the lethal effect of compaction. Survival has also been previously shown to vary with the size of the organism and not their heat sensitivity (Plumpton, 1982; Yanagita et al., 1978).

Dried yeast particles consist of agglomerates of cells and may be sieved to enable the influence of compaction pressure on the survival of different "sized" organism units to be modelled, without inherent differences in sensitivity to pressure and heat. Simultaneous variation of the particle size of both the yeast and the potassium chloride (Fig. 2) and variation of potassium chloride with *A. niger* (Fig. 4) showed that the greatest changes in colony-forming ability occurred when the differences in particle size were the greatest. The survival patterns obtained with the yeast particles will be a combination of activation and lethal processes. The void spaces in a powder bed of large particles will be of greater volume than those within a bed of smaller particles. Hence, in the early stages of compaction, the relative size between the potassium chloride and the yeast will determine the extent of stress on the yeast particles. Small yeast particles in a bed of large potassium chloride crystals will be subjected to the lowest stress and activation will be the predominant process. The survival levels of yeast (< 74 μm) in Emdex, Sta-Rx and lactose lends support to this (Fig. 3). Significant increases in colony-forming ability occurred in Emdex only and only this material of the three has a large proportion of particles of size greater than 100 μm.

The picture for *A. niger* was not complicated by an observable activation step. Thus, the degree of killing increased, for any given compaction pressure above 58 $MN \cdot m^{-2}$, with particle size. Beneath this pressure, no change in colony-forming ability was observed. Presumably, the spores were able to move into void spaces and escape mechanical damage. *A. niger* spores are approximately 4 μm in diameter, and at 58 $MN \cdot m^{-2}$, the results from the porosimetry studies showed the majority of the pore volume was greater than this size. After 58 $MN \cdot m^{-2}$, inactivation occurred, being greatest for the large size fraction of potassium chloride. This size fraction undergoes the greatest change in porosity and hence subjects the spores to the greatest degree of mechanical damage.

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