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Effect of spatial distribution of contaminant microorganisms within tablet formulations on subsequent inactivation through compaction

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Summary

A wet granulation process, employing an aqueous suspension of *Bacillus megaterium* spores as binder, was used to prepare contaminated granules of three direct compression vehicles, Emdex, lactose and potassium chloride. Granules were also prepared with sterile water as binder and directly contaminated by dry mixing with *B. megaterium* spores, as were the original direct compression vehicles. Particle size distributions for the granules and the direct compression vehicles were similar. Survival of *B. megaterium* was assessed, following compaction (500 mg) at various pressures (0–271 $MN \cdot m^{-2}$) using a 1 cm flat-faced punch and a 10 ton hydraulic press. In all cases the degree of killing was directly proportionate to the compaction pressure. For those materials compacting by fracture, lactose and Emdex, then the degree of killing was similar for the dry contaminated direct compression vehicle and those granules prepared with contaminated binding fluid, but significantly greater in the dry contaminated granules and greater overall for lactose rather than Emdex. For potassium chloride, a material which plastically deforms, then the degree of killing was significantly enhanced by incorporation of the contaminant into the binding fluid.

Introduction

Microbiological contamination of solid dosage forms may cause disease and under humid storage conditions lead to visible deterioration of the tablet. Granulation steps prior to tabletting often employ elevated temperatures (10–80°C) for prolonged periods of time and are inimical to vegetative forms of microorganisms (Fassihi and Parker, 1977). Increasing use of direct compression vehicles within the pharmaceutical industries has increased the likelihood of organisms surviving the initial stages of tablet manufacture. The effects of compaction per se upon microbial survival are therefore of increased significance. A number of studies have reported upon the incidence of contaminated batches of tablets and also upon the lethality of compaction processes towards microorganisms (Chesworth et al., 1977; Fassihi and Parker, 1977; Fassihi et al., 1977; Yanagita et al., 1978). Generally linear dependencies of the degree of killing upon compaction pressure have been observed and attributed mainly to shear stresses within the compact causing a direct mechanical rupture of contaminant microorganisms. The possibility of localized high temperatures, generated at points of contact within the compact, being

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partially responsible for the lethality of the process has been proposed (Fassihi and Parker, 1977), but recent work (Plumpton, 1982) failed to detect the presence of heat-injured cells amongst the survivors of compaction processes, and suggests that such effects are minimal.

In this study we examine the effects of spatial location of contaminant microorganisms within the formulation upon their subsequent killing by the compaction process. Microorganisms were either tightly bound onto and within granules composed of the direct compression vehicles or loosely associated by dry mixing with the direct compression vehicle or prepared granules thereof.

Materials and Methods

Chemicals

Three direct compression vehicles were used in the course of this study, Emdex (K.&K. Greeff Chemicals, Croydon, U.K.), spray-dried lactose (Unigate Foods, London, U.K.) and potassium chloride. Sodium starch glycollate (Explotab) and magnesium lauryl sulphate were kindly donated by Thomas Kerfoot (Ashton-under-Lyme, U.K.). All other reagents were obtained from BDH (London).

Tablet formulations

Lactose, Emdex and potassium chloride were used as direct compression excipients in tablet formulations in conjunction with magnesium lauryl sulphate (1% w/w) as a lubricant and sodium starch glycollate (1% w/w) as a tablet disintegrant. Granules (< 251 μm) of these direct compression vehicles were prepared (see below) and similarly used in tablet formulations. 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 compressed. The tablets so produced disintegrated upon shaking in distilled water within 10 min at all compaction pressures employed.

Preparation of inocula

Bacillus megaterium ATCC 8245 spores, were incorporated into the direct compression vehicles by dry mixing. *B. megaterium* cultures were streaked onto pre-dried potato-dextrose agar (Oxoid, CM139) plates, incubated at 30°C for 48 h and subsequently at room temperature for 72 h. Cultures were harvested in distilled water and the cells collected by centrifugation of the suspensions at 35°C (10,000 × g, 15 min). Pellets were resuspended in 20 ml of lysozyme solution (20 $\mu g \cdot ml^{-1}$) and the resultant spore suspension harvested after incubation for 1 h at 35°C and washed by successive centrifugation and resuspension in distilled water. Two ml volumes were placed in sterile glass mortars, covered and allowed to dry at 35°C for 48 h. Five grams of the appropriate direct compression vehicle was then gently mixed in the contaminated mortars. The contaminated mixes were incorporated into tablet formulations of the appropriate direct compression vehicle or sterile granules thereof, to a level of 0.5% w/w.

Granules, contaminated internally, were prepared by using *B. megaterium* spore suspension, in sterile water, as the binder during the granulation process (see below), and used directly in the tablet formulations without further contamination.

Granulation

The particle size of the direct compression vehicles was reduced to 104 μm prior to granulation, by milling in a vibratory ball mill (Griffin& George, U.K.). This material was placed in a mortar and moistened with a suspension of *B. megaterium* spores in sterile distilled water (10^7 spores/ml) and forced through a 700 μm sieve. Following drying at 35°C for 5 h, the granules was screened through a 251 μm sieve to break up large agglomerates. The particle size distributions of the prepared granules were similar to those of the original direct compression vehicles and the tablets formed from each at any given pressure were of a similar thickness and hardness.

Compaction of tablets

The formulations were poured, in 500 mg amounts, into a 12.6 mm diameter die and com-

pressed 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 maintained at this value for 30 s before release.

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 Tryptone soya agar plates, incubated at 30°C for 16 h. Survival, estimated as the mean of triplicate determinations, made upon each of three tablets for each compaction pressure, was expressed as a percentage relative to uncompressed control samples of the contaminated formulation.

Results

Tablets of three direct compression vehicles, Emdex, lactose and potassium chloride, were prepared over a range of compaction pressure (0–271 $MN \cdot m^{-2}$). Each of the tablet formulations included either a dry-contaminated direct compression vehicle, a dry-contaminated preparation of granules prepared from the direct compression vehicle or a preparation of granules contaminated by the addition of bacterial spores at the wet massing stage of granulation. In the former two cases the contaminant spores ought to coat the particles and granules of direct compression vehicle and also fill void spaces whilst in the latter case contaminant spores ought to form part of the intragranular bridges and be tightly bound to the particulate surfaces.

Survival of *B. megaterium* spores in the various tablet formulations following compaction is given in Fig. 1, for lactose and Emdex and in Fig. 2 for potassium chloride. In all cases the levels of survival were inversely proportional to the compaction pressure. For those materials which compact by a fracturing mechanism (Fig. 1), the degree of killing observed was greatest for the granule preparations to which dry contaminated compression vehicle had been added. Levels of survival were similar for any given compaction pressure for

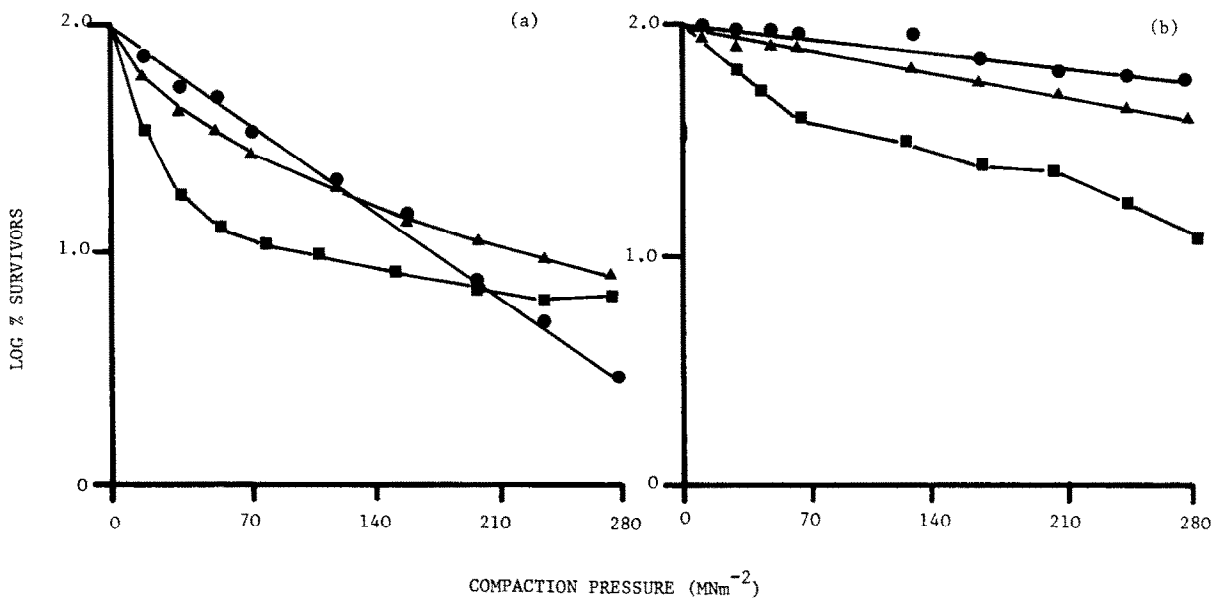

Fig. 1. Survival of *Bacillus megaterium* spores following compaction in variously contaminated (a) lactose and (b) Emdex formulations. ▲, dry contaminated direct compression vehicle; ■, dry contaminated granules; ●, granules prepared from contaminated granulating fluid.

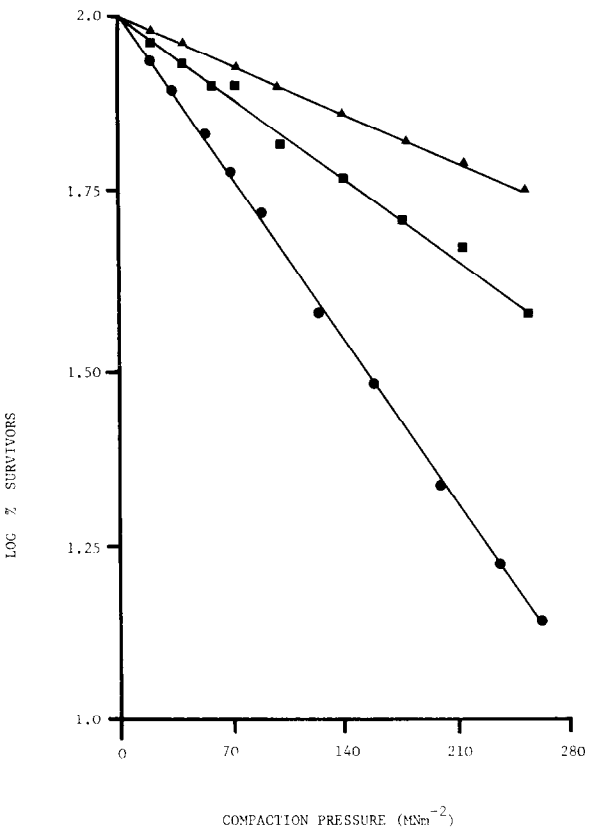

Fig. 2. Survival of *Bacillus megaterium* spores following compaction in variously contaminated potassium chloride. ▲, dry contaminated KCl; ■, dry-contaminated KCl granules; ●, KCl granules prepared from contaminated granulating fluid.

wet contaminated granules and for the direct compression vehicle itself. The extent of killing appeared to maximize for pressures 120 MN · m^{-2}, where mercury porosimetry had indicated that minimum porosity had been achieved. For the potassium chloride formulations, which compact by plastic deformation, then the degree of killing was least for the dry-mixed vehicle and greatest for contaminated granular preparation. The response of dry-contaminated granules was intermediate. In none of these cases did the dependency of killing upon compaction pressure deviate from linearity nor did the tablets achieve minimum porosity (Fig. 2).

Discussion

The origin of contaminants and the stage of the manufacturing process at which they are introduced will influence their spatial distribution in/on the granules. Contaminants arising from raw materials will be distributed randomly throughout the granules whereas contaminated granulating fluid will give rise to microorganism surface films and in interparticulate bridges. If the contaminants are introduced from excipients added after granulation (such as lubricants or disintegrants), they will be located on the surface of the granules only.

On compaction, granules will fracture at low pressures introducing an extra element of shear which could increase inactivation. For materials which fracture on compression, this shear will be more effective in inactivating microorganisms if they are situated on the surface of the granules. Microorganisms present within interparticulate bonds may be protected to some extent and may only be inactivated when directly fractured or exposed. Potassium chloride compacts by plastic flow after the initial fracture of the granules, and as the material as a whole is flowing, inactivation will be greater for contaminants present within the material.

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