Evaluation of Blow/Fill/Seal Extrusion through Processing Polymer Contaminated with Bacterial Spores and Endotoxin

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SHORT TITLE: Blow/Fill/Seal Extruder Challenges

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ABSTRACT: A collaborative study involving Cardinal Health, Inc. and Air Dispersions Ltd. has been carried out to further the understanding of the extrusion process and its impact upon the quality of Blow/Fill/Seal product. Controlled challenges to the extrusion system, comprising Low Density Polyethylene granulate contaminated with characterized levels of *Bacillus atrophaeus* (ATCC 9372) endospores and *Escherichia coli* 055:B5 bacterial endotoxin, have been conducted. Batches of spore contaminated polymer, at challenge levels varying from 10^3 to 10^6 spores g^{-1} polymer with derived D_{160} values ranging from 1.22 to 2.07 minutes' and endotoxin contaminated polymer, at challenge levels varying from 10^2 to 10^4 EU g^{-1} polymer, were processed through a Blow/Fill/Seal machine employing Tryptone Soya Broth and Water for Injection as the fill mediums, respectively. Relationships have been established between the levels of challenge for each of spores and endotoxin and the extent of product contamination. The relationships allow for prediction of microbiological and pyrogenic quality based upon the microbiological and pyrogenic attributes of unprocessed polymeric granulate and for rationalized choices of polymeric granulate acceptance limits. It is stressed that the findings apply only to the particular Blow/Fill/Seal machine and to the specific conditions of machine operation.

KEYWORDS: B/F/S, Blow/Fill/Seal, Extruder, Polymer extrusion, Spore challenge, Endotoxin challenge, Microbiological quality, Pyrogenic quality.

Introduction

Blow/Fill/Seal technology, originally developed in Europe in the 1930's and introduced in the U.S. in the 1960's, has emerged as a highly acceptable method for aseptic packaging of liquid pharmaceutical and healthcare products. The method provides flexibility in container design, high product output, low operational costs and assurance of product sterility. The Blow/Fill/Seal process enables a plastic container to be molded, aseptically filled and hermetically sealed in one continuous, integrated operation (1), with fill volumes ranging from 0.1 to 1000 cm^3. A variety of polymers may be used in the process, with low and high-density polyethylene and polypropylene being the most common. For processing, these polymers are typically supplied in granulate form.

During molding of the containers, the polymeric granulate is processed through an extruder held at elevated temperature. The combination of high temperature and pressure within the extruder provides a container "free" of viable microorganisms and with acceptable endotoxin levels, as demonstrated by the Blow/Fill/Seal operators through routine performance of media fills (2) and final product testing, respectively.

Because extrusion occurs within a closed system that is essentially inaccessible, it is not amenable to the usual direct methods for assessment of dry heat treatments used by the industry, circumstances that prompted the present experimental work. The objectives of the work were threefold;

(i) to challenge the extrusion process with polymeric granulate whose surfaces were contaminated with known levels of characterized bacterial endospores or pyrogens and measure the affect upon container/product contamination,

(ii) to gain a better understanding of the process by which extrusion influences risk of product contamination, and

(iii) to develop a rationale for setting microbiological and pyrogenic limits on virgin incoming polymeric granulate in order to control risk.

To achieve these objectives, multiple batches of spore or endotoxin contaminated polymeric granulate were processed on a Blow/Fill/Seal machine employing an appropriate fill medium for evaluation of the particular contaminant. Thereafter, for the spore challenge, measurements were made of the fraction of product exhibiting

microbial growth, and, for the endotoxin challenge, of the quantity of endotoxin present in the contained fill liquid.

The Extrusion Process

Thermoplastic extrusion is a complex process that involves heat, pressure, phase transition and melt rheology. During the extrusion process, the plastic melt is forced through a shaped orifice by a single screw extruder to produce a semi-solid continuous tube of plastic material with a constant cross section (the parison). This tube is cut to the desired length and then formed into the container(s) by a set of molds. Vacuum, applied through the mold face, is used to facilitate container formation and, for some container designs, it can be necessary to apply filtered air under pressure to blow open the container. A cooling liquid, circulated through a closed manifold within the mold, expedites solidification of the polymer so that, upon sealing and mold release, the container is robust and no longer malleable. The cycle time from parison exit from container release from the mold is primarily dependent upon container volume and typically ranges from 12 to 18 s.

For convenience, the process of polymer phase transition and conveyance is considered to occur over three distinct physical zones. These zones are known as the feed zone - solids conveying, transition zone - melting and metering zone - melt pumping (3). Figure 1 is a diagrammatic representation of a single screw extrusion system commonly used in Blow/Fill/Seal technology. As the polymeric granulate drops into the extruder barrel from the hopper, the feed zone conveys the granulate forward in the solid state. Several flights of constant depth are provided on the extruder screw to level out irregularities of feed. This zone is designed with a volume capacity higher than subsequent zones to ensure a constant flow of polymer free of air pockets. The transition zone is designed to apply increasing pressure to the granulate by decreasing screw flight depth. At the same time, the temperature of the granulate, especially near the wall of the extruder barrel, increases due to the heat supplied to the barrel wall and the heat generated by the friction between the granulate and the wall. Phase transition, solid to liquid, occurs as the now semi-solid polymer moves forward through the screw flights of the transition zone. The metering zone controls the uniformity of the output melt flow; a mixing tip installed on the end of the extruder screw enhances melt uniformity by providing intimate mixing of the various melt layers.

Following the metering zone the melt passes into the parison tooling which is used to dictate the tubular shape of the extrudate.

A critical design specification of an extruder is the Length/Diameter (L/D) ratio that describes the length of the flighted portion of the screw divided by the inside diameter of the barrel. This ratio dictates the design of the extruder screw. To process a given quantity of polymer within a set period of time, a low L/D ratio extruder, contrasted with a high L/D ratio extruder, must have increased screw flight depth to compensate for the reduction in length of the processing zones. The extruder barrel is usually equipped with electrical heaters that are mainly used during cold extruder start-up to melt solid material remaining from the previous operation. Temperature set points and screw rotational speed may be adjusted to process different types of polymer. Also in common use are adiabatic extruders that rely solely on friction heat to maintain temperature during the process. Adiabatic extruders normally have a low L/D ratio and operate at high rotational speeds to achieve the same or greater outputs of polymer compared with non-adiabatic extruders.

Materials and Methods

Blow/Fill/Seal Machine

State of the art Model 624 Blow/Fill/Seal machines (Weiler Engineering, Inc., Elgin, IL), designed to produce sterile liquid product, have been used for the present studies. Each 624 machine was equipped with a 24:1 L/D single screw non-adiabatic extruder designed for polyethylene or polypropylene extrusion. Variations in machine configurations and operating parameters were used to provide a comprehensive body of performance data.

Spore Polymer

(a) Test Spore

Endospores of *Bacillus atrophaeus* (ATCC 9372), formerly classified as *Bacillus subtilis* var. *niger* (ATCC 9372), were selected as the test spore; they exist as discrete cells, produce readily scoreable colonies and possess a high resistance to inactivation by dry heat. The USP and EP recommend this particular strain of *Bacillus*

atrophaeus for preparation of biological indicators for dry heat sterilization. The source of the test spores was a freeze-dried bacterial culture obtained from the American Type Culture Collection. 50 cm^3 quantities of 3x washed stock suspensions at concentrations of around 10^9 spores cm^{-3} were produced from the surface growth on nutrient agar supplemented with manganese to aid sporulation. Prepared spore suspensions were routinely characterized for dry heat resistance through determination of the D value at 160°C for spores dried on borosilicate glass surfaces. This characterization was carried out to ensure a consistent behaviour of test spores on a standard heated surface. Spores taken from suspensions used in the present study exhibited D_{160} values on glass that were close, ranging from 0.89 to 1.22 min.

(b) Preparation of Spore Polymer

Nine 35 kg batches of spore contaminated low density polyethylene granulate (Huntsman 6010, Huntsman Corp., Salt Lake City, UT) were prepared individually at levels of nominally 10^4 and 10^6 g^{-1} polymer. Preparation was achieved through controlled deposition of discrete air dispersed spores on all surfaces of polymer granules using a purpose built drum, mounted horizontally and rotating about its longitudinal axis; air dispersed spores were generated within the drum through aerosolization of a known volume of aqueous spore suspension of pre-selected concentration. The number of deposited spores per gram polymer (designated "spore challenge level") was determined through conducting a viable count on each of 5 x 1 g samples of spore contaminated granules taken randomly from a given spore polymer batch. Viable spores were recovered from polymer granules employing sonication of each sample in 10 cm^3 sterile solution of 0.1% Tween 80 for 30 minutes at a temperature of approximately 50°C; the method achieved a recovery efficiency of > 80% spores from polymer granules.

(c) Determination of D_{160} value of test spores located on polymer granules

A sample of granules of spore contaminated polymer was withdrawn from each batch as it was prepared. Single granules from this sample were put into separate 4 cm^3 thin walled glass tubes (i.d. 9mm). Immediately prior to undertaking a D_{160} value determination, the single granules, contained individually in tubes, were heated at 56 ± 1°C for 2 hours. This conditioning ensured that spores were at a constant low water content; heating at 56°C was shown to have no effect on the viability of test spores. Following conditioning, the individual granules

held in separate tubes were located in a heating block at 160 ± 1°C; oil in the wells of the block ensured rapid heat transfer. At timed intervals, tubes were removed from the heating block and immediately cooled by placing in a water bath at 10°C. Twenty replicate granules were heated at each of a minimum of eight time intervals. Following cooling, a 2 cm^3 aliquot of sterile nutrient broth was aseptically added to each tube. The tubes and contents were incubated at 37°C for 7 days and inspected for visible growth; appropriate controls showed that the presence of polymer granules had no inhibitory effect on the growth of test spores. For each spore polymer batch, the D_{160} value and its standard deviation were calculated using the Spearman-Karber estimation procedure (4) described in USP Chapter 1035 <Biological Indicators>.

Endotoxin Polymer

(a) Test Endotoxin

Bacterial endotoxin derived from E. coli 055:B5 (sourced from both Difco Inc or Sigma Chemical Ltd) was selected for test purposes. This bacterial endotoxin is commonly used in the pharmaceutical industry to validate dry heat sterilization processes. The USP specifies that a control standard endotoxin (CSE), appropriate for depyrogenation studies, should exhibit a potency of not less than 2 EU ng^{-1} and not more than 50 EU ng^{-1}. When tested by Associates of Cape Cod Int. Inc., an accredited test facility, against the USP Reference Standard Endotoxin (RSE) prior to its use in the preparation of contaminated polymer batches, the commercial bacterial endotoxin gave potencies between 10 and 11 EU ng^{-1}.

To characterize the dry heat resistance of the supplied endotoxin, thin walled glass tubes were individually inoculated with endotoxin at typically 10^4 EU per tube, and each tube heated at 200°C for a specified time up to 480 min. Following exposure to heat, levels of endotoxin recovered in LAL reagent water (certified $<10^{-3}$ EU cm^{-3}) were assayed employing the LAL kinetic turbidimetric method (5,6). The dry heat resistance of the endotoxin at 200°C was represented by a bi-phasic curve of log recovered endotoxin plotted against exposure time; such behavior is typical for reduction in recovered endotoxin consequent upon exposure to heat (7). Overall, test endotoxin exhibited a 3 log reduction following 90 to 100 min exposures at 200°C, times typical of dried lipopolysaccharide derived from *E.coli* treated under similar conditions (8).

(b) Preparation of Endotoxin Polymer

Seven batches of Huntsman 6010 granulate, each between 10 and 30 kg, were contaminated with test endotoxin at mean levels of Endotoxin Units (EU) per gram of polymer that ranged from 1.60×10^{2} to 1.15×10^{4}. Controlled deposition of the endotoxin onto granulate surfaces occurred through aerosolization of a solution of endotoxin of known potency in equipment similar to that developed for preparation of spore polymer batches. The EU g^{-1} polymer (designated “endotoxin challenge level”) was determined through the performance of assays on each of 5 x 1 g samples of endotoxin contaminated granules taken from throughout the given endotoxin polymer batch. The deposited endotoxin was recovered from the surfaces of the granules by immersing the granules in 10 cm^{3} of LAL reagent water and subjecting the sample to two successive recovery treatments done immediately after one another; a recovery treatment comprised sonication for 10 minutes followed by vigorous vortexing for 1 minute at room temperature. Determination of the EU content per cm^{3} of recovery water, and hence the EU per gram polymer, was carried out using the LAL Kinetic Turbidimetric Assay.

(c) LAL Kinetic Turbidimetric Assay

Endotoxin assays were carried out using an LAL-5000E series 2 system (Associates of Cape Cod Int. Inc.). The system was certified to determine endotoxin concentrations to a minimum of 10^{-3} EU $cm^{-3.}$ This lower limit was reduced twenty-five fold to allow assays to be undertaken over an endotoxin concentration range from 4×10^{-5} to 10^{2} EU cm^{-3}. The assay method was validated in accordance with the FDA Guideline on Validation of the Limulus Amebocyte Lysate Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products and Medical Devices (1987) and USP <85> Bacterial Endotoxins Test.

(d) Determination of D_{150} value of test endotoxin located on polymer granules

As for spore polymer, a sample of endotoxin polymer was drawn from each batch as the batch was prepared. Individual granules from the batch were each placed in separate 4 cm^{3} thin walled glass tubes. The tubes were located in a heating block at 150 ± 1°C. This heating temperature was selected for the determinations as prolonged exposures to levels above this temperature affected the integrity of the polymer granule and hence the ability to recover the endotoxin from the granule. Following timed intervals at 150 °C extending up to 480 min, tubes were removed from the heating block and immediately cooled by placing in a water bath at 10 °C.

Following cooling, a 1.2 cm^3 volume of LAL reagent water was added to each tube and endotoxin recovered from the heated granule, employing two successive recovery treatments comprising sonication followed by vortexing and the solution assayed using the LAL kinetic turbidimetric method. Generated data in the form of a semi-logarithmic plot of recovered endotoxin against exposure time, extending over 2 log cycles, yielded a linear curve of negative slope allowing the D_{150} value to be derived from the best-fit straight line.

Processing of Spore or Endotoxin Polymer

Spore and endotoxin polymer challenges to the extrusion process were carried out on a number of separate experimental occasions at Cardinal Health Biotechnology & Sterile Life Sciences, Woodstock, IL. On each experimental occasion, the Blow/Fill/Seal machine was set up according to standard operating procedures used for validation and routine processing. Product contacting surfaces were cleaned in place with Water for Injection (USP XXV) and then sterilized by exposure to saturated steam. Prior to filling, the fill liquid was passed through a sterilizing grade hydrophilic filter located upstream of the fill nozzles; after use, the filter was tested for integrity.

For spore polymer challenges, a minimum fill volume was set according to the vi al size being processed with Tryptone Soya Broth, demonstrated to be capable of sustaining the growth of the test spore, employed as the fill medium. The extruder was set and maintained at a rotational speed and temperature that would routinely be used for the particular fill configuration and polymeric granulate. The established extruder and temperature set points are associated with a given type of polymer and container design and cannot be significantly altered without impacting negatively upon the vial formation. Immediately after processing, all broth filled vials were incubated for a minimum of 7 days at 22.5°C, inverted and then incubated for a minimum of 7 days at 32.5°C so that contamination of the vials could be assessed by appearance of visible growth. The fraction of vials contaminated was derived for each of the different spore polymer challenges.

For endotoxin polymer challenges, set-up and processing procedures were similar to those used for spore polymer, except the fill medium was Water For Injection (USP XXV); a single vial size of 5.5 cm^3 was used throughout this work together with a nominal fill volume of 5 cm^3 except on one processing occasion, C, when a fill volume of 2.0 cm^3 was employed. To assess the level of recovered endotoxin in WFI fill medium, between 5

and 10 vials were selected from across the whole of the production which commenced after an initial processing period of 10 minutes that allowed the endotoxin polymer to enter fully the barrel of the extruder. Recovery of endotoxin in WFI was achieved through application of the previously described successive treatments of sonication and vortexing. Following recovery, the level of endotoxin in WFI was assayed employing the LAL kinetic turbidimetric method.

Results and Discussion

Spore Polymer Challenges

Table Ia) lists machine configurations and settings employed for processing the nine different spore polymer batches. The different batches were processed on separate occasions employing different machine configurations ranging from 24 x 0.5 cm^3 vials to 8 x 120 cm^3 vials [see columns 2 and 3 of Table Ia)]. Differences in extruder speed and temperature settings reflect typical differences in machine operating conditions that are required for the different machine configurations.

In preparing the spore polymer, the aim was to have available batches with spore challenge levels that varied widely and batches whose levels were close, for which an estimate of the D_{160} value of the deposited spores located on the polymer, specific to the batch, would be made. Columns 2 and 3 of Table II lists, in order of increasing spore challenge level, the characteristics of the nine challenge batches. For each batch, they give the mean spore challenge level and associated standard deviation, determined from at least 3 viable counts carried out on separate occasions, and the derived D_{160} value and its standard deviation. It can be seen that the extremes of challenge level cover a difference in level of about 100 fold, while four of the nine levels (3 through 6) are effectively the same. The D_{160} values also varied appreciably, falling within the range 1.22 to 2.07 min. The remaining columns of Table II provide the vial characteristics, vial processing data and the derived outcomes from the media fills undertaken when processing the nine batches employing a variety of vial sizes.

Consideration of the findings from batches 1, 2, 5, and 8, all of which were processed using a 2.4 cm^3 vial volume, allows a rational interpretation to be applied to the results. For a given test organism exposed to a

particular set of inimical conditions, the expectation is that smaller the challenge level, the lower the number of organisms surviving which, for extruded containers, would translate into a lower fraction of units contaminated. A comparison of the characteristics and outcomes of batches 1 and 5 enables this expectation to be examined directly. These batches had spores with very similar D_{160} values, but possessed spore challenge levels that differed 100 fold and, on processing, they gave a corresponding 100 fold difference in the fraction of units contaminated. On the other hand, batches 2 and 8, possessed spore challenge levels that were not widely different from that of batch 5, but took values of D_{160} of 1.28 and 1.56 minutes respectively, in other words, resistances appreciably less than that of batch 5. Inspection of the fractions of units contaminated for batches 2 and 8, relative to the fraction seen for batch 5, shows these to be wholly in accord with the magnitudes of the their D_{160} values. Thus, the indications are that the heat resistance of the microbial challenge in the extruder will also affect container contamination and that heat lethality is, to some degree, associated with the extrusion process.

Two other batches (4 and 9) were processed to give vials of the same volume, 20 cm^3, different from that previously considered. Batch 4 had a spore challenge level and a D_{160} value that was somewhat less than those of batch 9 (Columns 2 and 3, Table II). The observed outcomes of processing, given in column 7, are 3.95×10^{-3} fraction units contaminated for batch 4 and 1.50×10^{-2} for batch 9, a difference and direction that supports the above generalizations.

Without knowing the exact temperature and time combinations experienced by the polymer during its passage through the extruder and during molding, determination of the extent of inactivation of spores by the heating associated with container formation is not feasible. The thermal conditions in the extruder are complex and inaccessible, and consequently pursuit of this form of analysis was not an option. The alternative is to examine other possible fates of spores entering the extruder that might impact on the interpretation of the present findings.

Extrusions, carried out with coloured granules or finely divided particles mixed with virgin polymer, give melts exiting the parison tooling that are wholly homogenous. This suggests that the added spores too will be uniformly distributed throughout the melt, which, in turn, means throughout the solidified polymer comprising the containers. In such circumstances, it is to be expected that there will be, on or in the container, viable spores

and probably heat inactivated spores, either encapsulated in solid polymer or borne on the internal and external container surfaces. In this context, 'surface borne' spores refers to discrete spores that are free and held on the polymer by forces of adhesion or those that are partially encapsulated and, if viable, be capable of germination and outgrowth. Clearly, heat inactivated spores of either kind will not contribute to the fraction of units contaminated, nor is it likely that wholly encapsulated viable spores will either, since access to the liquid growth medium is totally excluded. Thus, the only spores to contribute to the fraction of vials contaminated are viable internal surface borne spores, whose frequency of occurrence per unit area of surface will be directly related to the level of the spore challenge and, possibly, spore heat resistance.

To take account of the above considerations in the analysis of the overall data given in Table II, an index, normalizing the fraction units contaminated with respect of challenge level and vial internal surface area, has been derived for each challenge batch. Values of this index are plotted against corresponding D_{160} values for spores on polymer granules for the nine processed batches in Figure 2.

The general conclusion drawn from inspection of the figure is that, over the range of D_{160} values examined, there is a definite relationship between the normalized fraction of vials contaminated and the D_{160} values of the spores contaminating the polymer used to make the vials. The best fitting asymptotic curve describing this relationship is concave relative to the D_{160} value axis and it shows that the rate of increase in the index is greatest at low values of D_{160} and least at high values. The inference from this finding is that heat lethality has a role in the extrusion process. At D_{160} values of around 2 min, it appears that the index is approaching a maximal value, which, when reached, would mean that heat lethality is not operating during the extrusion process. Nonetheless, this upper limit corresponds to a low contamination frequency and probably reflects the frequency of occurrence of viable surface borne spores on the vial internal surface area only, other viable spores present during BFS molding being encapsulated in and immobilized by polymer and thus inconsequential in respect of product contamination.

Endotoxin Polymer Challenges

Table Ib) lists machine configurations and settings employed in processing seven different endotoxin polymer batches. The batches were used on three separate processing occasions designated A through C. The three

processing occasions employed the same machine configuration of 24 x 5.5 cm^3 vials. The differences in extruder speed and temperature settings recorded in Table Ib) reflect typical differences in machine operating conditions that might be required from one processing occasion to another. On occasion C, batch 7 was processed as two lots employing fill volumes of 2.0 and 5.0 cm^3 and, for convenience, the two lots are designated 7a and 7b respectively.

Table III gives the characteristics of the endotoxin polymer and fill volumes employed in processing the seven endotoxin polymer batches, together with corresponding estimates of the amounts of endotoxin present in the fill WFI. Column 2 of the table lists the mean endotoxin challenge level and associated standard deviation derived from assays performed on each of five samples taken from a given endotoxin polymer batch. For the batches processed on occasions A and C, endotoxin challenge levels were deliberately set close at 10^4 EU g^{-1} polymer, whereas for occasion B, challenge levels varied over a 70 fold range. The conditions associated with occasion A allowed assessment of the repeatability of the levels of endotoxin recovered from vials extruded from different polymer batches, those for occasion B an assessment of the effect of changing endotoxin challenge level and, for occasion C, an assessment of the impact of fill volume and storage on recovered endotoxin.

Column 3 of Table III lists the derived D_{150} value for each endotoxin polymer batch. It can be seen that D_{150} values are high and somewhat different, falling within the range 212 to 382 min. However, examination of the curves used in the determination of the D_{150} values revealed a high degree of scatter of points around the best-fit line, which, in part, reflects the imprecision of the LAL assay. These high D_{150} values show that at 150 ^oC the reduction in recovered endotoxin with time of heating is extremely slow (a tenfold reduction requiring more than 3 hours of heating), a finding wholly in accord with the results of others who have studied endotoxin recovery following heating to similar temperatures (9). For present purposes, the seven values reported in Table III are regarded as estimates of a particular D_{150} value.

For the three endotoxin polymer batches nominally at 10^4 EU g^{-1} and processed under the same heating and extrusion conditions of occasion A, mean values of recovered endotoxin are reasonably consistent, ranging from 4.42×10^{-2} to 9.03×10^{-2} EU cm^{-3}. Given that the endotoxin challenge level was in the order of 10^4 EU g^{-1} polymer, recovered levels of endotoxin in the WFI fill of extruded vials are strikingly low. Bearing in mind the temperature to which the endotoxin is exposed during extrusion (temperature settings were mainly less than 170

^{o}C) and the short length of time over which exposure occurs (estimated to be less than 10 minutes), these low levels cannot principally be a consequence of the processes responsible for the slow reduction found on heating endotoxin at 150 ^{o}C. A more likely explanation resides in dispersal of endotoxin throughout the extruded polymer mass. Recovered endotoxin can then be attributed to that available for dissolution from a defined depth of the internal surface of the extruded vial. To account for the amounts of endotoxin recovered for the different challenges processed on occasion A, assuming homogenous dispersal of endotoxin throughout the extruded polymer, depths of polymer for recovered endotoxin were calculated to range from 9 to 16 nm; this calculation was achieved through dividing the amount of recovered endotoxin in WFI fill by the product of the derived endotoxin concentration in the extruded polymer mass and the vial internal surface area. The molecular order of these calculated depths suggests that lipopolysaccharide located directly on the internal surface of the extruded vial has the potential to contaminate the vial contents whereas endotoxin encapsulated within the polymer mass is immobilized and unavailable for dissolution and contamination of vial contents.

Findings from processing endotoxin polymer having different challenge levels (occasion B) show a direct relationship exists between the level of endotoxin on polymeric granulate and that recovered from extruded vials. For the three endotoxin polymer batches processed on occasion B, a 70 fold change in endotoxin challenge level brought about a 300 fold change in recovered endotoxin. The discrepancy from direct proportionality may well be explained by the imprecision associated with the LAL assay, particularly at the low levels of recovered endotoxin found for batches 4 and 5.

For the two sets of processing conditions employed on occasion C, mean recovered levels of endotoxin from extruded vials were found to fall close to one another, 2.05×10^{-3} and to 1.57×10^{-3} EU cm^{-3} for vial fill volumes of 2.0 and 5.0 cm^{3} respectively. The somewhat higher mean value for the lower fill volumes provides some support for the notion that total recovered endotoxin from an extruded vial is independent of fill volume (i.e. the product of mean recovered endotoxin and fill volume is constant for a given vial configuration).

Recovered endotoxin from vials produced from endotoxin polymer batch 7 were also assessed following 90 days storage under ambient, ‘real’ (40% RH at 25 °C) and ‘accelerated’ (90% RH at 40 °C) conditions. Table IV gives values of mean recovered endotoxin ± standard deviation for the vials subjected to the different storage conditions, together with the corresponding value for unstored vials taken from Table III. For the two fill

volumes, mean recovered endotoxin levels for vials following 90 days storage under ambient, ‘real’ and ‘accelerated’ conditions are seen to fall within a 5 fold range (i.e. well within the precision of the LAL assay) and are close to that recorded for vials at 0 days storage. This finding provides evidence that endotoxin encapsulated during the extrusion process remains immobilised and unavailable to contaminate the packaged WFI during storage.

To take account of differences in fill volume and endotoxin challenge level between the different endotoxin polymer challenges, an index designated Endotoxin Reduction Factor (ERF), allowing for fill volume and normalising for endotoxin challenge level, has been derived for each challenge. The ERF was derived as follows,

$$ERF = \frac{\text{mean recovered endotoxin x fill volume}}{\text{endotoxin challenge level}}$$

The value taken by the ERF index provides a simple quantitative measure of the impact of endotoxin present on polymeric granulate upon the fill volume packaged within extruded vials. Values of this index are listed in column 6 of Table III.

Inspection of values of ERF reveals that endotoxin polymer batches processed on the same occasion take values that generally fall close to one another. This includes occasion A which employed three endotoxin polymer batches prepared with bacterial endotoxin obtained from two different sources (Difco Inc. and Sigma Chemicals Ltd.) and with three different lots of polymeric granulate supplied by Huntsman Corp. For the three different processing occasions, mean values of ERF were 2.7×10^4, 2.4×10^5 and 1.2×10^6 for occasions A, B and C respectively. Clearly, on each occasion, values of ERF were high and exceeded 10^4, demonstrating a low impact of presence of endotoxin on polymeric granulate upon packaged WFI. In practice, the impact is such that WFI filled in vials extruded from low density polyethylene contaminated with endotoxin at levels up to 10^4 EU g^{-1} polymer is predicted to meet the USP Endotoxin Release Limit of 0.25 EU cm^{-3} for Water For Injection. Clearly, it must be stressed that this prediction holds for the particular Blow/Fill/Seal 624 machine and to the specific conditions of machine operation.

Summary of Findings and their Relevance to Setting Quality Acceptance Limits for Polymeric Granulate

Polymeric granulate is supplied to operators of Blow/Fill/Seal technology in large bulk quantities, typically in plastic lined corrugated containers capable of holding up to several tons of material. Until use, bulk material is generally stored in a controlled temperature/humidity environment. Control data generated for bulk material at the Cardinal Health suggest that bioburden levels are routinely low with not more than 5 CFU g^{-1} polymer. Endotoxin on bulk material was generally found to be no more than 1 EU g^{-1} polymer.

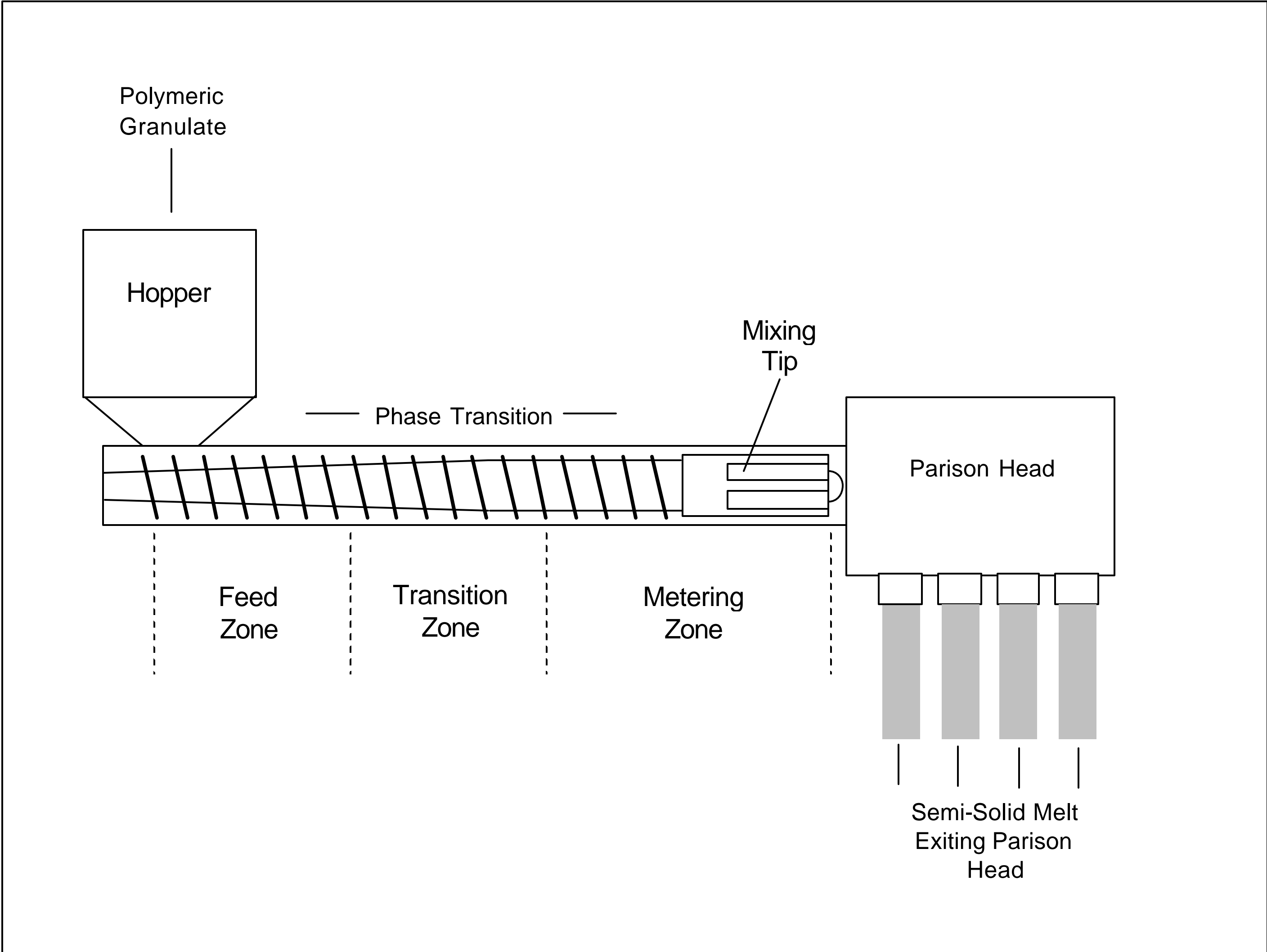
Extruder challenge studies, employing spore polymer and endotoxin polymer, have provided definitive evidence for polymer extrusion having the capability to produce vials "free" of viable microorganisms and possessing acceptable endotoxin levels. These studies have also demonstrated that the microbiological and pyrogenic qualities of the surfaces of virgin polymeric granules can impact upon the quality of the extruded vial. Thus, it is of fundamental importance in Blow/Fill/Seal processing that acceptance limits are established for bioburden and endotoxin levels on polymeric granulate to assure consistent production of vials of appropriate quality.

Through processing spore polymer, possessing known challenge levels and dry heat resistances, data have been generated to assess the extent of spore reduction associated with the extrusion process. Spore reduction is attributable to heat inactivation combined with encapsulation and immobilization of spores within the solidified polymer mass that makes up the container. Given this, it has been possible to develop a curve relating fraction of vials contaminated, normalized with respect of spore challenge level and vial internal surface area, against dry heat resistance of test spores. The nature of the curve reveals the role that heat inactivation plays in determining the fraction of extruded vials exhibiting contamination. The contribution of heat inactivation is seen to decrease with increasing heat resistance of test spores so that, at high levels of heat resistance, the fraction of vials contaminated is regarded as simply reflecting the frequency of occurrence of viable spores on internal vial surfaces. The derived relationship between vial contamination and dry heat resistance provides, for the first time, a rational scientific basis for setting bioburden limits on unprocessed polymeric granulate so that the risk of occurrence of a contaminated vial is low and acceptable. This approach of applying a bioburden limit to

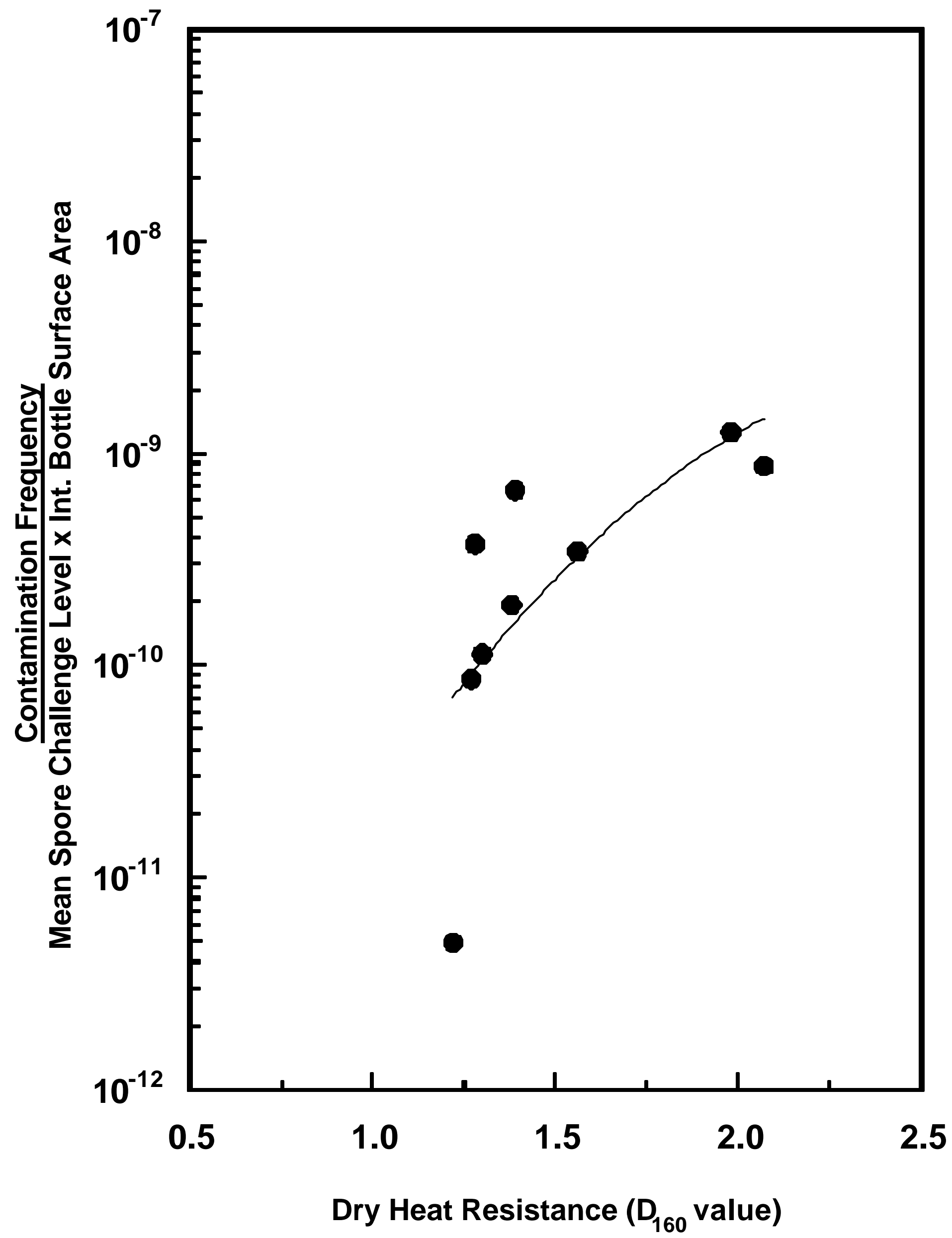
polymeric granulate to achieve the outcome of an extruded sterile vial is wholly analogous with application of bioburden limits to items to be processed by conventional methods of terminal sterilization.

Through processing endotoxin polymer, possessing known challenge levels and dry heat resistances, data have been generated to assess the extent of endotoxin reduction associated with the extrusion process. As for bacterial spores possessing high levels of heat resistance, endotoxin reduction associated with polymer extrusion is not primarily attributable to heat inactivation but rather explained by immobilisation within and on the extruded polymer. Recovered levels of endotoxin from processed vials were generally low and were directly related to the endotoxin challenge level on the unprocessed polymeric granulate. Levels of recovered endotoxin from vials were also little changed following 90 days storage, a finding illustrative of the immobilized nature of endotoxin encapsulated in extruded polymer. To quantify reductions in recoverable endotoxin consequent upon polymer extrusion, an index ERF, relating endotoxin challenge level to recovered endotoxin from extruded vials, has been devised. For the processing conditions of the present study, values taken by ERF consistently exceeded 10^4 and were found to be independent of endotoxin challenge level on unprocessed polymeric granulate. Knowledge of the ERF value allows endotoxin limits on unprocessed polymeric granulate to be rationally set so that the pyrogenic quality of the extruded Blow/Fill/Seal vial is appropriate for the intended use.

The above work is intended to provide a step forward in the understanding of the Blow/Fill/Seal extrusion process in relation to provision of a sterile vial of appropriate pyrogenic quality. However, it has to be stressed that the findings apply to the extrusion system peculiar to the Model 624 Blow/Fill/Seal machine and to the specific conditions of machine operation.



Polymeric Granulate
Hopper
Mixing Tip
Phase Transition
Parison Head
Feed Zone
Transition Zone
Metering Zone
Semi-Solid Melt Exiting Parison Head



10^{-7}
10^{-8}
10^{-9}
10^{-10}
10^{-11}
10^{-12}
Contamination Frequency
Mean Spore Challenge Level x Int. Bottle Surface Area
0.5
1.0
1.5
2.0
2.5
Dry Heat Resistance (D_{160} value)

Figure Captions

Figure 1

Typical Blow/Fill/Seal single screw extrusion system

Figure 2

Best fit asymptotic curve showing the relationship between fraction of vials contaminated, normalized for spore challenge level and internal bottle surface area, and dry heat resistance

Table I

BFS machine processing configurations and settings

a) *Spore polymer challenges*

Batch No.	No. Fill Cavities	Nominal Vial Volume (cm^3)	Extruder Speed (rpm)	Extruder Temp. Settings (°C)			
				Zone 1	Zone 2	Zone 3	Parison Head
1	24	2.4	62	155	160	163	166
2	24	2.4	62	155	160	163	166
3	8	120	103	160	163	166	168
4	15	20	90	155	163	166	166
5	24	2.4	62	155	160	163	166
6	24	0.5	83	155	157	163	166
7	24	5.5	70	155	160	163	166
8	24	2.4	62	155	160	163	166
9	15	20	90	155	163	166	166

b) *Endotoxin polymer challenges*

Proc. Occasion	Batch No.	No. Fill Cavities	Nominal Vial Volume (cm^3)	Extruder Speed (rpm)	Extruder Temp. Settings (°C)			
					Zone 1	Zone 2	Zone 3	Parison Head
A	1	24	5.5	86	157	160	163	166
	2	24	5.5	86	157	160	163	166
	3	24	5.5	86	157	160	163	166
B	4	24	5.5	67	163	166	166	171
	5	24	5.5	67	163	166	166	171
	6	24	5.5	67	163	166	166	171
C	7a	24	5.5	67	163	166	166	171
	7b	24	5.5	67	163	166	166	171

Table II

Characteristics of the nine different spore polymer challenges together with outcomes of the corresponding media fills

Batch No.	Spore Challenge Level ± S.D. (spores g^{-1})	D_{160} value ± S.D. (min)	Vial Internal Surface Area (cm^2)	No. Units Filled	No. Units Contaminated	Fraction Units Contaminated
1	$9.7 \pm 3.0 \times 10^3$	2.07 ± 0.04	12.9	9216	1	1.09×10^{-4}
2	$4.6 \pm 2.2 \times 10^5$	1.28 ± 0.02	12.9	11256	25	2.22×10^{-3}
3	$9.0 \pm 5.4 \times 10^5$	1.30 ± 0.03	158.7	1296	21	1.62×10^{-2}
4	$1.0 \pm 0.3 \times 10^6$	1.27 ± 0.03	45.8	3795	15	3.95×10^{-3}
5	$1.0 \pm 0.3 \times 10^6$	1.98 ± 0.02	12.9	7656	124	1.62×10^{-2}
6	$1.1 \pm 0.6 \times 10^6$	1.39 ± 0.02	3.0	6799	15	2.21×10^{-3}
7	$1.3 \pm 0.6 \times 10^6$	1.22 ± 0.02	28.7	5438	1	1.84×10^{-4}
8	$1.4 \pm 0.4 \times 10^6$	1.56 ± 0.02	12.9	9024	56	6.21×10^{-3}
9	$1.7 \pm 0.3 \times 10^6$	1.38 ± 0.02	45.8	2205	33	1.50×10^{-2}

Table III

Characteristics of the eight endotoxin polymer challenges together with outcomes of assaying the corresponding WFI fills

Batch No.	Endotoxin Challenge Level ± S.D. (EU g^{-1})	D_{150} Value (min)	Fill Volume (cm^3)	Mean Recovered Endotoxin in WFI ± S.D. (EU cm^{-3})	Endotoxin Reduction Factor (ERF)
1	$6.67 \pm 1.06 \times 10^3$	2.44×10^2	5.0	$4.42 \pm 0.96 \times 10^{-2}$	3.0×10^4
2	$7.29 \pm 0.20 \times 10^3$	3.82×10^2	5.0	$9.03 \pm 3.89 \times 10^{-2}$	1.6×10^4
3	$1.02 \pm 0.23 \times 10^4$	2.17×10^2	5.0	$5.84 \pm 3.14 \times 10^{-2}$	3.5×10^4
4	$1.60 \pm 0.79 \times 10^2$	2.60×10^2	5.0	$1.27 \pm 0.87 \times 10^{-4}$	2.5×10^5
5	$8.07 \pm 1.34 \times 10^2$	3.47×10^2	5.0	$3.97 \pm 2.29 \times 10^{-4}$	4.1×10^5
6	$1.15 \pm 0.11 \times 10^4$	2.30×10^2	5.0	$4.00 \pm 1.38 \times 10^{-2}$	5.8×10^4
7a	$6.48 \pm 0.87 \times 10^3$	2.12×10^2	2.0	$2.05 \pm 2.47 \times 10^{-3}$	1.6×10^6
7b	$6.48 \pm 0.87 \times 10^3$	2.12×10^2	5.0	$1.57 \pm 1.81 \times 10^{-3}$	8.3×10^5

Table IV

Endotoxin recovered from vials formed from endotoxin polymer batches 7a (2.0 cm^3 fill vol.) and 7b (5.0 cm^3 fill vol.) following 0 and 90 days storage under ambient, 'real' and 'accelerated' conditions

Batch No.	Mean Recovered Endotoxin in WFI ± S.D. (EU cm^{-3})			
	0 days storage	90 days storage		
		ambient	'real' (40% RH at 25°C)	'accelerated'(90% RH at 40°C)
7a	$2.05 \pm 2.47 \times 10^{-3}$	$4.12 \pm 10.79 \times 10^{-3}$	$7.85 \pm 7.03 \times 10^{-4}$	$1.20 \pm 0.66 \times 10^{-3}$
7b	$1.57 \pm 1.81 \times 10^{-3}$	$4.25 \pm 4.13 \times 10^{-3}$	$4.13 \pm 4.39 \times 10^{-3}$	$2.13 \pm 1.99 \times 10^{-3}$

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