Food Packaging and Shelf Life xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Food Packaging and Shelf Life

journal homepage: www.elsevier.com/locate/fpsl

Leakage analysis of flexible packaging: Establishment of a correlation between mass extraction leakage test and microbial ingress

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ARTICLE INFO

Keywords:
Microchannel defects
Mass extraction
Package integrity
Shelf-life
Food safety

ABSTRACT

Package integrity over the shelf life of a product is critical in order to demonstrate the ability to maintain product sterility. The development of a rational physical test method to evaluate the microbial barrier properties of sterile packages has necessitated its correlation to microbiological exclusion. In this study, the ability of a mass extraction leak detector to assess the integrity of multilayer pouches with a perforated microchannel was investigated as an alternative to the microbial challenge test. Results were obtained for defects of four different sizes (15 to100 μm, with 5 mm channel lengths within the sealing component). The results of the tests indicated that the leak tester possessed the ability to detect microchannel defects in sealed packages. Moreover, the mass extraction system was able to reliably distinguish between intact samples and samples with the aforementioned defects. The results of this study could be used to develop a time saving alternative method to analyze the sealing quality of flexible packages.

1. Introduction

Package integrity is a critical quality attribute for packaging of sterile products as it is important for the protection of both the environment and the product itself. Pouch sterility must be maintained to avoid product contamination; hence, any defects to the sterile condition are considered to be a serious risk and often result in the disposal of valuable products. In other words, it is important to maintain the shelf life of a product until it reaches the consumer. Multilayer flexible pouches have replaced some forms of heavy packaging such as glass and cans. All types of flexible packaging provide a seal to close the package. Typically, the sealing areas in flexible packaging are bonded together by the application of heat. Therefore, these areas are where most leaks would be expected to occur. A wrinkle in a sealing area can form a channel leak because the leak length is significantly larger than the diameter. However, sealing must be maintained to ensure product sterility.

The microbial barrier properties of sterile product packaging are typically determined through package integrity testing. This test method can be accomplished by challenging packages with microorganism exposure. However, FDA guidelines encourage the industry to develop package integrity and physical test methods in lieu of microbial testing (FDA, 2004, 2008). The use of physical methods to test package integrity depends on the establishment of a correlation between microbial failure and physical measurements (Morrical et al., 2007; Pethe and Dove, 2011; Singer, 2014).

Physical leak detection methods do not directly measure microbial ingress; rather, they measure some physical property of the leak that can be attributed to microbial failure. Additionally, validation studies supporting the microbial integrity of packages are required. Current physical technologies in the packaging industry are capable of detecting leak rates, which is equivalent to detecting the size of defects. Thus, the industry needs a method to test the integrity of flexible packaging that can detect defects corresponding to the minimum size needed to block waterborne microbes (FDA, 2004, 2008).

One of the major questions associated with package integrity and physical testing development is the detection limits (or sensitivity) that should be achieved by the method. Extensive studies have focused on determining the smallest defect sizes allowing for microbial penetration into packages, which have been shown to be as wide as 0.2–80 μm. Lampi (1980) established and Chen, Harte, Lai, Pestka, and Henyon (1991) and Keller et al. (1996) independently substantiated that the critical dimension for defects, allowing bacterial penetration into flexible pouches, was 11 μm or less, while Gilchrist, Shah, Radle, and Dickerson (1989) determined that the critical dimension was twice that (i.e., 22 μm). Blakiston et al. (1996) later established that the critical size for leaks was 7 μm. Furthermore, other physical test methods have demonstrated varying results for different defect shapes. Therefore, it is

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https://doi.org/10.1016/j.fpsl.2018.02.004
Received 14 June 2017; Received in revised form 26 January 2018; Accepted 14 February 2018

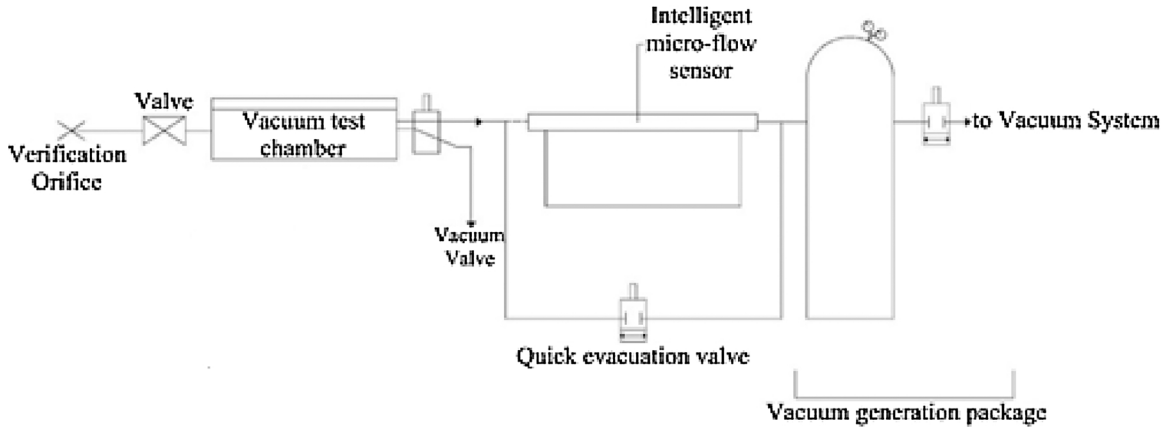

Fig. 1. Diagram of the mass extraction apparatus.

important that defects be defined when demonstrating the capabilities of a method (Yoon, Sagi, Goldhammer, & Li, 2012). A defect can be defined as an unintended crack, hole, or porosity in the walls of a material or a sealing part (Lampi, 1980), which must contain or exclude different fluids and gases while allowing the escape of a closed medium (Pethe and Dove, 2011). If a packaging system contains defects, channels can be created in the packaging, and microorganisms can ingress through the channels, affecting the safety of the product (Yoon et al., 2012).

This study focused on investigating the mass extraction package integrity test method and its application for testing the integrity of seals in pouch type packaging in combination with previously reported bioaerosol procedures (Moghimi, Kim, & Park, 2016). Therefore, mass extraction, a mass flow measurement technology, was introduced as a limit test; the detection limits were determined using the mass extraction measurement system. However, it was essential to determine the accuracy of the system by which the equipment could perform tests in order to develop confidence in its ability as an alternative to traditional microbial methods.

The objective of this study was to determine the critical defect dimensions at which sterile channels form in pharmaceutical/food packages. However, a bioaerosol challenge test has been used to determine the critical size of leaks in the seals of LLDPE/nylon laminated pouches. Furthermore, validation of the mass extraction-based procedure was evaluated in relation to the microbial challenge method on a significant scale. Therefore, an indirect correlation was established by comparing the results of physical and microbial tests on samples containing leaks of various sizes.

2. Materials and methods

2.1. Test pouches

The tests were performed on flexible laminated pouches with an overall dimension of 170 mm × 100 mm (BNA Co., Korea). The pouches were made from linear low-density polyethylene/nylon (LLDPE/nylon), with a nominal filling volume of 150 mL, and total thickness of 113 μm with 15 μm of nylon layer.

2.2. Defect simulation and sample preparation

It was necessary to produce defects that were repeatable and consistent to demonstrate the capabilities of the method when performing tests to quantify microbial ingress into sterile packages (Kassarjian, Bello, Bix, Burgess, & Linz, 2014). Channel leaks were intentionally prepared using a technique according to the ASTM F1929 (2015) test method. Only one leak was created by puncturing each pouch with a tungsten wire ranging in size from 100, 50, 25, and 15 μm in diameter (W558911, Oxford, England) across the sealing lip. Tungsten wire was placed at a right angle to the sealing line, sealed with a sealer (ISA-350-10, INNOSEAL, Korea) and then pulled through the seal after sealing. The microchannel length for defects of all sizes was about 5 mm.

2.3. Microbiological challenge test

A total of 1200 pouches were subjected to the bioaerosol challenge test. The testing unit and operating procedures to determine the critical leak size for flexible packaging sterility using the bioaerosol challenge test have been previously reported (Moghimi et al., 2016). Defective test pouches were aseptically filled with 135 mL of a microbial growth medium (Trypticase Soy Broth). Pouches attributed to the bioaerosol chamber were developed by Keller (1998) with an internal volume of 0.13 m^3 and were assembled with a nebulizer kit (Pari LC SPIRINT 0123, Germany) containing approximately 2.0×10^7 CFU m^{-3} of *Staphylococcus aureus* (*KCCM 11335*) and *Escherichia coli* (*DH 5α*). A negative control was prepared to verify the sterility of the pouches and to validate the aseptic filling process; a positive control was prepared to verify the growth of the test microorganisms inoculated into the pouches. For the positive control, a sample without defects was injected with 0.2 mL of a 10^3 CFU/mL solution of organisms. The test pouches were subsequently incubated at 37 °C. The growth medium inside the test pouches was periodically checked for microbial growth over a period of 14 days, indicating microbial ingress.

2.4. The mass extraction leak detector

A diagram of the mass extraction equipment system is shown in Fig. 1. The leak tester was a VE2 mass extraction detector manufactured by ATC Inc. (Advanced Test Concepts Inc., Indianapolis, IN, USA). A test sample (shown in the diagram) was placed inside a test chamber constructed of stainless steel. Yoon et al. (2012) described the equipment and its operation to test the integrity of vials, syringes, and cartridges. In the current study, the method was modified to leak test flexible pouch packages. In this flow regime, the micro-flow sensor (Intelligent Gas Leak Sensor or IGLS) physical signal was proportional to the volumetric flow rate. Therefore, the units in this study were volumetric flow units or mm^3/min. The mass extraction test possessed four major steps to assess package integrity. The approximate testing time required for each step should thus be determined.

1. Quick evacuation step: the chamber starts under barometric conditions, and then most of the air surrounding the packaging is removed (2–3 seconds is sufficient for flexible pouches). 2. Large leak step: a quick initial check for any gross leaks. If there are gross leaks, the sensor can detect the gross leak quickly via an excessive flow rate and/or pressure increase, and the instrument will stop immediately. 3. Evacuation step: the instrument performs a long evacuation step if there