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An innovative challenge test for solid cosmetics using freeze-dried microorganisms and electrical methods

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ABSTRACT

Freeze-dried bacteria and fungi were used as inoculum in 28 days' PET. An electrical method was used in replacement of the conventional plate count method. The use of freeze-dried microorganisms in association with the electrical method can minimize the workload and the variability involved in PET for cosmetic powders.

1. Introduction

The cosmetic industry has been expanding and growing steadily in recent decades. Sales in the beauty and personal care industry reached US$68.7 billion in the United States and US$433.4 billion globally in 2012. The US industry will reach US$ 81.7 billion by 2017 according to the Euromonitor International Forecast by Direct Selling News (Direct Selling News, 2014). In Brazil, according to the 2012 Catalogue of the Brazilian Association of Cosmetics, Toiletries, and Perfume Industry (ABIHPEC), this sector earned R$ 29.4 billion in 2011 ex-factory amounts, which represents 1.7% of Brazil's GDP. During this same period, the sector grew by 4.6%, more than Brazil's overall total GPD, which grew by only 0.1%. Brazil ranks 3rd in the global makeup market. A joint survey conducted by ABIHPEC and Booz & Company revealed that the consumption of products in the sector is expected to grow at around 5% per year by 2015 (ABIHPEC, 2014).

The cosmetic industry is a very dynamic segment that readily embraces innovation to maintain a competitive edge and must offer high quality products, involving esthetic considerations and microbiological quality. Cosmetics have multiple uses, and it is not unusual that they are stored under a variety of conditions, including warm and moist bathrooms, women's handbags as well as forgotten in cars and then used again. In this context, an adequate preservative system must be used to assure product integrity during its shelf life (Russel, 2003) and use (Orth, 1989; Magee et al., 1997; Russel, 2003). However, the preservative system should not replace good manufacturing practices or reduce the viable microbial population of a nonsterile product (USP, 35).

The preservative efficacy test (PET) is performed to determine the minimum effective concentration of antimicrobial preservatives required for adequate preservation of cosmetic and pharmaceutical products (Orth, 1991, 1997). This test assures the quality of the cosmetic before it is marketed. Despite all efforts to continuously improve the microbiological quality of cosmetics, microbial contamination of commercially available products are still reported in the literature of developing countries (Abdelaziz et al., 1989; Okeke and Lamikanra, 2001; Shaqra and Al-Groom, 2012; Tan et al., 2013), and even in Italy (Campana et al., 2006).

Part of PET involves challenging a sample using different microorganisms and determining survivors in specific time intervals by conventional microbial counting (challenge test). A challenge test is performed to verify if the preservative system (types of preservatives and their concentrations) is capable of inhibiting microbial growth. This method is applied only to liquid and semi-solid products, even though solid cosmetic products can also suffer microbial contamination with repeated use by consumers.

To evaluate samples, it is recommended to challenge them with microbial suspensions (Magee et al., 1997; British Pharmacopeia, 2010; Unites States Pharmacopeia, 2012). However, for solid powders it is difficult to guarantee microorganism homogeneity in the sample. This difficulty leads to high variability in the results. A freeze-dried inoculum could facilitate the sample homogeneity, since a mixture involving powder is much easier to obtain. Considering this, the use of freeze-dried microorganisms can be a practical alternative to PET.

Periodic evaluation of the survivors in preservative efficacy testing using the pour plate technique involves extensive work in preparing

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the material, executing the test and counting plates. Several alternatives to traditional colony-count techniques have been developed.

Electrical methods have been proposed as promising alternatives. This method is based on the modifications of electrical properties in culture medium due to metabolism of the microorganism (Jeffrey et al., 1989; Connolly et al., 1993, 1994; Zhou and King, 1995; Pinto et al., 1999; Basa and Flores, 2000; Chorianopoulos et al., 2008; Yang. and Bashir, 2008). Non-charged molecules are turned into charged molecules for microorganism growth, so these electrical changes may detect microbial growth by the automatic system using signals such as conductance, capacitance and impedance (Pinto et al., 1999; Szita et al., 2007). Among the advantages, this method requires less material, labor and time (Connolly et al., 1993; Connolly et al., 1994). Many authors found promising results using this method for bacteria determination (Connolly et al., 1993; Chorianopoulos et al., 2008; Priego et al., 2011).

In this context, the aim of this study is to evaluate the application of freeze-dried microorganisms as inoculum in the preservative efficacy test for a solid cosmetic and to verify the applicability of the impedance method to determine survivor microorganisms instead of using the plate count technique for bacteria.

2. Materials and methods

2.1. Freeze-dried microorganisms in preservative efficacy test

2.1.1. Microorganisms

The test organisms consisted of strains of freeze-dried *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404 (Souza and Ohara, 2003).

2.1.2. Cosmetic sample

The samples used in this study consisted of a powdered eye-shadow containing 1.0% (p/p) of Glydant plus® (DMDM hydantoin and iodopropynyl butylcarbamate).

2.1.3. Inactivation of the preservative system

The preservative system was inactivated by using the decimal dilution of three different diluents: 1) Peptone 1.0%, sodium thiosulfate 0.6%, sodium bisulfite 0.25%, soy lecithin 0.7%, and polysorbate 80 0.5% in distilled water to the bacteria (Diluent 1). 2) Peptone 2.0%, sodium thiosulfate 0.6%, sodium bisulfite 0.1%, soy lecithin 1.0%, and polysorbate 80 3.0% in distilled water was added to yeast (Diluent 2). 3) Casein soy broth 3.0%, soy lecithin 0.5% and polysorbate 20 4.0% in distilled water (Diluent 3) to the mold. All of the media were previously sterilized.

2.1.4. Challenge test

One-vial containing 10^7 CFU of the freeze-dried microorganisms (Souza and Ohara, 2003) was mixed gradually with 15 g of the sample. The inoculated samples were maintained in glass bottles at room temperature and samples were aseptically removed after 0 h, 2 h, 4 h, 8 h, 24 h, 48 h, 7 days, 14 days, 21 days and 28 days for viable counting. The sample was diluted as described in the inactivation of the preservative system, and after 30 min of contact, 10-fold serial dilutions were made.

The pour plate technique was performed on a 1-mL aliquot taken from the appropriate dilution using Tryptic Soy Agar for the bacteria and Sabouraud Dextrose Agar for the fungi. The incubation time was 48 h at 32 ± 2.5 °C for bacteria and yeast; for the mold it was 72 h at 22.5 ± 2.5 °C.

At least six tests were performed for each microorganism and the results were compared with the specifications of the official compendia and CTFA.

2.2. Challenge test using electrical methods to determine the survivors

2.2.1. Microorganisms

The test organisms consisted of strains of freeze-dried *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 9027, *A. niger* ATCC 16404 and *C. albicans* ATCC 10231.

2.2.2. Electrical device

A Bactometer® model-128 microbial monitoring system (Biolab Merieux®) was used. It has eight plastic modules in one unit. Each module contains sixteen molded wells. Each well contains two stainless steel electrodes holding up to 2 mL of culture medium. A total of 128 samples can be monitored simultaneously in one unit. The instrument uses three detection modes: impedance, capacitance and conductance.

2.2.3. Signal choice (impedance, capacitance and conductance)

Volumes of 1.5 mL of GPMplus Bactometer® medium were added to the wells at least 24 h before use.

A suspension containing 10% of eye shadow was prepared using Diluent 2 as described in the inactivation of the preservative system for both bacteria. This mixture was held stationary for 30 min before the test execution.

The freeze-dried microorganism was recovered in 10 mL of saline solution 0.9% (w/v), and was diluted to give a bacterial concentration range from 10^2 to 10^7 CFU/mL. A total of 0.1 mL of each dilution was withdrawn and transferred to a group of six wells containing the culture media in order to obtain 10 to 10^6 CFU/well. The amount of 0.1 mL of the diluted sample described above was added to each well.

The detection time (DT) was determined using impedance, capacitance and conductance signals for each two wells.

The incubation temperature was 35 °C for bacteria for 24 h and 28 °C for the fungi for 100 h.

Plate counting was performed in parallel using 1.0 mL of the same microorganism suspension used above diluted to present around 10^2 CFU/mL. Tryptic Soy Agar was used as media and the incubation time was 24–48 h at 32 ± 2.5 °C.

The choice of the better signal was based on the data obtained in this test and also a calibration was conducted to correlate plate count with the 3 signals used.

2.2.4. Challenge test and determination of the survivors by impedance method

The test was conducted as described in the challenge test using freeze-dried microorganisms in the preservative efficacy test. Samples were aseptically removed after 0 h, 2 h, 4 h, 8 h, 24 h, 48 h, 7 days, 14 days, 21 days and 28 days to determine the detection time (DT). Two tests were performed in duplicate for *S. aureus*, *P. aeruginosa* and *A. niger*.

The wells were incubated at 35 °C for 24 h to obtain the detection time (DT). The number of survivors was established using the calibration curve.

2.2.5. Statistical analysis

PET results obtained from electrical and pour plate methods were compared using a linear least square regression analysis. We assumed that both impedance and pour plate methods were equivalent if the confidential intervals for slope and intercept include the values 1.0 and 0.0, respectively.

3. Results

3.1. Use of freeze-dried microorganisms in the challenge test

Table 1 shows the average number of survivors of *S. aureus*, *P. aeruginosa*, *C. albicans* and *A. niger*. On the 7th day, the number of