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Strategies for the Microbiological Testing of Cosmetic Gels

Qasem M. Abu Shaqra[1*], Aseel Q. Abu Shaqra[2] and Mohammad Q. Abu Shaqra[2]

[1]Department of Allied Medical Sciences, Zarqa University College, Al-Balqa Applied University, Jordan.
[2]Quality Control Laboratories, Jordan Medical Solutions Mfg. Company, Zarqa, Jordan.

Authors' contributions

This work was carried out in collaboration between all authors. Author QMAS designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author AQAS managed the literature searches and performed the experimental investigations while author MQAS conducted the statistical analyses of the study. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Background: Official methods for the microbiological testing of cosmetics are available; evidence is required for the suitability of procedure employed particularly while dealing with thick or viscous preparations such as gel formulations.
Aim: To illustrate the inherent problems that exist in conventional methodologies used for the microbiological testing of cosmetic gels and to recommend new strategies which could yield more consistent results.
Place and Duration of Study: The work was carried out at the quality control laboratories in Jordan Medical Solutions Manufacturing Company over a period of 4 months
Methodology: A carbomer based hair styling gel formulation and a cell suspension of *Pseudomonas aeruginosa* ATCC 9027 were prepared following standard procedures. Homogeneous dispersion of the prepared microbial challenge into the gel was achieved by two approaches. The first depended on inoculating the gel preparation prior to the addition of alkali while the second relied on breaking down the gel matrix by acidification of the original gel to pH 4

**Corresponding author: E-mail: qabushaqra@hotmail.com;*

in order to allow the release of contaminants from the gel matrix. The traditional pour plate technique on 1 ml sample was used for microbial count.
Results: The percentage coefficient variation (PCV) in bacterial count recovered from the gel using the traditional approach was 30.58% whereas; when the proposed strategies were employed the PCV was 7.15%. The smaller the PCV, the better is the precision and repeatability of the assay.
Conclusion: Strategies presented herein are more suitable for the microbiological testing of cosmetic gels than the known routine methods.

Keywords: Cosmetic gels; microbiological testing; pH; homogeneity of inoculums; bacterial recovery.

1. INTRODUCTION

Many cosmetic products currently available in the market are in the form of gel. Most of these preparations are basically aqueous and contain carboxy- vinylpolymer which thickens upon the addition of alkali [1,2]. They also contain a variety of ingredients to provide the product with required functional properties. The pH of such products is typically within a degree on either sides of neutrality [3] and marketed brands are stored by consumers at room temperature or in slightly warmer places. Thus, physical and chemical factors required for microbial growth are all fulfilled by the environment of gel formulations.

As the case is with all cosmetics packed in multi use containers, gels are usually protected from microorganisms that might be inadvertently introduced during or subsequent to the manufacturing process by the addition of preservatives [4]. Despite precautions taken by cosmetics manufacturers to prevent occurrence of contamination, surveys conducted in different parts of the world demonstrated that this problem is far from being solved [5,6]. Sutton and Jimenez [7] showed that cosmetics constituted 7.0 % of products recalled from the market place in the United States of America due to microbiological problems and gels were among the products recalled.

Cosmetics are microbiologically tested prior to marketing in order to safe guard product from spoilage and consumers from possible health hazards. Such testing includes microbial count, isolation of specified organisms and preservative efficacy testing. Details of techniques for testing cosmetics has been published by regulatory and standardization bodies [ISO "8" and USP "9"]. Certain products may require special techniques while handling them in the laboratory and therefore, verification of procedures used should be established [10]. For instance adequate release of organisms from product matrix is essential for accurate microbial count particularly in viscous preparations such as gels. Official microbiological tests can be performed in a straight forward manner for water soluble formulations but those which are viscous may require extra experimental procedures. The objectives of this communication were to draw attention to the difficulties encountered in the microbiological testing of cosmetic gels and second to propose strategies for handling these formulations while processing in the microbiology laboratory.

2. MATERIALS AND METHODS

2.1 Gel Formulation

A simple hair styling gel formulation was prepared as follows: Forty gm (4%) of Polyvinyl-pyrrolidone was mixed with 920 ml of sterile distilled water, then 20 gm (2%) of Carbomer 934 fine powder was sprinkled slowly while mixing and the gel was formed after the addition of 20 gm (2%) triethanolamine. The final pH of the preparation was 7.2. Although this preparation did not contain any preservative, it was made up in a laminar flaw cabinet and precautions were taken to prevent contamination during preparations. Bio- burden of this product was then tested to establish the number of contaminants if at all detectable.

2.2 Preparation of Inoculums

The organism used throughout this investigation was *Pseudomonas aeruginosa* ATCC 9027. The inoculum was prepared following the technique described by Sutton [11] using 0.5 MacFarland standards as the index of turbidity. The cell suspension so prepared contained 2×10^8 Colony Forming Units ml^{-1} and this was confirmed by plate count. Further 10 fold dilutions of this suspension to contain specific bacterial numbers were made as required.

2.3 Microbial Challenge Distribution Study and Bacterial Count

2.3.1 Traditional methods for bacterial challenge and count

One ml aliquot of the above prepared bacterial suspension containing 2 $x10^6$ CFU ml^{-1} was used to inoculate 200 ml of the prepared gel placed in sterile 500 ml flask. The flask was shaken in an orbital shaker at 250 rpm for 5 minutes, allowed to set for 1 hour before 10 samples of 1 ml aliquot were withdrawn from the flask, each aliquot was dispersed in 9 ml phosphate buffer pH 7 and serial dilutions were made for the determination of microbial count using the traditional pour plate technique.

2.3.2 New strategy for bacterial challenge and count

The same experiment performed in 2.3.1 was repeated with slight modification. In this case, inoculum was introduced into the gel formulation prior to the addition of alkali and then shaken for 5 minutes. After 1 hour of gel formation (addition of triethanolamine), samples were taken as described above but the initial dilution was made in phosphate buffer pH 4. Other serial dilutions were carried out in the same buffer but with pH 7. The time of exposure to the acidified dilution did not exceed 3 minutes in order to prevent possible microbial injury due to low pH.

2.3.3 Testing ready made gel in accordance with the new strategy

For products that can't be prepared in the laboratory, the pH of the preparation was tested and the whole product was acidified to pH 4. At this point inoculum was introduced, homogenized by shaking and then the original pH of the product was restored by the addition of alkali. Samples were then collected for processing as described in 2.3.2.

2.4 Statistical Analysis

Statistical analysis was performed using online alcula statistics calculator (www.alcula.com/calculators/statistics/box-plot). This was employed to calculate standard deviation, mean values, quartiles and coefficient of variance. The percent coefficient of variation (PCV) represents the ratio of the standard deviation to the mean multiplied by 100.

3. RESULTS AND DISCUSSION

Unless properly preserved; cosmetic gels are susceptible to microbial contamination [6,7]. This short communication describes some of the inherent problems encountered in testing the microbiological quality of cosmetic gels and provides solutions to these problems. For this purpose, two strategies were adopted. The first was to handle gels as described by official guidelines [9] and the second was to test gels in accordance with new strategies. The variation in results obtained using the two approaches is presented and discussed below.

As indicated in the material and methods section, the gel was prepared under aseptic conditions. Bio-burden testing of this preparation revealed that the number of bacteria recovered from it was almost close to zero (never exceeded 10 CFU^{-ml}). The experiment described in 2.3.1 was performed to determine wither straight forward inoculation of readymade gel would result in an even distribution of microbial challenge throughout the inoculated product. The statistical variables presented in Table 1 clearly demonstrate that when inoculation and counting were performed in accordance with routine technique, results varied greatly from those which were obtained using the new approach. Although the table is self explanatory, some of the figures shown require clarification.

Quartiles of a ranked set of data values are the three points that divide the data set into four equal groups; each group comprises an equal quarter (25%) of the data. The first quartile value is defined as the middle numbers between the lowest and the median of the data set whereas; the third quartile is the middle value between the median and the highest value of the data set. Findings related to the third quartile of counts, obtained using the traditional method gave counts 11325 CFU^{-1}, whereas, the same quartile secured by the new strategy gave count of 9625 CFU^{-1}. The original number of bacteria inoculated into the gels was supposed to theoretically yield 10^4 CFU^{-1} but in order to eliminate the working error, the mean was taken as target count.

Table 1. Variation in statistical parameters calculated for bacterial counts obtained using routine and proposed strategies

Statistical parameters	Routine technique	New strategy
Lowest bacterial count	5800 CFU ml^{-1}	8200 CFU ml^{-1}
Highest bacterial count	13000 CFU ml^{-1}	9700 CFU ml^{-1}
Mean bacterial "count average"	8780 CFU ml^{-1}	9090 CFU ml^{-1}
Median	7450 CFU ml^{-1}	9425 CFU ml^{-1}
First quartile	6625 CFU ml^{-1}	8375 CFU ml^{-1}
Third quartile	11325 CFU ml^{-1}	9625 CFU ml^{-1}
Standard Deviation, σ	2684.85	650.55
Coefficient of variation	0.358	0.0715
Total number of samples	10	10
percentage coefficient variation	30.580%	7.158%

The great variation in microbial count from the mean when the conventional method was used indicated the agglomeration and concentration of bacterial cells in certain places of the gel at the expense of other places. These observations suggest one of two possibilities. The first is that homogeneity of inoculum throughout the readymade gel was not achieved by simple shaking and the second is related to the counting method employed. In case of the first possibility, lack of homogeneity can be attributed to product rheology. Carbomer based gel matrix is composed of cross-linked network structure which requires a high stress in order to flow or to break [12,13]. Therefore, shaking using the orbital shaker was not enough to break down the gel and consequently even dispersion of inoculum was not sustained. The second reason which is related to the microbial counting method suggests that microbial contaminants were not released from the gel matrix into the phosphate buffer (pH 7.0) used in making serial dilutions for plate counts. The pH of 7.0 could have favored microorganisms to remain entrapped within the gel matrix and therefore, microorganisms were not released into the diluent.

The approaches adopted in this study relied on the fact that Carbomer based gel formulations remain in the liquid state until the alkali is added. When the product is in the fluid consistency and is inoculated with microorganisms, simple mixing will ensure the homogeneity of the inoculum throughout the product. Afterwards, addition of the alkali will result in the formation of gel that contains evenly distributed microbial communities in its matrix. Data in Table 1 shows that samples handled using this approach and bacterial count carried out by diluting initial samples in phosphate buffer pH 4 resulted in more consistent microbiological results as compared to those obtained using routine methods. Percentage coefficient variation (PCV) is an indication to precision and repeatability of the assay; the smaller the value the better is the assay. The PCV calculated for the bacterial count obtained by the conventional method was 30.58% whereas; it was 7.157% when the new approach was used. These parametric percentages indicate that consistency of results was improved by at least 4 times when the new strategy was used for microbiological testing of gel formulations.

In the preliminary stage of this investigation it was found that adding acid to gels resulted in a rapid liquefaction of its matrix and consequently easy release of microorganisms. It is noteworthy that a pH 4 was not enough to completely liquefy the product but increased its fluidity; this increase in fluidity in addition to simple mixing using a shaker was enough for microbial homogeneity. Islam et al. [12] demonstrated that the viscosity of dispersed carbomer in water remained constant from pH 5 to 8 and then decreased outside this range. Results obtained showed that a pH below 4 is required to completely break down the gel, but the pH used here and the simple mixing were found to be good enough for microbiological testing.

Contamination testing of cosmetics is routinely performed as part of product quality control program. The experiment presented in 2.3.3 was designed to mimic a contaminated product which is to be tested for its bio- burden. Testing was performed after acidification of product and results were almost the same as those given in Table 1 for experiment 2.3.2. Therefore results are not shown. As indicated by Sutton [14] compendial methods are, by definition, validated but may require verification for their application to certain products. Therefore the entire work

presented in this communication should be considered as verification or suitability testing study.

Preservative efficacy test (PET) as described in official methodologies is performed along the following lines; inoculation of a readymade cosmetic preparation with a known number of organisms, mixing to homogenize the inoculum and collecting samples for bacterial count at different time intervals for as long as 28 days [15]. Although it was not among the aims of this work to deal with PET, data presented in this investigation demonstrates that microbial challenge used in gel PET should be introduced into the product before gelation. If this is not possible, then it is suggested that the finished product should be acidified to pH 4, inoculated, shaken and then alkali added to bring the product back to its original pH. This approach ensured equal bacterial distribution into the preparation which is a pre-requisite in PE testing.

Initial dilution which is typically followed in bacterial count should be done in a buffer of pH 4 to allow complete microbial release from the gel matrix in to the diluent; further dilution can be performed in similar buffer but with pH 7.0. Three minutes exposure time to pH 4 does not affect the number of the inoculated organisms and this was confirmed in a separate experiment (Un- published data).

It is evident from the results of this investigation that a major obstacle in the microbiological testing of gels is the quantitative recovery of product contaminants. Sutton [14] pointed out to the necessity of the procedure to recover 70 % of the total number of contaminants. The quartile values presented in Table 1 demonstrates that the routine procedure failed to recover 70% of the mean count in at least 25% of samples tested, whereas when the new approach was used, recovery rate was always better than 70%.

4. CONCLUSION

As established in this work, a snatched gel sample taken from a product and processed by traditional microbiological methodologies might not reflect the true extent of contamination level. A special treatment is needed as organisms are difficult to disperse evenly or recover quantitatively from the gel matrix. The approaches adopted herein are simple, easy to perform and can become part of the routine work in the microbiological quality control of cosmetic gels.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

DEDICATION

This communication is dedicated to the memory of the late Dr. Scott Sutton, founder of the Pharmaceutical Microbiology Forum Newsletter.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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