The development of self-preserving gels

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ABSTRACT: A lot of questions have arisen regarding the safety of chemical/traditional preservatives in cosmetics. The interest of using natural preservatives in cosmetic formulations has been grown extremely. The chemical preservatives are replaced by other cosmetic ingredients with anti-microbial properties that are not classified as preservatives in the Annex VI of the Commission Directive 76/768/EEC, in order to produce preservative-free or natural preserved or self-preserving cosmetics. This study aims to evaluate the efficacy and stability of alternative preservatives such as levulinic acid and anisic acid in order to produce natural preserved cosmetics for wound healing and moisturizing gel. The above mentioned alternative preservatives protected sufficiently both the healing and moisturizing gel against bacteria and fungi examined under the challenge test procedure, satisfying the A criteria of the European Pharmacopoeia, and also maintained their efficacy during the use of the products by consumers. The physicochemical stability of final products was proved through accelerated ageing test at 42°C, 5°C and cycle test.

INTRODUCTION

Although, traditional chemical preservatives have been used in cosmetics a lot of years, in order to protect them against microbial spoilage and infection, the safety is under question and is being examined very carefully. Contact dermatitis for formaldehyde and allergies for isothiazolinones have been mentioned [ref]. Furthermore, parabens have been associated with breast cancer (1-4). There fore, there is a big interest from the investigators to develop cosmetic products without using chemical/traditional constituents [5, 6] in order to increase the safety of the product. The chemical/traditional preservatives have been replaced by other anti-microbial ingredients, that are not classified as cosmetics preservatives in the Annex VI of the Commission Directive 76/768/EEC. The developed cosmetic products consisted of these alternative anti-microbial substances are characterized as preservative-free or natural preserved cosmetics. Alternative ingredients with anti-microbial properties are herbal essential oils and extracts, ethanol, medium polar compounds such as caprylyl glycol and monoglycerides of capric acid and caprylic acid i.e. glyceryl caprylate and glyceryl caprate [7-9]. Additionally, some fragrance ingredients such as p-anisic Acid and levulinic Acid [10, 11], which were found to be the main compounds of some herbs have shown excellent anti-microbial activity. Sufficient information and tests have to be provided in order to predict that a formulated product will be adequately preserved. Several factors affect the activity and the effectiveness of the preservative in the final product. These include pH, temperature of storage, the type of formulation; i.e., aqueous or oily, gel or emulsion, the water content of the formulation, other ingredients present, i.e., proteins, amino-acids, surfactants and the possible interaction of the preservative with containers. It is important to mention that the production of preservative-free cosmetics is more difficult because the above parameters have to be carefully considered and the principles of Good Manufacturing Practice (GMP) and Hurdle Technology have to be strictly followed. It is recognized that evaluation of the antimicrobial activity of a preservative in a cosmetic preparation can be made by testing its efficacy in the final product. The challenge test or Preservative Efficacy Test (PET) includes artificial contamination (challenge) of the intact product with a predetermined number of micro-organisms; it also involves periodic removal of samples at certain time intervals which, after recovery in suitable media, are used for the viable count of the micro-organisms present in the formulation at that time. There are, however, only a few reports referring to the efficacy of the preservatives to control the microbial contamination of the products during their use by consumers [12, 13]. This study aims to develop some stable and effective cosmetic products with alternative preservative systems instead of the chemical/traditional ones. For this purpose, we prepared a wound healing gel with glyceryl caprylate (1% w/w), levulinic acid (0.2% w/w), p-Anisic acid (0.1% w/w) and a moisturizing gel with levulinic acid (0.3% w/w) and p-anisic
Acid (0.15 % w/w).
Series of tests were done in order to confirm the anti-microbial efficacy both in intact products and in in-use state. A challenge test according to the European Pharmacopoeia criteria and a full microbiological analysis after 2, 4, 6, 12 months of use were performed. The physicochemical stability of the products and the preservative system compatibility were carried out using accelerated ageing tests at 42°C, 5°C, 25°C and cycle test.

**MATERIALS AND METHODS**

**Cosmetic formulations**

**Wound healing gel**
Ingredients: Aqua, glyceryl polymethacrylate, propylene glycol, aloe barbadensis leaf extract, glycerin, ammonium acryloyldimethyltaurate/VP copolymer, levulinic acid, p-anisic acid, ricinoleth-40, tocopheryl acetate, sodium hyaluronate, Ginkgo biloba extract, allantoin, sodium carboxylate beta glucan, glycyrrhetinic acid, sodium hydroxide, copper peptide.

**Moisturizing gel**
Ingredients: Aqua, glycerin, butylene glycol, panthenol, glyceryl caprylate, sodium carboxymethyl beta glucan, sodium hyaluronate, Saccharomyces lysate extract, glycyrrhetinic acid, epilobium angustifolium extract, aloe barbadensis extract, Centella asiatica extract, propylene glycol, Arctium lappa root extract, hydroxyethyl cellulose, carborner, TEA, sodium hydroxide, levulinic acid, p-anisic acid, sodium phytate, Syringa vulgaris extract.

**Preservative systems**
The composition of the preservatives systems used is presented in Table 1. Dermosoft GMCY® (Dr Straetmans Chemische Produkte GmbH, Hamburg, Germany) = glyceryl caprylate.

**ORGANISMS AND INOCULUMS PREPARATION**

**Organisms**
Staphylococcus aureus ATCC 6538 (S. aureus), Pseudomonas aeruginosa ATCC 9027 (P. aeruginosa), Escherichia coli ATCC 8739 (E. coli), Aspergillus niger ATCC 16404 (A. niger) and Candida albicans ATCC 10231 (C. albicans) were used.

**Inoculums preparation**
Bacteria were cultured on Tryptone Soya Agar (TSA, Oxoid) for 24 h at 37°C. C. albicans and A. niger were grown on Sabouraud Dextrose Agar (SDA, Oxoid) at 25°C for 48 h and 5 days respectively. For microbial inoculum, the cells were harvested into 0.1 percent peptone water by gentle agitation and adjusted to yield suspensions of approx. 10^8 cfu/ml. The peptone water used for harvesting A. niger contained 0.05% V/V of Tween 80 (Sigma-Aldrich).

**Microbial challenge tests (Preservative Efficacy Tests, PETs) according to European Pharmacopoeia (E. Ph.)**
Preliminary studies were performed in order to assure the ability of the unpreserved formulations to support the viability and/or microbial growth and also the effectiveness of the neutralizing medium for the inoculums recovery. The microbial challenge test was performed according to the standards proposed by European Pharmacopoeia (E. Ph., 1996) concerning topical preparations. The formulations (samples of 20 g) were placed in sterile containers and separately inoculated with bacterial and fungal suspensions to reach microbial levels of not less than 10^6 cfu/g for bacteria and 10^5 cfu/g for fungi. The test samples were mixed and assayed at 0, 2, 7, 14, 21 and 28 days. The assays were performed on 1 g of test sample serially diluted in Letheen broth and plated in suitable agarized media. Plates were incubated at 35°C for bacteria and at 25°C for fungi. After 5-day incubation, readings of the number of colonies per gram (cfu/g) were made. The results were confirmed in three different experiments. Products are judged adequately preserved when bacteria are reduced or more than 99 percent (2 log) after 2 days and more than 99.9 percent (3 log) after 7 days, yeasts and moulds should be reduced of more than 99 percent (2 log for criterion A and 1 log for criterion B) after 14 days (Table 2).
MICROBIOLOGICAL STABILITY IN THE IN-USE STATE

The samples of cosmetic products collected were analyzed for total bacterial count and the absence of pathogenic microorganisms after 2, 4, 6, 12 months of use. Each assay was performed in triplicate.

PHYSICOCHEMICAL STABILITY TEST

Room temperature (R.T)
The final product was kept in its packaging, in 20-25°C in an air-conditioned and dark area, where the humidity was 50-60% v/v, for 12 months.

Thermal Test (42°C)
The product was placed in a glass vessel (50 ml) and in its container, completely closed in an oven, where the temperature was kept at 42°C +/- 0.5°C, for 2 months.

Cooling Test (5°C)
The product was placed in a glass vessel (50 ml) and in its container, completely closed in a refrigerator, where the temperature was kept at 5°C +/- 0.1°C, for 1 month.

Cycle Test (C.T)
The product was kept in a glass vessel (250 ml), completely closed. At first, it is located in an oven, where the temperature was kept at 42°C +/- 0.5°C, for 8 h. After, the product was taken off the high temperature of oven, it was kept at room temperature, for 1 h. The product was then analyzed for its physicochemical properties (appearance, colour, odour, pH, viscosity and separation.) A full cycle test was thus performed. After 7 days of subsequent cycle tests and full physicochemical analysis, sufficient indications of product stability, during the alterations of temperature were evaluated.

RESULTS AND DISCUSSION

The results of E. Ph. challenge test regarding the two formulations of gels are presented in Table 3. Both alternative preservative systems I and II showed effective action, satisfying the A criteria, even against P. aeruginosa, a microorganism known to be very persistent and predominant in cases of drug and cosmetic contaminations [14]. The system consisting of glyceryl caprylate (1% w/w) and levulinic acid (0.2% w/w), p-anisic acid (0.1% w/w) was sufficient to protect the healing gel against contamination, in spite of the large variety of ingredients such as vitamins, peptides, glucans and high water content (63.45% w/w). Levulinic acid (0.3% w/w) and p-anisic acid (0.15% w/w), in the case of moisturizing gel with very high water content (82.5% w/w) were very efficient against the bacteria, yeast and fungi. The absence of glyceryl caprylate did not seem to decrease the preservative efficacy of system II, probably because of the increase of percentage of levulinic acid and p-anisic acid in it. Since, it is known that challenge test can not replace the consumer’s use [15], we analyzed the two different formulations of gels for the Total Microbial Count (TMC), yeast and moulds and the presence of pathogenic germs after of 2, 4, 6, 12 months of use.
The results (Table 4) were within the limits of the E. Ph. and confirmed that the efficacy of the alternative preservative systems was not decreased during the use of the products. The compatibility of alternative preservative systems I and II with the other ingredients of the formulations and the physicochemical stability of final products were examined during a long period of time, at different temperatures and in a cycle test procedures (Tables 5 and 6). Both the healing and moisturizing gels were extremely stable and no change in organoleptic and physicochemical properties were observed. The preservative systems I and II did not affect the critical parameters of products such as colour, odour, pH and viscosity. In conclusion, the alternative natural preservative systems used in the present study i.e. the combination of glyceryl caprylate with levulinic and anisic acid (system I) and the levulinic acid and anisic acid alone (system II) preserved adequately both gels under the challenge test and also maintained their efficacy during the use of the products in twelve months. Additionally, they were compatible with the other active ingredients and did not influence the physicochemical features of the products. It seems that the production of microbiologically and physicochemically stable naturally preserved products could be a real possibility, although the toxicity of the above system or other self-preserving system could not be absolutely eliminated.

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REFERENCES AND NOTES

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