Preservation in facial sheet masks: Biocellulose as polymeric matrix to avoid preservative release into the skin

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Abstract

Biodegradable material such as biocellulose has a lower environmental impact than the classical polymers used in cosmetic and medical field, in addition to all the beneficial properties for the skin, such as a deeply moisturizing and soothing effect. The focus of this work was to develop a new face Biocellulose Mask (BCM) embedded with cosmetic formulation, in which the matrix can act as a system to avoid the release of preservative into the skin.Two preservatives were chosen for this study: Levulinic Acid ("non-preservative preservatives") and Phenoxyethanol (classic chemical preservative). The choice of using Levulinic Acid permits to fulfill the claim "Preservative free", popular in the cosmetic field.

Specific adsorption studies aimed at determining the capability of these preservatives to bind the polymer, were set up by evaluating zeta potential variation of BCM. Subsequently, the evaluation of the quantity of preservative bound and released from the masks, were carried out using an HPLC technique.

The final goal was to evaluate which of the two preservatives showed the desired behaviour, that is being adsorbed by the mask, but not released on the skin, so as not to interfere with microbial skin ecosystem. The in vivo study was carried out on 5 selected volunteers. In conclusion, we can state that Levulinic Acid has proved to be an ideal candidate for the formulation because it is able to bind to BCM without being released on the skin. This work opens the door to a very promising use of this new system in formulation field.

Keywords: Biocellulose mask; preservatives; Levulinic acid; Phenoxyethanol; formulation

Introduction

The microbiological safety of cosmetic products has always been of special interest for industries as microbial spoilage can lead to product degradation, or in the case of pathogens, an intimate contact with broken or damaged skin can cause a hazard for the health of the consumer and potentially spread infection [1]. The most pursued and most effective strategy for the preservation of cosmetic products consists in the addition of preservatives during the formulation phase of the product itself. Preservatives are substances which, by virtue of their ability to prevent or reduce microbial proliferation, are added to cosmetic formulations in order to avoid contamination by microorganisms such as bacteria, yeasts and molds [2]. Their definition is reported in the European Regulation 1223/09 Art. 2, which defines them as “substances which are exclusively or mainly intended to inhibit the development of

microorganisms in the cosmetic product". The use of preservatives in cosmetics is essential to prevent alterations caused by microorganisms and contamination during formulation, shipping, storage or use by consumers. One of the most important documents when talking about preservatives is the fifth Annex of the European Regulation 1223/09, which contains the list of substances that can be used as antimicrobial preservatives and their maximum concentrations of use [3].

The focus of this work was to compare the behavior of two preservatives towards an innovative material, such as biocellulose, in order to find the one that best suited the formulation of a very popular cosmetic product from a commercial point of view, that are the disposable face masks. The formulation of sheet face masks using a biodegradable material such as biocellulose allows to have a lower environmental impact than the classical polymers used in this field, in addition to all the beneficial properties of this material for the skin, such as for example a deeply moisturizing and soothing effect. The two preservatives chosen for this study are different: Levulinic Acid falls into the category of "non-preservative preservatives", while Phenoxyethanol is a classic chemical preservative. The choice of using a non-preservative preservative such as Levulinic Acid allows us to fulfill the claim "Preservative free", which is very popular in the cosmetic field in recent years and it is linked to the growing awareness of the possible aggressive impact of classic chemical preservatives on the skin microbiota. Specific adsorption studies aimed at determining the capability of the two preservatives to bind the polymer, were set up by evaluating zeta potential variation of biocellulose tissue. Subsequently, quantitative analyzes, aimed at evaluating the quantity of preservative bound and released from the masks, were carried out using an HPLC technique. A different analytical method was obviously pursued for each of the two preservatives.

Materials and Methods

Biocellulose sheet masks were obtained by fermentation from the bacterium *Acetobacter xylinus*. The reagents used for zeta potential were an electrolyte solution of KCl 1mM, solution of Levulinic Acid 10 mg/mL, a buffered solution of Levulinic Acid (pH 5.27) and a solution of Phenoxyethanol 10 mg/mL (pH 5.02).

The reagents used for HPLC analyses were Acetic Acid 1.0 N, as mobile phase for the analysis of Levulinic Acid, a solution composed by the 21% of Acetonitrile (ACN), 13% of Tetrahydrofuran (THF) and 66% of MilliQ buffered water until a pH value of 3 is reached.

Adsorption study

In these analyses the Zeta Potential was used to evaluate the extent of the adsorption of two different preservatives to biocellulose masks. The Zeta Potential is a parameter related to the surface charge at a solid/liquid interface and it's important to understand the surface properties of a material. It allows a real-time control of the changes in cellulose fibers, during an adsorption process. To analyze the capacity of interaction there was exploited the shift in charge of the samples, at the time of adsorption. The Zeta Potential measurement was performed by using a high-performance electrokinetic analyzer model SurPASS™ 3 (Anton Paar,Rivoli, TO, Italy) which allows a completely automatic analysis of macroscopic solids' Zeta Potential under real conditions. For the direct analysis of the Zeta Potential of different surfaces, SurPASS™ 3 resorts to the classical streaming potential and streaming current method. Automatic pH readings and time-dependent recording of adsorption kinetic allow a

deep comprehension of the chemistry of biocellulose masks. Before proceeding with the studies of adsorption of substances to biocellulose masks, we have conducted some preliminary analyses. At first, we have made a pH scan from the initial pH value registered by the system, about six, first towards basic values and then towards acid values. That was the goal to locate the isoelectric point of the biocellulose masks. Then, we have measured the "basal" adsorption kinetics of the masks, with 200 mL of a 1mM KCl solution, at different pH values: 3, 5,5, and 7. We made this to identify the baseline condition of the adsorption of the masks. The procedure we followed is described below. At first, cellulose fibers (3,5 cm x 2 cm) were dipped and washed down for 30 minutes with Milli-Q water. After a first rinsing step with 200 ml of an electrolyte solution of KCl 1 mM, brought to pH 7, the Zeta Potential was measured by the instrument to stabilize the system. Then there were done three cycles of adsorption kinetics at the constant pressure value of 200 mbar.

At this time, we picked up 10 ml of the KCl 1mM solution and added 10 mL of a solution of Phenoxyethanol or levulinic acid. During this second part of the test the analytical solution was left to its pH value. Afterwards, we let the instrument esteem the Zeta Potential and measure 4 cycles of adsorption kinetics. Once the measurement finished, we took 2 mL of the analytical solution, filtered it and organized in HPLC's vials. So we proceed removing the analytical solution, and replacing it with a new one composed by KCl 1 mM. Then, we brough the pH of the solution to 7. Therefore, the instrument evaluated the Zeta Potential and made 4 cycles of adsorption kinetics. The analyses were carried out in triplicate. For each mask it was built a graph, that was constructed by placing the time (expressed in seconds) on the abscissa axis and the Zeta Potential (mV) measured by the SurPASS on the ordinate axis.

Quantitative analysis

Quantitative analyses, aimed at evaluating the quantity of preservative bound and released from the masks, were carried out using an HPLC instrumentation. HPLC apparatus model Vanquish ThermoFisher scientific (Waltham, USA) equipped with UV detector set at 258 nm for Phenoxyethanol and 280 nm for Levulinic acid was used. A different analytical method was obviously pursued for each of the two preservatives, starting from previous literature studies.

Standard Solutions and Sample preparation

For calibration studies, a calibration standard solution of Levulinic acid was prepared starting from 3 stock solutions of Levulinic Acid 5 mg/mL. From each of these solutions were made six solutions diluited with Acetic acid. The samples thus obtained, at different concentrations, were then analysed in HPLC, and for each were made two injections.

For the Calibration Curve of the Phenoxyethanol, we starts from 3 stock solutions of Phenoxyethanol 3 mg/ml From each solution there were made 6 dilutions. The samples thus obtained, at different concentrations, were then analysed in HPLC, and the calibration curve was obtained.

In vivo study

The in vivo studies was performed on 5 selected volunteers.The final goal was to evaluate which of the two preservatives showed the desired behaviour, that is being adsorbed by the

mask, but then not released on the skin of the volunteers subjected to the tests, so as not to interfere. During this test, placebo masks (containing a preservative-free serum), masks soaked with a serum containing Levulinic Acid (LA serum) and masks soaked with a serum containing Phenoxyethanol (PH serum) were applied on different area of the skin of the volunteers..

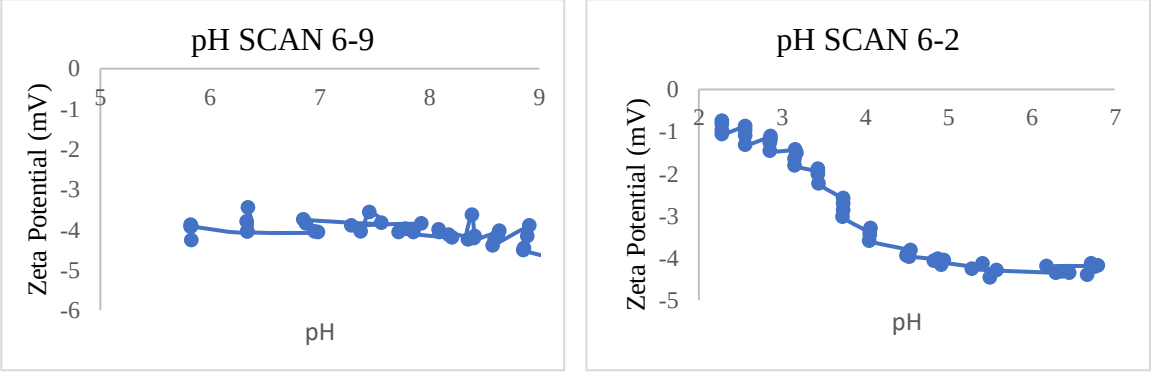
The pieces of the mask, soaked with the two serums, were placed on the arm of the volunteers, and left for 20 minutes (time of use of the mask). At the end of 20 minutes, the masks were removed and put in contact with a suitable solvent and the solutions were filtered and analysed by HPLC.

Once these masks were removed, it was therefore possible to quantify the presence of each of the two preservatives on the skin of the volunteers, by a stripping method. Briefly, in the application area of mask, an adhesive skin strips were placed by pressing for 10 seconds. Subsequently, the strips were removed and put in contact with suitable solvent. The solutions were filtered and analysed by HPLC. At the same time, as a control 20 µL of Levulinic Acid or Phenoxyethanol solutions and 600 µL of LA serum or PH serum were applied to the arm of the subjects. The solutions were left on the skin for 20 minutes and then operated with the strips as described above.

RESULTS AND DISCUSSION

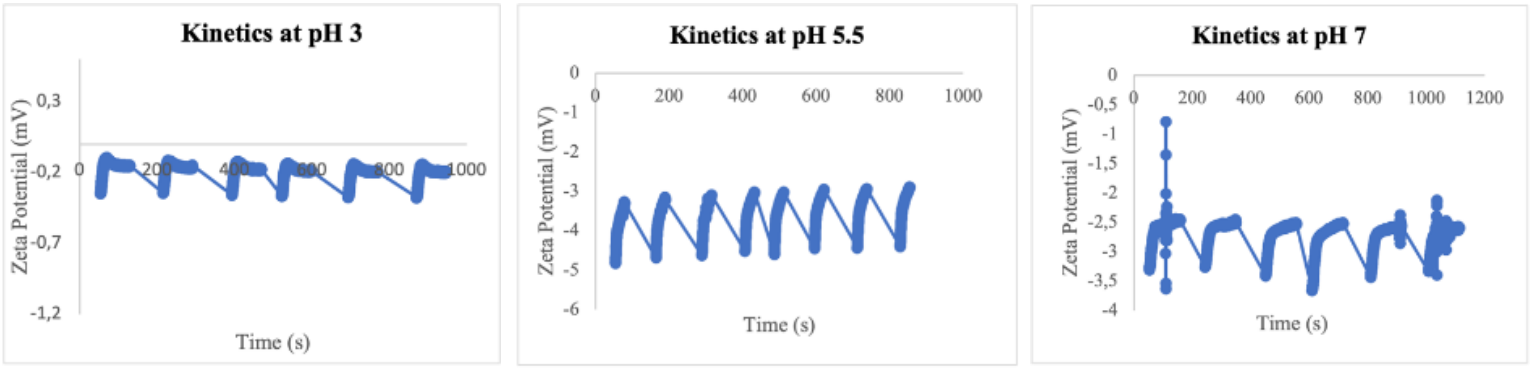
Absorption results

The first analysis had the goal of identifying the isoelectric point of the biocellulose masks. The two graphs obtained from this analysis are shown in the **Graphic 1.**



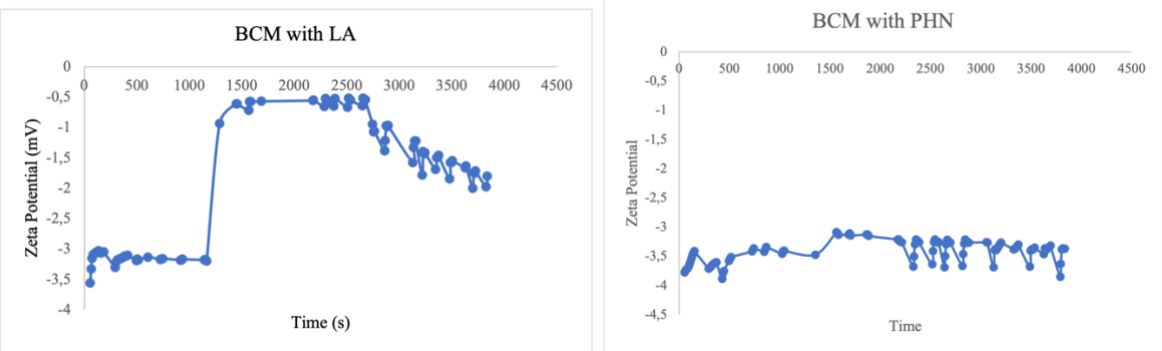
Graphic 1 BCM pH scan.

The system did not identify the isoelectric point, but despite this, it is possible to state that this condition occurs towards acid pH values. In fact, the Zeta Potential is stable in the pH range that goes from 9 to 6 and tends to decrease reaching 0 mV, when it moves towards acid pH values. The second part of the preliminary adsorption tests allowed us to identify the adsorption kinetics of biocellulose masks at different pH values, which mime the adsorption conditions in an acidic (pH 3 and 5.5) and neutral (pH 7) environment. In this way it was possible for us to determine the baseline adsorption condition of the masks (**Graphic 2).**



Graph 2. Baseline absorption kinetics pH 3, pH 5.5 and pH 7.

At pH 3, the Zeta Potential recorded by SurPASS, during the adsorption kinetics, is stable to a value of 0/0.2 mV. At pH of about 5. 5, the Zeta Potential recorded is included between -3 and -5 mV. At pH of about 7, the Zeta Potential recorded during the adsorption kinetics is very similar to the one recorded at pH 5.5. After having conducted these preliminary tests, we started analysing the adsorption kinetic, using Levulinic Acid. With the first general procedure we analysed 3 masks. For each mask it was built a graph, that was constructed by placing the time (expressed in seconds) on the abscissa axis and the Zeta Potential (mV) measured by the SurPASS on the ordinate axis (**Graphic 3**).



Graph 3. BCM with LA and PHN

As it can be seen in the **Graphic 3**, at a pH value of 7, the Zeta Potential is between -4 mV and -3 mV; once added the Levulinic Acid, Zeta Potential grew until -0,5 mV. However, when the system was brought back to pH 7, the Zeta Potential value decreased, but it never

reached the starting value. For this reason, we assumed that the mask adsorbed part of the Levulinic Acid, which prevents the system from returning to its initial Zeta Potential's value. The Levulinic Acid, whose pKa value is 4.6, is undissociated at pH 3.3, which represents the pH value reached by the system after having added the preservative to the analytical solution. So, its elevated lipophilicity may be the basis of its adsorption by biocellulose fibers. In fact, interactions of the hydrophobic type could be responsible for the adsorption.

The adsorption kinetics with Phenoxyethanol is totally different from the one shown by the analyzes previously conducted with Levulinic Acid. Indeed, the Zeta Potential is stable during the entire analysis and any evident change in its value after the addition of the preservative to the analytical solution is evident. For this reason, it is possible to conclude that the Phenoxyethanol is not able to bind to the biocellulose fibers, and therefore it isn't adsorbed by the mask (**Graphic 3**).

Thanks to these studies it was possible to state that Levulinic Acid is able to bind to biocellulose masks, both in dissociated form and in undissociated form. However, the bond to the mask is stronger when it is in dissociated form, as Levulinic Acid is able to give more stable chemical bonds with it. On the other hand, Phenoxyethanol is not able to bind to the biocellulose masks in a consistent manner.

In vivo analyses

The results obtained after in vivo application of sheet masks, serum containing only levulinic acid or phenoxyethanol or only solutions of preservatives, are reported in the tables 3 and 4.

Tab. 3 Amount of Levulinic Acid detected during in vivo experiment in the skin area treated with: Levulinic Acid serum (S-LA-S), BC sheet mask containing levulinic acid (S-LA-BCS). Biocellulose sheet mask were analyzed before (LA-BCS-b) and after skin application (LA-BCS -a) , as a controls

SAMPLE	AVERAGE (mg/mL)
S-LA-S	n.d
S-LA-BCS	n.d
LA-BCS-a	3,.98
LA-BCS-b	3. 070

In all samples after application of levulinic acid serum (S-LA-S) the Levulinic Acid was not detectable. This means that the preservative was completely absorbed by the skin. It appeared pretty evident during the test too, because before applying the strip the arm of the volunteers was almost dry and there was no trace of the serum.

Strips referring to the area treated with the mask soaked with SA serum (S-LA_BCS) reveal that the Levulinic Acid is not present. This means that either the Levulinic Acid was completely absorbed from the skin, or it was not released from the biocellulose mask.

At this point in order to discover if Levulinic Acid remains strongly bound to the BC sheet, the pieces of masks were put in solvent and after sonication, the solution was analysed by HPLC.

As it can be seen, the amount of Levulinic Acid present in the masks applied on the skin is almost identical to the one in these masks which were not applied on the skin. These results permitted to conclude that the entire amount of the Levulinic Acid remains bound to the masks and it was not released on the skin.

Results obtained after application of products containing Phenoxyethanol are reported in the Table 4

Tab. 4 Amount of Phenoxyethanol detected during in vivo experiment in the skin area treated with: Phenoxyethanol serum (S-P-S), BC sheet mask containing Phenoxyethanol (S-P-BCS). Biocellulose sheet mask were analyzed before (P-BCS-b) and after skin application (P-BCS -a) , as a controls

SAMPLE	AVERAGE (mg/mL)
S-P-S	0.026
S-P-BCS	n.d
P-BCS-a	0.500
P-BCS-b	0.020

The entire amount of Phenoxyethanol present in the serum or in the mask were adsorbed by the skin, since the amount detected on the skin surface was practically nil. It appeared pretty evident during the execution of the test too, because there was no more trace of the serum on the arm of the volunteers once the 20 minutes of application finished.

At this purpose pieces of masks soaked with the serum have been put in the mobile phase and analysed, without having applied them to the skin of the volunteers. This allowed to quantify the real amount of Phenoxyethanol actually bound to the surface of the biocellulose masks.

As shown, the amount of Phenoxyethanol present in the masks applied on the skin is strongly different to the one recovered in the masks not applied on the skin. For this reason, it is possible to deduce that the entire amount of the preservative present in the serum is released on the skin.

CONCLUSION

The focus of this work was to compare the behavior of two preservatives towards an innovative material, such as biocellulose, in order to find the one that best suited the formulation of a very popular cosmetic product from a commercial point of view, that are the disposable face sheet masks.

Results obtained from this study highlighted that Levulinic Acid is able to bind to biocellulose sheet masks, both in dissociated form and in undissociated form. However, the bond to the mask is stronger when it is in dissociated form, as Levulinic Acid is able to give more stable chemical bonds with it. On the other hand, Phenoxyethanol is not able to bind to the biocellulose masks in a consistent manner.

In vivo studies showed that Levulinic acid was mostly adsorbed by the masks, but not released on the skin of the volunteers, thus showing an ideal behavior. Phenoxyethanol remains on the surface of the biocellulose sheet masks and not adsorbed by the masks, if not minimally and consequently was not released on the skin of the volunteers.

In conclusion, it is therefore possible to state that Levulinic Acid has proved to be an ideal candidate for the stabilization of biocellulose sheet masks. Having demonstrated that Levulinic Acid is able to bind to biocellulose masks without being released on the skin opens the door to its very promising use in the formulation field.

Conflict of Interest Statement. NONE

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