DESIGN AND CHARACTERIZATION OF A BILAYER SODIUM ALGINATE/CARBOXYMETHYL CELLULOSE PATCH ENRICHED WITH CANNABIDIOL AND ACTIVATED CARBON FOR POTENTIAL USE IN ACNE TREATMENT

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ABSTRACT

This research explores the use of natural polymers as a sustainable alternative to synthetic polymers for developing patches with diverse applications. We created a bilayer patch utilizing sodium alginate (SA) and carboxymethylcellulose (CMC) as natural polymers. Each layer contained an active ingredient: cannabidiol (CBD) in the SA layer and activated carbon (AC) in the CMC layer. CBD and AC were chosen due to their potential as natural treatments for skin conditions like acne. The SA layer was formed using ionic gelation, while the CMC layer was created through compression, both considered eco-friendly methods.

The mechanical properties of the patch were evaluated, showing the SA layer had a breaking strength of 11.617 N ± 0.2839, and the CMC layer had a strength of 12.36 N ± 0.1300. Both layers exhibited effective swelling capacity for exudate containment, with swelling percentages of 75.78% ± 1.120 for SA and 76.84% ± 1.171 for CMC. The morphology of the layers met expectations.

To quantify the amount of CBD in the patch, high-performance liquid chromatography was employed, optimizing separation conditions based on the column used. The analysis revealed that the SA layer contained 2.08 mg of CBD. The analytical method proved accurate for quantifying CBD in various patch samples.

In summary, the developed bilayer patch, using natural materials and sustainable techniques, presents a viable alternative to conventional acne treatments. It demonstrates suitable mechanical properties and effective CBD release, aligning with greener and more sustainable practices.

Keywords: Cannabidiol, activated carbon, acne, patch, natural polymers, green methods.

1. INTRODUCTION

In the last years, the research about the possibility of use natural polymers in the pharmaceutical field as excipients in different formulations of pharmaceutical products has been arisen [1,2]. This kind of research is relevant to the pharmaceutical sciences field because it can be an alternative for the synthetic polymers which involve different synthesis processes that can be harmful for the environment[3]. Furthermore, these molecules are characterized by being biodegradable, inexpensive, most of them are biocompatible and in terms of production it may be sustainable, because they come from natural resources[4]. According to this, these kinds of materials can be used as excipients in different drug delivery systems[5].

One of the most studied natural polymers is sodium alginate, it comes from the alginates biopolymers family that usually can be obtained from brown algae (*Ochrophyta, Phaeophyceae*), and bacteria (*Azatobacter vineland* and *Pseudomonas* species)[6]. It is currently used in pharmaceutical technology due its properties like biocompatibility, biodegradability, non-antigenic and non-toxic. It has several uses as thickening, gelling and stabilizing agent, on the other hand, it has other uses in bone regeneration, neovascularization, drug delivery, controlled release system between others [7]. One remarkable feature of alginates is the ability to have ionotropic gelation which allows the formation of gel upon the contact with cations[8], this is one of the main properties that made this polymer eligible for this research, because, using this method it's possible to obtain a film or layer that be able to carry actives such as cannabidiol and release it in a controlled manner[6,9].

Other polymer that also has been extensively studied is carboxymethyl cellulose a derived of cellulose, it is categorized as one of the most important polysaccharides, due to its different applications in several fields, one of them is the pharmaceutical sciences as an excipient that is used in drug delivery, as a wound dressing, tissue engineering, cosmetics, among others [10]. Advantages of this polymer includes that it is nontoxic for humans, water soluble, abundant in nature, highly absorbent, mucoadhesive, biodegradable and biocompatible [11,12]. On the other hand, it has been used for the development of many medications materials and drug delivery systems like hydrogels, films and nanocomposites [13]. According to all this features it was chosen as another polymer that may have the ability to carry activated carbon and, in this way, potentiate the absorption of different impurities associated with the possible application of the layer obtained using this polymer.

Cannabidiol (CBD), is a non-psychoactive Phyto cannabinoid from cannabis, approved by the Colombian national framework for access, safe and informed use in 2016 [14]. CBD comes from a plant that had its natural origin and evolution with the beginning of the first agricultural human societies in Central Asia and that over the years spread throughout the world, thanks to its various recreational, medicinal, and cosmetic properties [15]. The growing interest in the properties of this plant led to the commercialization of cosmetic products, which include CBD as the main functional ingredient, a molecule analogous to endocannabinoids that presents ideal properties to improve the appearance of topical conditions, eliminating bacteria (*Cuntibacterium acnes*), controlling excess oil and reducing redness and imperfections, so it is feasible to consider it as a functional ingredient in a formulation for skin care[16,17]. On the other hand, there are other components such as zinc, sulfur and activated carbon that also have beneficial actions on the skin and its appearance, promoting the strengthening of the epidermis and removing impurities[18,19].

Currently, some of the cosmetic products marketed for the treatment of skin conditions whose composition includes CBD correspond to liquid or semi-solid products such as ointments, creams, gels, and emulsions of magistral preparation. Taking into account that the world is seeking to expand and maximize the industrial use of cannabis, it is necessary to develop innovative products with added value, such as the availability of new presentations as patches, which give the consumer a localized application and thus ensure that the product is in contact with the skin for a certain time without being affected by external agents, for this reason it is proposed to design and formulate a cosmetic patch that improves the appearance of the skin affected by rashes caused by acne [20].

The patches can be composed of different layers, in this case the use of two layers was proposed, one composed of a sodium alginate (SA) and the other of carboxymethyl cellulose (CMC) , which has been previously used for these products due to its mechanical properties that facilitate its manipulation [21]. Sodium alginate was chosen for the ability to cross-link with zinc sulfate and generate a three-dimensional network that allows the incorporation of the active ingredients, in this case CBD [21]. According to the above, in this research work a bilayer patch based on biodegradable natural polymers that allow the incorporation of CBD as a functional ingredient was developed and characterized. In addition, this product is intended to have mechanical, morphological, and functional characteristics suitable for use as a cosmetic.

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2. EXPERIMENTAL

2.1 Materials

The materials used for the research were Sodium Alginate (Madretierra), Zinc Sulfate (Sigma Aldrich), isolated CBD was supplied by Breeze laboratory S.A.S, high molecular weight Carboxymethyl cellulose (CMC) (ChemiPharma Batch 201910063), Activated carbon (MCKENNA GROUP S.A.S Batch August 2022), dimethicone (Momentive Performance Materials) and purified water for the preparation of the different dispersions. As for the quantification of CBD by liquid chromatography, the use of SHIMADZU LC-2030 liquid chromatograph with a UV-VIS detector, with an ultra C18 0,1500m x 0,0046m column, Thermo Scientific spectrophotometer (Genesys 10S UV-VIS), ultrasonic probe (QSONICA) and Ohaus analytical balance was required.

2.2 Methods

2.2.1 Obtaining the CMC layer:

The layer developed was obtained by a roller homogenization and compression methodology [22], with some modifications, using CMC at 43% as structural component. Different formulations of the CMC layer were obtained from preliminary tests to this research, which consisted of an no loaded system (CMC1), and two systems loaded with activated carbon (CMC2, CMC3). The composition of the different systems worked is listed in Table 1.

Table 1. Composition of the different formulations obtained for CMC layer.

Assay	CMC (%w/w)	Activated carbon (%w/w)	Dimethicone (%w/w)
CMC1	43%	-	-
CMC2	43%	5%	-
CMC3	43%	5%	5%

Note: CMC1= no loaded system; CMC2 = system loaded with activated carbon; CMC3 = system loaded with activated carbon and dimethicone. CMC= Carboxymethyl cellulose

The way in which the previously mentioned systems were obtained is reported below. Briefly, the CMC is dispersed in hot purified water (353,1 K) by means of a propeller agitator at 10 Hz, when a homogeneous mixture is obtained, it is taken to a drying stage in an oven for a period of about 12 hours at 315,1 K. Subsequently, a moldable dough is obtained, which is kneaded and compressed using a rolling pin until a thickness of approximately 1mm is achieved. Circular cuts of 1,0 cm in diameter were made, and finally the samples were frozen and then freeze-dried to obtain the dry product. The previously mentioned methodology was also applied to obtain the CMC layer loaded with activated carbon. The specific amount of activated carbon (5% w/w) was added to the hot purified water in which the CMC is dissolved. In some formulations the addition of dimethicone as elastomer was performed.

2.2.2 Obtaining the Sodium Alginate layer (SA):

The layer developed was obtained by an ionic crosslinking methodology using 3% w/w sodium alginate as structural component[18]. Derived from preliminary tests to this research, different formulations of the SA layer were obtained, which consisted of a no loaded system (SA1), and a system loaded with CBD (SA2). The composition of the different systems worked is listed in Table 2.

Table 2. Composition of the different formulations obtained for the SA layer.

Assay	Sodium Alginate (%w/w)	CBD isolet (%w/w)
SA1	3%	-
SA2	3%	5%

Note: SA1= no loaded system; SA2 = system loaded with CBD. CBD = cannabidiol

The way in which the previously mentioned systems were obtained is reported below. Briefly, sodium alginate is dispersed in water at a temperature of 353,1 K and stirred at 5,000 Hz until a homogeneous mixture is obtained, then, the dispersion obtained is poured into a container until a thin layer is obtained and 0,1M zinc sulfate is added to generate crosslinking and thus obtain the AS layer. The product formed is washed with purified water to eliminate excess zinc sulfate. Circular cuts of 1,5 cm in diameter were made, and finally the samples were frozen and then freeze-dried to obtain the dry product [18]. The previously mentioned methodology was also applied to obtain the CBD-loaded SA layer. For this, an ethanolic solution of isolated CBD (1mL) was added to the sodium alginate dispersion and mixed until a homogeneous mixture was obtained.

2.2.3 Characterization of the layers obtained:

2.2.3.1 Morphology:

The scanning electron microscopy (SEM) technique was used to evaluate the surface of the developed layers. The SEM analysis was performed by adhering the slices of each layer on a carbon tape. Briefly, each of the layers were coated with gold to improve the image quality, then, they were imaged at a voltage of 20,00V at different magnifications, using a SEM (TESCAN VEGA 3 SB).

2.2.3.2 Breaking strength:

For this test, layers were cut with the following dimensions: 4 cm long and 2 cm wide. These are placed in the texture analyzer (LAMY RHEOLOGY - TX-700) and the measurement is performed with the following parameters: starting force 0,1 N, distance of 20 mm and speed of 0,1 mm/s.

2.2.3.3 Swelling capacity:

2.2.3.3.1 Swelling Capacity for the CMC layer: To perform this test the loaded and dry CMC layers of 1,0 cm in diameter were weighed (Ps), then drops of mineral oil were slowly added without the material losing its initial characteristics. Finally, it is weighed again (Ph), and its swelling ratio (Rh) is calculated according to the following formula:

$$Rh = Ph - Ps(Ph) * 100 \quad \text{(Equation 1)}$$

2.2.3.3.2 Swelling capacity for the AS layer: To perform this test the circular SA layer (1,0 cm diameter) enriched with isolated CBD crystals previously weighed, was immersed in a phosphate buffer. Finally, it is weighed again before the layer changes its initial characteristics. The swelling capacity was calculated according to equation 1.

For the AS layer, additional characterizations were performed such as:

2.2.3.4 Compositional analysis: For the obtained layer the Energy Dispersive X-ray Compositional Analysis (EDX) technique was used to determine the presence of Zinc and Sulfur in the obtained layer, by means of a SEM (TESCAN VEGA 3 SB).

2.2.3.5 Functional component quantification: For the layer obtained, an analysis was performed by the high-performance liquid chromatography (HPLC) separation method with a column (Thermo Scientific C18 0,25 X 4,6 mm), using as mobile phase a mixture between acetonitrile and analytical grade water (90:10). The initial chromatographic conditions worked for the separation and quantification of the CBD analyte of interest were an isocratic elution on a C18 250 mm x 4,6 mm column, with a 75:25 acetonitrile-water mobile phase, flow rate of 1,5 mL/min and a wavelength detection of 214 nm for the UV/VIS detector based on previous consultations. To determine the total amount of CBD, it is required the construction of a calibration curve that allows observing the linearity of the quantification method by means of the equation of the straight line, for this study we worked with a wide range of known concentrations (2000;1500; 800,0; 600,0; 400,0; 400,0; 200,0; 100,0 ppm), this allows to quantified the functional component in the patch at different conditions.

2.2.3.6 *In vitro* release assay: For this determination, an AS layer loaded with CBD previously lyophilized was taken and placed in contact with 2,0 mL of mineral oil in a 15mL falcon tube. The sample was placed on an orbital shaker in an oven at 310,1K and constant agitation was maintained. To determine the release profile, samples were taken every 40 minutes until 160 minutes were completed. Each time sampling was performed, 1mL samples of mineral oil were withdrawn, and this volume was immediately replaced with fresh mineral oil.

The samples taken were subjected to liquid/liquid extraction with ethanol by vortex agitation. Subsequently, the dispersion obtained was centrifuged at 50,00 Hz for 15 minutes. The ethanolic phase was analyzed by HPLC methodology using the conditions optimized for the method, and with this to quantify the functional component in the patch at different conditions.

2.2.4 Assembly of the CMC - SA layers:

For the assembly of the final product, once each of the layers is obtained, the final assembly is performed, for this, a 0,08 cm hole is made in the AS layer to adjust a 0,08 cm layer of the CMC layer in the central part. Once the cut is made, the CMC layer is inserted and correctly adjusted. Finally, the samples were frozen and then freeze-dried to obtain the dry product

3. RESULTS AND DISCUSSION

3.1 Obtaining the CMC layer (CMC):

The CMC layer was obtained using the roller homogenization and compression methodology. This methodology was chosen based on a previous literature review where it is reported as the most used methodology for these systems[23]. On the other hand, the CMC polymer was selected due to its swelling characteristics that allow the formation of absorbent sheets that turn into gel in contact with exudate and other fluids present on the skin such as sweat [24]. It was considered that when working with high molecular weight CMC, a high viscosity mixture would be obtained due to the swelling of the polymer, tending to be semi-solid, so to reach the desired concentration, a backward mixing was performed, in which water was used in excess and then evaporated until the desired concentration was reached. The mass obtained was easily molded by the roller to obtain the film with the desired characteristics. Additionally, the use of activated carbon was implemented considering its characteristic of being a fine powder, whose particles have micropores (<2,0 nm) that give it a large surface area and therefore a high adsorption capacity mainly of organic compounds [19]. This allows the loaded layer to be able to absorb the exudate of skin imperfections without undergoing any appreciable physical change. Finally, the film formed was freeze-dried to remove the water from the system and obtain a dry product, which tends to be much more stable over time.

3.2 CMC layer characterization:

3.2.1 Morphology:

The study of the surface morphology of the films or layers obtained was carried out for the CMC1, CMC2 and CMC3 systems. The micrographs obtained by SEM at two magnifications (100,0x;250,0x) are reported in Figure 1. The CMC1 test, which corresponds to CMC only, shows a surface with some porosities and with some areas that look quite solid, which is ideal since due to its structure it would allow the adsorption of chemically related substances, however, in the CMC2 test, in which activated carbon was incorporated, a much more integral, solid surface with few porosities is observed, which is not desirable in this type of products since it could affect the adsorption capacity, additionally, This gives rise to the CMC3 test, which was the one in which we worked with the greatest amount of dimethicone and which, as will be studied later, yielded better mechanical properties and appearance. When complemented with the morphological analysis, it is evident that it also improves the porosity of the product, obtaining a structured network that would allow the absorption of the exudate from the skin imperfection.

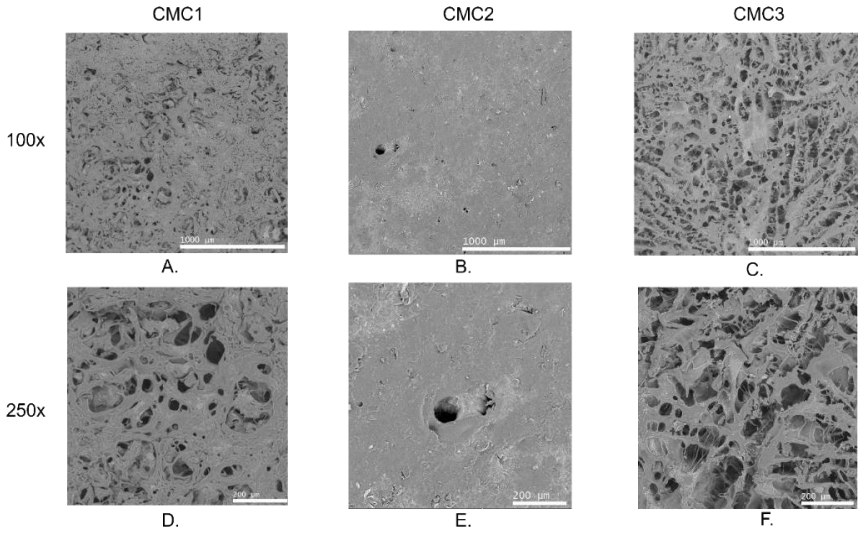

Figure 1. Morphological characterization of the developed assays by SEM. A and D CMC1 no loaded CMC layer, B and E. CMC2 CMC layer with activated carbon, C and F. CMC3 CMC layer with activated carbon and dimethicone.

3.2.2 Resistance to breakage:

The mechanical properties were evaluated by means of a texturometer, obtaining as a result that the activated carbon, due to its interactions with the polymeric network of carboxymethylcellulose, increased the rigidity and porosity of the CMC structure, since the maximum force necessary to break the freeze-dried CMC sheet increased to 16,43 N +/- 0,5609, without elongation and flexibility of the material as can be seen in table 3. It is for this reason that dimethicone is added; a silicone commonly used in cosmetic formulations capable of granting flexibility, elongation, and softness to the touch [25]. Then it was established that as the concentration of dimethicone increased in the formulation 1% < 3% < 5%, the mechanical properties such as resistance to breakage, elasticity and flexibility also increased, presenting a directly proportional behavior as shown in Table 3, however, the amount added was not higher than 5%, because silicone is a material that hinders the passage of oxygen and the exit of CO_2 through the patch. It is considered that dimethicone as a polydimethylsiloxane of hydrophilic nature, can form interactions and formation of bonds with the polymeric network of carboxymethylcellulose, conferring its mechanical properties of elasticity, allowing to obtain at a concentration of 5% in the formulation an adequate elongation for handling and application at the site of action [26].

Table 3. Data obtained from the breaking strength test of the carboxymethyl cellulose CMC layer.

Samples	CMC2	Standard deviation CMC2	CMC3	Standard deviation CMC3
Average maximum force (N)	16,43	+/- 0,5609	11,61	+/- 0,2839
Elongation	NA	NA	1,0 cm	+/- 0,0500

Note: CMC2 = system loaded with activated carbon; CMC3 = system loaded with activated carbon and dimethicone added.

NA: Not applicable since it did not show elasticity.

3.2.3 Swelling capacity:

To evaluate the adsorption characteristics, the swelling capacity test was performed by means of the ratio between the dry weight and the wet weight of the CMC with mineral oil; this was used to simulate the oily characteristics of the skin exudate. During this test it was evidenced that the no loaded CMC layer did not have the capacity to adsorb or absorb the mineral oil; however, after adding the activated carbon, a swelling capacity percentage of 75,78% +/- 1,120 was obtained, as can be seen in Table 4.

It is possible that the polymeric network of uncharged carboxymethyl cellulose does not have the capacity to adsorb or absorb the mineral oil due to its hydrophilic nature, since in its chemical structure there are functional groups such as hydroxy ethyl ether that interact with difficulty with non-polar groups such as those present in the solvent used, so it does not allow the entry of the mineral oil in its structure, nor generate an interaction at surface level, the opposite case happens when using solvents of aqueous nature. On the other hand, the CMC layers that are loaded with activated carbon allow both absorption (at the level of the layer) and adsorption at the level of the activated carbon, which allows trapping this solvent or, at the level of topical use, the exudates produced by the skin lesion [19].

Table 4. Data obtained to calculate the swelling capacity of the carboxymethylcellulose CMC layer of the patch.

Sample	Average Ps	Average Ph	Rh	Standard deviation
CMC1	0,0226 g	0,0226 g	0%	NA
CMC3	0,0115 g	0,04473 g	75,78 %	+/- 1,120

Note: CMC1= no loaded system; CMC3 = system loaded with activated carbon.

3.3 Obtaining the SA layer (SA):

The AS layer was obtained using the ionic crosslinking methodology between the divalent cation (Zn^{+2}) and sodium alginate as a natural polymer. This polymer is used, considering its cost, its natural origin and that additionally it has a considerable amount of carboxyl groups which makes it ideal for crosslinking at different points to give rise to bonds with the Zn^{+2} and with this build the structure that allows housing the CBD crystals [19]. At this point it is worth mentioning that it is important to study the solubility of CBD, which is not very good in aqueous solvents, for this reason it was necessary to previously dissolve CBD in a small amount of ethanol [18].

Subsequently, when added to the sodium alginate at 353,1 K, the CBD goes from being dissolved to being melted because of the temperature and with this it is possible to incorporate it into the SA structure. Controlled cooling will allow the CBD to crystallize properly; however, it would be worthwhile to carry out an X-ray scattering analysis to show if there is any change in the crystalline structure of the CBD. Temperature is a determining factor to obtain a thin and uniform layer, because as the temperature increases the viscosity decreases and facilitates its manipulation. Finally, the film formed was freeze-dried to remove the water from the system and obtain a dry product, which tends to be more stable as a function of time.

3.4 SA layer characterization (SA):

3.4.1 Morphology:

The study of the surface morphology of the films or layers obtained was carried out for the SA1 and SA2 systems. The micrographs obtained by SEM at two magnifications (100x and 250x) are reported in Figure 2. The SA1 test, which corresponds to the no loaded SA layer, shows a smooth surface with some imperfections on its surface, which turns out to be quite intact and with low levels of porosity. In the SA2 test, the aim is to study the effect that CBD has on the surface of the sodium alginate layer, in the micrographs it is possible to evidence an important change at surface level in which an important roughness stands out, on which the CBD crystals that will be in direct contact with the skin are believed to be lodged.

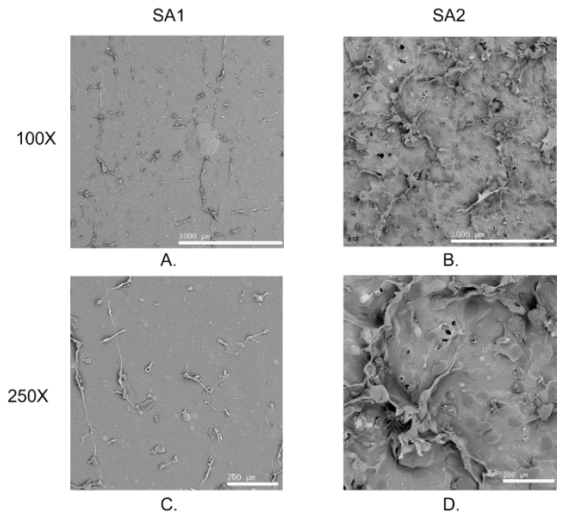

Figure 2. Morphological characterization of the tests developed by SEM. A. and C. AS1 No loaded SA layer, B. and D. SA2 SA layer loaded with CBD.

3.4.2 Resistance to breakage:

Regarding the breaking strength of the sodium alginate layer ionically cross-linked with Zinc sulfate, it is possible to state after the tensile test performed by the texturometer that the addition of CBD to the formulation does not alter the mechanical properties of the freeze-dried SA layer, indicating that the recrystallization of CBD takes place in the pores of the alginate-zinc polysaccharide polymeric polysaccharide network, and does not perform interactions that positively or negatively alter this structure, because as evidenced in Table 5, there is no significant variation in the maximum force to perform the breakage of the no loaded or loaded material. This may happen due to the contrast of the hydrophobic and hydrophilic natures of the CBD and the natural alginate polymer respectively. Finally, it is observed that the polymeric material based on alginate and zinc does not have elasticity, since during the test it did not present elongation or physical deformation, giving rise to a layer of plastic behavior.

Table 5. Data obtained from the SA layer breaking strength test.

Samples	SA1	Standard deviation SA1	SA2	Standard deviation SA2
Average maximum force (N)	12,23	+/- 0,1531	12,36	+/- 0,1300

Note: SA1= no loaded system; SA2 = system loaded with CBD. CBD = cannabidiol

3.4.3 Swelling capacity:

When performing the swelling capacity test of the loaded and no loaded SA layer in the phosphate buffer who simulated the sweating conditions presented by the skin, it is evident that CBD does not make changes in this mechanical property, since the swelling capacity of the SA layer remained at a ratio higher than 70% before and after the incorporation of the active ingredient as can be evidenced in Table 6, i.e. that is to say, the alginate layer will be able to continue trapping water droplets in its polymeric network, this allows the loaded SA layer to be able to absorb transpiration and maintain an ideal humid environment for the release of CBD [27].

Table 6. Data obtained to calculate the swelling capacity of the SA layer.

Sample	Average Ps	Average Ph	Rh	Standard deviation
SA1	0,0035 g	0,0146 g	74,48%	+/- 3,018
SA2	0,0060 g	0,0261 g	76,84%	+/- 1,171

Note: SA1= no loaded system; SA2 = system loaded with CBD. CBD = cannabidiol

3.4.4 Composition analysis:

To study the composition of the layers obtained, an EDX study was carried out, the spectra obtained are reported in Figure 3. When analyzing the images, it was possible to observe that the main elements of the SA layer are carbon and oxygen, considering that they are mainly organic compounds. On the other hand, it is also possible to evidence the presence of zinc and sulfur, which is attributed to the crosslinking phase of the polymer in which the sodium ions of the alginate are substituted by the zinc ions of the zinc sulfate used. On the other hand, when comparing the percentages of each element, it is possible to show that the proportion in which sulfur is found from $ZnSO_4$ are extremely low (SA2) or tend to be zero (SA1) compared to the other elements studied, this allows inferring that the $ZnSO_4$ in excess was removed almost entirely in the washing phase of the film obtained.

A comparison between the two tests performed shows a significant increase in carbon and oxygen atoms, which allows inferring that it was possible to incorporate the CBD to the surface of the carrier polymer, this will be confirmed later in the quantification test of the functional ingredient (CBD).

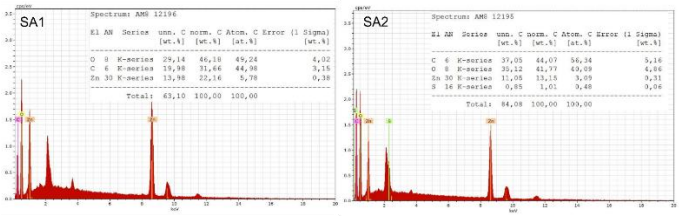

Figure 3. EDX spectra of SA layers obtained. SA1 no loaded SA layer, SA2 SA layer loaded with CBD.

3.4.5 Quantification of the functional ingredient (CBD) by high performance liquid chromatography (HPLC):

The initial chromatographic conditions used for the separation and quantification of the CBD analyte of interest were established because of a literature review. When using these conditions, it was found that the matrix where the samples were found presented another series of peaks that overlapped with the peak of interest for the study, which in this case is CBD, indicating the presence of other compounds. For this reason, some parameters were modified to optimize the method, such as the flow rate and the proportion of mobile phase used, to improve the selectivity and resolution of the peak and thus be able to correctly quantify CBD with reproducible and robust results. Because of the modifications, a better number of theoretical plates (2300) is evidenced by decreasing the flow to 0,7 mL/min and increasing the proportion of acetonitrile to 90 v/v improves the resolution, which must be greater than 2, these parameters comply with the acceptance criteria proposed in USP 42. With these final conditions it is possible to obtain a peak with a specific retention time of 3,27 minutes, which allows the quantification of the area under the curve to determine the concentration of the analyte at different controlled release times, as shown in Figure 4. To verify the specificity of the peak for CBD, free of interferences, 10 verification tests were performed with the addition of a standard to guarantee an increase in the intensity of the signal at the same retention time and verify that the peak corresponds to the analyte under study.

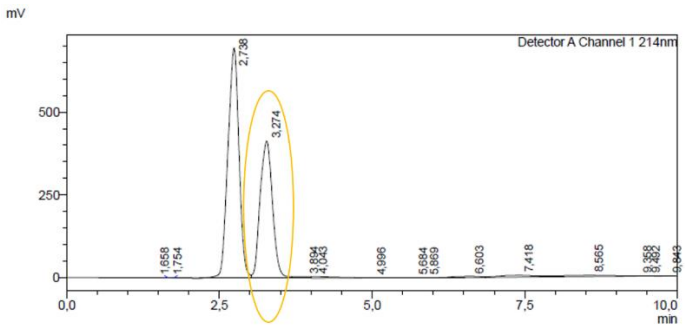

Figure 4. Chromatogram for identification and quantification of CBD by high-performance liquid chromatography.

With the parameters already defined, i.e., an isocratic elution flow, the use of a C18 column, mobile phase acetonitrile/water 90:10 at a flow rate of 0,7 mL/min and a variable wavelength detection using 214 nm, a calibration curve is constructed, as can be seen in Figure 5, using the previously mentioned concentrations of CBD, where a linear behavior is evident with R^2= 0,9918, the relationship between the concentration and the area under the curve is direct and allows obtaining reliable results. With the determination of the equation of the straight line, the concentration of CBD present in the study samples can be defined.

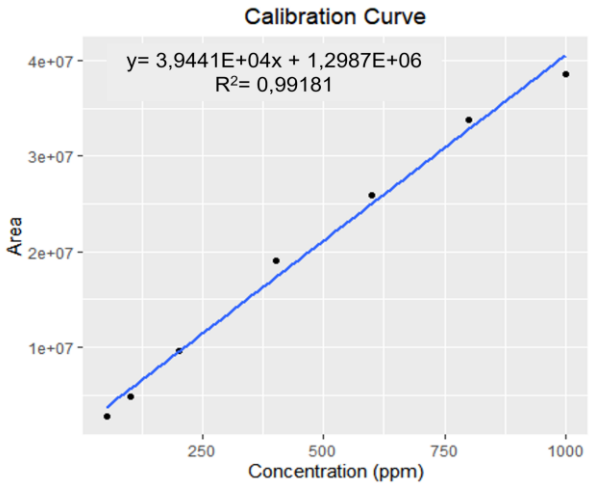

Figure 5. Calibration Curve of CBD standard

The patch was dissolved in an ethanolic medium to determine the total amount vehiculated in the SA2 layer. The 97% ethanolic solution was chosen since the certificate of analysis of the raw material supplied by Flora Med indicated a high solubility in hydroalcoholic solutions, and this was demonstrated during the tests carried out with the isolated CBD crystals. Based on the calibration curve, it is determined that the vehicleized amount of isolated CBD in the circular sodium alginate layer of 1cm diameter corresponds to 2,08mg.

3.4.6 In vitro release assay:

CBD release was evaluated in an oily medium that simulated the oily environment of the skin, for this purpose mineral oil was chosen to study the way in which CBD is released from the patch. Considering that the quantification method previously developed was by high-performance liquid chromatography, it was necessary to perform a liquid/liquid extraction in which the active diffuses to a suitable solvent for its quantification. For this purpose, 97% ethanol was used. Samples were taken at established times until completing 160 min, the minimum estimated time the patch remained on the skin. The samples obtained were analyzed by HPLC using the conditions described in the section on quantification of the functional ingredient and from this it was possible to obtain the profile observed in Figure 6.

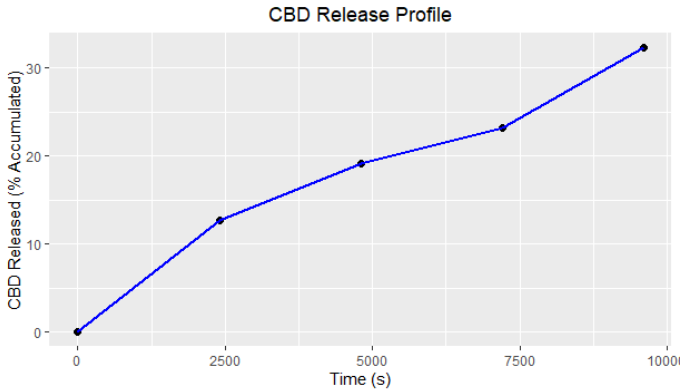

Figure 6. CBD release profile of CBD vehicleized in a sodium alginate layer between 0 and 160 minutes.

As can be seen, during the first 160 minutes, 32,35% of the total CBD present in the SA2 layer is released, which is equivalent to $6{,}8x10^{-7}$ kg of CBD. It is then that the amount released during the first 160 minutes is sufficient to fulfill a cosmetic function on the skin as mentioned in the literature[18,24]. Additionally, the in vitro release tests performed indicate that the SA2 layer shows a significant improvement in CBD release compared to other layers reported in the literature, which release approximately 20% of the CBD load in 60 minutes [18]. Regarding the release profile obtained for the SA2 layer, it is necessary to extend the release trials to determine the time required to fully release the CBD load and to establish whether a prolonged release is obtained, a plateau indicating a sustained release or otherwise the profile behavior is an immediate release.

3.5 Assembly of the SA- CMC layers:

To obtain the final product, it is necessary to assemble the previously obtained layers, for this it is important to mention that this must be done dry to prevent the CBD from migrating towards the activated carbon and being trapped by it. For this reason, a hole is made in the SA layer so that it surrounds the imperfection and helps to improve its reddened appearance; on the other hand, the CMC layer is placed in the center so that it adsorbs as much as possible the exudate coming from the skin lesion as shown in Figure 7.

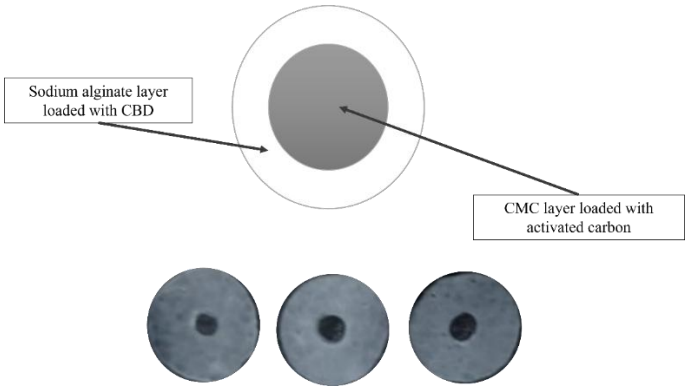

Figure 7. Graphical scheme of the assembly of the experimentally obtained layers and patches.

According to the results obtained and shown previously, it is considered that in the future, patches containing different layers, with different functionalities could be used to treat pathologies such as acne or other skin diseases, in such a way that we would be talking about an effective treatment, easily accessible and that probably does not generate considerable damage to the skin. Additionally, the use of natural products to obtain these pharmaceutical forms is highlighted, which makes it a proposal that could be considered biocompatible, biodegradable and with few effects on the environment, unlike the traditional products used to treat this type of pathologies.

CONCLUSION

In this study it was possible to successfully design and formulate a bilayer green patch based on sodium alginate and CMC that allowed trapping CBD to be used to improve the appearance of skin with imperfections caused by acne and be a greener and sustainable alternative to current acne treatments. The appropriate method to elaborate the SA layer was crosslinking with Zn^{+2} ions and the polysaccharide, allowing the CBD to lodge on its porous surface. While the CMC layer was obtained by the mixture of CMC, activated carbon and dimethicone which resulted in a surface with high porosity that allows cleaning by absorbing impurities present in the superficial imperfections of the skin, in addition to maintaining skin hydration by absorbing aqueous fluids transpired by the skin and avoiding dehydration due to exposure to the environment, The two layers behaved appropriately from the mechanical point of view, as they have the ability to resist external forces that could damage them during handling when placed on the skin. The patch was dissolved in a 97% ethanolic medium to determine the total amount of CBD in the layer, where 2,08 g of CBD was found by high-performance liquid chromatography. Similarly, it is evidenced by HPLC, that at 160 minutes 32.35% of the total CBD present in the SA2 layer is released, which is equivalent to 0,68 mg of CBD.

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CONFLICT OF INTEREST

The authors declare that they have nothing to declare.

AUTHOR CONTRIBUTIONS

María Angelica Velandia Paris: Conceptualization, methodology, writing – reviewing, original draft preparation, Investigation. **Emersón León Ávila:** Methodology, writing – reviewing and editing, **Hector David Garzón:** Investigation.

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