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ORIGINAL ARTICLE

Superabsorbent biodegradable CMC membranes loaded with propolis: Peppas-Sahlin kinetics release

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Abstract

Propolis is a resinous product collected by honeybees with a complex chemical composition. Sodium carboxymethylcellulose is a polymer commonly used in wound care. The goal of the present work was to produce and characterize NaCMC membranes loaded with extract of Brazilian brown propolis (CMC-P). Flavonoids and phenolic acids were identified in the propolis extracts, where the main identified substance was kaempferide. The brown propolis extracted was active against *S. aureus*. The low swelling capacity and high gel fraction of CMC-P would be the consequence of propolis (responsible for a hydrophobic barrier) filling the pores of the membrane. Propolis could be anchoring the NaCMC chains (as observed by FTIR) due to interaction between components, which is corroborated by the CMC-P sample degrading less than the CMC sample (>400ºC). There was non-linear diffusion release kinetics for most phenolic substances of the propolis extract. The CMC-P sample presents potential as a dressing material.

Keywords: *wound care, NaCMC hydrogel, propolis release.*

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1. Introduction

Wound healing is a complex process contemplating the following steps: hemostasis (vascular constriction, thrombus formation), inflammation (neutrophil, monocyte, and lymphocyte infiltration), proliferation (angiogenesis, collagen synthesis, and extracellular matrix formation), and remodeling (collagen remodeling).[1][2] Wound proper care should reach the previous steps without delay. However, the World Health Organization highlights the importance of wound proper care to avoid infection, since up to 30% of patients in intensive care develop an infection.[3][4] Wound infection can be considered a major cause of healing delay and high costs related to it.[5] Regarding skin wound infection microorganisms, *P. aeruginosa* (gram-negative) and *S. aureus* (gram-positive) are common bacteria that colonize wounds.[1] Infection control might be difficult since to avoid bacterial resistance, the rational usage of systemic antibiotics is advised.[6] Infection kinetics is also a variable process, but usually, Gram-positive bacteria are the first ones to colonize the wound site (1st week of infection), followed by gram-negative bacterial colonization. Regarding *P. aeruginosa* and *S. aureus* resistance, they usually are resistant to several antibiotics, e.g., methicillin,[7] carbapenems,[8] cephalosporins (3rd generation antibiotics)[9].

Bee products like honey are known to inhibit both, gram-positive and gram-negative resistant mechanisms, due to their broad spectrum of activity.[10] In addition, propolis, at proper amounts, can be bacteriostatic and even bactericide on wound infections without inducing bacterial resistance.[11] Propolis is a beehive product containing mainly beeswax and resins obtained from plants. *Apis Mellifera* bees produce propolis to seal the hive, protecting it from insects and pathogenic microorganisms. More than 300 substances have been identified in different propolis and their composition varies according to the region of collection, the season of the year, species of bees, and local flora.[12][13] Propolis presents antibacterial, antioxidant, antifungal, anti-inflammatory, and wound-healing properties.[14] Different classes of compounds, including hydrocarbons, fatty acids, fatty esters, flavonoids,

phenolic acids, and phenolic esters have been reported in propolis around the world.[15][16] The color of propolis varies from dark green to reddish, and its composition depends on the plant source of the resin. The most studied Brazilian propolis is the green type originating from *Baccharis dracunculifolia* and produced mainly in the southeast of Brazil.[12] Other types of Brazilian propolis are known, such as brown and yellow ones, but with undetermined plant origin. Although its geographical origin is unknown, Brazilian brown propolis is rich in terpenes,[17] while Brazilian yellow propolis can be considered rich in triterpenoids.[18]

Propolis' effects on wound healing are according to its composition. Nonetheless, flavonoids' bactericide mechanism includes damage to bacteria's cytoplasmic membrane and inhibition of nucleic acid synthesis.[19] Regarding propolis application in wound care, green and red propolis have been successfully applied in Wistar rats' wounds. Although red propolis presented high amounts of flavonoids, green propolis led to high reepithelization,[20] also controlling inflammatory response.[21] Red propolis was tested in male Wistar rats' wounds. It increased the wounds' contraction rate, as well as stimulated healing factors, and increased collagenase activity.[22] Brown propolis was compared to green propolis regarding their action on oxidative stress and inflammation. Brown propolis and green propolis present different metabolite profiles and mechanisms of action, but brown propolis was more active than a green one.[23] Brown propolis was tested against the biofilm formation of *S. aureus*. Propolis' prenylated phenylpropanoic acids were antibiofilm (*S. aureus'* colonies spread) probably due to artepillin C, drupanin, and baccharin metabolites.[24] Brown propolis was incorporated in alginate membranes to be used as a food covering when the membrane was active against gram-positive bacteria.[25]

Sodium carboxymethylcellulose (CMC) is one of the main polymers derived from cellulose. It is soluble in water, and its viscosity and adsorption capacity can be modulated by varying pH, concentration, and temperature. It is a hydrocolloid, forming a gel or a viscous dispersion in water.[26][27] Sodium carboxymethylcellulose has recently been used in dressings for the treatment of wounds and burns. Its membranes improve the healing process, and they can be used pure or in combination with other polymers. Due to its hygroscopic characteristic, carboxymethylcellulose promotes an autolytic debridement of wounds; facilitates cellular rehydration; and has bacteriostatic action. It is, therefore, applicable to wounds with scabs, fibrinous, devitalized, and necrotic tissues.[28][29][30] CMC hydrocolloids allow the incorporation and controlled release of different drugs or natural products. CMC-tamarind gum hydrocolloids were loaded with moxifloxacin hydrochloride, where the equimolar gel delivered moxifloxacin hydrochloride properly[31]. Red propolis extract from Alagoas/Brazil was added to CMC hydrogels, and they were effective against microbes' penetration towards the wound site, being considered promising materials for dressings.[32] The goal of the present work is to develop and characterize CMC membranes loaded with Brazilian southeast brown propolis for wound care.

2. Materials and Methods

2.1 Propolis analysis

Propolis extraction was performed by four methods: dynamic maceration at room temperature (DM) – 50ml of ethanol PA was used as solvent for 48h to extract 2g of propolis; dynamic maceration at 50ºC (DMT) - 50ml of ethanol PA was used, at 50ºC for 48h, to extract 2g of propolis; by ultrasound bath (US) – 50ml of ethanol PA in ultrasound bath for 2h at room temperature; and by immersion of ultrasound probe (USI) - 50ml of ethanol PA under ultrasound probe for 30min at room temperature. The samples were then characterized following their active compound amounts.

2.1.1 Propolis' phenols quantification

To a 50μL aliquot of methanolic solution (1.0 mg/mL) of propolis extract (triplicates), methanol from VETEC/Brazil, was added to 2.5 mL of the Folin-Ciocalteau reagent (1:10) (Sigma-Aldrich) and 2.0 mL of 4% sodium carbonate (Sigma-Aldrich) aqueous solution. After 5 minutes at 50°C, the color of the solution changed from greenish to blue, and the absorbance was recorded at 760 nm, equipment NOVA 2000UV.[33] In addition, a gallic acid (25, 50, 100, 200, 300, 400, 500 e 600 μg/mL) (Sigma-Aldrich) standard curve was plotted (Absorbance = 0.12497+ 0.12951 concentration of gallic acid (R^2 = 0.999)).

2.1.2 Propolis' flavonoids quantification

Aliquots of 400 μL of propolis extract (triplicates) and 200 μL of 2% aluminum chloride (Sigma-Aldrich) methanolic solution were mixed. The final volume was adjusted to 10 mL by adding methanol. After 30 minutes, the absorbance at 425 nm was measured. A standard curve of quercetin (Sigma-Aldrich) (50, 40, 30, 20, 10, 15, 5 e 1 μg/ml) was plotted (Absorbance = 0.04078 + 0.06553 concentration of quercetin (R^2 = 0.999)).

2.1.3 Propolis antioxidant activity (DPPH, FRAP, and ABTS)

The percentage of antiradical activity was calculated through the decolorization of the DPPH• radical (Sigma-Aldrich), according to Equation 1.[34] To determine the antioxidant activity (%AA) of each propolis sample, the absorbance of the solution of methanol + DPPH ($Abs_{control}$) was considered the negative control, and methanol was used as a reference (Abs_{REF}). This analysis was performed in triplicate. To calculate the amount of antioxidant (hydrogen donator) necessary to diminish the initial concentration of DPPH by 50% (CE_{50}), a stock methanolic solution of propolis was prepared (concentration of 1000 μg/mL). Then, 0.29μL of the DPPH solution was added to ELISA plate wells as well as the prepared solutions. After 30 minutes of incubation in the dark, the absorbance was measured at 520nm. Analysis was performed in triplicate.

$$AA(\%) = 100\left(\frac{Abs_{sample} - Abs_{REF}}{Abs_{control}}\right) \quad (1)$$

The antioxidant capacity can be evaluated by the reduction of the ferric complex 2,4,6-tripyridyl-s-triazine (Fe3+-TPTZ) to the ferrous complex 2,4,6-tripyridyl-s-triazine (Fe2+-TPTZ). A 0.5mL aliquot of each methanolic propolis

extract solution was added to 4.5mL of FRAP Reagent. Each mixture was heated at 37°C for 10 minutes, and then their absorbance was registered at 593nm. A standard curve based on an aqueous solution of heptahydrate ferrous sulfate (FeSO4.7H2O (Sigma-Aldrich)), concentrations of 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 μM was plotted (Absorbance = 0.00107 + 0.0018 concentration of FeSO4.7H2O (R^2 = 0.999)).

For the cation radical ABTS scavenging activity, 5ml of $ABTS^+$ solution (7mM $ABTS^+$) (Sigma-Aldrich) was mixed with 88μL of 140 mM potassium persulfate solution in the dark for 16h at room temperature. For the stock solution, to a 50μL aliquot of propolis solution, 5.0mL of ABTS reagent was added. The absorbance of the dilutions was recorded at 734nm, after 6 minutes. The reference was ethanol. A Trolox standard curve (ethanolic solution, 0,0; 0;3; 0,6; 0,9; 1,2; 1,5; 1,8; 2,1 e 2,4 mmol/L) was plotted (Absorbance = -26.37778 concentration of Trolox + 0.65164, R^2 = 0.999), and the results were expressed as mmol Trolox per 100 mg of extract.[35]

2.1.4 CLAE-DAD chromatography

Chromatographic analysis was performed on a C-18 reversed-phase analytical column (Betasil, Thermo, 5μm particles size), at 30 °C. The mobile phase used was water with 1% acetic acid (solvent A) and methanol (solvent B), a constant flow of 1.0 mL/min, and a volume of the injected sample of 20μL. The concentration gradient was performed from 35% of solvent B for 2min, followed by (35-80)% at 20min, (80-92)% at 25min, returning then to 35%B for 2min. The chromatograms were recorded at 280 and 340 nm since most of the phenolic acids and flavonoids in propolis are excited near these wavelengths. Data acquisition used the LCSolution (Shimadzu) software.

2.1.5 Antimicrobial analysis

The antimicrobial activity of propolis extracts against *S. aureus* was determined according to the agar diffusion method with modifications.[36] The *S. aureus* strain (ATCC 25923) was incubated at 36ºC for 24h. A suspension of cells was prepared in 3mL of peptone saline solution to reach $2x10^8$ cfu/mL (turbidity equivalent to Mc Farland scale number 5). This suspension was diluted 100 times and 0.1 mL was inoculated on Mueller Hinton agar plates. Wells (sterile drill, diameter of 0.75 cm) were drilled and, in each well, 50 μL of propolis extract (10 μg/mL) was inoculated. Ampicillin (10 μg/mL, positive control) was used, as well as a solution of 95% ethanol (negative control). The incubation occurred at 36°C for 20-24 hours, after which the inhibition diameter of each well was measured. All tests were performed in triplicates. The inhibition (I) was calculated according to Equation 2.

$$I(\%) = 100\left(\frac{Sample's\,inhibition\,halo - Negative\,control's\,halo}{Positive\,control's\,halo}\right) \quad (2)$$

2.2 CMC gels

The hydrogels were prepared according to the casting method[37]. 3g of sodium carboxymethyl cellulose - CMC (Sigma Aldrich) was diluted in 100 mL of deionized water under mechanical stirring (Fisatom brand, model 710) for 90 min at room temperature. The solution was then divided into 25 mL portions and, under magnetic stirring (Fisatom brand, model 752), 0.8g of citric acid (VETEC) was added [38][39]. The samples were dried in an oven (Nova instruments, model NI1512) for 24 h at 50 ºC. The CMC membranes obtained were immersed in deionized water for excess citric acid removal (10 mL of deionized water for 24 h in an oven at 50 ºC per sample). After this, the CMC membranes were subjected to the swelling process in propolis extracts (20 w/v%, resulting in 2.4 g of propolis per membrane) for 24 h.

2.2.1 Physical properties

The samples' physic-chemical analysis was conducted by FTIR. The analysis was performed on Bruker equipment (Vertex 70), in the range of 400 cm^{-1}-4000 cm^{-1}, 16 scans per sample. The microstructural analysis of the samples was performed by X-ray diffraction (XRD) using the equipment Brucker-AXS D8 Advance Eco diffractometer (CETEM -UFRJ), with CuKα radiation at 40 kV and 25 mA, angular diffraction range of 2θ = 5°-70°, a step of 0.02°, and step time of 2 seconds. The XRD plotted curves were then smoothed (Method Savitzky-Golay, 400 points per window). The thermogravimetric analysis used the equipment TGA Q500 (TA Instruments Co.), Catalysis Lab-UFRRJ. The samples (~10 mg) were loaded in an open platinum crucible, where an empty crucible was used as a reference, continuous flow of N_2 (30 $mL.min^{-1}$), a heating rate of 10 °C / min, between 25° C and 400° C [40]. For the mechanical tests (modified ASTM D882 - 02 standard), rectangular samples of (40x20x1) mm^3 in triplicate were evaluated (EMIC DL 10000 equipment, load cell Trd 21 / 50 kgf), strain rate of 3 mm/min until failure, at room temperature.

2.2.2 *In-vitro* properties

The swelling test was performed in triplicate, where the hydrogels were immersed in 25 mL of saline solution [41]. The sample weight was evaluated at predetermined time intervals (0.5h, 1h, 2h, 3h, 4h, 24h, and 48h of immersion). The samples' swelling degree (SG) was calculated according to $SD(\%) = 100(W_t - W_i / W_i)$, where W_t is the weight of the samples at each time interval and W_i, is the dry samples' initial weight. The samples' gel fraction (GF) and weight loss (WL) were calculated according to $GF(\%) = 100\left(W_f / W_i\right)$ and $WL(\%) = 100\left(W_i - W_f / W_i\right)$, respectively, where W_f is the final dry weight of the samples (after 96h of swelling).

The *in vitro* release study of phenolic compounds was carried out according to the shake-flask methodology [42]. Carboxymethylcellulose membranes impregnated with propolis extract were immersed in 100mL of phosphate buffer pH 6 with 1% Sodium Sulfate Luaril (Sigma Aldrich). They were stirred under 50 rpm for 96 h, at 32ºC. The aliquots of the samples were collected at the following times: 0.5; 1,0;2,0; 3,0; 4,0; 5,0; 24; 48; 72; 96h for analysis of the release kinetic profile by HPLC-DAD. Before analysis, the aliquots were filtered (0.45 μm membrane) and analyzed by HPLC.

The data acquisition was done through the LCSolution software (Shimadzu). The analyses were performed in a reverse phase analytical column C-18 (Betasil, Thermo), maintained at 40 ºC. The mobile phase used was ultrapure water with 1% acetic acid (solvent A) and methanol (100,

solvent B), with a constant flow of 1.0 mL min/4.6 mm, and the volume of the sample injected was 20μL. The propolis aliquots were solubilized in spectroscopic grade methanol, with a concentration of 1000 μg/mL, and the solution was filtered (0.45 μm, PVDF, Millipore). The substances identification was based on the comparison of retention times. The release kinetics of propolis substances was performed through non-linear regression analysis, applying two models: Korsmeyer-Peppas and Peppas-Sahlin models. The model with the highest R^2 value was considered the best fit.

3. Results and Discussions

3.1 Propolis

Brown propolis from Southeast Brazil was evaluated and its flavonoids varied from 37 mg quercetin/g propolis extract (MDT) up to 58 mg quercetin/g propolis extract (US), Figure 1. The minimum concentration of flavonoids required by Brazilian regulation is 0.25% (w/w).[43] All propolis extracts presented higher amounts of flavonoids than the minimum required. Nonetheless, the current propolis presents low amounts of flavonoids, since brown propolis from northeast Brazil presented ~14% flavonoids.[25]

The samples also presented phenolic acids (from 45 mg gallic acid / g propolis extract (DM) up to 100 mg gallic acid / g propolis extract (DMT), 4.5-10% phenolic acids), but this amount can be considered low.[25][44] Maceration can be considered the best process, Figure 1, although the ultrasound energy usually results in high content of phenolic acids and flavonoids in the propolis extract.[45]

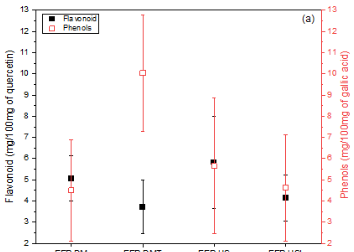
All samples presented antioxidant activity/scavenging activity. Regarding brown propolis,[46] low amounts of propolis extract were required to scavenge 50% of DPPH (from DMT (3.7 μg/mL) to USI (4.1 μg/mL)). The ABTS scavenging activity was in the expected range,[47] from 91 mmol trolox/100mg of propolis (DM) up to 103 mmol trolox/100mg of propolis (USI). DMT and USI techniques presented high FRAP results (264 mmol Fe(II)/100 mg propolis extract (DM) - 372 mmol Fe(II)/100 mg propolis extract (USI)), although these values can be considered low for propolis extracts.[46] The ultrasound extraction technique presented high values of antioxidant activity according to DPPH, FRAP, and ABTS methods.[48]

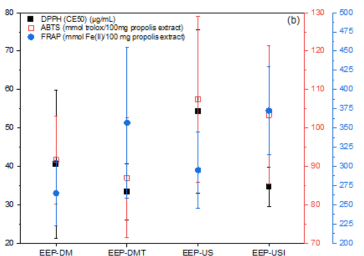
Regarding the identified phenolic compounds, there were chlorogenic, caffeic, ferulic, para-coumaric and rosmarinic acids, pinobanksin, kaempferol and kaempferide. The phenols amount in the propolis ethanolic extracts differ considerably, e.g., propolis from Brazilian South's region may present low amounts of artepilin C and coumaric acid.[18] Chromatographic analysis revealed the main active compounds in the propolis extract, Figure 1. Kaempferide was the main flavonoid in the propolis extracts, regardless of the type of extraction.[49] There were lower amounts of rosmarinic acid[50], p-coumaric acid[51][52], ferulic acid[53], and pinobanksin[54], mostly substances presenting antioxidant activity. Propolis extract also presented low amounts of chlorogenic acid, kaempferol, and caffeic acid.[55]

Propolis extract obtained through various techniques did not present significant differences regarding the active compounds. Then, macerated propolis extract was the one evaluated by antimicrobial activity. Propolis was placed in agar discs' wells, where *S. aureus* was incubated. The samples presented measurable halos, inhibiting approximately (40.97 ± 2.41) % of *S. aureus* microorganisms. The antimicrobial activity of propolis extracts is often related to their phenolic content.[56] Phenolic substances are capable of interfering with the structure and properties of bacterial membranes, increasing their susceptibility to proton permeation, and resulting in microorganisms' death.[57] The propolis extract activity against *S. aureus* might be related to the high amount of kaempferide, an antimicrobial substance related to a skin infection.[58]

3.2 CMC gels

Since brown propolis obtained through different techniques were similar regarding the active compounds, the direct macerated (DM) extract was used to incorporate in CMC membranes. Swelling capacity is a fundamental characteristic of hydrogel dressings, since they can absorb wound exudate.[59] Hydration content and swelling are relevant properties of dressings. Healing is improved by a moisturized environment. Wound dressings with high swelling capacity would be the best ones, they would absorb exudates and diminish the occurrence of infection.[60] CMC membranes presented high water absorption capacity compared to CMC-propolis (CMC-P) samples, Figure 2. Both samples reached the equilibrium of swelling degree (ESD) in 24h, CMC samples reached an ESD of ~449% while CMC-P samples reached an ESD of ~168%.[61], where CMC gels could be considered superabsorbent.[62] The low swelling capacity of CMC-P would be the consequence of propolis filling the pores of the membrane. Since the ethanolic extract of propolis is quite resinous, it may be responsible for a hydrophobic barrier formation. This barrier difficult the absorption of moisture by the pores of the polymer, leading to a low swelling degree and low weight loss.[63] The CMC-P samples presented a high gel fraction, where the presence of propolis extracts difficult the polymer's chains mobility, interfering with the





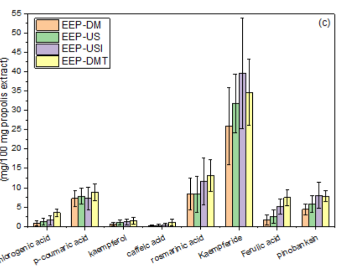


Figure 1. Propolis (a) phenols and flavonoids; (b) antioxidant activity; (c) active substances.

entanglement of amorphous chains. Due to the hydrophobic barrier of propolis, fewer chains could be leached out by the water entrance, and the CMC-P samples' weight loss was low, as well as its biodegradability rate in water.[64] Propolis extract, rich in phenolic substances, may be forming bonds with the polymer chains and thus contribute to the physical crosslinking of the gels, hindering the expansion of CMC chains. A low swelling degree allows a slow release of the active agents.[65]

The CMC sample, as well as the CMC-P sample, presented these materials' characteristic FTIR bands and vibration modes, in Table 1 and Figure 3. The CMC-P sample presented mainly bands related to both phases,

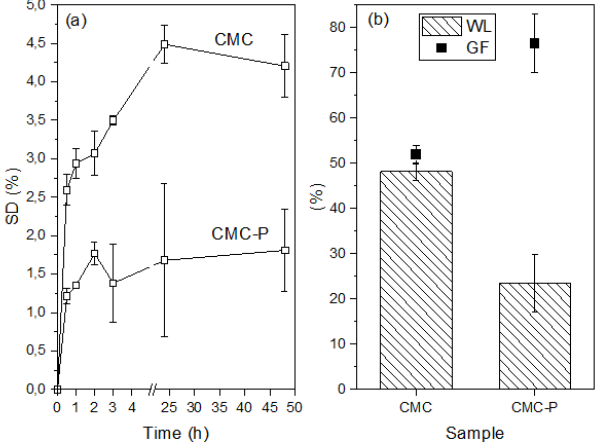


Figure 2 .(a) CMC and CMC-P (CMC-propolis) samples' swelling degree (SD) and (b) samples' gel fraction (GF) and weight loss (WL).

Table 1. CMC and propolis bands and their vibrational modes; CMC-P samples bands.

Sample				
CMC		Propolis		CMC-P
Band (cm^{-1})	**Vibration mode**	**Band (cm^{-1})**	**Vibration mode**	**Band (cm^{-1})**
3351	ν(-OH)[68]	3336	–OH group in -inter and intramolecular hydrogen bonds[69]	3386
-	-	2971	–OH groups[69]	-
2922	Aliphatic ν(C–H) vibrations[62]	2926		2922
-	-	2887		2855
1724	ester bond between anhydride of citric acid and non-substituted OH groups of cellulose[70]	-	-	-
-	-	1686	ν(carbonyl group)[71]	1687
-	-	1632	C=O of aldehydes, ketones, and carboxylic acids (isoflavones)[72]	1631
-	-	1601	ν(C=C), ν(C=O) of aromatic rings of polyols, flavonoids, and amino acids[69]	1602
1590	COO− of non-hydrated C=O groups[70]	-	-	1557
-	-	1515	polyphenolic ring vibration[73]	1511
-	-	1448	$\delta_{as}(CH_3)$[74]	1440
1416	$\sigma(–CH_2)$[70]	-	-	-
1372	δ(C-H)[75]	1379	$\delta_s(CH_3)$[74]	1376
1319	$\delta_{in\ plane}$(OH)[75]	-	-	-
-	-	1271	C−O−C bonds, related to phenolic acids and flavonoids[76]	-
1241	ν(C-O) of ether linkage[75]	-	-	1256
-	-	1183	ν(C-O), δ(C-OH) of lipids and alcohol groups[77]	1178
-	-	-	-	1132
-	-	1087	secondary alcohols; ν(C-O-) of ester group[77]	-
1054	ν_s(C-O) of primary alcohol[75]	1046	C-O folding of acids, alcohols, and esters[72]	-
1019	ν(carboxymethyl ether group)[70]	-	-	1026
-	-	983	$\delta_{out-of-plane}$(C-OH) and $\tau(-CH_2-)$[78]	984
893	β-Glucosidic linkages (sugar units)[75]	-	-	-
-	-	878	aromatic $\delta_{out-of-plane}$(C–H)[79]	-
-	-	-	-	835
-	-	802	aromatic ring $\delta_{out-of-plane}$(C–H)[80]	-
-	-	-	-	700

Vibrational modes: ν – stretching; ν_s – symmetric stretching; δ – bending/deformation, δ_{as} - asymmetric angular deformation/bending; δ_s - symmetric angular deformation/bending; τ – twisting; σ – scissoring.[81]

indicating proper incorporation. However, some band shifts were observed, indicating an interaction between propolis and CMC/citric acid.[66] Nonetheless, two bands, non-identified in the original materials, could be identified in the CMC-P sample (indicated by arrows in Figure 3), at 835 cm^{-1} and 700 cm^{-1}, probably related to new bonding between components' groups.[67] Further analysis would be required to properly identify the groups' interactions.

Regarding the XRD analysis, the CMC and CMC-P diffractograms were smoothed, then Gauss curves were fitted. Three crystalline peaks (2θ ~11°, ~21°, and ~35°) were identified, in Figure 3, related to CMC diffraction plans (110), (200), and (004) respectively.[82][83] The data (Half-width of the peak - FWHM, location of the peak, 2θ (rad)) related to the main peak (2θ ~21°) were the basis to calculate, by Scherrer equation, the crystallite size of CMC.[84] The CMC sample presented a crystallite size of 9.2 Å while the CMC-P sample crystallite size was 7.4 Å, which can be considered low-size crystallites.[85] Propolis seems to interfere with the bonds between CMC chains, leading to low crystallite size. Probably it breaks hydrogen bonds between CMC molecules; or the presence of propolis physically interferes with the CMC chains' mobility, diminishing the possibility of contact between chains.[86]

Regarding thermal properties, propolis increased the stability of the samples, Figure 4. The CMC hydrogel thermal degradation analysis began at 25° C, and the slight weight loss between 25 °C-200 °C would be related to volatile (H_2O, etc.) substances. The major loss is at 277 °C, due to the degradation of the CMC chains (bonds' cleavage related to functional groups and loss of weak groups of the main chains).[87] The degradation profile of both samples, CMC e CMC-P, are similar, but their behavior at high temperatures (above 400 °C) differs. The CMC-P sample degraded less than the CMC sample, probably due to propolis interaction with the CMC chains (which could be compared to the citric acid effect on CMC), where connection by hydrogen bonds would increase this sample's thermal stability.[88] The CMC sample's final residue was 1% while the CMC-P sample was 23%. The CMC sample weight loss in the first step was ~18% and in the second step, 49%. The CMC-P samples presented 14% and 37% of weight loss in the mentioned steps, where these two first degradation steps would be responsible for the samples' high degradation.[89][90] The increased stability of CMC-P was also shown by the high GF values and by the new FTIR bands, indicating an interaction between components of the CMC-P sample. The CMC sample presented many degradation steps above 400 °C, which might be related to the products of the 2nd stage degradation step, where the last steps would lead to gases (CO, CO_2, etc.) evolution and carbonaceous residue.[91]

The tensile tests of samples (triplicates for each composition) were performed until failure. CMC-P samples presented high strength compared to CMC samples, Figure 4. The samples' Young Modulus (E), elongation at break (e), and Failure strength (σ_f) were evaluated by ANOVA-1 way analysis (factor: composition; levels: CMC and CMC-P), with a confidence level of 95%. It was observed that the CMC sample presented Young Modulus (E) significantly lower than CMC-P (p-value = 0.01732). In addition, according to the ANOVA analysis, CMC-P showed failure strength significantly higher than CMC, with p-value = 0.04781. These results are in agreement with the finds reported in the FTIR and TGA analysis, where the propolis connection to the CMC

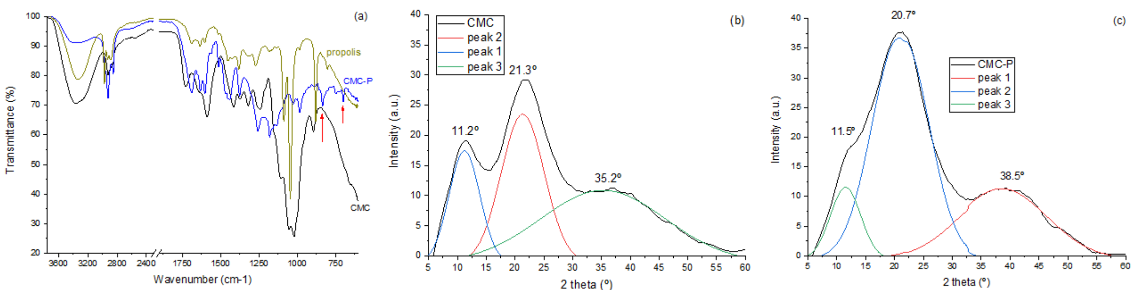


Figure 3. (a) FTIR spectra of CMC, propolis, and CMC-P samples, where new bands are indicated by arrows; XRD analysis of (b) CMC sample and (c) CMC-P sample, where fitted gauss curves ("peak") indicating the CMC diffraction peaks can be observed.

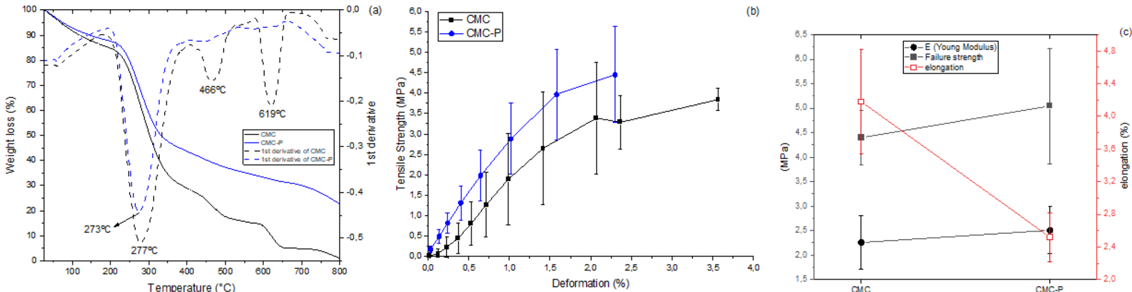


Figure 4. (a) Thermal degradation (TGA) curves of CMC and CMC-P samples and these curves' (1st) derivative curves; Samples (b) mechanical properties and (c) Young Modulus (E), elongation at break and failure strength.

chains could anchor the CMC molecules, diminishing the chains' mobility (and elongation at break), and increasing E and σ_f. Nevertheless, the samples' failure strength (CMC and CMC-P) can be considered low, as well as the young modulus.[92][93] The CMC-P samples presented elongation at break significantly lower than CMC samples (p = 0.02858). The elongation at the break would indirectly represent the samples' crosslinking, indicating effective interaction.[94]

Wound dressings have been designed to carry and to release drugs / antimicrobial agents locally. This property would be adequate for chronic lesions treatment, for wounds presenting prolonged inflammation step, as well as for delayed wound closure.[95] Mapping the drug release of the matrix (toward a controlled profile) would guarantee that the drug dose is within the therapeutical level. There are sustainable drug release systems (commercially available), e.g., Insulin Pen e Synchromate B., none of them for wound healing, though.[96] To highlight the importance of kinetics release knowledge regarding wound dressings, drug delivery can be the *sine qua non* condition to achieve robust delivery's steps and controlled amount of drug locally released. Local antibiotic sustainable release (within the dressing), compared to systemic antibiotic administration, led to efficient bactericide effect (against gram-positive and gram-negative bacteria) with low cytotoxicity to skin/eukaryotic cells.[97]

The delivered substances identified in the *in vitro* release studies were chlorogenic acid, caffeic acid, ferulic acid, *p*-coumaric acid, rosmarinic acid, and pinobanksin, Figure 5. The release profiles of all substances were similar (beginning of the release at 0.5 h and maximum release at 4h of testing, reaching a constant release/plateau from 4 h to 96 h).[98] The burst release is adequate for wound dressings since the delivery of the drug / active principle would prevent infection evolution. In pills, for example, it is expected that 75% of the active substance would be delivered within 45 minutes of administration.[99] Two release models were used to fit the results and the R2 values, Korsmeyer-Peppas (K-P) model (Equation 4)[100] and Peppas-Sahlin (P-S) model,[101] Figure 5. Peppas-Sahlin model is the best fit for the studied substances, but caffeic acid release was not properly adjusted by the studied models. All other substances were released according to the Peppas-Sahlin model, Equation 4.[101] This model consists of 2 terms on the right-hand side: the first term is the Fickian contribution, and the second term is the Case-II relaxational contribution.[102] A high k_1 value represents the drug diffusion release mechanism, while a high k_2 means polymer relaxation or heterogeneous erosion as the release mechanism.[103][104] Since negative k values should not be included in this analysis,[103] mainly non-linear Peppas-Sahlin propolis diffusion mechanism would be responsible for the release of the substances.

$$\frac{M_t}{M_\infty} = kt^m \quad (3)$$

$$\frac{M_t}{M_\infty} = k_1 t^m + k_2 t^{2m} \quad (4)$$

Mt = amount of substance released at time t; M∞ = amount of substance released at an ∞ period; m = release exponent; k = Korsmeyer–Peppas release constant; k_1, k_2 = rate constant and correlation coefficients.[105][106]

(a)

R2		
	P-S	K-P
Chlorogenic acid	0,99	0,33
Cafeic Acid	0,68	0,41
Ferulic Acid	0,99	0,02
p-Coumaric acid	0,97	0,12
Rosmarinus Acid	0,99	0,02
Pinobanksin	0,99	0,23

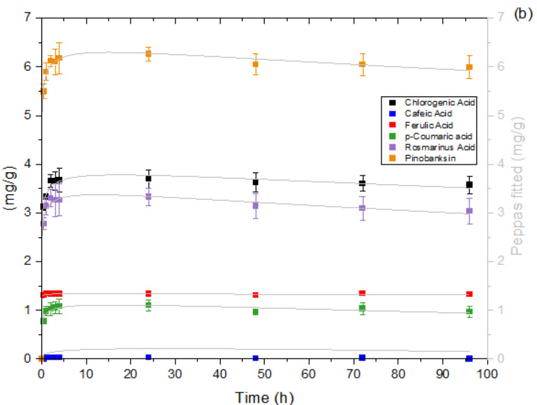


Figure 5. Kinetics of drug delivery: (a) R2 values of Korsmeyer-Peppas (K-P) and Peppas-Sahlin (P-S) models; (b) substances released – Peppas-Sahlin ("Peppas") fit.

4. Conclusion

Flavonoids and phenolic acids were identified in the studied propolis extracts, where the main substance was kaempferide, an antimicrobial substance. There were also considerable amounts of rosmarinic acid, p-coumaric acid, ferulic acid, and pinobanksin. Propolis had antioxidant properties, identified through DPPH, FRAP, and ABTS and it was active against *S. aureus*, no matter the extraction method. Propolis was successfully incorporated into CMC gels. The swelling capacity of the gels might be dose-dependent with the agent added, whereas CMC gels could be considered superabsorbent. The low swelling capacity and high gel fraction of CMC-P would be the consequence of propolis filling the pores of the membrane. Since the ethanolic extract of propolis is quite resinous, it may be responsible for the low absorption of moisture, leading to a low swelling degree and weight loss (biodegradability in water). Propolis could be anchoring the CMC chains, which was also observed by FTIR, where there was interaction and bonding between components. To corroborate the previous observation, propolis led to low CMC crystallite size formation (propolis could physically interfere with the CMC molecules, diminishing the possibility of contact between chains). The thermal degradation profile of CMC e CMC-P is similar, but the CMC-P sample degraded less than the CMC sample at temperatures above 400ºC. The CMC-P presented mainly a diffusion-controlled propolis release (Peppas-Sahlin model). The *in vitro* release studies showed a non-linear diffusion-based release kinetics for most phenolic substances of propolis extract (pinobankisin, rosmarinic acid, *p*-cumaric acid, ferulic acid, chlorogenic

acid), characterizing a diffusion-controlled release system. The CMC-P samples present potential as a dressing material.

5. Author's Contribution

- **Conceptualization** – Juliana Paes Leme de Mello Sousa; Rosane Nora Castro.
- **Data curation** – Juliana Paes Leme de Mello Sousa; Ormindo Domingues Gamallo; Leonardo Sales Araújo; Antonieta Middea; Yara Peluso Cid.
- **Formal analysis** – Juliana Paes Leme de Mello Sousa; Renata Nunes Oliveira; Rosane Nora Castro.
- **Funding acquisition** - Rosane Nora Castro; Renata Nunes Oliveira.
- **Investigation** – Juliana Paes Leme de Mello Sousa; Antonia Monica Neres Santos; Rosane Nora Castro.
- **Methodology** – Juliana Paes Leme de Mello Sousa; Antonia Monica Neres Santos; Rosane Nora Castro.
- **Project administration** – Rosane Nora Castro.
- **Resources** – Rosane Nora Castro.
- **Software** – NA.
- **Supervision** – Rosane Nora Castro; Renata Nunes Oliveira.
- **Validation** – Rosane Nora Castro.
- **Visualization** – Rosane Nora Castro.
- **Writing – original draft** – Renata Nunes Oliveira.
- **Writing – review & editing** – Renata Nunes Oliveira; Rosane Nora Castro.

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7. References

1. Guo, S., & DiPietro, L. A. (2010). Factors Affecting Wound Healing. *Journal of Dental Research*, *89*(3), 219-229. http://dx.doi.org/10.1177/0022034509359125. PMid:20139336.
2. Gonzalez, A. C. O., Costa, T. F., Andrade, Z. A., & Medrado, A. R. A. P. (2016). Wound healing - A literature review. *Anais Brasileiros de Dermatologia*, *91*(5), 614-620. http://dx.doi.org/10.1590/abd1806-4841.20164741. PMid:27828635.
3. World Health Organization (2013). *Prevention and management of wound infection*. Switzerland: Department of Violence and Injury Prevention and Disability - World Health Organization. Retrieved in 2023, February 05, from https://www.who.int/publications-detail-redirect/prevention-and-management-of-wound-infection
4. World Health Organization (2022). *Global Report on infection prevention and control.* Switzerland:World Health Organization
5. Cook, L., & Ousey, K. (2011). Demystifying wound infection: identification and management. *Practice Nursing*, *22*(8), 424-428. http://dx.doi.org/10.12968/pnur.2011.22.8.424.
6. Filius, P. M. G., & Gyssens, I. C. (2002). Impact of increasing antimicrobial resistance on wound management. *American Journal of Clinical Dermatology*, *3*(1), 1-7. http://dx.doi.org/10.2165/00128071-200203010-00001. PMid:11817964.
7. Chambers, H. F., & DeLeo, F. R. (2009). Waves of resistance: staphylococcus aureus in the antibiotic era. *Nature Reviews. Microbiology*, *7*(9), 629-641. http://dx.doi.org/10.1038/nrmicro2200. PMid:19680247.
8. Centers for Disease Control and Prevention – CDC (2019). *Multidrug-resistant pseudomonas aeruginosa*. Atlanta: CDC. Retrieved in 2023, February 05, from https://www.cdc.gov/drugresistance/pdf/threats-report/pseudomonas-aeruginosa-508.pdf.
9. Puca, V., Marulli, R. Z., Grande, R., Vitale, I., Niro, A., Molinaro, G., Prezioso, S., Muraro, R., & Di Giovanni, P. (2021). Microbial species isolated from infected wounds and antimicrobial resistance analysis: data emerging from a three-years retrospective study. *Antibiotics (Basel, Switzerland)*, *10*(10), 1162. http://dx.doi.org/10.3390/antibiotics10101162. PMid:34680743.
10. Mieles, J. Y., Vyas, C., Aslan, E., Humphreys, G., Diver, C., & Bartolo, P. (2022). Honey: an advanced antimicrobial and wound healing biomaterial for tissue engineering applications. *Pharmaceutics*, *14*(8), 1663. http://dx.doi.org/10.3390/pharmaceutics14081663. PMid:36015289.
11. Ghasemi, F. S., Eshraghi, S. S., Andalibi, F., Hooshyar, H., Kalantar- Neyestanaki, D., Samadi, A., & Fatahi-Bafghi, M. (2017). Anti-bacterial effect of propolis extract in oil against different bacteria. *Zahedan Journal of Researches in Medical Sciences*, *19*(3), e7225. http://dx.doi.org/10.5812/zjrms.7225.
12. Quintino, R. L., Reis, A. C., Fernandes, C. C., Martins, C. H. G., Colli, A. C., Crotti, A. E. M., Squarisi, I. S., Ribeiro, A. B., Tavares, D. C., & Miranda, M. L. D. (2020). Brazilian green propolis: chemical composition of essential oil and their in vitro antioxidant, antibacterial and antiproliferative activities. *Brazilian Archives of Biology and Technology*, *63*, e20190408. http://dx.doi.org/10.1590/1678-4324-2020190408.
13. Pinto, L. M. A., Prado, N. R. T., & Carvalho, L. B. (2011). Propriedades, usos e aplicações da própolis. *Revista Eletrônica de Farmácia*, *8*(3), 76-100.
14. Martinotti, S., & Ranzato, E. (2015). Propolis: a new frontier for wound healing? *Burns and Trauma*, *3*, 9. http://dx.doi.org/10.1186/s41038-015-0010-z. PMid:27574655.
15. Araujo, M. A. R., Libério, S. A., Guerra, R. N. M., Ribeiro, M. N. S., & Nascimento, F. R. F. (2012). Mechanisms of action underlying the anti-inflammatory and immunomodulatory effects of propolis: a brief review. *Revista Brasileira de Farmacognosia*, *22*(1), 208-219. http://dx.doi.org/10.1590/S0102-695X2011005000167.
16. Woźniak, M., Mrówczyńska, L., Waśkiewicz, A., Rogoziński, T., & Ratajczak, I. (2019). The role of seasonality on the chemical composition, antioxidant activity and cytotoxicity of Polish propolis in human erythrocytes. *Revista Brasileira de Farmacognosia*, *29*(3), 301-308. http://dx.doi.org/10.1016/j.bjp.2019.02.002.
17. Olegário, L. S., Andrade, J. K. S., Andrade, G. R. S., Denadai, M., Cavalcanti, R. L., Silva, M. A. A. P., & Narain, N. (2019). Chemical characterization of four Brazilian brown propolis: an insight in tracking of its geographical location of production and quality control. *Food Research International*, *123*, 481-502. http://dx.doi.org/10.1016/j.foodres.2019.04.004. PMid:31284998.

18. Machado, C. S., Mokochinski, J. B., Lira, T. O., Oliveira, F. C. E., Cardoso, M. V., Ferreira, R. G., Sawaya, A. C. H. F., Ferreira, A. G., Pessoa, C., Cuesta-Rubio, O., Monteiro, M. C., Campos, M. S., & Torres, Y. R. (2016). Comparative Study of Chemical Composition and Biological Activity of Yellow, Green, Brown, and Red Brazilian Propolis. *Evidence-Based Complementary and Alternative Medicine*, *2016*, 6057650. http://dx.doi.org/10.1155/2016/6057650. PMid:27525023.
19. Pontes, M. L. C., Vasconcelos, I. R. A., Diniz, M. F. F. M., & Pessôa, H. D. L. F. (2018). Chemical characterization and pharmacological action of Brazilian red propolis. *Acta Brasiliensis*, *2*(1), 34-39. http://dx.doi.org/10.22571/2526-433868.
20. Batista, L. L. V., Campesatto, E. A., Assis, M. L. B., Barbosa, A. P. F., Grillo, L. A. M., & Dornelas, C. B. (2012). Comparative study of topical green and red propolis in the repair of wounds induced in rats. *Revista do Colégio Brasileiro de Cirurgiões*, *39*(6), 515-520. http://dx.doi.org/10.1590/S0100-69912012000600012. PMid:23348649.
21. Moura, S. A. L., Negri, G., Salatino, A., Lima, L. D. C., Dourado, L. P. A., Mendes, J. B., Andrade, S. P., Ferreira, M. A. N. D., & Cara, D. C. (2011). Aqueous extract of Brazilian Green Propolis: primary components, evaluation of inflammation and wound healing by using subcutaneous implanted sponges. *Evidence-Based Complementary and Alternative Medicine*, *2011*, 748283. http://dx.doi.org/10.1093/ecam/nep112. PMid:19690045.
22. Conceição, M., Gushiken, L. F. S., Aldana-Mejía, J. A., Tanimoto, M. H., Ferreira, M. V. S., Alves, A. C. M., Miyashita, M. N., Bastos, J. K., Beserra, F. P., & Pellizzon, C. H. (2022). Histological, immunohistochemical and antioxidant analysis of skin wound healing influenced by the topical application of Brazilian red propolis. *Antioxidants*, *11*(11), 2188. http://dx.doi.org/10.3390/antiox11112188. PMid:36358560.
23. Zaccaria, V., Curti, V., Di Lorenzo, A., Baldi, A., Maccario, C., Sommatis, S., Mocchi, R., & Daglia, M. (2017). Effect of green and brown propolis extracts on the expression levels of microRNAs, mRNAs and proteins, related to oxidative stress and inflammation. *Nutrients*, *9*(10), 1090. http://dx.doi.org/10.3390/nu9101090. PMid:28974022.
24. Dembogurski, D. S. O., Trentin, D. S., Boaretto, A. G., Rigo, G. V., Silva, R. C., Tasca, T., Macedo, A. J., Carollo, C. A., & Silva, D. B. (2018). Brown propolis-metabolomic innovative approach to determine compounds capable of killing Staphylococcus aureus biofilm and Trichomonas vaginalis. *Food Research International*, *111*, 661-673. http://dx.doi.org/10.1016/j.foodres.2018.05.033. PMid:30007730.
25. Costa, M. C., Cruz, A. I. C., Ferreira, M. A., Bispo, A. S. R., Ribeiro, P. R., Costa, J. A., Araújo, F. M., & Evangelista-Barreto, N. S. (2023). Brown propolis bioactive compounds as a natural antimicrobial in alginate films applied to Piper nigrum L. *Ciência Rural*, *53*(5), e20210805. http://dx.doi.org/10.1590/0103-8478cr20210805.
26. Saha, D., & Bhattacharya, S. (2010). Hydrocolloids as thickening and gelling agents in food: a critical review. *Journal of Food Science and Technology*, *47*(6), 587-597. http://dx.doi.org/10.1007/s13197-010-0162-6. PMid:23572691.
27. Xu, H., Chen, G., Jin, R., Chen, D., Wang, Y., & Pei, J. (2014). Green synthesis of Bi2Se3 hierarchical nanostructure and its electrochemical properties. *RSC Advances*, *4*(17), 8922-8929. http://dx.doi.org/10.1039/c3ra46473c.
28. Waring, M. J., & Parsons, D. (2001). Physico-chemical characterisation of carboxymethylated spun cellulose fibres. *Biomaterials*, *22*(9), 903-912. http://dx.doi.org/10.1016/S0142-9612(00)00254-4. PMid:11311009.
29. Moseley, R., Walker, M., Waddington, R. J., & Chen, W. Y. J. (2003). Comparison of the antioxidant properties of wound dressing materials–carboxymethylcellulose, hyaluronan benzyl ester and hyaluronan, towards polymorphonuclear leukocyte-derived reactive oxygen species. *Biomaterials*, *24*(9), 1549-1557. http://dx.doi.org/10.1016/S0142-9612(02)00540-9. PMid:12559815.
30. Dhivya, S., Padma, V. V., & Santhini, E. (2015). Wound dressings – a review. *Biomedicine (Taipei)*, *5*(4), 22. http://dx.doi.org/10.7603/s40681-015-0022-9. PMid:26615539.
31. Mali, K. K., Dhawale, S. C., Dias, R. J., Dhane, N. S., & Ghorpade, V. S. (2018). Citric acid crosslinked carboxymethyl cellulose-based composite hydrogel films for drug delivery. *Indian Journal of Pharmaceutical Sciences*, *80*(4), 657-667. http://dx.doi.org/10.4172/pharmaceutical-sciences.1000405.
32. Silva, V. C., Silva, A. M. G. S., Basílio, J. A. D., Xavier, J. A., Nascimento, T. G., Naal, R. M. Z. G., del Lama, M. P., Leonelo, L. A. D., Mergulhão, N. L. O. N., Maranhão, F. C. A., Silva, D. M. W., Owen, R., Duarte, I. F. B., Bulhões, L. C. G., Basílio, I. D. Jr, & Goulart, M. O. F. (2020). New insights for red propolis of alagoas: chemical constituents, topical membrane formulations and their physicochemical and biological properties. *Molecules (Basel, Switzerland)*, *25*(24), 5811. http://dx.doi.org/10.3390/molecules25245811. PMid:33317120.
33. Papotti, G., Bertelli, D., Plessi, M., & Rossi, M. C. (2010). Use of HR-NMR to classify propolis obtained using different harvesting methods. *International Journal of Food Science & Technology*, *45*(8), 1610-1618. http://dx.doi.org/10.1111/j.1365-2621.2010.02310.x.
34. Mensor, L. L., Menezes, F. S., Leitão, G. G., Reis, A. S., Santos, T. C., Coube, C. S., & Leitão, S. G. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research*, *15*(2), 127-130. http://dx.doi.org/10.1002/ptr.687. PMid:11268111.
35. Embrapa Agroindústria Tropical (2007). *Comunicado técnico n. 128. Metodologia científica: determinação da atividade antioxidante total em frutas pela captura do radical livre ABTS°+*. Fortaleza: Embrapa.
36. Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, *45*(4), 493-496. http://dx.doi.org/10.1093/ajcp/45.4_ts.493. PMid:5325707.
37. Taylor, R. F., & Schultz, J. S., editors (1996). *Handbook of chemical and biological sensors*. USA: CRC Press. http://dx.doi.org/10.1887/0750303239.
38. Kamel, S., Ali, N., Jahangir, K., Shah, S. M., & El-Gendy, A. A. (2008). Pharmaceutical significance of cellulose: a review. *Express Polymer Letters*, *2*(11), 758-778. http://dx.doi.org/10.3144/expresspolymlett.2008.90.
39. Ghorpade, V. S., Yadav, A. V., & Dias, R. J. (2017). Citric acid crosslinked β -cyclodextrin/carboxymethylcellulose hydrogel films for controlled delivery of poorly soluble drugs. *Carbohydrate Polymers*, *164*, 339-348. http://dx.doi.org/10.1016/j.carbpol.2017.02.005. PMid:28325334.
40. Capanema, N. S. V., Mansur, A. A. P., Jesus, A. C., Carvalho, S. M., Oliveira, L. C., & Mansur, H. S. (2018). Superabsorbent crosslinked carboxymethyl cellulose-PEG hydrogels for potential wound dressing applications. *International Journal of Biological Macromolecules*, *106*, 1218-1234. http://dx.doi.org/10.1016/j.ijbiomac.2017.08.124. PMid:28851645.
41. Kokabi, M., Sirousazar, M., & Hassan, Z. M. (2007). PVA–clay nanocomposite hydrogels for wound dressing. *European Polymer Journal*, *43*(3), 773-781. http://dx.doi.org/10.1016/j.eurpolymj.2006.11.030.

42. Brasil. Ministério da Saúde. *Resolução-Re nº 90*. (2003, May 29). Guia para ensaios de dissolução para Formas Farmacêuticas Sólidas Orais de Liberação Imediata (FFSOLI). Biblioteca Virtual em Saúde. Retrieved in 2023, February 05, from https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2003/res0901_29_05_2003.html.
43. Brasil. Ministério da Agricultura e do Abastecimento. *Instrução Normativa nº3*. (2001, January 19). Diario Oficial da União, Brasília. Retrieved in 2023, February 05, from https://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=23/01/2001&jornal=1&pagina=46&totalArquivos=56.
44. Kurek-Górecka, A., Keskin, Ş., Bobis, O., Felitti, R., Górecki, M., Otręba, M., Stojko, J., Olczyk, P., Kolayli, S., & Rzepecka-Stojko, A. (2022). Comparison of the antioxidant activity of propolis samples from different geographical regions. *Plants*, *11*(9), 1203. http://dx.doi.org/10.3390/plants11091203. PMid:35567206.
45. González-Montiel, L., Figueira, A. C., Medina-Pérez, G., Fernández-Luqueño, F., Aguirre-Álvarez, G., Pérez-Soto, E., Pérez-Ríos, S., & Campos-Montiel, R. G. (2022). Bioactive compounds, antioxidant and antimicrobial activity of propolis extracts during in vitro digestion. *Applied Sciences (Basel, Switzerland)*, *12*(15), 7892. http://dx.doi.org/10.3390/app12157892.
46. Sousa, J. P. L. M., Pires, L. O., Prudêncio, E. R., Santos, R. F., Sant'Ana, L. D., Ferreira, D. A. S., & Castro, R. N. (2019). Chemical and antimicrobial potential study of Brazilian propolis produced by different species of bees. *Revista Virtual de Química*, *11*(5), 1480-1497. http://dx.doi.org/10.21577/1984-6835.20190103.
47. Muzzolon, A., Bicudo, Á. J. A., Oldoni, T. L. C., & Sado, R. Y. (2021). Dietary brown propolis extract modulated nonspecific immune system and intestinal morphology of Pacu Piaractus mesopotamicus. *Brazilian Archives of Biology and Technology*, *64*, e21200787. http://dx.doi.org/10.1590/1678-4324-2021200787.
48. Castro, R. N., & Salgueiro, F. B. (2016). Comparação entre a composição química e capacidade antioxidante de diferentes extratos de própolis verde. *Quimica Nova*, *39*(10), 1192-1199. http://dx.doi.org/10.21577/0100-4042.20160136.
49. Funari, C. S., Ferro, V. O., & Mathor, M. B. (2007). Analysis of propolis from Baccharis dracunculifolia DC. (Compositae) and its effects on mouse fibroblasts. *Journal of Ethnopharmacology*, *111*(2), 206-212. http://dx.doi.org/10.1016/j.jep.2006.11.032. PMid:17207952.
50. Ramanauskienė, K., Savickas, A., Inkėnienė, A., Vitkevičius, K., Kasparavičienė, G., Briedis, V., & Amšiejus, A. (2009). Analysis of content of phenolic acids in Lithuanian propolis using high-performance liquid chromatography technique. *Medicina*, *45*(9), 712-717. http://dx.doi.org/10.3390/medicina45090093. PMid:19834308.
51. Tomazzoli, M. M., Zeggio, A. R. S., Dal Pai Neto, R., Specht, L., Costa, C., Rocha, M., Yunes, R. A., & Maraschin, M. (2020). Botanical source investigation and evaluation of the effect of seasonality on Brazilian propolis from Apis mellifera L. *Scientia Agrícola*, *77*(6), e20180258. http://dx.doi.org/10.1590/1678-992x-2018-0258.
52. Calegari, M. A., Prasniewski, A., Silva, C., Sado, R. Y., Maia, F. M. C., Tonial, L. M. S., & Oldoni, T. L. C. (2017). Propolis from Southwest of Parana produced by selected bees: influence of seasonality and food supplementation on antioxidant activity and phenolic profile. *Anais da Academia Brasileira de Ciências*, *89*(1), 45-55. http://dx.doi.org/10.1590/0001-3765201620160499. PMid:28177054.
53. Chaa, S., Boufadi, M. Y., Keddari, S., Benchaib, A. H., Soubhye, J., Van Antwerpen, P., & Riazi, A. (2019). Chemical composition of propolis extract and its effects on epirubicin-induced hepatotoxicity in rats. *Revista Brasileira de Farmacognosia*, *29*(3), 294-300. http://dx.doi.org/10.1016/j.bjp.2019.01.005.
54. Xu, W., Lu, H., Yuan, Y., Deng, Z., Zheng, L., & Li, H. (2022). The antioxidant and anti-inflammatory effects of flavonoids from propolis via Nrf2 and NF-κB pathways. *Foods*, *11*(16), 2439. http://dx.doi.org/10.3390/foods11162439. PMid:36010439.
55. Lima, A. B. S., Santos, D. O., Almeida, V. V. S., Oliveira, A. C., & Santos, L. S. (2022). Quantificação de constituintes fenólicos de extratos de própolis vermelha de diferentes concentrações por HPLC. *Research. Social Development*, *11*(8), e1111830536. http://dx.doi.org/10.33448/rsd-v11i8.30536.
56. Torres, A. R., Sandjo, L. P., Friedemann, M. T., Tomazzoli, M. M., Maraschin, M., Mello, C. F., & Santos, A. R. S. (2018). Chemical characterization, antioxidant and antimicrobial activity of propolis obtained from Melipona quadrifasciata quadrifasciata and Tetragonisca angustula stingless bees. *Brazilian Journal of Medical and Biological Research*, *51*(6), e7118. http://dx.doi.org/10.1590/1414-431x20187118. PMid:29791598.
57. Sousa, A. K. A. (2018). *Atividade antibacteriana do extrato hidroalcoólico da própolis vermelha no semiárido paraibano sobre streptococcus pyogenes.* (Master''s Thesis). Campina Grande: Universidade Federal de Campina Grande.
58. Dégi, J., Herman, V., Igna, V., Dégi, D. M., Hulea, A., Muselin, F., & Cristina, R. T. (2022). Antibacterial activity of romanian propolis against Staphylococcus aureus isolated from dogs with superficial pyoderma: in vitro test. *Veterinary Sciences*, *9*(6), 299. http://dx.doi.org/10.3390/vetsci9060299. PMid:35737351.
59. Xiao, Y., Zhao, H., Ma, X., Gu, Z., Wu, X., Zhao, L., Ye, L., & Feng, Z. (2022). Hydrogel dressing containing basic fibroblast growth factor accelerating chronic wound healing in aged mouse model. *Molecules (Basel, Switzerland)*, *27*(19), 6361. http://dx.doi.org/10.3390/molecules27196361. PMid:36234898.
60. Berglund, L., Squinca, P., Baş, Y., Zattarin, E., Aili, D., Rakar, J., Junker, J., Starkenberg, A., Diamanti, M., Sivlér, P., Skog, M., & Oksman, K. (2023). Self-assembly of nanocellulose hydrogels mimicking bacterial cellulose for wound dressing applications. *Biomacromolecules*, *24*(5), 2264-2277. http://dx.doi.org/10.1021/acs.biomac.3c00152. PMid:37097826.
61. Hezaveh, H., Muhamad, I. I., Noshadi, I., Shu Fen, L., & Ngadi, N. (2012). Swelling behaviour and controlled drug release from cross-linked κ-carrageenan/NaCMC hydrogel by diffusion mechanism. *Journal of Microencapsulation*, *29*(4), 368-379. http://dx.doi.org/10.3109/02652048.2011.651501. PMid:22309480.
62. Akalin, G. O., & Pulat, M. (2018). Preparation and characterization of nanoporous sodium carboxymethyl cellulose hydrogel beads. *Journal of Nanomaterials*, *2018*, 9676949. http://dx.doi.org/10.1155/2018/9676949.
63. Oliveira, R. N., McGuinness, G. B., Rouze, R., Quilty, B., Cahill, P., Soares, G. D. A., & Thiré, R. M. S. M. (2015). PVA hydrogels loaded with a Brazilian propolis for burn wound healing applications. *Journal of Applied Polymer Science*, *132*(25), 42129. http://dx.doi.org/10.1002/app.42129.
64. Pereira, I. C. S., Santos, N. R. R., Middea, A., Prudencio, E. R., Luchese, R. H., Moreira, A. P. D., & Oliveira, R. N. (2019). *In vitro* evaluation of PVA gels loaded with Copaiba Oil and Duotrill®. *Polímeros: Ciência e Tecnologia*, *29*(3), e2019039. http://dx.doi.org/10.1590/0104-1428.03719.
65. Oshiro, J. A. Junior, Shiota, L. M., & Chiavacci, L. A. (2014). Desenvolvimento de formadores de filmes poliméricos

orgânico-inorgânico para liberação controlada de fármacos e tratamento de feridas. *Matéria (Rio de Janeiro)*, *19*(1), 24-32. http://dx.doi.org/10.1590/S1517-70762014000100005.

66. Alhazmi, H. A. (2019). FT-IR spectroscopy for the identification of binding sites and measurements of the binding interactions of important metal ions with bovine serum albumin. *Scientia Pharmaceutica*, *87*(1), 5. http://dx.doi.org/10.3390/scipharm87010005.
67. Yamakami, S. A., Ubaldini, A. L. M., Sato, F., Medina Neto, A., Pascotto, R. C., & Baesso, M. L. (2018). Study of the chemical interaction between a high-viscosity glass ionomer cement and dentin. *Journal of Applied Oral Science*, *26*(0), e20170384. http://dx.doi.org/10.1590/1678-7757-2017-0384. PMid:30020351.
68. Prasad, C. V., Sudhakar, H., Swamy, B. Y., Reddy, G. V., Reddy, C. L. N., Suryanarayana, C., Prabhakar, M. N., Subha, M. C. S., & Rao, K. C. (2011). Miscibility studies of sodium carboxymethylcellulose/poly(vinyl alcohol) blend membranes for pervaporation dehydration of isopropyl alcohol. *Journal of Applied Polymer Science*, *120*(4), 2271-2281. http://dx.doi.org/10.1002/app.33418.
69. Rajczak, E., Tylkowski, B., Constantí, M., Haponska, M., Trusheva, B., Malucelli, G., & Giamberini, M. (2020). Preparation and characterization of UV-curable acrylic membranes embedding natural antioxidants. *Polymers*, *12*(2), 358. http://dx.doi.org/10.3390/polym12020358. PMid:32041291.
70. Juncu, G., Stoica-Guzun, A., Stroescu, M., Isopencu, G., & Jinga, S. I. (2016). Drug release kinetics from carboxymethylcellulose-bacterial cellulose composite films. *International Journal of Pharmaceutics*, *510*(2), 485-492. http://dx.doi.org/10.1016/j.ijpharm.2015.11.053. PMid:26688041.
71. Silva, C., Prasniewski, A., Calegari, M. A., Lima, V. A., & Oldoni, T. L. C. (2018). Determination of total phenolic compounds and antioxidant activity of ethanolic extracts of propolis using ATR–FT-IR spectroscopy and chemometrics. *Food Analytical Methods*, *11*(7), 2013-2021. http://dx.doi.org/10.1007/s12161-018-1161-x.
72. Lapa, L. S. S., Silva, Y. R. O., & Sales, P. F. (2020). Aplicação das análises espectroscópicas e termogravimétricas em filmes biodegradáveis de amido de milho incorporados com extrato de própolis-verde. *ForScience*, *8*(2), e00712. http://dx.doi.org/10.29069/forscience.2020v8n2.e712.
73. Freitas, L. H., & Lima, L. S. C. 73. Freitas, L. H., & Lima, L. S. C. (2018). *Encapsulação de extrato de própolis utilizando soro de leite*. Fortaleza: Instituto Federal de Educação, Ciência e Tecnologia do Ceará - IFC. Retrieved in 2023, February 05, from http://prpi.ifce.edu.br/nl/_lib/file/doc4831-Trabalho/ENCAPSULACAOODEEXTRATODEPROPOLISUTILIZANDOSORODELEITE.pdf.
74. Claudino, G. P. (2011). *Estudo fitoquímico-biológico da madeira da espécie Dalbergia glaucescens (Mart. ex Benth.) Benth* (Doctoral Dissertation). Campos dos Goytacazes: Universidade Estadual do Norte Fluminense Darcy Ribeiro.
75. Yeasmin, M. S., & Mondal, M. I. H. (2015). Synthesis of highly substituted carboxymethyl cellulose depending on cellulose particle size. *International Journal of Biological Macromolecules*, *80*, 725-731. http://dx.doi.org/10.1016/j.ijbiomac.2015.07.040. PMid:26210036.
76. Scatolini, A. M., Pugine, S. M. P., Vercik, L. C. O., Melo, M. P., & Rigo, E. C. S. (2018). Evaluation of the antimicrobial activity and cytotoxic effect of hydroxyapatite containing Brazilian propolis. *Biomedical Materials (Bristol, England)*, *13*(2), 025010. http://dx.doi.org/10.1088/1748-605X/aa9a84. PMid:29135460.
77. Oliveira, R. N., Mancini, M. C., de Oliveira, F. C. S., Passos, T. M., Quilty, B., Thiré, R. M. S. M., & McGuinness, G. B. (2016). FTIR analysis and quantification of phenols and flavonoids of five commercially available plants extracts used in wound healing. *Matéria (Rio de Janeiro)*, *21*(3), 767-779. http://dx.doi.org/10.1590/S1517-707620160003.0072.
78. Tyliszczak, B., Walczyk, D., & Wilczyński, S. (2015). Acrylic hydrogels modified with bee pollen for biomedical applications. *Journal of Applied Pharmaceutical Science*, *5*(11), 010-014. http://dx.doi.org/10.7324/JAPS.2015.501102.
79. Nascimento, T. G., Silva, P. F., Azevedo, L. F., Rocha, L. G., Porto, I. C. C. M., Moura, T. F. A. L., Basílio-Júnior, I. D., Grillo, L. A. M., Dornelas, C. B., Fonseca, E. J. S., Oliveira, E. J., Zhang, A. T., & Watson, D. G. (2016). Polymeric Nanoparticles of Brazilian Red Propolis Extract: Preparation, Characterization, Antioxidant and Leishmanicidal Activity. *Nanoscale Research Letters*, *11*(1), 301. http://dx.doi.org/10.1186/s11671-016-1517-3. PMid:27316742.
80. Salmazo, P. S. (2019). *Biorremediação de solo e água contaminados por solventes aromáticos provenientes de combustíveis*. (Master's Thesis). Sorocaba: Universidade de Sorocaba.
81. Nasdala, L., Smith, D. C., Kaindl, R., & Ziemann, M. A. (2004). *Raman spectroscopy.* In A. Beran, & E. Libowitzky (Eds.), *Spectroscopic methods in mineralogy* (pp. 281-343). McLean: Mineralogical Society of Great Britain and Ireland. http://dx.doi.org/10.1180/EMU-notes.6.7.
82. Trilokesh, C., & Uppuluri, K. B. (2019). Isolation and characterization of cellulose nanocrystals from jackfruit peel. *Scientific Reports*, *9*(1), 16709. http://dx.doi.org/10.1038/s41598-019-53412-x. PMid:31723189.
83. Shetty, S. K., Ismayil, Hegde, S., Ravindrachary, V., Sanjeev, G., Bhajantri, R. F., & Masti, S. P. (2021). Dielectric relaxations and ion transport study of NaCMC:NaNO3 solid polymer electrolyte films. *Ionics*, *27*(6), 2509-2525. http://dx.doi.org/10.1007/s11581-021-04023-y.
84. Bokuniaeva, A. O., & Vorokh, A. S. (2019). *Estimation of particle size using the Debye equation and the Scherrer formula for polyphasic TiO 2 powder.* In *J, 6th International School and Conference "Saint Petersburg OPEN 2019": Optoelectronics, Photonics, Engineering and Nanostructures (012057)*. Moscow: Journal of Physics: Conference Series.
85. Poletto, M., Zattera, A. J., Forte, M. M. C., & Santana, R. M. C. (2012). Thermal decomposition of wood: influence of wood components and cellulose crystallite size. *Bioresource Technology*, *109*, 148-153. http://dx.doi.org/10.1016/j.biortech.2011.11.122. PMid:22306076.
86. Mohkami, M., & Talaeipour, M. (2011). Investigation of the chemical structure of carboxylated and carboxymethylated fibers from waste paper via Xrd and Ftir analysis. *BioResources*, *6*(2), 1988-2003. http://dx.doi.org/10.15376/biores.6.2.1988-2003.
87. Ahmad, N., Wahab, R., & Al-Omar, S. Y. (2014). Thermal decomposition kinetics of sodium carboxymethyl cellulose: model-free methods. *European Journal of Chemistry*, *5*(2), 247-251. http://dx.doi.org/10.5155/eurjchem.5.2.247-251.971.
88. Lee, J. Y., Im, J. N., Kim, T. H., Chung, D. J., & Doh, S. J. (2015). Structure and liquid handling properties of water-insoluble carboxymethyl cellulose foam. *Fibers and Polymers*, *16*(4), 726-734. http://dx.doi.org/10.1007/s12221-015-0726-1.
89. El-Sayed, S., Mahmoud, K. H., Fatah, A. A., & Hassen, A. (2011). DSC, TGA and dielectric properties of carboxymethyl cellulose/polyvinyl alcohol blends. *Physica B, Condensed Matter*, *406*(21), 4068-4076. http://dx.doi.org/10.1016/j.physb.2011.07.050.
90. Badry, R., Ezzat, H. A., El-Khodary, S., Morsy, M., Elhaes, H., Nada, N., & Ibrahim, M. (2021). Spectroscopic and thermal

analyses for the effect of acetic acid on the plasticized sodium carboxymethyl cellulose. *Journal of Molecular Structure*, *1224*, 129013. http://dx.doi.org/10.1016/j.molstruc.2020.129013.

91. Yaradoddi, J. S., Banapurmath, N. R., Ganachari, S. V., Soudagar, M. E. M., Mubarak, N. M., Hallad, S., Hugar, S., & Fayaz, H. (2020). Biodegradable carboxymethyl cellulose based material for sustainable packaging application. *Scientific Reports*, *10*(1), 21960. http://dx.doi.org/10.1038/s41598-020-78912-z. PMid:33319818.
92. Seki, Y., Altinisik, A., Demircioğlu, B., & Tetik, C. (2014). Carboxymethylcellulose (CMC)–hydroxyethylcellulose (HEC) based hydrogels: synthesis and characterization. *Cellulose (London, England)*, *21*(3), 1689-1698. http://dx.doi.org/10.1007/s10570-014-0204-8.
93. Tabari, M. (2017). Investigation of Carboxymethyl Cellulose (CMC) on mechanical properties of cold water fish gelatin biodegradable edible films. *Foods*, *6*(6), 41. http://dx.doi.org/10.3390/foods6060041. PMid:28555025.
94. Lan, W., He, L., & Liu, Y. (2018). Preparation and properties of sodium carboxymethyl cellulose/sodium alginate/chitosan composite film. *Coatings*, *8*(8), 291. http://dx.doi.org/10.3390/coatings8080291.
95. Laurano, R., Boffito, M., Ciardelli, G., & Chiono, V. (2022). Wound dressing products: a translational investigation from the bench to the market. *Engineered Regeneration*, *3*(2), 182-200. http://dx.doi.org/10.1016/j.engreg.2022.04.002.
96. Hassan, S., Ali, M. N., Mir, M., Ahmed, A., & Arshad, M. (2021). Development and evaluation of drug delivery patch for topical wound healing application. *SN Applied Sciences*, *3*(10), 825. http://dx.doi.org/10.1007/s42452-021-04809-9.
97. Miranda-Calderon, L., Yus, C., Landa, G., Mendoza, G., Arruebo, M., & Irusta, S. (2022). Pharmacokinetic control on the release of antimicrobial drugs from pH-responsive electrospun wound dressings. *International Journal of Pharmaceutics*, *624*, 122003. http://dx.doi.org/10.1016/j.ijpharm.2022.122003. PMid:35811042.
98. Basílio, J. A. D. (2018). *Desenvolvimento e avaliação in vitro da atividade cicatrizante de membranas poliméricas incorporadas com própolis vermelha* (Doctoral Dissertation). Maceió: Universidade Federal de Alagoas.
99. Brasil. Ministério da Saúde. *Resolução-RDC nº 31*. (2010, August 11). Dispõe sobre a realização dos Estudos de Equivalência Farmacêutica e de Perfil de Dissolução Comparativo. Biblioteca Virtual em Saúde. Retrieved in 2023, February 05, from http://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2010/res0031_11_08_2010.html.
100. Rozo, G., Bohorques, L., & Santamaría, J. (2019). Controlled release fertilizer encapsulated by a κ-carrageenan hydrogel. *Polímeros: Ciência e Tecnologia*, *29*(3), e2019033. http://dx.doi.org/10.1590/0104-1428.02719.
101. Peppas, N. A., & Sahlin, J. J. (1989). A simple equation for the description of solute release. III. Coupling of diffusion and relaxation. *International Journal of Pharmaceutics*, *57*(2), 169-172. http://dx.doi.org/10.1016/0378-5173(89)90306-2.
102. Ritger, P. L., & Peppas, N. A. (1987). A simple equation for description of solute release I. Fickian and non-fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs. *Journal of Controlled Release*, *5*(1), 23-36. http://dx.doi.org/10.1016/0168-3659(87)90034-4.
103. Chaiya, P., Rojviriya, C., Pichayakorn, W., & Phaechamud, T. (2022). New Insight into the Impact of Effervescence on Gel Layer Microstructure and Drug Release of Effervescent Matrices Using Combined Mechanical and Imaging Characterisation Techniques. *Pharmaceutics*, *14*(11), 2299. http://dx.doi.org/10.3390/pharmaceutics14112299. PMid:36365118.
104. Trucillo, P. (2022). Drug Carriers: A Review on the Most Used Mathematical Models for Drug Release. *Processes (Basel, Switzerland)*, *10*(6), 1094. http://dx.doi.org/10.3390/pr10061094.
105. Altun, E., Yuca, E., Ekren, N., Kalaskar, D. M., Ficai, D., Dolete, G., Ficai, A., & Gunduz, O. (2021). Kinetic Release Studies of Antibiotic Patches for Local Transdermal Delivery. *Pharmaceutics*, *13*(5), 613. http://dx.doi.org/10.3390/pharmaceutics13050613. PMid:33922739.
106. Liu, F., Wang, Z., Guo, H., Li, H., Chen, Y., & Guan, S. (2023). A Double-Layer Hydrogel Dressing with High Mechanical Strength and Water Resistance Used for Drug Delivery. *Molecules (Basel, Switzerland)*, *28*(2), 499. http://dx.doi.org/10.3390/molecules28020499. PMid:36677557.

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