Nanocellulose membranes loaded with vitamin B-based ionic liquids for skin care applications

G. Chantereau[a,b], M. Sharma[a], A. Abednejad[a], C. Vilela[a], E. M. Costa[c], M. Veiga[c], F. Antunes[c], M. M. Pintado[c], G. Sèbe[b], V. Coma[b], M. G. Freire[a], C. S. R. Freire[a], A. J. D. Silvestre[a,1]

[a]*CICECO – Aveiro Institute of Materials and Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal*

[b]*Laboratoire de Chimie des Polymères Organiques, University of Bordeaux, 33607 Pessac Cedex, France*

[c]*Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal*

[1] **Corresponding author:** Prof. Armando J. D. Silvestre
CICECO – Aveiro Institute of Materials and Department of Chemistry, University of Aveiro,
3810-193 Aveiro, Portugal
Tel: +351 234 370 711, E-mail address: armsil@ua.pt

Abbreviations:

IL	ionic liquid	B1	thiamine	[Ch][B1]	cholinium thiaminalate
BC	bacterial cellulose	B3	nicotinic acid	[Ch][B3]	cholinium nicotinate
CH	cholinium	B5	pantothenate	[Ch][B5]	cholinium pantothenate
VIT	vitamin B	B6	pyridoxine	[Ch][B6]	cholinium pyridoxylate

ABSTRAC[MF1]T

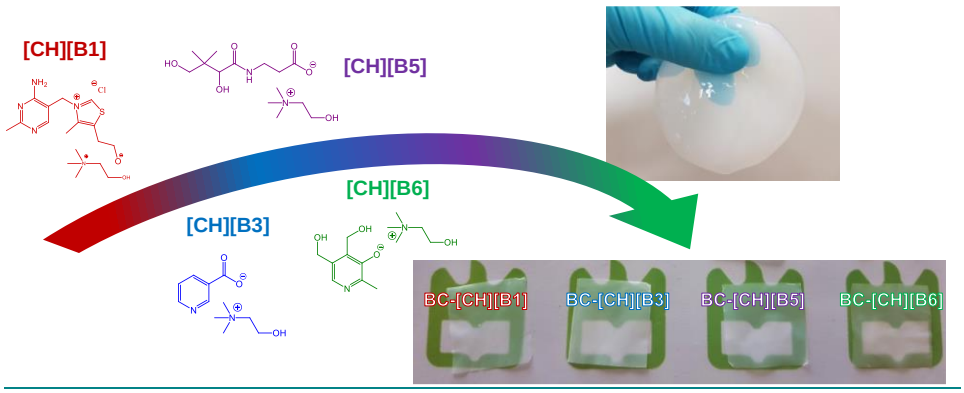
We report here the synthesis of original active principle ingredients (API) ionic liquids (ILs) with cholinium cation and vitamins B anions followed by their incorporation in bacterial nanocellulose (BC) membranes for topical applications. Four ILs were synthesized through a metathesis reaction namely, cholinium thiaminalate [Ch][B1], cholinium nicotinate [Ch][B3], cholinium pantothenate [Ch][B5] and cholinium pyridoxylate [Ch][B6]. The structure of these ILs was assessed through FTIR, ^{1}H and ^{13}C NMR and their thermal properties were investigated via DSC and TGA confirming their denomination as ionic liquids and their thermal resistance to autoclaving. Solubility assays in buffer solutions exhibited an increase of ILs solubility in comparison to their vitamin precursors, especially in the case of [Ch][B3] whose solubility increased up to 30.6-fold enhancing thus the bioavailability of the active principle. The evaluation of antioxidant activity of vitamins, ILs and formulations composed of mixtures of vitamins (B-complex) or ILs ([Ch][B-complex]) was used to determine the influence of ILs synthesis on the bioactive properties showing that the impact of ILs can be positive or negative depending on the vitamin but the synergetic effect of B-complex formulations is highly reinforced by ILs. The incorporation of ILs in BC membranes led to transparent and homogeneous membranes stable up to 190°C. ILs acted as plasticizers in BC reducing the brittleness of membranes and improving the contact with the skin. Moreover, the re-hydration ability of BC-ILs was improved 2.9 to 4.8-fold in comparison to BC, ensuring good absorption of exudates, while the release of ILs in buffer solutions was more complete and faster than the release of vitamins. Finally, we have demonstrated that BC-ILs are not cytotoxic to ~~dermal~~ skin epithelial cells ~~are~~ and thus are suitable materials for skin care applications.

HIGHLIGHTS

- Simple synthesis of original cholinium-vitamin B ionic liquids
- Successful design of non-cytotoxic IL-incorporated bacterial cellulose membranes
- Improved of vitamins solubility in buffer, especially for nicotinic acid IL
- Improvement of the synergetic effect of B-complex formulations on antioxidant activity
- Fast and complete release of ILs in buffer solutions and improved re-hydration capacity of membranes.

~~(graphical abstract: 5x13cm max)~~

Graphical abstract



~~Graphical abstract~~

KEYWORDS: bacterial nanocellulose, vitamin B, ionic liquids, cholinium-based, skin care application, antioxidant activity

1. INTRODUCTION

Vitamins are essential nutrients typically acquired through diet ~~that~~ since cannot be synthesized by human beings [1]. Among those nutrients, vitamins from complex B, namely thiamine (B1), riboflavin (B2), niacin or nicotinic acid (B3), pantothenic acid (B5), pyridoxine (B6), biotine (B8), folic acid (B9) and cobalamin (B12), are a class of vitamins acting as coenzymes in multiple enzymatic processes ~~of~~ in cells. The main roles of vitamin B are in catabolic metabolism for energy production, and in anabolic metabolism for the transformation of specific bioactive molecules [2]. The use of formulations combining several vitamins from complex B to reach synergetic effects is common in food supplementation [3] and skin care [4,5].

In the last decades, vitamins from complex B have been mainly used in the pharmaceutical industry as supplements, but also by cosmeceutical companies for skin care because of their reported beneficial properties. Vitamin B1 exhibits collagen production enhancement [6], vitamin B3 is used to prevent skin yellowing or hyperpigmentation, to increase collagen production and to reduce fine lines and wrinkles [7,8], vitamin B5 shows skin protection, *stratum corneum* hydration and anti-inflammatory properties [9], and vitamin B6 has demonstrated efficiency in the treatment of acne [10]~~, being present in skin care products~~. ~~Furthermore~~Additionally, B-complex vitamins formulations are recurrently used to prevent skin aging because of their antioxidant properties [11].

The design of drug-based ionic liquids (ILs) for tuned water-solubility and improved biological and/or therapeutic activities has recently been investigated for pharmaceutical and cosmeceutical applications [12–15]. ILs are organic salt-like compounds with a liquid domain below 100°C. They are promising compounds because of their high thermal stability, negligible vapor pressure and low flammability [12]. The cholinium cation, considered also part of the vitamin B group, has been largely investigated in the design of non-toxic and biocompatible ILs [16–18]. In 1962, the first cholinium-based IL, with the salicylate counter ion, showed improved water-

solubility and preservation of anti-inflammatory properties in comparison to salicylic acid [19]. Accordingly, in the current work, the design of cholinium-based ILs containing anions derived from vitamins of complex B is expected to lead to materials with enhanced bioactive properties.

Oral administration of vitamins B is widely spread, even for skin care, but might have several side-effects. For instance, excessive ingestion of vitamin B3 can lead to liver damage [20,21], while vitamin B6 can block nociception in the brain [22]. These problems can be overcome by topical delivery, in some cases even with stronger effect applying the same dosage [22]. Three different systems can be developed for topical delivery of vitamins B, namely creams, gels and patches. Creams and gels offer the advantage of simple usage for consumers, but present the drawback of small control of the amount of the active principle applied. On the other hand, patches permit a controlled drug release and easy posology control. Typically, patches have three layers, *viz* a drug-containing matrix, an outer membrane for easy handling and a rate controlling layer to slow down the diffusion of drugs [23–25]. An adhesive layer is also needed to guarantee the application onto the skin, but this layer tends to be replaced by matrices with inherent adhesiveness.

Bacterial nanocellulose (BC) is a nanofibrillar form of cellulose produced by several non-pathogenic bacteria of the ~~genus~~ *Gluconacetobacter*, *Sarcina* or *Agrobacterium* genus [26]. This biopolymer raised considerable interest in the pharmaceutical field, and particularly on drug delivery [27], due to its unique set of properties, such as nanofibrillar porous structure, high purity, high water-holding capacity, biocompatibility and high Young's modulus [26,28]. BC membranes have already been used to produce efficient topical drug delivery systems (TDDS) for the administration of different drugs or active molecules, namely lidocaine [29], ibuprofen [30], berberine hydrochloride [31] and also antioxidant-based ionic liquids [32], with the advantage of having a single layer structure, thus promoting a sustained release of drugs and absorption of exudates when applied in wounds.

Based on the exposed, the purpose of the current work was to synthesize novel vitamin B-based ILs, pairing the cholinium cation with anions derived from vitamins of complex B, namely cholinium thiaminalate[MF2][GC3][AS4] ([Ch[B1]), cholinium nicotinate ([Ch][B3]), cholinium pantothenate ([Ch][B5]) and cholinium pyridoxylate ([Ch][B6]). The obtained ILs were characterized by nuclear magnetix resonance (NMR), attenuated total reflexion-Fourier transformed infrared (FTIR-ATR), thermagravimetric analysis (TGA) and differential scanning calorimetry DSC. Then, their solubility in PBS aqueous solutions (pH 7.4, 0.1 M) was assessed, as well as their antioxidant properties. Finally, ILs were incorporated into BC membranes and the obtained ILs-incorporated membranes were characterized in terms of thermal and mechanical properties, and re-hydration ability to assess their suitability for skin care. The release of the ILs out of the BC membranes was investigated by dissolution tests in PBS aqueous solutions. Finally, the cytotoxicity of ILs-incorporated membranes was evaluated to confirm their feasibility to apply in topical applications. Most of these properties were also determined for the original vitamins from complex B for comparison purposes.[EMC5]

2. MATERIALS & METHODS

2.1. Materials

Cholinium (CH) bicarbonate (80% aqueous solution) was supplied by Sigma-Aldrich, thiamine hydrochloride (98.5-101.5%, vitamin B1) by Acros Organics, nicotinic acid (99%, vitamin B3) and calcium pantothenate (vitamin B5) by Carlo Erba Reagents, and pyridoxine hydrochloride (99.8%, vitamin B6) by Fagron. For the preparation of the phosphate buffer saline (PBS), sodium chloride (99.5%, NaCl) was purchased from Acros Organics, and potassium chloride (99%, KCl), disodium phosphate dodecahydrate (98-102%, $Na_2HPO_4 \cdot 12H_2O$) and potassium phosphate (99%, KH_2PO_4) from Sigma-Aldrich. PBS (pH 7.4, 0.1 M) was prepared with

8.010 g.L^{-1} NaCl, 0.200 g.L^{-1} KCl, 2.388 g.L^{-1} $Na_2HPO_4 \cdot 12H_2O$ and 0.270 g.L^{-1} KH_2PO_4. All other chemicals were used as received. Vitamins and vitamin solutions were protected from light due to the light sensitivity of vitamin B6 [33].

The preparation of Dulbecco's Modified Eagle's Medium (DMEM) was made with 4.5 g.L^{-1} glucose, L-glutamine without pyruvate (Lonza, Verviers, Belgium) containing 10% (v/v) fetal bovine serum (FBS, Biowest, Nuaillé, France) and 1% (v/v) Penicillin-Streptomycin-Fungizone (Lonza, Verviers, Belgium).

2.2. Synthesis of vitamin B-based ionic liquids

ILs comprising cholinium as the common cation and anions derived from vitamins of complex B ([Ch][VIT]) were synthesized by simple metathesis reactions [34]. After the synthesis, ILs were dried under vacuum at least for 72h at 35 ºC (water content < xx ppm[GC6]), and collected and stored in closed vials in the dark at 4°C up to use. Four ILs have been synthesized, namely cholinium thiaminalate ([Ch][B1]), cholinium nicotinate ([Ch][B3]), cholinium pantothenate ([Ch][B5]) and cholinium pyridoxylate ([Ch][B6]).

2.3. Bacterial cellulose production

BC membranes were prepared in our laboratory by incubating the bacterial strain *Gluconacetobacter sacchari* [35]. After 6 days of incubation, BC membranes were withdrawn from the culture medium, treated with a 0.5 M NaOH aqueous solution and repeatedly washed with water until neutral pH was reached. Wet BC membranes were stored in distilled water at 4°C until use.

2.4. Incorporation of vitamins and corresponding ILs in BC membranes

Wet BC membranes were cut into 2×2 cm² rectangular pieces (99.22±0.27 wt.% water content). Membranes were pressed at room temperature until at least 50% of their water content was drained. Then, BC membranes were soaked in 1 mL of PBS aqueous solutions containing

vitamins B1, B3, B5 and B6 at a concentration of 10 mg.mL^{-1}, as control samples. For ILs, the concentration was adapted to incorporate the same mass of each vitamin B in membranes, *i.e*. 13.4, 18.4, 14.8 and 16.1 mg.mL^{-1} for [Ch][B1], [Ch][B3], [Ch][B5] or [Ch][B6], respectively. After the complete absorption of the solutions by BC membranes, they were dried in a ventilated oven at 40°C. Dried membranes were kept in a desiccator until use.

After this, the sample holders used for the drying of membranes were rinsed with 4 mL of PBS aqueous solutions and the amount of the original vitamins or [Ch][VIT], not absorbed by the BC membranes, was determined by UV-vis spectroscopy or DAD-HPLC for vitamin B5 and [Ch][B5], as described below. By subtraction of the quantified amount of non-incorporated compounds, the mass of each vitamin and [Ch][VIT] incorporated in BC was determined.

For tensile tests, 7×7 cm² membranes were later prepared with ~~adapted~~ adjusted vitamin- or [Ch][VIT]-solution volumes to keep the same vitamin/BC or [Ch][VIT]/BC ratio.

2.5. Characterization of vitamins, ILs and BC

Quantification of ILs and BC-ILs. Ultraviolet visible (UV-Vis) spectroscopy, using a Thermo Scientific Evolution 600 spectrophotometer, was carried out to determine each vitamin and [Ch][VIT] concentration in aqueous PBS buffer solutions. The wavelengths of 231, 267, 262.5, 262.5, 254.5 and 222 nm for vitamin B1, [Ch][B1], vitamin B3, [Ch][B3], vitamin B6 and [Ch][B6] were used, respectively, using calibration curves previously established. Vitamin B5 and [Ch][B5] were quantified by ~~DAD-~~HPLC-DAD, ~~[Ch]~~using an Ultimate 3000, Dionex chromatographer (Germany) ~~with a reverse phase method~~ using a C18 column with a 5 µm pore dimension (Zorbax TMS 250×4.6 mm²). Mobile phase was acetonitrile:sodium phosphate pH 2.5 (10:90, v:v) solution, and the flow rate and the injection volume were set at 1.0 mL.min^{-1} and 10 µL, respectively. Quantifications and calibration curves were carried out at 198 nm.

The solubility of vitamins and [Ch][VIT] ILs was assessed in PBS aqueous solutions at pH 7.4. An excess of each compound was added to the PBS aqueous solution and allowed to equilibrate at 25°C under 750 rpm for 72h. After equilibration, solutions were centrifuged during 8 min at 8000 rpm. The ~~Supernatant~~ supernatant was recovered and centrifuged again for 8 min at 8000 rpm. Then, saturated solutions were diluted by successive 10-fold dilutions and their concentration was determined by UV-Vis or ~~DAD~~ HPLC-~~-~~DAD as described above). These experiments were performed at least in duplicate and the values expressed are an average of these results with the respective standard deviation.

Fourier transformed infrared-attenuated total reflection (FTIR-ATR). FTIR-ATR spectra of vitamins, [Ch][VIT] ILs and BC membranes loaded with [Ch][VIT] were obtained on a Perkin Elmer FT–IR System Spectrum BX spectrophotometer equipped with a single horizontal Golden Gate ATR cell. Thirty-two scans were acquired in the 4000–600 cm^{-1} range with a resolution of 4 cm^{-1}. Spectra were recorded at 3 different spots of the membranes surface and averaged.

Thermogravimetric analysis (TGA). Thermograms of vitamins, [Ch][VIT] ILs and BC membranes were conducted with a SETSYS Setaram TGA analyzer equipped with a platinum cell. Samples were heated at a constant rate of 10°C.min^{-1}, from room temperature up to 800°C, under a nitrogen flow of 20 mL.min^{-1}. The thermal decomposition temperatures (T_{dmax}) were taken as the maximum of the derivative of TGA curves. The residue fraction at 800°C was calculated in terms of weight percentage of the dry material.

Differential scanning calorimetry (DSC). DSC was used to determine the glass transition (T_g) and melting (T_m) temperatures of [Ch][VIT] ILs. Analyses were carried out in a power compensation differential scanning calorimeter, PERKIN ELMER model Pyris Diamond DSC, using hermetically sealed aluminum crucibles with a constant flow of nitrogen (50 mL.min^{-1}).

Samples of about 15 mg were used in each experiment. The temperature and heat flux scales of the power compensation DSC were calibrated by measuring the temperature and the enthalpy of fusion of reference materials, namely benzoic acid, 4-~~metoxibenzoic~~ methoxybenzoic acid, triphenylene, naphthalene, anthracene, 1,3,5-triphenylbenzene, diphenylacetic acid, perylene, *o*-terphenyl and 9,10-diphenylanthracene, at the scanning rate of 2 °C.min^{-1} and flow of nitrogen. The T_g and T_m were taken as the onset temperatures.

Antioxidant activity. The antioxidant activity of vitamins and [Ch][VIT] ILs was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The principle of the assay is based on the color change of the DPPH solution from purple to yellow, as the radical is quenched by the antioxidant. Change in color was monitored by visible spectroscopy at 517 nm [32]. Briefly, 3.34 mL of a DPPH solution (1 mM) in methanol was mixed with 50 mL of a vitamin solution (10 mg) in with a [Ch][VIT] solution (containing 10 mg of the vitamin IL anion) in methanol. A positive control (PC) was prepared with 10 mg of ascorbic acid and a negative control with 3.34 mL of DPPH solution (1 mM) in 50 mL of methanol. Samples were kept in the dark at room temperature and the decrease in the absorbance at 517 nm was determined by UV-vis spectroscopy [36]. DPPH radical scavenging activity, AA (%), was determined according to eq 1:

$$AA(\%) = \frac{(A_0 - A_1)}{A_0} * 100 \qquad (1)$$

where A_0 is the absorbance of the blank control and A_1 is the absorbance of the sample at 517 nm. Experiments were conducted in triplicate and the values given correspond to the average with the respective standard deviation.

Scanning electron microscopy (SEM). SEM analyses of the cross-sections of BC and BC loaded with vitamins (BC-VIT) of ILs (BC-[Ch][VIT]) were performed ~~after broken~~over breaked

~~the~~ membranes after immersion in liquid nitrogen. Samples were then covered with carbon and analyzed using a Hitachi SU-70 microscope at 4 kV and 10 mm focal distance.

Tensile assays. Tensile assays of BC, BC-VIT and BC-[Ch][VIT] membranes were performed using an Instron 5944 testing machine with Bluehill 3 software in tensile mode with a 1 kN load cell. ~~Strips~~ Specimens of 70×10 mm² and gauge length of 30 mm were used. The corresponding stress (MPa)–strain (%) curves were plotted to determine the Young's modulus (GPa) from the slope of the low strain region near 0.05%, maximum stress (MPa) and elongation at break (%). Experiments were conducted in quintuplicates and averaged with the respective standard deviation.

Re-hydration tests. Membranes (dried BC, BC-VIT and BC-[Ch][VIT]) were weighted and then soaked in individual containers with PBS aqueous solutions at room temperature, during 24h. At different times, samples were taken out, the excess of PBS was gently removed with an aluminum foil and membranes were weighted and re-immersed again. The absorbed PBS, in $g_{PBS}.g_{dry\ material}^{-1}$, was calculated according to eq 2:

$$PBS\ absorption = \frac{w_{wet} - w_{dry}}{w_{dry}} \quad (2)$$

where w_{dry} and w_{wet} are the weight of dried and wet BC samples, respectively. Experiments were conducted in triplicate and averaged with respective standard deviation.

Dissolution assays. Dissolution assays were conducted with BC-VIT and BC-[Ch][VIT] membranes. Samples were placed in a closed flask containing 200 mL of PBS aqueous solution (pH 7.4) under magnetic stirring. The vitamins or [Ch][VIT] ILs release was then evaluated. At determined time intervals (during 24h), 2 mL of solution was withdrawn, and the same volume of fresh buffer was added to maintain a constant volume. The vitamins and cholinium-based ILs

content in each aliquot was determined as described before. The vitamins and ILs content at each time was plotted as a cumulated concentration release (C_{cumul}), determined according to eq 3:

$$C_{cumul} = C_n + \sum_{k=0}^{n-1} \frac{C_k}{100} \quad (3)$$

where C_n is the IL concentration at time *n = 0, 5, 10, 15, 20, 30, 60, 120, 240, 360 and 480 min*. Weights of vitamins or [Ch][VIT] ILs leached out of the sample ~~was~~ were calculated and ~~divided by the initial mass incorporated to get~~ the released ratio (wt%) relative to the initial mass was calculated. Experiments were conducted in triplicate and averaged with respective standard deviation.

***In vitro* cytotoxicity assays.** Cytotoxicity was ascertained on human keratinocyte cell line (HaCaT cells) obtained from Cell Line Services (Appenheim, Denmark). The cells were cultured, at 37ºC in a humidified atmosphere of 95% air and 5% CO_2, using Dulbecco's Modified Eagle's Medium (DMEM). BC, BC-VIT and BC-[Ch][VIT] membranes cytotoxicity was assayed through adaptation of the procedure previously described by Sadeghian *et al.* [37]. Briefly, HaCaT cells were seeded in the wells of a 96 well microplate and allowed to adhere for 24h. Simultaneously, membranes were cut into 2×2 cm² pieces and then soaked in 10 mL of DMEM for 24h. After this period the media was removed and cells were washed with PBS aqueous solutions. Then, membranes were taken out of DMEM medium and the leached substances were added (direct and ½ diluted) to cells. After 24h incubation, 25 µL of sodium 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)-carbonyl]-2H-tetrazolium (XTT) working solution were added to each well and the cells were incubated, in the dark, for 2h. The optical density (OD) at 485 nm was then measured using a microplate reader (FLUOstar, OPTIMA, BMG Labtech, Ortenberg, Germany). Experiments were conducted in quintuplicate and averaged with respective standard deviation.

The cytotoxicity results were used to calculate the percentage of inhibition after incubation with the sample using eq 4:

$$Inhibition\ (\%) = 1 - abs_{sample}/abs_{control} * 100 \quad (4)$$

where abs_{sample} is the absorbance in a well containing sample and $abs_{control}$ is the absorbance of untreated control cells.

3. RESULTS

In this work, novel cholinium-based ILs paired with anions derived from vitamins B1, B3, B5 and B6, namely thiaminalate (B1), nicotinate (B3), pantothenate (B5) and pyridoxylate (B6), were synthesized and characterized by NMR, FTIR-ATR, DSC, TGA, solubility in PBS aqueous solutions and antioxidant activity. Most of these characterization studies were also conducted for the original vitamins. The obtained ILs and vitamin B precursors were then incorporated into BC membranes, characterized by NMR, FTIR-ATR, SEM, TGA, tensile tests, re-hydration capacity, dissolution and *in vitro* cytotoxicity assays, envisaging their use in skin care applications.

3.1. Synthesis and characterization of vitamin B-based ILs

The vitamin B-based ILs were synthesized by the neutralization of cholinium bicarbonate with different vitamins B. The full names, acronyms and chemical structures of the studied ILs and their vitamin precursors are depicted in Fig. 1~~Fig. 1~~.

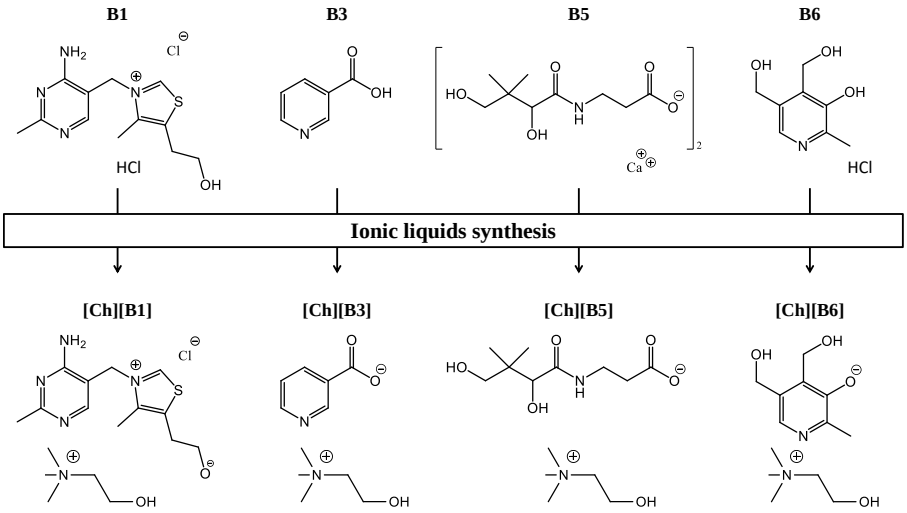


Fig. 1. Chemical structures of the vitamin B precursors (thiamine hydrochloride (B1), nicotinic acid (B3), calcium pantothenate (B5), pyridoxine hydrochloride (B6)) and the corresponding cholinium-based ILs (cholinium thiaminalate [Ch][B1], cholinium nicotinate [Ch][B3], cholinium pantothenate [Ch][B5], and cholinium pyridoxylate[Ch][B6]).

3.1.1. Structural and thermal characterization

After the synthesis of the [Ch][VIT] ILs, the cholinium:VIT molar ratios (Table S1) were confirmed based on their ^{1}H NMR spectra (^{1}H and ^{13}C NMR spectra are given in Fig. S1, S2, S3 and S4 in the Supporting Information, as well as the corresponding resonance assignments), confirming the ILs successful synthesis. The FTIR-ATR spectra of the [Ch][VIT] ILs and their vitamin precursors are displayed in Fig. S5 in the Supporting Information. The spectra of all [Ch][VIT] ILs showed the stretching δ(C–C–O) vibration of the cholinium ion at 955 cm^{-1} [38]. Additionally, the synthesis of [Ch][B1] was confirmed by the appearance of the C–O^{-} vibration band of the ethanolate at 1074 cm^{-1}. Regarding [Ch][B3], the disappearance of the bending ν(C=O) band of the carboxylic acid at 1708 cm^{-1}, typical of vitamin B3, and the appearance of a ν(C=O) stretching band of the carboxylate at 1611 cm^{-1} were observed. The ν(C=O) stretching band of carboxylate is shifted from 1556 cm^{-1} in vitamin B5 to 1585 cm^{-1} in [Ch][B5]. Finally, in the

spectrum of [Ch][B6], there is the appearance of the methoxylate C–O^- vibration at 1059 cm^{-1} is perceived [38]. All these data confirm the successful synthesis of [Ch][VIT] ILs.

The thermal characterization and thermal stability of the synthesized ILs was ascertained to confirm [Ch] if they are stable enough for the target application, and particularly to address if they support typical sterilization procedures such as autoclaving. The glass transition (T_g), melting (T_m) and decomposition temperatures (T_{dmax}) of the prepared [Ch][VIT] ILs were determined, being reported in

Table 1~~Table 1~~. All ILs showed low glass transition temperatures, in the range of -25 to -75°C. [Ch][B1] and [Ch][B6] ILs are solid at room temperature, with melting temperatures of 91 and 68°C, respectively, while [Ch][B3] and [Ch][B5] are liquid at room temperature and do not have any melting peak between glass transition and 100°C, meaning that these two ILs pass from the glass to the liquid state without a solid domain identified at the studied heating speed. This difference of thermal behavior is due to the presence of alkoxide ions in [Ch][B1] and [Ch][B6] and carboxylate ions in [Ch][B3] and [Ch][B5]. It is known that alkoxide ions form weaker ionic interactions with cholinium than carboxylate ions, leading to lower melting temperatures [39–41]. Overall, all synthesized compounds display a melting temperature below 100°C, meaning that they fit within the ILs class.

Regarding the decomposition temperatures, [Ch][B1] (256°C), [Ch][B5] (230°C) and [Ch][B6] (200°C) have decomposition temperatures slightly lower than those of their precursors B1 (264°C), (B5 (232°C) and B6 (212°C). This is due to the fact that B1, B5 and B6 are already in a salt form. Accordingly, the addition of an organic cation as cholinium is shown to not decrease significantly the decomposition temperatures. [Ch][B3] degrades at 224°C, which is ca. 100ºC below nicotinic acid. This decrease is coherent with other ~~works~~ studies on ILs compared with their acid precursors [16,17,42]. ~~Overall~~Nevertheless, all [Ch][VIT] ILs are thermally stable enough to

support ~~sterilization by~~ autoclaving at around 120°C to sterilize the patches for the~~and the~~ target skin care applications.

Table 1. Thermal properties of [Ch][VIT] ILs.

	T_g (°C)	T_m (°C)	T_{dmax} (°C)
B1		>248[c]	264
B3		232-263[d]	320[b]
B5		No defined melting[e]	231
B6		217±30[f]	212[a]
[Ch][B1]	-27	91	256
[Ch][B3]	-73	Liquid at RT	224
[Ch][B5]	-47	Liquid at RT	230
[Ch][B6]	-56	68	200

RT: room temperature, [a][43], [b][44], [c][45], [d][46], [e][47], [f][48]

3.1.2. Solubility assays

The solubility results ~~for~~of the original vitamins and [Ch][VIT] ILs in PBS (pH 7.4) aqueous solutions are reported in Fig. 2~~Fig. 2.~~ ~~Detailed data are given~~and Table S2 in the Supporting Information. The solubility values are 1721, 5224, 608 and 751 mM for [Ch][B1], [Ch][B3], [Ch][B5] and [Ch][B6], respectively. These are higher than ~~that~~ those of their vitamin precursors, namely 1515, 170, 160 and 683 mM for vitamins B1, B3, B5 and B6, respectively. For most [Ch][VIT] the improvements in solubility remain in the same order of magnitude. Nevertheless, it should be remarked that ~~most of~~ (with exception of nicotinic acid) these vitamins are in a salt form, being demonstrated here that the addition of an organic cholinium cation, which is also a vitamin and can be valuable to improve biological activities, still leads to ~~an improvement on~~ solubility improvements. The most remarkable increase (up to 30.6-fold) in solubility is observed ~~with~~ for nicotinic acid when converted to the respective IL, namely cholinium nicotinate, since there is the

conversion of an acid to a salt form. These results are in agreement with previous studies on the synthesis of cholinium-based ILs with phenolic acids [32,34]. Although most ~~works~~ studies on the solubility of novel ILs are carried out with water, ~~in this work~~here, aqueous PBS solutions were used ~~as more similar~~doe to their higher similarity to intra- and inter-cellular media in epidermis, while envisaging their use in transdermal delivery and skin care applications.

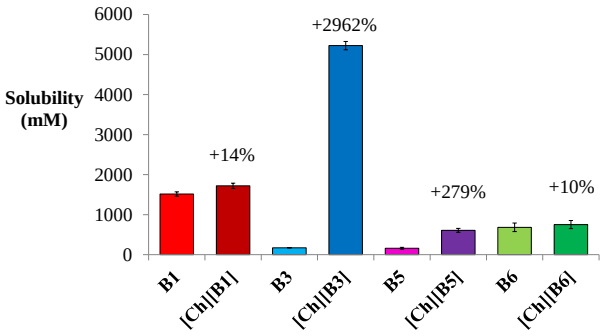


Fig. 2. Solubility of the original vitamins and [Ch][VIT] ILs in PBS (pH 7.4) aqueous solutions.

3.1.3. Antioxidant activity

The antioxidant activity of the prepared cholinium-based ILs and respective vitamin ~~percursors~~precursors was evaluated by the DPPH radical scavenging assay. Fig. 3~~Fig. 3~~ depicts the antioxidant activity (AA%), using ascorbic acid as positive control. The assays were carried for 3 days due to the slow kinetics of radical scavenging activity of vitamins B, ~~and~~ as reported by Higashi-Okai *et al.* [11]. Fig. S6 in the Supporting Information shows photographs of all samples after 3 days. However, in most cases, a maximum antioxidant activity plateau was reached after 2 days for both vitamin and [Ch][VIT] samples. At the studied concentration, ascorbic acid reaches a maximum antioxidant activity of 98.0 ± 0.6% ~~and~~ while all vitamins reached more than 50% of antioxidant activity, namely 95.0±0.7%, 70.4±2.8%, 55.4±0.6% and 83.7±0.6% for B1, B3, B5 and B6, respectively. The design of vitamin B-based ILs leads however to different effects on the antioxidant activity. While [Ch][B1] has an activity (96.0±0.1 AA%) similar to that of vitamin B1,

[Ch][B3] and [Ch][B6] have ~~smaller~~ lower activities (63.2±0.9 and 68.4±1.4%, respectively). On the other hand, [Ch][B5] ~~shows~~ showed a strong antioxidant activity increase (73.2±4.7%).

The use of vitamins in topical applications for daily skin care typically involves B-complex vitamin formulations because of potential synergetic effects on anti-aging or oxidative stress reduction [5,49,50]. Accordingly, we determined the antioxidant activity of a B-complex formulation, namely composed of 2.4 mg of B1, 32 mg of B3, 10 mg of B5 and 2.6 mg of B6, prepared based on the daily Dietary Reference Intake (DRI) approved by the Food and Drug Administration [51]. A formulation of ILs containing the same ~~mass of IL anions~~vitamin precursors was also prepared, here called as [Ch][B-complex]. The antioxidant activity of both formulations is very high, namely 97.8±0.4% and 97.9±0.1%, respectively, thus supporting synergetic effects that can be even more enhanced by the presence of cholinium. Furthermore, the kinetics of antioxidant activity is significantly faster for these formulations than for isolated vitamins and ILs. The vitamin B-complex formulation reaches its maximal activity after 18 h, whereas [Ch][B-complex] formulation reaches its maximal activity in less than 1 h. This remarkable improvement of antioxidant activity kinetics reinforces the potential of [Ch][B-complex] formulations as efficient as ascorbic acid, opening the path to interesting applications in oxidative stress reduction.

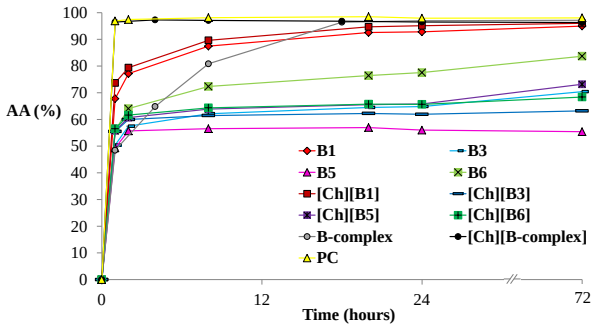


Fig. 3. Antioxidant activity of vitamins B1, B3, B5 and B6, [Ch][B1, [Ch][B3], [Ch][B5] and [Ch][B6] ILs, vitamin B complex (B-complex), [Ch][B-complex] and ascorbic acid as positive control (PC) along time.

3.2. Preparation and characterization of BC membranes enriched with vitamin B-based ILs

The obtained [Ch][VIT] ILs, and the original vitamins for comparison purposes, were incorporated into BC membranes aiming their application in skin care. The average weight of vitamins and [Ch][VIT] ILs incorporated into BC was determined by UV-vis spectroscopy or DAD-HPLC as described before. ~~The respective data are given in Table S3 in the Supporting Information.~~ In general, both vitamins B and ILs were incorporated in BC in high percentages, ranging from 86.9±1.7% to 99.3±0.5% of the initial amount poured on the top of BC (The respective data are given in Table S3 in the Supporting Information.). Furthermore, no significant differences in incorporation levels were observed between the original vitamins and ~~I~~resulting ILs ~~incorporation levels~~.

3.2.1. Structural and morphological characterization

The structural characterization of BC-[Ch][VIT] membranes was carried out by solid-state ^{13}C NMR (spectra given in Fig. S7, S8, S9 and S10 in the Supporting Information) and FTIR-ATR (Fig. S11 in the Supporting Information). The solid-state ^{13}C CP/MAS NMR spectra of BC-[Ch][VIT] samples show the typical resonances of BC and of the incorporated IL, whereas the higher intensity of BC resonances agrees with the relative proportion of BC and ILs. A more detailed analysis reveals shifts of some specific carbon resonances of [Ch][VIT] after incorporation in BC, namely: the resonance of (C–**C**H_2–O^-) carbon of [Ch][B1] that is shifted from 60.59 ppm to 62.36 ppm; the resonance of (C–**C**OO^-) carbon of [Ch][B3] that is shifted from 167.87 ppm to

173.54 ppm; the resonance of (CH_2–**C**OO^-) carbon of [Ch][B5] that is shifted from 175.17 to 165.18 ppm; and the resonance of (aromatic **C**–O^-) carbon of [Ch][B6] that is shifted from 139.17 to 134.41 ppm. These shifts suggest strong interactions between ionic liquids and bacterial cellulose nanofibrils. FTIR-ATR results further confirm this behavior. Although the ethanolate band of [Ch][B1] is overlapped with the (C–O–C) band of cellulose, it is observed a shift from 1682 to 1653 cm^{-1} of the protonated imine of thiaminalate after incorporation in BC; the carboxylate band of [Ch][B3] moves from 1613 to 1598 cm^{-1}; and the carboxylate band of [Ch][B5] shifts from 1585 to 1564 cm^{-1}. It should be remarked that in BC-[Ch][B6], the overlapping of the methanolate band of [Ch][B6] with the (C–O–C) band of cellulose does not allow to observe any significant shift.

Both BC-VIT and BC-[Ch][VIT] dried membranes (~~morphological characterization in~~ Fig. S12 ~~given~~ in the Supporting Information), with the exception of BC-B3, are transparent and homogeneous, similarly to BC, which is an indication of the good dispersion of the vitamins and of the corresponding ILs within the network of cellulose nanofibrils. In the case of VIT B3, the obtained BC membrane is heterogeneous, with the presence of agglomerates. On the other hand, the BC-[Ch][B3] is homogenous, avowing the formation of agglomerates. This is due to the considerable increase on the aqueous solubility when converting vitamin B3 to the respective IL, resulting in a higher affinity to the hydrophilic cellulose matrix and dispersion in the membrane.

SEM analysis ~~was~~ were performed on the cross-sections of BC, BC-VIT and BC-[Ch][VIT] membranes~~, with the respective SEM images given in~~ (~~-~~Fig. 4~~Fig. 4~~). All samples presented the characteristic lamellar structure of BC. Yet, BC-[Ch][VIT] membranes had less distinguishable nanofibrils, confirming improved affinity between ILs and cellulose nanofibrils and the complete filling of the spaces between the cellulose fibrils by ILs. The improvement of affinity promoted by the conversion of vitamins into the respective ILs is particularly notorious for vitamin B3, where

agglomerates are easily perceptible in the cross-section of the corresponding membranes. These results are in line with the macroscopic appearance of the membranes (Fig. S12 in the Supporting Information), and are in agreement with the low solubility of vitamin B3 in water and significant solubility enhancement ~~by~~ resulting from its conversion into a cholinium-based IL (~~shown in~~ Fig. 2~~Fig. 2~~).

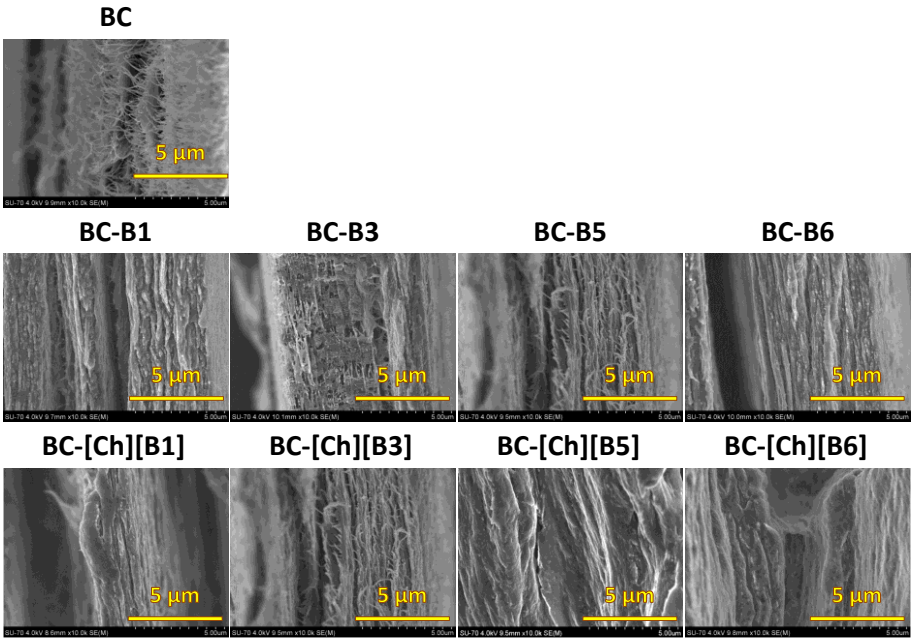


Fig. 4. SEM images of BC, BC-VIT and BC-[Ch][VIT] cross sections at ×10k magnification.

3.2.2. Thermal properties

The impact of the incorporation of vitamins and [Ch][VIT] on the thermal properties of BC membranes was investigated by TGA. Fig. S13 shows the thermographs of BC, BC-VIT and BC-[Ch][VIT] membranes, whereas Fig. S14 in the Supporting Information depicts the corresponding derivative curves. Table 2~~Table 2~~ summarizes the decomposition temperature of each material, ascertained by the T_{dmax} values. BC has a characteristic maximum weight loss at 339°C [52]. BC-B1, BC-B3, BC-B5 and BC-B6 samples present a 1st weight loss at 235, 213, 197 and 203°C, respectively, due to the decomposition of vitamins B (cf.

Table 1~~Table 1~~), and a 2nd weight loss at 341, 351, 355 and 327°C due to cellulose decomposition. Regarding the BC-[Ch][VIT] samples, the 1st decomposition step, assigned to the degradation of the ILs, is observed at 202, 236, 225 and 190°C, while the decomposition of the BC-enriched fraction is observed at 288, 317, 329 and 286°C for BC-[Ch][B1], BC-[Ch][B3], BC-[Ch][B5] and BC-[Ch][B6], respectively. The incorporation of ILs into BC leads only to slight differences on the decomposition temperatures of the [Ch][VIT] ILs. However, a decrease on the cellulose decomposition temperature is observed in all cases, being in agreement with the literature on the decomposition temperatures of other salt-incorporated cellulose materials [53,54]. This effect is stronger in the case of BC-[Ch][B1] and BC-[Ch][B6], probably because [Ch][B1] and [Ch][B6] have propanolate and ethanolate groups, respectively. The methanolate groups of the negatively charged surface of BC may have competitive interactions with cholinium cations [53]. ~~Although~~ Despite these differences, BC-[Ch][VIT] samples are thermally stable at least up to 190°C, allowing them to be submitted to thermal treatment such as autoclaving (ca. 120°C) necessary in many nutraceutical and cosmetic applications, and not compromising the envisioned skin care applications.

Table 2. Decomposition temperatures determined by DTG curves.

	T_{dmax} (°C)			
	T_{d1}	T_{d2}	T_{d3}	T_{d4}
BC	339			
BC-B1	235	341		
BC-B3	213	351		
BC-B5	197	231	267	355
BC-B6	203	251	327	
BC-[Ch][B1]	262	316		
BC-[Ch][B3]	236	271	317	450
BC-[Ch][B5]	225	329		
BC-[Ch][B6]	190	249	286	417

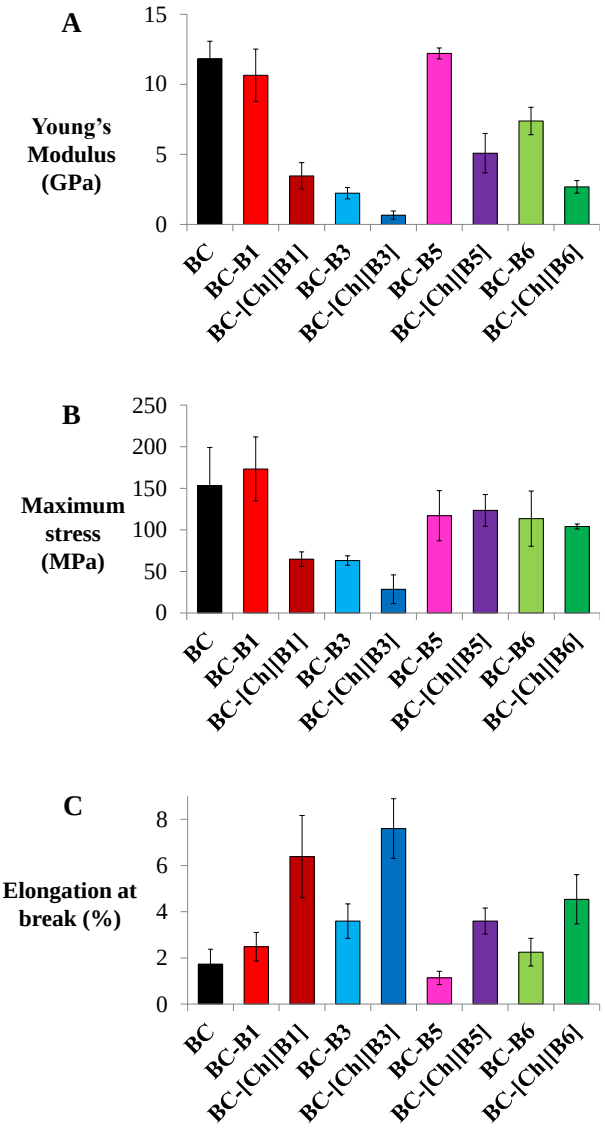
3.2.3. Mechanical properties

Mechanical properties of BC, BC-VIT and BC-[Ch][VIT] were investigated via tensile tests. Fig. 5~~Fig. 5~~ shows the Young's modulus, maximum stress and elongation at break calculated from stress/strain curves, given in Fig. S15 in the Supporting Information. Detailed data are given in Table S3 in the Supporting Information. The BC membrane has properties of brittle elastic materials, with 11.8±1.2 GPa of Young's modulus, 153±46 MPa of maximum stress and 1.7±0.6% of elongation at break [55]. The incorporation of vitamins B1, B5 and B6 lead to a small or non-significant loss of both Young's modulus and maximum stress, with 10.6±1.9, 12.2±0.4 and 7.4±1.0 GPa for Young's modulus and 173±39, 117±30 and 114±33 MPa for maximum stress, respectively. Similarly, BC-B1, BC-B5 and BC-B6 samples present similar values of elongation at break when compared with BC, specifically 2.5±0.6, 1.1±0.3 and 2.2±0.6%. On the other hand, the BC-B3 material shows a strong decrease of the Young's modulus (2.2±0.4 GPa) and maximum stress (63±6 MPa), and an increase on the elongation at break of 3.6±0.7%. This loss of elastic properties with small compensation of plastic properties can be explained by the low affinity between B3 and the BC matrix, which is mainly due to low water solubility of the vitamin, as discussed before, weakening the whole structure [REF[CV7]]. This[GC8] trend is in agreement with the morphology of BC-B3 (Fig. 4~~Fig. 4~~).

The incorporation of ILs into BC, in comparison with the BC-VIT membranes, leads to a significant decrease of the Young's modulus and maximum stress, with 3.5±0.9, 0.7±0.3, 5.1±1.4 and 2.9±0.4 GPa and 65±9, 29±17, 124±19 and 104±3 MPa for BC-[Ch][B1], BC-[Ch][B3], BC-[Ch][B5] and BC-[Ch][B6], respectively. Together, the elongation at break is significantly improved with values of 6.4±1.8%, 7.6±1.3%, 3.6±0.6% and 4.5±1.1% for BC-[Ch][B1], BC-[Ch][B3], BC-[Ch][B5] and BC-[Ch][B6], respectively. These results demonstrate a clear

plasticizing effect of [Ch][VIT] ILs, which is not observed with their vitamin B precursors due to the good affinity between BC and ILs as observed by solid-state NMR and SEM.

~~Overall~~~~d~~Despite of the decrease of the Young's modulus for~~,~~ the BC-[Ch][VIT] membranes ~~have sufficient~~ those values as well the corresponding ~~Young's modulus,~~ maximum stress and elongation at break are adequate for materials aiming at ~~to serve as relevant materials for~~ topical applications, when compared with other BC-based membranes [30,56]. Furthermore, these mechanical properties have been obtained without adding any ~~a~~ plasticizer (*e.g.* glycerol as typically carried out [57]), with this role being fulfilled by the ILs themselves.



480

481 Fig. 5. Young's modulus (A), maximum stress (B) and elongation at break (C) of BC, BC-VIT and

482 BC-[Ch][VIT] membranes.

483 ***3.2.4. Re-hydration and drug release capacity***

484 In order to evaluate the ability of membranes to absorb aqueous solutions when applied on

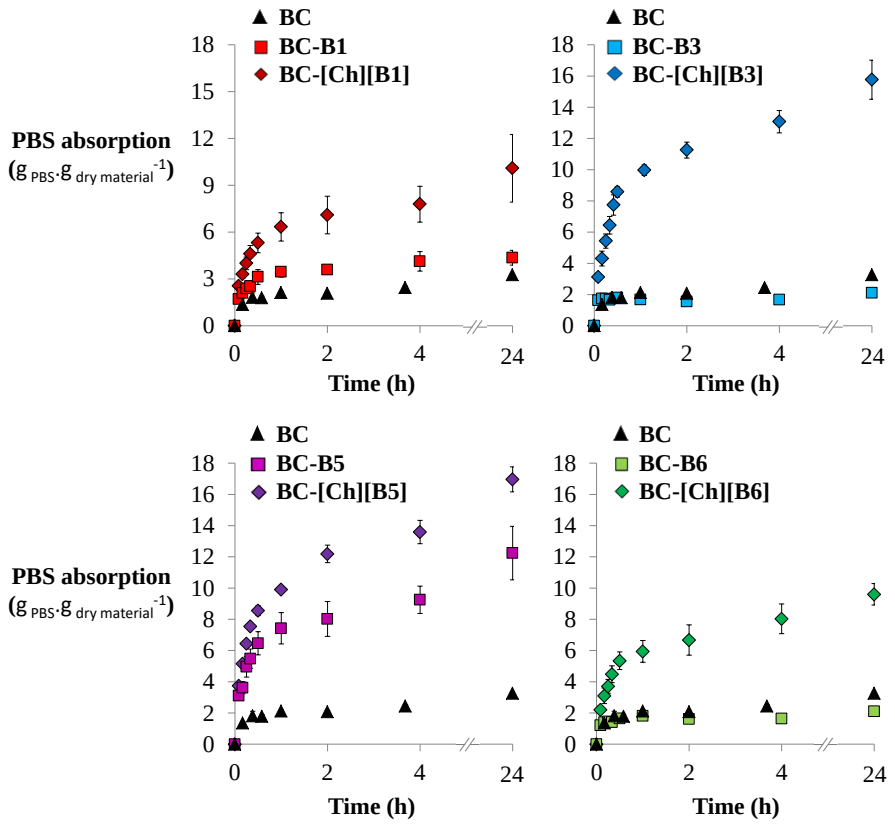
485 the skin, which is particularly relevant to absorb exudates, re-hydration tests in PBS (pH 7.4)

486 aqueous solutions were performed ~~for~~ during 24h ~~were performed~~. The respective results are given

487 in Fig. 6~~Fig. 6~~. BC membranes are able to absorb 3.3±0.1 times their dry weight of PBS aqueous

solutions. BC-B1 and BC-B5 present higher absorption capacity, namely 4.4±0.5 and 12.2±1.7 g $_{PBS}$.g $_{dry\ material}^{-1}$, respectively. On the other hand, BC-B3 and BC-B6 exhibits a lower absorption capacity, with 2.1±0.1 and 2.1±0.1 g $_{PBS}$.g $_{dry\ material}^{-1}$, respectively. ~~Interestingly, the re-hydration capacity seems to be driven by the molecular weight of vitamins than by their solubility in PBS, probably due to the steric hindrance [MF9] of the biggest molecules.~~

Remarkably, all BC-[Ch][VIT] membranes exhibit higher re-hydration values than BC and BC-VIT membranes, with values of 10.1, 15.8, 16.0 and 9.6 g $_{PBS}$.g $_{dry\ material}^{-1}$ for BC-[Ch][B1], BC-[Ch][B3], BC-[Ch][B5] and BC-[Ch][B6], respectively. This re-hydration improvement is in accordance with the increased solubility of [Ch][VIT] ILs in PBS aqueous solutions. FTIR-ATR and NMR spectroscopy observations suggest strong interactions between ILs and BC, while the SEM images show homogeneous distribution of ILs in the BC network. Therefore, the affinity between ILs and BC leads to less interlayer hydrogen bond~~s~~ formation during the drying of membranes, avoiding the BC structure to collapse and allowing an easier re-hydration [58]. This improvement in absorption capacity of the membranes afforded by ILs is highly relevant toward the envisioned skin care applications.



503

504 Fig. 6. PBS (pH 7.4) aqueous solutions absorption by BC, BC-VIT and BC-[Ch][VIT] dried
505 membranes.

506 The kinetics of release of vitamins and [Ch][VIT] from the corresponding BC membranes in
507 PBS aqueous solutions was assessed by dissolution assays~~, being given in~~(~~-~~Fig. 7~~Fig. 7~~). These
508 results are relevant because the buffer solution can serve as model for cytoplasmic medium and
509 intercellular medium and thus predict the behavior of our compounds in vivo. All samples show a
510 strong burst effect with at least 66% of the incorporated compound being released in the first 5
511 min. With the exception of [Ch][B1], the use of ILs leads to a faster and more complete release of
512 the target compounds when compared to the original vitamins. The Weibull model [59] was used
513 to fit the 1st order kinetics of the release profiles and the respective parameters and linear regression
514 coefficients are listed in Table S4 in the Supporting Information. All BC-VIT samples release

profiles are well described by the model, while for BC-[Ch][VIT] profiles only BC-[Ch][B5] follows this model (regression coefficients given in Table S4 in the Supporting Information). Samples described well by the Weibull model behave as if BC had no barrier properties in the release of the incorporated compounds. The other samples present an almost instantaneous dissolution in PBS.

If proper amounts are loaded in the membranes, the high percentage release of vitamin B-based ILs from BC is very interesting for topical skin care applications, particularly for short-term masks to be used in the cosmetic or pharmaceutical field, for moisturizing of the skin or curing burnt skin. Depending on the target application, it should be remarked that the fast release of [Ch][VIT] ILs can eventually be delayed by the addition [GC10] of compounds with tuned barrier properties, such as poly(~~ethylene—~~ethylene-co-vinyl acetate[AS11]), polyester urethanes or polyacrylates [23].

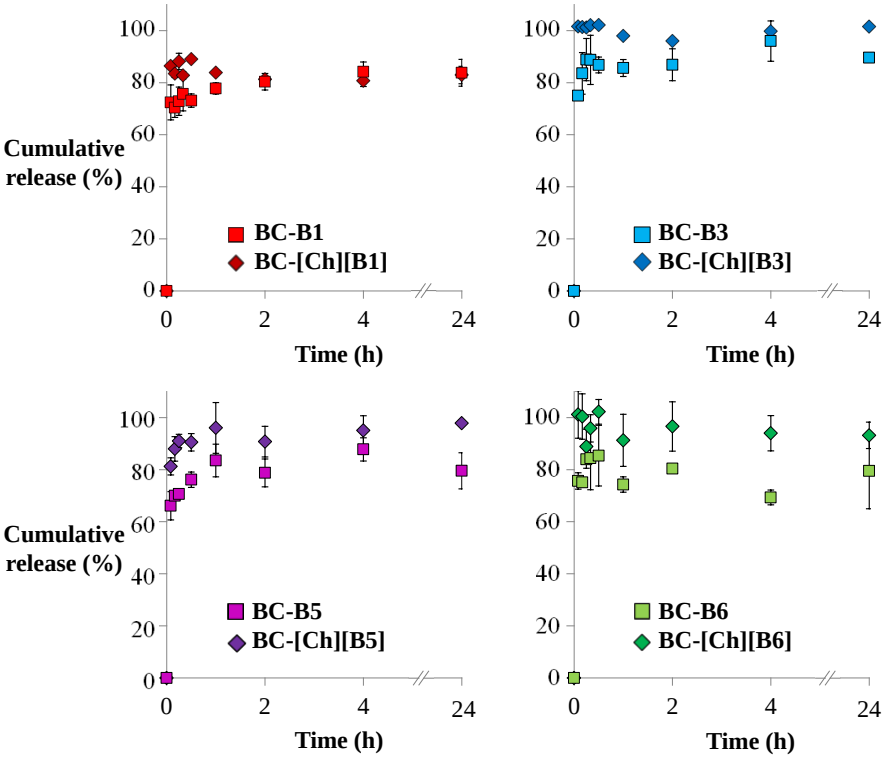


Fig. 7. Cumulative release of vitamins and [Ch][VIT] ILs in PBS (pH 7.4) aqueous solutions from BC-VIT and BC-[Ch][VIT] membranes.

3.2.5. In vitro cytotoxicity assays

In order to ensure the safety of the prepared membranes and the possibility for cosmeceutical and pharmaceutical application, the cytotoxicity of BC, BC-VIT and BC-[Ch][VIT] membranes was finally assessed ~~by the inhibition of the human keratinocyte cell line~~ against ~~(~~HaCaT cells~~) in contact with the compounds leached from the BC-based membranes~~. ~~The results are given in~~ (Fig. 8~~Fig. 8~~). As expected, the BC membrane ~~shows~~ are non-cytotoxic~~ity~~, with only 5.6±3.9 % of inhibition for the exposure time, being in agreement with its well-known non-cytotoxicity towards several cell lines [60] (including HaCaT cells [61]). The BC-VIT and BC-[Ch][VIT] membranes are also non-cytotoxic, with inhibition of HaCaT cells ranging from 3.7±0.5% to 12.1±4.5%. To be considered as non-cytotoxic materials, membranes have to exhibit an inhibition rate below 20% [62], which is the case of all membranes prepared and investigated. The non-cytotoxicity of the prepared BC-[Ch][VIT] materials demonstrates that they can be safely used in skin treatments.

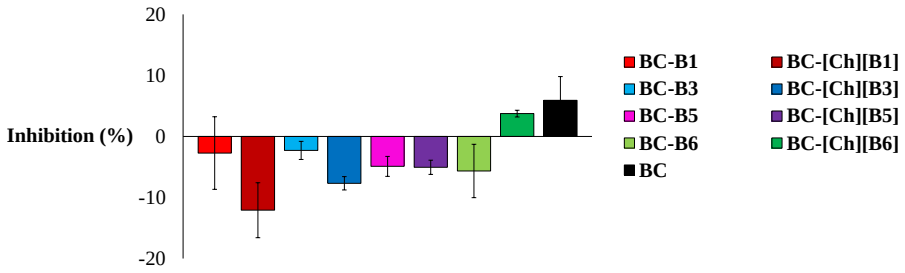


Fig. 8. Inhibition of HaCaT cells in contact with BC, BC-VIT and BC-[Ch][VIT] membranes.

4. CONCLUSIONS

~~We have successfully synthesized v~~Vitamin B-based ionic liquids were successfully synthesized and incorporated ~~them~~ into bacterial cellulose membranes envisioning their use in skin

care applications. These ILs exhibit high thermal stability (at least 120°C), display melting temperatures below 100ºC (where [Ch][B3] and [Ch][B5] are liquid at room temperature) and show increased solubility in aqueous PBS buffer solutions, particularly for vitamin B3 whose solubility increased 30.6-fold when converting it to an IL. The antioxidant properties of [Ch][VIT] revealed different behavior, compared ~~ILs were compared~~ to their ~~vitamin~~ precursors ~~with various results:~~namely an improvement of the radical scavenging activity for [Ch][B5], similar activity for [Ch][B1] and loss of activity for [Ch][B3] and [Ch][B6]. However the ~~The~~ use of a [Ch][B-complex] formulation~~s have~~ demonstrated a high antioxidant activity, similar to ~~the widely used antioxidant agent,~~ ascorbic acid. The incorporation of [Ch][VIT] ~~ILs~~ in BC led to transparent and homogeneous ~~membranes~~ materials with strong IL/BC interactions, good thermal stability and improved elongation at break due to the plasticizing effect of ILs which is particularly relevant to avoid the use extra plasticizers. Additionally, the ability of BC-[Ch][VIT] s~~ILs~~ membranes to absorb buffer aqueous solutions has been improved about 3 times in comparison to BC, allowing good absorption of exudates release by the skin. The release of ILs in ~~buffer~~ PBS has been more complete and faster than the release of vitamins, except for [Ch][B1] whose release was similar to B1. Finally, ~~neither BC,~~ BC-VIT ~~nor~~ and BC-[Ch][VIT] ~~exhibit significant toxicity on~~were shown to be non-cytotoxic for human dermal cells (HaCaT). Based on the overall results obtained, BC-[Ch][VIT] membranes appear to be a promising material for topical delivery of vitamins B in skin care applications. Further investigations should focus on the kinetic of release of ILs through the epidermis, and on the anti-aging properties of ILs, such as collagen production enhancement, moisturizing of the *stratum corneum* skin layer and wound healing.

SUPPLEMENTARY INFORMATION

Cholinium:vitamin B ratio in ILs evaluated by ^{1}H NMR, liquid-state ^{1}H and ^{13}C NMR spectra of ILs and peak attributions, FTIR-ATR spectra of ILs and their precursors, solubility of vitamins and ILs in PBS, image of DPPH mixed with ILs and vitamins in methanol after 3 days, average weight of each compound incorporated in BC membranes, solid-state ^{13}C NMR spectra of BC-IL membranes and peak attributions, FTIR-ATR spectra of BC-ILs, photographs of membranes, TGA and DTG curves of membranes, stress/strain curves of BC-IL and BC-VIT membranes after tensile tests as well as the Young's modulus, maximum stress and elongation at break data, and parameters related with the regression by the Weibull model.

ACKNOWLEDGMENTS

This work was developed within the scope of project CICECO-Aveiro Institute of Materials (FCT Ref. UID /CTM /50011/2019), financed by national funds through the FCT/MEC. G. Chantereau thanks the European Joint Doctorate in Functional Materials Research EJD FunMat (Grant agreement ID: 641640) for the PhD fellowship. Laboratoire de Chimie des Polymères Organiques (LCPO, CNRS UMR 5629) is also acknowledged. The research contract of C.Vilela is funded by national funds (OE), through FCT – Fundação para a Ciência e a Tecnologia, I.P., in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19. FCT is also acknowledged for the research contract under Stimulus of Scientific Employment 2017 to C.S.R. Freire (CEECIND/00464/2017).

REFERENCES

[1] G.F.M. Ball, Vitamins In Foods: Analysis, Bioavailability, and Stability, 1st ed., CRC

Press, Boca Raton, 2006.

[2] D.O. Kennedy, B Vitamins and the Brain: Mechanisms, Dose and Efficacy—A Review, Nutrients. 8 (2016) 1–29. doi:10.3390/nu8020068.

[3] M. Lettko, Additive clinical efficacy of B-vitamins orally co-administered with the NSAID diclofenac., in: H.U. Gerbershagen, M. VAN DER WENDE (Eds.), B-Vitamins Pain, 50th–54th ed., PMI Verlag., Frankfurt, 1988.

[4] Molketstr, Medical Beauty Forum: Vitamins in cosmetics, in: Kosmetik Konzept KOKO GmbH &Co. KG, Leichlingen, 2011: pp. 14–16.

[5] R. S, J. Kumar V, M. S B, R. M, N. G, M. Kapoor, Therapeutic Response of Vitamin A, Vitamin B Complex, Essential Fatty Acids (EFA) and Vitamin E in the Treatment of Phrynoderma: A Randomized Controlled Study., J. Clin. Diagn. Res. 8 (2014) 116–8. doi:10.7860/JCDR/2014/7086.3918.

[6] O.M. Alvarez, R.L. Gilbreath, Thiamine Influence on Collagen during the Granulation of Skin Wounds, J. Surg. Res. 32 (1982) 24–31. doi:10.1016/0022-4804(82)90180-9.

[7] Z.D. Draelos, The latest cosmeceutical approaches for anti-aging, J. Cosmet. Dermatol. 6 (2007) 2–6. doi:10.1111/j.1473-2165.2007.00313.x.

[8] D.L. Bissett, K. Miyamoto, P. Sun, J. Li, C.A. Berge, Topical niacinamide reduces yellowing, wrinkling, red blotchiness, and hyperpigmented spots in aging facial skin1, Int. J. Cosmet. Sci. 26 (2004) 231–238. doi:10.1111/j.1467-2494.2004.00228.x.

[9] E. Proksch, H.P. Nissen, Dexpenthenol enhances skin barrier repair and reduces inflammation after sodium lauryl sulphate-induced irritation, J. Dermatolog. Treat. 13 (2002) 173–178.

[10] N. Jolliffe, L.A. Rosemblum, J. Sawhill, The effects of pyridoxine (vitamin b6) on persistent adoslescent acne, J. Invest. Dermatol. (1942) 143–148. doi:10.1038/jid.1942.22.

[11] K. Higashi-okaii, H. Nagino, K. Yamada, Anti-oxidant and pro-oxidant activities of B group vitamins in lipid peroxidation, J UOEH. 4 (2006) 359–368.

[12] I.M. Marrucho, L.C. Branco, L.P.N. Rebelo, Ionic Liquids in Pharmaceutical Applications, Annu. Rev. Chem. Biomol. Eng. 5 (2014) 527–546. doi:10.1146/annurev-chembioeng-060713-040024.

[13] M.M. Santos, L.R. Raposo, G.V.S.M. Carrera, A. Costa, M. Dionísio, P. V. Baptista, A.R. Fernandes, L.C. Branco, Ionic Liquids and Salts from Ibuprofen as Promising Innovative Formulations of an Old Drug, ChemMedChem. 14 (2019) 907–911. doi:10.1002/cmdc.201900040.

[14] R. Ferraz, L.C. Branco, C. Prudêncio, J.P. Noronha, Z. Ptrovski, Ionic liquids as active pharmaceutical ingredients, ChemMedChem. 6 (2011) 975–985. doi:10.1002/cmdc.201100082.

[15] R. Ferraz, J. Costa-Rodrigues, M.H. Fernandes, M.M. Santos, I.M. Marrucho, L.P.N. Rebelo, C. Prudêncio, J.P. Noronha, Ž. Petrovski, L.C. Branco, Antitumor Activity of Ionic Liquids Based on Ampicillin, ChemMedChem. 10 (2015) 1480–1483. doi:10.1002/cmdc.201500142.

[16] M. Isik, R. Gracia, L.C. Kollnus, L.C. Tome, I.M. Marrucho, D. Mecerreyes, Cholinium lactate methacrylate: Ionic liquid monomer for cellulose composites and biocompatible ion gels, Macromol. Symp. 342 (2014) 21–24. doi:10.1002/masy.201300176.

[17] B.L. Gadilohar, G.S. Shankarling, Choline based ionic liquids and their applications in organic transformation, J. Mol. Liq. 227 (2017) 234–261. doi:10.1016/j.molliq.2016.11.136.

[18] R. Ferreira, H. Garcia, A.F. Sousa, M. Guerreiro, F.J.S. Duarte, C.S.R. Freire, M.J. Calhorda, A.J.D. Silvestre, W. Kunz, L.P.N. Rebelo, C. Silva Pereira, Unveiling the dual

role of the cholinium hexanoate ionic liquid as solvent and catalyst in suberin depolymerisation, RSC Adv. 4 (2014) 2993. doi:10.1039/c3ra45910a.

[19] R.H. Broh-Kahn, E.J. Sasmor, US Patent-3069321: choline salicylate composition and method of use, 3069321, 1962.

[20] M. Bassan, A case for immediate-release niacin, Hear. Lung J. Acute Crit. Care. 41 (2012) 95–98. doi:10.1016/j.hrtlng.2010.07.019.

[21] I. Reiche, S. Westphal, J. Martens-Lobenhoffer, U. Trger, C. Luley, S.M. Bode-Bger, Pharmacokinetics and dose recommendations of Niaspan® in chronic kidney disease and dialysis patients, Nephrol. Dial. Transplant. 26 (2011) 276–282. doi:10.1093/ndt/gfq344.

[22] M. Zimmermann, G.D. Bartoszyk, D. Bonke, I. Jurna, A. Wild, Antinociceptive Properties of Pyridoxine Neurophysiological and Behavioral Findings, Ann. New York Acad. Sci. 585 (1990) 219–230. doi:10.1111/j.1749-6632.1990.tb28055.x.

[23] S. Kandavilli, V. Nair, R. Panchagnula, Polymers in Transdermal Drug Delivery Systems, Pharm. Technol. 1 (2002) 62–80.

[24] C. Valenta, B.G. Auner, The use of polymers for dermal and transdermal delivery, Eur. J. Pharm. Biopharm. 58 (2004) 279–289. doi:10.1016/j.ejpb.2004.02.017.

[25] S. Lv, P. Quan, X. Liu, L. Fang, Effect of backing films on the transdermal delivery of cyclobenzaprine patch, Asian J. Pharm. Sci. 11 (2016) 780–783. doi:10.1016/j.ajps.2016.05.007.

[26] I. Reiniati, A.N. Hrymak, A. Margaritis, Recent developments in the production and applications of bacterial cellulose fibers and nanocrystals, Crit. Rev. Biotechnol. 37 (2017) 510–524. doi:10.1080/07388551.2016.1189871.

[27] A.J. Silvestre, C.S. Freire, C.P. Neto, Do bacterial cellulose membranes have potential in drug-delivery systems?, Expert Opin. Drug Deliv. 11 (2014) 1113–1124.

662 doi:10.1517/17425247.2014.920819.
663 [28] P.R. Chawla, I.B. Bajaj, S. a. Survase, R.S. Singhal, Microbial cellulose: Fermentative
664 production and applications, Food Technol. Biotechnol. 47 (2009) 107–124.
665 [29] E. Trovatti, N.H.C.S. Silva, I.F. Duarte, C.F. Rosado, I.F. Almeida, P. Costa, C.S.R.
666 Freire, A.J.D. Silvestre, C.P. Neto, Biocellulose membranes as supports for dermal release
667 of lidocaine, Biomacromolecules. 12 (2011) 4162–4168. doi:10.1021/bm201303r.
668 [30] E. Trovatti, C.S.R. Freire, P.C. Pinto, I.F. Almeida, P. Costa, A.J.D. Silvestre, C.P. Neto,
669 C. Rosado, Bacterial cellulose membranes applied in topical and transdermal delivery of
670 lidocaine hydrochloride and ibuprofen: In vitro diffusion studies, Int. J. Pharm. 435 (2012)
671 83–87. doi:10.1016/j.ijpharm.2012.01.002.
672 [31] S. Li, A. Jasim, W. Zhao, L. Fu, M.W. Ullah, Z. Shi, G. Yang, Fabrication of pH-
673 electroactive Bacterial Cellulose/Polyaniline Hydrogel for the Development of a
674 Controlled Drug Release System, ES Mater. Manuf. 1 (2018) 41–49.
675 doi:10.30919/esmm5f120.
676 [32] E.S. Morais, N.H.C.S. Silva, T.E. Sintra, A.O. Santos, B.M. Neves, F. Isabel, P.C. Costa,
677 I. Correia-Sa, S.P.M. Ventura, A.J.D. Silvestre, M.G. Freire, C.S.R. Freire, Anti-
678 inflammatory and antioxidant nanostructured cellulose membranes loaded with phenolic-
679 based ionic liquids for cutaneous application, Carbohydr. Polym. (2018).
680 doi:10.1016/j.carbpol.2018.10.051.
681 [33] T.E. Furia, Handbook of Food Additives, Second Edi, CRC-Press, Palo Alto, 1973.
682 [34] T.E. Sintra, A. Luís, S.N. Rocha, A.I.M.C.L. Ferreira, F. Gonçalves, L.M.N.B.F. Santos,
683 B.M. Neves, M.G. Freire, S.P.M. Ventura, J.A.P. Coutinho, Enhancing the antioxidant
684 characteristics of phenolic acids by their conversion into cholinium salts, ACS Sustain.
685 Chem. Eng. 3 (2015) 2558–2565. doi:10.1021/acssuschemeng.5b00751.

[35] E. Trovatti, L.S. Serafim, C.S.R. Freire, A.J.D. Silvestre, C.P. Neto, Gluconacetobacter sacchari: An efficient bacterial cellulose cell-factory, Carbohydr. Polym. 86 (2011) 1417–1420. doi:10.1016/j.carbpol.2011.06.046.

[36] D. Huang, O.U. Boxin, R.L. Prior, The chemistry behind antioxidant capacity assays, J. Agric. Food Chem. 53 (2005) 1841–1856. doi:10.1021/jf030723c.

[37] A. Sadeghian, M. Montazer, T. Harifi, M. Mahmoudi, Aged-look vat dyed cotton with anti-bacterial / anti-fungal properties by treatment with nano clay and enzymes, Carbohydr. Polym. 95 (2013) 338–347. doi:10.1016/j.carbpol.2013.02.063.

[38] M. Hesse, H. Meier, B. Zeeh, Infrared and Raman Spectroscopy, in: G. Thieme (Ed.), Spectrosc. Methods Org. Chem., Verlag Stuttgart, New York, 1997: pp. 40–55.

[39] W.H. Brown, J. March, Carboxylic acid, Encycl. Br. Inc. (2018). https://www.britannica.com/science/carboxylic-acid (accessed May 20, 2019).

[40] K. Fumino, R. Ludwig, Analyzing the interaction energies between cation and anion in ionic liquids: The subtle balance between Coulomb forces and hydrogen bonding, J. Mol. Liq. 192 (2014) 94–102. doi:10.1016/j.molliq.2013.07.009.

[41] W. Zhao, X. Chi, H. Li, J. He, J. Long, Y. Xu, S. Yang, Eco-friendly acetylcholine-carboxylate bio-ionic liquids for controllable: N-methylation and N-formylation using ambient CO 2 at low temperatures, Green Chem. 21 (2019) 567–577. doi:10.1039/c8gc03549k.

[42] M. Petkovic, J.L. Ferguson, H.Q.N. Gunaratne, R. Ferreira, M.C. Leitão, K.R. Seddon, L.P.N. Rebelo, C.S. Pereira, Novel biocompatible cholinium-based ionic liquids - Toxicity and biodegradability, Green Chem. 12 (2010) 643–649. doi:10.1039/b922247b.

[43] A. Fulias, G. Vlase, T. Vlase, Thermal degradation of B-group vitamins : B 1 , B 2 and B 6 Kinetic study, (2014) 1033–1038. doi:10.1007/s10973-014-3847-7.

[44] R. V Pinto, F. Antunes, J. Pires, V. Graça, P. Brandão, M.L. Pinto, Acta Biomaterialia Vitamin B 3 metal-organic frameworks as potential delivery vehicles for therapeutic nitric oxide, Acta Biomater. (2017) 1–9. doi:10.1016/j.actbio.2017.01.039.

[45] K. Wöstheinrich, P.C. Schmidt, Polymorphic changes of thiamine hydrochloride during granulation and tableting, Drug Dev. Ind. Pharm. 27 (2001) 481–489. doi:10.1081/DDC-100105172.

[46] S. Jingyan, L. Jie, D. Yun, H. Ling, Y. Xi, W. Zhiyong, L. Yuwen, W. Cunxin, Investigation of thermal behavior of nicotinic acid, J. Therm. Anal. Calorim. 93 (2008) 403–409. doi:10.1007/s10973-007-8593-7.

[47] K. Stiller, E.T., Stanton, A.H., Finkelstein, J., Keresztesy, J.C., Folkers, Pantothenic acid VIII. The total syntehsis of pantothenic acid, 246 (1940) 1785–1790.

[48] D. Han, X. Li, H. Wang, Y. Wang, S. Du, B. Yu, Y. Liu, S. Xu, J. Gong, Determination and correlation of pyridoxine hydrochloride solubility in different binary mixtures at temperatures from (278.15 to 313.15) K, J. Chem. Thermodyn. 94 (2016) 138–151. doi:10.1016/j.jct.2015.09.026.

[49] M. Manela-Azulay, E. Bagatin, Cosmeceuticals vitamins, Clin. Dermatol. 27 (2009) 469–474. doi:10.1016/j.clindermatol.2009.05.010.

[50] S. Mukul, K. Surabhi, N. Atul, Cosmeceuticals for the skin: An overview, Asian J. Pharm. Clin. Res. 4 (2011) 1–6.

[51] IOM, Dietary Reference Intakes Tables and Application : Health and Medicine Division, Copyr. © 2019 Natl. Acad. Sci. (2019) 1–2. http://nationalacademies.org/HMD/Activities/Nutrition/SummaryDRIs/DRI-Tables.aspx (accessed May 2, 2019).

[52] K.-C. Cheng, J.M. Catchmark, A. Demirci, Enhanced production of bacterial cellulose by

using a biofilm reactor and its material property analysis, J. Biol. Eng. 3 (2009) 12. doi:10.1186/1754-1611-3-12.

[53] R. Geoffrey, Z. Guangcheng, Influence of metal ions and of salts on products from pyrolysis of wood: Applications to thermochemical processing of newsprint and biomass ., J. Anal. Appl. Pyrolysis. 21 (1991) 133–146. doi:10.1016/0165-2370(91)80021-Y.

[54] J.T. Wanna, J.E. Powell, Thermal decomposition of cotton cellulose treated with selected salts, Thermochim. Acta. 226 (1993) 257–263. doi:10.1016/0040-6031(93)80227-2.

[55] S. Yamanaka, K. Watanabe, N. Kitamura, M. Iguchi, S. Mitsuhashi, Y. Nishi, M. Uryu, The structure and mechanical properties of sheets prepared from bacterial cellulose, J. Mater. Sci. 24 (1989) 3141–3145. doi:10.1007/BF01139032.

[56] N.H.C.S. Silva, I. Drumond, I.F. Almeida, P. Costa, C.F. Rosado, C.P. Neto, C.S.R. Freire, A.J.D. Silvestre, Topical caffeine delivery using biocellulose membranes: A potential innovative system for cellulite treatment, Cellulose. 21 (2014) 665–674. doi:10.1007/s10570-013-0114-1.

[57] N.H.C.S. Silva, A.F. Rodrigues, I.F. Almeida, P.C. Costa, C. Rosado, C.P. Neto, A.J.D. Silvestre, C.S.R. Freire, Bacterial cellulose membranes as transdermal delivery systems for diclofenac: In vitro dissolution and permeation studies, Carbohydr. Polym. 106 (2014) 264–269. doi:10.1016/j.carbpol.2014.02.014.

[58] W. Zhang, Y. Zhang, C. Lu, Y. Deng, Aerogels from crosslinked cellulose nano/micro-fibrils and their fast shape recovery property in water, J. Mater. Chem. 22 (2012) 11642–11650. doi:10.1039/c2jm30688c.

[59] S. Dash, P.N. Murthy, L. Nath, P. Chowdhury, Kinetic Modeling on drug release from controlled drug delivery systems, Acta Pol. Pharm. ñ Drug Res. 67 (2010) 217–223. doi:10.2307/3237001.

[60] I. Sulaeva, U. Henniges, T. Rosenau, A. Potthast, Bacterial cellulose as a material for wound treatment: Properties and modifications. A Review., Biotechnol. Adv. (2015). doi:10.1016/j.biotechadv.2015.07.009.

[61] C. Vilela, H. Oliveira, A. Almeida, A.J.D. Silvestre, C.S.R. Freire, Nanocellulose-based antifungal nanocomposites against the polymorphic fungus Candida albicans, Carbohydr. Polym. 217 (2019) 207–216. doi:10.1016/j.carbpol.2019.04.046.

[62] International Standard Organization, ISO 10993-10:2010, Biological evaluation of medical devices — Part 10: Tests for irritation and skin sensitization, (2010). https://www.iso.org/obp/ui/#iso:std:iso:10993:-10:ed-3:v1:en (accessed May 14, 2019).