

Bacterial nano cellulose as non-active pharmaceutical ingredient production optimization, quality control development and prototype design

Abstract

Materials from bio-based resources have attracted immense research interest in recent years as a result of their very high potentials for fabricating several high-value products with low impact on the environment. Cellulose produced by some bacteria has received great attention. Bacterial Nano Cellulose (BNC) imparts attractive combinations of biophysicochemical characteristics and improved mechanical properties. The aims of this work was to develop growth media for increase BNC production, characterize a non-active pharmaceutical ingredient (NAPI), design an efficient quality control method by FTIR and SEM by compared that NAPI with commercial ones and present a first prototype of a pharmaceutical product. LB and KB media were modified and BNC obtained had better quality respect its crystallinity and/or polymerization degree than the BNC from commercial media. Different extraction processes were proposed, in no case microbiological material remnants has been found. Films for controlled drugs release prototype were made and its physical properties such as water solubility, moisture content, swelling property, UV barrier capacity were evaluated. BNC films present an additional advantage, having lower UV permeability than films with cellulose of vegetal origin, adding potentialities to a patch design that protects the tissue and the Active Pharmaceutical Ingredients.

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Introduction

Cellulose is a linear polysaccharide consisting of a chain of β (1-4) linked D-glucose units.¹ Cellulose is the major component of wood and most natural fibers from vegetal kingdom. This natural polymer represents about a third of the plant tissues and can regenerate through photosynthesis.² Although vegetable origin cellulose is widely applied at industrial level, the productive scale generates serious problems on ecosystems where the pulp mills mega industries known as “pasteras” are located.³ In emerging countries such as Argentina and Uruguay, the location of these “pasteras”, generates great concern. A report presented by Uruguay Republic University (UDELAR) on 2006, summarizes the available scientific evidence about environmental impacts of the installation of pulp mills and the associated forestry model.⁴ The report presents an analysis and studies of the effects of the forest crop on the ecosystem, benefits provided by the natural pastures, and the effects generated by the liquid effluents of the pasteras at hierarchical levels (molecular, individual, population, community and ecosystem).⁴ Traditionally the Kraft method is used to obtain vegetable cellulose, the numerous processes that make up the method, where each one contributes its share in energy consumption, chemical compounds and pollution.⁵ Materials from bio-based resources have attracted immense research interest in recent years as a result of their very high potentials for fabricating several high-value products with low impact on the environment.⁶ Cellulose is also produced by some species of bacteria such as *Pseudomonas fluorescens*, *Gluconacetobacter xylinus*, *Gluconacetobacter hansenii* among others. In recent years another route for the production of micro/nanofibers of cellulose has received great attention.⁷ This is the microbial synthesis of cellulose in which specific bacteria synthesize cellulose microfibrils as a primary metabolite.⁸ Cellulose molecules are synthesized in the interior of the bacterial cell and spun out to form nanofibrils which then

aggregate in the form of ribbonshaped microfibrils.⁹ During biosynthesis of cellulose chains, van der Waals forces and hydrogen bonding between hydroxyl groups and oxygen of adjacent molecules promote parallel stacking of multiple cellulose chains forming elementary fibrils that further aggregate into larger microfibrils.¹⁰ Bacterial Cellulose impart attractive combinations of biophysicochemical characteristics such as biocompatibility, biodegradability, light weight, non-toxicity, stiffness, renewability, sustainability, optical transparency, low thermal expansion, adaptable surface chemistry, and improved mechanical properties.¹¹ Potential applications include barrier films, flexible displays, antimicrobial films, biomedical implants, transparent films, pharmaceuticals and drug delivery systems.^{1,12-14} One of the cellulose producer bacteria is *Pseudomonas fluorescens*.¹⁵ These bacteria present a convenient behavior as cellulose biofactorie. The space for cellulose attachment is between air and liquid interface, which when colonized, provides bacteria with access to both the gaseous (e.g. oxygen) and liquid (e.g. nutrient) phases.¹⁶ All so, these attachments facilitate its harvest from reactor surface, diminish handling errors and improve production recovery process. These main factors represent advantages thinking on scaling up process, but commercial growing media are expensive for industrial. Many authors previously reported the use of waste from other industries as a carbon source for the production of bacterial cellulose.¹⁷⁻¹⁹ However these practices, although very interesting and useful, adds steps to the process of product purification. In previous works,^{20,21} our research team presented new growing media designed as a clean production experience by recovering residual bacterial cell, from our lines. The main objective of this work was to develop growth media for increase bacterial nano cellulose (BNC) production, characterized it as non-active pharmaceutical ingredient (NAPI), design an efficient quality control method by compared our NAPI whit commercial ones and presented a first prototype of a pharmaceutical product.

Material and methods

Bacterial strains and culture conditions

A *Pseudomonas fluorescens* WS strain (wrinkly spreader) (donated by PhD Andrew Spears, School of Science, Engineering and Technology, Abertay University) It was grown in LB broth (Britania-C.A.B.A-Argentina) and kept at 25°C.

Culture media design

Commercial media were used as controls King B broth-(Britania-

-Buenos Aires-Argentina) and Luria Bertani broth (Britania-Buenos Aires-Argentina). PH was measured, by using a Lutron Germany PH-meter. To obtain high value products and allow us to reduce production cost, 11 culture media were designed.

Pseudomonas fluorescens static cultivation and BNC obtention

Research team designed several processes seeking that increase productivity and accomplish our ambient responsibility purpose.²¹ Figure 1 shows our more efficient process cycle.

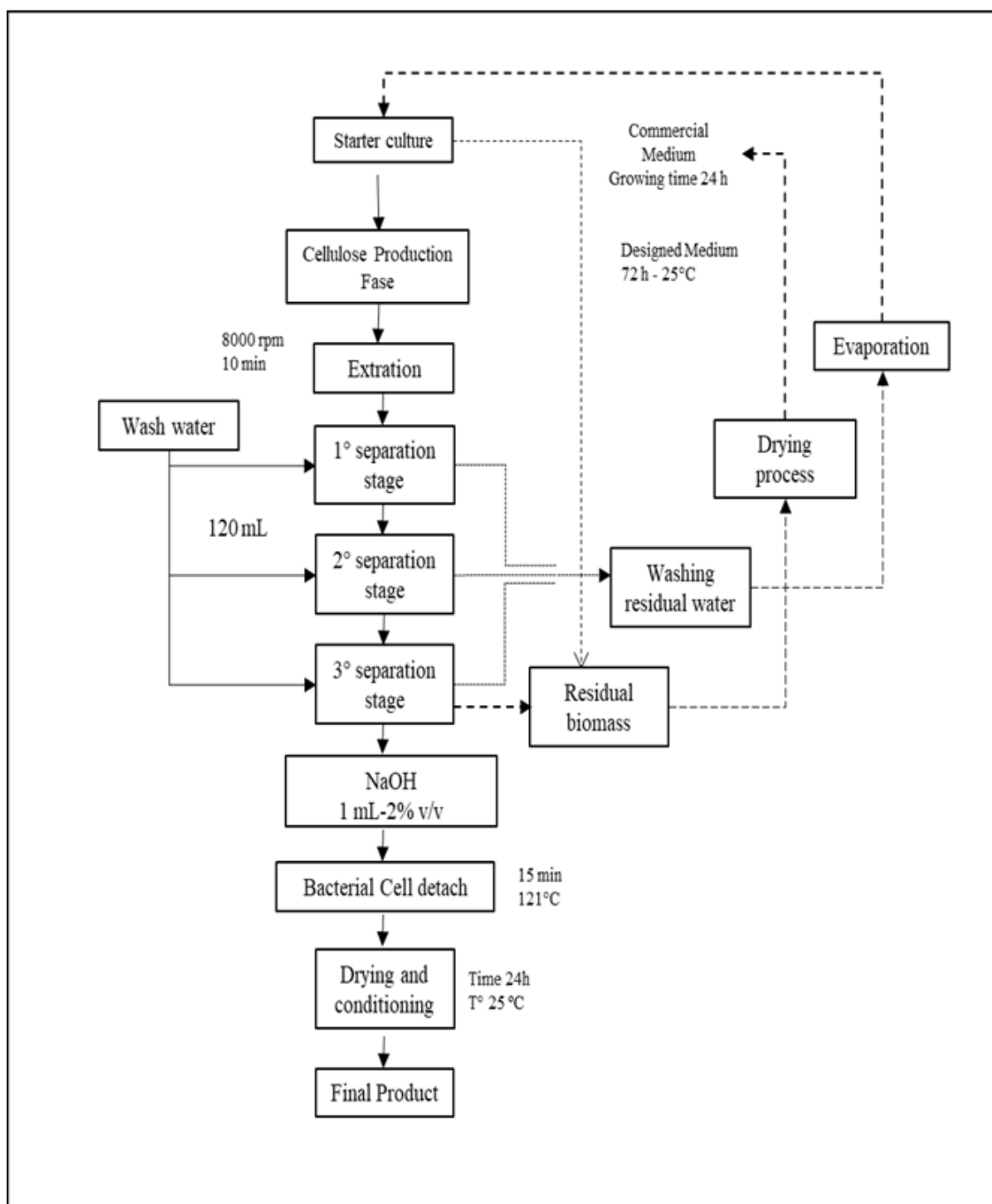


Figure 1 Clean production process flux diagram.

For BNC production, static cultivation was used. All growing test were made on petri dishes with 20mL of fresh medium for 72h at 28±1°C.²² For BNC production estimation, all cellulose pellicles were washing with distillate water to remove medium. BNC was centrifuged 20min at 8000rpm. Then 1mL of NaOH 2% v/v was added and autoclaved 15min at 121°C, these procedure is made for detach some bacterial cell which could by immerse on BNC. Then, films were washed with distilled water until neutralization.²³

BNC physical characterization- dry weight

Dry weight was measured after drying the films at 70°C until constant weight. The results were reported as 'production' and expressed in weight of dry cellulose per liter of original medium (g/L).²⁴

BNC Structure analysis and estimation of crystallinity index by FTIR spectroscopy

Cellulose characteristic bands were determinate at mid infrared region (4000cm⁻¹-500cm⁻¹). The BNC samples were processed in solid pills with KBr employing a Perkin-Elmer GX-1 spectrophotometer (Perkin-Elmer, Waltham, MA). The spectra were obtained for triplicate with 64 scans and 4cm⁻¹ of resolution. OMNIC software version 8.0 (Thermo Fisher Scientific, Waltham, MA) was used for the spectra analysis. Each spectrum was subjected to a baseline correction and an automatic smoothing prior to its analysis. Also, the crystallinity index (CI) of the final products was calculated based on the ratios between the absorbance at pre-established frequencies CI1 (A143-1419/A897-894) and CI2 (A1375/A2900).²⁵⁻²⁷

Scanning electron microscopy (SEM)

The dried samples were coated with gold using a cathode ion coater (sputter coater), and observed in a scanning electron microscope Zeiss Supra 55VP with field emission cannon operated at 3kV. The size of the cellulose particles was measured by the software of the equipment used.

Congo red tinction

Congo red tinction was made for optic microscope observation by adding one more step to Gram technique. For this purpose, amyloid stain Congo red was used (Sigma Aldrich INC. St. Luis-USA), all process was developed as described on Sigma Aldrich kit by setting gram tinction and then Congo red tinction for 20min. Then, by using a microscope camera (Digital Imager Usb Celestron-Buenos Aires-Argentina) pictures of stain crystals were taken.

Comparative evaluation with samples of commercial cellulose excipient by using electron microscope and FTIR spectroscopy techniques

Commercial cellulose samples

Commercial Non Active Pharmaceutical Ingredient (NAPI) was donated for: Cellint SA (Santa Fe – Argentina) and Cicloquimica SAC (Buenos Aires – Argentina) compared with BC, for these purpose, 3commercial products were tested microcristaline cellulose named Comprecel M101D+(MINGTAI chemical Co. Ltd. Bah-Der City, Taoyuan Hsien, Taiwan), PH-101-LD (Cellint-Avellaneda-Santa Fe-Argentina), PH-102-LD (Cellint-Avellaneda-Santa Fe- Argentina).

FTIR spectroscopy

The BNC obtained from design media based on King B and LB

and the BNC obtained from the King B and LB controls were compared with a commercial cellulose sample PH-101-LD taking as standard reference. Structure comparison was performed based on spectra obtained for each sample using FTIR spectroscopy. Spectra were processing with the same methodology described for the quality control.

Scanning electron microscopy (SEM)

Commercial samples and biotechnology produced samples, where compared by SEM technique as previously described.

Film for controlled drugs release prototype and first physical tests to characterize it

Pre formulation design protocol

Table 5 shows composition of films and commercial controls. For the pharmaceutical design, four commercial products were used: commercial cellulose (6.1) and hidroxypropil methyl cellulose 4KM (Cicloquimica SAC -Buenos Aires Argentina). Filmogenic solutions were prepared using cold mechanical method described.²⁵ According to the casting technique, for each formulation, 25mL of filmogenic solution was poured onto rectangular acrylic plates (106.25cm² of area) to obtain a constant thickness of 100±10µm, followed by drying at 37±1°C for 16h, in an oven with forced air circulation MiLaB Drying oven 101AB (Buenos Aires-Argentina). The filmogenic solution was degassed using a sonicator Cleanson (Buenos Aires-Argentina). After drying, all films were stored at a controlled relative humidity of 53% at 25±1°C for three days prior to testing. Since films have a hydrophilic character, high moisture ambient was chosen in order to evaluate film performance in a typical tropical weather condition.^{26,27}

Determination of physical properties

Water solubility

Water solubility was determined according to the method reported by Rubilar, et al.²⁸ The initial dry matter content of each film was determined by drying to constant weight in an oven at 105°C. Film disks (2cm diameter) were cut, weighed (Mi), and immersed in 50mL of water. After 24h of immersion at 20°C with agitation (60rpm), the samples were taken out and dried to constant weight (Mf) in an oven at 105°C, to determine the weight of dry matter that was not solubilized in water. The solubility of each film was then determined as follows:

$$\text{Solubility in water (\%)} = (W_i - W_f / W_i) \times 100 \quad (1)$$

Where WI is the initial mass and Wf is the final mass of the sample. Three replicates were obtained for each sample.

Moisture content

To determine the moisture content of films, about 50mg of film were dried at 105°C during 24h (until the equilibrium weight was attained). The weight loss of the sample was determined, and moisture content was calculated using the following equation.²⁹

$$\text{Moisture content (\%)} = (M_{wi} - M_{wf} / M_{wi}) \times 100 \quad (2)$$

Where Mwi and Mwf are the masses of initial and dried samples, respectively, Three replicates were obtained for each sample.

Swelling property

According to the method reported.²⁹ the films were cut into a piece of 2.5X2.5cm in size and weighted in air-dried conditions (w1). They

were then immersed in deionized water (25 °C) for 2 min. Wet samples were wiped with filter paper to remove excess liquid and weighed (w2).²⁹ The amount of adsorbed water was calculated as:

$$\text{Swelling (\%)} = 100(w2 - w1) / w1 \quad (3)$$

Where w2 and w1 were the weights of the wet and the air dried samples, The measurement was repeated three times for each type of film, and an average was taken as the result.

Opacity measurement

Film specimen was cut into a rectangle piece and placed in a spectrophotometer test cell directly, and air was used as the reference. A spectrum of each film was recorded on an UV-Vis spectrophotometer (Thermo Scientific Multi scan Go- Buenos Aires- Argentina). The area under the absorption curve from 400 to 800nm was recorded, and the opacity of film was calculated by the following equation:

$$\text{Opacity} = A500 / T \quad (4)$$

Where A500 was the absorption at 500nm, and T is the film thickness (mm).³⁰ The measurement was repeated three times for each type of film, and an average was taken as the result.

Statistical Analysis

Each treatment was conducted in triplicate and all experiments were repeated at least twice. The statistical significance of the evaluated data was analyzed by one-way analysis of variance. Differences

among the mean values were tested using the least significant difference multiple range test. Values were considered significant when $p < 0.05$, except when otherwise indicated.

Results and discussion

Productive activity is a fundamental column in country's economic development, also is one of the main factors of unsustainability. One of the greatest advantages of pharmaceutical biotechnology is the versatility. Although its approach to the production of drugs opens a plethora of green and sustainable possibilities, the generation of waste continues to represent a problem on a smaller scale, but it can be brought to near zero. Waste production and natural resources consume diminishing sustainability and economic growth. We are seeking to improve our own process and to insert Clean Production in our production lines. In previous work data from designed Clean Production protocol and growing media where reported.²¹

Table 1 shows media composition designed by replacing or added carbon fonts. By using King B broth as control 8 enriched media were designed. Table 2 shows 3 media designed by using LB medium as control. In previous work media design and growth kinetics were reported when *P. fluorescens* residual cells were used, 3media were presented and named GS1 (Gonzalez Sesto) to GS321, we choose GS1 for these work, as these presented the best cellulose productivity. Also we design GS4, which is GS1 with glucose added seeking to improve cellulose production yield. As media where designed, clean production protocol were also designed and time and volume of non-renewable resources were significantly diminished as we presented on Figure 1.

Table 1 Media culture's composition based on King's B media. Media design composition E1 to E4. King's B commercial media was used as reference. Trip: Triptein; BE: Beef extract; SP: Soya Peptone; Gluc: Glucose; Glic: Glicerol; R King B Medium: consumed culture media

Media	Trip	BP	K2HPO ₄	K2SO ₄	MgSO ₄	SP	NaCl	CaCl ₂	Gluc	Glic	R King B medium
Kings B	x	x	x	x							
KB1	x	x	x	x						x	
KB2	x	x	x	x					x	x	
KB3	x	x	x	x					x		
KB4	x	x	x	x							x
E1	x	x		x	x	x	x				
E2	x	x		x	x	x	x	x			
E3	x	x	x	x		x	x				
E4	x	x	x	x		x	x	x			

Table 2 Media culture's composition on Luria-Bertani media Media design composition The Luria-Bertani commercial media was used as reference. YE, Yeast extract; BE, Beef extract; BP, Beef peptone; BRC, Bacterial residual cells; Gluc, Glucose

Media	YE	BP	NaCl	BRC	Gluc
LB	x	x	x		
LI	x	x	x		x
GS1*		x	x	x	
GS4*		x	x	x	x

*Media design contains the same ingredients that LB broth but replacing 50% of proteins by BRC

‘The best solvent is no solvent and if a solvent is needed then water is preferred’.³¹ The use of water is environmentally favorable due to it is nontoxic and nonflammable nature, and it is economically available because of its low price and abundant reserves. However, water has the highest heat capacity among all materials. Intensive energy inputs associated with production using water make it a challenge towards the demand for energy-saving production. In the last decade, an extensive effort has been made in attempt to search for solvent alternatives to overcome the environmental problems. In this work our research team, develops a method for clean production Figure 1.

In all cases known volumes of water were diminish until the minimum volume limit consumed. Distillation was use as physical method for water recovery, and we managed for reuse 45% of our residual

recovered water. Residual biomass where separated, centrifuged at 8000rpm, and then by using a physical method for the obtention of residual bacterial cells (RBC) as described previously.²¹

Table 1 show all media designed by based on King B commercial culture media. Designed media E1 to E4 shows all compounds added. Divalent Mg⁺² and Ca⁺² cations were added, seeking to provide a basic structure on which cross-linking is facilitated to obtain tridimensional structure cellulose layers. It was previously established that biofilm architecture can also be strongly influenced by the interaction of anionic extracellular polymeric substances (EPS), containing carboxylic groups, with multivalent cations. For example, Ca⁺² can

form a bridge between polyanionic molecules, stimulating the development of thick and compact biofilms with increased mechanical stability.³² NaCl was added seeking saline stress and increased production of BNC. It is known that salt stress also biofilm formation and protects this mini assembly by retaining a water layer around the cells.³³ However when BNC was measured as dry weigh of BNCg/L of culture media, mono and di valent salts did not increase production. Being in all cases, BNC lesser than reference media King B productions yield Figure 2A. Our results are in line with previous studies where exopolysaccharides production reduced in the medium with the addition of salt.³⁴

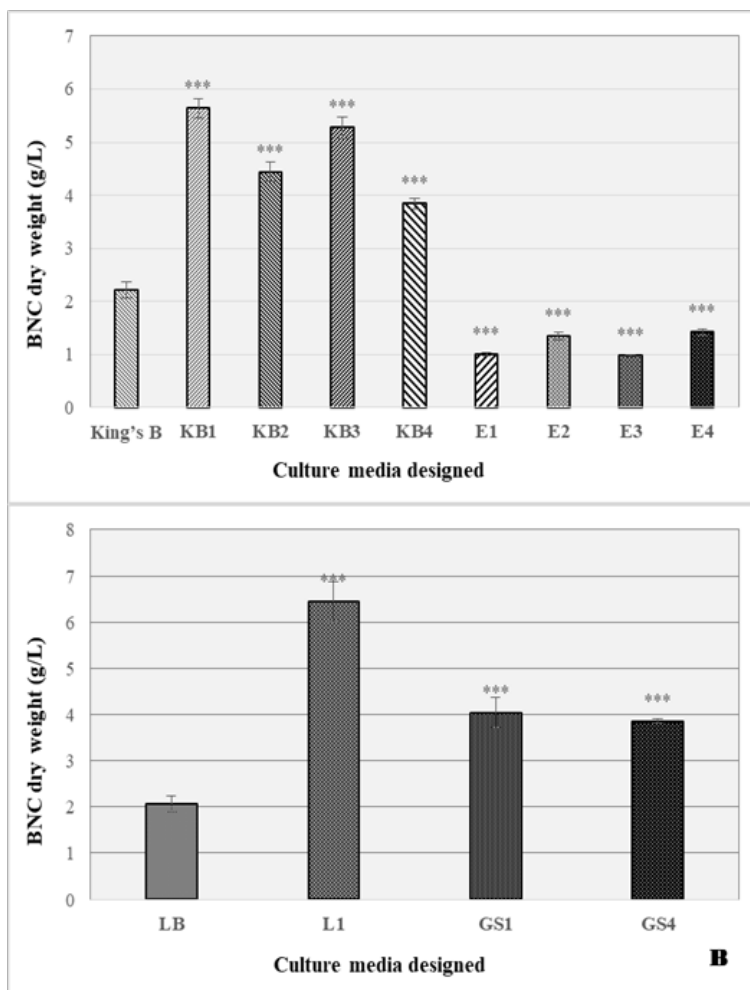


Figure 2 shows BNC dry weight obtained by using media designed showed in table 1 and 2.

When King B was added whit carbon sources KB1 to KB4 were designed. In all cases production yield was improve in reference to commercial media (2.21±0.15 g/L). When carbon source added was glycerol (KB1) production yield increases 155.2 %. When carbon source added was glucose and glycerol (KB2) production yield was 100.9% higher than reference medium. When glucose as carbon sources was added (KB3) production yield was 73.76 % higher than commercial media. When consumed culture media, was added as carbon source (KB4) non-significant differences were found between KB1 and KB4. This finding allows us to reduce even more residues in our own CP protocol and diminish cost with the mayor production of BNC.

Table 2 shows four media designed by using Luria Bertani culture media as commercial media control. Dry weight BNC production on LB maximum productivity was 2.07±0.18 g/L. Figure 2B shows all data obtain, in every case dry weight data was significantly different to control p<0.001. When glucose was added (L1) to LB commercial medium production yield was 211.6% being the mayor production of BNC from *P. fluorescens* presented on this paper. When residual bacterial cells were added to the culture media designed production increase over 96%. GS1 and GS4 can be a good replacement for carbon sources and its fount are strictly residues. This media are part on a national patent solicitude on the National Institute of Industrial Property N°AR20170123114 and our CP protocol.

Figure 3 shows micrographs obtained by using SEM (Material and Methods point 6). BNC scanning electron microscopy is a noninvasive quality control alternative. When BNC derived from King's B media was tested (A-F) the particles showed an arrangement in the form of aciculate crystals. When BNC derived from LB media was tested (G-I), the particles adopted flake format. In both cases the size of the BNC particles varies from 15µm to 30nm, arranged in a com-

plex, very compact reticulated structure. It should be noted that the thermal treatment used to dry the samples causes the compaction of the three-dimensional network of BNC as explained previously.²³ Figure 4 shows the acicular crystals of cellulose obtained using the KB3 medium. Gram staining with modified Congo red highlights the BNC crystals described in the Figure 3 G micrograph.

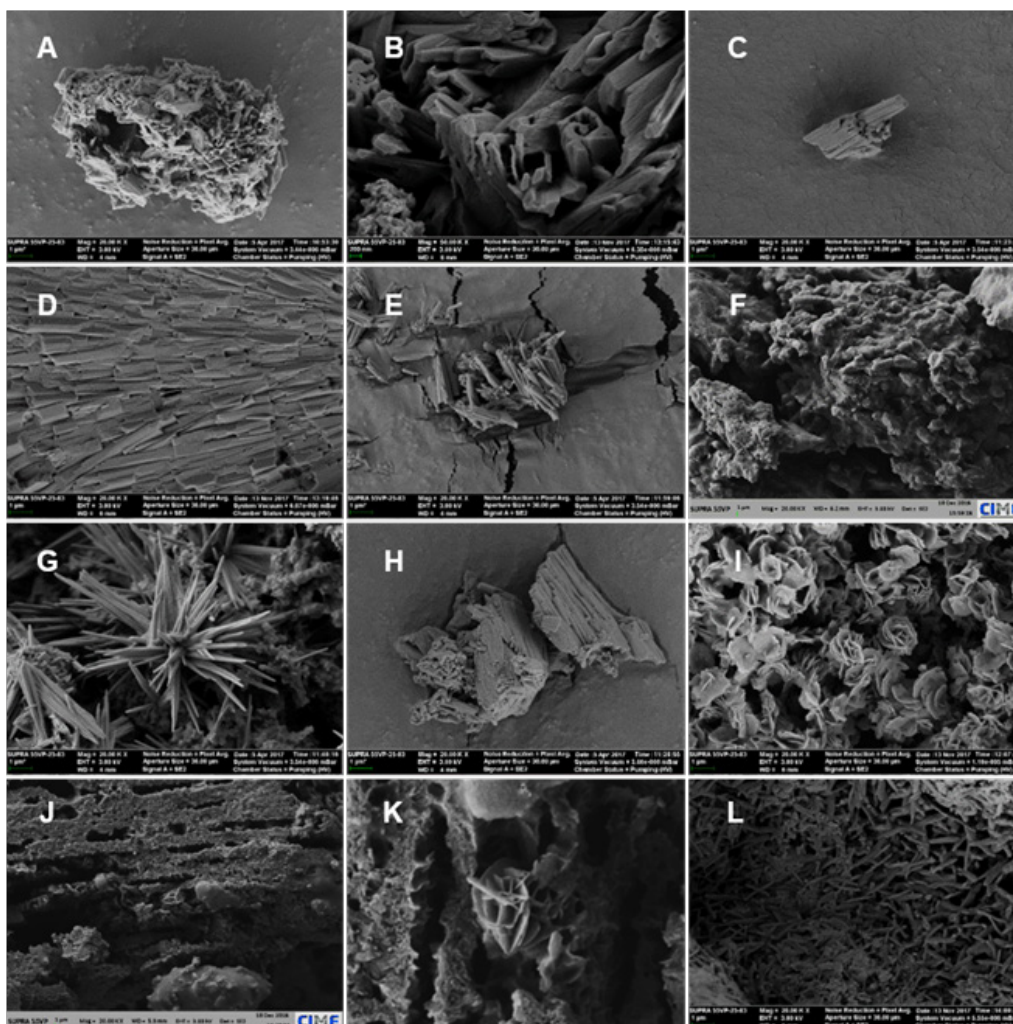


Figure 3 BNC Scan electron microscopy A) E1. B) E2. C) E3. D) E4. E) KB1. F) KB2. G) KB3. H) KB4. I) GS1. J) GS4. K) Amplified image from J. L) L1.

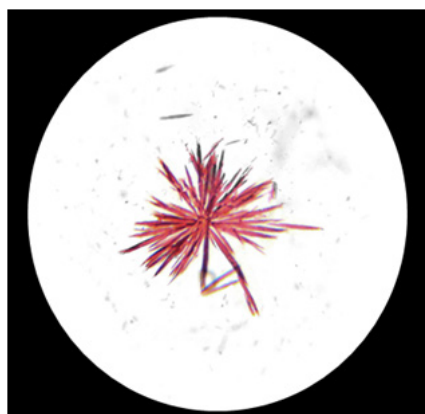


Figure 4 BNC Micrograph Congo red Tinction.

Quality control

Table 3 shows the main assignments described for cellulose found in the reference.³⁵⁻⁴¹ These bands were used for the subsequent analysis of the obtained products. BNC spectra obtained from design media based on King B, showed an increase in the quality of the final products against BNC obtained from King B controls. The absorbance at the glycosidic region increases considerably for all spectra which could be caused by a higher polymerization degree (Figure 5). The spectra of E2, E4, KB1 and KB3 showed intense bands at ~450cm⁻¹ and ~860cm⁻¹ (Figure 5 and Figure 6). This could be owed to the high crystallinity of these products.^{27,39} even above of the products obtained from commercial King B media. Conversely, E1, E3, KB2 and KB4 presented amorphous characteristics, showed intense peaks at ~2900cm⁻¹ and weak bands at ~1450cm⁻¹^{39, 42} (Figure 5 and Figure 6). Cellulose formed in LB media was less cohesive than King

B products and was dispersed in the medium. However, better results were found analyzing the BNC spectra obtained from design media based on LB (Figure 7). Similar patterns to the reference were found in these spectra. Although BNC spectra from design media based on LB showed bands at $\sim 1430\text{cm}^{-1}$ related to its crystallinity.²⁷ (Figure 7), an important band at 2900cm^{-1} was also observed, indicating that the BNC structure obtained from the design media had an amorphous structure compared to its analogues obtained from modified King B (Figure 7).²⁸ In addition, the absorbance in the glycosidic region was very low, indicating the low polymerization degree and the low cohesiveness of these products (Figure 7). GS3 showed a low absorbance at 1650cm^{-1} respect to the rest of BNC from modified LB media.

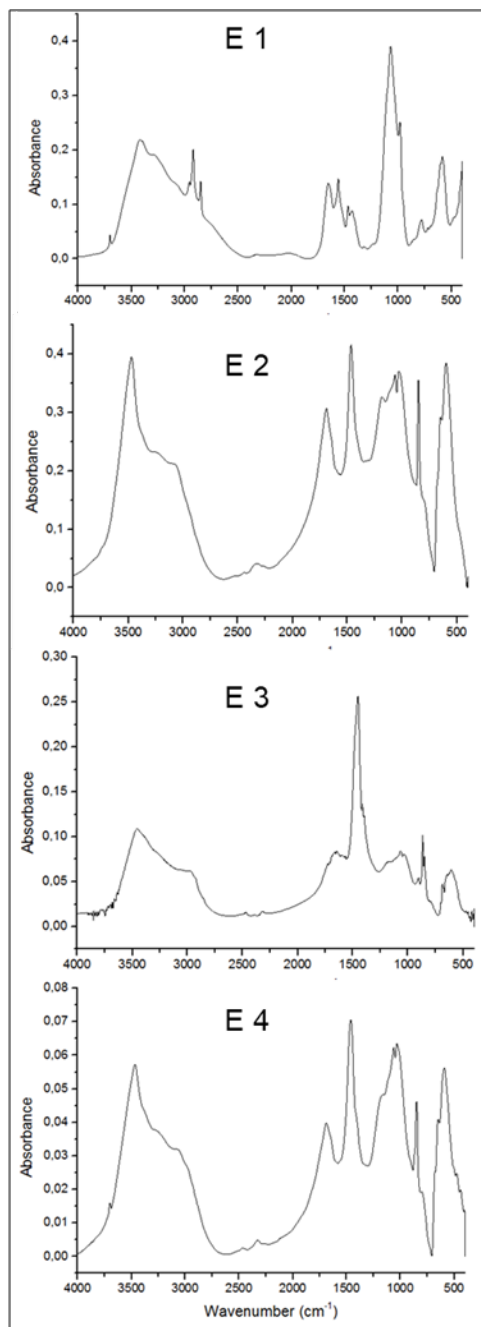


Figure 5 Spectra for BNC obtained from different design media based on King B added with salts. E1 and E3 showed amorphous characteristics, while E2 and E4 showed crystallinity properties.

Table 3 Main IR vibrational modes for cellulose. * 2900cm^{-1} is also known as “amorphous band”

Region (cm^{-1})	Vibrational modes
3175-3500	O-H stretching from intramolecular hydrogen bonds.
2850-2970*	symetric and asymmetric stretching of $>\text{CH}_2$ groups
1649 –1634	O–H bond stretching of the water
1430-1420**	$>\text{CH}_2$ groups scissoring
1382-1375	C-H linkages
1336	O-H stretching in the plane
1317	$>\text{CH}_2$ wagging
~ 1166	Band of C–C stretching for rings
~ 1116	Band of C–O–C ether glycosidic linkage
1058	C–O–C groups steching in pyranose rings
1034	C-O groups of C-OH linkage in carbohydrates
902 – 893	C-O-C deformation of the $\beta(1-4)$ glycosidic linkage

** 1430cm^{-1} also known as the “crystalline band”, * 2900cm^{-1} is also known as “amorphous band”.

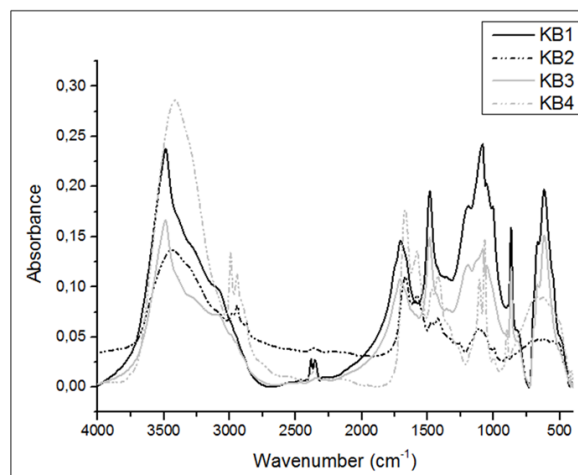


Figure 6 Spectra for BNC obtained from different design media based on King B added with carbon sources. KB2 and KB4 showed amorphous characteristics, while KB1 and KB3 showed crystallinity properties.

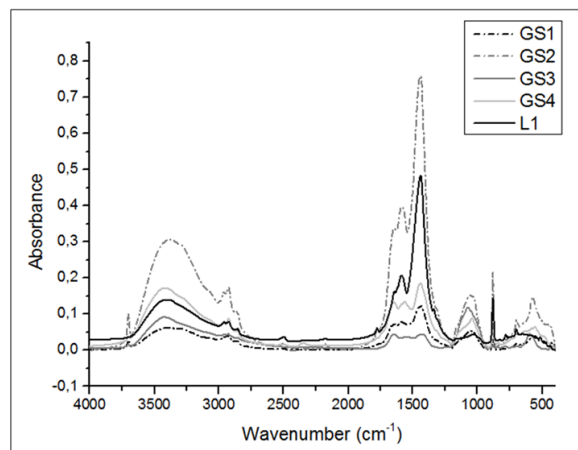


Figure 7 Spectra for BNC obtained from different design media based on LB.

Table 4 shows crystallinity index estimated from spectra. There was a total correlation with the bands found in the commercial cellulose spectrum and the reference described. The spectra obtained for all BNC products presented similar spectral patterns to the observed in commercial cellulose. However, certain BNCs showed a crystallinity degree higher than the commercial sample taken as a reference, as could be observed in E1, E3 and L1 crystallinity index 1 and 2 (Table 4).

Table 4 Crystallinity index estimated from FT IR spectra

Samples		CI ¹	CI ²
Comercial BNC	PH-101-LD	3,00	0,41
	E1	12,00	0,10
	E2	1,92	1,00
	E3	7,77	0,25
BNC from modified King B	E4	2,02	1,00
	KB1	0,82	1,00
	KB2	1,01	0,80
	KB3	0,88	1,00
	KB4	1,69	0,04
	GS1	2,31	0,13
BC from modified LB	GS2	2,71	0,28
	GS3	2,86	0,20
	GS4	3,50	0,11
	LI	3,06	0,64

Figure 8, shows commercial samples and biotechnology produced samples, where compared by SEM technique as previously described. The differences in appearance and degree of crystallinity previously discussed could be due to the extraction process used. When Kraft method was used,⁴³ strong acids and bases were employed; this generates the “rag appearance”. It is clearly observed that the particles, on a micro scale, are folded on themselves. This process that generates the folding influences the physical properties attributed to the cellulose particles obtained. The geometric properties of the nanocellulose structures (shape, length and diameter) depend mainly on the extraction process and on the origin of the cellulose raw material.⁴³ While the photos obtained at the same magnification scale of BNC (20000X), obtained by a green method, the crystals are arranged in

scales, they are needle-like as shown in Figure 4.⁴ The size at nano scale and its acicular composition and its arrangement in rosettes, they are related not only to the biotechnological process but also to the use of green solvents and diluted bases. These observations reinforce the findings and drive us to design pharmaceutical products to verify the difference in behavior of the same pure product, which was obtained by two different methods. KB3 was the designed media that complies with the prerogatives of maximum performance and high degree of crystallinity. Their aciculate crystals were shown in Figure 3 (J) and in Figure 4. Due to their characteristics, the BNC obtained using this culture medium was selected for the design of the coatings prototypes.

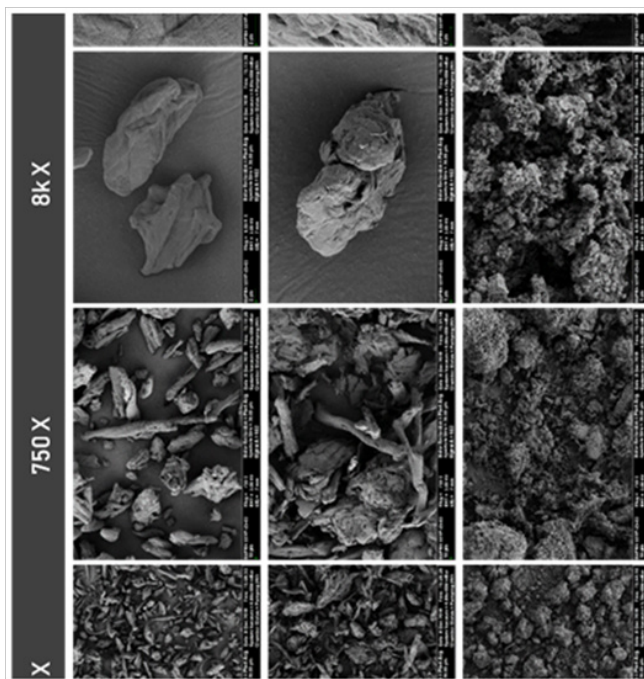


Figure 8 Shows SEM images from commercial samples: PH101 and Comprecel compared to BNC.

Prototype development

Physical properties analysis: Solubility is defined as the content of dry matter solubilized after 24h immersion in water. Table 5 shows HPMC films physical properties. The higher solubility of the film is attributed to the water binding capacity. By replacing cellulose for BNC on the original formula, we seek to establish how its configuration modifies the film behavior. F1 shows the lowest water binding capacity when it was compared to the control film and its water solubility was significantly different from control formulation (CF). F2 shows none significantly differences against control. Water solubility tests are determinant to establish the use of a prototype film.

Table 5 FFS, film forming solution; CF, control formulation; BNC, bacterial nano cellulose; HPMC, hydroxy propyl methyl cellulose; MCC, microcrystalline cellulose; WS, water solubility 25°C; SP, swelling power; MC, moisture content; UV BC,: UV barrier Capacity

Samples /Composition (g)				Film preformulation studies			Optical properties	
FSS	HPMC	MCC	BNC	WS%	SP%	MC%	Opacity	UV/BC
F1	1.43	0.04	0.04	92.52 ± 0.34***	36.43±1.51**	.44±0.41**	28.96±0.95*	90.66±0.52***
F2	1.43	0	0.08	95.02±1.65*	34.25±0.33***	4.68±0.03***	27.41±0.52*	95.11±1.71***
CF	1.43	0.08	0	98.96±1.48	42.42±2.06	6.36±0.22	27.82±0.37	52.96±0.43

Control film is made completely by using cellulose and derivatives from vegetal origin. As the fiber swells, intermolecular bonds are broken as a result of the internal stresses produced. The degree of order within the fiber is reduced and there is an increase in the surface area of the fiber.⁴⁴ BNC has a different structure that influence on swelling power. When BNC films swelling power was tested significantly differences against control was found. F2 who has high proportion of BNC shows lesser swelling power ($p < 0.001$). Robertson.⁴⁵ suggested that the factors that determine the effectiveness of the swelling liquid in cellulose include the hydrogen bonding properties and the existence of intermolecular forces that are summed up in the cohesive energy density. In addition, other factors such as molecular volume and steric effects are also of importance. The interaction with cellulose fibers depends additionally on the structure and porosity of the fiber. Moisture content is a measure of empty volume in the micro reticulates susceptible to being occupied by water molecules.⁴⁶ When F1 Y F2 were tested, moisture content was significantly different against control showing that films of vegetal cellulose are more water soluble, presents mayor swelling power and moisture content. These results shows that films designed entirely with vegetal origin cellulose are ideal for food products films such as coatings for fruits, which can be ingested with it. BNC increases properties such as lower solubility in water values, less swelling power and less moisture content, these are desirable when the application of the films is for the manufacture in controlled release patches design. It is also a measure of biodegradability of the films these property is useful for biodegradable packaging. BNC present an additional advantage, having lower UV permeability than films with cellulose of vegetal origin. Adds this potentiality in a patch design that protects the injured stratum, protecting the site from the wound and replacing the skin in its barrier function since the broken skin has lost its UV barrier action protecting the tissue. It also blocks UV light and protects Active Pharmaceutical Ingredients. On the other hand, these films are useful for the manufacture of containers for example to store vegetable or seed oils since they contain variable levels of unsaturated olefin acids that are susceptible to degradation by light. The incorporation of a low percentage BNC significantly reduces the transmission of UV light to the interior of the container and preserves the packaged product for longer.

Conclusion

Media designed allow as obtaining industrial interest products, exacerbating useful cellulose polymers properties. For both modified LB and modified King B, BNC obtained had better quality respect to its crystallinity and/or polymerization degree than the BNC from commercial media. The heterogeneity in the BNC properties found in this work allows designing procurement methods according to the final purpose of the desired product. In no case had remnants of microbiological material been found. For all cases, the BNC showed a crystallinity structure greater than the commercial cellulose. This characterization method could be used in the future for the quality control of the products in the production line. The prototypes of films obtained have advantages over those designed using cellulose of vegetable origin. The characteristics of the designed films significantly extend the applications of this type of topical pharmaceutical form.

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Conflicts of interest

Authors declare there is no conflict of interest.

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