

1 **TITLE Page**

2 **Title: Nanocellulose in Packaging: Advances in Barrier Layer Technologies**

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9 **Abstract:**

10 The review aims at reporting on recent developments in nanocellulose-based materials and their
11 applications in packaging with special focus on oxygen and water vapor barrier characteristics.
12 Nanocellulose materials, including cellulose nanocrystals (CNC), nanofibrillated cellulose (NFC),
13 and bacterial nanocellulose (BNC), have unique properties with the potential to dramatically
14 impact many commercial markets including packaging. In addition to being derived from a
15 renewable resource that is both biodegradable and non-toxic, nanocellulose exhibits extremely
16 high surface area and crystallinity and has tunable surface chemistry. These features give
17 nanocellulose materials great potential to sustainably enhance oxygen and water vapor barrier
18 properties when used as coating, fillers in composites and as self-standing thin films.

19 **Keywords:**

20 Cellulose nanocrystals; nanofibrillated cellulose; microfibrillated cellulose; packaging;
21 barrier films

22 **Graphical abstract:** (None; please let us know if you want us to create one.)

23 **Highlights:**

- 24
- Advances in the usage of nanocelluloses in packaging are reviewed in this article.
 - The nanocellulose can contribute strength of barrier films.
 - Films comprised of nanocellulose can effectively resist oxygen permeation.
 - Water vapor and moisture resistance are key areas of challenge.
 - A great many variables and processes can be considered in future research.
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30 **Nanocellulose in Packaging: Advances in Barrier Layer Technologies**

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38 and bacterial nanocellulose (BNC), have unique properties with the potential to dramatically
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40 renewable resource that is both biodegradable and non-toxic, nanocellulose exhibits extremely
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42 nanocellulose materials great potential to sustainably enhance oxygen and water vapor barrier
43 properties when used as coating, fillers in composites and as self-standing thin films.

44

45

46 **1. Introduction**

47 Nowadays, the use of packaging materials can be justified for preventing the deterioration of
48 food and drink, cosmetics, healthcare and other consumer goods due to physical, biochemical,
49 and microbiological factors. In addition, they should also provide sufficient barrier against
50 oxygen, water vapor, grease, and microorganisms, among others (Nair et al. 2014). The packaging
51 industry currently uses materials based on glass, aluminum, tin, and fossil-derived synthetic
52 plastics, which gives rise to concerns from both economical and environmental perspectives
53 (Johansson et al. 2012). It is true that the materials cited above show high strength and barrier
54 properties; however, they also have some drawbacks such as unsustainability, fragility (for
55 instance in the case of glass) and sometimes they are really heavy, and that increases energy
56 costs for shipping (Bayer et al. 2011; Priolo et al. 2010; Reis et al. 2011; Rodionova et al. 2011).
57 The continued use of petroleum-based products will mean eventually a decrease in availability
58 and thus an increase in the price of the raw materials. Additionally, because of their lack of
59 biodegradability, petroleum-based products can generate significant waste disposal problems in
60 some areas (Johansson et al. 2012).

61 The global consumer packaging demand is currently in the range of US\$400b-\$500b, and it is
62 worth highlighting that it is one of the fastest growing markets (Nair et al. 2014). In this sense,
63 the development and search for new materials, products, and processes have become a necessity
64 and should be based on the principles of sustainability, industrial ecology, eco-efficiency, and
65 green chemistry (Abdul Khalil et al. 2012). With the increased environmental concerns over
66 sustainability and end-of-life disposal challenges, materials derived from renewable resources
67 have been strongly advocated as potential replacements. In this sense, cellulose, whose structure

68 is shown in Figure 1, accounts for approximately 40% of lignocellulosic biomass (Li et al. 2015).
69 According to the literature, this polymer is the most abundant renewable organic material in the
70 biosphere, and its annual production is estimated to be more than 75 billion tons (French et al.
71 2004; Habibi, 2014). From all this annual cellulose production, only around 2 billion per year is
72 used for industrial conversion and human consumption, including all the possible uses (Keijsers
73 et al. 2013).

74 A large amount of cellulose-based materials have been used by the packaging industry for
75 different purposes such as wrapping materials and containers, primary and secondary packages
76 and flexible and rigid packaging (Lee et al. 2008). In fact, there are countless benefits when
77 cellulose is used for paper-based packaging, as for example, lightness in weight, low cost, and
78 most important, sustainability (Nair et al. 2010). Unfortunately, there are some drawbacks
79 inherent in the use of regular paper prepared from lignocellulosic fibers. These include paper's
80 low barrier ability against water, water vapor, oxygen or oil, and these deficiencies need to be
81 addressed in order to produce high-quality packages that meet various specifications (Hyden,
82 1929; Pal et al. 2006).

83 The packaging industry presently uses mainly unsustainable coatings of wax, plastics, or
84 aluminum, and many other materials in order to manufacture competitive packages based on
85 paper. In addition to all these unsustainable materials, the packaging industry also uses
86 cellophane, which is the only cellulosic material currently used as a film for packaging, as it
87 provides an effective gas barrier mainly in dry conditions. However, and despite the obvious
88 benefits of employing a product based on photosynthetically renewable cellulose, the production

89 of cellophane is harmful to the environment; the viscose route to cellophane production
 90 generates harmful byproducts and uses sulphur-based chemicals (Hyden, 1929).

91 **[[APPROXIMATE LOCATION OF FIGURE ONE]]**

92 **Figure 1.** The structure of cellulose.

93 In light of the issues just considered, nanofibrillated cellulose (NFC), cellulose nanocrystals (CNCs)
 94 and bacterial nanocellulose (BNC) have emerged as key components that should be seriously
 95 considered for the production of cellulose-based materials for packaging purposes (Hoeger et al.
 96 2013; Stelte and Sanadi, 2009). Nanocellulose can be extracted from various plant resources
 97 through mechanical and chemical operations (Klemm et al. 2011). A classification of
 98 nanocellulosic materials is given in Table 1.

99 **Table 1.** The family of nanocellulose materials (Data extracted from (Klemm et al. 2011)).

Type of nanocellulose	Synonyms	Typical sources	Formation and average size
Nano- or microfibrillated cellulose (NFC/MFC)	Microfibrillated cellulose, nanofibrils and microfibrils, nanofibrillated cellulose	Wood, sugar beet, potato tuber, hemp, flax	Delamination of wood pulp by mechanical pressure before and/or after chemical or enzymatic treatment Diameter: 5-60 nm Length: several micrometers
Nanocrystalline cellulose (NCC)	Cellulose nanocrystals, crystallites, whiskers, rodlike cellulose, microcrystals	Wood, cotton, hemp, flax, wheat straw, mulberry bark, ramie, Avicel, tunicin, cellulose from algae and bacteria	Acid hydrolysis of cellulose from many sources Diameter: 5-70 nm Length: 100-250 nm (from plant celluloses); 100 nm to several micrometers (from

			celluloses of tunicates, algae, bacteria)
Bacterial nanocellulose (BNC)	Bacterial cellulose, microbial cellulose, biocellulose	Low-molecular weight sugars and alcohols	Bacterial synthesis Diameter: 20-100 nm Different types of nanofiber networks

100

101 Cellulosic nanomaterials have large specific surface areas and also the ability to form hydrogen
 102 bonds. This hydrogen bonding ability allows the material to create a strong, dense network,
 103 which makes them very hard for various molecules to pass through (Nair et al. 2014). This
 104 property is excellent for barrier applications, and this is what the packaging industry is looking
 105 for. Nanocellulose has potential applications across several industrial sectors and allows the
 106 development of innovative materials, as well as the enhancement of conventional materials
 107 properties. The nanoscale cellulose can be utilized, in fact, as filler, in composites manufacture,
 108 as coating and as self-standing thin films, achieving very interesting and promising properties.
 109 These properties, together with fundamental aspects such as its renewable nature, non-food
 110 agricultural-based sources, biodegradability and/or biocompatibility, low cost and low energy
 111 consumption have attracted a lot of attention, which is motivated also by the general interest
 112 towards a sustainable economy able to overcome today's dependency on fossil sources (Li et al.
 113 2015).

114 This paper review aims to give an overview of the use of nanocellulose for packaging purposes
 115 as well as to summarize the recent developments in various barrier films based on nanocellulose
 116 with special focus on oxygen and water vapor barrier properties.

117 **2. Barrier properties within the nanocellulose film**

118 In order to understand the role of nanocellulose in the field of packaging, it is worth describing
119 the diffusion process of the molecules through this material (nanocellulose). The diffusion of
120 molecules between two adjacent volumes separated by a thin film of solid polymer or membrane
121 happens basically in three steps (Nair et al. 2014). In the first step, the sample surface adsorbs
122 the diffusing molecule. In a second step, the diffusing molecule will go through the film or
123 membrane. Finally, in a third step, the diffusing molecule will be desorbed from the film or
124 membrane surface on the other side. In many cases it is satisfactory to regard the adsorption and
125 desorption steps as being rapid relative to the diffusion through the film. Thus, according to this
126 three-step process, the gas permeability through a nanocellulose film will mainly depend on the
127 dissolution of gas and its rate of diffusion in the film. Thus, one can express the permeability of
128 gas molecules through various types of material, including thin films, as follows (Eq. 1):

$$129 \quad P = D \times S \quad (1)$$

130 where P is the permeability, D is the diffusion coefficient, and S is the solubility coefficient.

131 The permeability coefficient P can be obtained from the application of Henry's law of solubility
132 to Fick's law of diffusion (Eq. 2),

$$133 \quad P = D \times S = \frac{q \times l}{A \times t \times \Delta p} \quad (2)$$

134 where q is the amount of material passing through the film, l is the flow length or thickness, A is
135 the cross-sectional area, t is time, and Δp is the pressure difference between the two sides of
136 film.

137 It is worth mentioning that the gas molecules should be first dissolved in the membrane or film
138 before the diffusing step occurs. Although it is well known that the film surface influences the
139 permeating gas molecules, the rate of molecule diffusion in the membrane / film or, also called
140 bulk flow, is the most important factor in the molecular migration (Lagaron et al. 2004). Also, in
141 other words, bulk flow is commonly known as the movement of molecules from an area of high
142 concentration to an area of low concentration.

143 The fact that nanocellulose has good barrier properties, especially in terms of oxygen, can be due
144 to the dense network structure that is formed by nanofibrils. In the case of nanocrystalline
145 cellulose, the density can be attributed to the small and uniform particle sizes. In the case of
146 nanofibrillated cellulose, the densification is enhanced by the inherent flexibility of the wet
147 material. According to these explanations, the pores that might exist within the films serve as the
148 major path for permeating oxygen molecules (Nair et al. 2014). NFC, due to its inherent flexibility,
149 would be able to form a denser film, sealing up most of the spaces between the fibrils down to a
150 molecular scale. By contrast, CNC particles are inherently rigid, so it is reasonable to expect there
151 to be a lower density of packing and a higher porosity within a film composed only of CNC. The
152 concept of combining CNC (for high crystallinity) with NFC (for high conformability, favoring high
153 film density) merits research attention.

154 The definition of the term “pores” is worthy of receiving more attention in future research. At
155 one extreme, the term can refer to an unobstructed passage, or network of passages, through
156 which gas or liquid molecules can pass from one side of the film to the other. The sizes of such
157 pores can be specified according to typical size by using the IUPAC definitions of macropores,
158 mesopores, and micropores. In addition, future modeling studies need to take into account the

159 contributions to overall transport that can be attributed to the diffusion of O₂, H₂O, or other
160 molecules of interest through the amorphous polymeric matrix phases. If one compares cellulose
161 fibers to nanofibrils, one can conclude that the nanofibrils might form more complex and smaller
162 pores compared to their counterparts (cellulose fibers) due to significantly higher surface area
163 and high aspect ratio. This kind of network that the nanofibrils can originate basically decreases
164 the permeability by increasing the density within the film (Syverud and Stenius, 2009).

165 It is well known that both nanofibrillated cellulose and bacterial nanocellulose contain a high
166 proportion of crystalline regions in combination with disordered regions. Crystallinity values for
167 various types of nanocellulose have been reported ranging from 40-90%, showing cellulose
168 nanocrystals (CNCs) as having the highest crystallinity (higher than the nanofibrillated cellulose,
169 NFC) due to the strong acid used for their production, which hydrolyzes disordered cellulose (Guo
170 and Catchmark, 2012; Nair et al. 2014). The crystalline regions are essentially impermeable to
171 gas molecules (Lagaron et al. 2004; Saxena et al. 2010). However, because of the impermeable
172 nature of cellulose crystalline regions, the length of the effective flow path of the air, water vapor
173 or gas molecules through a nanocellulose film can be significantly greater than the measured film
174 thickness (Lagaron et al. 2004; Saxena et al. 2010). In other words, a dense, nonporous film of
175 nanocellulose may present a high tortuosity for the diffusion of gases. The concept of tortuosity
176 is represented in Figure 2.

177 **[[APPROXIMATE LOCATION OF FIGURE TWO]]**

178 **Figure 2.** Schematic representation of increased diffusion path within the nanocellulose films.
179 Figure is replotted from (Nair et al. 2014).

180 NFC films generally show better results in terms of barrier properties (more specifically, less
181 oxygen permeability) compared to CNC films, even though the latter have higher crystallinity. It
182 is also true that the solubility is very similar for both NFC and CNCs. However, in the case of the
183 NFC films, the oxygen molecules can penetrate more slowly and with more difficulties. This fact
184 can be explained by the structural organization within the films. More and higher entanglements
185 can be observed in the case of the NFC films, leading to a high density and a tortuous diffusion
186 path (Belbekhouche et al. 2011).

187 Nanocellulose may play a different role with respect to gas permeability when it is used as a
188 reinforcement in a polymer matrix. The extent to which tortuosity can account for reductions in
189 permeability through nanocellulose-containing polymer films is a topic that merits further
190 research attention. In such composite structures, the content of nanocellulose within the
191 polymer matrix is often rather low, often in the range of 0.5 to 20%. For example, Follain *et al.*
192 (2013) reinforced a poly-caprolactone film with 3 to 12% of isocyanate-grafted CNC, resulting in
193 increased resistance to the diffusion of water. This increased resistance was attributed to
194 tortuosity. However, other factors that might account from such observations include any
195 tendency for the nanocellulose to improve film uniformity or to make film tough enough to
196 decrease the frequency of cracks or other defects. Due to their fibrillar or columnar shapes,
197 nanocelluloses would not be expected to provide effective blockage of diffusion paths where
198 present at relatively low levels in a polymer matrix.

199 In summary, one can conclude that the barrier properties for oxygen are high for nanocellulose.
200 However, the same trend cannot be observed for the water vapor barrier properties, as they tend
201 to be low for unmodified nanocellulose material (Nair et al. 2014). One can attribute this to the

202 high affinity that exists between water and the nanocellulose fibrils. Nevertheless, if one
203 compares nanocellulose fibrils and cellulose fibers in terms of water vapor barrier properties,
204 there is a strong reducing effect on this barrier property in the case of the nanocellulose, and this
205 can be mainly attributed to its size and swelling constraints formed due to rigid network within
206 the films. However, when the relative humidity is high, the typical structure of the nanocellulose
207 fibrils within the film can be ruined because of the high swelling, and then the barrier properties
208 for both oxygen and water vapor can be lost (Spence et al. 2010).

209 The literature shows different examples for the nanofibrillated cellulose (NFC) and nanocrystals
210 (CNCs) regarding their application in barrier layers. According to the data that can be found in
211 the literature, NFC could be considered as a strong gas barrier material. As a matter of fact, films
212 made of NFC with a thickness around 21 μm show very high air and oxygen barrier properties, as
213 for example, the oxygen transmission rates (OTR) were around $17 \pm 1 \text{ ml m}^{-2} \text{ day}^{-1}$. If one compares
214 these values with those shown by synthetic polymers such as ethylene vinyl alcohol ($3\text{-}5 \text{ ml m}^{-2}$
215 day^{-1}) or polyvinylidene chloride coated polyester films ($9\text{-}15 \text{ ml m}^{-2} \text{ day}^{-1}$) of approximately same
216 thickness, one can conclude that the values shown by the NFC are very competitive (Syverud and
217 Stenius, 2009). In addition, there are reports, such as that of Rodionova et al. (2011), which have
218 demonstrated that the barrier properties can be tunable for the NFC. As a matter of fact,
219 Rodionova and co-workers showed that NFC films (both pure and partially acetylated) can be
220 utilized for modified atmosphere packaging (Rodionova et al. 2011). Table 2 reviews the oxygen
221 permeability of NFC films compared with those from commercially available petroleum-based
222 materials and other polymers.

223

224 **Table 2.** Oxygen permeability of nanocellulose film compared to those made from commercially
 225 available petroleum based materials and other polymers (Data extracted from (Nair et al. 2014)).

Material	Oxygen permeability (cc.µm/m².day.kPa)	Conditions	References
NFC	0.6	65% RH 23 °C	Österberg et al. 2013
NFC (carboxymethylated)	0.0006	0% RH 23 °C	Aulin et al. 2010
NFC (carboxymethylated)	0.85	50% RH 23 °C	Aulin et al. 2010
Cellophane	0.41	0% RH 25-80 °C	Wu and Yuan, 2002
Polyethylene (PE)	50-200	50% RH 23 °C	Lange and Wyser, 2003
Polyvinylidene chloride (PVdC)	0.1-3	50% RH 23 °C	Lange and Wyser, 2003
Polyvinyl alcohol (PVOH)	0.20	0% RH 23 °C	Lange and Wyser, 2003
Ethylene vinyl alcohol (EVOH)	0.01-0.1	0% RH 23 °C	Lange and Wyser, 2003

226

227 In summary, NFC films are competitive with commercial films made of synthetic polymers in
 228 terms of oxygen and air barrier properties especially in dry conditions; however, this trend was
 229 not observed for the water vapor barrier properties. Table 3 shows the water vapor transmission
 230 rate (WVTR) of NFC films and commercially available films from petroleum based materials and
 231 other polymers. The strong hydrophilic nature of nanocellulose could be the explanation for this
 232 difference in performance (Nair et al. 2014). However, this is not the only reason that we can find
 233 in order to explain these results. Also, the compactness of the film will significantly affect the
 234 WVTR as less amount of water can penetrate through the film, which is the case of the films made
 235 of NFC.

236 Lignin is a major component of the most widely available cellulosic resources, and it is even
 237 possible to skip the delignification step when preparing nanofibrillated cellulose by extensive
 238 mechanical shearing (Spence et al. 2010). Lignin is also a factor to be considered as it can create
 239 films with surfaces more hydrophobic, which means higher water contact angles but less water
 240 vapor barrier properties. In addition to this, when lignin is present, the compactness of the film
 241 is clearly reduced compared to films made of NRC. This observation can be explained by the fact
 242 that lignin might hinder the hydrogen bonding creating more hydrophobic pores which facilitate
 243 the water vapor transmission (Spence et al. 2010).

244 In spite of the poor water vapor barrier properties that the NFC films show, there are some
 245 authors that have been working on the potential improvement of these specific barrier
 246 properties. For instance, Sharma and co-workers reduced by 50% the water vapor permeability
 247 of NFC films by heating them at 175°C for 3 hours compared to untreated NFC films. This
 248 achievement could be explained by the increase in hydrophobicity, as evidenced by the increase
 249 in water contact angle and reduced porosity by heat treatment (Sharma et al. 2014). Further,
 250 researchers have shown improvement in water vapor and gas barrier properties by developing
 251 nanocellulose composite films (Salminen et al. 2013).

252 **Table 3.** Water vapor transmission rates of NFC films compared to commercially available
 253 petroleum based materials and other polymers (Data extracted from (Nair et al. 2014)).

Material	Water vapor transmission rate (WVTR) (g/m ² .day)	Average thickness of the film (µm)	Conditions	References
NFC	234	42	50% RH 23 °C	Rodionova et al. 2011

NFC (acetylated for 0.5 h)	167	46	50% RH 23 °C	Rodionova et al. 2011
Cellophane	350	27	85% RH ambient	Joshi et al. 1997
Polyvinylidene chloride (PVdC)	3.07	12.7	100% RH 27 °C	Steven and Hotchkiss, 2002
Polyethylene (PE)	16.8	18.3	100% RH 27 °C	Steven and Hotchkiss, 2002
Plasticized polyvinyl chloride (PVC)	118.56	12.7	100% RH 27 °C	Steven and Hotchkiss, 2002

254

255 On the other hand, very few studies have been carried out for CNC films. Belbekhouche and co-
 256 workers studied and compared the gas barrier properties between NFC and CNC films. They
 257 found out that the oxygen permeability was lower for the NFC films compared to the CNC films,
 258 and this can be attributed to the higher fibril entanglements and higher film density that happen
 259 within the NFC films (Belbekhouche et al. 2011).

260 **3. Technologies for nanocellulose-based materials production**

261 The diameter of the cellulose nanofibrils is in the range of 2 to 50 nm, and the lengths are up to
 262 several micrometers, depending on the raw material and process used for their extraction
 263 (Hoeger et al. 2013; Stelte; Sanadi, 2009; Hubbe et al. 2008). As it has been mentioned earlier,
 264 this material has extraordinary optical and mechanical properties, which allows the generation
 265 of a huge variety of high-performance materials (Abraham et al. 2011; He et al. 2012; Kaushik
 266 and Singh, 2011). For the conversion of cellulosic fibers into nanofibrils, it is required to use
 267 intensive mechanical treatments (Uetani and Yano, 2011); however, a chemical pretreatment
 268 such as enzymatic treatment (Henriksson et al. 2007) may be applied to reduce the energy demand,
 269 depending on the raw materials and process. In the literature, one can find several methods used

270 for the production of cellulose nanofibrils, such as refining and high-pressure homogenizers
271 (Chinga-Carrasco and Syverud, 2012, Karande et al. 2013), microfluidizers (Ferrer et al. 2012),
272 and grinders (Wang et al. 2012). By contrast, sulfuric acid or hydrochloric acid are needed in order
273 to prepare cellulose nanocrystals (Saxena et al. 2010).

274 Bacterial nanocellulose, which is lignin-free and does not require chemical treatments or
275 intensive milling to achieve nano-scale dimensions, so far has received much less research
276 attention as a way to prepare barrier films for packaging (Dobre et al. 2012; Padrao et al. 2016).
277 Padrao et al. showed that a film of BNC was able to act as a water vapor seal on the surfaces of
278 sausages, thus extending their shelf life. Because of such promising results, in addition to the
279 “generally regarded as safe” status of some BNC materials (FDA 2006), there is strong motivation
280 to consider BNC for food-contact packaging applications.

281 In recent literature, one can find different processes theoretically applicable for the production
282 of nanocellulose-based materials; however in this review, we will comment very briefly only on
283 those that appear as most promising for manufacturing packaging materials based on
284 nanocellulose, namely, layer-by-layer assembly, electrospinning, composite extrusion, casting
285 evaporation, and coating.

286 **3.1. Layer-by-layer (LbL) assembly**

287 Layer by layer (LbL) assembly is a generic technique for thin film coating of functional materials
288 onto surfaces (Decher, 1997). This technique basically allows the creation of multicomponent
289 films on solid supports by controlling adsorption from solutions or dispersions (Decher, 1997). In
290 this way it possible to preserve the properties that are very important for packaging applications,

291 as for example gas barrier (Aulin et al. 2013; Marais *et al.* 2014.) and wet-strength (Ankerfors et
292 al. 2016). To achieve the effects cited above, the layers of nanocellulose were alternated with
293 poly-(etheneimine) (Aulin et al. 2013), cationic starch or polyamido-amine wet-strength resin
294 (Ankerfors et al. 2016). Other researchers have prepared layer-by-layer films of nanocellulose
295 alternating with chitosan (de Mesquita et al. 2010 ; Li et al. 2013) or various cationic
296 polyelectrolytes such as poly-(ethyleneimine), poly(allylamine hydrochloride), and poly-
297 (diallyldimethylammonium chloride) (Wågberg et al. 2008; Marais et al. 2014). In principle the
298 self-assembly of nanomaterials, in response to electrostatic attraction, can be envisioned as a
299 mechanism potentially leading to a dense, ultra-thin nanocellulose layer during the alternating
300 steps of the layer-by-layer building process.

301 This technique can also create multiple-layer structures of very thin alternating films based
302 primarily on electrostatic interactions and hydrogen bonds between a polyanion and a polycation
303 (Jean et al. 2009). This approach shows different advantages as, for example, simplicity and
304 versatility, thickness control at the nanoscale and the potential of coating easily on tridimensional
305 objects, such as small bottles, cups and trays.

306 In view of the multiple steps required to implement the types of layer-by-layer structures in the
307 above-cited articles, there also has been strong interest in self-assembled structures. For
308 example, Liu and Berglund (2012) prepared nanocomposites with montmorillonite clay
309 nanoplatelets and nanofibrillated cellulose. This combination, facilitated by the co-addition of
310 chitosan, yielded a promising combination of high strength, toughness, fire-resistance, and
311 resistance to oxygen permeation. Layering involving the organic and inorganic nano-components
312 was evident in the composite.

313 **3.2. Electrospinning (ES)**

314 Electrospinning (ES) is a broadly used technology for electrostatic fiber formation that utilizes
315 electrical forces to produce polymer fibers with diameters ranging from 2 nm to several
316 micrometers using polymer solutions of both natural and synthetic polymers (Bhardwaj and
317 Kundu, 2010). The viscous flow is responsible for the alignment of the individual polymer chains.
318 In the literature there are a lot of works showing that ES can be used to combine different
319 polymers with nanocellulose, overcoming problems of compatibility and preserving the most
320 interesting properties of the biopolymer. More specifically, studies have been focused on the
321 production of nanocellulose composites with both bio-based and synthetic polymers. In addition
322 to this, the thermal and mechanical properties need to be enhanced, and basically this could be
323 achieved by the change in crystallinity and the alignment of nanocellulose along the fiber length
324 (Park et al. 2007). As an example, cellulose microfibrils were introduced into poly(ethylene oxide)
325 fibers by electrospinning (Fortunato et al. 2012), and in 2010, Peresin and co-workers electrospun
326 poly(vinylalcohol) with cellulose nanocrystals, obtaining reinforced nanofibers (Peresin et al.
327 2010).

328 **3.3. Composite extrusion**

329 The extrusion process transform materials from solid to liquid and back again with the desired
330 thickness. This approach seems to be more versatile and simpler than LbL or ES; however poor
331 compatibility of nanocellulose with synthetic polymers can cause process issues. The main target
332 of this technique is to incorporate nanocellulose (acting as a functional nanofiller) in different
333 polymeric matrices and taking advantage of all their extraordinary properties as, for example,

334 high aspect ratio, specific surface area and renewability, among others (Fortunati et al. 2014, Li
335 et al. 2015). Since 1990, one can find many different works related to this topic that explain in
336 detail how the nanocellulose can reinforce the polymeric matrix. Miao and Hamad (2013) gave
337 an overview about the cellulosic materials used so far and their reinforcement effects (Miao and
338 Hamad, 2013).

339 **3.4. Casting from solution and evaporation**

340 This technique is based on the use of moderate temperature and a solvent in order to control
341 the nanocellulose concentration (Aulin et al. 2010, Khan et al. 2012, Bhardwaj et al. 2014, Lu et
342 al. 2014, Li et al. 2015). Good dispersibility of the components needs to be achieved, and this is
343 basically the main drawback that can be found (Li et al. 2015). Rodionova et al. (2012), Pereda et
344 al. (2014) and Rafieian and Simonsen (2014) provide examples where researchers were able to
345 disperse cellulose nanocrystals from an aqueous solution containing inherently water-loving
346 matrix materials such as chitosan. Even though casting from solution is not a technique
347 commonly used in the packaging industry, there are some works in the literature that report how
348 the nanocellulose has successfully been casted. In this sense, Svagan and co-workers casted
349 nanocellulose combined with a 50/50 amylopectin-glycerol blend (Svagan et al. 2007, 2009).

350 **3.5. Coating**

351 This is a technique that is very commonly used in order to improve many properties of packaging
352 materials (Farris and Piergiovanni, 2012, Pal et al. 2008). When coating is used in the packaging
353 industry, the main aim that needs to be pursued is including thin layers, which may be either
354 external or sandwiched between two substrates (Li et al. 2015). These layers normally show

355 thicknesses that range from tenths nanometers to a few micrometers. The excellent dispersibility
356 of nanocellulose in water makes it very attractive as an aqueous coating that can be applied as a
357 pure nanocellulose thin layer or as a composite with other traditional coating materials. In
358 addition to this, currently there is a trend to try to reduce the thickness of oil-based plastic films
359 by using a thin layer of functional and high performing bio-based material. In this sense,
360 nanocellulose appears to be an ideal material due to its high crystallinity, among other
361 properties. In fact, there are some reports in the literature that have successfully tested the use
362 of nanocellulose for coating. Among all the examples that can be found in the literature, Aulin
363 and co-workers coated carboxymethylated microfibrillar cellulose on papers, and they reported
364 a low oxygen permeability of the films at low relative humidity values (Aulin et al. 2010). The
365 values were comparable to those shown by conventional synthetic films such as ethylene vinyl
366 alcohol. However, the same trend was not observed at high relative humidity values, as the
367 microfibers were swelled and plasticized (Aulin et al. 2010).

368 **4. Safety and biodegradability issues in nanocellulose applications within packaging field**

369 Nanotechnology implies research and development of particle sizes in the 1-100 nm range. Nano-
370 materials show novel properties that have different applications, owing to their composition,
371 small size and shape. Although clear benefits are expected from the on-going surge of
372 nanotechnology products, special attention must be paid to the potential hazards that
373 nanoparticles can have on human and environmental health (Blaise et al. 2008).

374 Safety, biodegradability, and sustainability are issues that need to be addressed in all types of
375 packaging materials, and these issues are of particular concern in food packaging (Li et al. 2015).

376 Therefore, factors such as biodegradability, cytotoxicity, genotoxicity and ecotoxicity of
377 nanocellulose have recently been explored.

378 Some challenges in the development of packaging materials can be attributed to complexity. For
379 instance, if the product contains cellulose, should it be nanocrystalline, highly fibrillated,
380 bacterial, or macroscopic fibers, *etc.*? Nanocrystalline cellulose might be preferred in certain (but
381 not all) extrusion applications due to the inherent uniformity and very small sizes of the individual
382 particles. In principle, the removal of non-crystalline material during preparation of CNC can be
383 expected to yield material that is inherently more resistant to degradation by heat or enzymes.
384 By contrast, highly fibrillated celluloses might be preferred in cases where the cellulosic
385 component needs to provide structure to a barrier layer (Spence et al. 2010, Johansson et al.
386 2012). The cellulosic component can be further differentiated by surface chemical modifications.
387 For instance, a lot of emphasis has been placed on derivatization of cellulosic surfaces to improve
388 their adhesion to hydrophobic matrix materials in composites (Habibi 2014). Furthermore, one
389 can choose between many different processing schemes, such as melt extrusion, melt
390 compounding, electrospinning, layer-by-layer deposition, solution casting, and aqueous-based
391 coating of suspensions. In each case, although the raw materials may be regarded as safe and
392 biodegradable on their own, it is important to ask whether the terms “safe” and “biodegradable”
393 still can be used when describing the manufactured composite product.

394 **4.1. Biodegradability**

395 Nanocellulose and its biodegradability have been studied, as it has been used as a reinforcement
396 agent for packaging materials, especially when nanoparticles were used as biodegradable

397 reinforcement for other polymers (Coma et al. 1994). According to what can be found in the
398 literature, the biodegradability is not lost because of nanometric dimensions (Kummerer et al.
399 2011).

400 As a matter of fact, Kummerer and co-workers reported that nanoparticles from cellulose and
401 starch were even more rapidly biodegradable than their macroscopic counterparts; such
402 observations contrasted with tests in which the nano-reinforcements were non-cellulosic
403 (Kummerer et al. 2011).

404 Though a great deal of packaging material ends up in landfills or as roadside litter, one can expect
405 more and more of it, in the future, to be recycled into useful products. In this regard, increasing
406 motivation can be anticipated for the preparation of packaging materials comprised of readily
407 repulpable materials. Most of the nanocellulose products discussed in this article can be
408 expected to be highly compatible with their later usage as a component in recycled paper.

409 **4.2. Cytotoxicity and genotoxicity**

410 Nanomaterials show properties that are not usual for bulk materials, and this is why studies in
411 the last years have been focused on the possible potential health hazards of nanocellulose (Li et
412 al. 2015). Pure cellulose is generally well known as a safe food substance, according to the Food
413 and Drug Administration, and longer cellulose fibers can provide structure and texture to baked
414 products (Li et al. 2015). Although nanoparticles present several positive sensory effects, such as
415 smooth consistency, mouth feel and stickiness, further studies are required in order to evaluate
416 their potential health hazards. In this sense, there are some works that can be found in the
417 literature, as for example, the work developed by Ni and co-workers who found that cellulose

418 nanocrystals obtained by acid-hydrolysis can be used as nanobiomaterials because of their low
419 cytotoxicity to L929 cells (cells extensively used for cytotoxicity testing), and to MTT assay
420 (common test for cell viability) (Ni et al. 2012). In another study, the potential genotoxicity of
421 bacterial cellulose was also tested (Moreira et al. 2009). In this work, they used in vitro analysis
422 and also techniques that were tested and successfully effective on fibrous nanoparticles, such as
423 comet assay (single-cell gel electrophoresis) and Ames test (Salmonella reversion assays to
424 evaluate the mutagenic potential of chemical compounds). According to their findings, bacterial
425 cellulose is not genotoxic under the conditions tested (Moreira et al. 2009).

426 Hannukainen et al. (2011) used two differently prepared nanofibrillar celluloses in order to
427 evaluate their potential genotoxicity in human bronchial epithelial BEAS 2B cells. Nanofibrillar
428 cellulose was obtained by grinding after an enzymatic pre-treatment and by microfluidization
429 after a TEMPO-mediated oxidation pre-treatment. According to the results, there was an induced
430 DNA damage in the target cells when the nanocellulose produced by grinding was used, but no
431 induction of oxidative DNA damage was seen. However, when the nanocellulose obtained by
432 microfluidization was used, there was a DNA damage in BEAS 2B cells and a sporadic indication
433 of oxidative DNA damage at 5 $\mu\text{g}/\text{cm}^3$ (Hannukainen et al. 2011). Nevertheless, it is worth
434 mentioning that this method has not been widely applied to studies with fibrous nanomaterials;
435 thus, the mechanism behind the results is still unclear (Li et al. 2015).

436 Vartiainen and co-workers studied the effect of different processes on the potential toxicity risk
437 of cellulose nanocrystals (Vartiainen et al. 2011). In their investigations, they considered the
438 worker exposures to particles in air during grinding and spray-drying of birch cellulose. In both
439 cases, there was not a significant exposure to particles during operation. In the case of grinding,

440 the particles were removed because of the use fume hoods, and the spray dryer is not supposed
441 to leak particles unless it is not correctly operated (Vartiainen et al. 2011). The health effects of
442 the nanocellulose were also considered. For this purpose, the viability and cytokine profile of
443 mouse macrophages and human monocyte derived macrophages were evaluated after 6 and 24
444 hours of exposure (Vartiainen et al. 2011). According to their findings, there were not
445 inflammatory effects or cytotoxicity on mouse and human macrophages (Vartiainen et al. 2011).
446 Apart from this, there are studies in the literature that report about the toxicity of modified
447 nanocellulose (Male et al. 2012). For example, the cited study used oxidized/carboxylic
448 nanocellulose from different biomass sources, and these materials were tested for their possible
449 cytotoxicity by electric cell substrate impedance sensing, using *Spodoptera frugiperda* insect cells
450 (Sf9) and *Cricetulus griseus* (Chinese hamster) lung fibroblast cells. The cells were exposed to
451 nanocellulose, and their spreading and viability were monitored and quantified by electric cell
452 substrate impedance sensing. On the basis of the 50% inhibition concentration, none of the
453 nanocellulosic materials produced had any significant cytotoxic effects (Male et al. 2012).

454 **4.3. Ecotoxicity**

455 This factor is also important to consider, especially when cellulose nanoparticles are used for
456 packaging purposes. As a matter of fact, there is a research group in Canada that has been
457 studying the potential environmental risks of cellulose nanoparticles and carboxymethyl cellulose
458 since 2010 (Kovacs et al. 2010). In their research, they used rainbow trout hepatocytes and nine
459 aquatic species and they could show that the hepatocytes were most sensitive to the cellulose
460 nanoparticles, although neither cellulose nanoparticles nor carboxyl methyl cellulose generated

461 genotoxicity (Kovacs et al. 2010). According to their findings, the toxicity potential and
462 environmental risk of nanocellulose are very low.

463 **5. Conclusions**

464 As is evident from the studies cited in this article, interest in nanocellulose-based applications in
465 packaging is accelerating rapidly but still early in the product life cycle. A variety of promising
466 strategies have been demonstrated or are in different stages of implementation. Oxygen barrier
467 properties represent a primary domain of strong potential for nanocellulose-based packaging
468 films. On the other hand, a key need for continuing research concerns inadequate resistance to
469 water vapor and to moisture. Also, the contrasts between various nanocrystalline cellulose
470 products, nanofibrillated cellulose products, and bacterial nanocellulose materials implies that
471 there are a great many combinations to consider. Nanocellulose materials also can be modified
472 by means of surface chemical treatments (Habibi 2014). The various nanocelluloses can be
473 incorporated into packaging products and barrier layers by diverse means, with particular
474 promise having been shown for solution casting, melt-extrusion, and electrospinning
475 technologies, among others. Such challenges and options imply that there will be a strong need
476 for continuing research in the coming years to attempt to achieve the full potential of
477 nanocellulose in a wide range of packaging applications.

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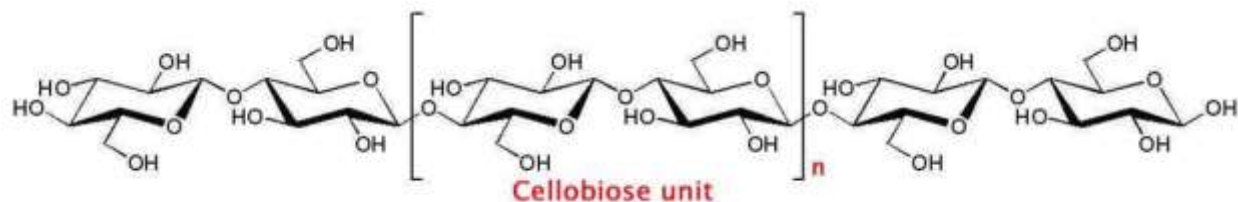
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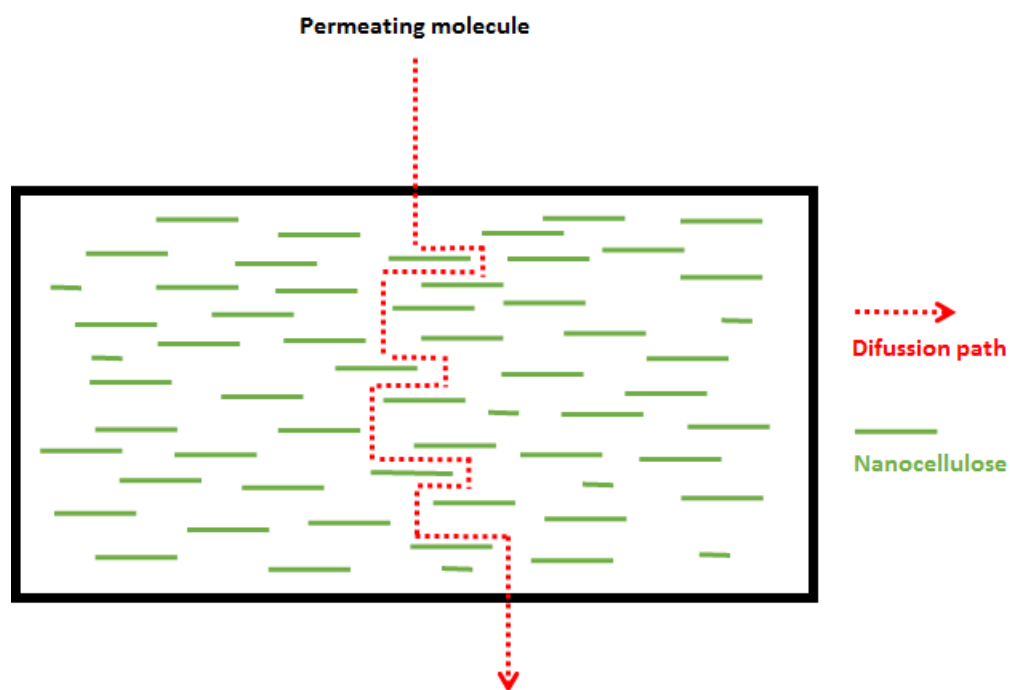
678

679 **FIGURES**



680

681 **Figure 1.** The structure of cellulose.



682

683 **Figure 2.** Schematic representation of increased diffusion path within the nanocellulose films.

684 Figure is replotted from (Nair et al. 2014).

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