

**ADSORPTION OF BARK DERIVED POLYPHENOLS ONTO FUNCTIONALIZED
NANOCELLULOSE: EQUILIBRIUM MODELLING AND KINETICS**

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Adsorption of bark derived polyphenols onto functionalized nanocellulose: Equilibrium modelling and kinetics

ABSTRACT

This paper describes the kinetics and capacity of adsorbing condensed conifer tannins onto cationic cellulose nanocrystals (CCNCs). Batch adsorption experiments were carried as a function of pH, contact time and initial tannin concentration with constant cationic cellulose nanocrystal concentration (0.01%). The adsorption process was highly pH dependent as adsorption capacities ranged from 13.2 mg/g to 112.7 mg/g at pH of 3–10. The amount of tannin adsorbed per unit mass of the cationic cellulose nanocrystals increased with increasing of tannin concentration until equilibrium was attained. The experimental data followed the Langmuir adsorption model, and the maximum experimental and theoretical adsorption capacities for the cationic nanocrystals reached 1008 mg/g and 1111 mg/g, respectively. The kinetics of adsorption was described best by the pseudo-second-order kinetics indicating a chemisorption process. The inherent adsorption has interesting applications for CCNC-complexes with natural polyphenolics in green chemical applications for e.g. adhesives, adsorbents, preservatives and packaging materials.

Keywords: Adsorption isotherm; Biomaterials; Cationic cellulose nanocrystals; Reaction kinetics; Tannin

1 INTRODUCTION

2 Proanthocyanidins such as tannins and other flavonoids are a group of bio-based chemicals
3 traditionally extracted from plant seeds, berry or fruit skins, tree heartwood or bark and novel
4 means to valorize these especially from industrial side streams are ongoing¹⁻⁵. These phenolic,
5 secondary metabolic compounds in plants have traditionally been used in leather industry due to
6 the protein binding capacity and more recently in colloidal complexes intended for food, pharma,
7 chemical and wood product industries⁶⁻⁹.

8

9 Tannin compounds are known for their ability to bind to proteins and compounds such as alkaloids
10 and amino acids, large-molecular compounds and metallic ions. They also present some anti-
11 oxidant activity that distinguishes them from other plant polyphenols¹⁰. Haslam found out that
12 tannins form complexes not only with proteins and alkaloids but also with certain
13 polysaccharides¹¹. Tannins have been used in glue-mix adhesives to improve internal cross-
14 linking and to reduce the required volume of adhesives¹². In addition, they have been used as
15 preservatives in wood and wood products offering protection against light and against deterioration
16 by insects, fungi and bacteria¹³⁻¹⁵. However, the main drawback for the use of tannins in wood and
17 wood products is their high leachability (i.e. tendency to be washed away) in outdoor
18 applications^{15,16}. Bonding between tannins and matrices must hence be strong enough to avoid
19 such leakage of tannin.

20

21 One route to achieve higher retention of tannins in chemical formulations is to combine them with
22 binding compounds^{17,18} or nanoparticles to complexes¹⁹ that can act as bridging or cross-linking
23 agents between the matrix and tannin. Immobilization of tannin, or specific hydrolysable tannin

24 compounds such as tannic acid, onto material surfaces has also been achieved using for instance
25 mesoporous silicate²⁰, silica microspheres²¹, collagen²², graphene²³ and activated carbon²⁴. As
26 reported by Xu et al. (2017), dialdehyde nanocellulose can be used to covalently immobilize tannin
27 molecules. In case of wood as matrix, carbohydrates with inherently good adhesion with wood can
28 be exploited²⁵. However, these carbohydrates must have a suitable structure, composition, as well
29 as a sufficient size and flexibility to be able to complex polyphenols such as tannins in the wood
30 structure²⁶⁻²⁹. Nanocelluloses (i.e. cellulose nanofibers or nanocrystals) with a high aspect ratio
31 and surface area combined with a chemically active structure, are potential agents to encapsulate
32 and complex with polyphenols, and allow them to be incorporated wood matrix. Apart from the
33 particle size, the surface chemistry also play an important role in surface interactions as too much
34 attraction of the complex formed may lead to surface coating and the desired impregnation of
35 voids, cavities and wood cells or lumen is not achieved.

36
37 The adsorption performance of modified nanocelluloses may be increased via high specific surface
38 areas and numerous reactive groups³⁰. Recent publications have shown uses of modified
39 nanocelluloses in water treatment in adsorption and removal of heavy metals and organic pollutants.
40 In these cases, the surface modification of the nanocellulose was obtained by adding specific
41 groups such as carboxyl^{31,32}, amine^{33,34}, ammonium³⁵ and xanthate³⁶ on the surface of cellulose.
42 There are several similar studies that report the use of nanocellulose or tannin layered particles in
43 wastewater purification³⁷ or metal ion scavenging systems²⁵ but to our understanding such analysis
44 on the kinetic rate and mode of adsorption between cationic nanocelluloses and wood bark-derived
45 tannins has not been published. In this study, we report the results of the interactions between
46 wood-derived condensed tannins and other polyphenols and cationic cellulose nanocrystals

47 (CCNC) obtained from deep eutectic solvent treatment, and especially address the adsorption of
48 bark chemicals onto cellulose nanocrystals. The effect of solution pH, contact time and initial
49 tannin extract concentration on the adsorption was studied in batch experiments in particular, and
50 the adsorption was further modelled using adsorption isotherms and kinetic data.

51

52 **MATERIALS AND METHODS**

53 **Raw materials and chemicals for making the CCNCs**

54 Dry sheets of bleached birch Kraft pulp (*Betula pendula*) were used as the fibre material for the
55 production of the cellulose nanocrystals. Lithium chloride (LiCl) (99%), sodium (meta) periodate
56 (NaIO₄), were from Sigma-Aldrich (Germany), ethanol (CH₃CH₂OH) (96%) and glycerol
57 (C₃H₈O₃) (97%) from VWR France, aminoguanadine hydrochloride (CH₆N₄·HCl) (>98%) from
58 Tokyo Chemicals Industry, Japan, hydrochloric acid (HCl) (0.1 mol/dm³) and sodium hydroxide
59 (NaOH) (0.1 mol/dm³) from Oy FF-chemicals AB, Finland. Acetate buffer (CH₃COOH /
60 CH₃COONa, 3·H₂O) (pH 3 and pH 5), phosphate buffer (NaH₂PO₄ / Na₂HPO₄) (pH 7 and 8) and
61 sodium carbonate buffer (NaHCO₃/ Na₂CO₃) (pH 10) were from CH₃COOH and CH₃COONa,
62 2·H₂O (Sigma-Aldrich), NaH₂PO₄ (Sigma), Na₂HPO₄ (Fluka), NaHCO₃ (Merck) and Na₂CO₃ (J.T.
63 Baker), all of which were p.a. grade, were used as received to prepare buffers. Deionized water
64 was used through the experiments.

65

66 **Cationization of cellulose**

67 A two-step method based on consequent periodate oxidation and cationization in deep eutectic
68 solvent was used to produce cationic cellulose. First, disintegrated birch Kraft pulp was oxidized
69 with sodium periodate at temperature 75°C to produce dialdehyde cellulose with aldehyde content

70 of 3.68 mmol/g³⁸. In brief, 9 g of lithium chloride (LiCl) and 4.1 g of sodium periodate (NaIO₄)
71 were added to water suspension of disintegrated pulp (5 g abs., temperature of pulp 75°C) in the
72 absence of light for 180 minutes and mixed with magnetic stirrer. The oxidized pulp was filtered
73 and washed with ethanol. The product (dialdehyde cellulose, DAC) was collected and stored at
74 4°C in a non-dried state.

75
76 Cationization of DAC was done in deep eutectic solvent (DES) according the method established
77 by Li et al. (2018). Cationization of DAC was done in DES formed between aminoguanadine
78 hydrochloride and glycerol in molar ratio of 1:2. A clear DES solution was obtained by melting
79 the compounds at 90°C after which the reaction temperature was adjusted to 70°C and DAC (2.5 g
80 abs.) was added and mixed for 10 minutes. The flask was removed from oil-bath and 25 ml of
81 ethanol was added. The cationized cellulose was filtered and washed with ethanol after which it
82 was dried in oven at 60°C. Finally, the product was mixed in 250 ml of deionized water for 1 h
83 and filtered.

84
85 The cationic charge density of cationized DAC (CDAC) was determined using polyelectrolyte
86 titration method by a particle charge detector (BTG Mütek PCD-03, Germany). The CDACs were
87 diluted by deionized water into 0.01% solution, along with 30 minutes magnetic stirring at room
88 temperature. Then, 10 ml of well dispersed CDAC suspension was titrated with sodium
89 polyethylene sulphonate (PES-Na) polyelectrolyte. The charge density was calculated by the
90 consumption of PES-Na. The charge of the cationic cellulose (in deionized water) was 0.76
91 mmol/g.

92

93 **Production of cationic cellulose nanocrystals**

94 Mechanical disintegration was used to liberate cationic cellulose nanocrystals (CCNC) from the
95 modified cellulose as described by our earlier study³⁹. First, 1% CDAC solution was stirred with
96 a magnetic bar for 10 min and then further disintegrated using a microfluidizer (Microfluidics M-
97 110EH-30, USA) with a pressure of 1000 bars. The suspension passed through the microfluidizer
98 chambers (400 and 200 μm) twice, yielding transparent and low viscous suspension.

99

100 **Determination of charge density, zeta potential and conductivity of tannin and cellulose**
101 **nanocrystals**

102 The anionic and cationic charge density of tannins and cationic cellulose nanocrystals (CCNC)
103 were determined using polyelectrolyte titration with particle charge detector (PCD). 0.01% (w/w)
104 tannin and cationic cellulose nanocrystals solution were prepared at different pH (3-10) and titrated
105 with poly-diallyldimethylammonium chloride and sodium polyelectrolyte sulphonate. All
106 dilutions were done using same buffer solutions used for tannin and nanocrystal sample
107 preparation.

108

109 Zeta potential and conductivity measurements were performed on a Zetasizer Nano ZS (Malvern
110 Instruments Ltd., UK). For the measurement, the CCNC suspension and tannin extract were diluted
111 (with 0.5 mM buffer solution at pH 3, 5, 7, 8 and 10) to a concentration of ~ 0.05 mg/mL. 1 mL of
112 cellulose nanocrystals suspension in DTS1070 disposable folded capillary cell was used for the
113 measurement. The average of three (3) replicates were computed for each pH measured.

114

115 **Extraction and quantification of spruce bark tannin extract**

116 Tannin extract (19.4% dry matter content) was obtained from Natural Resources Institute Finland,
117 Finland, following previously published method⁴⁰. The extract was the raw tannin complexes
118 obtained from spruce bark (*Picea abies*) using hot water extraction and subsequent spray drying
119 without purification. The tannin concentrate contained 21.3 mg/mL of soluble condensed tannins.
120 The average chain length of the tannin was six catechin-units, giving about 2320 for total molecular
121 mass. In addition, the concentrate was composed of many other low molecular weight phenols,
122 such as phenolic acids, flavonoids, lignans, neolignans and stilbenes (Table 1). There was also a
123 group of unidentified compounds with similar UV-spectra, which were likely polymers of
124 decomposed stilbenes with increasing mass of methyl-groups attached.

125

126 The acid-butanol assay for proanthocyanidin analysis⁴¹ was used to determine the amount of
127 soluble condensed tannins in the tannin extract. The samples of 10–30 microliters were diluted up
128 1 ml of methanol, 6 ml of HCl-butanol (20:1) solution and 2% FeNH₄(SO₄)₂ reagent was added.
129 The mixture was incubated for 50 minutes at 100°C. The absorbance of solutions was measured at
130 550 nm using a photometer (Spectronic 20 Genesys™), and the results were standardized with
131 purified tannins from the bark of spruce, *Picea abies*. The degree of the polymerization of the
132 tannins in the extract was determined by the phloroglucinol method with HPLC⁴¹. Shortly, the
133 samples of the tannin extract were incubated with phloroglucinol in acidic ethanol at room
134 temperature overnight. The formed phloroglucinol-derivatized extender units and unreacted
135 terminal units of flavanols were extracted into ethyl acetate and analysed by HPLC. Comparison
136 of the peak areas gave the average chain length for the proanthocyanidins (condensed tannins).

137

138 For the analysis of individual phenolic compounds, samples of 50 or 100 µl of concentrated tannin
139 extract was dissolved into 100 µl of methanol, identified UHPLC-qtof MS and quantified with
140 HPLC-DAD system (HP 1100 series, Agilent Technologies, Palo Alto, CA, USA) according to
141 Taulavuori et al.⁴². The column used in HPLC-DAD was Zorbax 3.5 µm, 4.6 x 75 mm, and
142 injection volume was 20 µl. The eluents used were aqueous 1.5% tetrahydrofuran + 0.25%
143 orthophosphoric acid (A) and methanol (B). The following gradient was used: 0–5 min 100% A,
144 5–10 min 85% A + 15% B, 10–20 min 70% A + 30% B, 20–45 min 50% A + 50% B, 45–65 min
145 100% B, 65–70 min 100% A. The flow rate was 2 ml/min, the injector temperature was 23°C and
146 the column temperature was 30°C. Detection wavelengths were 220 nm, 270 nm and 320 nm.

147
148 Identification and quantification of the compounds were based on their mass, retention times, UV-
149 VIS spectra and the commercial standard compounds: gallic acid (Sigma-Aldrich Finland Oy,
150 Helsinki, Finland); protocatechuic acid (Carl Roth, Karlsruhe, Germany); cinnamic acid (Sigma-
151 Aldrich Finland Oy, Helsinki, Finland); ferulic acid (Carl Roth, Karlsruhe, Germany); ampelopsin
152 (Carl Roth, Karlsruhe, Germany); taxifolin (Extrasynthèse, Genay, France); (+)-catechin (Fluka
153 Chemie AG, Buchs, Switzerland) for (+)-catechin and neolignans; piceatannol (Extrasynthèse,
154 Genay, France) for all stilbenoids. Analytical grade piceatannol (BioNordika Ltd, Finland) was
155 also used as monomer compound in adsorption reference studies.

156

157 **Adsorption experiments**

158 Figure 1 show tannin extract and CCNC used for the adsorption experiment. The adsorption
159 experiments were carried out at pH 3, 5, 7, 8 and 10 by mixing 25 ml of the 0.02% tannin solution
160 with 25 ml of 0.02% of cationic cellulose nanocrystals solution in Gallenhamp GWB flash shaker

161 at room temperature (25°C) for 24 hours. For the monomer compound, the experiments were made
162 using 0.02% concentrations of both nanocellulose and piceatannol at pH 5 and 8. The acetate (pH
163 3 and 5), phosphate (pH 7 and 8) and sodium carbonate (pH 10) buffer solutions (0.5 mM) were
164 used to adjust the pH. After adsorption, 10 ml of solution was filtered using a syringe filter (0.2
165 µm) and the concentration of tannin in the filtrate was determined via calibration curve. All
166 absorbance were determined using the Shimadzu UV-1800 spectrophotometer. Absorbance values
167 were measured at wavelength of 280–282 nm depending on the absorbance maximum at that pH.
168 For piceatannol the absorbance maximum was measured at wavelength of 322 nm. The amount of
169 tannin adsorbed per unit mass of cationic cellulose nanocrystals Q_e (mg/g) were calculated using:
170

$$171 \quad Q_e = \frac{C_o - C_e}{W} V \quad (1)$$

172
173 Where: Q_e = equilibrium adsorption capacity (mg/g), C_e = tannin concentration at equilibrium
174 (mg/L), C_o = Initial concentration of tannin (mg/L), W = mass of cationic cellulose nanocrystal
175 (g), V = volume of solution (L).

176 177 **Kinetics studies**

178 The kinetics studies were similar to the pH effect studies; however they were conducted at pH 8.
179 At different time intervals (5–1440 minutes), 5 ml of the solution in the Scott bottle was drawn,
180 filtered and analyzed using the Shimadzu UV-1800 spectrophotometer. The amount of tannin
181 adsorbed per unit mass of cationic cellulose nanocrystals Q_t (mg/g) were evaluated using:
182

183
$$Q_t = \frac{C_o - C_e}{W} V \quad (2)$$

184 Where: Q_t = adsorption capacity at time t.

185

186 **Effect of tannin concentration on the adsorption**

187 The effect of tannin concentration on adsorption was studied at pH 8 using contact time of 1440
188 minutes. The tannin concentration was varied from 0.002–1.0%. The amount of tannin adsorbed
189 per unit mass of cationic cellulose nanocrystals Q_e (mg/g) were evaluated using Equation 1.

190

191 Tannin solutions of different concentrations (0.001–0.05%) were prepared in 0.5 mM acetate
192 buffer (pH 3 and pH 5), phosphate buffer (pH 7 and 8) and sodium carbonate buffer (pH 10). The
193 UV absorbance of the samples was recorded at wavelength of 190–800 nm against a deionized
194 water as a blank or reference using a Shimadzu UV-1800 spectrophotometer. A 10 mm quartz
195 cuvette was used. There were three different absorption maximums or peaks for the different UV
196 spectrums (i.e. peaks were observed UV-C at 200 nm, UV-B at 280–282 nm and UV-A at 317 nm
197 and 318 nm depending on the pH of the tannin solution). Calibration curve were made for each pH
198 by plotting the absorbance at 280–282 nm against concentration.

199

200 **RESULTS AND DISCUSSION**

201 **Effect of the pH on the tannin and piceatannol adsorption on cationic cellulose nanocrystals**

202 Figure 2 shows the adsorption of the tannin on the CCNC as a function of pH. The adsorption was
203 found to be highly pH dependent and adsorption capacity increase from 13.2 mg/g to 86.36 mg/g
204 was observed when the pH was increased from 3 to 7. With higher pH values (from 7 to 10) the

205 adsorption capacity ranged from 86.4 mg/g to 112.7 mg/g, showing slight slower changes in the
206 uptake.

207
208 The adsorption of piceatannol to cationic cellulose nanocrystal were studied at pH 5 and pH 8.
209 Adsorption capacities were 3.98 mg/g at pH 5 and 11.90 mg/g at pH 8. At pH 8 some change in
210 the color due to piceatannol oxidation was observed but its impact on structure and adsorption
211 behavior could not be assessed. The tannin extract compounds were also subject to oxidation
212 during extraction, storage and usage.

213
214 The pH of the adsorption environment is crucial as it affects the surface charge or the ionic state
215 of the adsorbate (tannin extract) and adsorption capacity of the adsorbent (CCNC), due to acid-
216 base reactions between surface functional groups and solution⁴³. Here, the low adsorption capacity
217 at low pH values was likely attributed to protonation of tannins, which in turn reduced the
218 electrostatic attractive interaction with cationic nanocrystals.

219
220 At higher pH values, the degree of deprotonation of the phenolic hydroxyls in the tannins
221 increased^{44,45}, while nanocrystals still maintained their cationic charge. This charge behavior
222 resulted in attractive interaction between the tannins and CCNC, and increased the capacity of
223 adsorption. The dependency of tannin extract and CCNC charge in different pH is provided in
224 Table 2. The results clearly show that the anionic charge of tannin extract increased as a function
225 of pH, while the cationic charge of CCNC showed only slight decrease up to pH of 8. For studying
226 the influence of tannin concentration on CCNC adsorption and kinetics of adsorption, the pH value
227 of 8, which still showed a high capacity, was used.

228

229 The zeta potential and conductivity of CCNC and tannin extract at different pH values are shown
230 in Table 2. Values for piceatannol monomer were -10.11 mV and 0.063 mS/cm for pH 8,
231 respectively. The CCNC used in this work holds with amino group; whilst tannin extract contains
232 phenolic –OH groups that show ionization in solutions at high pH medium. From the Table 2, the
233 zeta potential of CCNC was positive across the pH range studied, which is attributed to the
234 presence of the protonated amino groups. The tannin compounds, on the other hand, had negative
235 charge across pH range and could therefore attach easily to the CCNC easily via electrostatic
236 attraction.

237

238 **Effect of the contact time on the tannin adsorption on CCNC**

239 The effect of contact time on adsorption of tannin on CCNC is presented in Figure 3. The
240 adsorption consisted of fast initial stage (first 15 minutes) and a slower plateau-stage after that.
241 The adsorption capacity of 75.7 mg/g was obtained already after 5 min of adsorption and relative
242 small differences were found in the adsorption capacities between 120 minutes to 360 minutes
243 (86–90 mg/g).

244

245 Similar observation⁴⁶ was reported on a two stage adsorption mechanism in the adsorption of
246 copper ions on cross-linked chitin-cellulose beads, chitosan-red soil beads and chitosan-banana
247 stem fiber beads with the first rapid and the second slower stages. Here the adsorption rate was
248 extremely high at the beginning, indicating that tannin was adsorbed by a readily available
249 adsorption sites^{30,47}, after which the sites with more difficult to achieve were occupied⁴⁶. This
250 implies that reasonable immobilization of tannin components onto nanocellulose can take place

251 significantly faster than used in most contemporary investigations where the adsorption process is
252 carried out for several hours^{18,25,48,49}. Process efficiency of a shorter reaction time was apparent in
253 the study by Li et al.⁵⁰ in which the production of nanocellulose-tannin films required only 10
254 minute reaction time between system components.

255

256 **Adsorption kinetics**

257 In order to describe the kinetics of tannin adsorption, the linear forms of the pseudo-first-order
258 (Equation 3) proposed by Lagergren⁵¹ and pseudo-second-order (Equation 4) by Ho et al.⁵², were
259 used:

260

$$261 \quad \ln(Q_e - Q_t) = \ln(Q_e) - K_i \times t \quad (3)$$

$$262 \quad \frac{t}{Q_t} = \frac{1}{K_a(Q_e)^2} + \frac{t}{Q_e} \quad (4)$$

263

264 Where Q_t and Q_e are adsorption capacities at time t and at equilibrium, respectively (mg/g), K_i =
265 rate constant of the pseudo-first-order adsorption (1/min), K_a = rate constant of the pseudo-
266 second-order adsorption (g/mg·min). The values of K_i and K_a were determined from the plots of
267 $\ln(Q_e - Q_t)$ vs t and t/Q_t vs t , respectively (Figure 4).

268

269 The results from the adsorption kinetics obtained from the two models are presented in Table 3
270 and Figure 4. The correlation coefficient (R^2) followed the order: pseudo-second-order > pseudo-
271 first-order (Table 3). The Q_e calculated from the pseudo-second-order adsorption matches well
272 with that from the experiment compared to Q_e of pseudo-first-order (Table 3). The pseudo-second-
273 order also had a correlation coefficient close to unity. This confirms that the data is well fitting by

274 the pseudo-second-order kinetics for the entire sorption period. The fitting model suggests that
275 adsorption step could be dominated by chemisorption, involving sharing or exchange of electrons
276 between adsorbent and adsorbate⁵³.

277

278 **Effects of the initial tannin extract concentration on the tannin's adsorption on CCNCs**

279 The adsorption of tannin on cationic cellulose nanocrystals was studied from initial tannin
280 concentration of 0.002–1%. The adsorption capacity ranged from 11.1 mg/g to 1007.9 mg/g and
281 increased as function of tannin concentration until equilibrium was attained (Figure 5). Simple
282 scheme to illustrate the electrostatic attraction between CCNCs and tannin extract constituents at
283 random bonding sites is shown in Figure 6. The lower relative removal (added vs. adsorbed tannin)
284 at higher concentrations resulted from an increased ratio of moles of tannin to the available
285 adsorption sites on nanocrystals surface; hence, fractional adsorption becomes dependent on initial
286 concentration. At optimal conditions the achieved tannin addition to CCNC (100% (w/w)) clearly
287 exceeds the previously reported yields of 67% by Xu et al.²⁵ and 42.3% by Huang et al.⁵⁴.

288

289 Mezenner and Bensmaili⁵⁵ stated that for a given adsorbent dose the total number of available
290 adsorption sites is fixed thereby adsorbing almost the same amount of adsorbate. The initial tannin
291 concentration provides an important driving force to overcome the mass transfer resistance of
292 tannin between the buffer solution and crystal phases. Thus, at higher initial tannin concentration,
293 the number of ions competing for the available adsorption sites on the CCNC was high, hence,
294 resulting in the higher adsorption capacity⁵⁶.

295

296 **Adsorption isotherms**

297 The adsorption equilibrium data was analyzed using Langmuir and Freundlich isotherms
298 expression. The Langmuir model is based on the assumption that the maximum adsorption
299 corresponds to a saturated monolayer of solute molecules on the adsorbent surface⁵⁷. The
300 Langmuir equation is given in the linear form as:

$$301 \quad \frac{C_e}{Q_e} = \frac{1}{Q_m} C_e + \frac{1}{Kl \times Q_m} \quad (5)$$

302

$$303 \quad \text{Slope} = \frac{1}{Q_m}, \text{ Intercept} = \frac{1}{Kl \times Q_m}$$

304 The separation factor, Rl is an important parameter in the Langmuir model indicating the
305 favorability of the adsorption and is calculated as:

$$306 \quad Rl = \frac{1}{1 + (Kl \times Co)} \quad (6)$$

307

308 where Kl is Langmuir constant and Co is the highest initial concentration (mg/L). $Rl > 1$ is
309 unfavorable, $Rl = 1$ is linear, $Rl = 0$ is irreversible, $0 < Rl < 1$ is favorable for the adsorption.

310

311 Freundlich model can be used for non-ideal sorption that involves heterogeneous surface energy
312 systems⁵⁸. It is expressed in the linear form as:

$$313 \quad \ln(Q_e) = \ln(K_f) + \frac{1}{nF} \ln(C_e) \quad (7)$$

$$314 \quad \text{Slope} = \frac{1}{nF}, \text{ Intercept} = \ln(K_f)$$

315

316 Where C_e = tannin concentration at equilibrium (mg/L), Q_e = equilibrium adsorption capacity
317 (mg/g), Q_m = maximum adsorption capacity (mg/g), K_l = Langmuir adsorption constant (L/mg),
318 K_f = Freundlich constant (L/mg), n_F = heterogeneity factor of adsorption sites (dimensionless).

319

320 K_f is a rough indicator of the magnitude of the adsorption capacity whereas $1/n_F$ describes the
321 adsorption intensity. Value of $n_F > 1$ indicates favorable adsorption conditions^{59,60}. In general, the
322 Langmuir model assumes that the surface of the adsorbent is energetically homogenous while the
323 Freundlich model considers for a multisite adsorption for heterogeneous surfaces and is
324 characterized^{61,62} by heterogeneity factor $1/n_F$.

325

326 Linearized Langmuir and Freundlich isotherm models for tannin adsorption on CCNC are
327 presented in Figure 7A and 7B, respectively, with details of the models outcomes given in Table
328 4. The value of R^2 for the Langmuir model (0.99) was found to be higher than that of the Freundlich
329 model (0.83) indicating that the data from this experiment is best fitting for the Langmuir isotherm.
330 This result suggests physical adsorption as well as homogenous distribution of active sites on the
331 surface of the cationic cellulose nanocrystals. The adsorption of tannin extract constituents and
332 piceatannol on cationic CNC surfaces can be described as an interaction between opposite charges
333 that tend to be mostly endothermic and purely entropy-driven⁶³. The maximum adsorption capacity
334 obtained from Langmuir model was also close to experimentally determined value (1111.1 mg/g
335 vs 1007.9 mg/g). The R_l value of 0.99 ($0 < R_l < 1$) also indicated favorable adsorption (Table 4).

336

337 **CONCLUSIONS**

338 Adsorption of condensed tannin extract from Norway spruce bark onto CCNC was found to be a
339 strongly pH dependent process that follows the Langmuir adsorption model of monolayer
340 adsorption with the highest adsorption capacity attained at 1111 mg/g. The adsorption of the
341 tannins to the nanocrystals was highly efficient due to electrostatic force of attraction between the
342 CCNC and the tannins, increasing up to pH 8 as a function of systems' pH. The kinetic modelling
343 studies also showed that the experimental data followed a pseudo-second-order kinetics indicating
344 a step-wise chemisorption process.

345

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351 analyzing the zeta potential and conductivity of CNCCs and tannin extract.

REFERENCES

- 1 Vivas N, Nonier M-F, Pianet I, De Gaulejac NV, Fouquet É. Proanthocyanidins from *Quercus petraea* and *Q. robur* heartwood: quantification and structures *Comptes Rendus Chim* 2006;**9**(1):120-126.
- 2 Chemler JA, Koffas MA. Metabolic engineering for plant natural product biosynthesis in microbes. *Current Opinion in Biotechnol.* 2008;**19**:597-605.
- 3 Pizzi A. *Tannins: major resources, properties and applications*. In: Belgacem, M N, Gandini, A (Eds), *Monomers, Polymers and Composites from Renewable Sources*. Elsevier; 2008.
- 4 Bianchi S, Kroslakova I, Janzon R, Mayer I, Saake B, Pichelin F. Characterization of condensed tannins and carbohydrates in hot water bark extracts of European softwood species. *Phytochem.* 2015;**120**:53-61.
- 5 Ding T, Bianchi S, Ganne-Chédeville C, Kilpeläinen P, Haapala A, Rätty T. Life cycle assessment of tannin extraction from spruce bark. *iForest J.* 2017;**10**:807-814.
- 6 Carn F, Guyot S, Baron A, Pérez J, Buhler E, Zanchi D. Structural Properties of Colloidal Complexes between Condensed Tannins and Polysaccharide Hyaluronan. *Biomacromolecules* 2012;**13**(3):751-759.
- 7 Anttila AK, Pirttilä AM, Häggman H, Harju A, Venäläinen M, Haapala A, Holmbom B, Julkunen-Tiitto R. Condensed conifer tannins as antifungal agents in liquid culture. *Holzforsch.* 2013;**67**(7):825-832.
- 8 Tondi G, Palanti S, Wieland S, Thevenon MF, Petutschnigg A, Schnabel T. Durability of tannin-boron-treated timber. *BioRes.* 2012;**7**:5138-5151.

- 9 Pranantyo D, Xu LQ, Neoh K-G, Kang E-T, Ng YX, Teo SL-M. Tea stains-inspired initiator primer for surface grafting of antifouling and antimicrobial polymer brush coatings. *Biomacromolecules* 2015;**16**(3):723-732.
- 10 Okuda T, Ito H. Tannins of constant structure in medicinal and food plants hydrolyzable tannins and polyphenols related to tannins. *Mol* 2011;**16**:2191-2217.
- 11 Haslam E. *Practical polyphenolics: from structure to molecular recognition and physiological function*. Cambridge University Press; 1998.
- 12 Moubarik A, Pizzi A, Charrier F, Allala A, Badia M, Mansouri HR, Charrier B. Mechanical characterization of industrial particleboard panels glued with cornstarch–mimosa tannin–urea formaldehyde resins. *J. Adhes. Sci. and Technol.* 2013;**27**:423-429.
- 13 Laks PE, McKaig PA, Hemingway RW. Flavonoid biocides: wood preservatives based on condensed tannins. *Holzforsch.* 1988;**42**:299-306.
- 14 Thevenon MF, Tondi G, Pizzi A. High performance tannin resin-boron wood preservatives for outdoor end-uses. *Eur. J. Wood Wood Prod.* 2009;**67**:89-93.
- 15 Tondi G, Schnabel T, Wieland S, Petutschnigg A. Surface properties of tannin treated wood during natural and artificial weathering. *Int. Wood Prod. J.* 2013;**4**:150-157.
- 16 Tondi G, Thevenon MF, Mies B, Standfest G, Petutschnigg A, Wieland S, Impregnation of Scots pine and beech with tannin solutions: effect of viscosity and wood anatomy in wood infiltration. *Wood Sci. Technol.* 2013;**47**:615-626.
- 17 Weckman NE, Olsson ALJ, Tufenkji N. Evaluating the binding of selected biomolecules to cranberry derived proanthocyanidins using the quartz crystal microbalance. *Biomacromolecules* 2014;**15**(4):1375-1381.

- 18 Wang G, Chen Y, Xu G, Pei Y. Effective removing of methylene blue from aqueous solution by tannins immobilized on cellulose microfibrils. *Int. J. Biol. Macrom.* 2019;**129**:198-206.
- 19 Zhou Y, Zhong J, Pan R, Wan Z, Guo J, Wang J, Yin S, Yang X. Zein/tannic acid complex nanoparticles-stabilised emulsion as a novel delivery system for controlled release of curcumin. *Int. J. Food Sci. Technol.* 2017;**52**:1221-1228.
- 20 Gao J-K, Zhang Z-J, Jiang Y-J, Chen Y, Gao S-F. Biomimetic-functionalized, tannic acid-templated mesoporous silica as a new support for immobilization of NHase. *Molecules* 2017;**22**:1597-1609.
- 21 Fan R, Min H, Hong X, Yi Q, Liu W, Zhang Q, Luo Z. Plant tannin immobilized Fe₃O₄@SiO₂ microspheres: A novel and green magnetic bio-sorbent with superior adsorption capacities for gold and palladium. *J. Haz. Mat.* 2019;**364**:780-790.
- 22 Huang X, Liao X, Shi B. Hg(II) removal from aqueous solution by bayberry tannin-immobilized collagen fiber. *J. Haz. Mat.* 2009;**170**:1141-1148.
- 23 Luo J, Zhang N, Lai J, Liu R, Liu X. Tannic acid functionalized graphene hydrogel for entrapping gold nanoparticles with high catalytic performance toward dye reduction. *J. Haz. Mat.* 2015;**300**:615-623.
- 24 Gong X, Weiguang L, Wang K, Jinhua H. Study of the adsorption of Cr(VI) by tannic acid immobilised powdered activated carbon from micro-polluted water in the presence of dissolved humic acid. *Biores. Tech.* 2013;**141**:145-151.
- 25 Xu Q, Wang Y, Jin L, Wang Y, Qin M. Adsorption of Cu(II), Pb(II) and Cr(VI) from aqueous solutions using black wattle tannin-immobilized nanocellulose. *J. Haz. Mat.* 2017;**339**:91-99.
- 26 Brook AJW, Munday KC. The interactions of phenols, anilines, and benzoic acids with sephadex gels. *J. Chromatogr.* 1970;**47**:1-8.

- 27 McManus JP, Davis K, Beart JE, Gaffney SH, Lilley TH, Haslam E. Polyphenol interactions: Part I Introduction Some observation on the reversible complexation of polyphenols with proteins and polysaccharides. *J. Chem. Soc. Perkins Transaction 2*, 1985;1429-1438.
- 28 Ozawa T, Lilley TH, Haslam E. Polyphenol interaction: astringency and the loss of astringency in ripening fruit. *Phytochem.* 1987;**26**:2937-2942.
- 29 De Freitas V, Carvalho E, Mateus M. Study of carbohydrate influence on protein-tannin aggregation by nephelometry. *Food Chem.* 2003;**81**:503-509.
- 30 Hokkanen S, Repo E, Suopajarvi T, Liimatainen H, Niinimaa J, Sillanpää M. Adsorption of Ni(II), Cu(II) and Cd(II) from aqueous solutions by amino modified nanostructured microfibrillated cellulose. *Cellul.* 2014;**21**:1471-1487.
- 31 Donia AM, Atia AA, Abouzayed FI. Preparation and characterization of nano- magnetic cellulose with fast kinetic properties towards the adsorption of some metal ions. *Chem. Eng. J.* 2012;**191**:22-30.
- 32 Yu X, Tong S, Ge M, Wu L, Zuo J, Cao C, Song W. Adsorption of heavy metal ions from aqueous solution by carboxylated cellulose nanocrystals. *J. Environ. Sci.* 2013;**25**:933-943.
- 33 Sun X, Yang L, Li Q, Zhao J, Li X, Wang X, Liu H. Amino-functionalized magnetic cellulose nanocomposite as adsorbent for removal of Cr(VI): Synthesis and adsorption studies. *Chem. Eng. J.* 2014;**241**:175-183.
- 34 Singh K, Arora JK, Sinha JMT, Srivastava S. Functionalization of nanocrystalline cellulose for decontamination of Cr(III) and Cr(VI) from aqueous system: computational modeling. *Clean. Technol. and Environ. Policy* 2014;**16**:1179-1191.
- 35 Lu M, Xu Y, Guan X, Wei D. Preliminary research on Cr(VI) removal by bacterial cellulose. *J. Wuhan Uni. Technol.–Mater. Sci.* 2012;**25**:572-575.

- 36 Saumya SP, Deepa B, Abraham E, Girija N, Geetha P, Jacob L, Koshy M. Biosorption of Cd(II) from aqueous solution using xanthated nano banana cellulose: Equilibrium and kinetic studies. *Ecotoxicol. and Environ. Saf.* 2013;**98**:352-360.
- 37 Sánchez-Martín J, Beltran-Heredia J, Delgado-Regaña A, Rodríguez-González MA, Rubio-Alonso F. Optimization of tannin rigid foam as adsorbents for wastewater treatment. *Ind. Crops. Prod.* 2013;**49**:507-514.
- 38 Sirviö J, Hyväkkö U, Liimatainen H, Niinimäki J, Hormi O. Periodate oxidation of cellulose at elevated temperatures using metal salts as cellulose activators. *Carbohydr. Polym.* 2011;**83**:1293-1297.
- 39 Li P, Sirviö JA, Asante B, Liimatainen H. Recyclable deep eutectic solvent for the production of cationic nanocelluloses. *Carbohydr. Polym.* 2018;**199**:217-227.
- 40 Kilpeläinen PO, Hautala SS, Byman OO, Tanner LJ, Korpinen RI, Lillandt MK, Pranovich AV, Kitunen VH, Willför SM, Ilvesniemi HS. Pressurized hot water flow-through extraction system scale up from the laboratory to the pilot scale. *Green Chem.* 2014;**16**(6):3186-3194.
- 41 Hagerman AE. *Tannin Handbook Department of Chemistry and Biochemistry*, Miami University, Oxford; 2011. <http://www.users.muohio.edu/hagermae/tanninpdf> (Accessed 18 January 2017).
- 42 Taulavuori K, Hyöky V, Oksanen J, Taulavuori E, Julkunen-Tiitto R. Species-specific differences in synthesis of flavonoids and phenolic acids under increasing periods of enhanced blue light. *Environ. Exp. Bot.* 2016;**121**:145-150.
- 43 Liu P. *Adsorption behavior of heavy metal ions from aqueous medium on nanocellulose*. Doctoral thesis Printed by Luleå University of Technology, Graphic Production; 2015.

- 44 Glazer AN, Smith EL. Phenolic hydroxyl ionization in Papain. *J. Biol. Chem.* 1961;**236**(11):2948-2951.
- 45 Cannas A. *Tannins: fascinating but sometimes dangerous molecules.* 2015 <http://poisonousplantsansicornelledu/toxicagents/tanninhtml> (Accessed 18 January 2017).
- 46 Thilagan J, Gopalakrishnan S, Kannadasan T. A comparative study on adsorption of copper (ii) ions in aqueous solution by; (a) chitosan blended with cellulose and cross linked by formaldehyde, (b) chitosan immobilized on red soil, (c) chitosan reinforced by banana stem fiber. *Int. J. Appl. Eng. Technol.* 2013;**3**(1):35-60.
- 47 Dong C, Zhang H, Pang Z, Liu Y, Zhang F. Sulfonated modification of cotton linter and its application as adsorbent for high-efficiency removal of lead (II) in effluent. *Biores. Technol.* 2013;**146**:512-518.
- 48 Pei Y, Wu X, Xu G, Sun Z, Zheng X, Liu J, Tang K. Tannin-immobilized cellulose hydrogel fabricated by a homogeneous reaction as a potential adsorbent for removing cationic organic dye from aqueous solution. *Int. J. Biol. Macrom.* 2017;**103**:254-260.
- 49 Dai H, Huang Y, Huang H. Enhanced performances of polyvinyl alcohol films by introducing tannic acid and pineapple peel-derived cellulose nanocrystals. *Cellul.* 2018;**25**:4623-4637.
- 50 Li P, Sirviö J, Haapala A, Khakalo A, Liimatainen H. Anti-oxidative and UV-absorbing biohybrid film of cellulose nanofibrils and tannin extract. *Food Hydrocolloids* 2019;**92**:208-217.
- 51 Lagergren S. Zur theorie der sogenannten adsorption gelöster stoffe. *Kungliga Sevenska Vetenskapakademiens Handlingar*, 1898;**24**:1-39.
- 52 Ho YS, McKay G, Wase DAJ, Forster CF. Study of the sorption of divalent metal ions on to peat. *Adsorption Sci. and Technol.* 2000;**18**:639-650.

- 53 Ho YS, McKay G. Pseudo-second order model for sorption processes. *Process Biochem.* 1999;**34**:451-465.
- 54 Huang X, Wang YP, Liao XP, Shi B. Adsorptive recovery of Au³⁺ from aqueous solutions using bayberry tannin-immobilized mesoporous silica. *J. Haz. Mat.* 2010;**183**:793-798.
- 55 Mezenner NY, Bensmaili A. Kinetics and thermodynamic study of phosphate adsorption on iron hydroxide-eggshell waste. *Chem. Eng. J.* 2009;**147**:87-96.
- 56 Idris MN, Ahmad ZA, Ahmad MA. Adsorption Equilibrium of Malachite Green Dye onto Rubber Seed Coat Based Activated Carbon. *Int. J. Basic and Applied Sci.* 2011;**11**:305-311.
- 57 Langmuir I. The adsorption of gases on plane surfaces of glass, mica and platinum. *J. Am. Chem. Soc.* 1918;**40**(9):1361-1403.
- 58 Freundlich H. Over the adsorption in solution. *J. Phys. Chem.* 1906;**57**:384-470.
- 59 Treybal RE. *Mass Transfer Operations* (second ed.). McGraw Hill (Part 4); 1968.
- 60 Ho YS, McKay G. Sorption of dye from aqueous solution by peat. *Chem. Eng. J.* 1998;**70**:115-124.
- 61 Bouberka Z, Khenifi A, Sekrane F, Bettahar N, Derriche Z. Adsorption of direct red on bentonite modified by cetyltrimethylammoniumbromide. *Chem. Eng. J.* 2008;**136**:295-305.
- 62 Gupta VK, Ali I, Saini VK. Adsorption studies on the removal of Vertigo Blue 49 and Orange DNA13 from aqueous solutions using carbon slurry developed from a waste material. *J. Colloid. and Interface Sci.* 2007;**315**(1):87-93.
- 63 Lombardo S, Thielemans, W. Thermodynamics of adsorption on nanocellulose surfaces. *Cellul.* 2019;**26**(1):249-279.

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Table 2. Charge dependency and zeta potential of tannin extract and CCNC particles (pH range 3-10)

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Figure 1. Materials used in the experiments, from left: tannin extract, water suspension of nanocellulose crystals (0.64%), TEM image on CCNCs used, below the chemical structure of CCNC.

Figure 2. Effects of pH on the adsorption capacity of tannin to CCNC.

Figure 3. Effect of the contact time on the adsorption capacity of tannin on CCNC (pH of 8).

Figure 4. Pseudo- first- order (4A) and Pseudo- second- order (4B) kinetics for the adsorption of tannin extract to CCNCs.

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Figure 6. Scheme representing the adsorption of tannin extract components on random sites of cationic cellulose nanocrystals driven by electrostatic force of attraction.

Figure 7. Linear fit for the Langmuir model (7A) and Freundlich model (7B) for the adsorption of tannins on CCNC.

Table 1. Concentrations of the main phenolic compounds in the concentrated tannin extract

Compounds	Rt	mg/ml
<i>Phenolic acids</i>		
Gallic acid	1.7	0.06
Protocatechuic acid	3.9	0.06
Cinnamic acid derivative	4.5	0.06
<i>p</i> -OH-Cinnamic acid	13.4	0.11
Ferulic acid	14.5	0.05
<i>Flavonoids</i>		
(+)-Catechin	9.9	0.04
Ampelopsin	12.4	0.09
Taxifolin	16.2	0.16
<i>Stilbenes</i>		
Piceatannol	17.0	0.74
Methyl-Piceatannol monoglucoside	17.8	0.77
Resveratrol	22.3	0.09
Methyl-Piceatannol	23.2	0.10
<i>Other phenols</i>		
Neolignan derivative 1	15.8	0.09
Neolignan derivative 2	21.7	0.07
Neolignan derivative 3	24.6	0.07
Unknown polymer	25.3	0.04
Unknown polymer	26.0	0.06
Unknown polymer	27.7	0.13
Unknown polymer	28.1	0.13

Unknown polymer	30.8	0.04
Unknown polymer	31.9	0.05
Unknown polymer	33.7	0.03
Unknown polymer	34.2	0.02
<i>Condensed tannins</i>		21.23

Table 2. Charge dependency, zeta potential and conductivity of tannin extract and CCNC particles (pH range 3-10)

	pH				
Charge	3	5	7	8	10
Tannin extract (- mmol/g)	0.05	0.11	0.38	0.52	1.19
CCNC (+ mmol/g)	2.24	2.46	2.20	1.98	1.33
ζ-potential					
Tannin extract (mV)	-17.90	-19.17	-11.56	-22.13	-15.50
CCNC (mV)	18.97	23.77	20.6	17.17	18.97
Conductivity					
Tannin extract (mS/cm)	0.16	0.09	0.13	0.14	0.11
CCNC (mS/cm)	0.30	0.14	0.18	0.20	0.15

Table 3. Estimated values of constants of kinetics for the adsorption of tannin extract to CCNCs

Pseudo- first- order kinetics model				Pseudo- second- order kinetics model		
$Q_e(Exp.)$	$Q_e(Calc.)$	K_i	R^2	$Q_e(Calc.)$	K_a	R^2
98.95	14.37	0.0013	0.32	99.01	0.00068	0.99

Table 4. The parameters of Langmuir and Freundlich isotherms for the adsorption of tannins to cationic cellulose nanocrystals

Langmuir isotherm				Freundlich isotherm		
<i>Rl</i>	<i>Qm (mg/g)</i>	<i>Kl (dm³/mg)</i>	<i>R²</i>	<i>Kf (dm³/mg)</i>	<i>nF</i>	<i>R²</i>
0.99	1111.1	0.018	0.98	2.46	1.63	0.83