



HAL
open science

Ozonized 2-hydroxypropyl- β -cyclodextrins as novel materials with oxidative and bactericidal properties

Elsi Haddad, Marielle Pagès, Frédéric Violleau, Olivier Marsan, Marie-Hélène Manero, Romain Richard, Jean-Philippe Torrè

► To cite this version:

Elsi Haddad, Marielle Pagès, Frédéric Violleau, Olivier Marsan, Marie-Hélène Manero, et al.. Ozonized 2-hydroxypropyl- β -cyclodextrins as novel materials with oxidative and bactericidal properties. Carbohydrate Polymers, 2022, 291, pp.119516. 10.1016/j.carbpol.2022.119516 . hal-03675958

HAL Id: hal-03675958

<https://hal.inrae.fr/hal-03675958>

Submitted on 18 Nov 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Ozonized 2-hydroxypropyl- β -cyclodextrins as novel materials with oxidative and bactericidal properties

Elsi Haddad^{a,c}, Marielle Pagès^{b,c}, Frédéric Violleau^{b,d}, Olivier Marsan^e, Marie-Hélène Manero^a,
Romain Richard^a, Jean-Philippe Torrè^{a,*}

^a *Laboratoire de Génie Chimique, Université de Toulouse, CNRS, INPT, UPS, 4 Allée Emile Monso, 31432
Toulouse, France*

^b *Plateforme TOAsT, Université de Toulouse, INP-PURPAN, 75 Voie du Toec, 31076 Toulouse, France*

^c *Physiologie, Pathologie et Génétique Végétales (PPGV), Université de Toulouse, INP-PURPAN, 75 Voie du
Toec, 31076 Toulouse, France*

^d *Laboratoire de Chimie Agro-industrielle, LCA, Université de Toulouse, INRA, 4 Allée Emile Monso, 31000
Toulouse, France*

^e *Centre Interuniversitaire de Recherche et d'Ingénierie des Matériaux, 4 Allée Emile Monso, 31432 Toulouse,
France*

* Corresponding author: Jean-Philippe Torrè. Email: jean-philippe.torre@ensiacet.fr

Abstract

Ozonized (2-Hydroxypropyl)- β -cyclodextrins (Oz-HPbCDs) were produced by direct gas/solid reaction between gaseous ozone (O₃) and solid HPbCD. The solid materials obtained were first characterized using physical and chemical methods and compared to the initial HPbCD. The main process parameters of the synthesis were studied independently to assess their effect on the oxidizing power of Oz-HPbCDs. The ability of the Oz-HPbCDs to retain their oxidative properties over time was evaluated, at different storage temperatures, for a period of at least two months. Lastly, aqueous solutions of HPbCD and Oz-HPbCD at different concentrations were contacted with bacterial strains of *Escherichia coli* and *Streptococcus uberis* to see whether these materials might have bactericidal properties. Since normal bacterial growth was noted with HPbCD, the antimicrobial efficiency of Oz-HPbCDs was clearly demonstrated on these two types of bacteria.

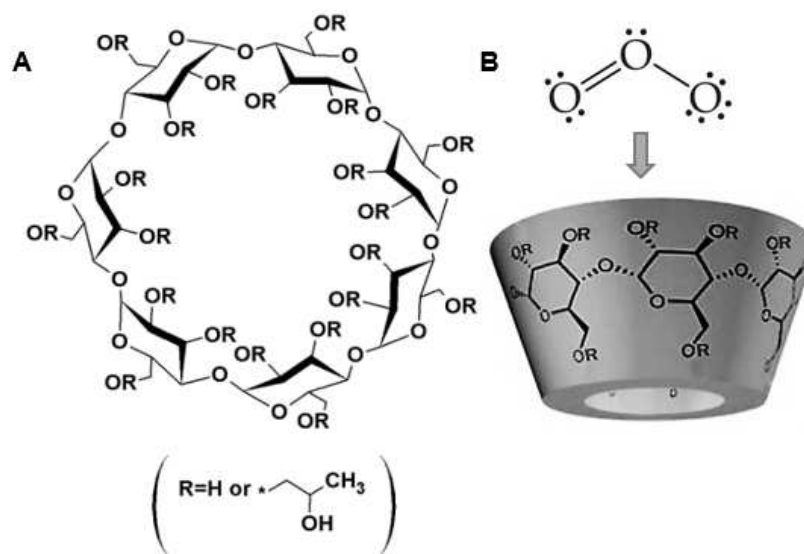
Keywords: Cyclodextrin; ozone; oxidant; material; antimicrobial activity; bactericide.

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of bridged glucopyranose subunits, shaped like a truncated cone or a lampshade (see **Figures 1A** and **1B**) and forming a cavity at the center (Morin-Crini et al., 2021; Szejtli, 1998; Szejtli & Osa, 1996). CDs are used for their versatile inclusion properties in the food, pharmaceutical and biological industries among others (Harada, Takashima, & Yamaguchi, 2009; Li et al., 2014; McCray, Boving, & Brusseau, 2000). Despite the fact that these materials have been used and studied for decades (Szejtli, 1997; Szente, Szemán, & Sohajda, 2016), understanding the mechanisms at play is still a scientific challenge in many respects.

There are different types of CDs, the most common being composed of 6, 7 and 8 glucopyranose units, named α -CD, β -CD and γ -CD, respectively (Ibrahim & El-Zairy, 2009; Szejtli, 1998). These native CDs are obtained from the enzymatic degradation of starch under the action of the enzyme glucosyl-transferase (also called CGT-ase), meaning they have the same advantageous qualities as bio-based products. The hydrophobic nature of the cavity is due to the non-polar carbon skeleton. Conversely, the hydroxyl groups are what give the CD its hydrophilic exterior, meaning that these compounds are particularly soluble in aqueous media (Amiri & Amiri, 2017; Crini, 2014). The water solubility of β -CD can be greatly improved by modifying certain hydroxyl groups to break this hydrogen bond network. For example, the modified CD (2-hydroxypropyl)- β -cyclodextrin (HP- β -CD, written HPbCD for convenience in the following) is obtained industrially by treating the β -CD with propylene oxide in an alkaline medium (Nasongkla et al., 2003). This process makes it possible to substitute part of the hydroxyl functions for hydroxypropyl (HP) ones on the CD faces, as shown in **Figure 1**. HPbCDs can be characterized by their degree of substitution (*DS*), corresponding to the average number of HP functions present on the CD (the *DS* of HPbCDs usually ranges from 2.8 to 10.5), or their degree of molar substitution (*MS*), which gives the number of substituents per glucose unit (ranging from 0.4 to 1.5 for HPbCDs). HPbCDs have a high solubility in water ($> 600 \text{ g.L}^{-1}$) (Loftsson & Duchêne, 2007), which is much greater than that of native β -CD (18.5 g.L^{-1}). As a high product solubility in water is advantageous for the biological applications targeted in this study, we therefore selected HPbCD as a relevant raw material for our experiments. Further information on the history, synthesis, properties and detailed characterizations of HPbCDs can be found in Malanga et al. (Malanga et al., 2016).

65
66



67
68

69 **Figure 1.** Example of the A) Molecular structure of (2-hydroxypropyl)-β-cyclodextrin, B) schematic view of the
70 truncated cone-shape of the CD in contact with the ozone molecule.

71 The resistance of bacterial pathogens is increasing dramatically, resulting in multidrug
72 resistance (MDR) to antibiotics. MDR bacteria are giving rise to serious health issues
73 identified by several organizations such as the World Health Organization (WHO) and the
74 European Centre for Disease Prevention and Control (ECDC). Studies are conducted by the
75 European Food Safety Authority (EFSA) on the growing hazards of MDR bacteria in the food
76 industry (Roca et al., 2015). The unreasonable use of antimicrobial agents has likewise caused
77 this problem to spread to the natural environment (Wellington et al., 2013). This buildup
78 should not be underestimated. The medical, food and agricultural sectors are faced with
79 microbial issues, which can be dealt with by using chemicals such as pesticides. The French
80 and European public authorities have introduced many regulations to control their use, but it
81 appears necessary to propose more healthy and environmentally friendly alternatives (e.g.
82 safer chemicals or less hazardous material) to better control microbial development.

83

84 Because of its oxidizing power and high effectiveness in killing bacteria, fungi, molds, and
85 viruses (Cristiano, 2020), ozone is currently used for many applications such as wastewater
86 treatment (Vittenet et al., 2015), air depollution (Vitola Pasetto et al., 2020) and is regarded as
87 a very promising option for disinfection and sanitation measures (Tizaoui, 2020). Ozone is

88 also being looked at for potential applications such as crop protection, including treating
89 plants and protecting fruit and vegetables like green peppers, raspberries and melons (Özen,
90 Koyuncu, & Erbaş, 2021; Piechowiak, Grzelak-Błaszczak, Sójka, & Balawejder, 2020; Zhang
91 et al., 2021). In practice however, spraying ozone-enriched water on outdoor crops is a very
92 difficult task due to the gas desorption phenomenon that occurs when the ozone-enriched
93 water droplets come in contact with the air. The efficiency of this treatment is limited because
94 ozone is so easily desorped from the water droplets (Canado et al., 2020). Moreover, ozone is
95 well-known for its high instability due to its specificity of decomposing into oxygen very
96 quickly (Muromachi, Ohmura, & Mori, 2012). Its half-life is about 20 minutes at ambient
97 temperature when it is dissolved in water. Although this property is interesting as it confers to
98 ozone a very low remanence (Pagès, Kleiber, Pierron, & Violleau, 2016), the gas' instability
99 is a severe disadvantage as it makes storage and transportation difficult. Ozone must be
100 produced continuously, as close as possible to the place where it is used. Ozone gas is
101 generally produced by a high-voltage electrical discharge (3 - 20 kV) through an oxygen flow
102 (O_2) or air in a continuous gas flow. Concentrations of up to 13 wt% of O_3 in the gas phase
103 can be obtained by means of ozone generators designed specifically for medium industrial
104 applications. Despite the fact that this type of ozone production system is very popular for
105 stationary applications — such as water treatment — these electrical devices are relatively
106 difficult to use in mobile applications, such as the in-field treatment of leaves, vegetables and
107 fruit.

108
109 The contacting of ozone with CDs is scarcely documented in the literature. Just a few authors
110 have proposed different concepts and protocols with CDs, the aim being to try and stabilize
111 the ozone molecule — due to its very short half-life — in the CD cavity. Wang et al. (Wang,
112 Peng, Li, Bai, & Huang, 2016) studied the complexation of HPbCD with O_3 in solution and
113 observed its oxidative properties on potassium indigotrisulfonate (indigo) and 2,2'-azino-bis
114 (3-ethylbenzothiazoline-6-sulfonate) (ABTS). They showed that only the solution containing
115 HPbCD/ O_3 decreased the indigo absorbance after 72 hrs. at 600 nm, and that the
116 decomposition of ABTS increased by increasing the doses of ozone/CD solution. Dettmer et
117 al. (Dettmer et al., 2017) concluded that O_3 can be stabilized with cyclodextrin in an aqueous
118 solution and they showed that the half-life of O_3 increases proportionally with the HPbCD/ O_3
119 molar ratio. The formation of an inclusion complex of O_3 , trichloroethene (TCE), 1,1,1-
120 trichloroethane (TCA) and 1,4-dioxane (1,4-D) with HPbCD was investigated by Khan et al.
121 (Khan, Johnson, & Carroll, 2018) to remove the guest contaminants (TCE, TCA and 1,4-D) or

122 apply reagents during water treatment. Recently, Fan et al. (Fan et al., 2021) used nanobubble
123 technology in an HPbCD inclusion to remove organic micropollutants from contaminated
124 water. Nanobubbles increased the solubilization of O₃ and, according to the authors, formed
125 an inclusion complex with HPbCD unlike macrobubbles. Using this technique, the removal
126 proficiency of the main micropollutant 4-chlorophenol was found to be 6.9 higher compared
127 to the standard macrobubble ozonation method. In all these studies, the CD-ozone contacting
128 was always done in an aqueous solution with a prior step of CD solubilization in the water.
129 Interestingly enough, to the best of the authors' knowledge, it is the first time that synthesis
130 and characterization experiments with gaseous ozone contacting solid HPbCDs followed by
131 tests of the ozonized CDs for biological applications, are presented.

132

133 The main target of this paper is therefore to report results on the synthesis and
134 characterization of novel materials obtained by contacting gaseous ozone and solid
135 cyclodextrins, and to evaluate their antimicrobial activity for potential biological applications.
136 The key hypothesis suggests that CDs are oxidative materials, that physical and chemical
137 changes are visible compared to native CDs, and that they have bactericidal properties. The
138 results of physical and chemical characterizations of the HPbCD and ozonized HPbCD
139 (denoted Oz-HPbCD) are discussed first. Then, the effect of the main process parameters on
140 the oxidative power of the Oz-HPbCD obtained by direct gas/solid reaction are presented. The
141 stability of the oxidative properties of the ozonized CDs stored at different temperatures for
142 several months is also evaluated. Finally, to illustrate potential applications of these types of
143 oxidative materials in microbiological treatments, the antimicrobial efficiency of ozonized
144 CDs is studied.

145

146

147 **2. Materials and methods**

148

149 **2.1. Materials**

150

151 (2-Hydroxypropyl)- β -cyclodextrin (HP β CD) (purity \geq 94%, $MS = 0.9$, produced by Wacker
152 Chemie AG, Burghausen, Germany) was purchased from Sigma-Aldrich, and used without
153 further purification. The chemicals used for the iodometric method were potassium iodide
154 (KI) (reagent grade), sulfuric acid at 1 mol.L⁻¹ (purity \geq 99.9%) purchased from Fisher
155 Scientific, and a sodium thiosulfate solution at 1 mol.L⁻¹ provided by Merck. The potassium
156 bromide (KBr) for IR spectroscopy Uvasol® (purity \geq 99%) was purchased from Merck
157 KBaA, Darmstadt, Germany. Ultra-pure water was used in all preparations with an ELGA
158 Purelab Option-Q 7 model from VWS (for chemical characterizations and oxidative power
159 determination) and with a Millipore Milli-Q Integral 15 water purification system from Merck
160 (for microbiological tests). Oxygen (purity $>$ 99.999%, purchased from Air Liquide, France)
161 was used to produce ozone in the experimental rig.

162

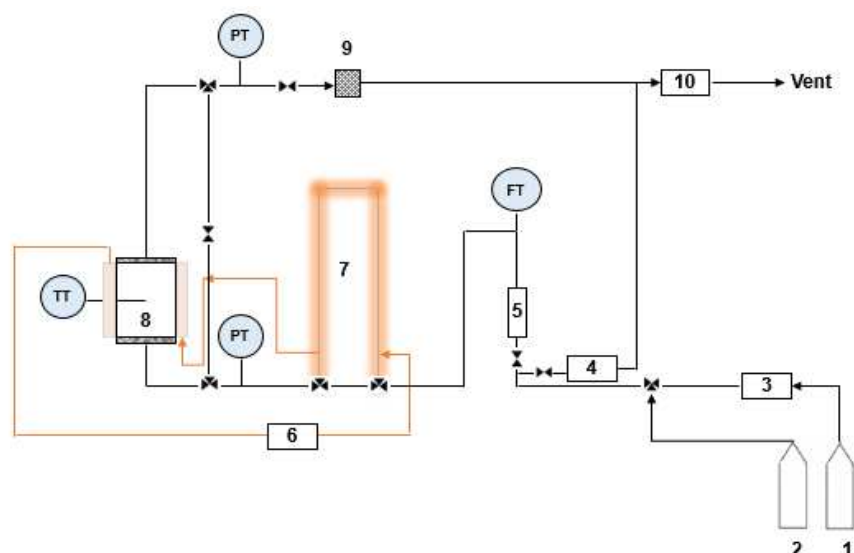
163 **2.2. Methods**

164

165 **2.2.1. Synthesis protocol**

166

167 The ozonized CDs are synthesized using an experimental rig at bench scale. A diagram of
168 the different elements of the experimental rig used in this study is shown in **Figure 2**, and
169 detailed technical information is given in **Appendix A (section A1)**.



170

171 **Figure 2.** Process and instrumentation diagram of the experimental rig used for the synthesis: 1. oxygen bottle; 2.
 172 nitrogen bottle; 3. ozonator; 4. ozone BMT analyzer; 5. float flowmeter; 6. thermostatic bath; 7. heat exchanger;
 173 8. reactor; 9. filter; 10. ozone destructor; FT. mass flowmeter, TT. temperature probe; PT. pressure sensor

174

175 The reactor is first loaded with the HPbCD, directly on a balance with a precision of $\pm 0,005$
 176 g. A mass of ~ 5 g is used for the process parameter study and ~ 7 g are used for all the
 177 characterization studies. The reactor is then closed, the temperature probe connected to the
 178 supervision system, the reactor mounted on the rig, and the coolant hoses connected to the
 179 reactor jacket. Before starting the synthesis, a 5-minute leak test is always performed with
 180 nitrogen at 2 bar to ensure perfect sealing of all the pieces composing the rig. All the process
 181 lines are flushed with oxygen and the reactor, regulated at temperature (T_r), is first bypassed
 182 to start the ozone production. The pressure drop is adjusted in the by-pass line to equalize the
 183 pressure drop in the reactor to avoid any change in the gas flow when this line is closed. The
 184 gas flow (Q_f) and ozone concentration (C_{O_3}) are then set to the correct values and stabilized
 185 using the reactor bypass line before the start of the synthesis. When all the process parameters
 186 are perfectly stable, the O_2/O_3 gas coming from the ozonator is sent to the reactor by turning
 187 the valves to shut the bypass line. The time $t = 0$ of the synthesis is defined at this moment.
 188 Contact between the ozone and the CD powder is then maintained for a given reaction time
 189 (noted t_r). At the end of the synthesis, the ozone concentration is gradually decreased to zero,
 190 and the ozonator is then switched off. After this, the reactor is vented with nitrogen,
 191 disassembled from the rig and transferred to a glove box (under air) to be opened safely. The
 192 ozonized CDs are finally discharged from the reactor into a capped glass vessel using a
 193 funnel. The product is immediately sampled for analysis and stored.

194 2.2.2. Oxidative power (*OP*)

195

196 The total oxidation capacity of the solid materials obtained from the synthesis is determined
197 by iodometric titration carried out according to the following procedure: 20 mL of an aqueous
198 solution of potassium iodide (KI) at 0.1 mol.L⁻¹ is stirred in a 125-mL Erlenmeyer flask; then,
199 the pH of the solution is adjusted to ~ 2 with few drops of sulfuric acid (1 mol.L⁻¹); finally, a
200 precise amount of ozonized CD ($m_{oz-CD} \sim 0.05-0.1$ g) measured at $\pm 10^{-4}$ g, is added to the
201 acidified KI solution. The reactants are kept in contact under stirring for 60 minutes before
202 titration.

203 The oxidative power of the material, noted *OP*, is therefore directly proportional to the
204 quantity of iodine generated. The *OP*, expressed here as the ratio of the mass of iodine
205 produced to the mass of *Oz-HPbCD*, is calculated by **Eq. (1)** as follows:

$$206 \quad OP\left[\frac{mg_{I_2}}{g_{Oz-HPbCD}}\right] = \frac{C_{thio} \cdot V_{thio} \cdot M_{I_2} \cdot 10^3}{2 m_{Oz-HPbCD}} \quad (1)$$

207 with M_{I_2} the molar mass of diiodine (253.81 g.mol⁻¹), C_{thio} the concentration of the thiosulfate
208 solution, V_{thio} the volume of the thiosulfate solution poured at equivalence, and $m_{Oz-HPbCD}$ the
209 mass of ozonized CDs.

210 The decrease in the oxidative properties of the material during the storage time (t_s) is
211 quantified by the rate at which oxidative power is lost (noted OP_{loss}). This indicator quantifies
212 the stability of the *OP* versus time: the lower OP_{loss} , the more stable the oxidative properties
213 of the product over time. OP_{loss} (in %) is defined as the difference in *OP* between the initial
214 *OP* (measured at $t_s = 0$) and the *OP* measured at the storage time t_s , normalized by the initial
215 *OP* obtained at $t_s = 0$. The OP_{loss} is expressed by **Eq. (2)** as follows:

$$216 \quad OP_{loss} [\%] = \left(1 - \frac{OP_{t_s}}{OP_{t_s=0}}\right) \times 100$$

217 (2)

218 With $OP_{t_s=0}$, *OP* at $t_s = 0$ and OP_{t_s} the *OP* at t_s .

219 To ensure a robust determination of the *OP*, the iodometric titration is triplicated for each
220 sample, and the given *OP* is the arithmetic mean of these 3 values.

221

222 2.2.3. Physico-chemical characterizations

223

224 The morphology of the particle before and after the synthesis is observed by scanning electron
225 microscopy (SEM), and the size distribution of the particles by granulometric analysis based
226 on light diffraction. Helium pycnometry is used to measure the real density (ρ) of the
227 particles, and the specific area ($a_{s,BET}$) of the samples is obtained from N₂ adsorption-
228 desorption isotherms at 77 K. The CDs before and after synthesis are characterized using FT-
229 IR and Raman vibrational spectroscopies. Details of the techniques and methods are given in
230 **Appendix A (section A2)**.

231

232 **2.2.4. Antimicrobial activity assessment**

233

234 **Preculture of bacteria.** The different bacterial strains (*Escherichia coli* L.1112 and
235 *Streptococcus uberis* L.1111) are stored in glycerol milk at -80°C . After thawing, 50 μL are
236 cultured in 5 mL of brain-heart broth (BHB) (Biokar diagnostics BK015HA). These
237 suspensions are incubated at 37°C under agitation (≈ 150 rpm) until bacterial growth is visible
238 (cloudy culture medium). On the day of the experiment, 100 μL of the bacterial preculture are
239 placed in 10 mL of BHB and incubated at 37°C under agitation at 150 rpm (in triplicate for
240 each strain). A tube of pure broth serves as a blank. The optical density at 600 nm (UV
241 Jenway ThermoFisher Scientific) is then measured for each suspension. The values obtained
242 are used to dilute bacterial suspensions in order to obtain bacterial inocula standardized at
243 5.10^5 CFU/mL.

244

245 **Minimum Inhibitory Concentrations (MIC)** are used to assess the antibacterial activity of the
246 ozonized powder and are determined by means of the microdilution method principle (CLSI,
247 2012). Solutions of Oz-HPbCD and HPbCD at seven different concentrations (between 30
248 and $0.003\text{ g}\cdot\text{L}^{-1}$) are prepared in brain-heart broth. The solutions are distributed over 48 wells
249 on 96-well microplates, as shown in **Appendix A (section A3, Figure A1)**. The highest
250 concentration of the powder (50 μL /well) is deposited in three wells in the first row (line 1).
251 Following the same configuration, decreasing concentrations are placed in wells from rows 2
252 to 7. 50 μL of bacterial suspension are added to the first two wells for each different dilution
253 (total volume 100 μL in each well) whereas 50 μL of BHB are added to the last well of each
254 row. The last row (no. 8) holds 50 μL of the bacterial suspension and 50 μL of the BHB
255 (positive control). The final concentration of the bacterial suspensions is therefore $2.5.10^5$
256 CFU/mL and those of the ozonized and non-ozonized HPbCD are between 15 and $0.0015\text{ g}\cdot\text{L}^{-1}$

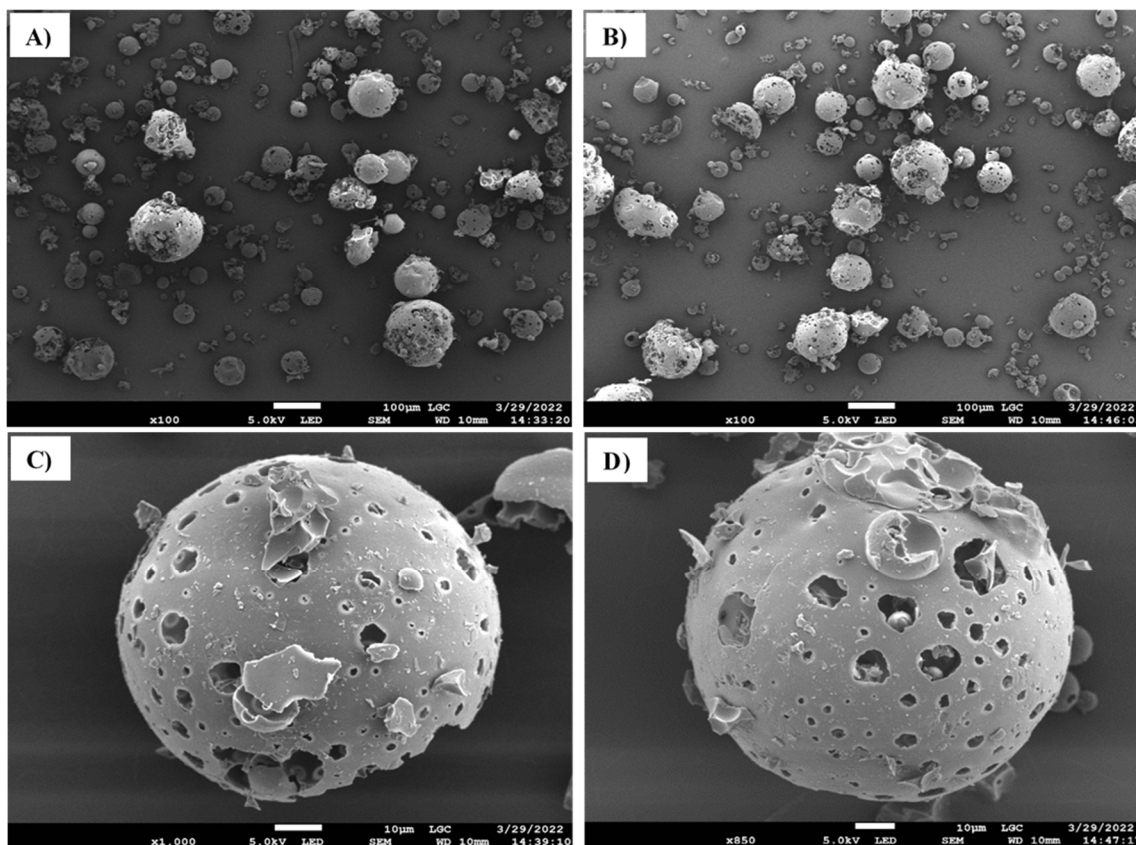
257 ¹. The microplates are placed in an incubator shaker (TECAN infinite M200 PRO) at 37°C
258 and 125 rpm for 24 hrs. After this period, the measured absorbance is 600 nm in each well.
259 This experiment is triplicated. Using this method, it is possible to simultaneously test the
260 effects of HPbCD and Oz-HPbCD on bacteria. Antimicrobial activity is assessed on two
261 bacterial strains: *Escherichia coli* and *Streptococcus uberis*. MICs are determined as the
262 minimum concentrations of HPbCD and/or Oz-HPbCD stopping the proliferation of bacteria.
263

264 **3. Results and Discussion**

265

266 **3.1 Physical and chemical characterization**

267 In order to compare the particles before and after ozonation (i.e. HPbCD and Oz-HPbCD,
268 respectively), the results of the physical characterizations of the powders are presented first.
269 Concerning the morphology of the powders, the SEM images of both samples presented in
270 **Figures 3A** and **3B**, show spheroidal faceted particles of various sizes, presenting (in most
271 cases) a smooth surface perforated by numerous holes, with the smallest particles visible
272 inside the largest spheres. No salient differences can therefore be noted between the particles
273 before (**Figure 3C**) and after ozonation (**Figure 3D**).



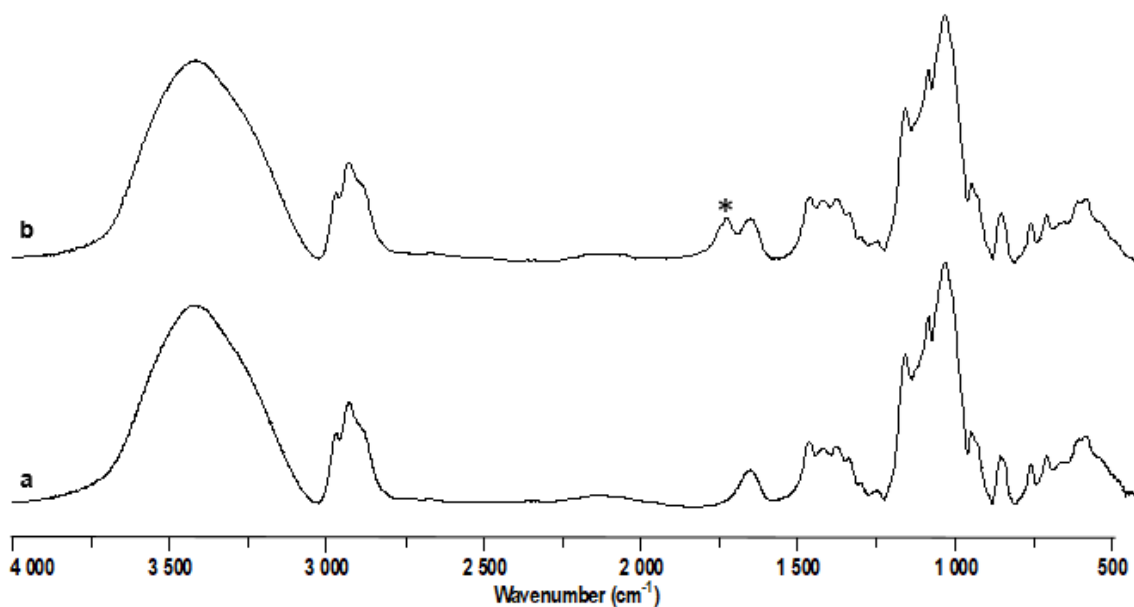
274
 275 **Figure 3.** SEM images of the materials. A) and C) : HPbCD; B) and D): Oz-HPbCD; the scale (horizontal white
 276 bar at the bottom of the picture) is 100 μm for A) and B), and 10 μm for C) and D).

277 **Table 1.** Densities, specific surface areas, and median particle diameters of *HPbCD* and *Oz-HPbCD*

Physical characterization	ρ ($\text{g}\cdot\text{cm}^{-3}$)	$a_{s,BET}$ ($\text{m}^2\cdot\text{g}^{-1}$)	$Dv(50)$ (μm)
HPbCD	1.3199 ± 0.0071	0.39 ± 0.01	42.3 ± 0.2
Oz-HPbCD	1.3062 ± 0.0073	0.43 ± 0.01	42.3 ± 0.2

278
 279 The real particle densities (ρ), specific surface areas ($a_{s,BET}$) and median particle diameters
 280 (D_{v50} , i.e. the point in size distribution below which 50% of the material is contained) of the
 281 HPbCD and Oz-HPbCD, are presented in **Table 1**. Given the very small differences between
 282 the values and considering their absolute uncertainties, it can be concluded that ozonation has
 283 no effect on the physical parameters in these conditions. No variation in the specific surface
 284 area proves that the contact of HPbCD with ozone creates no meso/microporosity inside the
 285 particles. In addition, the fact that the D_{v50} was exactly the same between the initial and final
 286 products demonstrates that the phenomena of fragmentation, attrition and dislocation of the
 287 initial particles during the synthesis are negligible in our case.

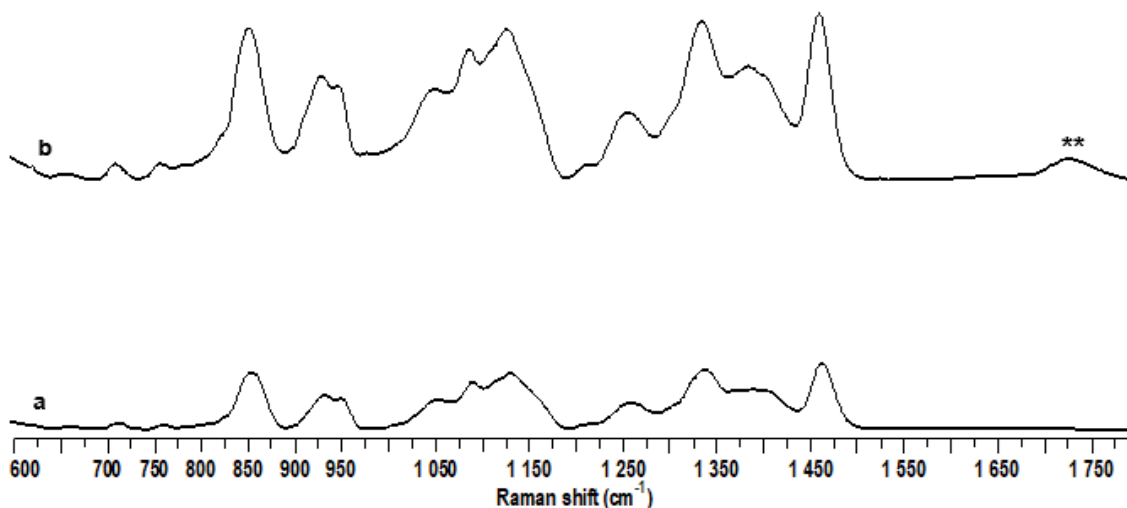
288 To characterize the possible chemical transformation after contact of the CD with O₃, HPbCD
289 and Oz-HPbCD were analyzed using FT-IR and Raman spectroscopy. Note that these two
290 techniques are complementary, particularly when the molecules are not symmetrical (Farber
291 et al., 2019). FT-IR and Raman spectra are shown in **Figure 4** and **Figure 5**, respectively.



292

293 **Figure 4.** FTIR spectra of a) HPbCD, b) Oz-HPbCD . (*) new peak identified on the spectrum.

294



295

296 **Figure 5.** Raman spectra of a) HPbCD, b) Oz-HPbCD. (**) new peak identified on the spectrum.

297

298 As shown in **Figure 4**, both the HPbCD and Oz-HPbCD spectra reveal strong bands at 3,300
299 cm⁻¹ (O—H stretching vibrations), 2,930 cm⁻¹ (C—H stretching vibrations), 1,160 cm⁻¹, 1,090

300 cm^{-1} and $1,031 \text{ cm}^{-1}$ corresponding to C—H, C—O stretching vibrations (Stancanelli et al.,
301 2008), and $1,650 \text{ cm}^{-1}$ corresponding to O—H bending vibrations of the water molecules
302 physisorbed or stabilized in the CDs (Yuan, Liu, & Liu, 2015). A new peak is clearly
303 noticeable at $1,730 \text{ cm}^{-1}$ in the spectrum of Oz-HPbCD (identified by the * in **Figure 4**),
304 which could be attributed to the —C=O stretching of carbonyl groups.

305 The Raman spectra of HPbCD and Oz-HPbCD shown in **Figure 5** exhibit strong peaks at
306 $1,460 \text{ cm}^{-1}$ and $1,330 \text{ cm}^{-1}$ corresponding to C—H bending and $1,135 \text{ cm}^{-1}$, $1,083 \text{ cm}^{-1}$ and
307 $1,048 \text{ cm}^{-1}$ which can be correlated with C—O stretching (Egyed, 1990; Martins et al., 2017).
308 Again, a clear difference exists between the two spectra with the presence of a new peak at
309 $1,728 \text{ cm}^{-1}$ in the Oz-HPbCD spectrum (identified by the ** in **Figure 5**). This contribution
310 may be attributed to the creation of novel carbonyl groups by ozonation of the native CD, in
311 agreement with the FT-IR results.

312

313 It can therefore be inferred from these characterization results that, in these conditions, the
314 ozonation of HPbCD did not induce noticeable changes in the physical parameters considered
315 for this study, i.e. particle morphology, density, specific surface area and median particle
316 diameter. However, as new vibrational contributions were detected both by FT-IR and Raman
317 spectroscopy on the spectra of the ozonized CD, this means that chemical changes did occur
318 in the product during ozonation. And it seems logical that, in the presence of ozone, some of
319 the primary and secondary alcohols initially present in the HPbCD could be oxidized to form
320 different types of new organic functions such as aldehydes, ketones, carboxylic acids,
321 peracids and so on. These functions could either be linked (i.e. chemically attached) to the
322 CDs, or found in organic by-products if some parts of the CDs were broken by ozonation.
323 However, we are aware that a detailed quantitative analysis will be necessary, using NMR for
324 example, to identify and precisely determine the possible chemical modifications of the CDs
325 and any by-product(s) that may form in these conditions.

326

327 **3.2 Influence of the process parameters on *OP* values**

328 In order to provide robust information as regards the reproducibility of the experiments, 5
329 syntheses were carried out in the same process conditions. The mean average values of the
330 synthesis parameters were: $Q_f = 49 \text{ NI/h}$, $C_{O_3} = 96 \text{ gO}_3/\text{Nm}^3$, $T_r = 25.8^\circ\text{C}$, and $t_r = 2 \text{ hrs}$. The
331 measurement uncertainties of the process parameters Q_f , C_{O_3} , and T_r , calculated as the average

332 of the standard deviations of each parameter obtained for the 5 experiments, were estimated to
333 be $\Delta Q_f = \pm 3 \text{ NL/h}$, $\Delta C_{O_3} = \pm 3 \text{ g}_{O_3}/\text{Nm}^3$, and $\Delta T_r = \pm 0.8^\circ\text{C}$.

334

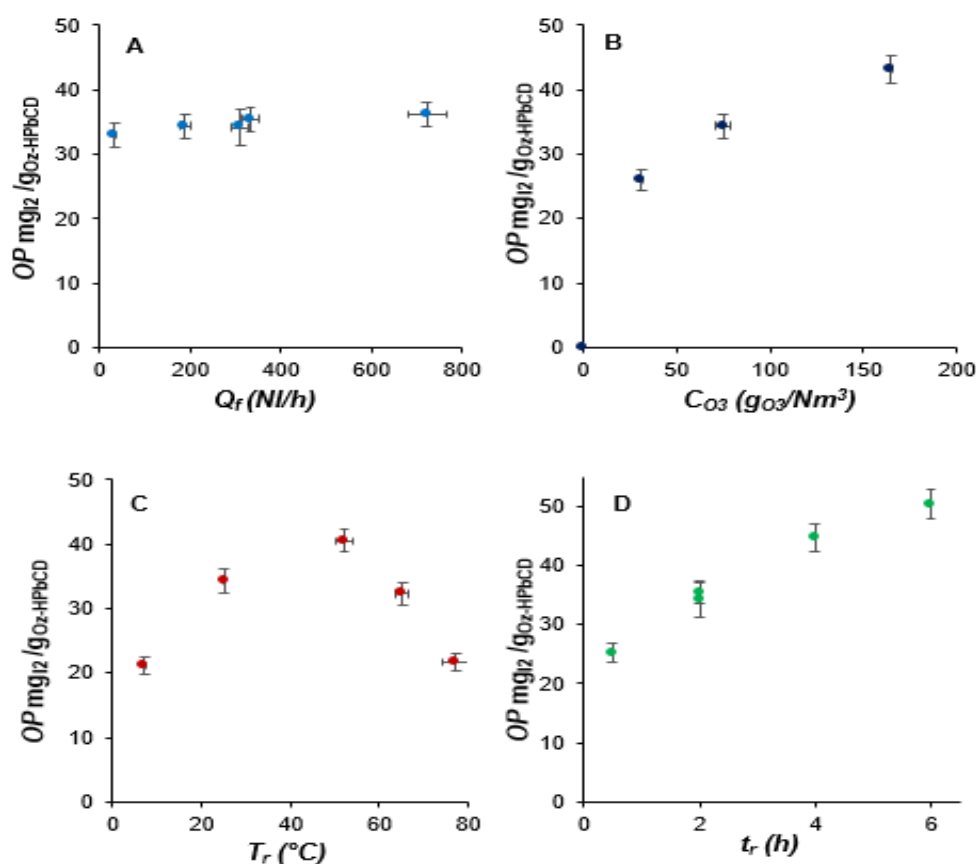
335 Based on all the experiments carried out, we noted that when the solid HPbCD was in contact
336 with gaseous ozone, an exothermic phenomenon occurred inside the reactor, leading to an
337 increase in temperature of about $5.0 \pm 1.5^\circ\text{C}$. Immediately after t_0 , the reactor temperature
338 increased rapidly, reached a maximum, and then progressively decreased to stabilize close to
339 the temperature set for the synthesis. In these conditions, the maximum peak temperature was
340 reached in a few minutes, and this exothermicity could be measured for a few dozen minutes
341 from t_0 . At the end of each synthesis, the oxidative power of the ozonized CDs was measured
342 by iodometry, and the mean value and standard deviation of OP were equal to $45 \text{ mg}_{I_2} / \text{g}_{Oz-}$
343 HPbCD and $\sigma = 3 \text{ mg}_{I_2} / \text{g}_{Oz-HPbCD}$, respectively. These observations and results highlight that: (i)
344 the contact between HPbCD and ozone leads to exothermic reaction(s); (ii) the synthesis
345 process and the OP determination method, both evaluated using a set of 5 independent and
346 identical runs, give good reproducible results, with a $\pm 7\%$ variation of the OP values ; and
347 (iii) as the $OP > 0$, it is clear that the process of contacting gaseous ozone with solid HPbCD
348 generates materials with oxidative properties.

349

350 The effect of the process parameters (i.e. the gas flow rate Q_f , the ozone concentration in the
351 gas C_{O_3} , the reactor temperature T_r) on the OP of the Oz-HPbCD was first studied more
352 precisely by maintaining the reaction time at $t_r = 2 \text{ hrs.}$: when one parameter varied (i.e.
353 ranging from 33 to 723 NL.h^{-1} for Q_f , from 31 to 165 $\text{g}_{O_3}/\text{Nm}^3$ for C_{O_3} , and from 7 to 77°C for
354 T_r), the others were maintained at constant values at $Q_f = 186 \text{ NL.h}^{-1}$, $C_{O_3} = 76 \text{ g}_{O_3}.\text{Nm}^3$, and
355 $T_r = 25.5^\circ\text{C}$. Then, to study the effect of the reaction time t_r , the same methodology was used,
356 using a higher gas flow rate value ($Q_f = 330 \text{ NL.h}^{-1}$). The results obtained are presented

357 **Figure 6.**

358



360
 361 **Figure 6.** Effects of the different process parameters on the oxidative power of the Oz-HPbCD: A. gas flow
 362 rate; B. ozone concentration in the gas; C. mean reactor temperature; D. reaction time.

363 As shown in **Figure 6A**, the gas flow rate has a negligible effect on the *OP*, as a variation in
 364 the *OP* of less than 10% (from 33 to 36 mg₁₂ / g_{Oz-HPbCD}) was measured across a very wide
 365 range of gas flow rates (from 33 to 723 NI/h). As expected however, the *OP* was found to be
 366 strongly dependent of the ozone concentration in the gas as shown in **Figure 6B**: stepping up
 367 the *C*_{O₃} from 31 to 165 g_{O₃}/Nm³ enhanced the *OP* of the Oz-HPbCD from 26 to 43 mg₁₂ / g_{Oz-}
 368 *HPbCD*. In an additional experiment performed using a gas containing only pure oxygen (*C*_{O₃} =
 369 0 g_{O₃}/Nm³), we found that the powder obtained at the end of the synthesis was not oxidant
 370 (the KI solution remained uncolored during the iodometric titration). As oxygen could also be
 371 a potential oxidizing agent, this latter point confirms with any doubt that the oxidative
 372 properties of the solid powder obtained at the end of the synthesis are due only to the presence
 373 of ozone in the O₂/O₃ gaseous mixture which contacts the CD. Interestingly enough, the mean
 374 reactor temperature had a singular non monotonic effect on the *OP* compared to the other
 375 parameters studied, as shown in **Figure 6C**: the *OP* variation curve first increases with *T_r* (*OP*
 376 = 21 to 40 mg₁₂ / g_{Oz-HPbCD} when the temperature *T_r* goes from 7 to 52°C), reaches a maximum
 377 (extremum obviously located between ~ 30 and ~ 50°C), and finally drops from 40 to 21 mg₁₂

378 $/g_{O_3-HPbCD}$ for temperatures between 52 and 77°C. Finally, it is clear from **Figure 6D** that
379 increasing the reaction time of the synthesis significantly enhances the *OP* of the final
380 ozonized product: the *OP* was increased from 25 to 50 $mg_{I_2} / g_{O_3-HPbCD}$ when the reaction time
381 was increased from 0,5 hrs. to 6 hrs., respectively.

382 As reaction kinetics are generally enhanced by a temperature increase, we initially expected
383 that the *OP* would be higher at high synthesis temperatures. The fact that we obtained the
384 exact opposite (i.e. we measured a drastic decrease in the *OP* for $T_r > \sim 50^\circ C$) reveals a
385 certain instability of the oxidative properties of the ozonized product at high temperature. This
386 can be correlated with the creation of oxidative chemical functions on the ozonized CDs,
387 and/or the encapsulation of oxidative species in the CD cavity, and/or the creation of by-
388 products, which are unstable in these temperature conditions. For example, it is a well-known
389 fact that the ozone molecule, as well as many peroxides (Batakliiev, Georgiev, Anachkov,
390 Rakovsky, & Zaikov, 2014), are naturally unstable from moderate to high temperatures.
391 Because the oxidative power of the ozonized CD increased with the reaction time, with the
392 ozone concentration in the gas and with the reactor temperature (for $T_r < \sim 50^\circ C$) while being
393 quasi-independent of the gas flow rate (i.e. the gas velocity inside the reactor), it is likely to
394 be directly correlated with kinetics. The reaction and/or encapsulation did not appear to be
395 limited by the external mass transfer (as the *OP* is independent of the velocity of the gas
396 through the particles in the reactor), but it is likely it was limited by the reaction/encapsulation
397 step and/or the diffusion of the gaseous ozone inside the solid CD particles. We therefore
398 believe that it is possible that CDs did not react completely when in contact with ozone in
399 these conditions. Unfortunately, it has been impossible to verify this assumption to date as the
400 conversion (i.e. the fraction of the initial ozonized CDs) could not be determined in this study
401 and requires additional specific characterizations with complementary analytical techniques,
402 such as NMR. Work is in progress in this respect.

403

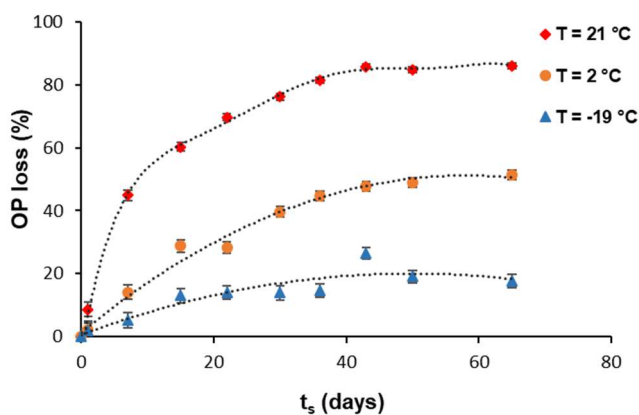
404 **3.3. Stability study**

405 In order to test the stability of the oxidizing power of the material over time, a synthesis was
406 carried out in the following conditions: $t_r = 6$ hrs., $Q_f = 335$ $Nl/hr.$, $C_{O_3} = 69$ g_{O_3}/Nm and $T_r =$
407 $27.1^\circ C$. The stability test was performed using the same ozone treated with HPbCD. A mass
408 of ~ 5 g of the powder obtained from the synthesis was separated into three equal parts (i.e. \sim
409 1.7 g), and each sample was stored in the same model of glass flask (sealed with a septum)
410 under different temperatures: at ambient temperature at $21 \pm 2^\circ C$, in a refrigerator at $2 \pm 2^\circ C$,

411 and in a freezer at $-19 \pm 2^\circ\text{C}$. A sample was taken periodically in each flask to measure the
412 *OP* by iodometric titration. The analyses were performed over a period of 65 days. The loss of
413 the *OP* of the ozonized CD over time (t_s) for the three different storage temperatures is
414 presented in **Figure 7**.

415

416



417

418 **Figure 7.** Evolution of the rate of the loss of OP (%) of the oxidative power over storage time (t_s) for Oz-
419 HPbCD stored at different temperatures

420

421 After 65 days, the ozone-treated powder lost 86% of its *OP* when stored at 21°C , 51% at 2°C
422 and 18% at -19°C . Looking at these results, it is obvious that the stability of the ozonized CDs
423 depends strongly on the storage temperature of the materials: the lower the temperature, the
424 more stable the oxidative material. As the chemical characterization of the material revealed
425 that ozonation creates new chemical functions on the CDs and/or forms by-products, it might
426 be possible that the ozonized CDs contain oxidative products or functions, such as ozone, an
427 ozone derivative, or oxidative organic species such as peroxides. Such species might be
428 unstable during long storage periods under moderate or high temperatures (Clark, 2001). This
429 assumption is in agreement with the previous conclusions made in the section relative to the
430 effect of the process parameters, where it was shown that the *OP* significantly decreased when
431 the synthesis temperature was increased to values greater than 50°C . To improve our
432 understanding of the product stability over time, it might be interesting to perform additional
433 analytical experiments on the cyclodextrin samples, using NMR. Accordingly, it can be
434 concluded that these ozonized CDs are unstable in certain conditions, but are nevertheless
435 able to hold on to the most of their oxidative properties for a relatively long period of time

436 (more than two months) if the product is stored in a freezer at $\sim -19 \pm 2^\circ\text{C}$. In the light of
437 these results, we might expect to achieve an even better long-term stability over time if the
438 product is stored in hermetically closed vessels (instead of semi-hermetic flasks opened
439 regularly for sampling purposes).

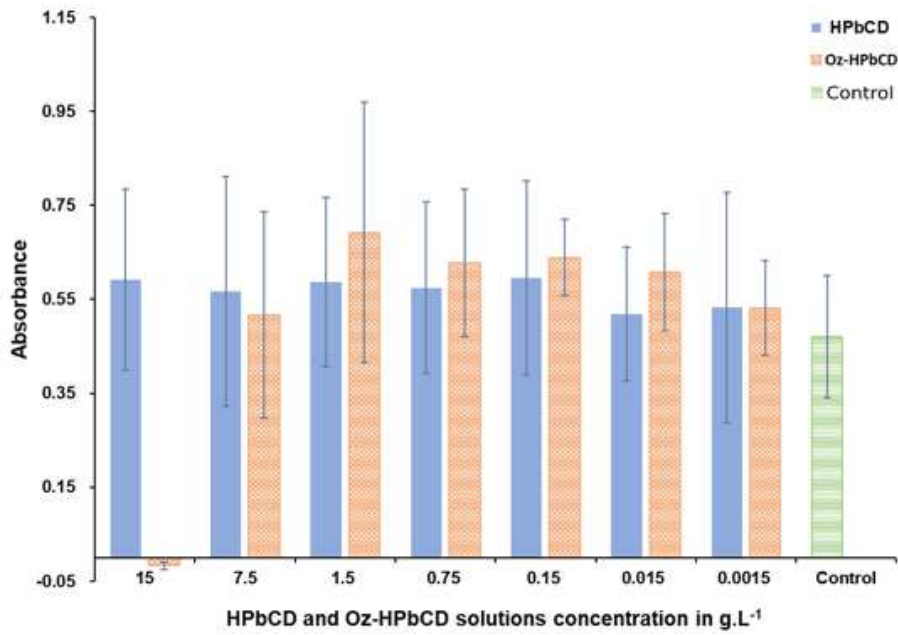
440

441

442 **3.4. Minimum inhibitory concentrations (MIC)**

443

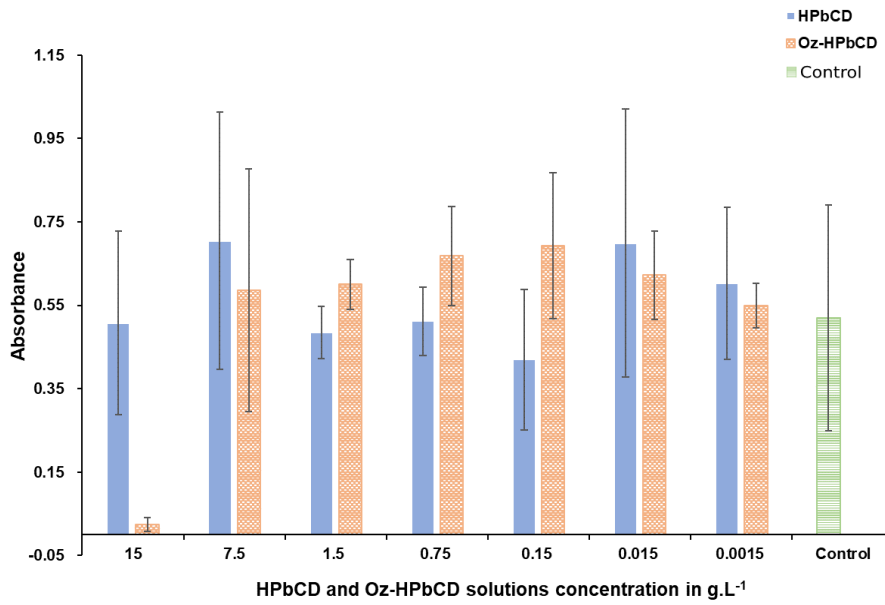
444 The results obtained for the MIC values are presented in **Figure 8** and **Figure 9** for the
445 bacterial strains *Streptococcus uberis* and *Escherichia coli* respectively. It is important to note
446 that these mean and standard deviation values result from 6 measurements, as each test
447 condition was replicated within the plate, and each plate was triplicated. The absorbance with
448 the different concentrations of powder for both strains was between 0.4 and 0.6. The positive
449 control, the bacterial suspension with the BHB, showed a normal bacterial growth. The well
450 with 15 g.L^{-1} of the Oz-HPbCD solution exhibited 0 absorbance for both strains *Streptococcus*
451 *uberis* and *Escherichia coli*. However, at the same concentration, the wells with HPbCD
452 presented a normal growth. Antibacterial activity was therefore clearly visible with the
453 ozonized powder, although no difference was recorded between the two kinds of powder at
454 any of the lower concentrations (between 7.5 and 0.0015 g.L^{-1}). As no concentrations were
455 tested in the range between 7.5 and 15 g.L^{-1} , the MIC value for Oz-HPbCD is considered to
456 be 15 g.L^{-1} and above.



457

458 **Figure 8.** Antimicrobial activity assessment of HPbCD and Oz-HPbCD against the *Streptococcus uberis* strain.

459



460

461 **Figure 9.** Antimicrobial activity assessment of HPbCD and Oz-HPbCD against the *Escherichia coli* strain.

462

463 **4. Conclusion**

464

465 The results obtained in this study demonstrate the possibility of producing solid oxidative
466 materials by contacting gaseous ozone with HPbCD powder. Comparing the physical
467 characterizations of the HPbCD used as raw material for the synthesis and that of the
468 ozonized HPbCD, there was no apparent modification of the morphology of the solid
469 particles, density, and specific area, leading to the conclusion that the contact with O_3 in these
470 conditions does not induce fragmentation or the creation of meso/micro porosity in the initial
471 particles. However, the characterization of Oz-HPbCD by FTIR and Raman spectroscopy
472 revealed a new band which could be attributed to the $-C=O$ stretching of carbonyl groups,
473 demonstrating that the contact with O_3 leads to chemical modifications of the CDs, which
474 may be directly related to new functions created on the CD and/or to the formation of by-
475 products. Further analytical work, in particular NMR studies, will be required to more
476 precisely identify and quantify both the by-products formed as a result of the synthesis and the
477 chemical changes to the solid CDs by reaction with the ozone. Looking at the influence of the
478 process parameters, the oxidative power (OP) of Oz-HPbCD was found to increase with the
479 ozone concentration in the gas, the reactor temperature, and the duration of the synthesis,
480 while the gas flow rate in the reactor had a low impact. These results seem to highlight the
481 fact that the OP values are directly correlated with the reaction kinetics between CDs and O_3 .
482 The global reactivity is likely to be more limited by the chemical reactions and diffusivity of
483 O_3 inside the solid particles than by the external mass transfer. It can therefore be
484 hypothesized that, in the conditions of this study, the HPbCD particles may have not reacted
485 completely. The Oz-HPbCDs, stored at low temperature were found to be relatively stable
486 products, as only 18% of their oxidative properties were lost after 65 days in a freezer at -
487 19°C . The microbiological results obtained by contacting Oz-HPbCDs and bacteria
488 demonstrated that the ozonized products have bactericidal properties, with a minimum
489 inhibitory concentration determined at $15\text{g}\cdot\text{L}^{-1}$ for both *Escherichia Coli* and *Streptococcus*.
490 We believe that these results may open up new avenues for developing novel solid materials
491 with tunable oxidative and antimicrobial properties, usable in biological applications.

492

493 **Funding sources**

494 This study benefited from funds provided by the French Region of Occitanie as part of the
495 MOZART project, and from state aid managed by the National Research Agency under the

496 program Investments for the Future with the reference ANR-18-EURE-0021. This work was
497 also supported by Toulouse Tech Transfer for potential valorization and technology transfer.

498

499 **Author Contributions**

500 The manuscript was written based on contributions from all the authors. All the authors have
501 given their approval to the final version of the manuscript.

502

503 **Declaration of competing interest**

504 The authors declare that they have no known competing financial interests or personal
505 relationships that could have appeared to influence the work reported in this paper.

506

507 **Acknowledgements**

508 We would like to acknowledge the French Region of Occitanie, the French National Research
509 Agency (ANR), and Toulouse Tech Transfer (TTT) for their financial support of this work.

510 We wish to thank the entire work group involved in the MOZART project, and more
511 particularly Marie-Line de Solan Bethmale, Christine Rey-Rouch and Gwenaëlle Guittier
512 from the SAP for the analyses performed. We also extend our thanks to Kevin Dolin-Dolcy
513 and Coralie Breton for their valuable help in the experiments. We would also like to
514 acknowledge the technical staff of the LGC for their participation, particularly J.-L. Nadalin
515 and Q. Ribière for prototyping and setting up instrumentation on the experimental rig.

516

517 **References**

518 Amiri, S., & Amiri, S. (2017). *Cyclodextrins: Properties and Industrial Applications* (1st
519 ed). John Wiley & Sons Ltd, Hoboken, NJ, (Chapter 2).
520 <https://doi.org/10.1002/9781119247609>.

521 Batakliiev, T., Georgiev, V., Anachkov, M., Rakovsky, S., & Zaikov, G. E. (2014). Ozone
522 decomposition. *Interdisciplinary toxicology*, 7(2), 47–59. [https://doi.org/10.2478/intox-](https://doi.org/10.2478/intox-2014-0008)
523 2014-0008.

524 Canado, A., Tournois, M., Pages, M., Roustan, M., Remus-Borel, W., Dietrich, N.,
525 Violleau, F., & Hébrard, G. (2020). The sudden decrease of the dissolved ozone
526 concentration in sprays: a mass transfer phenomenon? *Industrial & Engineering Chemistry*
527 *Research*, 59(33), 14914–14924. <https://doi.org/10.1021/acs.iecr.0c03216>.

528 Clark, D. E. (2001). Peroxides and peroxide-forming compounds. *Chemical Health &*
529 *Safety*, 8(5), 12–22. [https://doi.org/10.1016/S1074-9098\(01\)00247-7](https://doi.org/10.1016/S1074-9098(01)00247-7).

530 CLSI (2012). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That*
531 *Grow Aerobically*; Approved Standard—Ninth Edition. CLSI document M07-A9. Wayne,
532 PA: Clinical and Laboratory Standards Institute.

533 Crini, G. (2014). Review: a history of cyclodextrins. *Chemical Reviews*, 114(21), 10940-
534 10975. <https://doi.org/10.1021/cr500081p>.

535 Cristiano, L. (2020). Could ozone be an effective disinfection measure against the novel
536 coronavirus (SARS-CoV-2)? *Journal of Preventive Medicine and Hygiene*, 61(3), E301-
537 E303. <https://doi.org/10.15167/2421-4248/jpmh2020.61.3.1596>.

538 Dettmer, A., Ball, R., Boving, T.B., Khan, N.A., Schaub, T., Sudasinghe, N., Fernandez,
539 C.A. & Carroll, K.C. (2017). Stabilization and prolonged reactivity of aqueous-phase ozone
540 with cyclodextrin. *Journal of Contaminant Hydrology*, 196, 1-9,
541 <https://doi.org/10.1016/j.jconhyd.2016.11.003>.

542 Egyed, O. (1990). Spectroscopic studies on β -cyclodextrin. *Vibrational Spectroscopy*, 1(2),
543 225-227. [https://doi.org/10.1016/0924-2031\(90\)80041-2](https://doi.org/10.1016/0924-2031(90)80041-2).

544 Fan, W., An, W., Huo, M., Xiao, D., Lyu, T. & Cui, J. (2021). An integrated approach
545 using ozone nanobubble and cyclodextrin inclusion complexation to enhance the removal of
546 micropollutants. *Water Research*, 196, 117039.
547 <https://doi.org/10.1016/j.watres.2021.117039>.

548 Farber, C., Li, J., Hager, E., Chemelewski, R., Mullet, J., Rogachev, Yu. A., & Kurouski,
549 D. (2019). Complementarity of Raman and Infrared spectroscopy for structural
550 characterization of plant epicuticular waxes. *ACS Omega*, 4(2), 3700-3707.
551 <https://doi.org/10.1021/acsomega.8b03675>.

552 Harada, A., Takashima, Y., & Yamaguchi, H. (2009). Cyclodextrin-based supramolecular
553 polymers. *Chemical Society Reviews*, 38, 875-885. <https://doi.org/10.1039/b705458k>.

554 Ibrahim, N.A., & El-Zairy, E.M.R. (2009). Union disperse printing and UV-protecting of
555 wool/polyester blend using a reactive β -cyclodextrin, *Carbohydrate Polymers*, 76(2), 244-
556 249. <https://doi.org/10.1016/j.carbpol.2008.10.020>.

557 Khan, N. A., Johnson, M.D., & Carroll, K.C. (2018). Spectroscopic methods for aqueous
558 cyclodextrin inclusion complex binding measurement for 1,4-dioxane, chlorinated co-
559 contaminants, and ozone. *Journal of Contaminant Hydrology*, 210, 31-41.
560 <https://doi.org/10.1016/j.jconhyd.2018.02.002>.

561 Li, N., Chen, Y., Zhang, Y.M., Yang, Y., Su, Y., Chen, J.-T., & Liu, Y. (2014).
562 Polysaccharide-gold nanocluster supramolecular conjugates as a versatile platform for the
563 targeted delivery of anticancer drugs. *Scientific Reports*, 4, 4164.
564 <https://doi.org/10.1038/srep04164>.

565 Loftsson, T., & Duchêne, D. (2007). Cyclodextrins and their pharmaceutical applications.
566 *International Journal of Pharmaceutics*, 329(1-2), 1-11.
567 <https://doi.org/10.1016/j.ijpharm.2006.10.044>.

568 Malanga, M., Szemán, J., Fenyvesi, E., Puskás, E., Csabai, K., Gyémánt, G., Fenyvesi, F.,
569 & Szente, L. (2016). “Back to the future”: a new look at hydroxypropyl beta-cyclodextrins.
570 *Journal of Pharmaceutical Sciences*, 105(9), 2921-2931.
571 <https://doi.org/10.1016/j.xphs.2016.04.034>.

572 Martins, M.L., Eckert, J., Jacobsen, H., Dos Santos, E.C., Ignazzi, R., Ribeiro de Araujo,
573 D., Bellissent-Funel, M.-C., Natali, F., Marek Koza, M., Matic, A., De Paula, E., &
574 Bordallo, H.N. (2017). Raman and Infrared spectroscopies and X-ray diffraction data on
575 bupivacaine and ropivacaine complexed with 2-hydroxypropyl- β -cyclodextrin. *Data in*
576 *Brief*, 15, 25-29. <https://doi.org/10.1016/j.dib.2017.08.053>.

577 McCray, J., Boving, T., & Brusseau, M. (2000). Cyclodextrin-enhanced solubilization of
578 organic contaminants with implications for aquifer remediation. *Ground Water Monitoring
579 & Remediation*, 20, 94-103. <https://doi.org/10.1111/j.1745-6592.2000.tb00256.x>.

580 Morin-Crini, N., Fourmentin, S., Fenyvesi, É., Lichtfouse, E., Torri, G., Fourmentin, M., &
581 Crini, G. (2021). 130 years of cyclodextrin discovery for health, food, agriculture, and the
582 industry: a review. *Environmental Chemistry Letters*, 19, 2581–2617.
583 <https://doi.org/10.1007/s10311-020-01156-w>.

584 Muromachi, S., Ohmura, R., & Mori, Y. H. (2012). Phase equilibrium for ozone-
585 containing hydrates formed from an (ozone + oxygen) gas mixture coexisting with gaseous
586 carbon dioxide and liquid water. *The Journal of Chemical Thermodynamics*, 49, 1-6.
587 <https://doi.org/10.1016/j.jct.2012.01.009>.

588 Nasongkla, N., Wiedmann, A. F., Bruening, A., Beman, M., Ray, D., Bornmann, W. G.,
589 Boothman, D. A., & Gao, J. (2003). Enhancement of solubility and bioavailability of β -
590 lapachone using cyclodextrin inclusion complexes. *Pharmaceutical Research*, 20, 1626-
591 1636. <https://doi.org/10.1023/a:1026143519395>.

592 Özen, T., Koyuncu, M.A. & Erbaş, D. (2021). Effect of ozone treatments on the removal of
593 pesticide residues and postharvest quality in green pepper. *Journal of Food Science and
594 Technology*, 58, 2186–2196, <https://doi.org/10.1007/s13197-020-04729-3>.

595 Pagès, M., Kleiber, D., Pierron, R. J. G., & Violleau, F. (2016). Ozone effects on *Botrytis*
596 *cinerea* Conidia using a bubble column: germination inactivation and membrane
597 phospholipids oxidation. *Ozone: Science & Engineering*, 38(1), 62-69.
598 <https://doi.org/10.1080/01919512.2015.1074856>.

599 Piechowiak, T., Grzelak-Błaszczak, K., Sójka, M., & Balawejder, M. (2020). Changes in
600 phenolic compounds profile and glutathione status in raspberry fruit during storage in ozone-
601 enriched atmosphere. *Postharvest Biology and Technology*, 168, 111277.
602 <https://doi.org/10.1016/j.postharvbio.2020.111277>.

603 Roca, I., Akova, M., Baquero, F., Carlet, J., Cavaleri, M., Coenen, S., Cohen, J., Findlay,
604 D., Gyssens, I., Heur, O.E., Kahlmeter, G., Kruse, H., Laxminarayan, R., Liébana, E.,
605 López-Cerero, L., MacGowan, A., Martins, M., Rodríguez-Baño, J., Rolain, J.-M., Segovia,
606 C., Sigauque, B., Tacconelli, E., Wellington, E., & Vila, J. (2015). The global threat of

607 antimicrobial resistance: science for intervention. *New Microbes and New Infections*, 6, 22-
608 29. <https://doi.org/10.1016/j.nmni.2015.02.007>.

609 Stancanelli, R., Ficarra, R., Cannavà, C., Guardo, M., Calabrò, M.L., Ficarra, P., Ottanà,
610 R., Maccari, R., Crupi, V., Majolino, D., & Venuti, V. (2008). UV-vis and FTIR-ATR
611 characterization of 9-fluorenon-2-carboxyester/(2-hydroxypropyl)- β -cyclodextrin inclusion
612 complex. *Journal of Pharmaceutical and Biomedical Analysis*, 47(4-5), 704-709.
613 <https://doi.org/10.1016/j.jpba.2008.02.018>.

614 Szejtli, J. (1998). Introduction and general overview of cyclodextrin chemistry. *Chemical*
615 *Reviews*, 98(5), 1743-1754. <https://doi.org/10.1021/cr970022c>.

616 Szejtli, J. (1997). Utilization of cyclodextrins in industrial products and processes. *Journal*
617 *of Materials Chemistry*, 7(4), 575-585. <https://doi.org/10.1039/A605235E>.

618 Szejtli, J., & Osa, T. (1996). *Comprehensive Supramolecular Chemistry, Volume 3:*
619 *Cyclodextrins*. (1st ed.). Oxford: Pergamon.

620 Szente, L., Szemán, J., Sohajda, T. (2016). Analytical characterization of cyclodextrins:
621 *History, official methods and recommended new techniques. Journal of Pharmaceutical and*
622 *Biomedical Analysis*, 130, 347-365. <https://doi.org/10.1016/j.jpba.2016.05.009>.

623 Tizaoui, C. (2020). Ozone: a potential oxidant for COVID-19 Virus (SARS-CoV-2).
624 *Ozone: Science & Engineering*, 42(5), 378-385.
625 <https://doi.org/10.1080/01919512.2020.1795614>.

626 Vitola Pasetto, L., Simon, V., Richard, R., Pic, J.-S., Violleau, F., & Manero, M.-H.
627 (2020). A catalyst-free process for gas ozonation of reduced sulfur compounds. *Chemical*
628 *Engineering Journal*, 387, 123416. <https://doi.org/10.1016/j.cej.2019.123416>.

629 Vittenet, J., Aboussaoud, W., Mendret, J., Pic, J.-S., Debellefontaine, H., Lesage, N.,
630 Faucher, K., Manero, M.-H., Thibault-Starzyk, F., Leclerc, H., Galarneau, A., & Brosillon, S.
631 (2015). Catalytic ozonation with γ -Al₂O₃ to enhance the degradation of refractory organics
632 in water. *Applied Catalysis A: General*, 504, 519-532.
633 <https://doi.org/10.1016/j.apcata.2014.10.037>.

- 634 Wang, T., Peng, D., Li, Y., Bai, Y. & Huang, T. (2016). HP- β -Cyclodextrin assisted
635 ozonation. *Proceedings of the 2016 International Conference on Civil, Transportation and*
636 *Environment*, 1362-1365. Aug 28, 2016 - Aug 29, 2016, Phuket, Thailand.
637 <https://doi.org/10.2991/iccte-16.2016.239>.
- 638 Wellington, E. M. H., Boxall, A. B. A., Cross, P., Feil, E. J., Gaze, W. H., Hawkey, P.
639 M., Johnson-Rollings, A. S., Jones, D. L., Lee, N. M., Otten, W., Thomas, C. M., &
640 Williams, A. P. (2013). The role of the natural environment in the emergence of antibiotic
641 resistance in Gram-negative bacteria. *The Lancet Infectious Diseases*, 13(2), 155-165.
642 [https://doi.org/10.1016/S1473-3099\(12\)70317-1](https://doi.org/10.1016/S1473-3099(12)70317-1).
- 643 Yuan, C., Liu, B., & Liu, H. (2015). Characterization of hydroxypropyl- β -cyclodextrins
644 with different substitution patterns via FTIR, GC-MS, and TG-DTA. *Carbohydrate*
645 *Polymers*, 118, 36-40. <https://doi.org/10.1016/j.carbpol.2014.10.070>.
- 646 Zhang, X., Tang, N., Zhang, H., Chen, C., Li, L., Dong, C., & Cheng, Y. (2021).
647 Comparative transcriptomic analysis of cantaloupe melon under cold storage with ozone
648 treatment. *Food Research International*, 140, 109993.
649 <https://doi.org/10.1016/j.foodres.2020.109993>.

GRAPHICAL ABSTRACT

