

PLGA nanoparticles embedding molybdenum cluster salts: Influence of chemical composition on physico-chemical properties, encapsulation efficiencies, colloidal stabilities and in vitro release

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	Size (nm)	PDI	Zeta Potentiel (mV)
TBA2 - PLGAª	85.7 ± 4.9	0.12 ± 0.02	-25.8 ± 4.4
CMIF - PLGA ^a	102.0 ± 2.6	0.16 ± 0.01	-38.2 ± 1.7
CMB - PLGA ^a	75.7 ± 2.5	0.16 ± 0.02	-22.4 ± 9.8
TBA2 - PLGAb	95.0 ± 6.1	0.12 ± 0.01	-42.2 ± 8.0
CMIF - PLGA ^b	124.7 ± 2.5	0.08 ± 0.02	-47.8 ± 4.6
CMB - PLGA ^b	99.3 ± 4.6	0.12 ± 0.01	-47.8 ± 9.1
TBA2 - PLGAC	141.7 ± 6.4	0.09 ± 0.02	-24.8 ± 1.3
CMIF - PLGAc	144.7 ± 4.7	0.09 ± 0.03	-31.5 ± 3.6
CMB - PLGA ^c	118.3 ± 4.3	0.12 ± 0.01	-43.1 ± 6.5









24 ABSTRACT

We present a screening of poly (D,L-lactide-co-glycolide) (PLGA) nanoparticles embedding a 25 series of inorganic molybdenum octahedral clusters intended for photodynamic therapy (PDT) 26 of cancer. Three cluster compounds from 2 cluster units, $[{Mo_6Br}_8Br_6]^{2-}$ and 27 $[{Mo_6I_8}(OOC_2F_5)_6]^2$ were studied. $[{Mo_6Br}_8Br_6]^2$ cluster units are found in the soluble 28 ternary salt Cs₂[{Mo₆Br}₈Br₆] (CMB) prepared by solid state chemistry at high temperature. 29 In solution Cs^+ cations are replaced by tetrabutyl ammonium cations $(C_4H_9)_4N^+$ to form the 30 salt ($(C_4H_9)_4N_2$ [{Mo₆Br}₈Br₆] (TBA₂). [{Mo₆I₈}(OOC₂F₅)₆]²⁻ was prepared combining solid 31 state and solution chemistries; it is paired with Cs^+ cations to form $Cs_2[\{Mo_6I_8\}(OOC_2F_5)_6]$ 32 (CMIF). All tested cluster-based salts could efficiently be incorporated in PLGA nanoparticles 33 as seen with encapsulation efficiencies always higher than 60%. Cluster loaded nanoparticles 34 (CNPs) freshly prepared by solvent displacement method showed spherical shapes, zeta 35 potential values between -20 and -47mV, polydispersity index in the range 0.123-0.167 and 36 sizes in the range 75-150 nm according to the cluster compound and the polymer-to-cluster 37 mass ratio (P/C), suggesting a good cellular uptake. CNPs colloidal stability was maintened 38 for 3 months when they were stored refrigerated and protected from light but the chemical 39 stability was shorter, i.e. 4 weeks, 1 week and 1 day for CMIF, TBA2 and CMB, respectively, 40 CMIF penta-fluoropropionate apical ligands being less rapidly substituted by hydroxyles 41 groups than TBA₂ and CMB halogen apical ligands. FT-IR analysis revealed the lack of 42 strong chemical interaction between cluster compounds and polymer within the nanoparticles. 43 44 An interesting quick cluster in vitro release driven by diffusion outside the nanoparticles porous matrix was observed for all cluster compounds when P/C ratio was ≤ 2.5 and only a 45 higher P/C ratio not studied in this work (i.e > 5) could significantly affect the release of the 46 encapsulated cluster compound. Photophysical properties of cluster compounds were 47 preserved following PLGA incorporation. This work presents PLGA nanoparticles as a stable 48 and efficient cluster compound delivery systems for further in vitro and vivo evaluations in 49 cancer models. 50

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Key words: PLGA nanoparticle, molybdenum cluster, physico-chemical properties, colloidal
stability, chemical stability, *in vitro* release.

55 **1. INTRODUCTION**

56

Hexanuclear cluster of molybdenum with the general formula $[Mo_6(L^i)_8(L^a)_6]^{2-}$ (Figure 1) 57 have been widely studied due to their photo-physical and photo-chemical properties (Jackson 58 et al., 1996; Kirakci et al., 2012; Costuas et al., 2015; Dierre et al., 2017). In these clusters, 59 inner ligands (Lⁱ) are halogen atoms (Br, I, Cl) covalently attached to Mo, forming the stable 60 ${Mo_6L_8^i}^{4+}$ cluster core, while terminal or apical ligands (L^a) are labile inorganic or organic 61 ligands that are able to affect the physico-chemical properties of the cluster compound. When 62 they are photo-activated near 365 nm, the very highly chemical and photo-stable cluster cores 63 ${Mo_6L_8^i}^{4+}$ show bright photoluminescence with broad emission spectra in the red and near-64 infrared regions (from 550 to 900 nm) with high quantum yields (Vorotnikov et al., 2017; 65 Kirakci et al., 2012; Efremova et al., 2016). When the photo-activation occurs in the presence 66 of oxygen, they can act as powerful photosensitizers generating singlet oxygen efficiently 67 (Kirakci et al., 2012; Jackson et al., 1990; Amela-Cortes et al., 2015). Such physical and 68 chemical properties make molybdenum cluster compounds very attractive in many biological 69 sciences applications such as bioimaging, biolabeling, diagnostics, cell destructing or 70 antimicrobial agents (Vorotnikova et al., 2019; Solovieva et al., 2016; Neaime et al., 2016). 71 72 As a result, hexanuclear molybdenum halide cluster complexes are potential candidates for theranostic applications (Ahmed et al., 2012). Because of the poor solubility and stability of 73 these cluster compounds in water, the most physiological solvent for biological applications, 74 there is a need to incorporate them into biocompatible delivery systems such as polymeric 75 nanoparticles that will improve the cluster compound i) apparent solubility, ii) protection 76 against hydrolysis and iii) release in tumor areas. Several works have reported the use of 77 water soluble hybrid materials of molybdenum clusters (Kirakci et al., 2016; Svezhentseva et 78 al., 2017) or the encapsulation of molybdenum cluster compounds in matrixes less 79 biocompatible for parenteral injection such as silica nanoparticles (Aubert et al., 2013; 80 Solovieva et al., 2016) or nano-sized polystyrene beads (Vorotnikova et al., 2017). In a 81 previous work our team has shown the feasibility of incorporating a molybdenum cluster 82 compound into poly (D,L-lactide-co-glycolide) (PLGA) nanoparticles to maintain their 83 intrinsic photophysical properties and to use them as photosensitizer after entering the ovarian 84 cancer cells leading to their destruction (Brandhonneur et al., 2018). Nevertheless, the activity 85 and stability of the formulation can be improved by selecting the most appropriate cluster 86 compound and by an optimization of the polymer-to-cluster mass ratio (P/C). The aim of the 87 present study was to prepare PLGA nanoparticles (NPs) embedding a series of inorganic 88

hexanuclear molybdenum cluster compounds intended for PDT of ovarian cancer and to 89 select the most stable and efficient association for further in vitro and in vivo studies. The 90 polymeric nanoparticles were tested as a carrier system for molybdenum cluster compounds 91 to circumvent their poor aqueous solubility. Three cluster compounds from 2 cluster units, 92 $[\{Mo_6Br\}_8Br_6]^2$ and $[\{Mo_6I_8\}(OOC_2F_5)_6]^2$ were studied. $[\{Mo_6Br\}_8Br_6]^2$ cluster units are 93 found in the soluble ternary salt $Cs_2[\{Mo_6Br\}_8Br_6]$ (CMB) prepared by solid state chemistry 94 at high temperature. In solution Cs⁺ cations are replaced by tetrabutyl ammonium cations 95 $(C_4H_9)_4N^+$ to form the salt $((C_4H_9)_4N)_2[\{Mo_6Br\}_8Br_6]$ (TBA₂). $[\{Mo_6I_8\}(OOC_2F_5)_6]^{2-}$ was 96 prepared combining solid stae and solution chemistries; it is paired with Cs⁺ cations to form 97 $Cs_2[\{Mo_6I_8\}(OOC_2F_5)_6]$ (CMIF). These cluster compounds loaded nanoparticles (CNPs) were 98 prepared by solvent displacement method using polysorbate 20 as stabilizer and carboxylic 99 acid terminated poly (D,L-Lactide-co-glycolide) (PLGA) polymer with a 50:50 lactide to 100 gycolide ratio. Several polymer-to-cluster mass ratios (P/C) were tested, 1, 2.5 and 5. CNPs 101 were characterized using transmission electron microscopy or TEM (size and shape), dynamic 102 light scattering or DLS (size, polydispersity index, zeta potential, colloidal stability over 3 103 months), Fourier transform infrared spectroscopy or FT-IR (cluster/polymer interactions), UV 104 spectroscopy (encapsulation efficiency or EE, drug loading or DL, water stability and drug 105 release behavior). Finally, the effect of PLGA nanoparticles incorporation on cluster 106 compound photochemical parameters that can influence the generation of oxygen singlet, i.e. 107 absolute quantum yields and maximum emission wavelengths, were evaluated. 108

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111 **2. MATERIAL AND METHODS**

112 **2.1.** Chemicals

CMB was synthesized by solid state reaction techniques at high temperature whereas TBA₂ 113 and CMIF were prepared by combining solid state reactions and solution chemistry routes in 114 our laboratory (ISCR, CSM, Rennes, France) using reported procedures (Kirakci et al., 2005, 115 Amela-Cortes et al., 2015). Acid terminated PLGA with a 50:50 lactide to gycolide ratio, MW 116 24000-38000 (Resomer[®] RG503H) and polyoxyethylenesorbitan monolaurate or polysorbate 117 20 (Tween[®] 20) were purchased from Sigma Aldrich (St Louis, MO, USA). Analytical 118 reagent grade acetone, acetonitrile and absolute ethanol were obtained from VWR 119 international S.A.S. (Fontenay-sous-bois, FR). 120

121 **2.2.** Nanoparticles preparation

CNPs and cluster free nanoparticles or blank nanoparticles were prepared by solvent 122 displacement method or nanoprecipitation, described by Fessi et al. (1989). An organic 123 solution of PLGA polymer (10 to 50 mg) and cluster compound (9-13 mg according to its 124 molecular weight) in acetone (5 ml) was slowly added dropwise (1 ml/min) to a 0.5% w/v 125 aqueous polysorbate 20 solution (15 ml) with a 5 ml syringe and a 25G (0.5mm x 16 mm) 126 needle (Terumo Europe, Leuven, Belgium) under magnetic stirring (500 rpm) at room 127 temperature (20-25°C°). In these conditions, a bluish colloidal nanosuspension was 128 instantaneously formed owing to the ouzo effect (Francois et al., 2005). The acetone was then 129 removed under reduced pressure (200 mbar) by a rotary evaporator Laborata 4000 (Heidolph, 130 Schwabach, Germany) for 1h. The nanosuspensions ($\simeq 15$ ml) were then purified by a first 131 step of centrifugation (5000 g / 5 min) to eliminate polymer aggregates and by a second step 132 of dialysis against 1 liter of deionized water for 1h to eliminate free cluster, using 133 Spectra/Por®6 pre-wetted dialysis membrane with a molecular weight cut-off of 25kD 134 (Spectrum Laboratories, Inc, Rancho Dominguez, CA, USA). The resulting nanosuspensions 135 were used for size, PDI and zeta potential determinations, encapsulation efficiency, cluster 136 loading, water stability evaluation, release studies and quantum yields measurements. CNPs 137 physico-chemical characterizations performed in solid state, such as FT-IR, quantum yields 138 and emission spectra needed a freeze-drying stage of cluster loaded nanosuspensions for 24 h 139 using a Christ®Alpha laboratory freeze-drier, (Martin Christ, Germany). 140

141

142 **2.3. Nanoparticles characterization**

143 2.3.1. Particles size, polydispersity index and zeta potential

All three CNPs mean hydrodynamic diameters (nm) and polydispersity indexes (PDI) were determined by laser dynamic light scattering (DLS), and their zeta potentials (mV) were determined by laser droppler micro-electrophoresis technique using Malvern Zetasizer Nano ZS (Malvern Instruments, Malvern, United Kingdom). For each CNPs, all measurements were carried out in triplicate (from 3 different batches) following appropriate sample dilution with distilled water, immediately after preparation and following 1, 2, 7, 14, 30, 60 and 90 days storage protected from light, at room temperature and refrigerated between +2 and +8°C.

152 2.3.2. Encapsulation efficiency, cluster loading and mass yield

Cluster compounds encapsulation efficiency (EE%) as well as cluster compounds loading in 153 polymeric nanoparticles (CL%) were determined by UV spectroscopy at 250 nm following 154 appropriate dilution with acetonitrile (Specord®PC 205 UV VIS spectrophotometer, Analytik 155 Jena AG, Jena, Germany). Cluster calibration curve was linear from 2 to 20 μ g/ml (r² = 156 0.99991). Encapsulation efficiency determinations were performed immediately after 157 preparation following centrifugation of the CNPs suspensions at 40000 g for 30 min (Sigma 158 3K20 high speed refrigerated centrifuge). Free or non-encapsulated cluster compounds were 159 collected from supernatant, diluted with acetonitrile and concentrations were determined by 160 UV spectroscopy at 250 nm. 161

EE or the ratio of the experimental percent cluster loaded in the polymeric nanoparticles to the

initial amount of cluster in the formulation was calculated according the following equation:

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$$EE (\%) = \frac{\text{initial cluster amount (mg)} - \text{ non encapsulated cluster (mg)}}{\text{initial cluster amount (mg)}} \times 100$$

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166 Cluster loading (CL %) was performed by centrifugation of the CNPs suspension at 40000 *g* 167 for 30 min followed by freeze-drying of the sediment for 24h, after re-dispersion in distilled 168 water. A known amount of CNPs close to 5 mg was dissolved in 2 ml acetonitrile, diluted 169 (1/50) with acetonitrile and assayed for cluster quantification using the previous UV method. 170 CL or the percent cluster in the freeze dried polymeric nanoparticles was calculated according 171 the following equation:

$$CL(\%) = \frac{\text{encapsulated cluster in freeze dried nanoparticles (mg)}}{\text{freeze dried nanoparticles amount (mg)}} \ge 100$$

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173 Mass yields (MY%) of the formulations were also determined using the equation:

$$MY(\%) = \frac{\text{purified freeze dried nanoparticles amount (mg)}}{\text{initial freeze dried nanoparticles amount (mg)}} \times 100$$

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175 2.3.3. Transmission Electron Microscopy (TEM)

CNPs morphologies were examined with a transmission electron microscope (TEM, JEOL 2100, Japan) and charge coupled device camera Orius 200D (GatanInc, Pleasanton, USA).
Samples were prepared by placing a drop of CNPs nanosuspensiononto a carbon coated copper grid and air-dried, following negative staining during 3 min with one drop of 2%

aqueous solution of uranyl acetate for contrast enhancement. The air-dried samples were thendirectly examined under the transmission electronic microscope.

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183 2.3.4. Fourier Transform Infrared Spectroscopy (FT-IR)

PLGA Polymer (RG503H), cluster compounds TBA₂, CMB and CMIF, freeze dried blank nanoparticles, freeze dried CNPs as well as physical mixtures between cluster compounds and polymer with P/C ratios of 1, 2.5 and 5 were all analyzed by infrared spectroscopy using Universal Attenuated Total Reflectance Accessory (UATRA) Spectrum TwoTM FT-IR Spectroscopy (Perkin Elmer Inc., Massachusetts, USA). Samples were fixed on the diamond crystal and a pressure of 90 N/cm² was applied to each sample. Spectra were recorded in the range from 500 to 4000 cm⁻¹ and analyzed using Spectrum[®] Quant software.

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192 2.3.5. Cluster compound chemical stability in aqueous nanosuspensions

193 Chemical stabilities of cluster compounds in CNPs were shown with 2 complementary 194 approaches, i) their UV-visible absorption spectra evolution from preparation up to 48 hours 195 and ii) the evolution of cluster compound concentrations in aqueous nanosuspensions during 4 196 weeks by UV spectroscopy at 250 nm as described in section 2.3.2. In both studies 197 nanosuspensions were refrigerated and protected from light and measurements were 198 performed following appropriate dilution with acetonitrile using a Specord®PC 205 UV VIS 199 spectrophotometer (Analytik Jena AG, Jena, Germany).

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201 2.4 *In vitro* cluster release from CNPs

Cluster compounds release analysis from nanoparticles were carried out in absolute ethanol at 202 25°C, respecting the "sink" condition, i.e. cluster compound solubility in the dissolution 203 medium never exceeding 10% of its solubility. At the beginning of the experiment, 4.5ml of 204 freshly prepared CNPs corresponding to 2 to 4 mg of cluster compounds were placed in a 205 dialysis bag, a 25kD MWCO Spectra/Por®6 pre-wetted dialysis membrane (Spectrum 206 Laboratories, Inc, Rancho Dominguez, CA, USA) then sealed at its 2 sides and placed in a 207 150 ml beaker containing 100 ml release medium with continuous magnetic stirring (250 208 rpm). The sampling conditions were depending on the cluster compound chemical stability in 209 the aqueous nanosuspensions. The quick substitution of the halogen Br⁻ labile terminal ligands 210 of the [{Mo₆Br}₈Br₆]²⁻ cluster units with hydroxyl groups or water molecules described in 211

section 3.2 is leading to an apparent decrease in the percent released profile as a function of 212 time. To overcome this situation, percent cluster released was obtained from the percent 213 cluster remaining in the dialysis bag at the sampling time. At each sampling time (30, 60, 180, 214 360 and 1440 and 2880 min) the flask was centrifuged (5000 g/10 min) the supernatant was 215 discarded, the nanoparticles in sediment were solubilized in 5 ml acetonitrile and the percent 216 cluster released was evaluated by UV spectrophotometer at 250 nm after dilution (1/20) with 217 acetonitrile using the method mentioned in section 2.3.2. For CMIF, the apical ligand 218 substitution kinetics was much slower than for $[{Mo_6Br}_8Br_6]^{2-}$ cluster unit based salts and 219 direct sampling in the donor compartment was possible at each sampling time. Each point 220 represents the mean \pm SD of 3 different batches. 221

222

223 **2.5. Photophysical studies**

Absolute photoluminescence quantum yields, ϕ_{em} , were measured on powdered cluster compounds and on CNPs (as aqueous nanosuspensions or freeze-dried solid nanoparticles) using a Hamamatsu C9920-03G measurement system (Hamamatsu Photonics France, Massy, France) comprising a xenon excitation light source, an integrating sphere and a red-sensitive multichannel photodetector Hamamatsu PMA-12. The excitation wavelength was set at 365 nm. The same Hamamatsu set up was used to obtain emission spectra.

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231 **2.6. Statistical analysis**

All experiments were performed in triplicate. Results are presented as mean \pm SD. Mean values of nanoparticle size, PDI, zeta potential and encapsulation efficiency were compared using the Mann and Whitney test and differences were considered significant at the level of p < 0.05.

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242 **3. RESULTS AND DISCUSSION**

243 **3.1. Nanoparticles formulation and characterization**

PLGA nanoparticles were prepared by the solvent displacement method also known as nano-244 precipitation method (Fessi et al., 1989). The oily phase or organic phase is a solution of 245 acetone in which both cluster compound and PLGA polymer are dissolved while the aqueous 246 phase is a distilled water solution of surfactant polyoxyethylenesorbitan monolaurate. Cluster 247 loaded polymer nanosuspensions were obtained following dropwise addition of the organic 248 phase to the aqueous phase. Particle size is one of the most critical factor to consider for the 249 cellular uptake of colloidal system, sizes between 50-150 nm are needed for optimal uptake 250 by tumor cells (He et al., 2010). Following acetone removal under reduced pressure and using 251 the preparation conditions described in section 2.2, several cluster nanoparticles (CNPs) were 252 obtained with 3 different P/C ratios, i.e 1, 2.5 and 5. Table 1 shows the nanoparticle sizes, 253 polydispersity indexes (PDI) and zeta potential obtained for all freshly prepared formulations. 254 All nanoparticles sizes obtained were compatible with a cellular uptake, higher sizes were 255 obtained with CMIF the cluster salt with the higher atomic mass and significant variations in 256 nanoparticle sizes were observed when the tested P/C ratio was increased from 1 to 5, with 257 sizes increasing from the range 75-100 nm (P/C = 1) to the range 120-145 nm (P/C = 5). This 258 result is in accordance with other studieson PLGA concentration effect on nanoparticles size 259 and this may be due to an increase of the solution viscosity (Halayga et al., 2014; Homs et al., 260 2018). PDI values were always < 0.2 indicating a homogenous monodispersed profile of the 261 prepared nanoparticles but this time a significant decrease was observed when P/C ratio was 262 increased from 1 to 5, i.e. 33%, 77% and 33% for TBA2, CMIF and CMB, respectively, 263 indicating a narrowing of size distribution with higher P/C ratio. Zeta potentials were all 264 comprised between -25 and -45 mV, measuring the negative electric charge on the 265 nanoparticle surface resulting from the interaction between PLGA and the polysorbate 20 266 surfactant. 267

Zeta potential absolute values higher than 30 mV are generally related to better nanoparticle physical stabilities due to stronger electrostatic repulsion between nanoparticles (Gupta and Trivedi, 2018), the highest Zeta potential values were obtained with the P/C ratio of 2.5, whatever the cluster compound tested. The encapsulation efficiencies (EE%), cluster loadings (CL%) and mass yields (MY%) obtained for each CNPs were determined using UV spectrophotometry and the results are reported in Table 2. The EE was higher than 60% for all P/C ratios tested indicating an efficient cluster entrapment within the nanoparticles. Among the cluster compounds tested, only CMIF-PLGA reached an EE% higher than 95%. CLs were as expected significantly decreased when P/C ratio was increased. The MY% vas evaluated after freeze-drying of the samples and no statistically significant differences were observed between the different P/C ratios tested.

TEM was performed to examine CNPs morphology. For each cluster compound tested, all 279 P/C ratios showed the same morphology, therefore only nanoparticles with a P/C ratio of 2.5 280 were presented in Figure 2A. TEM images show the spherical shape and the porous structure 281 of CNPs prepared by nanoprecipitation, the particle size, around 50 nm were always lower 282 than those obtained with dynamic light scattering as already reported and commented in our 283 previous study with TBA₂ (P/C = 0.5) (Brandhonneur et al., 2018). Figure 2B presents freshly 284 prepared CNPs with a P/C ratio of 2.5 under daylight (left) and following photo-activation 285 under UV light at 365 nm (right) exhibiting eye-observable intensive red luminescence for 286 CMIF and less intensive pink to red luminescence, according to P/C ratio for CMB and TBA₂. 287 288

Fourier Transform Infrared spectroscopy was performed to investigate the polymer/cluster 289 interactions resulting from the cluster encapsulation in the nanoparticles. As PLGA is an 290 organic polymer, it is easy to follow the shifts concerning well characterized vibrations such 291 as C=O stretching or carbonyl bond at 1750cm⁻¹, C-O ester stretching at 1088cm⁻¹ and sp3 C-292 H stretching at 1750cm⁻¹. The results obtained for blank polymeric nanoparticles and CNPs 293 are given in Table 3. The C=O stretch recorded for the polymer at 1750 cm⁻¹ did not change in 294 CNPs while the peak representing the C-O ester stretching slightly moved from 1088 cm⁻¹ for 295 the polymer to 1078 cm⁻¹ and 1083 cm⁻¹ for both molybdenum halide CNPs with a P/C ratio 296 of 2.5, TBA₂ and CMB, respectively. The vibration corresponding to the sp3 C-H stretching 297 was detected at 2914 cm⁻¹ for the polymer and moved significantly between 2921 and 2924 298 cm⁻¹ for all CNPs tested, indicating the existence of interactions between the polymer and the 299 cluster compound. Nevertheless, FT-IR spectra show the presence of all polymer 300 characteristic peaks and the absence of new chemical modifications creation following cluster 301 302 compound incorporation into nanoparticles (data not presented), this clearly indicates the lack of strong chemical interactions formed but rather physical ones. This comes in accordance 303 with other studies on PLGA nanoparticles incorporating drugs such as methotrexate, garcinol 304 or hesperidin where authors concluded to the lack (Jang et al., 2019; Gaoncar et al., 2017) or 305 to minor (Ali et al., 2019) chemical interaction between drug and polymer. 306

307 3.2. CNPs Physico-chemical stability

CNPs physical stability is an essential parameter in formulation. The colloidal stability of 308 polymeric nanoparticles can be monitored by the change of particle size, PDI and zeta 309 potential at a function of time following storage in close vials protected from light, at ambient 310 temperature (+25°C) or refrigerated (between +2 and +8°C). For blank PLGA nanoparticles, 311 colloidal stability was conserved during the 3 months study whatever the temperature 312 (Supplementary material 1). Figure 3 shows the evolution of size, PDI and Zeta Potential 313 following dynamic light scattering measurements on a 3 months storage period for the 314 nanosuspensions refrigerated between +2 and +8°C and protected from light. Particle sizes 315 were not statistically modified during the 3 month of storage for all cluster compounds and all 316 P/C ratios tested, only a slight increase in size, always below 5%, was measured over the 317 period. Identically, PDI were not significantly modified, all values were comprised between 318 0.09 and 0.19, showing the conservation of size distribution over time. Zeta potential values, 319 predictor of nanoparticles medium or long term instability, increased rapidly after 320 nanoparticle preparation, up to 50% at day 7 and were approaching zero after 1 month. This 321 decrease in Zeta potential values not observed with blank nanoparticles may be related to 322 cluster compound hydrolysis and the creation of new chemical species perturbing the 323 nanoparticles environment thus limiting CNPs physical stability to 3 months under 324 refrigeration. These rapid decrease of zeta potential values were also confirmed when the 325 nanosuspensions were stored at ambient temperature $(25 \pm 1^{\circ}C)$ and protected from light 326 (Supplementary material 2) but this time, size was significantly increased after 2 months for 327 TBA₂ (8 fold increase) and CMB (2 fold increase) loaded nanoparticles only with P/C ratio of 328 1, while size was not modified during the period for CMIF. We can conclude that i) keeping 329 the nanosuspensions refrigerated is delaying the manifestation of instability; ii) zeta potential 330 is a critical parameter to predict a forthcoming instability; iii) increasing polymer 331 concentration improves nanoparticle stability and iv) using pentafluoro propionate as organic 332 apical ligand instead of brome seems to protect nanoparticles from aggregation. Chemical 333 stabilities of cluster compounds were also checked by following the evolution of absorption 334 spectra of aqueous nanosuspensions and by determination of cluster compounds 335 concentrations in nanosuspensions by UV spectroscopy at 250 nm. The UV-visible absorption 336 spectra of freshly prepared nanosuspensions of TBA₂, CMIF and CMB, as well as their 337 evolution for 48h (at 0, 3, 6, 24 and 48 hours) were recorded in acetonitrile and are shown in 338 figure 4. For CMIF all spectra from 0 to 48 hours are superposed signing the lack of chemical 339

modification during the period studied while for the 2 other clusters with brome as apical 340 ligand, the intensity of the UV absorption maximum around 250 nm was continually 341 decreasing during the same period and for TBA₂ a second UV absorption peak near 217 nm 342 was increasing, corresponding to the formation of a new chemical compound as shown with 343 the isosbestic point at 230 nm. In fact, molybdenum cluster compounds are known to be 344 unstable in water where they are converted into agua hydroxo complexes by substitution of 345 labile apical ligand by hydroxyl groups or water molecules leading to micron sized aggregates 346 with altered luminescence properties (Daigre et al., 2018; Kirakci et al., 2016, 2019; 347 Svezhentseva et al., 2017). Encapsulating the cluster in a matrix such as SiO₂ nanoparticles 348 (Aubert et al., 2013), polystyrene microparticles (Efremova et al., 2014) or nanoassemblies 349 with betacyclodextrins (Kirakci et al., 2014) was shown to be a solution to circumvent the 350 tendency to easily hydrolyse in water. In our study the use of a porous biocompatible 351 polymeric matrix is interesting with fluoropropionate organic apical ligand, as seen with 352 CMIF that can, contrary to halogen ligand protect the Mo₆I₈ cluster core from water 353 hydrolysis and this was also observed with a similar cluster core with fluorinated apical ligand 354 presented as colloidal nanodispersion (Kirakci et al., 2018). This was also confirmed by the 355 UV assay of cluster compound in the nanoparticles during 4 weeks (figure 5). 356

The percent cluster compound remaining unchanged at a function of time from CNPs was better for CMIF (lack of cluster loss after one week; <10% loss after 4 weeks) than for the two other cluster compounds, TBA₂ (10% and 30% loss after 1 and 4 weeks, respectively) and CMB (30% and > 50% loss after 1 and 4 weeks, respectively). This time again CMIF was the most protected within the PLGA matrix for up to 4 weeks due to the shielding effect of the organic apical ligand.

As shown in our previous study, release of cluster compound from CNPs is needed for an 363 optimal activity following photo-activation (Brandhonneur et al., 2018). Figure 6 presents the 364 percent cluster released as a function of time for the 3 clusters and the 3 P/C ratios. As 365 mentioned in material and methods section, in vitro release was performed in ethanol to 366 respect the sink condition for the duration of the test. From percent released curves we can see 367 a high initial burst for each cluster compound with more than 50% released at 180 min for all 368 P/C ratios, followed by a prolonged release phase during the 48h test. The initial quantity 369 released by burst effect is decreasing with P/C ratio for all cluster compounds, from 60-65% 370 (P/C = 1) to 50% (P/C = 5) and this can be explained by the mechanisms of cluster release 371 from PLGA nanoparticles that are drug diffusion in the porous matrix happening from the 372 373 beginning followed by late polymer degradation by ester linkages hydrolysis. The cluster

compound diffusion is improved with low P/C ratio due to a smaller polymeric matrix. In the 374 same way, the lower the P/C ratio (i.e.1), the higher the percent released at 24h, i.e. 88%, 80% 375 and 75% for CMIF, CMB and TBA₂, respectively and the higher the P/C ratio (i.e. 5), the 376 lower the percent released at 24h, i.e. 71%, 71% and 60%, for CMIF, CMB and TBA₂, 377 respectively. It is also possible to compare the different profiles by estimating the time needed 378 to obtain 50% of release or $t_{50\%}$, and this parameter was always increasing with P/C ratio, i.e. 379 from 77.2 to 138.9 min, 150.2 to 185.2 min and 24.8 to 171.2 min for CMIF, CMB and TBA₂, 380 respectively, when P/C ratio was increased from 1 to 5. There was a slight effect of P/C ratio 381 on the release of the cluster compound from nanoparticles, it seems that only a higher P/C 382 ratio that was not studied in this work (i.e > 5) could significantly decrease the release of the 383 encapsulated cluster compound thus affecting the production of singlet oxygen following 384 photoactivation. 385

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387 **3.3. Photophysical properties**

Molybdenum cluster compound are known to exhibit photoluminescence between 550 and 388 900 nm with quantum yield up to 100% and luminescence lifetimes up to 400 µs (Vorotnikov 389 et al., 2017; Kirakci et al., 2012; Efremova et al., 2016; Maverick et al., 1983). When metal 390 cluster compounds are embedded into a polymeric matrix there is a need to check if the 391 photophysical properties of the starting cluster compound are affected. For that purpose we 392 compared the luminescence properties of the molybdenum cluster compounds before and after 393 their incorporation into PLGA matrixes. Photophysical parameters for powdered pure cluster 394 compounds CMB, TBA2 or CMIF and for their PLGA nanoparticulate systems presented in 395 Figures 7, 8 and 9 (emission spectra) and Table 4 (photoluminescence absolute quantum 396 yields or AQY). The irradiation of CNPs in the UV-Blue region is leading to the cluster 397 emission in the red-near infrared (from 550 to 900 nm). Emission spectra were recorded from 398 CNPs nanosuspensions and from solid state CNPs obtained after freeze drying of the 399 nanosuspensions. From emission spectra (Figures 7, 8 and 9) we can observe a broad 400 emission signal typical of metal clusters phosphorescence when they are irradiated in the UV-401 blue region at 365 nm. Cluster compound incorporation in PLGA matrix does not lead to a 402 modification of the general shape of its emission band, only a slight blue shift of emission 403 signal could be guessed when CNPs were dispersed in water compared with their solid form, 404 but no significant shift of the emission maximum (figure 7). A slight red shift of cluster 405

406 compound emission maximum (15 nm) could only be seen with CMIF following its 407 incorporation into PLGA nanoparticles but no emission maximum shift could be detected 408 when P/C ratio was modified (Figure 8). All these results are evidencing that cluster 409 compounds emission properties are preserved when they are embedded into PLGA 410 nanoparticles.

411 AQY depend on the ratio of the emission and the absorption energy and thus characterize the ability of a sample to emit light following irradiation. An increase in the emission signal 412 intensity was observed with CMIF loaded nanoparticles (P/C ratio = 2.5) when passing from 413 an air to a N₂ saturated atmosphere in the integrating sphere (Figure 9) and this phenomenon 414 was reversible when N2 was replaced by air. Octahedral clusters compound excited state are 415 known to efficiently react with triplet oxygen to form emissive singlet oxygen (Jackson et al., 416 1990), resulting in a quenching of the cluster emission signal, i.e. its luminescence. The 417 decrease in atmosphere dioxygen concentration obtained with N₂ resulted in a decrease of 418 cluster luminescence quenching. This phenomenon is a totally reversible physical 419 phenomenon that does not lead to a chemical alteration of the emitting cluster, as seen with 420 the lack of emission maximum shift. Incorporation of molybdenum clusters in polymeric 421 PLGA network led to a decrease of AQY (Table 4) and this was also reported with several 422 cluster compounds, $((C_4H_9)_4N)_2[(Mo_6I_8)(NO_3)_6)]$ (Vorotnikov al., et 2016), 423 ((C₄H₉)₄N)₂[{Mo₆I₈}(CF₃CO₂)₆)] (Kirakci et al., 2012) and CMB (Aubert et al., 2013; Grasset 424 et al., 2008) following their incorporation into SiO₂ micro or nanoparticles and was also 425 reported for Mo₆ clusters after incorporation in PMMA (Amela-Cortes et al., 2016) or 426 PMMA-PEOMA copolymer (Robin et al., 2018) matrixes, these modifications being 427 attributed to perturbation of the ligand environment in the polymeric matrix or to cluster 428 compound instability during nanoparticles preparation, i.e. apical ligand substitution by water 429 molecules. The highest values were obtained for pure cluster compounds in solid state 430 compared to CNPs and, as reported by Amela-Cortes at al. (2015, 2016), molybdenum cluster 431 compound with organic apical ligand (CMIF) gave the higher AQY compared to clusters with 432 halogen apical ligands (CMB and TBA₂). AQY values were not significantly increased with 433 cluster compound concentration (i.e. when decreasing P/C ratio) showing that cluster 434 compound were absorbing most of the light at the excitation wavelength used, i.e. 365 nm. 435 Because of the existence of interactions between the triplet excited state of cluster compounds 436 and molecular oxygen, aerated water nanosuspensions were poorly emissive, as seen with 437 AQY values of 6.6, 1.5 and 2% for TBA₂, CMIF and CMB, respectively. For this reason 438

AQY values in freshly prepared nanosuspension were lower than those in solid state, excepted for CMIF for which no difference was seen between nanosuspension and solid form, the organic ligand giving a slightly higher protection against the quenching of luminescence by dioxygen. The luminescence quenching could also be reversed as seen with AQY value raising from 1.3 to 28% following 15 min N₂ bubbling of CMIF solid loaded nanoparticles. From this study we can conclude that intrinsic properties of molybdenum cluster compound are not significantly modified when they are incorporated in PLGA nanoparticles.

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447 Conclusion

Molybdenum cluster salts were successfully incorporated in PLGA matrixes leading to 448 homogenous cluster loaded nanosuspensions characterized as efficient delivery systems. All 449 nanoparticles had sizes compatible with cellular uptake, could quickly release their embedded 450 cluster compound and exhibited good colloidal stabilities upon refrigeration. However, the 451 tendency of cluster compounds to hydrolysis in water resulted in poor cluster chemical 452 stability especially when the apical ligand was a halogen, limiting the shelf-life of the cluster 453 loaded nanosuspensions. Thus, the development of a dry dosage form to be reconstituted 454 extemporaneously before injection, capable to maintain cluster salts physico-chemical as well 455 as photo-chemical properties for a longer period is of paramount importance. As cluster 456 compounds photophysical properties are preserved when they are embedded into PLGA 457 nanoparticles, their initial values are important to take into account before considering a 458 biological application such as PDT. In vitro biological properties of these CNPs, i.e. cellular 459 uptake, toxicity and photoactivity on ovarian cancer cells are currently under investigation. 460

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479 **References**

Ahmed, N., Fessi, H., Elaissari, A., 2012. Theranostic applications of nanoparticles in cancer.

481 Drug Discovery Today, 17, 928-934. https://doi.org/10.1016/j.drudis.2012.03.010

Ali, S.H., Sulaiman, G.M., Al-Halbosiy, M.M.F., Jabir, M.S., Hameed, A.H., 2019.
Fabrication of hesperidin nanoparticles loaded by poly lactic co-Glycolic acid for improved
therapeutic efficiency and cytotoxicity. Artificial cells, Nanomedecine and Biotechnology, 47,
378-394. https://doi.org/10.1080/21691401.2018.1559175

Amela-Cortes, M., Garreau, A., Cordier, S., Faulques, E., Duvail, JL, Molard, Y., 2014. Deep
red luminescent hybrid copolymer materials with high transition metal cluster content. J. Mat.
Chem. C, 2,1545-1552. https://doi.org/10.1039/c3tc31309c

Amela-Cortes, M., Paofai, S., Cordier, S., Folliot, H., Molard, Y., 2015. Tuned red NIR
phosphorescence of polyurethane hybrid composites embedding metallic nanoclusters for
oxygen sensing. Chem. Commun.,51, 8177-8180. https://doi.org/10.1039/c5cc01867f

492 Amela-Cortes, M., Molard, Y., Paofai, S., Desert, A., Duvail, J-L., Naumov, N.G., Cordier, 493 S., 2016. Versatility of the ionic assembling method to design highly luminescent PMMA 494 nanocomposites containing $[M_6Q_8^iL_6^o]^n$ octahedral nano-building blocks. Dalton Trans., 45, 495 237-245. https://doi.org/10.1039/c5dt03734d

Aubert, T., Cabello-Hurtado, F., Esnault, M-A., Neaime, C., Lebret-Chauvel, D., Jeanne, S.,
Pellen, P., Roiland, C., Le Polles, L., Saito, N., Kimoto, K., Haneda, H., Ohashi, N., Grasset,
F., Cordier, S., 2013. Extended investigations on luminescent Cs2[Mo6Br14]@SiO2

- 499 nanoparticles: physico-structural characterizations and toxicity studies. J. Phys. Chem. C, 117,
- 500 20154-20163. https://doi.org/10.1021/jp405836q

Brandhonneur, N., Hatahet, T., Amela-Cortes, M., Molard, Y., Cordier, S., Dollo, G., 2018.
Molybdenum cluster loaded PLGA nanoparticles: an innovative theranostic approach for the
treatment of ovarian cancer. Eur. J. Pharm. Biopharm., 125, 95-105. https://doi.org/
10.1016/j.ejpb.2018.01.007

- Costuas, K., Garreau, A., Bulou, A., Fontaine, B., Cuny, J., Gautier, R., Mortier, M., Molard, 505 Y., Duvail, J.-L., Faulques, E., Cordier, S., 2015. Combined theoretical and time-resolved 506 photoluminescence investigations of $[Mo_6Br_8^iBr_6^a]^{2-}$ metal cluster units: evidences of multi-507 Phys. Chem. emission. Chem. Phys., 17, component 28574-28585. 508 https://dx.doi.org//10.1039/c5cp03960f 509
- Daigre, J., Cuny, P., Lemoine, M., Amela-Cortes, S. Paofai, N. Audebrand, A. Le Gal La
 Salle, E. Quarez, O. Joubert, N. G. Naumov, S. Cordier, 2018. Metal-Atom clusters as
 building blocks for multifunctional proton-conducting materials: theoretical and experimental
 characterization. Inorg. Chem., 57, 9814-9825.https://doi.org/10.1021/acs.inorgchem.8b00340
- Dierre, B., Costuas, K., Dumait, N., Paofai, S., Amela-Cortes, M., Molard, Y., Grasset, F.,
 Cho, Y., Takahashi, K., Ohashi, N., Uchikoshi, T., Cordier, S., 2017. Mo₆ cluster-based
 compounds for energy conversion applications: comparative study of photoluminescence and
 cathodoluminescence. Science and Technology of Advanced Materials. 18, 458–466.
 https://doi.org/10.1080/14686996.2017.1338496
- Efremova, O.A., Shestopalov, M.A., Chirtsova, N.A., Smolentsev, A.I., Mironov, Y.V.,
 Kitamura, N., Brylev, K.A., Sutherland, A.J., 2014. A highly emissive inorganic
 hexamolybdenum cluster complex as a handy precursor for the preparation of new
 luminescent materials. Dalton Trans., 43, 6021-6025. https://doi.org/ 10.1039/c3dt53126k
- Efremova, O.A., Vorotnikov, Y.A., Brylev, K.A., Vorotnikova, N.A., Novozhilov, I.N.,
 Kuratieva, N.V., Edeleva, M.V., Benoit, D.M., Kitamura, N., Mironov, Y.V., Shestopalov,
 M.A., Sutherland, A.J., 2016. Octahedral molybdenum cluster complexes with aromatic
 sulfonate ligands. Dalton Trans., 45, 15427-15435. https://doi.org/ 10.1039/c6dt02863b
- 527 Fessi, H., Puisieux, F., Devissaguet, J-P., Ammoury, N., Benita, S., 1989. Nanocapsule

- formation by interfacial polymer deposition following solvent displacement. Int.J. Pharm. 55,
 R1-R4. https://doi.org/10.1016/0378-5173(89)90281-0
- Francois, G., Katz, J.L., 2005. Nanoparticles and nanocapsules created using the Ouzo effect:
 spontaneous emulisification as an alternative to ultrasonic and high-shear devices,
 Chemphyschem., 6, 209-216. https://doi.org/10.1002/cphc.200400527
- Gaonkar, R.H., Ganguly, S., Dewanjee, S., Sinha, S., Gupta, A., Ganguly, S., Chattopadhyay,
 D.,; Chatterjee Debnath, M., 2017. Garcinol loaded vitamin E TPGS emulsified PLGA
 nanoparticles: preparation, physicochemical characterization, *in vitro* and in vivo studies. Sci.
 Rep.,7, 530. https://doi.org/10.1038/s41598-017-00696-6
- Grasset, F., Dorson, F., Cordier, S., Molard, Y., Perrin, C., Marie, A-M., Sasaki, T., Haneda, H., Bando, Y., Mortier, M., 2008. Water-in-oil microemulsion preparation and characterization of $Cs_2[Mo_6X_{14}]$ @SiO₂ phosphor nanoparticles based on transition metal clusters (X=Cl, Br, I), Adv. Mater., 20, 143-148. https://doi.org/10.1002/adma.200701686
- Gupta, V., Trivedi, P., 2018. *In vitro* and *in vivo* characterization of pharmaceutical topical
 nanocarriers containing anticancer drugs for skin cancer treatment, in: Grumezescu, A.M. (Ed),
 Lipid nanocarriers for drug targeting. William Andrew applied Science Publisher, pp. 563627. https://doi.org/10.1016/c2016-0-04170-0
- Halayqa, M., Domanska, U., 2014. PLGA biodegradable nanoparticles containing
 perphenazine or chlorpromazine hydrochloride: effect of formulation and release, Int. J. Mol.
 Sci., 15, 23909-23923. https://doi.org/ 10.3390/ijms151223909
- He, C., Hu, Y., Yin, L., Tang, C., Yin, C., 2010. Effects of particle size and surface charge on
 cellular uptake and biodistribution of polymeric nanoparticles, Biomaterials 31, 3657-3666.
 https://doi.org/10.1016/j.biomaterials.2010.01.065
- Homs, M., Caldero, G., Monge, M., Morales, D., Solans, C., 2018. Influence of polymer
 concentration on the properties of nanoemulsions and nanoparticles obtained by a low-energy
 method, Colloids and surfaces A: physicochemical and engineering aspects, 536, 204-212.
 https://doi.org/10.1016/j.colsurfa.2017.06.009
- Jackson, J.A., Turro, C., Newsham, M.D., Nocera, D.G., 1990. Oxygen quenching of

- electronically excited hexanuclear molybdenum and tungsten halide clusters, J. Physic.
 Chem., 94, 4500-4507. https://doi.org/10.1021/j100374a029
- Jackson, J.A., Newsham, M.D., Worsham, C., Nocera, D.G., 1996. Efficient singlet oxygen generation from polymers derivatized with hexanuclear molybdenum clusters, Chem. Mater., 8, 558-564. https://doi.org/10.1021/cm950443f
- Jang, J.H., Jeong, S.H., Lee Y.B., 2019. Preparation and in vitro/in vivo characterization of
- 562 polymeric nanoparticles containing methotrexate to improve lymphatic delivery, Int. J. Mol.
- 563 Sci., 20, 3312. https://doi.org/10.3390/ijms20133312
- 564 Kirakci, K., Cordier, S., Perrin C., 2005. Synthesis and characterization of Cs₂Mo₆X₁₄ (X= Br
- or I) hexamolybdenumcluster halides: efficient Mo₆ cluster precursors for solution chemistry
 syntheses, Z. Anorg. Allg. Chem., 631, 411-416. https://doi.org/10.1002/zaac.200400281
- Kirakci, K., Kubát, P., Dušek, M., Fejfarová, K., Šícha, V., Mosinger, J., Lang, K., 2012. A
 highly luminescent hexanuclear molybdenum cluster a promising candidate toward
 photoactive materials, Eur. J. Inorg. Chem., 19, 3107-3111. https://doi.org /10.1002/ejic.
 201200402
- Kirakci, K., Kubát, P., Kučeráková, M., Šícha, V., Gbelcová, H., Lovecká, P., Grznárová, P.,
 Ruml, T., Lang, K., 2016. Water-soluble octahedral molybdenum cluster compounds
 Na₂[Mo₆I₈(N₃)₆] and Na₂[Mo₆I₈(NCS)₆]: Syntheses, luminescence, and *in vitro* studies,
 Inorganica Chimica Acta, 441, 42-49. https://doi.org/10.1016/j.ica.2015.10.043
- Kirakci, K., Zelenka, J., Rumlová, M., Martincik, J., Nikl, M., Ruml, T., Lang K., 2018.
 Octahedral molybdenum cluster as radiosensitizers for X-ray induced photodynamic therapy,
 J. Mater. Chem. B, 6, 4301-4307. https://doi.org/10.1039/c8tb00893k
- Kirakci, K., Zelenka, J., Rumlová, M., Cvačka, J., Ruml, T., Lang K., 2019. Cationic
 octahedral molybdenum cluster complexes functionalized with mitochondria-targeting
 ligands: photodynamic anticancer and antibacterial activities, Biomater. Sci., 7, 1386-1392.
 https://doi.org/10.1039/c8bm01564c
- 582 Maverick, A.W., Najdzionek, J.S., MacKenzie, D., Nocera, D.G., Gray, H.B., 1983. 583 Spectroscopic, electrochemical, and photochemical properties of molybdenum (II) and

- 584 tungsten (II) halide clusters, J. Am. Chem. Soc., 105, 1878-1882. https://doi.org 585 /10.1021/ja00345a034
- Nayak, S.K., Amela-Cortes, M., Roiland, C., Cordier, S., Molard, Y., 2015. From metallic
- cluster-based ceramics to nematic hybrid liquid crystals: a double supramolecular approach,
 Chem. Commun., 51, 3774-3777. https://doi.org/10.1039/c4cc10085
- Neaime, C., Amela-Cortes, M., Grasset, F., Molard, Y., Cordier, S., Dierre, B., Mortier, M., Takei, T., Takahashi, K., Haneda, H., Verelst, M., Lechevallier, S., 2016. Time-gated luminescence bioimaging with new luminescent nanocolloids based on $[Mo_6I_8(C_2F_5COO)_6]^{2-}$ metal atom clusters, Phys. Chem. Chem. Phys., 18, 30166-30173. https://doi.org/ 10.1039/c6cp05290h
- Robin, M., Dumait, N., Amela-Cortes, M., Roiland, C., Harnois, M., Jacques, E., Folliot, H., Molard, Y., 2018. Direct integration of red-NIR emissive ceramic-like $A_nM_6X_8^iX_6^a$ metal cluster salts in organic copolymers using supramolecular interactions, Chemistry, 24, 4825-4829. https://doi.org/10.1002/chem.201800860
- Solovieva, A.O., Vorotnikov, Y.A., Trifonova, K.E., Efremova, O.A., Krasilnikova, A.A.,
 Brylev, K.A., Vorontsova, E.V., Avrorov, P.A., Shestopalova, L.V., Poveshchenko, A.F.,
 Mironovand, Y.V., Shestopalov, M.A., 2016. Cellular internalisation, bioimaging and dark
 and photodynamic cytotoxicity of silica nanoparticles doped by {Mo₆I₈}⁴⁺metal clusters, J.
 Mater. Chem. B, 4, 4839. https://doi.org/10.1039/c6tb00723f
- 503 Svezhentseva, E.V., Solovieva, A.O., Vorotnikov, Y.A., Kurskaya, O.G., Brylev, K.A., 504 Tsygankova, A.R., Edeleva, M.V., Gyrylova, S.N., Kitamura, N., Efremova, O.A., 505 Shestopalov, M.A., Mironov Y.V., Shestopalov, A.M., 2017. Water-soluble hybrid materials 506 based on $\{Mo_6X_8\}^{4+}$ (X = Cl, Br, I) cluster complexes and sodium polystyrene sulfonate, New 507 J. Chem., 41, 1670-1676. https://doi.org/10.1039/c6nj03469a
- Vorotnikov, Y.A., Solovieva, A.O., Trifonova, K.E., Efremova, O.A., Chirtsova, N.A.,
 Brylev, K.A., Krasilnikova, A.A., Edeleva, M.V., Tsygankova, A.R., Smolentsev, A.I.,
 Vorontsova, E.V., Avrorov, P.A., Shestopalova, L.V., Kitamura, N., Poveshchenko, A.F.,
 Mironov, Y.V., Shestopalov, M.A., 2016. On the synthesis, characterization and biological
 properties of luminescent hybrid particles: Mo₆ metal cluster/SiO₂, RCS Adv. 6, 4336743375. https://doi.org/10.1039/c6ra04321f

Vorotnikov, Y.A., K.E., Efremova, O.A., Novozhilov, I.N., Yanshole, V.V., Kuratieva, N.V.,
Brylev, K.A., Kitamura, N., Mironov, Y.V., Shestopalov, M.A., 2017. Hexaazide octahedral
molybdenum cluster complexes: Synthesis, properties and the evidence of hydrolysis, Journal
of Molecular Structure, 1134, 237-243. https://doi.org/10.1016/j.molstruc.2016.12.052

Vorotnikova, N., Edeleva, M., Kurskaya, O., Brylev, K., Shestopalov, A., Mironov, Y.,
Sutherland, A., Efremova O., Shestopalov, M., 2017. One-pot synthesis of {Mo₆ I₈}⁴⁺doped
polystyrene microspheres via a free-radical dispersion copolymerisation reaction. Polymer
International, 10, 5473. https://doi.org/10.1002/pi.5473

622 Vorotnikova, N.A., Alekseev, A. Y., Vorotnikov, Y. A., Evtushok, D. V., Molard, Y., Amela-

623 Cortes, M., Cordier, S., Smolentsev, A. I., Burton, C. G., Kozhin, P., M., Zhu, P., Topham, P.

624 D., Mironov, Y.V., Bradley, M., Efremova, O.A., Shestopalov, M.A., 2019. Octahedral

625 molybdenum cluster as a photoactive antimicrobial additive to a fluoroplastic. Materials

626 Science & Engineering C, 105, 110150–110150. https://doi.org/10.1016/j.msec.2019.110150

Figure captions 628

Figure 1. General structure of hexanuclear molybdenum halide cluster unit $(Mo_6L_8^{i}L_6^{a})$ 629 where L^{i} and L^{a} represent inner (Br or I in our study) and apical (Br or $(OOC_{2}F_{5})_{6}$ in our 630 study) ligands, respectively and A⁺ represents the counter cation $((C_4H_9)_4N^+ = TBA^+ \text{ or } Cs^+ \text{ in }$ 631 our study). 632

Figure 2. Characterization of CNPs (P/C ratio = 2.5). A) TEM images of TBA₂ (left), CMIF 633

(middle) and CMB (right) and B) photographs of water suspensions under daylight (left

634 images / yellow colour) and UV light at 365 nm (right images / red phophorescence) for 635

TBA₂ (left), CMIF (middle) and CMB (right). 636

Figure 3. Water suspended CNPs stability upon 3 months storage at 4°C and protected from 637 light. Blank columns show particle size results, PDI are shown as lines and filled columns 638 show zeta potential values (each bar is the mean \pm SD of 3 determinations). 639

Figure 4. Water suspended CNPs UV-visible spectra evolution as a function of time (hour) 640 for TBA₂, CMIF and CMB (P/C ratio = 2.5). 641

Figure 5. Percent cluster compound remaining unchanged at a function of time from water 642 suspended CNPs with P/C ratios of 1 and 2.5. 643

Figure 6. In vitro release study of cluster compound TBA2, CMIF and CMB from water 644 suspended CNPs with 3 different P/C ratios. Each point represents the mean \pm SD of 3 645 646 differents batches.

Figure 7. Emission spectra recorded at λ_{exc} = 365 nm for TBA₂ in powder (black signal) and 647 for TBA₂ loaded nanoparticles (P/C ratio = 2.5) as nanosuspension in water (greysignal) or 648 solid form following freeze-drying of the nanosuspension (light grey signal). 649

Figure 8. Left: Solid state emission spectra of CMIF (black) and CMIF loaded nanoparticles 650

(P/C ratio = 2.5) (grey); Right: Solid state emission spectra of CMIF loaded nanoparticles 651

with 3 different P/C ratios. 652

Figure 9. Evolution of emission signal recorded at λ_{exc} = 365 nm for solid CMIF loaded 653

nanoparticles (P/C = 2.5) with saturation of atmosphere with N_2 654

Declaration of interests

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

□The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

	Size (nm)	PDI	Zeta Potentiel (mV)
TBA ₂ - PLGA ^a	85.7 ± 4.9	0.12 ± 0.02	-25.8 ± 4.4
CMIF - PLGA ^a	102.0 ± 2.6	0.16 ± 0.01	-38.2 ± 1.7
CMB - PLGA ^a	75.7 ± 2.5	0.16 ± 0.02	-22.4 ± 9.8
TBA2 - PLGAb	95.0 ± 6.1	0.12 ± 0.01	-42.2 ± 8.0
CMIF - PLGA ^b	124.7 ± 2.5	0.08 ± 0.02	-47.8 ± 4.6
CMB - PLGA ^b	99.3 ± 4.6	0.12 ± 0.01	-47.8 ± 9.1
TBA ₂ - PLGA ^c	141.7 ± 6.4	0.09 ± 0.02	-24.8 ± 1.3
CMIF - PLGA ^c	144.7 ± 4.7	0.09 ± 0.03	-31.5 ± 3.6
CMB - PLGA ^c	118.3 ± 4.3	0.12 ± 0.01	-43.1 ± 6.5

P/C ratio **a**) 1; **b**) 2.5 and **c**) 5

Table 1. Size (nm), polydispersity index (PDI) and zeta-potential (ZP) for all water suspended CNPs with 3 different P/C ratios (each value is the mean \pm SD of 3 determinations).

	EE %	CL	MY %
TBA ₂ - PLGA ^a	63.1 ± 7.5	50.9 ± 3.5	78.1 ± 10.2
CMIF - PLGA ^a	68.1 ± 5.2	73.6 ± 12.9	83.1 ± 12.8
CMB - PLGA ^a	61.7 ± 6.3	73.9 ± 9.4	69.2 ± 4.1
TBA ₂ - PLGA ^b	85.6 ± 12.1	38.0 ± 13.5	76.2 ± 4.8
CMIF - PLGA ^b	96.3 ± 1.8	69.8 ± 6.5	78.4 ± 7.0
CMB - PLGA ^b	70.3 ± 7.8	47.8 ± 12.0	67.7 ± 18.8
TBA ₂ - PLGA ^c	60.3 ± 4.5	29.2 ± 5.6	79.5 ± 7.1
CMIF - PLGA ^c	96.9 ± 2.3	55.0 ± 9.4	81.0 ± 2.2
CMB - PLGA ^c	81.4 ± 6.8	34.9 ± 3.0	72.1 ± 11.2

P/C ratio **a)** 1; **b)** 2.5 and **c)** 5

Table 2. EE%, CL and MY% for all CNPs with 3 different P/C ratios (each value is the mean \pm SD of 3 determinations).

$HO \begin{bmatrix} CH_3 \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\$	C=O stretching 1749 cm ⁻¹	C-0 ester stretching 1088 cm ⁻¹	Sp3 C-H bond 2914 cm ⁻¹
TBA ₂ - PLGA ^a	1752	1088	2924
CMIF - PLGA ^a	1750	1084	2922
CMB - PLGA ^a	1751	1085	2921
TBA ₂ - PLGA ^b	1752	1080	2923
CMIF - PLGA ^b	1752	1084	2922
CMB - PLGA ^b	1751	1083	n.d.
TBA ₂ - PLGA ^c	1750	1084	2924
CMIF - PLGA ^c	1749	1084	2923
CMB - PLGA ^c	1750	1084	2923

P/C ratio **a**) 1; **b**) 2.5 and **c**) 5; n.d: result not determined Bold values mean significant shifts

Table 3. FT-IR vibrations obtained with PLGA blank nanoparticles characteristic bonds and their mofification following cluster loading in PLGA nanoparticles.

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	$\lambda_{em}(\mathbf{nm})$	ϕ_{em} solid state (in air)	$\phi_{\rm em}$ water suspension (aerated)
TBA ₂	785	0.22 ^a	n.d.
TBA2 - NP 1	785	0.120	0.066
TBA ₂ - NP 2.5	785	0.137	0.066
TBA₂ - NP 5	785	0.143	0.067
CMIF	660	0.35 ^b	n.d.
CMIF - NP 1	675	0.013	0.014
CMIF - NP 2.5	675	0.013	0.016
CMIF - NP 5	675	0.017	0.016
СМВ	767	0.14 ^c	n.d.
CMB - NP 1	767	0.032	0.020
CMB - NP 2.5	767	0.032	0.019
CMB - NP 5	767	0.038	0.020

^{a, b, c}: values obtained previously in our team (same experimental conditions and apparatus) by Amela-Cortes (2014), Robin (2018) and Nayak (2015), respectively.

Table 4. Emission maximum wavelengths (λ_{em}) and absolute quantum yield (ϕ_{em}) values obtained at $\lambda_{exc} = 365$ nm for cluster compounds in solid state and CNPs in solid state or as nanosuspension in water.



A)



B)





















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