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Koceïla Doufène, Vincent Lapinte, Philippe Gaveau, Gautier Félix, Thomas Cacciaguerra, Joel Chopineau, Jean-Jacques Robin, Jean-Marie Devoisselle, Anne Aubert-Pouëssel

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6 Koceïla Doufène, Vincent Lapinte, Philippe Gaveau, Gautier Félix, Thomas Cacciaguerra, Joël

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Chopineau, Jean-Jacques Robin, Jean-Marie Devoisselle, Anne Aubert-Pouëssel\*.

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9

*ICGM, Univ Montpellier, CNRS, ENSCM, Montpellier, France.*

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\* **Corresponding Author Email Address:** [anne.aubert@umontpellier.fr](mailto:anne.aubert@umontpellier.fr)

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## 12 | **ABSTRACT**

13 To encapsulate and deliver poorly water-soluble drugs, castor oil/silica hybrid microparticles  
14 (HMP)s were synthesized. Green chemistries were used to silylate the oil and further cross-  
15 link it into solid microparticles by sol-gel reaction. Silylated castor oils (ICO)s at various  
16 silylation ratios were prepared and allowed the solubilization of ibuprofen at several  
17 concentrations up to 16 wt%. The HMPs were formulated by ThermoStabilized Emulsion (TSE)  
18 process which permits to “freeze” the oil-in-water emulsion while the sol-gel reaction occurs.

19 The hybrid mineral / organic composition and the morphology (spherical shape and  
20 micrometric size) of these HMPs were determined by complementary technics (SEM, TGA,  
21 EDX, <sup>29</sup>Si NMR and FTIR spectroscopies).

22 The HMPs reached a good ibuprofen loading efficiency regardless to the formulation used  
23 while the release kinetics in simulated oral administration exhibited a tunable release during 3  
24 hours according to the silylation ratio. The ibuprofen rate also influenced its own amorphous  
25 or crystalline character within the HMPs. For subcutaneous conditions, ibuprofen release took  
26 place over 15 days. Finally, biodegradability assays in simulated digestion medium suggested a  
27 surface-limited hydrolysis of the particles and cytocompatibility studies on NIH-3T3 and Caco-  
28 2 cells demonstrated an excellent cellular viability.

## 29 | **ABBREVIATIONS:**

30 API: Active Pharmaceutical Ingredient = drug; CO: Castor Oil; EDX: Energy Dispersive X-ray;  
31 (F)FA: (Free) Fatty Acid; FTIR: Fourier Transform Infrared; HMP: Hybrid Microparticle; ICO:  
32 silylated castor oil; LBDDS: Lipid-Based Drug Delivery System; NMR: Nuclear Magnetic  
33 Resonance; O/W: Oil-in-Water; SEM: Scanning Electronic Microscopy; SGF: Simulated Gastric  
34 Fluid; SIF: Simulated Intestinal Fluid; TG: Triglyceride; TGA: Thermogravimetric Analysis; TSE:  
35 ThermoStabilized Emulsion, wt%: weight percentage.

36

## 37 | **KEYWORDS:**

38 Vegetable oil; Sol-gel; Silica; Hybrid microparticle; Drug delivery system; Poorly water-soluble  
39 drug.

# 40 | ARTICLE

## 41 | 1. Introduction

42         The continuous improvement of health conditions over the last decades increased the  
43 incidence of so-called "ageing" and "civilization" diseases such as diabetes, hypertension,  
44 neurological degenerations and cancers. The pharmaceutical research and the development  
45 of Active Pharmaceutical Ingredients (API)s are therefore in great demand. However, the vast  
46 majority of these APIs have solubility issues and their "drug-like" profiles are compromised  
47 during early stages of the development (Kruse et al., 2008). Several formulations have been  
48 studied to overcome this limit. Lipid-based drug delivery systems (LBDDS) dominated these  
49 formulations owing to their high solvation capacity on poorly water-soluble APIs. The  
50 composition of LBDDS varied according widely to its use, ranging from simple lipids such as  
51 fatty acids (FA)s and triglycerides (TG)s to more complex systems like self micro- and nano-  
52 emulsifying LBDDS which contain phospholipids, surfactants, co-surfactants and co-solvents in  
53 addition to FA and TG. Liposomes were predominant as LBDDS for the parenteral  
54 administration and many formulations were commercialized such as Ambisome®  
55 (amphotericin B) or Doxil® (doxorubicin) (Chen and Yazdi, 2013). In addition, solid lipid  
56 particles showed a high potential of drug entrapment and delivery via parenteral route.  
57 Indeed a wide variety of chemotoxics (paclitaxel, camptothecin), corticoids (hydrocortisone,  
58 prednisolone) and anesthetics (tetracaine, etomidate) was loaded into solid lipid  
59 nanoparticles and their preclinical trials were promising (Wissing et al., 2004). For the oral  
60 administration, the Lipid Formulation Classification System divided the LBDDS into four types  
61 depending on their composition and their behavior in the gastrointestinal gut (Pouton and  
62 Porter, 2008). The type I consists in digestible oils without surfactants whereas the type II  
63 contains water-insoluble ones. The type III is made of oils, surfactants and cosolvents. It is  
64 subdivided into IIIA, which is predominantly made of oil, and IIIB, which contains more water-  
65 soluble components. The type IV is free of oils and contains only self-assembling surfactants  
66 and cosolvents, which makes it unlikely digestible. Despite the interest of LBDDS, they require  
67 large quantities of organic solvents that represent a non-valuable industrial input and  
68 required additional purification of the final product. On the other hand, many complex  
69 approaches were considered to solidify these LBDDS in order to improve their  
70 pharmacotechnical properties and enhance their stability (Kalepu et al., 2013). For example,

71 the spray-congealing consists in a molten lipid sprayed into a cooling chamber to form cooled  
72 solid particles (e.g., diclofenac in stearyl polyoxyglycerides “Gelucire® 50/13”) whereas the  
73 spray-drying consists on a solution of drug and lipid excipients sprayed inside an atomizing  
74 chamber (e.g., glibenclamide in silicon dioxide and Gelucire®), to form spherical solid particles  
75 in both cases. Carbon dioxide in supercritical physical state was also involved in the  
76 solubilization and reprecipitation of lipid excipients for drug coatings (e.g., carbamazepine in  
77 vitamin E, TPGS and Gelucire® 44/14). Unfortunately, these processes remain highly energy-  
78 intensive and require complex equipments.

79 To circumvent the use of organic solvents and complex manufacturing processes, an  
80 original approach combining vegetable oil recovery, eco-friendly chemistry and simple  
81 pharmacotechnology process was investigated. As firstly demonstrated by Gallon et al. (Gallon  
82 et al., 2017), bio-based hybrid microparticles for drug delivery have already been synthesized  
83 by this technology coupling castor oil silylation and a thermostabilized emulsion (TSE) process.  
84 Resulting microdroplets of silylated castor oil (ICO) were cross-linked by a sol-gel reaction  
85 inducing the solidification of the organic / inorganic hybrid microparticles (HMP)s at ambient  
86 temperature. Preliminary tests were carried out on the microparticles to optimize their  
87 preparation process, to characterize their structure and to assess their ability to entrap  
88 ibuprofen as model of poorly water-soluble API.

89 Herein, the HMPs drug delivery kinetics were investigated using several castor oil-based  
90 formulations that differ either in the organic/inorganic ratio of the ICO or the ibuprofen  
91 loading rate. The TSE process was optimized to improve the biocompatibility of the HMPs for  
92 a pharmaceutical application using a catalyst approved by the US Food and Drug  
93 Administration (US-FDA). Comprehensive and multi-scale physicochemical studies were  
94 achieved on synthesized particles regarding to their shape and size, condensation degree,  
95 organic/inorganic composition and atomic distribution. We designed several types of  
96 ibuprofen-loaded HMPs, and monitored their release kinetics in various simulated media. The  
97 drug release kinetics were characterized by mathematical modeling to prove the influence of  
98 the matrices on the API diffusion. To conclude this screening, the hybrid microparticles  
99 degradability in simulated digestion medium was studied and their biocompatibility assessed  
100 on NIH-3T3 (fibroblasts) and Caco-2 cells (enterocytes-like cells).

## 101 **2. Materials and methods**

### 102 **2.1. Materials**

103 Pharmaceutical grade castor oil (CO;  $934 \text{ g}\cdot\text{mol}^{-1}$ ) was purchased from Cooper  
104 Pharmaceutique. (3-Isocyanatopropyl)triethoxysilane (IPTES;  $247.3 \text{ g}\cdot\text{mol}^{-1}$ ), stannous octoate  
105 (SnOct;  $405.1 \text{ g}\cdot\text{mol}^{-1}$ ),  $\kappa$ -carrageenan, ibuprofen ( $206.3 \text{ g}\cdot\text{mol}^{-1}$ ) and flurbiprofen ( $244.3 \text{ g}\cdot\text{mol}^{-1}$ ),  
106 bile extract porcine, pancreatin from porcine pancreas ( $4 \times$  USP specifications) and  
107 polysorbate 80 (PS80; Tween<sup>®</sup> 80) were purchased from Sigma-Aldrich and Soybean  
108 phospholipids (S100) from Lipoid. Solvents such as acetonitrile HPLC plus Gradient (ACN),  
109 acetic acid, methanol (MeOH) and salts such as sodium chloride (NaCl), sodium phosphate  
110 dibasic ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ ) and potassium phosphate monobasic ( $\text{KH}_2\text{PO}_4$ ) were also supplied  
111 by Sigma-Aldrich, while potassium chloride (KCl) was provided by Panreac Quimica, calcium  
112 chloride ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ) by Merck and sodium acetate ( $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ ) by VWR chemicals.

### 113 **2.2. Silylation of the castor oil**

114 Castor oil, which contains 90 % of ricinoleic acid, was functionalized with IPTES for 72 hours at  
115  $60 \text{ }^\circ\text{C}$  under nitrogen atmosphere, following a solvent- and catalyst-free process (Fig. 1). Four  
116 ratios of silylation  $X_R$  between IPTES and CO were established: 1.05, 0.8, 0.6 and 0.4. These  $x_R$   
117 corresponded to the percentage of CO hydroxyl groups related to the isocyanate groups of  
118 IPTES (1.05 = 100 % with 5 % excess of free IPTES; 0.8 = 80 %; 0.6 = 60 % and 0.4 = 40 %). The  
119 silylated castor oils (ICO)s were aimed to synthesize several types of HMPs, designated with  
120 the same  $x_R$ . The completion of the reaction was checked by ATR-IR spectroscopy with a  
121 Spectrum 100 from Perkin Elmer (Li et al., 2016).

### 122 **2.3. Formulation of the hybrid microparticles**

123 As described in our previous paper (Gallon et al., 2017), HMPs were formulated according to  
124 an original process (Fig. 2) based on a thermostabilized oil-in-water emulsion (TSE). The TSE  
125 process was optimized for a pharmaceutical application by the use of SnOct, a US-FDA  
126 approved catalyst for food packaging (Department of Health and Human Services, 2018) and  
127 biomedical devices (Niemeyer and Mirkin, 2007, p. 296), instead of Dibutyltin dilaurate  
128 (DBTDL). The oil phase was composed of ICO with 1 % of SnOct  $\pm$  ibuprofen dissolved at  
129 several rates ranging from 4 wt% up to 16 wt% into the ICO, prior to its formulation. Thus  
130 each formulation differed either by the ICO amount (0.4, 0.6, 0.8, 1.05) or by the rate of  
131 ibuprofen dissolved (4, 8, 12 and 16 wt%). The aqueous phase consisted of an acetate

132 aqueous buffer (2N) at pH=2.8 with 0.5 wt% of  $\kappa$ -carrageenan (thermogelling agent) and 0.3  
 133 wt% of KCl (thermogel stabilizer (Mangione et al., 2005)). The O/W emulsion was formed at  
 134 60 °C with a T 18 digital Ultraturrax<sup>®</sup> (IKA) at 9000 rpm for 2 min, then a thermal shift (from 60  
 135 °C to 4 °C) was applied in order to gel the aqueous phase and stabilize the oil droplets in which  
 136 the sol-gel reaction occurred at 25 °C. The acetic acid within the acetate buffer leads to a  
 137 better hydrolysis of ethoxysilanes while SnOct improves the network condensation (Brinker,  
 138 1988; Shi et al., 2012). This combination of catalyst (SnOct) and co-catalyst (acetic acid)  
 139 permitted to accelerate the sol-gel reaction down to 8 days. Once HMPs were solidified, the  
 140 gel was disrupted by heating to 60 °C. The HMPs were washed with distilled water, separated  
 141 by centrifugation at 4000 rpm for 6 min and freeze-dried (Heto Powerdry<sup>®</sup> LL3000, Thermo  
 142 Fisher Scientific). After recovery, preparation yields  $\eta$  were calculated by dividing the weight  
 143 of dried particles by the initial mixture weight (ICO + catalyst  $\pm$  API) (eq. 1)

$$144 \quad \eta = \frac{\text{Weight of recovered particles}}{\text{initial oil phase weight}} \quad (1)$$

## 145 **2.4. Characterization of the hybrid microparticles**

### 146 **2.4.1. Physicochemical characterization**

147 Firstly, the HMP morphologies were analyzed both by optical microscopy (AxioLab<sup>®</sup> equipped  
 148 with AxioCam<sup>®</sup> ERc 5s model, Zeiss) and scanning electron microscopy (SEM, Hitachi S4800  
 149 high resolution). HMPs size measurements were assessed by laser diffraction (Mastersizer<sup>®</sup>  
 150 2000, Malvern instruments) in a Hydro 2000SM dispersion unit with absolute ethanol as a  
 151 dispersion medium. This technique allowed to draw volume size distributions of the HMPs, to  
 152 determine Volume Median Diameters\* (VMD) and to deduct Moment means (Rawle, 2018).  
 153 The volume moment mean  $D[4,3]^{\dagger}$  was interesting in our case because it takes into account  
 154 the spherical volume of HMPs. For the entrapped API, the distance to reach the surface  
 155 depends indeed on this volume. As well, surface area moment mean  $D[3,2]^{\ddagger}$  is even of  
 156 interest considering that API release is modulated by the extent of HMPs surface area. A  
 157 Kruskal-Wallis test in Anasats<sup>®</sup> software was used to compare the results and  $P < 0.05$  value  
 158 was considered statistically significant.

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\* The Volume Median Diameter refers to the midpoint particle size where half of the total volume is in HMP smaller, and half in HMP larger.

$\dagger D[4,3] = \frac{\sum d^4}{\sum d^3}$

$\ddagger D[3,2] = \frac{\sum d^3}{\sum d^2}$

159 The sol-gel cross-linking (hydrolysis and condensation) was monitored with IR-ATR  
160 spectroscopy (Spectrum 100, Perkin Elmer) and the condensation was quantified by solid state  
161 <sup>29</sup>Si-NMR (Varian VNMRS 400 MHz [9.4T] NMR spectrometer, with a 7.5 mm Varian T3 HX  
162 MAS probe spinning at 5 kHz). Two sequences of NMR at Magic Angle Spinning (MAS) were  
163 assessed: a Cross Polarization (CP-MAS) and a Single Pulse sequence (SP-MAS) with <sup>1</sup>H  
164 decoupling. CP-MAS experiments were performed with a 1.5 ms contact time and a recycle  
165 delay of 5 s in order to measure chemical shifts corresponding to silica fraction units present  
166 in each sample. For the quantitative determination of identified silica fraction units, SP-MAS  
167 experiments were carried out using a 2μs 30° pulse and a recycle delay of 60 seconds. Spectra  
168 were accumulated overnight in order to increase the signal-to-noise ratio. Dmfit program  
169 (Massiot et al., 2002) was used to fit peaks and areas under curves were integrated for each  
170 unit and expressed in percent.

171 In order to determine the atomic composition and the API disposition within particles,  
172 flurbiprofen-loaded HMPs were studied by energy dispersive X-ray (EDX, FEI Quanta 200 FEG  
173 SEM). One micrometer cube was analyzed at each dot and backscattered-electrons were  
174 recorded (Oxford Instruments, X-Max<sup>N</sup> Silicon Drift Detector). Flurbiprofen as well as  
175 ibuprofen is a nonsteroidal anti-inflammatory drug belonging to aryl carboxylic family (Fig.  
176 A.1). It was selected for its fluorine atom that can be mapped by EDX within HMPs. The  
177 mineral part of HMPs was also estimated by thermogravimetric analysis (TGA; STA 6000,  
178 PerkinElmer) from 25 to 900 °C at 10 °C.min<sup>-1</sup> under airflow.

#### 179 **2.4.2. Pharmaceutical characterization**

180 The loading efficiencies of ibuprofen-loaded HMPs at 4, 8, 12 and 16 wt% were determined  
181 by dispersing 50 mg of each formulation in 10 mL of methanol overnight, prior to an HPLC  
182 dosage (LC-2010HT Shimadzu, static phase: C18 Protonsil® column from Bischoff, mobile  
183 phase: acetonitrile/ 0.5 % acetic acid solution, 65/35 v/v). Encapsulation yields were  
184 calculated using eq. 2:

$$185 \quad \text{Encapsulation Yield} = \text{Loading rate after formulation} \times \eta' \quad (2)$$

186 Where  $\eta'$  is the corrected preparation yield (taking into account the hydroxyethyl loss – see  
187 results).



188 The crystalline state of the entrapped ibuprofen was also checked. Two analyses were done:  
189 X-Ray powder Diffraction (XRD; Bruker D8 advance generator equipped with copper tube and  
190 1d LYNXEYE detector,  $\lambda = 1,5406 \text{ \AA}$ , acquisition from 5 to  $40^\circ$  of  $2\Theta$  angle) to detect ibuprofen  
191 crystals and Differential Scanning Calorimetry (DSC; DSC4000, PerkinElmer, sample heated  
192 from 20 to  $95^\circ\text{C}$  at  $10^\circ\text{C}\cdot\text{min}^{-1}$  under  $50 \text{ mL}\cdot\text{min}^{-1}$  of nitrogen flow) to track the melting point  
193 of API crystals within the matrix.

194 Furthermore, *in vitro* releases were assessed from ibuprofen-loaded HMPs using flow-through  
195 cell apparatus (Sotax CE1) according to the United States Pharmacopoeia (USP) IV  
196 specifications for poorly water soluble APIs dissolution (Paprskářová et al., 2016). Three  
197 protocols were established for the purpose, depending on the buffer system used: in protocol  
198 **(1)**, 25 mL of Simulated Gastric Fluid (SGF, hydrochloride solution containing 0.1 wt% PS80, pH  
199 = 1.2 at  $37^\circ\text{C}$ ) were used as a release medium for 30 minutes, then 25 mL of an intermediate  
200 solution were added in order to reach 50 mL of a Simulated Intestinal Fluid (SIF, 50 mM of  
201 phosphate buffer containing 0.1 wt% of PS80, pH = 6.8 at  $37^\circ\text{C}$ ) whereas in protocols **(2)** and  
202 **(3)**, 50 mL of media were directly used as follows **(2)**: SIF, 50 mM of phosphate buffer  
203 containing 0.1 % of PS80, pH = 6.8 at  $37^\circ\text{C}$  and **(3)**: 12 mM of phosphate buffer without PS80,  
204 pH = 7.4 at  $37^\circ\text{C}$  (Stippler et al., 2004). PS80 is a hydrophilic surfactant used here at 0.1 wt%  
205 to decrease the surface tension of release media down to  $42 \text{ mN}\cdot\text{m}^{-1}$ . The low surface  
206 tensions of gastric juice reported between 35 and  $45 \text{ mN}\cdot\text{m}^{-1}$  (Efentakis and Dressman, 1998;  
207 Finholt and Solvang, 1968) and the intestinal fluid around  $50 \text{ mN}\cdot\text{m}^{-1}$  (Klein, 2010) were thus  
208 simulated, ensuring an optimal dispersion and wettability of HMPs. The system was then  
209 connected in closed loop through powder cells containing 50 mg of microparticles. 1 mL  
210 samples were collected at defined times prior to an HPLC dosage and 1 mL of fresh medium  
211 was added after each sampling to maintain the volume constant.

212 A mathematical model for 4 wt% ibuprofen-loaded HMPs releases in Simulated Intestinal Fluid  
213 (SIF) was established and described in Appendix B.

### 214 **2.4.3. Biological characterization**

215 Biodegradability and biocompatibility of HMPs were assessed. Biodegradability assays were  
216 achieved on the particles following a simulated digestion protocol. An AT7 smart Dissolution  
217 Apparatus from Sotax (USP II) was used as an incubation system: 500 mg of microparticles  
218 were dispersed in 50 mL of a pre-digestion medium at  $37^\circ\text{C}$ , with continuous stirring (see

219 Table A.1 for composition). As a reference, 500 mg of CO were emulsified in 50 mL of pre-  
220 digestion medium following the same protocol as used for the HMPs formulation (9000 rpm  
221 for 2 min with T 18 digital Ultraturrax®). The lipase was then added to initiate the digestion. 1  
222 mL samples were collected at defined times and their pHs were consecutively measured using  
223 a SevenCompact pH-meter equipped with an Inlab® Micro sensor (Mettler Toledo). 1mL of an  
224 aqueous solution of CaCl<sub>2</sub> ([Ca<sup>2+</sup>] = 36mM) was added in each medium in order to maintain  
225 media volumes constant while the Ca<sup>2+</sup> continuously precipitate the Free Fatty Acids (FFAs)  
226 liberated during the digestion (Zangenberg et al., 2001). These FFAs, composed at 90 % of  
227 ricinoleic acid (pKa= 4.74), 4 % of linoleic acid (pKa=4.77) and 3 % of oleic acid (pKa=5.02),  
228 were able to exhibit their acidities at pH of the medium (pH=6.8). Thus, a pH drop versus time  
229 curve was drawn for each sample in order to monitor the digestion (Mosgaard et al., 2015).

230 On the other hand, cytotoxicity studies of the HMPs were conducted according to the ISO  
231 10993 norm. The cytocompatibility of the particles was assessed by following the viability of  
232 two cell lines: Caco-2/TC7 enterocytes and NIH-3T3 fibroblasts. These cell lines were selected  
233 to simulate an intestinal environment for an oral administration of HMPs and a dermal  
234 environment for a subcutaneous administration, respectively. Cells were treated with  
235 microparticles extracts using a CellTiter 96® AQ cell proliferation assay (Promega) composed  
236 of a tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-  
237 sulfophenyl)-2H-tetrazolium, inner salt: MTS) and an electron coupling reagent (phenazine  
238 methosulfate: PMS). The cells were seeded at 5000/well density into a 96-well culture dish  
239 plate containing 200 µL/well of a culture medium (Dulbecco's Modified Eagle Medium:  
240 DMEM) and permitted to adhere at 37 °C and 5 % CO<sub>2</sub> for 24 h. Subsequently, they were  
241 treated with various samples (particle suspensions: 0.1, 1 or 10 mgmL<sup>-1</sup>, incubated 2 days at  
242 37 °C). After 24 h of exposure, 20 µL of a mixture MTS-PMS was added according to the  
243 manufacturer instructions for 4 h reaction with the cells. The assay plate was read at 490 nm  
244 using a microplate reader (Multiskan Go, Thermo Fisher Scientific). The absorbance of the  
245 untreated cells (control group) corresponded to the 100 %.

## 246 **3. Results and discussion**

### 247 **3.1. Castor oil functionalization and hybrid microparticles** 248 **formulation**

249 Castor oil was silylated with IPTES in solvent- and catalyst-free conditions corresponding to  
250 the first stage of the HMPs preparation. For the four ratios  $X_R$  studied, the silylation reaction  
251 was followed by IR-ATR spectroscopy (Fig. 3) wherein two chemical groups could be  
252 monitored: i) the disappearance of the isocyanate group labeling the IPTES (Socrates, 2010),  
253 which exhibits an asymmetric stretching vibration band at  $2270\text{ cm}^{-1}$ ; ii) the appearance of  
254 urethane group characterized by three vibration bands: (a) an amid-I band at  $1720\text{ cm}^{-1}$   
255 corresponding to the C=O stretching vibration; (b) an amid-II band at  $1700\text{ cm}^{-1}$   
256 corresponding to the N-H bending vibration (these two bands were shouldered by the C=O  
257 vibration band of the ricinolein ester function) and (c) a band at  $1240\text{ cm}^{-1}$  resulting from  
258 interaction between C-N stretching and N-H bending (Cannon, 1976; Silverstein et al., 2005, p.  
259 100). However, the disappearance of ricinolein hydroxyl groups cannot be confidently  
260 monitored because of the overlapping between O-H and N-H stretching bands at adjacent  
261 frequencies (around  $3400\text{-}3500\text{ cm}^{-1}$ ).

262 The completion of hydroxy-isocyanate reaction between the IPTES and the ricinolein was  
263 attested for the four silylation ratios. In all cases, isocyanate group transmittance ( $2270\text{ cm}^{-1}$ )  
264 was proportional to the IPTES engaged in each reaction. They were minimal at  $t=0$  and  
265 increased to a 100 % transmittance at  $t_{\text{final}}=72$  hours. By contrast, the urethane group  
266 transmittances ( $1720$ ,  $1700\text{ cm}^{-1}$  and  $1240\text{ cm}^{-1}$ ) decreased during the same period. Four ICOs  
267 with high purity were thus formed exhibiting an oily aspect and transparent to yellowish  
268 appearances. The physicochemical properties of these ICOs (Table 1) were consistent with  
269 their chemical structures: the silylation of the CO increased its molecular weight (from 932 to  
270  $1600\text{ g}\cdot\text{mol}^{-1}$  for the higher  $X_R$ ), which induced an increase of the viscosity (from 661 to 961  
271  $\text{mPa}\cdot\text{s}$ ) and density (from 0.907 to 0.999). During the HMPs formulation, the viscosity is an  
272 important parameter and difference in ICOs viscosities could affect emulsion properties.

273 The second stage of HMPs preparation consists in their formulation by a TSE process in  
274 aqueous medium (Fig. 2). To calculate relevant preparation yields, hydroxyethyl moieties  
275 released during hydrolysis (as described below) had to be taken into account. Therefore,  
276 assuming that the hydrolysis of ethoxysilanes was complete in our case, corrected preparation

277 yields  $\eta'$  were calculated (Table 2) and displayed excellent values ranging from 87.4 to 95.3 %.  
278 The robustness of the process was thus proven since there is no influence of the silylation  
279 ratio on the preparation yield.

### 280 **3.2. Physicochemical characterization of hybrid microparticles**

281 Hybrid microparticles were spherically shaped through the emulsification step of TSE process.  
282 According to ICO amount, various HMP surface states were observed by SEM: for HMPs 1.05,  
283 rough and irregular surface (Fig. 4-A) whereas more regular and smooth textures were  
284 exhibited at the surface of HMPs 0.8 to 0.4 (Fig. 4-B to D). However, some broken particles  
285 were noticed on the overview of HMP 0.4 (Fig. 4-D). It may be explained by the low  $X_R$  of  
286 these particles, which leads to less potential siloxane bonds and results in a weaker cohesion  
287 of these objects. A matrix structure was also uncovered on cross-sections of HMPs revealing a  
288 microsphere organization of the particles (Fig. 8). Furthermore, it can be noticed that the  
289 freeze-dried microspheres were well-isolated and formed powders with good rheological  
290 behaviors.

291 Size measurements put in evidence that for all of HMPs, volume size distributions (Fig. 5)  
292 followed an almost Gaussian curve around 50-60  $\mu\text{m}$  with reasonable spans between 1.38 and  
293 1.67 (Table 3). A slight asymmetry towards high values of size was also observed. We  
294 supposed that it was due to a coalescence phenomenon between the emulsification step and  
295 the thermogel freeze during the TSE process.  $D[4,3]$  ranged from 54.9 to 58  $\mu\text{m}$  and were  
296 quite close to the VMD. In contrast,  $D[3,2]$  were much lower (from 19.9 to 22.6  $\mu\text{m}$ )  
297 highlighting the presence of a significant number of small particles (Piacentini, 2014). A  
298 Kruskal-Wallis test on summarized results in Table 3 proved that there is no statistically  
299 significant difference between all HMPs, neither in  $D[4,3]$  nor  $D[3,2]$ . We concluded that  
300 particle sizes were homogeneous and independent from the physicochemical properties of  
301 the ICO engaged, underlining the predominance of the mechanical stirring influence.

302 The hybrid structure of HMPs was the result of the vegetable oil cross-linking by means of  
303 mineral bonds, during a sol-gel reaction. At the molecular scale, this chemical reaction  
304 proceeds in two phases: a first one of ethoxysilanes hydrolysis leads to free silanols which,  
305 during the second phase of condensation, can react between them to form siloxanes bonds  
306 (Si-O-Si). To offset the progressive inactivation of the catalyst SnOct in the TSE aqueous  
307 medium (oxidation of Sn II into Sn IV), the use of a co-catalyst was investigated to accelerate

308 HMP synthesis down to one week rather than one month. It was previously reported that the  
309 ethoxysilanes residue hydrolysis increases using acetic acid in a co-catalysis mechanism firstly  
310 described by Andrianov (Andrianov, 1965). An anionic substitution of the acetyl- moieties  
311 leads to an ethyl-acetate further eliminated during the microparticle recovery (Pope and  
312 Mackenzie, 1986). To confirm the influence of the acetic medium on the efficiency of cross-  
313 linking, infrared spectra were recorded on HMPs formulated in deionized water (HMPw) or in  
314 acetic buffer (HMPa) using ICO as reference (Fig. 6).

315 The absorbances at 952, 1075 and 1102  $\text{cm}^{-1}$  were assigned to the ethoxysilanes ( $\text{Si-O-CH}_2\text{-}$   
316  $\text{CH}_3$ ) (Leyden and Atwater, 1991; Silverstein et al., 2005, p. 124). An enhanced hydrolysis of  
317 the ethoxysilanes moieties was observed for HMPa related to HMPw. In addition, the broad-  
318 band around 1000  $\text{cm}^{-1}$  attributed to overlapping of the  $\text{-Si-O-Si-}$  and  $\text{-C-Si-O-}$  stretching  
319 peaks (Allauddin et al., 2013) underscores the efficiency of the condensation in HMPa  
320 spectrum. To ensure that the acidic pH did not alter the triglyceride structure, especially the  
321 ester bond between glycerol and ricinoleate, microdroplets of CO formulated in the same  
322 conditions were analyzed by  $^1\text{H}$  NMR spectroscopy and the result showed that the ester bond  
323 remained stable despite the low pH (data not shown). In addition and according to the  
324 literature (Shafiei et al., 2017), the ethyl-acetate generated during ICO formulation does not  
325 trans-esterify the ricinolein in mild conditions.

326 CP-MAS experiments in  $^{29}\text{Si}$ -NMR spectroscopy enhanced the response of silicon nuclei and  
327 perfectly identified the chemical shifts of non condensed ( $\text{T}^0$ ), partially ( $\text{T}^1$ ,  $\text{T}^2$ ) and fully  
328 condensed ( $\text{T}^3$ ) silica species (Fig. 1) in the hybrid network at -48, -53, -57 and -67 ppm,  
329 respectively. The different natures of silica bonds between the ICOs monomers were  
330 quantified by complementary SP-MAS experiments as summarized in Table 4 and Fig.A.2.

331 Unexpectedly, SP-MAS spectra presented a sharp peak (from 0.2 to 0.6 ppm wide) at -45 ppm  
332 with decreasing values from 39.9 % for HMP 1.05 to 1.7 % for HMP 0.6. As far as we searched  
333 in literature, we did not find any description on such thin peak during solid-state  $^{29}\text{Si}$  NMR  
334 experiment in SP-MAS conditions. We supposed that it was a liquid-state profile with  
335 averaged anisotropic interactions and a liquid-state  $^{29}\text{Si}$  NMR experiment was consequently  
336 conducted on uncross-linked ICO 1.05 (Fig. A.3). A strong peak was detected at -45.7 ppm and  
337 assigned to liquid-state ICO monomers of which none of the silanol functions had reacted  
338 (liquid peak = LP) whereas the  $\text{T}^0$  peak represent non-condensed silica species in solid-state

339 (i.e. connected to the hybrid network by other silica species present on the same ICO  
340 monomer). To quantify the sol-gel cross-linking, condensation yields (CY in eq. 3) were  
341 calculated:

$$342 \quad \mathbf{CY = 100\% - LP} \quad \mathbf{(3)}$$

343 A correcting factor (1/CY) was then used to calculate accurate condensation degrees (CD in  
344 eq. 4):

$$345 \quad \mathbf{CD = \frac{T^1+2T^2+3T^3}{3 \times CY}} \quad \mathbf{(4)}$$

346 Owing to the variable intensity of the liquid peak, CDs evolved from 60.1 to 98.3 % for HMP  
347 1.05 and HMP 0.6, respectively, meaning that high silylation ratios did not permit an efficient  
348 condensation. It would seem that the sol-gel reaction had not occurred in some domains  
349 within high silylated HMPs. The rough surface of HMP 1.05 (Fig. 4-A) could be thus explained,  
350 on the assumption that particles partially “collapse” above liquid domains. Nevertheless, CDs  
351 which represent the ability of silylated precursors to bind to each other, were not complete  
352 (around 70-80 %) and similar for the three HMPs (1.05, 0.8 and 0.6). Indeed it was  
353 documented that alkylsilanes with long organic tails don’t reach a complete condensation  
354 owing to an increase of steric hindrance (Delattre and Babonneau, 1994; Peeters et al., 1995).  
355 Moreover, multifunctional silica precursors are often limited to form single siloxane bonds  
356 (Brochier Salon and Belgacem, 2011).

357 TGA analyses were conducted in order to check the hybrid structure of HMPs (Fig. 7). For all  
358 samples, the weight-loss began approximately at 160 °C corresponding to the ibuprofen  
359 degradation (Lerdkanchanaporn and Dollimore, 1997). Then the dissociation of the urethane  
360 bond around 250 °C underscored the degradation onset of the hybrid matrix (Saunders,  
361 1959). From 300 to 600 °C corresponding to the triglyceride disruption, the four curves  
362 differed: higher was the organic part, faster was the weight-loss (HMPs 0.4 > 0.6 > 0.8 > 1.05).  
363 Finally, a stabilization of the weights occurred after 650 °C, highlighting the mineral residue of  
364 each HMP. As expected, the values ranged from 7.4 to 14 % for the highest silylated sample  
365 (HMP 1.05) related to the lowest one (HMP 0.4).

366 It is well known that the location of the API in microspheres governs release kinetics from the  
367 matrices. Therefore, EDX analyses were performed on flurbiprofen-loaded HMPs to study the

368 API location inside the particles using its own fluorine atom as probe. 8 wt% flurbiprofen-  
369 loaded HMP 0.4 were cryofreezed prior to a mechanical shock. Firstly, EDX spectra of fluorine  
370 (K-L<sub>2</sub> and K-L<sub>3</sub> transition<sup>§</sup>), carbon (K-L<sub>2</sub> and K-L<sub>3</sub> transition) and silicium (K-L<sub>3</sub> transition) atoms  
371 were recorded on three HMP 0.4 cross-sections as illustrated in Fig. 8 and summarized in  
372 Table 5. Several positions in the inner and outer regions of HMP were recorded and similar  
373 values ranging from 0.37 to 0.52 % were measured attesting an almost homogeneous API  
374 distribution in the HMPs.

375 The same conclusion was deduced from the silicium and carbon atoms on the homogenous  
376 composition of the hybrid matrix even if the carbon was slightly overestimated due to  
377 undetermined hydrogen atoms. To support this observation, retro-scattered electrons from  
378 carbon and silicium were recorded on HMP 1.05 during 20 min producing an atomic map  
379 depicted in Fig. 9. Despite the presence of carbon in background and some electronic shadows  
380 due to the eccentric position of the SD Detector (at the top-right of the pictures), the scans  
381 exhibited a uniform disposition of carbon (Fig. 9– C) and silicium (Fig. 9– D) on particle  
382 surfaces. It should also be noted that less than 0.3 % of catalyst's tin was detected. In contrast  
383 with the TGA results on HMP 0.4 (i.e. 7.4 % of mineral residue), a smaller part of silicium (1.8  
384 % in average of silicium, corresponding to 4 % in weight) was observed during EDX analyses on  
385 the same sample. The formation of silicon oxides throughout the thermal analysis under  
386 airflow was the cause of this overestimation.

### 387 **3.3. Pharmaceutical characterization of hybrid microparticles**

388 The drug delivery potential of HMPs was firstly explored measuring the encapsulation yield of  
389 ibuprofen in the particles. Two parameters varied in the formulations: the silylation ratio of  
390 the ICO (from 0.4 to 1.05) and the loading rate of ibuprofen (from 4 to 16 wt%). The silylation  
391 ratio was first tuned from 0.4 to 1.05 while the ibuprofen loading rate was set at 4 wt%.  
392 Ranging from 90 to 100 %, the encapsulation yields showed an almost complete encapsulation  
393 of the API with a slight downward trend with the decreased silylation ratio (Fig. 10). In fact,  
394 some ethoxysilanes might remained within highly silylated HMPs and the previous assumption  
395 (i.e. complete hydrolysis of ethoxysilanes) was not yet accurate in this case. Therefore, the  $\eta'$   
396 (and consequently the encapsulation yields) might be overestimated. Second, the loading rate

---

<sup>§</sup> K-L<sub>2</sub> and K-L<sub>3</sub> refer to the electronic transitions between atomic orbitals after the X-ray excitation.

397 of ibuprofen was tuned from 4 to 16 wt% while the silylation ratio of the ICO was set at 1.05.  
398 The results exhibited also very satisfying yields up to 16 wt% loaded-HMP and no trend to loss  
399 of the API was noticed.

400 The crystallinity of the API is a key parameter that modulates the drug dissolution. Indeed  
401 crystalline drugs are thermodynamically more stable than amorphous ones and are thus less  
402 able to dissolve *in vivo* (Hancock and Parks, 2000). XRD spectra were recorded on pure  
403 ibuprofen and ibuprofen-loaded HMPs at various loading rates (Fig. 11). 4 and 8 wt% loaded-  
404 HMPs had an amorphous organization (large halo) highlighting a molecular solubilization of  
405 the ibuprofen within the particles. By contrast, 12 and 16 wt% loaded-HMPs exhibited specific  
406 peaks of crystallized ibuprofen at 6, 16.5, 17.5, 20, 22 and 25° of 2theta underlining the  
407 formation of API microcrystals inside these HMPs.

408 The XRD results were corroborated by supplementary DSC experiments (Fig. 11). The  
409 endothermic peak of the ibuprofen melting (around 76 °C) was detected for 12 and 16 wt%  
410 loaded-particles whereas 4 and 8 wt% ibuprofen-loaded HMPs presented no peak of melting  
411 meaning the molecular solubilization of ibuprofen within the latter matrices.

412 The release kinetics of ibuprofen from the particles were widely investigated. In order to  
413 respect sink conditions, all the media used ensured more than tenfold of ibuprofen solubility  
414 (Finholt and Solvang, 1968; Avdeef, 2007). Therefore ibuprofen release kinetics from HMPs  
415 depended only on the particle properties. To investigate the oral route, the release properties  
416 of 4 wt% ibuprofen-loaded HMPs 1.05 were studied in simulated digestive medium added by  
417 a surfactant (protocol 1 – see Methods). The results depicted in Fig.A.4 showed a double stage  
418 release of the API. Ibuprofen being a carboxylic acid, it was protonated in acidic medium (SGF,  
419 pH= 1.2) whereas it lost its proton in neutral medium (SIF, pH= 6.8) accelerating its release  
420 from the matrix. The entire payload was then released after 3 hours.

421 A comprehensive study was done on the second stage of release (i.e. in SIF) using HMPs 0.4 to  
422 1.05 loaded at 4 wt% of ibuprofen in order to highlight the influence of the silylation ratio (Fig.  
423 12).

424 The results exhibited a definite trend in the release kinetics: the lower is the ratio, the faster is  
425 the release. The sizes of HMPs being statistically similar, the differences observed in release  
426 kinetics could only be explained by a difference in HMPs properties and their abilities to retain



427 the API. In case of LBDDS, the discussion remains open about the two models of “medium  
428 diffusion into the matrix” or the “API diffusion out of the matrix” as limiting, and thus  
429 controlling factors of release (Siepmann and Siepmann, 2011). However, the use of a  
430 biorelevant medium to mimic the physiological conditions especially in term of pH and surface  
431 tension overcomes these considerations as it ensures an optimal *in vitro* / *in vivo* correlation.  
432 In other hand, Xu (Xu et al., 2009) demonstrated that the change in surface state of  
433 mesoporous silica nanoparticles by grafting trimethylsilane (an hydrophobic agent) was  
434 sufficient to slowdown the release of API without changing the matrix composition, the  
435 wettability of the particles is consequently a significant factor. The potential impact of the  
436 HMPs surface states was annihilated in our study by optimizing the wettability of the HMPs  
437 and by setting the amount of the wetting agent at 0.1 wt% for all experiments. In order to  
438 quantify the differences in internal structure of the matrices, the release kinetics were  
439 mathematically simulated from which the diffusion coefficients (D) of ibuprofen were  
440 deduced. Taking into consideration EDX, XRD and DSC results (i.e. homogeneity of the API  
441 within the spheres and its molecular state), 4 wt% ibuprofen-loaded HMPs were related to  
442 monolithic solutions in which a molecular dissolution of the API happened (Siepmann and  
443 Siepmann, 2012). Therefore, an appropriate release model was implemented (Appendix B).  
444 The fitting optimization was carried out by least squares method (Fig. 13) and the diffusion  
445 coefficient (D) of ibuprofen in each HMP was then deduced (Table 6).

446 The diffusion coefficients of ibuprofen ranged from  $1.44 \cdot 10^{-9}$  to  $7.28 \cdot 10^{-9} \text{ cm}^2 \cdot \text{s}^{-1}$  according to  
447 the HMP type. They increased by a seven factor when the  $X_R$  decreased from 1.05 to 0.4.  
448 Considering  $^{29}\text{Si}$  NMR results, the condensation yields seemed to follow the same trend  
449 whereas the condensation degrees were similar. It suggests that the release from effectively  
450 cross-linked matrices is facilitated and the liquid fraction within ICO 1.05 and 0.8 exhibited a  
451 solubilization capacity towards ibuprofen and thus slowed down its release. These diffusion  
452 coefficients in our matrices are in same range with ones reported on classical materials such  
453 as the diffusion of ibuprofen in a mesoporous magnesium carbonate material “Upsalite®” ( $9.8$   
454  $\cdot 10^{-8} \text{ cm}^2 \cdot \text{s}^{-1}$ ) (Zhang et al., 2016) which permitted an extended-release on 24 h in phosphate  
455 buffer. Similarly, ibuprofen diffusion coefficients were determined in polyester-based  
456 microparticles and were found to be  $4.18 \cdot 10^{-12}$  and  $6.59 \cdot 10^{-10} \text{ cm}^2 \cdot \text{s}^{-1}$ , for PLLA and PLGA

457 respectively (Lee et al., 2012); ibuprofen release in these systems occurred over 70 days (in  
458 phosphate buffer).

459 Finally to consider the subcutaneous route as a potential administration route, release  
460 kinetics of 4 wt% ibuprofen-loaded HMPs 1.05 were recorded in PBS medium (Fig. 14). A first  
461 stage of “burst” effect was noted where 35 % (3.39  $\mu\text{mol}$ ) of the API was released in 50 h.  
462 However, a second stage of sustained release exhibited a slope of 5 % per 50 hours (9.7 nmol  
463 per hour). It can be explained by the poor dispersibility and wettability of the HMPs in PBS  
464 buffer that makes full sense to the hypothesis of water diffusion control (Siepmann and  
465 Siepmann, 2011). Indeed in absence of surfactant, HMPs tend to form a cluster that favors a  
466 sustained release by the slow diffusion of the API from the core to the surface of the insert-  
467 like material.

#### 468 **3.4. Biological characterization of hybrid microparticles:**

469 As noted above, HMPs presented an interesting potential for oral and subcutaneous drug  
470 delivery. Therefore, the digestibility and the cytotoxicity of these particles were studied. Fig.  
471 15 shows the pH drop of digestion media containing CO or the HMPs. The fitted curve of CO  
472 digestion was used as reference and it exhibited a linear decrease of pH during the 1.5 h of  
473 the experiment highlighting a constant degradation rate of the ester bonds. However, HMPs  
474 curves demonstrated an exponential decrease of pHs with an initial drop higher than the one  
475 of CO curve, followed by a slowing after 45 min of digestion. We supposed that the hydrolysis  
476 of the particles was limited to the surface due to the inability of lipase to catalyze the rupture  
477 of deeper ester bonds. Indeed silylated Fatty Acids (FA)s are linked to the matrix by two kinds  
478 of bonds: the ester and the siloxane. The ester bond could be hydrolyzed by the lipase while  
479 the siloxane remains stable preventing the catalytic progression of the enzyme. This  
480 hypothesis is in line with the more significant pH decrease recorded for HMP 0.4 compared to  
481 others, because of their high content of non-silylated FA.

482 In order to support this hypothesis, HMPs 1.05 loaded with 1 wt% of Sudan Black B (SBB) were  
483 synthesized and subjected to the same digestion protocol. The SBB allowed us to track the  
484 black HMPs within the digestion medium. After 2 h of digestion, the dye diffused in the  
485 medium but the morphology of HMP seemed to be preserved confirming the surface-limited  
486 digestion of the particles (Fig. 16).

487 The cytotoxicity studies of the four HMPs at different concentrations were conducted on two  
488 kinds of cells simulating the digestive (Caco-2) and the subcutaneous (NIH-3T3 fibroblasts)  
489 cellular environments. Briefly, satisfying results were reached and the elements extracted  
490 from HMPs had not induced cell death in both cases (Fig. 17). For Caco-2/TC7 enterocytes, the  
491 cell viability varied from 80 to 120 % without a trend dependent on microparticle  
492 concentration or  $X_R$  and this cellular type seems little sensitive. On the contrary, two trends  
493 were observable for the fibroblasts assays: the influence of the HMP concentration and their  
494  $X_R$ . The cell viability decreased from 100 to 70 % by increasing the concentration from 0.1 to  
495  $10 \text{ mg}\cdot\text{mL}^{-1}$  or by decreasing the silylation ratio from 1.05 to 0.4. An explanation to this slight  
496 viability decrease could be related to the release of FFA from 0.4 HMP leading to the  
497 acidification of the culture medium. Concerning the influence of the concentration that was  
498 already observed (Gallon et al., 2017), we could attribute it to the presence of carrageenan  
499 residues. However, viability results were higher than 84 % below this excessive concentration  
500 of  $10 \text{ mg}\cdot\text{mL}^{-1}$ . Thus we could consider that HMPs are well tolerated.

#### 501 **4. Conclusion**

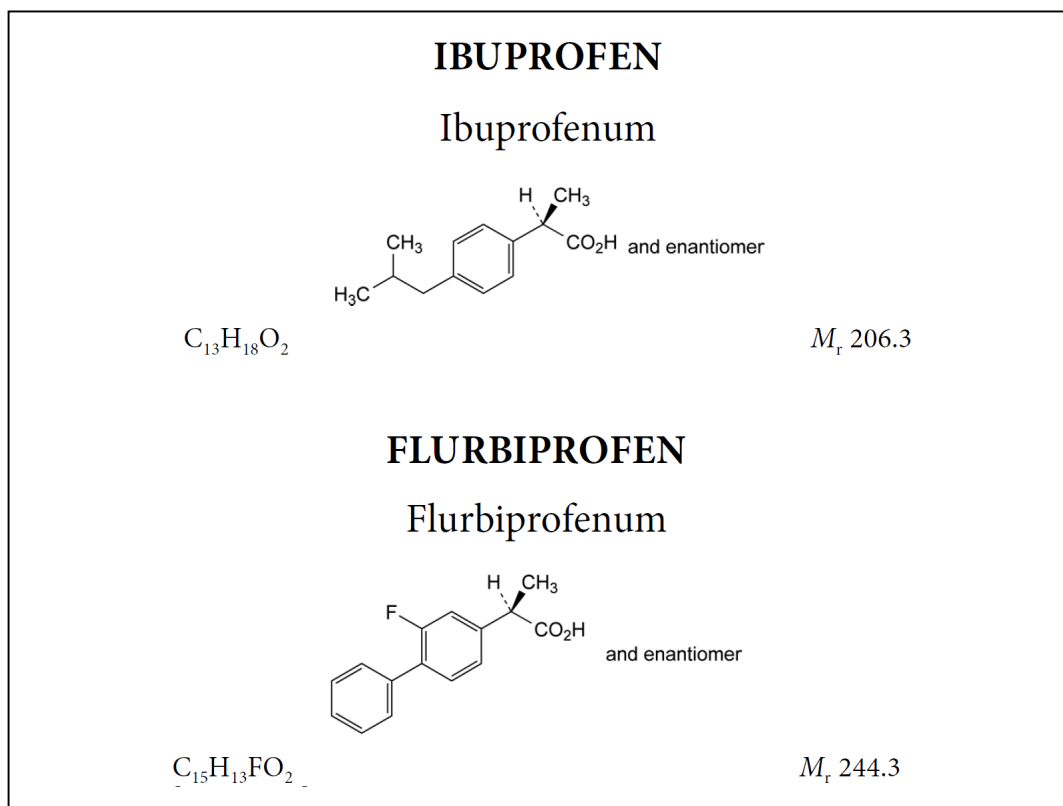
502 An original approach to formulate poorly water-soluble APIs in vegetable oil / silica hybrid  
503 microparticles (HMPs) was described. A complete oil silylation in solvent- and catalyst-free  
504 conditions was reached whatever the oil / silica ratio used, and the TSE process was optimized  
505 for pharmaceutical application exhibiting a good robustness with high preparation yields (> 85  
506 %) in all formulations. This process produced solid and microspheric particles in which the API  
507 was homogeneously disposed. For ibuprofen, excellent encapsulation yields were reached up  
508 to 16 wt% of ibuprofen-loaded HMPs. Furthermore, two behaviors of the loaded-API were  
509 highlighted: an amorphous solubilization up to 8 wt% then a microcrystalline dispersion up to  
510 16 wt% of ibuprofen. A tunability of API release from HMPs was underscored and depended  
511 on two physicochemical parameters. First, the wettability of the HMPs, which is connected to  
512 the biorelevant medium used, induced a complete API release ranging from 3 hours (in  
513 simulated intestinal fluid) up to 15 days (in subcutaneous simulated medium). Second, the  
514 structure of the HMP matrix, which depends on the silylation ratio of the CO and the sol-gel  
515 condensation yield and degree, led to a slowdown of API release when the uncross-linked  
516 amount of ICO increased. These releases were also mathematically fitted to the diffusion  
517 model from a sphere described by Crank and the diffusion coefficients  $D$  were then deducted.

518 Finally, a surface-limited digestion of the HMPs by means of lipases was highlighted and a  
519 good cytocompatibility was exhibited on fibroblasts and enterocyte-like cells.

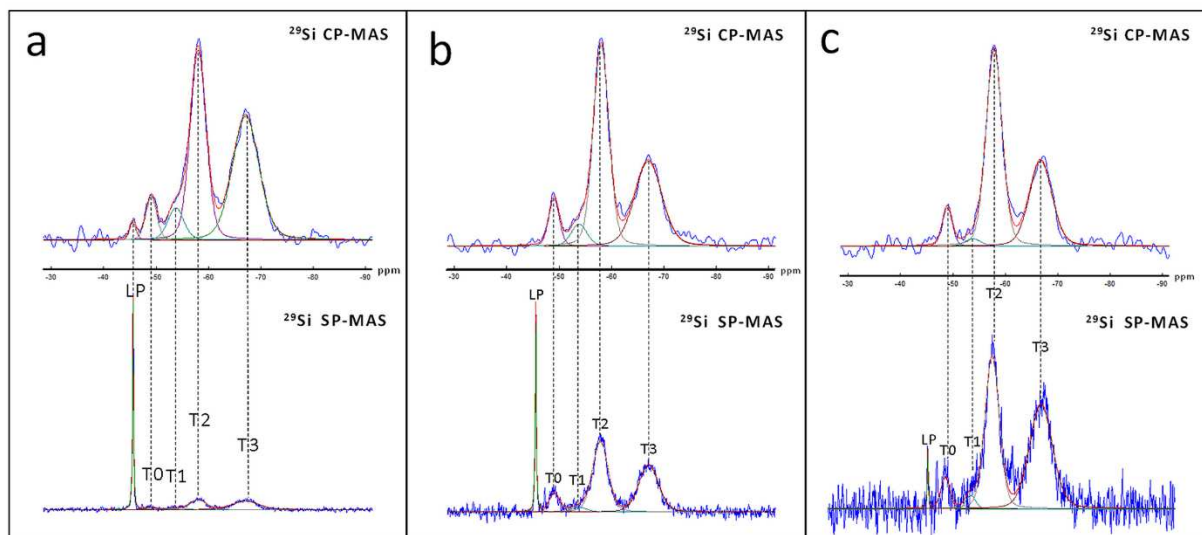
520

521 **5. Appendix A: Supporting Data**

522 **Fig. A.1. Chemical structures of ibuprofen and flurbiprofen (from European**  
 523 **Pharmacopeia 9.5)**



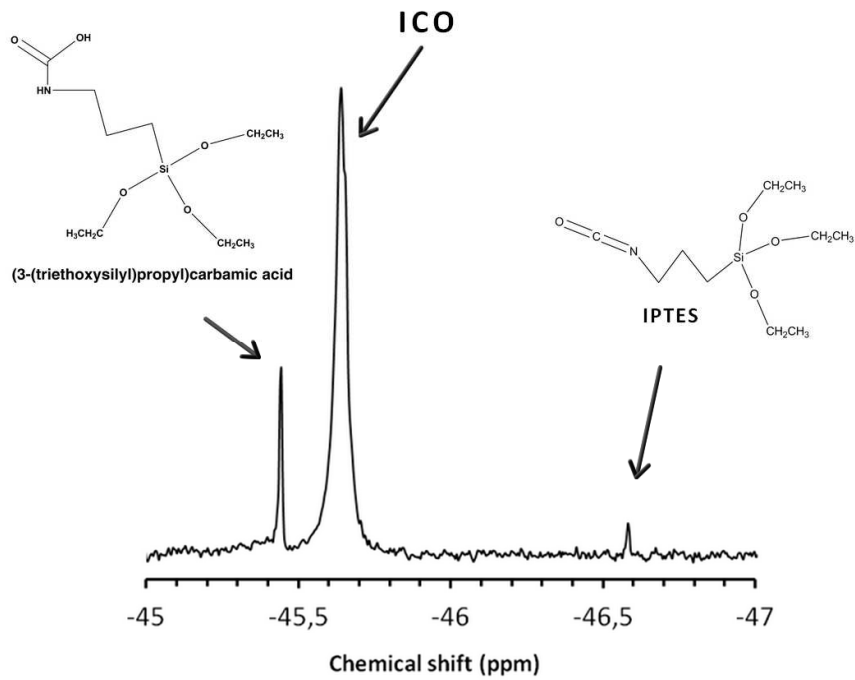
524  
 525  
 526 **Fig. A.2.  $^{29}Si$  NMR spectra of HMPs: a) 1.05 b) 0.8 c) 0.6 . Spectra were fitted and**  
 527 **area under curves for SP-MAS experiments were integrated in percent.**



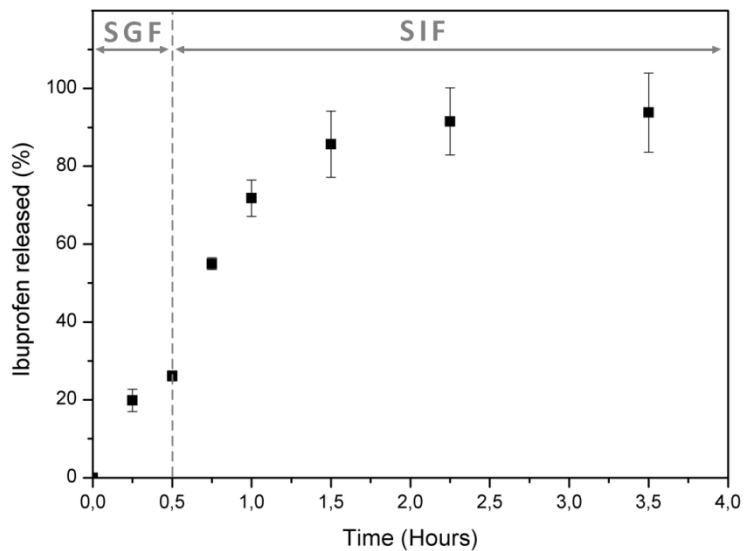
528

529

530 Fig. A.3.  $^{29}\text{Si}$  NMR of ICO 1.05. The peak at -46.6 ppm was assigned to the excess of  
531 IPTES, and the one at -45.4 ppm to an impurity initially present in the reagent.



532  
533 Fig. A.4. Release kinetics from 4 wt% ibuprofen-loaded HMPs 1.05 in Simulated  
534 Gastric and Intestinal Fluids (SGF and SIF respectively). n=3.



535

536

537

538 **Table A.1. Composition of the HMPs digestion medium for biodegradability assays**

		Component	Concentration	Purpose
		Digestion medium	Pre-digestion medium	PBS buffer
Porcine bile extract	5 mM			Emulsifying the substrate
Phospholipids (S 100 Lipoïd)	1.25 mM			
Pancreatin (4 × USP)	450 UA		Hydrolysis of glycerol-fatty acid bonds	
		Ca <sup>2+</sup>	36mM aqueous solution 1mL injected every 15mn	Precipitation of free fatty acids (FFA)s

539

## 540 **6. Appendix B: Mathematical Modeling**

541 Considering that:

- 542 • An optimal wettability has been ensured on HMPs;
- 543 • The API is homogeneously and amorphyously disposed within HMPs;
- 544 • The HMPs are not significantly swelling or eroding during API release (surface-limited  
545 digestion).
- 546 • Sink conditions are provided in the release media.

547 A mathematical model for 4 wt% ibuprofen-loaded HMPs releases in Simulated Intestinal Fluid  
548 (SIF) was established referring to the diffusion equation in spheres (Crank, 1975, p. 91):

$$M(R, t) = M_{\infty}(R) - M_{\infty}(R) \frac{6}{\pi^2} \sum_{n=1}^{N \rightarrow \infty} \frac{\exp\left(-\frac{Dn^2\pi^2t}{R^2}\right)}{n^2} \quad (\text{B.1})$$

549 Where  $M(t)$  and  $M_{\infty}$  are the cumulative amounts of ibuprofen released at time “t” and at  
550 infinite time, respectively. D is the diffusion coefficient of the ibuprofen within the particle,  
551 and R is the radius of this particle.

552 The cumulative amounts of ibuprofen at infinite time depends on three parameters,  $C_1$ ,  $C_0$  and  
553 R, which represent the concentration at the surface of the sphere, the initial concentration  
554 within the sphere and the radius of the particle, respectively (Zhang, 2008, p. 226).

$$M_{\infty}(R) = (C_0 - C_1) \times V = (C_0 - C_1) \frac{4\pi R^3}{3} \quad (\text{B.2})$$

555 It is important to note that  $M(t)$  and  $M_{\infty}$  are both R dependent. As the amount of released  
556 ibuprofen is additive, then the total of released ibuprofen depends on the size distribution of  
557 HMPs at a given radius ( $P(R)$ ):

$$M(t) = \overline{M(R, t)} = \int_{R_{min}}^{R_{max}} M(R, t) P(R) dR \quad (\text{B.3})$$

558



$$\overline{M(R, t)} = \overline{M_\infty(R)} - M_\infty(R) \frac{6}{\pi^2} \sum_{n=1}^{N \rightarrow \infty} \frac{\exp\left(-\frac{Dn^2\pi^2 t}{R^2}\right)}{n^2} \quad (\text{B.4})$$

559

$$\frac{\overline{M(R, t)}}{\overline{M_\infty(R)}} = 1 - \frac{1}{\overline{M_\infty(R)}} M_\infty(R) \frac{6}{\pi^2} \sum_{n=1}^{N \rightarrow \infty} \frac{\exp\left(-\frac{Dn^2\pi^2 t}{R^2}\right)}{n^2} \quad (\text{B.5})$$

560

$$\frac{\overline{M(R, t)}}{\overline{M_\infty(R)}} = 1 - \frac{1}{\overline{R^3}} R^3 \frac{6}{\pi^2} \sum_{n=1}^{N \rightarrow \infty} \frac{\exp\left(-\frac{Dn^2\pi^2 t}{R^2}\right)}{n^2} \quad (\text{B.6})$$

561 The size distribution of HMPs ( $N(R)$ ) can be obtained experimentally by Laser diffraction

562 particle sizing technique, and used to calculate the  $\overline{R^3}$  and  $R^3 \frac{6}{\pi^2} \sum_{n=1}^{N \rightarrow \infty} \frac{\exp\left(-\frac{Dn^2\pi^2 t}{R^2}\right)}{n^2}$ :

$$\overline{R^3} = \int_{R_{min}}^{R_{max}} R^3 P(R) dR \quad (\text{B.7})$$

563

$$\begin{aligned} R^3 \frac{6}{\pi^2} \sum_{n=1}^{N \rightarrow \infty} \frac{\exp\left(-\frac{Dn^2\pi^2 t}{R^2}\right)}{n^2} \\ = \int_{R_{min}}^{R_{max}} R^3 \frac{6}{\pi^2} \sum_{n=1}^{N \rightarrow \infty} \frac{\exp\left(-\frac{Dn^2\pi^2 t}{R^2}\right)}{n^2} P(R) dR \end{aligned} \quad (\text{B.8})$$

564

565 The eq. (B.6) was used to fit the experimental data points, using the non-linear least square  
566 method, and the GNU Octave software (leasqr function). To work out the eq. (B.6), we used  
567 the experimental size distribution of particles and the trapeze method to estimate integrals in

568 the eqs. (B.7) and (B.8). Finally, the solver was used in order to extract the diffusion coefficient  
569 (D) of ibuprofen in each HMP.

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707

Fig.1. At the top: the silylation process of castor oil by means of IPTES, the ICO 1.05 (fully silylated) is represented. At the bottom: chemical structure of silica species in various condensation states.

Fig. 2. Formulation of the HMPs by an optimized Thermo-Stabilized oil-in-water Emulsion (TSE) process. The blue dots and the purple dashes describe the evolution of the system temperature and the viscosity of the water phase, respectively.

Fig. 3. FTIR monitoring of the castor oil silylation at various  $X_R$ . Red: ICO 1.05; Brown: ICO 0.8; Blue: ICO 0.6; Purple: ICO 0.4.

Fig.4. Scanning electronic microscopy observations of: a) HMPs 1.05 b) HMPs 0.8 c) HMPs 0.6 d) HMPs 0.4.

Fig. 5. Volume size distributions of the HMPs with four  $X_R$ . Each curve displays mean of  $n=3$ .

Fig. 6. IR-ATR spectra of silylated castor oil (ICO) and hybrid microparticles formulated in acetic buffer (HMPa) or in deionised water (HMPw). The Fig. attest to the enhancement of ethoxysilanes hydrolysis when the acetic buffer was used.

Fig.7. TGA curves and mineral residues of 4 wt% ibuprofen-loaded HMPs formulated with multiple ICOs (from 0.4 to 1.05).

Fig.8. EDX spectra (sp.) recorded on cross-section of 8 % flurbiprofen-loaded HMPs. Each measurement was done on a  $1\mu\text{m}^3$  dot.

Fig.9. EDX analysis of HMP 1.05: a) SEM image b) carbon / silicium map c) carbon map d) silicium map (the background is made from carbon).

Fig.10. On the left: encapsulation yields of HMPs for various silylation ratios, loaded with 4 % of ibuprofen. On the right: encapsulation yields of HMP 1.05 loaded with various rates of ibuprofen.  $n=3$ .



Fig. 11. X-ray powder diffractograms and differential scanning calorimetry of ibuprofen-loaded HMPs at: a) 4 % b) 8 % c) 12 % d) 16 % and of e) pure ibuprofen. The Y scale in the X-ray diffractogram “e” was increased three folds for more convenience.

Fig. 12. Release kinetics from 4 wt% ibuprofen -loaded HMPs in Simulated Intestinal Fluid (pH=6.8). n=3 and the minus error bars were hidden for more clarity.

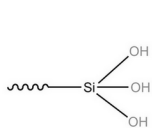
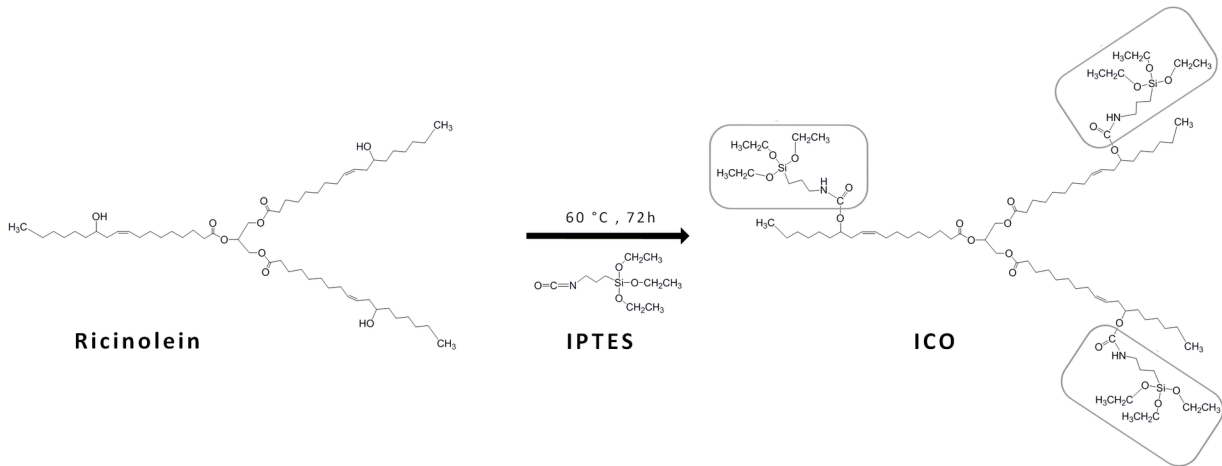
Fig. 13. Experimental and mathematical modeling release kinetics of ibuprofen from HMPs. squares: experimental data; line: computed fits.  $M_t$ = ibuprofen released at time “t”;  $M_{inf}$ = ibuprofen released at infinite time.

Fig. 14. Release kinetics of 4 wt% ibuprofen-loaded HMP 1.05 in PBS (pH=7.4). The experiment was stopped after 350 hours. n=3.

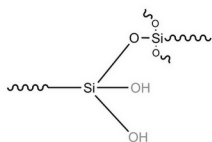
Fig. 15. pH drop versus time of digestion media containing ICO or HMPs. n=2

Fig.16. Sudan Black B-loaded HMP a) before and b) after 2 hours in a simulated digestion medium containing lipases.

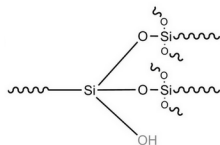
Fig.17. Cytotoxicity studies of HMPs conducted on Caco-2/TC7 enterocytes and NIH-3T3 fibroblasts.



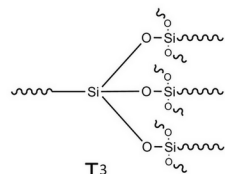
LP or T<sup>0</sup>



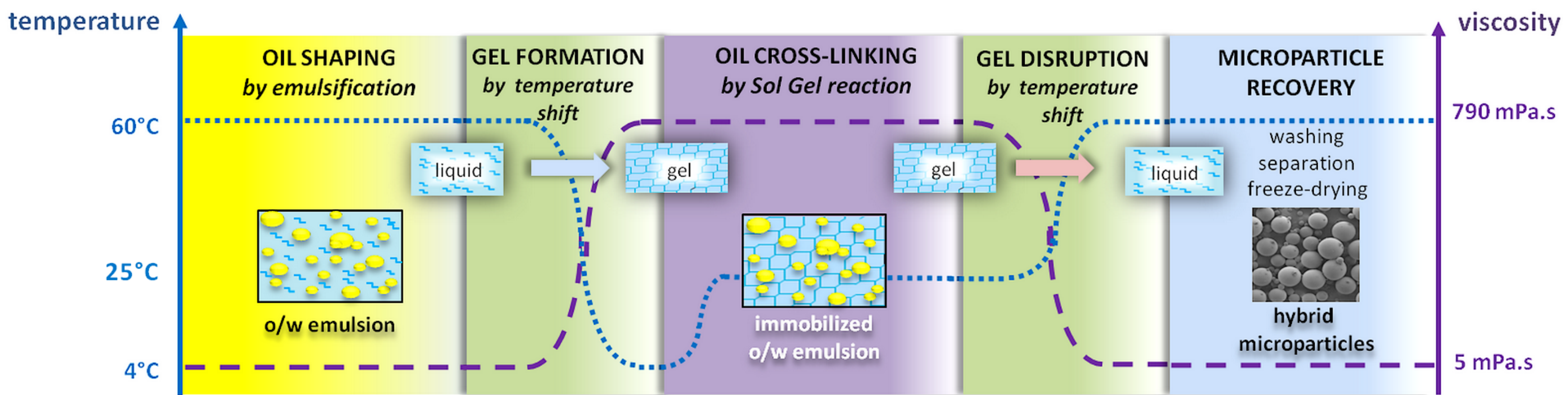
T<sup>1</sup>

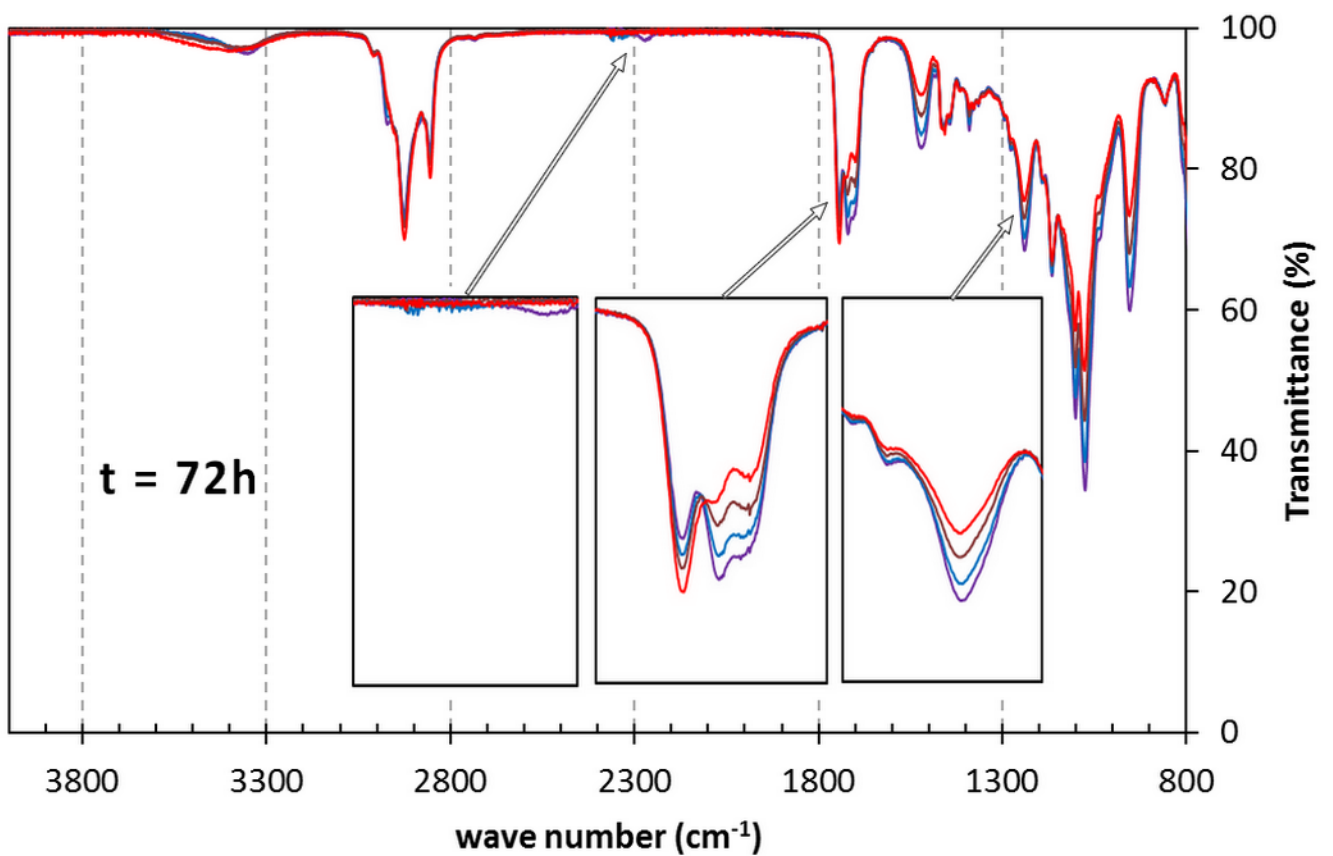
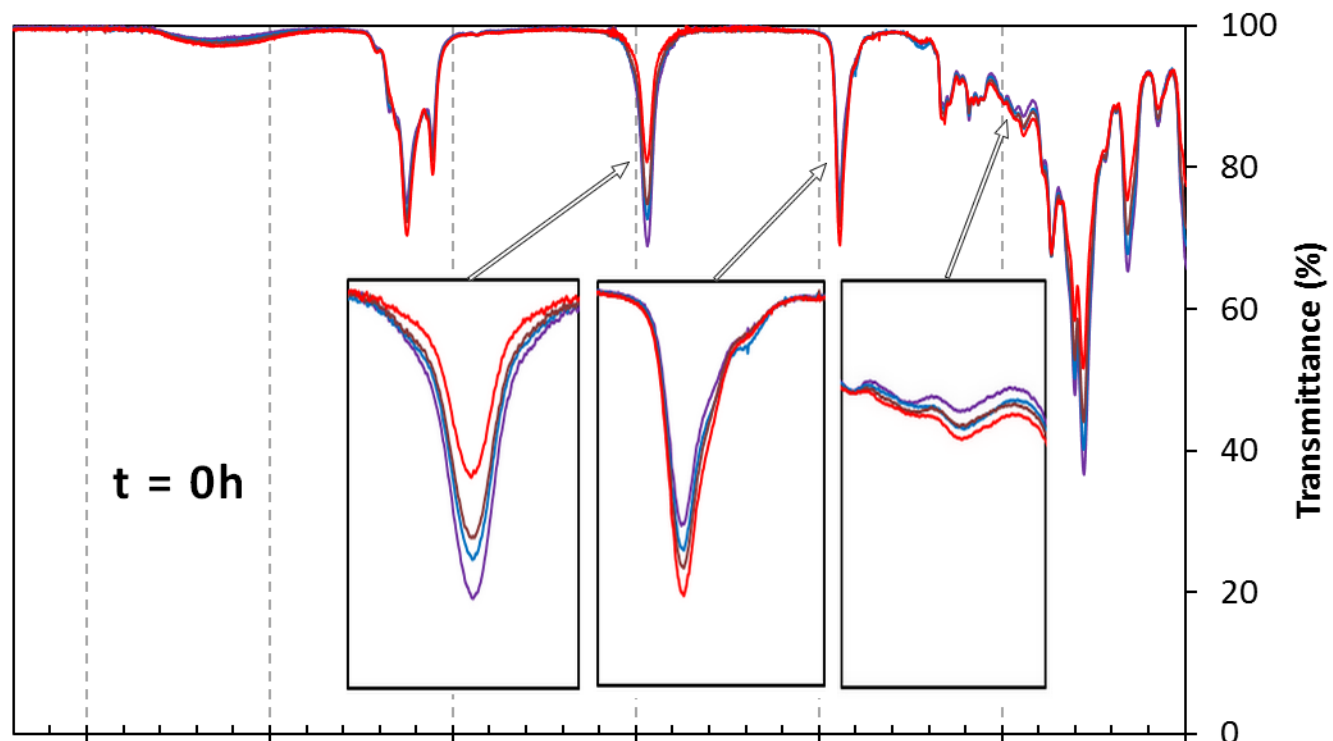


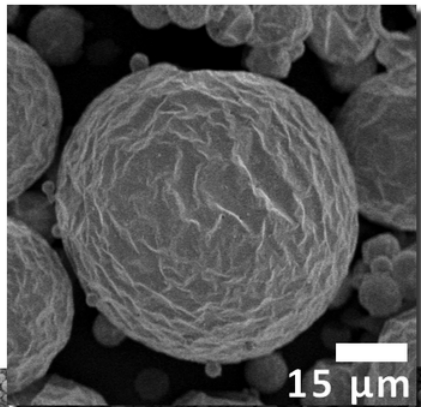
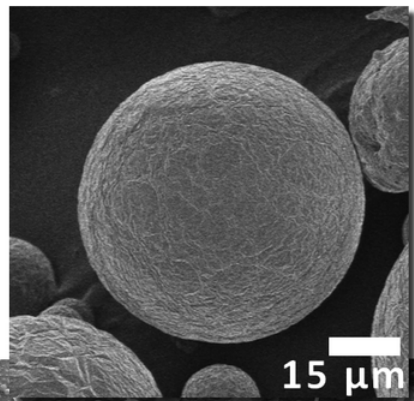
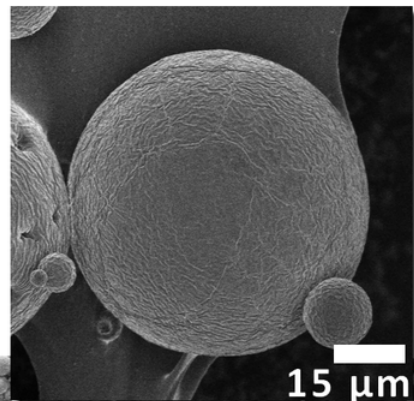
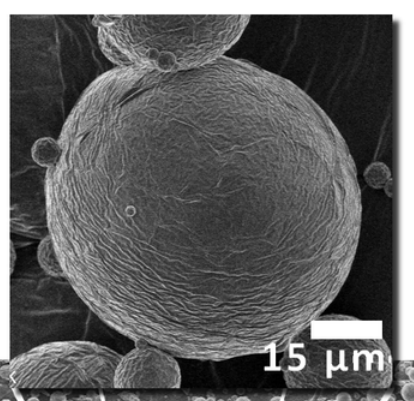
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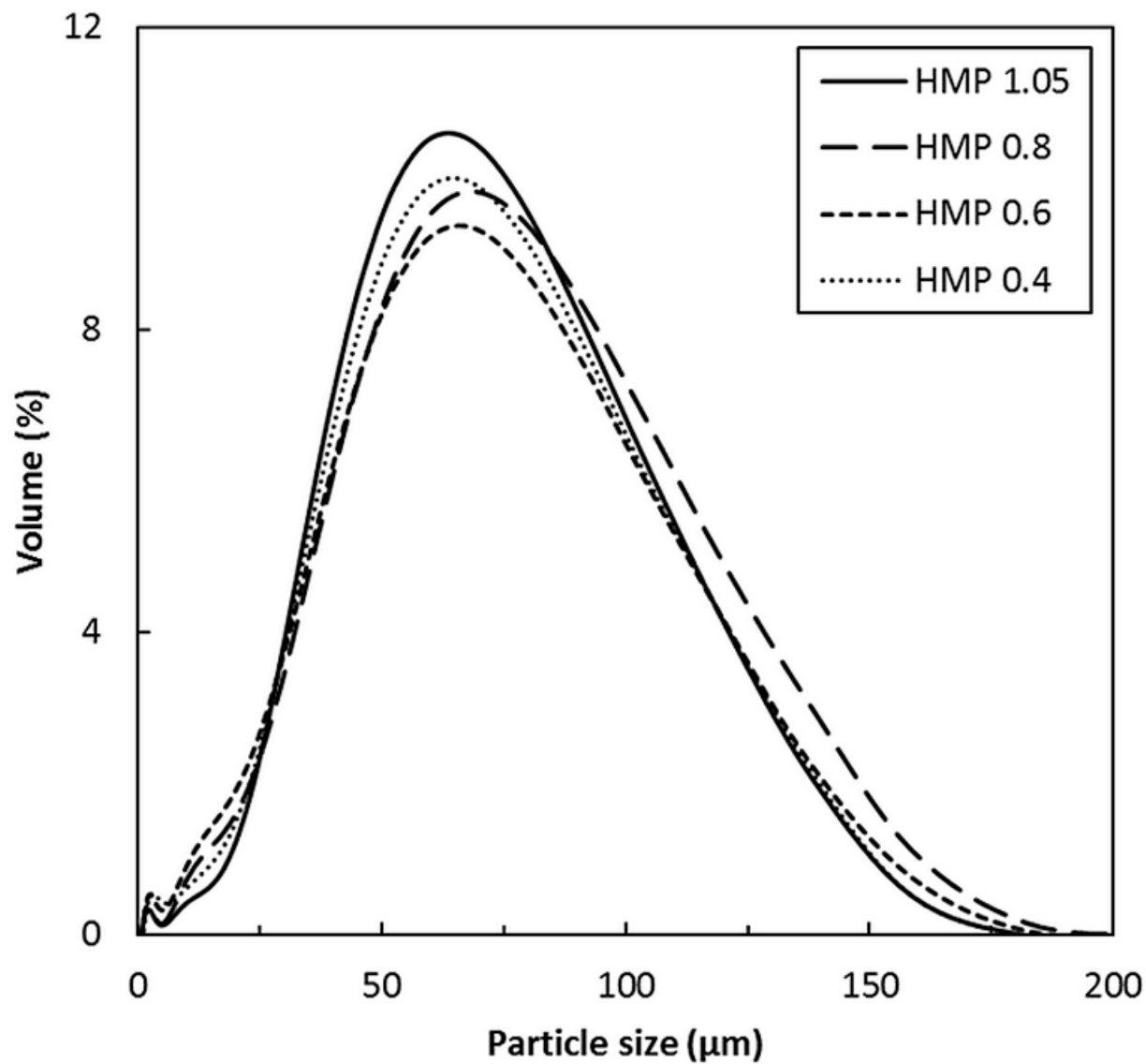


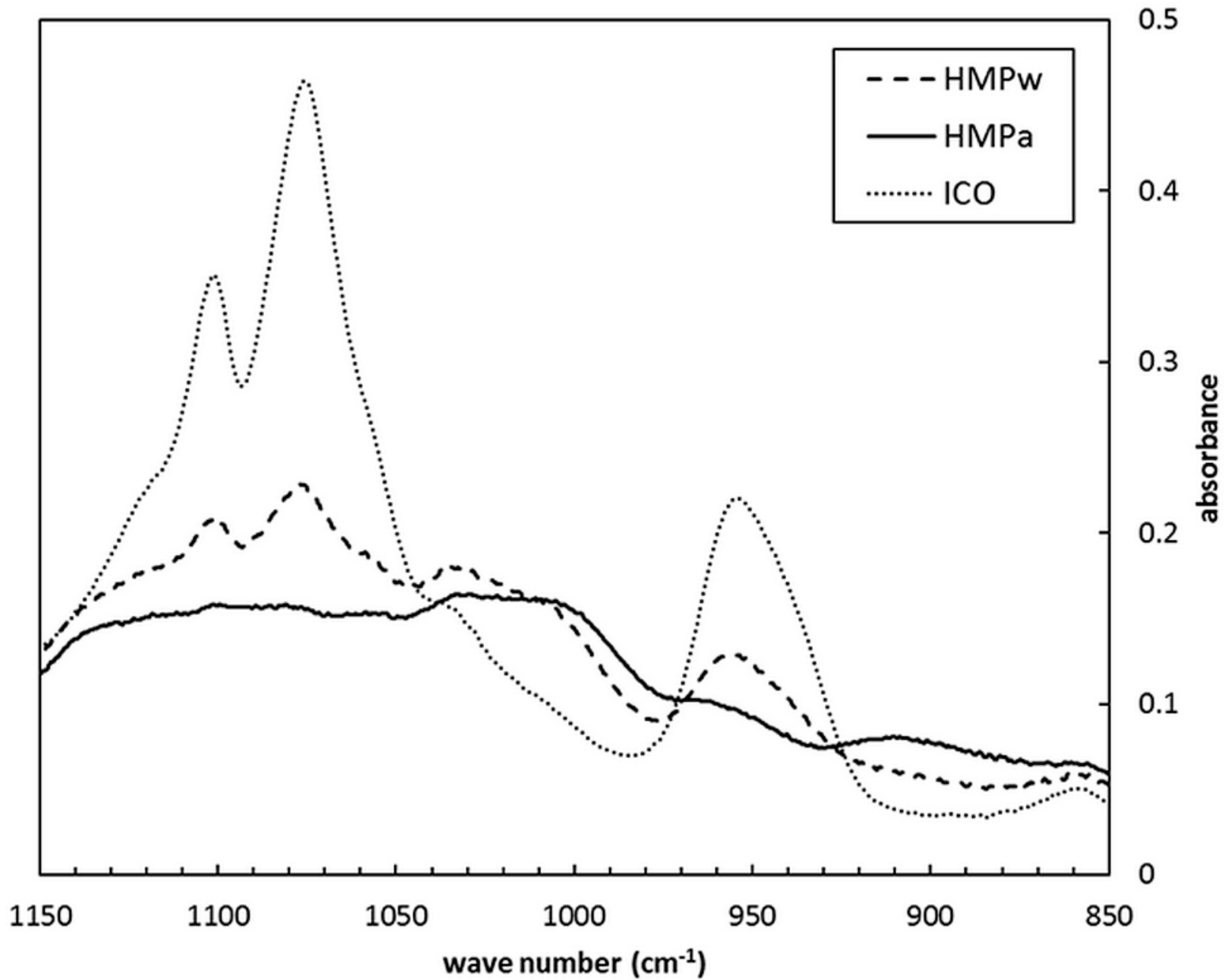
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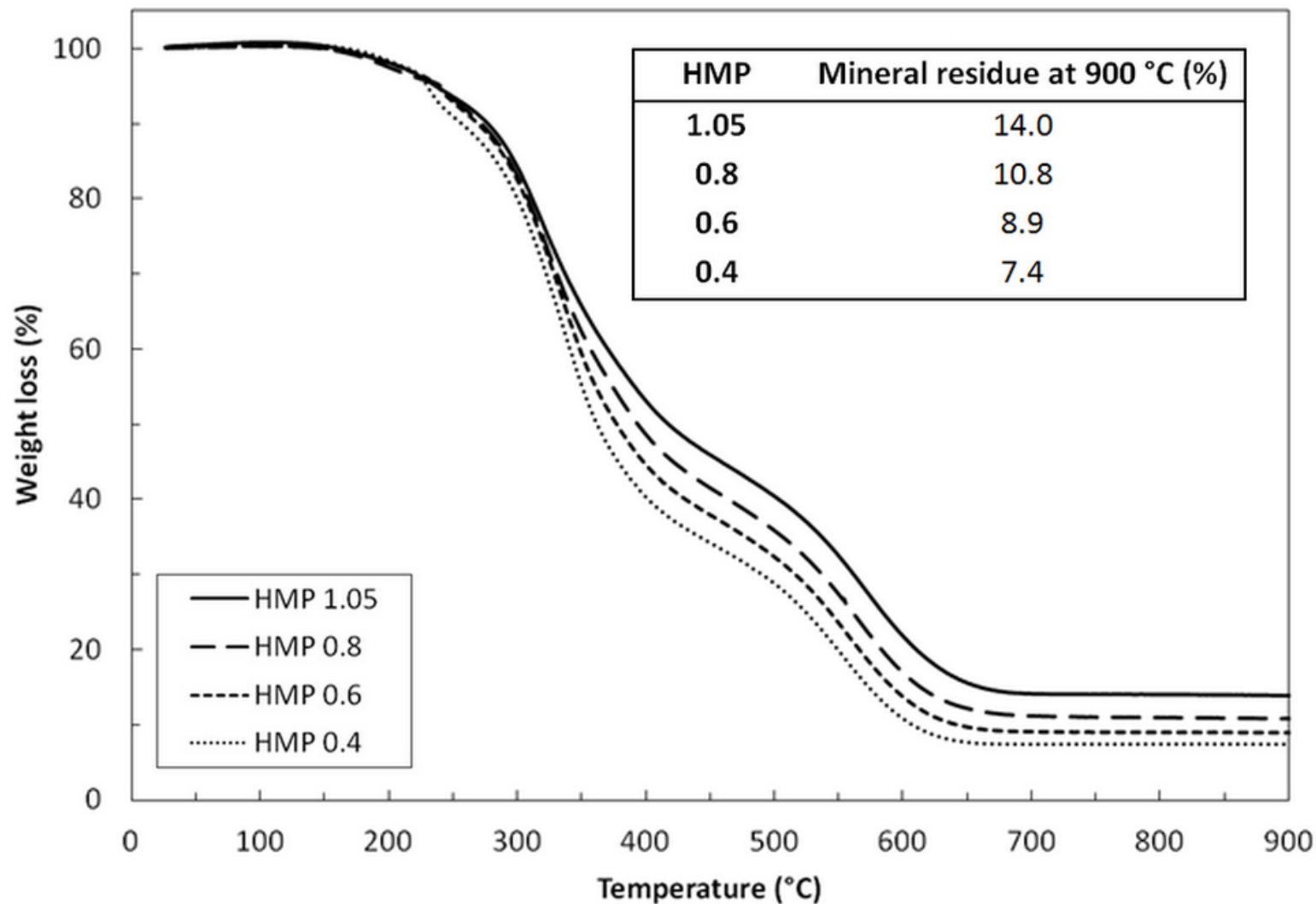




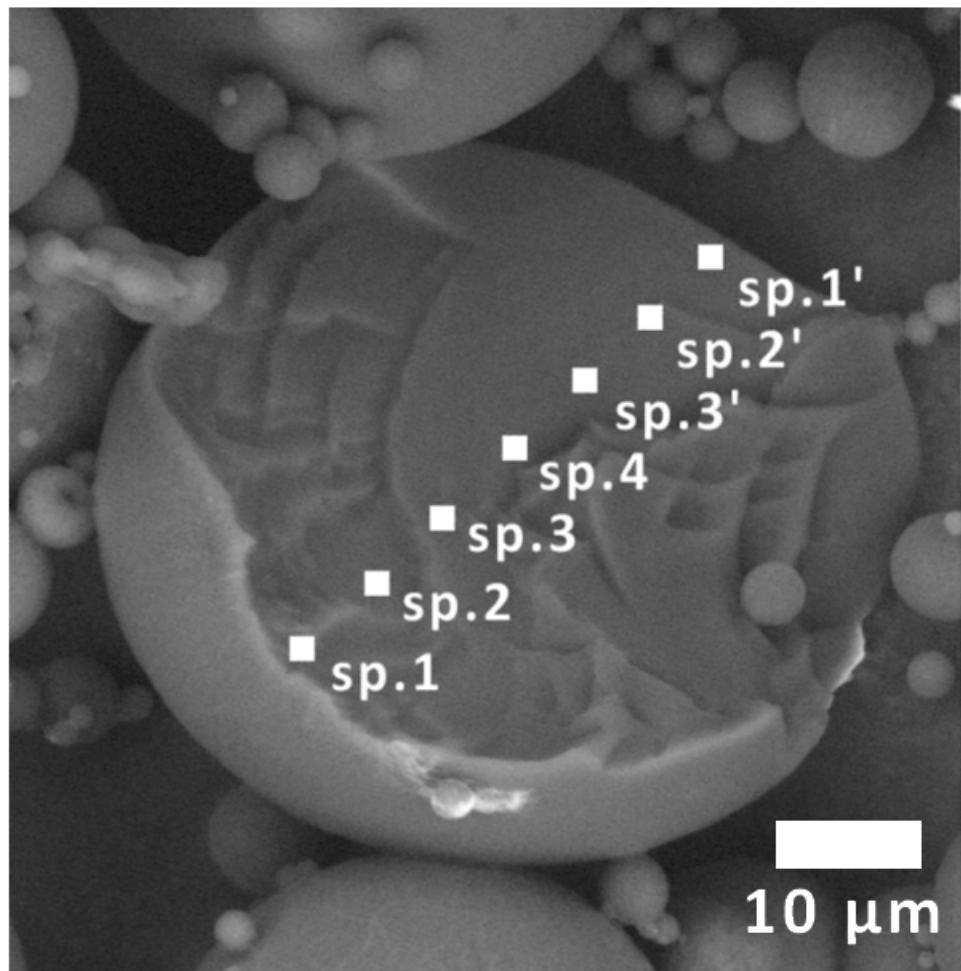
**a**15  $\mu\text{m}$ 300  $\mu\text{m}$ **b**15  $\mu\text{m}$ 300  $\mu\text{m}$ **c**15  $\mu\text{m}$ 300  $\mu\text{m}$ **d**15  $\mu\text{m}$ 300  $\mu\text{m}$

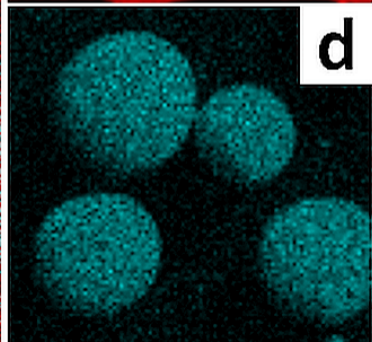
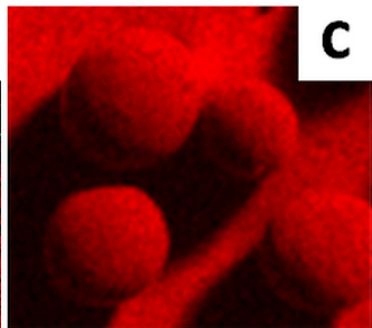
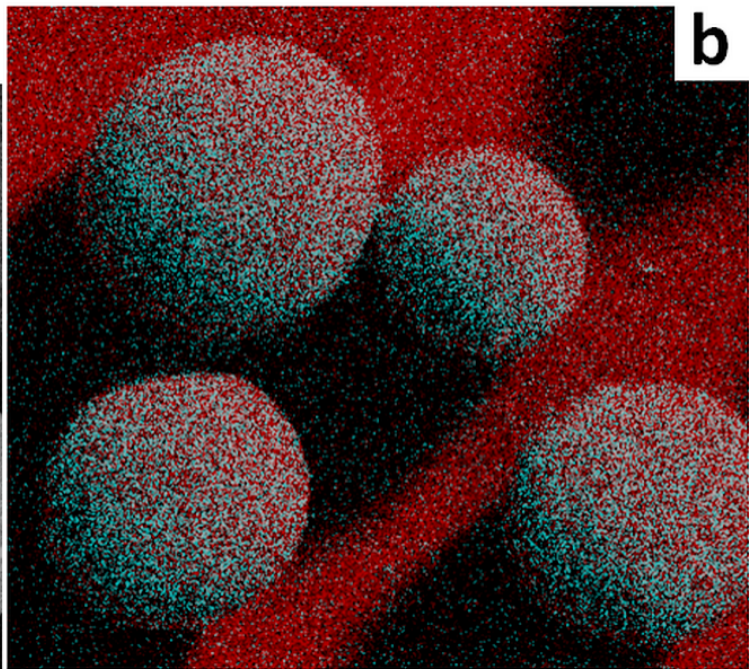
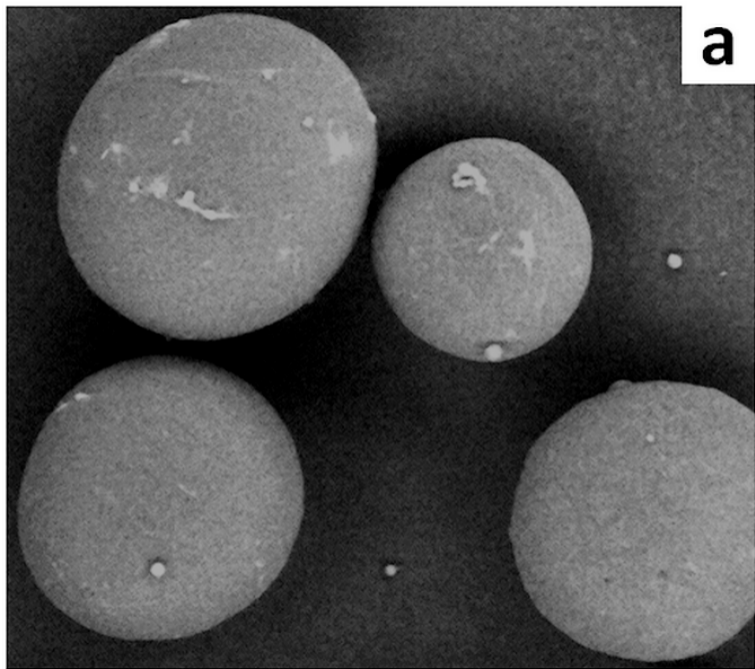


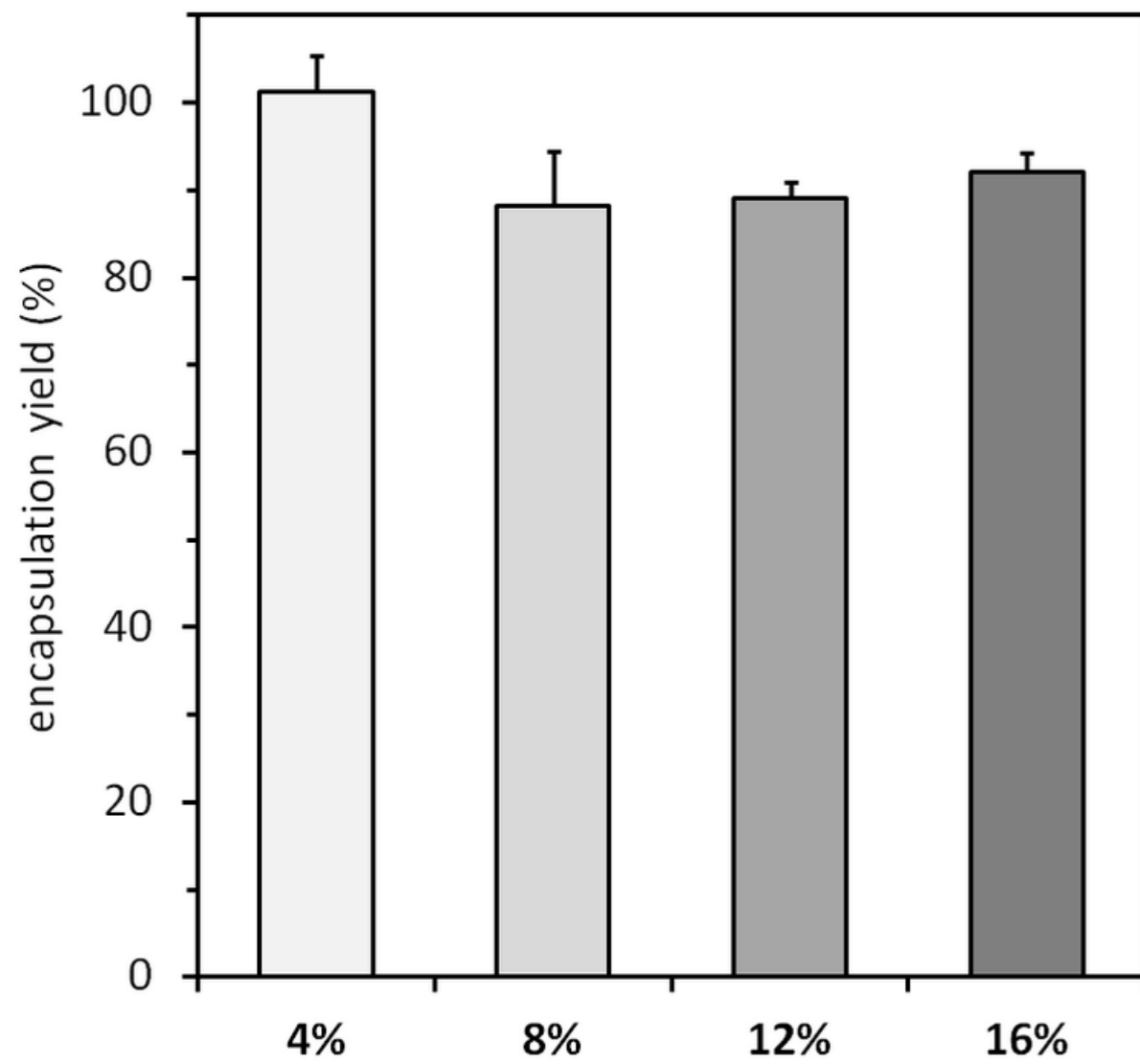
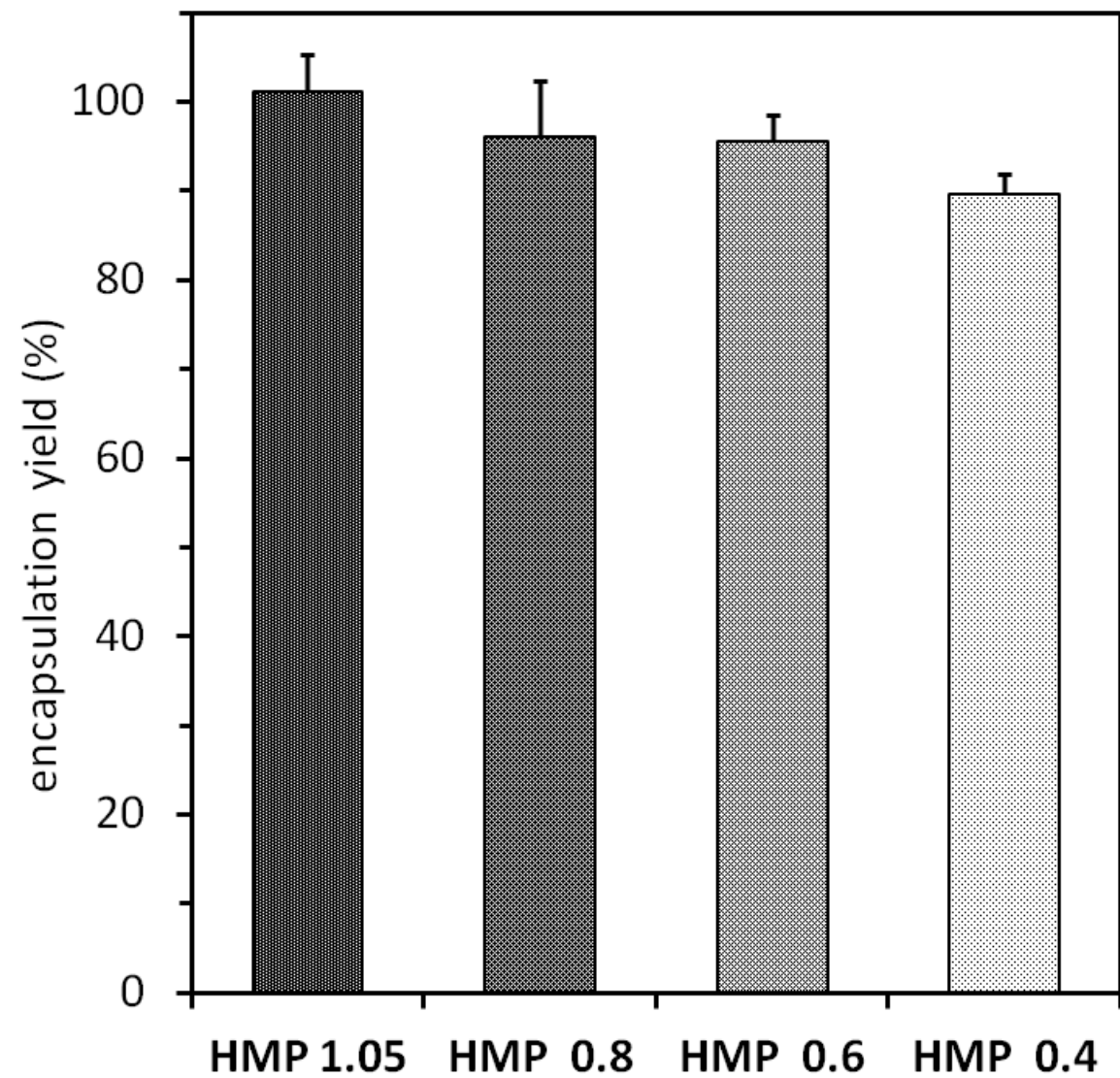




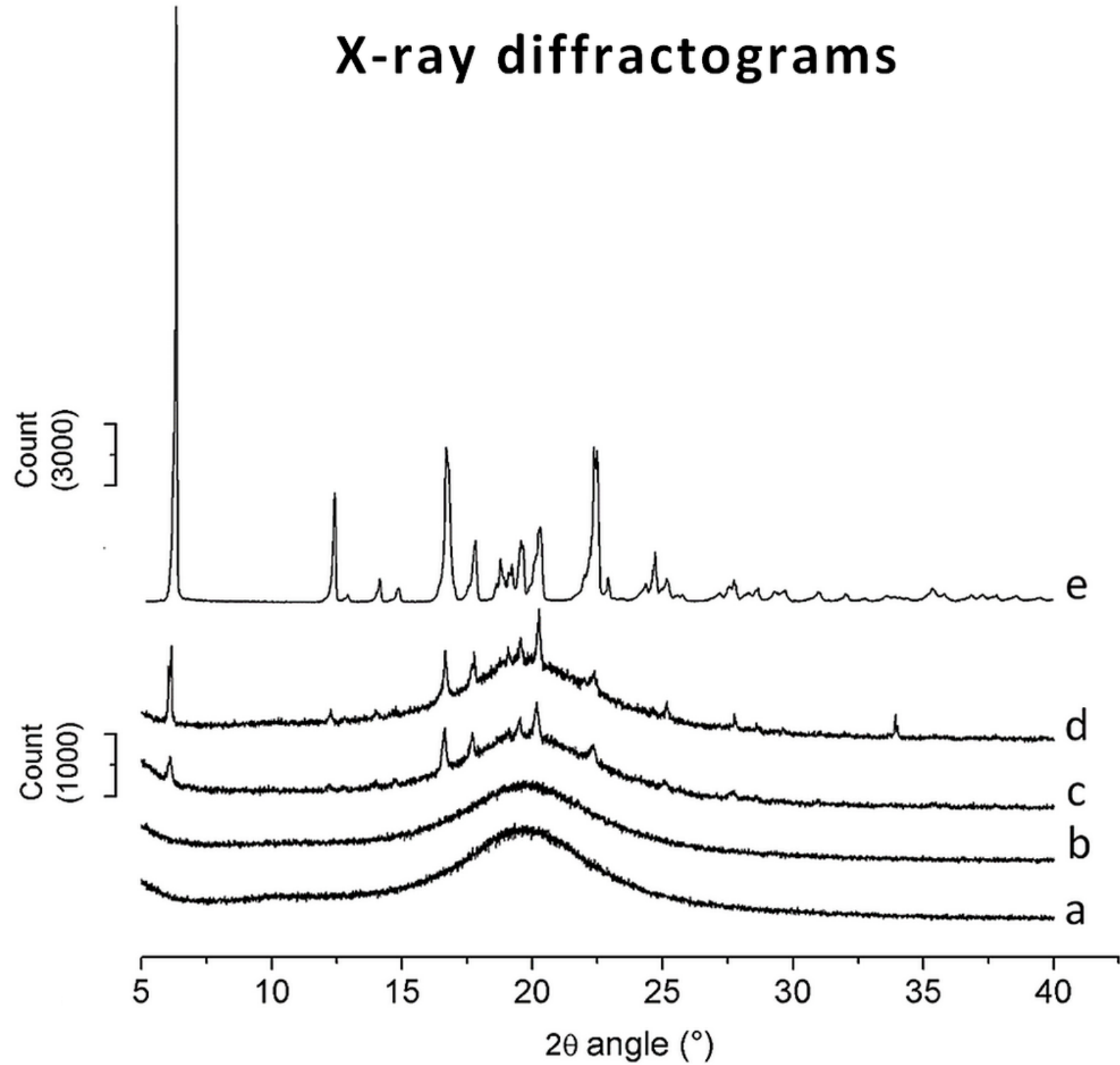




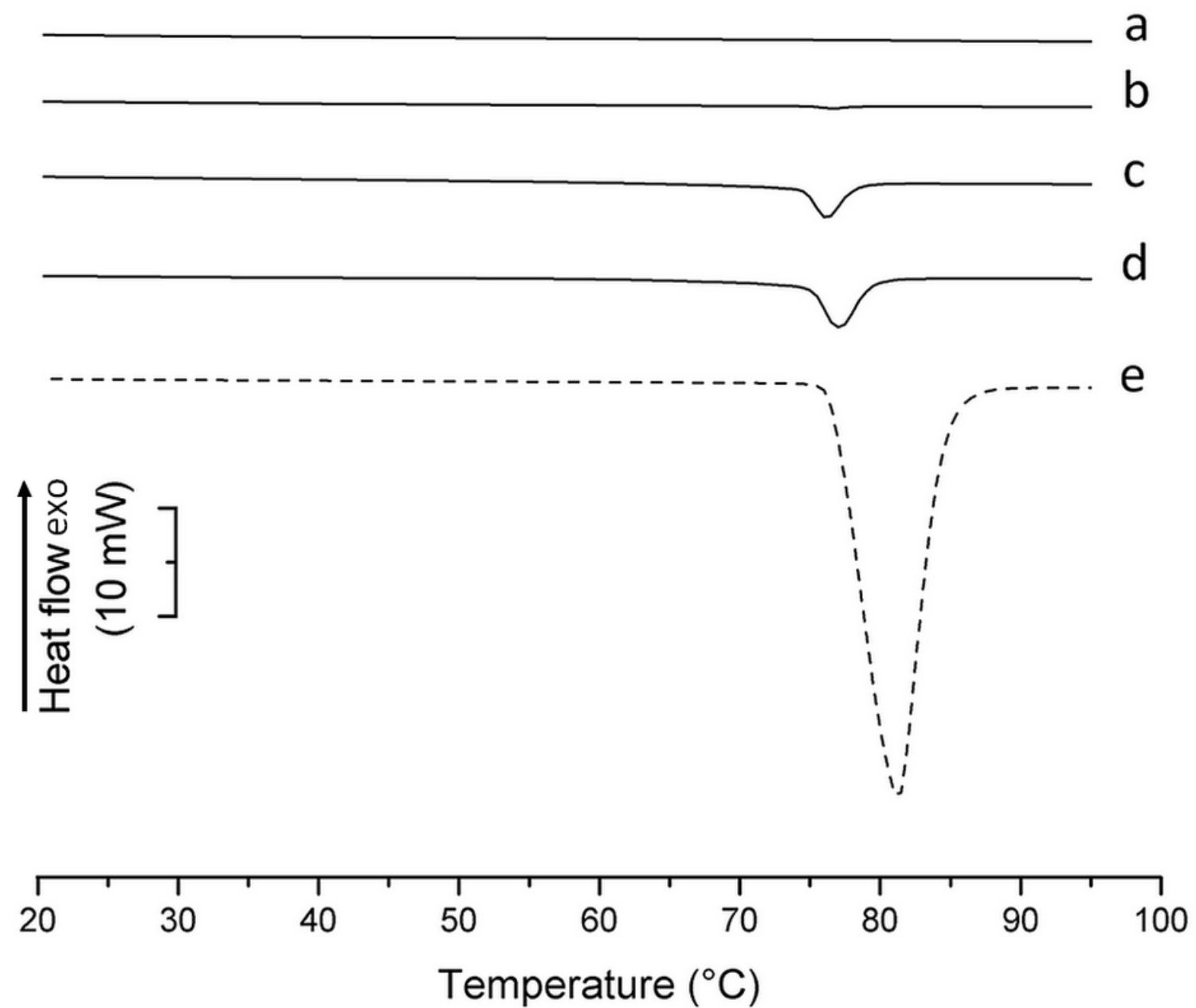


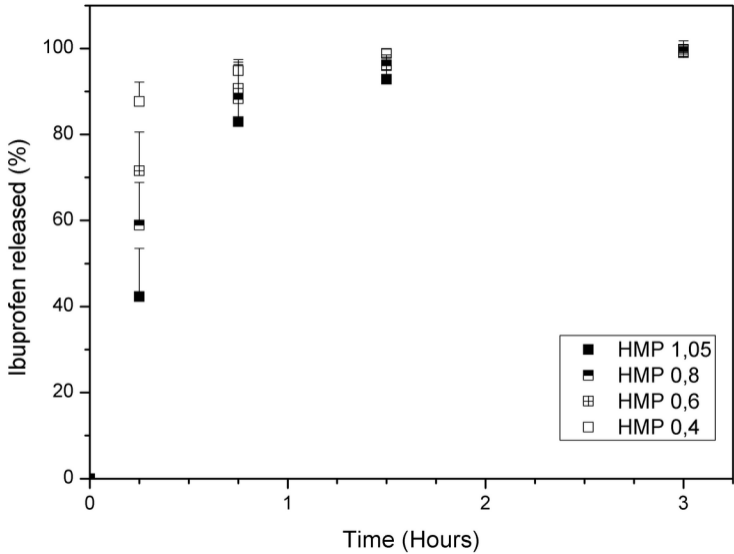


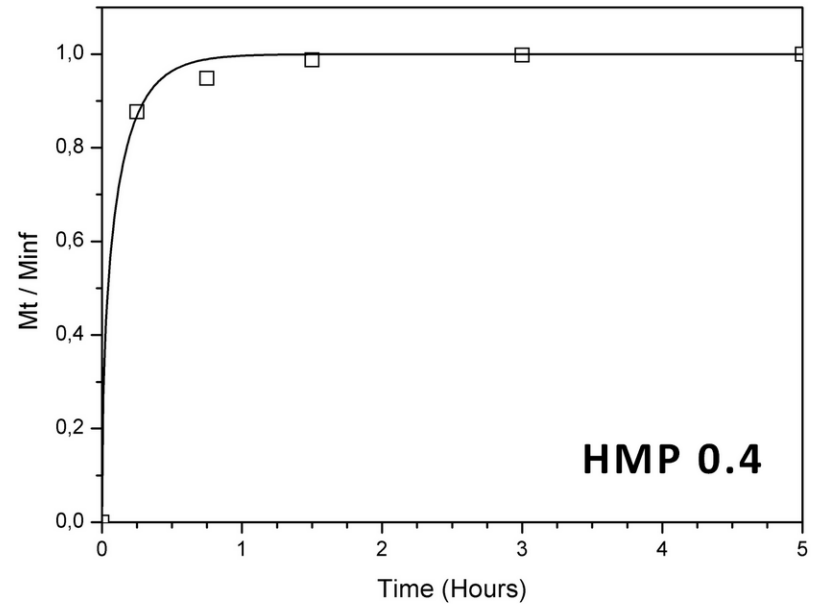
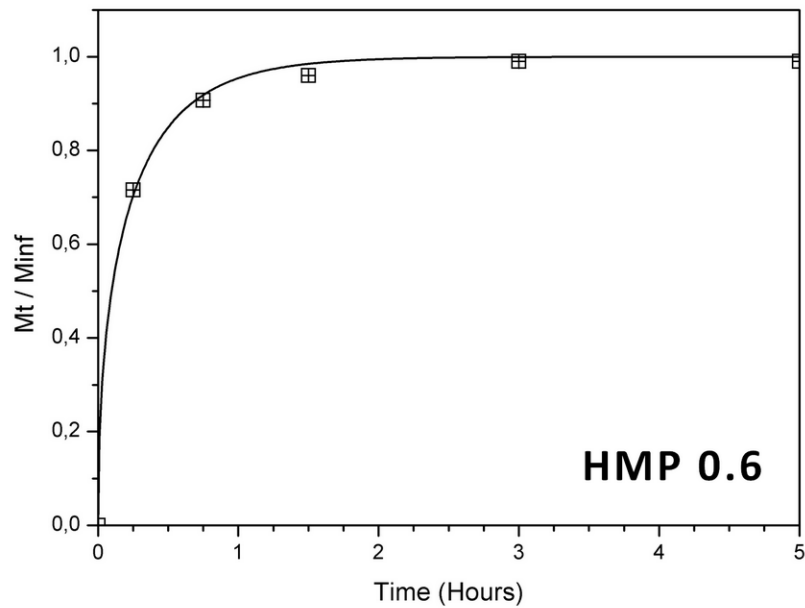
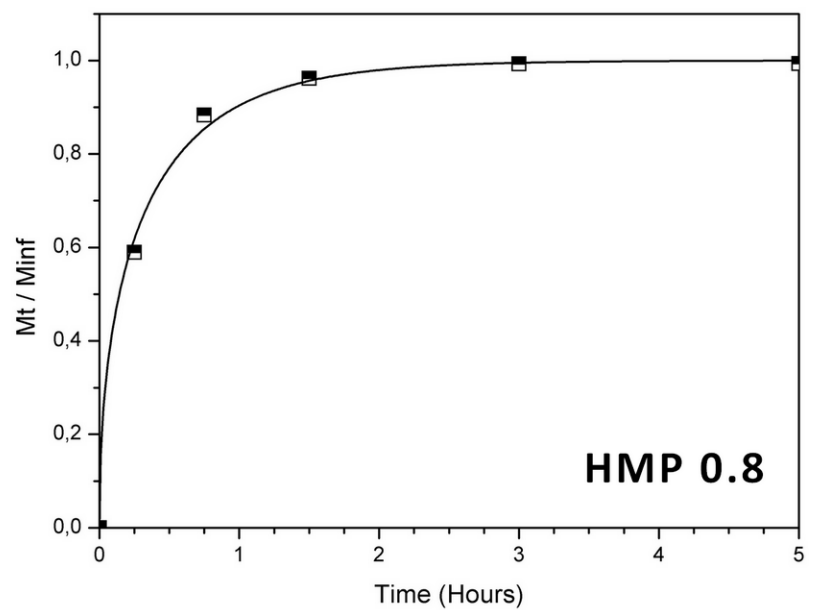
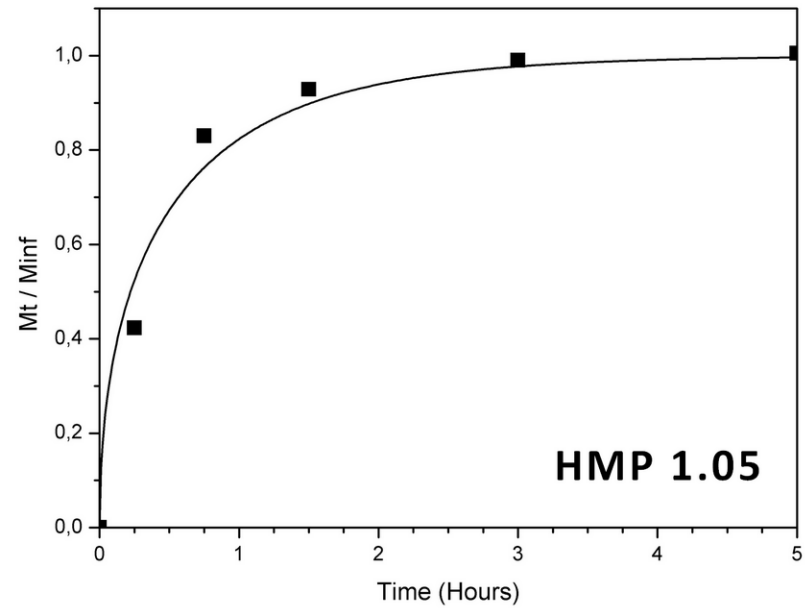
## X-ray diffractograms

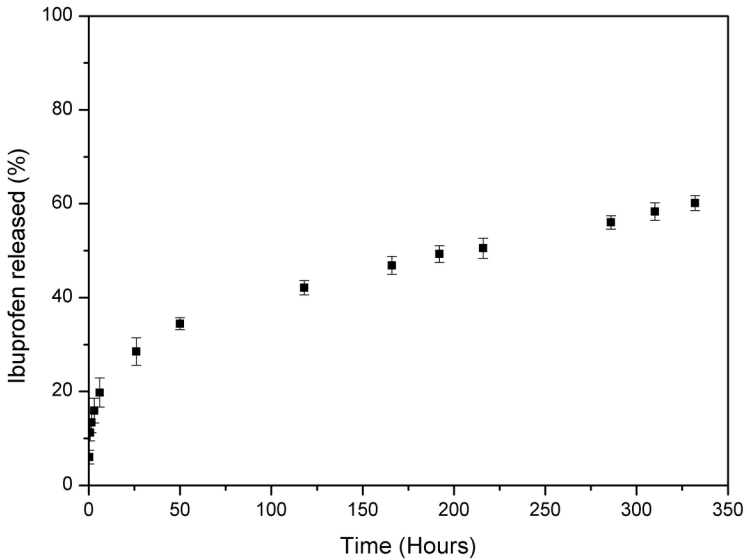


## DSC spectra

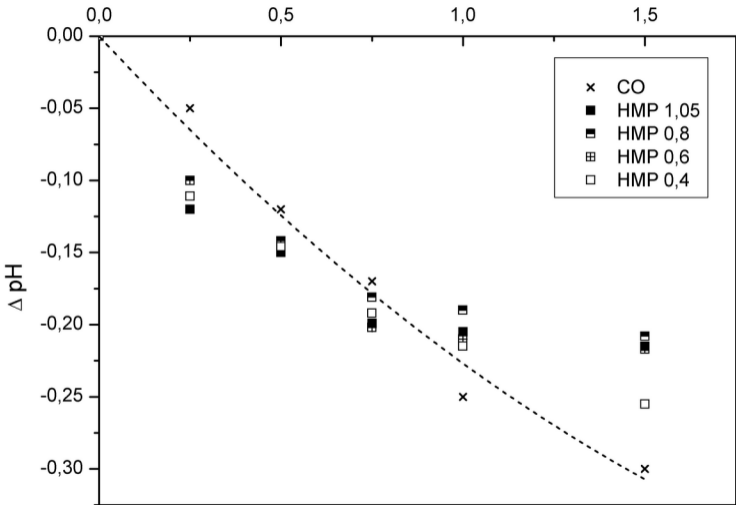




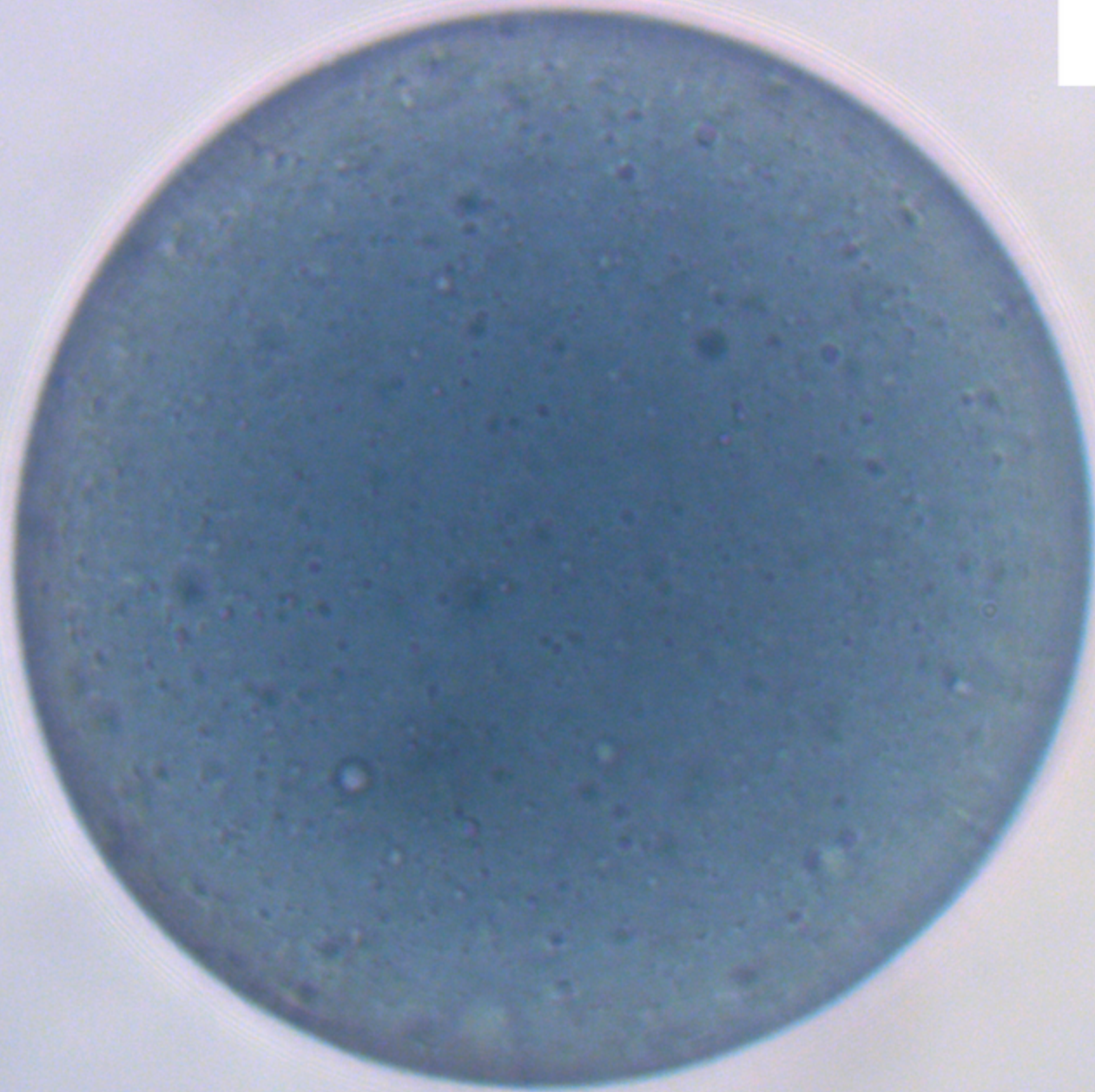
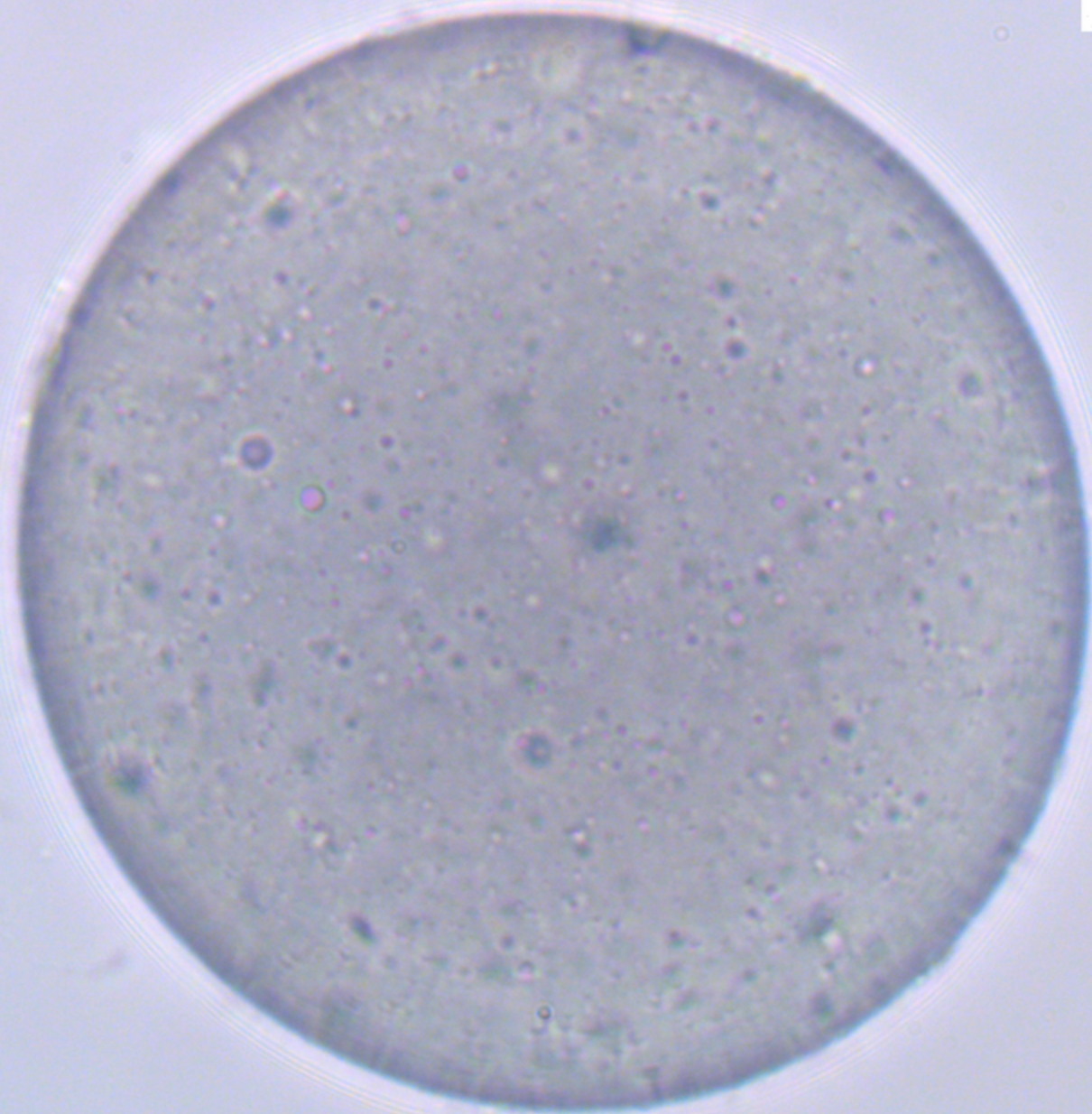




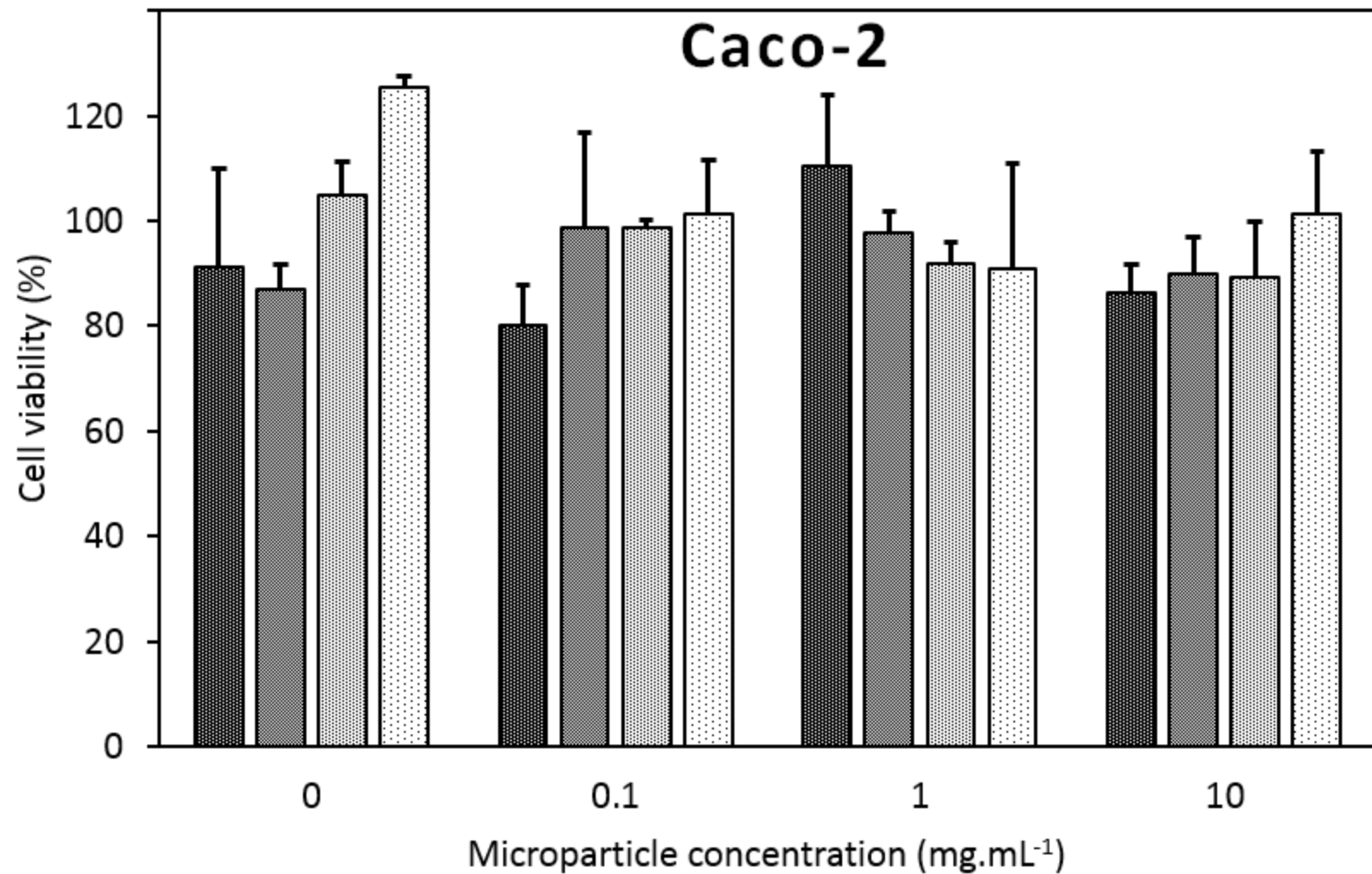
Time (Hours)





**a****b****10  $\mu\text{m}$**

### Caco-2



### NIH3T3

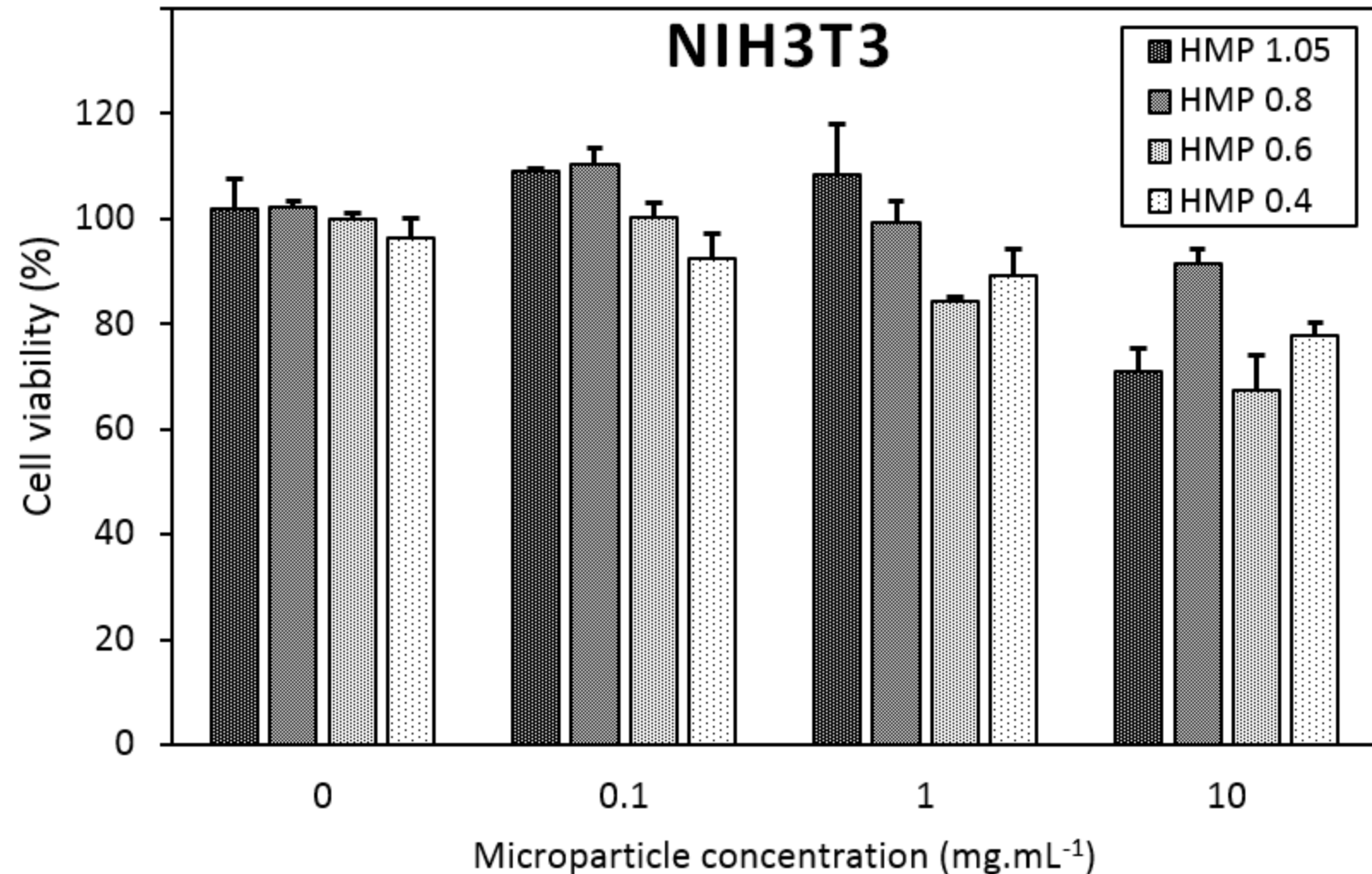


Table 1. Physicochemical properties of ICOs.

ICO	Molecular weight (g·mol <sup>-1</sup> )	Viscosity (mPa·s)	density
<b>1.05</b>	1600.9	961	0.999
<b>0.8</b>	1467.5	764	0.994
<b>0.6</b>	1334.1	709	0.986
<b>0.4</b>	1200.7	682	0.970
<b>Castor oil</b>	932.7	661	0.907

Table 2. Preparation yields of HMPs synthesized with the corresponding ICO. Data are shown as mean ± SD (n=3).

HMPs	Preparation yield $\eta$ (%)	Weight loss by ethoxysilanes hydrolysis (%)	Corrected preparation yield $\eta'$ (%)
<b>1.05</b>	73.4 ± 1.4	21.9	95.3 ± 1.4
<b>0.8</b>	70.0 ± 1.6	19.1	89.1 ± 1.6
<b>0.6</b>	75.3 ± 2.9	15.7	91.0 ± 2.9
<b>0.4</b>	75.7 ± 2.5	11.7	87.4 ± 2.5

Table 3. Volume Median Diameters (VMD)s, spans and deducted parameters (volume and surface area moment means) of HMPs with four  $X_R$ . Data are shown as mean±SD (n=3).

HMP	VMD ( $\mu\text{m}$ )	Span	D [4,3] ( $\mu\text{m}$ )	D [3,2] ( $\mu\text{m}$ )
<b>1.05</b>	53.8 ± 4.9	1.38 ± 0.05	56.7 ± 5.1	22.3 ± 1.8
<b>0.8</b>	55.0 ± 5.3	1.55 ± 0.15	58.0 ± 5.3	22.4 ± 2.5
<b>0.6</b>	51.5 ± 2.7	1.67 ± 0.09	57.1 ± 6.9	19.9 ± 0.2
<b>0.4</b>	52.3 ± 2.9	1.54 ± 0.11	54.9 ± 2.9	22.6 ± 1.0

Table 4. Condensation yields and degree of HMPs with various  $X_R$ .

HMPs <sup>a</sup>	<sup>29</sup> Si signal (%)					CY (%)	CD (%)
	LP (-45 ppm)	T <sup>0</sup> (-48 ppm)	T <sup>1</sup> (-53 ppm)	T <sup>2</sup> (-57 ppm)	T <sup>3</sup> (-67 ppm)		
<b>1.05</b>	39.9	3.3	3.4	21.8	31.6	60.1	78.6
<b>0.8</b>	11.4	7.5	2.8	42.5	35.8	88.6	73.4
<b>0.6</b>	1.7	5.5	4.4	42.4	46.0	98.3	77.0

<sup>a</sup>): HMPs 0.4 exhibited an insufficient signal-to-noise ratio to reach the confident accuracy of integrations (>95 %).

Table 5. Mass percentage of fluorine obtained from EDX spectra on a cross-section of 8 % flurbiprofen-loaded HMP. Data are shown as mean  $\pm$  SD (n=3) and the technical accuracy was  $\pm$ 10 % of each value.

Spectrum	Atomic rate (%)		
	Fluorine	Carbon	Silicium
<b>1</b>	0.44 $\pm$ 0.2	81.9 $\pm$ 4.0	1.87 $\pm$ 0.9
<b>1'</b>	0.49 $\pm$ 0.1	81.3 $\pm$ 0.7	1.73 $\pm$ 0.7
<b>2</b>	0.45 $\pm$ 0.1	82.7 $\pm$ 2.0	1.52 $\pm$ 0.4
<b>2'</b>	0.37 $\pm$ 0.1	82.7 $\pm$ 0.5	2.16 $\pm$ 0.9
<b>3</b>	0.52 $\pm$ 0.1	81.6 $\pm$ 3.0	1.75 $\pm$ 0.9
<b>3'</b>	0.39 $\pm$ 0.0	80.8 $\pm$ 1.1	1.77 $\pm$ 0.6
<b>4</b>	0.42 $\pm$ 0.2	81.7 $\pm$ 2.7	1.68 $\pm$ 0.6

Table 6. Diffusion coefficients (D) deducted from the mathematical model. Results are shown as value  $\pm$  probability of deviation

HMP	Diffusion coefficient ( $10^{-9} \text{ cm}^2 \cdot \text{s}^{-1}$ )
<b>1.05</b>	1.44 $\pm$ 0.23
<b>0.8</b>	2.96 $\pm$ 0.18
<b>0.6</b>	4.35 $\pm$ 0.23
<b>0.4</b>	7.28 $\pm$ 0.75

