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Tunable vegetable oil / silica hybrid microparticles for poorly water-soluble drug delivery

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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, ²⁹Si 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.

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; $405.1 \text{ g}\cdot\text{mol}^{-1}$), κ -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[®] 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 ($\text{Na}_2\text{HPO}_4, 12\text{H}_2\text{O}$) and potassium phosphate monobasic (KH_2PO_4) were also supplied
111 by Sigma-Aldrich, while potassium chloride (KCl) was provided by Panreac Quimica, calcium
112 chloride ($\text{CaCl}_2, 2\text{H}_2\text{O}$) by Merck and sodium acetate ($\text{CH}_3\text{COONa}, 3\text{H}_2\text{O}$) 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 κ -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[®] (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[®] LL3000, Thermo
 142 Fisher Scientific). After recovery, preparation yields η 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[®] equipped
 148 with AxioCam[®] ERc 5s model, Zeiss) and scanning electron microscopy (SEM, Hitachi S4800
 149 high resolution). HMPs size measurements were assessed by laser diffraction (Mastersizer[®]
 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 Anatsats[®] software was used to compare the results and $P < 0.05$ value
 158 was considered statistically significant.

* 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 ²⁹Si-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 ¹H
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^N 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⁻¹ 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 η' 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° of 2Θ angle) to detect ibuprofen
191 crystals and Differential Scanning Calorimetry (DSC; DSC4000, PerkinElmer, sample heated
192 from 20 to $95 \text{ }^\circ\text{C}$ at $10 \text{ }^\circ\text{C}\cdot\text{min}^{-1}$ 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 $37 \text{ }^\circ\text{C}$) 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 $37 \text{ }^\circ\text{C}$) 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 $37 \text{ }^\circ\text{C}$ and **(3)**: 12 mM of phosphate buffer without PS80,
204 pH = 7.4 at $37 \text{ }^\circ\text{C}$ (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 $37 \text{ }^\circ\text{C}$, 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 CaCl₂ ([Ca²⁺] = 36mM) was added in each medium in order to maintain
225 media volumes constant while the Ca²⁺ 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 % CO₂ for 24 h. Subsequently, they were
241 treated with various samples (particle suspensions: 0.1, 1 or 10 mgmL⁻¹, 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 cm^{-1} ; ii) the appearance of
254 urethane group characterized by three vibration bands: (a) an amid-I band at 1720 cm^{-1}
255 corresponding to the C=O stretching vibration; (b) an amid-II band at 1700 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 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 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 cm^{-1} and 1240 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 η' 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 μm 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 μm and were
296 quite close to the VMD. In contrast, $D[3,2]$ were much lower (from 19.9 to 22.6 μm)
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 cm^{-1} were assigned to the ethoxysilanes ($\text{Si-O-CH}_2\text{-}$
316 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 cm^{-1} attributed to overlapping of the -Si-O-Si- and -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 ^1H 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}Si -NMR spectroscopy enhanced the response of silicon nuclei and
327 perfectly identified the chemical shifts of non condensed (T^0), partially (T^1 , T^2) and fully
328 condensed (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}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}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 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-L₂ and K-L₃ transition[§]), carbon (K-L₂ and K-L₃ transition) and silicium (K-L₃ 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 η'
396 (and consequently the encapsulation yields) might be overestimated. Second, the loading rate

[§] K-L₂ and K-L₃ 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}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 μmol) 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**

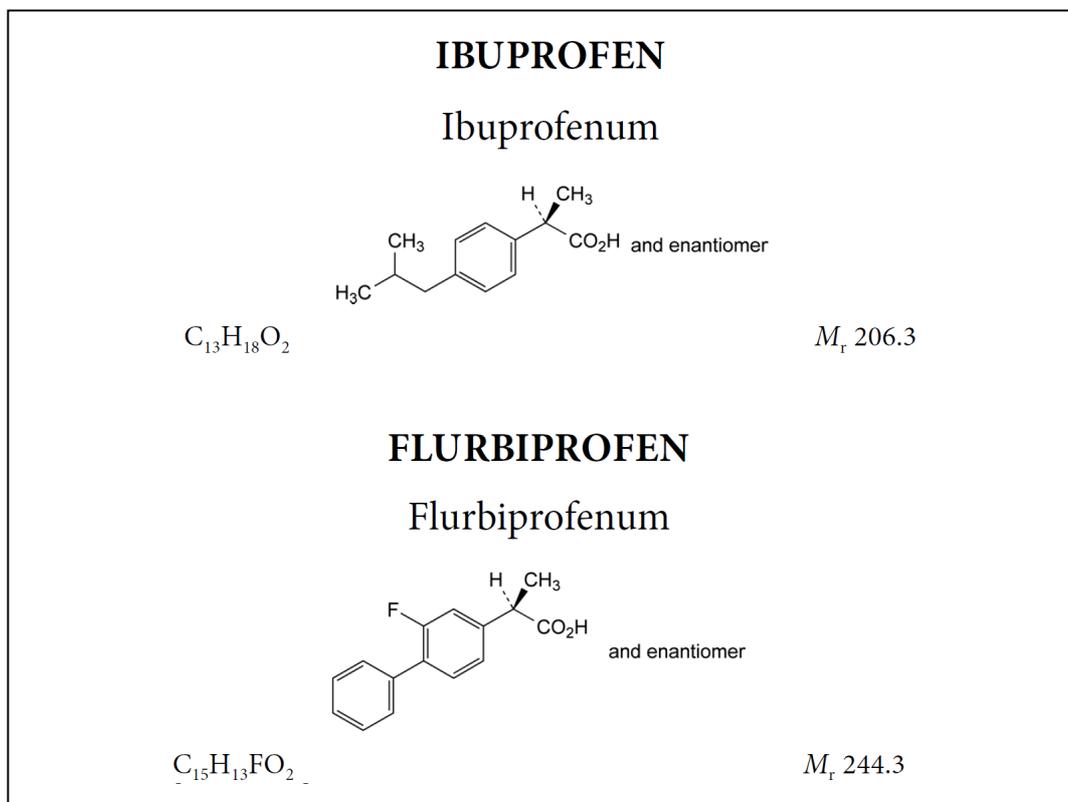
502 An original approach to formulate poorly water-soluble APIs in vegetable oil / silica hybrid
503 microparticles (HMPs) was described. A complete oil silylation in solvent- and catalyst-free
504 conditions was reached whatever the oil / silica ratio used, and the TSE process was optimized
505 for 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**

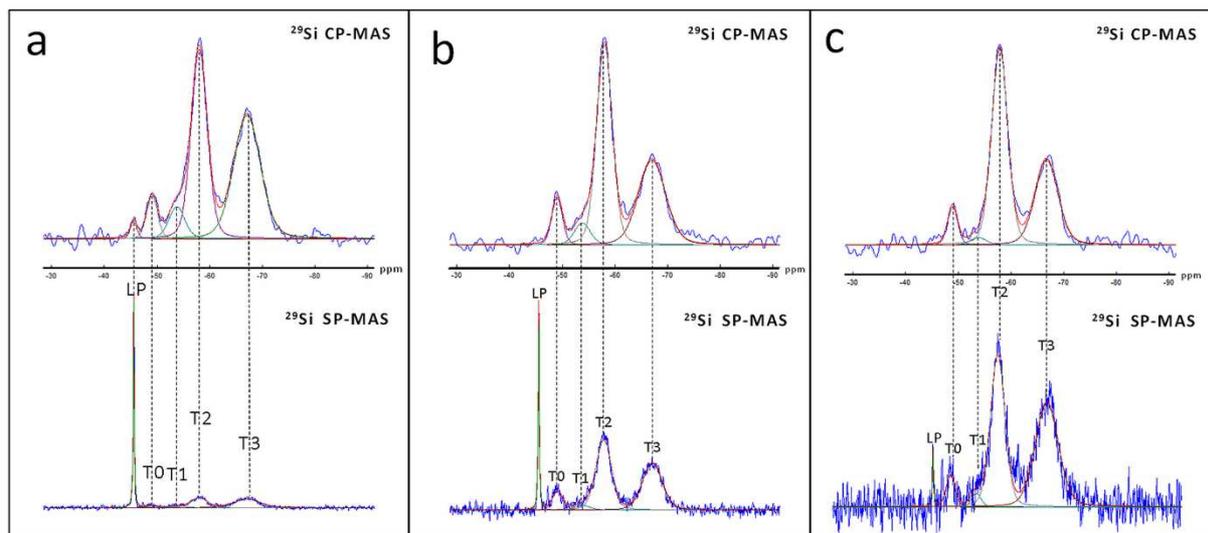
522 **Fig. A.1. Chemical structures of ibuprofen and flurbiprofen (from European**
523 **Pharmacopeia 9.5)**



524

525

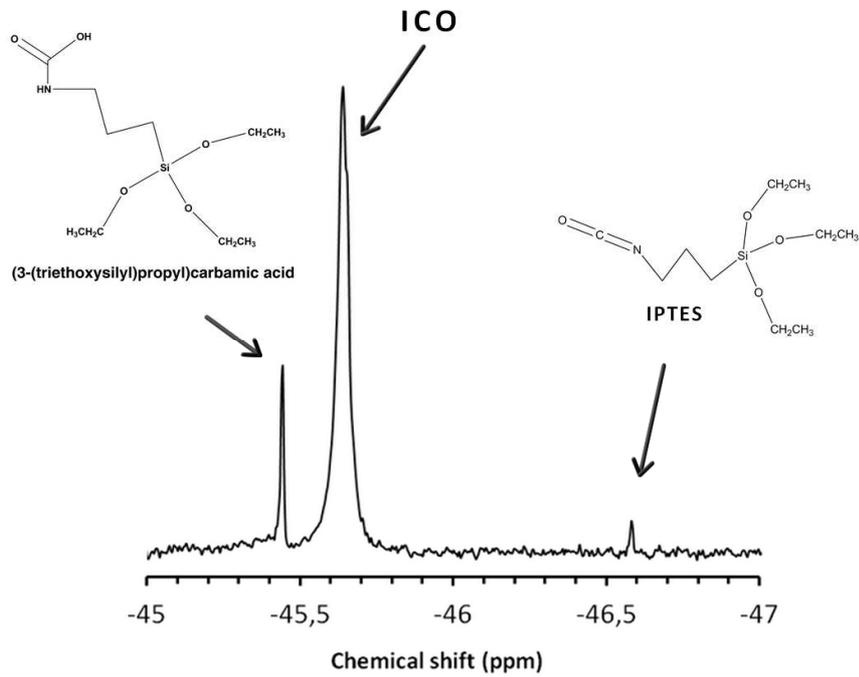
526 **Fig. A.2. ^{29}Si NMR spectra of HMPs: a) 1.05 b) 0.8 c) 0.6 . Spectra were fitted and**
527 **area under curves for SP-MAS experiments were integrated in percent.**



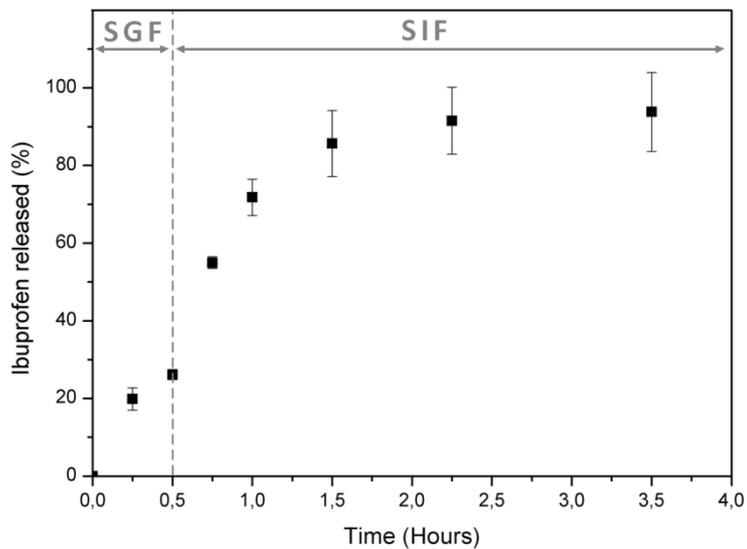
528

529

530 Fig. A.3. ^{29}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 ²⁺	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 amorphously 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_{∞} 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_{∞} 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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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.

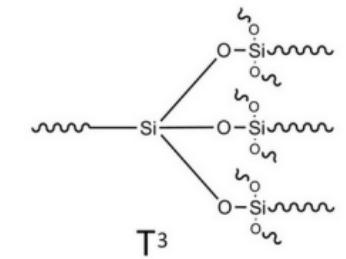
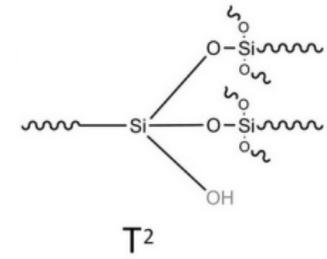
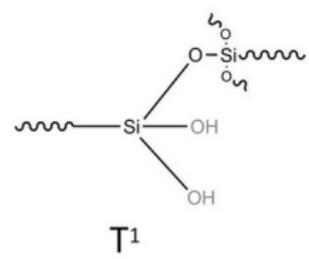
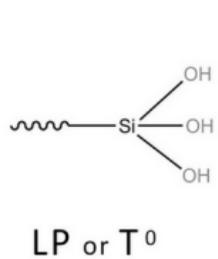
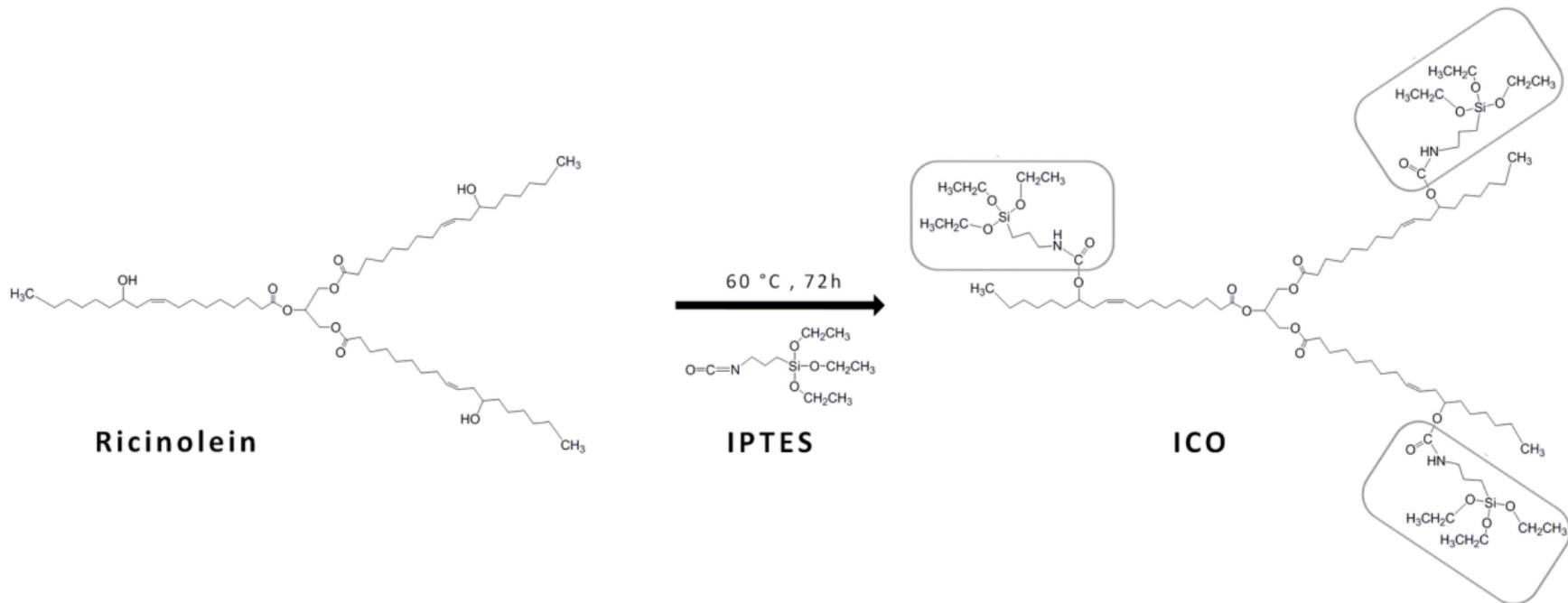
Fig. 13. Experimental and mathematical modeling release kinetics of ibuprofen from HMPs. squares: experimental data; line: computed fits. M_t = ibuprofen released at time “t”; M_{inf} = ibuprofen released at infinite time.

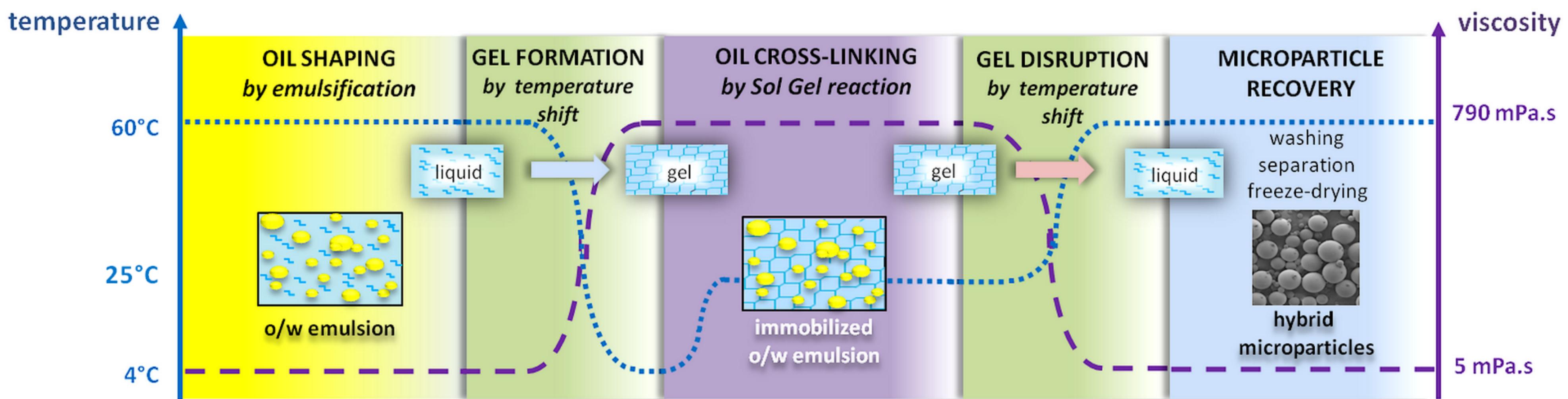
Fig. 14. Release kinetics of 4 wt% ibuprofen-loaded HMP 1.05 in PBS (pH=7.4). The experiment was stopped after 350 hours. n=3.

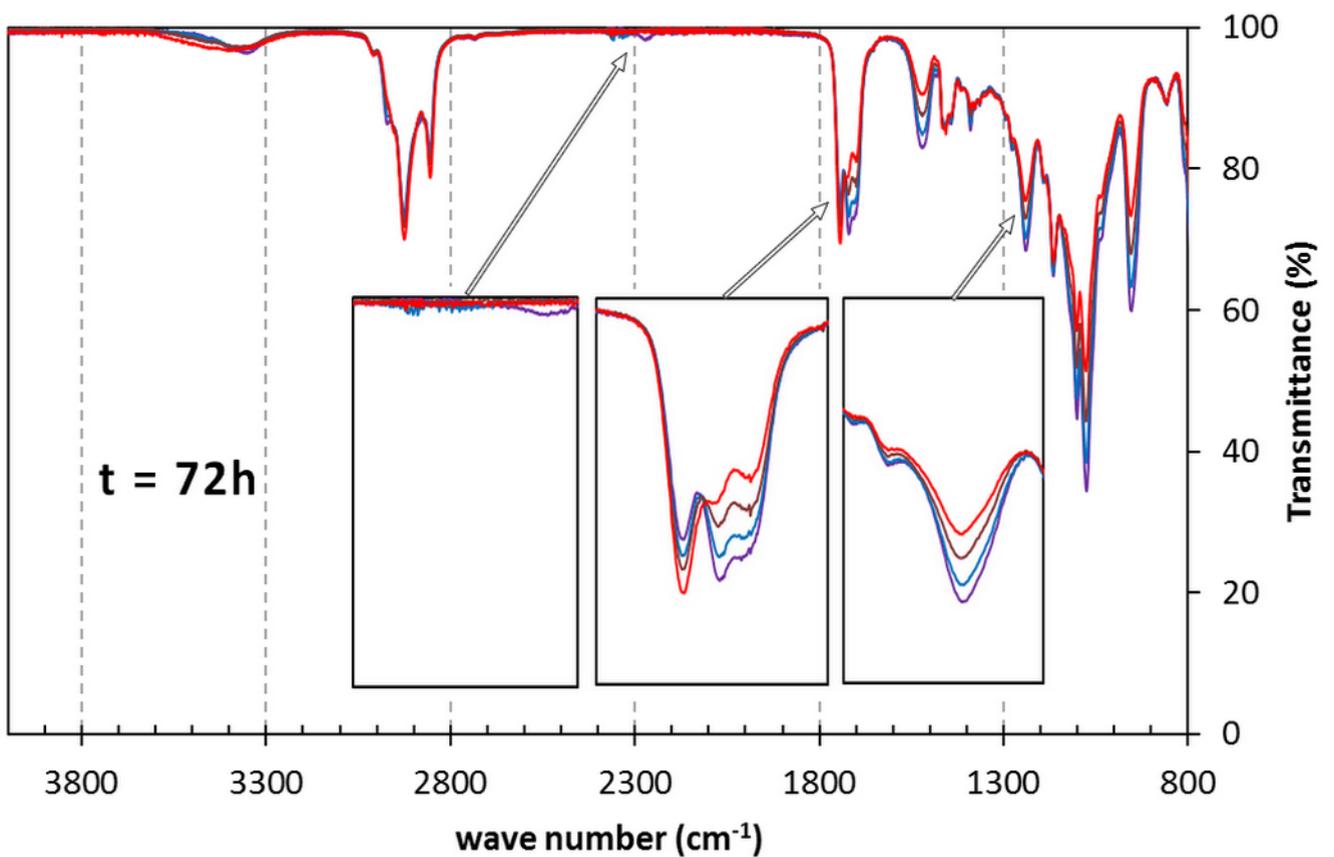
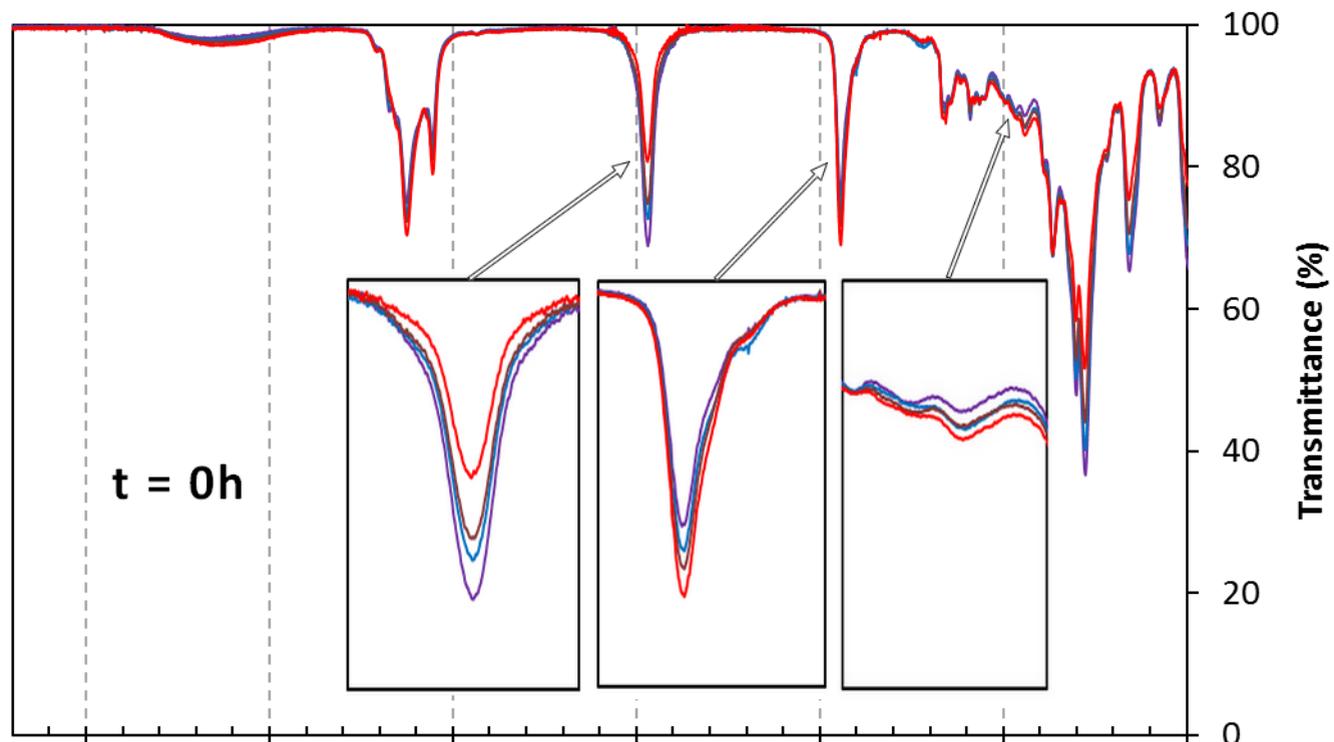
Fig. 15. pH drop versus time of digestion media containing ICO or HMPs. n=2

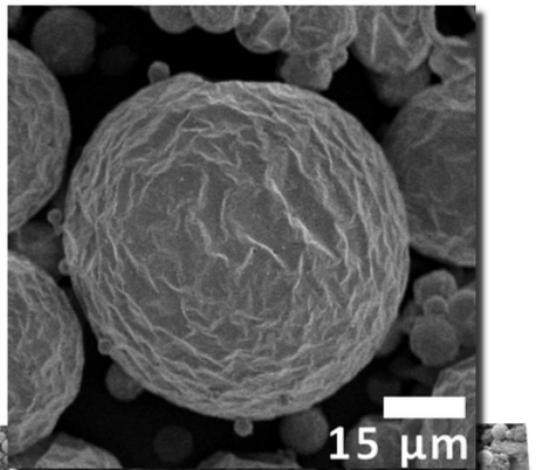
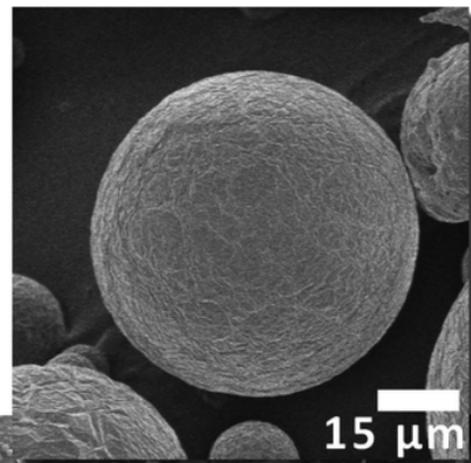
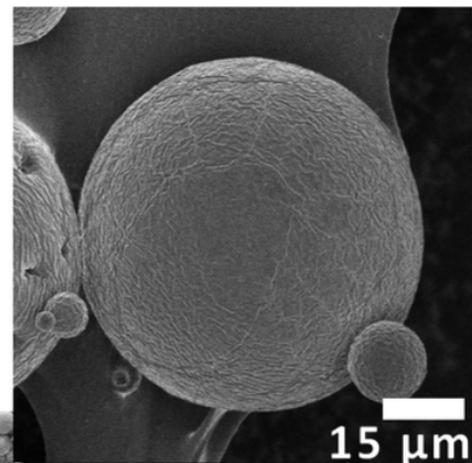
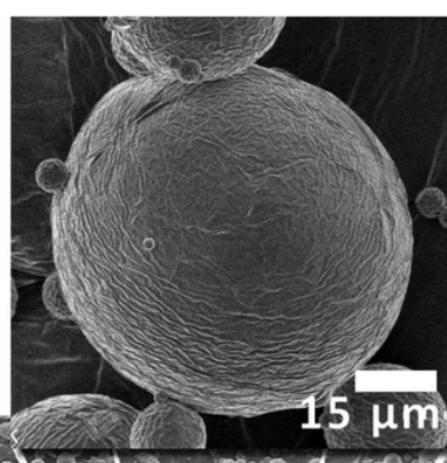
Fig.16. Sudan Black B-loaded HMP a) before and b) after 2 hours in a simulated digestion medium containing lipases.

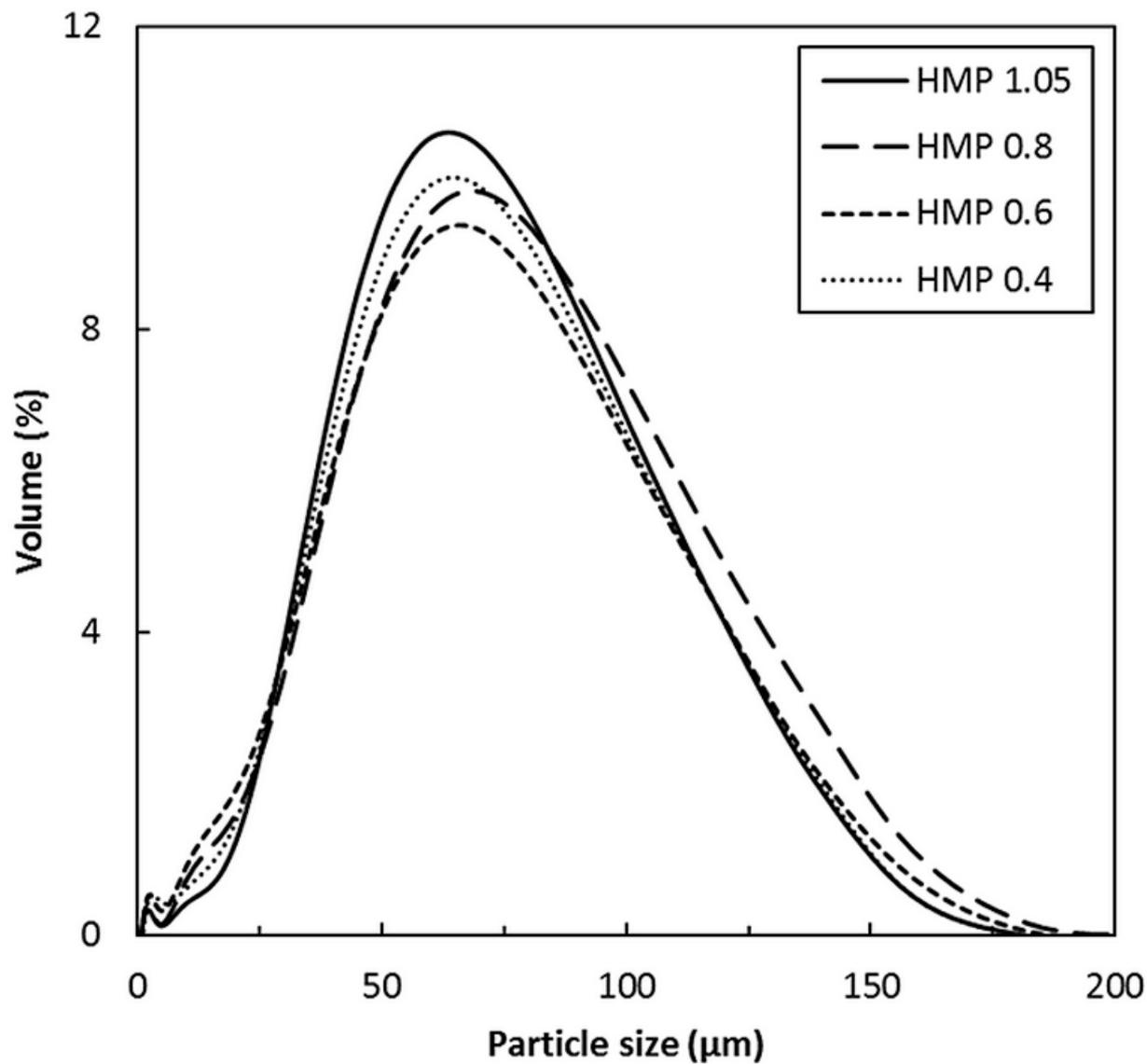
Fig.17. Cytotoxicity studies of HMPs conducted on Caco-2/TC7 enterocytes and NIH-3T3 fibroblasts.

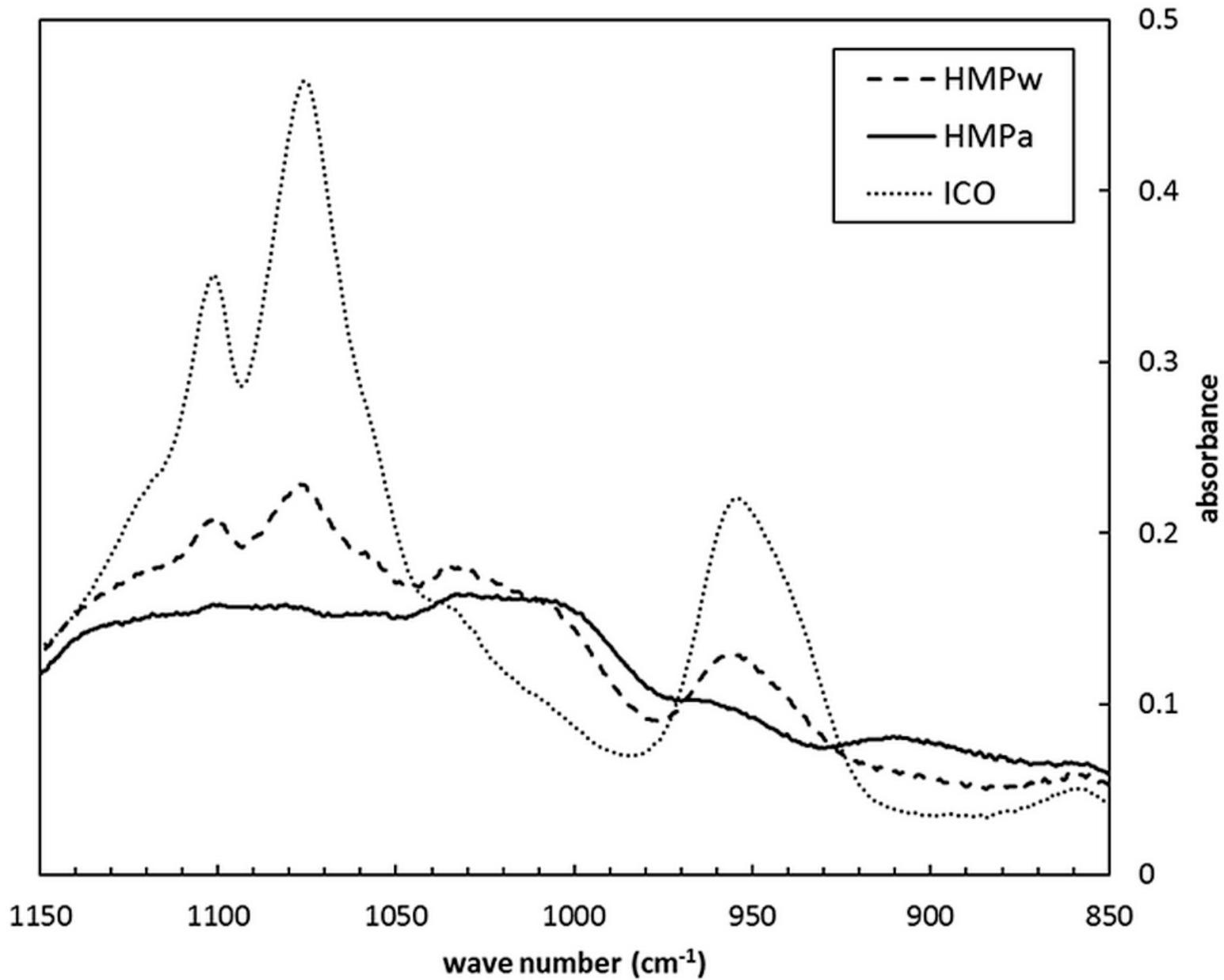


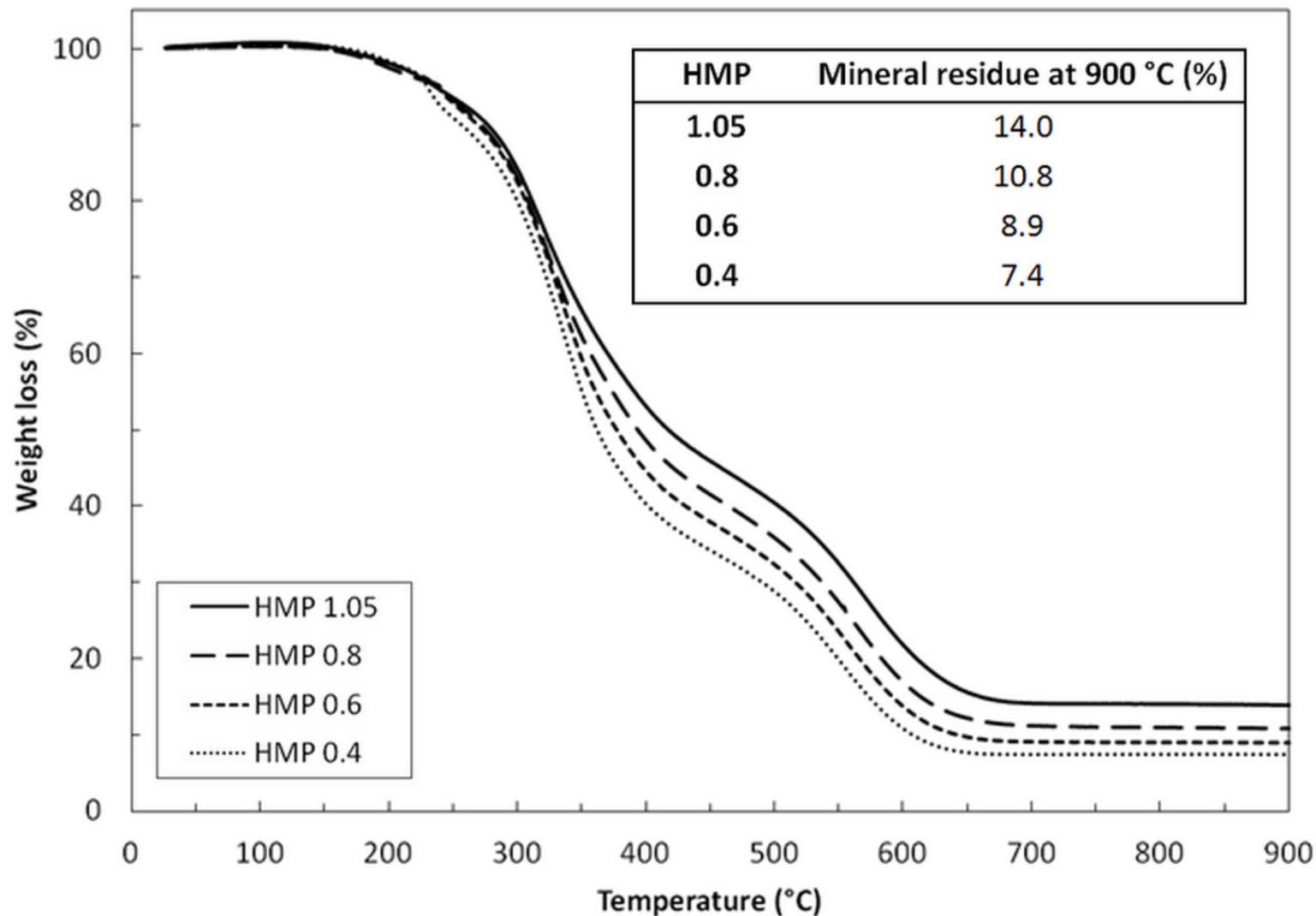


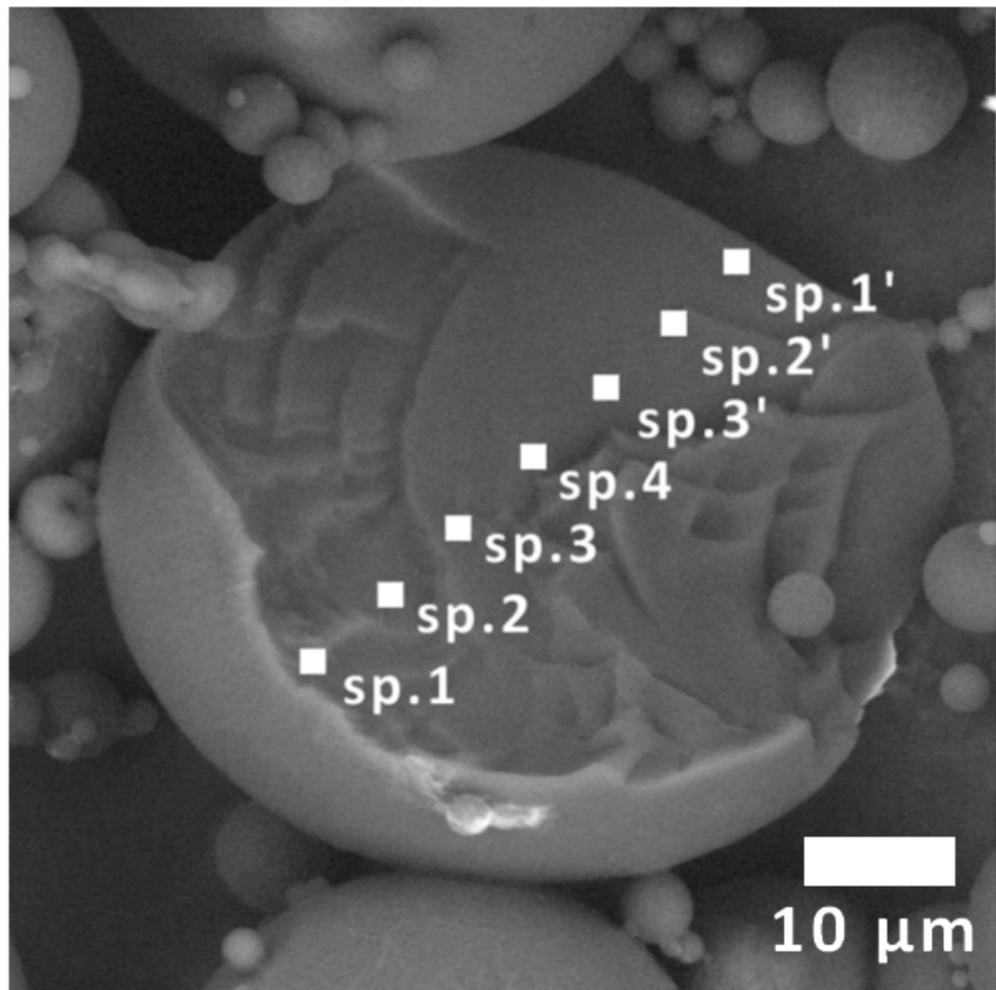


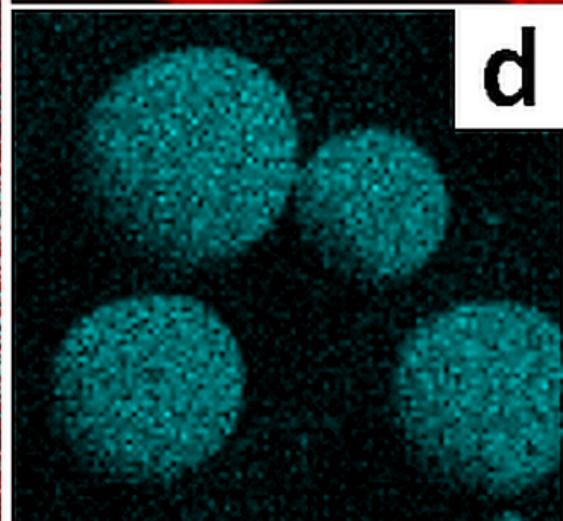
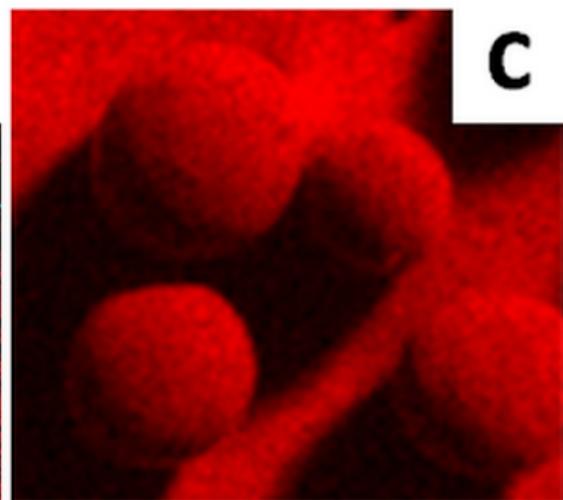
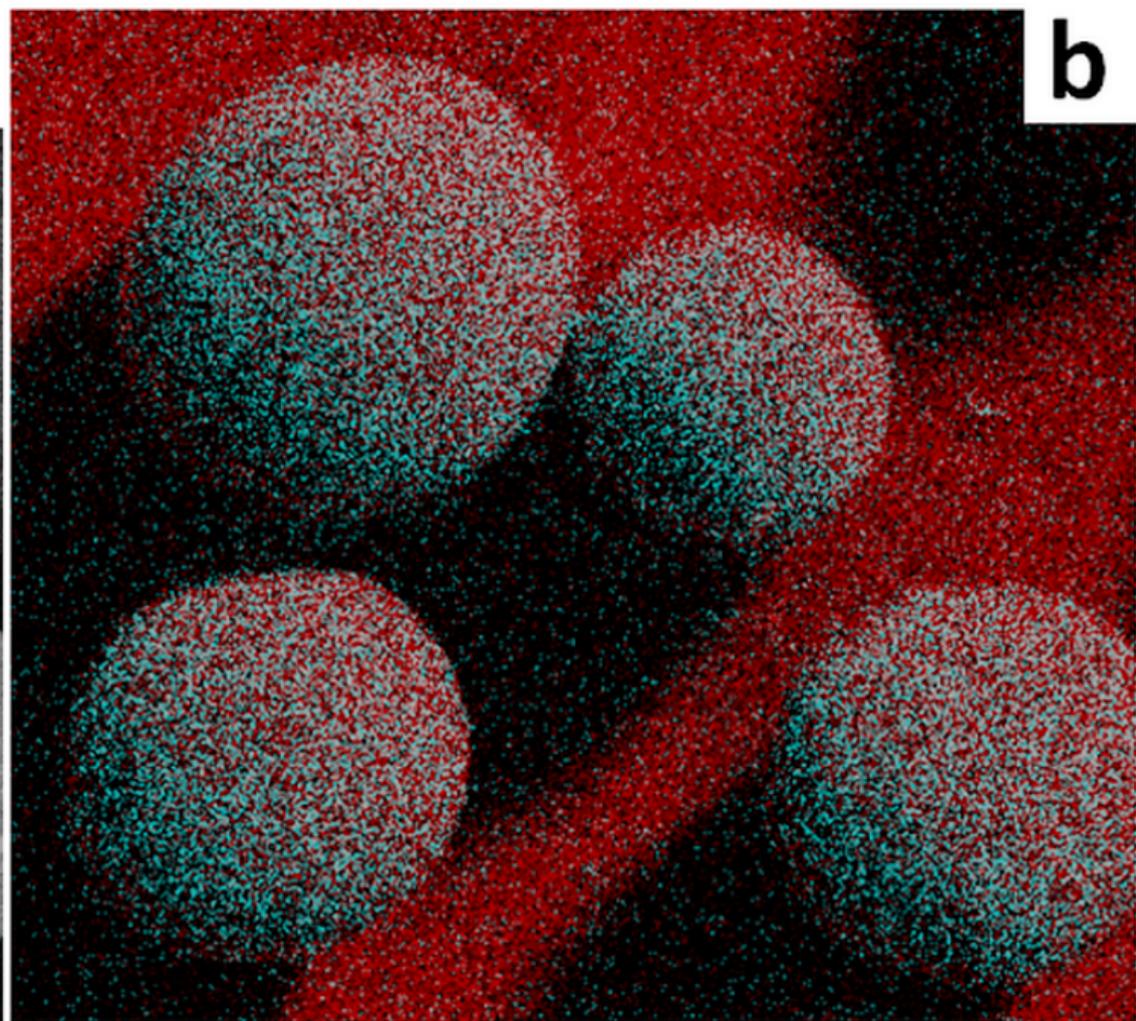
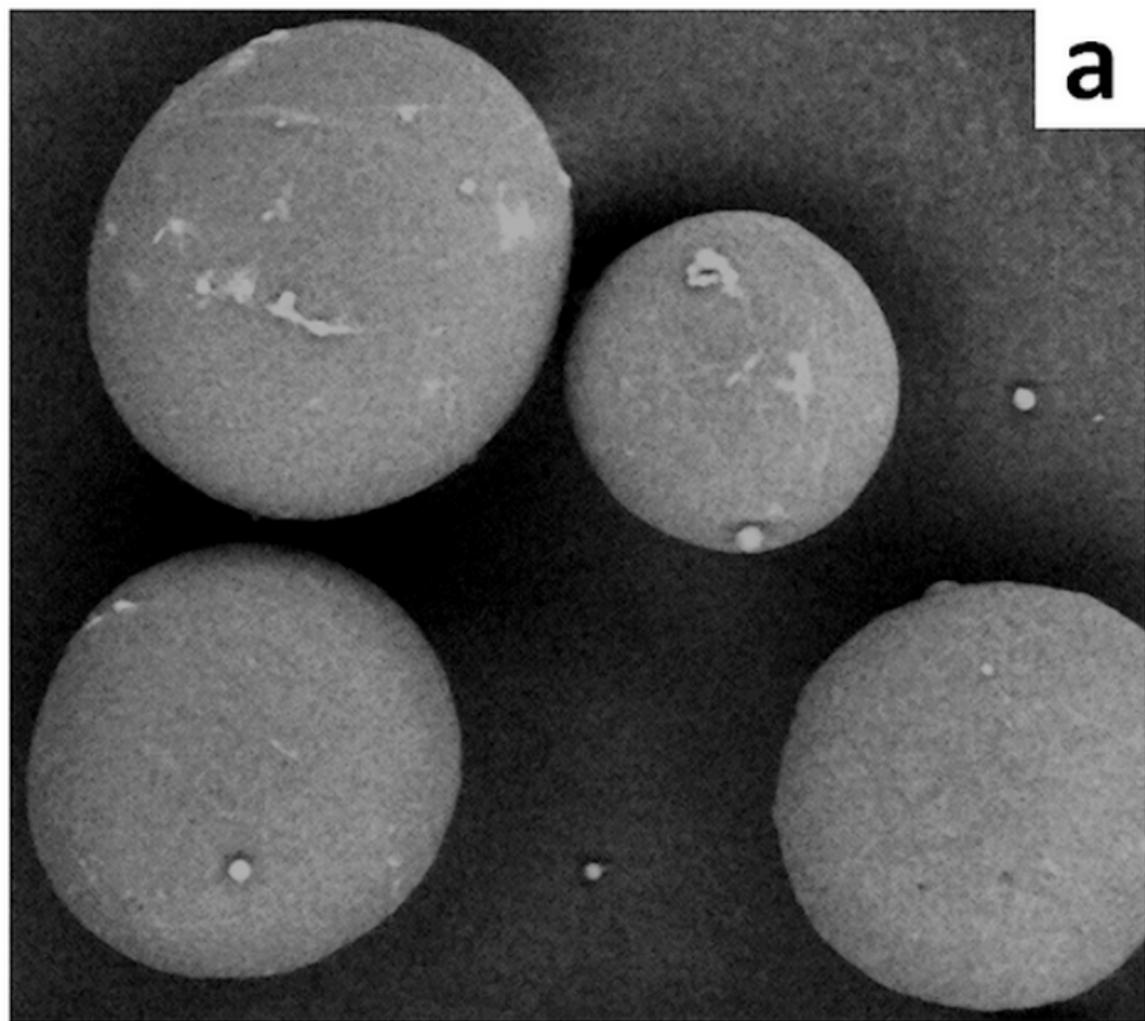
a15 μm 300 μm **b**15 μm 300 μm **c**15 μm 300 μm **d**15 μm 300 μm

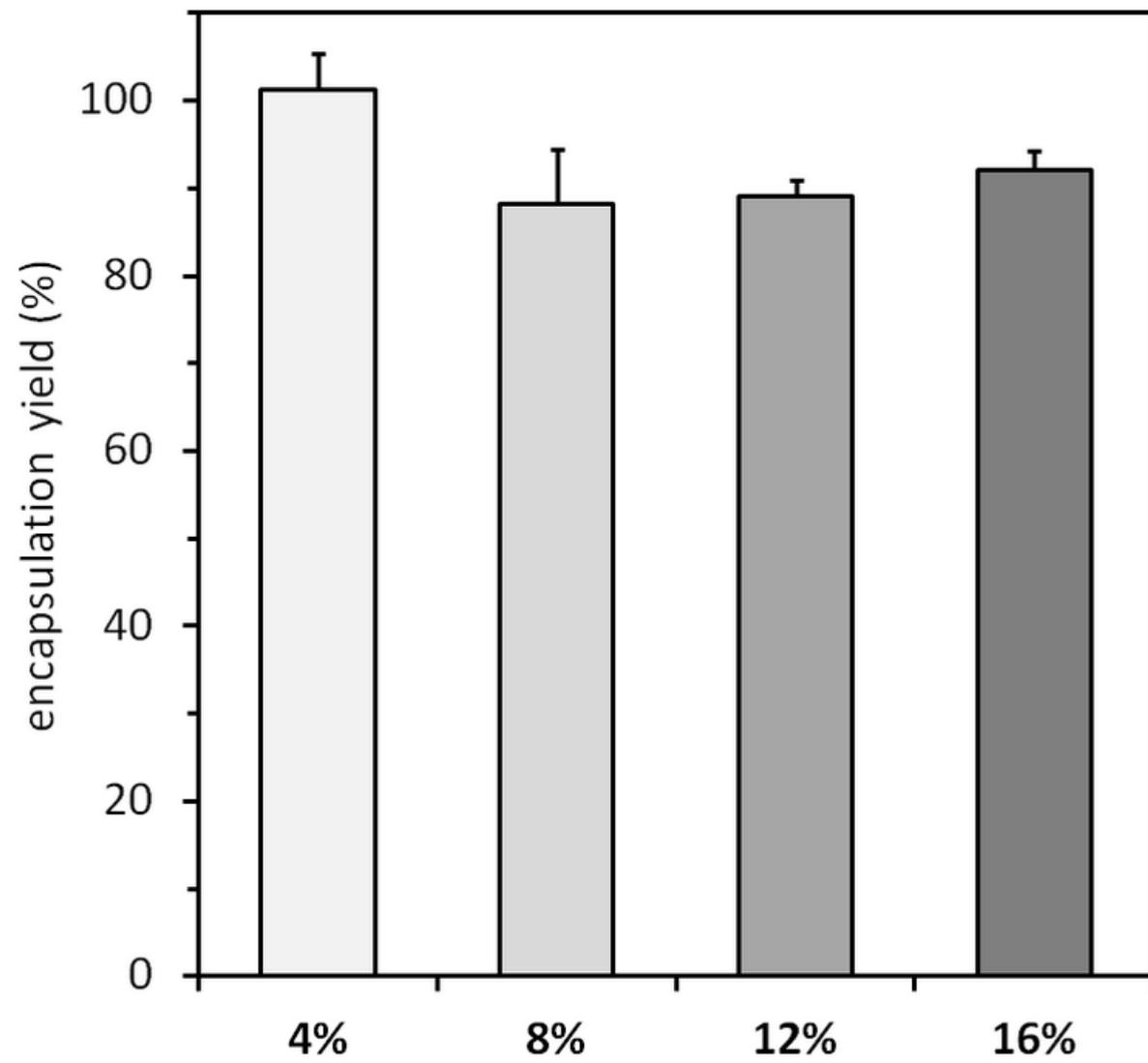
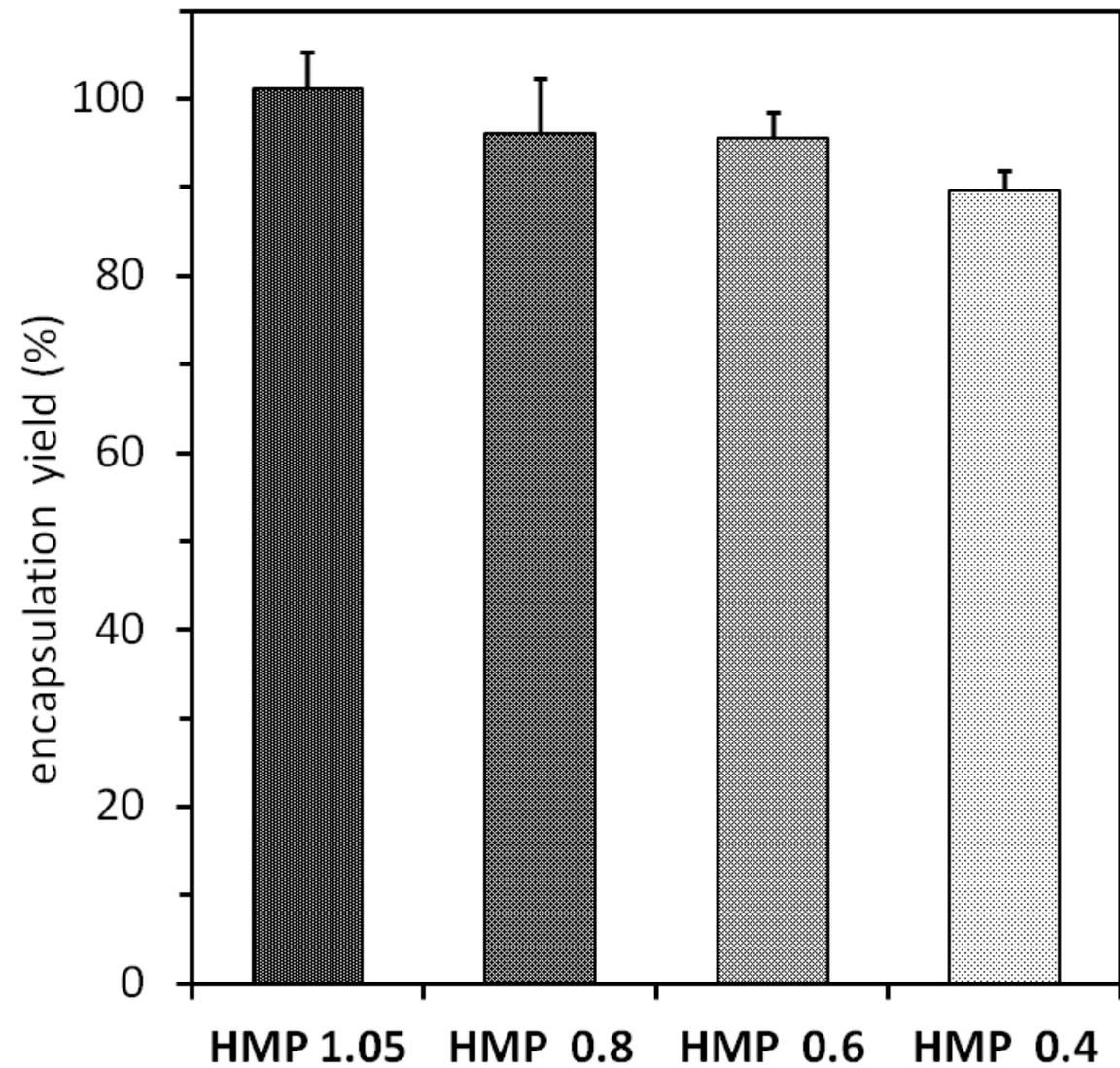




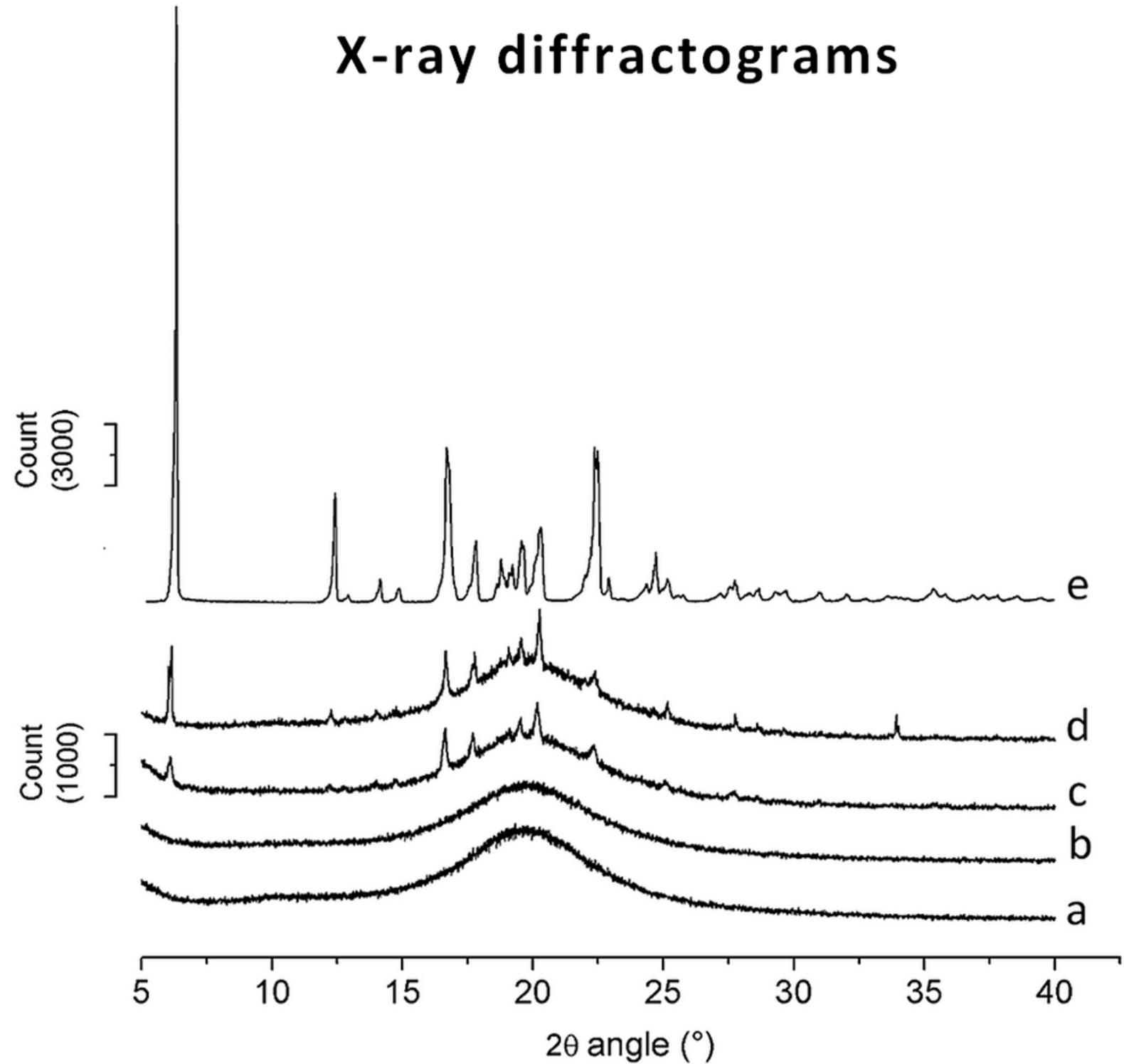




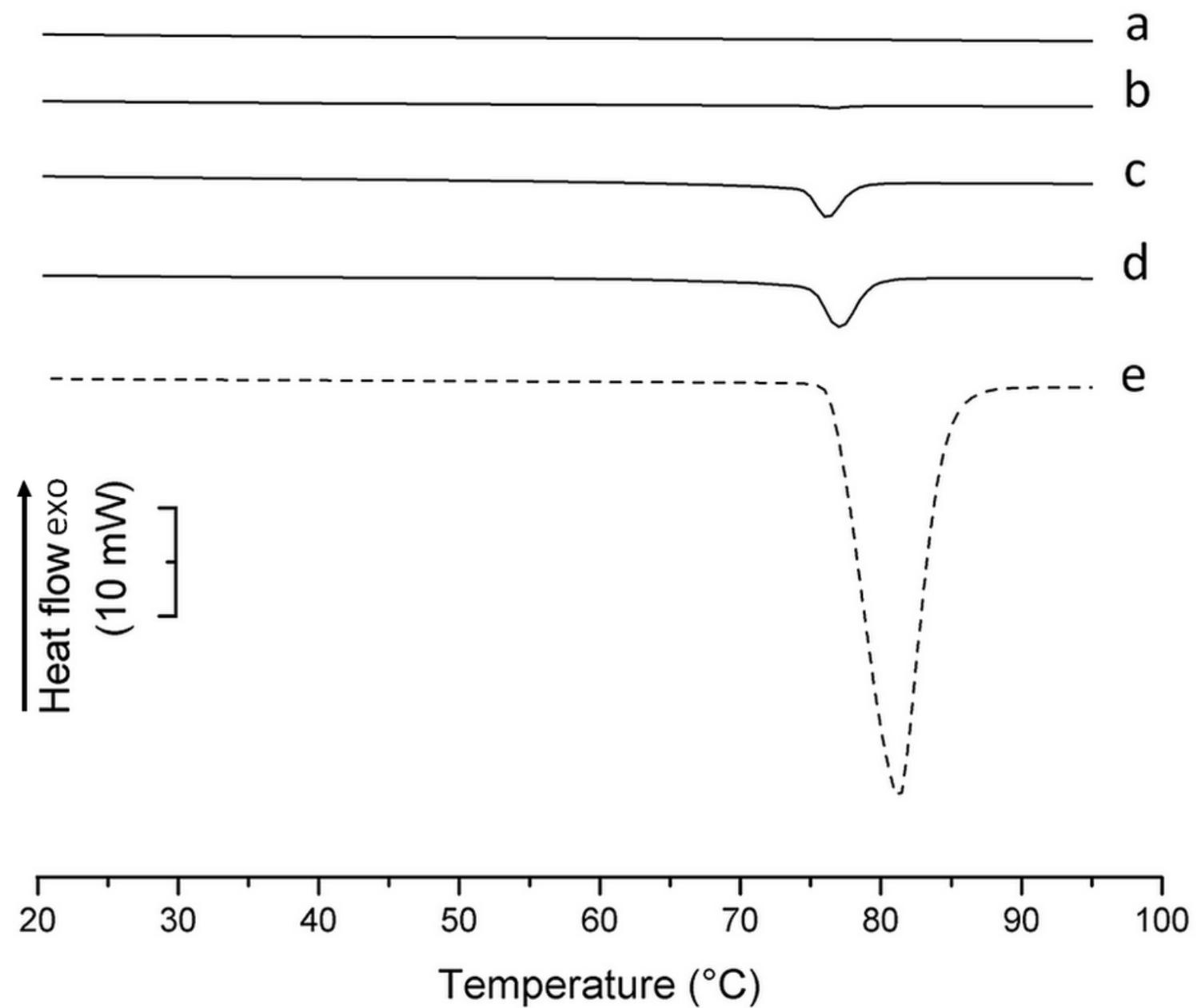


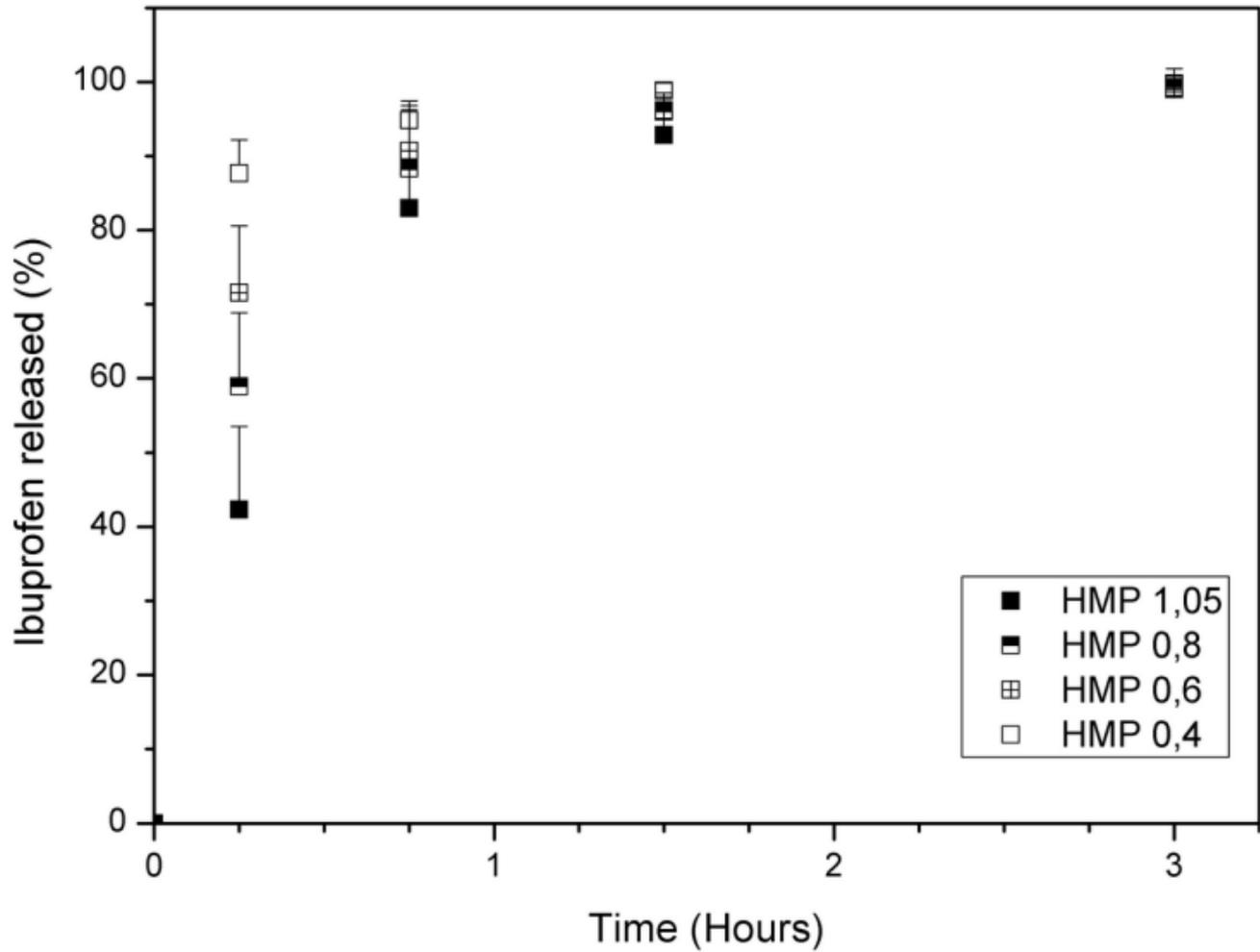


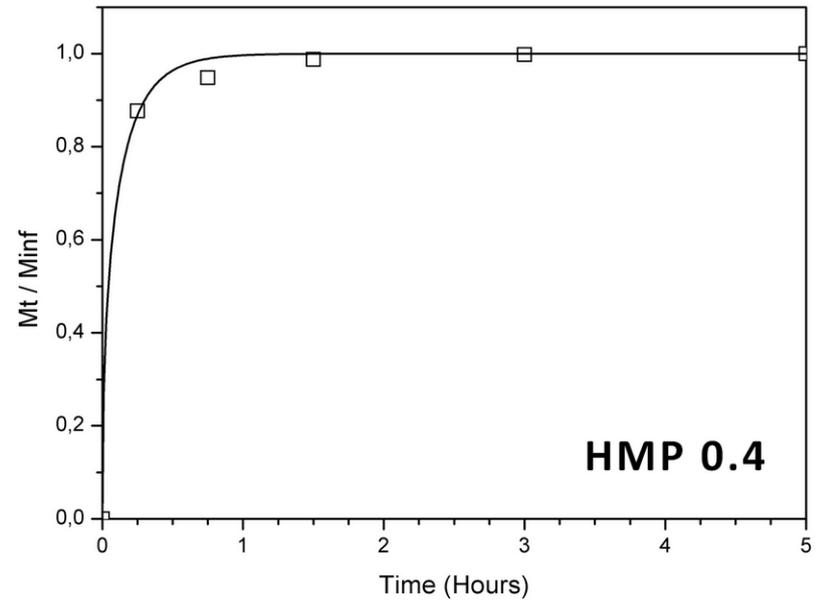
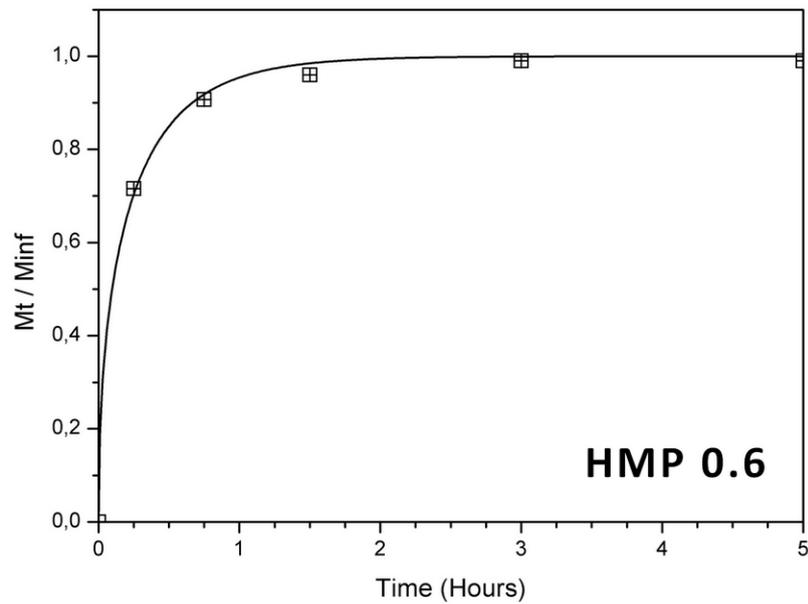
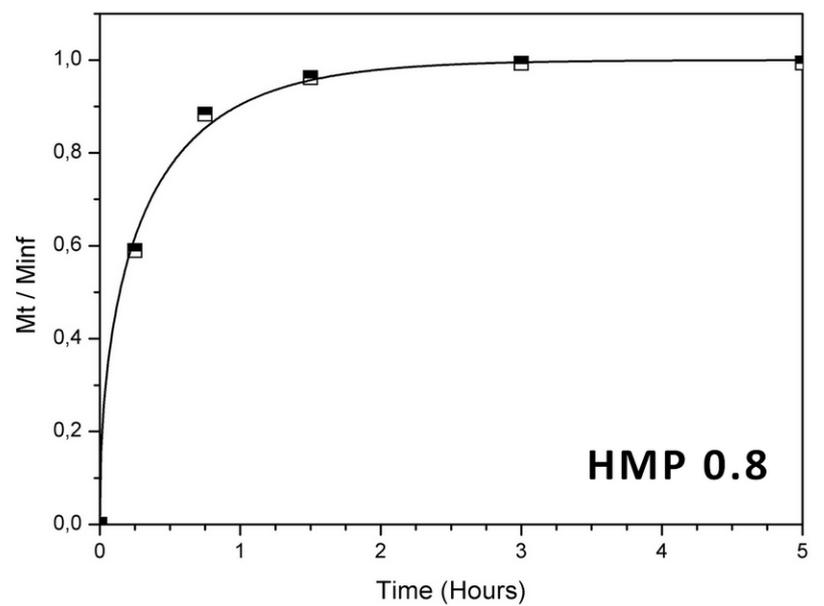
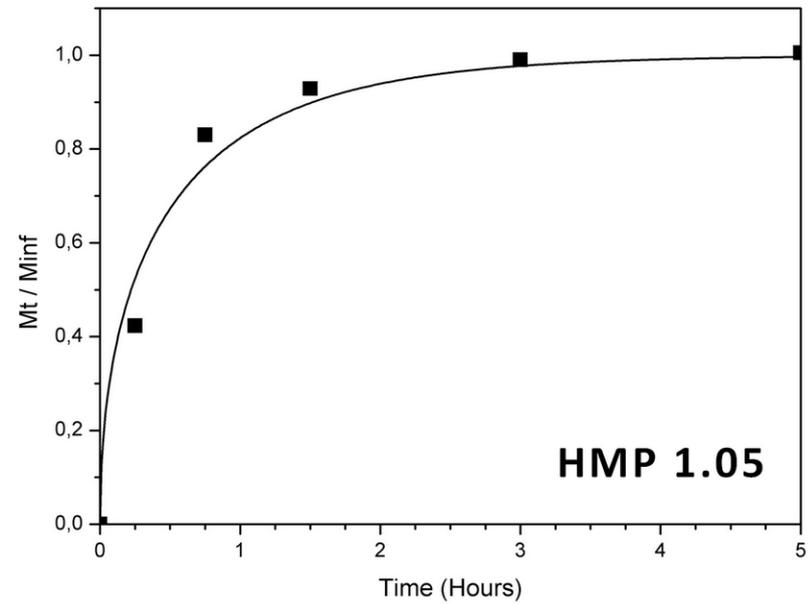
X-ray diffractograms

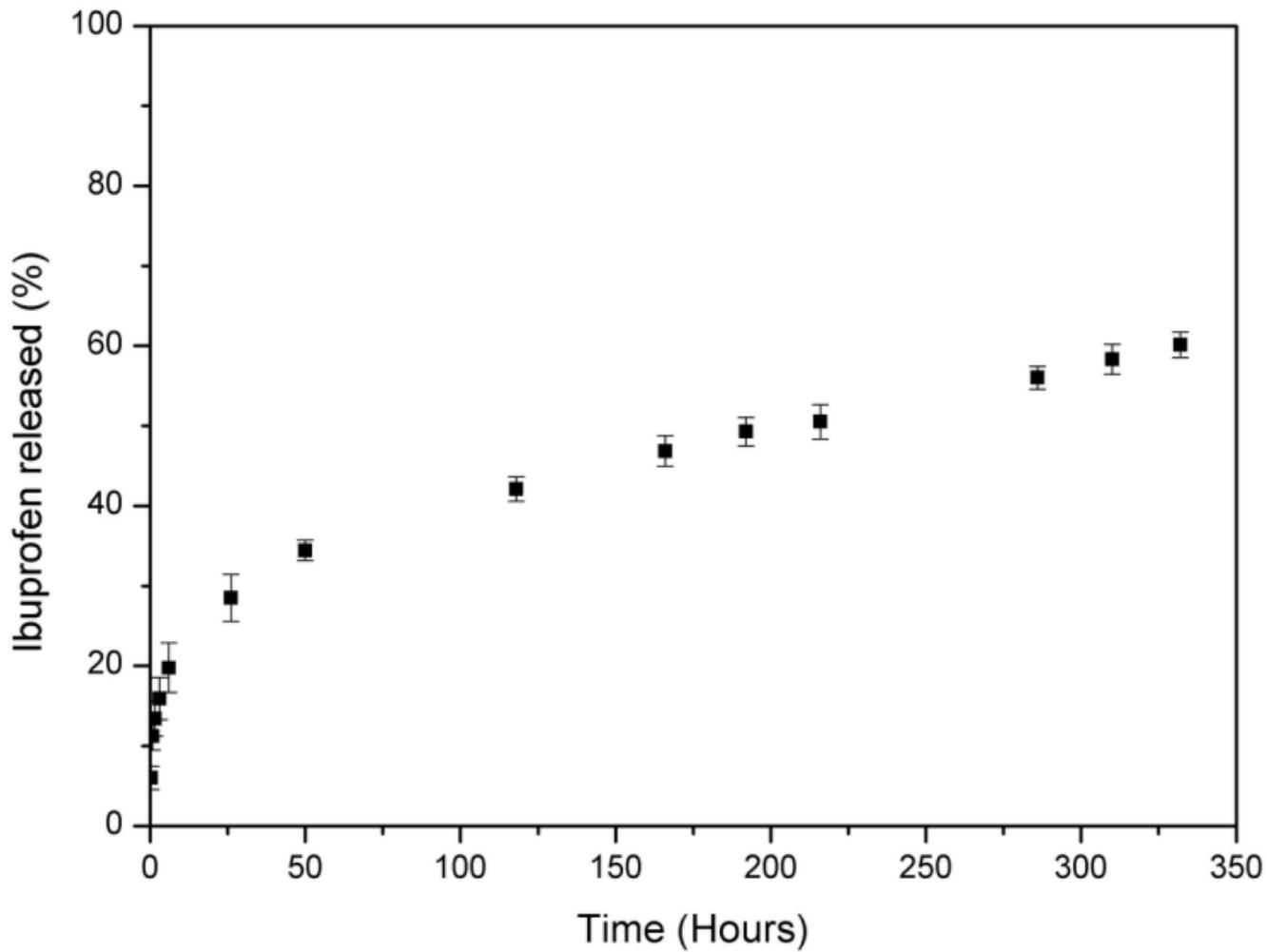


DSC spectra

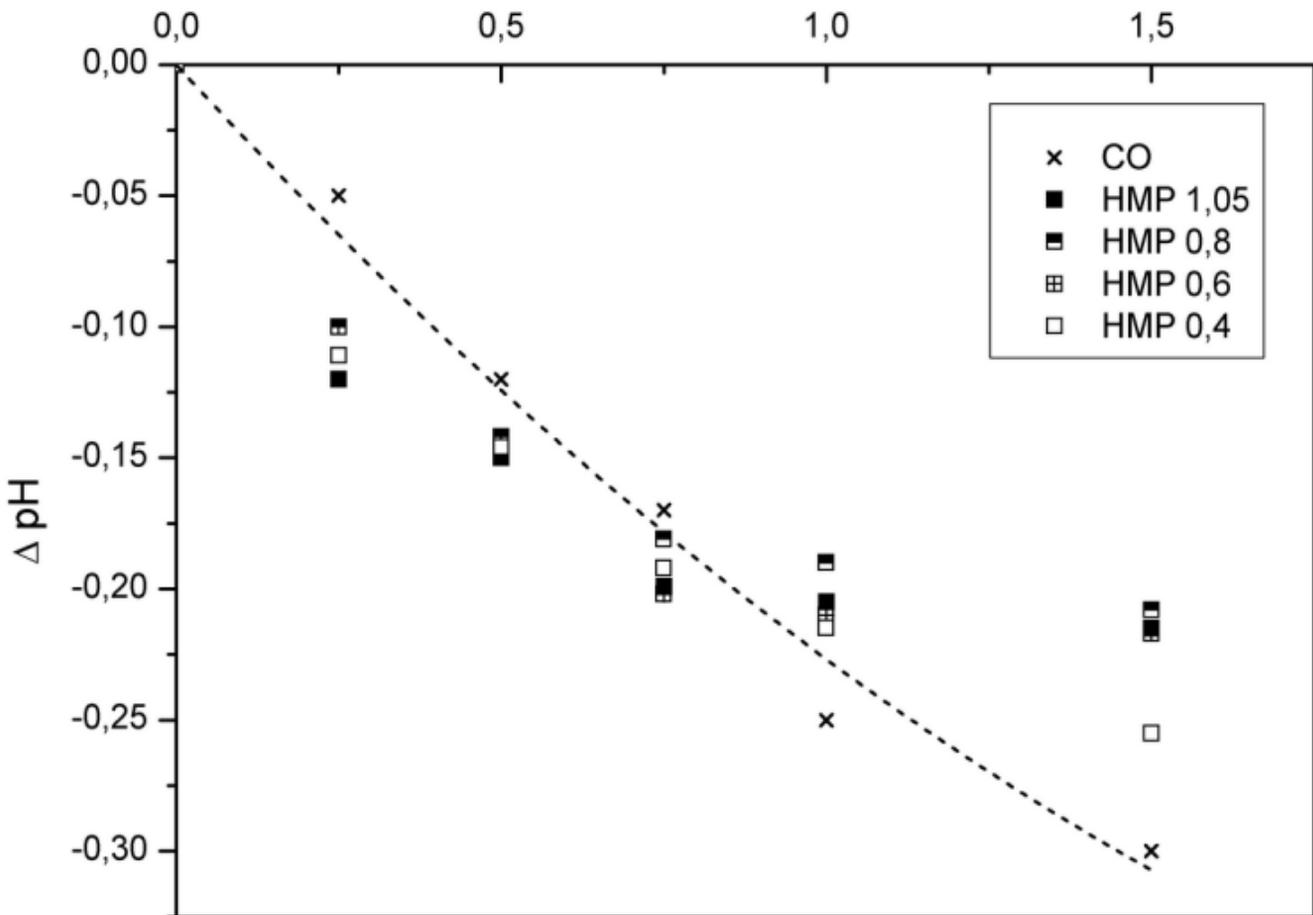


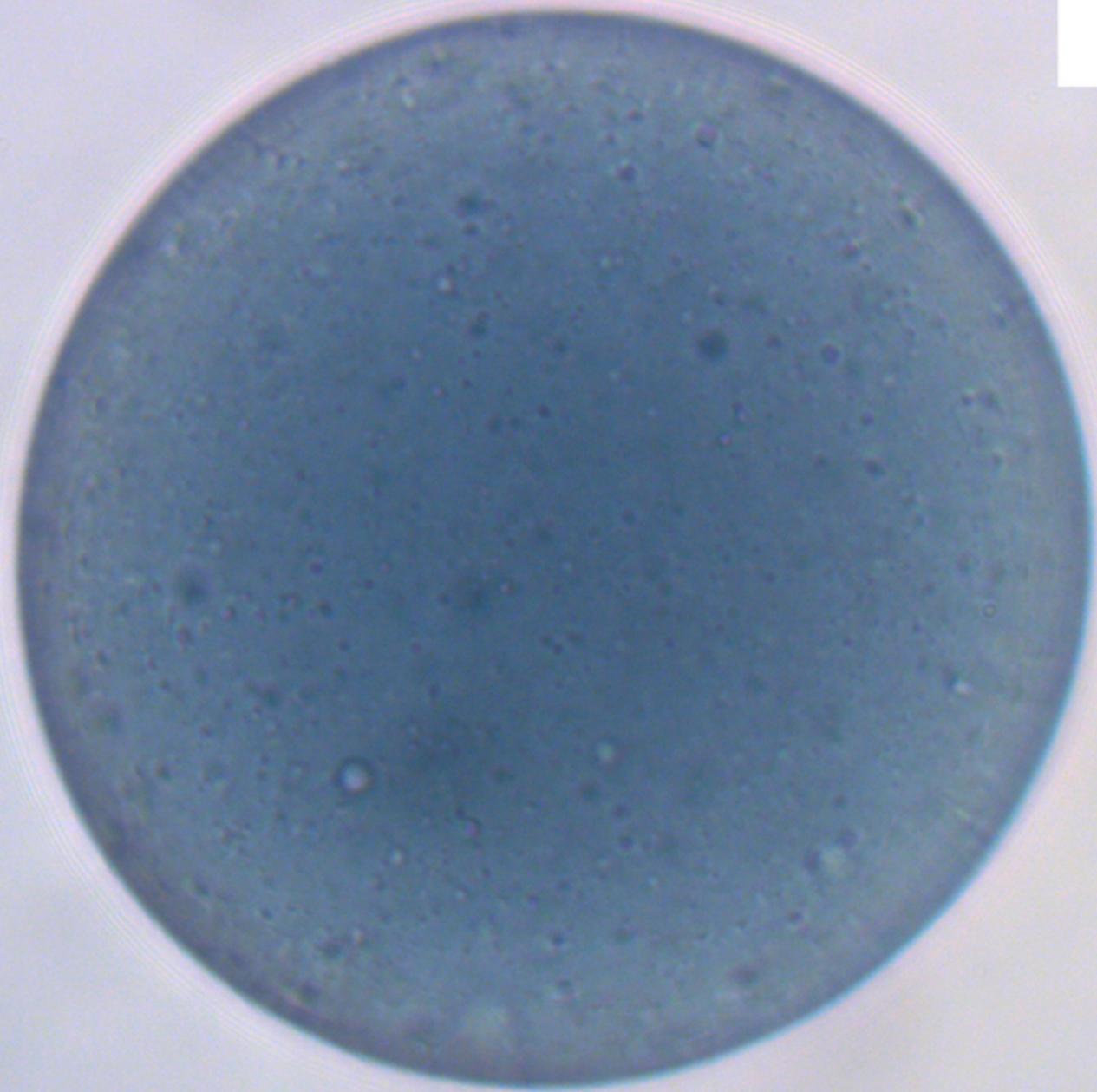
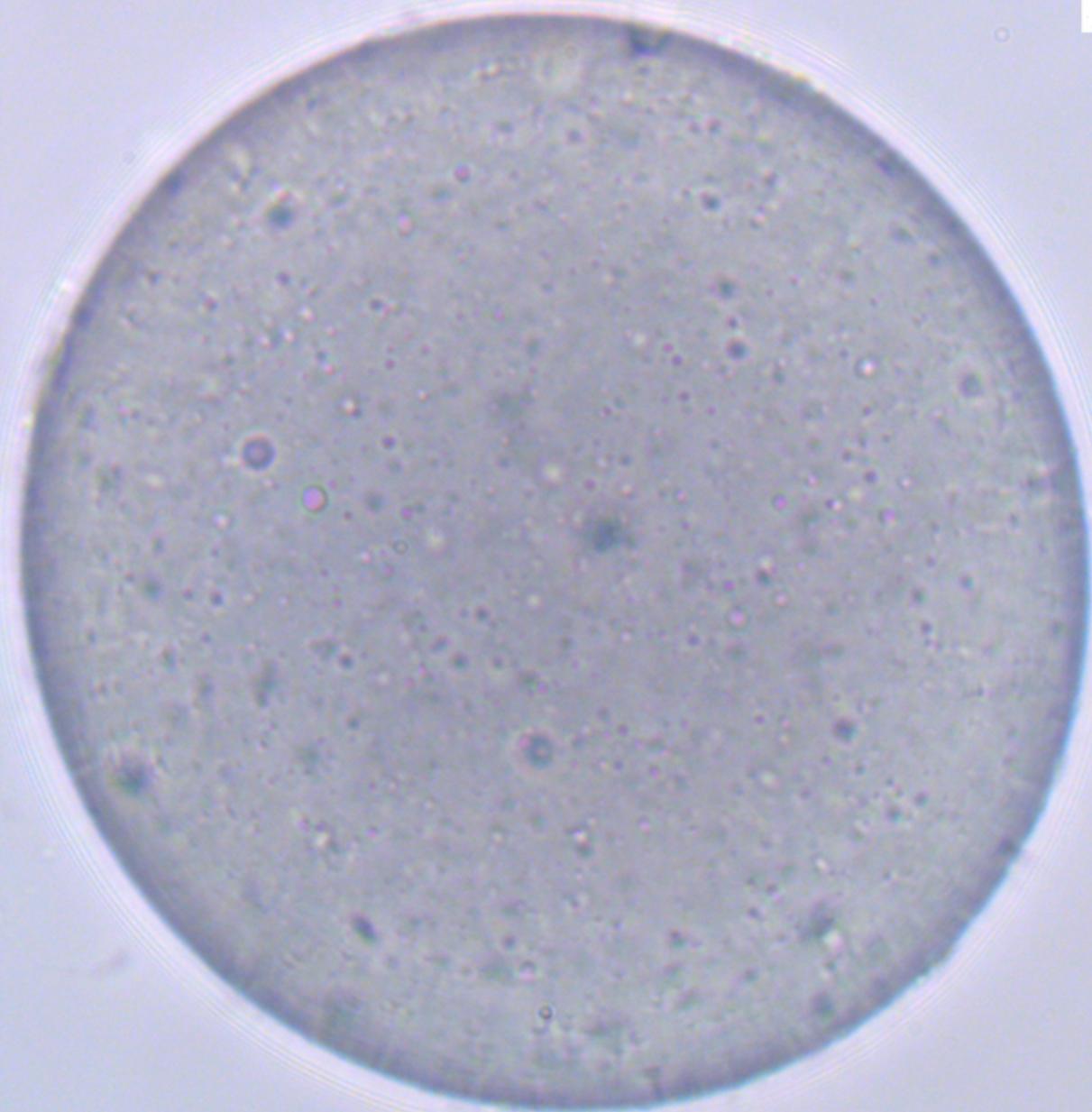




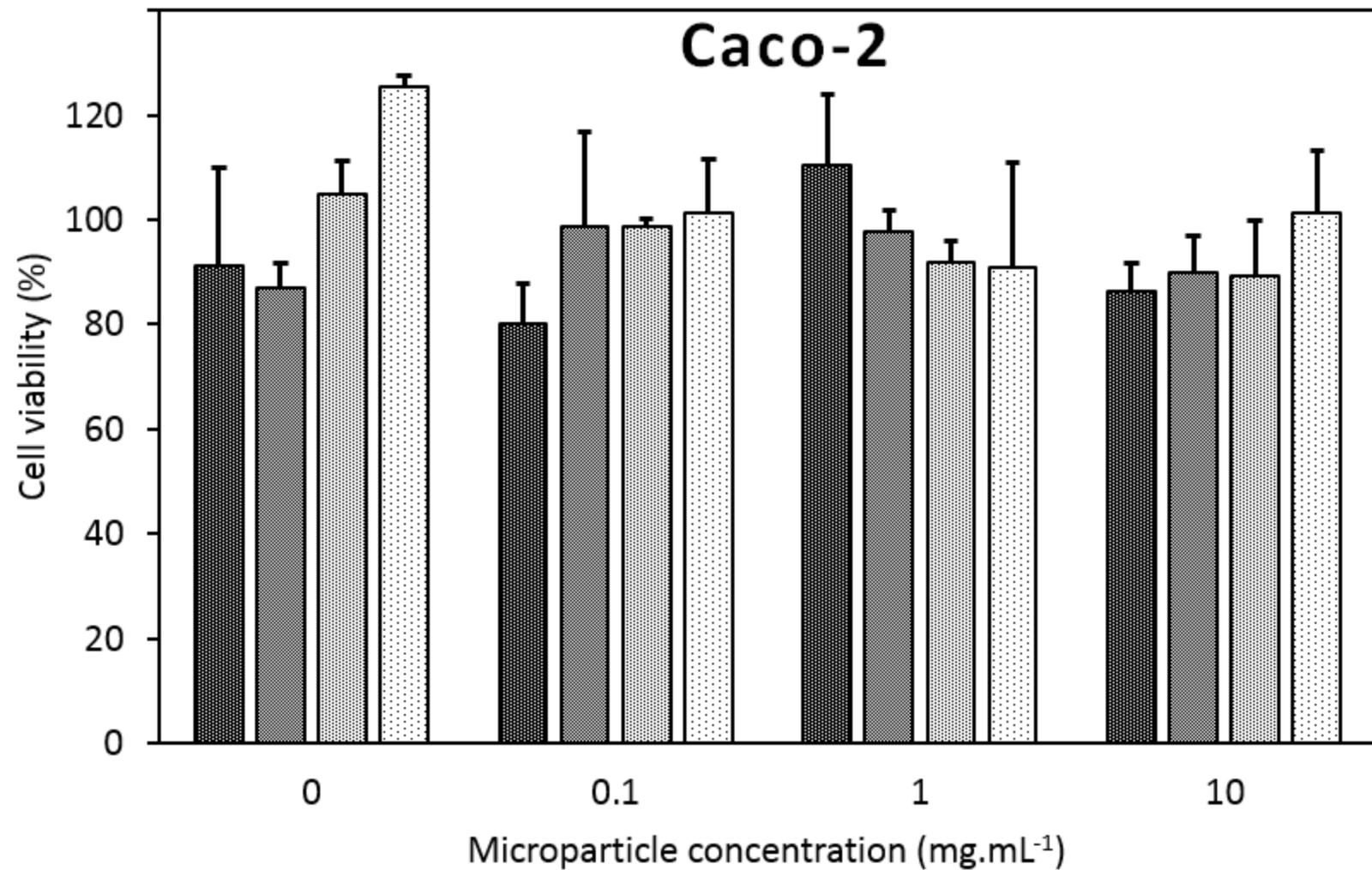


Time (Hours)



a**b**
10 μm

Caco-2



NIH3T3

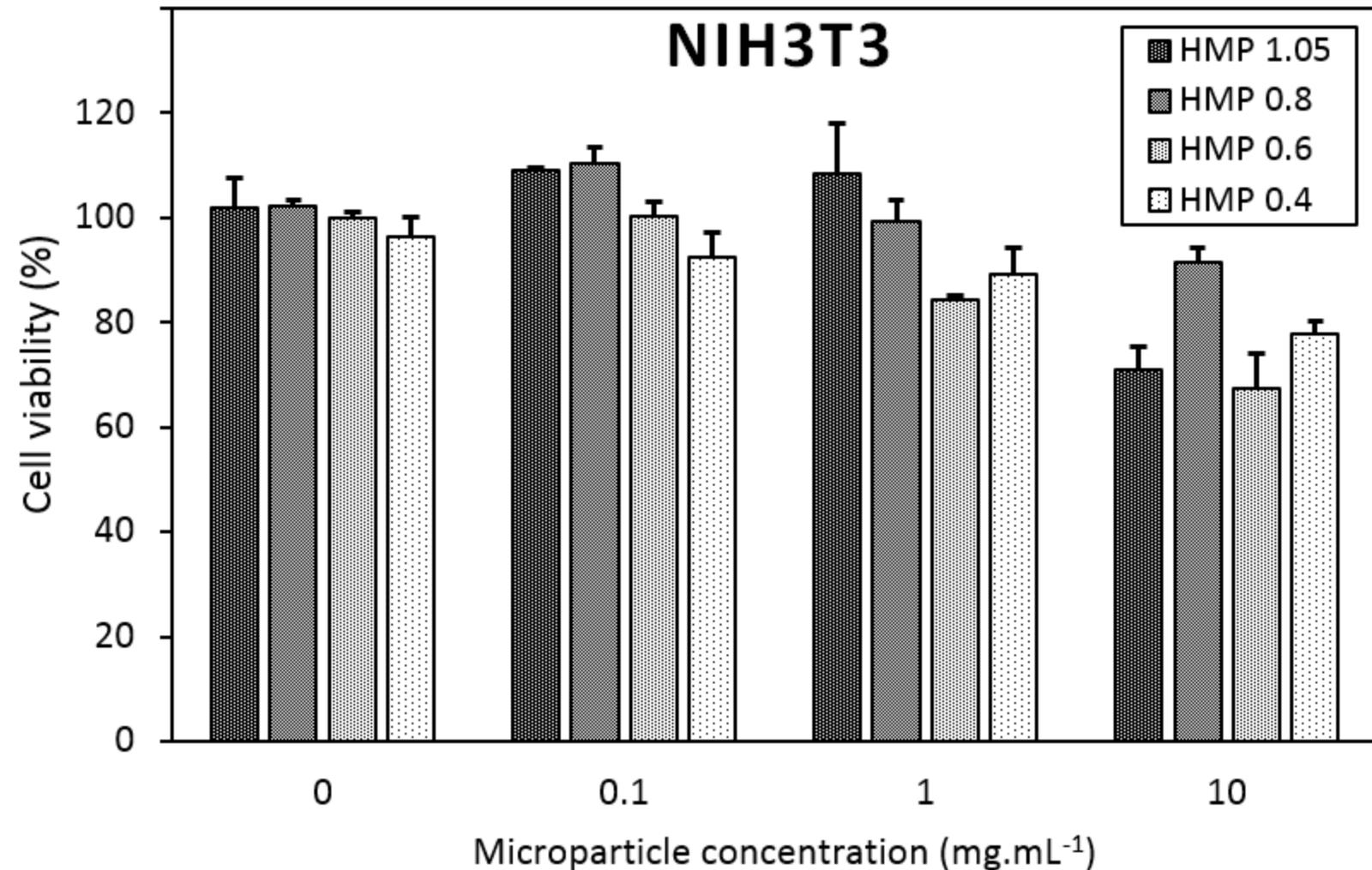


Table 1. Physicochemical properties of ICOs.

ICO	Molecular weight (g·mol ⁻¹)	Viscosity (mPa·s)	density
1.05	1600.9	961	0.999
0.8	1467.5	764	0.994
0.6	1334.1	709	0.986
0.4	1200.7	682	0.970
Castor oil	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 η (%)	Weight loss by ethoxysilanes hydrolysis (%)	Corrected preparation yield η' (%)
1.05	73.4 ± 1.4	21.9	95.3 ± 1.4
0.8	70.0 ± 1.6	19.1	89.1 ± 1.6
0.6	75.3 ± 2.9	15.7	91.0 ± 2.9
0.4	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 (μm)	Span	D [4,3] (μm)	D [3,2] (μm)
1.05	53.8 ± 4.9	1.38 ± 0.05	56.7 ± 5.1	22.3 ± 1.8
0.8	55.0 ± 5.3	1.55 ± 0.15	58.0 ± 5.3	22.4 ± 2.5
0.6	51.5 ± 2.7	1.67 ± 0.09	57.1 ± 6.9	19.9 ± 0.2
0.4	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 ^a	²⁹ Si signal (%)					CY (%)	CD (%)
	LP (-45 ppm)	T ⁰ (-48 ppm)	T ¹ (-53 ppm)	T ² (-57 ppm)	T ³ (-67 ppm)		
1.05	39.9	3.3	3.4	21.8	31.6	60.1	78.6
0.8	11.4	7.5	2.8	42.5	35.8	88.6	73.4
0.6	1.7	5.5	4.4	42.4	46.0	98.3	77.0

^a): 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 ± 10 % of each value.

Spectrum	Atomic rate (%)		
	Fluorine	Carbon	Silicium
1	0.44 \pm 0.2	81.9 \pm 4.0	1.87 \pm 0.9
1'	0.49 \pm 0.1	81.3 \pm 0.7	1.73 \pm 0.7
2	0.45 \pm 0.1	82.7 \pm 2.0	1.52 \pm 0.4
2'	0.37 \pm 0.1	82.7 \pm 0.5	2.16 \pm 0.9
3	0.52 \pm 0.1	81.6 \pm 3.0	1.75 \pm 0.9
3'	0.39 \pm 0.0	80.8 \pm 1.1	1.77 \pm 0.6
4	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 ($10^{-9} \text{ cm}^2 \cdot \text{s}^{-1}$)
1.05	1.44 \pm 0.23
0.8	2.96 \pm 0.18
0.6	4.35 \pm 0.23
0.4	7.28 \pm 0.75

