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1 **Formulation of monomyristin-loaded polyester nanoparticles with controlled**
2 **surface properties**

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24 **Abstract**

25 Monomyristin (MM), a monoacylglycerol with very low water solubility, has been reported to
26 exhibit anti-proliferative property against cervical cancer cells. In order to increase its
27 bioavailability, MM was encapsulated for the first time inside dextran-covered poly(lactic acid)
28 nanoparticles. The use of dextran-based stabilizers with convenient structural characteristics was
29 shown to provide important end-use properties to particle suspensions like colloidal stability
30 during extensive washing steps or in the presence of high ionic strengths, easy reconstitution
31 after lyophilization without particle aggregation in the absence of added cryoprotectant.
32 Furthermore, immobilization of proteins at the surface of loaded particles was investigated with
33 Bovine Serum Albumin and Transferrin using functionalized dextran-based stabilizers. The
34 nature of links (covalent or non-covalent) between proteins and dextran-based superficial layer
35 was discussed.

36 **Keywords**

37 Monomyristin, Poly(Lactic Acid), Hydrophobically-Modified Dextran, Encapsulation,
38 Transferrin.

39

40 **1. Introduction**

41 Chemotherapy has been reported as an effective treatment against cancer. Nevertheless, it can
42 destroy some normal cells and frequently leads to undesirable effects for patient. In the recent
43 decade, nanotherapeutics has drawn a great attention since it was shown to overcome several
44 limitations—such as poor water solubility, low biodistribution, and bioavailability—of the
45 effective anticancer drugs used in the conventional chemotherapy [1]. Design and preparation of
46 nanoparticles (NPs), the key components of nanotherapeutics, have been receiving a great
47 interest for obtaining promising drug delivery system as well as targeted drug delivery. The
48 followed strategy involved encapsulating anticancer drugs/agents into nano-size carriers and
49 adding specific targeting molecules onto NP surface. Surface characteristics appeared highly
50 important for delivering anticancer drugs passively or actively [1], passing through blood
51 fenestra to the sites of cancer cells or target tissues and internalization of drug-loaded NPs.
52 Accordingly, encapsulation has a potency to enhance drug residence time in blood circulation.
53 This led to reduced side-effects and increased drug biodistribution as well as bioavailability
54 without causing any serious problems to patients [2].

55 For preparing drug-loaded NPs, materials and processes are selected according to essential
56 properties targeted for application. For instance particle diameter and surface characteristics are
57 closely related to active/passive drug targeting, colloidal stability in aqueous medium, feasibility
58 of operations like suspension lyophilization and reconstitution, site-specific targeting through
59 ligand attaching onto particle surface. Similarly, particle diameter and matrix material are key
60 properties regarding drug loading capacity, controlled/sustained drug release profile,
61 biodegradability, biocompatibility, antigenicity, and suitability for administration [3].
62 Degradable materials used for preparing particulate systems include natural or synthetic aliphatic

63 polyesters such as poly(lactic acid) (PLA), poly(D,L-glycolide) (PLG), poly(D,L-lactide-co-
64 glycolide) (PLGA) and poly(cyanoacrylate) (PCA) [3-6]. Among these polymers, PLA and
65 PLGA have been the most widely used [7] due to the degradation rates of these polymers which
66 can undergo hydrolysis forming lactic and glycolic metabolites that can be easily eliminated.
67 Moreover, PLA and PLGA are approved by the U.S. Food and Drug Administration (FDA) as
68 well as the European regulatory authorities (EMA) and are considered as safe materials for
69 therapeutic applications [6,8]. PLA exhibits low immunogenicity and good biodegradability via
70 hydrolysis or enzyme degradation [9]. Because of the chemical nature of PLA, which is a polar
71 but hydrophobic material, dextran-covered PLA NPs have been used for encapsulation and
72 release of hydrophobic drugs exhibiting low water-solubility. Dextran and chemically modified
73 dextran derivatives have been widely used for the preparation of nanoparticles for biomedical
74 applications [10]. Covering the PLA core of NPs by a hydrophobically modified dextran (DexP),
75 has been shown to form a dense hydrophilic layer which ensured particle dispersion and
76 prevented non-specific protein adsorption. [11,12] In addition, this superficial hydrophilic layer
77 improved the colloidal stability of suspensions as a function of ionic strength. [11-16] Another
78 interest of dextran surface coverage is the presence of hydroxyl groups which may be used for
79 introducing functional groups for various goals like covalent anchoring of superficial layer onto
80 polymeric core or attachment of specific groups. [17] Regarding drug targeting, transferrin
81 protein (Tf) has attracted a great attention during the last decade because Tf receptors are over-
82 expressed at the surface of tumor or cancerous cells [18]. Thus NPs carrying Tf immobilized on
83 their surface could provide targeted and selective drug delivery to the cancer cells.
84 Monomyristin (MM) has been previously reported for its antiproliferative property against
85 cervical cancer cells with its apoptotic-inducing ability. [19] In addition, MM has low toxicity to

86 the normal cells [20]. Nevertheless, until now only a few works examined the encapsulation of
87 monoacylglycerols inside nano- or microparticles. [12,19,21] More particularly, we previously
88 demonstrated the feasibility of MM encapsulation into polymeric nanoparticles either by solvent-
89 evaporation procedure or by nanoprecipitation. [12] In addition, we previously reported the
90 interactions of monomyristin-loaded polymeric nanoparticles with cervical cancer cells.[19] It
91 was the aim of the present paper to report the physico-chemical aspects of nanoparticle
92 preparation as well as the colloidal stability of the suspensions. Particularly, we wanted to define
93 the most convenient experimental procedure for preparing polymeric particles with optimal
94 colloidal characteristics for biological applications.

95 The present work systematically investigated the preparation of MM-loaded PLA nanoparticles
96 covered by physically adsorbed dextran using emulsion/solvent evaporation procedure. In
97 addition, encapsulation of fluorescein isothiocyanate (FITC) into PLA nanoparticles was carried-
98 out for biological experiments. Bovine Serum Albumin (BSA, as a reference) and Transferrin
99 proteins were immobilized onto NP surface. The effects of various formulation parameters
100 (structure of dextran-based stabilizer, MM, FITC and protein load) as well as process parameters
101 (sonication conditions, washing step, lyophilization for storage) on particle size distribution were
102 discussed. Finally important end-use properties of NP suspensions like colloidal stability vs.
103 ionic strength and passive MM release upon diffusion were evaluated.

104

105 **2. Experimental**

106 *2.1. Materials and reagents*

107 Dextran T40 was purchased from Amersham Pharmacia Biotech AB (Uppsala, Sweden).
108 Poly(D,L-lactide) (PLA), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide (EDCI), N-

109 hydroxysuccinimide (NHS), succinic anhydride, anhydrous pyridine, and fluorescein
110 isothiocyanate (FITC), Bovine Serum Albumin (BSA) were purchased from Sigma-Aldrich (St.
111 Louis, USA). Dichloromethane (CH₂Cl₂) was purchased from Carlo Erba Reagents (Val de
112 Reuil, France). Deuterated dimethyl sulfoxide (DMSO-d₆, >99.8%) was purchased from
113 Amresco (OH, USA). Monomyristin (MM, C₁₇H₃₄O₄) was purchased from TCI
114 (Shanghai, China, >97.0%, MW 302.46 g/mol). Human holo-transferrin was purchased from
115 Calbiochem® (Millipore, USA, ≥95%). All reagents were used as received and all aqueous
116 solutions were prepared in deionized distilled water (DDW).

117

118 *2.2. Synthesis of phenoxy-modified dextran*

119 The attachment of aromatic groups on dextran backbone was adapted from previously described
120 protocol [12]. Typically, 10 g of dextran T40 was allowed to react with 1,2-epoxy-3-
121 phenoxypropane in 100 mL of 1 M NaOH (**Figure 1**). The heterogeneous medium was stirred
122 magnetically for 48 h at 37°C. Then the milky crude product was precipitated on ice with cold
123 ethanol and washed 2-3 times. The dried product was purified by dialysis against deionized
124 water by using Spectra/Por® membrane with molecular weight cut off 6-8 kDa, for 24 h to
125 eliminate the salt residues before lyophilization. Thereafter, the purified polymer was
126 characterized by ¹H NMR analysis after dissolution in DMSO-d₆ (>99.8%) and addition of a few
127 drops of deuterated water (D₂O) to suppress the signals from hydroxyl groups (**Figure 1S**).

128

129 *2.3. Synthesis of carboxylated dextran*

130 Phenoxy-modified dextrans were further carboxylated. Briefly, 1 g of dried DexP_τ was
131 redissolved in 20 mL DMSO in the presence of the required amount of succinic anhydride (for

132 instance 49 mg, $6.2 \cdot 10^{-4}$ mole) and pyridine (1:1 molar ratio with succinic anhydride, here 52
133 mg, $6.2 \cdot 10^{-4}$ mole). The reaction was allowed to occur under magnetic stirring for 48 h. The
134 reaction medium was dialyzed against ethanol/water mixture (50/50, v/v) for 48 h and against
135 pure water for 96 h before lyophilization.

136 The amount of attached COOH groups was determined by potentiometric titration with 0.01M
137 NaOH. Briefly, the polymer was dissolved in water and the solution was acidified with HCl
138 during 1 h. The acidified polymer was recovered by precipitation in ethanol before washing with
139 ethanol/water mixture (95/5, v/v) followed by pure water before lyophilization. The resulting
140 polymer was dissolved in water before titration with 0.01 M NaOH.

141

142 *2.4. Preparation and characterization of dextran-covered PLA nanoparticles*

143 The procedure for preparing dextran-covered PLA NPs was first optimized using the
144 emulsion/solvent evaporation protocol reported by Chiewpattanakul et al. [12]. Briefly, PLA was
145 dissolved in CH_2Cl_2 (50 g/L). One milliliter of previous solution was added into 10 mL of an
146 aqueous solution containing the dextran-based stabilizer (5 g/L) [11-16]. The heterogeneous
147 mixture of the two phases was sonicated (pulsed mode at 45 to 90 W) with 50 % active cycle, for
148 120 sec on ice using Ultrasonic Vibra Cell Meter, Bioblock Scientific-600W (Sonics&Materials
149 Inc., Danbury, CT). Thereafter, the CH_2Cl_2 was evaporated at 37°C for 4 h using a thermal
150 shaker (Thermo-Shaker, QSI, Gerhardt). Suspensions were washed thrice with DDW (18,000
151 rpm, 20°C for 45 min) to remove the excess chemicals, and then lyophilized (FreeZone® Model
152 77500, LabCongo). Aliquots of NP suspensions, before and after washing, were characterized
153 using the Mastersizer 2000 (Malvern-instruments Ltd., UK). The surface coverage (in mg of
154 stabilizer/m² of PLA) of each prepared suspension was calculated from ¹H NMR spectra obtained

155 from BRUKER spectrometer Avance 300 (Bruker AG, Switzerland), by using DMSO-d₆
156 (>99.8%) and deuterated water (D₂O) as a solvent **(Figure 2S)**.

157

158 *2.5. Preparation and characterization of MM- and FITC-loaded PLA nanoparticles*

159 The process in preparation of drug-loaded NPs was similar to that described in section 2.4 with
160 the added component (MM or FITC) in the organic phase. Briefly, PLA/MM and PLA/FITC
161 mixtures were dissolved in CH₂Cl₂ (50 g/L) and dextran-based stabilizer was dissolved in
162 ultrapure water (Milli-Q) (5 g/L). Then 1 mL of organic phase was mixed with 10 mL of aqueous
163 phase then emulsified for 120 sec on ice with the pulsed mode at 45 W, 50% active cycles using
164 the Ultrasonic Vibra Cell Meter, Bioblock Scientific-600W (Sonics&Materials Inc., Danbury,
165 CT). Next, the CH₂Cl₂ was removed in an incubator shaker at 37°C for 4 h (Thermo-Shaker,
166 QSI, Gerhardt). After washing with DDW using a centrifuge at 18,000 rpm, 20°C, all
167 suspensions were lyophilized in the FreeZone® Model 77500 (LabCongo, USA). All drug-
168 loaded NPs were characterized by performing size measurement and ¹H NMR analysis similar to
169 section 2.4. The amounts of MM or FITC loaded into NPs were estimated by using the ¹H NMR
170 spectra **(Figure 3S)**.

171

172 *2.6. Immobilization of BSA and Tf on the surface of dextran-covered nanoparticles*

173 For the human holo-transferrin (Tf) the method reported by Tsuji et al. [22] was followed.
174 Briefly, the suspensions of NPs covered by carboxylated dextran derivatives were reconstituted
175 by addition in ultrapure water (Milli-Q) (50 mg NPs added in 25 mL water) followed by
176 treatment in an ultrasonic bath (Branson® 2510 Ultrasonic Cleaner) for 30 min. Each suspension
177 of carboxylated NPs was adjusted at pH 6.8. Then the required volume of EDCI solution (20

178 mg/mL, pH 6.8) was added, with continuous stirring at 37°C. 10 min later, the required volume
179 of NHS solution (20 mg/mL, pH 6.8) was added, followed by 1 mL of Tf solution (5 mg/mL, pH
180 6.8). The suspension was stirred magnetically during 1 h. Then the solution was centrifuged at
181 18,000 rpm, 20°C three times, replacing the supernatant by pure water each time. Finally, the
182 recovered solid was lyophilized.

183 The same procedure was followed with BSA except that the first two centrifugations were
184 carried-out at pH 9 (because the pI of BSA was 4.7), while the final one was carried out in pure
185 water.

186

187 *2.7. Determination of immobilized BSA*

188 A known amount of dried nanoparticles (having undergone the washing steps and lyophilization
189 procedure described in section 2.6) was mixed with acetone (2 mL) during 4 h in order to
190 dissolve PLA. Then 10 mL of water was added and the solution was stirred overnight in order to
191 dissolve the BSA both physically and covalently bound to dextran-based stabilizer. Acetone was
192 evaporated at 40°C for 3.5 h. The precipitated PLA was removed by centrifugation (15,000 rpm,
193 1h) and the collected aqueous phase was lyophilized.

194 The concentration of recovered BSA was determined using the Bradford assay. Aqueous
195 solutions of BSA at known concentrations were prepared. 1 mL of each solution was added to 5
196 mL Coomassie blue and allowed to react 10 min. The amount of bound protein was determined
197 by using UV-Visible spectrometer at 587 nm (UVikonXL, Bio-Tek Instruments). Two BSA
198 calibration curves were established, i.e; from commercial BSA and from BSA after treatment in
199 acetone. The curves slightly differed and the data were interpreted according to the calibration
200 curve of BSA after treatment with acetone (Figure 4S).

201

202 *2.8. Characterization of colloidal stability of suspensions in the presence of NaCl*

203 Each suspension was diluted in NaCl solution at various concentrations (10^{-4} , 10^{-3} , 10^{-2} , 10^{-1} , 1,
204 2, and 4 M). The samples were analyzed by optical density (OD) measurements (450, 500, 550,
205 600, 650 nm) by using the spectrophotometer UVikonXL (Bio-Tek Instruments). For each NaCl
206 concentration, the variation of $\log OD$ vs. $\log \lambda$ was conveniently fitted by a linear equation of
207 which the slope was extracted and noted n . The graph showing $|n|$ vs. NaCl concentrations
208 allowed the detection of particle aggregations upon increasing salt concentration since it would
209 induce a significant decrease of n . [23]

210

211 *2.9. Drug release study*

212 The suspension of MM-loaded NPs was divided into 6 aliquots which were added in various
213 dialysis tubes with molecular weight cut off 12-14 kDa (Spectra/Por® 2 Dialysis Membrane
214 Tubing, MWCO: 12,000 – 14,000 Daltons). Then samples were dialyzed against Milli-Q water
215 (1 L) for 5, 16, 24, 48 and 72 h. External water was renewed periodically. At each time point one
216 sample of suspension was removed, lyophilized and subjected to 1H NMR analysis (after
217 dissolution in DMSO- d_6). The fraction of remaining MM into NPs was calculated by the ratio of
218 the composition of NPs at the time to the composition at initial time.

219

220 *2.10. Size distribution of particles*

221 Particle size distribution was determined using laser light scattering experiments (Mastersizer
222 2000© Malvern Instruments-Cellule Hydro 2000 μ p). The particle diameters at 90%, 50% and
223 10% cumulative volumes were determined and reported as $d(0.9)$, $d(0.5)$ and $d(0.1)$, respectively.

224 The average particle diameter was evaluated by the surface-average diameter, noted D[3,2]. The
225 width of the size distribution was evaluated by the Span value (**Equation (1)**).

$$226 \quad \text{Span} = [(d(0.9) - d(0.1)) / d(0.5)] \quad (1)$$

227 In what follows, the size distribution of particles will be characterized using the D[3,2] and Span
228 values. In the case of monomodal and narrow size distributions, the Span values ranged between
229 0.8 and 1.5. We observed that Span values higher than 1.5 corresponded to broad size
230 distributions which combined submicronic particles and microparticles.

231

232 *2.11. Surface tension measurements*

233 **Crystallizing dishes were used as the measuring cells.** Before the measurements, sulfuric acid
234 was used to eliminate **all organic traces**, then the crystallizing dish was rinsed abundantly with
235 water for cleaning, finally acetone was used to dry the crystallizing dish. Then 20 mL sample
236 solution was introduced into the crystallizing dish and kept for 12 h **at room temperature** to
237 stabilize the adsorption of the polymer at the air/water interface. Surface tension measurements
238 were carried out at 25°C using a K8 surface tensiometer (Krüss, Germany). Surface tensions
239 were measured using the Wilhemy technique. All samples were equilibrated during 30 min
240 before any measurement for temperature equilibration at 25 °C **with a circulating water bath**. For
241 each sample, six measurements were carried out and the average value was calculated.

242

243 *2.12. Differential scanning calorimetry*

244 Samples of 50 mg of mixtures of PLA/MM (5:5, 7:3, 8:2, 9:1, 9.5:5, wt:wt) and PLA/FITC (9:1,
245 wt:wt) were dissolved in 2 mL of acetone using glass vials. The vials were shaken for 2 h to
246 ensure the complete dissolution. Then solvent was evaporated at ambient temperature overnight

247 before complete drying using a dryer to eliminate the residual acetone and subsequently put in an
248 oven at 90°C overnight. Next, the dried samples were scratched from the vials using a sterile
249 spatula and cut into small pieces using sterile forceps and scissors before transferred to DSC
250 apparatus. Measurements were carried-out using a Q2000 TA Instruments apparatus. The heating
251 rate was 5°C/min by modulating $\pm 1^\circ\text{C}$ every 40 seconds under nitrogen, from -50°C to 190°C.

252

253 **3. Results and discussion**

254 *3.1. Preliminary experiments on the constituents of nanoparticles*

255 3.1.1. Synthesis of dextran-based functional stabilizers

256 Native dextran is highly hydrophilic and exhibits no capacity to adsorb at oil/water interfaces and
257 consequently cannot be used directly to produce dextran-covered NPs. On the basis of previous
258 work, native dextran was chemically modified in aqueous alkaline medium by reacting with 1,2
259 epoxy-3-phenoxypropane (see section 2.2). [15,24] Phenoxy groups were fixed onto a limited
260 number of glucose repeat units in order to induce physical adsorption at oil/water interface
261 (during emulsification) and onto PLA core (after evaporation) while preserving water-solubility.
262 For all samples of phenoxy-modified dextran, the molar ratio of the number of covalently linked
263 phenoxy groups to the total number of glucose repeat units was named “degree of substitution”
264 and noted τ . The degree of substitution was determined experimentally using ^1H NMR (see
265 section 2.2). Phenoxy-modified dextran derivatives were named DexP_τ in which τ was expressed
266 in percents. Varying the conditions of reaction, time and temperature, four dextran derivatives
267 were prepared with τ ranging from 15 to 27 %.

268 Carboxylic acid groups were further attached onto the polysaccharide backbone of DexP_τ
269 derivatives by reaction with succinic anhydride in DMSO in the presence of pyridine (see section

270 2.3). In order to keep sufficient ability to adsorb at oil/water interface despite the addition of
271 hydrophilic carboxylic groups, only DexP₂₆ and DexP₂₇ were further modified. Although using
272 ¹H NMR the signal of the proton of carboxylic acid group could be detected slightly above 12
273 ppm (in DMSO-d₆), it was not possible to use it to reliably quantify the extent of modification by
274 succinic anhydride. Thus the amount of covalently linked carboxylic groups was determined by
275 potentiometric titration (see section 2.3). Similarly to phenoxy groups, the molar ratio of the
276 number of covalently linked carboxylic groups to the total number of glucose repeat units was
277 noted x . Doubly modified dextran derivatives were named DexP _{τ} COOH _{x} in which x was
278 expressed in percents. Various derivatives were obtained with x ranging between 6 and 9 %.

279 The ability of adsorption of DexP₂₇ and DexP₂₇COOH₆ at air/water interface was evaluated by
280 surface tension measurements of aqueous solutions with polymer concentrations up to 10 g/L
281 (Figure 2). With aqueous solutions of native dextran up to 10 g/L, surface tension kept the same
282 value as pure water. [15] For DexP₂₇, experimental results were consistent with previous reports.
283 [15] In the case of DexP₂₇COOH₆ taking into account experimental uncertainty, higher values of
284 surface tension (as compared to those found with DexP₂₇) were measured for polymer
285 concentrations up to 0.1 g/L. This difference was consistent with the additional presence of
286 hydrophilic carboxylic groups within the chains of DexP₂₇COOH₆ as compared to DexP₂₇.
287 Nevertheless, for polymer concentration above 1 g/L, experimental values of surface tension
288 were similar for both carboxylated and non-carboxylated dextran derivative. These results
289 showed that both DexP _{τ} and DexP _{τ} COOH _{x} dextran derivatives could be used for preparing NPs
290 following the emulsion/solvent evaporation procedure.

291

292 3.1.2. Miscibility of loaded molecules and PLA in the solid state

293 DSC experiments were used to evaluate solid state miscibility of MM and FITC with PLA. [25]
294 Indeed, bulk PLA was an amorphous polymer which exhibited glass transition temperature (T_g)
295 around 51.9 °C. In the case of neat MM, melting peaks were observed: one single peak at 72 °C
296 during the first heating cycle and two melting peaks at 27 °C and 54 °C during the following
297 cycles. These results could be explained by the polymorphic state of MM and were consistent
298 with previous studies. [26]

299 Solid mixtures of PLA and MM were analyzed by DSC (**Figure 5S**). For mixtures containing up
300 to 10 wt% MM, a single T_g was observed. This was consistent with homogeneous solid mixtures
301 containing molecularly dispersed MM within polymeric matrix. Accordingly, the value of T_g
302 decreased when increasing MM content (from 52 °C for bulk PLA down to 31 °C for 9:1
303 PLA/MM mixture) which was interpreted as a plasticizing effect. For PLA/MM solid mixtures
304 containing higher weight fractions of MM, DSC experiments evidenced both glass transition and
305 melting peaks. This result was attributed to biphasic solid mixtures formed by amorphous phase
306 containing PLA and a part of initial MM load together with excess MM crystals. Thus, full
307 miscibility of PLA and MM corresponded to MM loads lower than 10 wt%. In the case of FITC,
308 a solid mixture with PLA containing 10 wt% FITC exhibited a single T_g and was thus considered
309 as homogeneous.

310

311 3.1.3. Chemical stability of MM and FITC during sonication and their solubility in water

312 Ultrasonication is a high energy emulsification procedure. In order to verify that MM or
313 not significantly degraded by application of ultrasounds, control experiments were carried out.
314 Solutions of MM and FITC in CH_2Cl_2 were emulsified in water following the same procedure as
315 for preparation of NPs using 45 W power (see section 2.4). Chemical modifications of MM and

316 FITC were investigated by comparing ^1H NMR spectra before and after emulsification. For both
317 molecules, no significant difference could be detected, which proved that no chemical
318 modification of MM or FITC occurred during the emulsification step.

319 In addition, during emulsification the volume ratio of organic to aqueous phase was 1:10. As a
320 consequence, because of the partition of MM or FITC between the two phases, part of the
321 molecules may be lost during the washing step. For checking residual solubility of MM and
322 FITC in the aqueous phase, powders were stirred during 24 h in an excess of D_2O . Then, after
323 separation of liquid phase, ^1H NMR analysis was performed. In the case of MM, residual peaks
324 could be detected which were attributed to the solubilization of a very limited amount. In the
325 case of FITC, no signal attributable to FITC molecules could be detected, demonstrating that the
326 solubilized amount, if any, was below the limit of detection.

327

328 *3.2. Nanoparticles of PLA covered by DexP_τ and $\text{DexP}_\tau\text{COOH}_x$*

329 Nanoparticles were prepared following the emulsion/solvent evaporation procedure and using
330 dextran derivatives as stabilizers of the intermediate emulsion. Because the stabilizer was loaded
331 in large excess as compared to the amount required to fully cover the surface of final particles,
332 washing operations followed the evaporation step (**Figure 3**). In addition to size distribution of
333 final suspensions, analysis of chemical composition of NPs was carried-out using ^1H NMR. To
334 that goal, suspensions were lyophilized and the resulting dried solid was dissolved in DMSO-d_6
335 which was a common solvent of PLA, dextran-based stabilizers and encapsulated molecules
336 (MM and FITC). This chemical analysis allowed determination of encapsulated amounts of MM
337 or FITC (in weight ratio to PLA) as well as surface coverage by the polymeric stabilizer (in mg
338 of stabilizer by m^2 of PLA, in combination with data about size distribution).

339 First experiments were carried out with PLA as the sole solute in the organic phase, so as to
340 check the effects of formulation and process parameters on particle characteristics. Using DexP₂₇
341 and with sonication power equal to 45 W, NPs with relatively narrow size distribution (Span =
342 1.0 to 1.3) were obtained after solvent evaporation as well as after centrifugation and washing
343 steps. Repeating the experiments led to similar results, which proved the repeatability was
344 correct taking into account the random nature of the emulsification step (**Table 1**).

345 Further experiments explored the influence of sonication power and that of the degree of
346 substitution of DexP_τ (**Table 2**). Similar size distributions were found after solvent evaporation
347 whatever the sonication power up to 90 W and with τ equal to 15, 21 and 26 %. This
348 demonstrated that, within the range of explored conditions, the size distribution of NPs resulting
349 from solvent evaporation was not significantly modified by increasing sonication power or by
350 changing the structure of polymeric stabilizer. Our interpretation was that all conditions led to
351 similar transient oil-in-water emulsions at the end of emulsification step and thus similar NPs
352 after solvent evaporation. Previously we showed that the weight ratio of DexP_τ to PLA had a
353 significant effect on the average particle diameter after evaporation. [12]

354 Apart from size distribution, the surface coverage of washed NPs was also investigated. Except
355 for one experiment, it was found to fall within the range 2.8 – 4.4 DexP_τ / m² PLA which was
356 consistent with previous results. [24] On the basis of loaded amounts of DexP_τ and PLA,
357 between 10 and 15 wt% of the initially added DexP_τ was still adsorbed on the final particles after
358 the washing step. A significant excess of stabilizer was required to provide a fast and full surface
359 coverage of initial oil droplets during emulsification step using sonication. Moreover, from ¹H
360 NMR spectra of washed particles, we estimated the molar ratio of phenoxy groups to glucose
361 repeat units of adsorbed DexP_τ macromolecules at the surface of final NPs. For all amphiphilic

362 dextrans, the value found from adsorbed molecules was about 30 % higher than the one
363 corresponding to the polymer recovered after the hydrophobic modification of native dextran
364 **(Figure 6S)**. In order to explain this observation, we considered **three aspects**. First, the
365 **adsorption of amphiphilic dextrans at the surface of PLA particles was entirely due to**
366 **hydrophobic phenoxy groups since native dextran had no tendency to adsorb at oil/water**
367 **interface**. Second, the chemical modification of native dextran did not ensure a fine control of the
368 **degree of substitution**. Thus, the value of τ determined from ^1H NMR with the purified polymer
369 **should be considered as an average value resulting from hydrophobically modified dextrans**
370 **covering an interval of degree of substitution**. Third, the mechanism of formation of NPs during
371 **emulsion/solvent evaporation process with PLA has been shown to involve transition from liquid**
372 **droplets to solid particles without aggregation**. [27] Consequently, we assumed that dextran
373 **derivatives with higher degree of substitution preferentially accumulated at oil/water interface**
374 **during emulsification step and remained strongly adsorbed during solvent evaporation as**
375 **expected while dextran derivatives with lower degrees of substitution were more easily expelled**
376 **from interface during solvent evaporation**. [28] This could explain why the degree of substitution
377 **of polymers covering the surface of final PLA particles was slightly higher than that of loaded**
378 **polymer**. Nevertheless we checked that using either the value of τ determined after the synthesis,
379 **or the value deduced from ^1H NMR spectra of washed NPs for calculating the surface coverage**
380 **did not significantly modify the found values**.

381 Changing DexP₂₇ by DexP₂₇COOH₆ as stabilizer did not give rise to significant modification
382 either of the distribution of particle diameter or of the surface coverage by dextran-based
383 stabilizer (**Table 1**).

384 Elimination of excess DexP_τ or $\text{DexP}_\tau\text{COOH}_x$ in the aqueous phase involved washing step which
385 comprised centrifugation and re-dispersion of NPs (**Figure 3**). These operations affected the size
386 distribution of NPs in a way that depended on the chemical structure of the stabilizer. For DexP_{15}
387 and DexP_{21} , limited amounts of aggregates were formed during centrifugation and re-dispersion
388 steps. As a result, $d(0.1)$ and $d(0.5)$ values remained almost unchanged while $d(0.9)$ increased
389 significantly. Consequently, $D[3:2]$ value varied slightly while Span became higher than 50
390 (**Table 2**). Whereas the formation of aggregates was detected with DexP_{15} and DexP_{21} , it was
391 almost undetected in the case of DexP_{26} , DexP_{27} and $\text{DexP}_{27}\text{COOH}_6$ (**Table 1 and 2**). Indeed, the
392 degree of substitution appeared to be the relevant parameter. DexP_τ and $\text{DexP}_\tau\text{COOH}_x$ stabilizers
393 comprised hydrophobic phenoxy groups randomly distributed within polysaccharide backbone
394 which provided multiple anchoring points onto oil/water and solid/water interfaces. Thus, the
395 higher the degree of substitution, the more anchoring points and the stronger the steric barrier
396 between NPs for preventing aggregation, especially when particles were close packed during
397 centrifugation.

398 The colloidal stability of suspensions of PLA nanoparticles as a function of ionic strength was
399 examined using turbidimetry. [23] NPs prepared with DexP_{15} , DexP_{21} and DexP_{26} exhibited good
400 colloidal stability up to 4 M NaCl (**Figure 4**). This result was attributed to the thick superficial
401 layer formed by adsorbed polysaccharides with hydrophilic loops protruding into aqueous phase,
402 which was at the origin of efficient steric repulsions. Previous work provided an estimation of 6
403 nm for the thickness of this superficial layer in the case of DexP_{15} dextran derivative. [24]
404 Similar colloidal stability was observed with suspensions of NPs stabilized by $\text{DexP}_{26}\text{COOH}_9$.
405 The normal Na^+ concentration in blood is 136-145 mM (0.136-0.145 M) and in the culture media

406 condition is 137-164 mM [29]. We could conclude that NPs exhibited convenient colloidal
407 stability for drug encapsulation and release applications.

408 On the basis of these results emulsification conditions with 45 W power for sonication and
409 dextran derivatives with $\tau \geq 0.26$ were selected for encapsulation of MM and FITC in NPs.

410

411 *3.3. Encapsulation of MM and FITC inside dextran-covered PLA nanoparticles*

412 In the case of MM, the main question was to determine the maximum load that could be
413 encapsulated into NPs while keeping similar characteristics as found with bulk PLA (see section
414 3.2). To that goal, a series of suspensions were prepared using the previously established
415 conditions and adding increasing amounts of MM in CH₂Cl₂ while keeping unchanged the
416 concentration of PLA (50 g/L). Except for the lowest weight fraction of MM i.e. 0.05, the
417 formation of aggregates was observed in all experiments, particularly after the washing steps.
418 Nevertheless, the amount of aggregates became really significant for weight fractions of MM
419 higher than 0.1, leading to a significant increase of the surface-average diameter during the
420 washing operations (**Figure 5**). The same tendency was observed using either DexP₂₇ or
421 DexP₂₇COOH₆. Interestingly, the value of 0.1 for the weight fraction of MM corresponded to the
422 upper limit of solid-state miscibility as determined by DSC experiments. A similar correlation
423 has been reported for the encapsulation of gallic acid esters inside PLA NPs. [25] Our
424 explanation was that phase separation during the formation of solid particles upon solvent
425 evaporation induced accumulation of excess MM close to interface which reduced the extent of
426 adsorption of DexP₂₇ or DexP₂₇COOH₆ and thus induced particle aggregation both after
427 evaporation (limited extent) and after washing steps (mainly). The increase of the surface-
428 average diameter with the loaded weight fraction of MM could be attributed, at least in part, to

429 the increase of the total amount of PLA + MM dissolved in CH₂Cl₂. As a result, the initial load
430 of MM was limited to 10 wt% (**Table 3**).

431 In addition to MM, the potential of PLA NPs to encapsulate FITC was investigated with the aim
432 of performing fluorescence microscopy. Similarly to experiments with MM, FITC-loaded NPs
433 were prepared with an initial load of FITC equal to 10 wt%. Only limited amounts of aggregates
434 were formed, which was consistent with DSC measurements indicating a homogeneous solid
435 mixture.

436 ¹H NMR analysis of MM- and FITC-loaded NPs demonstrated the encapsulation of both
437 compounds within the PLA core in the presence of DexP₂₆ in the aqueous phase. The contents
438 were evaluated and found equal to 0.023 g of MM/g of PLA and 0.100 g of FITC/g of PLA.
439 These values had to be compared to the initial loads which were equal to 0.11 g/g of PLA for
440 both molecules. Thus, for FITC the encapsulation efficiency was close to 100 %. On the
441 contrary, for MM the encapsulation efficiency was significantly lower, around 20 %. We had
442 previously showed that the presence of excess amphiphilic polymers in the aqueous phase
443 enhanced the aqueous solubility of hydrophobic molecules to be encapsulated. [30] Thus our
444 assumption was that MM was partly dissolved inside DexP₂₆ aggregates present in the aqueous
445 phase, while FITC was not because of low chemical compatibility.

446 Similarly, when using DexP₂₆COOH₉ as the stabilizer in the aqueous phase, ¹H NMR analysis
447 proved that MM and FITC were present in the recovered NPs (**Table 3**). The contents were
448 0.119 g of MM/g of PLA and 0.027 g of FITC/g of PLA. The efficiency of encapsulation was
449 significantly modified in comparison to previous experiments involving DexP₂₆. It was about
450 100 % for MM (to be compared to 20 % with DexP₂₆) while it was close to 25 % for FITC (to be
451 compared to 100 % with DexP₂₆). This point would require more experimental investigations to

452 be fully understood since several phenomena may be involved: modification of self-assembly of
453 amphiphilic dextran derivatives in aqueous phase changing the partition of encapsulated
454 molecules, specific interactions between carboxylic groups and encapsulated molecules...

455 Colloidal stability of MM-loaded NPs was similar to that of bulk PLA NPs and extended up to 4
456 M NaCl (**Figure 7S**).

457 Finally, suspensions of NPs stabilized with DexP₂₆COOH₉ could be lyophilized and
458 reconstituted by addition of an amount of water equivalent to initial and application of ultrasound
459 bath (**Table 3**). The surface-average diameter of the reconstituted suspension was close to the
460 one of the initial suspension. No addition of solid carrier like sucrose was necessary with that
461 stabilizer contrary to what has been reported with other dextran-based stabilizers. [31]

462 Passive release of MM out of NPs by diffusion of loaded molecules within PLA matrix was
463 investigated over a period of 72 h. MM release started to be detected after 24 h (**Figure 6**). This
464 result confirmed that MM was encapsulated into the core of NPs and not retained at the surface
465 otherwise an initial burst would have been observed. Thus, previous results about the efficiency
466 of encapsulation as well as its variation with the chemical structure of the drug or the stabilizer
467 should be further discussed taking into account this fact. The absence of passive diffusion during
468 the first 24 h appeared as an interesting aspect for further promoting MM specificity. Indeed,
469 during the first 24 h, MM release could only occur via close interactions between NPs and cancer
470 cells. Consequently, the presence of immobilized Transferrin at the surface of NPs could be of
471 particular importance.

472

473 *3.4. Immobilization of BSA and Transferrin at the surface of dextran-covered PLA nanoparticles*

474 The general strategy for immobilization of proteins at the surface of NPs was to use carboxylic
475 acid groups introduced into dextran chains and amine groups contained in residues of amino
476 acids. [22] Nevertheless, because of conformation limitations, carboxylic groups may not be
477 fully accessible for chemical reaction. In addition, other non-covalent interactions may take place
478 (electrostatic or hydrophobic) between protein and dextran-based stabilizers. As a result,
479 immobilization of protein on the surface of NPs may have different origins, covalent and non-
480 covalent, which can alter its resistance to dilution or to other constituents of biological
481 environment. Thus washing steps were carried-out after contacting protein and NPs, so as to
482 extract all protein macromolecules that were not strongly immobilized on the surface of NPs.

483 First experiments were carried-out with BSA with DexP₂₇COOH₉-stabilized particles. The
484 coupling procedure involved three steps: one step for activating the carboxylic acid groups
485 contained into the superficial layer, a second step of contacting particles with BSA for coupling
486 and the last step which included washing for extracting excess free BSA. Characteristics of
487 particles were controlled before and after the coupling procedure (size distribution and surface
488 coverage by DexP₂₇COOH₉) while the amount of immobilized BSA was determined after the
489 coupling procedure (**Table 4**). It was not possible to obtain a direct evidence of covalent
490 attachment of BSA at the surface of particles because the amounts of amide groups potentially
491 produced were in too low concentration.

492 Neither the size distribution of NPs nor their surface coverage by DexP₂₇COOH₉ was
493 significantly modified by the coupling procedure. This demonstrated that DexP₂₇COOH₉ was not
494 desorbed in the presence of BSA, which was consistent with previous results. [24] In addition,
495 the presence of BSA immobilized onto NPs after washing was verified by titration. The presence

496 of BSA even when no reactant was used during the coupling step (**exp. 4, Table 4**) indicated that
497 protein immobilization on the surface of NPs was partly of non-covalent origin. Washing steps
498 were performed at high pH (at which dextran-based stabilizer and protein macromolecules were
499 both negatively charged), thus electrostatic interactions could not be involved. **In addition,**
500 **previous results obtained with polystyrene NPs covered by physically adsorbed DexP_τ showed**
501 **that BSA adsorption was strongly reduced by the presence of the superficial layer. In addition,**
502 **the residual BSA adsorption became undetectable when the degree of substitution reached 16 %.**
503 **[13-15]** Other types of non-covalent interactions must have been at the origin of BSA
504 immobilization at the surface of NPs. Nevertheless, the presence of BSA even after extensive
505 washing was a good indication of the strength of protein binding.

506 On the basis of previous results, Tf immobilization was carried-out with DexP₂₇COOH₆-
507 stabilized particles following the same procedure as for BSA except protein titration. **We**
508 **observed that the size distribution remained unchanged after the coupling procedure while a**
509 **decrease of the surface coverage by DexP₂₇COOH₆ was observed (Table 5).** This fact could be
510 attributed to partial desorption of DexP₂₇COOH₆ in the presence of Tf. **In the presence of BSA,**
511 **no desorption of modified dextran could be detected (Table 4), which could result from the**
512 **difference of amphiphilic behavior between BSA and Tf. More experimental investigations about**
513 **competitive adsorption between Tf and dextran derivatives would be necessary to reach further**
514 **conclusions. The same procedure was followed with MM-loaded NPs leading to similar results**
515 **about the conservation of size distribution after the coupling procedure (Table 6).** The proof of
516 Tf immobilization at the surface of NPs was obtained by studying their interactions with
517 biological systems, which was reported elsewhere. [19] **Nevertheless, these results did not**
518 **provide information about the exact amount of Tf present at the surface of NPs.**

519 Our results demonstrated that protein immobilization on the surface of NPs was possible. Direct
520 evaluation of the extent of covalent binding was not obtained because of the low amounts of
521 functional groups. Nevertheless, colloidal characteristics of suspensions were kept unchanged
522 during the coupling procedure.

523

524 **4. Conclusion**

525 Dextran-covered PLA nanoparticles were prepared following emulsion/solvent evaporation
526 procedure. The use of dextran-based stabilizers led to rather narrow and well-defined size
527 distribution as well as dense surface coverage. The latter was at the origin of good colloidal
528 stability during washing steps as well as in the presence of high ionic strengths in the aqueous
529 medium, and of the ability to lyophilize and reconstitute suspensions. Our experiments showed
530 that the use of hydrophobically modified dextrans with an average of 26 % of repeat units
531 modified by one phenoxy group was convenient for reaching previous performances. In addition,
532 monomyristin and FITC were readily encapsulated into PLA core while keeping good colloidal
533 characteristics, provided that the initial load was in the full miscibility domain. Finally, using
534 hydrophobically modified dextran derivatives functionalized with carboxylic acid groups,
535 various proteins were immobilized at the surface of nanoparticles.

536 This work reported a detailed investigation about the colloidal characteristics of NPs and how the
537 use of amphiphilic dextran derivatives brought very interesting properties regarding colloidal
538 stability, protein immobilization on the surface and possible reconstitution of suspensions after
539 lyophilization. All these fundamental aspects would result in highly interesting properties for
540 practical developments of MM-loaded formulations.

541 On the basis of these results, interactions of these nanoparticles with biological systems have
542 been investigated in vitro and reported elsewhere. [19]

543

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550

551

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631

632

Table 1: Characteristics of PLA nanoparticles prepared with DexP₂₇ or DexP₂₇COOH₆ and sonication power set to 45 W. Experience with DexP₂₇ was repeated twice for checking repeatability.

| Stabilizer | Step of the process | Diameter (nm) | | | | | Span | Surface coverage (mg of DexP ₂₇ / m ² of PLA) |
|--------------------------------------|---------------------|---------------|--------|--------|--------|--------|------|--|
| | | d(0.1) | d(0.5) | d(0.9) | D[3:2] | D[3:2] | | |
| DexP ₂₇ | After evaporation | 66 | 124 | 225 | 109 | 1.3 | --- | |
| | | 65 | 113 | 194 | 102 | 1.1 | --- | |
| | After washing | 86 | 137 | 223 | 133 | 1.0 | 3.2 | |
| | | 63 | 113 | 205 | 100 | 1.3 | 3.0 | |
| DexP ₂₇ COOH ₆ | After evaporation | 65 | 126 | 282 | 112 | 1.7 | --- | |
| | After washing | 62 | 114 | 220 | 101 | 1.4 | 3.2 | |

Table 2: Characteristics of PLA nanoparticles prepared with different DexP_τ and various sonication powers. Size distributions were controlled after solvent evaporation and after centrifugation and washing.

| DexP _τ | Sonication | Size distribution | | | | Surface coverage (mg of DexP _τ / m ² of PLA) ^a |
|--------------------|--------------|-------------------|-------------------|---------------|-------------------|--|
| | power (W) | After evaporation | | After washing | | |
| | | D[3:2] (nm) | Span | D[3:2] (nm) | Span | |
| DexP ₁₅ | 45 | 125 | 1.1 | 129 | >> 1 ^b | 3.0 |
| | 60 | 131 | 1.2 | 160 | >> 1 ^b | 3.2 |
| | 75 | 130 | 1.0 | 137 | >> 1 ^b | 2.8 |
| | 90 | 144 | >> 1 ^b | 144 | >> 1 ^b | 2.8 |
| DexP ₂₁ | 45 | 108 | 1.4 | 132 | 1.2 | 3.6 |
| | 60 | 118 | 1.0 | 170 | >> 1 ^b | 4.4 |
| | 75 | 117 | 1.2 | 155 | >> 1 ^b | 3.8 |
| | 90 | 128 | 1.2 | 152 | 3.3 | 3.3 |
| DexP ₂₆ | 45 | 105 | 1.1 | 126 | 0.9 | 7.2 |
| | 60 | 107 | 1.3 | 122 | 1.0 | 3.7 |

| | | | | | |
|----|-----|-----|-----|-------------------|-----|
| 75 | 116 | 1.6 | 106 | 1.5 | 2.9 |
| 90 | 116 | 1.5 | 118 | >> 1 ^b | 3.2 |

^a Value determined after washing.

^b Presence of aggregates leading to meaningless value.

Table 3: Characteristics of MM- and FITC-loaded NPs prepared with DexP₂₆ and DexP₂₆COOH₉ as stabilizers.

| Stabilizer | Load (wt%) | | D[3:2] (nm) | Surface coverage (mg of stabilizer / m ² of PLA) ^a | Content (g of MM or FITC / g of NPs) ^a |
|--------------------------------------|------------|------|------------------|---|--|
| | MM | FITC | | | |
| DexP ₂₆ | 10 | 0 | 113 ^a | 3.2 | 0.020 |
| | 0 | 10 | 193 ^a | 5.9 | 0.080 |
| DexP ₂₆ COOH ₉ | 0 | 0 | 120 ^b | 4.5 | --- |
| | 10 | 0 | 133 ^b | 3.4 | 0.096 |
| | 0 | 10 | 195 ^a | 3.7 | 0.024 |

^a After washing.

^b After washing, lyophilization and reconstitution.

Table 4: Size and surface characteristics of DexP₂₇COOH₉-stabilized PLA nanoparticles before and after undergoing the procedure for BSA coupling.

| Exp. | Amount of reactant (mol / 100 mg PLA) | | Step ^a | D[3:2] (nm) | Span | Surface coverage (mg / m ² of PLA) | |
|------|--|-----------------------|-------------------|----------------|------|--|--------|
| | NHS | EDCI | | | | By DexP ₂₇ COOH ₉ | By BSA |
| | 1 | 1.08 10 ⁻⁴ | | | | | |
| | | | AC | 99 | 1.3 | 3.2 | 0.3 |
| 2 | 3.74 10 ⁻⁴ | 4.35 10 ⁻⁴ | BC | 90 | 1.3 | 2.9 | --- |
| | | | AC | 97 | 1.3 | 3.3 | 0.4 |
| 3 | 0 | 4.37 10 ⁻⁴ | BC | 98 | 1.2 | 3.2 | --- |
| | | | AC | 101 | 1.5 | 4.0 | 0.4 |
| 4 | 0 | 0 | BC | 95 | 1.4 | 3.0 | --- |
| | | | AC | 95 | 1.3 | 2.0 | 0.2 |

^a BC: Before coupling. AC: After coupling. For details see section 2.6.

Table 5: Size and surface characteristics of DexP₂₇COOH₆-stabilized PLA nanoparticles before and after undergoing the procedure for Tf coupling.

| Exp. | Amount of reactant | | Step ^a | D[3:2] (nm) | Span | Surface coverage by DexP ₂₇ COOH ₆ (mg / m ² of PLA) |
|------|-----------------------|-----------------------|-------------------|----------------|------|---|
| | (mol / 100 mg PLA) | | | | | |
| | NHS | EDCI | | | | |
| 1 | 4.34 10 ⁻⁵ | 4.17 10 ⁻⁵ | BC | 105 | 1.4 | 2.5 |
| | | | AC | 133 | 1.3 | 1.9 |
| 2 | 4.34 10 ⁻⁶ | 4.17 10 ⁻⁶ | BC | 115 | 1.4 | 2.7 |
| | | | AC | 138 | 1.3 | 1.1 |
| 3 | 0 | 4.37 10 ⁻⁴ | BC | 111 | 1.4 | 2.7 |
| | | | AC | 128 | 1.2 | 2.5 |
| 4 | 0 | 4.17 10 ⁻⁵ | BC | 110 | 1.5 | 2.7 |
| | | | AC | 138 | 1.4 | 2.2 |

^a BC: Before coupling. AC: After coupling. For details see section 2.6.

Table 6: Size and surface characteristics of DexP₂₆COOH₉-stabilized and MM-loaded PLA nanoparticles before and after undergoing the procedure for Tf coupling.

| Exp. | Load PLA:MM (wt:wt) | Amount of reactant (mol / 100 mg NPs) | | Step ^a | D[3:2] (nm) | Span | Surface coverage by DexP ₂₆ COOH ₉ (mg / m ² of PLA) |
|------|---------------------------|--|----------------------|-------------------|----------------|-------|---|
| | | NHS | EDCI | | | | |
| 1 | 100:1 | 3.5 10 ⁻⁴ | 2.6 10 ⁻⁴ | BC | 120 | 4.6 | 4.5 |
| | | | | AC | 120 | 18.1 | --- |
| 2 | 90:10 | 3.5 10 ⁻⁴ | 2.6 10 ⁻⁴ | BC | 133 | 7.9 | 3.4 |
| | | | | AC | 187 | 101.4 | --- |

^a BC: Before coupling. AC: After coupling. For details see section 2.6.

Figure captions

Figure 1: Chemical reaction between Dextran T40 and 1, 2-epoxy-3-phenoxypropane. For the sake of simplicity, the substitution was represented at the position of the most reactive hydroxyl group.

Figure 2: Surface tension at 25 °C of aqueous solutions of DexP₂₇ and DexP₂₇COOH₆. Lines are guides for the eye.

Figure 3: Schematic representation of the procedure for preparing MM- or FITC-loaded nanoparticles using dextran-based stabilizers. Analytical methods are indicated at the relevant steps.

Figure 4: Absolute value of n index as a function of NaCl concentration for suspensions of PLA nanoparticles stabilized by DexP₁₅ (bold circles), DexP₂₁ (open circles), and DexP₂₆ (bold squares). Dotted lines are the limits of normal sodium ion concentration in blood.

Figure 5: Surface-average diameter of nanoparticles after evaporation (bold circles) and after centrifugation and washing (open circles) as a function of the initial weight fraction of MM (in the MM+PLA mixture).

Figure 6: Fraction of encapsulated monomyristin as a function of time after immersion in a large excess of deionized water for DexP₂₆-stabilized nanoparticles prepared with an initial PLA/MM load of composition 9:1 wt:wt.













