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**Impact of polylactide fluorinated-end group length and their dynamics on
perfluohexane microcapsules morphology**

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Keywords: Perfluorocarbon microcapsules, perfluorohexane, fluorinated polymers, focused ultrasound, end-group dynamics, fluorous interaction

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Abstract

We have synthesized polylactide (PLA) polymers containing five distinct lengths of fluorinated end-groups (from C_3F_7 to $C_{13}F_{27}$). The influence of fluorinated end-groups length and their dynamics in chlorinated solvents (chloroform and dichloromethane) was investigated as a function of the presence of perfluorohexane (PFH) and related to the morphology of capsules of PFH obtained using these fluorinated polylactides. ^{19}F spin-spin relaxation time (T_2) measurements revealed a reduced mobility of the fluorinated units with dependency on fluorinated chain length in the order $C_3F_7 > C_8F_{17} > C_{13}F_{27}$. The presence of perfluorohexane (PFH) led to a further decrease on the segmental mobility, indicating the existence of fluororous interactions. The T_2 relaxation time of the CF_3 resonance of PLA- C_3F_7 decreased from 540 ± 50 ms in $CDCl_3$ to 81 ± 15 ms after the addition of PFH. Due to these fluororous interactions, PLA polymers containing short fluorinated groups (C_3F_7 and C_6F_{13}) led to microcapsules with core-shell morphologies, whereas those formulated with long F-units (C_8F_{17} , $C_{11}F_{23}$ and $C_{13}F_{27}$) favored the formation of multinucleated capsules as observed by confocal microscopy.

1. Introduction

Fully and partly fluorinated compounds are widely explored in the chemical industry and academic research for their inherent immiscibility with hydrophobic and lipophobic solvents. This so-called “fluorophilic” character is responsible for low or “gas like” intermolecular attractions which confer to such molecules inertness and biocompatibility, features that attracted special attention for a wide range of biomedical applications^{1,2}. In particular, liquid perfluorocarbons (PFCs), such as perfluorohexane (PFH) or perfluorooctyl bromide (PFOB) are described to be minimally absorbed, eliminated from the lungs by evaporation and did not lead to any significant histological, cellular or biochemical disturbance³. Therefore, these PFCs are intensively investigated for their potential to act as intravascular contrast agents⁴.

Recent reports describe the use of capsules containing PFOB to provide *in vivo* ¹⁹F-MRI or ultrasound detection and also passively deliver chemotherapeutics to cancer cells^{5–9}. Similar formulations use PFH as an active core for its lower boiling point, as it favors liquid-to-gas transition by acoustic and thermal stimuli, allowing real-time ultrasonic imaging as well as localized drug-release^{10–12}. Nonetheless, the entrapment of fully fluorinated PFCs, such as PFH, within a polymeric shell is proving to be difficult due to their dual lipophobic and hydrophobic character and their trend to easily evaporate during formulation¹⁴. Several groups have turned to the use of fluorinated surfactants or lipids to improve PFCs encapsulation^{15–17}. Literature also reports the functionalization of polymer with fluorinated end-groups to favor interactions with PFCs^{11,14,18–24}. It was previously demonstrated that a wide range of fluorinated end-groups linked to the PLA chain – from C₃F₇ to C₁₃F₂₇ – increased the entrapment of PFCs into nanocapsules, resulting in more efficient acoustic responses^{14,19}. However, no significant distinction regarding the morphology or PFC entrapment was observed by increasing the length of the end-fluorinated groups in nanoformulations.

The main objective of this study is to investigate deeper the influence and extent of fluororous interactions between fluorinated end-groups of PLA-C_xF_{2x+1} and PFH on the final morphology of microcapsules (MC) as a function of fluorinated end-group length. In particular, we examine the role of the solvent in which the polymer was dissolved on the strength of fluororous interactions by comparing chloroform and dichloromethane, two solvents often used to formulate polymer particles and capsules^{25,26}. To fully explore the impact of fluorinated end-group chain length and solvent, end-group dynamics and PFH entrapment efficiency were evaluated by NMR and MC morphology was assessed by confocal microscopy. This study provides information for the morphological design of new ultrasound activable systems formulated with fluorinated PLA polymers.

2. Materials and Methods

2.1 Materials

D,L-lactide was purchased from Polysciences (Germany) and perfluorohexane 98+% was acquired from Alfa Aesar (Germany). 1-hexanol was obtained from Acros Organics (Belgium), 2,2,3,3,4,4-heptafluoro-1-butanol, 1H,1H perfluoro-1-heptanol, 1H,1H perfluoro-1-nonanol, 1H,1H perfluoro-1-dodecanol and 1H,1H perfluoro-1-tetradecanol were acquired from Fluorochem (United Kingdom). Acetone, tetrahydrofuran (THF) and dichloromethane were purchased from Carlo Erba Reactifs (France), chloroform and diethyl ether from VWR (France). Nile red, stannous octoate, sodium cholate, trifluoroacetic acid (TFA), deuterated chloroform and deuterated dichloromethane were provided by Sigma-Aldrich (France). The ultrapure water was produced by a RIOS/Milli-Q system (Millipore, France), with a resistivity of 18.2 MΩcm. The NMR sample tubes and coaxial inserts were obtained from CortecNet (France).

2.2 Polymer Synthesis

All fluorinated (PLA-C₃F₇, PLA-C₆F₁₃, PLA-C₈F₁₇, PLA-C₁₁F₂₃ and PLA-C₁₃F₂₇) and non-fluorinated (PLA-C₆H₁₃) derivatives of polylactide polymers were synthesized by ring opening polymerization (ROP) with the presence of stannous octoate as catalyst^{27,28}. All glassware and stir bars were flame-dried and cooled under argon flow. Briefly, in a 10 mL schlenck tube equipped with a magnetic stir-bar, the D,L-lactide (10.4 mmol, 1.5 g) and corresponding initiator (0.075 mmol) – 1-hexanol for PLA-C₆H₁₃, 2,2,3,3,4,4-heptafluoro-1-butanol for PLA-C₃F₇, 1H,1H perfluoro-1-heptanol for PLA-C₆F₁₃, 1H,1H perfluoro-1-nonanol for PLA-C₈F₁₇, 1H,1H perfluoro-1-dodecanol for PLA-C₁₁F₂₃ or 1H,1H perfluoro-1-tetradecanol for PLA-C₁₃F₂₇ – were added to the flask under argon flow. The tube was sealed with a rubber cap and a stannous octoate solution (0.05 mmol, 20 mg) dissolved in 2 mL of dried toluene was added through the septum. The tube was purged with argon for 0.5 h and the polymerization reaction was conducted with continuous stirring at 130 °C for 55 minutes in an oil bath under

argon flow. The reaction was quenched by immersing the flask in a cold water bath. Afterwards, the solvent was evaporated under reduced pressure for 1h and the material was dissolved in 5 mL of chloroform. The product was purified by precipitation as previously described²⁹: all polymers were precipitated into cold diethyl ether (80 mL), next, PLA-C₆H₁₃ was dissolved into THF (5 mL), whereas the fluorinated polymers were dissolved in acetone (20 mL) and precipitated again in ultrapure water (150 mL). The samples were freeze-dried for 24 h and a white powder was obtained. Lactide conversion $\geq 95\%$ (¹H-NMR). ¹H-NMR [400 MHz, CDCl₃, 25°C] **PLA-C₆H₁₃**: δ_H 5.10-5.28 (PLA-CHCH₃COO-), 1.52-1.61 (PLA-CHCH₃COO-), 0.88-0.92 (-CH₂CH₃). **PLA-C₃F₇**, **PLA-C₆F₁₃**, **PLA-C₈F₁₇**, **PLA-C₁₁F₂₃** and **PLA-C₁₃F₂₇**: δ_H 5.10-5.28 (PLA-CHCH₃COO-), 4.50-4.70 (-OCH₂CF₂-), 1.52-1.61 (PLA-CHCH₃COO-). ¹⁹F-NMR [400 MHz, CDCl₃, 25°C] **PLA-C₃F₇**: δ_F -79.0 (-CF₃), -118.7 (-OCH₂CF₂-) and -125.7 (-CF₂CF₃); **PLA-C₆F₁₃**, **PLA-C₈F₁₇**, **PLA-C₁₁F₂₃** and **PLA-C₁₃F₂₇**: δ_F -78.9 (-CF₃), -117.7 (-OCH₂CF₂-), -120.0 (-CF₂)_nCF₂CF₃), -120.7 (-OCH₂CF₂CF₂CF₂-), -121.4 (-OCH₂CF₂CF₂-) and -124.2 (-CF₂CF₃).

2.3 Characterization

All polymers were characterized by size exclusion chromatography (SEC) in chloroform at 30 °C with a flow rate of 1 mL.min⁻¹ by two columns (PL-gel 5 μ m MIXED-D 300 x 7.5 mm) calibrated against a curve based on poly(methyl methacrylate) standards (PMMA, Polymer Laboratories, Varian Inc.) The system was coupled to a refractive index detector (Spectrasystem RI-150, Thermo Electro Corp.). ¹H and ¹⁹F-NMR spectra were obtained on a Bruker AVANCE III HD 400 MHz in CDCl₃ at 25°C.

2.4 Interfacial surface tension

The interfacial surface tension was performed in a Tracker tensiometer (Teclis, France). PFH was previously filtrated by a column of aluminum oxide. A constant 2 μ L PFH or sodium cholate drop was formed by a syringe and a G20 stainless steel needle inside an optical glass cuvette containing fixed polymer concentrations of 25 mg.mL⁻¹ in chloroform or

dichloromethane previously filtered by 0.22 μm (Millipore). The temperature was kept at 20 °C and the interfacial surface tension was determined from the drop profile employing the Laplace equation and the forces balance between capillarity and gravity.

2.5 ^{19}F -NMR T_1 and T_2 Relaxation

^{19}F Spin-lattice and spin-spin relaxation measurements were performed at 25 °C in CDCl_3 or CD_2Cl_2 with fixed polymer concentrations of 25 $\text{mg}\cdot\text{mL}^{-1}$. Samples containing 15 μL of PFH were also evaluated. The samples were not degassed. All experiments were performed on a Bruker AVANCE III HD NMR spectrometer operating at 9.4 T, equipped with a 5-mm multinuclear inverse detection probe, observing ^{19}F and ^1H nuclei at 376.49 and 400.13 MHz, respectively. ^{19}F T_1 was determined by the inversion-recovery pulse sequence ($T_1\text{IR}$). The delay (τ) between the inversion and read-pulse was varied along 16 values (1 to 22 s). The total number of scans was 32, spectral width = 100 ppm and relaxation delay = 22 s. ^{19}F T_2 values were measured by the PROJECT-CPMG pulse sequence along 15 values of τ (0.1 to 2 ms)³⁰. The number of scans was 128, spectral width = 100 ppm and relaxation delay = 10 s. Relaxation times were obtained by nonlinear least-squares fitting of a mono- exponential function with $r^2 \geq 0.998$ for all samples.

2.6 Microcapsules Formulation

Microcapsules were prepared by the emulsion-evaporation technique, as previously described¹³, with minor modifications. Briefly, the desired amount of polymer was dissolved into 2 mL of chloroform or dichloromethane in a 50 mL glass flask. Afterwards, 50 μL of Nile red (55 $\mu\text{g}\cdot\text{mL}^{-1}$) was added and the flask was sealed with a rubber cap. Next, 30 μL of PFH was introduced through the septum with constant stirring and the media was emulsified with 10 mL of sodium cholate 1.5% (w/w) at 4 °C. The mixture was submitted to high-speed homogenization at 8.000 RPM using an Ultra-Turrax T25 (IKA) coupled with an SN25-10G dispersing tool in an ice bath for 1 minute. The solvent was removed by rotary evaporation

(Büchi) with a 30 °C water bath and recirculation chiller at -10 °C. A vacuum gradient from 400 to 30 mbar in 7 steps of 5 minutes was employed. Afterwards, the total volume of each sample was completed to 10 mL with ultrapure water.

2.7 Confocal Microscopy

Microcapsules were washed twice with ultrapure water by centrifugation and resuspended in glycerol to minimize motion. All samples were observed in a Leica TCS SP5 confocal scanning microscope (Leica) with a 1 mW helium neon laser operating at excitation of 551 nm and 636 nm of emission, Plan Achromat 63X objective (NA 1.40 oil immersion) and pinhole diameter at 71 nm. For diameter analysis, the measurements were performed in the equatorial plane of each capsule as previously described ¹³.

2.8 Scanning Electron Microscopy

Scanning electron microscopy was performed in a Merlin 6040 (Carl Zeiss, Germany) operating at 3 kV. All microcapsules were washed twice with ultrapure water by centrifugation to remove the excess of sodium cholate, deposited on carbon conductive double-sided tape (Euro-medex, France) and dried at room temperature. Afterwards, they were coated with a palladium–platinum layer of about 3 nm using a Cressington sputter-coater 208HR with a rotary-planetary-tilt stage, fitted with an MTM-20 thickness controller.

2.9 PFH encapsulation efficiency by ¹⁹F-NMR spectroscopy

For PFH quantification, 1 mL of microcapsules was freeze-dried during 24 h employing an Alpha-1-2 LD apparatus (Christ). The flasks were hermetically sealed with a rubber cap and stored at -20 °C until analysis. The PFH encapsulation efficiency was determined as described by Diou *et al.* ²⁹. A total volume of 1 mL of chloroform was introduced into the sealed tube by a needle, the suspension was vortexed (5 cycles of 30 seconds) and centrifuged at 0 °C for 10 minutes at 5000 rpm (Jouan CR4I centrifuge, Thermo Electron Corporation). The organic solution was collected in a cold room at 4 °C and introduced into

an NMR tube that was afterwards loaded with a stem coaxial insert containing TFA in D₂O (12 μmol.mL⁻¹) as external standard ³¹. All spectra were obtained at 4 °C on a Bruker Avance 300 (400 MHz) spectrometer. The total amount of encapsulated PFH was determined based on a PFH calibration curve. For each MC sample, the integration of the TFA peak at -76.5 ppm was determined as 3 and the resulting integration of the PFH CF₃ at -81.2 ppm was used to calculate the PFH concentration in the NMR tube based to the equation of the calibration curve.

2.10 Statistical analysis

The statistical analysis was performed using Statistica™ 8.0 software (StatSoft Inc., Tulsa, USA). The data was analyzed by two-sided Student's *t*-test and a statistical significance was set at the level of $p < 0.05$.

3. Results and Discussion

3.1 Polymers synthesis and characterization

Six end-group fluorinated or alkylated polymers were synthesized by ring opening polymerization of D,L-lactide with appropriate initiator (hexanol or fluorinated alcohols) as previously described^{14,19,28,32} (reaction yield > 85%). The polylactide main chain is constituted by ~140 repetitive units containing different lengths of end fluorinated-groups: polymers are designated according to the terminal group chemistry and length of fluorinated units as PLA-C₆H₁₃, PLA-C₃F₇, PLA-C₆F₁₃, PLA-C₈F₁₇, PLA-C₁₁F₂₃ and PLA-C₁₃F₂₇. They were characterized by ¹H and ¹⁹F-NMR and exhibited a molecular weight around 18000-21000 g.mol⁻¹ as assessed by NMR, with a narrow dispersity (Supporting information **Figure S1 and Table S1**).

3.2 Polymers dynamics

To explore the role and extent of fluorous interactions between the different PLA-C_xF_{2x+1} and PFH during the formation of microcapsules, we have investigated interfacial properties, dynamics in solution as well as morphological features generated by each of these polymers. As fluorine recognition and compartmentalization are strictly dependent on the solvent's character, the behavior and impact of polymer end-group chemistry was studied in two distinct solvents usually employed in the formulation of MC, namely, chloroform and dichloromethane^{25,26}.

The interfacial activity of the polymers was determined at the solvent/PFH and solvent/sodium cholate (SC) interfaces by interfacial surface tension measurements (**Table 1**). At the solvent/SC interface, interfacial tension values were between 7–8 mN.m⁻¹ in CHCl₃ or CH₂Cl₂, for all polymers, suggesting no specific solvent or end-group contribution to the interfacial activity. At the PFH/solvent interface, polymers dissolved in CHCl₃ exhibited an interfacial tension average value of 3.0±0.05 mN.m⁻¹ whereas 6.0±0.1 mN.m⁻¹ was measured for polymers dissolved in CH₂Cl₂, indicating that CHCl₃ favors PFH spreading. Nonetheless,

none of the polymers was able to decrease the interfacial tension compared to the solvent itself regardless the end-group chemistry or the length of the fluorinated end-group.

Table 1. Interfacial tensions at the perfluorohexane/solvent and alkylated or fluorinated polymer/sodium cholate 1.5% (w/v) interfaces. All polymers were dissolved at 25 mg.mL⁻¹ in chloroform or dichloromethane. All results are expressed in mN.m⁻¹.

	Chloroform		Dichloromethane	
	PFH/solvent	Solvent/SC 1.5%	PFH/solvent	Solvent/SC 1.5%
Solvent	3.2±0.3	7.5±0.3	6.0±0.3	8.1±0.2
PLA-C₆H₁₃	3.0±0.5	7.5±0.2	6.2±0.5	8.0±0.2
PLA-C₃F₇	3.0±0.3	7.0±0.1	6.1±0.7	7.5±0.3
PLA-C₆F₁₃	3.0±0.5	7.1±0.5	6.2±0.4	7.7±0.5
PLA-C₈F₁₇	3.1±0.4	7.1±0.2	5.9±0.4	7.7±0.4
PLA-C₁₁F₂₃	3.0±0.3	7.2±0.3	5.9±0.3	7.8±0.2
PLA-C₁₃F₂₇	3.1±0.4	7.4±0.1	6.1±0.4	7.6±0.1

PFH-SC (1.5% w/v) interfacial tension was 22.5±0.2 mN.m⁻¹

The lack of adsorption at the PFH/solvent interface might be explained by the mass ratio difference between the relatively small fluorinated blocks compared to the PLA main chain (C₃ to C₁₃ for fluorinated block vs. 140 repetitive units for PLA) and the conformation assumed by the polymer in solution. Indeed, as the PLA polymers are well solvated in CHCl₃ and CH₂Cl₂ (Hildebrand solubility parameter (δ_T) of 20.8 MPa^{0.5} for unmodified PLA, 18.7 MPa^{0.5} for CHCl₃ and 20.2 MPa^{0.5} for CH₂Cl₂³³), a conformational rearrangement based on the higher solubility of the polymeric chains within the solvents may hide the fluorinated groups inside the PLA coil, hindering an effective interaction with the PFH phase¹⁹.

Although no interfacial activity was observed at the PFH/solvent interface during static measurements, the solvent-evaporation process is dynamic and associated with transitory interfacial instabilities due to system disorganization³⁴. Such constant rearrangement of water and organic solvent molecules may promote the formation of fluorinated domains

favorable for PFH encapsulation ³⁵. Indeed, many studies carried on mixed systems, containing both fluorocarbons and organic solvents, showed the tendency of perfluoralkyl moieties to self-associate and generate fluorous compartments. This aggregation in rich fluorous domains is generally associated to a reduced mobility of fluorinated groups ^{26,36,37}.

To assess the behavior of the fluorinated chains in a more dynamic media containing PFH – a situation similar to what happens prior to solvent evaporation – the end-group mobility of three fluorinated polymers, PLA-C₃F₇, PLA-C₈F₁₇ and PLA-C₁₃F₂₇, was analyzed by fluorine NMR spectroscopy employing spin-lattice (T₁) and spin-spin (T₂) relaxation measurements at fixed polymer concentration of 25 mg.mL⁻¹ and 15 µL of PFH (corresponding to the formulation conditions). Accordingly, the CF₃ group dynamics in each solvent was probed in presence and absence of PFH in the solvent. Unfortunately, some of the PFH resonances overlapped with the CF₃ polymer signals for PLA-C₈F₁₇ and PLA-C₁₃F₂₇ in both solvents. Only PLA-C₃F₇ exhibited well segregated resonance from those of PFH because of the different chemical environments, which permitted a resonance shift towards lower magnetic fields, not overlapping with PFH as displayed in **Figure 1**. The nomenclature and ¹⁹F-NMR peak labeling consists alphabetically with the position of the fluorinated resonance respective to the PLA chain ^{38,39}.

Figure 1. Magnified CF_3 and CF_2 regions of the ^{19}F -NMR spectra for PLA- C_3F_7 , PLA- C_8F_{17} and PLA- $\text{C}_{13}\text{F}_{27}$ in CDCl_3 . The insets (left) exhibit the CF_3 resonance in a mixed system of polymer and perfluorohexane. ^{19}F peak assignment is indicated in the top of each image.

T_2 analysis revealed that the CF_3 resonance mobility decreased strongly for polymers functionalized with longer fluorinated units in the order $\text{C}_3\text{F}_7 > \text{C}_8\text{F}_{17} > \text{C}_{13}\text{F}_{27}$ for both solvents (**Table 2**). Such reduction in the local fluorine dynamics according to chain length is related to the strong electronegativity of the C-F bond that generates linear and rigid chain conformations, reducing the segmental mobility as the chain length increases⁴⁰. Nonetheless, samples dissolved in CDCl_3 showed smaller relaxation values compared to their counterparts prepared in CD_2Cl_2 : this effect is clearly illustrated by the CF_3 resonance of PLA- C_3F_7 , with T_2 of 540 ± 50 ms in CDCl_3 and 1530 ± 150 ms in CD_2Cl_2 . Such results were also confirmed by T_1 measurements (Supporting information **Table S2**). These significant changes in relaxation times indicate that chloroform is not a good solvent for the fluorinated end-groups and drives self-association of ^{19}F containing units.

Table 2. ^{19}F spin-spin relaxation (T_2) values of CF_3 resonances for samples dissolved in deuterated chloroform or dichloromethane in presence or absence of perfluorohexane. All polymers were dissolved at fixed concentration of 25 mg.mL^{-1} (Experiment performed in duplicate, $n=2$).

Sample	T_2 (ms)		T_2 (ms)	
	CDCl_3	CDCl_3+PFH	CD_2Cl_2	$\text{CD}_2\text{Cl}_2+\text{PFH}$
PLA- C_3F_7	540 ± 50	81 ± 15	1530 ± 150	1710 ± 250
PLA- C_8F_{17}	210 ± 20	---	1263 ± 165	---
PLA- $\text{C}_{13}\text{F}_{27}$	76 ± 10	---	790 ± 50	---

Afterwards, PFH was added to each polymer solution to simulate the system behavior just before emulsification with sodium cholate. In CD_2Cl_2 , T_2 varied from $1530\pm150 \text{ ms}$ to $1710\pm250 \text{ ms}$ in presence of PFH, showing that the end-group dynamics is not significantly affected by PFH addition in the media ($p = 0.475$). However, in CDCl_3 , the CF_3 signal of PLA- C_3F_7 showed a significant T_2 decrease with PFH, from $540\pm50 \text{ ms}$ to $81\pm15 \text{ ms}$ ($p < 0.05$). This lower mobility denotes a polymer rearrangement in presence of PFH evidencing an increased tendency for the fluorinated segments to associate with each other (**Figure 2**). Indeed, in CHCl_3 , addition of PFH further decreases the solubility of the fluorinated segments which promotes dipolar interactions between the fluorinated end-group and PFH. These more efficient interactions in CHCl_3 suggest formation of F-rich domains where the reduced mobility of the fluorinated segment leads to shorter T_2 values.

Figure 2. T_2 decay profiles for the CF_3 resonance of PLA- C_3F_7 in CDCl_3 (A) and CD_2Cl_2 (B) without (open circles) and with perfluorohexane (dark circles).

According to those results, the introduction of PFH was able to reduce the local end-fluorinated group mobility of PLA- C_3F_7 in CDCl_3 . As PFH is well dissolved in chloroform, its contact with the fluorinated units may lead to the formation of fluorophilic domains, a process that might also depends on the length of the fluorinated group. This scenario, however, illustrates the system's behavior only in stable organic solutions; during the solvent-evaporation process in which MCs are formulated, transitory and thermodynamically unstable interfaces are created. Therefore, the ability of each fluorinated derivative to better stabilize and retain PFH as a liquid core was further studied in MCs formulations.

3.2 Solvent Influence on MC formulation

The impact of the fluorinated end-groups on the final morphology of microcapsules was then studied for all synthesized polymers. All microcapsules were initially formulated with fixed amounts of polymer (50 mg) that were dissolved either in chloroform or dichloromethane and

subsequently submitted to the same solvent-evaporation process. The encapsulation efficiency (EE) of PFH was quantified in freeze-dried samples by ^{19}F -NMR and the size of all MCs was measured by confocal microscopy as listed in **Table 3**.

Table 3. Encapsulation efficiency and size of microcapsules formulated with 50 mg of polymer prepared in chloroform and dichloromethane.

Sample	Chloroform		Dichloromethane	
	Size (μm)*	PFH EE (%)	Size (μm)*	PFH EE (%)
PLA-C ₆ H ₁₃	4.2 $\pm$ 1.0	5.5 $\pm$ 0.2	4.8 $\pm$ 0.8	12.3 $\pm$ 0.2
PLA-C ₃ F ₇	4.4 $\pm$ 0.6	10.1 $\pm$ 0.2	4.7 $\pm$ 0.8	9.1 $\pm$ 0.1
PLA-C ₆ F ₁₃	4.8 $\pm$ 1.0	12.7 $\pm$ 0.2	4.6 $\pm$ 1.0	13.1 $\pm$ 1.3
PLA-C ₈ F ₁₇	4.6 $\pm$ 0.8	10.3 $\pm$ 0.5	4.9 $\pm$ 1.2	11.1 $\pm$ 0.2
PLA-C ₁₁ F ₂₃	5.2 $\pm$ 1.4	11.1 $\pm$ 1.4	4.8 $\pm$ 0.9	7.1 $\pm$ 0.1
PLA-C ₁₃ F ₂₇	5.2 $\pm$ 1.0	11.4 $\pm$ 1.5	5.3 $\pm$ 0.6	10.0 $\pm$ 0.7

*All measurements correspond to observation and analysis of at least 100 microcapsules per each group.

As determined by ^{19}F NMR, the non-fluorinated polymer, PLA-C₆H₁₃, presented an encapsulation efficiency of 5.5 $\pm$ 0.1% when prepared in CHCl₃. The use of fluorinated polymers led to a 2-fold increase in PFH encapsulation efficiency, reaching 11 $\pm$ 3.0% without any significant distinction as a function of the fluorinated end-group length (**Table 3**). The reduced end-group mobility of fluorinated polymers in CDCl₃ containing PFH was translated into higher encapsulation efficiencies. Altogether, these results evidence the ability of fluorinated polymers to interact with the fluorocarbon phase and enhance its stabilization inside the organic phase, as previously reported for nanocapsules¹⁴. For microcapsules prepared in CH₂Cl₂, no trend was observed for the encapsulation efficiency, probably due the absence of specific interactions between PFH and the polymer (Table 2).

Nonetheless, PLA or PLGA polymers are able to encapsulate higher amounts of PFH into MCs (~40%)¹³, the discrepancy with our results may be related to the fast process of solvent

evaporation employed here, whereas Mousnier et al. have evaporated the organic solvent slowly at ambient pressure¹³.

Although all samples were predominately smooth as shown by SEM in **Figures 3 and 4 (bottom)**, a certain amount of rough shelled MCs were also observed in all images. According to Chung, *et al.*, this morphological variation might be related to the fast evaporation process used, which promoted an uneven polymer concentration gradient towards the interface⁴¹. Also, a partial PFH vaporization during the solvent evaporation step (performed at 30 °C) may have caused a volumetric expansion of the MCs and, as the temperature dropped, a subsequent shrinkage altered the continuous spreading of the polymer at the interface as previously reported by Lebeveda *et al.* for PFH containing microspheres⁴².

Figure 3. Confocal (top) and scanning electron microscopy (bottom) images of microcapsules produced in chloroform. Scale bar represents 10 µm.

Figure 4. Confocal (top) and scanning electron microscopy (bottom) images of microcapsules produced in dichloromethane. Scale bar represents 10 µm.

A clear and subtle distinction on MC morphology was observed according to the fluorinated end-group length in both solvents. In all cases, PLA-C₆H₁₃, PLA-C₃F₇ and PLA-C₆F₁₃ exhibited core-shell morphologies comprising a well-centered PFH phase; however, as the end-group length increased to C₈F₁₇, C₁₁F₂₃ and C₁₃F₂₇ higher extents of randomly distributed PFH cores were observed as displayed by the white arrows in **Figures 3 and 4 (top)**. In CH₂Cl₂, MCs still possess a distinctive core shell structure but the inner PFH core is displaced from the center of the MC. Such decentered morphology had already been observed¹³. Formulation in CHCl₃ leads to a mixture of structure with well centered MC and with MC having multi-cores of PFH.

Regardless of the solvent employed, samples prepared with short fluorinated or non-fluorinated end-groups, PLA-C₃F₇ and PLA-C₆F₁₃ or PLA-C₆H₁₃, presented the formation of core-shell capsules. Only MCs formulated with longer fluorinated groups, PLA-C₈F₁₇, PLA-C₁₁F₂₃ and PLA-C₁₃F₂₇, led to the appearance of different morphologies, either in chloroform or dichloromethane, indicating that the shell precipitates prior to complete PFH nucleation at the center of the organic droplet. Thus, the length of the fluorinated end-group played an important role in the fluorocarbon nucleation process, suggesting that longer fluorous chains were able to better interact and restrain the PFH diffusion as illustrated in **Figure 5**.

336

337 **Figure 5.** Schematic representation of the perfluorohexane phase-separation and nucleation
 338 process in the organic phase containing fluorinated polymers of distinct end-group lengths.

339 The different morphologies generated in each solvent are possibly related with the extent of
 340 fluorous interactions, PFH miscibility as well as solvent evaporation rate. In dichloromethane,
 341 the formation of decentered capsules for the longer F-units samples, such as $\text{PLA-C}_{13}\text{F}_{27}$,
 342 probably reflected the reduced extent of interactions between the end-groups and PFH.
 343 Nonetheless, such samples were still able to alter the nucleation pattern, inducing the PFH to
 344 accumulate towards the drop's periphery, also suggesting the fluorinated segregation effect.
 345 The obtained decentered morphologies are, however, favored by the solvent's lower boiling
 346 point (39.6 °C for CH_2Cl_2 vs. 61.2 °C for CHCl_3) that accelerates the onset of polymer
 347 precipitation.

348 In chloroform, the shell of polymers with longer fluorinated groups precipitates before the
 349 complete fluorocarbon nucleation restraining PFH diffusion and causes multiple PFH cores
 350 dispersed inside the rigid polymeric phase. As disclosed by T_2 results, the PFH is able to
 351 interact more efficiently with the fluorinated end-groups in this solvent. Thus, such structure

is probably attributable to formation of F-rich domains driven by the end-group polymer arrangement to minimize contact with the surrounding polymer. This effect indicates that longer fluorinated units interact more importantly with PFH.

3.3 Influence of polymer concentration on MC morphology

To further evaluate the end-group chemistry and length influence on microcapsules structure, samples PLA-C₆H₁₃, PLA-C₃F₇, PLA-C₈F₁₇ and PLA-C₁₃F₂₇ were prepared only in chloroform using different polymer masses (12.5, 25, 50 and 100 mg) and constant PFH volume of 30 μ L.

Samples prepared with 12.5 mg presented core-shell structures with thin and smooth polymer layers that were predominant for all polymers, independently of end-group chemistry or length as shown in **Figure 6**. The increase of polymer mass to 25 mg altered only the morphology of MCs having the longest fluorinated group: PLA-C₁₃F₂₇ by formation of multinucleated capsules (42 $\pm$ 10%) as displayed by the white arrow in **Figure 6**. The percentage of multiple PFH cores for PLA-C₁₃F₂₇ became more obvious for 50 and 100 mg, in which the total amount of multinucleated MCs increased from 60 $\pm$ 5% to 91 $\pm$ 3%, respectively. The same effect but at a lower extent was observed for PLA-C₈F₁₇, as thin-shelled MCs are observed in the formulations containing 25 mg and multinucleated morphologies increased from 10 $\pm$ 2% to 56 $\pm$ 7% for 50 and 100 mg, respectively. A similar behavior was observed for MC formulated with the polymer containing the shortest fluorine end-group, PLA-C₃F₇, as core-shell structures predominated until a polymer mass of 100 mg, in which a total of 45 $\pm$ 5% of multinucleated MC were found. In contrast, MC formulated with the non-fluorinated sample, PLA-C₆H₁₃, exhibited core-shell morphologies for all of the tested polymer masses.

Figure 6. Confocal microscopy images of microcapsules formulated in chloroform with non-fluorinated (PLA-C₆H₁₃) and fluorinated (PLA-C₃F₇, PLA-C₈F₁₇ and PLA-C₁₃F₂₇) polymers with varied polymer masses: 100, 50, 25 and 12.5 mg. Scale bar of 10 µm.

Despite heterogeneity of morphology within the same sample, a clear effect of the polymer end-group chemistry and fluorinated length was evidenced in the capsule's morphology. Compared to PLA-C₆H₁₃, polymers containing F-groups generated MCs with decentered and/or multiple PFH cores. At high fluorinated polymer mass, the dominant morphology whatever the fluorinated length block consists in multinucleated capsules. This increase in polymer mass favored the formation of richer fluorophilic domains – in the order of C₃F₇<C₈F₁₇<C₁₃F₂₇ – that altered the polymeric phase dynamics, reducing the migration of PFH to the center of the organic drop.

4. Conclusion

We have successfully synthesized and characterized poly(lactide) end-group fluorinated polymers for improved fluorous interaction with PFH. The presence of fluorinated end-groups

provided more favorable interfaces with perfluorohexane in organic solution of chloroform with fluorinated end-group length dependency, as revealed by spin-spin relaxation measurements. The formation of fluorophilic environment is reflected with improved perfluorohexane encapsulation efficiencies into microcapsules and by modifying the final morphology, particularly in the case of longer fluorinated end-groups and important polymer masses.

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Supporting Information.

¹H and ¹⁹F-NMR spectra of all synthesized polymers in CDCl₃.

SEC chromatogram of all polymers in chloroform at 30°C.

Number-average molar masses (M_n) obtained by SEC/¹H-NMR, dispersity and glass-transition temperature (T_g) of all polymers.

Spin-lattice relaxation (T1) values of correspondent ¹⁹F resonances for samples dissolved in CDCl₃ and CD₂Cl₂ with and without perfluorohexane.

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