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Design and formulation of nanoemulsions using 2-(poly(hexafluoropropylene oxide)) perfluoropropyl benzene in combination with linear perfluoro(polyethylene glycol dimethyl ether)

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Abstract

This is the first report where PFP AE aromatic conjugates and perfluoro(polyethylene glycol dimethyl ether) are combined and formulated as nanoemulsions with droplet size below 100 nm. A perfluoropolyalkylether (PFP AE) aromatic conjugate, 2-(poly(hexafluoropropylene oxide)) perfluoropropyl benzene, was used as fluorophilic-hydrophilic diblock (FLD) aimed at stabilizing perfluoro(polyethylene glycol dimethyl ether) nanoemulsions. Its effects on colloidal behaviors in triphasic (organic/fluorous/aqueous) nanoemulsions were studied. The addition of FLD construct to fluorous phase led to decrease in PFP AE nanoemulsion droplet size to as low as 85 nm. Prepared nanoemulsions showed high colloidal stability. Our results suggest that these materials represent viable novel approach to fluorous colloid systems design with potential for biomedical and synthetic applications.

Keywords

PFP AEs; perfluoro(polyethylene glycol dimethyl ether); PFPEs; nanoemulsions; fluorous; microfluidization

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1. Introduction

The initial development of poly(hexafluoropropylene oxide) [poly(HFPO)], a perfluoropolyalkylether (PFPAE), dates back to a United States priority patent application published in 1962 by Earl Philip Moore [1]. These fluoro-oligomers currently have a range from 332 to 16,600 Daltons and are extremely chemically stable; withstanding prolonged exposure to concentrated mineral acids, bases, and oxidative environments. They show relatively low temperature dependence in viscosity, have low volatility, and show temperature stability in a range of –100 to 400 °C [2]. Because of these robust properties, poly(HFPO) originally found itself in hundreds of tribiological applications [3,4,5,6,7,8,9,10,11,12,13,14]. Over time, new applications of PFPAEs were found outside of tribology. One example came in 1991 by Michael Vogt using poly(HFPO) in a biphasic catalysis system [15]. However the technology, known as fluorous biphasic catalysis, was further developed by others using simpler systems such as perfluorocarbons (PFCs) [16,17,18]. The reason the technology worked so well was for the fact that perfluorinated materials are amphiphobic, simultaneously hydrophobic and lipophobic. This means that when they are mixed with hydrocarbon oil and water, they separate into three distinct liquid phases, called the fluorous effect [19]. Overtime, perfluorinated materials were heavily exploited in catalysis and other areas of fluorous chemistry, where fluorocarbon constructs were used to facilitate organic separations.

PFPAEs were not completely overshadowed by PFCs in amphiphobic applications. For example, perfluoro(polyethylene glycol dimethyl ether) and other structurally similar linear perfluoropolyethers (PFPEs) have found broad biomedical and pharmaceutical applications in molecular imaging [20,21,22,23,24], drug delivery [25,26], and ophthalmology [27]. Another interesting example was syntheses of novel amphiphiles using poly(HFPO) acyl chloride and reacting it with didodecyl glutamate and bis(undecanoyl)diethanolamine. When the resulting product was placed in methyl perfluoro (2,5,8-trimethyl-3,6,9-trioxadodecanoate) it formed stable bilayer dispersions in the fluorocarbon media and was stable for one month at room temperature [28].

Nanoemulsions represent kinetically stabilized colloidal dispersions of oil in water. They are typically thermodynamically unstable and for formation typically require high energy input [29,30]. When nanoemulsions are destabilized that is seen as increase in droplet size over time. Perfluoro(polyethylene glycol dimethyl ether), commonly referred to as PFPE, a type of PFPAE, was used previously as the fluorous phase in a unique nanosystem termed triphasic nanoemulsions. In these formulations PFPE was at the core of the droplet, surrounded by a significant volume fraction of hydrocarbon oil and stabilized by surfactants in aqueous phase [25]. The addition of the hydrocarbon (organic) phase allowed for the incorporation of lipophilic drugs and fluorescent dyes which could be dissolved in the hydrocarbon oil before the nanoemulsion was made. The presence of PFPAE (fluorous phase) makes nanoemulsion droplets visible by ¹⁹F magnetic resonance and this feature can be used to track nanoemulsions or labeled cells *in vivo* non-invasively [31,32]. Further, these triphasic nanoemulsions have been shown to be taken up by macrophages *in vitro*, suggesting that they can be potentially used as a vehicle for anti-inflammatory drugs and

inflammation imaging reagents. [25,26] This report expands on earlier developed triphasic hydrocarbon/PFPAE/water nanoemulsions design.

We hypothesized that triphasic nanoemulsions can be further improved through the incorporation of a fluorophilic-lipophilic diblock (FLD) as a PFPAE- hydrocarbon oil interface stabilizers. Most reported PFPE surfactants are fluorophilic and hydrophilic, designed to stabilize water/fluorocarbon interface [33]. The fluorocarbon portion of the FLD is expected to facilitate interaction with fluorous phase (PFPAE), while the aromatic ring is expected to enhance oil solubility. A PFPAE aromatic conjugate, 2-poly(HFPO)-perfluoropropyl benzene, was synthesized as a model FLD construct and tested in several triphasic nanoemulsion formulations. Figure 1 shows the proposed structure of the triphasic nanoemulsion that incorporates FLD.

In the proposed model the PFPAE is immersed in the hydrocarbon oil and FLD is used to stabilize the hydrocarbon/fluorocarbon (PFPAE) liquid/liquid interface. These two phases are then stabilized in aqueous phase using Pluronic® P123 and P105 surfactants. In the pre-formulation development, sonication was used as the emulsification method in order to determine the optimum ratio of FLD to PFPE. Best candidate nanoemulsions with smallest droplet size and polydispersity identified from sonication screening were prepared on larger scale (20 mL) by microfluidization. Nanoemulsions were followed by dynamic light scattering (DLS) upon storage at different temperatures. Further, we exposed nanoemulsions to stressors such as high and low temperature and exposure to salts and serum in cell culture media. The purpose of these tests was to evaluate nanoemulsions potential as future imaging and drug delivery agents and test their stability under biologically relevant conditions.

2. Results and Discussion

2.1 Poly(hexafluoropropylene oxide) based Aromatic as FLD

Initially the poly(HFPO) based aromatics were designed and prepared as intermediates for use as both high temperature [34,35,36,37] and anticorrosion additives [38]. Later, it was realized that the aromatic group attached to a perfluoropolyalkylethers (i.e. 2-(poly(hexafluoropropylene oxide) perfluoropropyl benzene) could be exploited as surfactants in medical applications. Further, the chain length of the oligomeric poly(HFPO) can be adjusted to allow preferential solubility in organic or in fluorinated materials depending on the applications. It was also known that poly(HFPO) based aromatics readily formed emulsion in toluene:hexanes:Krytox (K6) [39]. The PFPAE derivatives have shown to be non-bioaccumulative and non-persistent compared to their perfluoroalkyl counterparts [40,41] and hence preferred candidates for stabilizing nanoemulsions for biomedical applications. To synthesize PFPAE constructs, free-radical perfluoroalkylation of aromatics was originally demonstrated by Minisci in 1997 [42]. Here we have extended the scope to PFPAE based iodides. In particular, we have found that the phenyl poly(HFPO) candidate can be prepared easily in high yields from poly(HFPO) primary iodide. Both benzene and poly(HFPO) primary iodide were added to the glacial acetic acid solvent (HOAc). A radical coupling reaction took place when benzoyl peroxide (BPO) and copper (II) acetate were added and then stirred at 95 °C. It is theorized that the copper (II) acetate behaves as a catalyst to increase the yield of the desired product while minimizing side products. Without

copper (II) acetate and anhydrous reagents, the poly(HFPO) radical attaches primarily to hydrogen radicals rather than attaching to benzene in any high yield (see Scheme 1).

The phenyl moiety imparted sufficient polarity to dissolve PFPAE construct (FLD) in organic media. This feature was expected to further facilitate interactions with hydrocarbon oils needed for triphasic nanoemulsion stabilization. Figure 1 shows 2-(poly(hexafluoropropylene oxide)) perfluoropropyl benzene accumulating at the interface of the PFPAE and hydrocarbon oil phases. The two PFPAEs (Figure 1) show significant similarities, indicating that they are likely highly miscible. This was also confirmed experimentally (data not shown). The presence of benzene was expected to increase mixing with hydrocarbon oils needed for future drug and fluorescent dye solubilization. As shown in Figure 1, we designed the FLD to populate the interface between PFPAE (fluorous oil) and hydrocarbon oils, further stabilizing the triphasic nanosystem. The FLD additive, 2-(poly(hexafluoropropylene oxide)) perfluoropropyl benzene, was synthesized in 84% yield and at sufficient scale (> 25g) needed for formulation development.

2.2 Nanoemulsion optimization studies by sonication

PFPAE nanoemulsions in water have been previously reported [43]. However, these systems do not have sufficient capacity to carry significant amounts of poorly water soluble drugs or lipophilic fluorescent dyes. Therefore, triphasic nanoemulsions represent a more attractive formulation for both drug delivery and imaging. From our earlier studies, we found that synthetic oils (e.g. Miglyol) are good carriers for poorly water soluble drugs in such systems [25]. Here we focused on establishing how well FLD can further stabilize triphasic nanoemulsions by gradually incorporating FLD into the PFPAE phase. To explore FLD utility more fully, we investigated two different hydrocarbon oils separately and a combination of those two. One was olive oil (super refined), which has been previously used in other formulations in our lab and has been found to contribute to colloidal stability of PFC nanoemulsions [44]. The other oil investigated was Capmul® PG-8 NF (Propylene Glycol Monocaprylate) which is known as a good solubilizer for highly water insoluble drugs.[45] In earlier studies it was shown that Pluronics have good stabilizing effects on PFPAE nanoemulsions [43]. Here we used a combination of two Pluronic polymers (hydroxy terminated triblock copolymers based on poly(ethylene glycol)-poly(propylene glycol)-poly(ethylene glycol)) in the surfactant commixture: Pluronic P123 and P105 at 4.0% and 2.4% w/v respectively. During initial optimization of FLD and PFPAE content we used sonication as nanoemulsion processing method. Sonication provides speed, ease of operation and ability to prepare large number of samples at low volume of 1 mL.

Nanoemulsions typically require low surfactant concentrations for oil droplet stabilization in water [46]. Following this rationale we aimed to find lowest FLD concentration needed to stabilize fluorocarbon/hydrocarbon interface. As shown in Figure 2, the Z average size (A) and the PDI (B) of the emulsions decreases as the FLD:PFPE ratio increases. The smallest droplet size was observed at FLD to PFPE 1:4 ratio (v/v) in the fluorocarbon phase of the triphasic nanoemulsions. This FLD:PFPE combination was then chosen for further studies and larger scale preparation by microfluidization.

2.3 Nanoemulsion studies by microfluidization

Sonication and microfluidization are two distinct high energy emulsification techniques. In a comparison study by Ma and Hsu [47], microfluidization was deemed more reliable and less heat generating producing more homogenous products. Microfluidization was chosen here as better method for larger volume emulsification testing. Triphasic nanoemulsions were prepared by microfluidization with three different hydrocarbon oil phases, Table 1. The hydrocarbon oil phase was composed of Olive oil (super refined), Capmul® PG-8 NF, and Capmul® PG-8 NF:olive oil mixture at a ratio of 1:1.

2.4 Effect of FLD on nanoemulsions characteristics

When olive oil alone was used in the triphasic nanoemulsions (nanoemulsions A and B), the addition of FLD had no significant effect on droplet size and polydispersity index (PDI), Table 2. This indicates that FLD can be introduced to fluorocarbon phase without negatively affecting the overall formulation. When the nanoemulsions with olive oil (emulsions A and B) were stored at 4 and 37 °C, there was also no significant effect of FLD on stability. Both formulations remained stable over 28 days showing no change in size and polydispersity, Figure 3A–B.

When Capmul® PG-8 NF was used as hydrocarbon phase in the triphasic nanoemulsion, the FLD led to decrease of droplet size from 102.6 nm to 92.8 nm and PDI decrease from 0.188 to 0.144, Table 2. This was an encouraging result. However, there was no effect of FLD on overall stability of Capmul® PG-8 NF formulations (nanoemulsions C and D) and these nanoemulsions were not stable beyond 48 hours. Stability of Capmul® PG-8 NF nanoemulsion was improved by introducing olive oil. This was evident from DLS follow up of nanoemulsions E and F. After 3 weeks at both 4 and 37 °C the nanoemulsion droplet size and PDI stayed unchanged, Figure 3C–D. In these nanoemulsions FLD led to decrease in droplet size which then remained consistent (8–10 nm) during entire course of measurements at both temperatures. This indicates that FLD size reduction effect was fairly robust and suggests that presence of simple aromatic ring in the fluororous phase of the droplet can affect its structure.

Therefore, the most significant finding of experiments with Capmul® PG-8 NF was substantial decrease in nanoemulsion droplet size compared to our earlier reports with PFPAEs [25,26,43]. Reported size range for PFPAE nanoemulsions was typically 150–200 nm, while formulations reported here fall below 100 nm, Table 2. Small droplet size is advantageous for future imaging and drug delivery applications. However, stability was a concern. To balance small size with stability we opted for testing a combination of olive oil and Capmul® PG-8 NF, where olive oil stabilizing effects are combined with synthetic oil solubilizing capacity. Nanoemulsions were prepared using a 1:1 mixture of Capmul® PG-8 NF and olive oil.

The production of the Capmul® PG-8 NF:olive oil (1:1) formulation (nanoemulsion D) yielded to much smaller droplet diameter (92.8nm) compared to olive oil alone (nanoemulsion B) with size of 144 nm, Table 2. This indicated that Capmul® PG-8 NF had significant effect on the triphasic nanoemulsion droplet size with and without FLD present.

When FLD was added to the mixed oil nanoemulsions droplet size reached 84.5nm (nanoemulsion F), which is the lowest reported PFPE nanoemulsion to date. When considering the effects of hydrocarbon oil on the triphasic nanoemulsion systems, we found the following trend for nanoemulsion droplet size: olive oil > Capmul® PG-8 NF > Capmul® PG-8 NF: olive oil (1:1). It was also evident that when Capmul® PG-8 NF was used either alone or in combination of olive oil, FLD had significant impact on droplet size, Table 2. The nanoemulsions prepared with Capmul® PG-8 NF/olive oil mixture in the organic phase were monitored for 21 days and both FLD containing formulation (nanoemulsion E) and control (nanoemulsion F) retained their small droplet size, Figure of 3C–D. The data indicates that no effect of FLD could be detected on nanoemulsion stability upon storage. This data led to conclusion that storage alone is not sufficient to fully assess FLD effects on colloidal behavior of the nanoemulsion systems. Therefore we opted to perform stress tests at elevated and low temperature on the prepared nanoemulsions.

2.5. Temperature and serum stability

Under typical pre-clinical testing conditions for either imaging or drug delivery applications, nanoemulsions experience stressors such as increased temperature (up to 37 °C), presence of salts (from cell culture media) and high protein content (serum present in culture media). The nanoemulsions are exposed to elevated temperature and presence of serum in cell culture media while droplet size (Z average) and PDI are monitored over by DLS. The nanoemulsion formulations were diluted 20 times in serum free media (SFM), 10% and 20% fetal bovine serum (FBS) containing media. As a control deionized water was used. Nanoemulsion samples were then stored at 4 °C and 37 °C and monitored for up to two weeks, Figure 4. These tests aim to model the environment that the emulsions would be subjected during biological *ex vivo* and *in vivo* testing. Under these conditions we investigated effects of FLD and hydrocarbon oil composition on triphasic nanoemulsion stability. In olive oil nanoemulsions (nanoemulsions A and B) there was no significant change in droplet size and PDI after two weeks of follow up, Figure 4A–B. The presence of FLD did not seem to have an effect on the nanoemulsion size change over time under these conditions. Further, the FLD containing nanoemulsion with combined hydrocarbon oils (nanoemulsion E) also showed no change under these conditions upon one week of measurements. These results suggested that for serum stability olive oil had most profound effect and presence of FLD did not affect stability when olive oil was present, though it did lead to decrease in droplet size. The nanoemulsions were then tested under more dramatic temperature changes and exposed to two temperature extremes 0 and 60 °C for 40 min. The nanoemulsion size (Z average) and PDI were measured before and after temperature change using DLS, Figure 5.

These formulations (nanoemulsion A and B) showed no significant change of droplet size and PDI after 40 minutes at either temperature, Figure 5A–B. FLD showed no impact on size or stability under these conditions. The combination formulations with Capmul® PG-8 NF: olive oil (1:1) as hydrocarbon phase showed only minor increase in droplet size after 40 minutes at 0 °C, Figure 5C–D. FLD seemed to not impact the change in size at low temperature (0 °C). However, the size was overall smaller in FLD containing samples at both temperatures tested by 15–20 nm. This means the size effect of FLD on Capmul® PG-8

NF containing nanoemulsions remains significant even under low temperature stress testing. When emulsions were tested at 60 °C, the change in size was more prominent reaching 30nm. Under these conditions the FLD did not suppress change in size. However, the overall effect on droplet size remained between FLD containing nanoemulsion and the control (without FLD), Figure 5C–D.

The temperature stress tests further confirmed that olive oil had most significant impact on colloidal stability while Capmul® PG-8 NF led to very small droplet size (< 100 nm). The effects of FLD on size remained present under stress, but there was no evidence FLD alone impacted the overall stability.

3. Conclusions

This is the first report of triphasic nanoemulsions prepared with PFPAEs and their aromatic conjugates. This work could be divided into two interesting and impacting research areas. First is in the advancements of imaging capable drug delivery devices where ¹⁹F Magnetic Resonance Imaging (MRI) can be used for *in vivo* tracking. Secondly, the presented nanoemulsions could lead to further advancements in nanodroplet synthetic approaches with added fluororous biphasic separation (FBS) capabilities. In both areas overall colloidal stability and ease of preparation are critical. Further, for these formulations to be practical in either application scalability is important. Microfluidization was demonstrated as the processing approach of choice with significant scalability of the formulations presented. Nanoemulsions reported with PFPAEs were prepared on at least 20 mL scale. We also aimed to explore if nanoemulsions could be prepared with PFPAEs and distinct hydrocarbon oils. If this technology is to have broader impact it was important to determine how well it can be adapted to diverse materials in the organic phase of the triphasic system. Two different oils, one natural and one synthetic were used as hydrocarbon phase and the ratio between FLD and PFPE was varied. Nanoemulsions were prepared by microfluidization and tested for droplet size, PDI and colloidal stability. The scope of the study was limited to only two hydrocarbon oils and one FLD representative construct. However, we were able to conclude that FLD can be used in triphasic nanoemulsion systems, and in this study their effect was most prominent on droplet size when synthetic oil was present in the hydrocarbon phase of the nanoemulsions. The stability tests and shelf life monitoring indicated that olive oil had most profound stabilizing effect on the PFPAE nanoemulsions, and FLD did not induce any changes as we originally expected. Based on these findings we conclude that PFPAE aromatic conjugates can be introduced to PFPE nanoemulsions without colloidal stability penalties. Aromatic rings in the fluororous phase of a nanoemulsion represent a potential site for future chemical modifications.

4. Material and methods

4.1 Materials

Pluronic® P105 was purchased from BASF Corporation (Florham Park, NJ, USA). Pluronic P123 and chremophor EL (ethoxylated castor oil) were obtained from Sigma Aldrich (St. Louis, MO, USA). Olive oil was a kind gift from Croda International Plc (Snaith, UK). Campul® PG-8 NF was from Abitec (Columbus, OH, USA). Perfluoro(polyethylene glycol

dimethyl ether), referred to as PFPE, $(\text{CF}_3\text{O}(\text{CF}_2\text{CF}_2\text{O})_n\text{CF}_3)$, where $n = 8-13$) was obtained from Exflor Research Corporation (Round Rock, TX, USA). Dulbecco's modified eagle medium (DMEM; GIBCO-BRL, Rockville, MD, USA). All reagents were used without further purification and the water that we used was deionized (DI).

4.2. Nanoemulsion formulations with FLD

The final formulation of the nanoemulsions was based on a pre-existing and optimized formulation from our lab [26]. A premixed solution of Pluronic surfactants was prepared for the final formulation purposes: 4 g P123 (4.0% w/v) and 2.66 g P105 (2.6% w/v) were dissolved in 100 mL volumetric flask filled with deionized water by slow stirring with a magnetic stir bar at room temperature. PFPE (0.8 mL) and FLD (0.2 mL) were transferred in a 50 mL test tube and mixed for 30 sec. Then, 1 mL of a premixed Capmul® PG-8 NF: olive oil (1:1) was added and mixed for an additional 30 sec. 9 mL of a premixed solution of Pluronic surfactants P123 and P105 and 9 mL of deionized water was added to a final volume of 20 mL and the mixture was mixed for an additional 30 sec. All the mixtures were performed with a vortex mixer on a maximum speed. Subsequently, the mixture was transferred to a pre-cooled microfluidizer (M110S, Microfluidics) and processed under recirculation of 30 pulses at a pressure of 80 psi. Emulsion samples to assess stability were placed at 4 °C and 37 °C. Bulk emulsions were stored at 4 °C.

4.3. DLS measurements for nanoemulsions

The size distribution data and Zeta potential for nanoemulsions were obtained using dynamic light scattering and Zeta potential, respectively, for all formulations (Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, UK). Sample preparation was done by adding 50 μL of concentrated emulsion to 950 μL deionized water. Diluted samples were then analyzed for size and zeta potential. Samples were analyzed at 25 °C with a scattering angle of 173°.

4.4. Temperature and serum stability tests

Temperature stability testing was done by placing each emulsion to three different stability chambers (25 °C, 37 °C and 60 °C) and to the refrigerator (4 °C). The emulsions were packed into glass vials. The emulsions were tested for particle size and PDI. Dynamic light scattering measurements were taken by diluting 50 μL of emulsion to 1 mL with DI water. Serum stability testing was done by diluting 250 μL of emulsion to 5 mL in serum free media (SFM), 10% fetal bovine serum (FBS), 20% FBS and water as a control. Serum stability samples were then placed in a chamber at 37 °C. Serum stability measurements were made at time zero and 24 hours.

4.5. Synthetic conditions

All manipulations were performed under an atmosphere of dry nitrogen using oven-dried glassware. Poly(hexafluoropropylene oxide) primary Iodide ($M_n = 2094$ g/mol based on MALDI-TOF-MS analysis) was received from DuPont (Experimental Station, Wilmington, DE) and used as received. All other starting materials are commercially available and were purchased from Sigma Aldrich. Benzene was distilled over Na/benzophenone and stored

over Na wire in a glove box. Solution state ^1H , ^{13}C , ^{19}F NMR spectra were recorded on a Bruker AVANCE III spectrometer running TopSpin 3.1.6 operating at 400.13 (^1H), 100.62 (^{13}C), 376.46 (^{19}F) MHz. For gas chromatography/mass spectrometry (GC/MS) analyses, an Agilent Technologies 6890N GC was coupled with an Agilent Technologies 7638B series injector and Agilent Technologies 5975B inert mass spectrometer detector (MSD) was employed with electron impact (EI) as the mode of ionization. Matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) experiments were determined with a Bruker Autoflex™ MALDI-TOF/TOF-MS spectrometer equipped with a 1 kHz smartbeam-II laser and reflector in positive ionization. For sample preparation, a 2 mg sample of 2-phenyl-poly(HFPO) was added to a 1 mL solution of 50:50 1% LiCl in MeOH and 2% perfluorocinnamic acid dissolved in 50:50 MeOH:Methoxynonafluorobutane (3M HFE-7100). A 2 μL solution was then pipette on to a ground steel plate, dried, and irradiated for a minimum of 5000 shots.

4.6. Preparation of 2-poly(HFPO)-perfluoropropyl benzene

In a typical synthesis, a dry 250 mL three necked round-bottomed flask was equipped with a magnetic stirrer and a reflux condenser adapted for a nitrogen blanket.

Poly(hexafluoropropylene oxide) primary iodide (34.519 g, 16.48 mmol, $M_n = 2094$ g/mol) was added to the reaction flask followed by 50 mL glacial acetic acid. The contents were degassed by refluxing under nitrogen for 1 hour. After cooling to 80 °C, anhydrous benzene (74.2 g, 0.95 mol) was then added followed by copper (II) acetate (0.208 g, 1.15 mmol). The reaction was heated in an oil bath at 95 °C under a flow of nitrogen and upon continued stirring, benzoyl peroxide was added every 45–60 min in 2 g portions. After a period of 4 hours (total 10.33 g, 0.0426 mol), the reaction was completed by GC/MS analysis and the poly(HFPO) primary iodide was completely converted to the desired product. The reaction was cooled to room temperature, the upper organic layer removed, and the fluorinated product was washed three times with 25 mL glacial acetic acid, followed by three times 25 mL of ice-cold diethyl ether. The product was heated under reduced pressure at 90 °C to remove any volatile materials for a period of 45 min to afford nearly colorless oil (28.327 g, 13.86 mol, 84.1% isolated yield, >99% purity).

^1H NMR (400 MHz, none) δ 7.18 (t, $^3J = 7.4$ Hz, 2H), 7.24 (t, $^3J = 5.1$ Hz, 2H), 7.38 (d, $^3J = 7.2$ Hz, 1H). ^{13}C NMR (101 MHz, none) δ 102.51 (qd, $^1J_{\text{CF}} = 270.7$, $^2J_{\text{CF}} = 36.7$ Hz, $-\text{OCF}(\text{CF}_3)\text{CF}_2-$), 106.34 (tsex, $^1J_{\text{CF}} = 267.03$, $^2J_{\text{CF}} = 36.68$ Hz, $\text{CF}_3\text{CF}_2\text{CF}_2\text{O}-$), 115.77 (td, $^1J_{\text{CF}} = 285.74$, $^2J_{\text{CF}} = 31.26$ Hz, $-\text{OCF}(\text{CF}_3)\text{CF}_2-$), 117.97 (qd, $^1J_{\text{CF}} = 285.37$, $^2J_{\text{CF}} = 31.54$ Hz, $-\text{CF}(\text{CF}_3)\text{CF}_2\text{Ph}$), 117.22 (qd, $^1J_{\text{CF}} = 290.9$, $^2J_{\text{CF}} = 28.2$ Hz $-\text{OCF}(\text{CF}_3)\text{CF}_2-$), 126.15 (s, C_{ortho} , 2C), 127.33 (s, C_{meta} , 2C), 128.81 (td, $^2J_{\text{CF}} = 24.2$, $^3J_{\text{CF}} = 3.7$ Hz C_{ipso}), 130.75 (s, C_{para} , 1C). ^{19}F NMR (376 MHz, CDCl_3), δ -81.46 (s, $-\text{CF}(\text{CF}_3)\text{CF}_2\text{O}-$), -81.79 (s, $\text{CF}_3\text{CF}_2\text{CF}_2\text{O}-$, 3F), -81.61 ($-\text{CF}(\text{CF}_3)\text{CF}_2\text{O}-$, -110.76 to -113.67 (m, $-\text{CF}(\text{CF}_3)\text{CF}_2\text{Ph}$, 2F), -131.43 (s, $\text{CF}_3\text{CF}_2\text{CF}_2\text{O}-$, 2F), -144.62 (m, $-\text{CF}(\text{CF}_3)\text{CF}_2\text{Ph}$, 1F), -145.98 (m, $-\text{CF}(\text{CF}_3)\text{CF}_2\text{O}-$, 9.69F). EIMS, 70 eV, m/z (rel. int.): = 69 (17) (CF_3^+), 77 (3) (C_6H_5^+), 119 (5) (CF_3CF_2^+) 127 (100) ($\text{C}_6\text{H}_5\text{CF}_2^+$), 169 (30) ($\text{CF}_3\text{CF}_2\text{CF}_2^+$), 227 (23) ($\text{C}_6\text{H}_5\text{CF}_2\text{CF}(\text{CF}_3)^+$), 393 (3) ($\text{C}_6\text{H}_5\text{CF}_2\text{CF}(\text{CF}_3)\text{OCF}(\text{CF}_3)\text{CF}_2^+$). MALDI-TOF-MS $[412 + 166n + \text{Li}]^+ = 1415.40$ ($n = 6$), 1581.557 ($n = 7$), 1747.62 ($n = 8$), 1913.68 ($n = 9$), 2079.738 ($n = 10$), 2244.80 ($n = 11$), 2410.86 ($n = 12$), 2577.923 ($n = 13$).

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Highlights

- The smallest PFPAE nanoemulsions ever reported with droplet size of 85nm
- The first report of PFPAE aromatic conjugates formulated with PFPEs as nanoemulsions
- Triphasic (organic/fluorous/water) nanoemulsions prepared with two distinct oils in the organic phase (olive oil and synthetic lipid oil)
- Nanoemulsions showed remarkable stability upon storage at elevated temperatures and when exposed to stress

Figure 1. Proposed model for triphasic (organic/fluorous/aqueous) nanoemulsion with combined PFPAEs in fluorous phase.

Figure 2.
(A) Size for the olive oil in water nanoemulsions at different ratios of FLD:PFPE. (B) PDI for olive oil in water nanoemulsions at different ratios of FLD:PFPE.

Figure 3.

Long term monitoring of size and PDI by DLS. A) Nanoemulsion A prepared with olive oil and without FLD; B) Nanoemulsion B prepared with olive oil and with FLD. C) Nanoemulsion E prepared with Capmul® PG-8 NF/olive oil mixture and without FLD; D) Nanoemulsion E prepared with Capmul® PG-8 NF/olive oil mixture and with FLD. Measurements were done by DLS repeatedly up to 28 days for emulsions A and B, and up to 21 days for emulsions E and F. Data represents Z average and error bars represent half width of polydispersity width (PDIw/2).

Figure 4.

Serum and temperature stability of olive oil nanoemulsions with/without FLD.

A) Nanoemulsion A with FLD; B) Nanoemulsion B without FLD, monitored for two weeks at 37°C diluted in water, serum free media (SFM), 10% FBS and 20% FBS containing media. Measurements were done by DLS. Data represents Z average and error bars represent half width of polydispersity width (PDIw/2).

Figure 5. Nanoemulsions temperature stress tests. Formulations with olive oil and with/without FLD (nanoemulsions A and B) incubated for 40 minutes at 0 °C (panel A) and 60 °C (panel B); Formulations with Capmul® PG-8 NF: olive oil mixtue (nanoemulsions E and F) incubated for 40 minutes at 0 °C (panel C) and 60 °C (panel D). Measurements were done by DLS. Data represents Z average and error bars represent half width of polydispersity width (PDI_w/2).

Scheme 1.

Radical coupling of poly(HFPO) primary iodide with benzene.

Table 1

Nanoemulsion formulations

Components ^a	A	B	C	D	E	F
PFPAE	0.80	1.00	0.80	1.00	0.80	1.00
FLD	0.20	–	0.20	–	0.20	–
Olive Oil	1.00	1.00	–	–	0.50	0.50
Capmul® PG-8 NF	–	–	1.00	1.00	0.50	0.50
P123/P105 (4.0/2.6% w/v)	9.00	9.00	9.00	9.00	9.00	9.00
DI water	9.00	9.00	9.00	9.00	9.00	9.00

^aValues represent mL of liquid: either neat hydrocarbon oils and fluorocarbon oils or water solutions of surfactants.

Table 2

Droplet size (Z. average) and PDI of created nanoemulsion formulations measured by DLS

Oil Phase	Z. Average (nm)		PDI	
	FLD	No FLD	FLD	No FLD
Olive Oil	144.0	145.1	0.163	0.170
Capmul® PG-8 NF	92.8	102.6	0.199	0.187
olive oil: Capmul® PG-8 NF (1:1)	84.9	93.8	0.141	0.188