International Journal of Pharmaceutics 369 (2009) 176–184

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Reverse aqueous emulsions and microemulsions in HFA227 propellant stabilized by non-ionic ethoxylated amphiphiles

Udayan Chokshi[c], Parthiban Selvam[c], Lionel Porcar[a,b], Sandro R.P. da Rocha[c,*]

[a] Materials Science and Engineering Department, University of Maryland, College Park, MD 20742, United States
[b] Centre for Neutron Research, National Institute for Standards and Technology, 100 Bureau Drive, Bldg. 235, Stop 8562, Gaithersburg, MD 20899, United States
[c] Department of Chemical Engineering and Materials Science, Wayne State University, 5050 Anthony Wayne Dr., Detroit, MI 48202, United States

ARTICLE INFO

Article history:
Received 10 July 2008
Received in revised form 23 October 2008
Accepted 28 October 2008
Available online 5 November 2008

Keywords:
Inhalation
pMDI
HFAs
Novel Formulations
Microemulsions
Emulsions
SANS

ABSTRACT

In this work we use *in situ* high-pressure tensiometry to screen non-ionic ethoxylated surfactants at the 1,1,1,2,3,3,3-heptafluoropropane (HFA227) propellant|Water (HFA227|W) interface. The $EO_nPO_{\sim 30}EO_n$ series, where *EO* stands for ethylene oxide and *PO* for propylene oxide, and *n* the number of repeat *EO* units, was selected for this study based on the favorable interactions reported between HFA propellants and the *PO* moiety. The surfactants used in FDA-approved pressurized metered-dose inhaler formulations were also investigated. Tension measurements provide not only information on the relative activity of the different surfactants in the series, but they also serve as a guide for selecting an appropriate candidate for the formation of reverse aggregates based on the surfactant natural curvature. Moreover, the effect of ethanol and the chemistry of the surfactant tail group on the surfactant activity were also investigated. Surfactants with hydrogenated tails are not capable of forming stable water-in-HFA227 microemulsions. This is true even at very low tensions observed when in the presence of ethanol, indicating the lack of affinity between HFA227 and hydrogenated moieties—the surfactant does not tend to curve about water. On the other hand, *PO*-based amphiphiles can significantly reduce the tension of the HFA227|W interface. Small angle neutron scattering (SANS) and UV–vis spectroscopy results also reveal that a selected ethoxylated amphiphile ($EO_{13}PO_{30}EO_{13}$ at 1 mM concentration), when in the presence of ethanol, is capable of forming stable cylindrical reverse aqueous microemulsions. $EO_{13}PO_{30}EO_{13}$ is also capable of forming emulsions of water-in-HFA227 that are fairly stable against coalescence. Such dispersions are potential candidates for the delivery of small polar solutes and larger therapeutic biomolecules to and through the lungs in the form of pMDI formulations, and in other medical sprays.

1. Introduction

The inhalation route has been traditionally used for delivering drugs that exert their therapeutic effect locally; i.e., in the lungs (Courrier et al., 2002). However, the large alveolar surface area, reduced thickness of the epithelial barrier, extensive vascularization, and relatively low proteolytic activity (Patton and Byron, 2007) also make the lungs an outstanding route for the delivery of therapeutics to the systemic circulation (Laube Beth, 2005; Owens et al., 2003). Within this context, there is a need for the development of novel inhalation formulations that can be used for the systemic delivery of both small molecular weight drugs and larger therapeutic biomolecules.

The most economical vehicles for the oral delivery of drugs to the respiratory tract are the pressurized metered-dose inhalers (pMDIs) (Bowman and Greenleaf, 1999; McDonald and Martin, 2000; Tarara et al., 2004). pMDIs use a propellant to expel the pharmaceutical product as an aerosol (Meakin et al., 2003). The propellant also works as the solvent medium where the drugs are either dispersed or solubilized. For environmental reasons, the hydrofluoralkanes (HFAs) (Chinet, 2000; Tansey, 1997) more specifically 1,1,1,2-tetrafluoroethane (HFA134a) and 1,1,1,2,3,3,3-heptafluoropropane (HFA227), have replaced the chlorofluorocarbon (CFC) propellants in pMDI formulations (McDonald and Martin, 2000). HFAs have been selected partly because of their exceptional inertness (McDonald and Martin, 2000). HFAs are also biocompatible, and non-ozone depleting (Alexander and Libretto, 1995; Emmen et al., 2000; Graepel and Alexander, 1991). However, due to differences in physicochemical characteristics compared to those of CFCs (Butz et al., 2002), most notably their higher polarity, the reformulation of pMDIs with HFAs has been a challenging

* Corresponding author. Tel.: +1 313 577 4669; fax: +1 313 578 5820.
E-mail address: sdr@eng.wayne.edu (S.R.P. da Rocha).

0378-5173/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2008.10.025

task (Wu et al., 2007c). A concerted effort is currently under way to understand the solvation properties of HFAs, and the characteristics of colloidal domains in such low dielectric propellant media that should aid not only in the reformulation of existing pMDIs but also in the design of novel HFA-based formulations (Peguin and da Rocha, 2008; Peguin et al., 2007; Selvam et al., 2008; Wu et al., 2007b, 2007c).

pMDIs can be formulated either as solution (drug is soluble in the propellant), or suspension (drug particles dispersed in the propellant) formulations. In general, polar therapeutics have low solubility in HFAs (Patton et al., 2004; Rau, 2005) and thus have to be formulated as dispersions, or as solutions with the aid of a co-solvent (Rogueda, 2005; Tarara et al., 2004). Suspension formulations, which correspond to approximately 50% of the commercially available pMDIs (Rogueda, 2005; Stefely, 2002), typically contain a surfactant to aid in the dispersion of the drug particles (Stefely, 2002; Stefely et al., 2000; Wu and da Rocha, 2007), and a co-solvent used to enhance the solubility of the amphiphile (Steckel and Muller, 1998; Vervaet and Byron, 1999). Several studies have been published in recent years addressing the design of surfactants with enhanced solubility in HFAs (Selvam et al., 2008; Stefely, 2002; Wu et al., 2007b, 2008; Wu and da Rocha, 2007), thus potentially avoiding the use of co-solvents in pMDI formulations (Rogueda, 2005; Wu et al., 2008). New particle (solid-based) engineering technologies that do not rely on the use of surfactants in solution have also been proposed (Dellamary et al., 2000; Edwards et al., 1997; Wu et al., 2007a, 2007c), some of which lend themselves to the formulation of therapeutic biomolecules (Rogueda, 2005; Wu et al., 2007a, 2007c).

Aqueous-based dispersions in the form of reverse emulsions (Butz et al., 2002; Krafft, 2001) and microemulsions (Meakin et al., 2003; Patel et al., 2003a, 2003b; Selvam et al., 2006, 2008; Sommerville and Hickey, 1998; Sommerville et al., 2000, 2002) have been previously suggested as an alternative way to formulate therapeutic biomolecules or small polar drugs that are not soluble in HFA propellants. Water-in-perfluorooctyl bromide (W/PFOB) emulsions stabilized with a fluorinated surfactant have been shown to be easily dispersible in both HFA134a and HFA227 (Butz et al., 2002). The external phase (in that particular study) is a combination of the propellant HFA and PFOB, which (the latter) may be acting as a co-solvent to the fluorinated surfactant. The study with the W/PFOB in HFAs is also relevant in that it demonstrates that various drugs, including antibiotics, vasodilators and anticancer agents can be incorporated in the aqueous phase of the W/PFOB emulsion, and thus potentially dispersed in propellant HFA (Sadtler et al., 1999). Thermodynamically stable water-in-HFA (W/HFA) microemulsions are another potential pseudo-solution formulation (Meakin et al., 2003; Patel et al., 2003a, 2003b; Selvam et al., 2008; Steytler et al., 2003), where water-soluble therapeutics could be formulated. We have recently shown that ethoxylated surfactants are capable of forming stable aqueous reverse aggregates in HFA134a (Selvam et al., 2008). Small angle neutron scattering spectra (SANS) indicated the formation of aggregates with a cylindrical geometry, and containing a water core radius of 12–14 and 50–95 Å in length. The uptake of a model biomolecule within the core of the aggregates was demonstrated, illustrating the potential applicability of such formulations (Selvam et al., 2008).

The objective of this work was to investigate the behavior of ethoxylated surfactants at the HFA227–water (HFA227|W) interface, and their ability to form reverse aqueous aggregates in HFA227 in the form of emulsions and microemulsions. We employed a combination of experimental techniques including *in situ* high-pressure tensiometry, molecular probe UV–vis spectroscopy small angle neutron scattering (SANS), and visual inspection of the contents of the pressure cells to probe the activity, structure and stability of the reverse aggregates. The relevance of this work stems from the fact that such dispersions in HFA propellants are potential formulations for the delivery of small and large polar therapeutics to and through the lungs. The results shown here are also of relevance to traditional solution and dispersion pMDI formulations where surfactants are generally required excipients (Blondino, 1995). Previous studies indicate significant differences in the behavior of colloidal dispersions in HFA134a and HFA227. For example, water in fluorocarbon emulsions was more stable in HFA227 than in HFA134a (Butz et al., 2002). Generally, non-ionic surfactants show higher solubility in HFA227 than compared to HFA134a due to stronger interactions between the surfactant and HFA227 (Peguin and da Rocha, 2008; Ridder et al., 2005), and this may also affect the stability of both solid and aqueous dispersions in the different propellant HFAs. This study will also help understand differences in how ethoxylated surfactants behave at the interface between water and these propellant HFAs (HFA134a and HFA227).

2. Materials and methods

2.1. Materials

2H,3H-perfluoropentane (HPFP, assay 98% min) was purchased from SynQuest labs Inc. HFA227 (assay 99.9%) was a gift from Solvay Fluor und Derivative GmbH & Co. KG. Acetone and ethanol (analytical grade) were purchased from Fischer Scientific. Pluronic L® surfactants with a general structure (ethylene oxide)–(propylene oxide)–(ethylene oxide) ($EO_nPO_mEO_n$, where m and n are the average number of repeat units) were a gift from BASF. Sorbitan trioleate (Span 85, >99%) was purchased from TCI America Inc. Poly(ethylene glycol) (300 g mol^{-1}) (PEG300) and poly(propylene glycol) (2000 g mol^{-1}) (PPG2000) were purchased from Acros Organics. Lecithin (refined, 100%) was purchased from Alfa Aesar. Oleic acid (>99%) was purchased from Sigma–Aldrich. All of the surfactants were used as received. Ethanol (100%) was purchased from AAPER Alcohol and Chemical Co., and methyl orange [$(CH_3)_2NC_6H_4N{=}NC_6H_4SO_3{\cdot}Na^+$, dye content, 95%] was purchased from Sigma–Aldrich. Deionised water (Nanopure: Barnstead) with surface tension of 73 mN m^{-1} at 295 K was used in all experiments. Pressure proof glass vials (68000318) were a gift from West Pharmaceutical Services. The metering valves (EPDM Spraymiser^{TM}, 50 μl) were a gift from 3 M Inc.

2.2. High-pressure tensiometry

A variable volume pendant drop tensiometer was used to measure the interfacial tension of the HFA227|water (HFA227|W) interface with or without the presence of interfacially active species. The instrument, which allows us to measure the tension at high pressures, was described in detail in a previous publication (Peguin et al., 2006). A very small droplet of water (~3–5 μl) was initially formed into HFA227 (3.3 ml), and allowed to equilibrate in the presence of surfactant. On average, three such droplets were formed for each measurement, in a way that the depletion on surfactant concentration from bulk HFA227 was minimized. Once the system is equilibrated, a droplet of water (or HFA227) was injected into a high-pressure cell as a pendant drop (hanging drop), in an HFA277 (aqueous) surfactant solution. Visual ports in the pressure cell allowed for the extraction of the droplet profile at 298 K and saturation pressure of the system. The reported values are averages of at least three independent measurements. The whole droplet profile was used to determine the γ with the Laplace equation (KSV, 2001).

2.3. Emulsion formation and stability

Surfactant and water were initially loaded in transparent aerosol pressure proof glass vials (68000318, West Pharmaceutical Services), and crimped with 50 μl metering valves (EPDM Spraymiser™, 3 M Inc.) using an aerosol can crimper (Model 3000-C, Aero-tech, L.L.C.). Subsequently, the desired amount of liquid HFA227 was injected into the vessel using a manual syringe pump (HiP 50-6-15®) and a home-built, high-pressure aerosol filler. The mechanical energy for forming the emulsions was provided by sonicating the mixtures (VWR Model p250D) at a power rating of 180 W, for 2 h. Emulsion curvature and stability were determined/inferred visually, based on the physical stability results (phase separation due to creaming/sedimentation and coalescence) observed as a function of the age of the emulsions-time after ceasing the input of mechanical energy.

2.4. UV-vis spectroscopy

In situ (under pressure) UV–vis spectra of HFA227 containing one or more of the following: surfactant, water, a solvatochromic probe (methyl orange), and ethanol, were obtained in a set-up described in detail elsewhere (Selvam et al., 2008). Briefly, pure HFA227 and ethanol were added to an 'HFA Saturation Cell' containing pure water (enough to be in excess) with the help of a manual pressure generator (HiP 50-6-15®). The water-saturated HFA227–ethanol solution was subsequently transferred to a 15 ml high-pressure glass vial (Chemglass) containing a known amount of surfactant, and a known volume of aqueous solution saturated with methyl orange. Care was taken to avoid the transfer of bulk excess water from the saturation cell by inserting the capillary into the saturated HFA–ethanol mixture phase. The contents of the high-pressure glass vials were equilibrated at 298 K and at the saturation pressure of the propellant mixture using a magnetic stir bar. After equilibration, the contents of the high-pressure glass vial were transferred to a home made high-pressure cell fitted with two sapphire windows (1 cm path length), the "Spectroscopic Cell". Spectra were obtained with an UV–vis spectrophotometer (Varian Cary 3E®). The baseline for this system was obtained from the spectrum of a water saturated HFA227–ethanol solution equilibrated with methyl orange. Because the water uptake was measured in a solution of HFA227 (+ethanol) pre-saturated with water, the corrected water-to-surfactant molar ratio (W_o) was directly assessed; i.e., it was not needed to rely on calculated solubility of water to estimate W_o. The pre-saturation of the system (HFA227 + ethanol) with water is expected to significantly improve the accuracy of the reported W_o.

2.5. Small angle neutron scattering (SANS)

Scattering experiments were performed on the NG7 30-m SANS instrument at the NIST (Center for Neutron Research in Gaithersburg, MD). Neutrons of wavelength $\lambda = 6$ Å with a distribution of $\Delta\lambda/\lambda = 11\%$ were incident on samples held in a custom-built high-pressure SANS cell. Sample to detector distance between 3 and 15 m were used to give a q range of $0.0035\ \text{Å}^{-1} < q < 0.45\ \text{Å}^{-1}$, where $q = (4\pi/\lambda)\sin(\theta/2)$ is the magnitude of the scattering vector. Sample scattering intensity was corrected for background, empty cell scattering and detector sensitivity. The scattering length densities (SLD) were obtained from the scattering length density calculator available at NIST. The SLDs for water, HFA227, $EO_{13}PO_{30}EO_{13}$ and HFA227 + 20% (w/w) ethyl alcohol are -5.6×10^{-7}, 2.77×10^{-6}, 4.53×10^{-7}, 2.08×10^{-6} Å^{-2}, respectively. Corrected data sets were circularly averaged, and placed on an absolute scale using standard samples. The raw scattering data were reduced and analyzed using the Igor Pro SANS software (Kline, 2006). Before the SANS experiments, the formulations were prepared in a similar fashion as described above (UV–vis spectroscopy), using the same high-pressure cells.

3. Results and discussions

3.1. Interfacial tension of the bare HFA227|Water interface

The determination of the tension of the bare interface is of great relevance since it is expected to dictate the behavior of the adsorbed amphiphiles (da Rocha and Johnston, 2000). The tension of the bare HFA227|W interface ($\gamma_{HFA227|W}$) at 298 K and saturation pressure is shown in Fig. 1 as a function of drop age.

It can be observed that long times are necessary in order to reach the equilibrium tension value. This has also been observed for the HFA134a|W (Selvam et al., 2006) and HPFP|W interface (Rogueda, 2003). Long equilibration times for the HFA134a|W interface were attributed to impurities in HFA134a, and disappear after purification with alumina (Selvam et al., 2006). Slow equilibration in the case of HFA227|W interface is also attributed to impurities in the commercial sample of the propellant. The equilibrium tension was found to be 32.8_7 mN m^{-1}. This value was obtained by averaging the tension at long times, when the tension is seen to fluctuate around an equilibrium value—inset in Fig. 1. The deviation, obtained from three such measurements, is ± 0.4 mN m^{-1}.

The tension value determined in this work for HFA227|W (first time it has been reported for that interface) is slightly higher than that for the pharma grade (without further purification) HFA134a|W interface of 31.8 ± 0.3 mN m^{-1} (Selvam et al., 2006). A larger tension value for the HFA227|W interface is expected based on the more polar nature of HFA134a—see Table 1. The tension for the purified HFA134a|W is reported to be 33.5 ± 0.5 mN m^{-1} (Selvam et al., 2006). The more favorable interaction between HFA134a and water is also reflected in the higher mutual solubility between HFA134a and water, compared with that between water and HFA227. The higher interfacial tension of the model propellant HPFP|W (33.7 mN m^{-1}), is also justified in the same way. Even though the dipole moment of HPFP is higher than HFA227, its more hydrophobic character (larger number of CF2 groups) is evidenced in the lower solubility in water. Based on the discussion above, we expect that the γ of the purified HFA227|W interface to be in the range between 34 and 35 mN m^{-1}.

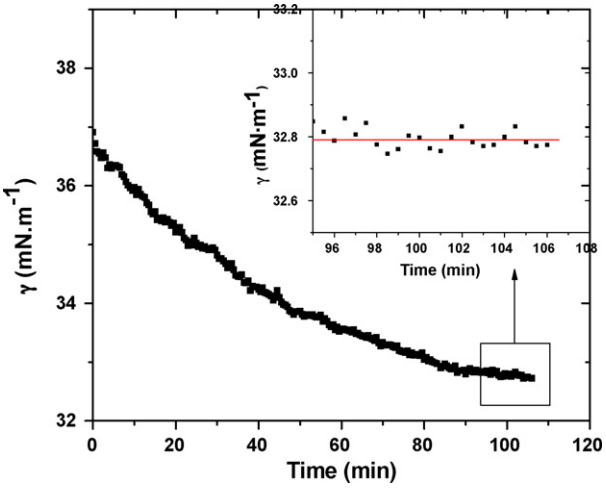

Fig. 1. Tension (γ) of the pharma grade HFA227–water interface as a function of droplet age at 298 K and saturation pressure of the propellant mixture. Inset: magnification of the tension at long times.

Table 1
Selected properties of HFA134a, HFA227 and HPFP.

Property	HFA227[ix]	HFA134a[ix]	HPFP[x]
Molecular weight (g mol^{-1})	170.03	102.03	252.05
Density (g cm^{-3})*	1.408[iii]	1.226[iv]	1.583[v]
Boiling point (°C)	−16.5	−26.3	53.6
Dielectric constant (ε)	4.07[iii]	9.46[iv]	15.05[v]
Dipole moment (D)	0.93	2.06	1.90
Surface tension (σ, mN m^{-1})[i]	6.96[iii]	8.69[iv]	13.59[v]
Interfacial tension (γ, mN m^{-1})[ii]	[vi]32.7 ± 0.5[iii]	[vii]31.8[iv]	33.7[v]
Water solubility (ppm)	610	2200	390 ± 40
Solubility in water (ppm)	58	193	140[xii]
PEG 1000 solubility (wt. %)[xi]	~2.0	~2.0	~0.7
PPG 2000 solubility (wt. %)[xi]	>50	~2.0	~0.7

[i] Density and surface tension at 293 K; [ii] interfacial tension at 298 K; [iii] saturation pressure 0.45 MPa; [iv] saturation pressure 0.665 MPa; [v] atmospheric pressure; [vi] This work; [vii] Selvam et al. (2006); [ix] Solvay (2005); [x] Rogueda (2003); [xi] Ridder et al. (2005); [xii] DuBoisson (2005).

3.2. Interfacial tension of the surfactant-modified interface

3.2.1. Effect of surfactants that can be found in commercial pMDI formulations

Surfactants that are excipients in commercial (FDA-approved) pMDIs have been screened in the past with regards to their ability to form and stabilize reverse aqueous aggregates in both HFA134a and HFA227 (Blondino and Byron, 1998). Within the conditions investigated, they were shown not capable of forming aqueous microemulsions in the semi-fluorinated propellants. It is worth noticing, however, that the screening process was based solely on phase behavior studies—both in the presence of water and with regards to the solubility of the amphiphiles in HFAs (Blondino and Byron, 1998). Their activity at the HFA|W interface, therefore, is not yet known.

In this work, three surfactants commonly used in FDA approved pMDI formulations (Wang and Kowal, 1980) (lecithin, oleic acid and sorbitan trioleate) were investigated for their interfacial activity at the HFA227|W interface at 298 K, saturation pressure of the propellant mixture, and 1 mM surfactant concentration-solubilized in water as they have limited solubility in HFA. The results are summarized in Table 2.

As can be seen from Table 2, a maximum reduction in tension (down to 11.5 mN m^{-1}) was achieved by oleic acid. Span 85 and lecithin were not as interfacially active. The final tension values are still much larger than what is typically required for the formation of microemulsions in both conventional and compressible systems (Aveyard et al., 1985; da Rocha et al., 1999; da Rocha and Johnston, 2000; Dickson et al., 2005; Sagisaka et al., 2004; Selvam et al., 2006, 2008). These results suggest that the tension reductions accomplished with surfactants in FDA-approved pMDI formulations alone are likely not enough to overcome the unfavorable contribution to the free energy of microemulsion formation due to the large increase in surface area that follows upon the formation of the nanometer size aggregates and the bulk water is dispersed. The relatively low interfacial activity can be attributed to the low interaction between HFA and the hydrogenated surfactant tails, as is reflected in their low solubility in HFAs (Blondino, 1995; Blondino and Byron, 1998; Byron and Patton, 1994; Courrier et al., 2002; Modi, 2000; Vervaet and Byron, 1999; Williams and Liu, 1998).

Table 2
Surfactant structure, corresponding wt% EO in the molecule, interfacial tension (γ) and surface pressure (Π) obtained at 298 K and saturation pressure of the system.

Surfactant	MW (g mol^{-1})	Structure[1]	% EO	$\gamma_{HFA227\|W\|S}$ (Π)[1]
Oleic acid	282	$C_{17}H_{33}COOH$	(1)[2]	11.5 (21.2)
Span 85	956	$C_{60}H_{108}O_8$	(1.8)[2]	13.8 (18.9)
Lecithin	731	$C_{40}H_{77}O_8NP$	(7)[2]	12.5 (20.2)
PPG 2000	2000	PO_{35}	0	12.7 (20.0)
Pluronic L61	2000	$EO_{2.5}PO_{31}EO_{2.5}$	10	9.30 (23.4)
Pluronic L62	2500	$EO_6PO_{34}EO_6$	20	8.30 (24.4)
Pluronic L64	2900	$EO_{13}PO_{30}EO_{13}$	40	7.80 (24.9)
Pluronic P65	3500	$EO_{20}PO_{30}EO_{20}$	50	6.90 (25.8)
Pluronic F68	8400	$EO_{76}PO_{30}EO_{76}$	80	11.9 (20.8)
PEG 300	300	EO_7	100	18.0 (14.7)
Oleic acid + 10% EtOH	–	–	–	1.40 (31.3)
Pluronic L64 + 10% EtOH	–	–	–	0.9 (31.8)

[1] $\Pi = \gamma_0 - \gamma$, where γ_0 is the tension of the bare HFA227|W interface. The surfactant concentration is 1 mM. [2] - HLB instead of % *EO*.

3.2.2. Activity of $EO_nPO_{\sim 30}EO_n$ surfactants and HFB

The activity of a series of $EO_nPO_{\sim 30}EO_n$ surfactants at the HFA227|W interface was also investigated, and the results summarized in Table 2. The results were obtained at 298 K and saturation pressure of the propellant mixture, and at 1 mM surfactant concentration. They are shown in Fig. 2. The results for the homopolymers PEG 300 (EO_7) and PPG 2000 (PO_{35}) are also included in the figure (100 and 0% EO, respectively), as are the results for the HFA134a|W (Selvam et al., 2006, 2008) and HPFP|W (Selvam et al., 2006) interfaces. $EO_{2.5}PO_{31}EO_{2.5}$ and PO_{35} have low solubility in water. These were originally solubilized in HFA227, and hanging drops of pure water were injected in the HFA + surfactant system. All other surfactants were solubilized in water, and tension measurements were performed with a hanging water droplet.

The results allow us not only to understand the balance of the $EO_nPO_{\sim 30}EO_n$ series at the HFA227|W interface, but also to understand subtle differences in the interaction between the propellants with the surfactant molecule, and the *PO* tail group. To that end, a new variable is defined. The surface pressure (Π), which is the difference between the surface tension of the bare interface ($\gamma_{HFA|W}$) to that of the surfactant-modified interface ($\gamma_{HFA|W|S}$); i.e., $\Pi = \gamma_{HFA|W} - \gamma_{HFA|W|S}$. The concept of Π allows us to concentrate on the surfactant activity alone, since it excludes any effect due to differences in the binary (HFA|W) tension. A large Π arises from a small $\gamma_{HPFP|W|S}$, thus indicating high interfacial activity.

As expected, HFAs interact more strongly with the *PO* than with methyl-based moiety. This is indicated by the overall large Π val-

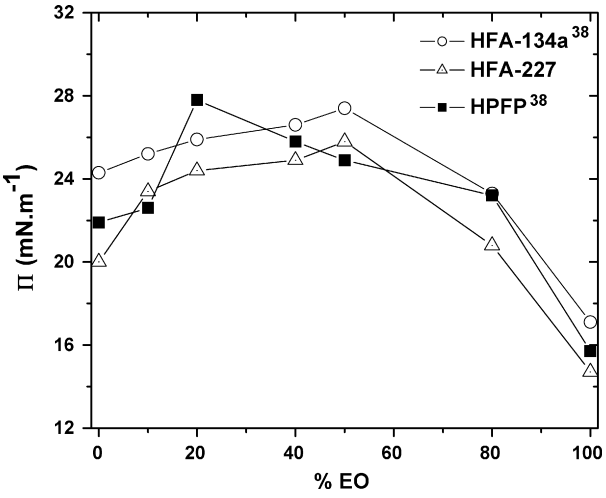

Fig. 2. Surface pressure (Π) of the surfactant-modified HFA134a|W, HFA227|W and HPFP|W interface (Selvam et al., 2006, 2008) as a function of the hydrophilic–HFA philic balance (HFB) for the $EO_nPO_{\sim 30}EO_n$ surfactant class. In this case, HFB is indicated as % EO. Conditions are 1 mM surfactant concentration, 298 K and saturation (HFA134a and HFA227) or ambient (HPFP) pressure. The lines serve as a guide to the eye. 0 and 100 % EO correspond to the PO and EO homopolymer, respectively.

ues. *PO* has significantly higher solubility in HFAs (Blondino and Byron, 1998; Ridder et al., 2005) and the presence of ether oxygen provides for a site that can interact with the dipole of the propellants and the model solvent (Peguin et al., 2007; Selvam et al., 2006, 2008; Wu et al., 2007b, 2007c). The results also show that the curves for HFA134a and HFA227 are qualitatively the same, except for the fact that the results for HFA134a are shifted up, indicating that the surfactants are more active at the HFA134a|W interface. This results is somewhat surprising based on the fact that the enthalpic interactions between HFA227 and the PO fragment is stronger that that between HFA134a and *PO*, as indicated in recent *ab intio* calculations (Peguin and da Rocha, 2008). It is worth noticing, however, that the same studies also indicate that both HFAs can also interact with the ether oxygen of *EO* (the surfactant head-group in this case), and that makes the interfacial activity analysis for the copolymers in questions confounded. The HPFP|W results do not follow either HFA134a or HFA227.

The shape of the curves seen here (an inverted 'V', or a 'V' in a tension plot) is typically observed for conventional interfaces (Aveyard et al., 1990; Aveyard et al., 1989) and has also been reported for interfaces with compressible solvents (da Rocha et al., 1999; Selvam et al., 2006, 2008). A maximum in Π is observed when the system is balanced with respect to the partitioning of the surfactant to the interface. The maximum in Π for HFA227|W and HFA134a|W happens at the same % *EO* (~50%), thus suggesting that both solvent-tail (HFA-*PO*) and water-head (W-*EO*) interact strongly with each other. However, on should note that HFAs are also known to interact favorably not only with the *PO* tail, but also with the *EO* moiety (Peguin et al., 2007; Wu et al., 2007b) the head-group of the surfactant in question.

The Π results for the mimicking propellant HPFP are somewhat intermediate between those at the HFA134a|W and HFA227|W interfaces. Interfacial activity thus correlates much better with the polarity of the semi-fluorinated fluids than with solubility arguments. While the dipole of HFA134a is larger than HFA227, that of HPFP is intermediate, as shown in Table 2. It is also worth noticing that minimum tension values for both propellant and liquid HFAs are similar in magnitude as those values observed at the critical aggregation concentration in other compressible solvents (da Rocha et al., 1999; da Rocha and Johnston, 2000; Dickson et al., 2005; Sagisaka et al., 2004; Selvam et al., 2006, 2008).

3.2.3. Effect of co-solvent

The effect of ethanol on the interfacial tension of the HFA227|W interface was also investigated. The tension of the HFA227|W interface in the presence of 1 mM oleic acid is reduced further by 10 mN m^{-1} upon the addition of 10% (w/w) ethanol, bringing the overall tension down to 1.4 mN m^{-1}—see Table 2. This value is similar to those where reverse aggregates have been observed in HFA134a (Selvam et al., 2008). Such low tensions should also provide for lower energy requirements during emulsification. The tension for 1 mM $EO_{13}PO_{30}EO_{13}$ in the presence of the same amount of ethanol is also significantly reduced to 0.9 mN m^{-1}. We focus our attention on $EO_{13}PO_{30}EO_{13}$ as it is one of the most interfacially active species at the HFA227|W interface. Moreover, it falls on the LHS of the inverted 'V'-shaped figure (Fig. 2) in the surface-pressure vs. % EO, indicating that if it can form aggregates, the natural curvature would be expected to be water-in-HFA227 (da Rocha et al., 1999; Selvam et al., 2008). It is worth noticing that similar tension reductions achieved with $EO_{13}PO_{30}EO_{13}$ in the presence of ethanol can be accomplished with surfactant alone ($EO_3PO_{40}EO_3$) at the HFA134a|W interface. For a similar system, containing 10–13% (w/w) $EO_{13}PO_{30}EO_{13}$ and 20–26% (w/w) ethanol, a recent patent describes the formation of a stable "cloudy" aqueous dispersion in HFA227 (Meakin et al., 2003). It is also known that the solubility of water in HFA227 increases from 610 to 11,000 ppm in the presence of 10% ethanol (Vervaet and Byron, 1999).

In order to shine some light into the effect of ethanol, the tension of the model propellant and water ($\gamma_{HPFP|W}$) with and without the modifier (ethanol) was evaluated. The tension of the binary HPFP|W interface decreased by ~ 9 mN m^{-1} when in the presence of 10% (w/w) ethanol. However, in the presence of 1 mM oleic acid or $EO_{13}PO_{30}EO_{13}$, the $\gamma_{HPFP|W}$ was reduced by 30.2 or 32.3 mN m^{-1}, respectively. The significant decrease in tension in the absence of the amphiphiles suggests that ethanol has a significant co-solvent effect; i.e., the lowering of the tension can be attributed to the fact that the propellant–ethanol combination is more polar, and thus more compatible (lower tension) with water. EtOH can also work as a co-solvent by aiding in the solvation of the surfactant tail groups, and thus affecting the surfactant natural curvature (da Rocha et al., 1999; da Rocha and Johnston, 2000). The co-surfactant effect of ethanol; i.e., how it can aid in the packing of the surfactant at the interface, is less clear cut (da Rocha and Johnston, 2000).

3.3. Microemulsion formation: UV-vis spectroscopy and SANS

The reduction in the tension of the HFA227|W interface in the presence of ethanol is very promising, and suggests the potential ability of those systems to form W/HFA227 microemulsions. Low interfacial tension alone, however, cannot guarantee the formation of the aggregates, as we observed in the case of HFA134a. 1 mM $EO_3PO_{43}EO_3$ was shown to reduce the tension of the HFA134a|W interface to 1.95 mN m^{-1}, but UV–vis and SANS studies confirmed that no microemulsions were formed with the surfactant alone (Selvam et al., 2008). Only in the presence of EtOH, reverse aggregates were seen in HFA134a (Selvam et al., 2008). Photon correlation spectroscopy studies (Meakin et al., 2003) also indicate that at 20% (w/w) *EtOH*, 10% (w/w) $EO_{13}PO_{30}EO_{13}$ and 5% (w/w) water, water-in-HFA227 reverse aggregates can be formed. However, the nature of the aggregates (emulsions or microemulsions) is not obvious, as cloudy dispersions were reported—microemulsions should form pseudo-solutions that are transparent due to their small (several nanometers) size; while emulsions, which are micron-submicron, form cloudy/white dispersions.

Using methyl orange as solvatochromic probe, we indirectly investigated the nature of the aqueous phase environment of the most promising candidates (from tension studies) using UV–vis spectroscopy. UV–vis spectroscopy has been previously used to probe the local environment of reverse aggregates in HFA134a (Li et al., 2000; Selvam et al., 2008) as well as compressed CO_2 (Johnston et al., 1996; Liu et al., 2004). In this study we focus again on the $EO_{13}PO_{30}EO_{13}$ copolymer, for the same reasons discussed above. In the absence of ethanol, 3 mM of $EO_{13}PO_{30}EO_{13}$ in water saturated HFA resulted in a white unstable (emulsion) dispersed phase. The same holds true for 1 mM oleic acid, and with 3 mM oleic acid in HFA227 in the presence of 10% (w/w) of ethanol. A cloudy solution was formed when 5% (w/w) of water was added to HFA227–ethanol mixture (90:10%, w/w) in the presence of 10% (w/w) of $EO_{13}PO_{30}EO_{13}$. However, when the ethanol concentration was raised to 20% (w/w), a clear single phase was formed. UV–vis results for 10% (w/w) of $EO_{13}PO_{30}EO_{13}$ with 20% (w/w) of EtOH in HFA227 are shown in Fig. 3a (inset).

When contrasting the λ_{max} of 417 nm observed in this system with that for MO in pure water (464 nm) (Johnston et al., 1996) we can conclude that the environment in which the MO probe is in is significantly different from that of bulk water (Lay et al., 1989). A departure in λ_{max} of the probe in the aqueous core of the aggregates compared to that in bulk water implies that a different solvent environment exist within the aggregates. This may lead to differences in solvation, and thus affect the chemistry in such environment (Li et

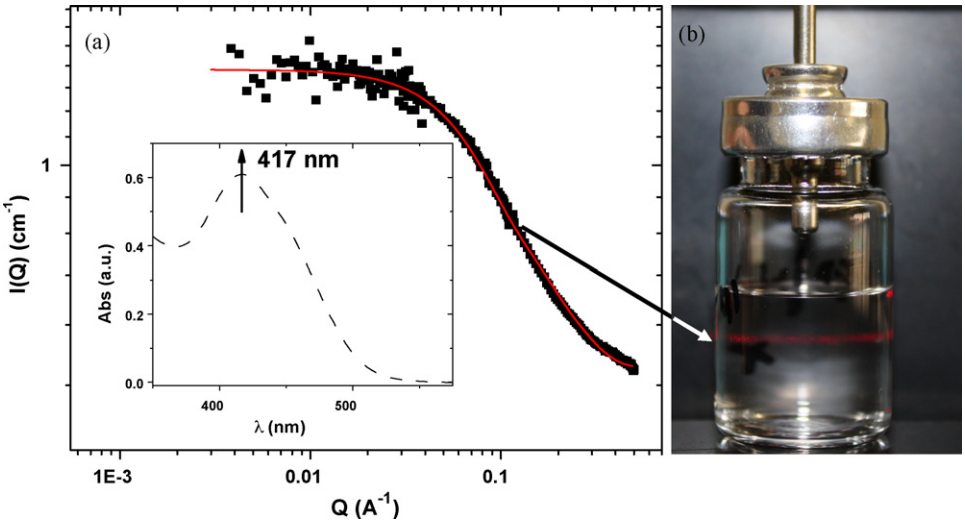

Fig. 3. (a) Neutron scattering curve for HFA227 with 20% (w/w) ethanol (10%, w/w, $EO_{13}PO_{30}EO_{13}$ and 5%, w/w, water; volume fraction of 15%) at 298 K and saturation pressure of the propellant mixture. Inset: UV–vis spectra for the same system as that for the SANS study. (b) Same formulation in the presence of a red laser light.

al., 2000). It is worth noticing, however, that in the HFA134a–EtOH (10%, w/w) system, with 5% (w/w) $EO_3PO_{43}EO_3$ and 2% (w/w) water (W_o = 18), which does form microemulsions, the determined λ_{max} (420 nm) was also far from that of the probe in bulk water (Selvam et al., 2008). A digital image of the microemulsion system containing 20% (w/w) ethanol, 10% (w/w) $EO_{13}PO_{30}EO_{13}$ and 5% (w/w) water (volume fraction of 15%) in HFA227 at 298 K and saturation pressure of the propellant mixture is shown in Fig. 3b, in the presence of a red laser light source. The formation of a microemulsion (pseudo-solution) is illustrated by the fact that no excess phase is observed inside the pMDI, and that the system was clear and yet capable of scattering the laser light. A formulation containing ethanol and HFA227 (solvent medium) at the same conditions (concentration, temperature and pressure) as in Fig. 3b, but no water or surfactant is characterized by the absence of scattered light, indicating the formation of a true homogeneous solution (no aggregates)—figure not shown.

UV–vis spectroscopy may suggest the existence of water-rich domains where the probe is being solvated, but does not provide any details on the microstructure of these reverse aqueous aggregates. SANS is used here to confirm the presence of microemulsions in HFA227, and to characterize their geometry. For the system with the same composition as that shown in Fig. 3 (same as in the UV–vis study), the microstructure of the polar core of the microemulsions can be obtained from scattering curves from SANS. The SANS spectrum, and the fit using the Igor Pro (Kline, 2006) is shown in Fig. 3a.

Assuming ethanol partitions equally between the water and HFA phase as established previously (Selvam et al., 2008); and fitting just the polar core, the SANS results indicate the formation of cylindrical aggregates with an aqueous-rich core radius of 6.9 ± 0.01 Å, and a core length of 62.2 ± 0.2 Å. SANS studies of aqueous reverse aggregates in HFA134a (10%, w/w, EtOH) using 5% (w/w) $EO_3PO_{43}EO_3$ and 2% (w/w) water (W_o = 18) also suggested the presence of cylindrical aggregates (Selvam et al., 2008), and capable of encapsulating a model biomolecule.

The smaller size of the reverse aggregates in this study compared to that at the HFA134a|W interface is attributed to the lower W_o used in this work. For water-in-xylene reverse micelles formed using $EO_nPO_mEO_n$ (at constant % EO), the core radius was found to increase with increasing block copolymer molecular weight, and with increasing water/copolymer volume fraction (Alexandridis and Andersson, 1997; Svensson et al., 1999).

These results indicate that aqueous reverse aggregates in HFA227 can be potentially used as oral inhalation formulations for the delivery of water-soluble compounds to and through the lungs (Butz et al., 2002; Lawrence and Rees, 2000; Liu et al., 2004; Meakin et al., 2003; Selvam et al., 2008; Sommerville and Hickey, 1998; Sommerville et al., 2000). Kinetically stable aggregates of water in the form of emulsions are also potential candidates for the delivery of polar therapeutics (Butz et al., 2002; Krafft and Riess, 1998). The ability of $EO_{13}PO_{30}EO_{13}$ in forming and stabilizing reverse aqueous emulsions in HFA227 is discussed below.

3.4. Emulsion formation and stability with $EO_{13}PO_{30}EO_{13}$

It has been demonstrated that reverse water-in-fluorocarbon emulsion stabilized by a fluorinated surfactant packaged in a pMDI containing HFA227 can be successfully used to deliver caffeine in a homogenous and reproducible way (Butz et al., 2002). It was also suggested that water in HFA emulsions are promising delivery systems for therapeutic biomolecules including enzymes (human recombinant deoxyribonuclease (rhDNAse), proteins, peptides like insulin, interferon, calcitonin, immunoglobulin, antibiotics), and oligonucleotides for either local or systemic administration (Butz et al., 2002; Courrier et al., 2002; Meakin et al., 2003; Sommerville and Hickey, 1998; Wu et al., 2007c). One should notice that the presence of large concentrations of water and other non-volatile excipients is expected to affect the aerosol characteristics of the pMDI (Brambilla et al., 1999), and should thus be considered when developing emulsion-based dispersions in propellant HFAs.

In this work, we have investigated the ability of $EO_{13}PO_{30}EO_{13}$ in forming and stabilizing emulsions of water and HFA227. $EO_{13}PO_{30}EO_{13}$ was selected for the emulsion studies based on the surface pressure vs. % *EO* diagram (Fig. 2). From Fig. 2, we can find that the surfactant balance is at ~50% EO. The balanced state region corresponds to the phase inversion point. Here emulsions are unstable since the Marangoni–Gibbs gradients become weak and the monolayer can be bent and ruptured easily. Moving the balance away from the phase-inversion point causes the surfactant to become preferentially soluble in one of the phases, resulting in a decrease in interfacial activity, and thus an increase in interfacial tension, which is accompanied by an increase in the stability of the emulsion (Aveyard et al., 1985; Psathas et al., 2000). $EO_{13}PO_{30}EO_{13}$ it is the most interfacially active species that lies on the LHS of the

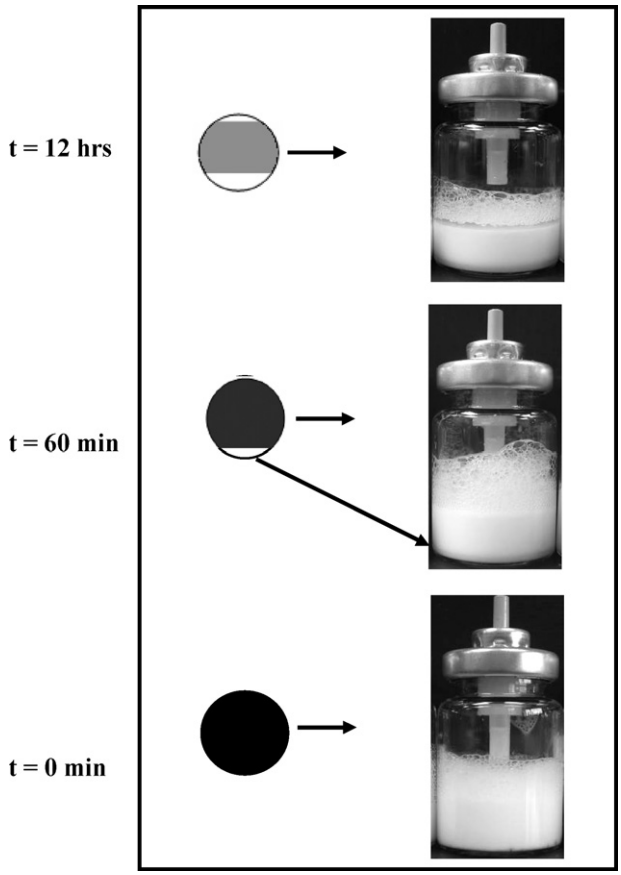

Fig. 4. Schematic diagram and digital images of the water-in-HFA227 emulsion formed at 30:70 (v/v) water/HFA227 (22:78%, w/w, of water/HFA227), 298 K, saturation pressure of the propellant mixture and 1% (w/w) $EO_{13}PO_{30}EO_{13}$. An arrow indicates the clearing front at 60 min.

pressure vs. % *EO* diagram, and was thus selected for the emulsion studies.

We selected a system consisting in 30:70% (v/v) water/HFA227 (22:78%, w/w, of water/HFA227), and 1% (w/w) surfactant overall for the studies. The experiments were run at 298 K and saturation pressure of the propellant mixture. After 2 h of sonication (180 W), both phases were completely emulsified—milky white. The emulsion was fairly stable against creaming, taking over 60 min for the formation of a weak clearing front, observed on the lower part of the cell. Stability against coalescence was much longer. A small clear phase on top of the cell (coalesced water droplets) took over 12 h to become visible, even at such low energy input levels. A creaming front moving upward, with a lower HFA phase changing from milky white → less white → clear phase is indicative of water dispersed droplets in HFA227 (Petros A., Psathas et al., 2000). The stability behavior is schematically shown in Fig. 4.

The relatively high stability against coalescence may be attributed to a favorable interaction between *PO* and HFA227, and good anchoring of the surfactant to the aqueous dispersed phase. At short times (less than 2 min), the non-coalesced droplets were fully redispersible simply by hand agitation of the container. These emulsions were stable up to 3 days. Water in fluorocarbon (W/FC) reverse emulsions stabilized by a fluorinated surfactant dispersed in HFA227 has been shown to be highly stable for a week, and remained fine and narrowly dispersed (Butz et al., 2002).

It is worth noticing that the energy input here was relatively low, and the emulsion stability is expected to improve as smaller droplets (higher mechanical energy and or higher surfactant concentration) are formed. In another compressible media, dilute W/CO_2 emulsions with $PO_{15}EO_{26}PO_{15}$ were found stable for shorter times (~10 min) (da Rocha et al., 1999), due to the low viscosity of CO_2.

4. Conclusions

In this work we demonstrate how ethoxylated surfactants can be successfully screened for the propellant HFA227|W interface. The measurement of interfacial tension of the modified interface is at the heart of the process, indicating the relative activity of the different surfactants in the series. A minimum tension at the HFA227|W interface of 6.9 mN m^{-1} was observed for $EO_{20}PO_{30}EO_{20}$, followed closely by $EO_{13}PO_{30}EO_{13}$ (at 1 mM surfactant). The surfactants in FDA-approved pMDI formulations were not very active at the HFA227|W interface. *In situ* tension measurements also allow us to investigate the effect of co-solvent, and the natural curvature of the amphiphiles. $EO_{13}PO_{30}EO_{13}$ has a very low tension, and falls to the LHS of the pressure vs. % *EO* (formulation diagram variable) curve. These results suggest that reverse microemulsions of the water-in-HFA227 type would be favored for that amphiphile, and it was thus selected to perform emulsion and microemulsion formation studies. In the presence of 10% (w/w) ethanol and 1 mM $EO_{13}PO_{30}EO_{13}$, the tension of the interface is furthered reduced to 0.9 mN m^{-1}, well below the tension at the critical aggregation concentration for other compressible solvents. It is interesting to observe that, even though similarly low-tension values were observed for a methyl-based amphiphile (oleic acid), no microemulsions were formed in that system. The results suggest that the better solvation of *PO* by HFA is indeed essential in helping the amphiphile to curve at the interface (form and stabilize the reverse aggregates). Overall the results suggest that this class of amphiphiles behave similarly in both HFA134a and HFA227. However, they seem slightly more active at the water interface with HFA134a. This result is somewhat counterintuitive based on the evidence that HFA227 interacts more strongly (enthalpically) with *PO*, the surfactant tail group. Some discrepancies may arise due to the fact that the propellants in question are also expected to interact with the surfactant head-group, and thus alter the balance (activity) in a non-predictable manner. The behavior of the amphiphiles at the model solvent (HPFP)–water interface cannot be directly extrapolated to the propellant systems.

With a combination of UV–vis spectroscopy and SANS, the presence and microstructure of the aggregates were determined. They follow similar trends to those for the HFA134a|W interface, where cylindrical aggregates were detected when in the presence of ethanol (co-solvent effect is essential). The ability of $EO_{13}PO_{30}EO_{13}$ in forming and stabilizing reverse emulsions of water-in-HFA227 was also demonstrated. While emulsions have been suggested as a potential route for formulating pMDIs, the effect of low volatility excipients (water and surfactant in this case) in the respirable fraction of the aerosol must be considered. Such emulsions may be also relevant to other medical sprays and related industries.

Acknowledgements

WSU for start-up funds and NSF-CBET grant no. 0553537, and a Thomas C. Rumble Fellowship for PS; Solvay Fluor und Derivate GmbH & Co., Hannover, Germany, for the HFA227 samples; BASF for surfactant samples; and West Pharmaceuticals and 3 M, for the glass vials and metering valves, respectively. We acknowledge the support of the National Institute of Standards and Technology, U.S. Department of Commerce, in providing the neutron-research facilities used in this work. The mention of commercial products does not imply endorsement by NIST, nor does it imply that the mate-

rials or equipment identified are necessarily the best available for the purpose.

References

Alexander, D.J., Libretto, S.E., 1995. An overview of the toxicology of HFA-134a (1,1,1,2-tetrafluoroethane). Human and Experimental Toxicology 14, 715–720.

Alexandridis, P., Andersson, K., 1997. Reverse micelle formation and water solubilization by polyoxyalkylene block copolymers in organic solvent. Journal of Physical Chemistry B 101 (41), 8103–8111.

Aveyard, R., Binks, B.P., Clark, S., Fletcher, P.D.I., 1990. Effects of temperature on the partitioning and adsorption of dodecyl pentaethylene glycol ether in heptane–water mixtures. Journal of the Chemical Society, Faraday Transactions 86 (18), 3111–3115.

Aveyard, R., Binks, B.P., Fletcher, P.D.I., 1989. Interfacial tensions and aggregate structure in pentaethylene glycol monododecyl ether/oil/water microemulsion systems. Langmuir 5, 1210–1217.

Aveyard, R., Binks, B.P., Lawless, T.A., Mead, J., 1985. Interfacial tension minima in oil + water + surfactant systems. Effects of salt and temperature in systems containing nonionic surfactants. Journal of the Chemical Society 81 (9), 2155–2168.

Blondino, F.E., 1995. Novel Solution Aerosols for Inhalation. Department of Pharmacy and Pharmaceutics, Virginia Commonwealth University, Richmond, Virginia, pp. 340.

Blondino, F.E., Byron, P.R., 1998. Surfactant dissolution and water solubilization in chlorine-free liquified gas propellants. Drug Development and Industrial Pharmacy 24 (10), 935–945.

Bowman, P.A., Greenleaf, D., 1999. Non-CFC metered dose inhalers: the patent landscape. International Journal of Pharmaceutics 186 (1), 91–94.

Brambilla, G., Ganderton, D., Garzia, R., Lewis, D., Meakin, B., Ventura, P., 1999. Modulation of aerosol clouds produced by pressurised inhalation aerosols. International Journal of Pharmaceutics 186, 53–61.

Butz, N., Porte, C., Courrier, H., Krafft, M.P., Vandamme, T.F., 2002. Reverse water-in-fluorocarbon emulsions for use in pressurized metered-dose inhalers containing hydrofluoroalkane propellants. International Journal of Pharmaceutics 238 (1–2), 257–269.

Byron, P.R., Patton, J.S., 1994. Drug delivery via respiratory track. Journal of Aerosol Medicine 7 (1), 49–75.

Chinet, T., 2000. Changes in metered dose inhaler propellants. Revue des Maladies Respiratoires 17 (1), 15–20.

Courrier, H.M., Butz, N., Vandamme, T.F., 2002. Pulmonary drug delivery system: recent developments and prospects. Critical Reviews in Therapeutic Drug Carrier Systems 19 (4–5), 425–498.

da Rocha, S.R.P., Harrison, K.L., Johnston, K.P., 1999. Effect of surfactants on the interfacial tension and emulsion formation between water and carbon dioxide. Langmuir 15 (2), 419–428.

da Rocha, S.R.P., Johnston, K.P., 2000. Interfacial thermodynamics of surfactants at the CO_2–water interface. Langmuir 16 (8), 3690–3695.

Dellamary, L.A., Tarara, T.E., Smith, D.J., Woelk, C.H., 2000. Hollow porous particles in metered dose inhalers. Pharmaceutical Research 17 (2), 168–174.

Dickson, J.L., Smith Jr., P.G., Dhanuka, V.V., Srinivasan, V., Stone, M.T., Rossky, P.J., Behles, J.A., Keiper, J.S., Xu, B., Johnson, C., DeSimone, J.M., Johnston, K.P., 2005. Interfacial properties of fluorocarbon and hydrocarbon phosphate surfactants at the water–CO_2 interface. Industrial and Engineering Chemistry Research 44 (5), 1370–1380.

DuBoisson, R., 2005. Surface Tension of HPFP. Synquest Labs, Alachua, FL.

Edwards, D.A., Hanes, J., Caponetti, G., 1997. Large porous particles for pulmonary drug delivery. Science 276 (5320), 1868–1871.

Emmen, H.H., Hoogendijk, E.M.G., Klopping-Ketelaars, W.A.A., Muijser, H., Duistermaat, E., Ravensberg, J.C., Alexander, D.J., Borkhataria, D., Rusch, G.M., Schmit, B., 2000. Human safety and pharmacokinetics of the CFC alternative propellants HFC 134a (1,1,1,2-tetrafluoroethane) and HFC 227 (1,1,1,2,3,3,3-heptafluoropropane) following whole-body exposure. Regulatory Toxicology and Pharmacology 32, 22–35.

Graepel, P., Alexander, D.J., 1991. CFC replacements: safety testing, approval for use in metered dose inhalers. Journal of Aerosol Medicine 4 (3), 193–200.

Johnston, K.P., Harrison, K.L., Clarke, M.J., Howdle, S.M., Heitz, M.P., Bright, F.V., Carlier, C., Randolph, T.W., 1996. Water-in-carbon dioxide microemulsions: a new environment for hydrophiles including proteins. Science 271, 674.

Kline, S.R., 2006. Reduction and analysis of SANS and USANS data using Igor Pro. Journal of Applied Crystallography 39, 895–900.

Krafft, M.P., 2001. Fluorocarbons and fluorinated amphiphiles in drug delivery and biomedical research. Advanced Drug Delivery Reviews 47, 209–228.

Krafft, M.P., Riess, J.G., 1998. Highly fluorinated amphiphiles and colloidal systems, and their applications in the biomedical field. A contribution. Biochimie 80 (5–6), 489–514.

KSV, 2001. CAM 200—Optical Contact Angle Meter., p Product manual.

Laube Beth, L., 2005. The expanding role of aerosols in systemic drug delivery, gene therapy, and vaccination. Respiratory Care 50 (9), 1161–1176.

Lawrence, M.J., Rees, G.D., 2000. Microemulsion-based media as novel drug delivery systems. Advanced Drug Delivery Reviews 45 (1), 89–121.

Lay, M.B., Drummond, C.J., Thistlethwaite, P.J., Grieser, F., 1989. ET(30) as a probe for the interfacial microenvironment of water-in-oil microemulsions. Journal of Colloid and Interface Science 128 (2), 602–604.

Li, J.-R., Lee, Y.-M., Yu, T., 2000. Solubilization of hydrophilic compounds in 1,1,1,2-tetrafluoroethane with a cationic surfactant. Analytical Chemistry 72, 1348–1351.

Liu, J., Ikushima, Y., Shervani, Z., 2004. Investigation on the solubilization of organic dyes and micro-polarity in AOT water-in-CO_2 microemulsions with fluorinated co-surfactant by using UV–vis spectroscopy. Journal of Supercritical Fluids 32 (1–3), 97–103.

McDonald, K.J., Martin, G.P., 2000. Transition to CFC-free metered dose inhalers—into the new millennium. International Journal of Pharmaceutics 201 (1), 89–107.

Meakin, B.J., Lewis, D.A., Berrill, S.A., Davies, R.J., 2003. Solubilization of drugs in HFA propellant by means of emulsions, Eur. Pat. Appl., pp. 14 (Chiesi Farmaceutici S.p.A., Italy).

Modi, P., 2000. Pulmonary Drug Delivery. Generex Pharmaceuticals Inc., Canada.

Owens, D.R., Zinman, B., Bolli, G., 2003. Alternative routes of insulin delivery. Diabetic Medicine 20 (11), 886–898.

Patel, N., Marlow, M., Lawrence, M.J., 2003a. Fluorinated ionic surfactant microemulsions in hydrofluorocarbon 134a (HFC134a). Journal of Colloid and Interface Science 258 (2), 354–362.

Patel, N., Marlow, M., Lawrence, M.J., 2003b. Formation of fluorinated nonionic surfactant microemulsions in hydrofluorocarbon 134a (HFC134a). Journal of Colloid and Interface Science 258 (2), 345–353.

Patton, J.S., Byron, P.R., 2007. Inhaling medicines: delivering drugs to the body through the lungs. Nature Reviews Drug discovery 6, 67–74.

Patton, J.S., Fishburn, C.S., Weers, J.G., 2004. The lungs as a portal of entry for systemic drug delivery. Proceedings of the American Thoracic Society 1, 338–344.

Peguin, R.P.S., da Rocha, S.R.P., 2008. Solvent–solute interactions in hydrofluoroalkane propellants. Journal of Physical Chemistry B 112, 8084–8094.

Peguin, R.P.S., Selvam, P., da Rocha, S.R.P., 2006. Microscopic and thermodynamic properties of the HFA134a–water interface: atomistic computer simulations and tensiometry under pressure. Langmuir 22 (21), 8826–8830.

Peguin, R.P.S., Wu, L., da Rocha, S.R.P., 2007. The Ester Group: how hydrofluoroalkanephilic is it? Langmuir 23 (16), 8291–8294.

Psathas, P., da Rocha, S.R.P., Ted Lee, C.J., Johnston, K.P., Lim, K.T., Webber, S., 2000. Water-in-carbon dioxide emulsions with poly(dimethylsiloxane)-based block copolymer ionomers. Langmuir 39, 2655–2664.

Rau, J.L., 2005. The inhalation of drugs: advantages and problems. Respiratory Care 50 (3), 365–382.

Ridder, K.B., Davies-Cutting, C.J., Kellaway, I.W., 2005. Surfactant solubility and aggregate orientation in hydrofluoroalkanes. International Journal of Pharmaceutics 295 (1–2), 57–65.

Rogueda, P.G.A., 2003. HPFP, a model propellant for pMDIs. Drug Development and Industrial Pharmacy 29 (1), 39–49.

Rogueda, P.G.A., 2005. Novel hydrofluoroalkane suspension formulations for respiratory drug delivery. Expert Opinion on Drug Delivery 2 (4), 625–638.

Sadtler, V.M., Krafft, M.P., Riess, J.G., 1999. Reverse water-in-fluorocarbon emulsions as a drug delivery system: an in vitro study. Colloids and Surfaces A: Physicochemical and Engineering Aspects 147, 309–315.

Sagisaka, M., Fujii, T., Ozaki, Y., Yoda, S., Takebayashi, Y., Kondo, Y., Yoshino, N., Sakai, H., Abe, M., Otake, K., 2004. Interfacial properties of branch-tailed fluorinated surfactants yielding a water/supercritical CO_2 microemulsion. Langmuir 20 (7), 2560–2566.

Selvam, P., Chokshi, U., Gouch, A., Wu, L., Porcar, L., da Rocha, S.R.P., 2008. Ethoxylated copolymer surfactants for the HFA134a–water interface: interfacial activity, aggregation behavior and biomolecule encapsulation. Soft Matter 4, 357–366.

Selvam, P., Peguin, R.P.S., Chokshi, U., da Rocha, S.R.P., 2006. Surfactant design for the 1,1,1,2-tetrafluoroethane–water interface: *ab initio* calculations and *in situ* high-pressure tensiometry. Langmuir 22 (21), 8675–8683.

Solvay, 2005. Solvay Fluor und Derivate GmbH & Co. KG, Hamburg.

Sommerville, M., Hickey, A.J., 1998. Microemulsion alternate propellant systems for aerosol drug delivery. In: Respiratory Drug Delivery VI: Biological, Pharmaceutical, Clinical and Regulatory Issues Relating to Optimized Drug Delivery by Aerosol, the International Symposium, Hilton Head, S.C, pp. 421–424.

Sommerville, M.L., Cain, J.B., Johnson Jr., C.S., Hickey, A.J., 2000. Lecithin inverse microemulsions for the pulmonary delivery of polar compounds utilizing dimethyl ether and propane as propellants. Pharmaceutical Development and Technology 5 (2), 219–230.

Sommerville, M.L., Johnson Jr., C.S., Cain, J.B., Rypacek, F., Hickey, A.J., 2002. Lecithin microemulsions in dimethyl ether and propane for the generation of pharmaceutical aerosols containing polar solutes. Pharmaceutical Development and Technology 7 (3), 273–288.

Steckel, H., Muller, B.W., 1998. Metered-dose inhaler formulation of fluticasone 17-propionate micronized with supercritical carbon dioxide using the alternative propellant HFA-227. International Journal of Pharmaceutics 173 (1–2), 25–33.

Stefely, J.S., 2002. Novel excipients for inhalation drug delivery: expanding the capability of the MDI. Drug Delivery Technology 2 (6), 64–69, 62.

Stefely, J.S., Duan, D.C., Myrdal, P.B., 2000. Design and utility of a novel class of biocompatible excipients for HFA-based MDIs. Respiratory Drug Delivery VII, 83–90.

Steytler, D.C., Thorpe, M., Eastoe, J., Dupont, A., Heenan, R.K., 2003. Microemulsion formation in 1,1,1,2-tetrafluoroethane (R134a). Langmuir 19 (21), 8715–8720.

Svensson, B., Olsson, U., Alexandridis, P., Mortensen, K., 1999. A SANS investigation of reverse (water-in-oil) micelles of amphiphilic block copolymers. Macromolecules 32, 6725–6733.

Tansey, I., 1997. The technical transition to CFC-free inhalers. British Journal of Clinical Practice 89, 22–27.

Tarara, T.E., Hartman, M.S., Gill, H., Kennedy, A.A., Weers, J.G., 2004. Characterization of suspension-based metered dose inhaler formulations composed of spray-dried budesonide microcrystals dispersed in HFA-134a. Pharmaceutical Research 21 (9), 1607–1614.

Vervaet, C., Byron, P.R., 1999. Drug–surfactant–propellant interactions in HFA-formulations. International Journal of Pharmaceutics 186 (1), 13–30.

Wang, Y.-C.J., Kowal, R.R., 1980. Review of excipients and pH's for parenteral products used in the United States. Journal of the Parenteral Drug Association 34 (6), 452–462.

Williams III, R.O., Liu, J., 1998. Influence of formulation additives on the vapor pressure of hydrofluoroalkane propellants. International Journal of Pharmaceutics 166, 99–103.

Wu, L., Al-haydari, M., da Rocha, S.R.P., 2008. Novel propellant-driven inhalation formulations: engineering polar drug particles with surface-trapped hydrofluoroalkanephiles. European Journal of Pharmaceutical Sciences 33 (2), 146–158.

Wu, L., Bharatwaj, B., Panyam, J., da Rocha, S.R.P., 2007a. Core–shell particles for the dispersion of small polar drugs and biomolecules in hydrofluoroalkane propellants. Pharmaceutical Research 25 (2), 289–301.

Wu, L., da Rocha, S.R.P., 2007. Biocompatible and biodegradable copolymer stabilizers for hydrofluoroalkane dispersions: a colloidal probe microscopy investigation. Langmuir 23 (24), 12104–12110.

Wu, L., Peguin, R.P.S., da Rocha, S.P.R., 2007b. Understanding solvation in hydrofluoroalkanes: *ab initio* calculations and chemical force microscopy. Journal of Physical Chemistry B 111 (28), 8096–8104.

Wu, L., Peguin, R.P.S., Selvam, P., Chokshi, U., da Rocha, S.P.R., 2007c. Molecular scale behavior in alternative propellant-based inhaler formulations. In: Hickey, A.J. (Ed.), Inhalation Aerosols—Physical and Biological Basis for Therapy. Informa Healthcare USA, New York, pp. 373–397.