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Reverse water-in-fluorocarbon emulsions for use in pressurized metered-dose inhalers containing hydrofluoroalkane propellants

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Abstract

Pulmonary administration of drugs has demonstrated numerous advantages in the treatment of pulmonary diseases due to direct targeting to the respiratory tract. It enables avoiding the first pass effect, reduces the amount of drugs administered, targets drugs to specific sites and reduces their side effects. Reverse water-in-fluorocarbon (FC) emulsions are potential drug delivery systems for pulmonary administration using pressurized metered-dose inhalers (pMDI). The external phase of these emulsions consists of perfluorooctyl bromide (PFOB, perflubron), whereas their internal phase contains the drugs solubilized or dispersed in water. These emulsions are stabilized by a perfluoroalkylated dimorpholinophosphate (F8H11DMP), i.e. a fluorinated surfactant. This study demonstrates the possibility of delivering a reverse fluorocarbon emulsion via the pulmonary route using a CFC-free pMDI. Two hydrofluoroalkanes (HFAs) (Solkane® 134a and Solkane® 227) were used as propellants, and various solution (or emulsion)/propellant ratios (1/3, 1/2, 2/3, 1/1, 3/2, 3/1 v/v) were investigated. The insolubility of water (with or without the fluorinated surfactant F8H11DMP) in both HFA 227 and HFA 134a was demonstrated. PFOB and the reverse emulsion were totally soluble or dispersible in all proportions in both propellants. This study demonstrated also that the reverse FC emulsion can be successfully used to deliver cafeine in a homogenous and reproducible way. The mean diameter of the emulsion water droplets in the pressured canister was investigated immediately after packaging and after 1 week of storage at room temperature. Best results were obtained with emulsion/propellant ratios comprised between 2/3 and 3/2, and with HFA 227 as propellant.

Keywords: Hydrofluoroalkane (HFA); Reverse emulsion; Fluorocarbon; Fluorinated surfactant; Aerosol; Pressurized metered-dose inhaler; Site specific-drug delivery

1. Introduction

In the past few years, the techniques for delivering drugs into the lung have progressed considerably. Inhalation of drugs allows their rapid

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deposit in the target organ, induces less side effects than their administration by other routes (Keller et al., 1999), and allows the deposit of larger drug concentrations at the sites of disease (Clifford Waldrep et al., 1997). The walls of the alveoli in the deep lung are extremely thin ($0.1-1$ μm) as compared with the capillary walls (7 μm). In addition, the surface area of the lung is extremely large, approximately 75 m^2 for a 70 kg male (Wearley, 1991).

The fraction of drug that reaches the various parts of the lung depends on a number of factors such as the amount and rate of air inhaled, the respiratory pause, and the vehicle's particle size and characteristics (Keller et al., 1999; Cripps et al., 2000). In spite of such complex mechanisms, pulmonary delivery of a number of drugs, such as bronchodilatators and steroids is increasingly favored (Purewal et al., 1998; Purewal, 1999). Progress has been achieved in the field of aerosol therapy (Keller et al., 1999), aerosol generators (Aiache and Aiache 1996), synthesis of new propellants (Purewal, 1998) and drug delivery systems (Gonda, 2000). The latter systems include liposomes, NanoCrystals™ technology, polymers, macromolecule-drug conjugates, microspheres, PulmoSpheres™, salts and precipitates.

For ecological reasons (Chinet, 2000; Tansey, 1997), it has recently been decided to package the pressurized metered-dose inhalers (pMDI) with propellants other than chlorofluoroalkanes (CFCs). Hydrofluoroalkanes (HFAs) were utilized for this purpose (Purewal, 1998). The HFAs selected for this study are 1,1,1,2-tetrafluoroethane (HFA 134a) and 1,1,1,2,3,3,3-heptafluoropropane (HFA 227). These compounds comprise C–F bonds, which are the strongest single bonds encountered in organic chemistry, and no chlorine. Their ozone depletion potential is considerably lower than that of CFCs and hydrochlorofluorocarbons (HCFCs). Liquid fluorocarbons (FCs) and HFAs are characterized by very high intramolecular bonding and very low intermolecular forces (Krafft et al., 1998). As a consequence, FCs and HFAs display exceptional biological inertness and absence of toxicity (Riess, 2001; Alexander and Libretto, 1995; Emmen et al., 2000; Graepel and Alexander 1991), including in the form of emulsions (Leese et al., 2000; Noveck et al., 2000). FCs are excreted unchanged (i.e. without being metabolized) through the lungs along with expired air (Riess, 2001). HFA 227 is biotransformed at a very low rate, but irreversible protein binding of the metabolite could not be demonstrated, even when using very sensitive methods (Köster et al., 1996). In addition, FCs are the best possible solvents of gases (Krafft and Riess, 1998; Riess, 2001). Their solubility for gases is related to the weakness of the intermolecular van der Waals forces that exist between fluorocarbon molecules. Such weak interactions allow easy insertion of gas molecules in the liquid. They also account for the exceptionally low surface tensions, low dielectric constants, high fluidities and high compressibilities of these unique compounds (Krafft and Riess, 1998). Neat perfluorooctyl bromide has been effective for the delivery of drugs (Cullen et al., 1999) and genes (Weiss et al., 2001) to the lung.

At present time, HFAs has become mandatory in the new pMDIs (Tansey, 1997). However, due to differences in physico-chemical characteristics with CFCs (Table 1), new formulations need to be devised (Vervaet and Byron, 1999). Most drugs and surfactants are insoluble in FCs and HFAs (Leach, 1995; Elveerog, 1997; Krafft et al., 1998; Dickinson et al., 2000) because these fluorinated compounds are simultaneously *hydro*phobic and *lipo*phobic. Suspensions of solid drugs in FCs lead to emission of non-homogeneous, hence non-reproducible amounts of drugs from the can when the valve is actuated. In order to overcome this problem, stable reverse emulsions consisting of suspensions of fine and narrowly dispersed droplets of water in a continuous FC phase have been elaborated (Sadtler et al., 1996; Krafft, 2001). Perfluorooctyl bromide (PFOB) emulsions containing 5% v/v of water were stabilized by perfluoroalkylated surfactants with a dimorpholinophosphate polar head group ($C_nF_{2n+1}(CH_2)_mOP(O)[N(CH_2CH_2)_2O]_2$, FnHmDMP) and prepared by high pressure homogenization (Sadtler et al., 1996). Furthermore, the mean droplet diameter of these new drug delivery systems can be adjusted to low sizes allowing targeting of the lower levels of the lung.

Table 1
Physical and chemical properties of the components of the pMDI

	Fluorinated surfactant	PFOB	Solkane® 227 pharma	Solkane® 134a pharma	CFC 11	CFC 12	CFC 114
Structural formula	$C_8F_{17}(CH_2)_{11}$ × OP(O) × $[N(CH_2CH_2)_2$ × $O]_2$	$CF_3(CF_2)_7Br$	CF_3CHFCF_3	CF_3CH_2F	$CFCl_3$	$CFCl_2$	CF_2ClCF_2Cl
Molecular formula	$C_{27}H_{38}F_{17}N_2PO_4$	$C_8F_{17}Br$	C_3HF_7	$C_2H_2F_4$	$CFCl_3$	$CFCl_2$	$C_2F_4Cl_2$
Chemical name	*11-(F*-octyl) × (undecyl) × -dimorpholinophosphate	1-Bromo-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadeca-fluorooctane	1,1,1,2,3,3,3,-Heptafluoropropane	1,1,1,2-Tetrafluoromethane	Monofluoro-trichloro-methane	Difluoro-dichloro	Tetra fluoroethane
Laboratory code	F8H11DMP	PFOB	HFA 227ea, HFC 227ea	HFA 134a, HFC 134a			
Physical form	Crystallized solid	Colorless liquid	Colorless gas	Colorless gas	Colorless gas	Colorless gas	Colorless gas
Storage directions	Stable at room temperature	Room temperature	Liquified by compression in steel cylinders	Liquified by compression in steel cylinders	Liquified by compression in steel cylinders	Liquified by compression in steel cylinders	Liquified by compression in steel cylinders
Boiling point	–	143 °C	−16.5 °C at 1013 bar	−26.1 °C at 1013 bar	+23.8 °C at 1013 bar	−29.8 °C at 1013 bar	+3.6 °C at 1013 bar
Vapor pressure	–	11 mmHg at 37 °C	3.90 bar at 20 °C	5.72 bar at 20 °C	0.87 bar at 20 °C	5.60 bar at 20 °C	1.81 bar at 20 °C
Density of liquid		1.918 g ml^{-1} at 25 °C	1.415 kg l^{-1} at 20 °C	1.23 kg l^{-1} at 20 °C	1.49 kg l^{-1} at 20 °C	1.33 kg l^{-1} at 20 °C	1.47 kg l^{-1} at 20 °C

The aim of this work was to evaluate and develop reverse water-in-FC emulsions at various emulsion/propellant ratios, intended to target hydrophilic drugs into the lungs and packaged in a pMDI containing an hydrofluoroalkane propellant.

2. Materials and methods

2.1. Materials

Solkane® 134a pharma and Solkane® 227 pharma were obtained from Solvay Fluor (Frankfurt, Germany). The fluorinated surfactant F8H11DMP was synthesized and thoroughly purified, according to Sadtler et al. (1998). PFOB was a gift from Alliance Pharmaceutical Corp. (San Diego, CA, USA). Monohydrate cafeine was provided by Cooper (77000 Melun, France). Cebal aluminium cans ($23.6 \times 60 \times 20$ mm, 20 ml) with standard inner varnish (when Solkane® 227 is used) or epoxyphenolic BASF 6256 ONC inner varnish (when Solkane® 134a is used) were obtained from Cebal (92115 Clichy, France). One milliliter valves (ST004, GL 001 and TD0033 for Solkane® 227; ST0018, GL0001 and TD0033 for Solkane® 134a) were purchased from Lablabo (74100 Annemasse, France). All other reagents were of analytical grade.

The reverse water-in-FC emulsion contained 95% v/v of PFOB, 5% v/v of a NaCl solution (0.9% w/v) in water, and 1.5% w/v of F8H11DMP. It was prepared as follows: F8H11DMP (1.5 g) was solubilized in PFOB (95 ml) by gentle stirring. The NaCl solution (5 ml) was added to the fluorocarbon phase, and the mixture was homogenized first at low pressure (Ultra-Turax fitted with the S25N25F probe, Ika-Labortechnik Stanfen), then under high pressure (Microfluidizer M110 fitted with a stainless steel cooling coil, Microfluidics Corp. Newton, MA 02164). The temperature was maintained below 40 °C. The mean droplet diameter was measured immediately after preparation by quasi elastic light spectroscopy (Zetasizer 3000 HS, Malvern Instruments). An emulsion containing cafeine (0.469 g l^{-1}) in the aqueous phase was also prepared for the assessment of the uniformity of the content (emulsion + propellant) of the pressurized canister.

All cans were crimped and filled up with a Pamasol P2016 apparatus. All weighings were done with a Ohaus Voyager V12140 balance.

2.2. Methods

2.2.1. Solubility assays

The solubility assays included studies of the pulverization weight uniformity and of the valve content uniformity. For each solution (water or PFOB) or dispersion (water-in-FC emulsion) that were investigated, the following solution (or dispersion)/propellant ratios were investigated: 1/3, 1/2, 2/3, 1/1, 3/2, and 3/1 v/v.

2.2.1.1. Pulverization weight uniformity study. Pulverization weight is the term used to indicate the weight of solution (water or PFOB), or dispersion (emulsion), that is emitted from the can when it is actuated. The aerosol canister was shaken prior to actuation. Each pulverization was done in a 150 ml erlenmeyer. The content of each shot was weighed 2 min after pulverization, i.e. after the propellant was evaporated but before the solvents (PFOB or water) began to evaporate significantly. The process was repeated until the canister was emptied. Two cans (19–24 pulverizations per can) were tested for each water/propellant or PFOB/propellant ratio, and for each propellant (Solkane® 134a or 227), except for the emulsion/propellant system, for which one canister was used for each ratio (1/3, 1/2, 2/3, 1/1, 3/2, and 3/1).

2.2.1.2. Valve content uniformity study. Valve content is the term used to indicate the total weight of contents (drug + propellant + excipient) that is emitted from the can. Valve content was measured by container weight loss. The aerosol canister was shaken prior to actuation. The can was weighed, then a shot was fired and the canister was re-weighed. The difference in weight gave the weight of the first shot. The process was repeated in order to obtain the

weight of each shot of the canister until it was emptied. Two cans (19–24 pulverizations per can) were tested for each water/propellant or PFOB/propellant ratio, and for each propellant, except for the emulsion/propellant system, for which one canister was used for each ratio (1/3, 1/2, 2/3, 1/1, 3/2, and 3/1).

2.2.2. Content uniformity assay

The content uniformity assay was evaluated by solubilizing a tracer, cafeine monohydrate (0.469 g l^{-1}) in the aqueous phase of the FC emulsion. The assay was realized with Solkane® 227. The emulsion/propellant ratios investigated were 1/3, 1/2, 2/3, 1/1, and 3/2 v/v. One canister was analyzed for each ratio. Each pulverization ($\sim$20 pulverizations per can) was then evaporated in a beaker in an incubator at 40 °C (Memmert B40). After 24 h, the residue was dissolved in 2 ml of distilled water. Cafeine concentration was quantified by UV spectrophotometry (UV-VIS Scanning Spectrophotometer UV-2101 PC Shimadzu). The detector was adjusted at 272 nm. Quantitations were realized using precision cells made of quartz suprazil (QS Hellma type 104 cells).

2.2.3. Mean diameter of the water droplets in the reverse emulsion after pulverization

The mean diameter ($\pm$10%) of the water droplets was measured with a Zetasizer 3000 HS (Malvern Instruments, ten successive measures) just after pulverization, and compared with the initial size of the emulsion droplets just after preparation. Pulverized solutions were allowed to degas for 5 min prior particle size analysis. Mean diameters were measured for 2/3 and 3/2 emulsion/propellant (Solkane® 227 and 134a) ratios. With Solkane® 134a, droplet mean diameters were also measured for 1/3 and 1/2 emulsion/propellant ratios. One canister was analyzed for each ratio and two samples of ten pulverizations were studied. For Solkane® 227, particle size analysis was also achieved after 1 week of storage, the emulsion being packaged in the pMDI. One canister was analyzed for each ratio. Four samples of six pulverizations were tested.

3. Results and discussion

Some difficulties have been encountered with Solkane® 134a which were not present with Solkane® 227. These problems were associated with the metered valve performance, and included valve sticking (valve did not spray) and leaking out (valve sprayed continuously). This is in agreement with Tiwari et al. (1998) who have reported that formulations containing HFA 134a instead of CFC adversely affected the functioning of valves. These authors showed that high concentrations of ethanol improved valve performance but increased leakage. Moreover, the use of Solkane® 134a requires specific compatible materials (special inner varnish for the canister, and special valves).

3.1. Water solubility in the propellants (Solkane® 134a and 227)

Knowing that the water solubility in the propellants investigated is very low (610 and 2220 ppm for Solkane® 227 and 134a, respectively) (Vervaet and Byron, 1999), it was of importance to determine the variation of the amount of the aqueous phase as a function of successive pulverizations.

When water was added to both propellants, three different phases were formed in the canister, this one being turned upside down. When the valve was actuated, propellant was expulsed first because of higher density, and the aqueous phase stood above HFA phase (Fig. 1a). Three different groups of weights corresponding to the various compositions were observed during assays of pulverization weight uniformity and valve content uniformity (Tables 2–5). Results confirm that water is neither soluble in Solkane® 227 nor in 134a, whatever the water/propellant ratio. As shown in Fig. 1a, the lowest phase contained a large amount of propellant. For both propellants, the pulverization weight was small ($\sim$0.20–0.30 g) due to the evaporation of the propellant, whereas the valve content is the highest of the three groups (around 1–1.1 g) since both propellants have a higher density than water (Table 1). The second group of

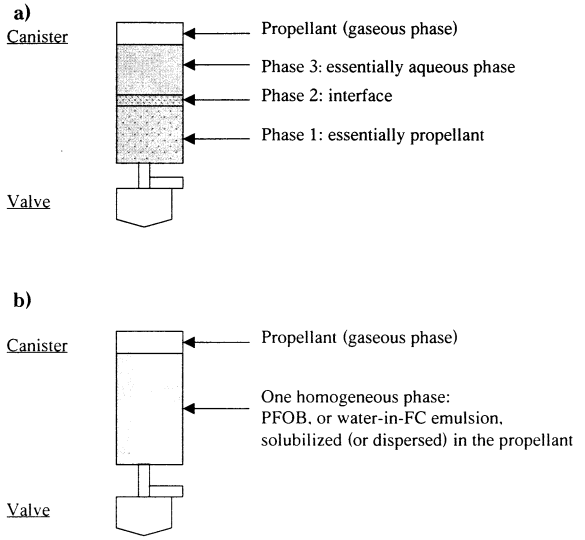

Fig. 1. Miscibility of the various components of pressurized metered-dose inhalers (pMDI) investigated. (a) Three phases were observed when the pMDI was filled with water and hydrofluoroalkane propellants (HFA 227 and 134a). (b) On the contrary, an homogeneous phase was found when the pMDI was filled with a FC (perfluorooctyl bromide, $C_8F_{17}Br$, PFOB), or with a water-in FC emulsion, and HFAs.

weights corresponds to the interface between the aqueous phase (third group of weights) and the propellant phase (first group of weights). For this second group, pulverization weights have values comprised between those of the first and second group. Finally, the third phase consists essentially of water. The pulverization weight is higher than that determined for the first group because there is less propellant that is evaporated, whereas the valve content is smaller.

Fig. 2 shows that the number of pulverizations of the first phase decreases when the water/propellant ratio increases. On the other hand, the number of pulverizations of the third phase increased then reached a plateau. Thus, the higher the amount of propellant in the canister, the higher the number of pulverizations that corresponds to the first group of weights and the smaller the number of pulverizations that corresponds to the third group of weights. This indicates that the first constituent emitted from the canister consists essentially of propellant, whereas the third phase consists essentially of water (Fig. 1a). The number of pulverizations that corresponds to the second phase was found to be very small for low water/propellant ratios (1/3 or 1/2).

It is noteworthy that when the fluorinated surfactant F8H11DMP was introduced with a mixture of water and propellant into a canister, it was not possible to create an emulsion spontaneously. In this case, only foam was emitted through the valve of the canister. This means that HFA propellants cannot act as a substitute for the external FC phase of the emulsion. The preparation of the reverse water-in-FC emulsion is necessary. It should be mentioned that Evans and Farr (1993) succeeded in solubilizing hydrophilic drugs like

Table 2
Solubility of water in Solkane® 227: uniformity of weight after pulverization

Water/propellant ratio	1/3	1/2	2/3	1/1	3/2	3/1
Group 1						
Mean weight (g)	0.3140	0.3120	0.3016	0.2795	0.3016	0.3125
Mean standard deviation	0.0259	0.0226	0.0420	0.0332	0.0453	0.0793
Mean number of pulverizations	18	14	12	10	7	6
Group 2						
Mean weight (g)	0.5659	0.5218	0.4469	0.415	0.5065	0.6222
Mean standard deviation	0.0112	0.0178	0.0574	0.0397	0.0466	0.0505
Mean number of pulverizations	2	3	4	3	3	4
Group 3						
Mean weight (g)	0.6999	0.6339	0.6635	0.7770	0.7798	0.8626
Mean standard deviation	0.0443	0.0481	0.0505	0.0766	0.0542	0.0434
Mean number of pulverizations	4	7	9	10	12	12

Table 3
Solubility of water in Solkane® 227: valve content uniformity

Water/propellant ratio	1/3	1/2	2/3	1/1	3/2	3/1
Group 1						
Mean content of the valve (g)	1.0883	1.0517	1.0476	1.0717	1.0759	1.0168
Mean standard deviation	0.0734	0.0807	0.0780	0.0797	0.0546	0.0410
Mean number of pulverizations	17	14	12	11	7	5
Group 2						
Mean content of the valve (g)	1.0623	0.9082	0.9160	0.9774	0.9406	/
Mean standard deviation	0.0678	0.0709	0.0337	0.0627	0.0503	/
Mean number of pulverizations	2	3	4	2	3	0
Group 3						
Mean content of the valve (g)	0.8244	0.8100	0.7727	0.8417	0.8238	0.8726
Mean standard deviation	0.0404	0.0674	0.0481	0.0673	0.0452	0.0706
Mean number of pulverizations	5	7	8	9	11	17

Table 4
Solubility of water in Solkane® 134a: uniformity of weight after pulverization

Water/propellant ratio	1/3	1/2	2/3	1/1	3/2	3/1
Group 1						
Mean weight (g)	0.2129	0.2226	0.2291	0.2355	0.2472	0.2292
Mean standard deviation	0.0225	0.0305	0.0258	0.0317	0.0495	0.0805
Mean number of pulverizations	16	13	8	6	3	2
Group 2						
Mean weight (g)	/	0.4856	0.4209	0.4148	0.3713	0.4708
Mean standard deviation	/	0.1346	0.0955	0.0827	0.0798	0.0520
Mean number of pulverizations	0	2	5	5	5	3
Group 3						
Mean weight (g)	0.6490	0.7025	0.6671	0.6240	0.6257	0.6634
Mean standard deviation	0.0499	0.0256	0.0429	0.0667	0.0840	0.0407
Mean number of pulverizations	4	6	8	11	14	19

polypeptides and proteins in reverse micelles formed from a combination of two or more surfactants. By controlling the water to surfactant molar ratio, the amount of polypeptide or protein could be controlled, thereby providing an accurate dosing mechanism. In addition, controlling the molar ratio of water to surfactant also could adjust the size and the shape of the reverse micelles which affected the degree and rate of penetration of the lung mucous for delivery of the drugs to the patient's blood stream.

3.2. Assessment of the solubility of PFOB in the propellants

Pulverization weight uniformity and valve content uniformity assays have shown homogeneous results with both propellants (Tables 6 and 7): standard deviation (S.D.) of the mean weight after pulverization and mean valve content did not exceed 0.08 and 0.12, respectively. Fig. 3 shows the variation of the mean weight after pulverization as a function of PFOB/propellant

Table 5
Solubility of water in Solkane® 134a: valve content uniformity

Water/propellant ratio	1/3	1/2	2/3	1/1	3/2	3/1
Group 1						
Mean content of the valve (g)	1.0543	1.0382	1.0341	0.9826	0.9430	0.9146
Mean standard deviation	0.0791	0.0796	0.0993	0.1013	0.0791	0.0503
Mean number of pulverizations	16	13	8	6	3	2
Group 2						
Mean content of the valve (g)	/	1.0007	1.0196	0.97102	0.9569	0.8758
Mean standard deviation	/	0.0612	0.0977	0.0716	0.0425	0.0906
Mean number of pulverizations	0	2	5	5	5	3
Group 3						
Mean content of the valve (g)	0.9430	0.8748	0.8591	0.7895	0.7825	0.7376
Mean standard deviation	0.0215	0.0691	0.0519	0.0699	0.0866	0.0511
Mean number of pulverizations	4	6	8	11	14	19

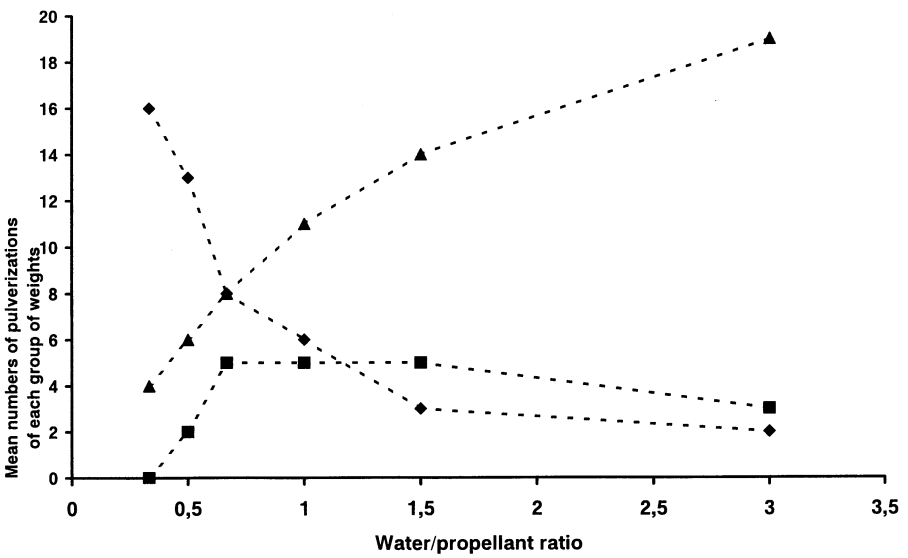

Fig. 2. Variation of the mean number of pulverizations of group 1 (…◆…), group 2 (…■…), and group 3 (…▲…) as a function of water/Solkane® 134a ratio.

Table 6
Solubility of PFOB in Solkane® 227: uniformity of weight and valve content after pulverization

PFOB/propellant ratio	1/3	1/2	2/3	1/1	3/2	3/1
Mean weight (g)	0.481	0.690	0.775	0.942	1.079	1.289
Mean standard deviation	0.012	0.039	0.061	0.069	0.067	0.030
Mean content of the valve (g)	1.456	1.439	1.489	1.437	1.453	1.500
Mean standard deviation	0.062	0.067	0.106	0.095	0.061	0.043
Mean number of pulverizations	20	19	19	19	19	18

Table 7
Solubility of PFOB in Solkane® 134a: uniformity of weight and valve content after pulverization

PFOB/propellant ratio	1/3	1/2	2/3	1/1	3/2	3/1
Mean weight (g)	0.5258	0.7030	0.7972	0.8735	0.9916	1.1286
Mean standard deviation	0.0474	0.0555	0.0707	0.0651	0.0576	0.0545
Mean content of the valve (g)	1.1916	1.3285	1.3369	1.2686	1.3353	1.2871
Mean standard deviation	0.1122	0.0976	0.1014	0.0857	0.0533	0.0608
Mean number of pulverizations	21	19	20	23	22	24

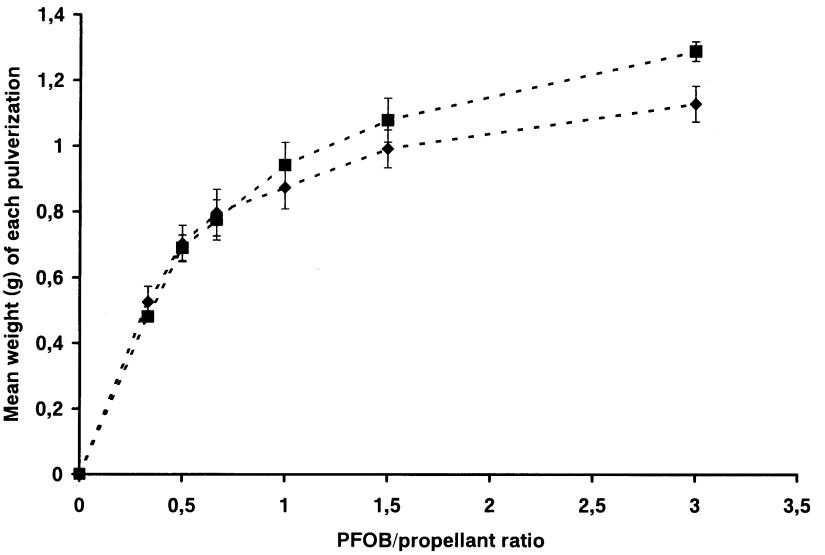

Fig. 3. Mean weight (g) of PFOB for successive pulverizations as a function of PFOB/propellant ratio, for Solkane® 227 (…■…) and Solkane® 134a (…◆…).

Table 8
Miscibility of the water-in-FC emulsion in Solkane® 227: uniformity of weight and valve content after pulverization

Emulsion/propellant ratio	1/3	1/2	2/3	1/1	3/2	3/1
Mean weight (g)	0.3462	0.5507	0.7290	0.8020	0.8787	1.1008
Standard deviation	0.0280	0.0386	0.0600	0.0410	0.0458	0.1050
Mean content of the valve (g)	1.2949	1.3633	1.3890	1.3900	1.4061	1.4797
Standard deviation	0.0630	0.0618	0.0232	0.0720	0.0968	0.0895
Number of pulverizations	20	20	22	20	20	22

ratio. It can be seen that the curves have identical exponential profiles for both HFAs, and are nearly superimposable. This shows that PFOB (the external phase of the emulsion) is completely miscible with both Solkane® 227 and 134a, and that each pulverization delivers almost the same amount of PFOB, which depends on the PFOB/propellant ratio (Fig. 3)

3.3. Assessment of the solubility of the reverse fluorocarbon emulsion in the propellants

Homogeneous results in terms of pulverization weight uniformity and valve content uniformity were obtained for both propellants with the reverse water-in-FC emulsion (Tables 8 and 9). S.D. of the mean weight after pulverization and mean

valve content did not exceed 0.11 and 0.15, respectively. Fig. 4 shows the variation of the mean weight after pulverization as a function of the emulsion/propellant ratio. As for PFOB (see above section), it can be seen that the curves are nearly superimposable with the same exponential profiles, allowing to conclude that the water-in-FC emulsion is totally miscible with both propellants. Each pulverization delivers almost the same amount of emulsion that depends on the emulsion/propellant ratio (Fig. 4). Solkane® 134a having, however, a tendency to form foam during pulverization, Solkane® 227 was used for the encapsulation of cafeine (see Section 3.4).

3.4. Cafeine encapsulation: pulverization content uniformity assay with Solkane® 227

Fig. 5 shows the variation of the measured of cafeine monohydrate solubilized in the water droplets as a function of the emulsion/propellant ratio. It can be seen that no significant variation could be observed between the experimental points and the theoretical curve. As for PFOB and water-in-FC emulsion, a curve with an exponential profile was obtained. Each pulverization delivered almost the same amount of cafeine, and this amount depends on the emulsion/propellant ratio (Fig. 5).

Table 9
Miscibility of the water-in-FC emulsion in Solkane® 134a: uniformity of weight and valve content after pulverization

Emulsion/propellant ratio	1/3	1/2	2/3	1/1	3/2	3/1
Mean weight (g)	0.5095	0.6369	0.7169	0.8968	0.9900	1.1604
Standard deviation	0.0434	0.0757	0.0502	0.0763	0.0758	0.0602
Mean content of the valve (g)	1.2546	1.2531	1.2030	1.3297	1.2865	1.2682
Standard deviation	0.1185	0.1216	0.0832	0.1062	0.0829	0.0634
Number of pulverizations	20	20	20	20	23	23

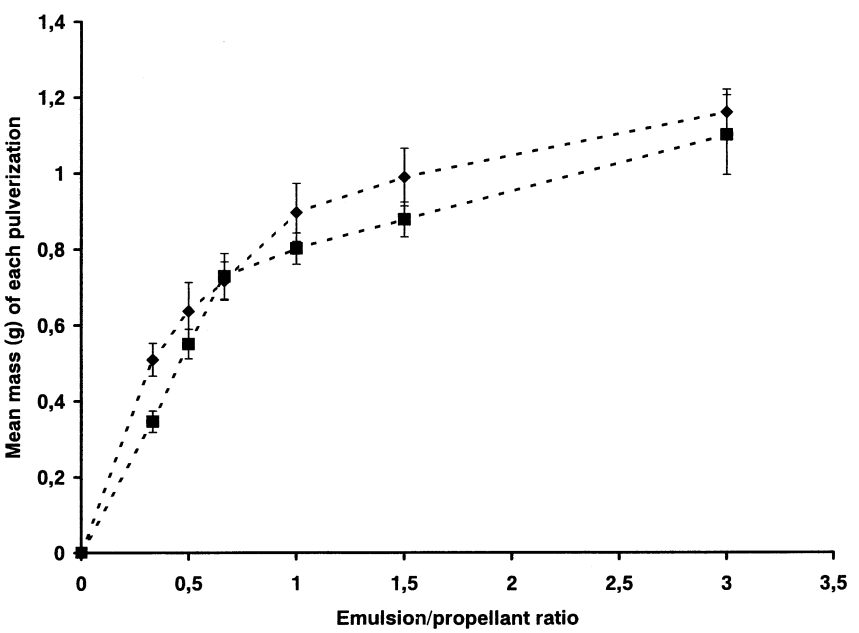

Fig. 4. Mean weight (g) of water-in-FC emulsion for successive pulverizations as a function of emulsion/propellant ratio, for Solkane® 227 (…■…) and Solkane® 134a (…◆…).

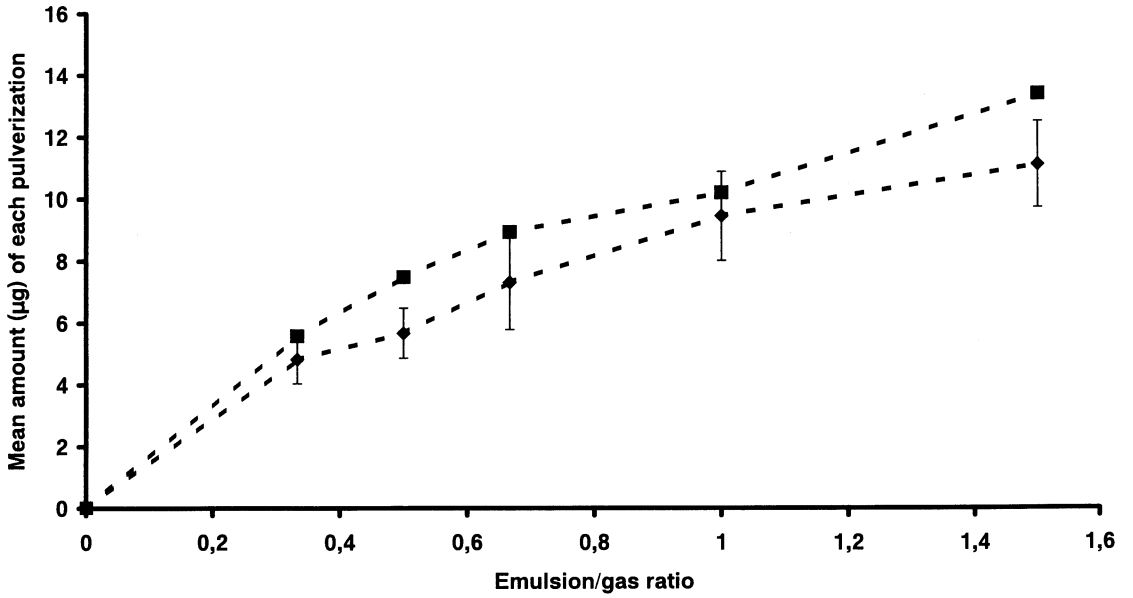

Fig. 5. Experimental (…◆…) and theoretical (…■…) mean amount of cafeine (µg) for successive pulverization as a function of emulsion/Solkane® 227 ratio.

These results indicate that the reverse water-in-FC emulsion allowed to deliver cafeine in a reproducible and homogeneous way, and that the development of such reverse FC emulsion packaged in a pressurized metered-dose inhaler can be contemplated for the administration of hydrophilic drugs.

3.5. Mean diameter of the emulsion droplets size after pulverizations

The mean diameter of the emulsion droplets after preparation was measured to be 57 ± 6 nm. Tables 10 and 11 show the variation of the mean diameter of the droplets as the function of successive pulverizations for both propellants. Results clearly showed that the reverse emulsion was more stable, i.e. the growth of the droplets was significantly reduced, with Solkane® 227 than in Solkane® 134a. This can be explained by the fact that molecular diffusion (Ostwald ripening), the main degradation mechanism of water-in-FC emulsion (Sadtler et al., 1996), depends on the intermolecular interactions of the dispersed phase. The weaker these interactions, the faster the aging rate.

For both propellants, the mean diameter of the droplets increased with successive pulverizations. For the above reason, however, the extent of the destabilization was higher for Solkane® 134a than for Solkane® 227. Finally, a strong effect of the emulsion/propellant ratio was also observed on the emulsion stability, the higher this ratio, the smaller the emulsion droplets after pulverization. Best results were obtained for the 3/2 ratio for which the mean diameter of the emulsion droplets was 122 nm only (i.e. very fine droplets), for the last set of pulverizations.

Table 10
Mean diameter of the water droplets in the reverse water-in-FC emulsion after pulverization with Solkane® 227

Emulsion/propellant ratio–[Pulverizations]	Mean size (nm $\pm$ 10%)
2/3–[1–6]	145
2/3–[7–12]	156
2/3–[13–18]	204
2/3–[19–21]	497
3/2–[1–6]	75
3/2–[7–12]	79
3/2–[13–18]	87
3/2–[19–24]	122

Table 11
Mean diameter of the water droplets in the reverse water-in-FC emulsion after pulverization with Solkane® 134a

Emulsion/propellant ratio–[Pulverizations]	Mean size (nm ± 10%)
2/3–[1–6]	202
2/3–[7–12]	309
2/3–[13–18]	329
2/3–[19–21]	728
3/2–[1–6]	156
3/2–[7–12]	157
3/2–[13–18]	134
3/2–[19–24]	378

Studies of the shelf stability of the water-in-FC reverse emulsion packaged in a pMDI are presently underway. Preliminary results show that, when packaged with a 3/2 emulsion/Solkane® 227 ratio, the average diameter of the water droplets was 122 ± 15 nm when measured immediately after packaging, and 200 ± 20 nm after 1 week in the pressurized canister stored at room temperature.

4. Conclusions and perspectives

This study allowed to optimize the critical packaging parameters for a reverse water-in-FC emulsion in a pressurized metered-dose inhaler (pMDI) loaded with hydrofluoroalkane propellants (Solkane® 227 and 134a). Best results were obtained with Solkane® 227 for which no sealing or leakage problems have been encountered, and that does not require use of a particular, compatible polymer coating the inner walls of the canisters.

We have shown that a reverse water-in-FC emulsion packaged in a pMDI containing Solkane® 227 can be successfully used to deliver cafeine in a homogenous and reproducible way. Homogenous results were obtained in assays of pulverization weight uniformity and valve content uniformity. It is noteworthy that it was not possible to form the emulsion spontaneously by simply introducing the fluorinated surfactant (F8H11DMP) with a mixture of water and propellant into the canister, which means that neither propellants can act as a substitute for the external FC phase of the emulsion, and that the use of the reverse water-in-FC emulsion is mandatory. It was determined that a high emulsion/propellant ratio ($\geq 3/2$) allowed to maintain a mean droplet size that was close to the initial emulsion droplet size. Compositions with a low emulsion/propellant ratio ($\leq 2/3$) showed improved content uniformity, to the expense, however, of the reproducibility of the droplets size. Promising preliminary results have shown that the emulsion droplets in the pressurized canister stored for 1 week at room temperature remained fine and narrowly dispersed (200 ± 20 nm). Shelf stability studies are underway.

This study allows to contemplate reverse water-in-FC emulsions packaged in a pMDI containing HFA (Solkane® 227) as a promising delivery system designed of drugs like enzymes (human recombinant desoxyribonuclease (rhDNAse), proteins, peptides (insulin, α-interferon, calcitonin, immunoglobulin, antibiotics) and genetic material to the lungs either for systemic or local administration. These active agents can, for example, be protected from proteolytic degradation by some inhibitors of proteases present at pulmonary sites.

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