Stability of Nonaqueous Suspension Formulations of Plasma Derived Factor IX and Recombinant Human Alpha Interferon at Elevated Temperatures

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Purpose. To identify a suitable nonaqueous, parenterally acceptable suspending vehicle whereby a therapeutic protein is delivered as a stable flowable powder, making it amenable to delivery from sustained delivery systems maintained at body temperature.

Methods. Formulations of plasma derived Factor IX (pdFIX) and recombinant human alpha interferon (rhα-IFN) were formulated as dry powders, suspended in various vehicles (perfluorodecalin, perfluorotributylamine, methoxyflurane, polyethylene glycol 400, soybean oil, tetradecane or octanol) and stored at 37°C. Stability was assessed by size exclusion chromatography, reverse phase chromatography, ion exchange chromatography, and bioassay, and was compared to the stability of dry powder formulations stored at 37°C and −80°C.

Results. PdFIX was stable when stored at 37°C as a dry powder, or when the dry powder was suspended in the pharmaceutically acceptable vehicles perfluorodecalin or perfluorotributylamine. Suspensions of the powder in other pharmaceutically/parenterally acceptable vehicles such as soybean oil or PEG 400 resulted in aggregation and loss of bioactivity. A dry powder formulation of rhα-IFN suspended in perfluorodecalin was also stable at 37°C.

Conclusions. This study shows the potential utility of perfluorinated hydrocarbons as nonaqueous suspending vehicles for long term in-vivo delivery of therapeutic proteins.

KEY WORDS: perfluorodecalin; nonaqueous suspension; protein; alpha interferon; factor IX; stability.

INTRODUCTION

For convenience and ease of administration, it is most desirable to formulate a given therapeutic protein as an aqueous solution. Unfortunately, when stored as aqueous solutions many proteins are susceptible to chemical degradation via a number of reactions including deamidation of asparagine and glutamine residues, oxidation of methionine (and to a lesser degree tryptophan, tyrosine and histidine) residues, hydrolysis of peptide bonds, disulfide interchange, and the racemization of chiral amino acid residues; and to physical denaturation, manifested by aggregation and/or precipitation (1,2). Since water is a participant in the mechanisms of all of the above transformations, reduction of the solution to a dry powder via lyophilization or spray drying can often enhance the stability of protein pharmaceuticals. However dried proteins (in general) must be reconstituted prior to use, thereby adding additional steps to dosing procedures and exposing the protein to potentially destabilizing conditions (3). In addition, it may be advantageous to administer therapeutic proteins in flowable form for extended periods of time (at either room temperature or body temperature), to allow their use in systemic drug delivery devices such as implantable pumps, as topical therapeutic agents, or as suspensions for inhalation. In most of these cases the protein must be in a formulation capable of flow, making a solid or a simple dry powder formulation unacceptable.

Other researchers have investigated dispersing powdered proteins or peptides in oleaginous vehicles to yield parenteral sustained release formulations (4–7). The vehicles used in these studies were either various vegetable (sesame, soy, peanut etc.) or synthetic (miglyol) oils gelled with aluminum mono-fatty acid esters such as aluminum monostearate, or with a polyglycerol ester. Although theoretically these vehicles might preclude solution denaturation and protect the drug from aqueous chemical degradation, the vehicles themselves are, to limited degrees, hydrophilic and unstable at higher temperatures. For example, the storage of liquid vegetable oils at elevated (physiological) temperatures can result in the formation of reactive species such as free fatty acids and peroxides (a process which is accelerated by the presence of traces of various metal ions such as copper or iron). Therefore the formulation of nonaqueous protein suspensions using these vehicles could require the inclusion of an antioxidant, many of which can adversely affect protein stability (8,9). In addition, the partial hydrophilicity of such vehicles could make it diffficult to guarantee that water mediated degradation and/or denaturation processes would not occur in environments where contact with and imbibement of water is possible.

It has been shown that several enzymes can function in a variety of organic solvent systems: aqueous-organic mixtures (10,11), organic solvents saturated with water (12,13), and anhydrous organic solvents (14,15). In attempting to maintain enzymatic activity at elevated temperatures, Klibanov and coworkers have shown that certain enzymes can be suspended as powders in anhydrous organic solvents and retain biological activity. In these studies, it was observed that the activity of chymotrypsin in organic solvents correlates with the amount of water retained by the enzyme in those solvents: the more water, the greater the enzymatic activity (15). With high enzyme hydration levels (> 2%) and/or the addition of low molecular weight protic solvents, these enzymes can have enough conformational mobility to exhibit appreciable enzymatic activity. However, the high hydration levels and/or the protic solvents used in these studies can result in unacceptable stability of generally labile protein drugs. Moreover, none of the organic solvents allowing the retention of the activity of select enzymes have been approved for pharmaceutical/parenteral use. Therefore a need exists to identify a suspending vehicle for therapeutic proteins which will provide sufficient stability of a suspended protein drug powder to allow long term delivery in an in vivo environment and which is acceptable for parenteral use. Examples examined here include perfluorodecalin and perfluorotributylamine, both of which are currently approved for parenteral use as artificial blood substitutes.

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MATERIALS AND METHODS

Perfluorodecalin, perfluorotributylamine, and n-octanol were purchased from Aldrich Chemical Company (Milwaukee, WI). Methoxyflurane was purchased from Abbot Laboratories (North Chicago, IL). Polyethylene glycol 400 was purchased from Spectrum Chemical Corp. (Gardena, CA). Super refined soybean oil was obtained from Croda Inc. (New York, NY). Recombinant human Alpha Interferon (rhα IFN), expressed in Escherichia coli, was purchased from Scitech Genetics Ltd. (Singapore), and was greater than 90% pure as determined by reverse phase and ion exchange chromatography. Plasma fractionated Factor IX (pdFIX) was purchased from Calbiochem-Novabiochem (San Diego, CA), and was greater than 98% pure as determined by size exclusion chromatography.

pdFIX Size Exclusion Chromatography (SEC)

Solutions of pdFIX were assayed for protein content and high molecular weight aggregates by size exclusion chromatography. Analyses were performed on a Hewlett Packard HP-1090 (Hewlett Packard, Palo Alto, CA) system using a ToSoHaas TSD G3000 swxl column (ToSoHaas; Montgomeryville, PA). The mobile phase consisted of 50 mM dibasic sodium phosphate, 150 mM sodium chloride buffered to pH 7. The mobile phase flow rate was 1 ml per minute and peaks were detected at 210 nm. A pdFIX reference standard solution was prepared, and its protein content calculated from the absorbance measurement at 280 nm (after subtracting scatter at 320 nm) assuming an A_{280} (of a 1% solution) of 13.3 (16). Three dilutions of this solution, representing 80%, 100% and 120% of the expected concentration of pdFIX in the samples, were run in triplicate at the beginning, middle and end of each run and used to calculate total protein content of the samples.

pdFIX Clotting Bioactivity Assay

Samples of pdFIX were assayed for bioactivity, using the one stage activated partial thromboplastin time assay of Biggs (17), on a Coag-A-Mate X2 photo-optical clot detection system (General Diagnostics Products, Oklahoma City, OK).

rhα-IFN Reverse Phase Chromatography (RP-HPLC)

Analyses were performed on a Hewlett Packard HP-1090 (Hewlett Packard, Palo Alto, CA) system using a Delta-Pak C-18 column (Waters Corporation), and gradient elution using mobile phase A of 70/30/0.2 water/acetonitrile/trifluoroacetic acid, mobile phase B of 20/80/0.2 water/acetonitrile/trifluoroacetic acid, a flow rate of 0.3 ml per minute, and quantitation at 210 nm. The gradient started at 25% B upon injection, increased to 35% B over 45 minutes, to 52% B from 45 to 55 minutes, and to 90% B from 55 to 90 minutes. Peaks were quantitated using an rhα-IFN stock as described for pdFIX above.

rhα-IFN Ion Exchange Chromatography (IEX)

Analyses were performed on a Hewlett Packard HP-1090 (Hewlett Packard, Palo Alto, CA) system using a TSK gel SP-5PW column (ToSoHaas or equivalent) and gradient elution. Mobile phase A consisted of 20 mM Sodium Phosphate (monobasic), at pH 5.2, mobile phase B consisted of 20 mM Sodium Phosphate (monobasic) and 0.5 M Sodium Chloride, also at a pH of 5.2. The gradient started at 0% B upon injection, increased to 20% B over 30 minutes, to 50% B from 30 to 40 minutes, and to 100% B from 40 to 50 minutes. The flow rate was 1.0 ml per minute, with peaks being quantitated at 210 nm as described under reverse phase chromatography (above).

Sodium Dodecyl Sulfate Poly-Acrylamide Gel Electrophoresis (SDS-PAGE)

Solutions of rhα-IFN were analyzed by reducing and non-reducing SDS-PAGE for possible cleavage products and aggregates using 15% tri-glycine gels. Gels were run at 100V for approximately 4 hours, stained with Coomassie Blue R-250, and analyzed using a GS-700 Imaging Densitometer (Bio-Rad, Richmond CA).

Karl Fischer Moisture Analysis

The moisture content of lyophilized samples and organic vehicles was determined by Karl Fischer moisture analysis, according to the USP Coulometric Titration method (18).

rhα-IFN Bioassay

Concentrations of biologically active rhα-IFN were determined using a Vesicular Stomatitis Virus MDKB cell bioassay. Samples were analyzed by Biomedical Laboratories (West Caldwell NJ).

Stability of pdFIX Suspension Formulations at 37°C

Formulations of plasma derived Factor IX (pdFIX) were prepared containing 1 mg/ml pdFIX, 60 mg/ml mannitol, 60 mg/ml sucrose, 0.001 mg/ml polysorbate 80 and 1.55 mg/ml histidine, buffered to pH 6.8. One ml aliquots of the above solution were pipetted into 3 ml glass vials, covered with lyophilization stoppers, and loaded into the freeze drying chamber of an FTS Dura Stop MP tray dryer coupled with a Dura Dry MP condenser (FTS Systems, Stone Ridge, NY). Vials were equilibrated to −5°C prior to initiation of freezing at 45°C. After 2 hours at −45°C, the chamber pressure was reduced to 100 millitorr and the shelf temperature increased to 25°C at a rate of 4°C per hour. After approximately 20 hours terminal drying at 25°C the vials were stoppered under vacuum. The resultant powder had a final moisture content of less than 1% (w/w) water, as determined by Karl Fischer analysis. Suspensions were prepared in a dry nitrogen glove box by adding 1 ml of suspending vehicle to the vials containing the dry pdFIX powder, and gently shaking to disperse the lyophilized powder. Vials containing either pdFIX suspensions or pdFIX dry powder were then incubated in a dry heat oven at 37°C. Control vials of the lyophilized powder were stored at −80°C. Samples were pulled at 0, 2.5, 4.5, 6.5, 8.5, 12.5, and 24.0 weeks. Protein recovery was measured by adding 1 ml of Water for Injection USP to vials containing suspension formulations, and the suspended powder was allowed to partition into the aqueous phase overnight at 5°C. Vials containing lyophilized powder were reconstituted with 1 ml of Water for Injection USP, and stored overnight at 5°C. All samples were analyzed for pdFIX activity

by clotting bioactivity assay, and for physicochemical stability (aggregation formation) by size exclusion chromatography.

Stability of rhα-IFN Suspension Formulations at 37°C

Formulations containing 5 mg/ml rhα-IFN, 5 mg/ml sucrose, and 0.96 mg/ml citric acid, were prepared at pH 4.5. Aliquots containing 0.2 ml of the above solution were pipetted into 1 ml glass vials, which were then covered with lyophilization stoppers, loaded into the freeze-drying chamber, and lyophilized according to the cycle described above. After removal from the lyophilizer vials were sealed with aluminum seals with flip-off caps. The resultant powder had a final moisture content of 2.6% (w/w) water, as determined by Karl Fischer analysis. Suspensions were prepared by adding 0.3 ml of a given suspending vehicle to the vials containing the rhα-IFN powder in a dry nitrogen glove box, and gently shaking to disperse the lyophilized powder. Vials containing either rhα-IFN suspensions or rhα-IFN dry powder were then incubated in a dry heat oven at 37°C. Control vials of the lyophilized powder were stored at −80°C. Samples were withdrawn at 0, 2.7, 4.5, 6.5, 10.0, 12.5, 16, 32 and 52 weeks and analyzed for rhα-IFN content and degradation/denaturation by reverse phase chromatography, ion exchange chromatography and SDS-PAGE. Additional samples were withdrawn at 8 and 52 weeks and submitted for bioassay (Biomedical Laboratories, New Jersey).

RESULTS

1. Recovery of pdFIX from Nonaqueous Suspensions

The amount of pdFIX that partitioned from the nonaqueous suspension vehicles into the aqueous phase over approximately 18 hours was measured by size exclusion chromatography. Examination of the initial stability timepoints of Figures 1 and 2 show that the amount of water soluble pdFIX partitioning from perfluorodecalin, methoxyflurane, octanol, perfluorotributylamine or tetradecane suspensions into bulk buffer was essentially 100%, identical to the recovery of pdFIX when the dried powder was merely reconstituted with water.

2. Stability of pdFIX in Nonaqueous Suspensions

No significant differences in stability were observed between pdFIX stored as a dry powder at either −80°C or 37°C, or as a suspension of dry powder in perfluorodecalin (also at 37°C) by either size exclusion chromatography (Figure 1A) or clotting bioassay (Figure 1B). In no case were higher molecular weight aggregates observed, nor was any loss in bioactivity measured. For suspensions in soybean oil however, a greater than 80% decrease in protein content was measured after only 1.5 weeks at 37°, significant amounts of both higher and lower molecular weight species were detected by size exclusion chromatography, and gross precipitation was observed in the aqueous solution following partitioning of the protein from the oil phase. In addition, a water insoluble oil/protein gel formed after 4 weeks storage at 37°C, indicative of extensive polymerization of the protein and/or vehicle. Significant activity loss occurred in methoxyflurane suspensions after 6 weeks storage at 37°C (Figure 1B), even though recovery of water soluble protein was approximately 90% (Figure 1A). In the

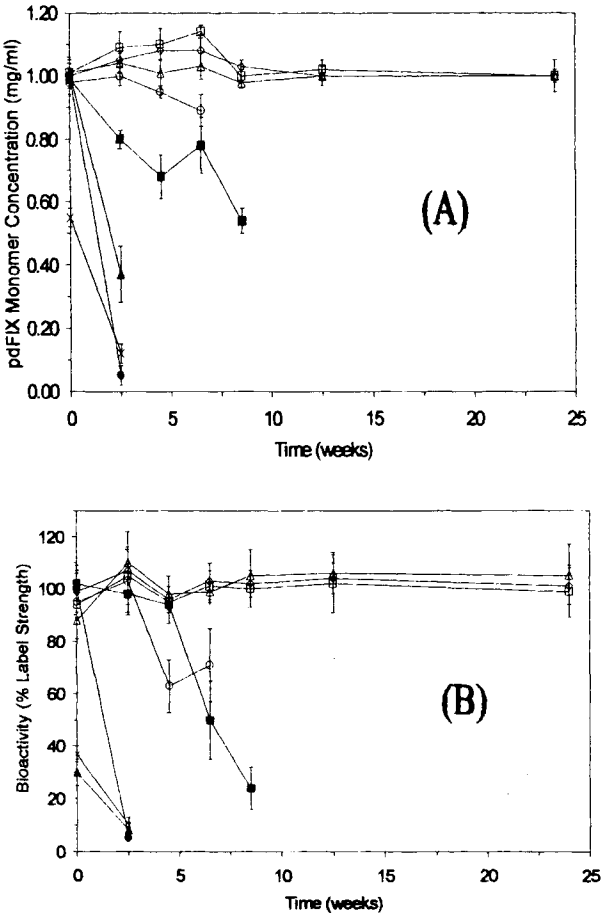

Fig. 1. Stability of pdFIX in aqueous solution, as a lyophilized powder, and as a suspension in water soluble and water insoluble vehicles as measured by (A) size exclusion chromatography, or (B) clotting bioassay. Key: (◇) lyophilized powder stored at −80°C; (□) lyophilized powder at 37°C; (△) perfluorodecalin suspension stored at 37°C; (○) methoxyflurane suspension stored at 37°C; (■) octanol suspension stored at 37°C; (▲) PEG 400 suspension stored at 37°C; (●) solution formulation stored at 37°C; (×) soybean oil suspension stored at 37°C. Target concentration of pdFIX monomer = 1.0 mg/ml. Each data point represents the mean ± standard deviation of 3 individual samples taken from 3 separate vials. % Label Strength = *measured pdFIX bioactivity (in IU/ml)* ÷ nominal pdFIX bioactivity (in IU/ml).

partially water soluble vehicle octanol, significant activity loss occurred after 8 weeks storage at 37°C (Figure 1B), and higher molecular weight aggregates were detected during size exclusion chromatography leading a loss of concentration of pdFIX monomer in the aqueous phase (Figure 1A). Additionally, there was visible precipitation of the protein in the aqueous phase after partitioning of pdFIX from octanol suspensions which were stored for 4 weeks at 37°C. Complete loss of activity and disappearance of soluble pdFIX was observed in both aqueous solution and PEG 400 suspension formulations after only 1.5 weeks at 37°C. In both cases (solution and PEG 400 suspension), a significant (~ 10%) amount of higher molecular weight aggregates were detected.

Good pdFIX stability was also measured in several inert, water insoluble suspending vehicles other than perfluorodecalin. No significant differences in stability between lyophilized

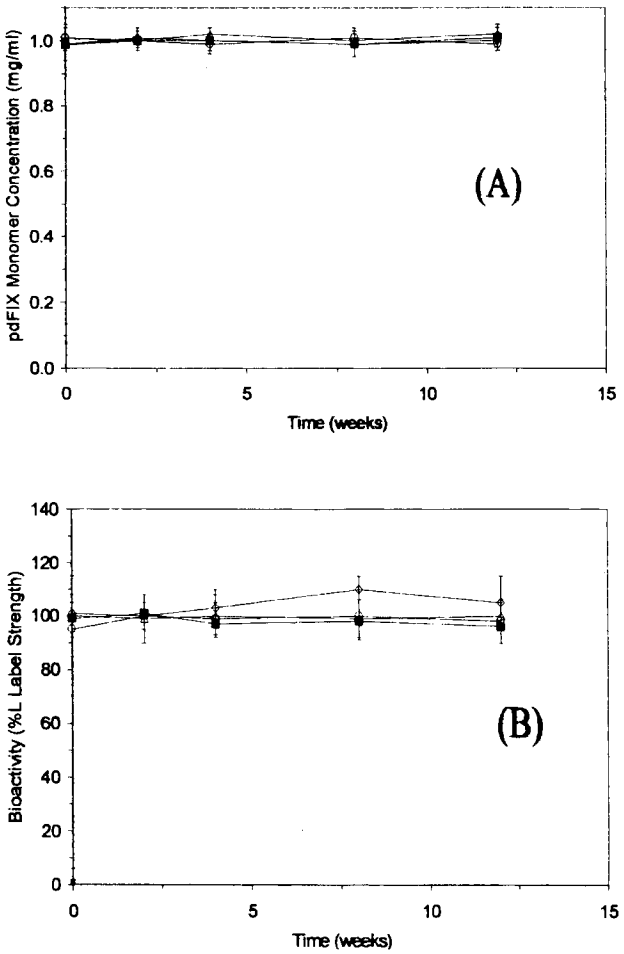

Fig. 2. Stability of pdFIX powder suspended in water immiscible vehicles and as a lyophilized powder stored at −80°C, as measured by (A) size exclusion chromatography, or (B) clotting bioassay. Key: (◇) lyophilized powder at −80°C; (△) perfluorodecalin suspension at 37°C; (○) perfluorotributylamine suspension stored at 37°C; (□) tetradecane suspension stored at 37°C. Each data point represents the mean ± standard deviation of 3 individual samples taken from 3 separate vials.

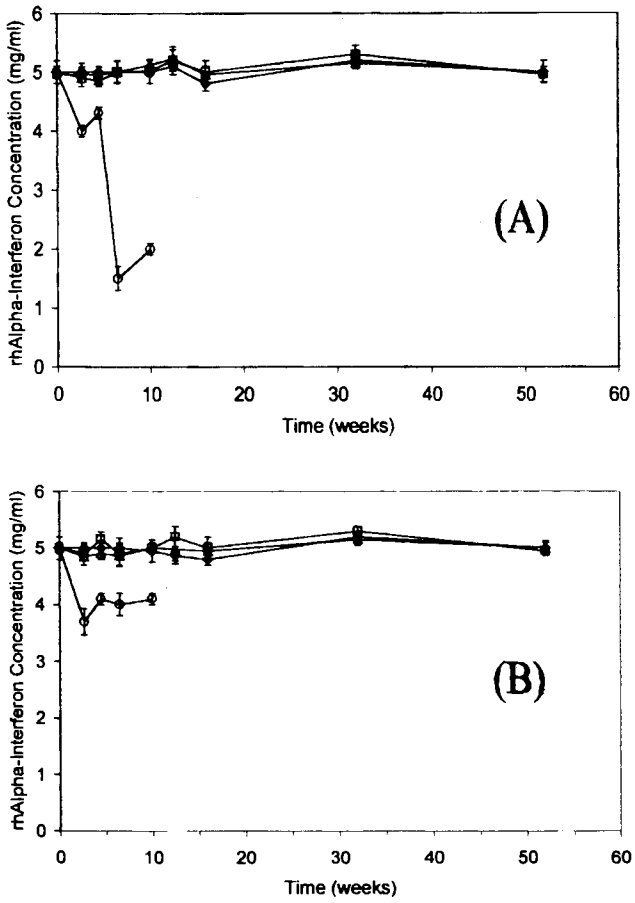

Fig. 3. Stability of rhα-IFN powder as measured by (A) reverse phase chromatography, or (B) ion exchange chromatography. Key: (◇) lyophilized powder stored at −80°C; (□)) lyophilized powder stored at 37°C; (△) perfluorodecalin suspension stored at 37°C; (○) methoxyflurane suspension stored at 37°C. Each data point represents the mean ± standard deviation of 3 individual samples taken from 3 separate vials.

powder stored at −80°C, lyophilized powder stored at 37°C, or lyophilized powder suspended in perfluorodecalin, perfluorotributylamine or tetradecane stored at 37°C were observed by either size exclusion chromatography (Figure 2A) or clotting bioassay (Figure 2B).

When pdFIX powder was equilibrated to 10% moisture content (rather than the <1% typical for normally lyophilized material) and subsequently incubated at 37°C for 1 week, neither the powder nor the perfluorodecalin suspension were stable as assessed by SEC or bioassay (Table I), with bioactivity decreasing to 47% and 35% of initial values in both the powder and suspension formulations respectively. High molecular weight aggregates (in amounts ranging from 20% to 25% of total peak area) were also measured by size exclusion chromatography in samples equilibrated to high moisture content.

3. Stability of rhα-IFN

To determine whether the high pdFIX stability measured in perfluorodecalin suspensions holds for other proteins, the stability of an interferon suspension was evaluated. Recombinant human alpha interferon suspended in perfluorodecalin at 37°C was found to be as stable as the same dry powder stored at 37°C or even at −80°C. Reverse phase (Figure 3A) and ion exchange HPLC analyses (Figure 3B) showed that no chemical degradation occurred after 52 weeks storage. Recombinant human α-IFN was less stable in the partially water soluble vehicle methoxyflurane, with 60% loss in soluble protein having occurred within 10 weeks. Bioassay results showed that no activity loss occurred after 12 months storage in perfluorodecalin at 37°C, with specific activity being equivalent between dry powder stored at −80°C and 37°C, and the suspension kept at 37°C (Table II). No bands other than the parent band were detected by SDS-PAGE (data not shown).

4. Suspension Vehicle Water Content

Water contents of suspending vehicles as employed in pdFIX and rhα-IFN stability studies, and after equilibration (at room temperature for 24 hours) with excess bulk water are shown in Table III. Water contents (as measured by Karl Fischer analysis) ranged from 0.0002 w/w% for perfluorocarbons to 4.3 w/w% for water saturated octanol.

Table I. Stability of pdFIX Lyophilized Powder Equilibrated to 10% (w/w) Moisture, as Measured by Size Exclusion Chromatography and Clotting Bioassay, After 1 Week Storage as a Powder at −80°C, as a Powder at 37°C, or as a Suspension of Powder in Perfluorodecalin at 37°C

Formulation	%LS by size exclusion chromatography	%LS by clotting bioassay
Powder stored at −80°C	98 ± 3	91 ± 23
Powder stored at 37°C	80 ± 3	53 ± 21
Powder suspended in perfluorodecalin and stored at 37°C	75 ± 4	65 ± 17

Note: Each data point represents the mean ± standard deviation of 3 individual samples taken from 3 separate vials.

DISCUSSION

The reduced stability exhibited by pdFIX in some of the vehicles studied can be explained by their properties. Methoxyflurane is a halogenated methyl ethyl ether, and as such it possesses a small net dipole moment and a fair degree of water solubility (19). These potentially high residual moisture levels can allow water mediated reactions or conformational changes to occur which could result in the observed activity loss of pdFIX. In addition, although as a class ethers are comparatively nonreactive and would seem good candidates for suspending vehicles, they form unstable peroxides upon storage—particularly when in contact with oxygen—and thus would be capable of degrading many proteins.

In the case of soybean oil, the cleavage and/or aggregation of pdFIX observed at the initial timepoint may be due to attack on the protein by peroxides that could have formed in the oil upon storage, or due to water mediated protein denaturation. On the other hand, both aggregation at the initial timepoint and the formation of an insoluble gel after four weeks at 37°C may be explained by the fact that soybean oil, like other pharmaceutically acceptable vegetable oils, is composed of a mixture of fatty acid glycerides. Such oils can contain aldehyde degradation products caused by attack of oxygen at the allylic positions of unsaturated fatty acid chains (20). The carbonyl groups of the aldehydic degradation products are able to react chemically with side chain amino groups of proteins, forming Schiff base adducts which in turn are able to undergo Amadori rearrangement to produce new carbonyl functional groups capable of forming a Schiff base with additional protein amino groups. Such reactions and subsequent rearrangements can therefore result in covalent cross-linking of proteins (21,22). In the case of polyethylene glycol 400 suspending vehicle, the instability of pdFIX could be due in part to oxidative attack on the protein by peroxides formed upon oxidation of the ether moieties during storage, or to water mediated reactions and/or aggregation due to the relatively high amount of water in the vehicle (Table III).

It would appear from the above discussion that the aggregation of pdFIX in soybean oil, methoxyflurane, and polyethylene glycol 400 could be caused by covalent reactions due to the presence of peroxides in these vehicles and/or, in the case of soybean oil, to the potential presence of aldehydes. Additionally, as alluded to above, all three of the "reactive" vehicles possess some degree of water affinity, with values measured prior to equilibration with bulk water ranging from 0.03 to 0.3 w/w% (Table III), allowing the possibility of water mediated reactions and/or conformational changes. In contrast, perfluorodecalin is incapable of peroxide or aldehyde formation and, as shown in Table III, has essentially no solvating power for water. These beneficial attributes are due in part to the large value of the carbon-fluorine bond strength (116 kcal/mol) and the shielding effect from the high electronegativity of the fluorine atoms (23).

Denaturation and aggregation of a protein suspension in contact with a given vehicle can be affected by the vehicle's water content, polarity (as measured by dielectric constant), and general solubilizing activity (as measured by Hildebrand solubility parameter). The results (Table III) show that the effects of vehicle water content and intrinsic solubilizing (i.e. denaturing) ability cannot readily be separated in that both actual (i.e. as employed in stability studies) and equilibrium water contents increase with either an increase in vehicle dielectric constant or solubility parameter, they nonetheless demonstrate a parallel trend between a vehicle's inability to interact

Table II. Stability of rhα-IFN, as Measured by Bioassay, After 12 Months Storage as a Dry Powder Stored at −80°C, as a Dry Powder Stored at 37°C, or as a Dry Powder Suspended in Perfluorodecalin Stored at 37°C

Formulation	rhα-IFN titer × 10^9 (units/ml)
Powder stored at −80°C	1.12 ± 0.43
Powder stored at 37°C	1.13 ± 0.88
Powder suspended in perfluorodecalin and stored at 37°C	1.40 ± 0.21

Note: Values represent the mean ± standard deviation of 20 samples.

Table III. Water Contents (Both Before and After Equilibration with Water), Dielectric Constant (ε), and Hildebrand Solubility Parameter (γ) of Suspending Vehicles Employed in Stability Studies

Vehicle	Pre-equilibrium water content of vehicle (%w/w)	Post-equilibrium water content of vehicle (%w/w)	ε	γ (cal cm^{-3})
Perfluorodecalin	0.0002	0.0003	1.82	6.54
Perfluorotributylamine	0.0002	0.0002	1.86	6.97
Tetradecane	0.0014	0.0035	2.01	8.33
Methoxyflurane	0.0300	0.0900	n.a	n.a.
Soybean Oil	0.0630	0.3200	n.a	8.45
Octanol	0.3200	4.3000	11.3	9.31
PEG 400	0.2600	miscible	n.a	10.0

with highly polar protein molecules (i.e. low dielectric constant and Hildebrand solubility parameter) and observed pdFIX stability. For example, considerable aggregation (with concomitant loss of activity) of pdFIX occurred when the powder was suspended in octanol. The instability of pdFIX in this vehicle, which does not form reactive peroxides or other species, can only be attributed to its relatively high hygroscopicity and/or polar solvent properties.

In the studies discussed above, it has been shown that powders suspended in perfluorodecalin were as stable as the dry powder stored under vacuum, however it was unclear whether an essentially anhydrous vehicle such as perfluorodecalin could increase the stability of an inherently unstable powder. From the results obtained with moist pdFIX powders, it is clear that while perfluorodecalin can maintain the stability of a dispersion of an inherently stable powder, it appears not to be able to stabilize an inherently unstable powder formulation.

The data presented thus far demonstrate the feasibility of using highly inert and water insoluble vehicles like perfluorodecalin to suspend pdFIX and maintain its stability at elevated temperatures. The data also indicate that this may extend to other proteins, such as rhα-IFN. Unlike pdFIX which is glycosylated, rhα-IFN isolated from Escherichia coli is non-glycosylated, and could be expected either to interact differently with perfluorocarbons or to partition differently from the organic to the aqueous phase. In fact it did neither, and perfluorodecalin was able to suspend rhα-IFN and maintain its stability at elevated temperatures for up to a year.

CONCLUSIONS

This study has shown the potential utility of perfluorinated hydrocarbons as nonaqueous suspending vehicles for proteins. Several rather unique physical and chemical properties of these molecules may make them ideal suspending agents for parenterally administered proteins, by precluding the formation of reactive species such as peroxides or aldehydes, which can occur in other parenterally acceptable vehicles during prolonged elevated temperature (37°C) storage, and by minimizing exposure to water in an aqueous environment. This combination of low water solubility in perfluorocarbons, low degree of interaction between perfluorocarbons and suspended materials (due in part to low vehicle polarity) and the chemically non-reactive and stable nature of these materials, and their parenteral acceptability, potentially allow their use as a novel and highly effective vehicles for sustained in vivo delivery of protein drugs.

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