Tetrafluoroethane (HFC 134A) Propellant-Driven Aerosols of Proteins

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Purpose. Develop metered-dose propellant-driven aerosols of proteins using tetrafluoroethane (HFC 134A) as propellant.

Methods. Proteins were lyophilized with the propellant-soluble surfactants Triton X-100, Triton X-405, Laureth-9, Brij-30, Nonidet-40, and diethylene glycol monoethylether and then charged with propellants.

Results. Small particle aerosols of the experimental protein bovine gamma globulin were produced. The fraction of aerosolized respirable-sized protein particles (<4–5 μm) increased after dispersion of particles in propellant with agitation by shaking. Scanning electron microscopy of respirable-sized protein aerosols demonstrated bead-like particles in grape-like clusters. Vigorous shaking of propellant-suspended particles for 2 minutes or more reduced the size of clusters and reduced the diameters of the protein-containing subparticles that constituted the clusters. A 50:50 ratio of HFC 134A and dimethylether (DME) propellants improved the respirability of protein aerosols compared to HFC 134A as the sole propellant. Protein/surfactant particles first dispersed in DME and then diluted in HFC 134A propellant most efficiently produced respirable-sized, propellant-driven, protein aerosols.

Conclusions. Metered-dose aerosols of respirable-sized proteins can be generated using HFC 134A and HFC 134A:DME blended propellants as an alternative to nebulized aqueous aerosols for delivering peptide-based pharmaceuticals to the respiratory tract.

KEY WORDS: propellant; aerosol; protein; tetrafluoroethane.

INTRODUCTION

Peptide and DNA-based therapeutics are gaining importance in the pharmaceutical industry (1,2). There is also an interest in alternative delivery methods that avoid painful parenteral injections, or that can target drugs to specific organ systems (3). The respiratory tract offers a large surface area for local and systemic delivery of protein and DNA pharmaceuticals (4,5). It also is a significant target organ for therapeutics aimed at respiratory infections, inflammatory diseases, and deficiencies (6–8). Pharmaceutical deliveries to the respiratory tract requires their aerosolization as small particles for inhalation. Propellant-driven aerosols generated by metered-dose inhalers (MDI) are a proven approach for delivering pharmaceuticals to the respiratory tract (9), and they are the preferred aerosolization method for nonhospital settings (10,11) . For the deepest penetration into the respiratory tract, aerosol particles should be of "respirable" size having aerodynamic diameters of less than 4–5 μm (12).

In previous work, we established that propellant-driven aerosols of functional proteins can be produced by lyophilizing propellant-soluble surfactants with proteins and then suspending them in propellant as reverse micelle-like particles. We have extended this approach to a range of proteins that include antigenically active bovine gamma globulin (BGG) (13), antigen-binding monoclonal antibody (14), whole bacterial vaccine particles (15), and DNA plasmid molecules (16). These aerosol systems employed dimethylether (DME) as the propellant. DME is an effective propellant (17) and is being used for diagnostic purposes targeted to the human respiratory system (18), but it is flammable (19) and not approved for human use by the FDA as a propellant for metered-dose inhalers. Tetrafluoroethane, also known as HFC 134A, is a nonflammable, low-toxicity (20), inhaler-propellant approved by the FDA for use with human medicinals. It is replacing chlorine-containing Freon propellants because of their ozone-damaging characteristics (21). Consequently, it was of interest to determine if we could prepare propellant-driven aerosols containing respirable-sized particles (<4–5 μm) of proteins using HFC 134A as propellant.

MATERIALS AND METHODS

Aerosol Preparations

Propellant-driven aerosols were prepared with bovine gammaglobulin (BGG) (Calbiochem-Behring, La Jolla, CA) as a large molecular weight model protein (≈150 kD). BGG as a sterile 50 mg/ml solution in saline was tested alone or combined with 25, 50, or 100 mg of one of the selected surfactants (100 mg/ml aqueous solutions). Each mixture was put into 20-mm-diameter, 10 ml, plastic-coated, aerosol vials (#WG-1045-005, Wheaton Coated Products, Mays Landing, NJ) and lyophilized for 36 to 48 hrs at a temperature of −4°C to remove water from the preparation (22). This procedure resulted in surfactant/protein preparations with residual water content of ≈1% or less (23). Lyophilized preparations were sealed and stored at −20°C until charged with propellant. Aerosol vials were "cold-filled" with 5 ml tetrafluoroethane (HFC 134A) propellant (local automotive outlet) or dimethylether (DME) (Aldrich, Milwaukee, WI) or their combination. Propellants were liquified by passage through a condenser chilled with dry ice and ethanol, measured, and placed into chilled vials. In most experiments, DME and HFC 134A gasses were passed through a filter containing calcium sulfate (Drierite, Hammond Drierite Co., Xenia, OH) and sodium borohydride (Eastman Kodak, Rochester, NY) prior to condensation into a liquid. This eliminated the possibility of any water vapor or peroxides in the propellants. The 20-mm diameter, metered-dose, aerosol valves (#MP-20CP) delivering 60 μl/actuation were kindly provided by Emson Research (Bridgeport, CT). Aerosol valves were crimped to the chilled propellant-containing vials as previously described (22).

Aerosol vials were warmed to room temperature and vortexed or shaken in a shaker/mill (#5100, Spex Industries, Metuchen, NJ) prior to use. Vials were agitated routinely prior to each actuation. One gram of 1-mm glass agitation beads was included in each aerosol vial when charged with propellant to improve the dispersion of BGG/surfactant particles when agitated in the propellant (24).

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Surfactants

The surfactants tested for solubility in HFC 134A propellant and their usefulness in preparing aerosols of BGG included: Arlacel-A [mannide monooleate] (Atlas Chemical Industries, Wilmington, DE); Triton X-100 (Biorad, Richmond, CA); and all of the following from Sigma Chemical Co., St. Louis, MO: Aerosol OT [AOT][sodium-bis(2 ethylhexyl) sulfosuccinate]; Brij 58 [Polyethylene 20 cetyl ether]; caprylic acid; deoxycholic acid; glycocholic acid; laureth-9 [polyoxyethylene 9 lauryl ether]; lethicin (Soy, #P3644); sodium lauryl sulfate; oleic acid; MOPS [3-(N-morpholino)propanesulfonic acid]; NP-40 [Nonidet P-40]; Sarcosyl [N-lauroyl sarkosine]; sorbitan trioleate [Span 85]; 5-sulfosalicylic acid; Triton X-405; Tween 20 [polyoxyethylenesorbitan monolaurate]; Tween 40 [polyoxyethylene sorbitan monopalmitate]; Tween 80 [polyoxyethylene sorbitan monooleate]; diethylene glycol monoethyl ether; diethylene glycol monpentyl ether; heptanoyl-n-methyglucamide [Mega-7]; cetylpyridium chloride; trialkyammonium bromide; N-octyl β-D glucopyranoside; N-octyl β-D thioglucopyranoside; polyoxyethylene W-1; and polyoxyethylene 4 lauryl ether [Brij 30].

Aerosol Sampling

Aerosolized BGG was assessed by two approaches. Total aerosolized BGG was quantitated by actuating aerosol releases through an A-2 actuator (Emson) that had a 22g needle affixed in its orifice into a 12 × 75-mm glass tube with two layers of parafilm covering its open end. The actuator's needle tip was inserted through the parafilm layer of the horizontally held tube. Two to four actuations were delivered into the tube. The pressure of expanding propellant was relieved by the expansion of the parafilm tube cover.

Particle size distributions of aerosolized BGG was determined by actuating aerosols into a previously described chamber (22). Ten to 30 actuations were released into the chamber through an Emson A-7 model aerosol actuator (3.81 mm depth, 0.330 mm orifice) at 30-second intervals with agitation between each actuation. Air flow through the chamber, as well as that for aerosol sampling, was provided by a vacuum pump attached to a Mercer 7-Stage Impactor (Intox, Albuquerque, NM). It fractionated particles between 12 μm and 0.6 μm in aerodynamic diameter into seven stages when air was drawn through both the impactor and chamber at 2.0 LPM (24). Aerosols in the chamber were sampled for an additional 10 minutes after the final aerosol actuation. Aerosol particles were captured at each stage on a 22-mm disc punched from a double layer of parafilm that was seated on each stage. The final end-filter (stage 8) of the impactor was a 0.8 μm pore-sided, 25-mm diameter, polyethersulfone filter (Supor 800, #60109, Gelman, Ann Arbor, MI). Impactor filters and discs were inserted into 12 × 75-mm tubes and analyzed directly for BGG content or eluted to recover the BGG. Alternatively, aerosols in the chamber were sampled with a Casella cyclone (BGI, Waltham, MA) at a flow rate of 2.2 LPM, with particles <4μm aerodynamic diameter (12) captured on a Supor 800 filter.

Quantitation of Aerosolized BGG Protein

Bovine gamma globulin in aerosols was quantitated using the bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL) by adding 2.0 ml BCA reagent to each tube followed by a 30 min incubation at 37°C. The developed reagent was mixed to ensure homogeneity, and two 100 μl samples were transferred into a polystyrene, 96-well, ELISA, microtiter plate. Optical density at 570 nm was determined with a Bio-Tek Model EL-309, microtiter, plate spectrophotometer (Bio-Tek, Winooski, VT). Duplicate readings for samples were averaged, and BGG protein quantities determined with a standard curve produced by a titrated set of known BGG standards.

Electron Microscopy

Tetrafluoroethane propellant-driven, metered-dose, BGG aerosols were released into the aerosol chamber and sampled with the cyclone containing a 25-mm 0.2 μm pore-sized polycarbonate filter (#GTTP02500, Milipore, Bedford, MA). Filters were mounted, fixed, and coated with gold, and examined with a Hitachi H300 Scanning Electron Microscope with a 3010 image scanning processor as previously described (13). Particle sizes were measured manually on photomicrographs and calculated based on the calibrated bar on each photomicrograph.

RESULTS

Surfactants Soluble in HFC 134A

We have established previously that successfully suspending proteins in the desired propellant was dependent on the surfactant having some solubility in that propellant. Surfactants were screened for their solubility in HFC 134A (Table 1). Seven of the 27 tested had a solubility of 10 mg/ml or more in HFC 134A propellant. Diethylene glycol monopentylether suspended BGG in HFC 134A; however, its high cost ($45/gm) relative to other surfactants (<$0.50/gm) discouraged its further analysis.

Table 1. Solubility of Surfactants in HFC 134A Propellant[a]

Soluble	Partially soluble	Insoluble
Laureth 9	Tween 20	Sarkosyl
Triton X-100	Tween 40	Glycocholic Acid
Brij 30	Span 85	MOPS 3
Nonidet 40	Arlacel A	Mega 7
Triton X-405	Oleic Acid	Brij 58
Diethylene glycol monopentyl ether		Deoxycholic Acid
Diethylene glycol monoethyl ether		N-octyl β-D thioglucopyranoside
		N-octyl β-D glucopyranoside
		AOT
		Sodium Lauryl Sulfate
		W-1
		5-sulfosalicilic acid
		Trialkylammonium bromide
		Cetylpyridium chloride
		Lethicin (Soy)

[a] 50 mg of surfactant (See Methods for alternative names for surfactants) was placed in an aerosol vial, which was cold-filled with HFC 134A propellant, capped, warmed to room temperature, and vortexed, and then assessed for solubility. Partial solubility <10 mg/ml was determined by rechilling vials and checking for turbidity from cold-induced loss of solubility.

Bovine Gamma Globulin Protein Aerosols Using HFC 134A as Propellant

Table 2 shows that total aerosolized protein was similar for BGG alone and BGG combined with all tested surfactants over a fourfold increase in surfactant concentrations. BGG lyophilized without surfactant required shaking with glass beads to disperse it as a suspension in HFC 134A. Although BGG could be dispersed sufficiently to be aerosolized, only 4% was respirable. In contrast, the percentage of respirable-sized (<4μm diameter, by cyclone) BGG formulated with 50 or 100 mg of the surfactants was 10 to 15% at 2 mg of protein/ml of propellant.

Prior studies using DME propellant demonstrated that shaking propellant suspensions of protein/surfactants for 5 minutes in a shaker/mill resulted in a greater proportion of aerosolized protein particles being of respirable size (23) . It was important to determine if the 5-minute shaking treatment used for all aerosols shown in Table 2 was essential for making BGG aerosols prepared with HFC 134A propellant. Identical aerosols, one shaken and one not, were formulated with surfactants Laureth-9, NP-40, and Brij 30, and they were analyzed for their aerosol particle distributions by sampling with a Mercer impactor. Fig. 1 shows a typical BGG protein particle-size distribution for BGG/Laureth-9; the distribution after shaking showed a modest 12% improvement in respirability. Shaking increased respirable BGG by 12% and 4%, respectively, in shaken NP-40 and Brij 30-containing aerosols (not shown). The peak of aerosolized BGG particles was in the respirable 2.5–5 μm size range for all aerosols.

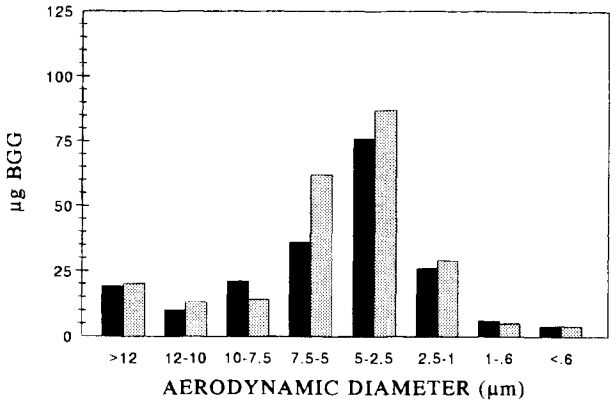

Fig. 1. Comparison of unshaken and shaken BGG aerosols using HFC 134A propellant. Ten mg of BGG lyophilized with 50 mg of Laureth-9 was charged with 5 ml of HFC 134A and 1 g of glass beads. The aerosol was vortexed after warming. Total released protein was determined by duplicate actuations in triplicate glass tubes. Fifteen aerosol actuations were released into the chamber while sampling with the Mercer impactor. An identical aerosol was shaken for 5 minutes and sampled. **Unshaken** (black bars), total aerosol-83 ± 2 μg/actuation, total impactor-198 μg, respirable-112 μg(10%). **Shaken** (white bars) total aerosol-81 ± 2 μg/actuation, total impactor-234 μg, respirable-125 μg(10%).

Table 2. Propellant-Driven Aerosols of Bovine Gammaglobulin Protein Using HFC 134a as Propellant

Surfactant (mg)	Aerosolized protein (μg)/ actuation ± S.D.[a] Total	Respirable	Respirable
None	86 ± 5	3.3 ± 0.4	4%
Triton-X100 (25)	87 ± 2	4.1 ± 0.7	5%
(50)	80 ± 1	6.4 ± 1.0	8%
(100)	79 ± 2	7.8 ± 0.7	10%
Laureth-9 (25)	93 ± 3	4.9 ± 0.4	5%
(50)	85 ± 2	9.2 ± 0.5	11%
(100)	80 ± 3	8.1 ± 0.4	10%
NP-40 (25)	82 ± 2	7.9 ± 0.6	10%
(50)	76 ± 2	10.3 ± 0.8	14%
(100)	75 ± 2	7.9 ± 0.3	11%
Brij 30 (25)	84 ± 11	11.0 ± 1.6	13%
(50)	76 ± 2	11.3 ± 0.1	15%
(100)	67 ± 7	6.0 ± 0.1	9%
Triton X-405 (25)	82 ± 4	5.5 ± 0.7	6%
(50)	85 ± 2	9.2 ± 0.1	11%
(100)	90 ± 2	9.1 ± 0.2	10%
Diethylene glycol monoethyl ether (25)	67 ± 4	5.5 ± 0.1	8%
(50)	55 ± 2	7.6 ± 0.4	14%
(100)	81 ± 3	12.9 ± 0.1	15%

[a] 10 mg of BGG protein was mixed with the noted mg of surfactants in 5 ml of water and lyophilized. Aerosol vials were charged with 5 ml of HFC 134A propellant. Aerosol vials contained 1 g of glass beads and were shaken mechanically for 5 min. Total aerosolized protein was determined by two actuations directly into tubes. Respirable-sized protein was determined by averaging duplicate deliveries of 15 actuations into an aerosol chamber while sampling by cyclone. S.D. = standard deviation.

We previously established that shaking DME propellant aerosols for more than 5 minutes failed to increase the respirability of the resulting aerosolized BGG particles. It was of interest to determine if shaking HFC 134A aerosols for less than 5 minutes would be equally effective in the formation of small particle BGG aerosols. Identical BGG/surfactant aerosols either were not shaken or shaken for 1, 2, or 5 minutes in a shaker mill, and each aerosol was sampled for total aerosolized BGG and respirable-sized BGG particles in a cyclone. Fig. 2 illustrates that 2 minutes of shaking for BGG/Brij-30/HFC 134A aerosol increased respirable BGG above the unshaken control ($p < 0.05$), as did 5 min. of shaking ($p < 0.001$). Five min. shaking of the Brij 30/BGG aerosol also significantly increased respirable BGG above that obtained with 2 min. of shaking ($p < 0.001$). However, similar experiments using aerosols formulated with Triton X-405, Triton X-100, and NP-40 surfactants did not result in significant differences between 2 min. and 5 min. of shaking (not shown). Together, these results indicated that shaking for 2 minutes was adequate to disperse most surfactant/BGG combinations in the propellant for subsequent dispersion as respirable-sized aerosol particles.

Particle Structure of BGG Protein Aerosols Generated with HFC 134A Propellant

Examination by scanning electron microscopy showed that metered-dose aerosols of proteins generated with DME propellant were composed of grape-like particle clusters (13,14) . It was of interest to determine if a similar particle structure would be produced when protein/surfactant particles were generated with the HFC 134A propellant. Fig. 3. shows an electron photomicrograph of BGG/NP-40 particles aerosolized with HFC

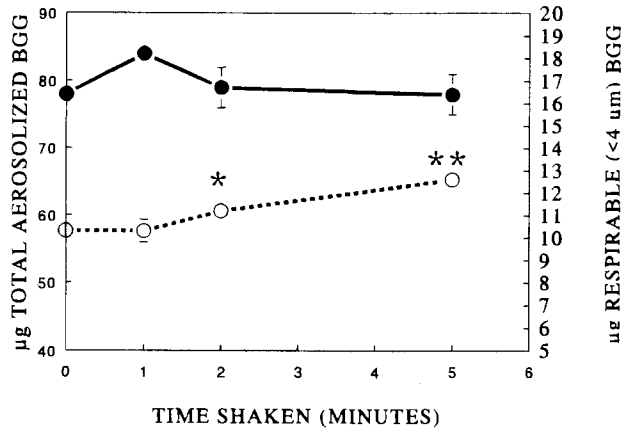

Fig. 2. Duration of shaking required to increase the respirability of 134A aerosolized BGG. Aerosols contained 50 mg of Brij 30 and 10 mg of BGG in 5 ml of 134A propellant. Separate aerosols for nonshaken, 1, 2, and 5 min. of shaking were aerosolized directly into triplicate tubes (two actuations/tube) to determine the total aerosolized protein/actuation (Left axis-solid lines and circles). Respirable protein/actuation (open circles, right axis), was determined by delivering 15 actuations into the chamber sampled by a Casella cyclone in duplicate determinations. Statistical analysis was performed using Instat biostatistical software (GraphPad Software Inc., San Diego, CA) using one way analysis of variance and the Student-Newman-Keuls multiple comparisons test of unshaken, 2 min. and 5 min shaken aerosols. * = different from unshaken ($p < 0.05$) ** = different from both unshaken and 2 min. shaken aerosols ($p < 0.001$).

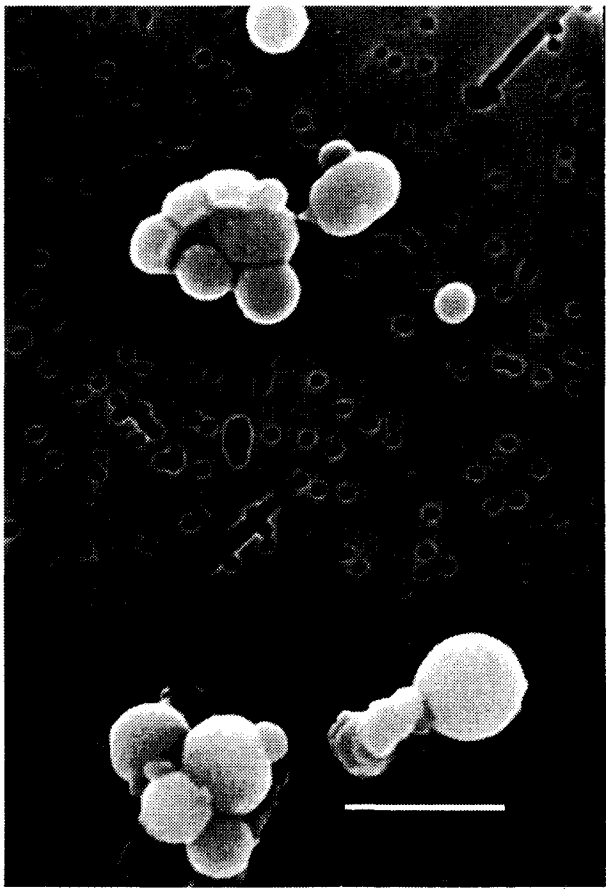

Fig. 3. Scanning electron micrograph of HFC 134A-aerosolized BGG. An aerosol comprised of 10 mg of BGG lyophilized with 50 mg of NP-40 surfactant was charged with 5.0 ml of HFC 134A propellant and shaken. Four aerosol actuations were released into the aerosol chamber, and particles (<4 µm) were recovered in a cyclone. (Bar = 2.0 µm)

134A. Individual or clusters of spheroid particles with a range of sphere diameters were produced in both unshaken and shaken aerosols. The aerodynamic particle size distribution for this NP-40/BGG/HFC 134A aerosol obtained by impactor showed a 42% increase in respirable protein (<5 µm diameter, by impactor) after shaking the same aerosol (not shown).

Because shaken HFC 134A propellant aerosols consistently produced aerosol particles with smaller aerodynamic sizes, we considered the possibility that shaking aerosols reduced the number of subparticles per cluster. Alternatively, vigorous agitation might disperse protein/surfactant complexes into smaller subparticles. The subparticle sizes and the number of aerosol subparticles/cluster of BGG/NP-40/HFC 134A aerosolized particles recovered in a cyclone were determined from electron photomicrographs. Fig. 4A shows the distribution of the number of subparticles composing each discrete particle. The percentage of nonclustered single subparticles nearly doubled with shaking, and the percentage of very large particles containing >8 subparticles was reduced by nearly half after shaking. Results demonstrate that shaken aerosols have smaller aerodynamic particles because of smaller subparticle clusters after shaking.

BGG/NP-40 particles in scanning electron photomicrographs were measured to determine if a difference occurred in the diameters of individual subparticles derived from unshaken and shaken aerosols. The average subparticle diameters were 0.95 µm ±0.42 for unshaken aerosol and 0.85 µm ± 0.37 for shaken aerosol. These averages are not significantly different because of the broad range of sizes in both groups. However, Fig. 4B compares the distribution of subparticle sizes between unshaken and shaken aerosols. It shows that shaking propellant suspensions caused a shift toward smaller particles. While 45% of nonshaken subparticles were <0.9 µm in diameter, 61% of the subparticles were <0.9 µm after shaking. Shaking nearly halved the percentage of large subparticles >1.5 µm in diameter, from 11% (nonshaken) to 6% (shaken). Results in Fig. 4 demonstrate that shaking aerosols dispersed clustered subparticles into individual subparticles or smaller clusters and also reduced the size of the subparticles.

Aerosols Prepared with Mixtures of HFC 134A and DME Propellants

An observation of aerosols prepared with HFC 134A propellant was that the particle suspensions in the liquified propellant were visually coarser and settled more rapidly than those previously observed using DME as propellant. Consequently, it was of interest to determine if the particle-suspending characteristics of surfactants with DME could be combined with the nonflammability of HFC 134A to produce better protein aerosols.

Our first objective was to determine what proportions of DME:HFC 134A would result in a nonflammable propellant. Aerosol vials charged with DME alone; HFC 134A alone; and

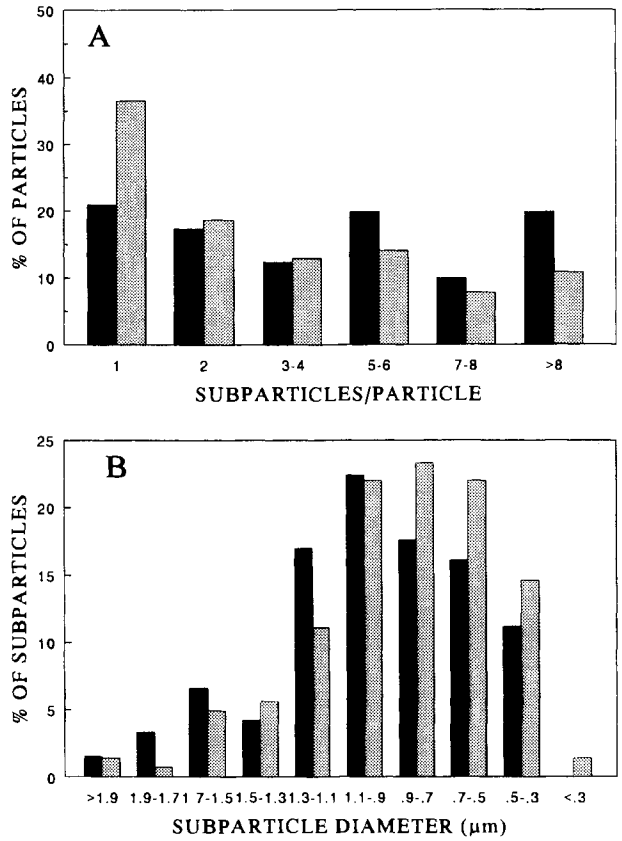

Fig. 4. The number and size of subparticles in aerosolized BGG analyzed by scanning electron microscopy. Eight photomicrographs of BGG/NP-40/HFC 134A aerosolized particles from four actuations captured on a Casella cyclone (aerodynamically <4 μm). Only four actuations were released to reduce the possibility of particles piling up and distorting the analysis of the diameter and the number of subparticles for separate aerodynamic particles. **Fig. 4A**-Distribution of the percentage of subparticles composing individual aerosolized particles for unshaken (black bars) and shaken (white bars) aerosols, analyzing 81 and 93 particles, respectively. **Fig. 4B** Distribution of the percentage of subparticles with a range of diameters for BGG/NP-40/HFC 134A unshaken (black bars) and after shaking (white bars), analyzing 330 and 287 subparticles, respectively.

DME:HFC 134A ratios of 25:75, 50:50, and 75:25 were actuated over a gas burner flame. DME alone and DME/HFC 134A 75:25 produced explosive flashing, whereas DME/HFC 134A 50:50, 25:75 and HFC 134A alone did not flash. We concluded that DME/HFC 134A mixtures with 50% DME or less were nonflammable.

Tween 40 and Tween 80 surfactants, which work well in producing protein aerosols with DME propellant (13,14,22–24), were tested for their capacity to aerosolize BGG in 50:50 DME/HFC 134A propellant. Lyophilized BGG/surfactant was charged first with DME, shaken, rechilled, charged with HFC 134A propellant to a 50:50 ratio, and shaken again. These mixed-propellant aerosols were compared to similar aerosols shaken twice with DME propellant alone. Table 3 compares impactor derived particle sizes for DME propellant alone with DME/HFC 134A mixed propellant for Tween 80 and Tween 40. Results showed that aerosolized BGG content of aerosols shaken first with DME and then HFC 134A was as good as or better than that of aerosols shaken in DME alone.

Table 3. Propellant-Driven Aerosols of Bovine Gammaglobulin Formulated with HFC 134A/DME Propellant Blends

Propellant[a]	Surfactant (mg)	Aerosolized protein (μg)/actuation ± S.D.[b] Total	Respirable	Respirable
DME only	Tween 80 (50)	34 ± 2	5.8	17%
DME → HFC 134A		37 ± 4	6.1	16%
DME only	Tween 40 (50)	37 ± 2	4.5	12%
DME → HFC 134A		36 ± 2	4.6	13%
HFC 134A/DME mix	Tween 80 (50)	44 ± 1	5.0	11%
DME → HFC 134A		48 ± 2	5.1	11%
HFC 134A/DME mix	Tween 40 (50)	40 ± 1	3.7	9%
DME → HFC 134A		45 ± 1	5.0	11%

[a] 10 mg of BGG protein was mixed with the noted mg of surfactants in 5 ml of water and lyophilized. Aerosols were charged with the 5 ml of either DME or 50:50 HFC 134A/DME mix as indicated, shaken 5 min., then charged with 5 ml more of DME (DME only), HFC 134A, or the HFC 134A/DME mix and reshaken for 5 min. All aerosols contained 1 g. of glass agitation beads.

[b] Total aerosolized protein was determined by four actuations directly into tubes. Respirable-sized protein was determined by adding the protein values recovered from impactor stages 5–8 (<5 μm) after delivering 30 actuations into the aerosol chamber.

Because 50:50 DME/HFC 134A, propellant-driven, aerosolized proteins were comparable to those prepared with DME alone, it was important to determine if BGG/surfactant preparations shaken with 50:50 DME/HFC 134A instead of being shaken first with DME and diluted in HFC 134A would be equally effective. Table 3 also compares BGG aerosols formulated with Tween 80 and Tween 40 shaken first with DME and then with HFC 134A with others shaken with a 50:50 DME/HFC 134A mixture. Shaking BGG/Tween 40 with DME first and then diluting with HFC 134A caused a 33% increase in both impactor-recovered and respirable (<5 μm, by impactor) particles. In contrast, aerosols prepared with Tween 80 were nearly equivalent whether shaken initially with DME or with the 50:50 DME/HFC 134A mix. Combined results demonstrate that BGG aerosols formulated with 50:50 DME/HFC 134A are nonflammable aerosols and perform as well as those formulated with flammable DME alone. Depending on surfactant, this was particularly the case when BGG/surfactant particles were dispersed by shaking in DME and then subsequently diluted in HFC 134A propellant.

DISCUSSION

Tetrafluoroethane (HFC 134A) has replaced ozone-damaging chlorocarbons as both a refrigerant and a propellant for metered-dose inhalers to deliver medicinals to the respiratory

tract (21) . This study demonstrated the aerosolization of a protein, bovine gamma globulin, as small particles using HFC 134A as propellant. Aerosols were formulated by lyophilizing BGG from aqueous solutions in the presence of surfactants soluble in liquified HFC 134A and then dispersing the BGG/surfactant complexes in liquified HFC 134A propellant. Nonionic surfactants capable of suspending BGG protein in HFC 134A included Triton X-100, Triton X-405, NP-40, Laureth-9, and diethylene glycol monoethyl ether. Agitation of BGG protein/surfactant suspensions in HFC 134A by mechanical shaking for as little as 2 minutes reduced the size of aerosolized BGG particles, increasing the fraction of respirable-sized particles with aerodynamic diameters less than 5 μm. Scanning electron microscopic analysis of aerosolized particles demonstrated bead-like round subparticles in grape-like clusters. Strong agitation dispersed subparticles into smaller clusters and reduced the average size of the subparticles. Our results are in general agreement with those of Bower *et al.* (25) who demonstrated that surfactants reduce diffusive cluster-cluster formation of drug particles suspended in propellants by reducing the attractive forces between particles.

Byron reported diethylene glycol monoethyl ether, Brij-30, and Tween 80 as being suitable for suspending pharmaceuticals in HFC 134A propellant (26) . Using our methods, Brij-30 and diethylene glycol monoethyl ether suspended BGG in HFC 134A, but shaking of aerosols to disperse BGG/surfactant particles in propellant was required. Tween 80 performed poorly with HFC 134A as the sole propellant, but effectively suspended BGG protein in 50:50 HFC 134A/DME blends.

Results for HFC 134A propellant-driven aerosols are consistent with principles established in our prior studies of propellant-driven protein and DNA aerosols using dimethylether (DME) as a propellant (13–16,22–24). In comparison, protein/surfactant particles are dispersed more evenly in DME propellant than are HFC 134A/protein/surfactant suspensions. The superior dispersing quality of DME may be due to its charge dipole moment, which likely improves its interaction with protein and surfactant charges. This improved dispersion likely explains the better protein aerosolization with DME. Drawbacks to the application of DME for aerosolized human medicinals is that it is not FDA approved for human use as a propellant. A second drawback is DME's flammability. We show here that 50:50 blends of HFC 134A/DME lack flammability, while maintaining the desirable aerosolization properties of DME. Blended DME/HFC 134A propellant permitted aerosol formulations with surfactants not compatible with HFC 134A propellant alone. Dispersing protein/surfactant particles in DME and then diluting them with HFC 134A propellant was particularly effective.

Overall, the results reported here demonstrate the application of tetrafluoroethane (HFC 134A) and HFC 134A/DME blends for propellant-aerosolization of small particles of proteins when formulated with selected surfactants. Metered propellant aerosolization offers a potential alternative to continuous flow, nebulized, aqueous aerosols for delivering therapeutic proteins/vaccines to the respiratory tract.

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REFERENCES

1. J. S. Barton, H. M. Gardineri, S. Cullen, P. C. Hindmarsh, C. G. Brook, and M. A. Preece. *Clin. Endocrinol. Oxf.* **42**:619–626 (1995).
2. F. D. Ledley. *Pharm. Res.* **13**:1595–1614 (1996).
3. A. P. Sayani and Y. W. Chien. *Crit. Rev. Ther. Drug Carrier. Syst.* **13**:85–184 (1996).
4. P. R. Byron. *Adv. Drug Delivery Rev.* **5**:107–132 (1990).
5. S. Wu-Pong and P. R. Byron. *Adv. Drug Deliv. Rev.* **19**:47–71 (1996).
6. C. M. O'Hara, W. R. Anton, F. X. Gormley, and C. Brazell. *J. Assoc. Nurses. AIDS Care*, **5**:41–48 (1994).
7. P. Chervinsky, A. van As, E. A. Bronsky, R. Dockhorn, M. Noonan, C. LaForce, and W. Pleskow. *J. Allergy Clin. Immunol.* **94**:676–683 (1994).
8. I. Gonda. *Adv. Drug Deliv. Rev.* **19**:37–46 (1996).
9. R. K. Wolff and R. W. Niven. *J. Aerosol Med.* **7**:89–106 (1994).
10. J. M. Hofford. *J. Fam. Pract.* **34**:485–492 (1992).
11. W. J. O'Donohue, Jr. *Chest*, **109**:814–820 (1996).
12. D. L. Bartley, C. C. Chen, R. Song, and T. J. Fischbach. *AIHA J.* **155**:1036–1046 (1994).
13. A. R. Brown and J. A. Pickrell. *J. Aerosol Med.* **8**:43–58 (1995).
14. A. R. Brown and J. G. Slusser. *Immunopharmacology,* **28**:241–257 (1994).
15. A. R. Brown, D. W. George, and D. K. Matteson. *Vaccine*, **15**: 1165–1173 (1997).
16. A. R. Brown and S. I. Chowdhury. *J. Aerosol Med.* **10**:129–146 (1997).
17. J. M. Bohnenn. *Aerosol Age*, **33**:26–29 (1988).
18. M. Scuri, V. McCaskill, A. D. Chediak, W. M. Abraham, and A. Wanner. *J. Appl. Physiol.* **79**:1386–1390 (1995).
19. M. A. Johnsen. *Aerosol Age*, **33**:32–39 (1988).
20. D. J. Alexander and S. E. Libretto. *Hum. Exp. Toxicol.* **14**:715–720 (1995).
21. J. J. Daly, Jr. and M. L. SanGiovanni. *Spray Technol. & Marketing*, **3**:34–38 (1993).
22. A. R. Brown and J. A. Pickrell. *J. Immunol. Meth.* **176**:203–212 (1994).
23. A. R. Brown and D. W. George. *J. Pharm. Sci.* **Submitted**: (1997).
24. A. R. Brown. *Aerosol Sci. Tech.* **24**:45–56 (1996).
25. C. Bower, C. Washington, and T. S. Purewal. *Int. J. Pharmaceutics*, **118**:229–235 (1995).
26. P. R. Byron and F. E. Blondino. *US Patent #5492688* (1996).