# Silicone Oil Microdroplets and Protein Aggregates in Repackaged Bevacizumab and Ranibizumab: Effects of Long-term Storage and Product Mishandling

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**Purpose.** To quantify levels of subvisible particles and protein aggregates in repackaged bevacizumab obtained from compounding pharmacies, as well as in samples of bevacizumab and ranibizumab tested in controlled laboratory experiments.

**Methods.** Repackaged bevacizumab was purchased from four external compounding pharmacies. For controlled laboratory studies, bevacizumab and placebo were drawn into plastic syringes and incubated at −20°C, 4°C, and room temperature (with and without exposure to light) for 12 weeks. In addition, mechanical shock occurring during shipping was mimicked with syringes containing bevacizumab. Particle counts and size distributions were quantified by particle characterization technology. Levels of monomer and soluble aggregates of bevacizumab were determined with size-exclusion high-performance liquid chromatography (SE-HPLC).

**Results.** Repackaged bevacizumab from the compounding pharmacies had a wide range of particle counts (89,006 ± 56,406 to 602,062 ± 18,349/mL). Bevacizumab sampled directly from the original glass vial had particle counts of 63,839 ± 349/mL. There was up to a 10% monomer loss in the repackaged bevacizumab. Laboratory samples of repackaged bevacizumab and placebo had initial particle counts, respectively, of 283,675 ± 60,494/mL and 492,314 ± 389,361/mL. Freeze-thawing of both bevacizumab and placebo samples led to >1.2 million particles/mL. In all repackaged samples, most of the particles were due to silicone oil. SE-HPLC showed no significant differences for repackaged samples incubated in the laboratory under various conditions, compared with bevacizumab directly from vial. However, repeated freeze-thawing caused a more than 10% monomer loss.

**Conclusions.** Bevacizumab repackaged in plastic syringes could contain protein aggregates and is contaminated by silicone oil microdroplets. Freeze-thawing or other mishandling can further increase levels of particle contaminants.

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Bevacizumab (Avastin; Genentech Technology, Inc., South San Francisco, CA) is a recombinant human monoclonal antibody that inhibits endothelial cell growth and subsequent vascularization. It was approved for intravenous (IV) treatment of metastatic colorectal cancer by the United States Food and Drug Administration (FDA) in 2004 and subsequently has been approved for IV treatment of non–small cell lung cancer, metastatic breast cancer, glioblastoma, and metastatic kidney cancer. Because of its antivascular activity, bevacizumab has also been used by ophthalmologists for the off-label treatment of wet age-related macular degeneration (AMD). This practice has grown rapidly because repackaged bevacizumab has been very effective in treating AMD[1] and because the cost per dose of bevacizumab is substantially lower than that of ranibizumab (Lucentis; Genentech), which is an FDA approved anti-VEGF agent specifically packaged and sold for the treatment of wet AMD.[1]

The apparent safety and efficacy of intravitreal bevacizumab have been supported by published peer-reviewed reports[2–5] and have led the National Eye Institute (NEI) to undertake a large randomized double-masked multicenter clinical trial (Comparison of AMD Treatments Trials [CATT]) to compare bevacizumab with ranibizumab.[6] However, reports of sustained elevation of intraocular pressure (IOP) and inflammation after the intravitreal use of bevacizumab and ranibizumab have been increasing.[7–13] Recently, we proposed that some increases in IOP could be due to particulate matter present in bevacizumab, which for off-label use is typically repackaged in plastic syringes.[14] In support of this hypothesis, our investigation documented that there were both protein aggregates and particles ≥1 μm in repackaged bevacizumab obtained from three external compounding pharmacies.

Many factors (e.g., storage time, type of syringe, freeze-thawing, and mechanical shock during shipping) that could affect particle and protein aggregate levels and sizes in repackaged bevacizumab have not been investigated. Furthermore, determining the ranges of values for these critical product characteristics in samples purchased from different external compounding pharmacies is important for the field. In the present study, we addressed these problems with repackaged bevacizumab. First, we quantified the levels of subvisible particles and protein aggregates in bevacizumab in plastic syringes purchased from four external compounding pharmacies. Second, we performed controlled laboratory experiments with bevacizumab repackaged in the same types of plastic syringes as those used by the external compounding pharmacies. We tested the effects of storage at room temperature (with and without exposure to light), −20°C, and 4°C on particle counts and protein aggregates and the effects of repetitive freeze-

thawing. In addition, we studied the potential for freeze-thawing of samples in shipping containers used by the external compounding pharmacies and the effects of mechanical shock due to handling mimicking that occurring during shipping.

We used a particle characterization technique (MicroFlow Imaging [MFI]; Brightwell Technologies, Ottawa, ON, Canada) to count and size particles ≥1 μm and size-exclusion high-performance liquid chromatography (SE-HPLC) to quantify levels of monomeric and aggregated bevacizumab. Also, we conducted analyses and experiments that documented that most of the particles were due to silicone oil microdroplets. Finally, for comparison to results with repackaged bevacizumab, we also quantified the subvisible particles present in ranibizumab samples.

In this article, we report that bevacizumab repackaged in plastic syringes was contaminated by silicone oil microdroplets. Freeze-thawing and mechanical shock can further degrade the product and increase levels of particle contaminants. Although we cannot directly link silicone oil contaminants and bevacizumab aggregates with sustained elevation of IOP and inflammation at this time, based on current studies, mishandling of repackaged bevacizumab should be and can be avoided.

## Materials and Methods

Bevacizumab was purchased from the University of Colorado Hospital Inpatient Pharmacy. It was supplied as 25 mg/mL × 4 mL in preservative-free, single-use glass vials. Unless otherwise indicated, for the controlled laboratory studies, 0.3-mL insulin syringes (BD Ultra-Fine Short; Cat. No. 08290-328438; BD Biosciences, Franklin Lakes, NJ) were used. All chemicals used were of reagent grade or higher.

Repackaged bevacizumab in insulin plastic syringes was purchased from four external compounding pharmacies (designated CP1–CP4) on two different occasions. For each compounding pharmacy, the details of the syringe configurations and the shipping materials used are described in the Results section.

Ranibizumab was obtained from the residual solution remaining in the vials after clinical administration at the University of Colorado Hospital Eye Center. The residual solution was collected on the day of administration, stored at 4°C in the dark, and used in laboratory experiments within 24 hours.

### Observations on Shipping Containers Used for Repackaged Bevacizumab

On arrival of the repackaged bevacizumab from each of the four compounding pharmacies, observations were made of the inside and outside of each shipping box; the orientation, weight, and surface temperature of the gel packs; the wrapping materials in which the syringes were placed; the relative position of syringes, gel packs, and wrapping materials; and the type of syringe. A thermocouple (Dual thermometer; Fisher Scientific, Pittsburgh, PA) was attached to the surface of a gel pack to determine the temperature on arrival.

### Testing of the Potential for the Formulation to Freeze in Syringes in Shipping Containers

First, the gel packs obtained from the respective shipping containers (Styrofoam boxes) used by CP1 and CP3 were placed in a −20° freezer overnight. Then, the gel packs were placed in the respective Styrofoam shipping boxes, in the orientation observed on arrival of the packages. A thin wire thermocouple was placed on the surface of a gel pack in each box, the box was closed with the lid, and the temperature on the surface of the gel pack was recorded for 45 hours.

To test the potential for repackaged bevacizumab to freeze during shipping, we inserted a thin wire thermocouple into 0.05 mL of placebo solution (60 mg/mL trehalose · $2H_2O$, 5.8 mg/mL $NaH_2PO_4$ · $H_2O$, 1.2 mg/mL $Na_2HPO_4$, and 0.4 mg/mL polysorbate 20; pH6.2) contained in 0.3-mL insulin syringes. The syringes were in direct contact with the surface of gel packs that had just been removed from the −20°C freezer. The gel packs and syringes were placed in the Styrofoam shipping boxes that were obtained from CP1 and CP3. The temperature of the placebo in the syringes was recorded with the dual digital thermometer as a function of time. In another experiment, to avoid potential nucleation of ice by the thermocouple immersed in the solution inside the syringes, we attached the thermocouple to the outside surface of the syringes at the level of the solution inside. The syringes were placed in contact with the gel packs in the Styrofoam shipping containers, as just described, and the temperature was recorded as a function of time. At various times, the syringes were taken out of the box for visual inspection to see whether the placebo had frozen. We also tested the temperature on the surface of the syringes (containing 0.05 mL placebo), which were wrapped with additional multilayers of bubble packing material and placed horizontally or vertically between the gel packs.

### Analytical Methods for Particles and Protein Monomer and Aggregates

Particle characterization technology (MicroFlow Imaging [MFI]; DPA4100 Flow Microscope, Series B; Brightwell Technologies) was used to capture digital images of particles, quantify particle counts, and determine size distributions (≥1 μm). The flow cell (100 μm) was used in SP3 mode after calibration with a 10-μm polystyrene microsphere standard (cat. no. 4210A; Duke Scientific Corp., Palo Alto, CA). Ultrapure water (0.22 μm filtered; Millipore, Billerica, MA) was used to check the background counts during MFI. The total volume of sample dispensed into the flow cell was 0.3 mL, and 0.15 mL was run into the cell before the start of data acquisition. The particle count for placebo was subtracted from that of the bevacizumab samples. Then, if applicable, to obtain the particle counts before sample dilution, the particle counts were multiplied by the dilution factor (see below) used in preparation of bevacizumab samples for analysis.

SE-HPLC was used to fractionate and quantify monomer and soluble aggregates in the bevacizumab samples. The samples were centrifuged (10 minutes at 14,000*g*) to pellet any insoluble material (potentially including protein precipitates). The supernatants were analyzed by using the SE-HPLC mode on an asymmetric flow field flow fractionation system (Eclipse AFFF; Wyatt Technology Corp., Santa Barbara, CA) that connected with a multiangle light-scattering detector (Dawn EOS; Wyatt Technology Corp.) and a UV detector (Gold Module 166; Beckman Coulter, Fullerton, CA). The mobile phase (0.182 M $KH_2PO_4$, 0.018 M $K_2HPO_4$, and 0.25 M KCl [pH 6.2]) was run at a flow rate of 0.5 mL/min through a gel column (TSK-GEL G3000SWxl; Tosoh Bioscience, Tokyo, Japan), and elution was monitored at 280 nm. The volume of injection was 100 μL, and the running time was 30 minutes. The sum of the peak areas (monomer and the earlier eluting oligomer) of the chromatograph for samples directly from the glass vial was calculated (ASTRA V software; Wyatt Technology Corp.) and served as the control value for the repackaged samples. The percentage of monomer and the earlier-eluting oligomer in repackaged samples was obtained via dividing the respective peak areas by the summed peak area for the control sample and multiplying by 100.

### Effects of Preanalysis Dilution on Particle Counts

The volume of repackaged bevacizumab obtained from compounding pharmacies was either 0.05 or 0.1 mL, and 0.05 mL was used in our controlled laboratory studies in insulin syringes, as described in the next section. Analysis by MFI requires at least 0.3-mL samples to give accurate particle counts. To be able to obtain data for samples from individual syringes, it was necessary to dilute the bevacizumab formulation to a sufficient volume so that the assay could be run properly. Dilutions were made in the placebo. To ascertain whether dilution changed the particle number and size distribution, MFI analysis was performed on undiluted bevacizumab formulation (from repackaged syringes) and samples diluted 1:10, 1:20, 1:30, 1:40, and 1:50 with placebo.

## Analysis of Repackaged Bevacizumab from Compounding Pharmacies

Particle counts and size distributions (≥1 μm) of the repackaged bevacizumab samples were determined by MFI after 1:30 dilution with placebo. Monomer and soluble aggregates in the diluted bevacizumab samples were measured by SE-HPLC. The solution in the syringe was expelled by pushing the plunger, and the sample was collected in a 2-mL cryogenic tube and then diluted with the appropriate volume of placebo. Shipments of syringes with repackaged bevacizumab were obtained on two separate occasions. For the first shipment, the samples were analyzed on receipt. For the second shipment, a set of syringes from each compounding pharmacy were analyzed immediately on receipt. Another set was stored at 4°C to within 14 days of the 3-month expiration date, which was used by all the external compounding pharmacies for repackaged bevacizumab.

## Controlled Laboratory Studies of Repackaged Bevacizumab

**Sample Preparation.** In a laminar flow hood, bevacizumab was drawn (0.05 mL, unless otherwise noted) into the 0.3-mL insulin syringes (cat. no. 328438; BD Biosciences) through the rubber closure of the vial. The 0.3-mL insulin syringes were used in all experiments unless otherwise indicated. For comparison, bevacizumab placebo (60 mg/mL trehalose · $2H_2O$, 5.8 mg/mL $NaH_2PO_4$ · $H_2O$, 1.2 mg/mL $Na_2HPO_4$, and 0.4 mg/mL polysorbate 20 [pH 6.2]) was prepared in the same type of syringe.

**Three-Month Incubation Study.** Syringes with 0.05 mL of bevacizumab were incubated at room temperature (with and without exposure to laboratory fluorescent lights), −20°C, and 4°C for 0, 1, 2, 4, 8, and 12 weeks. Particle counts and size distribution (≥1 μm) in the incubated bevacizumab samples were determined by MFI. Monomer and soluble aggregates in the bevacizumab samples were measured by SE-HPLC.

**Repeated Freeze-thawing.** Samples of 0.05 mL bevacizumab in syringes were frozen at −20°C and thawed at 4°C, 1, 5, and 10 times. MFI and SE-HPLC were used to characterize the freeze–thaw samples.

**Effect of Syringe Type during Freeze-thawing.** To compare 1.0-mL syringes with 0.3-mL syringes, we drew the bevacizumab into 1.0-mL tuberculin syringes (cat. no. 309602; BD Biosciences) through a dispensing pin (Mini-Spike Dispensing Pin, product code: DP1000SC; B. Braun Medical, Inc., Bethlehem, PA) which entered the rubber closure of the vial. The samples syringes were placed in a −20°C freezer and incubated for 2 weeks, and the solution was thawed at 4°C. MFI and SE-HPLC were used to characterize the freeze-thaw samples.

**Mechanical Shock of Syringes Containing Repackaged Bevacizumab.** Syringes containing 0.05 mL bevacizumab were placed between unfrozen gel packs in one of the Styrofoam shipping boxes obtained from CP3. To mimic the mechanical shock to which the samples could be subjected during shipping, the box was tossed horizontally at a height of 1.2 m and for a distance of approximately 2 m. This process was repeated 20 times. MFI and SE-HPLC were used to characterize the samples after the tossing.

**Characterization of Particles Measured with MFI.** To gain insight into the identity of the component material of these particles, we conducted experiments to confirm that most of the particles counted by MFI in the bevacizumab samples from plastic syringes were silicone oil. First, we prepared samples in syringes and incubated them for 3 weeks at 4°C and −20°C. We prepared a sufficient number of syringes so that, after incubation, pooling of the expelled bevacizumab provided enough sample volume that analysis could be performed without dilution. This method allowed us to observe in images from the MFI the full population of particles that were present without dilution (see the Results section, Fig. 6, for representative images) and to observe many more particles per image than noted after dilution. In addition, samples of bevacizumab or placebo were freeze-thawed in the plastic syringes and the results compared to those of the samples of placebo freeze-thawed in cryogenic vials, which are free of silicone oil. We directly drew 0.3 mL of bevacizumab or placebo into 0.3-mL insulin syringes. Placebo (0.3 mL) was pipetted into 2 mL cryogenic vials. Also, we prepared a suspension of microdroplets of silicone oil (160 μg/mL; 1000 cSt; Dow Corning, Midland, MI) in placebo with a vortex mixer (Vortex Genie2, cat. no. 12-812, Thermo Fisher Scientific, Inc., Pittsburgh, PA). The suspension of silicone oil microdroplets (0.3 mL) was pipetted into cryogenic vials.

To confirm that the spherical objects in the images were not air bubbles, we tested the effects of degassing. Samples (bevacizumab or placebo in plastic syringes, placebo in cryogenic vials, and placebo spiked with silicone oil in cryogenic vials) were frozen at −20°C and thawed at 4°C. For comparison, another set of samples was kept at room temperature. After that, each of the sample types, either with or without freeze-thawing, was degassed under vacuum (20 in. of mercury, i.e., 20 in. HgV) for 3 hours to remove any potential microbubbles of air before MFI analysis. For comparison, another set of each of the sample types was kept at room temperature without degassing. These studies allowed comparison of particles found in placebo samples taken from plastic syringes and cryogenic vials, as well as in an authentic silicone oil sample, with the particles found in the repackaged bevacizumab taken from plastic syringes.

**Particle Counts of Ranibizumab.** The particle counts and size distributions of the ranibizumab samples were obtained by MFI for the following preparations: removed directly from the glass product vials with a plastic tip on a pipetter; drawn without filtration from the vial into a 1-mL tuberculin syringe and then expelled for measurement; and according to preparation for administration as described in the ranibizumab prescribing information. In this case, the ranibizumab formulation was withdrawn from the vial through a filter needle (Nokor filter needle with 5-μm wall, 19-gauge × 1.5 in., reference No. 305200; BD Biosciences) attached to a 1-mL tuberculin syringe, and then, after removal of the filter, the solution was expelled for measurement. For all methods of sample handling, after the sample was obtained, the ranibizumab was diluted 1:30 with ranibizumab placebo (10 mM histidine HCl, 10% α-trehalose dehydrate, and 0.01% polysorbate 20 [pH 5.5]), and then the particles were counted.

# Results

## Observations on Shipping Containers Used for Repackaged Bevacizumab

The shipping systems for the repackaged bevacizumab varied among the compounding pharmacies (Table 1). In particular, the weight and orientation of the gel packs used as cold media, and the relative position of individual syringes to the gel packs varied greatly (Table 1). The materials wrapped around the syringes of the repackaged bevacizumab were also different. In some cases (CP1, -2, and -4), the syringes were first packed individually in a transparent plastic bag, which was then placed in a large brown or silver plastic ziplock bag. In one case (CP3), the syringes were initially packed individually in small black plastic bags, which were then placed inside a large transparent ziplock plastic bag. Samples from CP3 had an additional layer of bubble wrap packing materials outside the large ziplock bag. On arrival, after overnight shipping, the surface temperature of the gel packs from all four compounding pharmacies was 1°C.

The plastic syringes used by the four compounding pharmacies to repackage bevacizumab were also different (Table 1). CP2, CP3, and CP4 used 0.3-mL insulin syringes with permanently attached needles. The needle lengths were 5/16 in. (8 mm) on syringes obtained from CP3 and CP4 and ½ inch (12.7 mm) on those obtained from CP2. Only one compounding pharmacy (CP1) used 1-mL tuberculin syringes with a Luer-Slip fitting to which a new needle must be attached before intravitreal injec-

**Table 1.** Details of the Repackaged Bevacizumab

| Compounding Pharmacy | Total Weight of Gel Packs (kg) | Orientation of Gel Packs | Relative Position of Repackaged Bevacizumab | Syringe Reference Number |
|---|---|---|---|---|
| CP1 | 0.34 | Vertical | Between two gel packs | 309602 |
| CP2 | 1.14 | Vertical | Between three gel packs | 328440 |
| CP3 | 0.79 | Horizontal | Between two gel packs | 328438 |
| CP4 | 0.57 | Horizontal | Under two gel packs | 309301 |

Data show the weight and orientation of gel packs, the relative position of repackaged bevacizumab to gel packs, and the reference number of plastic syringes used by the four external compounding pharmacies to repackage bevacizumab.

tion. A black rubber cap on the Luer-Slip fitting was used to seal the 1-mL syringe.

### Testing of Potential for Formulation to Freeze in Syringes in Shipping Containers

After the gel packs equilibrated in the −20°C freezer, they were taken out and placed into their original shipping boxes. The surface temperatures of the gel packs remained below 0°C for up to 5 hours (Fig. 1A). Then, the temperature remained at 1°C for 20 to 40 hours, depending on the weight and initial temperature of the gel packs when they were placed into the shipping boxes. The temperature of the placebo in the syringes in contact with the gel packs—removed from the −20°C freezer and placed in the shipping container—cooled below 0°C, and the solution froze in less than 10 minutes (Fig. 1B). To avoid potential nucleation of placebo due to the thermocouple directly contacting the placebo solution, the thermocouple was attached to the outside surface of syringes. The sample temperatures dropped as low as −17°C (data not shown) and the samples froze within 30 minutes. Also, we tested placebo-containing syringes wrapped with an additional multilayer of bubble packing material. In syringes placed horizontally between the gel packs (CP3; Table 1), the placebo solution froze within 30 minutes. The temperature of samples placed vertically between the gel packs (CP1; Table 1) dropped to −12°C, but the solution did not freeze within 30 minutes (data not shown). Clearly, at this degree of supercooling, freezing would be expected to occur ultimately during actual shipping. The results presented in Figure 1 demonstrate that there is potential for repackaged bevacizumab to undergo freeze-thawing during the shipping process. However, unless temperature monitoring equipment is used during actual shipping from compounding pharmacies, the temperature profiles during the shipping of repackaged bevacizumab cannot be assessed.

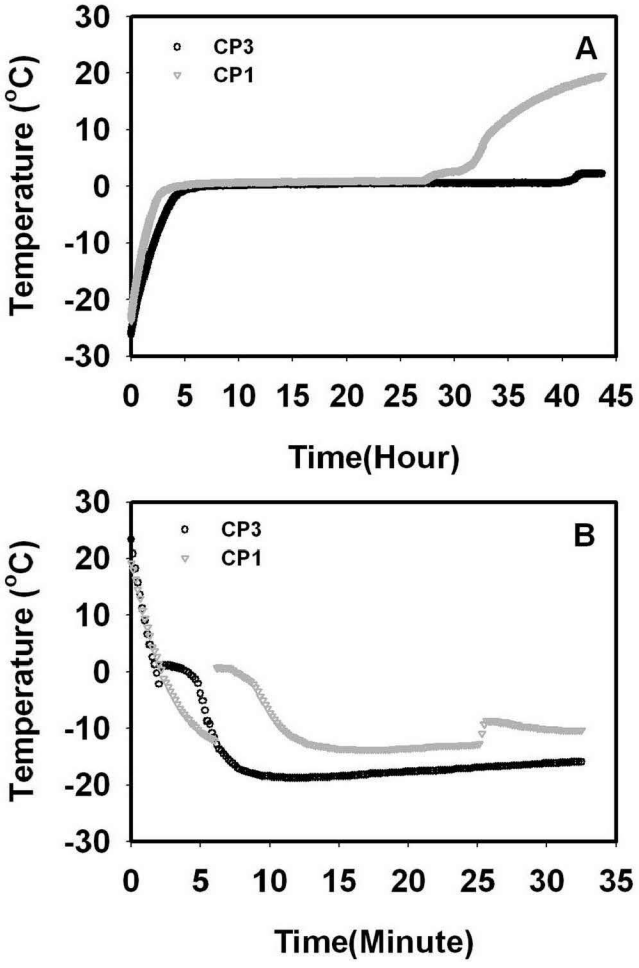


**Figure 1.** The temperatures of gel packs (**A**) and placebo in the syringe (**B**) as a function of time after the gel packs from CP1 and CP3 were kept in a −20°C freezer overnight and placed into their original shipping boxes. (**A**) Thermocouples were directly attached to the surface of the gel packs; (**B**) thermocouples were inserted into 50 μL of placebo solution in the syringes which directly contacted the surface of the gel packs.

### Effect of Preanalysis Dilution on Particle Counts

Compared with undiluted bevacizumab formulation, the particle number and size distribution of samples diluted 1:30, 1:40, and 1:50 with placebo did not show substantial differences from values obtained without dilution (data not shown). In contrast, the particle number and size distribution of samples diluted 1:10 and 1:20 did differ from the values obtained without dilution, but due to the variability of the results for individual replicates, the differences were not statistically significant ($P > 0.05$) when comparing the mean particle counts for each dilution to that for the undiluted sample by Student's *t*-test. We chose a 1:30 dilution for all repackaged bevacizumab samples, unless otherwise indicated.

### Analysis of Repackaged Bevacizumab from Compounding Pharmacies

The repackaged bevacizumab obtained in the first batch from the compounding pharmacies (no less than duplicate samples tested, except for CP2) had a wide range of particle counts (89,006 ± 56,406 /mL to 602,062 ± 18,349 /mL; Fig. 2A). For comparison, bevacizumab sampled directly from the original glass vials had a particle count of 63,839 ± 349/mL. Particle counts also varied greatly in the same batch of repackaged bevacizumab from CP3 (Fig. 3). Representative particle images of the CP3 and CP4 samples (Fig. 2) showed that there were some large size particles (e.g., 111 μm) in the CP3 samples and various sizes of dark spherical particles in the CP4 samples that were later confirmed to be silicone oil microdroplets.

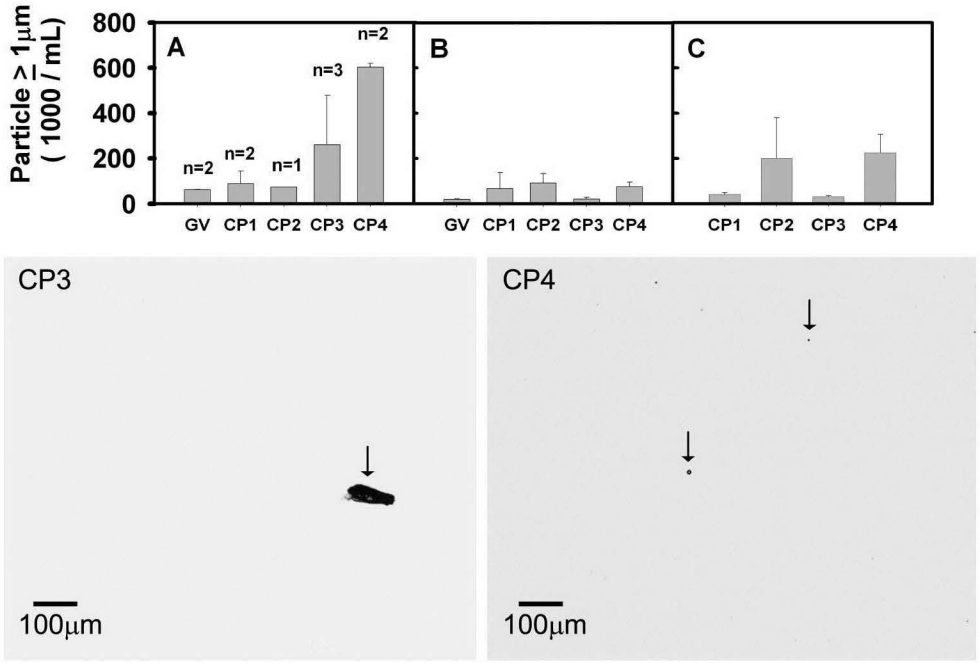


**FIGURE 2.** Particles per milliliter (≥1 μm) of bevacizumab directly from the original glass vial (GV) and repackaged bevacizumab ordered from four external CPs. (**A**) First batch; (**B**) second batch tested on receipt ($n$ = 3); (**C**) second batch tested within 14 days of the 3-month expiration date ($n$ = 2). Representative images of CP3 and CP4 samples in the first batch (*bottom*: 1:30 dilution; *arrows*: particles).

The same samples of repackaged bevacizumab were analyzed by SE-HPLC for monomer levels and soluble aggregates (Figs. 4, 5). Representative chromatograms are shown in Figure 4, with the peaks for high-molecular-weight (HMW) species 1 and 2 labeled (HMW1 and HMW2) in the Figure 4 inset. The weight-averaged molecular mass of the species in the chromatogram peaks was estimated with an online light-scattering detector. Based on analysis of the SE-HPLC results for all samples studied by this method (data not shown), we determined that the HMW1 species were dimers to tetramers and the HMW2 species were composed of higher-order oligomers ranging from octamers to decamers.

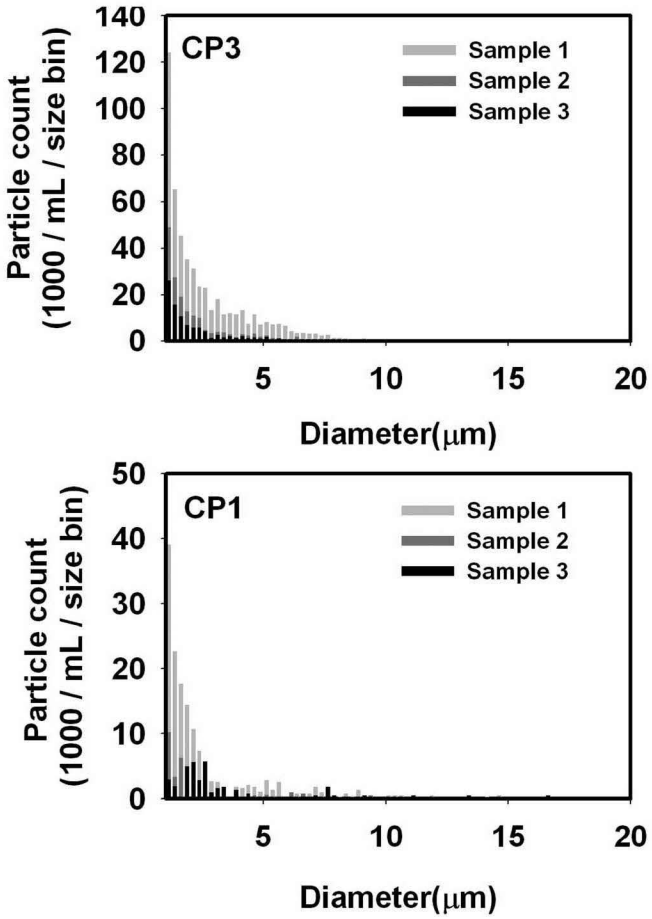


**FIGURE 3.** Particle size distribution (≥1 μm) of repackaged bevacizumab from three syringes from CP3 (first batch) and CP1 (second batch, tested on receipt).

SE-HPLC analysis showed that there was substantial monomer loss in the repackaged bevacizumab in batch one from CP2 and -3, whereas the levels in samples from CP1 and -4 were similar to that for bevacizumab taken directly from the drug product vials (Figs. 5A–C). For the repackaged bevacizumab in batch 2, the monomer level in samples from all the external compounding pharmacies was about the same as that measured in the bevacizumab taken directly from the drug product vials. In the samples in batches one and two, the levels of HMW1 ranged from approximately 2% to 4% and the levels of HMW2 from approximately 0.2% to 0.4%, respectively (Fig. 5).

Compared with the samples of repackaged bevacizumab obtained in the first batch, the range of particle counts was narrower among all the samples in the second batch when they were tested immediately on arrival (Fig. 2B). However, particle counts varied substantially between the samples taken from individual syringes in the second batch of repackaged bevacizumab from CP1 (Fig. 3). When we tested the second-batch samples that were stored at 4°C until 14 days before the 3-month expiration date, there was an increase in the total particles per milliliter in samples from CP2 and CP4 (Fig. 2C). There were no substantial changes in the levels of monomer or high-molecular-weight bevacizumab in the stored samples (Figs. 5D–F).

## Controlled Laboratory Studies of Repackaged Bevacizumab

**Three-Month Incubation Study.** Laboratory samples of repackaged bevacizumab and placebo had initial (after 2 hours at 4°C, used as time-0 data) particle counts, respectively, of 283,675 ± 60,494/mL and 492,314 ± 389,361/mL (Table 2). In

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