lation was measured using the Solo VP. The concentration of the sample was measured by added 50 to 100 μL of material into a SoloVP small UV disposable vessel. A new fibrette was installed and the sample absorbance was measured by the instrument, using an extinction coefficient of 1.55 mL. mg^{-1} cm^{-1}, and correcting background scattering. After analysis the sample is removed with a pipette the disposable vessel and fibrette are both disposed. This procedure is repeated for each sample.

[0322] J. pH Analysis

[0323] After sample preparation the pH will be checked for each formulation and be within ±0.1 of the target pH. Before the start of analysis, the pH probe was calibrated with three pH standards ordered from fisher. The pH of the formulation will be measured by inserting the pH probe in to the sample and waiting until the measured value has stabilized, which can take up to 1 to 2 minutes. After the analysis the pH probe is washed with 18Ω water for one minute and stored in the pH storage solution.

[0324] K. Osmotic Analysis

[0325] The osmotic analysis was performed using an Advanced Instruments, Osmo 1. At the start of analysis, a reference standard at 290 mOsm is analyzed to insure the instrument is working properly. After the reference standard has passed the samples are then analyzed. 20 uL of material is removed and analyzed by the Osmo 1, after analysis the chamber is cleared by a using a chamber cleaner. This procedure is repeated for each sample.

[0326] L. Formulating

[0327] The bulk processed aflibercept material was formulated following the procedures below. The aflibercept material was buffered exchanged following the dialysis procedure. After dialysis the osmotic pressure and the pH was checked, if the pH of the sample was not within ±0.1 pH units, the formulation buffer was made more acidic or basic, by repeat dialysis or by addition of HCl or NaOH, until the pH target was reached. Following pH adjustment if needed, the sample was concentrated above the target formulation concentration. The pH, osmotic pressure and the protein concentration was then measured once more. The sample was then diluted with the formulation buffer to reached the target formulation protein concentration within 10%. The protein concentration was then measured once more to insure the diluted sample was within 10% of the target concentration. The last step was the addition of the 20% PS 20 (diluted in water) to the sample based on weight, if required.

[0328] M. Sterile Filtering and Sample Filling

[0329] The samples were sterile filtered in a clean hood that was wiped down with 70% ethanol. Each formulation was loaded into a sterile syringe with sterile filter attached. The sample was then slowly pushed through filter into a sterile container. After the samples had been sterile filtered, they were loaded into autoclaved vials and caps

[0330] N. Size Exclusion Chromatography Method

[0331] Size exclusion chromatography (SEC) analyses were conducted on formulations to measure the changes in colloidal stability. SEC can quantify oligomers and soluble aggregates, as well as detect fragmentation of proteins.

[0332] The SEC analysis parameters are described below

[0333] Column: ACQUITY UPLC BEH SEC (CAT. 186005225), 220 Å 1.7 μm, 4.6 mm×150 mm

[0334] Mobile Phase: 20 mM Sodium Phosphate, 250 mM Sodium Chloride, pH 7.55*

[0335] Autosampler Temp: 5° C.±3° C.

[0336] Column Temp.: 30° C.±2° C.

[0337] Flow Rate: 0.25 mL/minute

[0338] Injection Vol: 5 μL

[0339] UV Setting: 280 nm

[0340] Data Collection Time: 15 minutes

[0341] O. Sub-Visible Particle Evaluation by MFI

[0342] In the examples below, screening studies were conducted to identify formulations with particle counts indicating that the formulation is sufficiently stable and therefore suitable as commercial aflibercept product. Formulations with exceptionally high particle counts (e.g. >1,000,000) may not be sufficiently stable. The particle counts reported in the examples below for a formulation may not be the particle counts for a commercialized aflibercept product. For example, a commercialized product is manufactured under strict GMP conditions which is expected to reduce the number of particles found in the formulation. Moreover, USP guidelines (e.g. USP <788>, <789>, which are hereby incorporated by reference in their entirety) limit the number of particles that can be present in certain products. When manufactured and handled under GMP conditions, it is expected that the formulations herein that are identified to be sufficiently stable based on the screening test of sub-visible particles will be suitable for a commercial aflibercept product.

[0343] To evaluate sub-visible particles in formulations Micro Flow Imaging analysis was conducted. The MFI analysis was performed on a MFI 5200 Protein Simple system following the procedure described below.

[0344] MFI System Suitability.

[0345] In order to ensure the MFI was counting and assessing particle size accurately, 5 uM size/concentration standards (5 um polystyrene beads for MFI system suitability Count-cal Cat # CC05) and 10 uM size standards (10 um polystyrene beads for MFI system suitability Duke Std Cat #4210A) were used. Triplicate analyses for each standard were performed and the average of each standard were within 10% of true/given manufacture value.

[0346] Sample Preparation.

[0347] Samples were placed in the BSC and allowed to equilibrate to room temperature for about 30 minutes. Using Neptune Barrier Tips (1000 uL Barrier Tip, Cat #1000.96N), samples were diluted 1:1 with MilliQ Water, specifically 190 uL MilliQ water+190 uL Samples. All samples were degassed for 20 min @ 70 cmHg.

[0348] MFI Analysis.

[0349] Prior to analysis, approximately 10 mL of MilliQ water was flushed through the MFI system using a 100 uM FlowCell (FlowCell, 100 uM, 1.6 mm, Silane Coating). For each sample, 350 μL was pipetted and sample-filled pipette was placed in the MFI inlet port. 0.03-0.05 mL manual purge (by volume) was performed followed by selecting "Optimize illumination" to calibrate and set the background. Analysis was started by selecting "Start analysis". A total of 180 ul of sample was analyzed for each measurement. After each measurement, 1-4 ml of MilliQ water was flushed through the flow-cell.

[0350] For the example below, the levels of subvisible particles (SVPs) were monitored for each of the formulations in Blocks A through G at all of the time points using micro-flow imaging (MFI). Total particle levels are reported, along with a corrected particle concentration once the circular particles likely due to air bubbles and oil droplets are