Journal of Pharmaceutical Sciences 114 (2025) 1605–1614

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutics, Drug Delivery and Pharmaceutical Technology

Developing ultra-high concentration formulations of human immune globulins for subcutaneous injectables

Jayprakash Yadav[a], Shihab Uddin[a], Francesco Civati[a], Wenchuan Ma[a], Andreas Liebminger[b], Wolfgang Teschner[b], Guillaume André[b], Bernhardt L. Trout[a], Richard D. Braatz[a], Allan S. Myerson[a,*]

[a] Department of Chemical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139-4307, United States
[b] Takeda Pharmaceuticals, Process Development, Plasma-derived Therapies R&D, Industriestraße 72, A-1220 Vienna, Austria

ARTICLE INFO

Article history:
Received 24 September 2024
Revised 28 January 2025
Accepted 29 January 2025
Available online 3 February 2025

Keywords:
Formulation
Antibodies
Spray drying
Viscosity
Rheology
Injectability

ABSTRACT

This work describes the first development of high-concentration suspension formulations of human immune globulin. Colloidal-level dispersions of immune globulin were achieved by suspending a spray dried solid powder of protein in a protein solution made saturated by the addition of pharmaceutical excipients. The spray drying process was used to generate ~90 % of particles below 20μ. The monomer and aggregates content of immunoglobulin were found to be 93 % and 0.3 %, respectively. The injection forces for the colloidal suspensions were characterized using a dynamic compression test. The concentrations of 300, 380, and 400 mg/mL formulations were injected at 3.8 N, 10 N, and 16.5 N of maximum injection forces, respectively, when a 24-gauge needle was used. The viscosity of a 300 mg/mL suspension was 128 cP. The viscosity of a 380 mg/mL suspension was 284 cP, and the viscosity was higher for the 400 mg/mL formulation; however, injectability was not an issue, which remains rare for non-Newtonian, shear-thickening systems. It is acknowledged that the 400 mg/mL suspension formulation remained relatively challenging as compared to other suspensions for injection because of its very high viscosity, and significant force was required to inject it. We show that where ultra-high-concentration immune globulin is being developed within reasonable constraints of pharmaceutical regulation, with an injectability parameter, formulations might make their way to the clinic when viscosity could say otherwise. However, further work should be conducted to assess chemical stability (using methods such as mass spectrometry) along with forced degradation studies prior any clinical use.

Introduction

Protein-based therapeutics have been widely developed over the past few decades. To be able to deliver a therapeutic protein, especially through the intramuscular and subcutaneous routes, an ultra-high concentration protein formulation is desired.[1] In highly concentrated protein therapeutics, the viscosity of the protein solution and aggregation remain crucial issues for the development of pharmaceutical formulations. With an increase in the concentration of protein in the aqueous solution, the viscosity of the solution significantly increases. The volume of formulation administered through the subcutaneous route is limited to 1.5 mL as per U.S. Food and Drug Administration regulation, and the viscosity of the solution should not exceed 50 cP.[2] This requirement presents a substantial challenge because highly concentrated antibody protein solutions are very viscous,[3] and this highly viscous behavior makes formulation challenging to administer to patients through a specified syringe and needle.

In the literature, different approaches have been used to reduce the viscosity of proteins in aqueous solutions. In a concentrated protein solution, the addition of hydrophobic salts[4,5] the use of amino acids lysine[6] and arginine,[6,7] bulky polar additives,[8] molecular crowding agents,[9,10] and organic electrolyte cosolutes[11] provided a reduction in viscosity. Recently, the synergistic effect of multiple excipients on controlling the viscosity using a microfluidic tool of the model protein bovine gamma globulin has also been found to be effective.[12] Another strategy employed crystalline suspensions instead of aqueous protein solutions[1,13] however, this approach is time-consuming and uncertain since crystallizing antibodies remains challenging because of their large molecular weight, numerous

* Corresponding author.
E-mail address: myerson@mit.edu (A.S. Myerson).

https://doi.org/10.1016/j.xphs.2025.01.028

glycosylation, and structural flexibility.[1,14] There have also been reports wherein the aqueous phase of a protein solution was replaced with an organic phase or non-aqueous phase in suspension to reduce the viscosity of γ-globulin.[15] A protein concentration up to 300 mg/mL was achieved[15]; however, absolute ethanol and other organic solvents are not permissible if the volume of formulation significantly exceeds the limit of subcutaneous injection volume of 1.5 mL.

The concentration of therapeutic protein, polyclonal antibody human immune globulin (h IgG), in Takeda Pharmaceuticals' market product, Cuvitru® for subcutaneous injection, is 200 mg/mL.[16] Whereas in Takeda's market formulation, Gammagard Liquid/KIOVIG for intravenous injection is 100 mg/mL.[17] At protein concentrations greater than 200 mg/mL, the viscosity of the protein solution increases and is significantly higher than 20 cP, which exceeds the acceptable viscosity range of 20–50 cP for a subcutaneous injection conventionally performed at the clinic. There is a need to deliver a large dose of this therapeutic protein, and therefore, there is a demand to develop a commercially viable formulation of highly concentrated h IgGs. The goal of this work is to develop suspension formulations of amorphous antibodies with concentrations up to 400 mg/mL. The work also addresses the issue of the viscosity of the dispersion medium with appropriate additives and allows them to be administered using the given injection setup. In order to achieve a higher protein concentration in a suspension formulation, the spray drying method is adopted. For suspension formulation, a suitable medium is identified with respect to its lower viscosity and lower aggregation requirements. The spray drying technique generates a solid powder of h IgG, which was then suspended in a dispersion medium saturated with additives to prevent the solid h IgG from dissolving in an aqueous medium. The content of aggregates, monomers, dimers, and fragments of protein is estimated using size-exclusion chromatography. Finally, the suitability of the formulation is determined based on syringeability and injectability studies, and a practical scenario is considered in which formulations are injected into patients at the clinic.

Materials and methods

Materials

The protein solutions of h IgG of 100 mg/mL (10 %, pH 4.8) and 200 mg/mL (20 %, pH 5.2) are provided by Takeda Pharmaceuticals, Process Development, Plasma-derived Therapies R&D, Industriestraße 72, Vienna. These solutions were in a glycine buffer of 250 mM L^{-1} and are designated as AFG (as-received formulation with glycine). Different grades of polyethylene glycols (PEGs) such as 600, 2040, 3350, 4000, 6000, 8000, and 10,000 were purchased from Sigma-Aldrich, St. Louis, MO, USA. NaCl, NaI, NaN_3 (sodium azide), Poloxamer 188, and Urea (URE) were purchased from VWR Chemicals, LLC, Ohio, and were of bioanalytical grade. Aspartic acid (Asp), KI, Arg HCl, NaH_2PO_4 monobasic, and Na_2SO_4 anhydrous were obtained from Fischer Scientific, Fair Lawn, NJ, USA. All chemicals were of analytical grade and had a minimum purity of 98.5–99 %.

Methods

Measurement of protein concentration

Protein concentration was estimated by using UV spectrometer (NanoDrop One^c, Thermo Scientific, Madison, WI-53711). This analysis is based upon the protein A280 method, and mass extinction coefficient, ε 1 %, of 13.2 L g^{-1} cm^{-1} was used for each measurement. Before analysis, water was used as a blank or background correction. Measurements were performed in triplicate for each sample (n = 3). Samples were appropriately diluted to the linear range of the method in order to achieve a high accuracy of the protein values determined.

Critical concentration of PEGs

Critical concentration is the amount of PEG required to precipitate human pAb IgG out of the AFG solution at the given concentration of 101.9 ± 1.0 mg mL^{-1} and ambient temperature, a RT of 22.5 ± 2.0 °C. Stock solutions of different grades of PEGs, 600 (80 % w/v), 2050 (60 % w/v), 3350 (60 % w/v), 4000 (50 % w/v), 6000 (40 % w/v), 8000 (30 % w/v), and 10,000 (20 % w/v), were prepared in 1.0 M NaCl. PEG 400 was used as received in liquid form, and NaCl was not used with it. In 500 μL of AFG, PEG that is dissolved in a 1.0 M NaCl solution was gradually added until it showed light to heavy precipitates. This point was determined by visual inspection. Below this critical point, lower concentrations of PEG and NaCl would yield protein solutions that are undersaturated. A rheometer was then used to measure the viscosity of these solutions, and a phase diagram was constructed, as shown in **Figure S1**, for example, for PEG 600. The details of the procedure for viscosity measurement are given in section 2.2.6.

Spray drying process

Spray drying was performed on a bench-top spray dryer (B-290 Mini Spray Dryer, Buchi Labortechnik AG, Flawil, Switzerland) provided at Takeda Pharmaceuticals (35 Landsdowne Street, Cambridge, MA). The concentration of protein in the feed solution was kept at 50 mg mL^{-1} unless otherwise specified. For this, an AFG 10 % solution with a pH of 4.8–5.0 was diluted with water and kept on continuous stirring overnight at 4.0 ± 0.2 °C in the cold room. The spray dryer was assembled with a spray chamber, cyclone, and sample collection vessel with a capacity of 0.3 L. The atomization of the solution was carried out using a 2-way spray nozzle with a tip diameter of 1.4 mm, and a peristaltic pump was used to feed the solution. The gas (air or nitrogen) flow on the rotameter was adjusted to 40. The spray drying parameters, inlet temperature, pump, and aspirator, were set to 120 ± 1.0 °C, 15 %, and 70 %, respectively, and these were optimized. The outlet temperatures recorded before and during the entire spray drying process were 60±2.0 °C and 54±3.0 °C, respectively. Before spraying the sample solution, purified water was sprayed for 5 min to ensure that there was no moisture accumulation in the cyclone and sample collection vessel. This is related to inlet and outlet temperatures.

Spray-dried product characterization. Product yield. Spray-dried solid powder was collected directly from the sample collection vessel, without scraping the inner surface of the vessel to avoid agglomeration of particles, and the PSD was determined using a particle size analyzer. To calculate the total recovery, the product that was stuck to the interior of the spray chamber, cyclone, and sample collection vessel was thoroughly washed with water and dissolved. This amount was estimated using the UV method as described in Section 2.2.1.

Moisture content. The moisture content of the sample was determined using a KF titration. About 300 mg of SDS was used in the KF titrator (C30, Coulometric KF Titrator, Mettler Toledo LLC, Columbus, OH, USA). Before analysis, the instrument was calibrated using a calibration standard, sodium tartrate dihydrate. The measurement was performed in triplicate (n = 3).

Particle size distribution analysis. The geometric PSDs were determined by laser diffraction using a Malvern Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, UK). Liquid measurements in neat heptane were carried out using a Hydro 2000 μP dispersion unit. For a small volume, wet sample dispersions were performed at 1000 rpm. Data were analyzed based on the D_{10}, D_{50}, D_{90} and the span of the PSD. Results reported are the average of three analyses. A span value was calculated using the formula, Span = D_{90} –D_{10}/D_{50}.

Protein estimation. 50 mg of accurately weighed SDS was dissolved in 1 mL of water using a speed shaker for 1 h, and the concentration was measured using a UV method as described in Section

Table 1
Obtained from qualitative precipitation assay. C_{PT-1} is the amount used for the preparation of the formulation that would yield an undersaturated solution with ease of addition of additives.

Grade of PEG	C_{PT} (% w/v)	C_{PT-1} (% w/v)
400	54.5	50
600	46.66	44
2050	13.12	12
3350	11.61	10
4000	10.93	8.33
6000	6.66	5.0
8000	5.80	5.0
10,000	4.37	4.0

2.2.1. The measurement was performed in triplicate (n = 3). The value was multiplied by 2 (dilution factor, D = 2) to obtain a concentration in 100 mg of solid powder.

Differential scanning calorimetry (DSC). Thermal analysis of spray dried powder was performed using DSC (Q2000, TA Instruments, New Castle, DE, USA) operating with Universal Analysis software, version 4.5A. 3–4 mg of sample was weighed accurately in aluminum pans and subjected to the thermal scan at a heating rate of 10 °C min^{-1}. During analysis, a dry nitrogen purge was maintained at 50 mL min^{-1}. The instrument was calibrated using a high purity standard of indium before analysis.

Preparation of suspension formulations

Formulations were prepared in AFG solution, 10 %, pH 4.8, called primary dispersion medium. This medium was saturated using a stock solution of PEG 6000 and sodium chloride below its critical point, as shown in Table 1. It was mixed with a magnetic stirrer at 400 rpm. PEG 6000 is an optimized grade from a lower viscosity, higher number of monomers, and lesser agglomeration point of view. This saturated solution is called a secondary dispersion medium. At this point, additives were also added, if any, for example, Asp and URE. Then an equivalent amount of SDS was dispersed (% suspended solid) to reach the target protein concentration in the solution. The solid was vortex mixed for 5 min at a vortex speed of 10 using a vortex mixer (VWR Analog Vortex Mixer, Mini 120 V, Henry Troemner, LLC, USA). The following micro-suspension level of dispersion was carried out using a magnetic stirrer overnight or 16–17 h at 400 rpm. The resultant formulations were left at room temperature for a day to remove or minimize the frothing generated on the top of the formulations.

Qualitative turbidity measurement

Turbidity was measured comparing the formulations with references, water, and AFG 10 %, and 20 % solutions. Formulations were faced against a black background to perceive their color, if applicable. The qualitative score was given based on their density and color, or opacity. The density of the solution was determined using a simple formula; density, ρ = mass (m)/volume (V).

Viscosity measurement

The rheological behavior of all formulations was determined using an instrumented rheometer (Discovery HR-3, Hybrid Rheometer, Thermal Advantage, TA, USA) at a temperature of 20±0.5 °C. The instrument was equipped with cone-and-plate geometry and a temperature-controlling unit. The cone had a dimension of 20 mm of diameter and cone angle of 1° (0.59′.53″). Viscosities at different shear rates (shear rate sweep) were measured using 100–200 μL of sample depending on the estimated viscosity of the samples. Before analysis, the instrument was calibrated. Values were expressed as mean of three determinations (n = 3).

Physical-chemical stability analysis

Mobile phase preparation. For chromatographic analysis, each liter of elution run buffer was prepared by dissolving 21.3 g of Na_2SO_4 anhydrous, 2.76 g of NaH_2PO_4 monobasic, and 1.3 g of NaN_3 in demineralized water. Then the pH of 6.8 was adjusted using a 1.0 N NaOH solution. 10 % DMSO was added, and the resultant solution was vacuum filtered through 0.45 μm membrane filter using a 250 mL disposable filter unit (Nalgene Filtration Products, Thermo Scientific Inc., USA).

Size-exclusion chromatography. The HPLC system (Agilent, 1200 Series) was equipped with a TSKgel™ $G3000SW_{XL}$ column 60 cm × 7.5 mm ID, 10 μm particle size (Tosoh Bioscience, Tokyo, Japan), binary pumps, and an Agilent variable wavelength DAD detector. The concentration of excluded proteins was determined at wavelength of 280 nm by UV detector with an injection volume of 10 μL. The column was preequilibrated with mobile phase. The elution was carried out with a mobile phase at ambient temperature with a flow rate of 0.7 mL min^{-1}. The total run time per sample was 60 min. Elution time for agglomerates, dimers, monomers and fragments were 14.41 min, 16.56 min, 19.48 min, and 25.08 min, respectively. The retention time uncertainties in this analysis were ±0.02 min. Chromatograms were analyzed using Agilent ChemStation software, and the retention time and percentage main peak were recorded.

Quantitative determination of injectability

Syringeability. The formulations were syringed from the vial using an 18G needle for the determination of the injection force, and the details for injectability analysis are provided in the section below.

Injectability analysis. The measurement of the injection force was performed in compression mode using a software-controlled (Bluehill® Universal, v4.37) compression testing machine (Instron 5580, Norwood, MA, USA). The syringe was fixed in the in-house-made syringe holder as shown in **Fig. S3**, and the needle (24G, 0.75 In) was positioned downward, which had an adaptor. The syringe was made of polypropylene and had a total capacity of 3 mL. The plunger end of the syringe was placed in contact with a 10 N loading cell. Injection testing was carried out at a crosshead speed of 0.83 mm s^{-1}, representative of the 3 mL min^{-1} flow rate. The aliquot volume for all formulations was 1.5 mL. The injection force required to maintain the constant flow rate of 3 mL min^{-1} of the formulation into air was continuously recorded against the resistance offered by the material. The function of plunger displacement (mm) was then transformed into the extruded volume of the formulation from the cross-sectional area of the cylindrical barrel.

Statistical analysis

Tests for significant differences and regression analysis were performed by using the software Origin® 8.5 (Origin Lab., USA). Differences were considered significant at the $p < 0.05$ level. Linear regression analysis was performed using the method of least squares by Microsoft Excel software. The frequency of the fit was assessed from the regression coefficient (R^2).

Results and discussion

The amount of PEG, C_{PT}, required to precipitate a 100 mg/mL concentration of h IgG in 250 mM glycine is enumerated in Table 1. As expected, to precipitate a given concentration of protein, a lower amount of PEG with a higher molecular weight was required. This behavior is consistent with the fact that the volume exclusion effect is related to their nominal mass.[18] The relationship between C_{PT} and

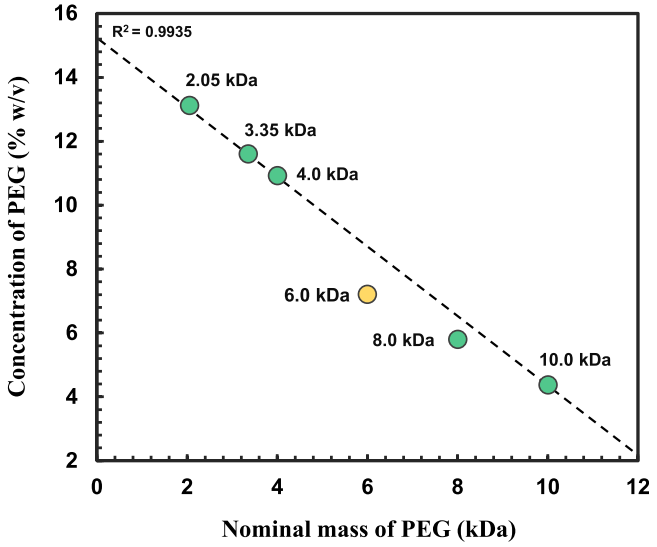

Fig. 1. The concentration of PEG and corresponding average molecular size obtained from the qualitative precipitation assay, with PEG 6000 (6.0 kDa) marked as an outlier. The lower molecular weight grades of PEGs, 400 and 600, are not shown. The R^2=0.99, is only for the data shown in the figure above, excluding PEG 6.0 kDa.

PEG molecular weight was linear except for PEG grades 400, 600, and 6000 (Fig. 1).

For suspension formulation, the objective is to optimize the dispersion medium in terms of viscosity and aggregation. The viscosities were measured for the secondary dispersion medium, which consists of PEGs and NaCl, with representative typical rheological behavior in **Fig. S2**. The viscosity of the dispersion medium increases exponentially with PEG concentration. Above the critical point, viscosity would be very high, and that solution is undesirable for the preparation of the formulation. Below this point, formulations can be prepared. In the literature, a viscosity of ~30 cP is considered the maximum acceptable for a formulation to be syringeable and injectable, with <20 cP being more preferable.[2] Fig. 2 shows the viscosity of different PEGs in a nearly saturated protein solution. The viscosity of the AFG 10 % solution is 3.97±0.08 cP. A larger size and a higher concentration of PEG contributed to a higher viscosity for the secondary dispersion medium, in the range of 4.2–7.5 (±2.5–3.3) cP. PEG 6000, an intermediate molecular weight, was selected for formulation development since the aggregation was minimal, <0.1 %, as compared to lower (400–3350) and higher (8000–10,000) molecular weight PEGs (Table S1).

A solid powder of h IgG was generated using spray drying for suspension preparation. Spray drying parameters are important critical process parameters to optimize. Based on spray conditions and atomization, the final product was deposited on different parts of the spray drying machine. In the case of product recovery, 85.5 % solids were collected directly from the sample collection vessel when the concentration of protein in the feed solution was 69.6 mg mL^{-1}. The rest of the product was recovered in wash solutions, and this contributed to 13.2 %. (Table 2) The wash solutions, comprising 13.2 % of product recovery and consisting of solutions from the spray chamber, cyclone, and sample collection vessel, had a product recovery of 2.4 %, 6.6 %, and 4.2 %, respectively. The protein content in the spray-dried solid (SDS) was estimated to be 80.21 ± 0.56 %. The amount of glycine was calculated by deducting the protein content and the moisture content. As mentioned before, spray drying was performed using different protein concentrations in the feed solution, and optimized spray drying parameters were used. To see the effect of different protein concentrations in the feed solution, the particle size distribution (PSD) of the product was determined. The total product yield was between 95.9–98.7 %. Their individual recoveries as solid powder and obtained from wash solutions are presented in Table 2. A unimodal PSD was found in all cases except for the AFG 10 % feed solution (Fig. 3).

A feed solution with a higher concentration, above 85 mg/mL, resulted in multimodal PSD, and most of the particles were found to be of larger size or agglomerates of particles when the concentration was 100 mg/mL. This is seen from their D_{10}, D_{50}, and D_{90} values (Table 2). More than 90 % of particles were below 20 μm. In a concentrated solution, the shear forces required to separate the proteins in a droplet at given process parameters are higher than for proteins in dilute solution. The 50 mg/mL concentration in the feed solution was found to be optimal for a slightly narrower PSD as compared to the rest of the protein concentration in the feed solution. This concentration was used to obtain SDS for further formulation preparations. Thermal analysis of spray dried powder showed broad endotherm due to water loss, and there was no degradation at 120 °C (Figure S1).

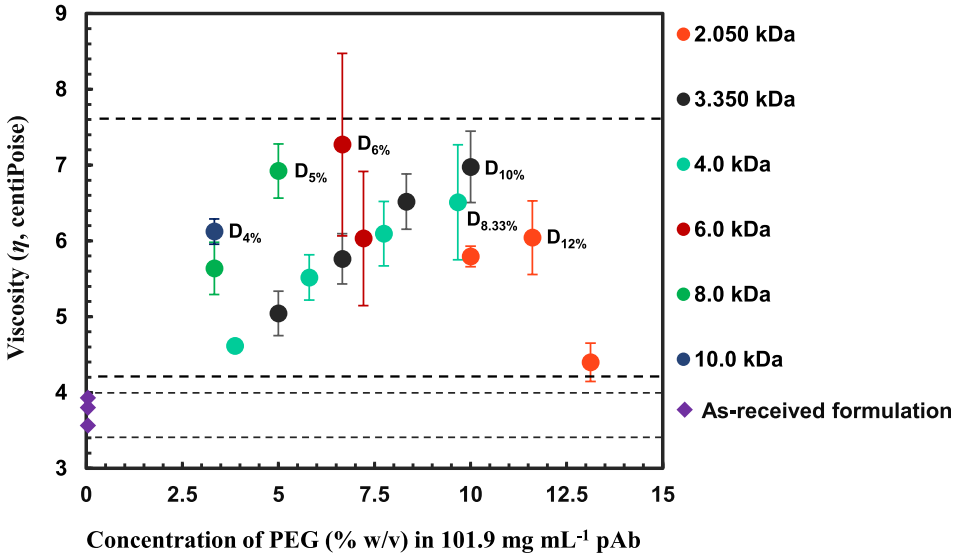

Fig. 2. Viscosity of different grades of PEG present in the 10 % solution of h IgG in 0.25 M glycine. For example, $D_{12\ \%}$ represents the concentration (12 %) of that particular PEG that can be used in the formulation preparation, and the viscosity should be below 20 cP.

Table 2
Spray-dried product characterization with different protein concentration in the feed solution.

Conc. (mg/mL)	Solid Powder (%)	Wash Solutions (%)	D_{10} (μm)	D_{50} (μm)	D_{90} (μm)	Span
100	79.74	16.18	57.4 ± 0.1	219.6 ± 0.4	608.9 ± 1.1	2.51 ± 0.49
83.6	88.0	9.0	4.1 ± 0.10	8.4 ± 0.2	16.9 ± 0.2	1.51 ± 0.02
69.6	85.5	13.2	7.7 ± 0.5	15.8 ± 1.7	29.7 ± 3.9	1.38 ± 0.09
54.3	85.0	12.0	3.7 ± 0.2	8.0 ± 0.1	16.4 ± 1.2	1.53 ± 0.14

This suggested that spray dried solid was physically stable at 120 °C. The physicochemical stability of proteins in SDS is reported in Table 3. In all cases, the h IgG was > 90 % monomers, < 7.5 % dimers, and < 0.3 % aggregates. SDS powder was found to be less dispersible in pure water, so a speed shaker was used to dissolve the solid before performing analysis.

Different electrolytes with PEG were studied in order to reduce the viscosity of primary and secondary dispersion mediums. Table 4 shows the amount of PEG 6000 (C_{PT1}) with different electrolytes (C_{PT2}) required to saturate the AFG 10 % solution. There was no significant change in the pH of the solution. Viscosity is higher for saturated solution than for 10 % solutions because of the presence of PEG. The reason both PEG and electrolytes are used is because PEG helps to quickly saturate the protein solution, and viscosity can be reduced by using electrolyte with it. NaI was a little more effective than NaCl and Arg HCl. The addition of 0.01 % of Poloxamer 188 did not have a statistically significant effect on the viscosity (Table 4). NaCl is commonly used as an electrolyte as in isotonic saline, and its amount was kept within the isotonic limit and selected for further experiments.

In order to understand the complete rheological profile with respect to the concentration of protein, a series of formulations were prepared using four optimized additives: NaCl, PEG 6000, Asp, and URE. The content of the second and third vials, 100 C and 200 C, respectively, is immune globulin in 250 mM glycine buffer, pH 4.6. Asp and URE were found to be effective when directly added to the protein solution (data not shown). The composition of formulations F1 to F7 is enumerated in **Table S2**. The role of each of these additives in the formulations has been mentioned before as being important in this work. As shown in

Fig. 4, all formulations form colloidal dispersion, and their turbidity was determined qualitatively. Qualitative turbidity depends on visual inspection, which can be subjective. According to USP Chapter ⟨855⟩, turbidity can also be determined using a visual comparison, but instrumental assessment provides a more discriminatory assessment.[19] Table S3 shows the qualitative turbidity measurement of the formulations captured in Fig. 4. The score of turbidity was given based on both density and color, or opalescence. Since the density of the formulations was also considered in the assignment of score, it can be a semi-quantitative measurement. The denser and darker color formulations scored higher, as shown in **Table S3**. The water is colorless, and 10 % and 20 % AFG solutions, designated as 100 C and 200 C, respectively, have a colorless to light yellow coloration (Fig. 4). The rest of the formulations have suspended SDS in them, and therefore, they scored higher. 100 C and 200 C are solutions; the formulations, F1 to F7, are micro-suspension or colloidal particulate dispersions.

Fig. 5 presents the rheological behavior of the formulations, F1 to F7. As anticipated, viscosity is higher for higher protein concentration; and is increasing by ~1.4 times from 250 to 280 mg/mL (Fig. 5A and B). On the other hand, the viscosity is higher with a higher protein concentration; and is increasing by ~1.6 times from 300 to 330 mg/mL (Fig. 5C and D). The viscosity was increased by 3.2-fold from 380 to 400 mg/mL (Fig. 6A and B). In both cases of relative change, F6 and F7, the difference in viscosity at the highest and lowest shear rates is 2.79 times greater, i.e., higher for F7, and lower for F6. This means formulations are better up to 380 mg/mL concentration, and beyond this concentration, viscosity drastically increases by more than 3-fold. Depending on their shear-thickening behavior, they can be further subcategorized. This subcategory is presented in Fig. 7. Except for F1, the rest of the formulations are non-Newtonian systems. The shear thickening in the case of F1 remained negligible. If the rheological behavior of F5 is compared with F6 (F6 is shown in Fig. 6) it is seen that the viscosity higher with a higher protein concentration; and was increased by only ~1.08 times from 350 (F5) to 380 mg/mL (F6).

Formulations (F1 to F7) were studied for injectability and also compared with references, AFG 10 % and 20 %, including water. Fig. 8 shows the typical injectability profile of the formulations, wherein the extruded volume of the formulation is plotted against injection force. The injection force was continuously recorded against the

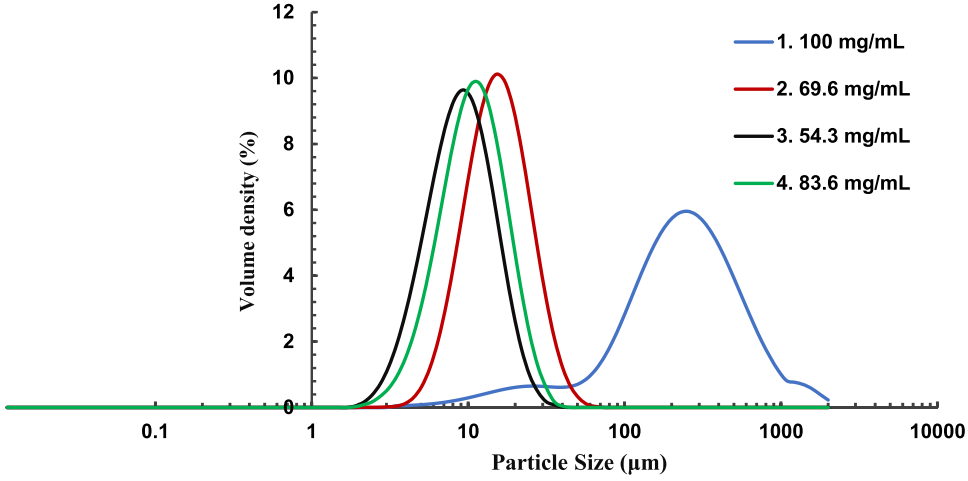

Fig. 3. Particle size distribution of spray-dried product for different values of initial protein concentration.

Table 3
Physical-chemical stability analysis of h IgG in SDS powder. The inlet temperature was 120 °C.

Conc. (mg/mL)	Aggregates (%)	Dimer (%)	Monomer (%)	Fragments (%)
100	0.263	7.371	90.979	1.387
83.6	0.204	5.357	93.097	1.341
69.6	0.164	6.592	91.865	1.379
54.3	0.233	5.852	92.564	1.352

Table 4
Concentration of components at the precipitation of AFG 10 % at RT and their viscosity.

Components	**pH**	**C_{PT1} (mMol)**	**C_{PT2} (%w/v)**	**Viscosity (cP)**	**Viscosity (cP)** with 0.01 % Poloxamer 188
AFG 10 %, A	4.8	–	–	3.97 ± 0.08	–
A + NaCl - PEG	4.9	182.3	6.66	7.93 ± 0.21	7.41 ± 0.74
A + NaI - PEG	4.9	166.6	5	4.14 ± 0.17	4.63 ± 0.23
A + Arg HCl - PEG	5.0	285.7	8.57	6.56 ± 0.19	7.89 ± 0.34

resistance offered by the formulations when the flow rate was constant at 3 mL/min. The entire injectability profile can be divided into four different phases: Phases I, II, III, and IV. Phase IV is not relevant to the flow behavior of the suspension since the syringe became empty and therefore can be ignored. Thus, phase IV has no practical relevance in a real-world scenario. Phase I is related to the initiation when the user starts pushing the plunger following phase II. The maximum force is the threshold at which the pain at the site of injection could become maximum; however, it is not easier to predict the threshold value for the pain at the site of injection based on Phase II and III. For the highly viscous formulation of 400 mg/mL, which shows maximum yield force in Phase II and ultimate force in Phase III, the injectability would become almost impractical from the pain at the site of injection viewpoint. It will be easier for user to inject the formulation with lower ultimate forces and therefore, 330 mg/mL and below this formulation would cause lesser pain at the site of injection. However, it remains difficult to directly link the Phase II and III with patients' response towards pain at the site of injection without studying at the clinic, which remains outside the scope of this work.

More details of Phase II and Phase III have been provided in Fig. 9. Phase III is called the plateau phase since most of the suspensions were injected at a constant force without any significant fluctuations in the recorded injection force. For low viscosity fluids, the ultimate force, f_u, is almost the same as yield force. This kind of formulation would not really cause any significant pain at the site of injection. f_u is the force recorded at the end of the plot, and is also called the maximum force offered by the material. Yield force, f_y, is the force or point after which formulations demonstrated continuous viscoplastic flow without any fluctuations in the injection force until it achieves the f_u. Almost all formulations showed this behavior in Phase III. Apparently, injectability or injection force is a function of the viscosity of the fluid, the type of the syringe (barrel and needle gauge used), as well as flow rate achieved. As the plunger moves in the barrel, stopper-plunger break-loose forces[20] are registered first, which are quite comparable regardless of the viscosity of the suspensions in all cases. Yield forces, f_y, vary substantially depending on the viscosity of the formulation, and their attainment has been delayed in Phase II or Phase III. For low-viscosity fluids, 100 C and 200 C, f_y are negligible. Moderately high viscosity fluids, from 250C to 330C, gained yield forces in Phase II, and the rest of the highest viscosity formulations, 350 C, 380 C, and 400 C inclusive, achieved their yield forces in Phase III.

Table 5 shows quantitative injectability parameters that were derived from the force-displacement profile of the suspension formulations shown in Fig. 8. As the concentration and therefore the viscosity of the formulations increased, the values of f_y, $f_{V\bullet 1/4}$, and f_u also increased accordingly. The reason these parameters are calculated is because they indicate the nature of the flow during injectability analysis. This means that for a given fluid, if the standard deviation between these parameters is minimal or close to zero, such systems are considered mechanically homogenous. If the value of the standard deviation is greater, those systems are mechanically inhomogeneous. Ideally, the value should decrease and approach close to zero, but it should not increase. And Pmax is the maximum pressure that was created inside the barrel, which is calculated from the value of f_u and the cross-sectional area of the cylindrical barrel.

Syringeability and injectability are key product performance parameters for any parenteral drug formulation. Although they seem very similar, they have considerable differences. Syringeability refers to the ability of an injectable formulation to pass easily through a hypodermic needle on transfer from a vial prior to an injection.[20] On the other hand, injectability relates to the performance of the formulation during injection and therefore has to do with injection force. At the clinic, an injection is conventionally performed by pushing the thumb on the plunger while the ipsilateral index and middle fingers are used to stabilize the syringe.[21] According to the literature, in this manner, the average maximum force that can be generated is 79.8 N. The males could generate a higher injection force, 95.4 N, than the females, 64.1 N.[22] Although the value in females is 64.1 N, it has also been recommended that, no matter what sort of formulation we are dealing with, the injection force should not exceed the limit of 40 N, otherwise, it will be difficult for a female physician to inject such a

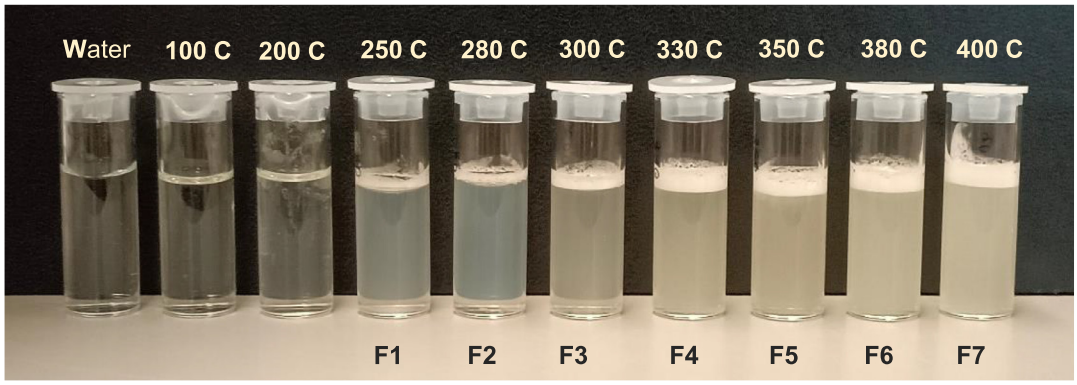

Fig. 4. Photographic image showing a series of formulations, from F1 to F7, with controls or references, water, 10 % AFG, and 20 % AFG, and were also considered for qualitative turbidity measurement.

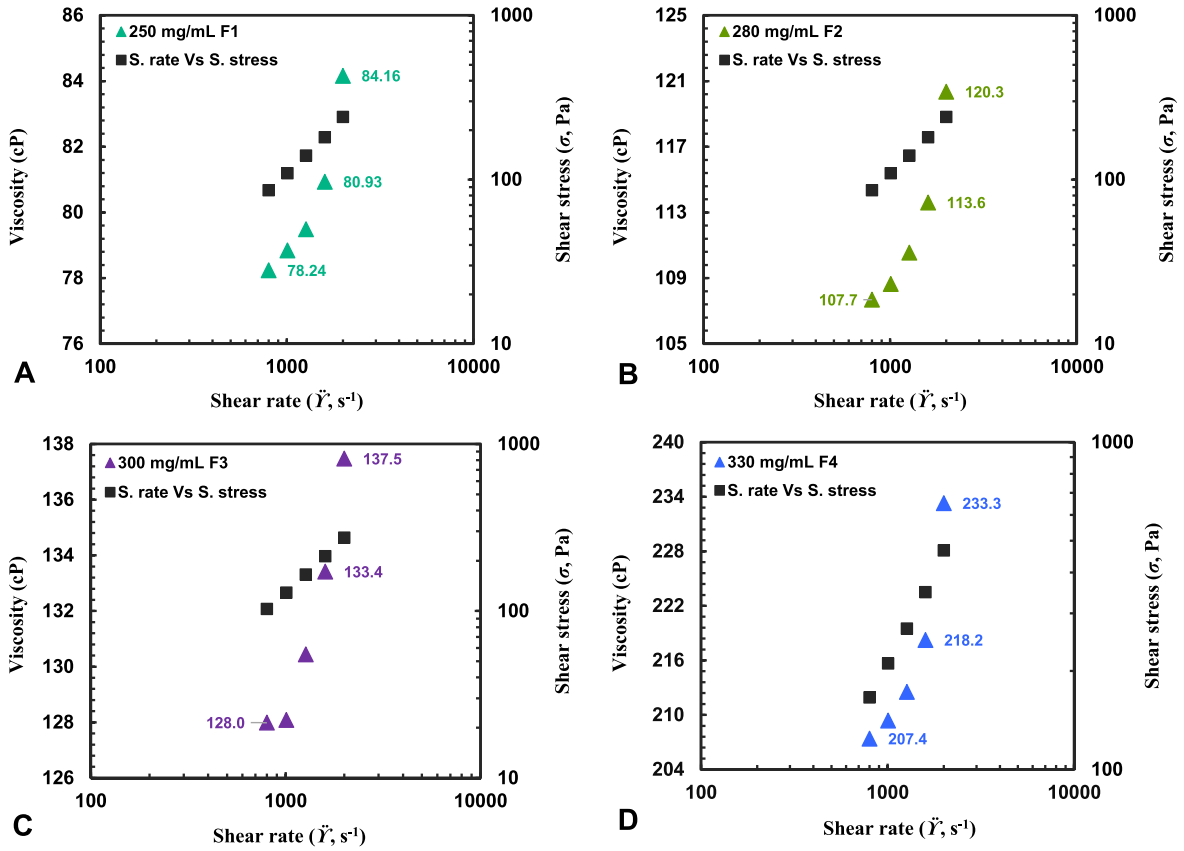

Fig. 5. Plots showing the rheological profile of formulations F1 (A), F2 (B), F3 (C), and F4 (D) The viscosity values are shown at the highest, lowest, and mid-point shear rates, respectively. Their concentration is also displayed in the legend of the plot.

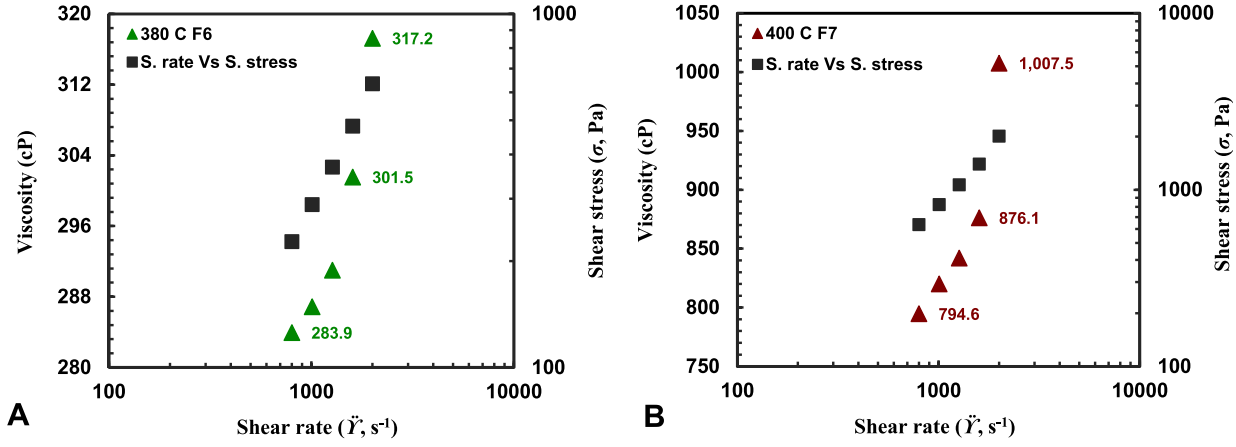

Fig. 6. Plots showing the rheological behavior of formulations F6 (A), and F7 (B). The viscosity values are shown at the highest, lowest, and mid-point shear rates applied. F25 and F26 has a protein concentration of 380 mg mL^{-1} and 400 mg mL^{-1}, respectively.

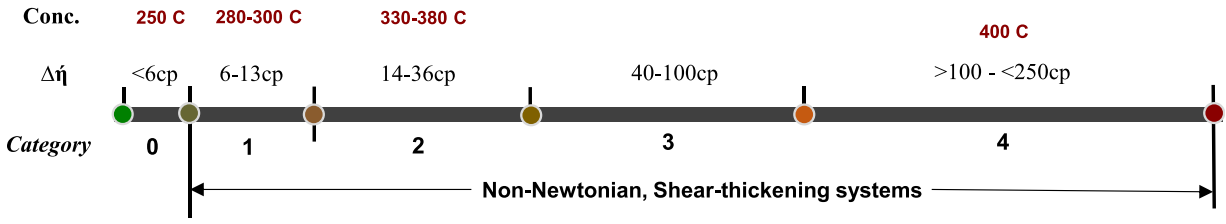

Fig. 7. Subcategory of rheological systems based on their shear thickening behavior. Category 0 stands for the Newtonian system, where shear thickening is negligible. Δή shows the magnitude difference of shear-thickening and viscosity differences at the lowest and highest shear rates applied.

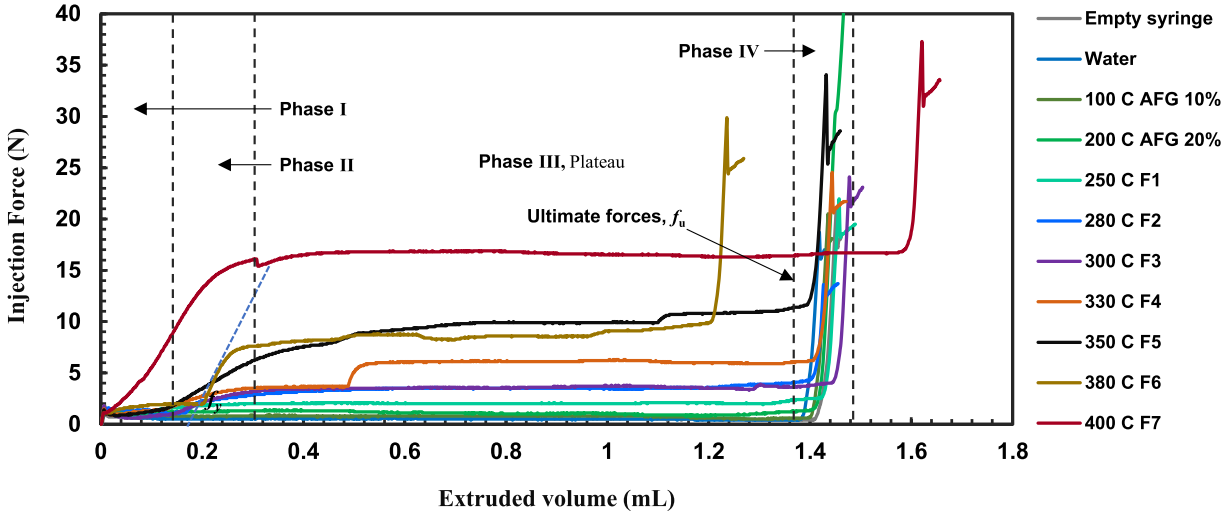

Fig. 8. The quantitative injectability of the formulations was also compared with empty syringes and reference protein solutions. Different regions of the plots are designated as Phase I, II, III, and IV depending on the injection force recorded.

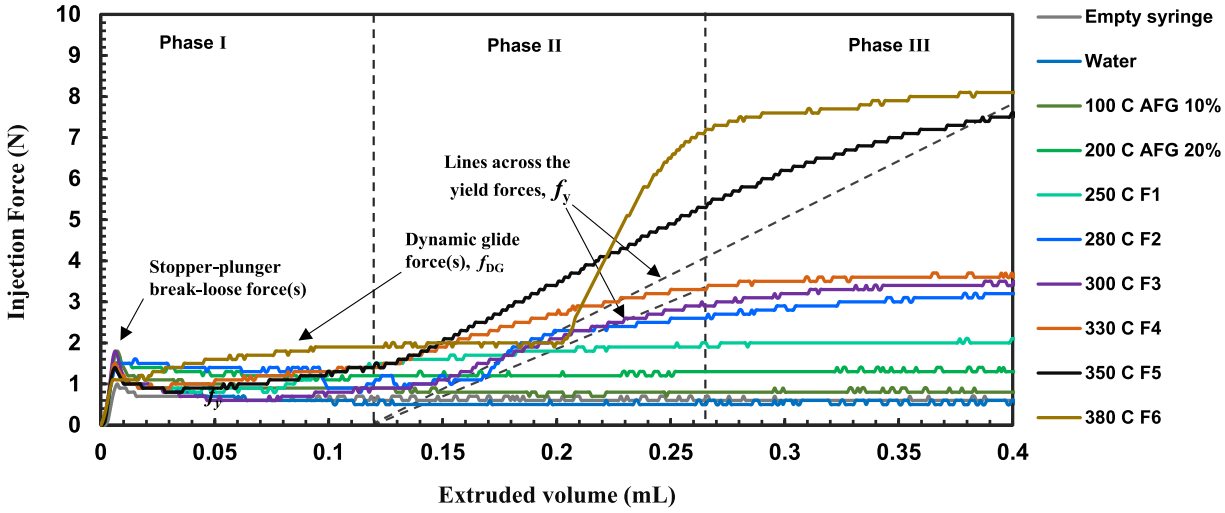

Fig. 9. Details of Phase I, and II, from Fig. 8. The plot characterizes behavior before fluid achieves a plateau, the highest concentration formulation, 400 C F26, is omitted. stopper-plunger break-loose force, following dynamic glide forces, f_{DG}, and a line across the yield forces, f_y. The forces at 1/4th of the volume shown at the end of the plot, have also been enumerated in Table 5.

formulation.[21] And, if we consider this 40 N cut-off instead of 64.1 N, all formulations, F1–F7, are well within range with respect to even the highest protein concentration formulation, F7 400 mg/mL (Table 5), with a maximum injection force of 16.5 N, which is 2.4 times lower than the injection force limit recommended for female physicians.

Despite high viscosity, specifically formulations F4 to F7 remained injectable. These high-viscosity, shear-thickening formulations were injected smoothly with the desired flow rate of 3 ml/min, and a syringe with a 24G needle was used. The applied forces recorded higher resistance as the formulations' viscosity increased. All formulations include additives such as PEG, NaCl, urea, and aspartic acid. PEG might contribute to the formulation's high viscosity, while the other additives (NaCl, urea, and aspartic acid) play a role in lowering the viscosity of the formulation and allowing the fluid to flow more easily through a 24G needle. PEG has polyhydroxy functional groups that might interact directly with water through H-bonds, and their very long polyethylene polymer chain might get entangled with large immune globulin molecules and contribute to higher viscosity. On the other hand, small molecules like aspartic acid (non-planar

Table 5
Quantitative injectability parameters obtained from force-displacement profile of the formulations.

Formulation	Protein Conc. (mg/mL)	f_y (N)	$f_{V•1/4}$ (N)	Ultimate Force, f_u (N)	Maximum Pressure, P_{max} (MPa)
100 C	101.9 ± 1.6	0.8 ± 0.01	0.8	0.7	0.012
200 C	207.2 ± 0.8	1.2 ± 0.03	1.3	1.4	0.024
F1	~250	2.0 ± 0.01	2.1	2.5	0.043
F2	~280	2.9 ± 0.01	3.2	4.3	0.075
F3	~300	3.5 ± 0.01	3.5	3.8	0.066
F4	~330	3.7 ± 0.05, 5.6 ± 0.10	3.6	6.2	0.108
F5	~350	7.9 ± 0.20	7.6	12.2	0.213
F6	~380	8.7 ± 0.10	8.1	10.0	0.175
F7	~400	15.6 ± 0.20	16.5	16.5	0.289

$f_{V•1/4}$ is the force or point at which 1/4th of the volume had already been injected in Phase II or Phase III.

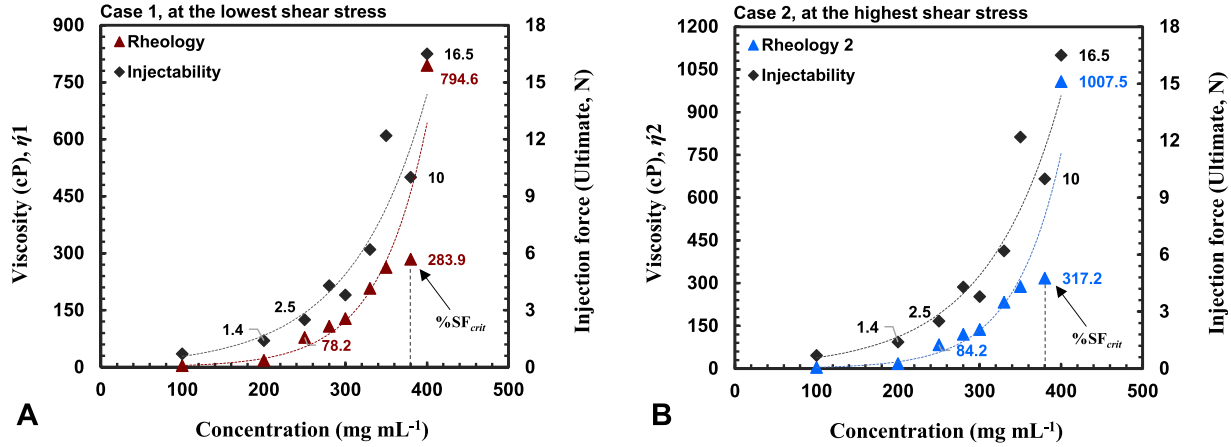

Fig. 10. Plots showing interdependency of protein concentration, viscosity, and associated injection force. A. at the lowest shear rates, B. At the highest shear rates. %SF is the critical solid fraction of spray-dried solid in suspension beyond which viscosity increases more than three times.

molecule) and urea (planar molecule) form stable H-bonds with water, PEG, as well as immune globulin molecules, and can act as molecular lubricants in order to reduce the viscosity. NaCl is responsible for solvation and stable ionic bonds that diffuse along the charged functional groups of amino acids of immune globulin molecules and water and thereby lessen the higher viscous forces. Also, under the applied force, shear-thickening systems would thicken and would resist the flow; under constant applied pressure, the design of the syringe-needle system might have played an important role in compensating for the high viscosity and allowing the suspension to flow under these specific conditions.

The interplay between protein concentration, viscosity, and injection force remains important to understand their interdependency. Fig. 10 captures this behavior, where viscosity and injection force both exponentially increased as protein concentrations were raised. %SF is the point or critical amount of SDS, 380 mg/mL, above which viscosity drastically increases and so does the injection force. However, apparently, in both Newtonian and non-Newtonian systems, the concentration dependence on the viscosity is much greater than the concentration dependence on the injection force. Rather, for the series of protein concentrations and at a given injection set-up, injection force relatively operates in a in a narrow range. This means such ultra-highly concentrated protein biologics, in this case, high-viscosity h IgG colloids, are syringeable and injectable up to 400 mg/mL concentration; however, their viscosity is not 20–50 cP.

Table S4 shows the physicochemical stability of h IgG in different formulations. In general, 10-fold dilution improves the dissolution of suspended solids and hence reduces the content of agglomerates. Suspensions, F1 to F7, had an aggregate content already close to the specification limit of Gammagaurd Liquid/KIOVIG. The higher content of monomers in the formulations is believed to be due to the high-quality spray-dried solids. All suspensions were prepared in an AFG 10 % solution, and in an AFG 20 % protein solution, dimers were found to be higher as compared to the 10 % solution. The aggregate amount remained low regardless of the concentration of the product.

Conclusion

The present work systematically developed a suspension formulation where simple additives, PEG 6000, NaCl, Aspartic acid, and Urea, were identified and optimized. These formulations investigated consist of additives that are pharmaceutically acceptable. Analytical techniques apart, formulation preparation involves unit operations like spray drying and mixing. We also demonstrated that highly concentrated, high-viscosity human immune globulin colloids are syringeable and injectable up to 400 mg/mL concentration, however, their viscosity is not 20–50 cP. This behavior creates a viscosity-injectability paradox (VIP), a very important parameter, and implies that highly concentrated protein biologics may not only be assessed from a viscosity point of view, but injectability or injection force should also be taken into account. The practical scenarios in which injections are performed at the clinic may supersede their highly viscous behavior. Finally, where stable, continuous, mechanically homogenous viscoplastic flow is achieved, injectability is not an issue, but pain assessment will remain an open issue. In addition, while the DSC analysis presented in this work provides information on chemical stability additional work such as mass spectrometry and force degradation studies should be performed to further characterize the stability.

Associated content

Supporting information for this article is provided and is available with the main text.

Declaration of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors thank the Central Machine Lab, MIT, and the Department of Materials Science and Engineering Lab, MIT, for providing the facility for injectability studies. We acknowledge Takeda Pharmaceuticals for providing funding for this work.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.xphs.2025.01.028.

References

1. Yang MX, Shenoy B, Disttler M, et al. Crystalline monoclonal antibodies for subcutaneous delivery. *Proc Natl Acad Sci*. 2003;100(12):6934–6939.
2. Shire SJ, Shahrokh Z, Liu J. Challenges in the development of high protein concentration formulations. *J Pharm Sci*. 2004;93(6):1390–1402.
3. Jezek J, Rides M, Derham B, et al. Viscosity of concentrated therapeutic protein compositions. *Adv Drug Deliv Rev*. 2011;63(13):1107–1117.

4. Du W, Klibanov AM. Hydrophobic salts markedly diminish viscosity of concentrated protein solutions. *Biotechnol Bioeng*. 2011;108(3):632–636.
5. Guo Z, Chen A, Nassar RA, et al. Structure-activity relationship for hydrophobic salts as viscosity-lowering excipients for concentrated solutions of monoclonal antibodies. *Pharm Res*. 2012;29:3102–3109.
6. Inoue N, Takai E, Arakawa T, Shiraki K. Arginine and lysine reduce the high viscosity of serum albumin solutions for pharmaceutical injection. *J Biosci Bioeng*. 2014;117(5):539–543.
7. Inoue N, Takai E, Arakawa T, Shiraki K. Specific decrease in solution viscosity of antibodies by arginine for therapeutic formulations. *Mol Pharm*. 2014;11(6):1889–1896.
8. Larson AM, Weight AK, Love K, Bonificio A, Wescott CR, Klibanov AM. Bulky polar additives that greatly reduce the viscosity of concentrated solutions of therapeutic monoclonal antibodies. *J Pharm Sci*. 2017;106(5):1211–1217.
9. Miller MA, Engstrom JD, Ludher BS, Johnston KP. Low viscosity highly concentrated injectable nonaqueous suspensions of lysozyme microparticles. *Langmuir*. 2010;26(2):1067–1074.
10. Johnston KP, Maynard JA, Truskett TM, et al. Concentrated dispersions of equilibrium protein nanoclusters that reversibly dissociate into active monomers. *ACS Nano*. 2012;6(2):1357–1369.
11. Dear BJ, Hung JJ, Laber JR, et al. Enhancing stability and reducing viscosity of a monoclonal antibody with cosolutes by weakening protein-protein interactions. *J Pharm Sci*. 2019;108(8):2517–2526.
12. Yang D, Walke LM. Synergistic effects of multiple excipients on controlling viscosity of concentrated protein dispersions. *J Pharm Sci*. 2023;112(5):1379–1387.
13. Basu SK, Govardhan CP, Jung CW, Margolin AL. Protein crystals for the delivery of biopharmaceuticals. *Expert Opin Biol Ther*. 2004;4(3):301–317.
14. Ahamed T, Esteban BN, Ottens M, et al. Phase behavior of an intact monoclonal antibody. *Biophys J*. 2007;93(2):610–619.
15. Srinivasan C, Weight AK, Bussemer T, Klibanov AM. Non-aqueous suspensions of antibodies are much less viscous than equally concentrated aqueous solutions. *Pharm Res*. 2013;30:1749–1757.
16. https://www.takeda.com/newsroom/shire-news-releases/2016/frj956/. Accessed in June 2023.
17. https://www.gammagard.com/primary-immunodeficiency. Accessed in June 2023.
18. Munakata S, Hatori K. The excluded volume effect induced by poly (ethylene glycol) modulates the motility of actin filaments interacting with myosin. *FEBS J*. 2013;280(22):5875–5883.
19. Barros M, Zhang X, Kenrick S, Valente JJ. Opalescence measurements: improvements in fundamental knowledge, identifying sources of analytical biases, and advanced applications for the development of therapeutic proteins. *J Pharm Sci*. 2021;110(11):3550–3557.
20. Cilurzo F, Selmin F, Minghetti P, et al. Injectability evaluation: an open issue. *AAPS PharmSciTech*. 2011;12:604–609.
21. Vo A, Doumit M, Rockwell G. The biomechanics and optimization of the needle-syringe system for injecting triamcinolone acetonide into keloids. *J Med Eng*. 2016;2016:1–8.
22. Astin AD. *Finger Force Capability: Measurement and Prediction using Anthropometric and Myoelectric Measures*, Masters thesis. Blacksburg, VA, USA: Faculty of the Virginia Polytechnic Institute and State University; 1999:1–103.