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Annamaria Lapenna, Camille Dagallier, Sylvain Huille, Christophe C. Tribet. Poly(glutamic acid)-Based Viscosity Reducers for Concentrated Formulations of a Monoclonal IgG Antibody. *Molecular Pharmaceutics*, inPress, 10.1021/acs.molpharmaceut.3c01159. hal-04406095

HAL Id: hal-04406095

<https://hal.sorbonne-universite.fr/hal-04406095>

Submitted on 19 Jan 2024

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Poly(glutamic acid)-based viscosity reducers for concentrated formulations of a monoclonal IgG antibody.

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KEYWORDS: antibody, viscosity, poly(amino acid), coacervate, polyanion:polycation complexes.

ABSTRACT. Above a concentration threshold the viscosity of solutions of proteins increases abruptly, which hampers injectability of therapeutic formulations. Concentrations above 200 g/L is an ideal goal for subcutaneous application of antibodies. Molecular additives, such as amino acids (ex. Arg) help to decrease viscosity, but they are used at concentrations as high as about 200 mmol/L. We addressed the question of whether poly(amino acids) could be more efficient than small molecular additives. We observed marked fluidification of a model therapeutic mAb solution by poly (D,L-glutamic acid) and poly(L-glutamic acid) derivatives added at < 6.5 g/L (i.e.,

mAb/polymer chain molar ratio between 4:1 and 1:1 mol/mol). The bare poly(glutamate) parent chains were compared with polyethyleneglycol-grafted chains as PEGylation is a common way to enhance stability. Viscosity could be lowered down to ~ 20 mPa.s as compared to values of ~ 100 mPa.s in the absence of polymers at 200 g/L mAb. Formation of complexes between the mAb and the polyglutamates was characterized by capillary electrophoresis analysis in dilute solutions (1g/L mAb) and by observation of phase separation at higher concentration, suggesting tight association at about 2:1 mol/mol mAb/polymer. Altogether these results show that polyglutamate derivatives hold an untapped potential as excipient for fluidification of concentrated protein solutions.

Introduction.

Since the commercialization of the first therapeutic monoclonal antibody (mAb) in 1986, this class of proteins has grown to become dominant in the drug development pipeline.¹ mAbs solutions combined with (bio)polymers are now a common chassis for developments of new therapeutic approaches, for immunotherapies (ex: cell-targeted controlled delivery),² intracellular targeting,³ oral or ocular delivery.^{4, 5} Their use is however hampered by obstacles, especially due to difficulties to control stability (protein aggregation), and solution viscosity. In practice, fluidification of highly concentrated mAb solutions (e.g., > 150 g/L) is in demand because of requirement for (i) syringeability (injection through usual syringe needs viscosity below ~50 mPa.s),⁶ and (ii) lowering injected volumes, specifically in the case of subcutaneous implementation (typically targeting < 2 mL). The viscosity issue and thickening effect observed in concentrated solutions of proteins is a key general question that has been discussed theoretically, with models coming from colloid science.^{7, 8} Viscosity-concentration relationships are now reliably used for small globular proteins.⁹ But it is still challenging to predict the viscosity pattern of non-spherical, flexible proteins such as mAbs. The complexity of viscosification mechanism of mAb and specifically a high sensitivity to concentration is presumably due to contributions of mAb-mAb contact points^{10, 11, 12} or formation of transient interprotein clusters.^{13, 14} In other words, a pronounced viscosity increase is generally associated with the presence of attractive interactions between mAbs, as suggested by experimental investigations on the correlation between viscosity and interaction parameters^{15, 16, 17}), or indications on the formation of interprotein reversible complexes at high concentrations.^{18, 19, 14, 20} But discrepancies with this simple rule are also reported.⁸

Fluidifying formulations of mAb aims to modulate these inter-protein interactions, via an introduction of molecular additives (excipients).²¹ Conventional additives can be amino acids^{22, 23, 24}, or surfactants such as polysorbates.⁸ These small molecules typically provide weak and labile perturbations of the interactions, either by affecting ionic strength or by screening specific patches onto mAbs. In situ PEGylation of IgGs has similarly been reported to improve fluidity.¹⁹ In other words, protein-protein attractions may be discouraged, by using transient (dynamic) competitive binding with additives. In particular charged amino-acids reduces viscosity, which was tentatively explained by their ability to shield coulombic interactions and to disrupt mAb-mAb associations. Arginine is the most prevalent amino acid additive in literature,²² although various cationic or anionic amino-acids impact viscosity as well.²¹ But high concentrations of up to 0.1 - 1 mol/L of these small molecular additives are required, making these formulations not always suitable for clinical trials or commercialization.. Molar amounts of amino acids makes it complicated to adjust pH, osmolarity and may introduce practical problems of cost or toxicity.

We hypothesized that tighter interactions could enhance the efficiency of viscosity-reducing agents. Here, we addressed the question of whether polymers of amino-acids, that afford a multiple binding pattern, offer more stable binding onto mAb while preserving the favorable effect of their monomeric amino-acids precursors. Short, ionized poly(amino acids) can bind onto mAbs of unlike charge.²⁵ A few studies are reported in literature, in which poly(glutamic acid) (PLE) and poly (γ -glutamic acid) bind onto mAbs. Association with PLE was used to trigger phase separation of native mAbs.²⁶ Enhancement of thermal stability of antibodies was achieved using poly (γ -glutamic acid),²⁷ or poly(glutamic acid)-b-polylysine diblocks.²⁸ The present work aims to interrogate the use of PLEs additives as viscosity reducers. As our goal was to establish a proof of principle, we included in the study both modified and unmodified poly(glutamates) whose G.R.A.S.

status (generally recognized as safe) may not be established. Specifically, in this work PLE parent chains were grafted with polyethylene glycol (PEG) strands i.e., a common enhancer of colloidal stability. We evaluated poly(glutamic acid) derivatives of either short (45 monomers) or longer (90 monomers) chains. We studied (i) the impact of PLE derivatives on viscosity of a model mAb and (ii) their binding to the mAb in dilute conditions. Viscosity was measured by capillary viscosimetry and by using tracer beads together with light scattering. Capillary electrophoresis enabled to characterize binding equilibrium.

Materials & Methods.

Preparation of mAb solutions.

For viscosity measurements, samples of concentrated mAbs were prepared by dialysis and reconcentration of stock solutions obtained from SANOFI (i.e., a 93 g/L IsatuximAb in 20mM Histidine-HCl buffer 10% sucrose pH 6.3, or the formulated 140 g/L mAb in the same buffer but with no sucrose and with excipients). Aliquots of 1-3 mL were dialyzed overnight (slide-A- Lyzer device 7-20 kDa MWCO, from Pierce, ThermoFisher Scientific), at 8°C against either 5mM Histidine-HCl + 10% sucrose (pH values of either 5.2 or 4.8) or 5 mM NaH₂PO₄-Na₂HPO₄ pH 5.2. An upshift in pH, compared to the dialyzing buffer, was systematically observed due to Donnan effect: the pH measured after dialysis in the sample was of 6.2-6.3 (resp. 5.9) when dialyzed against pH 5.2 (resp. 4.8). To prepare stock solutions at 180 g/L, the sample was kept in the slide-A-lyzer cassette that was deposited on an aluminium foil and covered by dry powder of poly(oxyethylene) (PEO 200 000 g/mol, Sigma) for 30 minutes. To prepare mAb solutions at ca. 250 mg/mL, the dialyzed samples were ultrafiltrated on Amicon Ultra 0.5 mL 10 kDa Centrifugal filters (from ThermoFisher, 30 min. at 12 000 g, 10°C). The final concentration of the mAb was determined from the absorbance at 280 nm (Nanodrop One, Thermo scientific) on undiluted samples, using eq. 1:

$$[\text{mAb}] \text{ (g/L)} = 1.65 \times [\text{absorbance}(280\text{nm}) - \text{absorbance}(600)] \quad \text{eq.1}$$

Where the coefficient 1.65 was obtained from a calibration curve measured on the used spectrometer.

For binding isotherm measurements by electrophoresis or to prepare coacervates at 20 g/L mAb, we used a stock solution at 20 g/L in 20mM Histidine, 10% sucrose, pH 6.3 that was dialyzed in similar condition as above, followed by pH adjustment with a solution of 5mM Histidine + 10% Sucrose (resp. phosphate buffer), and eventually concentration by ultrafiltration in Amicon Ultra 0.5 mL 100 kDa Centrifugal filters (15 min. at 15000 g).

Preparation of polymer:mAb mixed solutions.

If not specified, mixtures were prepared by adding aliquots of concentrated polyglutamate stock solutions (NaCl 2M, polyglutamate derivatives of 10 g/L to 120 g/L) in a concentrated mAb solution and dilution with the ad hoc buffer at the same pH as the mAb solution. The pH of polyglutamate stock solutions in water was adjusted using 0.1 M HCl or NaOH to the final pH (6.3 or 5.9) before mixing.

Capillary electrophoresis

Measurements were performed using a Beckman P/ACE system MDQ instrument equipped with a diode array UV/visible detector (Beckman Instruments, Fullerton, CA), operating at 25 °C, and fitted with bare silica capillaries of either 50 µm or 75 µm internal diameter (Polymicro Technologies CM Scientific). The capillary was flushed daily with 0.1 M NaOH (5 min., 10 psi), followed by a water rinse. Bare silica capillary was used to analyze PLE derivatives in the absence of mAb. To analyze mAb-containing samples, the capillary was coated by flushing (5min. 2 psi) 1.5 g/L PLL(20)-g[3.5]-PEG5 (a comb-like PEGylated poly(lysine) of 20 kDa from Susos Dübendorf Switzerland) before being conditioned by flushing the running buffer (5mM NaH₂PO₄-Na₂HPO₄ pH 6.3) for 5min. 2 psi. Frontal analysis (FACCE) consisted of running the separation with the inlet plunged in the sample, and the capillary outlet and initial filling being the analyte-free buffer. A voltage of 20 kV was applied in addition to a small pressure of 0.1 psi used to push the solution toward the detection window. In conditions of “positive” voltage (anode at the inlet), migration of the cationic mAb was the fastest. In condition of “negative” voltage (cathode at the inlet), the anionic polyglutamates were detected first. Possibly a small zone of neutral marker (0.1 g/L mesityloxyde in the run buffer) was injected (1 psi, 5s.) prior to running the separation.

The elution time of this zone, t_{eo} was used to calculate the effective electrophoretic mobility μ of analytes from eq 2, where t_m is the sample migration time, L_t is the total length of the capillary (31 cm), L_d is the length to the detector (21 cm), and E the electric field.

$$\mu = \frac{L_t \cdot L_d}{E} \left(\frac{1}{t_m} - \frac{1}{t_{eof}} \right) \quad \text{eq. 2}$$

Capillary viscosimetry

Viscosity measurements were performed at 25 °C, using the Beckman P/ACE system MDQ instrument as for electrophoresis. The capillary was first conditioned by rinsing with water, 0.1 M HCl, and 0.1 M NaOH at 2 psi (2 min. each), then filled with the sample (injection at 5 psi for 5-10 minutes). A small zone of buffer was formed by injecting a solution of buffer (15 s., 1 psi). Then a constant injection pressure ΔPa of 1 psi or 5 psi was applied, with the inlet plunged in the sample, to push the zone toward the detection window. Absorbance at 280nm or 200 nm enabled to detect the time, t_b , of elution of this zone of buffer as a negative peak. The viscosity, η , is calculated using the Hagen–Poiseuille’s law (with D the capillary inner diameter)²⁹:

$$\eta = \frac{\Delta Pa \cdot D^2}{32 \cdot L_t \cdot L_d} \cdot t_b \quad \text{eq. 3}$$

Calibration was done by measurements of Sucrose solutions in water (40% - 60%).

Tracer beads microviscosity by DLS

The protocol was adapted from ref³⁰. Polystyrene Carboxylate Microspheres (2.6% solid latex Polybeads, Polyscience) of 200 nm diameter were used as tracers. A volume of 99 μ L of concentrated mAb solution was supplemented with 1-2 μ L of a solution of Polybeads diluted to 1% solid latex in water. The scattering intensity was measured at 25 °C and at an angle of 90° in a BI-200SM Brookhaven Instrument system equipped with a 30 mW, 637 nm laser and photomultiplier detection. Effective relaxation time (τ_{bead}) was calculated by fitting the field correlation functions to a biexponential decay model (using nonlinear fitting of $y = A1 \cdot \exp(-x/t1) + A2 \cdot \exp(-x/t2) + y0$ in the software OriginPro 2022 from Origin Lab Corp.). The slower decay mode was ascribed to beads, whereas the faster one was ascribed to the mAb. The effective viscosity then reads:

$$\eta = \eta_{\text{ref}} \cdot \tau_{\text{bead}} / \tau_{\text{ref}} = \eta_{\text{ref}} \cdot Rh_{\text{bead}} / Rh_{\text{ref}} \quad \text{eq. 4}$$

where η_{ref} is the viscosity of water (0.89 mPa.s), τ_{ref} (resp. Rh_{ref}) the reference relaxation time (resp. hydrodynamic radius) of the beads in water. An illustration of correlograms and fits is shown in figure SI.1. in SI.

Results.

Preparation of Poly(amino acid) derivatives.

The poly(D,L-glutamic acid) parent chains of molecular weight 7500 or 15000 g/mol were random copolymers of D and L glutamic acids, under the form of sodium salt, with average degrees of polymerization of 45 or 90 monomer units respectively ; below they are named accordingly **45PLE** and **90PLE**. We used in addition a poly(L-glutamic acid) acid of Mw 6450 g/mol (45 monomers), quoted **45PLEL**. Derivatives of the parent chains were prepared by acid-amine coupling of short poly(ethylene glycol) side chains (PEG of Mw 3000 g/mol, designated as PEG3) as described in section SI.2 in SI. We prepared PEGylated derivatives with either 3 mol% or 10 mol% PEG compared to glutamate units. Capillary electrophoresis enabled to separate the products as a function of their effective mobility, which decreased with increasing degree of PEGylation (because of the drag added by PEG side chains). Electrophoresis separated PEG-modified chains from residual ungrafted parent chains and was capable to resolve single-grafted chains from multiple-grafted ones (Fig. SI.2 in SI). Both NMR (Fig. SI.3 in SI) and electrophoresis analysis indicated that the effective coupling of PEG was poor at 3 mol%. A significant amount of residual ungrafted 45PLE was observed when 3 mol% PEG was introduced (Table SI.1 in SI). In contrast, near quantitative grafting yield were obtained for the 10 mol% PEGylation and the fraction of ungrafted chains were low in that case. To evaluate the effect of PEGylation, we studied here the

10 mol% derivatives of 45PLE, 90PLE and 45PLEL chains that are named below as 45PLE-10PEG3, 90PLE-10PEG3 and 45PLEL-10PEG3.

Evidence for mAb:polyglutamate association

In dilute conditions, formation of mAb:polymer complexes was assessed by capillary electrophoresis. The (free/unbound) mAb was cationic in the acidic pH conditions used here, whereas the PLEs were anionic. Coulombic mAb:polymer complexes are expected to display a more balanced charge. Accordingly, the polyglutamate, polyglutamate:mAb complexes, and the free mAb shall have very distinctive electrophoretic migration rates. To avoid perturbation of association equilibria during the analysis, we implemented a continuous injection scheme (method called frontal analysis by continuous injection electrophoresis, FACCE ³¹, see method section). The continuous injection and migration of analytes resulted in adjacent zones detected as plateaus of absorbance. The first zone contained the fastest analyte (here mAb free or resp. polymer free in positive, resp. negative field conditions). Absorbance at the plateaus varied in proportion to concentrations (cf calibration curves in Figure SI.4 in SI). We analyzed samples prepared at a fixed total mAb concentration of 1 g/L (6.67 μ M) and varying polymer concentration. Representative electropherograms of mixtures are shown in Fig. 1. The fast drop in Fig. 1A of plateau height upon addition of micromolar concentration of 45PLE suggests that binding was very effective in these dilute conditions. Namely, above 10 micromolar 45PLE, about 80% of the 1 g/L mAb was “captured” under the form of complexes with polyglutamate chains. When represented as a function of electrophoretic mobilities (eq. 2 in “Materials & Methods” section) electropherograms confirmed that at pH 6.3 the mAb was weakly cationic (mobility at half plateau height of $+3.3 \cdot 10^{-5} \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$ in Fig.1C) and polyglutamate was anionic (mobility of ca. $-35 \cdot 10^{-5} \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$). Interestingly, detection of 45PLE(L):mAb complexes started under the negative field conditions

near zero mobility suggesting slightly negative complexes (ex: mobilities between 0 and $-5 \cdot 10^{-5} \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$ in Fig. 1D). The jump of absorbance associated to the detection of the mAb:PLE complexes was close to the time of electrosmotic flow, and broad enough to reach the zero axis. Accordingly, complexes between mAb and 45PLE(L) likely have compositions near charge cancellation.

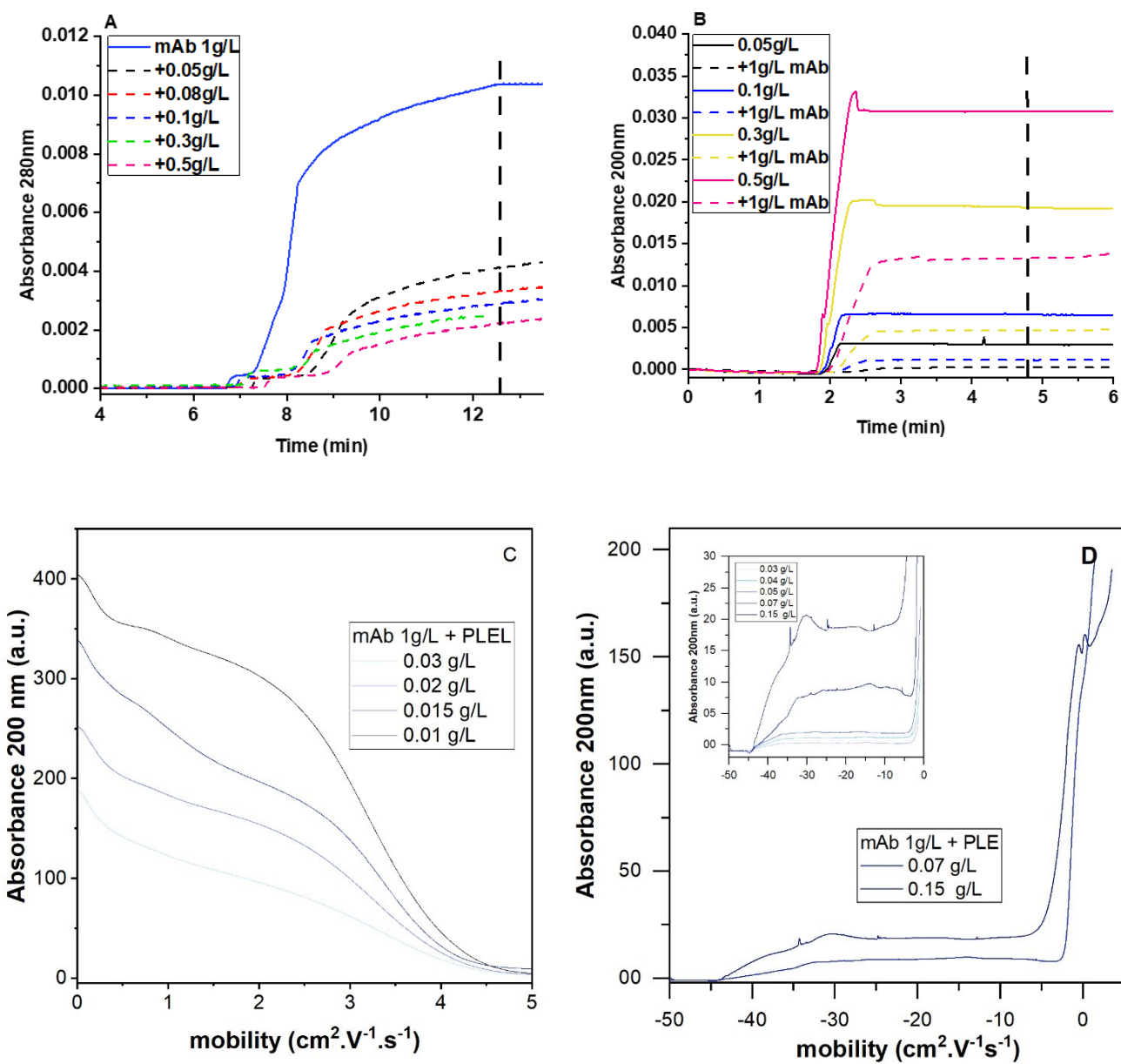


Figure 1. Representative electropherograms of mAb:polyglutamate mixtures at pH 6.3, 1 g/L mAb and concentration of polyglutamate varied between 0.05 g/L and 0.5 g/L. A, B) 50 mm ID capillary and 5mM Histidine + 10% Sucrose buffer, application of +/-12kV, A) anode at the outlet, i.e. free mAb migrating toward the detector. The solid blue line corresponds to the mAb solution without 45PLE. The dashed dotted lines are mAb + 45PLE mixtures as quoted. B) cathode at the outlet, i.e., free 45PLE migrating toward the detector. The solid lines correspond to the polymer with no mAb in the solution. The dashed dotted lines are mixed samples as quoted. C,D) 75 mm ID capillary and 5mM Na-phosphate buffer, application of +/-20kV + 0.1 psi pressure and x-axis calculated as the electrophoretic mobility.

Figure 2A compares the binding efficacy of 45PLE and 45PLEL in 5mM phosphate buffer pH 6.3 and in the presence of a total concentration of 1 g/L mAb. The decrease of concentration of free (unbound) mAb with increasing polymer concentration was more rapid in the case of 45PLEL suggesting a lower affinity of the racemic 45PLE. Estimation of affinity however requires assumption on association stoichiometry. The effective mean binding ratio, $R = [\text{polyglutamate}]_{\text{bound}} / [\text{mAb}]_{\text{bound}}$ was calculated, based on measurements of both unbound mAb and of unbound polymer in the samples (Fig. 2B). In the experimental window (total mAb of 1g/L and polymer varying between 0.01 g/L and 1 g/L), R typically varied from 0.5 to ~ 3 mol/mol). The smaller ratios were measured at the lower polymer concentrations. But when the amount of free polyglutamate was close or below the sensitivity of the technique, apparent R values were with no surprise close to the initial ratio of the mixture (dotted line in Fig. 2B). In mixtures containing reliably measured amounts of both free PLEs and free mAb, the data suggest that equilibrium corresponded to a minimum of R of 0.4-0.7 with 45PLEL, and of 0.8-1.2 in the case of 45PLE. Similarly, the lowest R values were in the window of 0.75-1.25 with 45PLE-10PEG3 or 45PLEL-10PEG3 suggesting a mAb/polymer ratio of 2:1 - 1:1 mol/mol association window in equilibrium with measurable unbound mAb. Interestingly, the 45PLE isotherm in Fig 2A was fitted to a conventional binding equilibrium with a binding constant $K = [\text{45PLE}]_{\text{free}} \cdot [\text{mAb}]_{\text{free}} / [\text{mAb}]_{\text{bound}}$. A similar fit of the 45PLEL data however did not converge. Assuming a 2:1 ratio, and accordingly $K' = [\text{45PLEL}]_{\text{free}} \cdot [\text{mAb}]_{\text{free}}^2 / [\text{mAb}]_{\text{bound}}$, enabled a suitable fit (Fig. 2A). The matching between simple stoichiometric models and data are however likely incidental, due to a focus into a limited and specific range of concentrations (corresponding here to 0 – 80% bound protein). Upon increasing the polymer concentration, a gradual and significant increase of R was always observed.

R of up to 3 mol/mol was reached with 45PLE (ex: 2.6 in 0.5 g/L 45PLE + 1 g/L mAb, or 2.8 mol/mol in 0.2 g/L mAb + 0.04 g/L 45PLE). Accordingly, $R < 1$ and mAb:polyglutamate ratio of 2:1 – 1:1 may be a situation only observed in a limited range of composition. In the presence of excess free polymer, the mAb could accommodate several polyglutamate chains, a possibility that presumably corresponded to binding with lower affinities (ex: contribution of association of polymer chains onto weaker binding sites). In summary, all polymer chains (45PLE, 45PLE(L)-10PEG3, 45PLEL) qualitatively bind mAb with a similar association pattern: a fast capture of up to 80% of the mAb at $[\text{polymer chain}] < 10 \mu\text{M}$, with a mean binding ratio increasing from $R=0.5$ -1 mol/mol at low polymer concentration to 3-4 mol/mol in the presence of excess polymer.

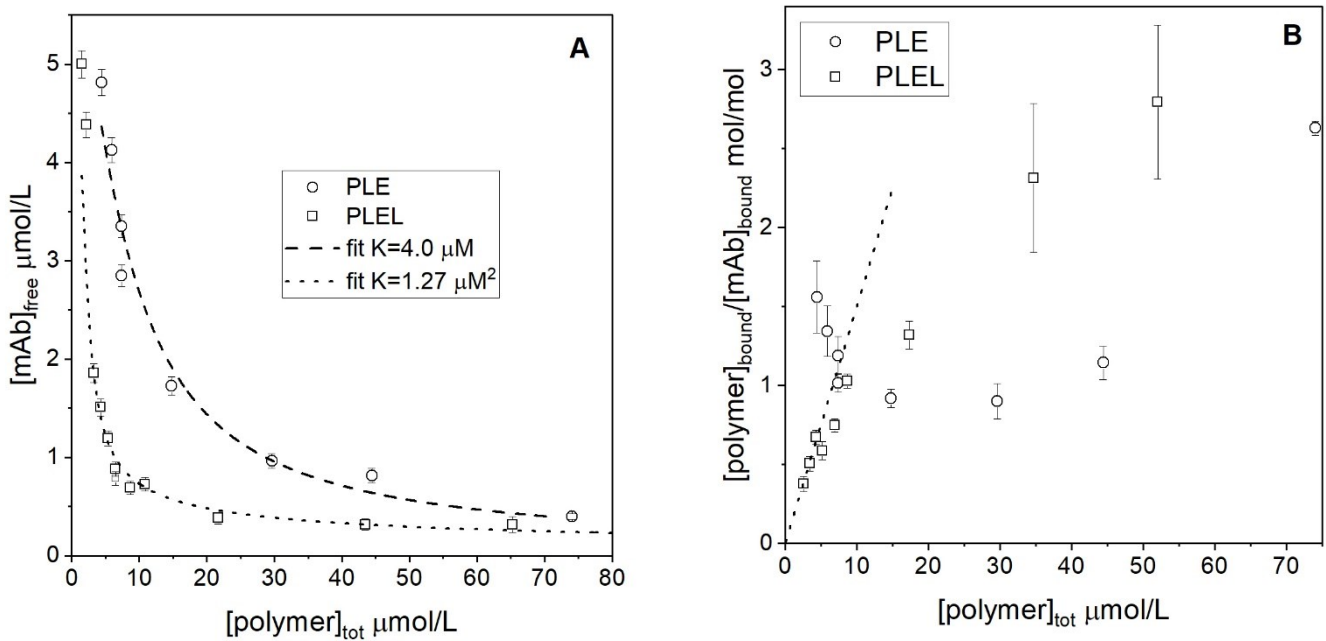


Figure 2. Association of mAb with 45PLE or 45PLEL as measured by FACCE in mixtures of 1g/L mAb, in 5mM phosphate buffer pH 6.3 and varying polymer concentration between 0.01 g/L and 0.5 g/L. (A) Free (unbound) mAb as a function of total (bound and unbound) polyglutamate chains. Dashed line shows a fit by a model of 1:1 complex with binding constant $K = 4.06 \mu\text{M}$, dotted line shows a fit by a model of 2:1 complex with binding constant $K' = 1.27 \mu\text{M}^2$ (see text); (B) Binding ratio R of bound polymer / bound mAb vs the total concentration polymer in the same samples.

At higher mAb concentrations, mixed mAb:polyglutamate solutions underwent associative liquid-liquid phase separation which is an additional evidence for the formation of complexes. Complex coacervation of protein:polyelectrolyte of unlike charge is well documented.³² It has been reported recently by Shiraki and coll on a similar example of a monoclonal mAb with a polyglutamate chain.^{33, 34} We observed this phase separation with all the polymers studied here, including the PEGylated ones, both at pH 6.3 and 5.9 (5mM buffers). To quantify the amount of mAb involved in the dense phase, samples were prepared at 20 g/L mAb and varying polymer concentrations between 0.1 g/L and 1 g/L. These conditions corresponded to ratios of (total) 45PLE / mAb in the mixture varying between 0.1 and 5.5 mol/mol. Samples were incubated for 1 hour and ultracentrifuged (Airfuge 78 000 rpm, 10min.) to separate the two fairly transparent phases. Measurements of the volume of the phases and absorbance at 280 nm of the upper, more dilute phase enabled to calculate the compositions reported in Table 1. The mAb concentration in the dense phase increased with increasing the polyglutamate (60 g/L – 177 g/L mAb), while the supernatant concentration decreased (20 – 2 g/L mAb). The maximum fraction of mAb entering in the dense phase (up to 93% with 45PLEL, or 73% with 45PLE) was observed at a similar molar concentration of polymer for both 45PLE and 45PLEL, corresponding to about 0.5-0.7 mol of polyglutamate chains per mol of mAb (total amount in the mixture). Addition of polymer beyond this optimum increased the fraction of mAb in supernatant. An excess of polyglutamate presumably favors formation of soluble complexes having a significant anionic charge because of excess PLE, thus creating repulsion between the complexes.³³ Finally, addition of 50 mM NaCl in phase-separated samples enabled to recover a single phase, transparent solution. Altogether, these observations are consistent with the formation of coulombic complexes between the mAb and polyglutamate chains at pH 6.3, the tighter association being observed near a 2:1

mAb/polyglutamate molar ratio, which is expected to reflect a situation close to charge cancellation in the complexes.

Table 1. Compositions of two-phases mixtures formed by addition of 45PLE or 45PLEL in a 20 g/L mAb solution in 5 mM phosphate-NaOH buffer pH 6.3.

	45PLE			45PLEL		
Polyglutamate total g/L	% mAb dense phase	[mAb] _{supernatant} g/L	[mAb] dense phase g/L	% mAb dense phase	[mAb] _{supernatant} g/L	[mAb] dense phase g/L
0.1	18%	17.7±0.1	49±4	23%	16.6±0.2	65±5
0.2	31%	15.3±0.3	62±4	58%	9.3±0.2	105±7
0.35	64%	8.3±0.3	83±5	93%	1.8±0.1	108±8
0.5	73%	5.9±0.2	177±16	92%	2.0±0.1	128±10
1	59%	8.7±0.3	177±17	66%	7.4±0.2	156±15

Viscosity.

Viscosity in the absence of polyglutamates was determined as a function of mAb concentration either by capillary viscosimetry (CV) or dynamic light scattering with polystyrene tracer beads of known diameter (DLS, see method section). A rapid increase of viscosity with increasing concentration was observed by both methods (Fig. 3A) in the concentration window of 40-200 g/L (in 5mM Histidine + 10% sucrose buffer). However, the DLS method systematically returned a viscosity 1.5-1.6 times higher than CV measurements (Table SI.2 in SI). The origin of a constant shift between the two methods is presumably the use of an inappropriate reference relaxation time

(i.e., incorrect bead's reference diameter). The reference relaxation time used in eq. 4 was here measured in water and in the histidine buffer and corresponded to an effective hydrodynamic radius of 185 ± 10 nm. It matched with the expected diameter of 180 nm claimed by the supplier. In addition, calibration using sucrose solutions (50 wt% - 60 wt% in the range of viscosities of 12 mPa.s to 44 mPa.s) returned the same values of the viscosity by either DLS or CV, suggesting that the reference diameter was correctly introduced in eq. 4. The difference observed in the presence of mAb, together with the constant ratio between effective "DLS" and "CV" viscosities (Table SI.2 in SI) suggest accordingly that the tracer beads were covered by adsorbed mAbs, which increased their effective diameter up to 270 nm. Adsorption of cationic mAb molecules onto the negatively charged beads is not surprising. Of note, effective viscosities as determined by DLS were stable for hours. And the same ratio of 1.5-1.6 between CV and DLS methods were also observed on mixtures of mAb with polyglutamate additives (Fig. SI.5 in SI). For the sake of consistency, we nevertheless show below the viscosity calculated from the reference bead diameter of 185 nm, that thus overestimate actual values. In the absence of polyglutamate, a rapid increase of viscosity was observed above 80 g/L (*cf* break in Fig. 3A), which is typical of molecular attraction and/or clusterization of the antibody molecules.^{35,36, 14} At 200 g/L mAb, the viscosity was above the practical injectability threshold of 50 mPa.s, that was crossed near/above 140 g/L mAb. Viscosity also increased with decreasing pH (Fig. 4) making Histidine buffer at pH 6.3 (i.e., pH of the « as prepared » mAb stock solutions) slightly more fluid than preparations at pH 5.9 (pH adjusted after dialysis and concentration, see method section). Gradual dilution in the buffer was used to obtain data in Figure 3B, showing a roughly exponential increase of viscosity with mAb concentration. Linear variations in the semi-log representation of Fig. 3B characterizes the effect of concentration variation, irrespective of the presence or absence of additives (NaCl,

polyglutamates). This suggests that measurements at one reference concentration can be representative of the role of polymers in a larger, practically relevant range of 100 – 250 g/L mAb.

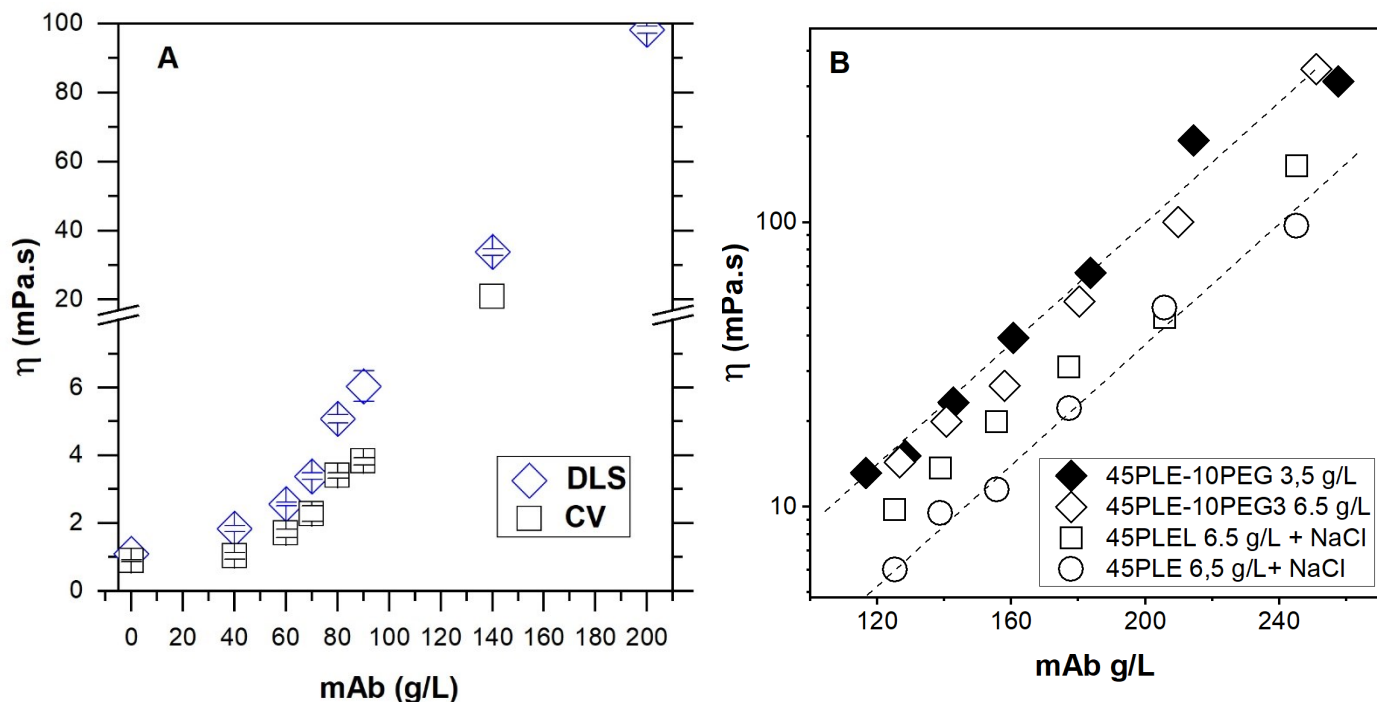


Figure 3. Viscosity of mAb in Histidine-HCl buffer pH 6.3 + 10% sucrose at 25°C (A) 20mM Histidine with no polymer, as measured either by capillary viscosimetry (CV) or using tracer beads and light scattering (DLS). The viscosity of 10% Sucrose solution is reported as a control at 0 g/L. error bars are the maximum half difference measured between 3 replicates (B) 5mM Histidine, DLS-measured viscosity in the presence of additives as quoted (+NaCl meaning in 50mM NaCl); lines are guide for the eye.

To assess the effect of polyglutamates, we compared the viscosity at 140 g/L mAb (Fig. 4). A mixture of 3.5 g/L 45PLE with 140 g/L mAb corresponds to an average mol/mol ratio of ~ 2 mAb monomer per polymer chain ($R=0.5$). Irrespective of the polymer used, supplementation of the mAb solution with 3.5 g/L or 6.5 g/L polymer markedly fluidified the solutions and decreased viscosity by up to three fold (Fig. 4A and Table 2). Viscosity of polyglutamate solutions (no mAb) was negligibly low in this concentration range (viscosity of 40 g/L polymer in water with no mAb

< 2 mPa.s, Table SI.3 in SI). Increasing the ionic strength by addition of NaCl further decreased the viscosity, suggesting the importance of coulombic contribution to the thickening effect observed in mAb solutions without additive. To assess the role of small ions having similar acid-base properties as the glutamic acid unit, we measured the decrease of viscosity achieved by addition of a stock solution of acetic acid, adjusted at pH 6.3 with NaOH. The equivalent concentration of glutamate units in 3.5 g/L (resp. 6.5 g/L) 45PLE was of about 20 mM (resp. ~ 40 mM). Of note, the ionic strength in 45PLE solution is expected to be lower than the one achieved in sodium acetate at the same molar concentration, because of counter ion condensation onto the polyelectrolyte chains. In the absence of NaCl, 45PLE decreased the viscosity slightly more than what was observed with molar equivalent sodium acetate addition (Table 2). This suggests that the contribution of the 45PLE chains cannot solely be explained by variation of the ionic strength. The PEGylated polyglutamates (45PLE-10PEG3 or 90PLE-10PEG3) have a molar mass about 3 times higher than their parent chains. Namely, a 6.5 g/L solution of 45PLE-10PEG corresponds to 14.5 mM glutamate units (resp 7.7 mM at 3.5 g/L). In comparison to similar concentration of sodium acetate, the PEGylated chains are shown in Table 2 to be either slightly more effective or of similar fluidification efficiency, though they were not as effective as 45PLE. Finally, comparison of polyglutamate derivatives having 45 or 90 monomer units (Fig. 4) suggested a higher efficiency of shorter polymers : in the presence of NaCl viscosity of samples containing 90PLE and 90PLE-10PEG3 was significantly higher than without polymer, or with shorter polymers (45PLE and 45PLE-10PEG3).

Table 2. Viscosity in the presence of sodium acetate or PLE additives of 140g/L mAb in 10% sucrose, 5mM Histidine buffer pH 6.3 for (a) commercial PLE and (b) PEG-modified PLE. For reference viscosity with no additives and no NaCl = 20.2 mPa.s, or 50mM NaCl = 9.0 mPa.s. n.d. = not determined.

(a)

	Weight concentration of PLE/PLE derivative (g/L)	Charge concentration of PLE or sodium acetate (mM)	Viscosity (mPa.s)		
			Sodium acetate	45PLE	45PLEL
Without NaCl	3.5	21	11.0	8.4	2 phases 1.6 / 24.2
	6.5	40	8.0	6.6	9.0
With 50 mM NaCl	3.5	21	9.2	7.2	6.6
	6.5	40	8.2	9.0	10.5

(b)

	Weight concentration of PLE/PLE derivative (g/L)	Charge concentration of PLE or sodium acetate (mM)	Viscosity (mPa.s)		
			Sodium acetate	45PLE-10PEG3	45PLEL-10PEG3
Without NaCl	3.5	7.7	16.7	12.6	16.0
	6.5	14.5	15.4	15.1	13.6
With 50 mM NaCl	3.5	7.7	n.d.	10.7	10.4
	6.5	14.5	n.d.	9.8	nd

Liquid-liquid phase separation as observed at 20 g/L mAb could also occur at 140 g/L mAb. At pH 6.3, this was the case of the mixture with 3.5 g/L 45PLEL (no NaCl) that formed a dense phase of high viscosity (Table 2, Fig. SI.6. in SI) dispersed in a very fluid (1.6 mPa.s) “light” phase. The dispersion was not stable enough to enable viscosity measurement of the full mixture. But as suggested by Shiraki and coll.³⁴, emulsion-like dispersions of droplets of a dense phase of mAbs can fulfill practical requirements of injectability. The 45PLE at 3.5 g/L + mAb mixture at pH 6.3 was opalescent, suggesting the onset of clusterization of mAbs with polyglutamate chains. However no obvious sedimentation neither phase separation took place with PLE during 24h (Fig. SI.6. in SI). At pH 5.9, fast demixing was generally hampering stable measurements of samples containing either 45PLE or 45PLEL. In contrast, the PEGylated polymers were forming transparent mixtures at either pHs, with or without NaCl, enabling viscosity measurements on single-phase systems. Finally, to assess the effect of all polymers in homogeneous, one-phase, stable samples, we added NaCl in all mixtures (Fig. 4 and Table 2). At pH of 5.95, increasing the polymer concentration from 3.5 to 6.5 g/L decreased the viscosity (Fig. 4B). However, at pH 6.3 (Table 2), the presence of polymers at 6.5 g/L did not allow better fluidification in the presence of NaCl than compared to 3.5 g/L polymer. The viscosity of polymer-containing samples with NaCl at pH 6.3 may be higher or lower than the viscosity of reference samples containing both NaCl and sodium acetate (Table 2). As pointed in the paragraph on mAb:polyglutamate association, the coulombic complexes are sensitive to ionic strength and are likely dissociated by increasing the concentration of NaCl, which may counteract their fluidification effect. In summary, the results above point to a capacity of 45PLE to fluidify mAb solutions of low ionic strength beyond the effect due to variation of ionic strength. The other short polymers (*i.e.*, 45 monomer units) also significantly decreased viscosity, though with magnitudes that were closer to that observed upon

addition of sodium acetate at similar (molar) concentration of glutamate units. Because all polymers were associated with mAb at low ionic strength, the mechanism of viscosity reduction by polymer may however differ and mAb-mAb interactions shall be affected differently upon binding of poly(glutamate). The association with PEGylated chains could in this line contribute to adding repulsive interaction between the complexes. Our results points in addition that cautious adjustment of the ionic strength shall be considered to avoid losing the coulombic complexation between the polyglutamate chains and the antibody.

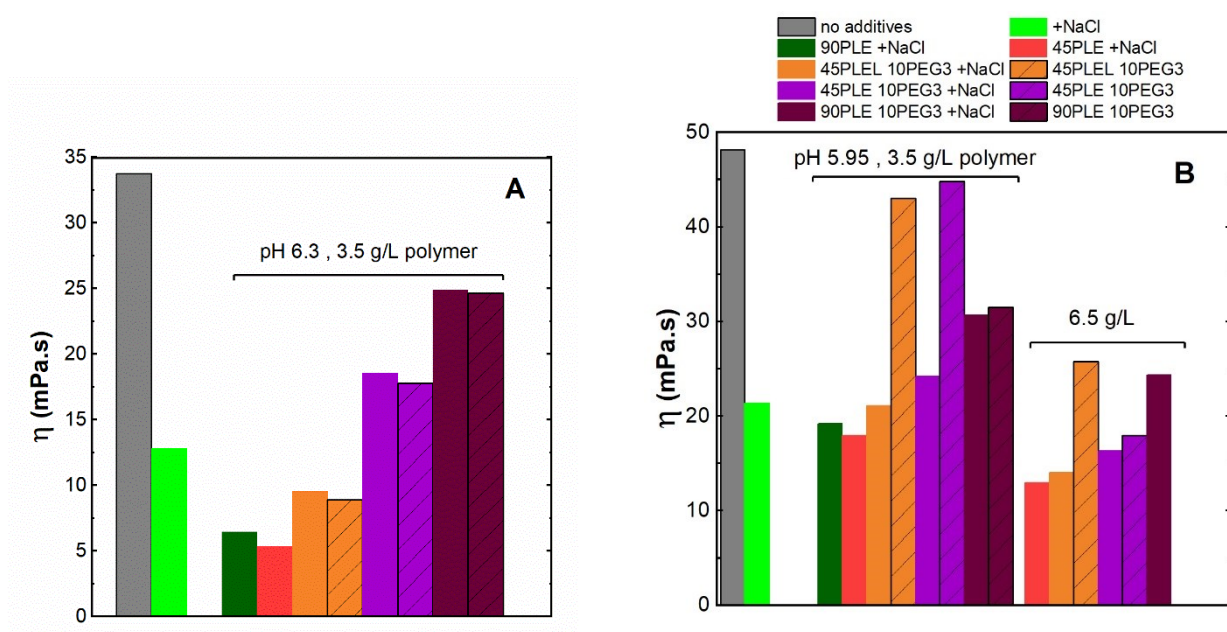


Figure 4. Viscosity of 140 g/L mAb solutions as measured by DLS method in 5 mM Histidine-HCl buffer + 10% sucrose with or without polymer additive, (A) pH 6.3, (B) pH 5.95. "+ NaCl" quotes for solutions with 55 mM NaCl (no NaCl otherwise). Concentration of polymer was either 3.5 g/L or 6.5 g/L.

Eventually, we prepared samples at 200 g/L mAb to assess whether injectable formulations (< 50 mPa.s) could be obtained. Figure 5 shows measurements of microviscosity (DLS) as obtained by mixing concentrated stock solutions (mAb ~ 250 g/L) with polymers and diluting in buffer down

to 200 g/L mAb. The 5 g/L polymer concentration corresponds here to the same mAb:polyglutamate ratio as in 140 g/L mAb + 3.5 g/L polymers. All polymer additives among 45PLE, 90PLE, and 45PLEL-10PEG3 were capable to decrease the viscosity of the mAb below the practical threshold of 50 mPa.s. Of note, the addition of 45PLE-10PEG3 without NaCl (11 mM eq glutamate units) also provided a fluidification near 50mPa.s, more effective than NaCl 55mM with no polyglutamate. As PEGylated copolymers may in addition contribute to stability (in contrast to the use of NaCl that can impair stability by enabling sticky collisions between mAbs and faster irreversible aggregation rate¹⁹) the trends observed suggest the interest of further studies of the ageing and formulations using PLEs and/or PEGylated PLE derivatives. In this line, the use of buffers of low ionic strength (in term of simple salt concentration) has been documented to provide better long term stability of highly concentrated solution of mAbs.³⁷ 45PLE and its PEGylated forms when used at low concentrations deserve accordingly to be considered as their impact on ionic strength shall be lower than simple salts.

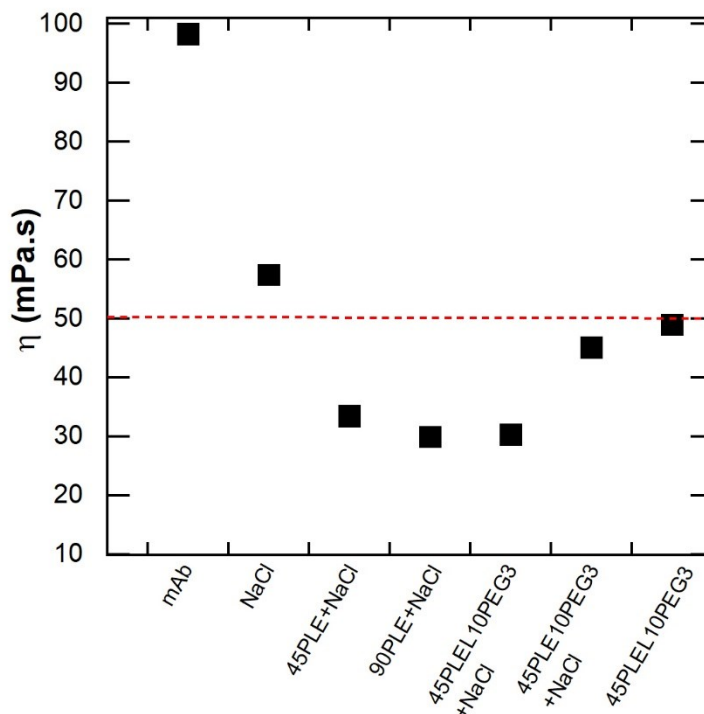


Figure 5. Viscosity measured by DLS of 200 g/L mAb solutions in 5 mM Histidine-HCl pH 6.3, 10% Sucrose. « mAb » is a solution with no additives, « NaCl » is for 55mM NaCl and no polymer, other samples contained 5 g/L polymer additive with or without 55 mM NaCl as quoted in x-axis. The dashed line is a typical acceptable viscosity threshold for injection.

Conclusion.

The main result of the present work is that a commercial poly(glutamate), PLE, and PEGylated derivatives of PLE can decrease the viscosity of a model therapeutic mAb, Isatuximab. At the highest concentration assessed (200 g/L) the viscosity was decreased to values that compared with a conventionally formulated sample of mAb, making the mixed solutions compatible with syringe injection in terms of viscosity. A very low amount of PLE additive was

required: we explored polymer/mAb ratio of the order of 2.5/100 – 5/100 wt/wt, which corresponds to polymer/mAb of $\frac{1}{2}$ – 3/1 mol/mol (depending of polymer's Mw). As compared to molecular amino acids such as Arginine, often used at >100 mM concentration, PLE was here of 23 - 43 mM eq. glutamate units (resp 7.7 – 14 mM for PEGylated derivatives). We determined in addition using capillary electrophoresis that association took place at millimolar concentration of the polyglutamates in dilute conditions (mAb of 1 g/L). At low ionic strength and in a range of PLE/mAb mol/mol ratios of 0.5 – 1, a predominant fraction of the mAb formed complexes with the polyglutamate additives.

A likely origin of the impact of PLEs on viscosity is a change of interactions developed between the mAbs as compared to interaction between polymer-bound mAbs (ex: perturbation of inter-mAb clusterization). Because coulombic effects significantly contribute both to viscosification of the bare mAb in solutions without PLE and to PLE:mAb association, it was however difficult to decipher the exact origin of fluidification. Present results point nevertheless to examples where fluidification was higher than what could be achieved by using a simple 1:1 salt such as sodium acetate or NaCl. It opens a yet poorly explored field of using poly(aminoacids) instead of small molecular excipients to modulate inter-mAb interaction in concentrated conditions. In formulation of mAbs, stability issues encountered with addition of simple salts, including irreversible mAb aggregation, shall not be overlooked. This motivates the interest for a diversity of viscosity-reducing additives, and we accordingly believe that polyglutamate derivatives deserve studies along this line.

Supporting Information. Supporting Information is available free of charge at <https://doi.org/10.1021/acs.chemmater.1c01111>. It contains a description of the PEGylation procedure of polyglutamates and characterization by ^1H NMR and electrophoresis, representative autocorrelation functions from light scattering of

concentrated mAb solutions, calibration data of FACCE method, supplementary viscosity data, and a photograph of concentrated mAb:PLE(L) mixture with/out phase separation.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding Sources

The work was supported by SANOFI research grant iTech award to C. Tribet, ANR Labex “Dynamo” ANR-11-LABX-0011-01, and ANR Fliposomes 20 CE06-007.

ABBREVIATIONS

FACCE, frontal analysis continuous capillary electrophoresis ; CV, capillary viscosimetry; DLS, dynamic light scattering ;

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REFERENCES

- (1) Lu, R.-M.; Hwang, Y.-C.; Liu, I. J.; Lee, C.-C.; Tsai, H.-Z.; Li, H.-J.; Wu, H.-C. Development of therapeutic antibodies for the treatment of diseases. *Journal of Biomedical Science* **2020**, 27 (1), 1. DOI: 10.1186/s12929-019-0592-z.
- (2) Miura, R.; Sawada, S.; Mukai, S.; Sasaki, Y.; Akiyoshi, K. Antigen Delivery to Antigen-Presenting Cells for Adaptive Immune Response by Self-Assembled Anionic Polysaccharide

Nanogel Vaccines. *Biomacromolecules* **2020**, *21* (2), 621-629. DOI: 10.1021/acs.biomac.9b01351.

(3) Davis, H. C.; Posey, N. D.; Tew, G. N. Protein Binding and Release by Polymeric Cell-Penetrating Peptide Mimics. *Biomacromolecules* **2022**, *23* (1), 57-66. DOI: 10.1021/acs.biomac.1c00929.

(4) Nikkhah, S. J.; Thompson, D. Copolyelectrolyte-Based Nanocapsules for Oral Monoclonal Antibody Therapy: A Mesoscale Modeling Survey. *Biomacromolecules* **2022**. DOI: 10.1021/acs.biomac.2c00699.

(5) Ilochonwu, B. C.; Mihajlovic, M.; Maas-Bakker, R. F.; Rousou, C.; Tang, M.; Chen, M.; Hennink, W. E.; Vermonden, T. Hyaluronic Acid-PEG-Based Diels-Alder In Situ Forming Hydrogels for Sustained Intraocular Delivery of Bevacizumab. *Biomacromolecules* **2022**, *23* (7), 2914–2929. DOI: 10.1021/acs.biomac.2c00383.

(6) Deokar, V.; Sharma, A.; Mody, R.; Volety, S. M. Comparison of Strategies in Development and Manufacturing of Low Viscosity, Ultra-High Concentration Formulation for IgG1 Antibody. *J. Pharma. Sci.* **2020**, *109* (12), 3579-3589. DOI: 10.1016/j.xphs.2020.09.014.

(7) Stradner, A.; Schurtenberger, P. Potential and limits of a colloid approach to protein solutions. *Soft Matter* **2020**, *16* (2), 307-323. DOI: 10.1039/c9sm01953g.

(8) Xu, A. Y.; Castellanos, M. M.; Mattison, K.; Krueger, S.; Curtis, J. E. Studying Excipient Modulated Physical Stability and Viscosity of Monoclonal Antibody Formulations Using Small-Angle Scattering. *Molecular Pharmaceutics* **2019**, *16* (10), 4319-4338. DOI: 10.1021/acs.molpharmaceut.9b00687.

(9) von Bulow, S.; Siggel, M.; Linke, M.; Hummer, G. Dynamic cluster formation determines viscosity and diffusion in dense protein solutions. *Proceedings of the National Academy of Sciences of the United States of America* **2019**, *116* (20), 9843-9852. DOI: 10.1073/pnas.1817564116.

(10) Arora, J.; Hickey, J. M.; Majumdar, R.; Esfandiary, R.; Bishop, S. M.; Samra, H. S.; Middaugh, C. R.; Weis, D. D.; Volkin, D. B. Hydrogen exchange mass spectrometry reveals protein interfaces and distant dynamic coupling effects during the reversible self-association of an IgG1 monoclonal antibody. *Mabs* **2015**, *7* (3), 525-539. DOI: 10.1080/19420862.2015.1029217.

(11) Yadav, S.; Laue, T. M.; Kalonia, D. S.; Singh, S. N.; Shire, S. J. The Influence of Charge Distribution on Self-Association and Viscosity Behavior of Monoclonal Antibody Solutions. *Molecular Pharmaceutics* **2012**, *9* (4), 791-802. DOI: 10.1021/mp200566k.

(12) Yadav, S.; Sreedhara, A.; Kanai, S.; Liu, J.; Lien, S.; Lowman, H.; Kalonia, D. S.; Shire, S. J. Establishing a Link Between Amino Acid Sequences and Self-Associating and Viscoelastic Behavior of Two Closely Related Monoclonal Antibodies. *Pharmaceutical Research* **2011**, *28* (7), 1750-1764. DOI: 10.1007/s11095-011-0410-0.

(13) Johnston, K. P.; Maynard, J. A.; Truskett, T. M.; Borwankar, A. U.; Miller, M. A.; Wilson, B. K.; Dinin, A. K.; Khan, T. A.; Kaczorowski, K. J. Concentrated Dispersions of Equilibrium Protein Nanoclusters That Reversibly Dissociate into Active Monomers. *Acs Nano* **2012**, *6* (2), 1357-1369. DOI: 10.1021/nn204166z.

(14) Skar-Gislinge, N.; Camerin, F.; Stradner, A.; Zaccarelli, E.; Schurtenberger, P. Using Cluster Theory to Calculate the Experimental Structure Factors of Antibody Solutions. *Molecular Pharmaceutics* **2023**, *20* (5), 2738-2753. DOI: 10.1021/acs.molpharmaceut.3c00191.

(15) Roche, A.; Gentiluomo, L.; Sibanda, N.; Roessner, D.; Friess, W.; Trainoff, S. P.; Curtis, R. Towards an improved prediction of concentrated antibody solution viscosity using the Huggins coefficient. *Journal of Colloid and Interface Science* **2022**, *607*, 1813-1824. DOI: 10.1016/j.jcis.2021.08.191.

(16) Kingsbury, J. S.; Saini, A.; Auclair, S. M.; Fu, L.; Lantz, M. M.; Halloran, K. T.; Calero-Rubio, C.; Schwenger, W.; Airiau, C. Y.; Zhang, J. F.; et al. A single molecular descriptor to predict solution behavior of therapeutic antibodies. *Science Advances* **2020**, *6* (32). DOI: 10.1126/sciadv.abb0372.

(17) Neergaard, M. S.; Kalonia, D. S.; Parshad, H.; Nielsen, A. D.; Moller, E. H.; van de Weert, M. Viscosity of high concentration protein formulations of monoclonal antibodies of the IgG1 and IgG4 subclass - Prediction of viscosity through protein-protein interaction measurements. *European Journal of Pharmaceutical Sciences* **2013**, *49* (3), 400-410. DOI: 10.1016/j.ejps.2013.04.019.

(18) Zidar, M.; Rozman, P.; Belko-Parkel, K.; Ravnik, M. Control of viscosity in biopharmaceutical protein formulations. *Journal of Colloid and Interface Science* **2020**, *580*, 308-317. DOI: 10.1016/j.jcis.2020.06.105.

(19) Gong, Y. H.; Abyaneh, H. S.; Drossis, N.; Niederquell, A.; Kuentz, M.; Leroux, J. C.; de Haan, H. W.; Gauthier, M. A. Ultra-sub-stoichiometric "Dynamic" Bioconjugation Reduces Viscosity by Disrupting Immunoglobulin Oligomerization. *Biomacromolecules* **2019**, *20* (9), 3557-3565. DOI: 10.1021/acs.biomac.9b00867.

(20) Godfrin, P. D.; Zarraga, I. E.; Zarzar, J.; Porcar, L.; Falus, P.; Wagner, N. J.; Liu, Y. Effect of Hierarchical Cluster Formation on the Viscosity of Concentrated Monoclonal Antibody

Formulations Studied by Neutron Scattering. *Journal of Physical Chemistry B* **2016**, *120* (2), 278-291. DOI: 10.1021/acs.jpcc.5b07260.

(21) Wang, S. J.; Zhang, N.; Hu, T.; Dai, W. G.; Feng, X. Y.; Zhang, X. Y.; Qian, F. Viscosity-Lowering Effect of Amino Acids and Salts on Highly Concentrated Solutions of Two IgG1 Monoclonal Antibodies. *Molecular Pharmaceutics* **2015**, *12* (12), 4478-4487. DOI: 10.1021/acs.molpharmaceut.5b00643.

(22) Dear, B.; Hung, J. J.; Truskett, T. M.; Johnston, K. P. Contrasting the Influence of Cationic Amino Acids on the Viscosity and Stability of a Highly Concentrated Monoclonal Antibody. *Pharmaceutical Research* **2017**, *34* (1), 193-207. DOI: 10.1007/s11095-016-2055-5.

(23) Dear, B. J.; Hung, J. J.; Laber, J. R.; Wilks, L. R.; Sharma, A.; Truskett, T. M.; Johnston, K. P. Enhancing Stability and Reducing Viscosity of a Monoclonal Antibody With Cosolutes by Weakening Protein-Protein Interactions. *Journal of Pharmaceutical Sciences* **2019**, *108* (8), 2517-2526. DOI: 10.1016/j.xphs.2019.03.008.

(24) Inoue, N.; Takai, E.; Arakawa, T.; Shiraki, K. Specific Decrease in Solution Viscosity of Antibodies by Arginine for Therapeutic Formulations. *Molecular Pharmaceutics* **2014**, *11* (6), 1889-1896. DOI: 10.1021/mp5000218.

(25) Izaki, S.; Kurinomaru, T.; Maruyama, T.; Uchida, T.; Handa, K.; Kimoto, T.; Shiraki, K. Feasibility of Antibody-Poly(Glutamic Acid) Complexes: Preparation of High-Concentration Antibody Formulations and Their Pharmaceutical Properties. *Journal of Pharmaceutical Sciences* **2015**, *104* (6), 1929-1937. DOI: 10.1002/jps.24422.

(26) Matsuda, A.; Mimura, M.; Maruyama, T.; Kurinomaru, T.; Shiuhei, M.; Shiraki, K. Liquid Droplet of Protein-Polyelectrolyte Complex for High-Concentration Formulations. *Journal of Pharmaceutical Sciences* **2018**, *107* (10), 2713-2719. DOI: 10.1016/j.xphs.2018.06.021.

(27) Lee, E.-H.; Tsujimoto, T.; Uyama, H.; Sung, M.-H.; Kim, K.; Kuramitsu, S. Enhancement of enzyme activity and stability by poly(γ -glutamic acid). *Polymer Journal* **2010**, *42* (10), 818-822. DOI: 10.1038/pj.2010.71.

(28) Li, B.; Wu, Y.; Zhang, W.; Zhang, S.; Shao, N.; Zhang, W.; Zhang, L.; Fei, J.; Dai, Y.; Liu, R. Efficient synthesis of amino acid polymers for protein stabilization. *Biomaterials Science* **2019**, *7* (9), 3675-3682, 10.1039/C9BM00484J. DOI: 10.1039/C9BM00484J.

(29) Allmendinger, A.; Dieu, L.-H.; Fischer, S.; Mueller, R.; Mahler, H.-C.; Huwyler, J. High-throughput viscosity measurement using capillary electrophoresis instrumentation and its application to protein formulation. *Journal of Pharmaceutical and Biomedical Analysis* **2014**, *99*, 51-58. DOI: <https://doi.org/10.1016/j.jpba.2014.07.005>.

(30) He, F.; Becker, G. W.; Litowski, J. R.; Narhi, L. O.; Brems, D. N.; Razinkov, V. I. High-throughput dynamic light scattering method for measuring viscosity of concentrated protein solutions. *Analytical Biochemistry* **2010**, *399* (1), 141-143. DOI: <https://doi.org/10.1016/j.ab.2009.12.003>.

(31) Seyrek, E.; Dubin, P. L.; Tribet, C.; Gamble, E. A. Ionic strength dependence of protein-polyelectrolyte interactions. *Biomacromolecules* **2003**, *4* (2), 273-282.

(32) McTigue, W. C. B.; Perry, S. L. Protein Encapsulation Using Complex Coacervates: What Nature Has to Teach Us. *Small* **2020**, *16* (27). DOI: 10.1002/smll.201907671. Sing, C. E.; Perry,

S. L. Recent progress in the science of complex coacervation. *Soft Matter* **2020**, *16* (12), 2885-2914. DOI: 10.1039/d0sm00001a. Kayitmazer, A. B.; Strand, S. P.; Tribet, C.; Jaeger, W.; Dubin, P. L. Effect of polyelectrolyte structure on protein-polyelectrolyte coacervates: Coacervates of bovine serum albumin with poly(diallyldimethylammonium chloride) versus chitosan. *Biomacromolecules* **2007**, *8* (11), 3568-3577. DOI: 10.1021/bm700645t.

(33) Mimura, M.; Tsumura, K.; Matsuda, A.; Akatsuka, N.; Shiraki, K. Effect of additives on liquid droplet of protein–polyelectrolyte complex for high-concentration formulations. *The Journal of Chemical Physics* **2019**, *150* (6), 064903. DOI: 10.1063/1.5063378.

(34) Tsumura, K.; Hsu, W. L.; Mimura, M.; Horiuchi, A.; Shiraki, K. Lowering the viscosity of a high-concentration antibody solution by protein-polyelectrolyte complex. *Journal of Bioscience and Bioengineering* **2022**, *133* (1), 17-24. DOI: 10.1016/j.jbiosc.2021.09.011.

(35) Chari, R.; Jerath, K.; Badkar, A. V.; Kalonia, D. S. Long- and Short-Range Electrostatic Interactions Affect the Rheology of Highly Concentrated Antibody Solutions. *Pharmaceutical Research* **2009**, *26* (12), 2607-2618. DOI: 10.1007/s11095-009-9975-2.

(36) Yadav, S.; Liu, J.; Shire, S. J.; Kalonia, D. S. Specific Interactions in High Concentration Antibody Solutions Resulting in High Viscosity. *Journal of Pharmaceutical Sciences* **2010**, *99* (3), 1152-1168. DOI: 10.1002/jps.21898.

(37) Bahrenburg, S.; Karow, A. R.; Garidel, P. Buffer-free therapeutic antibody preparations provide a viable alternative to conventionally buffered solutions: From protein buffer capacity prediction to bioprocess applications. *Biotechnology Journal* **2015**, *10* (4), 610-622. DOI: <https://doi.org/10.1002/biot.201400531>.

SYNOPSIS (Word Style "SN_Synopsis_TOC").

