Pharm Res
DOI 10.1007/s11095-012-0802-9

RESEARCH PAPER

Structure-Activity Relationship for Hydrophobic Salts as Viscosity-Lowering Excipients for Concentrated Solutions of Monoclonal Antibodies

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Received: 25 April 2012 / Accepted: 6 June 2012

ABSTRACT

Purpose To discover, elucidate the structure-activity relationship (SAR), and explore the mechanism of action of excipients able to drastically lower the viscosities of concentrated aqueous solutions of humanized monoclonal antibodies (MAbs).

Methods Salts prepared from hydrophobic cations and anions were dissolved into humanized MAbs solutions. Viscosities of the resulting solutions were measured as a function of the nature and concentration of the salts and MAbs.

Results Even at moderate concentrations, some of the salts prepared herein were found to reduce over 10-fold the viscosities of concentrated aqueous solutions of several MAbs at room temperature.

Conclusions To be potent viscosity-lowering excipients, the ionic constituents of the salts must be hydrophobic, bulky, and aliphatic. A mechanistic hypothesis explaining the observed salt effects on MAb solutions' viscosities was proposed and verified.

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KEY WORDS excipients · monoclonal antibodies · proteins · rheology · salts · structure-activity relationship (SAR) · viscosity

ABBREVIATIONS

CHO	Chinese hamster ovary
MAb	monoclonal antibody
SAR	structure-activity relationship
SC	subcutaneous
SD	standard deviation

INTRODUCTION

Due to relatively low specific activities of monoclonal antibodies (MAbs), high doses thereof (milligrams per kg of patient weight) are often needed, which requires their concentrated aqueous solutions for parenteral administrations (1). This is especially true for subcutaneous (SC) injections (a preferred route in a physician's office or by the patient at home), where for a given dose the volume to be injected is inversely proportional to the concentration of the protein in that solution; thus with the allowable SC administration volume of under 1.5 mL, over 100-mg/mL MAbs must often be employed (2). Such concentrated protein solutions are usually very viscous, making them difficult to handle and administer (2). For example, the syringeability of protein solutions appears to be inversely correlated with their viscosities, consequently rendering SC injections challenging (3,4).

Recently, we reported that some hydrophobic salts can substantially lower the viscosity of concentrated solutions of the model proteins bovine serum albumin and γ-globulin (5). In the present work, we have expanded these studies to humanized MAbs, as well as including much broader and diverse range of salts. Certain salts afforded MAb solution viscosity reduction levels of over 10-fold. Based upon the findings made herein, a detailed structure-activity relationship (SAR) has

Published online: 13 June 2012

been established and rationalized between the nature of the salt's cation and anion and the magnitude of this effect.

MATERIALS AND METHODS

Four humanized monoclonal antibodies (MAbs) were kindly provided by Novartis Pharma AG (Basel, Switzerland) and arbitrarily designated by us herein as MAbs 1, 2, 3, and 4. These antibodies were cloned, expressed in Chinese Hamster Ovary (CHO) cell lines, purified by standard Novartis purification processes, and prepared at different concentrations by tangential flow filtration. MAb concentrations were determined spectrophotometrically using the extinction coefficients at 280 nm of 206, 213, 201, and 238 mM^{-1} cm^{-1}, respectively. The molecular weights and calculated isoelectric points, respectively, of the MAbs were as follows: MAb 1, 143,500 Da and 7.8; MAb 2, 143,600 Da and 8.7; MAb 3, 145,200 Da and 8.6; and MAb 4, 146,600 Da and 7.2. The MAbs were obtained as the following aqueous solutions: MAb 1 at 150±1 mg/mL in a 20 mM histidine·HCl buffer (pH 6.1); MAb 2 at 86±1 mg/mL in a 10 mM histidine·HCl buffer (pH 5.4); MAb 3 at 186±7 mg/mL in a 15 mM histidine·HCl buffer (pH 6.4); and MAb 4 at 180±2 mg/mL in a 10 mM histidine·HCl buffer containing 85 mM trehalose (pH 4.8). They were all used for further studies as received, unless stated otherwise below.

Reagents for preparing salt excipients (see Fig. 1 for chemical structures) were obtained from commercial vendors as follows: trimethylphenylammonium iodide (**1**) from Cole-Parmer Instruments (Vernon Hill, IL); 5-amino-1-pentanol (**11**), camphorquinone-10-sulfonic acid (**33**), 3-hydroxypropane-1-sulfonic acid (**37**), 1,2-ethanedisulfonic acid (**38**), and homopiperazine-1,4-*bis*(2-ethanesulfonic acid) (**39**) from Santa Cruz Biotechnology (Santa Cruz, CA); and (+)-camphor-10-sulfonic acid (**2**), as well as all other chemicals (**3** through **10**, **12** through **32**, **34** through **36**, and **40** through **43**) and solvents used, from Sigma-Aldrich (St. Louis, MO).

For preparing hydrophobic salt excipients and to obtain a cation-to-anion stoichiometry, the following procedure was typically employed, as exemplified by **7** as a base and **2** as an acid: (i) 2 g of **7** was dissolved in 20 mL of water; (ii) a 0.1 M solution of **2** was added to adjust the pH to 6.0, and the volume of the acid necessary to achieve that was measured; and (iii) based on the molar ratio of the base to the acid, the stoichiometry was calculated.

Solution viscosities were measured using a Brookfield DV-II Pro viscometer equipped with a cone-and-plate geometry, a CPE 40 spindle, and a Brookfield TC-602 temperature-controlling water bath. The viscometer was pre-calibrated using water and the CAPOL (supplied by Brookfield) viscosity standards. Then 0.5-mL aliquots of aqueous solutions were loaded into the sample cup and incubated for 5 min to allow equilibration to 25°C. The measurements were conducted in two distinct modes designated by the instrument's manufacturer as "standalone" and "external". In the former, a constant shear rate fixed at 22.5 s^{-1} (3 rpm) was used; the solution was incubated at this shear rate for 3 min prior to the measurement. (Note that we selected this relatively modest shear rate for this study because SC injections, mixing, and ultra- and diafiltrations—all being operations adversely affected by high viscosity—are typically low-shear processes (2)). In the external mode, varying shear rates programmed by a remote-control computer were employed and solution viscosities were measured at shear rates increasing from 10% to 90% of the instrument torque, which is equivalent to from 3.8 s^{-1} to 90 s^{-1} depending on the viscosity (6). Samples were held for 30 s at each shear rate before making a measurement. The viscosity *vs.* shear rate plots exhibited a non-linear dependence characteristic of shear thinning (6,7), as illustrated in Fig. 2. Typically, the values at the highest three shear rates were linearly extrapolated to a zero-shear (Fig. 2) as a normalization technique with an acceptance criterion of $R^2 > 0.95$ (6). The comparison of the solution viscosity values obtained by the two modes revealed that the differences between them were always less than 5%. Therefore, the standalone mode was often used as a fast alternative to the external mode.

For measurements of viscosities of MAb solutions in the presence of salt excipients, a desired amount of the salt (rotary-evaporated from an aqueous solution of the pH adjusted to the value corresponding to that of the antibody solution to avoid changing the pH of the latter) was weighed and added to 1.5 mL of an antibody solution (the resultant volume change was always below 20% even for the highest-molecular-weight salts at a 0.5 M concentration, and below 10% for a 0.25 M concentration). The resulting mixture was gently shaken for 40 min at room temperature to allow complete dissolution of the salt, followed by centrifugation to eliminate bubbles prior to the measurement.

RESULTS AND DISCUSSION

For this study, four humanized MAbs were obtained from Novartis Pharma AG and designated by us as MAb 1, MAb 2, MAb 3, and MAb 4 (see Materials and Methods for their characteristics). Concentrated aqueous solutions of MAbs can be very viscous (8). For example, the viscosity of a 150 mg/mL solution of MAb 1 was found to be 63.2 mPa·s (cP) at 25°C (Table I, 1st entry), *i.e.*, some 71 times greater than water's under the same conditions. Since this type of value would be unacceptably high for SC injections (2), developing simple and effective approaches for lowering

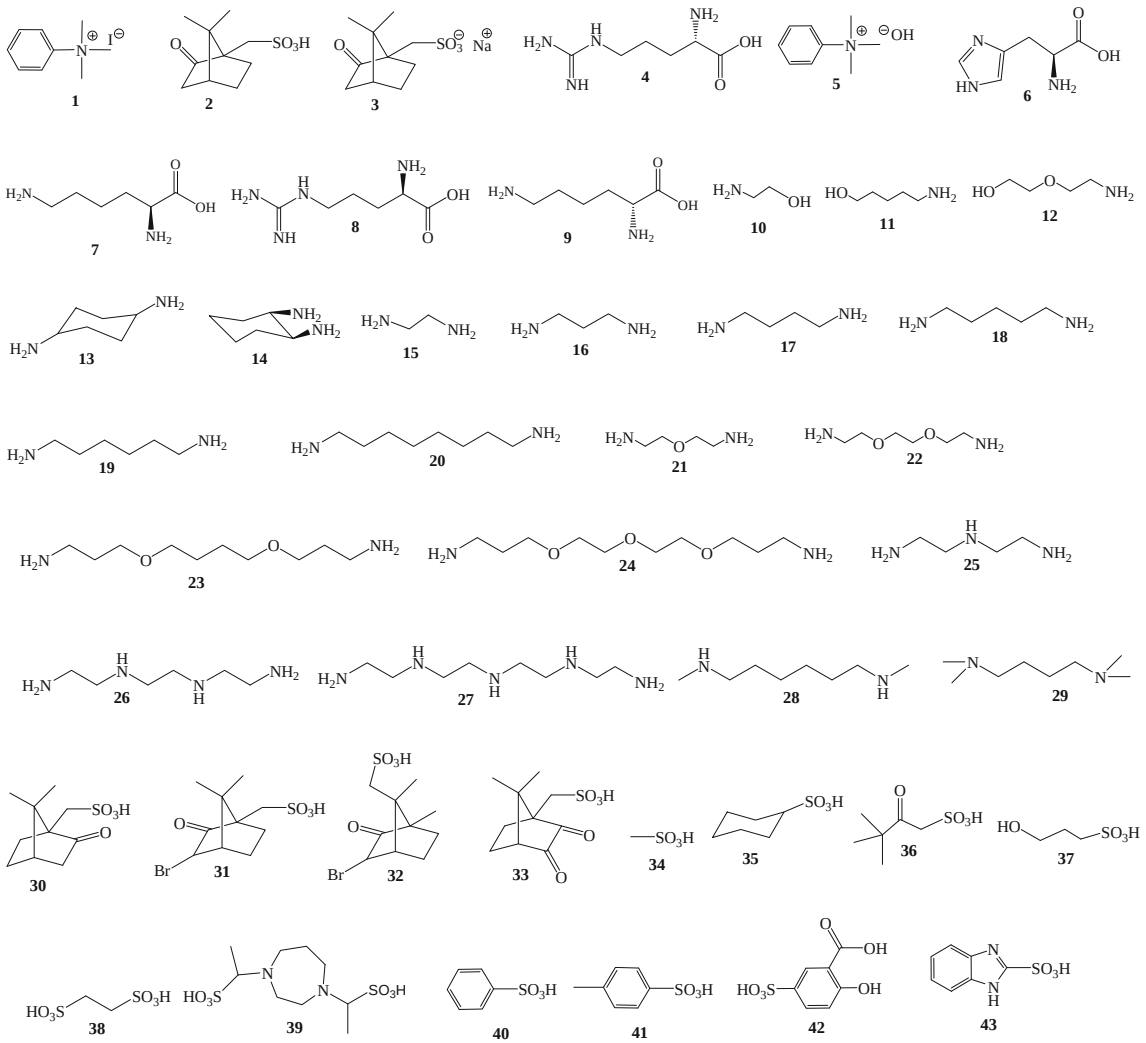

Fig. 1 Chemical structures of the salt excipients and their starting materials used in this study: phenyltrimethylammonium iodide (**1**), (+)-camphor-10-sulfonic acid (**2**), Na salt of **2** (**3**), L-arginine (**4**), phenyltrimethylammonium hydroxide (**5**), L-histidine (**6**), L-lysine (**7**), D-arginine (**8**), D-lysine (**9**), ethanolamine (**10**), 5-amino-1-pentanol (**11**), 2-(2-aminoethoxy)ethanol (**12**), *trans*-cyclohexane-1,4-diamine (**13**), *trans*-cyclohexane-1*R*,2*R*-diamine (**14**), ethylenediamine (**15**), propane-1,3-diamine (**16**), butane-1,4-diamine (**17**), pentane-1,5-diamine (**18**), hexane-1,6-diamine (**19**), octane-1,8-diamine (**20**), 2-(2-aminoethoxy)ethanamine (**21**), 2-(2-(2-aminoethoxy)-ethoxy)ethanamine (**22**), 3-(4-(3-aminopropoxy)butoxy)propan-1-amine (**23**), 3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propan-1-amine (**24**), *N*-(2-aminoethyl)ethane-1,2-diamine (**25**), *N*-(2-(2-aminoethylamino)ethyl)ethane-1,2-diamine (**26**), *N*-1-(2-(2-(2-aminoethylamino)ethylamino)-ethyl)ethane-1,2-diamine (**27**), *N,N*-dimethylhexane-1,6-diamine (**28**), *N,N,N,N*-tetramethylbutane-1,4-diamine (**29**), (−)-camphor-10-sulfonic acid (**30**), (+)-3-bromocamphor-10-sulfonic acid (**31**), (+)-3-bromocamphor-8-sulfonic acid (**32**), camphorquinone-10-sulfonic acid (**33**), methanesulfonic acid (**34**), cyclohexanesulfonic acid (**35**), 3,3-dimethyl-2-oxobutane-1-sulfonic acid (**36**), 3-hydroxypropane-1-sulfonic acid (**37**), 1,2-ethanedisulfonic acid (**38**), homopiperazine-1,4-*bis*-(2-ethanesulfonic acid) (**39**), benzenesulfonic acid (**40**), 4-methylbenzenesulfonic acid (**41**), sulfosalicylic acid (**42**), and benzimidazole-2-sulfonic acid (**43**).

viscosities of concentrated MAb solutions is a high-priority objective in biopharmaceutical formulations (2,9,10).

Recently, we identified phenyltrimethylammonium iodide (**1**) and sodium (+)-camphor-10-sulfonate (**3**) as capable of substantially reducing viscosities of concentrated solutions of model non-MAb proteins (5). Therefore, the present study was begun with applying these two excipients to a 150 mg/mL solution of MAb 1. As seen in Table I (2nd and 3rd entries), 0.5 M **1** and **3** indeed lowered the MAb 1 solution viscosity 3.2- and 3.0-fold, respectively, which made them a good starting point for further structure-activity relationship (SAR) investigation; this is particularly true for **3** which has been approved for use in injectable dosage forms (11).

In a recent study, Kanai *et al.* (4) observed a striking reduction in viscosity of concentrated aqueous solutions of one of Genentech's MAbs using sodium chloride. In our case, however, 0.5 M NaCl exerted no appreciable effect on the MAb 1 solution viscosity (Table I, 4th entry), suggesting that the previous observation (4) and its mechanistic rationale were limited to one particular MAb (12). Furthermore, this finding indicates that Na^+ ion itself was not responsible for the viscosity-lowering effect of salt **3** in Table I.

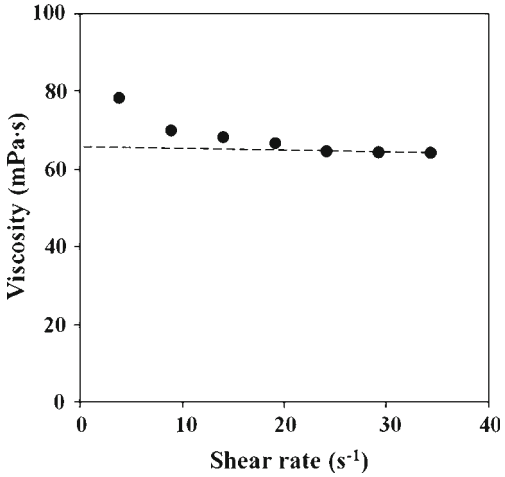

Fig. 2 The dependence of the viscosity of a 150 mg/mL buffered (pH 6.1) aqueous solution of humanized MAb 1 measured by the cone-and-plate technique as a function of the shear rate. The viscosities were measured in the external mode at 25°C and shear rates increasing from 3.75 to 34.5 s^{-1}. The true viscosity value determined in such an experiment and reported in the manuscript is the intercept of the dashed line with the Y-axis. See Materials and Methods for details.

Since L-arginine hydrochloride (**4**·HCl) is a frequently used stabilizer in pharmaceutical protein purifications and formulations (10), we examined its effect on the viscosity of MAb 1 aqueous solution. As seen in Table I (5th entry), 0.5 M **4**·HCl afforded a 2.2-fold reduction in viscosity. To test whether the viscosity-lowering effects of 3 (a pharmaceutically acceptable excipient (11)) and **4**·HCl were additive, we prepared and tested a salt combining **4** and **2** as cationic and anionic counterparts, respectively. Inspection of Table I reveals that the resultant salt **4·2** was indeed much more effective than either **4**·HCl or **3** alone and lowered the viscosity of a 150 mg/mL MAb 1 solution 5.9-fold. The same marked reduction in viscosity was obtained with **8·2** (Table I). These encouraging observations prompted us to undertake a systematic SAR investigation of a series of excipients with respect to their ability to lower MAb 1's solution viscosity; since numerous structural analogs of **4** are commercially available, **4·2** was selected as a lead salt toward that end.

Table I The Effect of Certain Salts on the Viscosity of a 150 mg/mL Aqueous Solution of Humanized MAb 1 at 25°C and pH 6.1

Excipient	Concentration of excipient (M)	Viscosity[a] (mPa·s)	Viscosity-lowering effect[b] (fold)
None	–	63.2 ± 0.8	1.0
1	0.5	19.5 ± 1.6	3.2 ± 0.3
3	0.5	21.1 ± 0.3	3.0
NaCl	0.5	68.9 ± 2.1	0.9
4·HCl	0.5	29.1 ± 0.4	2.2
4·2	0.5	10.7 ± 0.3	5.9
8·2	0.5	10.6 ± 0.2	6.0
5·2	0.5	10.8 ± 0.3	5.8
6·2	0.5	19.5 ± 0.4	3.2
7·2	0.5	7.7 ± 0.1	8.2
9·2	0.5	7.5 ± 0.1	8.4
7·2	0.25	13.4 ± 0.2	4.7
18·2	0.5	11.9 ± 0.2	5.3
18·2	0.25	6.4 ± 0.2	10

[a] Viscosity measurements were carried out at a 22.5 s^{-1} (3 rpm) shear rate. The data presented are mean values ± standard deviations (SDs) of three separate measurements

[b] The mean values of three separate determinations; unless indicated otherwise, all SD values did not exceed 0.1

First, the L-arginine portion in **4·2** was replaced with two other basic proteinogenic amino acids, **6** and **7**, to yield the salts **6·2** and **7·2**, respectively. While the former was much less effective than **4·2** (presumably due to a relatively weak basicity of the imidazole side chain), **7·2** was even superior to **4·2** affording an 8.2-fold reduction in the viscosity of a 150 mg/mL MAb 1 solution (Table I). Interestingly, the cation's stereochemistry in the salt was found to play no appreciable role: as seen in Table I, the D enantiomers (**8** and **9**) were essentially as effective as their L counterparts.

In view of the foregoing, we proceeded to "dissect" the structure of L-lysine in **7·2**, by first "cutting out" its carboxyl group to form pentane-1,5-diamine (**18**). The viscosity-lowering effect of the resultant **18·2** at 0.5 M was 5.3 (Table I), *i.e.*, below that of its parent compound **7·2**. Since the net charges of the cations formed by **7** and **18** are distinct (+1 and +2, respectively), we reasoned that different cation-to-anion stoichiometries might apply in the final salts **7·2** and **18·2**. Therefore, we examined the effect of the concentrations of **7·2** and **18·2** on the viscosity of a MAb 1 solution. It was found that while **7·2** was expectedly less effective at 0.25 M than at 0.5 M, the opposite was true for **18·2**; in fact, 0.25 M **18·2** was the most potent salt excipient thus far, reducing the viscosity of MAb 1 by an order of magnitude (Table I). This finding suggests that the optimal concentration of the excipient may depend on the cation/anion stoichiometry in the salts. Subsequently, we examined structural variants of salt **18·2** because (i) it was more potent than **7·2** at a lower concentration and (ii) **18** is structurally simpler than 7.

When one of the amino groups of **18** was replaced with a hydroxyl group, a 0.5 M salt formed by the resultant 5-amino-1-pentanol (**11**) with **2** yielded a 9.3-fold drop of viscosity (Table II). This effect was superior to that of 0.5 M **18·2** but somewhat inferior to that of 0.25 M **18·2** (5.2- and 10.0-fold, respectively). Likewise, the viscocity-lowering effects of salts of 2 with two other simple amino-alcohols, namely **10** or **12**, while high (some 8-fold), were below that afforded by diluted **18·2**, making the latter salt at a 0.25 M concentration the leading MAb 1 solution viscosity-lowering excipient thus far.

Table II The Structure-Activity Relationship (SAR) of Various Salts of **2** with Respect to Their Ability to Reduce the Viscosity of a 150 mg/mL Aqueous Solution of Humanized MAb 1 at 25°C and pH 6.1

Excipient	Concentration of excipient (M)	Viscosity[a] (mPa·s)	Viscosity-lowering effect[b] (fold)
10·2	0.5	7.9 ± 0.1	8.0
11·2	0.5	6.7 ± 0.2	9.3
12·2	0.5	8.0 ± 0.2	7.9
19·2	0.25	5.9 ± 0.1	10.7
13·2	0.25	6.7 ± 0.1	9.4
14·2	0.25	8.3 ± 0.1	7.7
21·2	0.25	6.5 ± 0.1	9.7
22·2	0.25	6.4 ± 0.1	9.8
23·2	0.25	6.6 ± 0.2	9.5
24·2	0.25	6.5 ± 0.1	9.6
25·2	0.25	6.5 ± 0.1	9.7
26·2	0.25	7.6 ± 0.1	8.4
27·2	0.25	7.4 ± 0.1	8.5
28·2	0.25	6.6 ± 0.1	9.6
29·2	0.25	10.2 ± 0.2	6.3

[a] Viscosity measurements were carried out at a 22.5 s^{-1} shear rate. The data presented are mean values ± SDs of three separate measurements

[b] The mean values of three separate determinations; all SD values did not exceed 0.1

As seen in Fig. 3, upon increasing the carbon-chain length (n) of a linear aliphatic diamine, the viscosity-lowering potency of the corresponding salt with **2** rose from n=2 to n=4 but reached a saturation point thereafter (note that salts formed by diamines with n>8 were insufficiently soluble in water). Converting a linear diamine, namely **19**, into its cyclic isomer had only a marginal negative effect as long as the amino groups

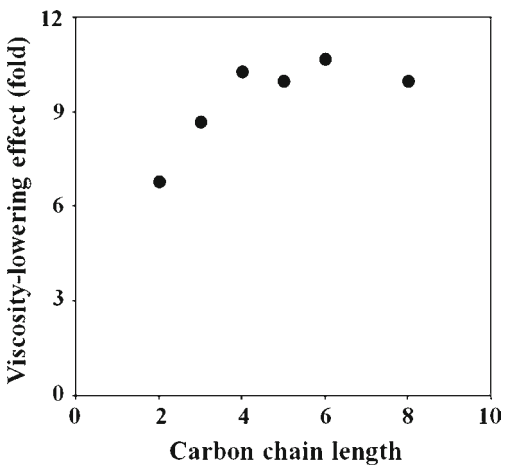

Fig. 3 The effect of the carbon chain length of linear diamines (**15** through **20**) in their salts with **2** on reduction in viscosity of a 150 mg/mL buffered (pH 6.1) aqueous solution of humanized MAb 1 at 25°C. The presented data points are mean values of three independent measurements; standard deviations (SDs) were below the diameters of the data points.

were spacially distant from each other (as in **13**) but a more pronounced one when they were immediately adjacent to each other (as in **14**) (Table II), presumably due to steric hindrances in forming the corresponding salt with **2**. As also seen in Table II, inserting one, two, or three oxygens (compounds **21**–**24**) or NH groups (compounds **25**–**27**) in linear primary diamines, or using secondary amines as terminal groups (compound **28**), had little influence on viscosity-lowering effects of the corresponding excipients; however, when the alipahtic chain was terminated with the tertiary amines (compound **29**), a marked decrease in the viscosity-lowering effect of the corresponding excipient was observed.

These observations provide a new class of excipients, namely salts of **2** with various amine compounds, which dramatically (some 10-fold) reduce the viscosity of a 150 mg/mL solution of MAb 1. That the magnitude of this effect is only slightly dependent on the specific structural features of the cation points away from its subtle molecular origin.

Next, by replacing **2** in salt **18·2** with other sulfonic acids, we investigated the role of the chemical structure of the latter. The results presented in Table III afford several insightful SAR conclusions. First, even significant structural alterations had a relatively minor influence on the viscosity-lowering effect as long as the anion remained aliphatic and bulky (**30**–**33** and **35**–**36**, as compared to **34** and **38**–**39**). In contrast, all aromatic sulfonic acids tested (**40**–**43**) were inferior, presumably due to their planar rings resulting in more compact structures. Finally, the viscosity-lowering effects of salts of **18** with simple inorganic acids (the last three entries in Table III) were all marginal.

To test the generality of our findings, we investigated the effect of several representative salt excipients tested above for MAb 1 on the viscosity of aqueous solutions of three other Novartis' MAbs. One can see in Table IV that the specific viscosities (*i.e.*, the viscosity divided by the protein concentration) varied widely among the antibodies suggesting significant differences in their physicochemical characteristics (8,13,14). Importantly, however, the three previously identified viscosity-lowering salts tested, **3**, **7·2**, and **18·2**, afforded a several-fold reduction of viscosity for all four MAbs. Furthermore, the relative order of their efficacy was the same for all the MAbs, with the 0.25 M salt **18·2** invariably being the most potent. These data bode well for the generality of hydrophobic salts developed in this work as viscosity-lowering excipients for humanized MAbs. Note that none of the four examined MAbs in aqueous solutions exhibited an aggregation upon addition of any of the excipients evaluated in this study.

We propose the following two-pronged mechanistic hypothesis to explain our findings. First, in concentrated MAb solutions in water protein molecules reversibly associate with each other through a combination of hydrophobic and ionic interactions resulting in transient three-dimensional protein

Table III Structure-Activity Relationship of 0.25 M Salts of **18** with Respect to Their Ability to Lower the Viscosity of a 150 mg/mL Aqueous Solution of Humanized MAb 1 at 25°C and pH 6.1

Group	Excipient	Viscosity[a] (mPa·s)	Viscosity-lowering effect[b] (fold)
Analogues of **2**	**18·30**	6.0 ± 0.1	10.5
	18·31	8.4 ± 0.2	7.5
	18·32	6.7 ± 0.2	9.3
	18·33	9.0 ± 0.2	7.0
Aliphatic sulfonic acids	**18·34**	15.1 ± 0.3	4.2
	18·35	9.6 ± 0.3	6.6
	18·36	6.8 ± 0.1	9.3
	18·37	9.8 ± 0.3	6.4
	18·38	18.6 ± 0.1	3.4
	18·39	11.5 ± 0.1	5.5
Aromatic sulfonic acids	**18·40**	13.3 ± 0.2	4.8
	18·41	13.0 ± 0.2	4.9
	18·42	11.0 ± 0.4	5.8
	18·43	14.9 ± 0.1	4.2
Inorganic acids	**18·HCl**	18.5 ± 0.1	3.4
	18·HI	17.5 ± 0.3	3.6
	18·H_2SO_4	24.2 ± 0.2	2.6

[a] Viscosity measurements were carried out at a 22.5 s^{-1} shear rate. The data presented are mean values ± SDs of three separate measurements

[b] The mean values of three separate determinations; all SD values did not exceed 0.1

networks, creating a strong resistance to flow and, in turn, leading to high viscosities (4). Second, hydrophobic salts, when added to such MAb solutions, compete with these hydrophobic/ionic protein-protein interactions, thereby disrupting the putative transient networks and hence lowering the viscosity. This hypothesis was verified in the following experiments with representative salt excipients.

In agreement with the first prong of the aforementioned mechanism, the viscosity of MAb 1 sharply increased as the concentration of the antibody was raised from 25 to 150 mg/mL (triangles in Fig. 4a). The observed almost exponential dependence, characteristic of a non-ideal (non-Newtonian) behavior (4,12,15), presumably reflect greatly intensified protein-protein interactions at the highest concentration where the average distance between MAb molecules is estimated (assuming a spherical 150-kDa protein) to be below the molecular radius (8). In addition, not only were the viscosity values in the presence of 0.25 M **19·2** expectedly dramatically diminished at all MAb concentrations, but the resultant dependence was almost linear (circles in Fig. 4a), which is typical for an ideal behavior of a Newtonian fluid (13,16,17). Furthermore, the higher the protein concentration, the greater was the viscosity-lowering effect of the hydrophobic salt (Fig. 4b); this phenomenon is consistent with a highly developed protein network at, for example, 150 mg/mL MAb disrupted by the excipient.

The second prong of the proposed mechanism predicts that lower concentrations of the hydrophobic salt excipient should be less effective in competing for protein-protein interactions and thus impart a smaller effect on the MAb solution viscosity. Indeed, inspection of Fig. 5 reveals that as the concentration of **19·2** was reduced below 0.25 M, its effect on the viscosity of a 150 mg/mL solution of MAb 1 gradually declined. (It is worth noting, however, that the data in this figure indicate that **19·2** affords some 9-fold reduction in viscosity of MAb 1 solutions even at a 0.15 M concentrations corresponding to an isotonic saline solution.)

We also reasoned that the putative intermolecular association of MAb molecules in aqueous solutions might manifest itself in characteristic rheological properties. For an ideal (Newtonian) liquid, such as a very dilute protein solution, the viscosity should be essentially independent of the shear rate (16). In contrast, for a concentrated protein solution, the viscosity typically decreases as the shear rate is increased, resulting in a shear-thinning pseudoplastic behavior (14,16,18,19). This is because as shear is applied, the protein-protein interactions are disrupted, thus leading to an alignment of protein molecules in the direction of shear which, in turn, decreases solution's resistance to flow, *i.e.*, viscosity (14,16,17).

Table IV The Effects of the Salts **3**, **7·2**, and **18·2** on the Viscosities of Aqueous Solutions of Four Humanized MAbs at 25°C (see Materials and Methods for Experimental Details)

Excipient	MAb 1		MAb 2		MAb 3		MAb 4	
	Viscosity[a,b] (mPa·s)	Viscosity-lowering effect[b] (fold)	Viscosity[a,b] (mPa·s)	Viscosity-lowering effect[b] (fold)	Viscosity[a,b] (mPa·s)	Viscosity-lowering effect[b] (fold)	Viscosity[a,b] (mPa·s)	Viscosity-lowering effect[b] (fold)
None	63.2 ± 0.8	1.0	10.3 ± 0.3	1.0	32.9 ± 2.2	1.0	72.8 ± 0.5	1.0
0.5 M **3**	21.1 ± 0.3	3.0 ± 0.1	3.3 ± 0.1	3.1 ± 0.1	9.8 ± 0.6	3.3 ± 0.2	17.9 ± 0.1	4.1 ± 0.1
0.5 M **7·2**	7.7 ± 0.1	8.2 ± 0.1	3.0 ± 0.1	3.4 ± 0.1	7.6 ± 0.2	4.3 ± 0.1	12.8 ± 0.1	5.7 ± 0.1
0.25 M **18·2**	6.4 ± 0.2	10.0 ± 0.1	2.6 ± 0.1	4.0 ± 0.2	7.1 ± 0.5	4.6 ± 0.3	11.6 ± 0.1	6.3 ± 0.1

[a] Viscosity measurements were carried out at a 22.5 s^{-1} shear rate

[b] The data presented are mean values ± SDs of three separate measurements

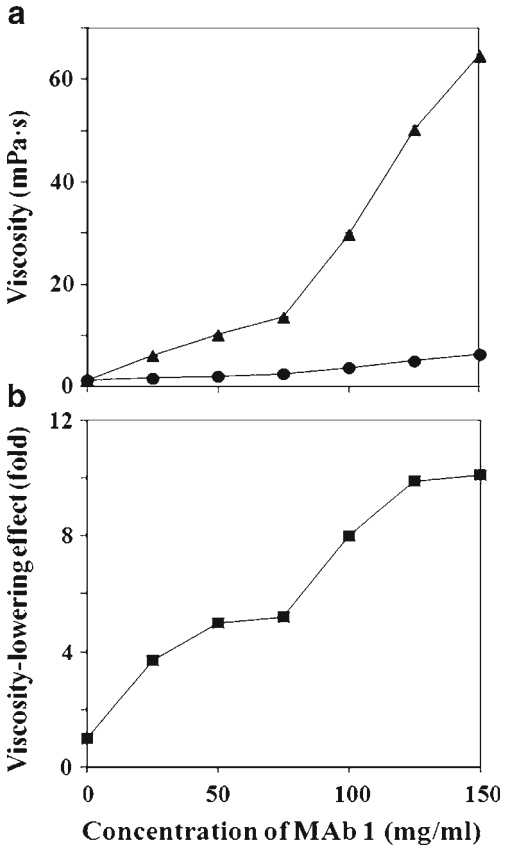

Fig. 4 The effect of the concentration of humanized MAb 1 (**a**) on the viscosity of its buffered (pH 6.1) aqueous solution at 25°C in the absence (triangles) and presence (circles) of the 0.25 M salt **19·2**, as well as (**b**) on the viscosity-lowering effect exerted by that excipient. The presented data points are mean values of three independent measurements; SDs were below the diameters of the data points.

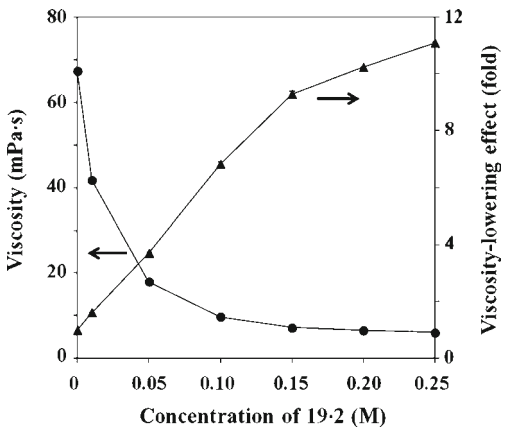

Fig. 5 The effect of the concentration of the salt **19·2** on both the viscosity (circles; left Y-axis) of a 150 mg/mL buffered (pH 6.1) aqueous solution of humanized MAb 1 at 25°C and the viscosity-lowering effect (triangles; right Y-axis) of the salt excipient. The presented data points are mean values of three independent measurements; SDs were below the diameters of the data points.

Indeed, we found that a 150 mg/mL MAb 1 solution in the absence of excipients or in the presence of a relatively impotent 0.5 M **3** displayed a pronounced non-ideal behavior (Fig. 6a). In stark contrast, however, when the potent viscosity-lowering excipients **7·2** and **18·2** were used instead, a classical Newtonian rheological behavior was observed (Fig. 6b), likely due to diminished protein-protein interactions afforded by them (12,14,16).

CONCLUSIONS

In this work, we systematically identified and investigated a number of new excipients highly effective in reducing the viscosities of concentrated MAb solutions. The descriptive SAR for the viscosity-lowering salts was established, and a mechanism of their action proposed and verified. The next logical step in exploring the practicality of the viscosity-lowering excipients identified herein may be examining their safety and effect on MAb stability.

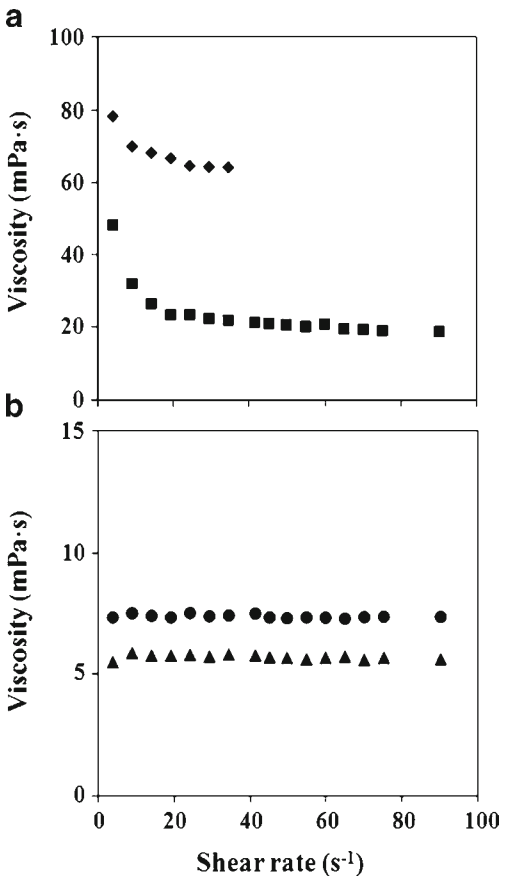

Fig. 6 The dependence of the viscosity of a 150 mg/mL buffered (pH 6.1) aqueous solution of humanized MAb 1 at 25°C on the shear rate (**a**) in the absence of excipient (*diamonds*) and in the presence of the 0.5 M salt 3 (*squares*) (a *non*-Newtonian rheology) and (**b**) in the presence of the 0.5 M salt **7·2** (*circles*) and the 0.25 M salt **18·2** (*triangles*) (a Newtonian rheology).

ACKNOWLEDGMENTS AND DISCLOSURES

This study was financially supported by Novartis AG, which also kindly supplied the humanized MAbs used by us.

REFERENCES

1. Yang MX, Shenoy B, Disttler M, Patel R, McGrath M, Pechenov S, Margolin AL. Crystalline monoclonal antibodies for subcutaneous delivery. Proc Natl Acad Sci USA. 2003;100(12):6934–9.
2. Shire SJ, Shahrokh Z, Liu J. Challenges in the development of high protein concentration formulations. J Pharm Sci. 2004;93 (6):1390–402.
3. Liu J, Nguen MDH, Andya JD, Shire SJ. Reversible self-association increases the viscosity of a concentrated monoclonal antibody in aqueous solution. J Pharm Sci. 2005;94(9):1928–40.
4. Kanai S, Liu J, Patapoff HW, Shire SJ. Reversible self-association of a concentrated monoclonal antibody solution mediated by Fab-Fab interaction that impacts solution viscosity. J Pharm Sci. 2008;97 (10):4219–27.
5. Du W, Klibanov AM. Hydrophobic salts markedly diminish viscosity of concentrated protein solutions. Biotechnol Bioeng. 2011;108 (3):632–6.
6. He F, Becker GW, Litowski JR, Narhi LO, Brems DN, Razinkov VI. High throughput dynamic light scattering method for measuring viscosity of concentrated protein solutions. Anal Biochem. 2010;399 (1):141–3.
7. Papanagopoulos D, Dondos A. Difference between the dynamic and static behavior of polymers in dilute solutions: The critical concentrations. Polymer. 1995;36(2):369–72.
8. Saluja A, Kalonia DS. Nature and consequences of protein–protein interactions in high protein concentration solutions. Int J Pharm. 2008;358(1–2):1–15.
9. Harris RJ, Shire SJ, Winter C. Commercial manufacturing scale formulation and analytical characterization of therapeutic recombinant antibodies. Drug Dev Res. 2004;61(3):137–54.
10. Frokjaer S, Otzen DE. Protein drug stability: a formulation challenge. Nat Rev Drug Discov. 2005;4:298–306.
11. Paulekuhn GS, Dressman JB, Saal C. Trends in active pharmaceutical ingredient salt selection based on analysis of the Orange Book database. J Med Chem. 2007;50(26):6665–72.
12. Yadav S, Shire SJ, Kalonia DS. Factors affecting the viscosity in high concentration solutions of different monoclonal antibodies. J Pharm Sci. 2010;99(12):4812–29.
13. Burckbuchler V, Mekhloufi G, Giteau AP, Grossiord JL, Huille S, Agnely F. Rheological and syringeability properties of highly concentrated human polyclonal immunoglobulin solutions. Eur J Pharm Biopharm. 2010;76(3):351–6.
14. Tung MA. Rheology of proteins dispersions. J Texture Stud. 1978;9(1–2):3–31.
15. Hall CG, Abraham GN. Reversible self-association of a human myeloma protein. Thermodynamics and relevance to viscosity effects and solubility. Biochemistry. 1984;23(22):5123–9.
16. Kinsella JE. Milk proteins: physicochemical and functional properties. Crit Rev Food Sci Nutr. 1984;21(3):197–262.
17. Lee C-H, Rha C. In: Sherman P, editor. Food texture and rheology. London: Academic; 1979. p. 245–63.
18. Sharma V, Jaishankar A, Wang Y-C, McKinley GH. Rheology of globular proteins: apparent yield stress, high shear rate viscosity and interfacial viscoelasticity of bovine serum albumin solutions. Soft Matter. 2011;7(11):5150–60.
19. Patapoff TW, Esue O. Polysorbate 20 prevents the precipitation of a monoclonal antibody during shear. Pharm Dev Technol. 2009;14(6):659–64.