

High-dose Monoclonal Antibodies Via the Subcutaneous Route: Challenges and Technical solutions, an Industry Perspective

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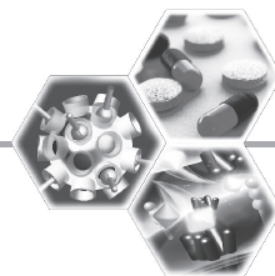
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High-dose monoclonal antibodies via the subcutaneous route: challenges and technical solutions, an industry perspective

This review summarizes the various challenges in product development involved in subcutaneous administration of high-dose monoclonal antibodies and attempts to provide an industry perspective of some of the available technologies and potential avenues to overcome these challenges.

The route of administration is a major consideration during pharmaceutical product development from a therapeutic, patient-care and economic point of view. The most common routes for injectable drugs are subcutaneous (sc.), intramuscular (im.) and intravenous (iv.). From a patient perspective, sc. is the preferred route over im. or iv., as it usually incurs less pain during injection, aided by shorter and smaller-gauged needles. In addition, postinjection muscle pain is usually less with the sc. route than with im. injection due to a lower injection volume and less tissue invasiveness with the sc. The most significant advantage of giving drugs by the sc. route is patient compliance; treatment can happen at home using simple prefilled administration devices. Trained healthcare professionals are needed for both im. and iv. administration including complicated setup for iv. infusion. For patients requiring multiple and frequent doses, for example insulin therapy, sc. injections offer a broader range of alternative sites [1].

Monoclonal antibody (mAb) drugs have become the fastest growing in the biopharmaceutical sector. Four mAb products were in the list of top ten selling drugs in 2010 – small molecules, biologics and vaccines combined [201]. Out of 26 approved mAb drugs in the USA, four are given via the sc. route, one via the im. route and the rest via the iv. route [2]. For chronic diseases, for example inflammatory disorders, sc. administration of mAb is certainly convenient and economical as it allows self-administration in a home setting. Elimination of hospital visits brings down the overall treatment costs associated with personal time, supplies and equipment (iv. sets, diluent bags, pumps and filters) required for

iv. administration. All the marketed sc. mAbs are high-concentration products and the new ones currently under development are expected to be the same for maximum patient benefits. Efforts are currently being made to convert the existing low-concentration iv. mAb products for high-concentration sc. products. For example, Herceptin®, Rituxan® and alemtuzumab (Campath®) are the iv. drugs currently in clinical trials for sc. formulation [3]. Alemtuzumab given iv. at the dose of 30 mg, three-times a week has been studied for sc. administration at the same dose as iv. administration in an attempt to reduce side effects and make the treatment more manageable [4].

The mAbs are usually low-potency drugs, less frequently administered due to longer half-lives and, hence, require high dose. For the iv. route, a high dose can be delivered by large volume administration. For the sc. route, a high-concentration product must be developed to deliver a high dose as administration volume is limited. The majority of the sc. injected mAbs already on the market achieve 50–65% bioavailability, yet the fate of the portion that is lost remains unknown [5–6,202]. This consistently incomplete systemic absorption further increases the demand of higher dose and overall cost of the drug product. There are many potential factors that might influence the absorption from sc. site, such as charge, hydrophobicity, formulation variables, and the depth and volume of the injection [6,7]. However, the typical injection volume for sc. administration is limited to 1–1.5 ml, thus requiring the development of solution or suspension products with high concentrations in the range of 50–300 mg/ml. At this concentration, viscosity and concentration-driven aggregation become

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Key Terms

Phase separate: Formation of distinct regions within the solution that have different compositions.

Opalescence: Hazy, turbid or blue-like appearance.

Subvisible particles: Particles that are imperceptible by eye, typically having diameter of 100 μm or less.

serious development issues. The principles governing the solubility of mAbs (and proteins in general) are more complicated than for synthetic small molecules as they depend on a number of variables, including excipient levels, formulation conditions (e.g., ionic strength, pH and surface tension), protein purity, protein–protein interactions and processing conditions [8]. The high-solution viscosity causes serious delivery problems from syringes and other dosing devices even when high concentration is achieved. High-concentration products also bring challenges to analytical testing, often requiring complex analytical tools (e.g., AUC and dynamic light scattering) and sample dilution for certain methods (e.g., SDS-capillary electrophoresis and MALDI-TOF MS). Dilution sometimes may change protein conformation and/or cause dissociation of aggregates, further complicating measurement and interpretation of data [9]. Although many of these technical challenges are less visible to clinicians but the goal to treat a patient optimally remains a common mission to both clinicians and pharmaceutical scientists. While innovation is required to overcome these challenges, active collaborations between different groups are necessary to develop a robust product.

Challenges

■ Viscosity

The increased protein concentration associated with the limited volume of sc. doses for monoclonal antibodies inevitably leads to elevated viscosity, which in turn poses problems with the processing of the viscous solutions during the manufacturing process. More importantly, problems arise with the force and time needed for the injection [10–13]. In addition, significant discomfort is thought to be associated with the injection of viscous liquids. Consequently, considerable efforts have been undertaken to elucidate the origin of the viscosity at high protein concentrations, as the models developed for more dilute solutions do not apply [13–15]. More specifically, the term ‘viscosity’ as applied to various proteins and their formulations is used to refer to so-called reduced viscosity (η_{red}), in that the specific increment in viscosity as a function of protein concentration [14]. At relatively low concentrations (<50 mg/ml), electrostatic effects appear to determine viscosity, due to the resistance of the diffuse double layer of counterions surrounding the macromolecule (‘primary effect’), in addition to the repulsion between the oppositely charged

layers (‘secondary effect’), and finally due to the potential charge-related shape distortions (‘tertiary effect’) [10,13,14,16]. Consequently, the viscosity is minimal at the isoelectric pH (pI) or in the presence of high ionic strength [17,18]. For higher protein concentrations (>100 mg/ml), however, an opposite behavior is often observed. For example, MAb-1 (IgG1) with a measured pI of 7.8, exhibited maximum viscosity at a pH two units lower than pI [19,20], in contrast to MAb-2 (IgG1) with a theoretical pI of 6.5 [21], which was less viscous and had a lower tendency to self-associate at comparable conditions [22]. The analysis of MAb-1, -2 and a series of relevant mutants indicated that the spatially complementing attraction forces between specific charged amino acid residues in both VL and VH chains within Fab domains played a primary role in the increase of solution viscosity [23]. Overall, these pioneering discoveries gave a sense of potential for the attempts to modulate viscosity through protein engineering, as the authors believe that no specific case of such a development has been disclosed to date. More detailed studies on the potential correlation with the self-association propensity as measured by the second virial coefficient [24], as well as the propensity to **phase separate** at low temperatures [25] will facilitate more complete understanding of the forces governing the behavior at high concentrations, and increase the probability of success when rationally designed mutations are tested. Parallel research related to the charge phenomena will complement the description of the protein behavior at high concentrations [25,26].

Investigations of the viscosity effect at high concentrations have been hampered not only by the limited availability of protein samples, but also by the large sample and time consumption associated with available methods. Originally, viscosity measurements were based on the time needed for flow through a glass capillary tube under gravitational force [27], a method still used to study more ubiquitous proteins [28–31]. Conventional plate-and-cone rheometers, while primarily designed for use with non-protein samples, have often been applied despite large sample consumption and manual operation [13]. Over time, falling-ball or piston-based capillary systems that utilize relatively small volume (hundreds of milliliters) and slow flow rates have been adopted [32]. More recently, a dedicated system based on ultrasonic detection [32] has been developed. Additionally, a capillary system that utilizes backpressure measurements has been

made available commercially (Rheosense, CA, USA). For ultra-low sample consumption and high throughput, a combination of a capillary tubing and a high-pressure chromatography system can be utilized to estimate viscosities of samples that can be injected from an autosampler [25]. High-throughput and low sample consumption can also be achieved by the use of an extrinsic size standard that is an order of magnitude larger than prevailing protein molecules present in solution, monitored by a commercially available plate-based dynamic light-scattering system [33].

■ Opalescence & phase separation

The nature of **opalescence** is sometimes described after defining the therapeutic protein molecules in aqueous media as though being in a gas phase. The analogy is based on poor correlation of their positions in the 3D space, as the electrostatic interactions that are common in a high dielectric constant medium are minimized by large distances between molecules. Just like water molecules suspended in humid air, protein molecules will start dynamically self-associating as the concentration is increased and/or temperature is decreased. Transient micro-regions of increased density will produce additional light scattering, a phenomenon first described in 1908 by Smoluchowski [34] and further characterized by Einstein in 1910 [35]. The resulting blue-like, hazy or even turbid appearance [36] is often perceived as not pharmaceutically elegant [16,37], and its acceptable intensity range is often included in the label. However, the opalescent appearance is not generally associated with an imminent risk of formation of potentially immunogenic visible and **subvisible particles**, as filtering does not generally change their appearance [16,37]. Nevertheless, the factors affecting opalescence are of interest to the protein formulator. Besides protein concentration and temperature these may include excipients affecting intermolecular electrostatic interactions either through direct effect on bulk ionic strength [16,36,38] or through binding of polyvalent counterions [6]. Repulsive forces, further manipulated by adjusting the solution and local pH, generally lead to the attenuation of opalescence [16,36].

With an increase in concentration and a decrease in temperature, local dynamic density fluctuations that give rise to opalescence may become long-lived, resulting in the appearance of physically distinct droplets of highly

concentrated protein. Since the collections of micro-droplets that were originally causing opalescent appearance will coalesce and then sediment at a faster rate than their isolated constituents; cloudy appearance of the protein may gradually subside, resulting in a presence of two transparent or slightly opalescent regions (phases) as the more dense droplets sediment toward the bottom of the container [39]. The presence of two separate phases may impede bioprocess operations and affect the ability to withdraw the liquid from the vial and subsequently inject the dose owing to elevated viscosity of the more dense regions. For therapeutic monoclonal antibodies, phase separation phenomena are typically observed at concentrations exceeding 100 mg/ml, with the denser phase being typically at 200 mg/ml or more, with factors analogous to those causing opalescence [21,40–45]. Since phase separation is a thermodynamic process and is in a state of equilibrium, the effect of various excipients and formulation conditions can be studied. In particular, the potential correlation of the position of present ions in the Hoffmeister series with the tendency to phase separate (kosmotropic effect) can be studied, as well as the effect of polyvalent counterions that tend to alter surface charge potential [7]. Freeze–thaw phenomena, where the local concentration fluctuations at low temperatures may further induce phase separation, should also be taken into account when optimizing the formulation. While the initial turbidity associated with phase separation can be monitored with any optical density or light scattering instrument, more systematic studies may involve accelerating the process by decreasing the dielectric constant of the solution and simultaneous detection of phase separation ('cloud point') and thermal unfolding with extrinsic fluorescent dye [25]. Minimizing the sample consumption and time needed to evaluate the propensity to phase separate may allow more effective lead identification and optimization through early candidate rejection and/or rational sequence design to attenuate short-range attractive forces.

■ Aggregation & subvisible particle formation

One of the critical quality attributes defining therapeutic protein products is the amount of aggregates that potentially compromise their safety and efficacy. Despite a number of biologics being on the market for decades,

until recently there were substantial analytical gaps present related to the ability to detect the presence of subvisible particles [46]. These gaps prompted coordinated efforts to formulate adequate strategies within the industry [47]. It was acknowledged that the widely applied size-based methods, such as size-exclusion chromatography, require not only orthogonal methods such as analytical ultracentrifugation to validate their output within their size range [48–52], but also adoption of new approaches to address the shortcomings related to sensitivity [48], size range [53] and material interactions [54,55]. To this end, several dedicated imaging methods have been developed and made available commercially. In addition to the standard obscuration-based instrument (HIAC), a microflow imaging (MFI) instrument (Brightwell Technologies) was adopted for measuring size distributions of particles larger than 2 μm [56–58], and has become a standard method for formulation development. For particles smaller than 1 μm , a real-time particle tracking system (NanoSight) is often used to determine size distributions [59,60]. In addition, other optical approaches, such as microscopy methods for particle assessment (e.g., fluorescence microscopy imaging) [61], fluorescence-based flow imaging [203], differential scanning fluorimetry [62], flow cytometry [63,64] and atomic force microscopy [65] have been demonstrated to be useful in protein aggregate characterization. Consequently, the regulatory agencies generally expect that the size range exceeding 1 μm is monitored during clinical material development and production [66]. In addition, the use of orthogonal methods is encouraged as multiple techniques are available for any given size range, as summarized in several recent reviews [67–69]. Technological advances, however, can only partially negate the protein aggregate sizing paradox: the particle counts and size distribution of submicron particles cannot be determined simultaneously using practical acquisition and computation time limitations. For example, the particle tracking system (NanoSight) can reliably measure the distribution, but the observation volume that can be scanned in a reasonable amount of time (minutes) is miniscule (fraction of nanoliter). In order to obtain statistically significant data, a total volume of approximately 100 μl would have to be scanned to properly describe a population in the range of thousands particles per milliliter. Thus a compromise must be reached, for example, providing a count of particles larger

than 1 μm after protein-specific staining with extrinsic dye in a high-throughput mode (96-well autosampler) using a commercially available flow cytometer [63].

With the analytical gaps closed, it is generally assumed that the appearance of the protein aggregates can be adequately monitored, and attention now becomes increasingly focused on the qualitative factors. It has been long recognized that chemical modification that may include deamidation and oxidation [70,71], as well as fragmentation [72–75], among others, can induce aggregate formation. Screening of formulations that would minimize these adverse processes is generally performed [76], often employing thermal unfolding assays [62,77–81]. Computational approaches to predict the propensity for aggregation are also seriously considered [82]. The increase of a mAb circulation time through Fc engineering can indirectly decrease the load of aggregated proteins through dose reduction. [83]. The formation of aggregates is also closely monitored during process development, as purification and filling steps can reduce their amount, but also can introduce new ones [84–88]. Packaging components can compromise protein stability leading to aggregation [89–91].

All these degradative processes can potentially lead to increased immunogenicity of protein drugs. In fact, several mechanisms have been identified. Use of surfactants in the absence of protective albumins was shown to increase the amount of aggregates and subsequent hypersensitivity reactions to erythropoietin [89,92]. Specific glycosylation patterns of a therapeutic monoclonal antibody (cetuximab) matched those of pre-existing antigens, resulting in high hypersensitivity reaction rates in some regions of the USA [93]. The presence of specific T-epitope sequences is thought to increase risk of immunogenicity, aiding in lead identification selection prior to expression of the protein [94]. However, the presence of aggregates is thought to be a dominant cause of the appearance of anti-drug antibodies. It has been demonstrated that a group of at least ten identical epitopes in a closely spaced array is necessary to induce the immune system [95]. Importantly, the conformational state of these epitopes plays a critical role. Non-native aggregates, for example, those partially unfolded [96] may induce potent immune response, but generally do not result in production of relevant inhibitory antibodies since they cross-react with inactive (unfolded) forms of the proteins [97,98]. Native-like aggregates, on the other hand, result in antibodies cross-reacting

with original epitopes and their appearance is correlated with the loss of therapeutic protein efficacy [46,99], and facilitates breaking of tolerance in case of endogenous therapeutic proteins [100,101]. In addition to conformation, the exact topography of the epitopes, dependent on the nature of the chemical or physical stress, has an effect on the magnitude of the response [102]. In addition, contact with equipment and/or packaging components can lead to enhanced immunogenicity [98,103].

The sc. route – increasingly popular due to the possibility of self-administration – is also the most provocative to the immune system (aside from intradermal) due to the rich network of antigen-presenting cells that are known to rapidly absorb aggregates by endocytosis [97,104]. Moreover, with the volume of the sc. injection typically limited to 1 ml, concentrations exceeding 50 mg/ml increase the probability of aggregate formation [67,105,106]. Therefore, the aggregation processes in the high concentration regime are being characterized [107–109].

■ Processing limitations

mAbs are typically purified and concentrated by tangential flow filtrations, ultrafiltration (UF) and diafiltration (DF), which can be challenging at a commercial scale for a high concentration product (>100 mg/ml). The membrane flux required during filtration may lead to protein concentration (higher than target) at the membrane surface, potentially leading to protein unfolding and aggregation [110]. Higher shear and longer residence time during UF/DF may also lead to similar protein degradations and the formation of subvisible particles. High concentration can impart high viscosity causing backpressure leading to diminished pump flow and loss of yield. High viscosity can be offset by operating at elevated temperatures which needs to be balanced with a potential increase in product degradation [111]. Many of these processing problems are intrinsic to the biophysical properties of mAbs (e.g., isoelectric point), concentration used and equipment capacity. Nevertheless, the proper selection of buffers (type, ionic strength and pH) and addition of stabilizers (surfactants, amino acids and sugars) in the final buffer can significantly improve processing conditions [112].

■ Pharmacokinetic effects

Many marketed monoclonal antibodies delivered via sc. route exhibit moderate bioavailability (50–65%), despite relatively high concentrations (50–125 mg/ml) [6,204–206]. However, the local

non-specific and electrostatic interactions appear to be relatively insignificant at these concentrations [7]. The known dependence of the antibody half-life on plasma concentration [113], combined with the dependence of bioavailability on the presence of FcRn receptors [114] points out to the possibility that an incomplete recovery from the FcRn cycle during lymphatic transport is the primary cause of protein loss during sc. delivery [7]. Indeed, a shortened lymphatic transport time after intradermal delivery resulted in substantial increase of bioavailability in a swine model [115]. Potential improvements in the pharmacokinetic delivery time may lead to dose sparing, which is beneficial from the cost-of-goods and efficacy standpoints.

■ Technical solutions

Several new formulation technologies and administration tools are under development to enable efficient sc. administration of mAbs that require high doses. This is an emerging field and only a few technologies have been introduced in clinical trials. A brief review of these formulation technologies and administration tools are provided in the following sections, highlighting advantages and disadvantages based upon available information and scientific principles.

■ Convenience of administration

Resistance to self-injection is a significant disadvantage of parenteral administration of chronically administered therapeutics. Although introduction of autoinjectors have alleviated this problem in part, physiological barriers related to needles and self-injection still exist. Several technologies are now either available or being actively pursued that strive to help patients to overcome this psychological barrier through improved convenience of administration.

■ Disposable autoinjectors

The evolving autoinjector technology for self injection of therapeutics for chronic diseases attempts to address patient concerns through the critical variables listed below:

- ‘Painless’ experience;
- Elimination of disruptive injection behavior;
- Site-specific targeting;
- Needle exit-pressure;
- Flow-rate accuracy and injection volumes;
- Patient-treatment documentation;

Key Terms

Implantable minipump:

Surgically implanted pump under the infraclavicular fossa or in the abdominal wall for continuous subcutaneous infusion of drugs.

Enhance™: Technology by Halozyne Corporation used to increase the dispersion of subcutaneously injected drugs.

- Ergonomics;
- Elimination of needle deflection;
- Advanced tactile-needle control;
- Precision fluid metering.

Several advanced autoinjectors such as, CompuFlo® and DPS Dynamic Pressure Sensing® technology are available today that address the variables outlined in the list above. These autoinjectors afford multiple unique benefits that the manual syringes cannot provide [207]. Other manufacturers of autoinjectors (e.g., Scandinavian Health Ltd) manufacture a range that include features such as enhanced safety, ergonomic design, reduced number of components, two- or three-step operation and interlock systems. Autoinjectors such as the precision pen injector (PPI) address the need for an extremely high-precision device with dosage accuracy that can handle highly viscous formulations. Dual-chamber and shield-activated autoinjectors are also either available or in advanced stages of development.

■ Patch pump

Patch pump technology has been maturing over the years and is becoming a commercial reality. Patch pumps are worn directly on the body, discretely attached at the infusion site and wirelessly controlled with a separate device [116]. Omnipod® is a patch pump that is commercially available for insulin administration while others such as JewelPump™, Cellnovo, V-Go™, Solo™ and BetaWedge are in different stages of development. While the focus has primarily been on insulin delivery, recent acquisition of Medingo Solo by Roche potentially paves the way for sc. delivery of mAbs using patch pumps.

■ Implantable minipump

A sc. infusion **implantable minipump** (IIP) is intended to provide long-term continuous or intermittent drug infusion [117,118]. Surgically implanted under the infraclavicular fossa or in the abdominal wall, the drug is infused through a catheter threaded into the desired position. The pump operates by peristalsis, fluorocarbon propellant, osmotic pressure, piezoelectric disk benders, or the combination of osmotic pressure with an oscillating piston.

Several implantable infusion pumps have been approved by the US FDA through the premarket approval process, including the SynchroMed family of pumps (Medtronic), the Codman

3000 (Codman), Model 3000 Constant Flow Implantable Infusion Pump (Arrow International) and the Infusaid implantable infusion pump (Strato/Infusaid, a subsidiary of Pfizer). Additional work is still needed to increase the reservoir volume in these minipumps in excess of 2 ml to enable administration of large volumes and doses.

Enabling technologies

The increased need for high-concentration mAb products places considerable demands on technologies that will enable development of formulation of stable drug products with longer shelf lives. This section discusses some of those enabling technologies driven by these demands. It should be mentioned that many of these technologies are at the exploratory stages with respect to therapeutic mAbs.

■ Crystallization

Of the many therapeutic proteins and peptides that are available in the market, only insulin is available in the injectable crystalline form. Use of crystalline full-length mAbs as therapeutics is very much in the early stages of development [119,120]. Crystallization of large proteins such as mAbs is challenging primarily due to their size and dynamic structure in solution. In some cases, presence of exposed oligosaccharides presents additional difficulties for crystallization.

Choosing to use therapeutically acceptable 'generally regarded as safe' excipients and optimization of conditions suitable for large-scale crystallization in the batch mode are key to developing crystalline mAbs for therapeutic use. Marketed mAbs, such as rituximab, trastuzumab and infliximab have been shown to be amenable to batch crystallization processes in acceptable yields and quality. Furthermore, these crystals seem to afford crystalline suspensions to concentrations in excess of 100 mg/ml so that high doses can be injected sc. with acceptable viscosity, stability, pharmacokinetics and efficacy in animal models. Challenges with this technology include development of aseptic processes suitable for appropriate scale of manufacture and availability of regulatory guidance for such product approval.

■ Halozyne

Halozyne technology is based on the use of recombinant human hyaluronidase (rHuPH-20) that depolymerizes the viscoelastic component (hyaluronan) of the interstitial matrix. The enzyme rHuPH-20 increases the dispersion of sc.

injected drugs across a broad range of molecular weights without tissue distortion. Significant improvements in pharmacokinetics and increase in bioavailability have been observed for sc. injected therapeutics with poor bioavailability. This technology affords a potential opportunity to deliver high doses of sc. mAb [121]. The mechanism of action of Halozyme's Enhance™ technology is depicted in **FIGURE 1**. The Enhance technology has been successfully applied to therapeutic biologics including Herceptin, MabThera®, Cinyze®, iv. IG and recombinant human α 1-antitrypsin. Halozyme's Ultrafast Insulin program combines its rHuPH20 enzyme with mealtime insulins, which may produce more rapid absorption, faster action and improved glycemic control.

■ Nanosuspension

Nanocluster dispersions of antibody therapeutics with low viscosity that may afford high-dose injection and enable self administration by sc. injection are being developed by Johnston *et al.* [122]. Using proprietary technology, Johnston *et al.*, have developed conformationally stable nanoclusters of antibodies in solution

using extrinsic crowders, such as trehalose, at concentrations exceeding 250 mg/ml. Although in its infancy, the authors have shown that these nanoclusters retain biological activity *in vitro* and *in vivo* in suitable animal models. Pharmacokinetic studies in mice established feasibility of this approach for sc. administration of high-dose mAbs. However, necessary attributes for a therapeutic, such as stability, shelf life, process material and dosing device compatibility need to be demonstrated for commercial viability of this technology.

■ Molecular design including glycoengineering

Glycoengineering is an evolving approach and has been employed to modulate the physical, chemical and physiological properties of therapeutic biologics, such as peptides, hormones and proteins [123,124]. Results from the work of Kuroda *et al.* suggest that the content of negatively charged sialic acids in oligosaccharide side chains is one of the critical factors to determine IgG3 cryoglobulin activity, combined with optimized amino acid sequences of the IgG3 variable regions [123]. The presence of high sialic acid

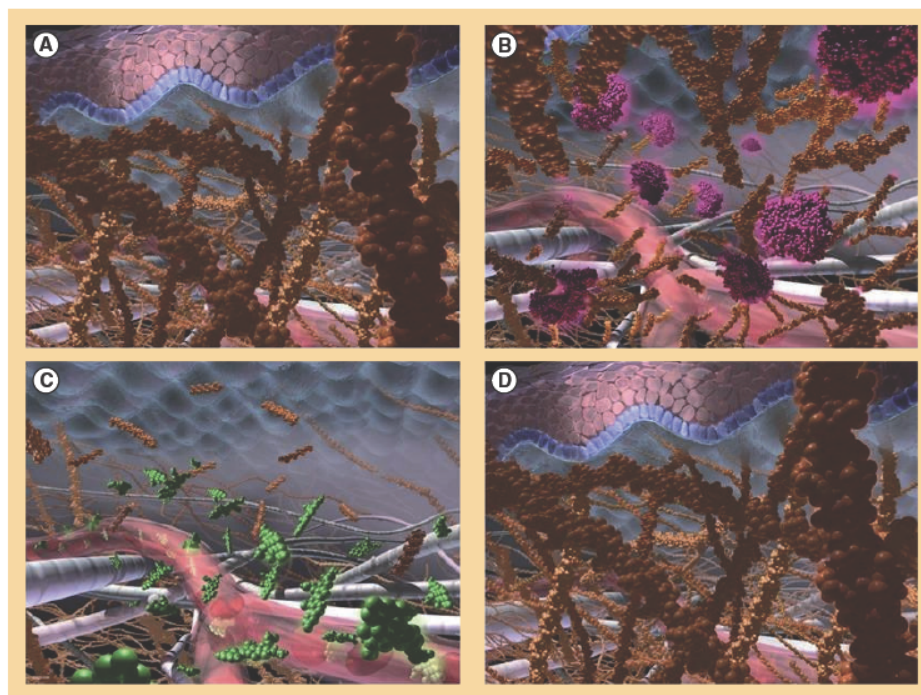


Figure 1. Cross-sectional view of skin dermis. (A) The hyaluronan matrix creates resistance to bulk fluid flow. **(B)** Hyaluronidase decreases local resistance to injection of other molecules, **(C)** allowing exposure to capillaries and lymphatics. **(D)** The barrier is reconstituted rapidly. Reproduced with permission from Halozyme.

Key Term

Nanobodies: Stable fragments of variable regions of monoclonal antibodies.

content is thought to increase solubility of the mAbs. However, implication of increased sialic acid in many neurological diseases and brain development warrants careful evaluation of this approach for improving 'druggability' of mAbs.

■ Spray drying & lyophilization

Spray-drying technology has been explored to develop high-concentration antibody formulations. Dani *et al.* have developed a spray-dried formulation of IgG using glass-stabilization technology [3]. Here, use of sugar excipients (sucrose) stabilized the glasses containing antibody during the spray-drying process and concentrations up to 200 mg/ml were achieved with low viscosity and good syringeability. Schuele *et al.* demonstrated feasibility of employing mannitol to spray-dry antibodies [125]. Control of particle size and moisture is critical for the stability of spray-dried products. Maintaining aseptic conditions during filling of spray-dried powder is essential for product quality and safety. Shire *et al.*, have demonstrated lyophilization of antibodies in the presence of suitable sugars and surfactants followed by reconstitution to lower volumes as a means to achieve high-concentration formulation suitable for sc. injection [9,106]. In these methodologies attention must be paid to minimize degradation such as aggregation of the target antibody molecule. Choice of suitable stabilizing agents is very critical through rational formulation design approaches.

■ Nanobodies

Although several monoclonal antibodies have been successfully developed and are in the market, challenges do exist for sc. administration of high doses. Accessibility to certain disease tissue types (e.g., tumor or Alzheimer's) and cost of production add to the development challenge. **Nanobodies** seem to provide an attractive option to obviate these challenges.

As fragments derived from the variable regions of the heavy chains of single domain antibodies, nanobodies are monomeric, relatively more stable entities with improved solubility [126]. With a molecular mass of only approximately 15 kDa, nanobodies possess fully functional antigen-binding properties that allow sc. injection of efficacious doses. Additionally, their circulating half-lives can be modulated using a range of methods, such as PEGylation, albumin conjugation or directed binding to serum protein. Their size and robustness (e.g., stability at pH extremes) make them particularly suitable for targeting antigens in obstructed locations, such as tumors, where tissue penetration is critical. At the time of this review, clinicaltrials.gov listed five clinical trials that are either in recruitment of patients or active for a variety of disease indications.

Several of the enabling technologies discussed above are futuristic with respect to therapeutic mAbs but possess great potential for delivering sc. high doses. However, since many of these technologies are in early stages of development their value and applicability will be determined by more preclinical and, ultimately, clinical data to ensure patient safety.

Future perspective

Antibody portfolios will continue to expand globally; entry of biosimilars will drive this growth further. Emerging protein engineering and modification tools (e.g., antibody-drug conjugates and nanobodies) will likely introduce next-generation sc. products with improved solubility, bioavailability, efficacy and safety profiles. Development of high-concentration products, including converting marketed iv. into sc. products, will be a key focus for formulation and delivery systems. Thermal stabilization to achieve room temperature storage of these products will be a key driver for product improvements and launching in emerging markets.

Executive summary

- The proportion of subcutaneously delivered therapeutic monoclonal antibodies continuously increases.
- High viscosity, opalescence, aggregation and detrimental pharmacokinetic effects are common challenges associated with subcutaneous monoclonal antibody formulations.
- Numerous approaches in formulation development, with emphasis on both bioprocess development and analytical aspects are undertaken to address subcutaneous development issues.
- The improvements in convenience of administration include technical solutions, such as the use of disposable autoinjectors, with newer technologies, such as patch pumps or implantable minipumps, being developed.
- In addition to commonly used lyophilization technology, a number of enabling technologies are under development that includes crystallization, enzymatic depolymerization of subcutaneous networks, nanosuspensions, molecular design and glycoengineering and nanobodies.

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