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Research paper

High-concentration protein formulations: How high is high?

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ABSTRACT

High-concentration protein formulation (HCPF) is a term that is used to describe protein formulations, mostly monoclonal antibody (mAb) drugs, at high protein concentration. The concentration is rarely defined, with typical ranges varying between 50 and 150 mg/ml for mAbs. The term HCPF is meant to include and express specific solution properties of formulations that are prone to appear at high protein concentrations such as high viscosity, high opalescence, phase separation, gel formation or the increased propensity for protein particle formation. Thus the term HCPF can be understood as a descriptor of protein formulations, usually at high protein (monoclonal antibody) concentrations, which have specific solution, stability and colloidal properties that differ from formulations at low protein concentration (e.g. at 10 mg/ml).

The current paper highlights in brief the development challenges that might occur for high-concentration protein/monoclonal antibody formulations. In particular, the maximum concentration regimes achievable in HCPF remained unclear. Based on geometrical considerations involving packing of monoclonal antibodies in a lattice we map out a maximum concentration range that might be theoretically achievable. Different geometrical assumptions and packing models are compared and their relevance is critically discussed, in particular concerning the influence of the physicochemical properties of the monoclonal antibodies on their solubility, which is neglected in the simple geometrical model. According to our estimates, monoclonal antibody concentration above 500 mg/ml will be very challenging to achieve. Our results have implications for setting up realistic drug product development strategies and for preparing convincing drug target product profiles for development.

1. Introduction

Currently most of the commercially approved monoclonal antibody drug products are formulated at low protein concentration (below 30 mg/ml) and administered intravenously through infusions, especially for oncology drugs [69,57,15]. For specific therapeutic indications, especially chronic diseases such as asthma, psoriasis, or arthritic diseases, however, the alternate delivery route, sub-cutaneous administration, has advantages and becomes more and more of relevance. The main reasons for developing sub-cutaneously administered formulations are: (i) self-administration, especially in the context of home medications, (ii) ease of use, (iii) reduction of hospitalization and thus treatment costs, and (iv) increased patient compliance.

The typical injection volume for sub-cutaneous administration is limited to 1 to 1.5 ml [41]. This volume limitation is due to sub-cutaneous tissue back pressure that might expel the injected drug as well as observed injection pain.

However, the volume restriction can be overcome by using drug product co-formulations with the enzyme hyaluronidase being co-injected. This approach allows the delivery of up to 10 ml via the sub-cutaneous route [14]. Based on this technology, sub-cutaneous administration was reconsidered for treatments in the focus of sub-cutaneous delivery. Notably, even in the context of oncology drugs, the sub-cutaneous route is now envisaged as a valuable alternative [60,35,24]. This is attributed to potential PK/PD (pharmacokinetic and –dynamic) benefits, but also to the ease of drug administration [62]. Jackisch and co-workers [24] showed that it is possible to co-formulate a therapeutic monoclonal antibody (trastuzumab®) with recombinant human hyaluronidase and to generate a stable formulation. They demonstrated the relatively pain-free administration of larger fluid volumes of 10 ml via the sub-cutaneous route [39], hence allowing for injecting biologics at lower concentrations.

It was concluded from these studies, that the co-formulation with hyaluronidase constitutes a less invasive, time-optimised

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and a flexible administration form for oncology patients (HER2-positive breast cancer), with fixed dosing possibilities, leading to improvement in therapeutic safety [60,24]. The main challenges with this approach are to show overall stability of the formulation for the co-formulated drug. In addition, studying tissue back pressure, injection site leakage, local tolerability, and injection-related adverse events, such as injection pain, have to be carefully considered for the development of larger sub-cutaneous injection volumes.

Up to now, however, most clinical protocols involve a sub-cutaneous administration of max. 1.5 ml. One has to keep in mind that the clinical doses for monoclonal antibody drugs are usually quite high, about 5–700 mg drug per patient (up to 10 mg per kg bodyweight). Considering the high doses needed for monoclonal antibody therapies and the low volume that can be injected via the sub-cutaneous route, high-concentration protein formulations are requested, implying a number of challenges.

2. Challenges with high-concentration protein formulations

The development of highly concentrated protein formulations above 150–200 mg/ml is associated with a number of challenges, which have been discussed for example in: [57,70,17,18,1]. In the following, we shall briefly discuss the issue from the HCPF CMC (Chemistry Manufacturing Control) development perspective.

Intrinsic protein properties: Protein solubility and hydration, colloidal and structural stability, and solution properties are key factors that govern the development of a high-concentration formulation [53,17,18,27,63]. At high protein concentration >100 mg/ml, the solution becomes crowed and protein-protein interactions become more relevant. As a consequence of increased protein concentration, opalescence and especially viscosity may strongly increase [53,52,73,45]. Liquid-liquid phase separation becomes more likely with increasing protein concentration ([49] and references cited therein). The critical density, at which phase separation is observed, often corresponds to the density showing maximum opalescence, as density fluctuations are maximal at this point.

However, it should be noted, that the formation of separated phases has also been observed for lower protein concentrations [46,47]. Recently, Raut and Kalonia have shown for a dual variable domain immunoglobulin, that phase separation already occurs at ca. 10 mg/ml forming a protein rich phase of ca. 125 mg/ml and a protein depleted phase of ca. 4–5 mg/ml [46]. Another aspect that is relevant for high-concentration protein solutions is the potential of the formation of a gel phase [11,8], which will also impair drug delivery via pre-filled syringes or devices. The outcome depends strongly on the amino acid sequence and chemical structure of the antibody [27,63,65]. Various studies are known, showing for monoclonals, that already changes of a few amino acids in the primary sequence of proteins may induce pronounced differences in solution properties of antibodies, especially at high protein concentrations [61,57,76,77].

To reach high protein concentration, solubility is a key parameter. Different definitions of solubility have to be considered, namely kinetic solubility (amorphous versus crystalline phase formation) and thermodynamic solubility [2,19]. This differentiation depends on how the solubility is measured [13,2,19]. Kinetic solubility described the extent to which a protein precipitates (amorphous or crystalline protein phase formation) when added to a new solvent. This means, that kinetic solubility is determined by preparing a concentrated stock solution in a specific solvent, after which the solution is diluted in another aqueous solution to a desired concentration. Protein solubility is then determined experimentally, and usually prior to the measurement, insoluble protein is removed by filtration or centrifugation.

From the thermodynamic point of view, solubility is handled as an equilibrium constant. Thus, solubility as a thermodynamic characteristic of proteins involves the chemical potential of the molecules. If the chemical potential of the protein molecule in solution exceeds the chemical potential of the protein of a specific solid phase, crystalline or amorphous protein precipitates. Protein solubility is therefore defined as the protein concentration, at which the chemical potential of the dissolved protein is the same as the chemical potential of the solid protein phase, under the given environmental conditions (e.g. pH, ionic strength, temperature) [2,19]. Therefore, thermodynamic solubility determines how much of a compound dissolves, i.e. transfers from a solid to a liquid phase, and thus is the concentration reached in a specific liquid phase under specific environmental conditions [19].

As denoted by Trevino et al. [64] solubility experiments in the presence of an amorphous solid phase are more useful, because mostly, amorphous protein is precipitated [13,64]. In this context, it should be remembered that, under the same experimental and environmental conditions, the solubility of a protein solution in equilibrium with an amorphous solid phase will be higher than the solubility of a protein solution in equilibrium with its crystalline solid phase [64].

Measuring protein solubility is very challenging, and often surrogate parameters are used, such as opalescence or protein-protein interaction parameters, to obtain or derive apparent solubility data [64,33,67,22]. At best comparative solubility experiments are performed that allow ranking different solution conditions with regards to protein solubility [64,34,19,6,22]. Kramer et al. [31], however, emphasised the difficulty of obtaining quantitative solubility data, because in solubility experiments it is often highly demanding to get reproducible and reliable measurements due to potential protein gel or supersaturated solution formation [31].

Manufacturing: There are different methods available for concentrating proteins, but from an industrial perspective and handling, UF/DF (ultra-filtration/dia-filtration) is the most used and appropriate method, considering GMP (Good Manufacturing Practice) and cost aspects [42,58]. However, for HCPF, the strong, non-linear dependence of viscosity on protein concentration sets limits to the application range of UF/DF procedures. The high viscosity induces strong backpressures in the UF/DF systems and the filtration flow is strongly reduced, thus making the process challenging to develop.

Other process steps that might be impaired by high viscous protein solutions are filtrations such as sterile filtration, pumping of the liquid during the fill and finish process or the filling itself. For example, Allmendinger et al. [1] investigated sterile filtration of highly concentrated protein formulations and the impact of protein concentration, formulation composition, and filter material. They observed differences in filtration behaviour of concentrated protein formulations during aseptic drug product manufacturing of biologics dependent on formulation composition. Furthermore, the filtration behaviour was influenced by the presence of specific excipients in the formulation, which defines the interaction between filter membrane and surface active formulation components [1]. It was also confirmed, that filtration behaviour was additionally defined by rheological non-Newtonian flow behaviour.

Pharmaceutical development: Rheological and syringeability properties of high-concentration formulations may impair application via a syringe or injection device [5,9]. In most cases the key parameter is solution viscosity (η). Due to the use of extremely thin needles for sub-cutaneous administration (27 G up to 30 G) to meet patient convenience, the pressure decrease ΔP during the injection is proportional to $\Delta P \propto \eta\, r^{-4}$ (with r being the needle radius). Thus, injecting high-concentration protein solutions with a viscosity of ca 20–30 mPa·s (20 °C) through a 30 G needle already requires an injection force above 80 N.