International Journal of Pharmaceutics 548 (2018) 682–688

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Novel salts of dipicolinic acid as viscosity modifiers for high concentration antibody solutions

Peng Ke[a], Iris L. Batalha[a,b], Andrew Dobson[c], Esther Tejeda-Montes[a], Sofia Ekizoglou[a], Graham Christie[b], James McCabe[c], Christopher F. van der Walle[a,*]

[a] Dosage Form Design & Development, MedImmune, Ltd., Aaron Klug Building, Granta Park, Cambridge CB21 6GH, UK
[b] Department of Chemical Engineering and Biotechnology, University of Cambridge, Philippa Fawcett Drive, Cambridge CB3 0AS, UK
[c] Pharmaceutical Development, AstraZeneca, Macclesfield SK10 2SN, UK

ARTICLE INFO

Keywords:
Antibody formulation
Anionic excipients
Crystallinity screening
Salt selection

ABSTRACT

Concentrated monoclonal antibody (mAb) solutions can lead to high viscosity as a result of protein-protein interactions and pose challenges for manufacture. Dipicolinic acid (DPA, pyridine-2,6-dicarboxylic acid) is a potential excipient for reduction of protein solution viscosity and here we describe new DPA salts with improved aqueous solubility. Crystallinity and solubility screens identified ethanolamine and diethanolamine as two promising counterions which generated crystalline, high melting point, anhydrous salt forms of DPA at 2:1 M stoichiometry. These salts significantly reduced the solution viscosity of five mAbs, equal to or better than that for the addition of arginine hydrochloride at equivalent osmolality. The presence of the DPA salts in solution did not significantly perturb the melting point of the mAbs, as determined by calorimetry, indicating an absence of any destabilization of protein conformation. Addition of the DPA salts to the mAb solutions stored at 5 °C over 6 months did not cause additional loss of the monomer fraction, though evidence of increased aggregation and fragmentation for three of the five mAbs was observed during 40 °C (accelerated and stressed) storage. Overall, this study demonstrates that ethanolamine-DPA and diethanolamine-DPA can serve as two novel excipients for viscosity reduction and could be considered by formulation scientists when developing highly concentrated mAb formulations.

1. Introduction

Highly concentrated monoclonal antibody (mAb) solutions often encounter high viscosity issues which could impact the manufacturing process as well as their injectability during subcutaneous (SC) administration (such as injection time, volume and force) (Gibson, 2009). Additionally, at high concentrations, mAbs are more prone to aggregation, reversible self-association and particulate formation (Yang et al., 2017). In order to reduce viscosity as well as mitigate instability risks associated with highly concentrated mAb solutions, various viscosity reducing agents are used in conjunction with modifications to the pH and buffer systems of the final formulation (Yadav, 2012). For example, derivatives of some charged amino acids such as Arg.HCl and Lys.HCl have established their merit as viscosity modulators and are extensively used in the pharmaceutical industry (Bowen et al., 2013). The use of amino acid combinations, such as the equimolar combination of L-Arg and L-Glu, has been reported to have a synergistic effect by reducing intermolecular attractions and aggregation propensity to a greater extent than Arg.HCl alone (Kheddo et al., 2014, 2016). Clinical introduction of novel excipients is of course controlled by strict regulations on safety, though formulation scientists continue to search for novel excipients which may yield protein products with superior performance, such as increased shelf life and temperature stability. Some examples include the utilisation of low molecular weight multi-ions (Maclean et al., 2002) and natural compounds from thermophilic microorganisms (Apte and Ugwu, 2003; Lentzen and Schwarz, 2006).

In a recent study, we investigated the use of dipicolinic acid (DPA, pyridine-2,6-dicarboxylic acid) as a new class of organic acid excipients for use in biologics formulations (Batalha et al., 2017). DPA is a small molecule, ubiquitously present in the core of bacterial spores, which for a long time has been thought to play a role in the regulation of spore metabolic activity and heat resistance (Church et al., 1959). More recent work showed that in addition to protecting bacterial DNA from damage, DPA displaces water from the spore core, thus shielding core proteins from denaturation by wet heat (Setlow et al., 2006). In light of this, we investigated the ability of calcium salts of DPA to confer

* Corresponding author.
E-mail address: wallec@medimmune.com (C.F. van der Walle).

https://doi.org/10.1016/j.ijpharm.2018.07.034
Received 8 March 2018; Received in revised form 11 July 2018; Accepted 11 July 2018

stability to high concentration liquid mAb formulations. For example, 10 mM Ca.DPA prevented the phase separation of a highly concentrated solution of 'mAb A' (150 mg/mL in 25 mM His, 120 mM NaCl, pH 6) and also reduced its viscosity from 54 to 26 cP (Batalha et al., 2017). However, the use of DPA as a pharmaceutical excipient is limited by its poor water solubility. DPA free acid (and its chemical analogues) presents an aqueous solubility of around 30 mM, while its calcium salt (Ca-DPA) – the prevalent chelate form in bacterial spores – was reported to have water solubility of well below 100 mM (Huang et al., 2007). The solubility reported for Ca-DPA is variable and in a separate study, the solubility was reported as 11 mM (Lewis, 1972). This is expected to hamper the potential application of DPA as a novel excipient in protein formulations, since a relatively high excipient concentration is often required to maximise its effect. The low aqueous solubility of DPA is likely to be related to the existence of strong intermolecular forces, which prevent the solvent to effectively disrupt solute-solute interactions (Williams, 2013).

A strategy that can be used to improve water solubility of DPA is through the use of salts (Williams, 2013). Complexation of DPA with suitable counterions has the potential to generate novel salt forms, which easily dissociate in water to form ionised species and lead to increased solubility compared with the un-ionised free acid. However, the choice of a suitable counterion for DPA is non-obvious, hence a counterion screening study was conducted to identify the most suitable counterions for solubility enhancement of DPA free acid. Salt screening was conducted with a focus on selecting the most suitable counterions to provide good 'handleability' (e.g. stable crystalline salts) of DPA which could allow its ease of practical application. Once the counterions that could simultaneously improve solubility and enable good handleability were identified and characterised, the resultant DPA salts were added to high concentration solutions of five therapeutic mAbs to determine their impact on solution viscosity and mAb stability.

2. Materials and methods

2.1. Materials

The following reagents used in this study were > 99% pure (apart from DPA, which was > 98% pure). DPA, dibenzylamine, dicyclohexylamine, diethanolamine, ethanolamine, benethamine, potassium chloride (KCl), magnesium chloride ($MgCl_2$), sodium hydroxide (NaOH), zinc chloride ($ZnCl_2$), meglumine, piperazine, ethyl acetate (EtOAc), acetonitrile (MeCN), and methanol (MeOH), were all purchased from Sigma-Aldrich (Sigma-Aldrich Company Ltd., Gillingham, Dorset, UK). L-Arg, Arg.HCl, L-His, His.HCl, tris(hydroxymethyl)aminomethane (Tris), L-Lys, and sodium chloride (NaCl) were acquired from J. T. Baker (Avantor Inc. Arnhem, Netherlands). All mAbs were provided by MedImmune Ltd (Cambridge, UK) (Table 1).

Table 1
General properties of the mAb molecules used in this study.

Molecule	mAb class	MW (kDa)	measured, isolectric point (pI)
mAb 1	IgG1 YTE[1] (λ)	147.7	7.3–8.0
mAb 2	IgG1 TM[2] (λ)	148.0	7.5–7.8
mAb 3	IgG1 (κ)	144.5	7.0–7.3
mAb 4	IgG1 TM (λ)	148.9	8.2–8.8
mAb 5	IgG1 (κ)	148.2	8.5–8.8

[1] YTE triple mutation in the Fc C_H2 (fragment crystallisation, second constant, heavy domain) increasing binding to the neonatal Fc receptor (Dall'Acqua et al., 2006).

[2] TM triple mutation in the Fc C_H2 increasing binding to human Fc gamma RIIIA (Oganesyan et al., 2008).

Table 2
Solubility of DPA at room temperature with different counterions.

Sample number	Counterion	DPA solubility (mM)
1	Dibenzylamine	N.D.
2	Dicyclohexylamine	N.D.
3	Diethanolamine	~500
4	Ethanolamine	~500
5	Benethamine	N.D.
6	$MgCl_2$	< 50
7	KCl	< 50
8	NaOH	< 50
9	Meglumine	~500
10	Piperazine	~250
11	Tris	~500
12	L-Lys	> 500
13	$ZnCl_2$	< 50
14	L-Arg	> 500

2.2. Methods

2.2.1. Counterion screening for crystalline salt forms of DPA

In the initial screen, 0.1 M solutions of DPA and the counterions listed in Table 2 were prepared. From these solutions, 50 µL of DPA and 50 µL of counterion (equimolar stoichiometry) were added to each well in a 96-well plate, in duplicate. The wells were sealed with foil, pinholed to allow slow evaporation of solvent, and the plates placed at 25 °C and 50 °C for 3–4 days until evaporation of the solvent was complete. Each well was then analysed using polarised light microscopy to detect the potential presence of crystalline, birefringent material. Since the presence of crystalline material at this stage is only indicative of the formation of a true salt, two further rounds of solvent addition and evaporation were applied to more rigorously test the nature of the crystalline material. Thus, 200 µL of EtOAc and MeCN were next added to the wells, allowed to evaporate and analysed by polarised light microscopy. This was followed by the addition to each well of a further molar equivalent of counterion in MeOH, generating a 2:1 M stoichiometry of counterion:DPA, and a final round of evaporation was induced prior to the final microscopic analysis.

2.2.2. Counterion screening for DPA salt solubility

The counterions listed in Table 2 were further screened to identify the most suitable candidates to improve the room temperature solubility of DPA; 25 mM L-His/His.HCl, pH 6.0 (hereafter termed 'histidine buffer') was used to control the pH during the solubility study. A 100 mM counterion solution was dissolved in histidine buffer, followed by equimolar addition of DPA (100 mM). The suspensions were sonicated for 30 min at room temperature and inspected visually. If a clear solution was observed, the molar concentration of the counterion and DPA was increased to 250 mM by the addition of the solid. If a clear solution remained after sonication, this step was repeated, increasing the molar concentration of counterion and DPA to 500 mM. Conversely, if a clear solution was not achieved at the initial 100 mM concentration, histidine buffer was added until a clear solution was observed and the final concentration noted.

2.2.3. Preparation of concentrated mAb solutions with DPA salts for viscosity assessment

The five mAbs listed in Table 1 were used to test the ability of the selected DPA salts to reduce solution viscosity. The mAb solutions were exchanged into histidine buffer alone (control) and histidine buffer containing: i) 200 mM Arg.HCl, ii) 200 mM ethanolamine-100 mM DPA, iii) 200 mM diethanolamine-100 mM DPA, by dialysis (Slide-A-Lyzer G2 cassette, 10,000 MWCO, Thermo Fisher, Loughborough, UK). In each case the cassette was dialysed against a 300-fold excess volume of buffer while stirring for 8–16 h (manufacturer's instructions), repeating this process twice to allow complete buffer exchange. The