Accepted Manuscript

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PII: S0939-6411(17)31478-9
DOI: https://doi.org/10.1016/j.ejpb.2018.07.011
Reference: EJPB 12834

To appear in: *European Journal of Pharmaceutics and Biopharmaceutics*

Received Date: 19 December 2017
Revised Date: 9 July 2018
Accepted Date: 10 July 2018

Please cite this article as: V. Gervasi, R. Dall Agnol, S. Cullen, T. McCoy, S. Vucen, A. Crean, Parenteral protein formulations: An overview of approved products within the European Union, *European Journal of Pharmaceutics and Biopharmaceutics* (2018), doi: https://doi.org/10.1016/j.ejpb.2018.07.011

Parenteral protein formulations: An overview of approved products within the European Union

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Declaration of Interest: Timothy McCoy is an employee of Sanofi Global R&D, Framingham, USA and Sean Cullen an employee of Sanofi, Waterford, Ireland.

Abstract

The study presented is a comprehensive overview of commercial parenteral protein formulations, approved by the European Medicines Agency (EMA), 1995-2018. The objective of this overview was to analyse current trends in the design of commercial parenteral protein products and thereby support formulation scientists in the design of new formulations.

The main data source was the publicly available European Public Assessment Reports (EPARs) published by the EMA for each authorised product. An analysis of the percentage of formulations in a liquid and lyophilised form was conducted. In addition, the number of products containing individual excipients, classified into functional categories is provided. Finally, the overview includes comprehensive details of product compositions obtained from EMA, US Food and Drug Administration (FDA) and product Marketing Authorisation Holder.

Data analysis highlighted trends in the number of products approved, and the higher percentage of liquid parenteral protein formulations (66%) compared to lyophilised formulations (34%). This overview identifies the most commonly incorporated excipients employed as buffering agents, stabilisers/bulking agents, surfactants, preservatives and tonicifiers, including their concentration ranges of use in both liquid and lyophilised formulation approaches. Finally, antibody-based formulations were a particular focus of this overview. The relationship between parenteral routes of administration and antibody concentrations in approved products was also investigated.

Keywords

Excipients; proteins; antibodies; formulation; lyophilisation; high concentration; European Medicines Agency

1. Introduction

The focus of parenteral protein formulation design is to identify an excipient composition that will stabilise proteins against stresses experienced during processing, storage, and administration. Excipients also aid reconstitution of lyophilised formulations, maintain sterility of multi-dose products, provide isotonicity, and in a small number of cases alter pharmacokinetics (1, 2). The majority of parenteral protein formulations consist of proteins and excipients in an aqueous-based solution or suspension. Processing conditions and external factors such as shifts in pH, changes in temperature, surface interactions and extraneous impurities can destabilise proteins, provoking their chemical and physical structural degradation (3, 4). In some cases, aqueous formulations of therapeutic proteins do not provide adequate stability and therefore, a dried state formulation is a favoured, alternative approach which can aid the stability and prolong the shelf-life of protein products (5). Lyophilisation, the process of subliming water from frozen solutions under low pressure (vacuum), is a widely employed technique for the manufacture of dried biological materials (6). However, lyophilisation has the potential to cause protein damage due to stresses during both the freezing and drying phases (3, 7). Hence, an appropriate excipient composition is required to protect proteins from stresses experienced during the lyophilisation process.

The function and behaviour of excipients in protein formulations is widely reported (8-14). Despite the wide range of formulation information available, it can be challenging for a formulation scientist to get an overview of how frequently excipients are included in commercial protein products. For example, prior publications focus on the function of excipients and provide a range of products as examples of their application. What differentiates this overview from previous work is that it builds on earlier literature and provides up to date, comprehensive information of the frequency of excipients use in approved protein products. It also presents an analysis of the quantitative excipient composition in the majority of the commercial protein products. The compilation of such information creates a valuable source for formulation scientists regarding the regulatory acceptance of excipients and their prior history in commercial formulations.

Approval of therapeutic protein products for use within the European Union is via a centralised procedure (15). The European Medicines Agency (EMA) publishes a European Public Assessment Report (EPAR) for every medicine assessed (approved

or refused), providing the public with information regarding the product. The EPAR is not a single document but a resource containing a set of regulatory documents related to authorisation details, product information and assessment history. The EPAR is one of the most informative and up to date public sources of information on a large number of commercial therapeutic proteins. The overview presented is a summary of the wealth of the formulation data available in the EPARs in relation to approved parenteral protein formulations. Specifically, data was gathered and analysed to provide a breakdown of products according to protein type, formulation approach (aqueous-based liquid or lyophilised formulations), the most frequently included excipients classified in functional categories, with a more detailed look at antibody formulations.

In reviewing EPARs' data, the split of protein formulations between the liquid and dried (lyophilised) state was investigated and the types of excipients incorporated in both formulation approaches (liquid or lyophilised) are discussed. Qualitative and quantitative composition of protein formulations can be influenced by the process selected for manufacturing products in a certain dosage form (liquid or lyophilised). The overview provides details of the excipient concentrations employed in approved protein products in the European Union (EMA). Due to the limited data related to the excipient quantitative composition in the EPARs, this information has been supplemented by using FDA sources (16) and product information documents published by the Marketing Authorisation Holder (17). The excipient quantitative composition is provided for 215 out of 264 protein products and 88 out of 94 antibody products approved in the European Union.

The final part of the overview focuses specifically on monoclonal antibody (Mab) products, since Mabs are currently the largest class of therapeutic proteins (18-21). Mab doses required for the treatment of chronic diseases are relatively high (usually 50-200 mg) compared to the majority of the other therapeutic proteins (22). Intravenous infusion administration is mostly used for the delivery of a number of these products. However, an alternate treatment approach is to formulate Mabs at high concentrations to enable administration of the required dose in smaller volumes (1-2 ml) subcutaneously (SC). Employing this approach can avoid intravenous infusions and allow patients to be treated at home rather than in a hospital setting. However, the formulation of proteins in high concentrations represents a challenge (20, 23-27). Proteins at high concentrations have an intrinsic tendency to form aggregates (23) that

have the potential to stimulate an immune-response following administration (24-26). Furthermore, high concentration protein formulations can be challenging to lyophilise due to the high total solute concentration, which can prolong the reconstitution time of lyophilised products and increase the viscosity of liquid and reconstituted formulations (23, 24, 27). Therefore, the selection of appropriate excipient compositions is required to address these challenges when designing high concentration protein formulations (21, 23).

2. Methodology

A review of the EPARs available on the EMA website was conducted for all parenteral protein products authorised centrally in the European Union between 1995-2018 (up to June 2018) (22). For each of these products, the following information was collected: commercial name, active pharmaceutical ingredient (API) and related quantitative composition, class of therapeutic protein, therapeutic area, dosage form, route of administration, excipient composition, date of issue of marketing authorisation and marketing authorisation holder (pharmaceutical company). Information was compiled in an MS Excel database (included in supplementary information). The accuracy of data transferred to the database was assured by two researchers checking 100% of the data entries against the EPARs.

Formulations were divided into two groups: liquid (L) and lyophilised (LYO), based on their manufacturing process detailed in the EPARs. Commercial products having the same name but different excipient composition, different formulation approaches (e.g. liquid versus lyophilised) or a different liquid formulations' format (e.g. concentrate, solution or suspension) were considered as distinct products in this overview.

All excipients were categorised considering their potential roles in protein formulations and were assigned to one of seven functional categories. In the case of multi-functional excipients, they were listed in one category but referred to in the discussion under all relevant categories. Excipients can possess diverse roles when added at different concentrations or to different formulation approaches. This multi-functional nature of excipients influenced their classification in this publication. For example, amino-acids were designated as a single category with a range of functions (buffers, stabilisers, bulking agents). Non-amino acids stabilisers/bulking agents

category comprise excipients (mainly carbohydrates) that in the liquid dosage form serve as stabilisers. However, some of these excipients in the lyophilised dosage form, have the potential to act also as bulking agents. Furthermore, as formulation data does not specify the excipients' solid form (amorphous or crystalline) it was not possible to separate the stabilising and bulking functions of some excipients (amino acids and carbohydrates) in lyophilised formulations. Therefore, the following seven categories were designated 1) non-amino acids buffers 2) amino acids buffers/stabilisers/bulking agents; 3) non-amino acids stabilisers/bulking agents; 4) surfactants; 5) preservatives; 6) tonicifiers and 7) other excipients.

The 'other excipients' category consisted of excipients present in a relatively low number of products, this included complexing agents, antioxidants, solubilising agents and excipients exclusively present in specific types of formulations, for example zinc salts in insulin products. The hypothesis and assumptions regarding the role of these excipients in a specific product were stated only in presence of available information (16, 22, 28-30) or individual specific references which are detailed in section 3.2.8.

The analysis of the percentage and frequency at which individual excipients were included in the products was conducted using IBM SPSS Statistics v.23 software. Percentage of products containing a specific excipient or excipient category is calculated as percentage within the type of dosage form (liquid or lyophilised).

Quantitative composition of protein products is also reported in this overview as a guideline for the range of protein and excipients concentrations employed in marketed formulations. This information was gathered from accredited sources: FDA label (FDA) (16) and product information document (Marketing Authorisation Holder) (17), when data was not provided in the EPAR's scientific discussion (Assessment history, EMA) (28). These primary information sources are dynamic databases; hence this overview is a snapshot of the marketed products information available in the specific timeframe. Furthermore, to guarantee accuracy of the dataset, only information that could be verified against the primary sources was included in this overview. Again, the accuracy of data transferred was assured by two researchers checking 100% of the products' information against these sources. It is important to note that information listed in this overview relates only to products approved by the EMA. Other formulations with the same product name may be approved in other

jurisdictions, for example, products with different strengths or excipient compositions.

Analysis of monoclonal antibody products was also conducted to show the relationship between concentration of protein administered and route of delivery.

3. Results and discussion

3.1 Approved protein formulations

EPAR data showed that approximately 440 parenteral products were approved by the EMA via its centralised procedure in the period 1995-2018 (June). More than half of these products (n=264) contained therapeutic proteins and a greater number of them were formulated as liquid dosage forms (66%; n=174), compared to lyophilised forms (34%; n=90). **Figure 1** shows that the number of liquid parenteral protein formulations is consistently higher than the number of lyophilised formulations approved in the timeframe studied. Since 2013, approximately 20 parenteral protein products have been approved per year.

Therapeutic proteins included in liquid and lyophilised products, were divided into their functional classes, **Figure 2a and 2b.** Antibodies (36%; n=94) and hormones (27%; n=71), represent the two main classes, and are more commonly formulated as liquid formulations (antibodies: n=69 and hormones: n=61) compared to lyophilised formulations (antibodies: n=25 and hormones: n=10). Insulin and its analogues are the most frequent therapeutics in the class of hormone products (n=26 out of 61), and they are formulated exclusively as liquid dosage forms (solutions for infusion or injection, and suspensions for injection). In contrast, coagulation factors are manufactured only as lyophilised formulations (n=24) and represent the second largest class of therapeutic proteins in this dosage form following antibodies.

3.2 Excipients included in approved protein formulations

3.2.1 Excipient categories

Excipients were subdivided into seven categories: non-amino acids buffers; amino-acids buffers/stabilisers/bulking agents; non-amino acids stabilisers/bulking agents; surfactants; preservatives; tonicifiers and other excipients as detailed in section 2. The percentages of liquid and lyophilised products containing excipients which fall into each of these categories are shown in **Figure 3**. Most categories of excipients are

frequently employed in both liquid and lyophilised products, whereas others, such as preservatives, are more specific to one formulation approach (e.g. multi-dose liquid formulations).

The majority of the formulations: liquid (70%; n=122) and lyophilised (61%; n=55) contain non-amino acids buffers. However, amino acids are present in a large number of products (L: 39%; n=68; LYO: 51%; n=46) and can have different functions as buffering agents or stabilisers/bulking agents depending on the concentration incorporated. A small number of products are formulated in the absence of buffering agents. This may be due to the ability of the protein to maintain the critical pH, especially if formulated at high concentrations (e.g. FLEBOGAMMA DIF® (*human normal immunoglobulin*)) (31).

Non-amino acids stabilisers are present in a large number of liquid products (64%, n=111). Excipients included in the non-amino acids stabilisers/bulking agents category are also incorporated in most of the lyophilised products (93%, n=84). Surfactants are present in a similar percentage of liquid (65%; n=113) and lyophilised (62%; n=56) formulations. Preservatives are included in liquid (29%, n=50) and lyophilised (6%, n=5) products. As expected, all multi-dose products (n=53) contain preservatives. Multi-dose products comprise 20% of all products (liquid and lyophilised) and are more commonly formulated in the liquid dosage form (n=48). All lyophilised formulations intended for multiple use contain preservatives in the diluent for reconstitution and none in the lyophilised product. The reason for including preservatives directly in the diluent for reconstitution is to minimise any possible preservative-protein interactions (4, 32). Antibodies are exclusively formulated in a single dosage form, whereas insulin in a multiple dosage form. Tonicifiers are incorporated in a high percentage of both liquid (36%; n=62) and lyophilised (42%; n=38) formulations to achieve the iso-osmolarity recommendations for parenteral administration (33-35). Excipients classified in the 'other excipients' category were present in a similar percentage of liquid (27%; n=47) and lyophilised (22%; n=20) products.

Table I. summarises the more common excipients included in liquid and lyophilised products for each functional category. This table also presents the concentration ranges employed for each of these excipients in the liquid and lyophilised protein products analysed. Non-amino acids buffers are usually included in a range of concentrations between 0.2 and 14.8 mg/ml (usual ionic strength: 5-25 mM).

However, ATRYN® (*antithrombin alfa*) contains sodium citrate at a higher concentration of 135 mM (34.8 mg/ml). Amino acids can have different functions; hence they can be used in a wide range of concentrations (0.1 and 52 mg/ml) within the formulations. The usual range of concentrations at which amino acids are employed is between 0.1-25 mg/ml, with an ionic strength of 5-260 mM. However, arginine is added in higher concentrations compared to other amino acids, acting mainly as a stabiliser. For example, METALYSE® (*tenecteplase*) contains arginine at a concentration of 52 mg/ml to increase the solubility of the protein as reported in the product EPAR's scientific discussion (36). Histidine at low concentrations between 0.7-8.9 mg/ml is included in the majority of the products, serving mainly as a buffer. Histidine and arginine are frequently used in their hydrochloride salt form in protein products. Glycine is often employed as a stabiliser/bulking agent besides its role as a buffering agent at concentrations between 0.1-25 mg/ml; whereas methionine at low concentrations (0.06-3 mg/ml) is usually selected for its antioxidant properties. Non-amino acids stabilisers and bulking agents are incorporated at concentrations up to 200 mg/ml to maintain the required molar ratio with the protein. Surfactants are generally present in a different range of concentrations in liquid (0.01-2 mg/ml) and lyophilised (0.04-1.2 mg/ml) products. However, the liquid formulation ORENCIA® (*abatacept*), with a high concentration of the fusion protein (125 mg/ml), contains 8 mg/ml of Poloxamer 188. Preservatives are employed in concentrations between 0.7 and 14.9 mg/ml. Sodium chloride and potassium chloride are included as tonicifiers and/or stabilisers in a variable range of concentrations between 0.2 to 27 mg/ml.

The following sections provide greater details and discussions regarding individual excipients divided into each functional category.

3.2.2 Non-amino acids buffers

Buffers are required to adjust and maintain the pH to a value at which the specific protein has maximal stability. The optimum pH value is selected considering the protein's isoelectric point (pI) as a critical physical parameter that can affect protein solubility, aggregation and degradation (37). Furthermore, the selected pH needs to be in a physiological range in order to avoid irritation, pain or extravasation during injection into the patients. For intramuscular and subcutaneous administration the acceptable pH range is between pH 4 and 9 (38). Of the protein products where pH is

reported, the values range from pH 3.1 for JETREA® (*ocriplasmin*) to pH 8.15 for XULTOPHY® (*insulin degludec*), which are respectively administered through intravitreal and subcutaneous routes.

The percentage of approved liquid and lyophilised parenteral products containing non-amino acids buffers are shown in **Figure 4**. The most common buffers (excluding amino acids) employed in liquid protein formulations are: sodium phosphate (33%; n=58), sodium acetate (19%; n=33) and sodium citrate (17%; n=29). Sodium phosphate (32%, n=29), and sodium citrate (20%; n=18) are also frequently added to lyophilised protein products (12). However, acetate buffer was found not to be incorporated in any lyophilised products identified as part of this overview. Acetic acid is volatile and can be converted to a gaseous state and hence be lost from the formulation during lyophilisation (39, 40). Sodium phosphate buffers employed in commercial products are usually composed of two salt forms: sodium dihydrogen monohydrate and disodium dihydrate. The different sodium phosphate components were grouped together under the name of sodium phosphate in this overview.

Despite their wide use as buffers, sodium phosphate salts present some drawbacks, especially when included in formulations intended to be lyophilised. Highly concentrated buffer systems can crystallize and provoke changes in pH during freezing (39). Sodium phosphate is prone to crystallize during freezing, leading to a pH shift of up to four units. Furthermore, sodium phosphate crystallization and precipitation are severely influenced by salt components solubility and concentration, temperature, cooling rate, volume of solution and initial pH value (41, 42). The presence of other compounds can influence buffer crystallization, precipitation and consequently cause pH shifts which can accelerate drug degradation in frozen solutions. For example, crystallizable solutes, such as glycine, mannitol or sodium chloride can modify ion activity, facilitating crystallization of buffers; while non-crystallizable excipients such as sucrose or trehalose can inhibit buffer crystallization (39). The citrate salts remain in an amorphous state upon freezing and drying, minimizing pH shifts, compared to succinates and tartrates (31, 43).

A concept that should also be taken into account during pre-formulation studies is that the pH of the solution before drying has an influence on the rate of chemical reactivity in the resulting dried material (43). In lyophilised formulations, buffers tend to depress critical temperatures for the lyophilisation process; glass transition of the maximally freeze-concentrated solution (Tg') and collapse temperature (Tc).

However, buffering agents are usually used in low concentrations (5-25 mM) (44). TRIS buffer which is included in a small number of liquid (3%; n=6) and lyophilised (8%; n=7) formulations has been shown to release formaldehyde in peptide formulations stored at 70°C. However, this does not occur at the lower temperatures normally employed for formulation processing or storage (43).

Individual acids and bases can also be incorporated as pH modifiers to adjust the pH to a target value. Sodium hydroxide (L: 46%; n=80; LYO: 20%; n=18), hydrochloric acid (L: 36%; n=62; LYO: 11%; n=10) and phosphoric acid (L: 3%; n=5; LYO: 6%; n=5) are frequently added to liquid and lyophilised formulations, to modify their pH to a desired value and/or to create a salt form in combination with other buffer components.

Key considerations: The choice of the optimal buffer system for a specific formulation needs to be conducted by performing compatibility studies between the excipient and the specific protein. However, some general rules need to be considered, especially for lyophilised protein products. Salts that can provoke significant pH shifts during freezing should be used with caution (e.g. sodium phosphate, sodium acetate). Despite its disadvantages, sodium phosphate is present in a high number of liquid and lyophilised products. Most of the commercial lyophilised products contain amino acids (histidine) or salts with low pH shift tendency (sodium citrate) as buffer systems. The absence of buffers in protein formulations can be considered when this does not negatively impact the quality and stability of the product.

3.2.3 Amino acids buffers/stabilisers/bulking agents

Amino acids play several roles in parenteral protein formulations as buffers, stabilisers or bulking agents. They can influence tonicity of the formulation and some have antioxidant properties (45-48). The solid state (amorphous or crystalline) and concentration of an amino acid can determine its role in a specific product. Amino acids use as excipients in biopharmaceutical products has increased in recent years, due to their multi-functionality (108 products (2007-2018, June) vs 54 products (1995-2007) contain amino acids). Amino acids tend to stabilise proteins by hydration or direct interactions (46). However, the mechanism of interaction between the different amino acids with proteins is complicated and not always completely

understood. Furthermore, amino acids have a complex chemistry (acidic or basic, multiple functional groups) and can be included in formulation as different salt forms; all these factors can further impact the type of protein-amino acid interactions. Most of the amino acids are included in formulations in their salt form, in order to increase their solubility. The effect of the counter-ion, can also impact the stability of the protein (49).

As shown in **Figure 5**. the most commonly employed amino acids in liquid and lyophilised commercial products are histidine (L: 16%; n=27; LYO: 34%; n=31), methionine (L: 12%; n=21; LYO: 6%; n=5) and glycine (L: 9%; n=15; LYO: 13%; n=12), followed by arginine (L: 8%; n=14; LYO: 4%; n=4). Histidine as a buffer is reported to provide maximal stability (50), provoking minimal pH shifts during freezing (51). Histidine, is a multi-functional excipient, capable in some cases of reducing protein aggregation and functioning as cryo/lyo-protectant during the lyophilisation process in addition to acting as a buffer (52, 53). Al-Hussein et al. revealed how the role of histidine as a stabiliser was particularly important when formulated in combination with sugars and when maintained in the amorphous state (47). The concentration at which the excipient is used in different formulations determine its main role, i.e. as a stabiliser/bulking agent when present in high concentrations or as a buffer in low concentrations. Furthermore, histidine has antioxidant properties, it can act as scavenger of hydroxy radicals in solution (45, 46). Methionine is present in a high number of liquid and lyophilised products; this excipient can be selected for its antioxidant properties (46, 54). Methionine is added to commercial products often in combination with other amino acids (e.g. histidine or arginine). Glycine is present in lyophilised products where it can act as a bulking agent, in addition to its potential buffering properties in liquid and lyophilised formulations (46).

The capability of positively charged amino acids to particularly enhance the stability of protein formulations and suppress aggregation is reported (55). Arginine is present in a relatively low number of products. However, its trend of use has increased between 2014-2018. Amongst the 18 products containing arginine, 10 were approved since 2014. Arginine and its salt forms have been shown to be capable of reducing protein aggregation, increasing protein solubility and reducing viscosity of protein solutions in some cases (46, 56, 57). This effect is particularly important when formulating proteins at high concentrations. However, the mechanism of interaction

between arginine and proteins is not completely understood. Trout et al. (58-60) proposed a hypothesis according to which arginine molecules self-associate in clusters. These amino acidic clusters create weak hydrophobic interactions (hydrogen, electrostatic, cation- or Van der Waals) with guanidinium and aromatic groups of the protein, crowding out protein-protein interactions and avoiding aggregates formation. Furthermore, arginine and glutamic acid mixtures are shown to have a synergistic effect in increasing the solubility of proteins; this is due to the formation of additional hydrogen bonds with the protein in the presence of both excipients (61).

Other amino acids such as alanine, isoleucine, leucine, lysine, phenylalanine, proline, threonine and valine are added to a small number of commercial products; they can contribute to the stability of the protein in formulation through specific interactions (46, 54). Arginine, histidine and lysine are reported to be amorphous in the solid state, while all the other amino acids are observed to be in a crystalline form after lyophilisation (48, 62). Concentrations of amorphous amino acids in formulations intended to be lyophilised should be carefully selected, since they tend to suppress critical temperatures (Tg' and Tc) for lyophilisation process, increasing time and processing costs (49).

Key considerations: Amino acids are a varied class of excipients with multi-functional roles and mechanisms, which are not yet completely understood especially when formulated in the dried state. The role of these excipients in protein formulation can be altered by adjusting the concentrations employed. From the analysis of this database, the trend of use of amino acids in protein formulations has increased in recent years probably due to their multi-functionality. In particular, the effect of combining different amino acids can provide a synergistic effect. Furthermore, some amino acids (basic amino acids) have properties that could be noteworthy when formulating proteins at high concentrations (e.g. to increase solubility, to reduce aggregation and viscosity) (46, 55-57, 63).

3.2.4 Non-amino acids stabiliser/bulking agents

The percentage of approved liquid and lyophilised parenteral products containing individual stabilisers and bulking agents are shown in **Figure 6**. The excipients included in this category are predominantly carbohydrates that can function as

stabilisers in liquid and lyophilised protein products. Additionally, some of these excipients can act as bulking agents, maintaining the structure of lyophilised cakes (e.g. mannitol) (43, 64). In this case, the solid state of the excipient within the formulation determines its role. For example, mannitol which tends to crystallise is used as a bulking agent, while sucrose, which maintains its amorphous state, acts mainly as a stabiliser in lyophilised products. Due to the lack of this solid-state information in the EPAR data, it was difficult to determine the specific role of these excipients in commercial products. For this reason, stabilisers have been combined with bulking agents in a single category. The list of excipients that have been referenced as bulking agents (excluding amino acids) is broad, consisting of human albumin, maltose, mannitol, sorbitol, sucrose and trehalose (38, 43, 45, 65-67). However, sucrose and trehalose are predominately incorporated in lyophilised products as stabilisers in an amorphous state, rather than bulking agents in their crystalline state.

Three different hypotheses have been proposed to explain the mechanism by which excipients are able to physically stabilise proteins (vitrification, exclusion and water replacement theories) (68-70). For many formulations the stabilisation may be due to the cumulative effect of these three different mechanisms. Excipients contribute to protein stabilisation by a range of mechanisms including direct interactions, minimising protein-protein interaction and aggregation, and stabilising the folded state of the protein (71-73).

Bulking agents are employed in lyophilised formulations of low dose (high potency) drugs that do not have the necessary bulk to support their own structure (total solid content < 2% (w/v)) (43, 64). While protein integrity and stability are not necessarily related to the cake structural defects, requirements for an intact cake appearance tend to be observed for commercial lyophilised products (74, 75). A review by Patel et al. (75) establishes some guidelines of what is acceptable from a product quality and regulatory perspective in terms of visual cake appearance. The presence of a crystalline compound in formulation can also reduce the reconstitution time of lyophilised products containing high concentrations of protein (76).

The most common stabilisers included in liquid formulations are glycerol (17%; n=29), sucrose (16%; n=28), and mannitol (14%; n=24). Due to the increased requirement for physical stabilisation of the protein during the lyophilisation process, non-amino acids stabilisers/bulking agents are added to the 93% (n=84) of the

lyophilised formulations. Non-amino acids stabilisers/bulking agents most frequently present in lyophilised products are sucrose (59%; n=53), mannitol (33%, n=30) and in a lower number of products trehalose (10%; n=9) and human albumin (7%; n=6). Glycerol is exclusively included in liquid products where it can have multiple roles; this excipient is a co-solvent/solubilising agent, which can serve as a tonicifier as well. Absence of glycerol in lyophilised commercial products may be attributed to its plasticising effect on the product's glass transition and to the stability issues observed in some formulations, where the increase in protein mobility provokes deamidation (68). Sucrose can act as a cryo/lyo-protectant in lyophilised formulations; it is maintained in an amorphous state after lyophilisation with moisture contents lower than 2.5% (68). On the contrary, mannitol is widely present in lyophilised products as a bulking agent due to its capability to crystallize and support the lyophilised cake.

Sugars and polyols are frequently used as stabilisers (cryo/lyo protectants) and bulking agents in lyophilised products. However, carbohydrates with low Tg' values, such as sorbitol (Tg'= -45 °C) (77) can increase lyophilisation process time. This explains the absence of sorbitol as an excipient in lyophilised commercial formulations. Furthermore, sorbitol can crystallize over time and this needs to be considered in formulating a product intended to be lyophilised (77).

Despite several reported advantages, trehalose use is not widespread amongst commercial products compared to other sugars (L: 6%; n=10 ; LYO: 10%; n=9). However, eight new products containing trehalose were approved between 2017-2018. Trehalose has good aqueous solubility, low hygroscopicity, high hydration number (due to its hydrophilicity), good hydrolytic stability in extreme pH and upon beta-glycosidase action. This sugar has a higher Tg compared to other carbohydrates; Tg value in presence of 0.3% residual water content was reported to be approximately 111°C for trehalose, while it is approximately 65°C for sucrose (43, 69, 70). However, the following reasons may limit trehalose use as an excipient. Firstly, business reasons can determine a company's choice of excipient; trehalose is more expensive than other stabilising sugars, such as the more commonly employed sucrose (78). Where comparative stability can be achieved with sucrose, logistic and business rationale would influence excipient choice. Secondly, the use of trehalose as stabiliser in some cases was observed to be less effective in comparison to sucrose. Jovanović et al. (79) reported significant changes in the tertiary structure of lysozyme and myoglobin in formulations containing trehalose. Finally, trehalose can be present

in formulation as an heterogenous system formed by different crystalline phases. This polymorphism renders its behaviour in formulation difficult to predict, with a potential impact on stability (79-81). The transformation paths that trehalose can undergo depend on several factors, including the solid-state (amorphous or crystalline) and the dosage form (liquid or solid) as well as the dehydration process and residual moisture levels in the product (80, 82). The use of trehalose could be preferred over other carbohydrates if there is a significant improvement in terms of product stability or process efficiency.

Reducing sugars (e.g. lactose and maltose) should be avoided due to potential interactions with amino acid side chains, which can cause chemical alteration of the proteins (Maillard reaction or glycation) (68). Indeed, maltose is present in only one lyophilised commercial product (ORENCIA® (*abatacept*)) at a concentration of 50 mg/ml and at a ratio of 2:1 maltose to *abatacept* in the lyophilised form prior to reconstitution (16). Maltose is reported on the EPAR's scientific discussion to be used as a stabiliser/bulking agent at the concentration in formulation (83). This sugar is included in the ORENCIA® *(abatacept)* lyophilised product intended to be administered intravenously but replaced by sucrose in the corresponding subcutaneous liquid product. It is important to note that the Maillard reaction is favoured in alkaline or acidic conditions, and that the pH of this product after reconstitution is maintained neutral (pH 7.2-7.8). Trace level reducing sugars can also be found in non-reducing excipients such as mannitol, maltitol and sucrose. Hence, care should be taken regarding the quality of the excipients selected (68, 84).

Recrystallisation of sugars and polyols during manufacture and storage should be avoided, because conversion between amorphous and crystalline states can compromise the protein stability. Mannitol at high concentrations can provoke vial breakage due to recrystallisation during the lyophilisation process (primary drying phase) and storage (68). For these reasons, the introduction of an annealing step in the lyophilisation cycle is required when crystalline components are present in protein formulations. In some formulations, different sugars/polyols are used in combination, so one excipient behaves as a stabiliser and the other as a bulking agent, for example ENBREL® (*etanercept*) contains sucrose and mannitol. The former is capable of stabilising the protein, whereas the latter prevents the collapse of the cake. Pikal et al. (85) reported similar examples of formulations containing glycine as a stabiliser and mannitol as a bulking agent. The main advantage in the employment of a mixture of

amorphous and crystalline compounds is the possibility to reduce the lyophilisation cycle time, conducting the primary drying above the Tg' value of the amorphous phase (86). The recommended molar ratio of protein to stabiliser is 360:1 (weight ratio 1:1), whereas it is usually higher for bulking agents (44, 51, 64, 87).

Human albumin can have a number of functions in parenteral formulations, as a stabiliser (38, 45), bulking agent and tonicifier (38), but it is not employed in recent products possibly due to its potential risk of introducing contaminants (e.g. viruses) (38). Dextran 40 is included in one lyophilised antibody-drug conjugate product MYLOTARG® (*gentuzumab ozogamicin*), approved in 2018. Dextran at concentrations of 9.1 mg/ml is reported to act as a bulking agent (EPAR's scientific discussion) (88).

Key considerations: The physical state of the excipient and the dosage form (liquid or lyophilised) need to be evaluated in the selection of a stabiliser/bulking agent. Sorbitol and glycerol can be used in liquid formulations, but they are not recommended in lyophilised products. Reducing sugars should be avoided in both liquid and lyophilised products because of the possibility to undergo Maillard reaction, altering the chemistry and the activity of the protein. Mannitol and sucrose are widely used especially in lyophilised commercial products. Mannitol as a bulking agent is in a crystalline form, hence the introduction of an annealing step in the lyophilisation process is required. Trehalose is a promising excipient, but its employment is limited partially due to its relative high costs.

3.2.5 Surfactants

Surfactants in both liquid and lyophilised formulations stabilise the protein, increasing its solubility and minimising interface interactions (89). Surfactants stabilise proteins by the following mechanisms; a) direct interactions of the surfactant molecules with hydrophobic domains exposed on the protein surface and b) interfacial competition, i.e. surfactant occupancy of the surface is more thermodynamically favoured compared to the protein occupancy (89). The use of surfactants in lyophilised products reduces the surface tension at the ice-water interface, promotes protein refolding and prevents aggregation (25, 70). High concentration protein formulations require surfactants in order to improve solubility of the protein and overcome problems related to their high tendency to form aggregates. Surfactants can also

protect highly concentrated proteins from mechanical agitation and manipulation (e.g. during syringeability) and reduce the reconstitution time of lyophilised products (9, 90).

A relatively small number of surfactants are included in the liquid and lyophilised products. The main excipients are polysorbate 80 (L: 32%, n=55; LYO: 41%, n=37), polysorbate 20 (L: 26%, n=45; LYO: 18%, n=16) and poloxamer 188 (L: 7%, n=12; LYO: 3%, n=3). Several of both formulation types (L: 35%; n=61; LYO: 38%; n=34) do not contain any surfactants, most of which are insulin-based products. The reason for the absence of surfactants in these commercial products was investigated. However, no clear trend was observed when evaluating products by year of approval, class of therapeutic protein or dosage form. The majority of surfactants included in protein formulations are non-ionic. Non-ionic surfactants are preferred over ionic surfactants which can denature proteins (89); but they are also selected for their low toxicity and reduced sensitivity to the presence of electrolytes (91). Non-ionic surfactants are normally employed in a concentration range between 0.0003-0.3% (w/v) (70). Polysorbates are composed of fatty acid esters of polyoxyethylene sorbitan monolaurate (polysorbate 20) and polyoxyethylene sorbitan monooleate (polysorbate 80). The main disadvantage of polysorbates is their ability to undergo hydrolysis and autoxidation of the side-chains, resulting in hydrogen peroxide formation and development of short chain acids (formic acid). These sub-products can compromise the stability of a biopharmaceutical formulation (e.g. increasing the oxidation rate of proteins) and the safety of the product if accumulated in high amounts (89, 91, 92).

The concentration of polysorbates in a pre-formulation stage is selected considering: a) critical micelle concentration (CMC) and b) the possibility of degradation through the manufacturing process or during storage of the product. The concentration of surfactant needs to be carefully determined at the pre-formulation stage because of these disadvantages. An alternative surfactant is poloxamer 188, a triblock copolymer, included in a small number of liquid and lyophilised protein products (89). In comparison to polysorbates, poloxamer 188 inhibits protein adsorption through a different mechanism; which is independent from the interface affinity and allows formation of protein-surfactant complexes (93). The mechanism by which surfactants reduce protein adsorption can also impact the concentration used in formulation, which does not always depends exclusively on the CMC (93). Poloxamer 171 was

added to INSUMAN® solution for infusion (400 IU/ml) or injection (100 IU/ml) in the new formulation (EPAR updated 04/06/2018).

Key considerations: The most commonly employed surfactants are polysorbates. However, the impact of the formation of degradation sub-products on protein stability should be evaluated at the pre-formulation stage. If their employment is required, the minimum functional concentration should be included and the impact of their variability on product and process stability be assessed. Poloxamers represent an alternative to the polysorbates.

3.2.6 Preservatives

Parenteral liquid products in multi-dose vials require the presence of a preservative to minimise microbial contamination. One of the main drawbacks of antimicrobial preservatives is their significant volatility and reactivity. Furthermore, many examples of interactions between various preservatives and drugs, excipients, packaging and filter materials are reported in literature (32, 68, 94). Preservatives are usually used in low amounts (0.002-1% (w/v)) (32), however, concentrations above 1% (w/v) have been noted in commercial products. Metacresol, phenol, benzyl alcohol and benzalkonium chloride were identified as the main preservatives included in protein products. Preservatives are mainly incorporated in multi-dose products (L: n=48; LYO: n=5) and in multi-dose lyophilised products they are always added to the solvent for reconstitution. Metacresol (L: 19%, n=33; LYO: 1%, n=1) and phenol (L: 14%, n=24; LYO: 0%) are the most frequently used preservatives, they are included respectively in 69% and 50% of the liquid multi-dose products. Metacresol is more active against gram +ve than gram -ve bacteria (45).

Phenol is the most common preservative in liquid insulin-based formulations and is active against a broader spectrum of microorganisms including viruses and mycobacteria. Phenol activity increases in acidic and concentrated solutions as well as at higher temperature. It has been reported that monoclonal antibody formulations containing phenol can lead to soluble and insoluble aggregates formation (4, 38, 45). Benzyl alcohol is present in four liquid multiple use products (8% of the multi-dose liquid products) However, it is also added to two liquid single use products (PEGASYS® (*peginterferon alfa-2a*) and REBIF® (*interferon beta-1a*)), where it acts

as stabiliser to prevent oxidation (EPAR's scientific discussion) (95). Benzyl alcohol is also added to four lyophilised formulations (80% of the multi-dose lyophilised products). Benzalkonium chloride (L: 1%, n=1; LYO: 1%, n=1) is a quaternary ammonium compound active against gram +ve and gram -ve bacteria. Both the products containing benzalkonium chloride are intended for multiple use.

Key considerations: Metacresol and phenol are the most common preservatives employed in liquid commercial products. The use of preservatives is particularly required in multi-dose preparations. Metacresol, benzyl alcohol and benzalkonium chloride were observed to be added to the diluent for reconstitution of a low number of lyophilised products (all for multiple use).

3.2.7 Tonicifiers

Tonicifiers are added to protein formulations to create isotonic solutions for parenteral administration. The delivery of a non-isotonic solution through a parenteral route of administration can cause damage to the tissue and pain at the site of administration. Osmolality values between 280-300 mOsm/Kg and <600 mOsm/Kg for intravenous and subcutaneous administration respectively represent the osmolality limitations in developing parenteral protein formulations (35). The usual range of osmolality observed in commercial protein products is between 210-440 mOsm/Kg (22). The achievement of iso-osmolar biopharmaceutical products is particularly challenging for high concentration protein formulations due to the high amount of protein and the overall high solute concentration. Sodium chloride (NaCl) and potassium chloride (KCl) are the two main tonicifiers, however all formulation components can contribute to the product tonicity. NaCl is used in a relatively high percentage of liquid (36%; n=62) and lyophilised (42%; n=38) commercial products and is added to formulations, especially in the liquid form, also as a stabiliser. Sodium chloride was reported to reduce the viscosity of a reconstituted high concentration protein formulation (90) and to have a stabilising effect on insulin based formulations (96). This excipient was also observed to inhibit mannitol crystallization in frozen solutions (97). However, the use of NaCl is not optimal for lyophilised formulations, due to the ability of water and NaCl to form an eutectic mixture at -21 °C that can enhance

protein mobility (38, 81). This could be the justification for six lyophilised products, including BETAFERON® (*interferon beta-1b*) and ALPROLIX® (*eftrenonacog alfa*), wherein sodium chloride is added to the diluent provided for reconstitution rather than to the lyophilised product (98). KCl is exclusively present in a low number of liquid formulations (L: 1%, n=2). The inclusion of sugars, polyols, amino acids and salts all increase the tonicity of a protein formulation. Hence, excipients reported in sections 3.2.3 and 3.2.4 contribute to the tonicity of the product. Furthermore, the impact of these excipients on the ionic strength of the formulation should be evaluated, since a high ionic strength can compromise the protein stability, promoting protein aggregation (99).

Key considerations: Achieving iso-osmolarity is recommended for products intended to be administered through parenteral routes. Tonicity of a product can be adjusted using NaCl or KCl, but also by using specific concentrations of sugars, polyols and amino acids in formulation. NaCl is present in a high number of lyophilised products, however care should be taken during lyophilisation process design due to its suppression effect on the eutectic temperature of the formulation.

3.2.8 Other excipients

The 'other excipients' category contains mainly complexing agents, antioxidants and solubilising agents. All these excipients have stabilising properties, but are not included in the main category stabilisers/bulking agents for two reasons: 1) they are not bulking agents (usually used in low concentrations); 2) they are present in a low number of products and/or they are stabilising agents via specific mechanisms. The percentages of approved liquid and lyophilised parenteral products containing 'other excipients' are listed along with their main functions in **Table II.** (38, 45, 96, 100-106).

Edetic acid or edetate salt (EDTA) is used as a complexing agent in liquid formulations (L:3%, n=6;). EDTA can form complexes (chelates) with metal ions which are removed from the solution in a process defined as sequestering. Heavy metals have the capability to catalyse autoxidation, so their removal can be required for stabilisation. Usually employed EDTA concentrations are between 0.005-0.1% (w/v) (45). EDTA also possess antioxidant and antimicrobial properties and it can be used in combination with other antioxidants and preservatives for a synergistic effect

(45). Calcium chloride (L: 3%; n=5; LYO: 19%; n=17) is usually employed as a complexing agent in lyophilised products containing coagulation factors. Coagulation factors is a class of therapeutic proteins whose activity and stability is promoted in presence of calcium ions (38).

Antioxidants minimise oxidative reactions of the API or excipients over the shelf-life of the product. Glutathione is an antioxidant found exclusively in two lyophilised products, ADVATE® (*octocog alfa*) and ADYNOVI® *(rurioctocog alfa pegol)*, both based on coagulation factors. Glutathione behaves as a reducing agent creating disulphide bonds with cysteine residues of proteins, preventing their oxidation. This process is aided by the thiol groups of glutathione which are oxidised forming glutathione disulphide (GSSG) (107-109).

Nicotinamide is included in one recent liquid product, FIASP® (*insulin aspart*). This excipient is reported to reduce the self-association of insulin, promoting the rapid absorption of the monomeric form, which results in a faster action. Protamine sulphate (L: 5%, n=9) is a specific excipient employed exclusively in liquid insulin products to prolong the action of the therapeutic protein. Zinc acetate (L: 3% n=5), zinc chloride (L: 10% n=17) and zinc oxide (L: 3% n=5) are also additives used specifically in liquid formulations based on insulin. The presence of zinc ions in the formulation at specific concentrations promote the association of insulin molecules in hexamers, increasing the protein stability and prolonging its activity (96).

Finally, recombinant human hyaluronidase (rHuPH20) is a novel excipient that enhances protein bioavailability following subcutaneous administration. It modifies and creates conduits in the interstitial matrix to promote dispersion of molecules including proteins (105). This excipient, exclusively employed in three liquid antibody products intended to be administered subcutaneously, is reported to reduce administration times in comparison to the corresponding intravenous products (110).

Key considerations: The employment of 'other excipients' can be due to the necessity to further improve the performance of the product, overcoming specific issues. Their inclusion in formulation should be evaluated case by case.

3.2.9 Additional considerations in excipient selection

Data analysis showed how qualitative and/or quantitative composition can vary for protein products according to different factors: 1) formulation approach (liquid or

lyophilised); 2) route of administration; 3) API concentration; 4) primary packaging container; 5) different formats of liquid formulations (concentrate vs solution). Commercial examples are provided for each of the listed factors. BENLYSTA® *(belimumab)*, COSENTYX® *(secukinumab)*, ENBREL® *(etanercept)* and XOLAIR®, *(omalizumab)* are all examples in which the excipient composition differs between the liquid and lyophilised product. In particular, a replacement of other stabilisers (amino acids or trehalose) with sucrose in the lyophilised form was observed in BENLYSTA® *(belimumab)*, COSENTYX® *(secukinumab)* and XOLAIR® *(omalizumab)*. In ENBREL® *(etanercept)*, instead, the sodium phosphate salt included in the liquid form is replaced by the TRIS salt in the lyophilised form. Mannitol is also added, in combination with sucrose, to the lyophilised product. HERCEPTIN® *(trastuzumab)* contains methionine and rHuPH20, which are not present in the lyophilised product. The route of administration of a specific product can also impact the choice of the excipients; MABTHERA® *(rituximab)* is an antibody product whose subcutaneous formulation contains rHuPH20. This excipient is excluded from the product for intravenous use for reasons related to its function and discussed in section 3.2.8. The different API concentrations can require varied qualitative and quantitative excipient compositions to reach an adequate formulation stability, this is the case of HUMIRA® *(adalimumab)* and OMNITROPE® *(somatropin)*. Furthermore, LANTUS® *(insulin glargine)* and TOUJEO® *(insulin glargine)* liquid products do not contain polysorbate 20 in the cartridges; however, the surfactant is present in the vials. Some excipients can be added to specific types of liquid formulations, for instance, STELARA® *(ustekinumab)* concentrate for solution for IV infusion (20 mg/ml) contains EDTA, which is not included in the highly concentrated (80 mg/ml) subcutaneous liquid solution. Therefore, excipients should be carefully selected considering several factors with the aim of improving the stability of a target/final product with specific characteristics.

Regarding lyophilised products, the addition of specific excipients to the diluent for reconstitution could improve stability of the product during its life span between manufacture and administration. As discussed before, sodium chloride or preservatives can be included directly in the diluent for reconstitution. Furthermore, REFIXIA® *(nonacog beta pegol)* contains 4.2 ml of 10 mM histidine solution in a prefilled syringe as diluent for reconstitution which was observed to improve the stability of the final product (111). BLINCYTO® *(blinatumomab)* includes a stabiliser

solution (containing lysine, citric acid, polysorbate 80 and sodium hydroxide) to prevent adsorption of *blinatumomab* to the surfaces of administration; hence, it is added to the IV infusion bag (112).

3.3 Approved antibody products

3.3.1 Antibody concentration and relationship with the route of administration

The number of antibody products approved up to June 2018 was 94 (L: 73%, n=69; LYO: 27%, n=25). **Figure 7** shows the trend in the number of liquid and lyophilised parenteral antibody products approved per year from 1998, when the first antibody product was approved, to June 2018. Liquid formulations are more common than lyophilised formulations (11) and the number of marketed products per year has significantly increased in 2017. The most common class of antibodies in approved products is IgG1 (L: 70% n=48; LYO: 84% n=21). A lower number of liquid products contain IgG4 (L: 10% n=7), human normal IgG (L: 9% n=6), and IgG2 (L: 7% n=5) as API. Two liquid products contain Fab fragments (PRAXBIND® (*idarucizumab*) and CIMZIA® (*certolizumab pegol*)) and one liquid product contains an IgG2/4 (SOLIRIS® (*eculizumab*)). Lyophilised products are predominately based on IgG1 with three products containing IgG4. Only two bispecific antibody products are currently approved by the EMA. One HEMLIBRA® (*emicizumab)* is formulated as a liquid and the second BLINCYTO® (*blinatumomab)* as a lyophilised product. The four approved antibody-drug conjugates (ADC) are all in a lyophilised dosage form. No particular trend was observed in the use of specific excipients for the different classes of antibody. However, human normal immunoglobulin products tend to have a low number of excipients in formulation (1 or 2).

Figure 8 shows the antibody concentration (mg/ml) in liquid products before dilution and in lyophilised products following reconstitution. Approximately half of these products (L: 48%; LYO: 68%) contain antibodies at a concentration ≤ 50 mg/ml. **Figure 9** reports the amount of therapeutic protein per vial (mg/vial) for lyophilised products. Information regarding the fill volume of the vials before lyophilisation was not provided in the EPAR data, therefore it is not possible to determine the initial concentration of protein or the total solute concentration prior to lyophilisation. However, a higher percentage of products contain protein in amounts ≥100 mg/vial. Analysis of products' protein concentration (lyophilised products after reconstitution) reveals a clear relationship with the route of administration, **Figure 10.** More than

half of the commercial antibody products are intended to be administered intravenously (60%). Antibody formulations with high concentrations of protein (>50-mg/ml) are more commonly administered subcutaneously. The antibody concentrations in formulation depends on the therapeutic effect and the route of administration selected to deliver the dose. For example, LUCENTIS® (*ranibizumab*), a product for the treatment of macular degeneration and edema, myopia and diabetes complications, is formulated at a relatively low concentration (10 mg/ml) and is intended to be administered by intravitreal route (113).

3.3.2 Qualitative and quantitative excipient composition

The categories of excipients included in liquid and lyophilised antibody products are shown in **Figure 11**. Antibody formulations contain non-amino acids buffers in approximately half of both liquid (54%; n=37) and lyophilised (56%; n=14) commercial products. Amino acids are also added to approximately 50% of the products in both dosage forms (L: 54%; n=37; LYO: 52%; n=13). A high number of liquid formulations contain non-amino acids stabilisers (L: 59%, n=41), whereas all lyophilised products contain non-amino acids stabilisers/bulking agents (LYO: 100%, n=25). Most of the liquid and lyophilised products include surfactants (L: 87%, n=60; LYO: 88%, n=22). Preservatives are not present in any antibody formulations, which are always provided in a single use dosage form. Sodium chloride and potassium chloride are more frequently added to liquid products (L:36%, n=25) where they can act as tonicifiers and/or stabilisers. Excipients classified as 'other excipients' are only included in a small number of liquid products (13%; n=9).

Figure 12 shows the percentage of liquid and lyophilised antibody formulations that contain individual excipients. Amongst non-amino acids buffers the most commonly employed excipients are: sodium citrate (L: 20%, n=14; LYO: 20%, n=5) and sodium phosphate (L: 12%, n=8; LYO: 28%, n=7). As for protein products, sodium acetate (L: 22%, n=15) is observed to be included exclusively in liquid products. Histidine (L: 39%; n=27; LYO: 40%; n=10) is the most common amino acid added to liquid and lyophilised antibody products, followed by the amino acid glycine (L:12%; n=8; LYO: 8%; n=2). Arginine is more frequently used in the liquid forms (10%; n=7) than the lyophilised forms (4%; n=1). Methionine is included exclusively in liquid products (9%; n=6).

Sucrose is the most common non-amino acid stabiliser and is present in most of the lyophilised products (L: 20%, n=14; LYO: 72%, n=18). Sorbitol is included exclusively in liquid products (10%; n=7). Surfactants employed comprise polysorbate 80 (L: 55%, n=38; LYO: 64%, n=16), polysorbate 20 (L: 29%, n=20; LYO: 24%, n=6) and to a lesser extent poloxamer 188 (L: 3%, n=2; LYO: 0%). Sodium chloride is present in a high number of liquid products (L: 36%, n=25) and only three lyophilised products with low concentrations of antibodies, two of which are antibody-drug conjugates (ADC). Finally, excipients classified as 'other excipients' and employed in antibody formulations include chelating agents (EDTA and pentetic acid), calcium chloride as complexing agent and rHuPH20. rHuPH20 is exclusively present in MABTHERA® (*rituximab*), HERCEPTIN® (*trastuzumab*) and HYQVIA® (*human normal immunoglobulin*), all liquid products based on antibodies.

The quantitative composition of 88 out of 94 liquid and lyophilised antibody commercial products was investigated consulting accredited sources and summarised in **Table III**. (8-10, 114). Some of the products for which the quantitative composition was not available are biosimilars to other reference products. Biosimilars in some cases can have the same qualitative and/or quantitative excipient composition. For example, MVASI® *(bevacizumab)* is a biosimilar, having the same formulation of the reference product AVASTIN® *(bevacizumab)* (115). On MVASI®'s scientific discussion (EMA) it is reported: 'The finished product was developed to have the same formulation, route of administration, dosage form and strength as the reference product Avastin' (116). Furthermore, some biosimilars possess identical excipient composition (e.g. *rituximab* biosimilars: RITUZENA®, RITEMVIA® and BLITZIMA®) and have been approved by the EMA following a multiple marketing authorisation application (117).

The pH values at which listed antibody formulations were buffered are in a range between pH 4.6 and 8.2. The trend observed in the use of excipients for antibody formulations matches with the results obtained for protein formulations, probably because antibodies form the main class of therapeutic proteins. Hence, additional information is reported below only for other excipients added to antibody products and not discussed in previous sections. For example, proline (24-28.78 mg/ml) is reported in the EPAR's scientific discussion of some liquid antibody products to be a viscosity and tonicity modifier or a stabiliser (118, 119). EDTA and pentetic acid are added as chelating agents only to liquid products at concentrations usually in a range

between 0.02-1 mg/ml and 0.01-0.04 mg/ml, respectively. Calcium chloride is reported to be at concentrations of 0.4 mg/ml in the antibody product HYQVIA® *(human normal Ig)*. Finally, rHUPH20 is added at concentrations of 2000 units/ml in MABTHERA® *(rituximab)* and HERCEPTIN® *(trastuzumab).* However, in HYQVIA® (*human normal Ig*) rHUPH20 (160 units/ml) is provided in a separate vial containing other excipients, and added to the antibody product prior to administration.

4. Conclusions

The information summarised in this overview aims to update formulation scientists on the trends of excipients' use in approved protein products, aiding them in the selection of excipients for the development of new formulations. The data presented details the most common excipients included in liquid and lyophilised formulations classified into functional categories, focusing in particular on antibody products. The discussion also provides information and key considerations in the use of specific excipients, analysing their role and rationale of use in protein formulations with different dosage form (liquid or lyophilised). A shortcoming of the EPARs data is the limited quantitative compositional information on approved products. However, this information was collected for most of the protein formulations using other publicly available sources (FDA and Marketing Authorisation Holder). Of note from this overview is the low number of 'novel' excipients introduced within the products approved over the period analysed. This may be due to many factors; such as the employment of an usual 'platform approach' formulation strategy (9), the regulatory requirements of approving new excipients for parenteral formulations (71), as well as companies' marketing/commercial reasons that can drive the choice of one excipient over another (9, 120).

Acknowledgements

This publication has emanated from research conducted with the financial support of the Synthesis and Solid State Pharmaceutical Centre, funded by Science Foundation Ireland (SFI) under Grant Number [12/RC/2275] and Enterprise Ireland under Grant Number [IP/2015 0358]

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Figures captions:

Figure 1. Trend of liquid and lyophilised parenteral protein products approved per year by the EMA between 1995-2018 (June) *(*Last updated: 18/06/2018).*

Figure 2. Classes of therapeutic proteins included in liquid and lyophilised parenteral products. 2a) Pie chart of the total number of products; 2b) Pie chart of the split between liquid and lyophilised products.

**'Other' class includes types of therapeutic proteins present in a percentage of commercial products <3% (analgesic peptide, antiangiogenic agent, anticoagulant, antiplatelet, antithrombin/thrombolytic, growth factor, HIV infusion inhibitor, muramyl peptide derivative, toxin, calcimimetic peptide)*

Figure 3. The percentage of liquid and lyophilised parenteral protein products that contain excipients from each of the excipient categories: Non-amino acids BUFF (buffers); Amino acids BUFF/S/BA (buffers/stabilisers/bulking agents); Non-amino acids S/BA (stabilisers/bulking agents); Surfactants; Preservatives; Tonicifiers and Other excipients.

Note: The function of these excipients as bulking agents is only relevant for lyophilised products

**'Other excipients' category consists of complexing agents, antioxidants, solubilising agents and excipients specific to individual formulations*

Figure 4. The percentage of approved liquid and lyophilised parenteral protein products containing individual non-amino acids buffers.

Figure 5. The percentage of approved liquid and lyophilised parenteral protein products containing individual amino acids buffers/stabilisers/bulking agents.

Note: The function of these excipients as bulking agents is only relevant for lyophilised products

Figure 6. The percentage of approved liquid and lyophilised parenteral protein products containing individual non-amino acids stabilisers/bulking agents.

Note: The function of these excipients as bulking agents is only relevant for lyophilised products

Figure 7. Trend of liquid and lyophilised parenteral antibody products approved per year by the EMA between 1995-2018 (June) *(*Last updated: 18/06/2018).*

Figure 8. Range of therapeutic concentrations (mg/ml) of liquid and lyophilised parenteral antibody products.

**for lyophilised formulations intended after reconstitution*

Figure 9. Amount of antibody per vial (mg/vial) for lyophilised parenteral antibody products.

Figure 10. Relationship between ranges of antibody concentrations (mg/ml) and route of administration.

**for lyophilised formulations intended after reconstitution*

Figure 11. The percentage of liquid and lyophilised parenteral antibody products that contain excipients from each of the excipient categories: Non-amino acids BUFF (buffers); Amino acids BUFF/S/BA (buffers/stabilisers/bulking agents); Non-amino acids S/BA (stabilisers/bulking agents); Surfactants; Preservatives; Tonicifiers and Other excipients.

Note: The function of these excipients as bulking agents is only relevant for lyophilised products

**'Other excipients' category consists of complexing agents, antioxidants, solubilising agents and excipients specific to individual formulations*

Figure 12. The percentage of liquid and lyophilised parenteral antibody products that contain individual excipients from each of the excipient categories: Non-amino acids BUFF (buffers); Amino acids BUFF/S/BA (buffers/stabilisers/bulking agents); Non-amino acids S/BA (stabilisers/bulking agents); (SURF) Surfactants; (T) Tonicifiers; (OE*) Other excipients.

Note: The function of these excipients as bulking agents is only relevant for lyophilised products

** (OE) 'Other excipients' category consists of complexing agents, antioxidants, solubilising agents and excipients specific to individual formulations*

Supplementary information captions:

Table 1. Database of approved liquid parenteral protein products listed alphabetically *(Last updated 18/06/2018)*

Table 2. Database of approved lyophilised parenteral protein products listed alphabetically *(Last updated 18/06/2018)*

Table I. More common individual excipients classified into functional categories and listed by descending frequency of use in liquid and lyophilised parenteral protein products (percentage and number of formulations containing each excipient and range of excipient concentrations included in approved products)

Excipient class	*Liquid*	*Lyophilised*
Non-amino acids *Buffering agents*	Sodium phosphate (33%; n=58) *(0.2-14.8 mg/ml)*	Sodium phosphate (32%; n=29) *(0.2-14.4 mg/ml)*
	Sodium acetate (19%; n=33) *(0.4-6.8 mg/ml)*	Sodium citrate (20%; n=18) *(1.1-34.8 mg/ml)*
	Sodium citrate (17%; n=29) *(0.5-14.7 mg/ml)*	Tris (8%; n=7) *(0.8-3 mg/ml)*
Amino acids *Buffering agents/stabilisers/bulking agents*	Histidine (16%, n=27) *(0.9-4.3 mg/ml)*	Histidine (34%; n=31) *(0.7-8.9 mg/ml)*
	Methionine (12%; n=21) *(0.1-3 mg/ml)*	Glycine (13%; n=12) *(0.2-25 mg/ml)*
	Glycine (9%; n=15) *(0.1-18.8 mg/ml)*	Methionine (6%; n=5) *(0.06-0.5 mg/ml)*
	Arginine (8%; n=14) *(4.4-42.1 mg/ml)*	Arginine (4%; n=4) *(5.4-52 mg/ml)*
Non-amino acids *Stabilisers/bulking agents*	Glycerol (17%; n=29) *(16-20 mg/ml)*	Sucrose (59%; n=53) *(1.9-160 mg/ml)*
	Sucrose (16%; n=28) *(10-200 mg/ml)*	Mannitol (33%; n=30) *(10.6-80 mg/ml)*
	Mannitol (14%; n=24) *(1.9-54.6 mg/ml)*	Trehalose (10%; n=9) *(8-70 mg/ml)*

	Sorbitol (10%; n=17) *(20-50 mg/ml)*	Human albumin (7%; n=6) *(8-15 mg/ml)*
Surfactants	Polysorbate 80 (32%; n=55) *(0.01-2 mg/ml)*	Polysorbate 80 (41%; n=37) *(0.05-0.7 mg/ml)*
	Polysorbate 20 (26%; n=45) *(0.01-2 mg/ml)*	Polysorbate 20 (18%; n=16) *(0.04-0.4 mg/ml)*
	Poloxamer 188 (7%; n=12) *(0.1-8 mg/ml)*	Poloxamer 188 (3%; n=3) *(1-1.2 mg/ml)*
*Preservatives**	Metacresol (19%; n=33) *(1.5-3.2 mg/ml)*	Benzyl alcohol (4%; n=4) *(9-14.9 mg/ml)*
	Phenol (14%; n=24) *(0.8-5.7 mg/ml)*	Benzalkonium chloride (1%; n=1) *(NA)*
	Benzyl alcohol (3%; n=6) *(9-10 mg/ml)*	Metacresol (1%; n=1) *(3.2 mg/ml)*
Tonicifiers	Sodium chloride (36%; n=62) *(0.6-11.7 mg/ml)*	Sodium chloride (42%; n=38) *(0.3-27 mg/ml)*
	Potassium chloride (1%; n=2) *(0.2 mg/ml)*	-

**Preservatives in lyophilised products are added to the diluent for reconstitution*
Note: the function of these excipients as bulking agents is only relevant for lyophilised products

Table II. Examples of other excipients with specific functions included in liquid and lyophilised parenteral protein products

	Function	***Liquid***	***Lyophilised***	***Reference***
Calcium chloride	Complexing agent; preservative	3%; n=5	19%; n=17	(38, 45)

EDTA	Complexing agent (chelating agent)	3%; n=6	0%; n=0	(45)
Glutathione	Antioxidant	0%; n=0	2%; n=2	(100)
Nicotinamide	Antioxidant; solubilizing agent	1%; n=1	0%; n=0	(45)
Pentetic acid	Complexing agent (chelating agent)	2%; n=3	0%; n=0	(45)
Protamine sulphate*	Complexing agent to prolong insulin activity	5%; n=9	0%; n=0	(101, 102)
Recombinant human hyaluronidase	Bioavailability enhancer following SC administration	2%; n=3	0%; n=0	(104, 105)
Urea	Stabiliser (dissolving aggregates)	1%; n=1	1%; n=1	(103)
Zinc acetate*	Complexing agent to prolong insulin activity	3%; n=5	0%; n=0	(38, 96)
Zinc chloride*		10%; n=17	0%; n=0	
Zinc oxide *		3%; n=5	0%; n=0	
1-palmitoyl, 2-oleoylphosphatidylcholine (POPC), 1,2-dioeloylphosphatidylserine (OOPS)	Liposomal targeting	0%; n=0	1%; n=1	(106)

**Specific for products containing insulin*

Table III. Quantitative composition of individual excipients included in 88 approved liquid and lyophilised parenteral antibody products, listed by ascending values of concentration (16,17,22,28) *(Last updated 18/06/2018)*

Excipient name	***Excipient quantitative composition of approved LIQUID antibody products***	***Excipient antibody p***
Potassium phosphate (Non-amino acids BUFF) [1]	**LEMTRADA® (alemtuzumab)** 0.2 mg/ml	**SIMULEC**
Sodium acetate (Non-amino acids BUFF) [1]	**AMGEVITA® (adalimumab)** 0.6 mg/ml acetic acid; **BAVENCIO® (avelumab)** 0.6 mg/ml; **SOLYMBIC® (adalimumab)** 0.6 mg/ml acetic acid; **TECENTRIQ® (atezolizumab)** 0.83 mg/ml acetic acid; **DUPIXENT® (dupilumab)** 1 mg/ml; **REPATHA® (evolocumab)** 1.2 mg/ml; **CIMZIA® (certolizumab pegol)** 1.36 mg/ml **PROLIA® (denosumab)** 17 mM (1.4 mg/ml); **XGEVA® (denosumab)** 18 mM (1.5 mg/ml); **OCREVUS® (ocrelizumab)** 20 mM (EMA); 2.14 mg/ml, 0.25 acetic acid (FDA); **CINQAERO® (reslizumab)** 2.45 mg/ml; 0.12 mg/ml acetic acid; **PRAXBIND® (idarucizumab)** 2.95 mg/ml, 0.20 mg/ml acetic acid; **DARZALEX® (daratumumab)** 2.96 mg/ml, 0.18 mg/ml acetic acid; **CYLTEZO® (adalimumab**) 3 mg/ml; 0.16 mg/ml acetic acid, **ARZERRA® (ofatumumab)** 6.8 mg/mL; **VECTIBIX® (panitumumab)** 6.8 mg/ml	None
Sodium citrate (Non-amino acids BUFF) [1]	**HUMIRA® (adalimumab, 50 mg/ml)** 1.3 mg/ml citric acid, 0.3 mg/ml; **ERBITUX® (cetuximab)** 10 mM **(**1,92 mg/ml) citric acid; **PORTRAZZA® (necitumumab)** 10 mM (EMA), 2.55 mg/ml, 0.26 mg/ml citric acid (FDA); **ZINPLAVA® (bezlotoxumab)** 4.75 mg/ml; 0.8 mg/ml citric acid, **TALTZ® (ixekizumab)** 5.11 mg/ml, 0.51 mg/ml citric acid; **OPDIVO® (nivolumab)** 5.88 mg/ml; **BLITZIMA® (rituximab)** 25 mM (7.35 mg/ml) tri-sodium dihydrate; **RITEMVIA® (rituximab)** 25 mM (7.35 mg/ml) tri-sodium dihydrate; **RITUZENA® (rituximab)** 25 mM (7.35 mg/ml) tri-sodium dihydrate;	**BLINCY EMPLIC BENLYS ADCETR** mg/ml, 0.2

Excipient name	*Excipient quantitative composition of approved LIQUID antibody products*	*Excipient antibody p*
	TRUXIMA® (rituximab) 25 mM (7.35 mg/ml) tri-sodium dihydrate; **MABTHERA® (rituximab, 10 mg/ml)** 7.35 mg/ml	
Sodium phosphate (Non-amino acids BUFF) [1]	**TYSABRI® (natalizumab)** 1.13 mg/ml monobasic monohydrate; 0.48 mg/ml dibasic heptahydrate; **LEMTRADA® (alemtuzumab)** 1.15 mg/ml dibasic dihydrate; **HUMIRA® (adalimumab, 50 mg/ml)** 1.53 mg/ml dibasic dihydrate, 0.85 mg/ml monobasic dihydrate; **HYQVIA® (human normal Ig)** 1.78 mg/ml dibasic; **SOLIRIS® (eculizumab)** 1.78 mg/ml dibasic, 0.46 mg/ml monobasic; **AVASTIN® (bevacizumab)** 51 mM (EMA), 5.8 mg/ml monobasic monohydrate, 1.2 mg/ml dibasic anhydrous (FDA); **MVASI® (bevacizumab)** 5.8 mg/ml monobasic monohydrate, 1.2 mg/ml dibasic anhydrous; **ROACTEMRA® (tocilizumab, 20 mg/ml)** 15 mM*	**SIMULEC** 0.56 mg/m heptahydra mg/ml dib **INFLECT** mg/ml mo mg/ml dib **REMICA** mg/ml mo mg/ml dib
Sodium succinate (Non-amino acids BUFF) [1]	None	**KADCYL**
Tris (Non-amino acids BUFF) [1]	**YERVOY® (ipilimumab)** 3.15 mg/ml HCl	**BESPON**
Arginine (Amino acids BUFF/S/BA) [2]	**DUPIXENT® (dupilumab)** 5.25 mg/ml HCl monohydrate; **BENLYSTA® (belimumab)** 5.3 mg/ml HCl monohydrate; **KEVZARA® (sarilumab)** 7.84 mg/ml; **ARZERRA® (ofatumumab)** 10 mg/ml; **HEMLIBRA® (emicizumab)** 150 mM (26.13 mg/ml); **XOLAIR® (omalizumab)** 42.1 mg/ml HCl monohydrate	**ENTYVI** monohydr
Glycine (Amino acids BUFF/S/BA) [2]	**SYNAGIS® (palivizumab)** 0.1 mg/ml; **ERBITUX® (cetuximab)** 100 mM (7.5 mg/ml); **LARTRUVO® (olaratumab)** 7.5 mg/ml; **CYRAMZA® (ramucirumab)** 133 mM **(**9.98 mg/ml); **PORTRAZZA® (necitumumab)** 133 mM (9.98 mg/ml); **KIOVIG® (human normal Ig)** 250 mM (18.77 mg/ml); **HyQVIA® (human normal Ig)** 250 mM (18.77 mg/ml)	**SYNAGIS (basilixim**
Glutamic acid (Amino acids BUFF/S/BA) [2]	**KYNTHEUM® (brodalumab)** 4.33 mg/ml	None
Histidine (Amino acids BUFF/S/BA) [2]	**SIMPONI® (golimumab)** 0.87 mg/ml; **PRALUENT® (alirocumab, 75 mg/ml)** 8 mM (1.24 mg/ml); **PRALUENT® (alirocumab, 100 mg/ml)** 6 mM (0.93 mg/ml); **STELARA® (ustekinumab, 90 mg/ml)** 1 mg/ml; **STELARA® (ustekinumab, 5 mg/ml)** 1.04 mg/ml HCl monohydrate, 0.77 mg/ml,; **BENLYSTA® (belimumab)** 1.2 mg/ml HCl monohydrate, 0.65 mg/ml; **CYRAMZA® (ramucirumab)** 10 mM (EMA), 1.22 mg/ml HCl monohydrate, 0.65 mg/ml (FDA); **FASENRA® (benralizumab)** 1.4 mg/ml, 2.3 mg/ml HCl monohydrate; **TREMFYA® (guselkumab)** 1.5 mg/ml HCl monohydrate, 0.6 mg/ml; **KEYTRUDA® (pembrolizumab)** 1.55 mg/ml; **CRYSVITA® (burosumab)** 1.55 mg/ml; **LARTRUVO® (olaratumab)** 1.7 mg/ml HCl monohydrate, 0.3 mg/ml; **ILARIS® (canakinumab)** 2.1 mg/ml, 1.3 mg/ml HCl monohydrate; **XOLAIR® (omalizumab)** 2.34 mg/ml HCl monohydrate, 1.37 mg/ml; **COSENTYX® (secukinumab)** 20 mM (3.1 mg/ml); **DUPIXENT® (dupilumab)** 3.1 mg/ml; **HEMLIBRA® (emicizumab)** 20 mM (3.1 mg/ml); **QARZIBA® (dinutuximab beta)** 20 mM (3.1 mg/ml); **TECENTRIQ® (atezolizumab)** 20 mM (3.1 mg/ml); **KEVZARA® (sarilumab)** 3.25 mg/ml; **MABTHERA® (rituximab, 120 mg/ml)** 3.47 mg/ml HCl monohydrate, 0.53 mg/ml; **SYNAGIS® (palivizumab)** 3,9 mg/ml; **PERJETA® (pertuzumab)** 20 mM (4.28 mg/ml) acetate; **LUCENTIS® (ranibizumab)** 10 mM**; **GAZYVARO® (obinutuzumab)** 20 mM**; **HERCEPTIN® (trastuzumab)** 20 mM**	**HERCEP** 0.31 mg/m monohydr mg/ml HC **(trastuzu SYLVAN (pembroli** mg/mL H0 2.8 mg/ml **(secukinu** 7.3 mg/ml mg/ml, 4.2
Lysine (Amino acids BUFF/S/BA) [2]	None	**BLINCY**
Methionine (Amino acids BUFF/S/BA) [2]	**STELARA® (ustekinumab, 5 mg/ml)** 0.4 mg/ml; **COSENTYX® (secukinumab)** 5 mM (0.75 mg/ml); **HERCEPTIN® (trastuzumab)** 10 mM (1.49 mg/ml); **CRYSVITA® (burosumab)** 1.49 mg/ml; **MABTHERA® (rituximab, 120 mg/ml)** 1.49 mg/ml	None
Proline (Amino acids BUFF/S/BA) [2]	**KYNTHEUM® (brodalumab)** 24 mg/ml; **REPATHA® (evolocumab)** 25 mg/ml; **PRIVIGEN® (human normal Ig)** 250 mM (28.78 mg/ml); **HIZENTRA® (human normal Ig)** 250 mM (28.78 mg/ml)	None
Dextran (Non-amino acids S/BA) [3]	None	**MYLOTA**
Human albumin (Non-amino acids S/BA) [3]	**HYQVIA® (human normal Ig)** 1 mg/ml	None

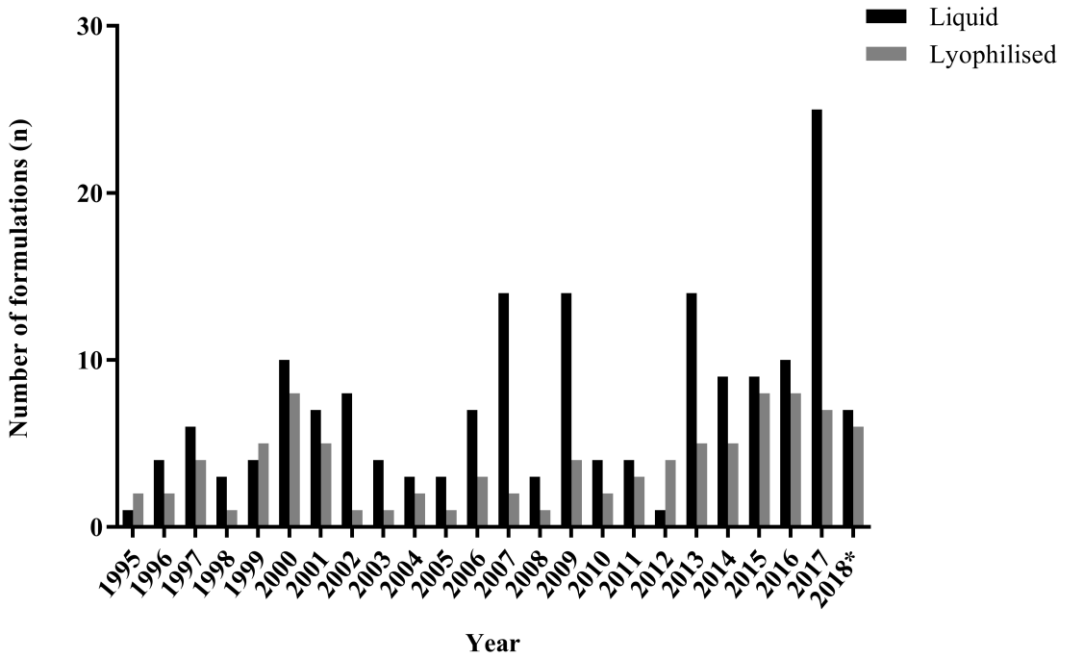
Excipient name	*Excipient quantitative composition of approved LIQUID antibody products*	*Excipient antibody p*
Mannitol (Non-amino acids S/BA) [3]	**PORTRAZZA® (necitumumab)** 50 mM (9.1 mg/ml); **YERVOY® (ipilimumab)** 10 mg/ml; **HUMIRA® (adalimumab, 50 mg/ml)** 12 mg/ml; **LARTRUVO® (olaratumab)** 13.7 mg/ml; **DARZALEX® (daratumumab)** 25.5 mg/ml; **OPDIVO® (nivolumab)** 30 mg/ml; **HUMIRA® (adalimumab, 100 mg/ml)** 42 mg/ml; **ILARIS® (canakinumab)** 49.2 mg/ml; **BAVENCIO® (avelumab)** 51 mg/ml	**SIMULEC (palivizum**
Sorbitol (Non-amino acids S/BA) [3]	**PRAXBIND® (idarucizumab)** 40.08 mg/ml; **SIMPONI® (golimumab)** 41 mg/ml; **CRYSVITA® (burosumab)** 45.91 mg/ml; **XGEVA® (denosumab)** 46 mg/ml; **PROLIA® (denosumab)** 47 mg/ml; **FLEBOGAMMA DIF® (human normal Ig, 50 mg/ml)** 50 mg/ml	None
Sucrose (Non-amino acids S/BA) [3]	**PERJETA® (pertuzumab)** 120 mM (41.08 mg/ml); **TECENTRIQ® (atezolizumab)** 120 mM (41.08 mg/ml); **DUPIXENT® (dupilumab)** 50 mg/ml; **KEVZARA® (sarilumab)** 50 mg/ml; **QARZIBA® (dinutuximab beta)** 50 mg/ml; **ROACTEMRA® (tocilizumab, 20 mg/ml)** 50 mg/ml; **CINQAERO® (reslizumab)** 70 mg/ml; **KEYTRUDA® (pembrolizumab)** 70 mg/ml; **STELARA® (ustekinumab, 90 mg/ml)** 76 mg/ml; **TREMFYA® (guselkumab)** 79 mg/ml **STELARA® (ustekinumab, 5 mg/ml)** 85 mg/ml; **AMGEVITA® (adalimumab)** 90 mg/ml; **SOLYMBIC® (adalimumab**) 90 mg/ml; **PRALUENT® (alirocumab)** 100 mg/ml	**SIMULEC (gemtuzu (siltuxima** mg/ml; **BE FLIXABI** 50 mg/ml; **(infliximа** 60 mg/ml; **BENLYS (canakinu** mM (EMA mg/ml; **XC (mepolizu**
Trehalose (Non-amino acids S/BA) [3]	**OCREVUS® (ocrelizumab)** 106 mM (EMA), 40 mg/ml (FDA); **AVASTIN® (bevacizumab)** 60 mg/ml**; MVASI® (bevacizumab)** 60 mg/ml; **COSENTYX® (secukinumab)** 200 mM (EMA), 75.67 mg/ml (FDA)**; HERCEPTIN® (trastuzumab)** 210 mM (71.88 mg/ml); **MABTHERA® (rituximab, 120 mg/ml)** 79,45 mg/ml; **CYLTEZO® (adalimumab)** 81.25 mg/ml; **GAZYVARO® (obinutuzumab)** 240 mM (82.15 mg/ml); **FASENRA® (benralizumab)** 95 mg/ml; **LUCENTIS® (ranibizumab)** 100 mg/ml	**HERCEP (trastuzu** 19.05 mg/ **ADCETR** mg/ml (FD
Polysorbate 80 (Surfactants)	**HIZENTRA® (human normal Ig)** 0.02 mg/ml (EMA), 0.008-0.03 mg/ml (FDA); **STELARA® (ustekinumab, 90 mg/ml)** 0.04 mg/ml; **BENLYSTA® (belimumab)** 0.1 mg/ml; **ERBITUX® (cetuximab)** 0.1 mg/ml; **YERVOY® (ipilimumab)** 0.1 mg/ml; **LEMTRADA® (alemtuzumab)** 0.1 mg/ml; **CYRAMZA® (ramucirumab)** 0.1 mg/ml; **REPATHA® (evolocumab)** 0.1 mg/ml; **PORTRAZZA® (necitumumab)** 0.1 mg/ml; **SIMPONI® (golimumab)** 0.16 mg/ml; **ARZERRA® (ofatumumab)** 0.2 mg/mL; **COSENTYX® (secukinumab)** 0.2 mg/ml; **KEYTRUDA® (pembrolizumab)** 0.2 mg/ml; **OPDIVO® (nivolumab)** 0.2 mg/ml; **TYSABRI® (natalizumab)** 0.2 mg/ml; **SOLIRIS® (eculizumab)** 0.22 mg/ml; **ZINPLAVA® (bezlotoxumab)** 0.25 mg/ml; **TALTZ® (ixekizumab)** 0.3 mg/ml; **ILARIS® (canakinumab)** 0.4 mg/ml; **STELARA® (ustekinumab, 5 mg/ml)** 0.4 mg/ml; **CRYSVITA® (burosumab)** 0.5 mg/ml; **ROACTEMRA® (tocilizumab, 20 mg/ml)** 0.5 mg/ml; **TREMFYA® (guselkumab)** 0.5 mg/ml; **MABTHERA® (rituximab, 120 mg/ml)** 0.6 mg/ml**; MABTHERA® (rituximab, 10 mg/ml)** 0.7 mg/ml; **BLITZIMA® (rituximab)** 0.7 mg/ml; **RITEMVIA® (rituximab)** 0.7 mg/ml; **RITUZENA® (rituximab)** 0.7 mg/ml; **TRUXIMA® (rituximab)** 0.7 mg/ml; **AMGEVITA® (adalimumab)** 1 mg/ml; **CYLTEZO® (adalimumab)** 1 mg/ml; **HUMIRA® (adalimumab, 50 mg/ml)** 1 mg/ml; **HUMIRA® (adalimumab, 100 mg/ml)** 1 mg/ml; **SOLYMBIC® (adalimumab)** 1 mg/ml; **DUPIXENT® (dupilumab)** 2 mg/ml	**FLIXABI (infliximа REMSIM (inotuzum** 0.16 mg/m **KEYTRU (blinatum** mg/ml; **BE (canakinu** mg/ml; **CC (mepolizu**
Polysorbate 20 (Surfactants)	**FASENRA® (benralizumab)** 0.06 mg/ml; **LUCENTIS® (ranibizumab)** 0.1 mg/ml; **KYNTHEUM® (brodalumab)** 0.1 mg/ml; **PROLIA® (denosumab)** 0.1 mg/ml; **PRALUENT® (alirocumab)** 0.1 mg/ml; **QARZIBA® (dinutuximab beta)** 0.1 mg/ml; **XGEVA® (denosumab)** 0.1 mg/ml; **OCREVUS® (ocrelizumab)** 0.2 mg/ml; **PERJETA® (pertuzumab)** 0.2 mg/ml; **PRAXBIND® (idarucizumab)** 0.2 mg/ml; **LARTRUVO® (olaratumab)** 0.2 mg/ml; **AVASTIN® (bevacizumab)** 0.4 mg/ml; **DARZALEX® (daratumumab)** 0.4 mg/ml; **HERCEPTIN® (trastuzumab)** 0.4 mg/ml; **TECENTRIQ® (atezolizumab)** 0.4 mg/ml; **MVASI® (bevacizumab)** 0.4 mg/ml; **XOLAIR® (omalizumab)** 0.4 mg/ml; **BAVENCIO® (avelumab)** 0.5 mg/ml **KEVZARA® (sarilumab)** 2 mg/ml	**HERCEP (trastuzu** mg/ml; **OI KADCYL (omalizum**
Poloxamer 188 (Surfactants)	**GAZYVARO® (obinutuzumab)** 0.2 mg/ml; **HEMLIBRA® (emicizumab)** 0.2-0.5 mg/ml	None
Potassium chloride (Tonicifiers)	**LEMTRADA® (alemtuzumab)** 0.2 mg/ml	None

Excipient name	*Excipient quantitative composition of approved LIQUID antibody products*	*Excipient antibody p*
Sodium chloride (Tonicifiers)	**PORTRAZZA® (necitumumab)** 40 mM (2.34 mg/ml); **LARTRUVO® (olaratumab)** 2.9 mg/ml; **OPDIVO® (nivolumab)** 2.92 mg/ml; **ARZERRA® (ofatumumab)** 2.98 mg/mL; **DARZALEX® (daratumumab)** 3.5 mg/ml; **CYRAMZA® (ramucirumab)** (75 mM) 4.38 mg/ml; **VECTIBIX® (panitumumab)** 5.8 mg/ml**; ERBITUX® (cetuximab**) 100 mM (5.84 mg/ml); **YERVOY® (ipilimumab)** 5.85 mg/ml; **HUMIRA® (adalimumab, 50 mg/ml)** 6.18 mg/ml**; BENLYSTA® (belimumab)** 6.7 mg/ml; **CIMZIA® (certolizumab pegol)** 7.31 mg/ml; **LEMTRADA® (alemtuzumab)** 8 mg/ml; **TYSABRI® (natalizumab)** 8.2 mg/ml; **HYQVIA® (human normal Ig)** 8.5 mg/ml; **SOLIRIS® (eculizumab)** 8.77 mg/ml; **ZINPLAVA® (bezlotoxumab)** 8.77 mg/ml; **MABTHERA® (rituximab, 10 mg/ml)** 9 mg/ml; **TRUXIMA® (rituximab)** 154 mM (9 mg/ml); **BLITZIMA® (rituximab)** 154 mM (9 mg/ml); **RITEMVIA® (rituximab)** 154 mM (9 mg/ml); **RITUZENA® (rituximab)** 154 mM (9 mg/ml); **TALTZ® (ixekizumab)** 11.69 mg/ml	**SIMULEC (inotuzum (gemtuzu**
Calcium chloride (Other excipients)	**HYQVIA® (human normal Ig)** 0.4 mg/ml	None
EDTA (Other excipients)	**STELARA® (ustekinumab, 5 mg/ml)** 0.02 mg/ml; **ARZERRA® (ofatumumab)** 0.02 mg/mL; **LEMTRADA® (alemtuzumab)** 0.02 mg/ml; **HYQVIA® (human normal Ig)** 1 mg/ml	None
Pentetic acid (Other excipients)	**OPDIVO® (nivolumab)** 0.01 mg/ml; **ZINPLAVA® (bezlotoxumab)** 0.01 mg/ml; **YERVOY® (ipilimumab)** 0.04 mg/ml	None
Recombinant human hyaluronidase (rHuPH20) (Other excipients)	**MABTHERA (rituximab, 120 mg/ml)** 2000 units/ml; **HERCEPTIN® (trastuzumab)** 2000 units/ml; **HYQVIA® (human normal Ig)** 160 units/ml	None

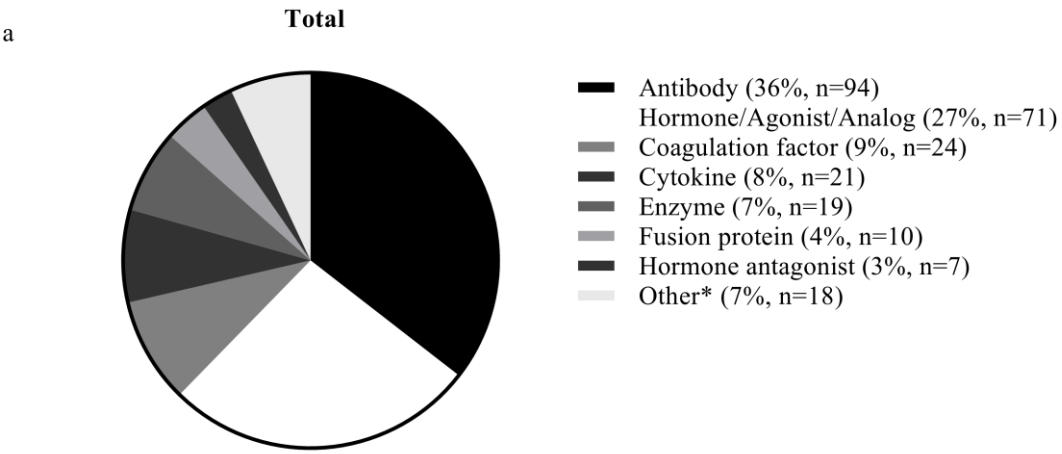
**Disodium phosphate dodecahydrate/Sodium dihydrogen phosphate dihydrate; **Histidine/Histidine-HCl; ***Sodium citrate/Citric acid*

1) Non-amino acids BUFF (buffers); 2) Amino acids BUFF/S/BA (buffers/stabilisers/bulking agents); 3) Non-amino acids S/BA (stabilisers/bulking agents)

Note: The function of these excipients as bulking agents is only relevant for lyophilised products



a

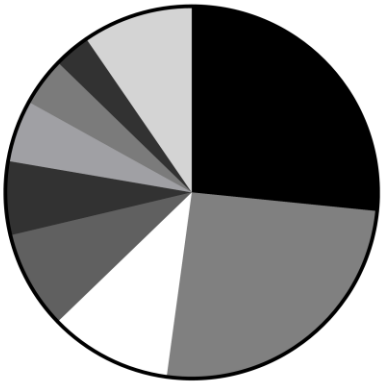


b

Liquid

- Antibody (40%, n=69)
- Hormone/ Agonist/ Analog (35%, n=61)
- Cytokine (9%, n=15)
- Enzyme (6%, n=11)
- Other* (5%, n=9)

Lyophilised



- Antibody (28%, n=25)
- Coagulation factor (27%, n=24)
- Hormone/ Agonist/ Analog (11%, n=10)
- Enzyme (9%, n=8)
- Cytokine (7%, n=6)
- Fusion protein (6%, n=5)
- Enzyme inhibitor (4%, n=4)
- Hormone antagonist (3%, n=3)
- Other* (10%, n=9)