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Formulation and delivery issues for monoclonal antibody therapeutics☆

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Abstract

Antibodies can have exquisite specificity of target recognition and thus generate highly selective outcomes following their systemic administration. While antibodies can have high specificity, the doses required to treat patients, particularly for a chronic condition, are typically large. Fortunately, advances in production and purification capacities have allowed for the exceptionally large amounts of highly purified monoclonal antibodies to be produced. Additionally, genetic engineering of antibodies has provided a stable of antibody-like proteins that can be easier to prepare. Together, these advances have made antibody-based therapies one of the most commonly pursued pharmaceuticals in biotechnology pipelines. With this success, however, has come a series of technical challenges in the formulation of antibody-based materials to maintain sufficient stability in a variety of configurations and sometimes at particularly high concentrations. This review focuses on issues related to identifying and verifying stable antibody-based formulations.
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Keywords: Antibody; Stability; Delivery; Formulation

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1. Introduction

Therapeutic and diagnostic antibodies have become the fastest growing area of biopharmaceutical applications. There are now 18 monoclonal antibodies on the market and over 100 in clinical trials, which highlights the significance of these therapeutics and the advances made in antibody engineering. Further, by 2008, engineered antibodies are projected to be the source of over a third of the revenues from biotechnology [1,2].

A few seminal events have led to the current and projected prominence of antibodies as biopharmaceuticals include the identification of methods to generate murine monoclonal versions of antibodies [3], the cloning of human antibody sequences to allow for humanization of murine monoclonal antibodies through complementary-determining region (CDR) grafting [4] and even the establishment of fully humanized systems to generate monoclonal antibodies [5]. With the sequential identification of these technological advances, antibodies for therapeutic and prophylactic indications have moved from fully murine, to partially murine (mostly human) to completely human constructions. Added to these events, dramatic advances in production have led to the ability to prepare monoclonal antibodies at scales that can provide sufficient material at costs that make this area appealing to pharmaceutical companies. One important outcome of these various advances is a greater potential to use antibody drugs in chronic settings, tremendously expanding their biopharmaceutical applications.

The majority of currently approved antibody drugs address previously unmet medical needs in the areas of cancer and inflammation. These indications came from practical considerations related to earlier forms of murine and partially murine antibody therapeutics; the first monoclonal to be approved (OKT-3®, muromonab-CD3) was approved in 1986 for a single treatment regimen to protect from life-threatening tissue rejection following kidney transplant [6]. Adverse reactions due to acute immunogenicity problems due to delivery of a non-self protein limited the use of OKT-3® to only acute application [7,8]. Subsequent partially murine antibodies such as Panorex® (17-1A), Zevalin® (ibritumomab tiuxetan) and Bexxar® (tositumomab) were approved for a limited number of administrations to cancer patients who had failed frontline therapies [2]. With the advent of fully humanized antibodies, chronic therapeutic or prophylactic applications for infectious agents have become a realistic clinical option [2]. Another critical aspect to the success of antibodies as therapeutic agents involves improved methods to express, purify and characterize these proteins [9]. In general, antibody therapeutics are

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large (typically >150 kDa), complex in nature (most are glycosylated) and must be administered in stoichiometric rather than catalytic quantities (nearly a gram per dose is required for many antibodies to be effective). Production and purification scales have thus reached levels of production that were previously assumed impossible in biotech companies [10]. Novel concerns then related to the development of stable formulations and delivery strategies for such large amounts of a complex molecule. Understanding product instability with regard to physicochemical and thermodynamic events becomes more and more critical to ensure market success [11]. The required high concentrations of antibodies in these formulations reduced the need to add a carrier protein, as is the case where serum albumin is commonly added in formulations of highly potent proteins such as erythropoietin alpha (Epogen®) and Factor VIII (Kogenate®) to enhance stability and reduce loss to vial surfaces. Simultaneously, the large amounts of an antibody protein used in these formulations allowed degradation products to be more readily detectable particularly when using the highly sensitive methods designed for evaluating hormone biotherapeutics.

Finally, the ability to prepare large quantities of an antibody that could be administered for chronic therapy resulted in a plethora of new and novel applications [9,10] involving the delivery of chemotherapeutics, radionuclides and imaging agents — to name a few. New issues, related to identifying stable formulations such as high concentration formulations that might reduce the volume of antibody administrations, arose in the move from intravenous infusions to the realm of subcutaneous injection strategies. In some cases the combination of agents with antibodies produced conflicting stability profiles. Additionally, potential new indications for antibody drugs increased the desire to find novel methods for sustained release, intracellular targeting [12] or delivery via methods not involving an injection. Thus, as is the case in most scientific advances, technological advances opened up possibilities for therapeutic opportunities that were not possible previously as well as a totally new set of problems that required solving. In this review we have attempted to identify the major issues associated with formulating and delivering antibody drugs, to recognize challenges already identified accompanied when possible by solutions, as well as to discuss concerns that will need to be addressed in the future as the use of antibody drugs continues to evolve.

2. Antibody characteristics relevant for formulation and delivery

The human body produces several classes of antibodies that appear to perform specific functions under different conditions and at discrete anatomic sites. Most of the currently approved antibody-based drugs are of the IgG1 class [13] and are glycosylated on the Fc (fragment crystallizable) domain. Glycosylation has been shown to participate in specific cell response events mediated by antibodies [14–16]: antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cell-mediated cytotoxicity (CDCC) through receptor recognition events. Individual glycoforms of antibodies can provide improved efficacies for certain clinical outcomes [17]. In general, IgG1 and IgG3 antibodies are effective at initiating ADCC while IgG2 readily fixes complement but is poor at inducing ADCC. IgG4 appears to be inefficient at both ADCC and CDCC [13]. Interestingly, antibodies can be re-engineered to increase their capacity to recruit complement [18] or to adjust their affinities to the various classes of Fc receptors and thus ADCC function through mutation of specific amino acids within the Fc domain [19]. Molecular engineering has also provided a spectrum of new antibodies (Fig. 1). Other chapters in this issue delve deeply into the many characteristics of antibodies that make them remarkable and diversely applicable treatment agents: ligand binding properties, effector functions and potential for modification in their design. Other chapters will also address applications of newly engineered antibody-like molecules that move beyond the realm of intact, full-length IgG antibodies. In the current review, attention will be focused primarily on IgG1 since this is where the majority of published information exists for antibody drug formulation and delivery.

Antibodies, due to their large size (being greater than the filtration cut-off size of the kidney) and their ability to be sequestered for periods of time from the blood, via interactions with FcRn receptors present on

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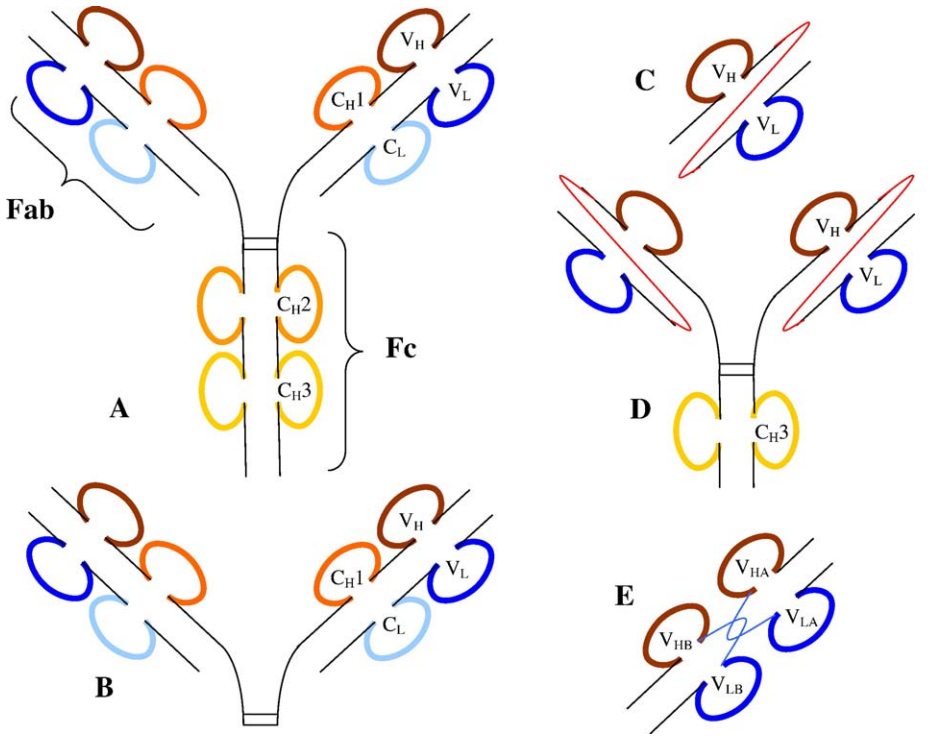

Fig. 1. General antibody structure and some antibody-based protein constructions. A) Human antibodies have a common framework that can be described as Fc (fragment crystallizable) and Fab (fragment antigen binding) domains that are derived from the folded structure of heavy (H) and light (L) chain proteins. Multiple constant (C) regions on both the H and L chain proteins are donated as are the variable (V) segments involved in antigen-specific recognition. B) Removal of the Fc domain produces a F(ab)2 structure. C) Single chain structures of the antigen binding region (scFv) can be prepared by themselves or D) in a construct that includes Fc domain elements. E) Bi-specific antibody elements (diabodies) can be prepared to bring two different antigens into close proximity. C_H, heavy chain constant regions; C_L, light chain constant region; V_H, heavy chain variable region; V_L, light chain variable region.

endothelial cells, can have extended serum profiles in the range of weeks. A number of engineered antibody-like molecules are now being examined for therapeutic applications [20]. Some engineered fragments, such as diabodies and minibodies, are much smaller than intact, full-length IgG1 and have very different properties of systemic distribution and clearance. Additionally, methods to prepare other antibody types such as dimeric secretory IgA [21] and methods to prepare antibodies using non-mammalian cell systems, such as plants [22], from the eggs of chimeric chickens [23] and from transgenic animals [24], are now being explored by academic and industrial groups. As these new approaches gain prominence, more information will be acquired about their unique requirements for successful formulation and delivery. At present, however, there is little information on formulation approaches for antibodies produced using these production processes.

3. General concerns for antibody formulations

Due to their proteinacous nature, antibodies present generic formulation issues that are similar for most protein therapeutics [11]. Proteins, in general, can be degraded under conditions when they are exposed to heat, freezing, light, pH extremes, agitation, sheer-stress, some metals and organic solvents. With ever advancing analytical technologies, the ability to assess the physicochemical and thermodynamic instability of antibody drugs has also led to the identification of several events that are more specific to the unique nature of this particular class of proteins: variations in

Fc glycosylation, partial heavy chain C-terminal Lys processing, Fc methionine oxidation, hinge-region cleavage and glycation of Lys residues [25]. In addition to these issues is the difficulty of preparing dosing materials that might have protein concentrations in the range of 100 mg/ml or greater. Since early preclinical assessment is so critical to successful identification of a new biotherapeutic product, general pre-formulation strategies are usually employed for initial studies with new antibody drugs. An initial formulation effort will likely employ an isotonic, non-hemolytic, slightly acidic solution that can be administered by intravenous infusion or subcutaneous injection in studies employing a relevant animal model. In some cases, some stability information for the antibody drug can be obtained from initial in silico and in vivo testing during this pre-clinical phase that aids in the selection of a formulation that is used in clinical trials [26].

Since most early pre-clinical studies attempt to emulate the delivery strategy that will be used in the clinic, initial studies frequently identify problems with either the initial pre-formulation approach and/or stability issues with the antibody drug itself. In the case of the formulation, protein precipitation can occur with refrigerated storage or with repeated freeze–thaw cycles; events that may be facilitated by an inappropriate pH or buffer selection. Analysis of antibody drugs stored in this initial pre-formulation system may also identify unstable sites within the protein or its linkage to attached molecules (chemotherapeutics, imagining agents, etc.). In the case of the antibody, this information is frequently evaluated to determine if re-engineering of the protein could be performed, for example, to provide greater thermodynamic stability or folding efficiency [27]. Such changes in the Fc region are typically conservative, but might act to modify Fc receptor binding properties to provide a different biological outcome [28,29]. Amino acid substitutions can also be made within the Fv region to improve thermal stability [30], but such changes might also alter antigen binding specificity [31].

4. Stability issues for antibody formulations

If one examines the amino acid sequences for currently approved IgG1-based antibody drugs, it becomes quite clear that only a small segment of these proteins are dramatically different from one another, being the Fv segments that are involved in antigen binding. It should be pointed out, however, that there are some minor differences in conserved regions as well. A priori, one might assume that by finding a stable formulation for one of these antibody drugs, that such a formulation would be good for most if not all, similar antibodies. If this were borne out by experience, there would be no need for a review such as this. Instead, each antibody seems to have a unique personality related to its requirements for stability; a phenomenon derived from the fact that the small differences between these antibodies are focused on surface-exposed amino acid differences that stipulate antigen specificity. Thus, the interfacial surface of each antibody drug is unique and thus requires specific formulation components to provide maximal stability and retention of activity.

Probably the most important aspect of identifying a successful antibody drug formulation is in the identification of truly detrimental alterations rather than all of the modifications that might occur. Although, from a regulatory standpoint, it may be beneficial to understand all of the potential degradation events associated with an antibody formulation, it is the recognition of a stability-indicating parameter (or several parameters) that allows the formulation scientist to focus in on what is critical to development of a successful product. Here, a series of physico-chemical and bioanalytical assays can be used to identify these parameters [32]. Although in vivo bioassays may ultimately be required to validate a stability-indicating formulation parameter, the most critical tools at the disposal of the formulation scientist are rapid and reproducible assays to measure protein changes due to oxidation, deamidation, aggregation, fragmentation and other forms of chemical modification that could lead to a loss of function of the antibody drug.

4.1. Oxidation

Methionine and cysteine residues are frequently a site of oxidation in protein drugs and this is also the case for antibody drugs [33]. In the case of antibody-based drugs, specific methionine residues within the Fc domain may be prone to oxidation, resulting in the

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production of methionine sulfoxide [25]. While cysteines are present in the Fc framework as disulfide pairs, unpaired cysteines in the variable region may also be sites of oxidation. Besides methionine and cysteine residues, oxidation of histidine, tyrosine, tryptophan and phenylalanine residues can also occur [34]. A number of methods can be used to monitor oxidation of amino acid residues. As a starting point, the number of reactive protein thiols can be monitored using reagents such as 5,5′-dithionitrobenzoic acid or iodoacethyl dansylcadaverine [35], providing a simple readout on the number of protein thiols per protein. Total amino acid analysis can also be used to monitor reductions in methionine, tyrosine and phenylalanine residues. Tryptic digestion and peptide separation by liquid chromatography followed by mass spectroscopy analysis (LC-MS) can be used to identify specific sites of amino acid oxidation.

A careful study of methionine oxidation has been performed for the humanized monoclonal antibody that recognizes the her2/neu gene product. For this protein it was determined that a methionine at position 255 in at heavy chain of the Fc region was the primary site of oxidation and that a methionine at position 431 could also oxidize under more aggressive conditions. In this study, a chromatography assay was used to detect oxidation events following papain digestion to separate Fab and Fc segments of the antibody [36]. Another study on the stability of this same protein provides a valuable template for dealing with antibody oxidation: identification of a critical oxidation event, validation of a robust assay for monitoring that event and comparison of formulation parameters to determine conditions that facilitate oxidation. Addition of antioxidants or stoichiometric levels of free methionine or thiosulfate were found to reduce the extent of oxidation in formulations of this antibody [37].

Issues associated with administration of oxidized forms of an antibody may extend beyond concerns of reduced efficacy. Systemic lupus erythematosus (SLE) is a disease characterized by the induction of anti-double-stranded DNA antibodies. Studies have shown that increased levels of markers of protein oxidation, including those of methionine sulfoxide and 3-nitrotyrosine, correlated with SLE disease activity [38]. Additionally, oxidative modifications of antibodies isolated from synovial fluid samples collected from patients with rheumatoid arthritis suggested alteration of histidine, methionine, tyrosine and cysteine residues [39]. While no direct causal association is shown or implied, these observations bring attention to the issue that we do not fully understand the clinical impact of antibody oxidation events.

4.2. Deamidation

Glutamine and asparagine residues show a propensity for deamidation [40]. Both light and heavy chains of antibodies can undergo deamidation of these amino acids [41] and deamidation events are considered to be one of the major sources of charge heterogeneity of monoclonal antibodies [42]. Deamidation events appear to be highly selective events for individual antibodies. For example, analysis of several monoclonal antibodies that bind the her2/neu gene product demonstrated asparagine 30 deamidation on one or both light chains and asparagine 55 on the heavy chain [43]. Deamidation events lead to more acidic forms of the antibody through the acquisition of additional carboxylic acid groups. Conversely, aspartic acid residues can also undergo modification to a succinimide moiety that results in a basic antibody isoform of the antibody by removal of a carboxylic acid group [43]. In general, deamidation events appear to be relatively slow but are accelerated by unfolding of the antibody [44]. Initial detection of deamidation in antibody preparations is typically identified by differences in charge distribution or content using methods such as isoelectric focusing (IEF) or high-performance cation-exchange chromatography [43].

High performance chromatography can then be used to separate succinimide, isoaspartic and aspartic acid isoforms of tryptic digest fragments of the antibody which can be unambiguously characterized by tandem mass spectroscopy (MS). Correlation between tryptic digest tandem MS results and some other more readily accessible method, such as IEF, can provide a rapid format to follow antibody deamidation events. Using these techniques, asparagine deamidation events residues appears to occur more frequently than glutamine deamidation and specific asparagine residues appear to be selectively targeted for this event [33]. Deamidation of asparagine is frequently associated with rearrangement

events that lead to the formation of isoaspartic acid [42] and this outcome is correlated to amino acids adjacent to the asparagine prone for deamidation [44] that provide a method for prediction of potential deamidation sites (BOX). In particular, asparagine–glycine sequences can demonstrate accelerated deamidation rates since glycine residues lack a side-chain structure that would otherwise act to decreased conformation interference for backbone-associated isoaspartyl formation [45].

Box 1

Amino acid* sequences predicted to deamidate
SNG, LNG, LNN and ENN
(Note these have a following amino acid of G or N)
and those that are less prone to deamidation
GNT, GNS, GNV, TNY, SNY, SNF, SNT, SNK, SNL,
WNS, WNS, FNW, FNS, FNR HNA, HNH, LNW,
CNV, NNF, DNA, and YNP
*standard single amino acid letter nomenclature.

Non-enzymatic parameters of asparagine and glutamine deamidation in proteins have been defined in detail for factors such as pH, temperature and ionic strength [46,47]. The primary sequence dependence of these deamidation events can be explained by a simple steric and catalytic model [46,47]. Prediction of deamidation events based upon three-dimensional structures have also been described but the data set for these studies is not yet sufficient to produce a general model [48]. That asparagine and glutamine deamidation appear to be a constant phenomenon of proteins and correlate with their turnover provides the basis for the hypothesis that the presence and distribution of these amino acids within a protein can act as a molecular clock for its biological turnover [49,50]. For the formulation scientist, empiric assessment of factors such as pH and ionic strength provide the best strategy to identify a formulation that minimizes deamidation events. In general, deamidation does not result in a decrease in potency and deamidation may be hard to detect as the charge difference is small. At times, however, there can be a detrimental effect on potency through the introduction of an unfavorable negative charge that can have further ramification on biological function, particularly when deamidation occurs in the binding regions [51]. As particularly reactive residues are identified, it may be possible to have the antibody engineered to remove the problematic amino acid or to change adjacent residues to reduce its reactivity.

4.3. Aggregation

Liquid formulations of protein therapeutics frequently exhibit a decrease in concentration due to adsorptivity to container walls at low concentration and can show aggregation events at high concentrations. Initial preparations of antibody drugs were administered in acute situations by IV infusion that could allow for medium protein concentration (1–10 mg/ml) formulations not dramatically affected by adherence or aggregation losses. As antibody-based drugs became more widely used in chronic care, high concentration formulations that would allow for clinically acceptable SC injection volumes became desirable and aggregation became an important concern for designing formulations. Along with aggregation events, protein solutions at concentrations > 50 mg/ml may become sufficiently viscous as to no longer readily pass through the gauge of a needle that would be comfortable for patients' use. Some antibodies demonstrate high concentration viscosity issues while others do not. For example, the anti-respiratory syncytial viral antibody Synagis® is formulated at 100 mg/ml protein and is administered successfully by IM injection. Thus, addressing viscosity, adsorptive loss and aggregation in any specific antibody formulation will likely be performed on a case-by-case basis.

As protein–protein contact frequency increases at high concentrations, the opportunity for stable aggregate formation increases proportionally. Proteins are folded in such a way as to internalize hydrophobic domains and surface-expose more hydrophilic domains in a thermodynamically stable situation. As folded proteins undergo normal molecular motion transient surface exposure of hydrophobic domains can lead to protein–protein contacts that hide these

surfaces in a more energetically favorable manner. The outcome of these events is the stabilization of these protein–protein contacts with the ultimate outcome of dimerization, trimerization and so on — leading to extended aggregation events [52]. As protein concentration and temperature goes up, so does the probability of energetically favorable contacts leading to aggregation with increased frequency and duration of reversible protein–protein contacts leading to a greater extent of stable (even irreversible) aggregation events. Aggregation (covalent, non-covalent, dissociable, non-dissociable) must be considered a likely degradation event for high concentration antibody formulations.

For some antibodies reduced temperatures can incite reversible self-associations; these molecules are known as cryoimmunoglobulins [53]. IgG3 cryoimmunoglobulins have been shown to self-associate through sugar structures of the Fc domain [54]. Self-association events involving IgG1 antibodies seem to occur primarily through weak ionic and hydrophobic interactions [55]. More recent studies have examined kinetic and thermodynamic aspects of antibody solution dimerization using a recombinant human monoclonal antibody that recognizes vascular endothelial growth factor (rhMab-VEGF) as a model [56]. It was found that for this IgG1 class antibody, aggregation rates were greater in slightly alkaline (pH 7.5–8.5) compared to slightly acidic (pH 6.5–7.5) conditions. A high salt environment (1 M NaCl) also enhanced dimerization. Not surprisingly, self-association events were accelerated as the storage temperature was increased. One of the most striking aspects of these dimerization studies on rhMab-VEGF is that trastuzumab, another geneticall-ly engineered antibody with the same IgG1 framework and a 92% sequence homology with rhMab-VEGF, fails to demonstrate aggregation events under the same conditions.

A number of additives have been identified that can reduce the rate of protein aggregation [52,57]. A rather wide spectrum of agents can reduce protein aggregation rates: urea, guanidinium chloride, amino acids (in particular glycine and arginine), various sugars, polyalcohols, polymers (including polyethylene glycol and dextrans), surfactants and even antibodies themselves. Studies have shown that different proteins benefit from different types of anti-aggregating factors, consistent with the notion that the interfacial surfaces that drive interactions leading to aggregation are protein-specific. Conceptually, anti-aggregating agents might fall into one of two categories: small agents that fit into channels or grooves and large agents that might interact with lower-curvature surfaces of the protein. In essence, the small agents would act to stabilize a protein from acquiring a conformation that might be more reactive toward aggregation while a large agent might act to reduce the frequency of surface contacts that lead to an aggregation event. In the case of antibody formulation, either or both may be important. As we will discuss, agents found to effectively protect an antibody from aggregation may differ for various stress conditions; antibodies in solution that are undergoing agitation and thermal stress may differ from those of the same antibody as it undergoes lyophilization and reconstitution.

Protection from aggregation can be limited in antibody formulations by the addition of surface-active agents such as detergents, both charged and uncharged. As an interesting aside, such surface-active agents can help reduce protein loss due to adherence to surfaces that can occur with low concentration protein formulations. Polysorbate 20 and 80 (now available from a vegetal source) are examples of detergents that are widely used to reduce the rate of protein aggregation in high concentration antibody drug formulations. A precautionary note must be sounded, however, as detergents perform this function optimally at low concentrations (0.01% to 0.05%) and can act detrimentally to participate in protein unfolding at higher concentrations, e.g., 1%.

That antibody aggregation can be accelerated by several physical conditions provides the potential for multiple methods to determine if a particular antibody drug is prone to aggregation and to rapidly test formulations for their ability to impede aggregation. Agitation studies are done, in addition to thermal stability studies, to determine if aggregation can occur in a given formulation. Different test methods are used, employing a shaker or a stirring apparatus. In one study done on an IgG1 antibody found sizes of aggregates produced by the two methods were comparable [58]. They concluded that a higher throughput test could be constructed using stirring analyzed by absorbance measurement. In their work,

the stirred samples exhibited much higher absorbance and second particle species in DLS, indicating that stirring stress produces a higher amount of smaller protein aggregates. While Mahler found polysorbate 80 protects against aggregation, the stirred samples had an increase in turbidity and more aggregate products were detected by DLS as compared to a surfactant-free formulation. Polysorbate 80 stabilizes small aggregates and prevents further progress in the aggregation process.

4.4. Fragmentation

Intact, full-length antibodies are composed of several segments that have natural flexure sites. One site, in particular, exists at the flexure point between the Fc and Fv domain. Cleavage at this hinge region is a common concern when assessing antibody drug stability [25]. Molecular sizing methods such as sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and size-exclusion high performance liquid chromatography (SE-HPLC) can be used to rapidly identify fragmentation events in antibody drugs [59]. Fragmentation of full-length antibody drugs is a common occurrence and invariably affects their function. In general, cleaved forms are present in such low amounts that it is likely that little change in efficacy would be seen [60]. At the very least, the antibody drug may have some small reduction in efficacy following fragmentation or may be more prone to proteases, degradation and clearance events that would limit the duration of its efficacy. At the worst, a fragmented antibody preparation may result in a different biodistribution and thus a different safety profile. This can be of particular concern if the antibody is being used to delivery a cytotoxic chemotherapeutic agent. Fortunately, the extent of fragmentation in an antibody can be easily limited in the formulated product.

5. Strategies to stabilize antibody formulations

While liquid antibody formulations are less expensive, faster to develop and generally easier to prepare for administration than alternative formulation approaches, liquid antibody formulations are prone to oxidation, deamidation, aggregation and fragmentation as discussed above. In each of these events, water is the common culprit: water mediates electron transfer during oxidation and deamidation events and its required addition across a peptide bond can be a critical step in fragmentation. The thermodynamic stresses that lead to protein aggregation are caused by hydrophobic protein surfaces being exposed to water and trying to find a lower energy state by non-native protein–protein interactions. Thus, water is a big problem for stabilizing antibody-based drugs. Lyophilization or introduction into hydrophobic polymer systems can reduce the impact of water on antibody drug formulations.

5.1. Lyophilization

Although some antibodies have demonstrated a propensity to undergo precipitation as they are cooled from 37 °C [53], antibodies are typically robust to freeze/thaw cycles [61]. Early studies reported that antibodies were stable to the freeze-drying (lyophilization) process in the absence of excipients that function as cryoprotectants [62], however, with the technical advancement of certain analytical methodologies, became evident that antibodies are generally damaged in some way as a result of lyophilization as demonstrated by extensive formation of insoluble aggregates [61]. More recently, studies have shown that freeze-drying antibodies to a specific percent of residual water (usually between 1 and 8%) allows for optimal stabilization in the dry state and stability during reconstitution [63]. Higher moisture content in lyophilized antibody preparations correlates with increased aggregation (as seen by SEC) and asparagine deamidation and isomerization (as measured by HIC). Studies have also been performed to identify optimized lyophilization schedules where the secondary drying phase is performed at an elevated temperature [64]. This technique allows the lyophylized cake to achieve optimum moisture content, allowing the final product to be conformationally stable (as measured by DSC) during extended storage. Solid-state structural studies can be used to monitor conformational changes and aggregation events during the storage of a lyophilized monoclonal antibody preparation [65]. Improved native-like structure and a reduction in aggregation can be obtained by incorporation of a carbohydrate excipient in sufficient

quantities to fulfill the hydrogen bonding requirements on the protein surface, suggesting a critical role for non-water molecules to act as placeholders for water molecules during drying and in the dry state. That the addition of excess carbohydrate failed to improve the stabilizing function of this excipient, reaffirms the conclusion that antibodies have a limited number of sites that must be protected in this fashion [65]. A variety of carbohydrates or polyol compounds such as sucrose, trehalose and mannitol have been shown to provide this stabilizing function [66,67].

The rate of rehydration when water is reintroduced during reconstitution of lyophilized formulations is a critical parameter. As water is removed during the different drying stages of the lyophilization cycle and replaced by a shell of non-water excipients, the antibody drug can undergo structural modifications that result in a non-native conformation as may be seen by differential scanning calorimetry [63]. If the rate of rehydration is sufficiently slow to allow recovery of native conformation as water replaces non-water excipient molecules, reconstitution will typically provide a satisfactory outcome for the antibody. If, however, the antibody does not have sufficient time or capacity to recover its native state during reconstitution, extensive aggregation can result. Efforts to protect monoclonal antibodies from the rigors of freeze-drying and reconstitution have been aided by a number of analytical methods that can examine protein modifications. In the solid state, circular dichroism (CD) and Fourier transform infrared (FTIR) spectroscopy may be used to evaluate the protein; after reconstitution in the liquid phase, SEC or SDS-PAGE have been utilized to gather information from lyophilization studies [68,69]. A microscopic apparatus to visually monitor freeze-drying events has even been described [70]. Excipients that have been reported to slow the reconstitution rate and thereby preventing such changes in the rehydrated antibody are glycerol [71,72] and polymeric materials such as polyethylene glycol [73].

Pharmaceutical scientists frequently use increased temperatures for accelerated formulation stability studies. Although such studies can be used to assess formulation parameters such as pH and ionic strength, antibodies lyophilized in the presence of a cryoprotective sugar excipient provide a more complicated picture. Lyophilized amorphous solids composed of a monoclonal antibody and a sugar cryoprotectant material such as sucrose or trehalose form glassy structures, liquids that are too viscous to flow. That antibody-excipient complexes exhibit a glass transition temperature (Tg) provides the basis for observations showing that these materials have unique properties above and below this value. Thus, in predicting shelf life in accelerated degradation studies, it is important to use testing temperatures that do not exceed the Tg threshold of the complex [67]. In general, lyophilized formulations containing trehalose may likely show less tendency toward antibody aggregation than those with sucrose, since trehalose preparations have a higher Tg [67]. With this in mind, a promising approach to assess secondary structural parameters in evaluating antibody formulation stability is the use of amide I band Raman spectroscopy. This method demonstrates a good correlation between structural protein perturbations immediately after lyophilization and the rate of aggregation during long-term storage under accelerated conditions [74].

5.2. Polymer delivery systems

Removal of water from antibody preparations, either by lyophilization or spray freeze-drying, provides a provides a starting point for incorporation of this dried material into a hydrophobic polymer matrix such as polylactide-co-glycolide (PLGA) that can be made into microspheres generated through a solid-in-oil-in-water (S/O/W) encapsulation process [75]. Although both mannitol and trehalose can stabilize human IgG during spray freeze-drying, the double-emulsion solvent evaporation method used to load spray freeze-dried human IgG into PLGA microspheres has been shown to produce extensive antibody aggregation [75]. Such an outcome is likely the result of thermodynamic stress experienced at the water/organic solvent interface and a variety of process methods and excipients can be use to minimize this concern [76].

One feature of PLGA microparticle preparations is their recognition by immune elements and their capacity to stimulate immunization against incorporated proteins [77–79]. While this has clear advantages for vaccines, it poses a serious concern for therapeutic protein delivery. Hydrophobic microparticles have been reported to incite local inflammation

at subcutaneous injection sites [80], events that may be partially mediated through macrophage recruitment [81]. Not only is the potential for antibody formation increased with local inflammation, the resulting injection site reaction may be clinically unacceptable.

In spite of some serious caveats, polymeric microspheres and delivery systems can be successful antibody formulations if administered to the appropriate sites, using certain types of polymers or preparing microspheres or polymeric formulations by methods that are more protective of the antibody. An example of a certain delivery site that may have less proclivity towards an inflammatory response is reported, where PLGA microspheres loaded with an anti-VEGF antibody were injected into the intravitreal humor of the eye, an immune-privileged site, for the treatment of age related macular degeneration (AMD) [82]. Resorbable polymers that show less potential to incite inflammation, e.g., have smoother surface characteristics, might be used to deliver antibodies in a sustained manner. Direct, local delivery of antibodies using carboxymethylcellulose aqueous gels can be an effective post-surgical anti-infective strategy [83]. Antibodies covalently coupled to a biodegradable hyaluronic acid hydrogel through a labile hydrazone linkage have been used for the sustained delivery of antibodies to sites within the central nervous system [84]. Introduction of dry powder IgG into ethylene–vinyl acetate copolymer (EVAc) has also been examined as a potential polymer-based antibody delivery system [85]. Similar to PLGA systems, EVAc microspheres can extend the time course of IgG release. This property makes polymers loaded with antibodies interesting opportunities for injectable sustained release delivery formats but also for topical application at mucosal surfaces. For example, long-term vaginal delivery of an antibody can be achieved in a mouse model using polymer vaginal rings [86,87]. Formulations of a biomedical grade polyurethane hydrogel with an antibody coating have also been prepared and shown to release bioactive protein [88]. It is important to note that one complicating aspect of many of these approaches is the requirement of a polymer solubilization step using a solvent, such as methylene chloride or isopropanol, which can compromise protein drug stability. Sustained release formulation approaches such as microspheres prepared using water, such as the PROMAXX microspheres (Epic Therapeutics) or crystallization of proteins [89] can bypass the use of solvents that are incompatible with proteins and antibodies.

6. Formulation issues for alternative forms of antibody delivery

To date, antibody development has focused primarily on injectable routes of administration: intravenous (IV), intramuscular (IM) and subcutaneous (SC). A long history of IV antibody infusions exists [90] and the advantages are clear for antibody-based drugs that are used in cancer treatments in order to reach tumor sites that may be diffuse or not otherwise accessible. In fact, the majority of the approved antibody-based drugs are administered by IV infusion (Table 1) with the total dose administered approaching one gram of protein per treatment in some cases. For many antibody-based therapies a critical safety concern involves the onset of acute or delayed systemic reactions following infusion [91,92]. Parameters and characteristics of the “infusion reaction” as well as treatment protocols have been well described with acute events commonly occurring within 10 min to 4 h and delayed events occurring 24 h to 4 days post dosing [93]. Although formation of antibodies to the administered antibody drug appear to be a risk factor for infusion reaction events [94], infusion reactions can occur following the initial exposure [93] and these reactions do not appear to be due to IgE responses or mast cell activation [95]. Most importantly for the patient, there appear to be treatment protocols to provide re-treatment of patients even after an infusion reaction event [93] with concomitant administration of immunomodulation molecules [94]. There are many unanswered questions about the mechanism and prevention of adverse infusion reaction events associated with IV infusion of antibody drugs. Certainly, one method is to explore IM and SC delivery when possible, although these delivery approaches may also have confounding aspects of tissue response and volume limitations.

Other routes are also amenable to antibody delivery. The increased number of antibody therapeutics on the market and the greater exploration of non-life threatening indications being treated by antibodies, drive efforts to deliver these agents by routes other than IV

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Table 1
Formulations of approved products

Antibody product; company	Generic name; description	Delivery route	Formulation concentration	Buffer components; pH[a]	*Excipients*
Othroclone OKT3®; J & J-Ortho Biotech	Muromonab-CD3; Murine IgG2a, anti-CD3	IV	1 mg/ml	Na,K phosphate; pH 7	NaCl, polysorbate 80
Reopro®; Centocor-Lilly	Abciximab; Chimeric IgG1, anti-GPIIb/IIIa, Fab	IV	2 mg/ml	Na phosphate; pH 7.2	NaCl, polysorbate 80
Rituxan®; Idec-Genentech	Rituximab; Chimeric IgG1, anti-CD20	IV	10 mg/ml	Na citrate; pH 6.5	NaCl, polysorbate 80
Zenapax®; Roche	Daclizimab; Humanized IgG1, anti-CD25	IV	5 mg/ml	Na phosphate; pH 6.9	NaCl, polysorbate 80
Simulect; Novartis[b]	Basilixamab; Chimeric IgG, anti-CD25	IV	4 mg/ml	Na,K phosphate, glycine	NaCl, sucrose, mannitol, polysorbate 80
Synagis®; MedImmune[b]	Palivizumab; Humanized IgG1, anti-RSV	IM	100 mg/ml	Histidine, glycine	Mannitol
Remicade®; Centocor[b]	Infliximab; Chimeric IgG1, anti-TNFα	IV	10 mg/ml	Na phosphate; pH 7.2	Sucrose, polysorbate 80
Herceptin®; Genectech[b]	Trastuzumab; Humanized IgG1, anti-HER2	IV	21 mg/ml	Histidine; pH 6	Trehalose, polysorbate 20, benzyl alcohol[c]
Mylotarg®; Wyeth[b]	Gemtuzumab ozogamicin; Humanized IgG4, anti-CD33, immunotoxin	IV	4 mg/ml	Na phosphate	NaCl, sucrose, dextran 40
Campath®-1H; Millennium-ILEX	Alemtuzumab; Humanized IgG1, anti-CD52	IV	Unclear — 30 mg /vial	Na,K-phosphate pH 7	NaCl, KCl, NaEDTA, polysorbate 80
Zevalin®; Biogen Idec	Ibritumomab tiuxetan; Murine IgG1, anti-CD20; radiolabeled (^{90}Y or ^{111}In)	IV[d]	1.6 mg/ml	Na Acetate, Na, K-phosphate pH 7	NaCl, human albumin
Humira®; Abbott	Adalimumab; Human IgG1, anti-TNFα	IV	50 mg/ml	Na phosphate, Na Citrate; pH 5.2	Mannitol, polysorbate 80
Xolair®; Genentech[b]	Omalizumab; Humanized IgG1, anti-IgE	SC	125 mg/ml	Histidine	Sucrose, polysorbate 20
Bexxar®; Corixam-GSK	Tositumomab-I131; Murine IgG2a, anti-CD20, radiolabeled (^{131}I)	IV	14 mg/ml	Na phosphate; pH 7.2	NaCl, maltose
Raptiva®; Genentech	Efalizumab; Humanized IgG1, anti-CD11a	SC	100 mg/ml	Histidine; pH 6.2	Sucrose, polysorbate 20
Erbitux®; Imclone Systems	Cetuximab; Chimeric IgG1, anti-EGFR	IV	2 mg/ml	Na phosphate; pH 7.2	NaCl
Avasatin®; Genentech	Bevacizumab; Humanized IgG1, anti-VEGF	IV	25 mg/ml	Na phosphate; pH 6.4	Trehalose, polysorbate 20
Tysabri; Biogen Idec	Natalizumab; Humanized IgG4, anti-4α-integrin	IV	20 g/ml	Na phosphate; pH 6.1	NaCl; polysorbate 80

Ordered by approval dates in the US.
Abbreviations: RSV=respiratory syncytial virus; TNFα=tumor necrosis factor alpha; EGFR=epidermal growth factor receptor; VEGF=vascular endothelial growth factor; NaEDTA=sodium edentate.
[a] Some lyophilized formulations do not stipulate a pH (or range) following reconstitution.
[b] Lyophlized product, concentration, pH, etc. values are obtained after reconstitution.
[c] Added as a preservative to generate a multi-dose vial configuration.
[d] Kit where components are mixed just prior to administation.

infusion. These routes may be more effective, less invasive, provide easier delivery, permit self-administration and thereby be more convenient and possibly have lower costs if less active agent can be used, as in the case where the alternate route of administration delivers the therapeutic agent to the intended site directly. Interestingly, some studies have suggested that antibodies administered by IV infusion are more

susceptible to proteolysis than via IM injection [59] and sucrose-stabilized immunoglobulins have been associated with acute renal failure following IV infusion [96]. The antibody used to protect neonates from respiratory syncytial viral (RSV) infection (Synagis®) is one example of an antibody administered by IM injection. For reasons discussed above, the majority of antibody-based drugs have moved toward SC delivery with formulations efforts being focused to this end. Based upon the desired actions for a number of antibody-based drugs, however, routes of administration other than IV, IM or SC may be desirable. These alternative routes can provide unique formulation and stability issues.

6.1. Oral

Uptake of antibodies from the intestinal lumen can occur through fetal Fc receptors (FcRn) expressed at the apical surface of enterocytes [97]. This natural antibody uptake mechanism was initially identified in the gastrointestinal (GI) tract of neonatal rodents and its expression shown to vanish following "closure" of the intestine after weaning. It was only recently that the continued expression of this receptor in the adult intestine was demonstrated and this finding has prompted efforts to examine this receptor system as a means of delivery antibodies after oral administration. Although this pathway appears to be a viable route for the delivery of therapeutic antibodies, issues associated with antibody stability in the GI tract [98] and competition from endogenous antibodies can dramatically reduce the efficiency of uptake after oral delivery. Outside of this specific mechanism, no other transcellular mechanism of efficient antibody transport across epithelial cell of the GI tract has been demonstrated to date. Topical application of antibodies to produce a desired clinical outcome have been shown, however, with the oral delivery of a chicken-derived (IgY) antibodies for treating and preventing infectious diseases of the GI tract [98–100]. It should be noted that IgY antibodies have been shown to not bind to (or be transported by) FcRn [97].

6.2. Respiratory tract

Intranasal and pulmonary administration of protein therapeutics has shown more promising clinical outcomes than oral delivery. While the surfaces of both the respiratory tract and the gastrointestinal tracts are readily accessible, the respiratory tract is a far less hostile environment for an administered protein therapeutic. Nonetheless there are physical clearance mechanisms in place to remove foreign particles which act to reduce residence time dramatically following administration. In the case of nasal administration the use of a cream emulsion and a polymer delivery system has been shown to significantly decrease the clearance rate for a monoclonal antibody when applied to the nasal mucosa [101]. This study provides one approach for the topical application of a therapeutic antibody designed to provide clinical benefit at the mucosal surface. It does not, however, suggest that an appreciable amount of an antibody might be absorbed into the systemic circulation from this site. The nasal mucosa affords only a small absorptive surface area compared to the epithelial linings of the lung.

Aerosol delivery of antibodies to the lung has been explored as either a liquid or dry powder formulation, where in both cases the droplets or particles must be of sufficiently small size that they are respirable perhaps to the level of the deep lung but large enough that they are not readily exhaled or just impact the back of the throat [102]. The sizes of particles that provide this performance have a mass mean aerodynamic diameter and density such that they enter the alveoli and remain in the lung. This size window has been reported to be between 1 to 5 μm depending on the formulation used and in one case, far larger, 20 μm where the particles are dense (<0.4 g/cm^3) and highly porous [102].

Formulation efforts were made for producing fine dry powder particles of an anti-IgE monoclonal antibody (E25) in the size range desired for delivery to the lung [103]. These studies showed that sodium phosphate was capable of extending the usefulness of mannitol as a protective excipient in spray-dried antibody formulations and that mannitol can play a role both in maintaining protein stability as well as producing a suitable aerosol preparation. Further studies to assess carbohydrate excipients for spray-dried aerosol E25 formulations showed that excipient to protein ratios were critical. Degradation issues involved not only protein aggregation but also protein glycation or lactosylation, a protein modification

event where a reducing sugar can add covalently at a free amine [104]. The ability to formulate and delivery an antibody-based drug to the lung provides the potential for systemic delivery of that protein via uptake through the FcRn expressed at this mucosal surface. Elegant studies showing the distribution and function as well as the application of this transport route for systemic deliver of an Fc-containing protein chimera has been demonstrated [105,106]. Thus, the potential or systemic delivery of antibody-based drugs via the respiratory tract looks promising.

6.3. Miscellaneous delivery sites of the body

Unique aspects of antibody–polymer formulations designed for intravitreal injection [82] or topical vaginal delivery [86,87] have been discussed above. Additionally, antibody formulations using hyaluronic acid hydrogels for sustained brain delivery have been noted [84]. These methods focus on strategies to administer large amounts of an antibody at a specific site in order to provide a sustained level to improve efficacy and/or to minimize injection frequency. Other strategies have been described to deliver antibodies to miscellaneous sites throughout the body, For example, an anti-transferrin antibody (OX-26) has been described as a carrier for delivery of covalently attached therapeutic molecules across the blood–brain barrier following IV injection [107]. An anti-tumor necrosis factor antibody has been adsorbed onto cardiovascular stent wires for local neutralization of this cytokine in order to limit restenosis events following stent placement [108]. A CMC gel containing antibodies to prevent post-surgical infections has been administered to the intraperitoneal cavity [83].

6.4. Intracellular targeting

Some promising antibody drug targets are intracellular. Although techniques exist where a cell can synthesize an antibody and secrete it into the cell cytoplasm, successful use of this technology requires gene therapy. Instead, it is possible to chemically couple an antibody to a pH-sensitive polymer that functioned to disrupt membranes at acidic pH. Many antibodies are internalized by cells through a receptor-mediated uptake process that leads to their delivery into early and then late endosomes. During endosomal maturation after receptor–antibody internalization the pH of these vesicles will drop from near neutral to below pH 6. This approach was demonstrated in a format where the pH-responsive polymer poly(propylacrylic acid) was covalently attached to a monoclonal antibody to facilitate its delivery to the cytoplasm of target cells [12].

7. Other delivery issues

A wide range of novel drugs and drug conjugates are being developed using monoclonal antibodies and engineered antibody fragments [20]. For some of these novel therapeutic approaches to be successful, they require additional or non-traditional delivery strategies. A cursory discussion of some of these approaches as they relate to intracellular targeting, site placement and engineered antibodies fragments follows.

7.1. Antibody fragments

An entire spectrum of antibody fragments have been identified through protein engineering studies with many of these showing remarkable potential for unique and novel clinical opportunities [109]. These smaller recombinant antibody fragments, the monovalent antibody fragments, Fab and single chain Fv and the engineered variants, diabodies, triabodies, minibodies and single-domain antibodies are an emerging form of antibody therapy that are less costly to manufacture while still retaining the targeting specificity of full length antibodies. In fact some may have greater efficacy and more applications than whole monoclonal antibodies. It may be anticipated that this new subset of antibodies are posing new formulation challenges, however, to date, publications detailing stability studies and formulation development for these molecules are rare in the scientific literature so formulation data on antibody fragments is scant. The single V domains have been found to be poorly soluble and have a tendency to aggregate [109], but these problems are being overcome by the identification of mutants that minimize the hydrophobic interface and selected by phage display [110]. Five Fab molecules have been approved as of this writing with the one humanized

Fab (ranibizumab rhFab) awaiting approval in the very near future, but many are in early clinical trials and most in preclinical development. One study has been described that focused on Fab fragments. In general, the binding of an antibody to an effector protein does not necessarily inactivate that target's function [111]. When an antibody is effectively inactivating, however, the Fab fragments in the absence of an Fc domain can frequently function as highly potent binding agents capable of masking binding sites or enzymatic elements on macromolecules. For example, Fab fragments prepared from sheep anti-snake venom antibodies (Digibind, from GSK) can be an effective anti-venom. Storage of liquid formulations of such anti-venoms in sub-tropical countries where refrigeration may not be possible provides a significant formulation challenge. Although lyophilized products can perform well under these conditions, a pH 4.0 (acetate buffer) formulation was found to also be stable and potent for at least one year at room temperature [112], presumably room temperatures characteristic of the tropical and subtropical regions where the material would be used. Strikingly, this stable formulation did not require the presence of a protective carbohydrate excipient. To improve drug delivery, antibody fragments have been fused to many types of molecules, such as radionuclides, cytotoxic drugs, toxins, peptides and proteins, enzymes and liposomes [113]. An immunoliposome made with a Mab fragment is reported to deliver drugs to the brain as such a molecule is able to cross the formidable barrier of the blood brain barrier [114].

7.2. Immunoconjugates

Arming antibodies with materials useful for detection or cell killing has become a prominent approach to increase the utility of these proteins, reviewed by Wu and Senter, [20]. Antibodies provide a useful format for the production of radiopharmaceuticals for site- or tissue-specific localization using gamma scintigraphy [10,115] or for enhanced cell killing. There are currently three approved antibody–radionuclide drugs: two are murine radiolabeled Ab for B cell lymphomas ibritumomab tuixetan (^{90}Y, Zevalin) and tositumomab (^{131}I Bexxar) and the third is a humanized, gemtuzumab ozogamicin (calicheamicin, Mylotarg) for leukemia. The presence of a high energy emitting radionuclide, however, will compromise long-term stability of any antibody formulation; not only would the released energies act to damage the protein but there is strong preference to use short half-life isotopes for these procedures [116]. Thus, in these instances, a stable formulation based upon the principles discussed above can provide a product that can be radiolabeled and then used soon after this step. One example of this approach is a lyophilized antibody formulation that is reconstituted and prepared by a kit method prior to patient dosing [117].

Antibodies coupled with cytotoxins for targeted cell killing [118] are particularly useful in antibody-based cancer therapies [119]. Beyond the identification of a stable formulation for the antibody, such materials have several additional problems for the formulation scientist to solve: stability issues of the attached agent and stability of the linker used to join the attached agent to the antibody. In the case of a monoclonal antibody-vinca alkaloid conjugate, a pH range of 4.5–5.5 in a phosphate buffer showed improved solution stability compared to material stored at pH 6.5–7.4 as evidenced by antibody aggregation and hydrolysis of the chemical linker used to conjoin the alkaloid to the antibody [120]. In most cases, cytotoxic materials are coupled to antibodies using reversible chemistries to provide a release mechanism for maximal activity at the target site. Many of these antibody conjugates use a disulfide bond linkage that can be reduced by a novel redox enzyme present in endosomes and lysosomes of target cells [121]. Slightly oxidizing conditions that would provide added stability of this linker in the formulation could, over time, act to cause detrimental oxidation events in the antibody and/or the linked compound. Other conjugates use an acid-labile linker [122]. In this case, formulation pH must be restricted to either neutral or alkaline conditions. Enzymatic separation of antibody and a cytotoxic material could also be achieved by coupling the two through an amino acid linker that is recognized as a substrate by a specific protease or peptidase [123]. Formulation instability of this linker sequences can be corrected to some extent by selective changes of the amino acids used.

Thus, the challenges of immunoconjugates include maintaining both drug and antibody potency and stability. While some antibodies are efficacious by

themselves, many are much more therapeutically active after being armed with a toxin, drug and radionuclide [124]. These changes make the resulting product more difficult to prepare, stabilize and deliver. Yet, there is tremendous enthusiasm for these approaches as they provide the promise for some companies to transform their antibody into a potentially curative drug. For this reason many antibodies are combined with a generic delivery system, such as a liposome, that can be used to delivery a variety or even multiple agents simultaneously through antibody-directed targeting [125]. Examples of such an approach include studies performed using an antibody directed against the Her2/neu antigen to delivery liposomes containing a chemotherapeutic have been described [126], antibodies used to deliver cationic liposomes for the administration of nucleic acid material for gene therapy [127] and antibodies used in the targeted delivery of modified gelatin nanoparticles [128] that can be coupled through a disulfide linkage [129].

8. Conclusions — challenges and opportunities

As antibody-based drugs come to the forefront of therapeutics derived from biotechnology, they provide a range of new and exciting opportunities to treat a number of currently unmet medical needs. Successful clinical application of these novel agents requires the development of stable formulations that can be used for specific delivery methods. Antibodies, because of their endogenous nature, have built-in features that may pose problems for stability as biotherapeutics, that function in the normal clearance of these proteins from the body [40]. Thus, despite our best efforts to prepare stable antibody-based drug formulations, the ultimate stability of these products may be limited to inherent mechanisms of degradation pre-designed for the rate of clearance optimal for their function and turnover in the body. Protein engineering has led to significant advances in improving some of these parameters and the trend would predict that additional advances will be made in the near future (reviewed in [109]). Protein modifications can be engineered such that stability and efficacy can be improved in ways that cannot be achieved solely, if at all, with formulation alterations. While it is clear that more research is needed in the relatively new area of engineered antibody fragments for clinical use [109], they have great potential for diagnostic and therapeutic application.

In general, successful antibody formulation designs are simply the best compromise of minimizing several competing degradation pathways. One approach is to find a pH where the minimal amount of degradation events such as deamidation and oxidation will occur. Another is to identify a buffer system that can stabilize the formulation at that pH without damaging the antibody. One common tactic taken to reduce the rate of many of these chemical degradation events is to prepare lyophilized or freeze-dried antibody preparation but even these can be compromised by aggregation issues due to thermodynamic stress that occurs during freezing, drying and reconstitution. A number of protective excipients, mostly carbohydrates, can reduce the propensity of antibodies to aggregate during these stressful events. Another issue is to produce a formulation that is compatible with delivery — neither hypotonic — nor hypertonic solutions are well-tolerated upon injection. Added to this is the potential use of a surface active agent to help with liquid stability such as a non-ionic detergent at a low concentration [58] as well as the possible addition of preservatives required for multi-use systems [130].

The future of antibody based drugs is likely to include more complex systems than those currently approved for use in man. Possibly the most critical aspect of generating a pharmaceutically successful formulation for antibody based drugs is an understanding the critical aspects of stability for the protein or the materials associated with the protein. It has been known for some time that even a single amino acid change in the Fv region of an antibody can result in dramatic differences in antigen-binding specificity [31]. Also, alterations at specific amino acids in the Fc domain can dramatically change functional aspects of an antibody. Thus, these kinds of changes must be considered more important in the search for a successful formulation than those changes that do not appear to affect function. In the case of antibody or antibody-like agents being used to delivery a payload such as a toxin or chemotherapeutic, the stability of the material to be delivered and its continued association with the delivery antibody is crucial.

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