Formulation strategies in immunotherapeutic pharmaceutical products

Yajie Zhang, Robert O Williams III, Haley Oana Tucker

ORCID number: Yajie Zhang (0000-0001-5570-5436); Robert O Williams III (0000-0003-4993-6427); Haley Oana Tucker (0000-0001-7735-2862).

Author contributions: Zhang Y drafted the manuscript; Williams III RO and Tucker HO contributed in editing and modifying the paper.

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

Manuscript source: Invited Manuscript

Received: January 19, 2020
Peer-review started: January 19, 2020
First decision: April 12, 2020
Revised: May 5, 2020
Accepted: May 12, 2020
Article in press: May 12, 2020
Published online: May 24, 2020
P-Reviewer: Qin R, Trkulja V

Yajie Zhang, Robert O Williams III, Division of Molecular Pharmaceutics and Drug Delivery, College of Pharmacy, The University of Texas at Austin, Austin, TX 78712, United States

Haley Oana Tucker, Departments of Bioengineering and Molecular Biosciences, The University of Texas at Austin, Austin, TX 78712, United States

Corresponding author: Haley Oana Tucker, PhD, Professor, Departments of Bioengineering and Molecular Biosciences, The University of Texas at Austin, 1 University Station A5000, Austin, TX 78712, United States. haleytucker@austin.utexas.edu

Abstract

Development of immunologic-based biopharmaceutical products have strikingly increased in recent years and have made evident contributions to human health. Antibodies are the leading entity in immunotherapy, while chimeric antigen receptor T cells therapies are the advent of a novel strategy in this area. In order to enable antibody candidates or cells available as products, formulation is critical in terms of stabilize molecules or cells to achieve practical shelf life, storage and handling conditions. Here we provide a concise and contemporary review of ongoing formulation strategies and excipients used in approved antibodies and cellular therapeutic products. Excipients are categorized, and their function in formulations are discussed.

Key words: Immunotherapeutic; Pharmaceutical products; Formulation; Excipients; Cell therapy; Antibody

©The Author(s) 2020. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: In this review, we have focused on the formulation strategies and excipients that have been used in commercialized antibody products as well as the formulation concerns for immuno-cell therapy. Development of immunologic-based biopharmaceutical products have strikingly increased in recent years and have made evident contributions to human health. Antibodies are the leading entity in immunotherapy, while chimeric antigen receptor T cells therapies are the advent of a novel strategy in this area.

Citation: Zhang Y, Williams III RO, Tucker HO. Formulation strategies in immunotherapeutic pharmaceutical products. World J Clin Oncol 2020; 11(5): 275-282
URL: https://www.wjgnet.com/2218-4333/full/v11/i5/275.htm
DOI: https://dx.doi.org/10.5306/wjco.v11.i5.275
INTRODUCTION

The approval of the first therapeutic monoclonal antibody (mAb) in 1986, Orthoclone OKT3, “opened the gate” of antibody therapy. Since then, more than 70 mAbs has been approved continuously and applied in both diagnose and therapeutics[1]. The performance of these products has proved to be remarkable in terms of minimized adverse effect and outstanding efficacy, which results from their unparalleled specificity and avidity. Yu et al[2] reported that progression-free survival and overall survival were greatly improved in lung cancer patients by immunotherapies as compared to chemotherapy without suffering the associated adverse reactions of chemo-patients. In addition, the half-life of mAbs are typically much longer than small molecules. For instance, the half-life of the anti-IgE mAB omalizumab (Xolair®) is 26 d[3]. This allows for once-monthly dosing, thereby avoiding the need of twice-daily doses of antihistamine agents for chronic idiopathic urticaria patients[3].

The year 2017 was celebrated within the pharmaceutical industry because of the approval of the first gene therapy product and the first two cellular therapy products, Yescarta and Kymriah™[4]. This historic action not only set forth the application of cellular immunotherapy but buttressed the success of biotechnology in disease treatment. Yescarta™ and Kymriah™, developed by Kite and Novartis, respectively, were based on chimeric antigen receptor (CAR) T-cell therapy of hematological cancers. In CAR T-cell therapy, patient’s autologous T cells are collected and genetically modified by either viral or non-viral methods to express CARs specific for given tumor antigens. The modified cells are subsequently sorted and expanded ex vivo before re-infusion back into patients. CAR is a fusion of two domains: An extracellular domain for tumor antigen recognition and an intracellular signaling domain that mediates T-cell activation[5]. Recently, anti-CD19 CAR T cells have been demonstrated to be remarkably effective for the treatment of relapsed or refractory B-cell malignancies in pediatric and adult patients[5,6].

Indeed, the growing market of Ab-based drugs and the advent of CAR T cell therapy have illustrated the success of the application of basic immunology to disease treatment. However, several issues have to be addressed to improve the “drugability” of new entities and to develop more candidates into products. An approved drug product must possess stable shelf-life and to endure the stresses of handling and transportation. Thus, stability and preservability have become a major challenge to Abs and cell therapies due to their relative unstable nature. Biologics are sensitive to external conditions, such as temperature changes, agitation, moisture (for solid forms), pH changes, and exposure to interfaces or denaturants[7]. Therefore, appropriate formulation is needed to enhance the stability of active pharmaceutical ingredients to maintain their potency and safety by directly or indirectly interacting with the active pharmaceutical ingredient to prevent them from being damaged by harmful factors.

In this review, we have focused on the formulation strategies and excipients that have been used in commercialized Ab products as well as the formulation concerns for immuno-cell therapy.

FORMULATIONS AND EXCIPIENTS IN ANTIBODY-BASED BIOPHARMACEUTICAL PRODUCTS

As shown in Table 1, Ab formulations are mostly in liquid form and occasionally in solid forms such as lyophilized powders. The excipients selected for Ab formulations can be categorized into 5 classes: Sugars and polyols, amino acids, surfactants, buffer and tonicifying agents, and others (preservatives, antioxidants, and chelators) (Figure 1).

Sugars and polyols

Sugars have been identified as one of the intracellular solutes (osmolytes) that stabilize microorganisms under harsh conditions such as serious dehydration and elevated temperature. Being wisely utilized in pharmaceutical industry, sugars and polyols are effective in stabilizing therapeutic Abs thereby protecting them from aggregation, denaturation and other degradative pathways in both dried and solution states.

In solution, sugars can stabilize Abs via increasing their melting temperatures (Tm), raising water surface tension, excluded volume effects, and preferential hydration at high concentrations[8-9]. For instance, sorbitol has been shown to increase the Tm of human IgG and reduce its aggregation during the heating process, which is employed for viral inactivation[10]. Sek[11] studied the effect of polyols in increasing the unfolding
Table 1 List of antibody products approved by the United States Food and Drug Administration in 2018 and through May 2019. Information source: www.fda.gov and each product’s package insert

| Trade name  | API                   | Yr | Sponsor               | Excipients                                                                 | Form               | Storage condition                  |
|-------------|-----------------------|----|-----------------------|-----------------------------------------------------------------------------|--------------------|-----------------------------------|
| Skyrizi     | Risankizumab-rzaa     | 2019 | Abbvie                | Disodium succinate hexahydrate, polyosorbate 20, sorbitol, and sucinic acid | Liquid             | 2-8 °C, avoid light/shake/freeze   |
| Evenity     | Romosozumab-aqqg      | 2019 | Amgen                 | Acetate, calcium, polyosorbate 20, and sucrose                              | Liquid             | 2-8 °C, avoid light/shake/freeze   |
| Cablivi     | Caplacizumab-yhdp     | 2019 | Ablynx/Ablynx         | Citrate dihydrate, polyosorbate-80, sucrose, and trisodium citrate dihydrate | Lyophilized Powder | 2-8 °C, avoid light/freeze         |
| Trogarzo    | Ibalizumab-uyyk       | 2018 | TaiMed Biologics/Theratechnologies | L-histidine, polyosorbate 80, sodium chloride, sucrose                   | Liquid             | 2-8 °C, avoid light/shake/freeze   |
| Ilumya      | Tildrakizumab         | 2018 | Sun pharma            | L-histidine, L-histidine hydrochloride monohydrate, polyosorbate 80, sucrose | Liquid             | 2-8 °C, avoid light/shake/freeze   |
| Crysvita    | Burosumab-twza        | 2018 | Ultragenyx pharmaceutical/kyo wa hakko kirin | L-histidine, L-methionine, polyosorbate 80, D-sorbitol                | Liquid             | 2-8 °C, avoid light/shake/freeze   |
| Aimovig     | Erenumab-aooe         | 2018 | AmgenNovartis         | Acetate, polyosorbate 80, sucrose                                         | Liquid             | 2-8 °C, avoid light/shake/freeze   |
| Poteligeo   | Megamulizumab-kpck    | 2018 | Kyowa hakko kirin    | Citric acid monohydrate, glycine, polyosorbate 80                         | Liquid             | 2-8 °C, avoid light/shake/freeze   |
| Takhyro     | Lanadelumab           | 2018 | Dyax/ Shire           | Citric acid monohydrate, L-histidine, sodium chloride, sodium phosphate dibasic dihydrate | Liquid             | 2-8 °C, avoid light/shake/freeze   |
| Lumoxiti    | Moxetumomab pasudotox-tdfk | 2018 | AstraZeneca          | Glycine, polyosorbate 80, sodium phosphate monobasic monohydrate, sucrose | Lyophilized Powder | 2-8 °C, avoid light/shake/freeze   |
| Ajovy       | Fremanezumab-vfrm     | 2018 | Teva                 | Disodium ethylenediaminetetraacetic acid dihydrate (EDTA), L-histidine, L-histidine hydrochloride monohydrate, polyosorbate-80, sucrose | Liquid             | 2-8 °C, avoid light/shake/freeze   |
| Emgality    | Galcanezumab-gnlm     | 2018 | Eli Lilly            | L-histidine, L-histidine hydrochloride monohydrate, polyosorbate 80, sodium chloride | Liquid             | 2-8 °C, avoid light/shake/freeze   |
| Libtayo     | Cemiplimab-rwlc       | 2018 | Regeneron/SanoPharma | L-histidine, L-histidine monohydrochloride monohydrate, sucrose, L-proline, polyosorbate 80 | Liquid             | 2-8 °C, avoid light/shake/freeze   |
temperature of several Abs and reported that the extent of stabilization improved with increasing polyl concentration or with larger polyls conferring greater stability\(^{[1]}\).

It has been widely demonstrated that solidifying biologics can improve the long-term storage stability of the biopharmaceutical product as well as ease shipping and storage related problems. Lyophilization or freeze-drying is the most commonly used technique to produce protein and peptide solids\(^{[12]}\). There are three major steps during lyophilization: Freezing, primary drying and secondary drying. During the processes, sugars and polyls can exert significant stabilizing effects via mechanisms such as water replacement and vitrification\(^{[13]}\). Moreover, sugars and polyls act as bulking agent to maintain the integrity of lyophilized “cake” structures\(^{[14]}\).

Sucrose, trehalose, mannitol, and sorbitol are the most frequently selected additives for protein formulations, acting as the stabilizer in both solid and liquid forms as well as lyoprotectants and/or bulking agents in solid form\(^{[15]}\). Reducing sugars, comprised of monosaccharides and most disaccharides (including glucose, lactose, fructose, maltose, and maltodextrins) should be avoided in Ab formulations. This group of compounds can degrade Abs via the Maillard reaction during storage which leads to degradation and deactivation of the Abs\(^{[16]}\).

### Amino acids

The amino acid seems an ideal excipient in pharmaceutical development due to its natural origin, safety within the human body, and other functions that benefit formulations. Thus far, the most frequently used amino acids that stabilize Ab molecules in pharmaceutical products include histidine, arginine, and glycine. Amino acids have been reported to stabilize proteins by various mechanisms, including buffering capacity, thermal stabilization, antioxidant properties, preferential hydration and direct/indirect interaction with proteins\(^{[17-19]}\).

For example, the stabilizing effect of an equimolar mixture of L-Arg and L-Glu on colloidal and conformational stability of four monoclonal antibodies (mAb1–mAb4) at different pH was examined\(^{[20]}\). L-Arg and L-Glu increased the aggregation temperature of all four mAbs in a concentration-dependent manner and elevated the unfolding temperature of the least thermally stable mAb3, without direct effects on the Tm1 of other mAbs. Consequently, aggregation is suppressed with increasing temperature/pH and, importantly, under accelerated stability conditions at weakly acidic to neutral pH\(^{[20]}\).

### Surfactants

Surfactants are one of the routine additives in biopharmaceutical products (Table 1). Non-ionic surfactants are formulated with Abs to specifically assist protein refolding and non-specifically suppress surface interaction-related aggregation against various stresses, including increasing temperature, freezing, dehydration, rehydration, and agitation. The fundamental pathway of the surfactant stabilization effect is to prevent surface adsorption and subsequent denaturation of Abs via competing with the protein for container surface, air-water interface, ice-water interface, solid-air interface and any other non-specific adsorption\(^{[21-23]}\). Certain surfactants also can directly and specifically bind noncovalently to the hydrophobic region of Abs. Stabilization results when the binding of the surfactant ligand is weaker in the non-native state than in the native state. This allows binding to hydrophobic sites of the protein to protect it from interacting with other Abs or surfaces\(^{[23]}\). Most commonly added surfactants are polysorbate 20, polysorbate 80, and poloxamer 188, regardless liquid or solid forms\(^{[23]}\).

### Buffer agents and tonicifying agents

Buffer systems are typically comprised of two chemical species that are related to a change in protonation state. The major function of a buffering agent system in a formulation is to provide a relatively consistent pH at which the active ingredient is physically and chemically stable. Several chemical degradation pathways are pH dependent for example, deamidation and oxidation. An arginine-acetate buffer was found to stabilize an IgG1 Ab against deamidation and aggregation at pH 4.5 to 6.0\(^{[24]}\). In addition, buffer agents also influence the electrostatic interaction both inter- and intra-molecularly by controlling solution pH. Otherwise, intramolecular charge repulsion can compromise the native structure of Abs, leading to protein unfolding\(^{[25]}\). Alternatively, intermolecular charge repulsion can protect the native structure, resulting in increasing Ab colloidal stability and solution phase stability\(^{[26,27]}\). Commonly used salt buffer systems are listed in Table 2.
Besides maintaining pH, as mentioned above, salts also can act to resolve “tonicity” (i.e., osmotic pressures differences between two solutions). Sodium chloride is the most commonly used of the iso-tonicizing agents. Other than salts, excipients like mannitol, lactose, and glycerin, are also incorporated into Ab formulations (mostly parenteral) to prevent tonicity related symptoms, including pain, irritation or tissue damage at the administration site\cite{21}.

Preservatives, antioxidants, and chelators
Chelators and antioxidants are typically used to prevent the oxidation of Abs and other excipients. Several conserved amino acid residues encoded within Abs, such as methionine and cysteine, are prone to oxidative degradation. During stages of production, purification, formulation, and storage, three sources can provide oxidative molecules to product formulations, including trace metal ions from containers or handling tools (during the extended production process), hydrogen peroxide from sanitizing agents, and additional oxidant impurities from other excipients\cite{29,30}. Besides, antimicrobials are typically added to the formulations, especially when employing multi-dose vials, as preservatives to inhibit microbial proliferation. These frequently employed antioxidants, chelators and antibiotics include edetic acid/or edetate salts (e.g., EDTA), glutathione, metacresol, phenol, benzyl alcohol, benzalkonium chloride, and certain amino acids such as methionine and cysteine\cite{25}.

IMMUNO-CELL THERAPY FORMULATIONS
Currently, the typical CAR-T manufacturing process involves blood collection, apheresis, T-cell activation, gene modification, cell expansion, formulation and packaging, cryopreservation, and eventually injection into patients\cite{31}. During these steps, cells experience multiple transportation. They also are exposed to processes such as separation, transduction, expansion and freeze-thaw. Each of the several steps of synthesis and operation require specific environments for the cells which expose them to different compositions in the formulation\cite{24,25}. Similar to other biopharmaceutical products, cells need ancillary materials to provide necessities for stability, including non-oxidative/reducing environment, proper pH, and other critical factors\cite{34}. However, unlike biologics, cell-based products also need nutritional components to keep them alive and to maintain robust metabolism as well as cryoprotectant agents (CPA) to protect them from the stresses caused by dramatic temperature fluctuations during their processing.

CPAs are typically necessary in cell-based products to support cells for surviving freeze-thaw processes that facilitates transportation. Often non-electrolytes are added as CPAs, including low molecular molecules such as sugars, glycerol (trehalose and sucrose) and dimethyl sulfoxide (DMSO), as well as large polymeric molecules (e.g., polyvinylpyrrolidone and hydroxyethyl starch)\cite{35}. Since the discovery of the
Table 2 Non-amino acid buffer systems frequently used in antibody parenteral products

| Buffer system | Controlled pH range (25 °C) | Acid           | Base          | Example product |
|---------------|-----------------------------|----------------|---------------|-----------------|
| Phosphate     | 5.8-7.8                     | Monosodium phosphate | Disodium phosphate | Tysabri®        |
| Acetate       | 3.8-5.8                     | Acetic acid      | Sodium acetate | Amgevita®       |
| Citrate       | 3.0-7.4                     | Citric acid      | Sodium citrate | Humira®         |
| Succinate     | 3.3-6.6                     | Succinic acid    | Sodium succinate | Kadcyla®       |
| Tris          | 7-9                         | Tris-HCl        | Tris           | Besponsa®       |

cryoprotective property of DMSO in 1959,[36] it has been investigated and routinely employed as a cryoprotectant in cellular products. For example, DMSO is used in majority of mostly approved cell products, HPC cord blood, as well as current CAR-T cell formulations to enable short term storage and transportation between the hospital and the CAR-T cell manufacturer.[37]

There are a number of potential issues that concern drug developing organizations and regulatory agencies for CAR-T cell application.[38,39] The exposure of cells to a variety of formulations during the multiple steps of processing and manufacturing may cause the final product to carry residual amounts of the unintended components. These could be potential hazards in a drug product and thus, requires risk assessment. Yet, the limited shelf life of some cellular products and the impact of extensive tests on their quality hinders the removal, or at least the assessment, of the residuals.[39-41]

Another issue results from the complexity of ancillary materials or excipients. Even subtle change within culture supplies can be influential to cellular physiology and may lead to the changes in their functional characteristics and performance. Also, serum and recombinant proteins might carry pathogen contamination. Therefore, the quality and stability of ancillary/excipient materials are crucial and need to be strictly controlled.[40]. Further studies to improve excipient/ancillary materials, both systemic and detailed, are urgently needed. These include determination of the correlation of excipient with cell density and process parameters (primarily freezing and thawing) as well as container-excipient compatibility. Finally, the developments of novel excipient and even new dosage forms for CAR T-cells are anticipated. For example, a recent patent reported that T cells can be kept activated via cross-linking when mixed with biodegradable nanospheres/microspheres.[43]

CONCLUSION

The discovery and invention of immunotherapies is a milestone in the history of the battle between humans and diseases. Inactive ingredients (e.g., excipients) are critical component of a successful immune-biopharmaceutical product. This review offers a brief and concise introduction to the currently used excipients and formulation strategies for antibody drugs and immune cell-based therapeutics. Knowledge about formulation compositions for Abs injectables has significantly matured, and the understanding of mechanisms of excipients is increasing. However, more dosage forms are anticipated for mAbs, especially the ones that are less or not invasive to patients, resulting in an improved patient compliance. For example, administration routes such as nasal, respiratory and oral can be promising options. As mentioned previously, the development of immune cell therapy is only in its infancy, future investigation remains. There are still many aspects of issues urgently need to be addressed by formulation scientists, such as manufacture process optimization, excipient choice, and stability of formulation or environment cells are exposed to.

REFERENCES

1 Administration U.S. Food and Drug. FDA Approved Drug Products. 2019. Available from: https://www.fda.gov/home
2 Yu DP, Cheng X, Liu ZD, Xu SF. Comparative beneficiary effects of immunotherapy against chemotherapy in patients with advanced NSCLC: Meta-analysis and systematic review. Oncol Lett 2017; 14: 1566-1570 [PMID: 28793581 DOI: 10.3892/ol.2017.6277]
3 Genentech. Package Insert of Xolair®. 2003. Available from: https://www.gene.com/download/pdf/xolair_prescribing.pdf
4 Mullard A. 2017 FDA drug approvals. Nat Rev Drug Discov 2018; 17: 81-85 [PMID: 29348678 DOI: 10.1038/nrd.2018.4]
5 Miliotou AN, Papadopoulou LC. CAR T-cell Therapy: A New Era in Cancer Immunotherapy. Curr Pharm Biotechnol 2018; 19: 5-18 [PMID: 29667553 DOI: 10.2174/138920101966818041890526]

6 Zheng PP, Kros JM, Li J. Approved CAR T cell therapies: ice bucket challenges on glaring safety risks and long-term impacts. Drug Discov Today 2018; 23: 1175-1182 [PMID: 29509111 DOI: 10.1016/j.drudis.2018.02.012]

7 Carpenter JF, Chang BS, Garzon-Rodriguez W, Randolph TW. Rational design of stable lyophilized protein formulations: theory and practice. Pharm Biotechnol 2002; 13: 109-133 [PMID: 11987749 DOI: 10.1007/s10895-005-0577-0]

8 Ohtake S, Kita Y, Arakawa T. Interactions of formulation excipients with proteins in solution and in the dried state. Adv Drug Deliv Rev 2011; 63: 1053-1073 [PMID: 21756953 DOI: 10.1016/j.addr.2011.06.011]

9 Kamerzell TJ, Esfandiary R, Joshi SB, Middaugh CR, Volkin DB. Protein-excipient interactions: mechanisms and biophysical characterization applied to protein formulation development. Adv Drug Deliv Rev 2011; 63: 1118-1159 [PMID: 21855564 DOI: 10.1016/j.addr.2011.07.005]

10 González M, Murature DA, Fidelio GD. Thermal stability of human immunoglobulins with sorbitol. A critical evaluation. Vox Sang 1995; 68: 1-4 [PMID: 7725666 DOI: 10.1111/j.1423-0410.1995.tb02535.x]

11 Sek DC, inventor, Wyeth, assignee. Protein Formulations Containing Sorbitol. United States patent US 20080200655A1. 2008 February 15. Available from: https://patents.google.com/patent/US20080200655A1/en

12 Remmele RL, Krishnan S, Callahan WJ. Development of stable lyophilized protein drug products. Curr Pharm Biotechnol 2012; 13: 471-496 [PMID: 22283723 DOI: 10.2174/1389201012799316990]

13 Kasper JC, Friess W. The freezing step in lyophilization: physico-chemical fundamentals, freezing methods and consequences on process performance and quality attributes of biopharmaceuticals. Eur J Pharm Biopharm 2011; 78: 248-263 [PMID: 21426937 DOI: 10.1016/j.ejpb.2011.03.010]

14 Rayaprolu BM, Swarz Jrr, Aniyambatlal G. Excipients in parenteral formulations: selection considerations and effective utilization with small molecules and biologics. Drug Dev Ind Pharm 2018; 44: 1565-1571 [PMID: 29663908 DOI: 10.1080/03639045.2018.1483592]

15 Pramanick S, Chandel V, Singhodia D. Excipient Selection In Parenteral Formulation Development. Pharma Times 2013; 45: 65-77

16 Carpenter JF, Pikal MJ, Chang BS, Randolph TW. Rational design of stable lyophilized protein formulations: some practical advice. Pharm Res 1997; 14: 969-975 [PMID: 9279875]

17 Kato Y, Matsuda T, Kato N, Nakamura R. Maillard reaction of disaccharides with protein: suppressive effect of nonreducing end pyranoside groups on browning and protein polymerization. J Agr Food Chem 1989; 37: 1077-1081 [DOI: 10.1021/jf00088a057]

18 Bözhøgmør MR, Monhemiu H. How Can a Free Amino Acid Stabilize a Protein? Insights from Molecular Dynamics Simulation. J Solution Chem 2015; 44: 45-53 [DOI: 10.1007/s10953-015-0291-1]

19 Taneja S, Ahmad F. Increased thermal stability of proteins in the presence of amino acids. Biochem J 1994; 303: 147-153 [PMID: 8945233 DOI: 10.1042/bj3030147]

20 Kheddo P, Tracka M, Armer J, Deerman RJ, Uddin S, van der Walle CF, Golovanov AP. The effect of arginine glutamate on the stability of monoclonal antibodies in solution. Int J Pharm 2014; 473: 126-133 [PMID: 24992318 DOI: 10.1016/j.ijpharm.2014.06.053]

21 Chi E. Excipients Used in Biotechnology Products: Properties, Functionality, and Applications in Research and Industry. In: Pharmaceutical Excipients. John Wiley Sons, Inc 2016; 145-198 [DOI: 10.1002/9781118992432.ch4]

22 Lu D, Liu Z, Liu Z, Zhang M, Ouyang P. Molecular simulation of surfactant-assisted protein reflowing. J Chem Phys 2005; 122: 134902 [PMID: 15847497 DOI: 10.1063/1.1866052]

23 Mollmann SH, Ellofsson U, Bukinsky JT, Frojkaer J. Displacement of adsorbed insulin by Tween 80 monitored using total internal reflection fluorescence and ellipsometry. Pharm Res 2005; 22: 1931-1941 [PMID: 16088428 DOI: 10.1023/b:aipm.0000010955-7249-1]

24 Carpenter JF, Manning MC. Rational Design of Stable Protein Formulations: Theory and Practice. US: Kluwer Academic/Plenum Publishers, 2012. Available from: https://www.springer.com/us/book/9781349369453

25 Gervasi V, Dall Agnol R, Cullen S, McCoy T, Vuca S, Crean A. Parenteral protein formulations: an overview of approved products within the European Union. Eur J Pharm Biopharm 2018; 131: 8-24 [PMID: 30006264 DOI: 10.1016/j.ejpb.2018.07.011]

26 Gokarn YR, Kamerzell TJ, Li M, Cromwell M, Liu H, inventor. Antibody formulation. United States patent US 20160368999A1. 2016 December 22. Available from: https://patents.google.com/patent/US20160368999A1/en

27 Zbancik TJ, Holcomb RE, Katayama DS, Murphy BM, Payne RW, Coccaro RC, Evans GJ, Matsuura JE, Remmele RL, Strawser JJ, Anyarambhatla G. Excipients in parenteral formulations: selection considerations and effective utilization with small molecules and biologics. Drug Dev Ind Pharm 2018; 44: 1263-1272 [PMID: 29894867 DOI: 10.1016/j.jb.pharmaceuticals.2016.11.014]

28 Ugo SO, Apte SP. The effect of buffers on protein conformational stability. Pharm Technol 2004; 28: 86-109

29 Akers MJ. Excipient-drug interactions in parenteral formulations. J Pharm Sci 2002; 91: 2283-2300 [PMID: 12379914 DOI: 10.1002/jps.10154]

30 Hada S, Kim NA, Lim DG, Lim JY, Kim KH, Adhikary P, Jeong SH. Evaluation of antioxidants in protein formulation against oxidative stress using various biophysical methods. Int J Biol Macromol 2016; 82: 192-200 [PMID: 26499868 DOI: 10.1016/j.ijbiomac.2015.10.048]

31 Kolhe P. Formulation and fill finish process development: CAR-T cell therapy case study. Proceedings of the AAPS Annual Meeting; 2017; San Diego, CA. Available from: https://patents.google.com/patent/US20160368999A1/en

32 Tyagarajan S, Spencer T, Smith J. Optimizing CAR-T Cell Manufacturing Processes during Pivotal Clinical Trials. Mol Ther Methods Clin Dev 2020; 16: 136-144 [PMID: 31988978 DOI: 10.1016/j.omtm.2019.11.018]

33 Levine BL, Miskin J, Woinacko C, Keir C. Global Manufacturing of CAR T Cell Therapy. Mol Ther Methods Clin Dev 2017; 4: 92-101 [PMID: 28344995 DOI: 10.1016/j.omtm.2016.08.008]

34 Atout F. Cell-Based Therapies Formulations: Unintended components. AAPS J 2016; 18: 844-848 [PMID: 27233803 DOI: 10.1016/j.aapsj.2016.06.032]

35 Woods EJ, Thirumala S, Badhe-Buchanan SS, Clarke D, Mathew AJ. Off the shelf cellular therapeutics: Factors to consider during cryopreservation and storage of human cells for clinical use. Cytotechnology 2016; 18: 697-711 [PMID: 27177474 DOI: 10.1007/s10611-016-9935-9]

36 Yu ZW, Quinn PJ. Dimethyl sulphoxide: a review of its applications in cell biology. Biosci Rep 1994; 14:...
259-281 [PMID: 7620078 DOI: 10.1007/BF01199051]

37 Administration U.S. Food and Drug. Approved Cellular and Gene Therapy Products. 2019. Available from: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products

38 Marks P. The FDA’s Regulatory Framework for Chimeric Antigen Receptor-T Cell Therapies. Clin Transl Sci 2019; 12: 428-430 [PMID: 31328862 DOI: 10.1111/cts.12666]

39 Seimetz D, Heller K, Richter J. Approval of First CAR-Ts: Have we Solved all Hurdles for ATMPs? Cell Med 2019; 11: 2155179018822781 [DOI: 10.1177/2155179018822781]

40 Read E. Ancillary Materials for Cell and Tissue-Based Products. Available from: www.factwebsite.org

41 Pessina A, Bonomi A, Baglio C, Cavicchini L, Sisto F, Neri MG, Gribaldo L. Microbiological risk assessment in stem cell manipulation. Crit Rev Microbiol 2008; 34: 1-12 [PMID: 18259977 DOI: 10.1080/10408410701683399]

42 Li Y, Hao Y, Yu L, Wang J. Quality Control and Nonclinical Research on CAR-T Cell Products: General Principles and Key Issues. Engineering 2019; 5: 122-131 [DOI: 10.1016/j.eng.2018.12.003]

43 Har-Noy M, inventor; Immunovative Therapies Ltd assignee. Method of making a cell therapy formulation. Europe patent EP1749090B1. 2004
