Journal of Pharmaceutical Sciences 106 (2017) 1722-1735

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Review

Trends on Analytical Characterization of Polysorbates and Their Degradation Products in Biopharmaceutical Formulations

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ARTICLE INFO

Article history:
Received 30 November 2016
Revised 1 March 2017
Accepted 3 March 2017
Available online 14 March 2017

Keywords:
excipients
protein formulation
stability
surfactants

ABSTRACT

Among many other applications, polysorbates (PSs) are used as the most common surfactants in biopharmaceutical products in particular to protect proteins against interfacial stress. Structural heterogeneity, presence of degradants and other impurities, and tendency for degradation are interrelated features found in commercial PSs with a direct impact on their functional properties in biopharmaceutical products. These pose a challenge for the analytical characterization of PSs at different stages of product development. This review article focuses on methods and strategies reported in the recent years for the analytical characterization of PSs, their degradants and other impurities within neat PS (i.e., PS raw materials), diluted PS solutions, as well as in biopharmaceutical formulations. The use of versatile and complementary methods applied in a systematic approach is crucial to understand the impact of the concentration, composition, and degradation of PSs on the quality of biopharmaceutical products.

Introduction

Polysorbates (PSs) are a family of nonionic surfactants widely used as excipients in food and pharmaceutical products. Polysorbate 20 (PS20) and polysorbate 80 (PS80)—also known as Tween® 20 and Tween® 80—are the most common surfactants used to protect therapeutic proteins against adsorption to interfaces and related instabilities. The contribution of PS20 and PS80 to protein stabilization in biopharmaceutical products is well accepted, and both are excipients for parenteral administration approved by regulatory agencies. Most biopharmaceutical products containing peptides, proteins, antibodies, and vaccines are formulated with PSs. For example, about 80% of the commercial mAbs contain PS20 or PS80. Their high hydrophilic-lipophilic balance value and low critical micelle concentration (CMC) account for their high surface activity even at low concentrations. Typical PS concentrations in biopharmaceuticals are between 0.001% and 0.1% (w/v), corresponding to 0.01 and 1 mg/mL. Examples of these are ReoPro® (abciximab) and HUMIRA® (adalimumab) for low and high PS content, respectively. PSs are often preferred over other stabilizers against surface-induced adsorption and related instabilities because of their low toxicity[1] and good stabilizing properties. Actually, there are quite a number of alternative surfactants for parenteral applications, including poloxamers, sodium dodecyl sulfate, Solutol HS 15, Cremophor, lecithin, and alkylsaccharides. For therapeutic proteins in commercial products, however, mainly poloxamer (i.e., GAZYVA®, ORENCIA®, and REBIF®) and sodium dodecyl sulfate (i.e., PROLEUKIN®) are found.

Along production, distribution, storage, and administration to the patient, protein pharmaceutical solutions are constantly exposed to a plethora of interfaces (e.g., glass, plastic polymers, stainless steel, air, ice crystals, silicone oil…) which can lead to adsorption, denaturation, aggregation, and decrease of the effective protein concentration. When combined with mechanical stress,[2-5] interfacial adsorption might act as a trigger for aggregation and

Abbreviations used: CMC, critical micelle concentration; HSA, human serum albumin; FFA, free fatty acids; FID, flame ionization detector; ICH, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; MALDI-TOF, matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry; PS, polysorbates; PS20, polysorbate 20; PS80, polysorbate 80.

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http://dx.doi.org/10.1016/j.xphs.2017.03.001
0022-3549/

Table 1
Relevant Application Areas of Analytical Methods for Characterization of PS Constituents

Neat PS and diluted PS solutions
Concentration (quantification of PS concentration)
Composition (analysis of PS-related molecules or structural variants)
Purity (analysis of PS degradants and impurities)
PS in biopharmaceutical formulations (e.g., in the presence of API and other excipients)
Concentration (quantification of PS concentration)
Composition (analysis of PS-related molecules or structural variants)
Purity (analysis of PS degradants and impurities)
Functionality (assessment of protective property)

particle formation.[6] This is particularly crucial for hydrophobic proteins[7] formulated at low-concentration doses in which the loss of protein due to surface adsorption can be significant.[2-5]

Although the molecular details of stabilization are not yet fully understood, PSs are known to contribute to protein stability by 2 different mechanisms, that is, through (1) competitive adsorption to the hydrophobic interfaces and (2) direct binding to the protein.[8] In the first case, PSs show higher adsorption energies per unit area than proteins, thereby efficiently competing with proteins and protecting them against adsorption to hydrophobic interfaces.[9] Moreover, PSs may interact directly with the protein increasing its stability in solution by binding to and protecting exposed hydrophobic regions.[10] This prevents aggregation by reducing protein-protein interactions. Which of these 2 mechanisms prevails within the stabilization of a particular protein is protein dependent and PS dependent. For instance, the binding between PS20 or PS80 and several mAbs was reported to be negligible, indicating that mAbs stabilization by PS occurs mainly via a competition mechanism.[11]

Besides stability of the therapeutic protein, stability of the PSs must be preserved during a product's shelf-life, at least from a functional point of view. Despite their successful use in marketed therapeutic protein products, PSs can undergo a variety of degradation reactions.[12] This can result not only in a loss of the functional properties of PSs in the formulation but also in the formation of degradants that may induce protein instability.[13] The last is a key area of concern because aggregates and chemically modified protein molecules are considered critical quality attributes and may be related to enhanced immunogenicity.[14,15] Moreover, PS degradation may lead to unwanted PS-related particles.[16-18] Therefore, it is crucial to set up analytical approaches to characterize and quantify PSs and their degradation products during formulation development, manufacturing, and the shelf-life of the product. The final goal should be to understand, anticipate, and prevent unwanted impact of PS degradation on the stability of the protein drug.

For the pharmaceutical industry, the characterization of neat PS (i.e., raw material) or aqueous diluted PS solutions—and as part of a biopharmaceutical formulations—that is, in the presence of active pharmaceutical ingredient and other excipients—is highly relevant (Table 1). The inherent complexity of PSs, which are mixtures of chemically related molecules and impurities, makes their analytical characterization challenging. Incidentally, the terminology used in the literature to describe all the components related to PSs is arbitrary and therefore ambiguous. For this review, we use the following nomenclature to refer to PS constituents: PS-related molecules (structural variants), PS degradants (altered PS-related components, e.g., oxidized PS species, fatty acid esters, free fatty acids, peroxides, organic aldehydes, ketones, acids…), and impurities other than degradants (e.g., peroxides, metal traces, byproducts, leachables from container closures).

The study of PS degradation is therefore complex due to the heterogeneity of the species inherently present in PSs. Fortunately, several recent publications have contributed to a better understanding of the complexity of PSs and their degradation mechanisms, in particular with regard to pharmaceutically relevant conditions.[16,17,19-22] The complexity of the PS constituents was reduced in recent publications by using customized PS20 and PS80 containing ~99% and ~98% lauric and oleic acid esters, respectively.[23-25] In one of these publications, unique degradant patterns of all-laurate PS20 were observed which, in combination with ^{18}O-labeling, provided a direct approach to differentiate the mechanism of PS degradation.[23]

Our review article aims to give a comprehensive and up-to-date summary on the state-of-the-art toolbox for the analytical characterization of PS-related molecules and their degradation products in biopharmaceuticals.

Overview on the Chemistry and Major Degradation Products of PSs

PSs are amphiphilic molecules that share a common sorbitan head group where each of the 4 hydroxyl groups is bound to a polyethylene glycol (PEG, also known as polyethylene oxide chain, POE) chain. The types of PSs mainly differ in the fatty acid side chain (hydrophobic fraction) esterified with one of the PEG side chains (hydrophilic fraction). However, this is just an idealized structure, and commercially available PSs consist of a mixture of structurally related molecules (Fig. 1). For instance, the theoretical expected structure for PS20 and PS80, according to their formal names polyoxyethylene (20) sorbitan monolaurate and polyoxyethylene (20) sorbitan monooleate, respectively, were reported to only account for about 20% (w/w) of the total PS material.[26]

The heterogeneity found in PSs arises from variabilities within both the sorbitan-PEG and the fatty acid side chain, in detail (1) the length of the PEG chains, (2) the esterification in more than 1 hydroxyl group of the side chains, (3) the variations in the head group, and (4) the fatty acid composition. The length of the PEG chains is variable (Fig. 1a) and provided that all together do not exceed 20 carbons, this results in a number of possible structures greater than 1500.[27] As obvious from the nomenclature monooleate/laurate, 1 of the 4 hydroxyl positions of the PEG side chains is randomly esterified in PS20 and PS80. In addition, mixtures of isomeric di-, tri- and tetra-esters are commonly found (Fig. 1a). The PS core is a mixture of sorbitans and isosorbides, the last one presenting only 2 hydroxyl positions (Fig. 1a).[28] On top of that, sorbitol, nonesterified sorbitan/isosorbide-PEG as well as PEG residues, which are all hydrophilic byproducts of the manufacturing process,[29] can be present in commercial PSs. In 1 example, a significant amount (<17%) of PS20 corresponded to nonesterified sorbitan-PEG.[30] Finally, the main fatty acids are lauric acid for PS20 and oleic acid for PS80. However, the composition varies from manufacturer to manufacturer and even may vary from batch to batch (Fig. 1b).[26,31,32] Traces of free fatty acids can also be found as PS-related molecules (byproducts) in commercial neat PSs. Altogether, this structural heterogeneity presumably accounts for PS' great features as emulsifiers[28] but also hampers their quantification and may affect their stability during storage.

PS instability is accounted by 2 main degradation pathways: oxidation and hydrolysis (chemically induced or enzyme mediated).[12,21,33] Oxidation was found to be the most common degradation pathway under pharmaceutically relevant conditions (e.g., pH 5.5 and 5°C-25°C).[13] It is initiated in the presence of oxygen by UV light or metal catalysis, leading to the formation of peroxides as the main product of the reaction. The highly reactive peroxides promote the formation of other secondary oxygenated products