European Journal of Pharmaceutics and Biopharmaceutics xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Predicting critical micelle concentration and micelle molecular weight of polysorbate 80 using compendial methods

Alexandra C. Braun [a], David Ilko [a], Benjamin Merget [a], Henning Gieseler [b], Oliver Germershaus [a], Ulrike Holzgrabe [a], Lorenz Meinel [a,*]

[a] Institute for Pharmacy and Food Chemistry, University of Wuerzburg, Wuerzburg, Germany
[b] GILYOS GmbH, Wuerzburg, Germany

ARTICLE INFO

Article history:
Received 25 June 2014
Accepted in revised form 1 December 2014
Available online xxxx

Keywords:
Polysorbate 80
Critical micelle concentration
Micelle molecular weight
Cloud point
Hydrophilic–lipophilic balance
Fatty acid composition
Peroxide value

ABSTRACT

This manuscript addresses the capability of compendial methods in controlling polysorbate 80 (PS80) functionality. Based on the analysis of sixteen batches, functionality related characteristics (FRC) including critical micelle concentration (CMC), cloud point, hydrophilic–lipophilic balance (HLB) value and micelle molecular weight were correlated to chemical composition including fatty acids before and after hydrolysis, content of non-esterified polyethylene glycols and sorbitan polyethoxylates, sorbitan- and isosorbide polyethoxylate fatty acid mono- and diesters, polyoxyethylene diesters, and peroxide values. Batches from some suppliers had a high variability in functionality related characteristic (FRC), questioning the ability of the current monograph in controlling these. Interestingly, the combined use of the input parameters oleic acid content and peroxide value – both of which being monographed methods – resulted in a model adequately predicting CMC. Confining the batches to those complying with specifications for peroxide value proved oleic acid content alone as being predictive for CMC. Similarly, a four parameter model based on chemical analyses alone was instrumental in predicting the molecular weight of PS80 micelles. Improved models based on analytical outcome from fingerprint analyses are also presented. A road map controlling PS80 batches with respect to FRC and based on chemical analyses alone is provided for the formulator.

1. Introduction

Polysorbate 80 (PS80) is a frequently used surfactant for biopharmaceutical product formulation. This non-ionic emulsifier is typically formulated at concentrations of 0.01–0.1% (v/v) for active pharmaceutical ingredient (API) stabilization, reduction of surface adsorption, or to avoid stress-induced aggregation (e.g., freezing, storage, transport, reconstitution of lyophilized products) [1–3]. Compendial grade PS80 is composed of polyoxyethylene sorbitan esters with fatty acids, at least 58% of which being specified as oleic acid along with myristic, palmitic, palmitoleic, stearic, linoleic, and α-linolenic acid esters, respectively [4,5]. Critical PS80 material attributes were derived from a focus on its impact on API or excipient stability, as e.g., residual peroxides within PS80 batches may drive oxidation. However, PS80 attributes were to a lesser extent selected based on galenical considerations/functionality related characteristics (FRC), an aspect which is thoroughly addressed here within. The batch-to-batch variability e.g., in residual peroxides was linked to the supplier's manufacturing and purification processes, packaging, or storage [6]. Polysorbates are inherently prone to radical autoxidation, leading to hydrolysis [2,7–9], and placing formulated proteins at risk of oxidative damage [10–12]. Apart from peroxides, variability is introduced by the type and amount of esterified and free fatty acids, unbound ethoxylates as well as the level of impurities [13–16]. Consequently, the United States Pharmacopeia (USP) and the European Pharmacopeia (Ph.Eur.) specify the entire (free and esterified) fatty acid composition, the peroxide value as well as the acid, saponification, and hydroxyl value, respectively. In addition, ethylene oxide, dioxin and heavy metal content are specified [4,5]. In more recent efforts, both pharmacopoeias allude to functionality related characteristics (FRCs; Ph.Eur. 5.15) or excipient performance (USP <1059>) in non-mandatory sections, detailing an approach for reliable excipient performance through additional specifications designed on top of compendial requirements. Several studies within the context of PS80 suggest a need for such additional specifications. For example, the stability of biologic formulations during processing or

* Corresponding author. Institute for Pharmacy and Food Chemistry, University of Wuerzburg, Am Hubland, DE-97074 Wuerzburg, Germany. Tel.: +49 931 318 54 71; fax: +49 931 318 46 08.
E-mail address: l.meinel@pharmazie.uni-wuerzburg.de (L. Meinel).

http://dx.doi.org/10.1016/j.ejpb.2014.12.015
0939-6411/

storage has been linked to the surface activity of polysorbates [17]. Other reports detailed the impact of polydispersed oxyethylenes on colloidal properties [18,19]. However, neither surface activity nor colloidal properties are monographed at present. It is for these exemplary selected reports on PS80 that compliance with compendial specifications alone has been questioned before yielding stable formulation outcome [20,21].

Consequently, we are addressing the need to set additional PS80 specification. We are also addressing the hypothesis, that monographed methods are sufficient in specifying PS80 batches for stable outcome with respect to FRC, when these are released based on adequate models. In order to identify possible input parameters for model building, PS80 batches were broadly characterized by numerous methods. For that, we correlated galenical functionality from sixteen PS80 batches (CMC, cloud point, hydrophilic–lipophilic balance (HLB) value and micelle molecular weight) with batch composition from thorough analytical studies (PS80 fatty acid composition before and after hydrolysis [22], unbound PEGs, sorbitan polyethoxylates, and mono- and diesters) as well as with peroxide value.

2. Experimental details

2.1. Materials

Sixteen PS80 batches of 5 different qualities (qualities being different suppliers or different supplier grades being distributed from one supplier) were used for the study. The polysorbate samples were from Croda (East Yorkshire, UK), Kolb (Hedingen, Switzerland), Merck (Darmstadt, Germany) and NOF (Tokyo, Japan). The order of the supplier names does not necessarily coincide with the order of the codes used within the manuscript. Grade, supplier and date of manufacture from each sample were detailed (Table 1). All samples were stored at room temperature, under nitrogen and protected from light and the experiments were conducted after storage times as indicated. Sorbitan monooleate 80 (Span 80), methylene blue, paraffin oil were from Sigma–Aldrich (Taufkirchen, Germany). Type 2 water (ASTM D1193, ISO 3696) was used (Millipore, Billerica, MA). All other chemicals or solvents were of at least analytical or pharmaceutical grade and obtained from Sigma–Aldrich or VWR (Darmstadt, Germany).

2.2. Sample preparation

For the cloud point determination, the polysorbate 80 samples were dissolved at 3% (w/v) in freshly prepared 1 M sodium chloride in water on a roller mixer to minimize foaming (SRT1, Sigma–Aldrich, Germany) until the solution was visibly clear and free from foam before further processing. Samples for surface tension measurement were dissolved in water.

Table 1
Overview of the PS80 batches.

Quality	Batch	Months of storage	Peroxide value at time of release[a]	Peroxide value after storage
A	1	26	0.6	8.2
A	2	18	1.3	8.2
A	3	21	0.2	9.2
A	4	21	0.5	16.5
B	1	17	0.4	21.2
B	2	20	0.0	19.7
B	3	31	0.6	16.1
C	1	27	1.0	4.8
C	2	27	1.0	3.7
C	3	16	0.0	4.4
D	1	10	0.1	4.9
D	2	20	0.1	4.4
D	3	18	0.8	2.9
E	1	6	0.2	11.6
E	2	18	1.3	3.9
E	3	14	1.7	8.7

[a] Taken from CoA.

2.3. Critical micelle concentration (CMC) by surface tension measurement

The CMC was determined by surface tension measurements using the Wilhelmy plate method with a Krüss K12 (Hamburg, Germany). Temperature was controlled at 20 ± 0.5 °C (Fryka, G. Heinemann, Schwäbisch-Gmünd, Germany) and experiments were conducted at atmospheric pressure. The surface tension of water was determined prior to measurements of the surfactant samples and to ensure agreement (±5 mN/m) with the reference value of 72.75 mN/m [23]. Freshly prepared, serial dilutions of the surfactants each in about 75–80 mL of water were equilibrated at 20 ± 0.5 °C for at least 30 min and then stirred for 60 s [24], and again equilibrated for 5 min before measurement ($n = 3$). The surface tension was recorded from ten different dilutions per sample and the CMC was fitted from the intersection of the straight lines for the linear concentration-dependent section and the concentration-independent section using Krüss tensiometer software (version 5.05) [25–27].

2.4. Cloud point

The cloud point was turbidimetrically determined from a 3% (w/v) PS80 sample solution in 1 M sodium chloride, measured in a water bath at increasing temperature. Vials with 8 mL of the surfactant solution were placed in the water bath with heating at a rate of 1.2 °C/min. from room temperature to 45 °C and then at a rate of 0.3 °C/min. until the cloud point, controlled with a thermometer accuracy of 0.2 °C. The cloud point was visually assessed by phase separation. Furthermore, the cloud point was confirmed by microcalorimetry. For that, 3 mL of identically prepared samples as used for the turbidimetric method were heated at a rate of 0.5 K/min from 35 to 90 °C in the small volume sample vessel of a C80 calorimeter (Setaram, Caluire, France) and recorded against 3 mL of the identical solution without PS80 in the reference cell. Cells were equilibrated at 35 °C until heat flow between the cells was constant.

2.5. Hydrophilic–lipophilic balance (HLB) value

Determination of HLB values was performed using the "Blender-Centrifuge Method" [28], combining polysorbate 80 sample (HLB ~ 15), sorbitan oleate (Span 80; HLB ~ 4.3), paraffin (required HLB (RHLB) ~ 10.5) and water. In brief, stock emulsions of 25 mg emulsifier per gram emulsion were prepared by diluting the polysorbate sample with water and diluting Span 80 with the paraffin oil and mixing them in varying proportions, with stock A yielding a HLB of 12.33 and stock B yielding a HLB of 6.98, respectively. Stocks were homogenized for 2 min after addition of a small amount of methylene blue. A series of emulsions were prepared from stocks, bracketing the RHLB by weighing each stock emulsion into a 15 mL centrifuge tube to yield a total amount of 10 g emulsion. Tubes were shaken to ensure mixing, centrifuged at 4000 r.p.m. for 20 min and stored at room temperature. After 15 days, the heights of the aqueous phase were measured and the HLB value of the emulsion showing the least phase separation was recorded as the RHLB value, from which the PS80 HLB value was calculated.

2.6. Static light scattering

All static light scattering experiments were conducted at 23 °C using a CGS-3 MD Goniometer (ALV, Langen, Germany). The laser

Please cite this article in press as: A.C. Braun et al., Predicting critical micelle concentration and micelle molecular weight of polysorbate 80 using compendial methods, Eur. J. Pharm. Biopharm. (2014), http://dx.doi.org/10.1016/j.ejpb.2014.12.015