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Choosing an Optimal Deaeration Protocol: A Revisit

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Abstract: The dissolution testing procedure encompasses a strictly controlled setup in regard to instrumentation and test conditions. While deaeration is acknowledged as important for accurate and reproducible dissolution testing, the deaeration protocols themselves are not as extensively studied or standardized as other dissolution parameters. The pharmacopoeial standard remains the time-consuming, heating and vacuum filtering deaeration procedure, while it is stated that other ways of deaeration, if shown to be equivalent, can be used. Nevertheless, there is little guidance on what is considered an equivalent method of deaeration and acceptable level of deaeration. In this study, our goal was to compile published information and provide keypoints when considering deaeration. Based upon these insights 13 candidate deaeration protocols were defined which would provide adequate deaeration with minimum effort/time. Dissolved oxygen measurement served as means for evaluation if optimum level of deaeration of dissolution media was achieved by each deaeration protocol.

Keywords: dissolution testing, deaeration protocol, time and labour efficient deaeration procedure, optimum level of deaeration, dissolved oxygen measurments

I. INTRODUCTION

The dissolution testing procedure, harmonized through the main pharmacopoeias ever since 2006 [1] encompasses a strictly controlled setup in regard to instrumentation and test conditions [2][3][4]. One of the parameters that plays a key role in dissolution testing is dissolution medium deaeration as it can affect the dissolution process and the results obtained. Deaeration ensures a more uniform, stable and controlled environment for drug release, providing improved reproducibility, reliability and minimized overall measurement uncertainty of the dissolution results. The effects of dissolved gasses on the performance of dissolution testing are well studied and established [5][6][7]. Furthermore, a multitude of studies and publications exists that evaluate different deaeration techniques and their effectiveness [8][9][10][11][12][13][14][15][16]. Even though the study of methods of deaeration in dissolution testing has been a subject of interest, it is not as extensively researched or standardized as some other aspects of dissolution testing. The proper way to remove dissolved gasses is given in pharmacopoeial monographs as exemplary. It is stated in the monographs that other ways of deaeration, if shown to be equivalent, can be used [2][3]. Although pharmacopoeias detail other dissolution testing conditions such as apparatus design, temperature, rotation speed and so on, there is little guidance on what is considered an equivalent method of deaeration and acceptable level of deaeration. Other guidelines on dissolution testing [7] also do not provide more data on deaeration. The procedure for deaeration recommended in the United States Pharmacopoeia (USP) and the European Pharmacopoeia (Ph. Eur.) consists of a few steps including heating, filtering and stirring of the dissolution medium, under vacuum. In our laboratory, we have been using the pharmacopoeial deaeration method, but often found it too laborious, especially when preparation of a larger amount of dissolution medium was needed. Since pharmacopoeial guidance on deaeration methods is scarce, in search of a simpler, suitable deaeration method, an extensive literature survey revealed that most of the studies on deaeration have been published up until 2006 and data on important aspects of deaeration is scattered throughout the published studies. In recent years dissolution media deaeration seems to be a somewhat forgotten topic and we consider it is worth a revisit. Our goal was to synthesize published information and provide main points when considering deaeration. Based upon these insights several deaeration protocols chosen in terms of simplicity of performance and good time management were devised, based on deaeration techniques that were shown in literature to provide optimal deaeration levels. Adequate deaeration for each deaeration protocol was experimentally evaluated using dissolved oxygen content measurements.

II. MATERIALS AND METHODS

Instruments:

Oxygen measurement instrument: Dissolved oxygen levels were measured using an HQ40d Portable Multi-Meter with an Intellical LDO101 Field Luminescent/Optical Dissolved Oxygen (DO) Sensor, fitted with an automatic pressure sensor module and a temperature sensor (Hach, USA).

Deaeration apparatus: automated heated vacuum degassing system, Distek Model ezfill Media Preparation (Distek, Inc., USA).

Dissolution testing system: SOTAX AT 7 Smart (offline) dissolution testing unit (Donau Lab Zurich, Switzerland).

Dissolution media:

Purified water was obtained with a WATERRO direct flow reverse osmosis system, model: OPTIMUM 400G (5 stage direct flow reverse osmosis system) (Waterro, Latvia), subjected to respective deaeration protocols.

Deaeration protocols:

- *Pharmacopoeial deaeration procedure (USP/Ph. Eur.)*: 1 L purified water was heated with gentle stirring to 41 °C, then filtered on glass funnel/support assembly with 1 L thick glass container, through a 0.45 µm filter (Durapore® PVDF membrane, 47 mm diameter, Merck, Germany) under vacuum with vigorous stirring, followed by additional 5 minutes of stirring under vacuum (deaeration protocol No.2). Generated vacuum pressure was less than 100 mbar, using Sartorius Laboratory Vacuum Pump (type 16692, Germany). Another 1 L of purified water was subjected to the same procedure, only this time a 0.45 µm cellulose acetate (CA) membrane filter (47 mm diameter, PratDumas, France) was used (deaeration protocol No.3) [2][3].
- *Deaeration apparatus*: Purified water was subjected to treatment on the automated degassing system. 1 L of deaerated water, at 37 °C, was obtained (deaeration protocol No.4).
- *Pharmacopoeial deaeration procedure (USP/Ph. Eur.), omitting the heating step*: 1 L purified water was filtered on glass funnel/support assembly with 1 L thick glass container, through a 0.45 µm filter (CA filter, 47 mm diameter, PratDumas, France) under vacuum with vigorous stirring, followed by additional time of stirring under vacuum, varied from 5 min, 10 min to 15 min (deaeration protocol No.5, No.6 and No.7, respectively). Generated vacuum pressure was less than 100 mbar, using Sartorius Laboratory Vacuum Pump (type 16692, Germany).
- *Preheating and temperature equilibration/deaeration with stirring in dissolution vessel*: 1 L purified water, heated to 38 °C, was filled in a dissolution container, on a previously heated dissolution instrument, and left for a specified amount of time with stirring by paddle (Apparatus 2). Stirring speed investigated was 50 rpm for a time of 60 minutes, and 100 rpm for a time of 30 min, 45 min and 60 min (deaeration protocol No.8, No.9, No.10 and No.11, respectively).
- *Filtering under vacuum at room temperature, using a coarse filter*: 1 L purified water, at room temperature, was filtered under vacuum in a 10 L thick glass container through a sintered glass filter (Filter Support with Glass Frit and O-Ring 6983004, Sartorius, Germany). The funnel and sintered glass filter were placed on the neck of the glass container and hermetically sealed with parafilm. After filtering the medium was left under vacuum for 10 min, 15 min or 20 min (deaeration protocol No.12, No.13 and No.14, respectively). Generated vacuum pressure was less than 100 mbar, using Sartorius Laboratory Vacuum Pump (type 16692, Germany).

Dissolved oxygen measurements:

Immediately after deaeration, deaerated media were transferred to sample bottles and closed tightly. Measurements were performed by the optical measurement method for determining dissolved oxygen in aqueous solutions (ISO 17289:2014), at room temperature. Conversion of results accounting for temperature of 37 °C was performed according to Henry`s law.

III. RESULTS

Results for dissolved oxygen content of water deaerated with respective deaeration protocols are presented in Table 1.

Table 1: Results for dissolved oxygen content of water deaerated with respective deaeration protocols.

#	Deaeration protocol	Measured dissolved oxygen (mg/L)

		Measurement temperature (°C)	Dissolved oxygen (mg/L)	Dissolved oxygen (mg/L), adjusted to 37°C
No.1	Non-deaerated water	23.8	4.46	3.55
No.2	Pharmacopoeial (USP/Ph. Eur.) procedure (PVDF filter)	23.5	7.11	5.63
No.3	Pharmacopoeial (USP/Ph. Eur.) procedure (cellulose acetate filter)	23.3	6.88	5.43
No.4	Deaeration apparatus			
No.5	Pharmacopoeial (USP/Ph. Eur.) procedure wo* heating, 5 min additional vacuum	22.9	6.46	5.06
No.6	Pharmacopoeial (USP/Ph. Eur.) procedure wo* heating, 10 min additional vacuum	22.9	6.39	5.00
No.7	Pharmacopoeial (USP/Ph. Eur.) procedure wo* heating, 15 min additional vacuum	22.9	6.39	5.00
No.8	Preheating/stirring in dissolution vessel, 50 rpm, 60 min	23.2	7.16	5.64
No.9	Preheating/stirring in dissolution vessel, 100 rpm, 30 min	23.6	6.73	5.34
No.10	Preheating/stirring in dissolution vessel, 100 rpm, 45 min	23.4	7.03	5.56
No.11	Preheating/stirring in dissolution vessel, 100 rpm, 60 min	23.1	7.11	5.59
No.12	Vacuum filtration (coarse filter) wo* heating, 10 min additional vacuum	23.5	7.72	6.11
No.13	Vacuum filtration (coarse filter) wo* heating, 15 min additional vacuum	23.1	7.64	6.01
No.14	Vacuum filtration (coarse filter) wo* heating, 20 min additional vacuum	23.1	7.35	5.78

*without

IV. DISCUSSION

Based upon information in published studies, we have assembled a few main points to consider when choosing a deaeration procedure. To commence, published data points to the importance of establishing the dissolved gas content of the water source in the laboratory, since it influences degassing effectiveness [10][14]. Furthermore, it is essential to not only choose an efficient deaeration method, but an optimal one, that should be performed at temperatures close to the target temperature of the dissolution test (37 °C). The pharmacopoeial deaeration procedure (USP/Ph. Eur.) has become an industry standard, and pharmacopoeia-equivalent media should only be produced using methods that achieve near equilibrium in both temperature and dissolved air [10]. Dissolved air in dissolution media tends to achieve equilibrium and over the course of an entire dissolution test more efficient deaeration of the dissolution media had no discernible effect on the final level of aeration. Both low level deaeration and aerated media will reach an equilibrium level of aeration after a certain period [9][10].

As a reliable measure of adequate degassing, PV test with 10 mg prednisone calibrator tablets was established [10][11][13][14][15][16][17]. In addition, as a relevant means of measurement of deaeration, dissolved oxygen content was monitored in several published studies [8][9][10][12][14][15]. In the study of Curley et al. [10] an acceptable range of dissolved oxygen in deaerated media that was found to produce an equilibrium effect was 4-7 mg/L, equivalent to the USP-recommended procedure. This is supported by data in another study by Nithyananadan et al [14], whereas criteria for suitable level of deaeration of water for PV test with USP Prednisone Tablets, an oxygen concentration threshold of about 6 mg/L has been found effective. The published findings by Nithyananadan et al. are also included in chapter <1092> of the USP [5]. It can be concluded that measurement of dissolved oxygen is a reliable means of evaluating the extent of deaeration for different deaeration protocols, except for inert gas sparging where measurement of total dissolved gas is necessary [5][11][14]. Helium sparging was demonstrated to achieve lowest amounts of dissolved oxygen in deaerated media, but as previously mentioned it was also demonstrated that low amounts of dissolved oxygen eventually attained state of equilibrium during the dissolution test [10].

Over the course of 30 years, several types of deaeration protocols comprising different conditions and procedures were studied, including: vacuum filtration (with and without heating), sonication/vacuum, heating and/or stirring, inert gas sparging, overnight equilibration at 37 °C, equilibration at 37 °C while stirring in dissolution vessels, USP deaeration procedure, FDA deaeration procedure, automated degassing systems (deaeration apparatus), etc. [8][9][10][11][12][13][14][15][16].

Considering these insights, inert gas sparging was immediately eliminated as a deaeration technique of choice, due to the financial burden of the needed equipment and helium supply. In terms of best effort/time/efficacy management, the most appealing procedure that stood out right away was equilibration at 37 °C, while stirring in dissolution vessels. Under the same criteria, pharmacopoeial deaeration procedure (USP/Ph. Eur.) without the heating step and room temperature vacuum filtration through sintered glass filter were also included. Pharmacopoeial deaeration procedure (USP/Ph. Eur.) using different types of filters was included as a benchmark. Media produced by a deaeration apparatus was also regarded as a benchmark, as this method has consistently been shown to produce media within the desired oxygen range [8][9][10][15]. The non-deaerated medium was measured to determine the starting point of the water source provided by the equipment in the laboratory. The devised deaeration protocols were evaluated against each other using measurement of dissolved

oxygen. Deaeration protocols providing deaerated medium with oxygen concentration ≤ 6 mg/L were considered acceptable.

All 13 tested deaeration protocols provided suitably deaerated media (Table 1). Results seem to be consistent with previously published data for similar deaeration protocols. As demonstrated in previous studies, effective deaeration was achieved by using the pharmacopoeial vacuum filtration procedure where the heating step was omitted [8][11]. Our results are in line with these findings. One further investigation distinct to our deaeration protocol for this technique was that we have varied the additional stirring under vacuum step for 5 min, 10 min and 15 min. It appears that the prolongation of this step provides only insignificant further deaeration. In correlation to this, prolonged stirring under vacuum (5 min, 10 min, 15 min and 20 min) after heated vacuum filtration through a 0.45 µm filter, was also shown to provide only slight further decrease of the level of dissolved oxygen, in a study by Degenhardt et al [9]. Room temperature vacuum filtration through a sintered glass filter provides slightly higher levels of dissolved oxygen in comparison to filtration through a 0.45 µm filter, but nevertheless manages to achieve equilibrium levels of dissolved oxygen. Prolonged time of stirring under vacuum, after filtration, provides only slight further decrease of dissolved oxygen levels. We encountered only one study that has examined deaeration effectiveness of room temperature vacuum filtration through a coarse filter (40-60 µm pore size), where similar results were obtained [8].

USP has indicated that allowing media to sit in the dissolution vessels while stirring may not be a suitable means of deaerating and believe that air is actually reintroduced by stirring [5][18][19]. A few studies investigated the effect of reaeration of previously deaerated media, left unstirred or stirred in dissolution vessels, at 37 °C, with different combinations of paddle/basket and rotation speed. The obtained results demonstrated that reaeration occurred even in unstirred media, while when stirring was applied, faster reaeration occurred, but stayed well below 100% saturation, even up to 6 hours [8][10][11][12]. Only one published study was found having explored the method of heating and stirring of media on a dissolution bath, as a deaeration procedure. In the study of Curley et al. [10], the media (deionized water) was heated and stirred on a dissolution bath with USP dissolution apparatus 2 (paddle), at 50 rpm, 37 °C, and was shown to produce comparable levels of dissolved oxygen to the USP deaeration procedure and gas permeable membrane deaeration apparatus. We considered this the most effort/time efficient method, so we wanted to examine it further, in regard to paddle speed and time. In the mentioned study of Curley et al., the equilibrium level of dissolved oxygen was achieved after stirring the dissolution media at 50 rpm, at 37 °C, for 5.5 h. In our study, suitable dissolved oxygen level was measured for all combinations of paddle speed and time (100 rpm for 30 min, 100 rpm for 45 min, 100 rpm for 60 min and 50 rpm for 60 min). Prolonged stirring time did result with higher oxygen levels, but within the defined threshold of 6 mg/L. One distinction in our study compared to Curley et al., is that the starting level of oxygen in non-deaerated water was lower (3.55 mg/L). Curley et al. found that media with an initial measurement of dissolved oxygen level of 5 mg/L, showed little change over time and can be considered at equilibrium throughout the dissolution process. Furthermore, these authors establish an acceptable range for the equilibrium state of 4-7 mg dissolved oxygen/L, allowing for oxygen meter and lab variability. Our results appear to be consistent with these findings, since stirring of the dissolution media at different combinations of paddle speed and time, at 37 °C, kept the equilibrium level of dissolved oxygen. Results also indicate that stirring media in the dissolution vessel will reaerate media, but the level of reaeration will not be significant if the starting oxygen concentration is near the equilibrium state. Indeed, from looking at results of all 13 examined deaeration protocols, it seems that further deaeration of incoming media whose initial dissolved oxygen level is near the equilibrium state (around 6 mg/L), does not lower dissolved oxygen content, but will keep this state after deaeration. In this way, if the purified water system at disposal in the laboratory provides water with levels of dissolved oxygen in the equilibrium concentration range, the media can be measured and preheated to 38 °C, then filled directly into dissolution vessels. Stirring for 30 minutes with paddle, at 100 rpm, will provide reliable temperature equilibration without disturbing the oxygen equilibrium state (deaeration protocol No. 9). This optimizes the performance of the dissolution test in regard to time and effort.

It must be pointed out that our results represent preliminary data. The discussion is an interpretation of our preliminary data in line with similar published results. We acknowledge that further investigation for each deaeration protocol should continue using PV test with 10 mg prednisone calibrator tablets in order to establish correlation between the dissolved oxygen measurements and the PV test results. This would provide much more reliable data upon which acceptable dissolved oxygen content for each deaeration protocol can be confirmed.

V. CONCLUSION

Overall, while deaeration is acknowledged as important for accurate and reproducible dissolution testing, the deaeration procedures themselves are not as extensively studied or standardized as other dissolution parameters. The topic is worth a re-visit, since any simplification of the deaeration process could compound to reduced cost and time for the dissolution testing procedure.

In this study, a thorough literature review regarding deaeration was conducted. Observations made from

this review were the foundation for several examined deaeration protocols. Preliminary data from 13 candidate deaeration protocols indicate that all deaeration protocols provide optimum level of deaeration, which is consistent with previously published data for similar deaeration protocols. Also, results point out that one important factor for the obtained oxygen level of the deaerated media in our study is the dissolved oxygen content of the incoming water, which resulted near the equilibrium dissolved oxygen concentration. Further confirmation of the success of the examined deaeration protocols should be obtained by correlating dissolved oxygen measurements with PV test results using prednisone calibrator tablets.

Although deaeration protocols have been somewhat extensively studied over the course of 30 years, the pharmacopoeial standard remains heating and vacuum filtering procedure. Standardization of deaeration protocols, particularly through regulatory bodies like the pharmacopoeias could help refine best practices. Further development in this area can be made by including alternate methods of deaeration that have been shown to provide equivalent deaeration.

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