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Research paper

Investigation of drug product and container-closure interactions: A case study of diluent containing prefilled syringe

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ABSTRACT

Prefilled syringes (PFS) constitute a widely used medical device for drug delivery particularly for the drugs of biological origin. Interactions between the product contents and the components of the PFS play a critical role in determining the suitability of selected PFS. A diluent (with benzyl alcohol/BzOH as a preservative) containing PFS used for reconstitution of the lyophilized product revealed a systematic decrease in the BzOH content during accelerated and stress stability program. Investigation was carried out to understand and identify the underlying causes of this phenomenon. BzOH has a varying propensity to bind to the rubber components (stopper and tip-cap) of the PFS. Vapor permeation behavior across the tip-cap of the PFS was studied via headspace-gas chromatography-mass spectroscopy (HS-GC-MS) enabled analysis. Depending on the properties of the rubber components, BzOH can not only bind but also traverse across them, resulting in a systematic loss during the course of the stability. PFS can allow not only water vapor permeation across the tip-cap as shown in previous studies, but also molecules like benzyl alcohol. This phenomenon stresses the need for careful selection of the components of the primary packaging and also provides an opportunity to deploy novel tools like HS-GC-MS in the early selection of the optimal primary packaging configuration.

1. Introduction

With the advent of the biotechnology based approaches for therapeutics, a larger number of monoclonal antibodies (mAbs) are under development. Around 40% of the developed monoclonal antibodies are lyophilized to obtain the final drug product in a powder (cake) form [1,2]. Gervasi et al. recently reported that in the European Union alone, around 34% of the parenteral protein formulations are lyophilized [3]. Lyophilization ensures that the solution mediated degradation pathways are circumvented so that the stability of the product is assured over the duration of the shelf life [4,5]. A lyophilised product necessitates the use of a diluent/vehicle to reconstitute the lyophilisate into a parenterally administrable solution. Typically, a diluent can be water for injection or a bacteriostatic water entailing use of a preservative [6] that can afford the microbiologically stable reconstituted solution for a defined period of use. BzOH containing water for injection is widely used as a diluent for protein lyophilisate and are mostly available in vials for multiple-use. Development of a diluent contained in PFS, co-packaged with lyophilizate offers advantages in terms of dose accuracy and patient safety, as the amount of the diluent to be used for reconstitution is ensured and errors due to variability inherent in the handling of different syringes is minimized [7,8].

Drug administration using PFS systems have become increasingly crucial, particularly for those drugs that require repeated or chronic administration [9]. The primary factors driving the growth of PFS is the ease of administration, dosing accuracy and their compatibility with auto injectors which further improves the patient compliance [10,11]. Development of a drug product based on PFS entails the technical challenges owing to the complexity of the device compared to vials. These challenges are often reflected in the form of an impact on the drug product quality (e.g: tungsten [12,13]/silicone oil induced aggregation or particle formation [14–17]) or device functionality (needle clogging, high break-loose/gliding force etc. [18,19]) resulting in adverse influence on product quality and performance. Vapor transmission across the needle shield of the PFS has recently been reported to result in clogging of the needle for protein containing drug product [20,21]. Comprehension of such interactions between the drug product solution and PFS components must be considered while selecting the

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primary container for drug products. This is not only relevant for biologic products with high concentration and viscosity but also to the non-biologic products containing BzOH. As far as PFS are concerned, BzOH is used as a preservative concomitantly in the small molecule based sterile formulations (e.g: Falsodex®) and also used as a preservative component of the diluent/vehicle for protein lyophilisates (e.g: Extavia® and Enbrel®) to ensure the sterility of the reconstituted product for the duration (inclusive of the hold time) until the last dose of product is administered to the patient. A detailed overview of the preservatives used in small molecule and protein parenteral formulations can be found in review article published by Brian and co-workers [22].

As a part of the stability program for the technical development of one of our projects, entailing diluent PFS, testing was conducted at 'accelerated conditions' and 'stress conditions' (along with real time storage conditions), which are typically higher than the recommended storage temperatures and relative humidity values. During the course of these studies, a decrease in the BzOH content of the diluent syringes was observed. A decrease in the preservative content of the diluent can result in a reconstituted product with potentially altered preservative efficacy. This can have a profound impact on the microbiological quality of such products particularly if they are intended for multiple administrations. Increased use of PFS [23] coupled with upsurge in the number of lyophilized biological products [24] compels and provides motivation for assessment and investigation of this phenomenon. Identification of such phenomena early in the development can serve as a key indicator for selection of optimal primary packaging components that afford expected shelf-life.

PFS are available in staked-in needle and luer based formats (Fig. 1). The luer formats can be based on a 'Luer-slip' (LS) wherein the tip-cap simply slips/fits onto the syringe tip (Fig. 1B) or it could be a 'Luer lock' (LL), where a rotating collar drives the tip-cap to fit/lock onto the syringe tip (bearing the collar) (Fig. 1C). The tip-caps used for luer-slip syringes are made of elastomer/rubber, whereas the tip-caps for LL syringes typically constitute the elastomer/rubber housed in a rigid plastic shield (e.g. polypropylene). The LL and LS syringes are available for pharmaceutical applications from many companies worldwide [25]. The elastomeric synthetic rubbers and butyl thermoplastic elastomers used for manufacturing of tip-caps are often gas-permeable wherein this permeability facilitates sterilization (by either steam or ethylene oxide) of PFS system [26]. Apparently, this permeability can also result in water vapor permeation across the tip-cap from the drug product contained in the syringe to the external environment [21]. We demonstrate that this phenomenon is not only responsible for the escape of the water molecules but also the small molecules like BzOH. In this research article, we systematically investigate the decrease in the benzyl alcohol content of the PFS and show that BzOH contained in the diluent can not only bind but also traverse across the rubber tip-cap of the PFS resulting in the loss of preservative, without any concomitant degradation.

2. Materials and methods

2.1. Materials

BzOH was purchased from Merck KGaA (Darmstadt, Germany). Ultrapure water obtained from Milli-Q device (Millipore, model Advantage A10) was used for compounding the BzOH containing solution (diluent). Dow Corning® 360 Medical Fluid (polydimethylsiloxane/silicone oil) with a viscosity of 1000cST was purchased from Dow Corning (USA). Syringe number 1: 2.25 mL LL-PFS samples were composed of a 2.25 mL long, Type I borosilicate clear glass barrel with finger flange and tip-cap made of synthetic isoprene-bromobutyl elastomer rubber blend shielded by a plastic (polypropylene) casing. The syringes were stoppered using teflon-coated (product contact surface) bromobutyl plunger stoppers. Syringe number 2: 2.25 mL LS-PFS samples were composed of a 2.25 mL long, Type I borosilicate clear glass barrel with a finger flange and tip-cap made of synthetic polyisoprene-proprietary elastomer rubber blend. The syringes were stoppered using teflon-coated (product contact surface) bromobutyl plunger stoppers. The syringes (with tip-caps) and plunger stoppers were received as ready-to-use (RU) components from the manufacturer (washed, siliconized, and sterilized). The information on the physico-chemical attributes of the above mentioned primary packaging components were available from the certificate of analysis/specification provided by the respective manufacturers. The BzOH containing solutions were prepared at the concentration of 10 mg/mL or 9.7 mg/mL in deionized water, filtered (0.2 μm), and filled (1 mL) into glass syringes and plungered. Each PFS had a target headspace of 5 mm (limits: 2–5 mm) between the liquid surface and syringe stopper, when held in vertical (flange up) position. The syringe filling operation was conducted under controlled environment of laminar flow. All chemicals used in this study were compendial grade. Teflon tape (width: 12 mm; thickness: 0.1 mm, article no. 7500001008) was purchased from Teguma GmbH, Germany. Pipette, pipette tips (1 mL) and sample tubes (Safe lock tubes 1.5 mL cat. No. 0030120.86) were procured from Eppendorf, Germany. Glass bottles used in the binding

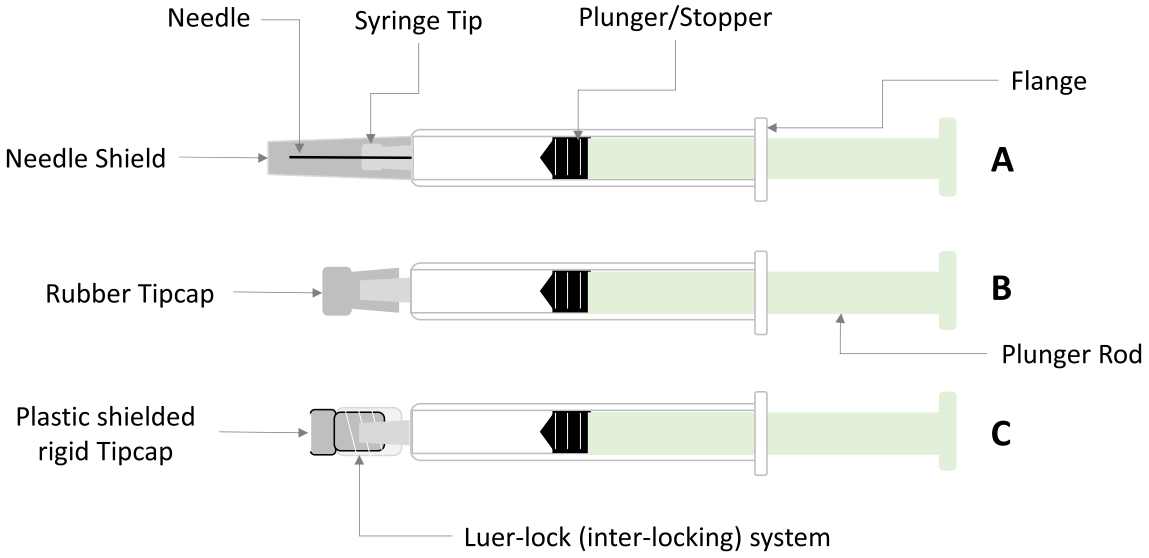

Fig. 1. Prefilled syringe formats used widely: (A) Staked in needle PFS, (B) Luer-slip PFS, (C) Luer-lock PFS.

studies were procured from Schott-Duran®, Germany and were made of clear borosilicate glass (EP/USP type I) provided with a lip sealed blue polypropylene caps.

2.2. Stability studies

Stability studies were performed as a part of the technical development on the diluent filled syringes (Syringe 1 and Syringe 2). Briefly; both syringe types were subjected to long term (5 ± 3 °C), accelerated (25 ± 2 °C/60 ± 5% relative humidity (RH)) and stress (40 ± 2 °C/ 75 ± 5% RH) storage conditions for up to 3 months with testing points at 0 months, 1 month and 3 month. One diluent syringe for each type was tested at every testing point per stability condition. At the designated pull-point, the tip-cap was unscrewed/removed and the entire contents of the diluent syringe were expelled (no needle used) into a 1.5 mL Eppendorf tube using a plunger rod. An aliquot was then transferred from the Eppendorf tube into the HPLC vial using a 1 mL pipette before analysis.

2.3. HPLC method for BzOH

The content of BzOH and impurities (benzaldehyde and benzoic acid) was quantified using a validated HPLC method. The system (Agilent LC 1200) was composed of a HPLC pump equipped with a pump, injection system, UV detector with standard flow cell capable of monitoring absorbance 254 nm and a column heater. A reversed phase column (Nucleosil 100-10C18, length 250 mm, diameter 4.0 mm) with a column temperature of 30 ± 2 °C was used. The mobile phase comprising 60% v/v of 10 mM KH_2PO_4 in water and 40% v/v of methanol was pumped at a flow rate of 1.5 mL/ min. Benzyl alcohol (t_R = 4.3 min), benzaldehyde (t_R = 6.7 min) and benzoic acid (t_R = 2.8 min) were detected at a wavelength of 254 nm. The diluent solution (aliquoted from respective study/stability program into HPLC vials) was injected directly with the injection volume of 10 µL. The method demonstrates excellent precision/very low method variability corresponding to relative standard deviation of 0.11% justifying the use of one syringe/sample at each testing condition.

2.4. Ishikawa diagram

A systematic analysis of the different variables that could result in the decreased BzOH content was performed and an Ishikawa diagram was designed. The design of Ishikawa/fish bone helped to graphically highlight the different potential causes of a specific problem or observation. It is particularly useful for classifying the risks and to promote actions for mitigating or eliminating risks. Upon the detection of any unexpected incident/observation it is imperative to implement the corrective and preventive actions. However, this is feasible only if the root cause has been identified and the Ishikawa diagram tool is a key enabler for this [27].

2.5. Material compatibility studies

Silicone rubber tubing (ID/inner diameter: 9.5 mm, OD/outer diameter: 16.6 mm, length: 159 cm, Saint Gobain) and fluoropolymer tubing (ID: 4.8 mm, OD: 6.4 mm, length: 995 cm, Saint Gobain) were rinsed with WFI. Tubing was then filled with 121 mL and 43 mL of 9.7 mg/mL of BzOH solution respectively. The open ends of tubes were closed with stainless steel plugs along with a parafilm. The respective tubing was incubated at room temperature and 5 mL of sample was withdrawn at 24 h and 48 h from each tubing. A diluent control was included (untreated) which was not in contact with any tubing but stored in glass bottles. The samples were analyzed for BzOH content and degradation impurities using HPLC.

2.6. Silicone binding studies

Silicone oil (Polydimethoxysilane, 1000cSt, Dow Corning, USA) corresponding to 0 mg, 70 mg and 140 mg was weighed into three glass bottles (100 mL). The silicone oil in each bottle was dissolved in dichloromethane (5 mL) and the dichloromethane was allowed to evaporate while forming a thin layer of silicone oil over the internal walls of the bottles (dried overnight). The diluent solution (9.7 mg/mL) was added to each of these bottles, resulting in a final silicone oil concentration of 0 mg/mL (control), 0.7 mg/mL and 1.4 mg/mL of diluent. The bottles were sealed with Teflon tape (Ulith-Fluoropolymer-Gewindedichtband, type A/E1, 100 g/m^2) wrapped around bottle thread and were incubated at 40 ± 2 °C under ambient humidity conditions. Aliquots of 1 mL were withdrawn from the bottle via a 1 mL pipette at time points of 0 week, 2 weeks, 4 weeks in Eppendorf tubes, centrifuged and the aqueous phase was tested for BzOH content and impurities using HPLC. One measurement was performed at every testing point per condition.

2.7. Surface area determination

The surface area of the rubber component of the tip-cap and stopper was computed using computer aided design (Solidworks 2014, Dassault Systèmes, France). The tip-cap and the stopper engineering designs were simulated based on the respective configurations of the primary packaging components as available from the manufacturers. The overall surface area values computed by the software were used for subsequent calculations.

2.8. Stopper binding studies

Ready to use stoppers were incubated in 9.7 mg/mL BzOH solution at the proportion of 1 stopper/mL (n = 100) in glass bottles (100 mL). The bottles were sealed with parafilm and were incubated at 40 ± 2 °C under ambient humidity conditions. Aliquots of 1 mL were withdrawn from the bottle via a 1 mL pipette in Eppendorf tubes at the time points of 0 week, 1 week, 2 week, 4 week and tested for BzOH content and impurities using HPLC. One measurement was performed at every testing point per condition. Since, both syringe 1 and syringe 2 have same stopper the outcome of this study is applicable for both syringe types.

2.9. Tip-cap binding studies

The luer-lock tip caps were unscrewed from the syringe barrel of syringe 1 and the rubber component of the tip-cap was easily separated (pushed through) from the polypropylene casing without affecting its integrity or causing any surface bruise. The tip-caps (rubber component) were incubated in 9.7 mg/mL BzOH solution at the proportion of 1 tip-cap/mL (n = 50) in glass bottles (50 mL). The bottles were sealed with parafilm and were incubated at 40 ± 2 °C. Aliquots of 1 mL were withdrawn from the bottle via a 1 mL pipette in Eppendorf tubes at the time points of 0, 1 week, 2 week, 4 week and tested for BzOH content and impurities using HPLC. One measurement was performed at every testing point per condition. Tip-cap binding study was only performed on syringe 1, as the tip-caps for syringe 2 were not available at the time of investigation.

2.10. Vapor permeation assessment using HS-GC-MS

Diluent containing syringes; Syringe 1 (n = 3) and syringe 2 (n = 3) were individually sealed into the GC-MS headspace vial and one syringe from each type was stressed at 5 °C, 40 °C and 60 °C for 14 days. At the end of 14 days, the headspace of the syringe containing vials was analyzed for BzOH. Unpackaged diluent (naked solution) and water in headspace vials were used as positive and negative control respectively.

The Headspace-GC-MS analysis was performed on an Agilent GC 7890A equipped with an Agilent headspace instrument 7697A coupled to an Agilent triple quadrupole MSD 7000. A 30 m Agilent HP-5MS column with 0.25 µm film thickness and an inner diameter of 0.25 mm was used. The headspace sample was incubated at 40 °C in the oven of the headspace sampler" for 15 min. The GC injector/headspace inlet of the GC was heated to 230 °C while the MS interface was set to 240 °C. Temperature gradient used was: 35 °C for 1 min with 20 °C per minute to 240 °C for hold for 1 min. The MS scanning was performed in the range of 33–400 *m/z* with 5 scans/s.

2.11. Gravimetric analysis of the syringes

The PFS (syringe 1, n = 5) were manually filled with approximately 1 mL of 9.7 mg/mL BzOH solution and stoppered with uniform headspace of 5 mm. The syringes were ensured to not have any adhering droplets onto its surface. The diluent containing syringes were placed horizontally in the incubator maintained at 40 °C at ambient humidity conditions. The syringes were withdrawn at t0, 1 week, 2 week 4 week, 6 week, 8 week, and 12 week, equilibrated to the room temperature for an hour, followed by weighing using the weighing analytical balance (Sartorious GmbH, Germany). After weighing, syringes were returned to the incubator chamber. Gravimetric analysis was performed only on syringe 1, as enough quantities of syringe 2 were not available at the time of investigation.

3. Results and discussions

3.1. Stability studies

Stability studies are typically performed during the early stage development for the selection of the optimal primary packaging material for the drug product. During the technical development of a diluent PFS for one of our lyophilisates, we observed that there was decrease in the BzOH content of the PFS particularly at the accelerated (25 ± 2 °C/60 ± 5% RH) and stress (40 ± 2 °C/75 ± 5% RH) conditions (Table 1) for two type of luer-lock syringes that were evaluated as a part of primary packaging selection. Quantitatively this decrease was 0.3%, 0.8% and 3.8% at the real time, accelerated and stress conditions respectively (for syringe 1) at the end of 3 months. For another tested PFS (syringe 2), this decrease was correspondingly; 0.6%, 10.9% and 12.7%. Accelerated and the stress conditions serve to provide the faster read-outs for product quality and help predict any unforeseen issues that can be potentially encountered at real time storage (5 ± 3 °C) conditions. The stress conditions may not be experienced by the product in a real time setting but they help reveal the unexpected observations early during development. The observed magnitude of BzOH decrease might not be very significant at 3 months, but can compromise the preservative content and potential preservative efficacy (offered after the reconstitution) over the course of product shelf-life. Moreover, the relative differences in decrease of BzOH between syringe 1 and syringe 2 were quite intriguing. These observations triggered a detailed scientific investigation to better understand the drug product and container closure interactions. In case of the proposed product, the BzOH decrease was deemed moderate for syringe 1 which was eventually chosen as primary packaging material. Nonetheless, the tentative shelf-life afforded to the product was conservatively reduced from 60 months to 48 months to have a product that meets its desired specifications in terms of BzOH content. In course of the following discussion; approaches, experimental data and corresponding observations directed towards investigation of the BzOH decrease have been described. Although the investigation aims to assess the factors particularly associated with syringe 1 being the chosen packaging configuration, data from syringe 2 have been compared to that of syringe 1 to support the hypothesis and inference of the experimental/investigation findings.

3.2. Fish bone analysis

As a part of the quality risk management (QRM) framework, it is important to assess and investigate the observations that pose risk to the quality of a pharmaceutical product [28]. QRM emphasizes on the structured process for analyzing the risks with respect to their probability of occurrence and potential consequences before deciding whether additional measures are warranted. One central element of QRM, is to identify the root cause(s) and contributing factors for an observation/incident. Different procedures depending on the nature of the observations can prove useful in the handling of an incident or observations. Fish bone/Ishikawa diagram is one of the widely used tools to assess and identify the root cause for an impending issue. As depicted in Fig. 2, a systematic analysis of the potential root causes that could lead to a decrease in BzOH content (within very narrow limits) was made based on the cross-functional inputs. The method of analysis used for BzOH content measurement was evaluated for the potential discrepancies associated with sample preparation, nature of the gradient, system suitability, carry over effects and method capability. This was observed to be well within control with a very low method variability. Effect of the environmental factors was evaluated in terms of the temperature, pressure and humidity excursions during the stability and these found to be within the limits. Errors in lab measurements resulting due to miscalculations and uncalibrated instruments were ruled out after verification of the records. Human intervention related causes like wrong labelling, analyst training and use of wrong primary packaging materials were assessed and confirmed to be nil. Probable causes attributed to materials factors like syringe barrel binding, stopper and tip-cap binding were hypothesized to be responsible for the observation as it could not be ruled out on any basis. Molecular phenomenon like BzOH transmission, polymerization/degradation were considered, of which degradation was ruled out as the impurity profile of the diluent syringe remained unchanged (or < LOQ) during the course of stability program. As a result of this assessment, some of the factors were excluded from being the probable root cause or from being contributing factors and the direction of the investigation could be defined. Studies were narrowed down and performed to understand the nature of the container-closure interactions with the product (diluent) that potentially resulted in a BzOH content decrease. These studies have

Table 1
Stability summary of the benzyl alcohol (10 mg/mL) containing diluent in the syringe 1 and syringe 2.

Stability time point	Benzyl alcohol content at 5 ± 3 °C (mg/mL)		Benzyl alcohol content 25 ± 2 °C/60 ± 5% RH (mg/mL)		Benzyl alcohol content 40 ± 2 °C/75 ± 5% RH (mg/mL)	
Syringe Type	Syringe 1	Syringe 2	Syringe 1	Syringe 2	Syringe 1	Syringe 2
0 months	10.00	9.94	10.00	9.94	10.00	9.94
1 months	NA	NA	9.95	9.73	9.86	9.54
3 months	9.97	9.88	9.92	8.86	9.62	8.68
Loss of BzOH at 3 months	0.03	0.06	0.08	1.08	0.38	1.26

One diluent syringe for each type was tested at every time point per condition. NA - these time points were not tested.

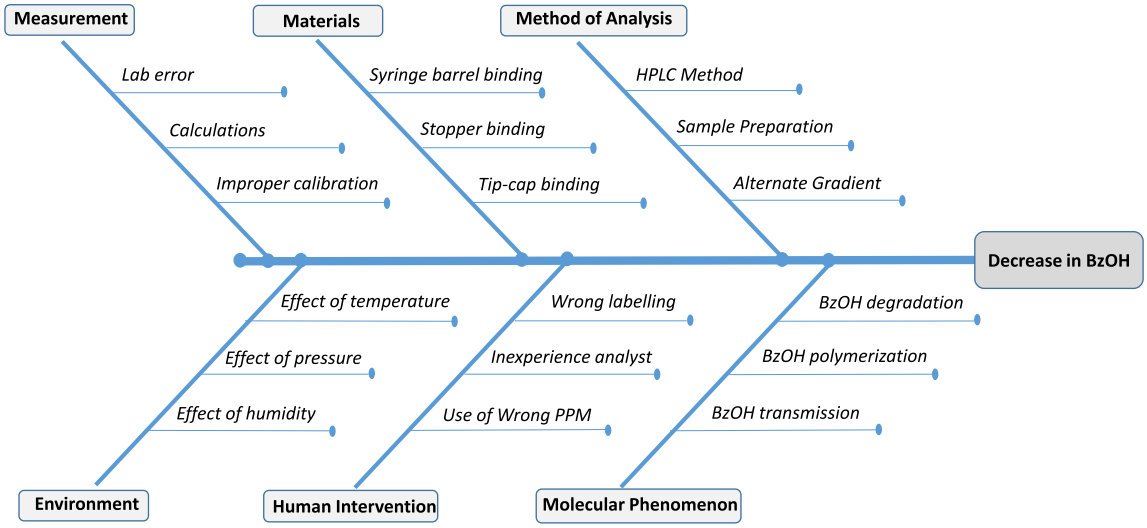

Fig. 2. A systematic analysis of the potential variables that could affect the BzOH content was taken into account (PPM – primary packaging material).

been described in greater details in the following sections of the manuscript..

3.3. Material compatibility studies

Material compatibility studies were undertaken to ascertain the nature of interactions of BzOH with materials like silicone rubber and fluoropolymer. These materials were chosen as they are routinely used in pharmaceutical manufacturing for transfer of the bulk solutions across the tanks/vessels including filling into vials/syringes and represent product contact materials not only during manufacturing but also to certain extent during their storage in primary packaging as some these components are made of silicone/fluoropolymer. To determine the binding/adsorption potential of BzOH containing diluent, silicone and fluoropolymer tubing were filled with diluent (9.7 mg/mL BzOH) solution and clipped at the ends. The filled tubing were incubated at room temperature and aliquots were withdrawn at 24 h and 48 h and analyzed for BzOH content and impurities. Benzyl alcohol (BzOH) content determination indicated strong loss of BzOH for silicone tubing leading to a decrease of 44% and 48% of BzOH after incubation for 24 h and 48 h, respectively (Table 2). This decrease did not correlate with degradation products; benzaldehyde and benzoic acid which remained unchanged (or < LOQ).

Despite the 10 fold higher contact surface area per unit volume of the diluent, fluoropolymer tube did not reveal any adsorption and the content of BzOH remained practically same at the end of 48 h compared to silicone tubing. These observations were in line with previous independent reports by Saller et al. and Bahal et al. on such behavior [29,30]. Bahal et al. have extensively investigated the binding behavior of preservatives like parabens, benzyl alcohol, benzoic acid, sorbic acid and benzalkonium chloride with different tubing; silicone, polyvinyl chloride and fluoropolymer employed in the pharmaceutical manufacturing. All the preservatives except benzalkonium chloride showed a time dependent and concentration independent losses with silicone and polyvinyl chloride tubing without any degradation. Whereas, none of the fluoropolymer based tubing tested caused any loss of the preservatives [30–32]. Likewise, Saller et al. have demonstrated that preservatives like BzOH, phenol and m-cresol when present in aqueous solution can bind, partition into and traverse across the matrix of the permeable silicone (polymer) tubing resulting in a significant loss of preservative [29]. This clearly demonstrates the inert nature of fluoropolymer and affirms the heavy adsorption potential of silicone rubber to preservative like BzOH.

3.4. Silicone binding studies

Silicon binding studies were performed based on the analogy drawn from the reported and observed interactions between silicone tubing and preservatives. It led us to the testing of potential interaction between BzOH and the silicone present on the inner walls of the syringe barrel based on the hypothesis that BzOH in diluent solution can bind to silicone on the inner walls of syringe causing decrease in its content. Silicone oil/polydimethylsiloxane is typically applied/sprayed onto the inner walls of the glass syringe (during PFS manufacturing) to facilitate the smooth sliding/lubrication of the plunger stopper during administration of the drug solution contained in the PFS [33]. The amount of the silicone applied per syringe depends on the properties of syringe (glass/plastic) [34] and the process of siliconization (diving/stationary nozzle [35], silicone crosslinking [19] etc.). The silicone content attribute can be requested from the PFS manufacturer and for the tested syringe 1, an average silicone content of 0.7 mg/syringe was reported. Commensurate with this content, silicone oil was spiked into diluent solution to evaluate the partitioning induced BzOH loss at two concentration; target 0.7 mg/mL and worst case (2 times target - 1.4 mg/mL). No decrease in the BzOH content (and impurities) was observed after incubation (at 40 °C) of the diluent with silicone oil at the concentration corresponding to 0.7 mg/mL and 1.4 mg/mL compared to the control sample (no silicone oil) (Fig. 3) at the end of 4 weeks. This observations are not similar to the ones reported for the silicone tubing and can be explained by the differences in the nature of silicone matrix afforded by a silicone tubing as against the silicone oil. Silicone tubing provides a physical matrix (polymer and fillers) with a certain porosity/

Table 2
BzOH content of the diluent in different tubing after incubation at room temperature.

Sample	BzOH content [mg/mL] at t = 0	BzOH content [mg/mL] at t = 24 h	BzOH content [mg/mL] at t = 48 h
Control	9.69	9.69	9.69
Silicone tubing	9.69	5.43	4.15
Fluoropolymer tubing	9.69	9.68	9.66

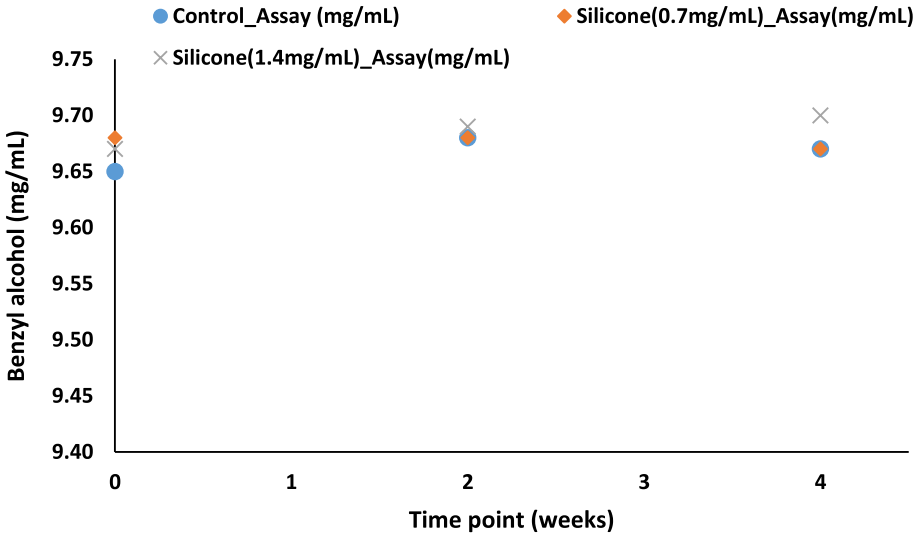

Fig. 3. Effect of the spiked silicone oil on the BzOH content of the diluent at 40 °C (in glass bottles).

permeability which causes the BzOH molecules to get physically lodged into its interstitial spaces which is reinforced by the chemical interactions between the silicone polymer and BzOH. Furthermore, it has been demonstrated that benzyl alcohol not only traverses across the wall of the tubing but is also released into environment at the external wall of the tubing creating a gradient that drives its permeation across it [30,36]. A lack of such a matrix effect in silicone oil spiked diluent solution along with the lower interfacial area afforded by low quantities of silicone oil can likely explain this phenomenon. Moreover, the outcome of the experiment might also be due to the fact that the amount of silicone oil (maximum 1.4 mg/mL) was much less compared to BzOH (9.7 mg/mL). However the tested silicon oil amount corresponds to the maximum amount present in the syringes evaluated and ensures representativeness of the study. Thus, the silicone oil on the syringe barrel was excluded to be a probable cause for the decrease in BzOH content.

3.5. Stopper binding studies

Preservatives are known to bind to the rubber components of the primary packaging. Different rubber formulations can have varying propensity to bind to the preservative [37,38]. Thus, stoppers were identified to be another potential source of BzOH reduction and hence preservative binding studies were undertaken. Incubation of the stoppers (1 unit of stopper/mL of diluent corresponding to 480 mm^2 of stopper surface area per unit volume of diluent, see Table 3 for surface area) into diluent solution resulted in a 5% decrease (see Fig. 4) of the BzOH at the end of 4 weeks as against no change in the control sample wherein, diluent solution was without any stoppers. This translates into a BzOH decrease of 1% per unit surface area of the stopper.

Although the experimental set-up represents a closed system with an inability to determine diffusion across the rubber material, it determines the adsorption/binding affinity of BzOH to rubber surface which is the first step that precedes transmission/diffusion. These experimental observations were compared with the relevant physico-chemical attributes of the rubber formulation like moisture vapor transmission (MVT) and oxygen transmission (OT) values which were determined on the sheet of the rubber of standard thickness of approximately 0.035 in. using methods available at the manufacturer (see Table 4). The stopper rubber formulation employed in the study has a moisture vapor transmission (MVT) of 0.1 g/(m^2 * day) and oxygen transmission (OT) of 63.6 cc/(m^2 * day) which is significantly lower compared to the other rubber components employed in the study (see syringe 1 in Table 4). Moreover, the stopper employed in this study, bears a fluoropolymer film which is present on the product contacting part of the stopper (filled syringe). The fluoropolymer film amounts to 27% (130 mm^3) of the total product exposed surface area of stopper (480 mm^2) in this study (see Table 3 for surface area) and presents an inert surface to BzOH. The relatively lower decrease of BzOH per unit exposed surface area, presence of an inert fluoropolymer film on product contacting surface and that fact that the stopper is common to both syringe type precludes the potential of the stopper to reduce the BzOH content or any vapor transmission across it [30]. Thus, rubber stopper/plunger was expected to have a minimal impact on the BzOH content of the PFS.

Table 3
Surface area (approximate) of the rubber components of syringe 1.

Rubber Component	Surface Area (mm^2)
Tip-cap	450
Tip-cap product contact surface area	1
Stopper	480
Stopper product contact surface area (fluoropolymer coated)	130

3.6. Tip-cap binding studies

The tip-cap represents another rubber component that comes into contact with the drug product. Needle clogging has been reported before for the staked-in needle PFS owing to the moisture vapor transmission across the needle shield resulting in poor functionality of the PFS system/device [20,21]. In this study, the rubber component of the tip-cap (PRTC) from syringe 1 was separated from the polypropylene casing to rule out any potential interactions with plastic casing and only assess interaction between rubber formulation and diluent. Incubation of the diluent with the tip-cap (1 unit of rubber tip-cap/mL of diluent corresponding to 450 mm^2 of tip-cap surface per unit volume of diluent, see Table 3 for surface area) at 40 °C resulted in around 14% decrease of the benzyl alcohol content at the end of 4 weeks as against control which showed no decrease (see Fig. 5). This corresponds to a BzOH decrease of 3% per unit exposed surface area of the tip-cap, which is 3 times higher compared to the one observed for the stopper. This observation was in line with the permeation properties of the tip-cap rubber formulation (as available from the respective manufacturer) which had a high moisture vapor transmission (MVT) of 1.5 g/(m^2 * day) and oxygen transmission (OT) of 1607.4.2 cc/(m^2 * day) compared to that of the stopper (Table 4). Above results together with the permeation data from the manufacturer corroborate to substantiate

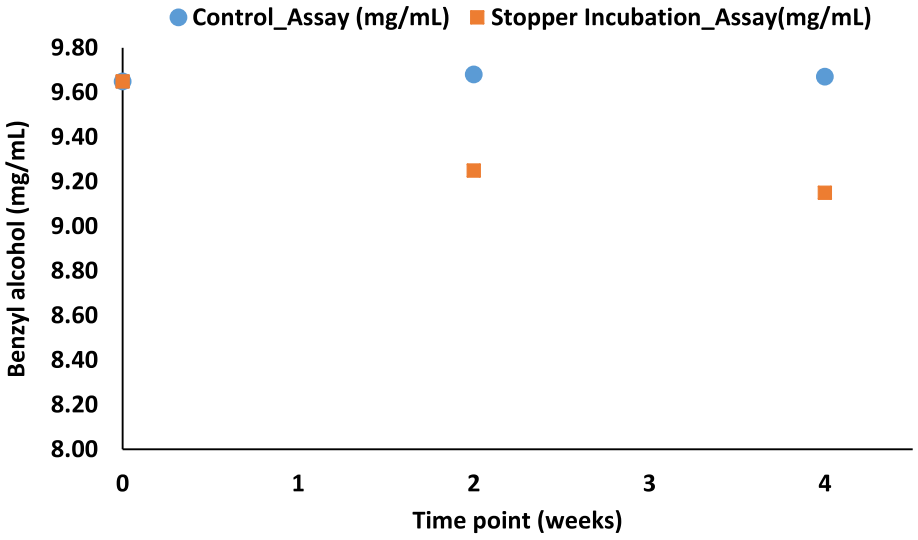

Fig. 4. Effect of the presence of the stoppers (1 unit/mL) on the benzyl alcohol content of the diluent at 40 °C (Approx. surface area of stopper: diluent volume = 48000 mm^2:100 mL).

Table 4
Physico-chemical properties of rubber formulations evaluated in the investigation.

Syringe	Rubber formulation	Moisture vapor transmission [g/(m^2 * day)]	Oxygen transmission [(cc/m^2 * day)]
Syringe 1	Synthetic isoprene – bromobutyl elastomer (Tip-cap)	1.5	1607.4
	Bromobutyl (stopper)	0.1	63.6
Syringe 2	Synthetic polyisoprene – proprietary elastomer (Tip-cap)	2.5	4077.2
	Bromobutyl (stopper)	0.1	63.6

Above information is available from the certificate of analysis/specification provided by the manufacturer of the respective primary packaging component.

the link between surface binding/adsorption and transmission/diffusion across the material, as it is necessary that the compound getting diffused (across the material is first bound to the material surface. This phenomena can be considered to be analogous to the one demonstrated by Saller et al. The rubber formulation of the tip-cap/needle shield often possesses a certain level of permeability to the gases (e.g: ethylene oxide), to ensure its sterilization (chemical) in conjunction with syringe barrel [26]. However, this can have a detrimental impact on the drug product manifested in the form of a moisture loss (resulting in needle clogging for biologic containing PFS) or loss of preservative and hence needs due consideration during development. For a PFS, the product contact surface area of the tip-cap is only a small percentage of the total contact area of a liquid product with primary packaging components. However, the uniform material characteristics of the rubber formulation (and surface thereof, unlike stopper) helps us extrapolate these observations to the actual scenario when product is contained in the syringe. This is also corroborated by the reports from previous studies where, relatively small tip-cap product contact surface area can cause enough water vapor transmission to cause clogging of the staked-in PFS needle [20,21]. The measurements and outcome from the binding

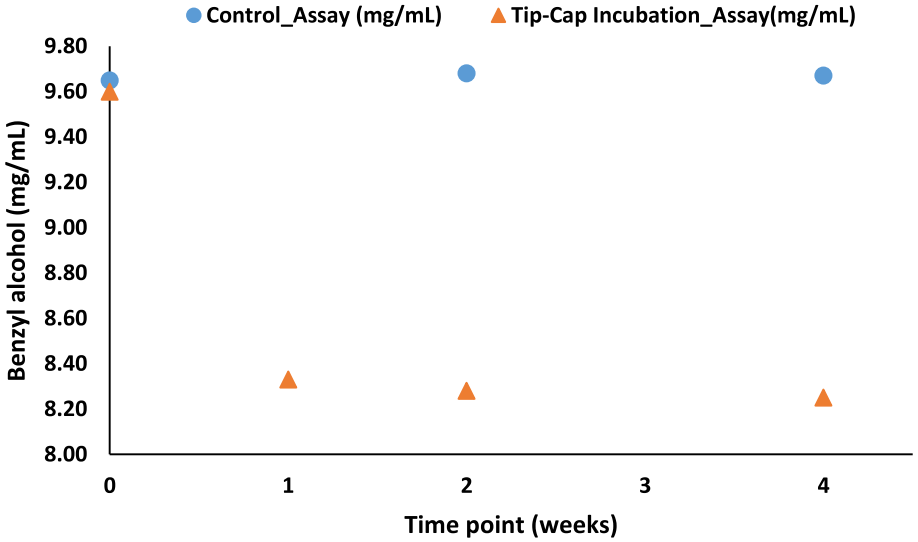

Fig. 5. Effect of the presence of the rubber components of the tip-cap (1 unit/mL) on the BzOH content of the diluent at 40 °C (Approx. surface area of rubber tip-cap: diluent volume = 45000 mm^2:50 mL).

studies, are based on the saturation kinetics but nonetheless reflect the adsorption/binding affinity of BzOH to the rubber surface. The studies were conducted at stress conditions (40 °C) to enable the quick read-out in terms of the BzOH behavior with rubber in an attempt to investigate these interactions. As is the case for the stopper binding study, the experimental set-up for binding studies represent a closed system, as a result it can only explain the affinity of the preservative to bind to the rubber component surface and not the ability of the preservative to traverse across the rubber formulation and its subsequent exchange with the environment. Nonetheless, binding represents the primary and the rate determining step prior to transmission/diffusion across the rubber system [29] and hence these studies are important to support our further inferences.

3.7. Vapor permeation assessment using HS-GC-MS

Having studied the binding behavior of the BzOH to rubber components and the properties of the rubber components used in the study, we hypothesized that tip-cap has a high likelihood of contributing to the loss of BzOH via adsorption-diffusion-desorption across its matrix. To evaluate the hypothesis of a potential exchange of BzOH between the diluent contained in the syringe and the external environment, a closed system containing the syringe system is necessary. A sealed HS-GC-MS vial afforded this condition, wherein a syringe system containing diluent was sealed into the vial (Fig. 6) and incubated at varying temperatures (2–8 °C, 40 °C and 60 °C) for 14 days. The temperature conditions (2–8 °C and 40 °C) were chosen to be representative (minimum and maximum) as that of the routine stability program. An additional evaluation at 60 °C was performed that served as an extreme worst case and helped assess the effect of temperature. As the temperature rises, the vapor pressure of the moisture and BzOH inside the syringe rises driving it across the rubber components (particularly the tip-cap), so that it appears in the headspace of the GC-MS vial, which can be then detected at the end of the incubation period. As can be seen from Fig. 6, the signals corresponding to BzOH were detected from the headspace vials when the diluent containing syringe system was contained in it. This demonstrates that BzOH traverses across the primary packaging materials under the conditions of given temperature and duration. Generation of a calibration curve to perform an accurate quantification of BzOH emerging from the syringe was quite challenging due to complexity in simulating the transmission process at predetermined concentrations. However, the area corresponding to the peaks of BzOH signals can be used as a metric for the relative quantification and understanding the differences between the syringe systems.

Since, the plunger stopper shows lower propensity to bind BzOH compared to the tip-cap and the product contact part of the stopper is made of fluoropolymer, BzOH is expected to emerge predominantly from the tip-cap. This is augmented by the observations that benzyl alcohol is very inert to fluoropolymer and does not bind to it [29,30]. The area of the benzyl alcohol peaks emerging from syringe 2 was many folds higher than the one observed for syringe 1 at all the temperature conditions (Fig. 7). This trend in the loss of BzOH between syringe 1 and syringe 2 can be well correlated to their rubber properties (Table 4), where the tip-cap of syringe 2 has a MVT (1.6 times) and OT (2.5 times) higher than that of the tip-cap of syringe 1. Given that the properties (MVT and OT) of the stoppers for both syringes are same and the product contact surface is made of fluoropolymer, it can be inferred that the loss of BzOH is predominantly due to the vapor transmission/diffusion across the tip-cap.

3.8. Gravimetric analysis of the syringes

A gravimetric method was used to assess the vapor (water/BzOH) transmission across syringe 1. Five syringes each filled with diluent were stored horizontally at 40 °C at ambient humidity conditions. The average weight of the syringes was plotted against the time points at which they were weighed. There was a linear decrease (approximately 0.075 mg/week) in the average syringe weight during the course of the study with the average weight difference amounting to 1 mg at the end of 12 weeks compared to the average weight at the start of the study. The loss of BzOH at the end of 3 months during the stability program at stress conditions was estimated to be 0.38 mg (see Table 1). Assuming that the lost BzOH is either bound to the rubber components or released into the environment, the remaining loss of weight in this study can

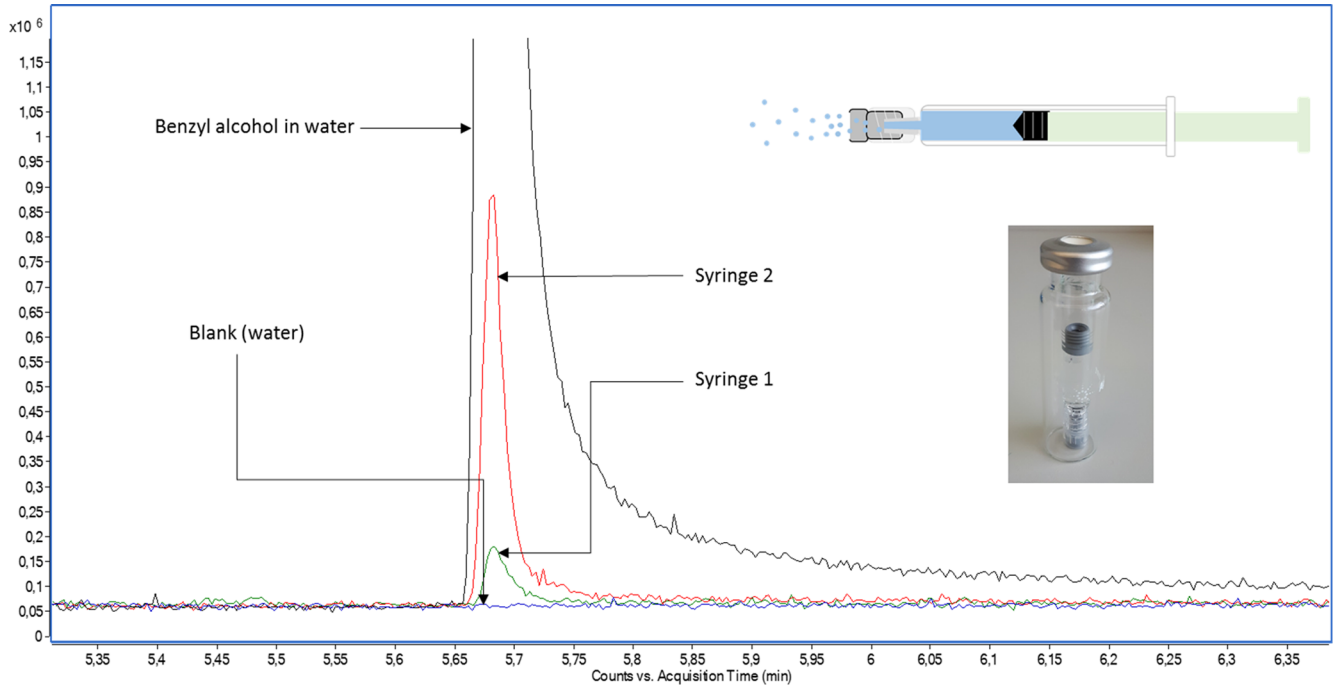

Fig. 6. Representative HS-GC chromatograms of the syringe 1 (green) and syringe 2 (red) incubated in sealed headspace vial at 40 °C after 14 days (x-axis: counts in arbitrary units; y-axis: acquisition time in minutes). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

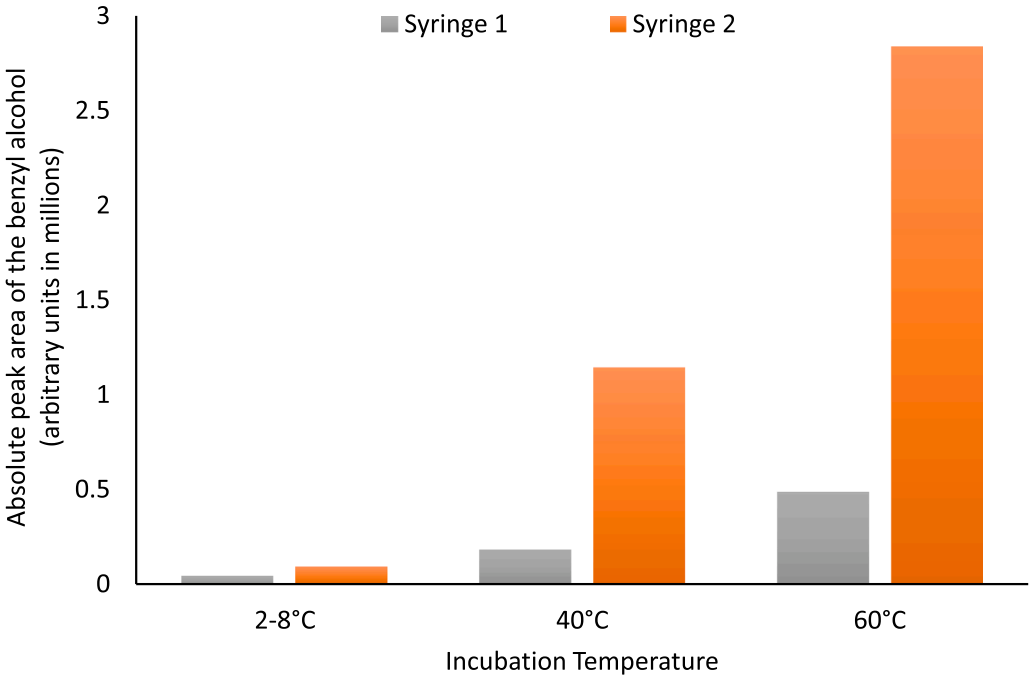

Fig. 7. Benzyl alcohol transmission across the tip-cap of syringe 1 (grey) and syringe 2 (orange) at 2–8 °C, 40 °C and 60 °C as analyzed by the HS-GC-MS at the end of 14 days of incubation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

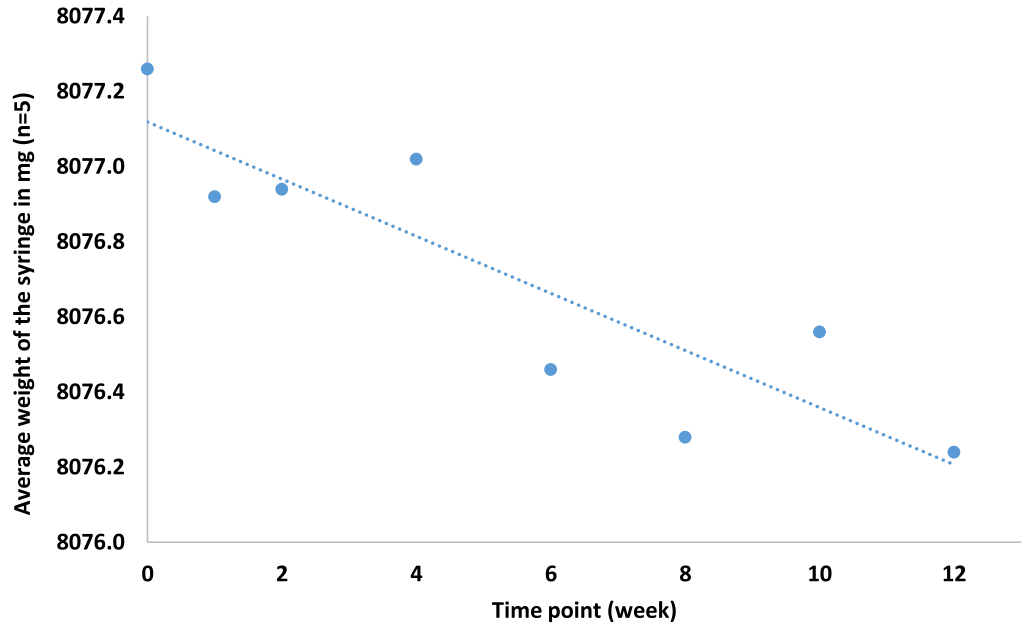

Fig. 8. Plot of average weight of syringe v/s time point for syringe 1 (n = 5) incubated at 40 °C under ambient humidity conditions.

thus be attributed to the loss of water vapor. This behavior is similar to the one reported by Bardi et. al for the staked-in needle PFS, where water vapor transmission has been demonstrated to take place across the rigid needle shield of the PFS [21]. The weight loss, as depicted in Fig. 8 for syringe 1 along with the BzOH vapor transmission as demonstrated by HS-GC-MS analysis strongly compound to the inference that evaluated PFS allows water as well as BzOH vapor transmission across its tip-cap.

To summarize, we systematically demonstrate through the series of experiments (Sections 3.2–3.7), the most probable cause of the BzOH decrease and process by which it can happen. The binding experiments serve to highlight the key aspect of the surface adsorption which is the foremost step before the transmission/diffusion across the rubber matrix can take place. The HS-GC-MS experiments particularly demonstrate that the transmission/diffusion (also referred to as permeation) and release of BzOH into the environment can indeed take place and is dependent on the temperature. The magnitude of the binding (Sections 3.4 and 3.5) and the relative diffusion across the rubber components (Section 3.6) correlate well with the permeation attributes (Table 4) of the primary packaging components and the same is reflected during the read-outs of the stability program (Section 3.1). The investigation aimed at unraveling the nature and the means by which the decrease in BzOH can take place across the PFS. The overall observed phenomenon can be expected to take a following course; 'binding/adsorption - diffusion/transmission – desorption', based on the findings described in the respective sections.

4. Conclusions

Selection of the primary packaging of the drug product is often empirical and based on the time consuming stability studies. A prior understanding and evaluation of the physico-chemical properties of the container closure system can help anticipate and mitigate the unforeseen problems encountered only much later during the course of the stability studies. In this article, we present a case study with a decrease in the BzOH content of the prefilled syringes during the stability. A reduced BzOH content can compromise the preservative content and affect the preservative efficacy afforded by the diluent to the reconstituted product. We have shown that, BzOH can not only bind to the rubber components of the PFS but also traverse across the rubber formulation resulting in the decrease of the preservative content over a period of time. Furthermore, the observations described (for BzOH) could as well be applicable for other preservatives (like phenol, m-cresol and parabens) that share similar physico-chemical properties and are used in parenteral formulations. It is imperative to identify such signals early on and correlate its relevance to expected shelf-life conditions. We demonstrate in a first of its kind study as to how HS-GC-MS can be used to better understand the interplay between the product and the container closure. Deployment of such tools in the early development coupled with detailed understanding of the primary packaging can help to select the optimal primary package right first time. For the development of the PFS containing preservatives particularly BzOH, it is recommended that the rubber formulation with lower values of MVT and OT for the tip-cap and stopper be chosen. Moreover, the unintended interaction between the preservatives and the container closures can be prevented by employing the components coated with inert materials like fluoropolymer at the product contacting surfaces. The impact of the decrease in the BzOH content can be influenced by the concentration of the BzOH employed as well as the duration of the intended shelf-life and this needs to be assessed on a case-by-case basis. Our observations also underscore the need for manufacturers of the packaging components to develop materials with improved properties that ensure product quality and ultimately the patient safety.

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Declaration of Competing Interest

All the authors and persons mentioned in the manuscript are employed by Novartis.

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