Research paper

Preservative loss from silicone tubing during filling processes

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ABSTRACT

Significant loss of preservative was observed during filling of drug products during filling line stops. This study evaluated the losses of three commonly used preservatives in protein drugs, i.e. benzyl alcohol, phenol, and m-cresol. Concentration losses during static incubation were quantified and interpreted with regard to the potential driving forces for the underlying sorption, diffusion, and desorption steps. Partitioning from the solution into the silicone polymer was identified as the most decisive parameter for the extent of preservative loss. Additionally, the influence of tubing inner diameter, starting concentration as well as silicone tubing type was evaluated. Theoretical calculations assuming equilibrium between solution and tubing inner surface and one-directional diffusion following Fick's first law were used to approximate experimental data. Since significant losses were found already after few minutes, adequate measures must be taken to avoid deviations during filling of preservative-containing protein solutions that may impact product quality or antimicrobial efficacy. As a possible alternative to the highly permeable silicone tubing, a specific make of fluoropolymer tubing was identified being suitable for peristaltic pumps and not showing any preservative losses.

1. Introduction

Preservatives are used in multi-dose formulations in order to ensure microbial quality after opening and during multiple use. In general, since mostly single-dose parenteral products are developed – also employing advanced device or pump systems – preservatives become less employed (Table 1). In case a preservative is required, commonly employed preservatives include benzyl alcohol, phenol, and m-cresol, especially for protein drug products. Thiomersal and chlorobutanol have also been used as preservatives for peptide or protein multi-dose formulations, however, less frequently [1]. Examples for protein drug formulations containing above mentioned excipients include Pegasys® from Roche,

Norditropin® from Novo Nordisk, Nutropin AQ® from Genentech, and Sandostatin® from Novartis as well as most insulin formulations. For proteins, the preservative has to be chosen very carefully since protein stability can be significantly impacted e.g. leading to aggregation by inducing partial protein unfolding [2–4]. In contrast, a positive effect of phenolic preservatives on the self-assembly and thus stability of some insulin analogs was reported [5]. Interestingly, benzyl alcohol also has antioxidative properties and thus can support protein stability.

Silicone tubing is typically used for filling processes, in connection with peristaltic pumps, due to its advantageous mechanical properties. During filling of a benzyl alcohol-containing drug product, the authors observed significant losses in benzyl alcohol, especially during line stops. In this study, we thus evaluated possible losses in excipient concentration of three commonly used preservatives for protein formulations, namely benzyl alcohol, phenol, and m-cresol. Silicone tubing with two different inner diameters (ID), as well as fluoropolymer-based tubing have been evaluated. Previous, unpublished results showed that preservative losses were independent of the presence of a protein drug (data not shown). Hence, preservative formulations in a placebo buffer only were employed for this study. With this approach, simple UV

Table 1
Number and type of marketed parenteral products containing preservatives, adapted from Meyer et al. [1].

Preservative	Product type			Frequency of use		
	Peptide/protein	Vaccines	Small molecule	1996	2001	2006
Chlorobutanol	x	–	x	17	13	3
Methylparaben	–	–	x	50	40	9
Propylparaben	–	–	x	40	33	9
Benzyl alcohol	x	–	x	74	69	19
Phenol	x	x	x	48	30	15
Thiomersal	x	x	–	46	20	6
m-Cresol	x	–	–	3	7	11
Phenoxyethanol	–	x	–	3	4	5

measurements could be performed for quantification of preservative without any need for further chromatographic separation steps. Formulation parameters were chosen according to Table 2. Since preservative losses are expected to be especially critical during filling line stops, static incubation experiments were performed to simulate worst-case conditions. It was the aim of this study to quantify preservative losses and also generate a deeper understanding of the underlying mechanism and influencing factors. Therefore, not only the known molecular characteristics of Table 2 were used for the interpretation of the obtained data. The three preservatives were additionally characterized for their partitioning, diffusion, and evaporation behavior in conjunction with the silicone polymer matrix, and additionally, a mathematical model was established. Sorption data for silicone tubing was finally compared to sorption data for a fluoropolymer tubing.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

m-Cresol and benzyl alcohol (abbreviated with BA) were purchased from Merck KGaA (Darmstadt, Germany), phenol was obtained from AppliChem (Darmstadt, Germany). NaCl, Na_2HPO_4, and NaH_2PO_4 were supplied by VWR International (Darmstadt, Germany). NaOH (1 M) and HCl (1 M) were obtained from Sigma-Aldrich (Taufkirchen, Germany). Highly purified water used for buffer preparation was taken from an arium® pro DI Ultrapure Water System (Sartoris Stedim Biotech GmbH, Goettingen, Germany) or a USF ELGA PURELAB Plus UV/UF purification system (ELGA LabWater, Celle, Germany). Buffer filtration was performed with pressurized nitrogen and 0.2 μm cellulose acetate filters (47 mm ø, Sartorius Stedim Biotech GmbH).

2.1.2. Tubing

The two employed platinum-cured silicone tubing types from different manufacturers (type A and B) with an ID of 6.0 mm and 1.6 mm were purchased from Watson-Marlow/Flexicon (Ringsted, Denmark). Wall thicknesses were 2.1 mm and 1.6 mm, respectively. Fluidvit FPM tubing made from fluoropolymer (Viton®), with an ID of 6.4 mm (OD of 9.6 mm) and a hardness of 60 Shore (suitable for peristaltic pumping) was obtained from ProLiquid GmbH (Überlingen, Germany).

2.2. Methods

2.2.1. Sample preparation

The three preservatives were dissolved in filtrated 10 mM phosphate buffer containing 145 mM NaCl at the pH and concentration stated in Table 3. The formulation was chosen due to its low interference with UV measurements, and not in order to reflect an actual protein formulation.

2.2.2. Sorption experiments

40 cm long pieces of tubing with an ID of 6.0 mm were filled with 10 mL of preservative solution (n = 3). For tubing with an ID of 1.6 mm, three 20 cm long pieces per analysis time point were filled with 300 μL. Ends were clamped and the tubing pieces were incubated at room temperature for 5, 10, 15, 30, 60, 120, 240, and 360 min. For the 6.0 mm ID tubing, samples were withdrawn from the filled tube at every analysis time point. The exact volume of the sampling aliquots is provided in Table 3. 1.6 mm tubing pieces were completely emptied for sampling. Sample aliquots were diluted (Table 3) and stored in 2R glass vials (Schott AG, Mainz, Germany) prior to analysis. For phenol and *m*-cresol samples that were incubated in 1.6 mm tubing for 4 and 6 h, the dilution factor was reduced to 1:5.

2.2.3. Preservative quantification via UV absorption measurements

200 μL of each diluted sample as well as of each standard concentration (Appendix, Table A.1) and the corresponding blank buffer were analyzed in triplicates in a 96-well quartz well plate (Hellma Analytics, Müllheim, Germany) for UV absorption with a FLUOstar Omega well-plate reader and Omega software version 1.01 (BMG Labtech GmbH, Ortenberg, Germany). A positioning delay of 0.5 s and analysis with 20 flashes per well in 'Absorbance endpoint' mode was applied. Absorption maxima as stated in Table 3 were determined from UV absorption scans in the range of 220–350 nm. Linear regression fit with the 'average data based on blank corrected' raw data was performed within the MARS Data Analysis Software version 1.01 (BMG Labtech).

2.2.4. Determination of the partition coefficient k

Approx. 600 mg of cut silicone tubing pieces were incubated with 2.0 mL of preservative solution in stoppered 10R glass vials (Schott AG, Mainz, Germany). Solutions with concentrations of 1, 2, 4, 6, 8, and 10 mg/mL preservative were employed (n = 3). For every concentration, a blank glass vial containing preservative solution without any added silicone tubing pieces was prepared. After overnight equilibration, preservative concentrations of the incubation and blank solutions were determined after dilution. From the concentration difference between the blank and the incubation solutions, the lost amount of preservative per unit volume of silicone tubing was calculated assuming a specific gravity for silicone tubing of 1.1 as stated by the manufacturer. The calculated preservative concentrations in the silicone tubing were plotted against the measured preservative concentrations in solution. Linear regression analysis (no error weighting, y-intercept of 0) was performed in Origin 8G (OriginLab Corporation, Northampton, MA, USA). The obtained slope represents the partitioning coefficient *k*.

2.2.5. Determination of the diffusivity D

Permeation tests were conducted with jacketed Franz-type diffusion cells (diameter of 15 mm, volume of 12 mL, Gauer Glas,