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Original Article

IMPACT OF FILLING PROCESS INTERRUPTIONS ON *m*-CRESOL CONCENTRATION IN PARENTERAL DRUG FORMULATIONS

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ABSTRACT

Ensuring the appropriate concentration of antimicrobial preservatives in multi-dose sterile pharmaceutical formulations is critical for maintaining sterility, quality, and stability throughout the product's shelf life. However, preservatives such as *m*-cresol and phenol are known to adsorb onto silicone components used during manufacturing, leading to potential preservative loss. This study investigates the impact of manufacturing process stops, particularly during the filling stage, on *m*-cresol concentration in dosed cartridges. Laboratory-scale experiments and full-scale manufacturing studies were conducted using solutions containing recombinant human insulin (RHI), insulin lispro, and a placebo solution to evaluate the effects of interruptions in the filling process—such as internal process control (IPC) checks—on the adsorption of *m*-cresol from pharmaceutical formulations.

KEYWORDS: preservative, insulin, filling process, parenteral formulation, *m*-cresol

1. Introduction

The sterile medicinal product in the final multi-dose container must comply with the appropriate guidelines, such as European Pharmacopoeia or the United States Pharmacopeia, ensuring an appropriate level of antimicrobial preservative. This is essential for maintaining sterility, quality, and stability throughout the product's use and until the end of its shelf life — a critical criterion for multi-dose formulations. The drug product must remain protected from microbial contamination throughout the entire administration period. According to the Ph. Eur., the antimicrobial efficacy test must be conducted on the final container both immediately after manufacturing and near the end of its shelf life [1–3].

Manufacturers of sterile pharmaceutical products ensure an optimal level of antimicrobial preservation through the development and optimization of the manufacturing process. Antimicrobial activity is enhanced by the addition of common preservatives, such as *m*-cresol, which typically is used at concentrations of 0.15%–0.3% [4]. Another common preservative used in parenteral formulations is phenol. Moreover, the combination of both compounds exhibits a synergistic antimicrobial effect [3]. Various licensed insulin solutions or suspension formulations include preservative combinations, such as NovoRapid (*Novo Nordisk*), Gensulin N (*Bioton*), and Humalog NPL (*Eli Lilly*). *m*-Cresol is used as the sole preservative in licensed biological formulations such as Humulin R, Humalog 100 (Eli *Lilly*), and Gensulin R (*Bioton*).

Apart from functioning as preservatives, *m*-cresol and phenol promote the formation of insulin hexamers, which are essential in pharmaceutical formulations. Although hexamers are not the active form, they play a crucial role in maintaining the overall stability of insulin and enabling its controlled release upon injection [5–7].

Due to their critical role in preventing microbial growth and ensuring product safety, pharmaceutical producers must maintain optimal levels of preservatives. This might become challenging during the manufacturing step since preservatives like *m*-cresol and phenol have been reported to be adsorbed on the surface of silicone components such as tubing or storage bags leading to decrease of their concentration in the solution [8,9]. Adsorption is a time-dependent process; therefore, its effects may be most significant during production stops—whether planned or unplanned—when the liquid formulation remains in prolonged contact with silicone components.

In this report, we present the results of our investigation into the impact of stops during the filling process of parenteral formulations on *m*-cresol concentration in dosed cartridges. Being the final step in manufacturing, the filling process is critical to ensure the accuracy, consistency, and stability of the final product, making any potential preservative loss a key concern.

2. Materials and Methods

2.1. Tubing

Two types of pharmaceutical-grade, platinum-cured silicone tubing (A6093), both conforming to industry standards, were utilized.

Type A Pre-fill hose, responsible for 90% of the final product volume, with an inner diameter (ID) of 2.38 mm, outer diameter (OD) of 7.14 mm, wall thickness of 2.38 mm and hardness of 65 shore.

Type B Final fill hose, responsible for 10% of the final product volume, with an inner diameter (ID) of 1.59 mm, outer diameter (OD) of 4.76 mm, wall thickness of 1.59 mm and hardness of 65 shore.

2.2. Chemicals

The active substances used in the formulations were produced in compliance with the standards of the European Pharmacopoeia (EP) at Bioton. The following reagents were utilized: *m*-cresol and anhydrous glycerin (purchased from Auq. Hedinger GmbH & Co. KG), $Na_2HPO_4 \cdot 12H_2O$, ZnO, glycerin 85%, NaOH, and HCl (purchased from Merck KGaA)

Water for injection (WFI), compliant with the European Pharmacopoeia (Ph. Eur.), was sourced from an in-house water system for both buffer and solution preparation. Sterility was ensured through filtration using 0.2 µm pore size capsule filters.

2.3. Test solutions

For the purpose of this study, three test solutions were prepared: one containing recombinant human insulin (RHI), the second containing insulin lispro, and the third without any recombinant protein (Table 1). Test solutions No. 1 and 2 respresent commercialy available formulations cointaing RHI and fast-acting insulin lispro, respectively. Each solution contained glycerin, zinc ions and *m*-cresol (conc. 3.15 mg/mL). According to the literature, the *m*-cresol content in parenteral preparations as a preservative typically ranges between 0.15% and 0.3% [4]. However, while the European Pharmacopoeia provides guidelines for the use of *m*-cresol, it does not specify an exact concentration for its use as a preservative [1]. During the development of the parenteral formulations within the scope of this study, it was demonstrated that a concentration of 3.15 mg/mL ensures the microbiological stability of the product while maintaining compliance with regulatory guidelines. Also, this is the same concentration used in the commercial formulation of insulin lispro Humalog 100 (*Eli Lilly*) [10].

2.4. Adsorption of *m*-cresol under laboratory conditions

Before conducting tests on a manufacturing scale, it was necessary to evaluate whether *m*-cresol is adsorbed by silicone tubing and to what extent. Each test solution was prepared separately in a 1 L glass bottle and stored until use.

For the tests, two types of silicone tubing were used:

- Pre-fill hose (total volume: 4.8 mL, marked as A)
- Final fill hose (total volume: 2.1 mL, marked as B)

This type of silicone tubing was selected as it is used routinely in manufacturing process. It serves as a part of the filling machine and it is the last silicone component that comes into contact with the finished product before it is dispensed into glass cartridges.

Each hose was filled with the respective solution and held for 0.25, 2, 3, 5, and 45 minutes. After the designated time, the solution was removed, and samples were taken to determine the *m*-cresol content using HPLC analysis. Each solution was tested only once for m-cresol adsorption using a new set of tubing.

2.5. Adsorption of *m*-cresol during manufacturing process

Preparation and dosing of 75 L scale solutions were carried out as a routine manufacturing process in accordance with Bioton's internal Standard Operating Procedures and Manufacturing Recipes. Each solution was prepared in an industrial partial mixer and sterilized via filtration while being transferred to the final mixer. Once the entire sterile solution was placed in the final mixer, it was dispensed into 3 mL glass cartridges using an industrial dosing machine equipped with 24 filling needles. The machine dispenses the final product in dispensing cycles, each consisting of 24 glass cartridges. Cartridges are filled in two stages: first, up to 90% of the volume via the pre-fill hose, and second, the remaining 10% via the final-fill hose. Sealed cartridges are then collected in cassettes, each capable of holding around 615 pieces.

In a routine manufacturing process, samples from the first three cassettes are taken to perform in-process control (IPC). It is a series of tests designed to confirm the correct insertion depth of the rubber plunger into the cartridge, the product dose, and the proper sealing. This takes around 30-45 minutes, during which the filling is halted. Once the correct results are confirmed, the process is resumed. During this period, the pharmaceutical formulation is exposed to prolonged contact with silicone tubing, which can lead to the adsorption of *m*-cresol from the solution.

To examine changes in *m*-cresol concentration over time during the filling process, cartridge samples were collected at various time points throughout the process, and the preservative content was determined using HPLC. During run tests, each halt for IPC lasted 45 minutes to minimize variability in results related to the dependence of m-cresol adsorption on contact time. All conditions, including the type of equipment used, the filling machine setup, the filling speed, and the method of solution preparation, mimicked the standard manufacturing process performed at Bioton's facility. Each run with a different solution was performed only once, resulting in a total of 3 test runs.

Table 1. Compositions of test solutions used in the study.

Solution	API	Glycerin	Zinc ions	*m*-Cresol
No.1	RHI	+	+	3.15 mg/ml
No. 2	Lispro	+	+	3.15 mg/ml
No. 3	-	+	+	3.15 mg/ml

2.6. High-Performance Liquid Chromatography (HPLC) Analysis

The *m*-cresol concentration in the cartridge samples was determined using an HPLC system equipped with a UV detector set at 235 nm. The separation was achieved on a C18 reverse-phase column (Waters XBridge BEH, 3.5 µm, 130 Å, 4.6 × 50 mm). The mobile phase consisted of a mixture of 0.2 M sodium sulfate/acetonitrile (82:18, v/v) solution (A) and 0.2 M sodium sulfate/acetonitrile (50:50, v/v) solution (B), using an isocratic elution program with the following composition: 76.2% A and 23.8% B (v/v) for 4.5 minutes. The flow rate was maintained at 2.0 mL/min, and the column temperature was set to 40°C. The injection volume was 5 µL.

3. Results

3.1. Adsorption of *m*-cresol under laboratory conditions

Studies performed under laboratory conditions has proven that *m*-cresol content in solution decreases over time when exposed to contact with silicone tubing (Fig. 1 and Fig. 2). Adsorption of the preservative begins almost immediately, as a slight drop in relative concentration is observed at the 0.25-minute timepoint in each test solution for both tubing A and B. After 45 minutes relative *m*-cresol content decreased at least two times for each test solution for both A and B tubing (Table 2). Tubing B exhibits higher adsorption levels than tubing A. In both experiments, the greatest decrease in *m*-cresol was observed in solution No. 1, while the lowest decrease occurred in solution No. 2.

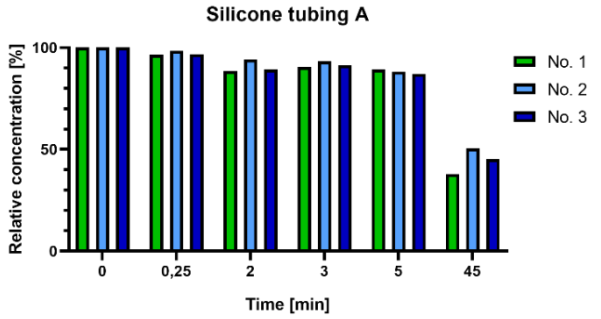

Fig 1. Adsorption of *m*-cresol by silicone tubing A over time under laboratory conditions.

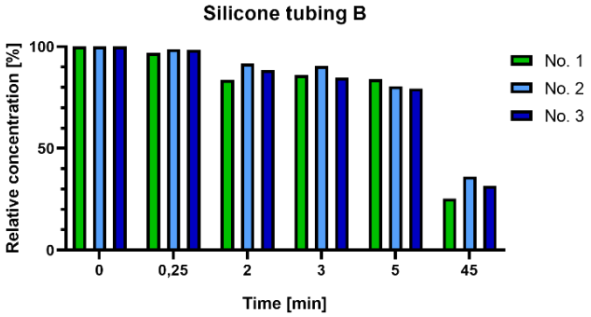

Fig 2. Adsorption of *m*-cresol by silicone tubing B over time under laboratory conditions.

Table 2. Relative concentration of *m*-cresol in test solutions after 45 minutes.

Solution	Tubing A	Tubing B
No. 1	37,8%	25,4%
No. 2	50,5%	36,2%
No. 3	45,1%	31,7%

3.2. Adsorpion of *m*-cresol during manufacturing process

The change of *m*-crezol in dosed cartridges the course of filling process is presented in a form of heatmaps (Fig. 3). For each manufactured batch, samples were taken at three stages of the filling process: at the beginning, when the machine was started; immediately after the IPC was completed and the machine restarted; and during the actual filling process. A total of 42 samples were collected and tested for each batch.

It can be observed that a drop in *m*-cresol content occurs after the IPC. Over time, as the tubing is flushed with a new portion of solution, the content increases to reach a stable level for the remainder of the filling process. However, this is not true for solution No. 2. During the filling process, the cartridges dispensed immediately after the IPC were discarded for not meeting the requirements (i.e., dose), and the first ones tested had already reached the regular *m*-cresol content level.

The greatest drop in *m*-cresol concentration after the IPC is observed for solution No. 3. As a result, it also required a longer time to restore its concentration compared to solution No. 1. This can be explained by the fact that the IPC for solution No. 3 lasted longer, leading to a higher amount of preservative being adsorbed to the silicone tubing.

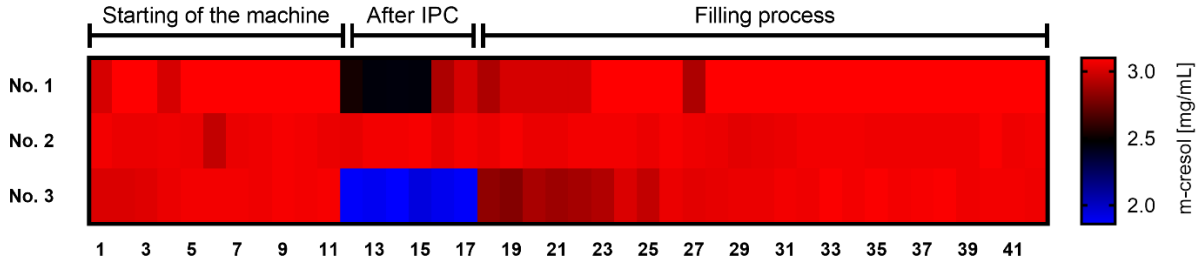

Fig 3. Change of *m*-cresol content in dosed cartridges over the course of the filling process.

4. Discussion and conclusions

Adsorption of *m*-cresol during the manufacturing process poses a significant risk to product quality and, consequently, to patient safety. In this study, we report that prolonged exposure of solutions to silicone tubing can affect the *m*-cresol content in parenteral formulations.

Tests performed with pre-fill and final-fill hoses have demonstrated that, over time, *m*-cresol adsorbs onto the surface of the tubing in contact with the solution. Several factors influence the degree of adsorption, including contact time, the surface-to-volume (S/V) ratio, and the composition of the solution.

Longer contact times result in a greater amount of *m*-cresol being adsorbed from the solution. Additionally, a higher S/V ratio leads to increased adsorption. In our study, the final-fill hose exhibited a higher S/V ratio compared to the pre-fill hose (2.52 m^{-1} vs. 1.68 m^{-1}). After 45 minutes, the degree of adsorption was 12–14% higher in tubing B, depending on the tested solution. Furthermore, solution composition appears to play a role in preservative adsorption; Solution No. 1 exhibited the highest decrease in *m*-cresol content, while Solution No. 2 showed the lowest.

These findings should be carefully considered when maintaining routine manufacturing processes or developing new pharmaceutical formulations. Proper *m*-cresol levels are essential for ensuring the sterility of parenteral products and, in the case of insulin-based formulations, for maintaining stability and proper mode of action. Although there is limited literature on the microbiological stability of drug products in relation to m-cresol concentration specifically, a study conducted by Abd-Elsalam and coworkers demonstrated that reducing the m-cresol concentration to 0.15% in antivenom formulations does not affect their microbiological stability over their shelf life [4]. During the development of test formulations, a series of studies confirmed that a 3.15 mg/mL m-cresol concentration, with a ±10% acceptance range, provides microbiological stability to the product. From a biopharmaceutical manufacturer's perspective, while it is not fully certain that an *m*-cresol concentration below 2.84 mg/mL would compromise microbiological safety, this value falls out of the tested range. Therefore, releasing a product outside of specification poses a risk to both patients and the manufacturer, and it is not permitted. As shown in the heatmaps (Fig. 3), the tested products fell out of specification after a filling halt for IPC, which clearly indicates that prolonged stops during the manufacturing process pose a realistic threat of *m*-cresol adsorption by silicone elements present in the technological line.

To mitigate *m*-cresol loss, several approaches can be employed. For instance, replacing silicone tubing with alternative materials that do not adsorb *m*-cresol, such as fluorinated polymers, may prevent losses [9]. Additionally, a thorough understanding and comprehensive characterization of the manufacturing process can help minimize both planned and unplanned production stops, during which adsorption may occur.

Any research in this regard should focus on evaluating alternative materials for tubing and container systems to minimize *m*-cresol adsorption while maintaining compatibility with pharmaceutical formulations. Additionally, real-time monitoring of *m*-cresol levels during manufacturing could provide valuable insights into adsorption dynamics and allow for process adjustments to ensure consistent preservative concentrations.

Moreover, optimizing manufacturing conditions — such as temperature, flow rate, and tubing surface modifications — may help reduce adsorption. Implementing stricter process control measures and predictive modeling could further enhance manufacturing efficiency and product quality.

Ultimately, a multidisciplinary approach involving material science, process engineering, and pharmaceutical formulation development should be taken to mitigate the impact of *m*-cresol adsorption and ensure the safety, efficacy and regulatory compliance of parenteral products.

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Conflicts of Interest: The authors declare no conflict of interest.

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