Sanitization of BPG 450 column with sodium hydroxide

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The efficiency of sodium hydroxide (NaOH) as an antimicrobial agent for a BPG 450 chromatography column has been tested. The column chosen for the study, a BPG 450/500 packed with Q Sepharose™ Fast Flow chromatography resin, was subjected to microbial challenge tests using strains of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* as recommended by the United States Pharmacopoeia (USP XXI). After infected with bacteria, the column was sanitized by washing with 2.5 column volumes of 1 M NaOH in forward and reversed flow directions followed by 60 min forward recirculation. Thereafter, sampling was carried out at predetermined test points, including the effluent at the column outlet, the resin, and surfaces inside the column. No viable test organisms were detected at any of these test points after sanitization. The results indicate that the sanitization method used is highly efficient for reducing populations of vegetative bacteria in a contaminated BPG 450 column. The observations support the findings of previous microbial challenge studies on similar columns and thus provides additional evidence of the hygienic design of BPG columns and the suitability of NaOH as a sanitizing agent.

Introduction

The production of biopharmaceuticals for clinical applications is governed by regulations that impose high standards on levels of purity. Sanitization is one method of reducing a population of microbial organisms to a level acceptable for production. Regulations require the efficiency of sanitizing agents to be evaluated. The microbial challenge tests described here measure this efficiency.

From previous studies, we know sodium hydroxide to be an effective antimicrobial agent (1). Furthermore, NaOH is both inexpensive and readily available. NaOH-based sanitizing procedures for chromatography equipment and resins have thus proved popular with large-scale manufacturers seeking high levels of process economy and process hygiene.

Fig 1. This study tested the efficiency of NaOH for sanitizing a BPG 450/500 column packed with Q Sepharose Fast Flow resin.

Microbial challenge tests

The principle of microbial challenge tests is to introduce a high concentration of contaminants into the equipment or resin and thereafter treat the equipment with an antimicrobial agent. After a specified time, the number of surviving organisms in terms of colony forming unit (CFU) is counted. In this study, a BPG 450/500 column (Fig 1) packed with the ion exchange resin Q Sepharose Fast Flow was contaminated three times with solutions containing high numbers of different microorganisms, one of which belonged to the Gram-negative Pseudomonas group. This group of endotoxin-producing bacteria is characterized by its ability to proliferate in low nutrient water-based systems, such as the buffers used in process chromatography. After contamination, the column was treated with NaOH as antimicrobial agent.

Sanitization

Sanitization, the use of chemical agents to reduce microbial populations, is commonly used in chromatography systems to maintain microbial presence at a level that will minimize the risk of contaminating the product being processed. Note that a very low microbial level is generally acceptable by process operators and to regulatory bodies, who do not require that systems or equipment be sterile or sterilizable.

Materials and methods

The study evaluated the sanitization effect of NaOH on a BPG 450/500 column packed with Q Sepharose Fast Flow resin to a bed height of 15.4 cm. Table 1 summarizes equipment and conditions.

Table 1. Equipment and conditions used in the microbial challenge tests

Column:	BPG 450/500
Resin:	Q Sepharose Fast Flow, bed height 15.4 cm, bed vol. 24 L
Pump:	Screw pump, 10 mm
Valves:	Membrane valves, Salvi 10 mm i.d.
Sterile filter:	MilliPac™ 200, 0.22 µm
Sanitizing agent:	1 M NaOH
Buffer:	0.9% NaCl
Sampling:	Tryptic soy agar (TSA)
	Sterile 0.45 µm membrane filters
	Sterile calcium alginate swabs
	Sterile 1% sodium hexametaphosphate in 25% Ringer's solution
Test organisms:	
Staphylococcus aureus	ATCC 6538 Gram-positive bacteria
Escherichia coli	ATCC 8739 Gram-negative bacteria
Pseudomonas aeruginosa	ATCC 9027 Gram-negative bacteria

Preparation of the test strains

Pure cultures were prepared for each experiment. The bacterial strains were stored on nutrient agar slants at 4°C. Before each test, the organisms were inoculated in 50 mL tryptic soy broth (TSB) and incubated at 30°C–32°C for 16 h followed by transfer to one liter TSB and incubation for a further 8 h.

Preparation of the column

The column was dismantled, checked and cleaned with 20% ethanol solution. Following reassembly, the column was rinsed with distilled water. Thereafter, the column was packed with Q Sepharose Fast Flow using the single-step flow packing method. A solution of 0.1 M NaOH was circulated through the packed column, which was thereafter left overnight in the same solution.

Infection

The bacterial solution was diluted to 28 L with sterile 0.9% NaCl. One column volume (24 L) of diluted suspension was pumped into the column via the bottom outlet. The column was left at room temperature for 16–18 h. This procedure was repeated for each test strain. The concentration of organisms in each suspension was approximately 10^9 CFU/mL.

Sanitization procedure

The column was sanitized by circulating 2.5 column volumes of 1 M NaOH at a flow rate of approximately 120 L/h and a pressure of 1 bar (0.1 MPa) in an upward direction. After approximately 30 min, the direction of flow was reversed. After a further 30 min, the direction was reverted to upward flow and the NaOH solution was recirculated through the column for 60 min.

Microbial testing

Following sanitization, the NaOH was washed out by pumping a sterile saline solution through the column until the pH of the eluent fell below pH 8. Thereafter, column and connections were dismantled and microbial samples taken at the predetermined sites. Figure 2 shows the positions of the sites in the column. Microbial sampling was performed by one of the following methods, depending on the sample site:

Test method 1 (direct filtration)
Solutions were passed through a sterile 0.45 µm membrane filter, which was thereafter incubated on TSA plates at 30°C–32°C. After five days, the number of CFU was counted and the number of viable microorganisms in the sample estimated. This method was used for 0.9% saline solution prior to rinsing, and afterwards at the column outlet when the pH of the eluent had fallen to below pH 8.

Test method 2 (agar plate)
A 1 mL sample of Q Sepharose Fast Flow from the sanitized column was mixed with 30 mL melted TSA (45°C). Thereafter, the mixture was poured into a sterile 9 cm petri dish. When the mixture had set, the dish was incubated at 30°C–32°C and inspected after five days for CFU.

Test method 3 (swab)
Surface samples were taken with calcium-alginate swabs, which were thereafter dissolved in 4.5 mL 1% sterile sodium hexametaphosphate in 25% Ringer's solution. The swab extract was passed through a sterile 0.45 µm filter and thereafter incubated on TSA at 30°C–32° for five days before inspection.

Table 2. Test points, test methods and the numbers of viable organisms after sanitizing the packed BPG 450/500 column with 1 M NaOH

Test point	Location	Test method	*S. aureus* (CFU/mL or CFU/unit)	*E. coli* (CFU/mL or CFU/unit)	*P. aeruginosa* (CFU/mL or CFU/unit)
1	0.9% saline solution	Direct filtration	0 per 5 mL	0 per 5 mL	0 per 5mL
2	Outlet sample	Direct filtration	0 per 5 mL	0 per 5 mL	0 per 5 mL
3	Resin (1 mL)	Agar plate	0	0	0
4	Hole in adaptor plate for attaching snap plug	Swab	0	0	0
5	Inside of adaptor net ring	Swab	0	0	0
6	Adaptor O-ring	Swab	0	0	0
7	Inside of U-shaped seal	Swab	0	0	0

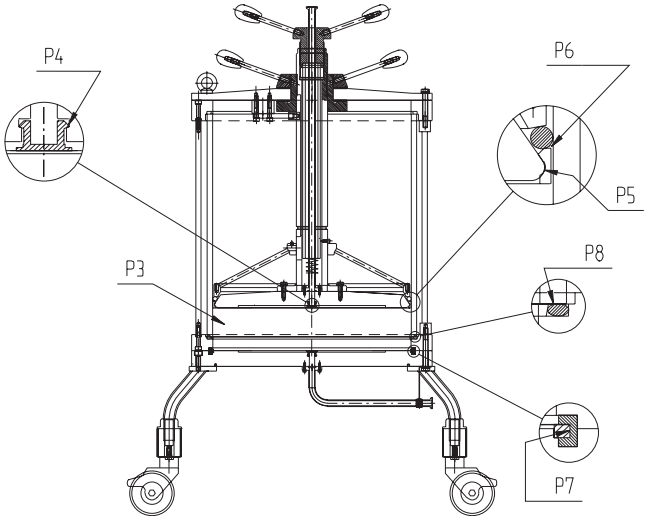

Fig 2. Location of test point in the BPG 450 column. Samples of the sterile saline solution were taken prior to and after rinsing.

Results

Table 2 shows the results of the sampling. No viable test organisms were detected at any of the test points after sanitization. The described sanitization method was thus very effective in reducing the level of microbial contamination in the packed BPG 450/500 column.

Conclusions

This study shows that a packed BPG 450/500 column is effectively sanitized with NaOH. Despite the high initial levels of microbial contamination, no surviving organism was found after treatment with 1 M NaOH. The results indicate that NaOH is suitable as an antimicrobial agent for vegetative bacteria and that the design, components and construction of BPG columns are well suited to sanitization with NaOH.

The three test strains selected were representatives of different microbiological species and included the Gram-negative *Pseudomonas aeruginosa*, which is a producer of endotoxins and frequently found in water-based systems. Even when initially present at high levels of contamination to represent a "worst case" scenario, this Gram-negative test strain did not survive treatment with NaOH.

These results also support the findings of similar studies and thus provide further evidence that NaOH is an effective and easy to use antimicrobial agent. However, the sanitization method described cannot alone guarantee good hygienic status of a chromatographic process. Sanitization methods should always be applied in conjunction with other well designed and carefully controlled hygienic routines, together with rigorous control of buffers, water, and other input material. Note also that the lack of viable organisms should not be interpreted as meaning that sterility has been attained.

Disclaimer

The information contained herein is not representative of any specific claims or any relevant environment, health and safety laws and regulations, including use authorization, product registration or application licensing or similar legal requirements.

Reference

1. Application note: Sanitization of BPG columns with sodium hydroxide. GE Healthcare, 18102086, Edition AC (2017).

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