Sanitization of BPG columns with sodium hydroxide

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We have tested the efficiency of sodium hydroxide (NaOH) as an antimicrobial agent for BPG chromatography columns. The column used in the study, BPG 100, was subjected to microbial challenge tests. The different microbial strains selected for the tests are recommended by the United States Pharmacopoeia (USP XXI). The column was packed with SP Sepharose™ Fast Flow chromatography resin and infected with solutions containing bacteria, yeast, or mold. Sanitization was performed by circulating 1 M NaOH for 60 min. The efficiency of NaOH as an antimicrobial agent was evaluated by microbial sampling at predetermined test sites in the column. No surviving organism of any of the test strains was detected after sanitization. This indicates that the sanitization method used is sufficient for the decontamination of BPG columns contaminated with vegetative bacteria, yeast and mold.

Fig 1. This study tested the efficiency of NaOH for sanitizing a BPG 100 column (far left) packed with Q Sepharose Fast Flow resin.

Introduction

Production of biopharmaceuticals for clinical applications, for example, monoclonal antibodies, vaccines, growth hormones and therapeutic enzymes is governed by regulatory authorities who impose very high standards on levels of purity and microbial presence. One method of reducing the amount of microbial organisms to a level acceptable for pharmaceutical production is sanitization. For regulatory purposes, however, the efficiency of the sanitizing agent needs to be evaluated and this can be conducted through microbial challenge tests. From previous studies, we know NaOH to be an effective antimicrobial agent frequently used to sanitize chromatography equipment and resins. Furthermore, NaOH is both inexpensive and readily available. For large scale manufacturers, sanitizing procedures that are economical, easy to operate, and provide high levels of hygiene are a key aspects of process economy and process hygiene.

Microbial challenge tests

The principle of microbial challenge tests is to introduce a high concentration of contaminants into the equipment or resin, and then treat with an antimicrobial agent. After a specified time, the number of surviving organisms in terms of colony forming units (CFU) is counted. In this study, a BPG 100 column (Fig 1) packed with SP Sepharose Fast Flow was infected with five solutions containing high numbers of microorganisms. After each infection, the column and resin were sanitized by allowing 1 M NaOH to circulate for 60 min, where after the system was dismantled and microbial samples taken at predetermined sites.

Microbial presence and survival

A variety of microorganisms, for example, bacteria and molds, are found in every “clean” environment. This is easily demonstrated by leaving agar plates uncovered for a day on an ordinary laboratory bench.

Not all microorganisms, however, require rich nutrients or elevated temperatures. Many inhabit water-based systems such as the buffers used in process chromatography. The most frequent contaminants of such water-based systems are certain kinds of Gram-negative bacteria. These bacteria, mostly belonging to the *Pseudomonas* group, are characterized by their ability to proliferate under low nutrient conditions; moreover, they are producers of bacterial endotoxin.

Sanitization and other terms

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 to process operators and regulatory bodies, who do not require that systems or equipment be sterile or sterilizable. Sanitization, sterilization, and other related terms are explained in Table 1.

Table 1. Explanation of relevant and related terms

Sanitization is the use of any chemical agent to reduce a microbial population to an acceptable predetermined level.
Sterilization is the destruction or elimination of all forms of microbial life in the inanimate environment.
Disinfection is the destruction of potential pathogens.
Antimicrobial agents are agents that minimize or destroy microorganisms *in vitro*. The term antimicrobial is general and all inclusive: antimicrobials include sanitizers, sterilizers, and disinfectants. However, sanitizers, sterilizers, and disinfectants are not necessarily the same and sanitization, sterilization, and disinfection are not interchangeable terms.

Materials and methods

The study evaluated the sanitization effect of NaOH on BPG 100 packed with SP Sepharose Fast Flow to a bed height of 15 cm. A µ-zero Triflow valve (T.H. I. Systems Co.) was connected to the bottom of the column and a Watson-Marlow™ X-800 peristaltic pump was used to pump the contaminating solutions through the column. Each of the five test strains was introduced to the column separately. After infection with each strain, the column was cleaned with 1 M NaOH for 60 min before sampling for viable organisms at the predetermined test sites. The sampling methods differed slightly depending on the test site.

Preparation of the test strains

Test organisms are listed in Table 2. The bacterial strains and the yeast *Candida albicans* were stored on nutrient agar slants at 4°C. The day before each test, the organisms were inoculated in tryptic soy broth (TSB) and incubated at 30°C–32°C for 18–24 h. Pure cultures were prepared for each experiment. The mold *Aspergillus niger* was cultivated on malt extract agar (MEA) and spores were harvested by flushing the one-week old culture with sterile saline, followed by mechanical treatment with a sterilized platinum loop, and finally, filtration through sterilized glass wool. The spore suspension was stored at 4°C.

Table 2. Test organisms

Staphylococcus aureus ATCC 6538 Gram-positive bacteria
Escherichia coli ATCC 8739 Gram-negative bacteria
Pseudomonas aeruginosa ATCC 9027 Gram-negative bacteria
Candida albicans ATCC 10231 yeast
Aspergillus niger ATCC 16404 mold

Test procedure

For each strain, a standardized volume of broth culture, (or in the case of *Aspergillus niger*, a suspension volume) was added to 2 L of sterile peptone water. Peptone water was used because it is a low nutrient solution that maintains cell viability. For each test, the concentration of microorganisms in the infecting solution was determined by counting the number of CFUs on tryptic soy agar (TSA) plates. The concentration was in the order of 10^4–10^5 CFU/mL.

Infection

The contaminated peptone water was pumped into the column with an upward flow at a flow rate of 370 mL/min. Thereafter, the filled column was left at room temperature for 18 h. This procedure was repeated for each test strain.

Sanitization

The column was sanitized by circulating 2 L of 1 M NaOH for 60 min at a pressure of 1 bar (0.1 MPa) and flow rate of 280 mL/min.

Microbial testing

Following the sanitization cycle NaOH was washed out of the column using a solution of sterile saline. The elimination of NaOH was determined by measuring the pH of the rinsing solution at the column outlet. Thereafter, the column and connections were dismantled and microbial samples taken at the predetermined sites. Microbial sampling was performed by one of the following methods, depending on the sample site:

Test method 1
Surface samples were taken with alginate swabs dissolved in 15 mL sterile citrate solution. The entire solution was passed through a sterile 0.45 µm membrane filter that was thereafter incubated on TSA for three to five days before inspection.

Test methods 2 and 3
Detachable parts were aseptically removed from the system and put into 10 mL (method 2) or 30 mL (method 3) of sterile physiological saline containing 0.1% Tween™, and thereafter vigorously shaken. The solutions were passed through a sterile 0.45 µm membrane filter, which was thereafter incubated on TSA plates at 30°C–32°C. After three to five days, the number of CFUs were counted.

Test method 4

The outlet sample from test site 8 (Fig 2) was taken by collecting 20 mL at the end of the saline wash and tested by membrane filtration as in Test method 2 and 3

Test method 5

The resin was tested by taking 1 g aliquots and mixing with 30 mL of melted TSA (45°C). The mixture was thereafter poured into a sterile 9 cm petri dish. When the mixture had solidified, the agar dish was incubated in an inverted position at 30°C–32°C and inspected after five days.

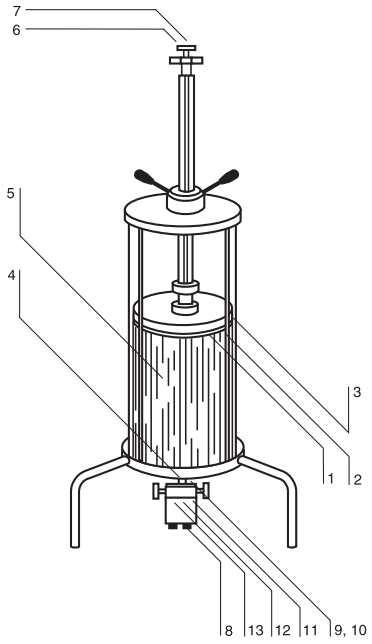

Fig 2. Location of test sites.

Results

As can be seen from the results in Table 3, sanitizing with NaOH for 60 min was very effective in reducing the microbial strains in BPG 100 column.

Conclusions

We have shown that a packed BPG 100 column is effectively sanitized with NaOH. Despite the high initial levels of microbial contamination, no surviving organisms were found after treatment with 1 M NaOH for 60 min. The results indicate that NaOH is suitable as an antimicrobial agent for the decontamination of vegetative bacteria, yeast, and mold in BPG columns and that the design, components and construction of BPG columns are well suited to sanitization with NaOH. The test strains selected were representatives of different microbiological species, and included the Gram-negative *Pseudomonas aeruginosa*, bacteria that are frequently found in water-based systems. It should be noted, however, that even when contaminating the column with high levels of microorganisms to represent a "worst case" situation, the Gram-negative test strains did not survive treatment with NaOH.

The results also support the findings of similar studies, that NaOH is an effective and easy to use antimicrobial agent. The sanitization method we have described, however, cannot alone guarantee good hygienic status of a chromatographic process. Sanitization with NaOH 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.

Table 3. Test sites and number of CFU after NaOH treatment

		S. aureus	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
Challenging concentration (CFU/mL)		1.3×10^5	1.1×10^5	8.9×10^4	1.0×10^5	1.0×10^4
Test sites	**Location in column**	**(CFU/unit or CFU/g)**	**(CFU/unit or CFU/g)**	**(CFU/unit or CFU/g)**	**(CFU/unit or CFU/g)**	**(CFU/unit or CFU/g)**
1	Column support net (fine)	0	0	0	0	0
2	Support net (rough)	0	0	0	0	0
3	O-ring	0	0	0	0	0
4	Inlet	0	0	0	0	0
5	Resin (1 g)	0	0	0	0	0
6	Outlet tubing	0	0	0	0	0
7	Gasket	0	0	0	0	0
8	Outlet	0	0	0	0	0
9	Inlet valve A	0	0	0	0	0
10	Gasket	0	0	0	0	0
11	Membrane	0	0	0	0	0
12	Metal part, inner	0	0	0	0	0
13	Metal part, middle	0	0	0	0	0

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Related literature

Application note: Sanitizing of BPG 450 columns with sodium hydroxide. GE Healthcare, 18111776, Edition AC (2017).

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