

Use of sodium hydroxide for cleaning and sanitizing chromatography media and systems

Sodium hydroxide is widely accepted for cleaning, sanitizing and storing chromatography media and systems. The benefits of its use include efficacy, low cost, ease of detection, removal, and disposal. As with any sanitizing agent, certain precautions should be taken and compatibility with both chromatography media and systems determined.

This Application Note examines these aspects of using sodium hydroxide as a cleaning and sanitizing agent. It includes many examples of particular interest to producers of recombinant proteins, monoclonal antibodies and oligonucleotides, since designing and scaling up validatable cleaning processes is a critical issue in the commercial manufacture of these products (1).

Efficacy

Sodium hydroxide has been shown to be effective in removing proteins and nucleic acids. It is also effective for inactivating most viruses, bacteria, yeasts, and endotoxins. It is common practice in industrial manufacturing to save time by adding a salt, such as sodium chloride, to the sodium hydroxide solution to combine cleaning with sanitization.

Removal of proteins and nucleic acids

As a cleaning agent, sodium hydroxide saponifies fats and dissolves proteins (2). In general, it can solubilize precipitated proteins. Its hydrolyzing power is enhanced by the presence of chlorine (3).

The ability of sodium hydroxide to remove proteins and nucleic acids from chromatography media depends on the nature of the media, the sample, and sample contaminants that may interfere with the cleaning efficiency. For example, a higher concentration of sodium hydroxide may be required if lipids are bound to a protein. To demonstrate the effectiveness of sodium hydroxide, run blank gradients after cleaning and sample storage solutions periodically (4).

Proteins

Sodium hydroxide has been used extensively to remove proteins from ion exchange, hydrophobic interaction and gel filtration media. In contrast, its use with affinity media has been restricted due to the limited stability of most immobilized ligands. However, the removal of proteins from Protein A Sepharose™ Fast Flow used to purify monoclonal antibodies has now been described (5). The authors repeatedly used 0.5 M sodium hydroxide with a contact time of 15 minutes and noted that they could run the affinity column for 85 cycles over more than two years with no loss of capacity. More importantly, they found that this routine eliminated detectable cross-contamination from previous batches of monoclonals, which had been observed when other cleaning procedures were employed. In addition, there was no endotoxin contamination and only very low levels of leakage, which were deemed unlikely to pose a clinical hazard.

Nucleic acids

Nucleic acids can bind tenaciously to anion exchangers. However, work in our laboratories has shown that a combination of 1 M sodium hydroxide and 3 M sodium chloride with a total contact time of one hour effectively removes radiolabelled calf thymus DNA from DEAE Sepharose Fast Flow, a weak anion exchanger. A small percentage of the radiolabelled DNA was retained, and could not be eluted under any conditions tested. Others have found that lower concentrations or shorter contact times are insufficient to remove nucleic acid from DEAE Sepharose Fast Flow and restore the medium's separation capabilities.

Further work on Q Sepharose Fast Flow, a strong anion exchanger, has shown that 1 M sodium hydroxide combined with 1 M sodium chloride effectively removes DNA, but that the level of removal is dependent on the nature of the sample (6). The contact time for cleaning-in-place (CIP) in these experiments was 2 hours. For one sample, DNase was required to completely remove DNA from the anion exchanger.

Inactivation of virus, bacteria, yeasts and endotoxin

Virus

Experiments performed by a testing laboratory showed that 0.1 M sodium hydroxide was sufficient to inactivate the murine leukemia virus, a commonly used model enveloped virus (7). More recently, Q-One Biotech Ltd. has made available its data on the ability of sodium hydroxide to inactivate eight different viruses. Both 0.1 M and 0.5 M sodium hydroxide were tested and the kinetics of inactivation were reported (Table 1). It is worth noting that even highly resistant, non-enveloped viruses, such as canine parvovirus and SV-40, are inactivated by sodium hydroxide. Furthermore, recent reports of new forms of Creutzfeldt-Jakob Disease (CJD) and its potential link to bovine spongiform encephalopathy (BSE) have raised further concerns about adventitious agents. Sodium hydroxide has been shown to be effective in inactivating the BSE agent, which is otherwise extraordinarily resistant to most treatments, including ashing at 360 °C for one hour (8,9).

Bacteria and yeasts

Large amounts of microorganisms such as yeasts and bacteria can destroy the function of chromatography columns. They may also have indirect effects, such as clogging filters and other system components. In addition, they may produce harmful substances such as endotoxins (see later), enterotoxins and proteases. Table 2 shows that sodium hydroxide is very effective in inactivating a number of yeasts and bacteria and that this inactivation is dependent upon concentration, contact time, and temperature.

It is clear from Table 2 that spores may not be totally inactivated by sodium hydroxide. However, good manufacturing practice (GMP) should eliminate the admission of spores into a manufacturing environment.

Peracetic acid has been suggested by some as an effective sanitization and even sterilization agent for chromatography processes (10). While there is no doubt that peracetic acid is an effective sterilizing agent, the extractables that may result from exposing the chromatography system to this agent can change the function of chromatography media and even adulterate a product.

		Virus titres ^{a)} and inactivation values (log ₁₀)							
		HIV	BVD	CPV	BHV	POL	SV-40	MLV	ADV
0.1 M NaOH	Spike	2.0 × 10 ⁶	9.5 × 10 ⁶	2.0 × 10 ⁹	6.9 × 10 ⁹	7.1 × 10 ⁸	1.7 × 10 ⁸	2.6 × 10 ⁵	2.2 × 10 ⁸
	<i>t</i> = 0 min	^{b)} 5.9 × 10 ²	2.7 × 10 ⁷	1.9 × 10 ³	1.2 × 10 ²	3.5 × 10 ⁴	1.5 × 10 ⁵	3.7 × 10 ¹	1.7 × 10 ²
	<i>t</i> = 10 min	5.7 × 10 ²	2.7 × 10 ⁵	2.4 × 10 ³	1.5 × 10 ¹	2.7 × 10 ³	3.6 × 10 ⁵	3.8 × 10 ¹	6.0 × 10 ¹
	<i>t</i> = 20 min	5.8 × 10 ²	1.5 × 10 ⁴	9.6 × 10 ²	4.5 × 10 ¹	2.0 × 10 ⁴	4.7 × 10 ⁴	4.0 × 10 ¹	6.3 × 10 ¹
	<i>t</i> = 60 min	5.8 × 10 ²	2.7 × 10 ⁴	5.0 × 10 ³	4.5 × 10 ¹	2.1 × 10 ³	2.0 × 10 ⁴	4.3 × 10 ¹	2.9 × 10 ¹
Inactivation (log ₁₀)		> 3.5	2.5	5.6	8.2	5.5	3.9	> 3.8	> 6.9
0.5 M NaOH	Spike	2.0 × 10 ²	9.5 × 10 ⁶	2.0 × 10 ⁹	6.9 × 10 ⁹	7.1 × 10 ⁸	1.7 × 10 ⁸	2.6 × 10 ³	2.2 × 10 ⁸
	<i>t</i> = 0 min	5.7 × 10 ²	1.9 × 10 ⁴	9.4 × 10 ²	5.9 × 10 ¹	1.1 × 10 ⁵	1.5 × 10 ⁵	6.3 × 10 ¹	9.4 × 10 ¹
	<i>t</i> = 10 min	5.6 × 10 ²	1.3 × 10 ²	1.2 × 10 ³	5.9 × 10 ¹	1.1 × 10 ⁵	1.7 × 10 ³	4.7 × 10 ¹	7.5 × 10 ¹
	<i>t</i> = 20 min	5.6 × 10 ²	1.7 × 10 ²	1.5 × 10 ³	5.9 × 10 ¹	2.0 × 10 ⁴	8.4 × 10 ³	4.7 × 10 ¹	2.0 × 10 ¹
	<i>t</i> = 60 min	6.7 × 10 ²	1.7 × 10 ²	1.5 × 10 ³	5.9 × 10 ¹	6.2 × 10 ³	1.0 × 10 ²	5.5 × 10 ¹	2.2 × 10 ¹
Inactivation (log ₁₀)		> 3.5	> 4.7	6.1	> 8.1	5.1	6.2	> 3.7	> 7.0

a) Virus titres expressed in TCID50 (Tissue Culture Infective Dose) units for all viruses except BHV and MLV (expressed in plaque forming units – pfu).

b) Italicised titres: No virus was detected in these samples and values listed are theoretical minimum detectable titres.

HIV: human immunodeficiency virus type 1; BVD: bovine viral diarrhoea virus; CPV: canine parvovirus; BHV: bovine herpes virus type 1; POL: human poliovirus type 2; SV-40: simian virus-40; MLV: murine leukemia virus; ADV: human adenovirus type 2. Data from Q-One Biotech Ltd., Todd Campus, West of Scotland Science Park, Glasgow G20 0XA, Scotland.

Table 1. The inactivation of 8 different viruses in 0.1 M and 0.5 M sodium hydroxide.

A) Types of microorganisms tested and the American Type Culture Collection (ATCC) Number.

Microorganism	ATCC No.	Type
<i>Escherichia coli</i>	8739	bacteria gram –
<i>Staphylococcus aureus</i>	6538	bacteria gram +
<i>Pseudomonas aeruginosa</i>	9027	bacteria gram –
<i>Candida albicans</i>	10231	yeast
<i>Aspergillus niger</i>	16404	mould

B) Test results at different times and temperatures.
(From Amersham Biosciences.)

Organism	Conc. NaOH	Time*	Temp.
<i>E. coli</i>	0.01 M	2 hours	4 or 22 °C
<i>S. aureus</i>	0.1 M	1 hour	4 or 22 °C
<i>C. albicans</i>	0.5 M	1 hour	4 or 22 °C
<i>A. niger</i>	0.5 M	1 hour	4 or 22 °C
<i>B. subtilis</i> spores	1.0 M	48 hours**	22 °C
<i>B. subtilis</i> spores	1.0 M	8 days***	4 °C
<i>P. aeruginosa</i>	1.0 M	1 hour	22 °C

* for reduction to below detection limit of <3 organisms /mL.

** <10 organisms/mL.

*** <100 organisms /mL.

Table 2.

For chromatography media that cannot tolerate high concentrations of sodium hydroxide, adding 20% ethanol can enhance the inactivation of microorganisms. Table 3 illustrates this effect for the yeast *C. albicans*.

Microbial challenge tests are performed as part of a continuing effort to design systems and columns that meet sanitary design requirements. These challenge tests should be performed by a vendor, rather than a user, to illustrate optimal sanitization procedures and direct attention to system components that require special care during

sanitization. For example, we have found that in-line filters may retain a heavy bioburden load when systems are deliberately contaminated with test microorganisms.

We have found that 1 M sodium hydroxide for 1 hour is an excellent sanitization procedure for a BioProcess™ chromatography system (11). Similarly, we found that 0.5 M sodium hydroxide with a contact time of one hour is sufficient for sanitizing a BioProcess stainless steel column (12).

Endotoxins

Figure 1 illustrates the effectiveness of sodium hydroxide in inactivating a very high load of endotoxins in solution. Note that much longer contact time is required when 0.1 M sodium hydroxide is used compared with a concentration of 0.5 or 1.0 M.

An endotoxin challenge study has been performed on a BioProcess System connected in-line with a BPG™ column packed with Q Sepharose Fast Flow. The system and packed column were filled with a solution containing 1200 EU per milliliter and allowed to stand for 23 hours at room temperature. They were then sanitized with 1 M sodium hydroxide circulated for one hour to remove pyrogens. After depyrogenation, the system was rinsed with sterile, Limulus Amebocyte Lysate (LAL)-negative water and tested by both the LAL test and total organic carbon (TOC). TOC analysis was used to show that no carbon-containing inactivated endotoxin fragments remained in the system. The column was unpacked, stirred, and an aliquot of the Q Sepharose Fast Flow tested by LAL. The results demonstrated that such a system can be depyrogenated within one hour with 1 M sodium hydroxide, even after an extremely high load of endotoxin (13).

It is important to recognize that each feedstream is unique, and there may be a protection effect provided by other feedstream substances such as lipids and proteins. We recommend, therefore, that endotoxin testing be part of a routine evaluation of the effectiveness of a depyrogenation regime.

Organism	0.01 M NaOH	Organisms*	0.01 M NaOH with 20% ethanol	Organisms*
<i>E. coli</i>	3–6 hours	< 3		
<i>P. aeruginosa</i>	3–6 hours	< 3		
<i>S. aureus</i>	24 hours	102–103		
<i>C. albicans</i>	7 days	102–103	7 days	0
<i>A. niger</i>	28 days	103	7 days	< 100

* per ml

Table 3. Addition of 20% ethanol can enhance the effectiveness of NaOH. (From Amersham Biosciences.)

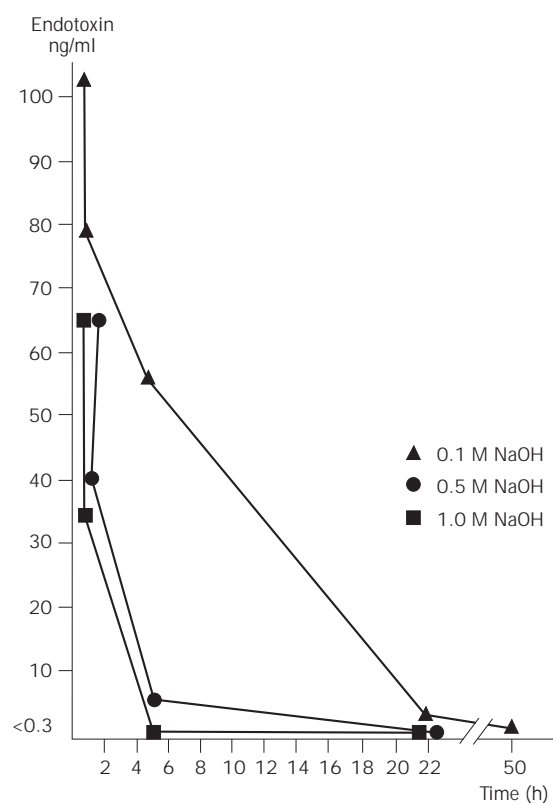


Fig. 1. Inactivation of endotoxin by NaOH. (From Amersham Biosciences.)

Other benefits of sodium hydroxide

Compared with other sanitizing agents, sodium hydroxide is inexpensive. Since it is a bacteriostat, it is also recommended for storage. According to the PDA Biotechnology Cleaning Validation Committee (see Reference 3), concentrations of 0.1 to 1.0 M sodium hydroxide are common for storing packed chromatography columns. Removal of sodium hydroxide can be determined by simple in-line pH and conductivity measurements. Furthermore, the disposal of sodium hydroxide solutions is relatively straightforward, requiring no special measures.

Precautions

Always take care to ensure that chromatography media, columns, systems and auxiliary components are compatible with sodium hydroxide at the concentration, time, and temperatures used. Also keep in mind that sodium hydroxide may be corrosive to both metal and skin (see Reference 2).

Compatibility

The concentration of sodium hydroxide employed for CIP and/or sanitization will often depend on the level of contamination. For chromatography media, the ability to withstand stringent sanitizing conditions depends on the functional groups, attachment chemistries, and the stability of base matrices to alkaline conditions. This aspect of compatibility has been studied extensively and is well documented in numerous references (14–18). Table 4 lists the general stability of a wide range of media as a function of pH.

Table 5 illustrates the functional stability of Butyl Sepharose 4 Fast Flow after extended exposure to 1 M sodium hydroxide. There is no significant change in the retention time of four different standard proteins after 4 weeks storage of the medium in sodium hydroxide at room temperature.

Weeks in 1 M NaOH at room temperature	Retention Time, min			
	A	B	C	D
0	8.50	23.80	37.40	51.65
3	8.40	23.08	37.13	51.48
4	8.44	23.26	36.84	51.47
Pooled S.D. ($n = 9$)	0.12	0.36	0.17	0.08

A: Cytochrome C; B: Ribonuclease A; C: Lysozyme; D: Chymotrypsinogen.

Table 5. Functional stability of Butyl Sepharose 4 Fast Flow after CIP with NaOH. (Adapted from ref. 17.)

Description	Working	Stability range		Storage ¹
		Short term CIP	Long-term operational	
Gel filtration				
Sephadex™ G-25	2–13	2–13	2–13	0.01 M NaOH
Sephacryl™ High Resolution	3–11	2–13	3–11	20% ethanol
Superdex™ prep grade	3–12	1–14	3–12	0.01 M NaOH
Sepharose Fast Flow	3–13	2–14	3–13	0.01 M NaOH
Ion exchange media				
STREAMLINE™ SP	4–13	3–14	4–13	0.01 M NaOH
STREAMLINE DEAE	2–9 ^{a)}	1–14	2–13	0.01 M NaOH
SP Sepharose Big Beads	4–13	3–14	4–13	0.01 M NaOH
Q Sepharose Big Beads	2–12	2–14	2–12	0.01 M NaOH
DEAE Sepharose Fast Flow	2–9 ^{a)}	1–14	2–13	0.01 M NaOH
CM Sepharose Fast Flow	6–10 ^{a)}	2–14	4–13	0.01 M NaOH
SP Sepharose Fast Flow	4–13	3–14	4–13	0.01 M NaOH
Q Sepharose Fast Flow	2–12	1–14	2–12	0.01 M NaOH
SP Sepharose High Performance	4–13	3–14	4–13	0.01 M NaOH
Q Sepharose High Performance	2–12	1–14	2–12	0.01 M NaOH
SOURCE™ 15/30S	2–13	1–14	2–13	0.01 M NaOH
SOURCE 15/30Q	2–12	1–14	2–12	0.01 M NaOH
Q Sepharose XL	2–12	2–14	2–12	0.01 M NaOH
SP Sepharose XL	4–13	3–14	4–13	0.01 M NaOH
STREAMLINE Q XL	2–12	2–14	2–12	0.01 M NaOH
STREAMLINE SP XL	4–13	3–14	4–13	0.01 M NaOH
Hydrophobic interaction media				
Phenyl Sepharose Fast Flow (h/l sub)	3–13	2–14	3–13	0.01 M NaOH
Phenyl Sepharose High Performance	3–13	2–14	3–13	0.01 M NaOH
Butyl Sepharose Fast Flow	3–13	2–14	3–13	0.01 M NaOH
Octyl Sepharose Fast Flow	3–13	2–14	3–13	0.01 M NaOH
Reversed phase chromatography media				
SOURCE 15 RPC	1–12	1–14	1–12	0.01 M NaOH
SOURCE 30 RPC	1–12	1–14	1–12	
Affinity media				
Blue Sepharose 6 Fast Flow	4–12	3–13	4–12	0.01 M NaOH
Chelating Sepharose Fast Flow	4–8.5 ^{b)}	2–14	3–13	0.01 M NaOH
STREAMLINE Chelating	4–8.5 ^{b)}	3–14	3–13	0.01 M NaOH
STREAMLINE Heparin	4–12	4–13	4–12	0.01 M NaOH
Heparin Sepharose 6 Fast Flow	4–12	4–13	4–12	0.01 M NaOH
Protein A Sepharose 4 Fast Flow	2*–9 ^{c)}	2*–10	3–9	20% ethanol
MabSelect™	2*–9 ^{c)}	2*–11	3–10	20% ethanol
rProtein A Sepharose Fast Flow	<u>2*–9^{c)}</u>	<u>2*–11</u>	<u>3–10</u>	<u>20% ethanol</u>
STREAMLINE rProtein A	<u>2*–9^{c)}</u>	<u>2*–11</u>	<u>3–10</u>	<u>20% ethanol</u>
Protein G Sepharose 4 Fast Flow	<u>2*–9^{c)}</u>	<u>2*–10</u>	<u>3–9</u>	<u>20% ethanol</u>
rpm Protein A Sepharose Fast Flow	<u>2*–9^{c)}</u>	<u>2*–11</u>	<u>3–10</u>	<u>20% ethanol</u>

* pH below 3 is sometimes required to elute strongly bound Ig's. However, protein ligands may hydrolyse at very low pH.

¹⁾ Many of these media are supplied wet in 20% ethanol. As an alternative to NaOH, 20% ethanol can be used for storage. Storage in ethanol should be buffered for some products such as SP ligands (0.2 M sodium acetate) and Heparin ligands (50 mM sodium acetate, pH 7).

Data underlined are estimates to the best of our knowledge and experience, complete studies on stability as a function of pH have not yet been performed.

Working pH: pH interval where the medium binds protein as intended or is needed for elution, without adverse long term effect. This differs from operational due to a) uncharged weak ion exchanger, b) broken complex with metal ions and c) no useful increase in binding.

Short term pH: pH interval where the medium can be subjected to, for cleaning- or sanitization-in-place (accumulated 90–400 hours at room temperature) without significant change in function.

Long term: pH interval where the medium can be operated without significant change in function.

Storage: Recommended storage conditions to prevent microbial growth. The medium can be stored up to one year without significant change in function.

The data presented here are an overview over recommended ranges. for detailed information on chromatographic stability and leakage on Amersham Biosciences media, please contact our specialists in process chromatography or regulatory affairs. 'Significant change' means that the media will pass our QC test again.

Table 4. pH ranges for operational, cleaning and storage of media.

More fragile affinity ligands may not tolerate such harsh conditions and the concentration of sodium hydroxide may have to be reduced. Figure 2 shows that Heparin Sepharose 6 Fast Flow withstands exposure to 0.1 M sodium hydroxide for long periods with no loss of binding capacity for antithrombin III. When contamination is severe, 0.5 M sodium hydroxide can be used effectively over shorter periods. However, a decrease in function will be seen over time, as is also shown in Figure 2 (19).

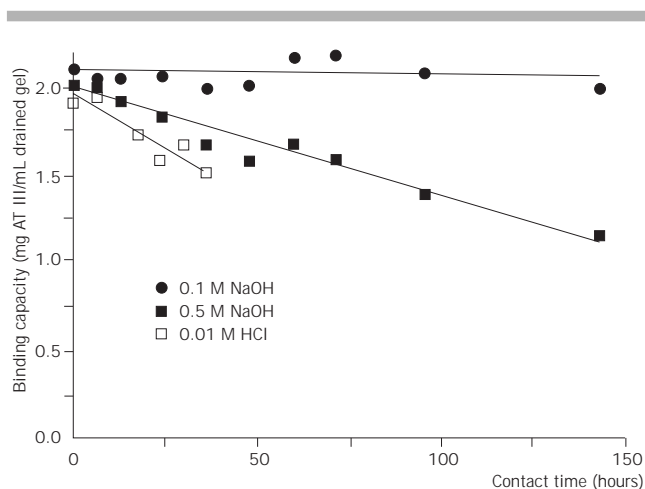


Fig. 2. Functional stability of Heparin Sepharose 6 Fast Flow after CIP with NaOH and HCl. (From Amersham Biosciences.)

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