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⟨1051⟩ CLEANING GLASS APPARATUS

INTRODUCTION

Success in conducting Pharmacopeial assays and tests depends on the cleanliness of the glassware apparatus used. For this reason, commercial detergents or inorganic reagents for cleaning should be used when necessary.

In all cases, it is important to verify that the cleaning procedure is appropriate for the particular test or assay used. This can be accomplished in a number of ways, including use of experimental controls or verification of cleaning by utilization of residue/residual testing to ensure removal of any potential contaminants. The cleaning protocol should include a statement describing how the success of the cleaning procedure will be assessed.

Examples of particular tests where clean glassware is critical for success include but are not limited to: pyrogen and total organic carbon tests and assays of heparin sodium and vitamin B_{12} activity. For optical measurements, special care is required for cleaning containers; however, the use of chromic acid or highly alkaline solutions should be avoided.

Additional references on cleaning glass apparatus are listed in the *Appendix*. USP does not endorse these citations, and they do not represent an exhaustive list. Further information about the effectiveness of the glassware apparatus cleaning procedures mentioned in this chapter may also be found in most quantitative chemical analytical textbooks.

Change to read:

CLEANING VALIDATION BEST PRACTICES

The validation of a cleaning process may involve both a qualitative and quantitative assessment of cleaning performance and may be followed by specific assessments of cleanliness appropriate for the intended use. An established cleaning procedure may require revalidation when significant changes to the procedure are made or when the intended use of the glassware changes. The following outline illustrates this strategy:

General Cleaning Validation Procedure for General Laboratory Use

1. Qualitative assessment of cleaning process
    A. Take a soiled worst-case load according to intended use or prepare a load by applying formulation with fluorescent indicator (see *Table 1*)
    B. Inspect glassware with a UV lamp or black light to verify suitable coverage with fluorescent indicator
    C. Perform cleaning with process to be validated
    D. Inspect glassware with a UV lamp or black light to qualitatively evaluate cleanliness

2. Quantitative assessment of cleaning process
    A. Perform rinse of glassware with *Purified Water*
    B. Collect rinse water
    C. Test per *Total Organic Carbon ⟨643⟩* and *Water Conductivity ⟨645⟩*

3. Perform additional (chemical) validation evaluations as appropriate for the intended use (see *Table 2*)

Qualitative Assessment of Cleaning Process

A fluorescent dye/indicator is useful for qualitatively determining if a cleaning process or procedure is able to apply cleaning solution where required and remove the solution completely. Two fluorescent indicators are commonly used for this type of qualitative assessment of a cleaning process: riboflavin and fluorescein. The fluorescent indicator solution in *Table 1* may be prepared with little or no viscosity-increasing agent (e.g., hydroxyethyl cellulose [HEC]) if the solution will completely fill the equipment to be cleaned. A viscosity-increasing agent (e.g., 5 g/L of HEC) is recommended if the solution will be sprayed into or onto the equipment. The concentration of the fluorescent indicator may be increased if more sensitivity is required. The presence or absence of the fluorescent indicator can be assessed using a black light or UV lamp with excitation (output) of about 365 nm (UVA range).

Table 1. Recommended Recipe for Fluorescent Indicator Solutions Used in Cleaning Validation Experiments

Ingredient	CAS	Function	Concentration (g/L)
Riboflavin or *Disodium Fluorescein*	[83-88-5] [518-47-8]	Fluorescent indicator	0.2
Hydroxyethyl Cellulose[a]	[9004-62-0]	Viscosity-increasing agent	0–5
Purified Water	[7732-18-5]	Solvent	*Quantum satis*

[a] Natrosol 250 HHR or equivalent.

Quantitative Assessment of Cleaning Process

For quantitative assessment of cleaning, the recommended approach is to follow the cleaning process by rinsing the glassware (or other laboratory equipment) with water of appropriate quality (e.g. *Purified Water*) and collecting the rinse water. The properties of this rinse water are evaluated to assess whether any contaminants are present at sufficient levels to significantly affect the properties of the rinse water and the involved analytical use of glassware. The amount of rinse water utilized should be commensurate to the quantities described in the methods in ⟨643⟩ and ⟨645⟩, which have been found to be good for assessing the cleanliness of the rinsed glassware. The results from these general chapter tests should show that the properties of the rinse water are not significantly changed after rinsing the glassware or remain compliant with the requested cleanliness level for the glassware. Determination of necessary cleanliness level should be risk-based and consider the intended use and the analytical test(s) involved.

ADDITIONAL CLEANING VALIDATION ANALYSES

Depending on the intended use of the glassware (or other laboratory equipment), it may be advisable to perform additional experiments to verify suitable cleanliness after the proposed cleaning process. For example, glassware used to contain samples for gas chromatography (GC) analysis should also be shown to be free from residual volatiles that may interfere with the GC method. This may be assessed by sealing the top of the glassware and performing a headspace GC analysis to confirm that there are no residual volatiles that will interfere with planned analyses. See *Table 2* for additional validation experiments that are recommended for intended uses.

Table 2. Additional Evaluation Recommended for Specific Use in Sensitive Analyses

Intended Use	Recommended Additional Cleaning Validation Experiment
General laboratory use	Default evaluation according to 1 and 2 of the outline in *Cleaning Validation Best Practices*
GC samples	Headspace GC analysis of residual volatile compounds
Liquid chromatography samples	Rinse with organic solvent (e.g., as the mobile phase) and assess for unknown peaks with similar retention times.
Elemental impurities samples	Evaluate Class 1 metals according to *Elemental Impurities—Procedures* ⟨233⟩ and verify levels are below ▲limit of detection.▲ (ERR 1-Sep-2021)
Particulate analysis samples	Rinse with particle-free water and assess for extrinsic particles in size range of interest.
Surface tension/surface energy analysis	Evaluate surface tension of rinse water.
Protein binding/activity	Evaluate rinse water per *Protein Determination Procedures* ⟨507⟩.

VALIDATION OF AUTOMATED GLASSWARE CLEANING

In laboratories that use automatic washers to clean glassware, it is important to conduct a validation procedure to demonstrate the effectiveness of the cleaning cycles by conducting a suitable validation procedure. One way this can be done is by first selecting a suitable molecule that is likely to be difficult to clean from soiled glassware. A suitable molecule is most relevant to the risks involved for each laboratory. It is recommended that laboratories choose a molecule that represents a worst-case scenario of the materials the glassware may be exposed to (e.g., poorly soluble molecules in common solvents). Next, items of glassware from a selection of different shapes and sizes (e.g., beakers, conical flasks, volumetric flasks) are spiked with known quantities of this analyte (i.e., analyte is dissolved into a good solvent and applied uniformly) and then allowed to dry. The spiked glassware is then distributed to selected positions in the glassware washer, which is then fully loaded with unspiked glassware so that all locations in the washer are populated. The wash cycle is then run, and

all spiked items are extracted separately with a suitable solvent. Finally, the sample preparations are analyzed quantitatively to determine the residual contaminants in each item, where application of the quantitative method is based on the chosen soil test case.

FAILURES OF CLEANING VALIDATION

If the cleaning procedures are not suitable to clean glassware to meet the desired validation criteria, it will be necessary to consider alternative cleaning procedures, additional rinsing procedures, or evaluate the use of disposable equipment.

Alternative Cleaning Procedures

Typical cleaning solutions contain surfactants in either an acidic or alkaline buffer solution. The selection of either an acidic or alkaline cleanser is typically based on whether the compound to be removed has better solubility at low or high pH or is easily degraded at low or high pH. However, if the glassware becomes unduly clouded or dirty, or contains coagulated organic matter, it may be cleansed with chromic acid cleaning solution. The dichromate should be handled with extreme care because it is a powerful corrosive and carcinogen.

When these more aggressive cleaning solutions are used, the item may be rinsed with the cleaning solution or it may be filled and allowed to stand. The length of time it is allowed to stand depends on the amount of contamination on the glassware. Relatively clean glassware may require only a few minutes of exposure; however, if debris is present, such as blood clots, it may be necessary to allow the glassware to stand overnight. Due to the intense corrosive action of the chromic acid solution, it is good practice to place the stock bottle and the glassware being treated in flat glass pans made from lead or coated with lead or in plastic polymer pans compatible with the concentration of chromic acid used. These more aggressive cleaning solutions should be disposed of properly.

Special types of precipitates may require removal with nitric acid, aqua regia, or fuming sulfuric acid. These are very corrosive substances and should be used only when required. Grease is best removed by boiling in a weak solution of sodium carbonate. Acetone or any other lipophilic solvent may be used. Strong alkalis should not be used. Silicone grease is most easily removed by soaking the stopcock plug or barrel for 2 h in warm decahydronaphthalene.

Alternative Rinsing Procedures

Generally, it is best to rinse glassware with purified or distilled water, and allow it to dry with or without blowing air into it to force it to dry. In some applications, it is important to avoid any nonvolatile residues on the glassware after cleaning (e.g., surface energy measurements). In these cases, it is recommended to use a procedure of alternatively rinsing with distilled water and a suitable volatile organic solvent (e.g., acetone). It is best to perform this final rinsing procedure just prior to use of the clean glassware. For example, a recommended rinse procedure would be as follows: water, acetone, water, acetone, water, acetone. This provides three rinses with each solvent and finishes with the last rinse being the volatile organic solvent, which will evaporate relatively quickly and leave the glassware clean, dry, and ready for immediate use.

Sterilization of Laboratory Equipment and Glassware

When sterile or depyrogenated laboratory equipment is required, it is best to first clean and rinse the glassware thoroughly and then sterilize. Dry heat sterilization (e.g., >160° for 2 h) is generally recommended as it will provide both sterilization and depyrogenation. Autoclaving (e.g., >121° for 30 min) is recommended if the laboratory equipment is incompatible with the temperature of dry heat sterilization. Chemical sterilants (e.g., peracetic acid and hydrogen peroxide solutions) should be avoided, if possible, because they require extensive rinsing procedures to avoid residual oxidants that may lead to undesirable chemical reactions in subsequent use.

Use of Disposable Laboratory Equipment

It is recommended that cost and environmental impact are assessed for the decision of whether to clean and reuse laboratory glassware or to use disposable laboratory plasticware. The environmental impact of cleaning and reusing laboratory glassware includes the cost of the production of the cleaning chemicals as well as the cost of proper disposal of any hazardous solvents and chemicals used or produced in the cleaning procedure. The environmental impact of the disposable plasticware includes the environmental impact of the production and disposal of the plasticware; however, it will reduce the production and disposal of any hazardous chemicals required for the cleaning process. In some cases, the use of disposable equipment may result in less of an environmental impact.

APPENDIX

Additional Sources of Information

Additional information and guidance can be found in the references listed below or in many quantitative chemical analytical textbooks:

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