Validation Case Study: Erroneous Negative Cleaning Validation Results | IVT

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“Validation Case Studies” provides a forum for validation practitioners to share information about actual validation experiences. Previous discussions addressed a wide range of activities. Previous case study titles discussed in this series include the following:

2. Equipment Qualification, JVT, Volume 16, #1.
3. Identical mixing Tanks, JVT, Volume 16, #3.
6. Yield, JVT, Volume 17, #2.
7. Like-for-Like Changes, JVT, Volume 17, #2.

Readers are invited to participate and contribute manuscripts for this series -- we encourage sharing successful practices with others. Please contact journal editor-in-chief Paul Pluta at paul.pluta@comcast.net or content specialist Dustin Henderson at dustin.henderson@cbinet.com with comments or submissions for publication.

ABSTRACT

This case study describes a cleaning validation event in which failing results for API residue from a small molecule extended release tablet dosage form were observed. The initial two lots in the cleaning validation were successful. The third lot failed acceptable residue limits. Investigation of the failure comprised cleaning process development and performance; residue sampling, sample handling, sample analysis, and evaluation of the analytical method. Investigation of this event initially involved interviews of relevant personnel and reviews of associated documentation. Two areas were identified for further evaluation – residue sampling and the cleaning process. Regarding sampling, a newly trained technician, working alone, sampled the first two acceptable lots, while an experienced technician working with a colleague sampled the third failing lot. Evaporation of sampling solvent occurred causing residue to be insufficiently recovered from the equipment surface resulting in erroneous false negative test results. Regarding the cleaning process, manufacturing operators commented that the new extended release formulation was more difficult to clean than the original immediate release formulation although the same cleaning procedure was utilized for both products. Evaluation of the cleaning process indicated that the process parameters were not optimal to clean the new extended release product. An improved cleaning process with increased cleaning agent concentration, increased cleaning time, and higher temperatures was developed, implemented, and ultimately validated.
Cleaning validation sampling personnel must have good technical understanding of their work, and must know the technical reasons for the procedures they perform, and potential problems if procedures are not correctly performed. Sampling personnel training for cleaning validation should include a quantitative demonstration of acceptable cleaning by means of analytical testing. Training exercises must also include worst-case sampling such as with volatile solvents, multiple equipment, and other potential variations in sampling. SOPs must be carefully written to describe potential problems and include performance constraints to minimize variation and risks. There is an inherent danger when variation is not deliberately introduced into a validation project – material variation, manufacturing operator variation, and in this case study, sampling personnel variation. Sampling by two different technicians enabled erroneous results to be discovered. Regarding the cleaning process, inactive ingredients in a formulation may have very significant effects on cleaning processes. Cleaning of residues does not depend solely on the properties of the API.

**BACKGROUND**

The process of validation typically comprise the following sequence of activities:

1. **Change desired.** An equipment or process change is needed or required. This may be a necessary change or a desirable improvement.
2. **Development work.** Appropriate Stage 1 development work is completed in support of the change.
3. **Validation plan.** A formal request to initiate the validation process is submitted to the validation approval committee (VAC). Development reports may be included in the request in support of the change. The change request includes a proposed level of work to confirm the acceptability of the change. The level of work is based on risk to the patient and to the organization. The VAC approves the change request. The approved change request document is stored in the validation library.
4. **Protocol.** A protocol is written specifying detailed sampling and testing to confirm the acceptability of the change. The VAC approves the protocol. The approved protocol is stored in the validation library.
5. **Validation work.** Stage 2 PPQ validation work is performed according to the protocol. Sampling and testing are completed. Data and other results are generated and recorded.
6. **Validation report.** A report containing all test results with discussion and conclusions is prepared and submitted to the VAC for approval. The report is approved and the process or equipment change is implemented. The approved validation report is stored in the validation library.
7. **Validation closure.** If no other work is needed, the validation project initiated by the change request is closed.
8. **Continued verification.** Stage 3 post-validation monitoring confirming acceptability of the change continues throughout the product/process lifecycle.

The issue addressed in this case study occurs in #5 and #6 above. The actual work conducted to confirm the acceptability of the validation project is performed by technical people. In this case study, samples were removed and tested for residue content.

**VALIDATION EVENT**

A small molecule pharmaceutical company conducted initial cleaning validation on a new extended release tablet product containing a water-insoluble API as active ingredient. The new product was a line exten-
sion -- an extended release formulation of a marketed immediate-release tablet product. The new product contained a polymeric matrix to enable prolonged release and once-daily dosage to patients.

The cleaning validation exercise was expected to be successful. Although the product contained a highly potent active drug which required low residue levels on cleaned equipment, the company had extensive experience with the cleaning procedure over several years. The original immediate-release product cleaning was relatively easy and had a long successful history of performance. Several previous cleaning validations had been successfully accomplished. The analytical method for residual API from swab samples was easily performed and very reliable.

Sampling of three lots of new product was planned for cleaning validation. The manufacturing process comprised several unit operations. Sampling of unit operations for cleaning validation was performed on multiple days for each lot. The first lot was manufactured and cleaning completed on all equipment. Equipment was visually clean. Swab sampling was done by the sampling technician. Cleaning validation analytical test data indicated no active drug present in all swab samples -- all acceptable results. A second lot was manufactured. Cleaning was completed. Swab sampling was done. Cleaning validation analytical test data again indicated no levels of residual drug in all swab samples. A third lot was manufactured. Cleaning was completed. Swab sampling was done. Cleaning validation analytical test data indicated extremely high residue levels significantly above the required acceptance criteria. Test results on the third lot indicated a significant failure of the cleaning process.

This event prompted multiple questions to be investigated and answered.

1. Cleaning process performance. Did manufacturing personnel correctly perform the cleaning process in the third (failing) lot? Which operator cleaned the equipment? Were manufacturing personnel adequately trained in the cleaning procedure? What was past history with use of this cleaning process? Were repeat cleanings required in past cleaning? Were deviations required?

2. Cleaning process development. How were the cleaning process parameters developed? What was the history of this method with the immediate release product? We any changes made for cleaning the extended release product?

3. Sampling. Did the sampling technician correctly sample the recommended equipment surfaces? Were sampling personnel adequately trained?

4. Residue samples. Was the integrity of residue samples adequately protected during transport to the lab? Could samples have become contaminated causing the test failures? Were samples correctly and quickly transferred to the lab for analysis? Were samples handled during transport and storage according to procedures? Were samples exposed to high temperatures during transport and storage?

5. Analytical laboratory. Was the analysis correctly performed? Which technician performed the analysis? Were laboratory technicians adequately trained? Was analytical equipment qualified for use for the API analysis? Was system suitability below required limits?

6. Analytical R&D. Were there any problems with the analytical method? Was the analytical method correctly developed? Was the analytical method validated?

INVESTIGATION

Investigation of this compliance event initially involved interviews of relevant personnel and reviews of associated documentation. Personnel related to the compliance event included manufacturing personnel, QC personnel, cleaning sampling technicians, and the technical personnel responsible for product formulation and process, cleaning method development, technical support, and analytical testing. There were many details and variables that needed to be investigated and/or confirmed. Personnel from all groups interacted to address the above issues.

Documentation reviews included manufacturing documentation, cleaning documentation, equipment inspection records, laboratory records, analytical method development reports, validation reports, and other records. All applicable manufacturing SOP’s and analytical SOPs were reviewed.

Cleaning Process Performance

Manufacturing personnel correctly performed the cleaning process in all three lots. Cleaning procedure documentation for all lots was reviewed and found to be perfectly executed. No deviations were issued. Different operators executed cleaning of multiple equipment in the validation lots. An experienced operator executed cleaning in the third (failing) lot. Training records for all operators were reviewed and found to be acceptable. Operators commented that the new extended release product was more difficult to clean than the original immediate release product. The extended release polymer made removal of the product residue more difficult that was typical with the original immediate-release product. This observation reflected operator experience with manual cleaning of small parts. Product was able to be removed from
equipment surfaces and yield visually-clean surfaces. No repeat cleanings were required. No deviations were issued.

Cleaning Process Development
The cleaning process for the immediate release product had been previously developed. An alkaline cleaning agent that was used on several other products in the plant was used. Because the API in the new extended release product was the same as in the immediate release product, no changes were made to the cleaning method. Technical personnel were unaware of any difficulty in cleaning the extended release product.

Sampling
Two different sampling technicians performed sampling in the three lots. A newly-trained technician sampled lots #1 and #2, both of which had acceptable low residue levels. The newly-trained technician worked alone to accomplish sampling since no other sampling technicians were available. Lot #3 was sampled by an experienced technician. The experienced technician worked with a colleague who helped sample the recommended equipment surfaces and complete required documentation. Both sampling personnel were adequately trained as evidenced by training documentation.

Residue samples
Residue samples were packaged in protective wrapping for transfer to the lab. Samples were immediately closed and not contaminated. Transport to the lab was rapid and without exposure to unusual environmental conditions or excessive heat.

Analytical laboratory
The laboratory analysis was correctly performed. Experienced technicians performed the analysis. All technicians were adequately trained. Analytical equipment was qualified for use for the API analysis. Lab documentation indicated acceptable execution of the analytical procedure.

Analytical R&D
The same analytical method was used for the original irradiate-release product and for the new extended-release product. Analytical R&D verified that the test method performed acceptably for the new product. There were no problems with the analytical method. The analytical method was validated.

DISCUSSION
Interviews and discussion of the above questions did not clearly indicate an obvious cause for the problem. Manufacturing personnel confirmed that they performed cleaning as required by procedure. Equipment was cleaned by automated methods wherever possible. All associated small parts were manually cleaned as required by procedure. The manufacturing supervisor verified that procedures were followed and that the equipment was visually clean. Quality unit personnel who inspected the equipment also verified that all equipment and small parts were visually clean. All inspections were conducted after the equipment was dry. Samples were transported to the lab quickly and according to procedure. Samples were also quickly stored in the laboratory upon receipt and under specified security conditions. Laboratory personnel confirmed acceptable performance of analytical procedures. Analytical standards over a range of concentrations tested along with the actual cleaning validation samples yielded accurate results. Analytical R&D scientists confirmed acceptable performance of the validated test method. Two areas were identified for further investigation. These included:

1. Swab sampling. A newly trained technician, working alone, sampled the first two acceptable lots. All samples in these lots were acceptable. An experienced technician, working with a colleague, sampled the third failing. Was something different about the third lot, or was the failing data due to the difference in sampling personnel?
2. Cleaning process. Manufacturing operators commented that the new extended release formulation was more difficult to clean than the original immediate release formulation. The same cleaning procedure was utilized for both products.

Swab Sampling
Swab sampling for the three lots was done by two different sampling technicians. The first two lots were sampled by a newly-trained person. Data for these lots indicated minimal or no residual soil – acceptable results. The third lot with failing data was sampled by an experienced technician who worked with a colleague.

The sampling method required wetting of the swab with organic solvent to dissolve residue from the equipment surface. The new technician did all sampling alone. The experienced technician performed sampling with a colleague to accomplish the sampling procedure in minimum time. She explained the necessity of the rapid sampling technique because evaporation of the sampling solvent must be minimized. The new technician was not aware of the time limitation in sampling. Although not conclusively proven, it was suspected that evaporation of solvent
occurred causing residue to not be adequately recovered from the equipment surface. The new technician worked slowly and carefully, and completed all necessary steps. However, the time required for performance, especially since she worked alone, may have caused residue recovery to be incomplete or minimal. The analytical lab confirmed that if sufficient solvent was not present on the swab, residue recovery would be unsuccessful.

Cleaning Process
Technical personnel responsible for the cleaning process had no previous experience with the cleaning method. The cleaning method for the original product had been established many years ago and never required new technical evaluation. Manufacturing management decided to use the well-established cleaning method without involvement of technical personnel. Management’s rationale was that since the API in the original product had been reliably cleaned for many years, there was no need to evaluate the cleaning process for the new product. Technical personnel had not been requested to evaluate the cleaning process used in the failed cleaning validation. In light of the cleaning failure, technical personnel recommended laboratory studies to evaluate available cleaning agents, cleaning process parameters, and related factors in a systematic way. Evaluation of the cleaning process indicated that the process parameters were insufficient to clean the new product. The polymeric matrix in the new product (methylcellulose mixture) was much more difficult to clean than the original immediate release product. Technical personnel conducted studies to establish new cleaning process parameters suitable for the extended release product. A new cleaning method with increased cleaning agent concentration, increased cleaning time, and higher temperatures was developed.

CORRECTIVE ACTION / PREVENTIVE ACTION (CAPA)
Two CAPA activities corrected the problems experienced in the original cleaning validation. These involved new training of swab sampling personnel and a modified cleaning process for the extended release product.

Swab Sampling Training
Personnel who perform cleaning residue sampling using swabs wetted with volatile solvents were taught the importance of rapidly performing swab sampling. Many of the swab sampling technicians did not have a technical background and did not understand solvent volatility and the consequences for swab sampling. Studies confirmed that the new technician, who worked alone in the sampling activity, did not perform swab sampling quickly. When sampling was not performed quickly, solvent evaporated and surface residue was not able to be dissolved. Analytical results on evaporated swab samples indicated extremely low or no levels of residue which erroneously passed cleaning validation acceptance criteria – a false negative due to solvent evaporation.

Future training of swab sampling technicians included new test procedures to require rapid performance of sampling procedures. The previous qualification test did not utilize a volatile solvent and did not require rapid performance. The new qualification test required technicians to demonstrate rapid sampling in order to become a qualified sampling technician. Sampling teams (two technicians) were required when volatile solvents were used in sampling. Technicians were required to quantitatively recover residue in training to be qualified for residue sampling. Training was repeated on an annual basis. SOPs describing cleaning sampling methods using volatile solvents were strengthened to require rapid sampling and working in teams. The combined emphasis of new training and new procedures that both underscored the risks and potential variation in residue sampling strongly addressed the issues described in this case study.

Modified Cleaning Process for Extended Release product
Technical personnel evaluated the cleaning process and determined that process parameters were not optimal to reliably clean process residues. The cleaning agent concentration was increased, the temperature was increased, and the cleaning time was increased in the new procedure. These parameters enhanced the cleaning process to more effectively and more efficiently remove the polymeric residue.

CLEANING VALIDATION OF MODIFIED CLEANING PROCESS
The new cleaning process was implemented. Manufacturing operators confirmed that new cleaning process parameters significantly improved the cleaning process. Three product lots were manufactured. Cleaning was performed on required equipment in three lots. Worst-case locations on equipment were swab sampled by two-person teams of sampling personnel. Two-person teams ensured minimal solvent evaporation and rapid sampling procedures. All test results passed the acceptance criteria.

SUMMARY AND FINAL THOUGHTS
A case study describing a compliance event in which erroneous false negative analytical data was generated in cleaning validation. These data caused a mistaken conclusion that a cleaning process for a new modified release dosage form was acceptable. The cause
of the problem was not easily determined – all test
data were acceptable. Initial investigation of potential
problem areas indicated that everything was done
according to procedure – nothing was done incor-
rectly. It was ultimately determined that the sampling
process for product residue was not sufficiently con-
trolled, and that the equipment cleaning process was
not adequate for the modified release formulation.
The sampling error, i.e., loss of solvent in sampling,
had a major effect on cleaning validation. The sam-
pling technician did not understand the importance
of working quickly to minimize solvent loss. This
lack of understanding resulted in a false negative test
result and an erroneous conclusion that the cleaning
process was acceptable. Fortunately the error was
discovered when a different technician correctly and
rapidly sampled the equipment surfaces. The com-
bined emphasis of new training and new procedures
that both emphasized the risks and potential varia-
tion of sampling strongly addressed the sampling
issues described in this case study. Observations by
manufacturing personnel caused the cleaning process
to be evaluated by technical personnel, and a new
cleaning process with optimized process parameters
was developed. The new cleaning process was ultim-
ately validated.

Lessons Learned
Several important lessons may be learned from this
case study.

• **Sampling personnel understanding of sam-
ing process and training.** Sampling person-
nel must have good technical understanding
of their work. They must know the reasons for
the procedures they perform. They must know
potential problems if procedures are not correctly
performed.

• **New procedures.** SOPs describing cleaning
sampling methods using volatile solvents were
strengthened to require rapid sampling and
working in teams. Time constraints were added
to all affected procedures. SOPs must be carefully
written to identify potential risks and minimize
variation.

• **Sampling personnel training.** Training of
cleaning validation sampling technicians is a
critical activity. Training exercises must include
a quantitative demonstration of acceptable
cleaning by means of analytical testing. Training
exercises must also include worst-case sampling
such as with volatile solvents, multiple sampling
equipment, and other potential variations used
in sampling. Retaining of technicians at some
defined and reasonable frequency should be
considered.

• **Inactive ingredients effects on cleaning.** Inac-
tive ingredients may have very significant effects
on cleaning processes. Cleaning of residues
does not depend solely on the properties of the
API. Formulation ingredients may significantly
affect the cleaning process. In this case study,
an extended release polymer in the formulation
caused difficulty in the cleaning process. Inactive
ingredients such as dyes and flavors may also
greatly influence cleaning, and may actually be
the most difficult ingredients to clean in a formu-
lation. All components in a formulation must be
considered when developing a cleaning process.
Experimental Parameters for Small-scale Cleaning Characterization Part I: Dilution of Process Fluids During Cleaning | IVT
Rizwan Sharnez, Ph.D., Angela To, Arun Tholudur, Ph.D.

CLEANING VALIDATION
Methodologies for estimating experimental parameters for small-scale cleaning characterization studies are described in this series: dilution of process fluids (e.g., process soil, cleaning solution, or rinse water) during cleaning is discussed in this paper; worst-case fluid velocity and soil load will be discussed in subsequent parts.

Dilution of the process fluid during cleaning was estimated to be on the order of 10^{16} for a typical cleaning cycle and 10^{5} for intermediate cleaning steps. These dilution factors are used to estimate the concentration of impurities in the final rinse and to simulate worst-case cleaning conditions for cleanability and inactivation studies.

INTRODUCTION
Small-scale cleaning characterization (CLC) studies are used to identify suitable cleaning chemistries (1, 2), optimize cleaning parameters and processes (3, 4), establish cleaning times for manufacturing equipment (5, 6), and streamline validation requirements for multiproduct equipment (7, 8).

Experimental models for small-scale CLC have been described in the literature (9-12). In these experiments, the kinetics of soil removal (mass transfer) from a surface is measured under simulated cleaning conditions. A critical step in the development of these models is to identify and scale down the hardest-to-clean (worst-case) location in the equipment (13). Note that it is not necessary to simulate the entire cleaning process; instead, it is only necessary to simulate the location within the equipment that is the hardest to clean. If the process soil can be adequately removed from the worst-case location, it follows that it can also be adequately removed from the other locations in the equipment. Further, with this approach, the cleaning times obtained at small-scale would be indicative of those at full scale assuming that there is adequate coverage at the surfaces that need to be cleaned.

The scalability of small-scale CLC data depends on the accuracy with which the experimental parameters are estimated. Experimental parameters for simulating the worst-case location fall into two categories (14):

- Parameters that can be readily determined from process data such as:
  - Material of construction and surface smoothness of coupons or parts used to simulate large-scale equipment
  - Post-soiling parameters such as hold time, temperature, and humidity
  - Cleaning parameters such as rinse or wash time, temperature of rinse solvent, and temperature and concentration of cleaning solution.
- Parameters that typically need to be determined from first principles such as:
  - Dilution of the process fluid during cleaning
Residual Process Fluid
The volume of residual process fluid (VR) can be estimated from the surface area of the equipment that makes contact with the process fluid (SA) and the residual volume of the process fluid per unit surface area (R):

\[ VR = SA \times R \quad \text{[Equation 1a]} \]

R can be determined experimentally by measuring the amount of process fluid that remains on a surface after it is drained. An experimental method for estimating R is described in the next section.

VR for a CIP circuit can be estimated by dividing the equipment into the following sections: vessel walls (W); bottom dish of vessel (D); and associated piping, filter housings, and other miscellaneous parts (P). Thus,

\[ VR = SA_w \times R_w + SA_D \times R_D + SA_P \times R_P \quad \text{[Equation 1b]} \]

For a tank, the surface area of the sidewall that makes contact with the process fluid is

\[ SA_w = \pi dh \quad \text{[Equation 2]} \]

Where d is the diameter of the tank and h is the height up to which the process fluid wets the sidewall.

The bottom dish surface area (SAD) is estimated from ASME engineering tables (15). The surface area of piping, filter housings, and other miscellaneous components (SAP) is estimated with a 15% overage factor. Thus,

\[ SA_P = 0.15 (SA_W + SA_D) \quad \text{[Equation 3]} \]

Estimation of Residual Process Fluid
The experimental method for estimating the residual volume of a process fluid (R) is described in this section. Two flanged stainless steel sections of piping and an end cap were connected as shown in the Figure. The pipes were one inch in diameter and four inches in length. The bottom section of piping was used to model the continuous nature of surfaces at full scale; it was not part of the surface area that was used to estimate the residual volume. The piping assembly was filled with the process fluid, turned to the appropriate angle (5° or 90°), and then drained by removing the end cap. The amount of residual process fluid in the top section was measured by gravimetry.

Residual volumes were estimated for rinse water and process soil. These fluids were simulated with deionized (DI) water (<1 µS/cm) and a 56% glucose solution (w/w), respectively. Due to its very high concentration and viscosity, the glucose solution was representative of a worst-case process soil from the standpoint of drainage. Also, residual volumes obtained with curved surfaces (pipes) were significantly greater than that for flat surfaces (plates). Consequently, pipes were used to estimate the worst-case residual volumes.

The experimentally determined residual volumes for the configurations that were tested are given in Table I.

Note that impellers, filter housings, and other components associated with the vessel may contain surfaces at a range of angles, typically between 5° and 90°. The residual volume for these components may be set to the R values for the smaller angle of repose (5°) because it represents a worse case from the standpoint of drainage.

Table I: Residual Volumes per Unit Surface Area (R) for Deionized Water (<1 µS/cm) and Process Soil.

Dilution of Process Fluid During Cleaning
For a given step in the cleaning process, the dilution of the process fluid (DS) is determined by the ratio of the volume of the cleaning solvent or solution (VS) to the volume of the residual process fluid on the surface that is being cleaned (VR):

\[ DS = \frac{VS}{VR} \quad \text{[Equation 4]} \]

VS can be obtained from the cleaning cycle parameters, and VR can be estimated as described in the previous section.

Equipment cleaning cycles typically consist of multiple steps such as a pre-rinse, alkaline wash, post-alkaline rinse, acidic wash, post-acidic rinse, and final rinse. The residual process fluid is serially diluted during each step of the cleaning cycle. The cumulative dilution of the
process fluid \( (D_{C}) \) is the product of the \( D_{i} \) values for all of the subsequent cleaning steps to which the process fluid is subjected:

\[
D_{C} = \prod_{i} D_{Si}
\]  

[Equation 5]

Where \( i \) denotes the \( i^{th} \) step and \( n \) is the total number of subsequent cleaning steps.

Note that the above equations do not account for any change in the volume of the residual process fluid \( (\Delta V_{R}) \). Thus, this approach is only applicable when \( \Delta V_{R} \) is negligible.

**Estimation of Dilution During CIP**

In this section, the dilution of a process fluid is estimated for a CIP circuit for a 10,000 liter (L) vessel. The vessel has a diameter (d) of 84 inches (2.14 m), and the process fluid wets the side wall of the vessel up to a height (h) of 120 inches (3.04 m). The cleaning steps and solution volumes for the circuit are given in Table II.

The surface area of the vessel in contact with the process fluid is estimated from Equations 2 and 3 and the engineering table for ASME domed heads (15):

\[
SA_{W} = \pi dh = 20.4 \text{ m}^2
\]

\[
SA_{D} = 4.24 \text{ m}^2
\]

\[
SA_{P} = 0.15 (SA_{D} + SA_{W}) = 3.70 \text{ m}^2
\]

The residual volume per unit area \( (R) \) for the above surfaces is obtained from the \( R \)-values for 56% glucose (representative of the worst-case process soil) in Table I. The \( R \) value for a 90° angle of repose \( (R_{W}) \), and the \( R \) value for a 5° angle of repose \( (R_{D}) \) is used to estimate the residual volumes for the vessel dish and miscellaneous parts \( (R_{P}) \).

The total residual volume of process fluid in the circuit \( (V_{R}) \) is calculated by substituting the above surface areas and \( R \) values into Equation 1b:

\[
V_{R} = SA_{W} \times R_{W} + SA_{D} \times R_{D} + SA_{P} \times R_{P}
\]

\[
= (20.4 \text{ m}^2) (6.60 \text{ mL/m}^2) + (4.24 \text{ m}^2) (26.5 \text{ mL/m}^2) + (3.7 \text{ m}^2) (26.5 \text{ mL/m}^2)
\]

\[
= 345 \text{ mL}
\]

The dilution of the process soil for each cleaning step \( (D_{i}) \) and the cumulative dilution \( (D_{C}) \) are calculated from the residual volume of process fluid calculated above and the volumes of cleaning solution given in Table II. \( D_{S} \) and \( D_{C} \) for the pre-rinse and alkaline wash steps are calculated as follows:

**Pre-rinse (PR):**

\[
D_{S,PR} = \frac{V_{S,PR}}{V_{R}} = \frac{125 \text{ L}}{0.345 \text{ L}} = 362
\]

\[
D_{C,PR} = D_{S,PR} = 362
\]

**Alkaline wash (AW):**

\[
D_{S,AW} = \frac{V_{S,AW}}{V_{R}} = \frac{312 \text{ L}}{0.345 \text{ L}} = 904
\]

\[
D_{C,AW} = D_{S,PR} \times D_{S,AW} = 362 \times 904 = 3.27 \times 10^5
\]

**Table II: Dilution of Process Fluid During Cleaning.**

Assuming that an impurity has negligible affinity for the equipment surfaces and is miscible, solubilized, or otherwise homogeneously distributed in the process fluid, the cumulative dilution factors in Table II can be used to estimate the concentration in the final rinse. For example, a miscible impurity such as hydrogen peroxide, an oxidizing cleaning agent, would be diluted by a factor of \( 2 \times 10^{16} \) during the six steps of the above cleaning cycle. Thus, if the concentration of peroxide before the pre-rinse is 10,000 ppm, its concentration in the final rinse would be \(<1 \text{ part per quintillion (i.e., 1 part per } 10^{18})\).

The step dilution factors for intermediate steps of the cleaning cycle are summarized in Table III.

**Table III: Cumulative Dilution of the Process Fluid for Intermediate Cleaning Steps.**

The above step dilution factors for intermediate steps can be used to simulate intermediate cleaning steps at small scale. For example, if the pre-rinse and alkaline wash steps are to be simulated for a product inactivation study (16, 17), the experimental parameters should be set to limit the dilution of the product to less than \( 3.8 \times 10^5 \) for these steps. This would ensure that the inactivation rate of the product at small scale for these steps represents a worst-case condition relative to that at full scale. This is because for a given pH, temperature, and shear...
rate, the inactivation rate of the product is determined by the concentration of cleaning agent and the concentration of the product, both of which depend on cumulative dilution.

Note that the above dilutions are based on the residual volumes for 56% glucose, a relatively viscous process soil. This represents a worst-case condition from the standpoint of estimating the residual volume (VR). Since cleaning is typically performed with relatively dilute aqueous solutions, VR for most cleaning solutions would be substantially smaller, and therefore the dilution of the process fluids during cleaning would be commensurately higher. Thus, the step and cumulative dilution factors for most CIP systems are likely to be much higher than those estimated in this example.

Conclusion
A methodology for estimating the residual volume and dilution of the process fluid during cleaning was described. Depending on the viscosity of the process fluid and the angle of repose, the residual volume of a process fluid (R) that remains on the equipment surface after drainage was estimated to be between 4.3 and 26.5 mL/m². These estimates are applicable only to systems with negligible pooling of the process fluid. Systems without dead-legs and with surfaces sloped at an angle of at least 5° generally meet this criterion.

Based on the worst-case R value of 26.5 mL/m² and typical rinse and wash volumes, the cumulative and step dilution of the process fluid during cleaning were estimated to be on the order of 1016 and 105, respectively. The cumulative dilution factor is used to estimate the concentration of an impurity in the final rinse. The step and cumulative dilution factors are used to simulate worst-case cleaning conditions for intermediate cleaning steps.

Methodologies for estimating fluid velocity and soil load for small-scale cleaning characterization studies will be described in subsequent parts of this series.

ARTICLE ACRONYMS LISTING

CLC Cleaning characterization
D Diameter of vessel
Dc Cumulative dilution of process fluid for multiple cleaning steps
Ds Step dilution of process fluid for an intermediate cleaning step
DI Deionized
h Height up to which the process fluid wets the side wall
R Residual process fluid per unit surface area
Rb R for vessel bottom dish
Rp R for miscellaneous parts of vessel
ppq Parts per quintillion (one part per 10^29)

SA Surface area of equipment that comes into contact with process fluid
SAd Surface area of bottom dish of vessel
SAp Surface area of vessel piping, filter housings, and other miscellaneous components
SAw Surface area of vessel side wall that is wetted by the process fluid
VR Residual volume of process fluid in equipment
Vs Volume of cleaning solution

REFERENCES

Experimental Parameters for Small-Scale Cleaning Characterization. Part II: Effect of Fluid Velocity on the Kinetics of Cleaning | IVT

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ABSTRACT
Methodologies for estimating experimental parameters for small-scale cleaning characterization are described in this series: dilution of process fluids during clean-in-place (CIP) operations was discussed in Part I (1); the effect of fluid velocity on the kinetics of cleaning is described in this part; the effect of humidity, hold time and soil load on cleanability will be discussed in Part III.

The kinetics of cleaning under worst-case conditions is modeled from first principles. The model is based on diffusion-controlled mass transfer in a laminar falling film, which typifies worst-case cleaning conditions for CIP operations. The effect of flowrate per unit width (Q/W) and fluid velocity (V) on mass transfer rate in film flow is characterized. An experimental approach for optimizing Q/W and V for identifying worst-case soils for cleaning validation is described. The model is also used to estimate fluid velocity, film thickness and Reynolds Number for a range of values of Q/W and angle of inclination (α).

The results indicate that Q/W and V have a relatively weak effect on the kinetics of cleaning. For instance, when these parameters are doubled, the mass transfer rate increases only by a factor of 8% and 12%, respectively. The results also indicate that for $5^\circ < \alpha < 90^\circ$ and Q/W < ~1 gpm/ft (~2 mL/s/cm), the flow would be laminar and the thickness of the film would be < ~1 mm. Further, under these worst-case conditions, V would be < ~52 cm/sec, which is substantially less than the design criterion for minimum fluid velocity in pipes and hoses – viz. 150 cm/sec (5 ft/s).

INTRODUCTION
Small-scale cleaning characterization data can be used to streamline validation requirements for multiproduct equipment – i.e. equipment that is used to manufacture or clean more than one product. This is accomplished by ranking process soils associated with a given cleaning circuit based on the relative cleanability of the soils. The hardest-to-clean or worst-case soil for the circuit is then used to validate that circuit. This approach obviates the need to validate the cleaning of every soil associated with a circuit. It also facilitates the introduction of a new product into an existing multiproduct facility. If it can be shown that the process soils associated with the new product are easier to clean than the corresponding soils of the previously validated product, the new product can be introduced into the facility without revalidating the cleaning procedures (2).

In addition to streamlining validation requirements for multiproduct equipment (3, 4), small-scale cleaning characterization studies can also be used to identify suitable cleaning chemistries (5, 6), optimize cleaning parameters and processes (7, 8) and estimate cleaning times at full scale (9, 10).
Experimental models for small-scale cleaning characterization have been described in the literature (11-14). In these studies, the rate at which the process residue is removed from the surface – i.e., the mass transfer rate – is measured under simulated cleaning conditions. A critical step in the development of these models is to identify and scale down the hardest-to-clean (worst-case) location in the equipment (15). The worst-case location is typically an area with poor circulation, such as a shadowed or occluded area. Note that it is not necessary to simulate the entire cleaning process at small scale; instead, it is sufficient to simulate the worst-case location within the equipment. If the process residue can be adequately removed from the worst-case location, it follows that it can also be adequately removed from other locations in the equipment. Thus, with this approach, the cleaning times obtained at small scale would be indicative of those at full scale, provided that there is adequate spray coverage at all surfaces that need to be cleaned, and the worst-case location is appropriately identified and simulated at small scale.

The scalability of small-scale cleaning characterization data depends on the accuracy with which relevant experimental parameters are estimated. Experimental parameters for simulating the worst-case location fall into two distinct categories (1):

- Parameters that can be readily determined from process data such as:
  - Material of construction and surface characteristics such as roughness and curvature of coupons or parts used to simulate large-scale equipment;
  - Post-soiling parameters such as hold time, and ambient temperature and humidity; and,
  - Cleaning parameters such as rinse or wash time, temperature of rinse solvent, and temperature and concentration of cleaning solution.

- Parameters that typically need to be determined from first principles, such as:
  - Dilution of the process fluid during cleaning (i.e. soil to rinse solvent or cleaning solution ratio).
  - Velocity of rinse solvent or cleaning solution at the worst-case location – the subject of this paper.
  - Soil load.

**Flowrate and Fluid Velocity at Worst-Case Location**

Engineering standards provide guidelines for setting the minimum average velocity or minimum volumetric flow rate of rinse solvent or cleaning solution in various sections of a CIP circuit. For instance, it is recommended that for pipes and hoses the minimum fluid velocity \( V_{\text{MIN}} \) be \( \geq 5 \text{ ft/s} \) (1.5 m/s), and for film flows in vessels the minimum flow rate per unit width \( (Q/W)_{\text{MIN}} \) be \( \geq 2.5 \text{ gpm per ft of vessel circumference} \) (31 L/min/m) (16). These criteria are designed to ensure turbulent flow during CIP; the 5 ft/s criterion for \( V_{\text{MIN}} \) is also designed to ensure flooding in pipes and hoses.

For pipes and hoses, the 5 ft/s criterion for \( V_{\text{MIN}} \) can be readily met at all locations and is therefore relatively straightforward to simulate at small scale. For film flows, however, the flow rate per unit width at the worst-case location \( (Q/W)_{\text{WCL}} \) – such as the underside of an impeller blade or a magnetically coupled bottom-mounted impeller – is likely to be substantially less than \( (Q/W)_{\text{MIN}} \). Design and operational variables that contribute to \( (Q/W)_{\text{WCL}} \) being less than \( (Q/W)_{\text{MIN}} \) are summarized in Table 1.

**Table 1:** Design and operational variables that contribute to the flow rate per unit width at the worst-case location \( (Q/W)_{\text{WCL}} \) being substantially less than the recommended minimum operating value \( (Q/W)_{\text{MIN}} \). For vessels, the recommended value for \( (Q/W)_{\text{MIN}} \) is 2.5 gpm per foot of vessel circumference.

**Effect of Flowrate and Fluid Velocity on Kinetics and Cleaning**

The mass transfer rate from a stationary surface into a laminar falling film of a Newtonian fluid was investigated by Kramers and Kreyger for a flat rectangular layer (17, 18) and by Blount for viscous drops (19, 20). Their results indicate that the mass or molar flux \( N_{\text{AX}} \) of the solute (A) into the fluid (X) is a function of the solubility \( S_{\text{AX}} \) and diffusivity \( D_{\text{AX}} \) of the solute in the fluid; the density \( \rho \), dynamic viscosity \( \mu \) and flow rate per unit width \( (Q/W) \) of the fluid; and the length \( L \) of the surface along the direction of the flow (Figure 1):

\[
N_{\text{AX}} = k \cdot S_{\text{AX}} \cdot (D_{\text{AX}} L)^{2/3} \cdot (Q/W)^{1/9} \cdot (\nu)^{-2/9} \quad \text{Equation [1a]}
\]

where \( \nu = \mu / \rho \), the kinematic viscosity; and \( k \) is a constant that includes the acceleration due to gravity \( g \), the angle of inclination \( \alpha \), and the width \( W \).

**Figure 1:** Mass transfer in gravity-driven film flow: Solute A diffusing into a laminar falling film of fluid X, moving with a fully developed parabolic velocity profile.
For a surface of given length, and a given fluid viscosity and angle of inclination,
\[ N_{AX} \propto (S_{AX} D_{AX}^{2/3}) \cdot (Q/W)^{1/6} \]  
Equation [1b]

Further, for laminar falling films,
\[ Q/W \propto V^{3/2} \]

where \( V \) is the average fluid velocity (21); thus,
\[ N_{AX} \propto (S_{AX} D_{AX}^{2/3}) \cdot V^{1/6} \]  
Equation [1c]

The flux \( (N_{AX}) \) of the solute into the fluid is the rate at which the solute is removed from the surface. Thus, \( N_{AX} \) is effectively a measure of the kinetics of cleaning. Note that the effect of \( Q/W \) and \( V \) on the mass transfer rate – and therefore the kinetics of cleaning – is relatively weak. For example, when \( Q/W \) or \( V \) is doubled, the mass transfer rate increases only by a factor of \( 2^{1/6} \) (8%), and \( 2^{1/6} \) (12%), respectively.

Equation 1 is valid when (1) the Reynolds Number \( Re = 4(Q/W)\nu < 1500 \), the criterion for laminar flow in a falling film; (2) the velocity profile in the falling film is fully developed, a condition that holds when \( L > \delta \), the thickness of the film; and (3) the distance over which the solute diffuses into the film \( (d) \) is << \( \delta \), and as a result, the velocity profile for \( 0 < y < d \) can be approximated as a linear function of distance from the surface being cleaned \( (y) \) (Figure 1). In terms of the parameters in Equation 1, the third condition is satisfied when the solubility \( (S_{AX}) \) and/or diffusivity \( (D_{AX}) \) of the solute in the fluid are low enough for the mass transfer to be diffusion controlled.

The above conditions would be satisfied at the worst-case location in the equipment for a process soil that is difficult to clean, as this represents a worst-case scenario from the standpoint of cleaning – viz. diffusion-controlled mass transfer in a laminar falling film. Thus, Equation 1 can be used to characterize the effect of process parameters such as \( Q/W \) and \( V \) on the kinetics of cleaning under worst-case conditions and for design purposes. It should also be noted that since Equation 1 is derived for mass transfer of a single component \( (A) \) into a pure solvent \( (X) \), its applicability to complex multicomponent process soils or solvents containing formulated cleaning agents would require the use of an effective solubility and diffusivity in the cleaning solution. Consequently, Equation 1 cannot be readily used to predict the cleanability of multicomponent soils; nonetheless, it can be used to characterize the effect of process parameters on the kinetics of cleaning, and to thereby identify and establish meaningful operating ranges for critical process parameters. Further, this equation can also be used to develop experimental models for cleaning (2).

An experimental approach for optimizing flowrate and fluid velocity for evaluating relative cleanability at small scale is described in the next section.

**Flow Rate and Fluid Velocity for Evaluating Relative Cleanability**

Consider a system that is validated to manufacture and clean product \( A \). A new product \( (B) \) needs to be manufactured and cleaned in the same equipment. A small-scale study is performed to evaluate the cleanability of \( A \) relative to that of \( B \). If \( A \) is harder to clean than \( B \), the new product could be introduced without revalidation. The objective is to determine the optimum flowrate per unit width \( (Q/W) \) and fluid velocity \( (V) \) for the small-scale study.

For products \( A \) and \( B \), Equation 1b can be written as follows:
\[ N_{AX} \propto (S_{AX} D_{AX}^{2/3}) \cdot (Q/W)^{1/6} \]  
\[ N_{BX} \propto (S_{BX} D_{BX}^{2/3}) \cdot (Q/W)^{1/6} \]  
Equation [2a]
\[ N_{AX} \propto (S_{AX} D_{AX}^{2/3}) \cdot (Q/W)^{1/6} \]  
\[ N_{BX} \propto (S_{BX} D_{BX}^{2/3}) \cdot (Q/W)^{1/6} \]  
Equation [2b]

Thus,
\[ N_{AX} / N_{BX} = (S_{AX} D_{AX}^{2/3}) / (S_{BX} D_{BX}^{2/3}) \]  
Equation [2c]

Since the cleaning time \( (t) \) is inversely proportional to the mass transfer rate \( (N) \), the cleanability of \( B \) \( (t_B) \) relative to that of \( A \) \( (t_A) \) can be expressed as
\[ t_B / t_A = N_{AX} / N_{BX} = (S_{AX} D_{AX}^{2/3}) / (S_{BX} D_{BX}^{2/3}) \]  
Eq [2d]

Equation 2d indicates that relative cleanability \( (t_B / t_A) \) depends on the physical properties of \( A \) and \( B \) (viz. the solubility and diffusivity of \( A \) and \( B \) in the fluid). Further, relative cleanability is independent of \( Q/W \), and therefore of \( V \). Thus, if the objective of the study is to rank \( A \) and \( B \) based on cleanability, \( Q/W \) and \( V \) can be set to any reasonable value, provided that the resulting flow is laminar – in this case \( Re = 4(Q/W)\nu < ~1000 \).

In practice, however, a lower value of \( Q/W \) is preferable because the absolute difference between \( t_A \) and \( t_B \) \( (\Delta t_{AB}) \) is amplified, which in turn makes it commensurately easier to differentiate between the two soils based on the larger magnitude of \( \Delta t_{AB} \). If necessary, \( \Delta t_{AB} \) can be amplified by reducing \( Q/W \) up to the point where the film is still intact and uniform, i.e. it does not disintegrate into slower-moving unsteady drops.

An equation for estimating fluid velocity of a laminar falling film from \( Q/W \) and \( \alpha \) is derived in the next section.

**Estimation of Fluid Velocity in a Laminar Falling Film**

A laminar falling film of a Newtonian fluid flowing primarily under the influence of gravity is shown in Figure 1. The flow is delineated as a thin sheet of liquid flowing down an inclined flat plate of length \( L \) and width \( W \). As
the liquid flows down the plate, it forms a film of thickness and develops a parabolic velocity profile, with the maximum velocity at the film surface. For this type of flow, the average fluid velocity ($V$) and film thickness ($\delta$) can be expressed as follows (21):

$$V = \frac{\omega}{\rho}$$

$$\delta = \sqrt{\frac{2\rho g \sin \alpha}{\mu}}$$

Where $\rho$ is the density of the liquid, $g$ is acceleration due to gravity, $\alpha$ is the angle of, $\mu$ is the dynamic viscosity of the fluid, $\omega$ is the mass flow rate, and $W$ is the width of the film.

Equations 3 and 4 can be combined to eliminate $\delta$ and express $V$ in terms of measurable parameters:

$$Q = \frac{\omega}{\rho}$$

$$\nu = \frac{\mu}{\rho}$$

$$V = \frac{Q}{\rho W \sqrt{\frac{2g \sin \alpha}{\mu}}}$$

Where $Q$ is $\omega/\rho$, the volumetric flow rate, and $\nu$ is $\mu/\rho$, the kinematic viscosity of the fluid.

Equation 5 is valid under the following conditions: (1) when edge effects are negligible, a condition that is valid when $L$ and $W$ are $>> \delta$, and (2) when viscous forces are large enough to prevent continued acceleration of the liquid along the length of the plate – i.e., at a low Reynolds Number, when the flow is laminar. Under these conditions, $V$ is independent of the distance traversed along the incline ($L$). Note that at the worst-case location the above conditions would be satisfied because (a) the surfaces being cleaned are relatively large, and thus $L$ and $W$ would be $>> \delta$, and (b) the flow would be laminar.

The Reynolds number (Re) is used to classify a falling film into three flow regimes: (a) laminar flow with negligible rippling ($Re < 20$); (b) laminar flow with pronounced rippling ($20 < Re < 1500$); and (c) turbulent flow ($Re > 1500$). When $Re$ is less than 20, the ripples are very long and grow slowly down the surface of the film. As $Re$ increases above 20, the ripple growth increases rapidly. Because of the assumptions made in developing the above model (21), the error in using Equation 5 to estimate velocity increases with ripple growth and $Re$. The velocities estimated using Equations 3 and 4 have been shown to be in good agreement with experimentally observed velocities when $Re$ is less than 1000 (22, 23).

The average velocity of the cleaning solution is estimated from the flowrate per unit width ($Q/W$) and the angle of inclination ($\alpha$) using Equation 5. The estimates are based on the kinematic viscosity ($\nu$) of water at 20°C (0.01 cm²/s), acceleration due to gravity ($g$) of 981 cm/s², and a range of values of $Q/W$ and $\alpha$. The results, summarized in Table 2, indicate that for $5^\circ < \alpha < 90^\circ$ and $Q/W < \sim 1$ gpm/ft (~2 mL/s/cm), the flow would be laminar and the thickness of the film (would be $< \sim 1$ mm, if the film were stable (i.e. if it did not disintegrate into unsteady drops). Further, under these conditions, the average velocity of the fluid would be $< \sim 52$ cm/sec, which is substantially less than the design criterion for $V_{MIN}$ in pipes and hoses – viz. 150 cm/sec (5 ft/s).

![Table II: Average velocity, film thickness, Reynolds Number and relative mass transfer rate in a laminar falling film for a range of values of flowrate and angle of inclination.](image)

**CONCLUSION**

Small-scale experimental models are used to determine worst-case soils for cleaning validation, and estimate cleaning times and other performance parameters. A critical step in the development of these models is to identify and scale down the hardest-to-clean or worst-case location in the equipment. For cleaning operations, the worst-case location is typically an area within the equipment with poor circulation, such as a shadowed or occluded area. Examples of such locations include the underside of a probe or an impeller blade where there is no direct or indirect impingement of the cleaning solution during CIP operations. Instead, the flow of the fluid at the worst-case location is in the form of a laminar falling film.

A mathematical model for diffusion-controlled mass transfer in a laminar falling film was used to characterize the effect of flowrate per unit width ($Q/W$) and fluid velocity ($V$) on the kinetics of cleaning under worst-case conditions. The results indicate that the effect of these parameters on the rate of mass transfer – and therefore the kinetics of cleaning – is relatively weak. The mass transfer rate increases only by a factor of 8% and 12%, respectively, when $Q/W$ or $V$ is doubled.

An experimental approach for optimizing $Q/W$ and $V$ for evaluating relative cleanability at small scale was described. Relative cleanability was shown to depend on the solubility and diffusivity of the soils being compared. Further, for diffusion-controlled mass transfer – which typifies worst-case cleaning conditions for CIP opera-
tions – relative cleanability was found to be independent of $Q/W$ and $V$. Thus, if the objective of the study is to rank coils based on cleanability, $Q/W$ and $V$ can be set to any reasonable value, provided that the resulting flow is laminar. In practice, however, a lower value of $Q/W$ is preferable because the absolute difference between the cleaning times of the soils ($\Delta t_{AB}$) is amplified, and as a result, the ability to differentiate between the soils based on the larger magnitude of $\Delta t_{AB}$ is enhanced commensurately. If necessary, $\Delta t_{AB}$ can be amplified by reducing $Q/W$ up to the point where the film is still intact and uniform – i.e. it does not disintegrate into slower-moving unsteady drops.

The laminar falling film model was also used to estimate fluid velocity ($V$), film thickness ($\delta$) and Reynolds Number ($Re$) from $Q/W$ and the angle of inclination ($\alpha$). The results indicate that for $5^\circ < \alpha < 90^\circ$ and $Q/W < ~1$ gpm/ft ($~2$ mL/s/cm), the flow would be laminar and would be $< ~1$ mm, if the flow was stable. Further, under these conditions, $V$ would be $< ~52$ cm/sec, which is substantially less than the design criterion for $V_{MIN}$ in pipes and hoses – viz. 150 cm/sec (5 ft/s). The calculated values of $V$ have been shown to be in good agreement with experimentally observed velocities when $Re$ is less than 1000.

### SYMBOLS AND ACRONYMS

| CIP | Clean-in-place |
| D  | Diffusivity    |
| g  | Acceleration due to gravity |
| L  | Length of object being cleaned |
| N  | Mass or molar flux |
| Q  | Volumetric flow rate |
| Re | Reynolds number |
| S  | Solubility |
| t  | Time |
| V  | Average velocity of cleaning solution |
| W  | Width of laminar falling film |
| $\alpha$ | Angle of inclination (slope) |
| $\delta$ | Film thickness |
| $\mu$ | Dynamic Viscosity |
| $\nu$ | Kinematic viscosity |
| $\rho$ | Density |
| $\omega$ | Mass flow rate |

### SUBSCRIPTS

| A  | Product A |
| B  | Product B |
| MIN | Minimum |
| WCL | Worst-case location |
| X  | Fluid X |

### REFERENCES

Methodology for Assessing Product Inactivation during Cleaning Part I: Experimental Approach and Analytical Methods | IVT

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ABSTRACT
For multiproduct cleaning validation, the conventional approach for setting an acceptance limit for the process residue is based on the maximum allowable carryover (MAC) of the active pharmaceutical ingredient (API) depending on the process soil, API refers to the active pharmaceutical ingredient in the drug product, drug substance, or drug substance intermediate). However, if the API becomes pharmacologically inactive during cleaning the acceptance limit does not need to be based on active product. This is an important consideration in biopharmaceutical manufacturing because the cleaning conditions are generally aggressive enough to inactivate the product.

The experimental approach and analytical methods for assessing inactivation of the API during cleaning are described in Part I. A rational approach for setting safety-based acceptance limits for inactivated product and process residuals is described in Part II. The scope of this paper is limited to biopharmaceutical cleaning processes; nonetheless, the underlying concepts may be useful in designing inactivation studies and setting acceptance limits for other types of pharmaceutical cleaning processes.

INTRODUCTION
An important regulatory expectation for multiproduct cleaning validation is to demonstrate that potential carryover of the previously manufactured API (Product A) into the subsequently manufactured product (Product B) is below an acceptable level. This criterion is often assessed through a maximum allowable carryover (MAC) calculation for the previously manufactured API (1-5). The MAC calculation is typically based either on the minimum therapeutic dose (1), or the acceptable daily exposure (ADE) (2) of the previously manufactured API.

Limitations of the MAC Approach
A limitation of the conventional MAC approach is that it is based on the assumption that the product is active after the cleaning. This has important implications for biopharmaceutical manufacturing because the API is often inactivated by the cleaning process (6, 7).

Another limitation of the MAC approach is that the calculated acceptance limits are often below the limit of quantitation (LOQ) of non-specific analytical methods, such as total organic carbon (TOC). The LOQ of TOC-based methods is typically between 0.05 and 0.2 ppm. The large surface areas and small batch sizes involved in biopharmaceutical manufacturing further exacerbate this issue. Product specific immunoas-
 says (PSIA) such as ELISA and EIA have been used to address this issue; the LOQ of most PSAs is on the order of 10 ppb. PSIAs detect activity indirectly by recognizing specific epitopes (short sequences of amino acids) in the API; however, epitopes are known to degrade during cleaning, and thus the results can be misleading (8, 9).

Other limitations of the MAC approach are discussed in the literature (9).

**PRODUCT INACTIVATION APPROACH**

With the product inactivation approach, if the API is inactivated during cleaning, the acceptance limits may be set based on the inactivated product instead of the API. The product inactivation approach is therefore more reflective of the phenomenological aspects of the cleaning process. Additionally, the acceptance limits based on inactivated product are very unlikely to be below the LOQ of TOC (refer to Part II). Thus, the product inactivation approach alleviates the limitations of the MAC approach described in the previous section.

The methodology described in Part I includes experimental simulation of the cleaning processes at small scale and analytical methods to evaluate inactivation of the API during cleaning. A rational approach for setting safety-based acceptance limits is described in Part II.

**PROPOSED METHODOLOGY**

Inactivation of the product during cleaning has important implications for cleaning validation of multiproduct equipment. If it can be demonstrated that the product becomes pharmacologically inactive during cleaning, there is limited value in verifying removal of the active ingredient. Instead, it is more appropriate to demonstrate that the inactivated product has been removed below a predefined acceptance limit. This is consistent with the expectation that the carryover of an extrinsic impurity into a subsequent batch should be justified from the standpoint of the safety and efficacy of the product. It also obviates the need to develop PSIAs for cleaning validation.

Biopharmaceutical cleaning cycles are generally designed to expose product contact equipment to extremes of pH (<2 and >13) and temperature (60-80°C) for several minutes. Under these conditions monoclonal antibodies, therapeutic proteins, and other biopharmaceuticals are known to degrade and denature rapidly, and are therefore likely to become pharmacologically inactive (6, 7). The product inactivation approach should therefore be considered for biopharmaceutical cleaning validation.

**GUIDANCE FOR DESIGNING INACTIVATION STUDIES**

Fragmentation and inactivation of an API during cleaning can be assessed by exposing the process soil to worst-case cleaning conditions at bench scale (10, 11). The results of the bench-scale studies can justifiably be extrapolated to the full-scale cleaning process. That is because under worst-case cleaning conditions of laminar flow and low shear rate fragmentation and inactivation are independent of scale (i.e., they depend on cleaning parameters that are not a function of the spatial coordinates of the system, such as time, temperature, concentration, and the ratio of cleaning solution to process soil).

The bench-scale experiments are typically performed in a vial or dialysis cassette, and are designed to simulate full-scale cleaning conditions that are least conducive (worst-case) for inactivation. For example, for a chemical wash, the lowest applicable concentration of cleaning agent, temperature, duration, and ratio of cleaning solution to residual process soil should be considered in simulating the cleaning cycle at bench scale. Other operating parameters that can contribute to product inactivation include dirty hold time (DHT) and associated drying conditions (humidity and air circulation rate), and shear rate due to impingement and turbulence.

An operating parameter or step can be eliminated from the experimental design if its elimination represents a worst-case scenario from the standpoint of inactivation. This approach can be leveraged to simplify the bench-scale studies. For instance, if it is reasonable to assume that product inactivation increases with shear rate, then it can be eliminated from the experimental design (i.e., the shear rate need not be simulated in the experiment). Similarly, the ratio of cleaning solution to process soil can be reduced, and the acid wash and rinse steps can be eliminated to minimize dilution of the process soil, and facilitate detection of the process residue in the sample. When making such changes, unexpected effects such as aggregation of the API can occur. It is therefore important to make sure that the modifications do not result in experimental artifacts.

If the cleaning cycles are being developed or modified, the inactivation study should be designed to evaluate the effect of key operating parameters on the fragmentation and inactivation rate of the API. This information, together with data from cleanability studies (12, 13), can be used to identify cycle parameters that are effective in inactivating the API.

For existing cleaning cycles, the cleaning conditions for the inactivation study should be based on worst-case operating parameters for all systems involved. For instance, if different systems are cleaned with different cleaning solutions and at different temperatures, then the study should be performed with the mildest cleaning solution, at the lowest cleaning agent concentration, and the lowest temperature, if these conditions are least conducive for inactivation. Further, for clean-in-place (CIP) systems with multiple toggle paths,