

Cleaning Memo for November 2019

Issues in Rinsing – Part 1

In this Cleaning Memo and in next month's, we'll cover issues in the rinse process itself, in rinse sampling, in limits for rinse sampling, and finally in rinse recovery studies. However, before we get to those issues, we'll first need to cover what actually (or hopefully) happens in the washing step of a cleaning process (note that I am making a distinction between the washing step, which typically involves use of cleaning agent such as a detergent, and the rinsing step, which generally involves use of only a solvent such as water). So, for purposes of this discussion *cleaning* process is composed of a *washing* step and a *rinsing* step. Note that a cleaning process may also use a "pre-rinse", which is typically just a solvent (such as water) to physically remove as much "soil" (that is the product you are trying to clean from the vessel) as practical by a mainly physical process. Note further that if you are just cleaning with water alone, it may be more difficult to distinguish what the "break" (or dividing line) might be between the washing step and the rinsing step.

The objective of the wash step is to get the entire product in the vessel after the pre-rinse dissolved, emulsified or suspended in the washing solution. Generally, we don't design cleaning processes so that we are leaving a significant amount of product still adhered to the equipment surfaces. Why is that the case? Think of it this way: if I leave a significant amount of product on the equipment surfaces, why am I expecting the rinsing process to remove that product from the equipment surfaces? If the rinse process was that effective, I would just use the rinse itself (for example, water alone) as both my wash solution and my rinse solution. [Okay, I know chemically it is not a "solution", but I think you know my intention]. So, in the ideal world, I am using the washing solution to remove everything from the equipment surfaces. If I am not doing that, then I should consider changing my washing solution or my washing solution parameters to make the washing step more effective. For example, I might use a higher wash temperature, or a longer wash time, or a higher concentration of detergent, or a different detergent. What I think I am trying to convince you (and myself) of is that I want the wash solution to "capture" in some way all product left on the surface. (I use the term "capture" to include dissolving, emulsifying, and suspending in the washing solution).

You might ask, how can I determine that occurs except by rinsing the equipment and sampling the surfaces? If that is required, then I am not looking at just the effectiveness of the washing step, but the effectiveness of both the washing step and the rinsing step *in combination*. One way to address only the washing step is to monitor the concentration of the active (assuming my target residue is the active) in the wash solution as a function of time. This may be possible in a recirculating CIP process using a spray device or in a "fill and dump" process (where there are not spray devices). What I do is sample the wash solution as a function of time, and then measure at each time interval something that is an indication of the degree of removal of product from the equipment surfaces into the washing solution. For a small molecule active, this might be a simple UV measurement. Yes, it might pick up UV absorbance from the excipients, but that *may* not matter. What I am looking for is an increase in UV absorbance in the wash solution as a function of time

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until that absorbance levels off, at which point I am no longer removing product from the surfaces (having captured it in the wash solution). I know you are probably thinking that the reliability of UV absorbance in an emulsified or suspended wash solution might not be so great. In that case, you might have to consider some modification of the sample taken (such as extraction with a solvent) to get a reliable and consistent UV readout. For a biotech active, it might be use of TOC as a measurement technique. One could expect the TOC value in the wash solution to increase, and then eventually level off when all the organic materials are removed from the surface and captured in the wash solution. Realize in such tests, you may have to use an element of creativity to get reliable measurements. Furthermore, realize that in certain situations, such as in some manual cleaning processes, this technique may not be very practical or very reliable.

Furthermore, realize there may be situations where determining that the measured value in the wash solution *appears* to level off, but is not really an indication that all surfaces are acceptably clean. One case is where there is a deadleg in the equipment. In this situation, you might remove 99.99% of the product, and the response curve appears to be leveling off, but due to the normal analytical method variability, you are still very slowly increasing in the response. Another case is where there may two different chemical species with significantly different kinetics of solubility in the wash solution. In that situation, you would see an initial leveling off of the response. If you stopped there, you would miss the later jump in response due to the slower dissolving species. It may be the case that when you perform rinsing and examine the equipment either visually or analytically, it would be obvious that something else is going on and that a different approach is needed. I guess that's why we call these *experiments!!!*

The point of discussing the washing process is to set the stage for talking about the rinsing process. If what I say about the wash process is true, and that ideally the wash solution has *captured* the entire product on the surfaces, what is going on in the rinse process? For this discussion I could use the example of either a CIP process or a "fill and dump" process. In either case, what is happening is that I am adding more rinse solvent to dilute out the concentration of active (or product) in the wash solution to bring it eventually to an acceptable level. Furthermore, what is left on the equipment is essentially dependent on the amount of final rinse solution left in the equipment and the concentration the target residue in that final rinse solution. Remember, this is based on the ideal situation of all product being captured in the wash solution.

There is another hitch, however. There may be situations where as rinsing continues, I achieve a state where the residue (product) previously captured by the wash solution is no longer still captured. For example this may happen in cases where the primary cleaning mechanism in the wash step is emulsification. At high concentrations of surfactants in the wash solution, the target residue is trapped in micelles. As rinsing continues and the "available" surfactant concentration drops, the emulsifying ability of the surfactant *may* be lost, and the target residue is no longer emulsified (and therefore no longer captured in the rinse solution). To balance this, it *may* be the case that at very low concentrations the target residue may now be below its solubility limit; the result may be that the residue is now captured by a *dissolving* process. Another example might be a case where solubility

of the target residue depends on the presence of an alkali in the wash solution (here I'm talking not about alkaline solutions that degrade the target molecule, but rather a situation where the molecule is intact, but may be more readily soluble at a higher pH). In this situation, as rinsing occurs, it may be the case that as the alkalinity drops, the solubility of the molecule changes significantly. So, absent a significant drop in concentration below the solubility limit at that pH, the target species drops out of solution. As with the previous example of loss of emulsification, it may be that the resulting concentration is below the solubility limit and will continue to be rinsed appropriately.

So, how does this discussion affect limits for *rinse* sampling? That takes us to the discussion of taking a sample of the final process rinse as compared to performing a separate sampling rinse following completion of the process rinse (see the Cleaning Memo of September 2002 for earlier consideration of this issue). The important point is this. If what was earlier said about the washing step and the rinsing step is true, then what is measured in the final portion of a "fill and dump" process rinse is a direct measure of the residue present on equipment surfaces at the *beginning* of that final "fill and dump" process rinse. Furthermore, what is measured in the *final* portion of a CIP process rinse is likely to be a much lower value as compared to the residue present in the equipment at the *beginning* of that final CIP process rinse. Since what we really want in either situation is a measure of what might be left on equipment surfaces *after completion of the process rinse*, taking rinse samples of the final process rinse represents a worst case.

On the other hand, if we truly want an "accurate" measurement by a rinse procedure of residues on the equipment surfaces *after completion of the process rinse*, a separate sampling rinse (SSR) is preferred. In a SSR, a fixed volume of rinse solution is contacted or passed through the equipment, and the *entire* volume captured to provide a more accurate measurement. In the case of a "fill and dump" rinse, the vessel is filled with rinse solution, agitated for a fixed time, and then any portion of that total volume is analyzed for the presence of residue. For a CIP rinse, a fixed volume of rinse solution is passed through the equipment and the *entire volume* is collected and then agitated for uniformity. A small sample of that uniform solution may then be analyzed for the target residue. The difficulty of a separate collection vessel for the CIP sampling rinse may be avoided by allowing the separate sampling rinse to collect in the bottom of the vessel, agitating it using appropriate means, and then taking a sub-sample for analysis.

Note that this consideration of washing and rinsing also impacts swab limits. If residues on equipment surfaces at the end of the process rinse are directly related to the amount of rinse solution left in the equipment at the end of the final process rinsing step, then more efficient rinsing of equipment should result in lower carryover. Furthermore, areas where rinse solution can accumulate and dry are more likely to have higher levels of residue than areas where the rinse solution has effectively drained from equipment surfaces. This suggests that locations where rinse solution can accumulate after the process rinse are candidates for worst case swab sampling locations. I realize that in one sense this might appear to fly in the face of the conventional wisdom that worst case swab locations are those locations based on geometry and design that are more likely to have higher amounts of residues (for example, at stainless steel/gasket junctures) or more difficult to clean

residue (for example, at air-liquid interfaces). Rather this analysis suggests that the more important role for these worst case swab locations is that they should be the locations that drive the *design* of the cleaning process itself. Furthermore, I am not suggesting that these swab sampling locations be discarded. They are still important in confirming that our cleaning process design was, in fact, successful.

If this is an accurate description of what happens in a cleaning process, then the value of designing a washing process that effectively captures all the product provides more assurance that residue levels can be consistently achieved both in validation protocols and in subsequent routine production.

Next month we will continue along these same lines and discuss rinse sampling recovery studies.