

November 2014 White Rings in Buffer Tanks

It isn't often that I hear a cleaning validation presentation or read a cleaning validation article and say "Wow, I really learned something new." However, that was the case in a recent presentation by Jenna Carlson of Genentech at an IVT conference in San Diego. The title of the presentation was "Cleaning and Passivation Program for Buffer Prep Tanks". It dealt with two topics for buffer tanks for biotechnology manufacture. One topic was that of rouging, derouging and passivation. The second half dealt with "white rings" in buffer tanks. It is this second part that I found fascinating. In this Cleaning Memo I will explore the issues related to "white rings in buffer tanks".

A fairly common problem in the manufacture of buffers (which are primarily inorganic salts and/or water soluble organics) is the formation of a white ring in the tank used to manufacture the buffer. This white ring appears on the tank walls and/or on the agitator shaft at the air/liquid interface. It does not necessarily appear after manufacture of one batch. It might take multiple batches before the white ring was clearly visible to the unaided eye. Furthermore, it was not just one buffer formulation that results in these white rings, but it can happen to varying degrees with a variety of buffers.

A common industry practice is to clean these buffer tanks with just hot Water for Injection (WFI). The rationale for that cleaning approach is that these buffers all contain only water soluble materials, so the buffer materials should be readily removed. But, there must be some other material (not water insoluble) that is somehow present in the formulation mix for these white rings to form.

Those other materials are in many (if not all) cases a combination of various slip agents used for manufacture of the polyolefin (most likely polyethylene) film used in bags in which the buffer raw materials were received. Somehow, those slip agents were transferring to the product in the bags. Then when added to a tank for buffer manufacture, the slip agents, which are insoluble in water, float to the top and attach to the tank walls and the agitator shaft. In one sense these residues could be considered extrinsic residues, since they don't represent manufacturing materials deliberately added to the equipment; manufacturing materials in this case would be buffer components, water, and cleaning agent (if used).

The white material is generally identified as one of several slip agents used in the manufacture of the polyolefin film component of the bag that the buffer component is packaged in. Those slip agents are commonly fatty acid amides, such as erucamide, oleamide and stearamide. Furthermore, even though these materials are found on the buffer tank, there is no evidence that they carry forward to subsequent process steps (which makes sense because those fatty amides are not water soluble).

You might ask what a slip agent is. A slip agent is something added to plastics prior to film formation to allow two pieces of polyolefin film to slip over each other during processing. The slip agents also serve as anti-blocking agents; blocking is the phenomenon in which two plastics will stick to one another and not easily separate. Both of these phenomena are related to frictional forces. The slip agents are added to the plastic, and then after film formation the slip agent "blooms", which means they tend to migrate to the surface (which is where you want to overcome the frictional forces so that films slide over one another and don't stick to one another). The polyolefin film is then a liner in a bag which contains the individual buffer component. Apparently, during shipping, handling and storage, the slip agent on the surface of the polyolefin film transfers to the material inside the bag. And the rest is history (as they say).

The most common slip agents for polyethylene are erucamide and oleamide. Stearamide is also used, but less often compared to the other two. Sometimes erucamide and oleamide are used in combination, because they have slightly different properties in terms of how fast the material “blooms” and offers protection.

Both erucamide and oleamide are unsaturated fatty amides, with melting points around 70-80°C. Stearamide is a saturated fatty amide, with a much higher melting point. All such amides are subject to alkaline or acidic hydrolysis, but the resulting fatty acids are not water soluble at a neutral pH and at ambient temperature.

It appears that the most effective cleaning solutions for preventing white rings is the use of a formulated alkaline detergent, used either alone or followed by an acidic detergent, at a temperature of at least 70°C. Cleaning with just NaOH at elevated temperature is generally not effective. I hesitate to give a time or detergent concentration level, because the amount of slip agent that could possibly transfer from the bags to the buffer component and then to the equipment surfaces will not be the same in all situations. For example, more concentrated buffers are more likely to provide the potential for greater deposition of white rings. Larger batch sizes are also more likely to have greater potential for transfer because of the batch size to surface area ratio (although this could be modified by considering the batch size to tank circumferences ratio, since it is at the air liquid ration where white rings form). But, also take into consideration that different buffer components (and/or different lots of a given buffer component) may have different propensities to leave white rings because of how much slip agent was transferred from the polyolefin surface to the buffer component.

Which brings me to the place where I can start speculating about how formulated detergents are effective in preventing the deposition of the white rings (which presumably are the fatty acid amides or the fatty acids themselves). There are at least two possible mechanisms.

One mechanism is that as the temperature approaches the melting point of the fatty amide, it can be liquefied and then emulsified by the alkaline detergent.

A second is that at a high (or low) pH and elevated temperature, the fatty amide is hydrolyzed off the surface, with a fatty acid being the result of the hydrolysis. Of course, these fatty acids are not soluble in water at a neutral pH. However, in an alkaline solution, it is the salt of the fatty acid which forms, and depending on the alkalinity source and temperature, that salt may be soluble. Other things being equal, KOH as an alkalinity source is preferred over NaOH because as a general principle, potassium salts are more soluble than sodium salts. Now it may also be the case that the fatty acid salt that is formed is still insoluble under the cleaning conditions, and that what is happening is that the fatty acid salt is being emulsified by the surfactant in the formulated detergent.

Of course, it might be that both mechanisms are in play. There certainly could be some relatively straightforward experiments to check out these theories. For example, add a fatty amide to either KOH or NaOH at 70-80°C, and after a given exposure time, determine whether the fatty amide has “dissolved” or been emulsified, and analyze the resultant mixture to see the extent of hydrolysis of the fatty amide.

Another experiment would be to do the same thing with just the fatty acids themselves, with observation of the extent of dissolution or emulsification (or possibly precipitation as the sodium or potassium salt).

One additional study might be to evaluate the effect of an acid detergent alone as compared to an alkaline detergent alone. I’m sure that those involved in this can envision further experiments to better understand what is occurring in the cleaning process.

As a final note, let me reiterate that there may be (and probably will be) a difference between what cleaning procedure is necessary to remove a buildup of fatty amides in a buffer tank, and what cleaning procedure is necessary to prevent a buildup of fatty amides in a buffer tank. Clearly, it is likely that alkaline detergents will be effective in both situations. However, one can expect that cleaning for a longer time and/or at a higher detergent concentration will be required for the situation where removal of a white ring is the issue (as compared to prevention of the formation of the white rings). However, it clearly should be the long-term objective to have a cleaning process that prevents deposition of the white rings.