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Limits for Bulk Biotech Manufacture – Part 1

A common issue in the manufacture of the bulk active in biotech manufacturing is setting of appropriate limits for the active (which for purposes of this Cleaning Memo we will call the "protein"). Can limits be set based on the standard maximum allowable carryover (MAC or MACO) calculation? After all, all you need to calculate the limit per surface area is the dose of the two actives (the one cleaned and the one subsequently manufactured), the batch size of the next bulk active, and the shared surface area of the manufacturing equipment train. Right? And the answer is, "Yes, you can calculate limits based on MAC principles". The next question is "Is that limit useful?" And for that question, the answer is generally "No". The reason it is not useful is that the result of the MAC calculation usually results in a value significantly below 0.01 $\mu g/cm^2$ of protein. If we could use a specific analytical technique (such as ELISA) to measure this protein, we could certainly detect surface levels of 0.01 $\mu g/cm^2$ and below. However, that assumes that the residue left on surfaces after the cleaning process is the protein itself. In most situations (where cleaning is done with hot, aqueous alkaline cleaning agents), the residue left on surfaces is degraded protein. What is left is not the protein, but rather protein fragments. Those protein fragments are not typically measured by a specific ELISA procedure for the native protein. One way to view this is to say that in this case, the ELISA method is not a "clean indicating" analytical technique. Even if the cleaning process were performed with no rinsing (thus leaving gross amounts of soil), the ELISA method specific for the native protein would not be able to detect such a cleaning failure.

For those reasons, in bulk biotech the measure of the protein is performed by a non-specific method, which can measure those protein fragments. Most commonly that non-specific method is total organic carbon (TOC), although some companies use total protein. For this discussion, we will focus on TOC, but the principles would also apply if total protein were used. Why use a method like TOC? One reason is that TOC is a good measure of the overall cleanliness of the equipment. It measures native protein (if it survives the cleaning process) and degraded protein, as well as other organic components of media, buffers, and/or cell materials.

The next question is "Okay, let's use TOC. Can't we still set limits based on MAC calculations and measure residues by TOC?" The answer again is that we can set limits that way, but we will not be able to measure the residues using TOC at the calculated limit. Let's assume that the MAC calculation results in a value as high as 0.01 $\mu g/cm^2$, and that we sample 100 cm^2 , and desorb it into 20 mL of water. Assuming that the protein contains 50% carbon, the concentration limit of TOC in the water sample would be 0.025 ppm (25 ppb). For cleaning validation purposes, TOC instruments cannot quantify at levels of 25 ppb; that level is essentially background noise. (A typical quantitation level is closer to 200 ppb TOC).

One option is to say something like this: "Well, only a percentage of the measured carbon is due to the protein (including protein fragments) itself; other organic components such as stabilizers will also contribute to TOC of the bulk active. Can I adjust my calculated MAC by dividing that limit by the percentage (expressed as a decimal) of the TOC in the bulk active due to the protein itself?" If the 25 ppb level is a realistic level, then my bulk active would have to have a relative percentage concentration due to the protein itself of around 10% (that is 10% of the carbon is due to the protein and 90% is due to other organics) in order to have a "corrected" limit of around 250 ppb. That probably is an unreasonably low relative carbon contribution for the protein itself. However, such a correction has not been allowed under the "rules" of using TOC. The FDA's "Q&A on

cGMP" (updated May 18, 2005) states that one of the "requirements" for using TOC is that "any detected carbon is to be attributed to the target compound(s) for comparing with the established limit." Therefore such an upward adjustment of the TOC limit is not permitted.

Since a carryover calculation is not feasible for bulk biotech manufacture, the approach used is typically based on setting limits on any analytical sample (rinse or swab) based on "industry standard practice". This is essentially setting limits based on the capability of the process (but not on a true process capability study). The "industry standard practice" is limits of about 5-10 ppm TOC for all equipment up to the first purification step, and a limit of about 1 ppm TOC for subsequent equipment Note there are some exceptions to the last rule, for items such as UF skids where higher TOC limits may be allowed because of capabilities, but these exceptions are outside the scope of this Cleaning Memo). Note that this is not an allowance of 1 ppm (or 5-10 ppm) in the next product, but is based on setting those limits for any analytical sample.

There are additional things to consider in justifying these limits, which will be covered in next month's Cleaning Memo.