DESIGN OF A CLEANING MONITORING METHODOLOGY FOR A CLEAN IN PLACE (CIP) FLUID BED DRYER (FBD) USING "RAPID TESTING" APPLICATIONS

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ABSTRACT

Over the past years, rapid testing technologies have been developed for the purpose of changing the way microbiology and chemical analysis is done. Rapid testing technologies are a valuable tool for the pharmaceutical industry to monitor process efficiency in significantly less time than typical technologies. Manufacturing equipments cleaning is a process that can be monitored using rapid testing technologies. The high capital costs and product demands require that the equipment be reused in a continuous way. Reducing downtime of the laboratory evaluations for equipment release brings substantial cost improvements for the manufacturing activities. This article describes a cleaning monitoring methodology for a CIP-FBD that incorporates rapid testing technologies for the verification of the cleaning process efficiency. Based on the proposed design, it is shown the possibility to configure a cleaning monitoring methodology for the CIP-FBD consisting of an assembly that allows for a rapid evaluation of the cleaning process.

BACKGROUND

Cleaning is performed within a manufacturing industry to protect product integrity, reuse equipment, and to comply with regulatory requirements for equipment cleaning. Cleaning validation, cleaning verification, and cleaning monitoring are methods to show that an equipment cleaning process is performed adequately so as not to affect the safety or efficacy of the next drug product manufactured in the cleaned equipment [13]. The cleaning validation involves setting acceptance criteria for at least three consecutive cleaning runs. Cleaning verification involves setting acceptance criteria for one specific cleaning event. Cleaning monitoring is implemented as an In-Process control of the cleaning performance, once a cleaning procedure has been validated. The high capital costs and the product demands require that the equipment be reused in a continuous way. Reducing downtime of the laboratory evaluations for equipment release after a cleaning process brings substantial cost improvements for the manufacturing activities. Good manufacturing practices (GMP) require that the manufacturing equipment be cleaned to certain low levels specifications. After performance of a cleaning process, samples (swabs or rinse) are taken from the equipment surfaces for the analysis of residues of drug product (Active Pharmaceutical Ingredients, API), detergent, and microbial bioburden, to evaluate cleaning process effectiveness. Chemical testing is performed for the analysis of API and/or detergent residues. The microbiological analysis evaluates the presence of bioburden (colony forming units).

It is valuable to establish adequate (efficient and rapid) cleaning monitoring programs that incorporate the use of rapid testing methodologies, to evaluate the cleaning efficiency of manufacturing equipments. Depending only on a visual inspection after a cleaning process as part of the monitoring program can represent a risk for the operation. Visual inspection relies on the visual ability of the operator. Some manufacturing equipments are large and complicated (too many parts), which makes difficult to perform a successful visual

inspection. Equipments used in automated process (FBD, Solution Tanks, Bioreactors), which are not disassembled, the performance of a solely visual inspection is at risk. In addition, insufficient illumination in the manufacturing area or equipment worsens the situation. Sampling after each cleaning event and laboratory testing is time consuming and represent an additional cost to the operation. Laboratory samples analysis includes sample preparation (extractions and or dilutions), analysis, data gathering, calculations, results verifications and report. This can increase the production cycle time and equipment offline period.

This article describes a cleaning monitoring methodology for a CIP-FBD that incorporates rapid testing technologies for the verification of the cleaning process efficiency. The objectives are:

- To design a cleaning verification methodology for a CIP-FBD for determination of the efficacy of the cleaning process in "*Rapid-Real Time*".
- To include in the cleaning verification methodology, applications for the analysis of Active Pharmaceutical Ingredient, Cleaning Agent and Microbial residues from equipment surfaces, using rinse sampling analysis.

The Fluid Bed Dryer

The Fluid Bed Dryer (FBD) is a process equipment that can be used for the manufacture of solid oral dosage formulations [10].

All the internal FBD surfaces are exposed to processing material and depending on the process type (drying, coating, and granulation) material in direct contact with the equipment may accumulate, and residues accumulation is an important issue when developing the equipment cleaning procedure.

Modern FBD have been developed with the required components to allow the implementation of Clean in Place (CIP) technology [8]. The CIP is an automated cleaning system that involves spray devises to distribute the cleaning solution to all process vessel surfaces without disassembly of the equipment.

Traditional Analytical and Microbial Testing Applications for Cleaning Sample

The most common techniques currently used for the chemical analysis of cleaning samples are: The High Performance Liquid Chromatography (HPLC), the Ultraviolet Visible Spectroscopy (UV/VIS) and the Total Organic Carbon (TOC). These methodologies are efficient for the analysis of rinse and swab samples.

Although these techniques are currently used in the manufacturing industry, such techniques have limitations. The disadvantage of HPLC and UV is that a new analytical method must be developed and validated for every new product manufactured. In addition, HPLC analysis must be performed in a relatively short time period to avoid sample degradation and appearance of unknown peaks in the chromatogram. For the HPLC a single measurement can take up to 60 minutes, thus laboratory time associated with the measurement of several samples and reporting can take few days. The UV/VIS is a tool acceptable for products having chromophores (majority of surfactants). In the case of TOC, the sample measured by this technique must be soluble in water (no organic solvents).

Classical microbiological test methods are divided in three categories: presence or absence of microorganism, enumeration of microorganisms, and identification [14]. Such methods are old methodologies, labor intense, and time consuming. The microbiological analysis includes the Screening of Indicators Organisms and the Quantitation by Pour Plate or Membrane Filtration Method [4]. To have an idea of the time consuming process, for example, the Screening of Indicators Organisms involve: dilution of sample in broth, incubation at specified temperature for a predetermined period of time (for example: 30-35°C, 24-48 hours), sub-culturing onto selective agars, incubation for a pre-determined period of time, assessing for characteristic growth, and further identification of suspected colonies; for a delay of about of 4 to 7 days.

ANALYTICAL AND MICROBIAL RAPID TESTING TECHNIQUES FOR CLEANING MONITORING

New analytical and microbial techniques for rapid testing have been developed. Some these techniques can be integrated within a cleaning process to determine in rapid time the effectiveness of a cleaning procedure.

The analytical techniques developed for rapid testing includes:

Middle-Infrared (mid-IR) Grazing-Angle Fiber Optics Probe: a technique based on Furier Transform Infrared Spectroscopy (FTIR) in the middle infrared (mid-IR) range using reflection at a grazing angle and can be used to detect small amounts of analytes from the equipment surfaces during cleaning verification [18]. A specially configured probe head illuminates the sample surface by an IR beam and maximize the distance traveled through the surface by the IR beam before it returns to the detector. The grazing-angle head uses carefully aligned mirrors to deliver the mid-IR beam to the sample surface at the grazing angle to collect the reflected beam and to return it to the mid-IR detector.

Ion Mobility Spectroscopy (IMS) and Ion Trap Mobility Spectroscopy (ITMS): Are used in trace determinations (narcotics and explosives) in military and security application. These techniques are ideally suited for use in cleaning verification and, in comparison to HPLC, offer advantage [15]. Swabs or rinse samples can be used. In *IMS* the analyte is ionized and the ions are gated into a drift tube and accelerated by an electric field to the detector. Ions drift depend mass, size, and shape of the analyte. The specificity of the *IMS* technique is based on the movement of the ions under the influence of the electric field, what identifies the original substance. *ITMS* differs in the use of "ion trap mechanism", which consist in the "build up" of the ions in a trapped area and then be periodically released into drift tube for measurement. These techniques are suitable to detect small molecules, reduce samples analysis downtime, and do not require the use of columns or mobile phase, when compared with HPLC. With these technique results can be obtained in less than an hour.

Remote Visual Inspection: work by placing an ultraviolet light inside a vessel after it's cleaning and adding riboflavin solution prior to vessel rinse. Riboflavin glows in places in were it wasn't rinsed off thus an indicator of cleaning efficiency [2].

HPLC with charged aerosol detection (CAD): Virtually universal (except for volatiles) in its detection properties and with a response independent of chemical structure, CAD can provide a level of information regarding cleaning samples that has until now been lacking with methods such as HPLC, UV and even TOC [5].

On-Line TOC: Oxidation of the sample and measure the CO_2 generated by using: Non dispersive infrared (NDIR), Direct Conductometric, and Membrane Conductometric Detection. The measured CO_2 is proportional to the amount of species in the sample (API or Detergent).

The rapid microbiological methods rely on microbial viability and growth. A summary of some of the available microbiological techniques for rapid testing is as follows.

VITEK System: Automated identification and susceptibility system based on microbial utilization of substrates and enzymatic reactions. Pure culture suspensions are tested with a series of biochemical substrates [4]. Uses disposable test cards containing the biochemical substrate to react with the sample and produce the microbial identification.

MIDI System: Analyze the composition of the fatty acids in the cell wall of an organism for microbial identification. Using pattern recognition, this profile is compared to the fatty acids present in the database, producing a microbial identification [4]. Use gas chromatography for detection and comparison of chromatographic profile.

Biolog System: Based on Redox chemistry to perform carbon utilization tests for bacteria and yeast [4]. Microorganisms identification based on their respiration.

RiboPrinter: Microbial Characterization. Molecular typing system that produces genetic

fingerprints based on microorganism's ribosomal RNA genes in about 8 hours [4] [14].

Bioluminescence: Microorganisms growth based technology (Adenosine Tri-Phosphate [ATP] Bioluminescence). ATP is present in all living cells. Under certain conditions, when ATP combines with Luciferase enzyme, it is converted to a photon having a yellow green color [14]. The luciferase hydrolyzes the ATP to AMP (Adenosine Monophosphate), and the energy is released as light. The amount of light or bioluminescence produced can be measured by sensitive luminometers and is proportional to the amount of ATP in the sample. The emitted light is usually expressed as relative light units (RLU) rather than as direct estimates of microbial numbers. This principle of bioluminescence can be used to detect microbial contamination by using correlations between the RLU readings and the approximate number of organisms (Colony Forming Units). Used to detect the presence or absence of microorganisms.

The VIDAS: (Vitek ImmunoDiagnostic Assay System) is a fully automated enzyme-linked fluorescent assay (ELFA) technology [4] [14]. Assay based on antigen-antibody technology, with specific reactions that occur between antibodies and their corresponding antigenic site. The VIDAS is used for pathogen screening of *Salmonella, Listeria, Escherichia coli, Listeria monocytogenes, Campylobacter*, and Staphylococcal enterotoxins A-E. The end result is a fluorescent product, and the VIDAS reader measures the amount of fluorescence generated by such final result.

METHODOLOGY

The methodology used consisted of an observational research including prospective, retrospective, and cross-sectional studies. Throughout the research study the following analysis were performed: (1) Searched analytical applications for the rapid testing of chemical traces from cleaning procedures, and microbial analysis techniques that permit the microbial rapid testing of rinse samples; (2) Compared the techniques advantages and disadvantages and selected the most suitable rapid testing applications; (3) Evaluated the FBD physical components/parts necessary to integrate the testing applications and design the methodology assembly; (4) Guidance development for application implementation; and (5) Evaluation of the Qualification and Validation Requirements.

DESIGN RESULTS

FBD-CIP Overview. The FBD contact parts includes the: Product Bowl (contains the product), Expansion Chamber (Transition between the product bowl and the filters), and Inlet Plenum (where air enters the equipment).

For cleaning purposes a skid is used for controlling the sequence of operation where it uses city water, cleaning agent, and purified water. The basic CIP cycle sequence consists of:

- First Cleaning Step of the Cycle: involves a wash down of the product contact surfaces and filters of the FBD. It can be performed by using hot water or cold water (purified or tap water).
- Second Cleaning Step of the Cycle: includes Chemical Cleaning and Sanitation (Alkaline and/or Acidic agents).
- Third Cleaning Step of the Cycle: includes a rinse with Purified Water to remove most cleaning/sanitizing agents and contaminants.
- Fourth Cleaning Step of the Cycle: is the final rinse step. This rinse removes last traces of residuals and can be used for rinse sampling after completion of the cycle.

The rapid testing applications selected for this design are: On line TOC analyzer and Bioluminiscence (ATP assay) using a Luminometer.

The On line TOC analyzer will monitor the effectiveness of the CIP system in removing the product and the cleaning agent.

The Bioluminiscence ATP assay will be used within the same room as the CIP FBD, but not on line. The ATP assay will monitor the presence or absence of microorganisms (Colony Forming Units, CFU).

The rinse sample for the cleaning monitoring will be taken from the FBD inlet plenum drain. The rinse sample taken from the plenum drain is representative for all product contact parts, since this water has traveled from the upper part of the equipment toward the lower part of the equipment, covering all surfaces. The plenum drain is in the lowest part of the equipment. This design requires High Purity Water for the TOC analysis (<500 ppb TOC).

On-Line TOC Analyzer: The TOC analyzer selected in this design is a *Sievers® 500 RL On-Line TOC Analyzer*. This equipment uses membrane conductometric detection methodology. The Sievers technology utilizes a gas-permeable membrane that selectively passes only the CO_2 produced from the oxidation of organics. By preventing acids, bases and halogenated compounds from interfering with the measurement of CO_2 from oxidation, the membrane conductometric method delivers unmatched selectivity, sensitivity, stability, accuracy, and precision [7]. The *Sievers® 500 RL On-Line TOC Analyzer* is connected to the FBD by means of a return CIP line, refer to Figure 1 and Figure 3. The 500 RL receives rinse samples from the last rinse of the cleaning cycle automatically and perform an analysis of TOC and conductivity.

Bioluminiscence ATP Assay: For the ATP assay a luminometer was selected for samples analysis. Although not on-line, this technique is classified as a rapid testing application due to simplified methodology it uses for microbial monitoring. For this design, the luminometer is incorporated within the same room as the FBD and the 500 RL. A sample from the last rinse of the cleaning cycle is taken from the FBD inlet plenum drain and pre-treated in a luciferase reaction for the analysis of the ATP. Measurement of the light intensity using a luminometer permits direct quantitation of ATP. The bioluminiscence ATP assay uses recombinant luciferase to catalyze the following reaction:

$$ATP + D\text{-}Luciferin + O_2 \rightarrow Oxyluciferin + AMP + PPi + CO_2 + Light\ (560nm)$$

From the above reaction, the intensity of the emitted light is proportional to the concentration of ATP. With a Luminometer the emitted light is expressed as relative light units (RLU). This principle of bioluminescence is used to detect microbial contamination by performing correlations between the RLU readings and the approximate number of organisms. Standard curves are used to translate the raw RLU data to more meaningful organism-quantitation data by correlating the RLU with the Colony Forming Units (CFU).

The luminometer selected for this design is a Modulus™ Single Tube Luminometer [19]. This equipment accommodates luminescent assays such as ATP measurements, or other bio-and chemiluminescent measurements. The Modulus™ Single Tube Luminometer has a detection limit of 1 x 10^{-20} moles of luciferase or 1 x 10-16 moles ATP.

The Modulus™ Single Tube Luminometer technology is combined with an ENLITEN® ATP assay system bioluminescence detection kit [17] for the sensitive and rapid detection of adenosine 5'-triphosphate (ATP).

DESIGN METHODOLOGY ASSEMBLY

- The automated cleaning process is activated from a CIP remote system.
- Purified Water (PW) supplied from the PW tank enters circulates through the CIP skid piping, and enters the FBD for a wash down of the product contact surfaces and filters of the FBD (brief rinse), then exit through the waste line.
- PW enters the cleaning agent mix tank where appropriate concentrations of alkaline and / or acidic solutions are added. The solution then passes through the CIP skid piping and washes the FBD. After a preset time, valves open and the FBD content exit through the waste line.

- The entire CIP skid piping and equipment are rinsed with PW, removing most cleaning agents and contaminants.
- Prior to final rinse, a rinse is performed using PW to remove last traces of residues.
- A final rinse with PW (Rinse Tank) is performed to monitor cleaning effectiveness. After water exits the FBD.
- After water from final rinse exits the FBD, the PLC/SCADA (Programmable Logic Control/ Supervisory Control and Data Acquisition) system activates the TOC Analyzer from a standby mode. Water passes by the TOC analyzer on the CIP return line and a TOC (and conductivity) measurement is taken, refer to Figure 3. The cycle can be repeated as needed if TOC measurement is unacceptable.
- Once a compliance TOC measurement is obtained a rinse sample is taken from the FBD micro sampling point (Luminometer sampling port), refer to Figure 1 and Figure 2, and processed according to Bioluminiscence ENLITEN® ATP Assay and Luminometer procedure [17]. The operator or designee will take a sample from the last rinse step of the cleaning cycle and analyze it for ATP assay (microbial bioburden). This analysis is designed to be performed within the same room as the FBD.

The cleaning monitoring process flow is summarized as follows:

- Preparation of the rL/L Reagent (incubate for 1 hour at room temperature) - recombinant luciferase to catalyze the ATP reaction
- CIP Cleaning Cycle
- On-line TOC Analysis
- ATP Extraction and Assay in the Luminometer
- Equipment Release

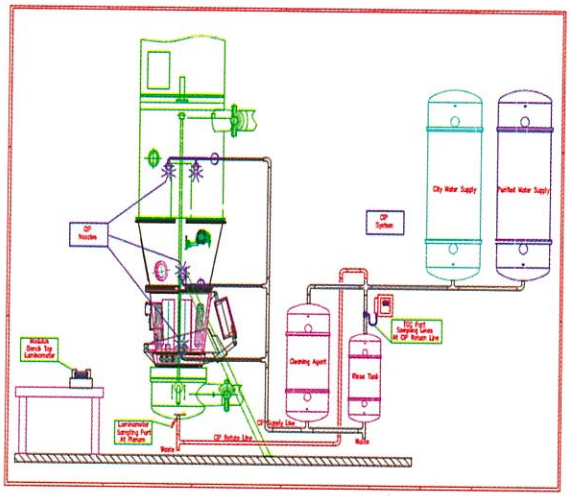

Figure 1: *Design Assembly*

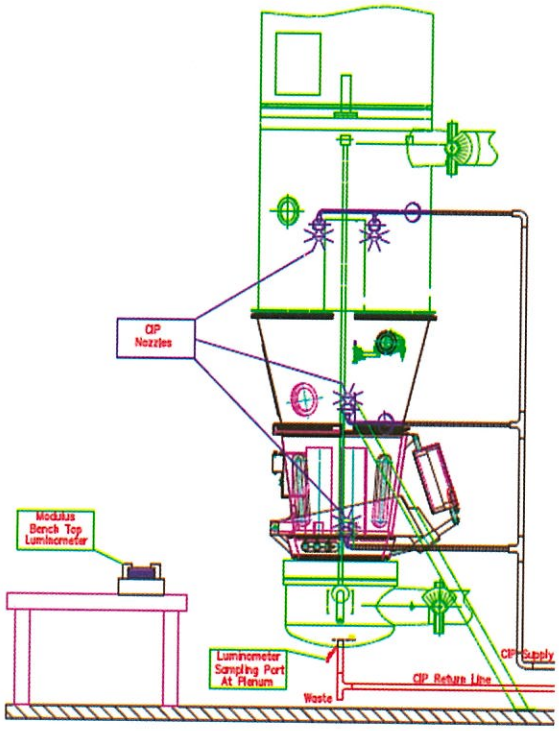

Figure 2: *Expanded Figure Of The Design Assembly Including FBD, CIP Supply Line And Luminometer*

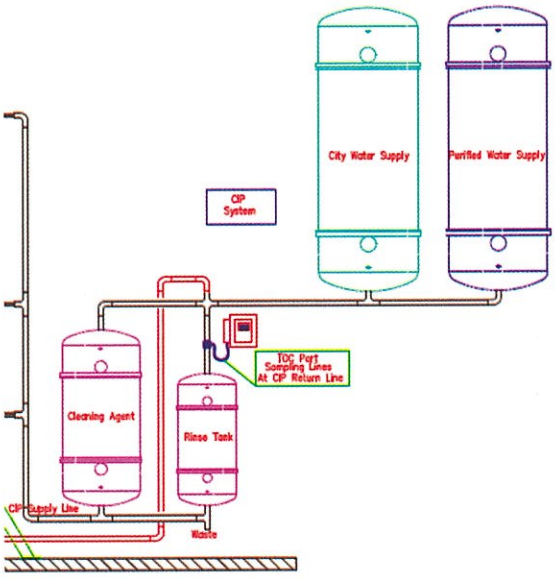

Figure 3: *Expanded Figure Of The Design Assembly Including CIP System Tanks And TOC Sampling Port And Analyzer*

GUIDANCE FOR APPLICATION IMPLEMENTATION

Pre-validation, validation and qualification studies are essentially necessary for this design implementation. All the equipments included in the design (CIP-FBD, TOC Analyzer and Luminometer) shall be subjected to validation/qualification evaluations including: Instrument Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ). The TOC analyzer qualification shall include validation of the automated sampling system.

The CIP-FBD validation must include the validation of the computerized system that controls the clean in place procedure and the validation of the cleaning process itself (concentrations of the cleaning agent, rinse time, cleaning solution flow thru equipment surfaces, cleaning cycles sequence, water quality: Purified Water or In-house Water or Tap Water), water temperature, and cleaning cycles time intervals).

A second non automated analytical technique (such as HPLC) shall be used to compare results obtained by the automated TOC analysis. After completion of equipment use and CIP cleaning direct surfaces sampling is performed to evaluate the cleaning process using a non-automated analytical technique to make correlation and comparison studies with the automated technique.

The CIP-FBD PLC/SCADA system validation must include the communication of the CIP system with the TOC analyzer.

The analytical methods (for direct surface sampling) used for the correlation studies and comparison, and the microbiological methods shall be validated to demonstrate suitability of the applications.

The microbiological rapid testing technique and sample preparation process shall be validated.

Standard curves shall be performed to translate the raw RLU data to more meaningful organism-quantitation data.

Studies need to be conducted to correlate the RLU with the Colony Forming Units (CFU). These studies must be product specific and can be carried out by inoculating the product in question with levels of organisms (such as 10, 100, 500, and 1000 CFU), processing the sample according to the testing protocol and ATP assay procedure, and observing the resulting RLU for a possible correlation with the known level of CFU added. Different type of organisms must be used in such validation studies, such as *Escherichia coli, Pseudonomas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Pseudomonas cepacia, etc.* [4]. For an accurate bioload determination alternate plate counting methods may be needed.

Personnel using the design equipment and instruments shall be trained in the technique to be used and qualified and the personnel performing the micro sampling shall be properly trained in aseptic techniques for micro sampling.

CONCLUSION

Based on the proposed design, it is shown the possibility to configure a cleaning monitoring methodology for the CIP-FBD consisting of a cleaning verification assembly that allows for a rapid (*real time*) evaluation of the efficacy of the cleaning process. The technologies incorporated in the design are consider rapid testing applications

since they are used to perform an analysis of a sample in significantly less time than typical analytical and microbial technologies and are capable of being integrated into a SCADA system. The analysis of cleaning samples and the release of the equipment may be reduced from days to hours by using such design. This cleaning monitoring methodology is designed to perform a rapid evaluation of a cleaning process without compromising products quality and integrity.

The design incorporates the tests necessary to evaluate the effectiveness of a cleaning process while complying with cleaning verification regulatory agencies requirements. The evaluation of the cleaning process effectiveness is performed by testing a rinse sample from the rinse cycle of the FBD CIP process. The tests include monitoring for product (Active Pharmaceutical Ingredient) residues, detergent (Cleaning Agent) residues, and microbial bioburden (colony forming units). The TOC analysis will monitor for residues of API and/or cleaning agent while establishing cleaning process end point. The ATP assay will monitor for microbial colony forming units based on the completion of the cleaning process steps.

All equipments/instruments incorporated in the design shall be qualified to demonstrate system suitability and compliance.

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