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It is very important to biopharmaceutical manufacturing that processing equipment be cleaned using validated procedures to avoid cross contamination from material remaining from a previously manufactured product (1–3). Potential residues remaining on the surfaces of equipment following processing steps (fermentation, for example) include, in addition to the product itself, a multitude of organic cellular components such as nucleic acids, proteins, and carbohydrates.

Together with appropriate surface sampling procedures, analytical techniques that can determine trace levels of

determined trace levels of proteins, nucleic acids, amino acids, sugars, and detergents (9).

In this article we present a procedure for the TOC analysis of swab samples for the cleaning validation of fermentation equipment used in the production of biopharmaceuticals. This procedure is based in part on a method developed previously for the colorimetric determination of total protein in swab samples (10).

Experimental

Materials. Potassium biphthalate and sodium hydroxide were purchased from Sigma (St. Louis, MO) and Mallinckrodt (Paris, KY), respectively. Texwipe Alpha Swab polyester swabs were acquired from Baxter Scientific Products (McGaw Park, IL), and *E. coli* cells were obtained from Eli Lilly and Company (Indianapolis).

It is important to note that the heads of the polyester swabs used in this study were thermally bonded to the handles, eliminating the need for adhesives that can cause contamination during extraction. The swabs were also laundered by the manufacturer to minimize inherent nonvolatile residues or particulates that might otherwise have significantly decreased the sensitivity of the analysis (11).

Equipment. The TOC analyzer used in this study was a TOC-5000 (Shimadzu, Columbia, MD) equipped with a 74-position

Total Organic Carbon Analysis of Swab Samples for the Cleaning Validation of Bioprocess Fermentation Equipment

Validated cleaning procedures are needed to ensure the absence of contaminants from bioprocessing equipment, and these procedures must be supported by appropriate analytical methodology. This article describes the development of a quantitative total organic carbon (TOC) assay for residual carbon-containing materials on stainless steel surfaces using *E. coli* cells as a model substance.

contaminants are required to support the validation of equipment cleaning procedures. Currently, the preferred method of sampling is to directly swab the equipment surface (4). In this approach, the swab head is wetted with an effective agent to facilitate transfer of contaminants from the surface to the swab, a defined area of the equipment surface is swabbed, and contaminants acquired by the swab are extracted into solution before analysis. Analytical methodology is then needed to determine trace levels of contaminants in the presence of both a solubilizing medium and a swab.

Total organic carbon analysis (TOC) is a method that has been used successfully for monitoring water quality (5–7), including the quality of water for injection in pharmaceuticals (8). TOC analysis offers a number of distinct practical advantages over other commonly used residual testing methods such as enzyme-linked immunosorbent assays (ELISA) and protein assays (Lowry, Bradford, and bicinchoninic acid, for example) because of its high sample throughput, lack of interfering substances, and inherent sensitivity. One study demonstrating the potential application of TOC for the analysis of rinse water samples for cleaning validation

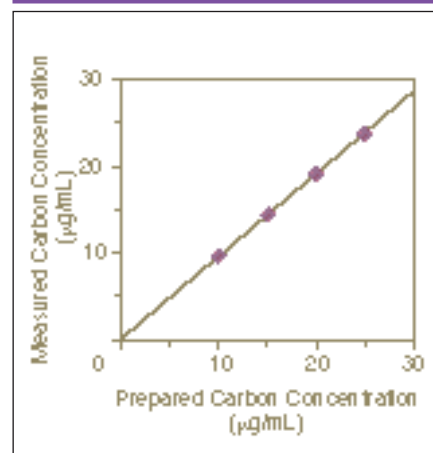


Figure 1. Plot of TOC-measured carbon concentration compared with prepared carbon concentration (in aqueous sucrose solutions) that demonstrates method linearity from 0 to 30 µg/mL carbon.

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autosampler. The TOC-5000 employs acidification of the sample with hydrochloric acid followed by sparging with purified air to remove inorganic carbon from solution as carbon dioxide gas. The organic compounds remaining in solution are then oxidized to carbon dioxide in a combustion tube packed with a catalyst and incubated at 680 °C. The carbon dioxide produced in this manner passes through a dehumidifier and halogen scrubber before it reaches a cell where it is quantified by a nondispersive infrared detector tuned to the highly specific carbonyl absorption frequency in the 1,700-cm⁻¹ range. The signal response was stored in a microprocessor and compared with a calibration curve generated by analysis of solutions of 0, 26.6, and 53.2 ppm potassium biphthalate corresponding to levels of 0, 12.5, and 25 ppm organic carbon, respectively. Between three and five replicate measurements were obtained for each sample so that in all cases, coefficients of variation of less than 2% were obtained for the series of replicate injections.

Methods. Potassium biphthalate standards were made up and diluted in purified water in glass volumetric flasks that had been previously rinsed with 50% nitric acid and copious amounts of purified water to remove any trace of organic contaminants. Purified water was obtained through the use of a Milli-Q water system (Millipore, Bedford, MA). All standards, blanks, spikes, and controls were prepared at least in triplicate.

Stainless steel pans were coated with *E. coli* cells in the following manner. Wet cell paste (1.43 mL), obtained after centrifugation of fermentation broth in a TJ-6 centrifuge (Beckman Instruments, Fullerton, CA) for 20 minutes at 1,000× g, was suspended to volume with purified water in a 100 mL volumetric flask to make a 70×-diluted cell suspension. A 3.825 mL aliquot of well-mixed 70×-diluted cell suspension was then diluted to 4.0 L final volume with purified water, making a 73,200×-diluted cell suspension. A 1.0 L volume of well-mixed 73,200×-diluted cell suspension was transferred to a 25.2 × 50.6-cm stainless steel pan and was then lyophilized to dryness, depositing *E. coli* cells corresponding to approximately 267 µg of wet cell mass per 25 cm² area.

Ten 25 cm² sections of the stainless steel pan surface were sampled with swabs using

ANALYTICAL PERFORMANCE PARAMETERS

Accuracy. The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often be expressed as percent recovery by the assay of known, added amounts of analyte. Accuracy is a measure of the exactness of the analytical method.

Limit of detection. The limit of detection is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. Investigators may measure the magnitude of analytical background response by analyzing a number of blank samples and calculating the standard deviation of this response. The standard deviation multiplied by a factor, usually 3, provides an estimate of the limit of detection.

Limit of quantitation. Limit of quantitation is a parameter of quantitative assays for low levels of compounds. It is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. Investigators may measure the magnitude of analytical background response by analyzing a number of blank samples and

calculating the standard deviation of this response. The standard deviation multiplied by a factor of 10 provides an estimate of the limit of quantitation.

Linearity. The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Linearity is usually expressed in terms of the variance around the slope of the regression line calculated according to an established mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analyte.

Precision. The precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample. The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation).

Source: *United States Pharmacopeia XXII, National Formulary XVII*, pp. 1710–1712 (1990).

the following protocol. The fabric head of a swab was wetted in 1 N sodium hydroxide, and excess liquid was removed by gently pressing the swab head against the walls of the reagent reservoir. 5 cm × 5 cm sections of the pan were swabbed in their entirety, after which the swab stem was cut approximately 1 cm above the swab head, dropping each swab head into a glass 5 mL TOC autosampler vial (Shimadzu) also containing a small stir bar. Before analysis, 1.0 mL of 1 N sodium hydroxide, 3.0 mL of Milli-Q water, and a small stir bar were added to tubes containing swab samples. The samples were then stirred on a stir plate at room temperature for one hour, after which time 1.0 mL of 2 N hydrochloric acid was added before sparging and injection by the instrument.

Swab blanks were prepared in a manner similar to that described above for the preparation of stainless steel pan samples,

with the exception that clean swab heads not exposed to the pan surface were used. For the determination of a limit of detection and a limit of quantitation, a set of 10 swab blanks were prepared and analyzed. Linearity was evaluated by analyzing four levels of sucrose standards in purified water corresponding to concentrations of 0, 10, 20, and 25 µg/mL carbon.

Assay precision was determined by preparing a set of 25 swabs by spiking 100 µL of 233× diluted cell suspension onto the swab heads and then analyzing five spiked swabs per day for five days. To validate recovery, two sets of three levels of diluted cell paste (100 µL volumes of 70×-, 140×-, and 280×-diluted cell paste were aliquoted four times each to sample tubes) were prepared, one set in the presence of swab heads (spikes) and one set in the absence of swab heads (controls).

Table 1. Precision of the analysis of five replicates of swabs spiked with diluted *E. coli* cell paste analyzed on each of five days.

Day No.	Precision (% RSD)
1	6.87
2	2.05
3	2.74
4	7.18
5	5.83
Pooled five-day precision = 7.15% RSD.	

Results and Discussion

We determined the TOC signals generated by the sucrose standards prepared in the absence of swab heads to increase linearly with carbon concentration, with a simple curve fit correlation coefficient of 0.999 (Figure 1). Because the preparation of swab samples required extraction and dilution to a 5.0 mL volume before analysis, the linearity determined for the concentrations of 0–25 µg carbon/mL from the sucrose solutions corresponded to levels of 0–125 µg/swab. In comparison with a standard curve, the 10 swab blanks generated signals equivalent to a mean background of 8.0 µg/swab. All sample determinations discussed in the remainder of this section were corrected for the swab blank background. A limit of detection and limit of quantitation were established at 9.2 µg/swab and 12.1 µg/swab, respectively, for this analysis.

A determination of analysis precision indicated relative standard deviations (RSD) to range between 2.05% and 7.18% within a set of five replicate analyses of swabs spiked with diluted cell paste, as assayed daily for five days (Table 1). Day-to-day precision was determined to equal 7.15% RSD for the

Table 3. Stainless steel pan swab sample recovery determined by the analysis of *E. coli* cell paste deposited on a stainless steel pan. The pan swab signal was compared with that generated by an aqueous suspension of the cell paste and is expressed as percent recovery.

Replicate	TOC Recovered (µg/swab)	% Recovery
1	48.1	74.8
2	60.0	93.3
3	57.4	89.3
4	55.0	85.5
5	59.1	91.9
6	46.1	71.7
7	47.9	74.5
8	44.1	68.6
9	38.2	59.4
10	71.3	110.9

pooled five-day data set.

The results of the evaluation of accuracy using diluted cell paste are presented in Table 2. At the three levels of dilution (70×, 140×, and 280× in purified water), the TOC signals from *E. coli* cell paste were determined to range from approximately 20 to 100 µg/swab, corresponding to 74.9–103% recovery in the presence of swab heads.

Table 3 shows the recoveries from each of the 10 swab samples taken from the surface of the stainless steel pan, which had been coated with a layer of *E. coli* cells. As described above, we calculated that approximately 267 µg of wet cell mass was deposited per 25 cm² area on the pan surface. A direct TOC determination of the 73,200× diluted cell suspension revealed that 267 µg of wet cells corresponded to 64.3 µg of organic carbon, against which number the percent recoveries of the assayed

swab samples were then calculated. As displayed in Table 2, recovery ranged from 59.4% to 110.9% for the set of 10 replicates, with a mean recovery of 81.9%. A lower TOC recovery (67%) was achieved by other researchers who used a swab wetted with dilute phosphoric acid to sample a surface upon which whole cells had been applied (12). A similar degree of high variability of recovery was also encountered in a previous study (10), in which it was believed to reflect limitations associated with precise sampling as well as the limitations in the establishment of a consistent layer of cells on the surface during drying.

General Applications

The method described in this article was developed as a quantitative assay for trace amounts of carbon-containing components that may be present on the stainless steel surfaces

TOC is less labor intensive and offers greater general application than other assays that have been used to support cleaning validation.

of bioprocess equipment. *E. coli* cells, which are present at levels of approximately 10¹³–10¹⁴ cells/L, together with a multitude of soluble carbon-containing components during a typical fermentation, were chosen to represent a “most challenging case” model for this study because of their insoluble particulate nature. In comparison with the fermentor cell concentration mentioned above, the TOC method accuracy was validated by the recovery of levels of carbon corresponding to approximately 10⁹ cells/25 cm² surface area. It is important to note that the method will determine the presence of cellular material in swab samples irrelevant

Table 2. Method accuracy determined by the analysis of diluted *E. coli* cell paste both in the presence (spikes) and absence (controls) of swab heads expressed as percent recovery of spike signal compared with control signal.

Dilution of Cell Paste	Mean Control Signal (µg)	Mean Spike Signal (µg)	Mean % Recovery (5 Replicates)
70×	97.8	100.6	102.9
140×	50.4	44.7	88.7
280×	25.9	19.4	74.9

of the condition of the cells (intact compared with lysed, for example).

TOC is less labor intensive and offers greater general application than other assays that have been used to support cleaning validation. For example, TOC can be used to determine the presence of compounds such as amino acids, nucleic acids, and sugars that are incapable of generating a response in assay methods such as a total protein determination. At Eli Lilly and Co., TOC has been routinely used for the analyses of swabs taken from process equipment exposed to a variety of water-soluble drug substances (such as antibiotics), excipients (such as lactose), and formulated cleaning agents (such as surfactants), and the methods used for these assays have undergone general

validation, with a specific focus on recovery, in a manner similar to that described in this article (13).

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