COMPRESSED GAS MONITORING OF MICROBES: THE CONTAMINATION CONTROL STRATEGY IN PHARMACEUTICAL MANUFACTURING ENVIRONMENTS

Abstract

Compressed gases, such as nitrogen, carbon dioxide, and oxygen, are used for a variety of applications in pharmaceutical manufacturing. These applications, such as aseptic packaging, purging, and filtration, are critical processes monitored for their efficacy. To avoid sampling compressed gases, it has been speculated by some manufacturers that the rapid decompression of a gas when exiting its container kills any microbial contamination. However, it has been shown by multiple studies that microbial survival is not impacted by the typical compression or decompression seen in pharmaceutical process gases.

Many GMP standards recommend sampling process gases for contamination before use in critical areas of manufacturing. This step, along with many others that make up a contamination control strategy, contribute to the purity and quality of the final product.

Introduction

The quality attributes of manufactured pharmaceutical products include the physical, chemical, and microbiological characteristics of the raw materials, excipients, active pharmaceutical ingredients (APIs), and final drug products. Here, absence of microbiological contamination is critical because it can dramatically impact a drug's safety. As a result, the cleanliness of compressed gases, which often come into contact with pharmaceutical products, is also critical.

For the variety of gases used in manufacturing, their compressed state refers to how they are contained. Compressed gases are typically sampled by taking a small amount from a gas line and drawing it into a smaller space (i.e., a sampler). The decrease in volume that the gas occupies increases its pressure. Gases are decompressed when exiting whatever is containing them for use in a

CRITICAL POINT OF A FILLING LINE

manufacturing line. Process gases are therefore more likely to be decompressed before coming into contact with the product.

Regulatory Requirements

The latest draft of *Annex 1, Manufacture of Sterile Medicinal Products*, introduced the concept of a "contamination control strategy" which limits particulate and microbial contamination and promotes process understanding[1]. A manufacturer's contamination control strategy must demonstrate:

- A thorough understanding of potential sources of contamination.
- Regular trend analysis is performed, ensuring appropriate critical quality attributes of high-risk utilities.
- Gases and other high-risk utilities that come in direct contact with the product or primary container are of appropriate chemical, particulate and microbial quality.

Specifically, actions should be taken to ensure the sterility of process gases, including filtration through a sterilizing filter at the point where the gas is used in production, and sterilization of any subsequent piping or tubing. Filtration should be part of batch standards, with certification guaranteed before release. Integrity testing should be performed for both critical and non-critical gas filters. Care should be taken to avoid introducing moisture to filtration systems, as this promotes the growth of microbes. In lines 715 - 716, Annex 1 states:

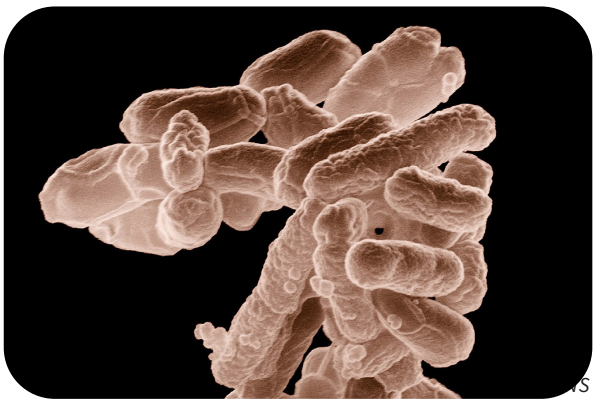
> **"When gases are used in the process, microbial monitoring of the gas should be performed periodically at the point of use."**

In a similar vein, the FDA's *Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing* makes specific mention of the need for purity in a compressed gas. Its microbiological and particle quality after filtration should also be equal to or better than where it's going[2]. ISO 8573 consists of nine separate parts pertaining to the quality of compressed air, with the first specifying the quality requirements and parts two through nine concerning the methods of testing for a range of contaminants. The test method for microbial monitoring of compressed gases is provided in ISO 8573-7.

All the data generated from testing should be recorded to show proof of conditions for any generated product. Auditors will want to see the end results of your testing in addition to the systems in place to verify your claims.

Microbial Survival

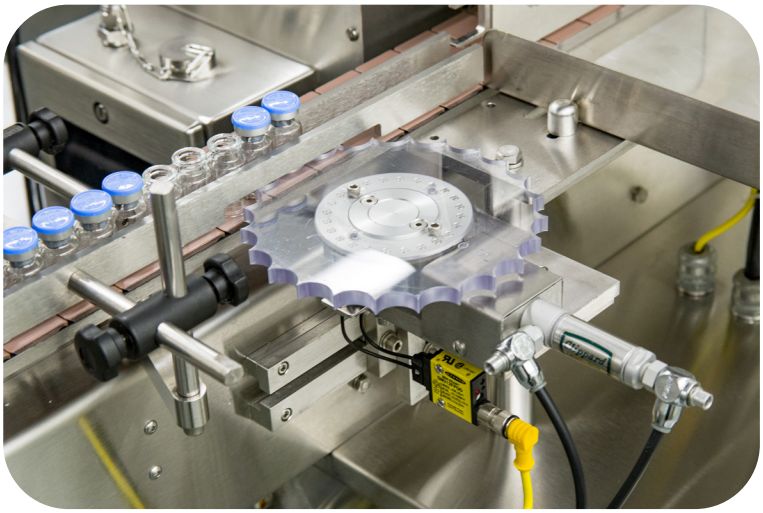
The microbial component and, more particularly, the sampling methodology of compressed gases, has been the subject of extensive discussion. It was assumed that the sudden decompression of a compressed gas before sampling was considered to have a deleterious effect on microbial survival, thereby voiding sampling results. This claim has been proven false with extensive study.



In an FDA study from 2014, bacterial cultures in food products were found to require 2500 to 3000 bar of pressure to inactivate. Cells subjected to pressures less

than 1000 bar had no significant loss in viability[3]. In another study, *Serratia* and *Carnobacterium* strains were found to survive conditions similar to those found on Mars[4]. Lessened microbial growth and metabolism in vivo was only seen in pressures higher than 1000 bar. In fact, microbial cells have been found to survive volatile external pressures found in the harshest environments Earth has to offer, including those in deep-sea environments. In these extremes, microbes conserve their ability to sustain life and reproduce.

In a study of decompression, *Escherichia coli* and *Corynebacterium xerosis* were shown to survive rapid decompressions from 300 to 0 bar[5]. In a typical compressed gas sampler, decompressions are on a much smaller scale (i.e., from around 10 bar to 1 at most). Only the viable counts of gram-negative, gas vacuolate bacteria types, such as *Marmoricola aquaticus*, *Prosthecomicrobium pneumaticum* and *Meniscus glaucopis* were shown to be affected by minimum decompression pressures from 25 to 50 bar[5].



TYPICAL DECOMPRESSION IN MOST PHARMA ENVIRONMENTS: 2.5 TO 1.1 BAR

Instrument Selection

The effects of compressed gas sampling on microbes are insignificant and should not affect instrument selection. Helpful parameters include ease of cleaning and disinfection, associated data management tools, and simplicity. Cost is also one to consider when the monitoring compressed gases is infrequent[6]:

- Classification frequency: Performed monthly or quarterly.
- Routine testing frequency: In Europe, twice a year. The US requires it to be once a year.

Investment into dedicated monitoring equipment can be hard to justify. Alternatives include hiring a service provider for scheduled sampling, and using other equipment that can be multipurposed for compressed gas sampling (i.e., the **MiniCapt® Mobile Microbial Air Sampler** with compressed gas kit). Deciding on the most feasible solution will require a comparison of these solutions.

Conclusion

Regulations have maintained their stance on the importance of contamination control, and continue to be a necessary tool to ensure products meet key standards for safety. GMPs are regularly updated and reflect the modernization and improvements made to manufacturing systems made in recent years. Knowing these guidelines and requirements will expand their reach, it is practical and responsible for companies to seek forward-thinking solutions to better their own processes. In the case of microbial monitoring, taking steps to ensure sterility of equipment and process gases is a vital part of the contamination control strategy. The methods for monitoring contamination levels should not be determined with speculation and antiquated reasoning, but with constructive comparison and validated study.

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