

# Diatomaceous Earth – Celpure® C300

Validation Guide

SARTORIUS

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# 1. Introduction

Diatomaceous Earth is composed of the siliceous shells or exoskeletons of diatoms, a diverse array of microscopic, single-cell algae. The big variety of species all have its unique 3-dimensional shapes which differ from perforated discs and balls to ladders, feathers and needles. The skeletons differ in 1 µm to 1 mm sizes and mainly consist of $SiO_2$.

Because of its very porous structure and its inert character, diatomaceous earth (DE) is often used in filtration applications. In the biotech industry DE is present in depth filters for harvesting applications where it provides high biomass retention capacities. In body feed filtrations the DE is added to the cell culture broth where it has a dual functionality:

- Creation of a permeable filter cake to improve the filter throughput
- Sieving out of sub-micron particles

A wide range of DE grades which differ in purity and permeability are available for different applications. Sartorius selected Celpure® C300 as the best filter-aid for mammalian cell culture harvests. Celpure® C300 is a highly purified form of DE and especially designed for pharmaceutical applications. The patented manufacturing process provides a highly pure DE and improved consistency.

Below you will have a detailed description of the Celpure® C300 production process including its product release specifications.

# 2. Production Process of Celpure® C300

In most cases DE is collected from marine deposits. For standard qualities, calcination is usually the first processing step, in which the DE is heated in an oven of around 1000 °C. The aim of this process is to remove organics and to reduce surface area. However, during calcination process filter aid impurities are fused into the diatom surface (see Figure 1 RIGHT). During the manufacture of Celpure® C300 the DE undergoes an extensive washing procedure before calcination to avoid such impurities (see Figure 1 LEFT).

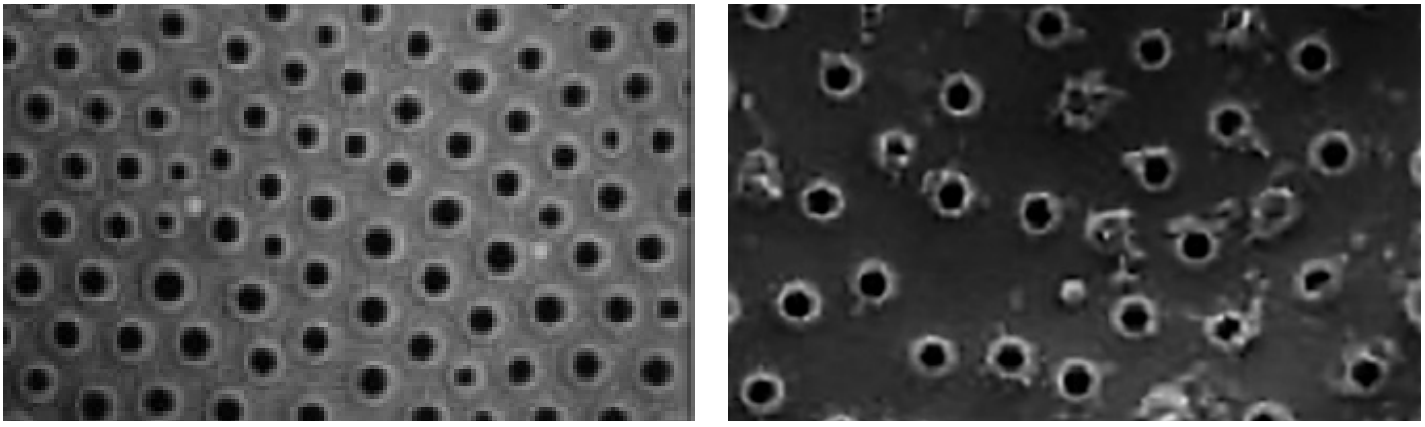


Fig. 1: Scanning electron micrographs (5000 ×) of a diatom surface of conventional DE RIGHT and Celpure® LEFT. Source: Advanced minerals

After the calcination process the DE is acid washed to remove any residual surface extractables. Trace amounts of acid are removed with deionized water and dried at 200 °C (400 °F) before it is packaged.

$SiO_2$ can occur in many different modifications and natural DE mostly consists of amorphous $SiO_2$ phases. During the calcination process, crystalline phases of $SiO_2$ are formed out of the amorphous phases. C300 can contain up 16 % of crystalline silica in total (up to 1 % quartz and 15 % cristobalite). Prolonged and repeated exposure to small respirable particles of crystalline silica (RCS) is known to cause silicosis and associated diseases. Therefore in nearly all countries and regions exposure limits are installed. As Celpure® C300 is a potential source of RCS, the Sartorius products were designed to prevent any RCS exposure.

# 3. Purity of Celpure® C300

The result of the extensive purification process is a high purity product. As Celpure® C300 is derived from siliceous exoskeletons of diatoms, its purity is reflected by its overall silica content (determined as $SiO_2$). The $SiO_2$ content of Celpure® C300 is 98 %. See also table 1:

**Table 1: Composition of Celpure® Grades (Source: Advanced Minerals)**

| | [%] |
|---|---|
| $SiO_2$ | 98.65 |
| $Al_2O_3$ | 0.60 |
| $Fe_2O_3$ | 0.27 |
| $Na_2O$ | 0.14 |
| $K_2O$ | 0.10 |
| MgO | 0.08 |
| CaO | 0.08 |
| $TiO_2$ | 0.03 |
| $P_2O_5$ | 0.03 |
| $MnO_2$ | Nd |
| $SO_3$ | Nd |
| Cl | Nd |
| **Total** | **99.98** |

**Extractables**

In order to generate a constant high quality filtrate, the level of extractables must stay below specified limits. The extractable levels of the most relevant compounds are part of the batch release specifications which are described in paragraph 4.

# 4. Product Release Specifications for Celpure® C300

Prior release all production batches of Celpure® filter aid must fulfill specified acceptance criteria as described below:

## 4.1 pH

The procedure for the determination of pH of diatomite filter aids is according to the USP|NF method. 10.0 g Celpure® C300 is suspended in 100 mL distilled water and boiled for 30 minutes before it is filtered. The pH of the filtrate is measured. A batch passes the test when the pH value is between 5.0 and 8.0.

## 4.2 Conductivity

10.0 g Celpure® C300 is suspended in 100 mL distilled water. After the DE settles down the conductivity of the sample can be measured. Only batches with conductivity of 20 µS/cm or below pass the test.

## 4.3 Acid Soluble Iron

1.0 g Celpure® C300 is mixed in 50 mL 0.1 N HCL for one hour and measured by inductively coupled plasma (ICP) spectroscopy. A batch passes the test when the value for acid soluble iron is 7.0 mg/kg or smaller.

## 4.4 Acid Soluble Aluminum

The soluble aluminum in a filter aid is leached with a citric acid ethanol solution. The amount of soluble aluminum content of each batch is measured by graphite furnace absorption.

A batch passes the test when the value for acid soluble aluminum is 4.0 mg/kg or smaller.

## 4.5 Endotoxines

The endotoxin content is determined with a kinetic-chromatographic test method as described in the United States Pharmacopoeia (USP <85>).

A batch passes the test when the total endotoxin content is 1.0 EU/g or lower.

## 4.6 Loss on Drying (LOD) and Loss on Ignition (LOI)

All Celpure® batches are tested on loss on drying (LOD) and loss on ignition (LOI) according to the US Pharmacopoeia | National Formulary (NF) Monographs for purified siliceous earth.

The amount of volatile matter in a siliceous earth sample will be expelled at elevated temperature.

Loss on drying of a powdered filter aid is determined by the weight difference before and after the sample being heated in a 105 °C oven for two hours, while loss on ignition is determined by the weight difference before and after the dried sample being heated at 980 °C (± 25 °C) to a constant weight.

The NF requirements for purified siliceous earth are as follows:
Loss on Drying: not more than 0.5 %
Loss on Ignition: not more than 2.0 %

## 4.7 Acid Soluble Substances

Acid-soluble substances are determined according to the U.S. Pharmacopoeia|National Formulary Monographs for purified siliceous earth.

The amount of acid-soluble matters in a purified siliceous earth sample is determined by digesting 10.0 g of a sample with 50 mL of 0.5 N hydrochloric acid at 70 °C for 15 minutes, and filter. The weight of the dried residue obtained after evaporating the filtrate at 110 °C and corrected for reagent blank represents the amount of acid-soluble substances.

The NF requirements for purified siliceous earth are as follows:
Acid Soluble Substances: not more than 200 mg or 2.0 %.

## 4.8 Water Soluble Substances

Water-soluble substances are determined according to the U.S. Pharmacopoeia|National Formulary Monographs for purified siliceous earth.

The amount of water-soluble matter in a purified siliceous earth sample is determined by mixing 12.5 g of a sample with 250 mL of water for 2 hours, and filtered with a 0.45 µm membrane. The weight of the dried residue obtained after evaporating the filtrate at 110 °C represents the amount of water-soluble substances. The maximal allowable value for water soluble substances is 0.20 %.

## 4.9 Leachable Arsenic and Lead

The amount leachable arsenic and leachable lead in purified siliceous earth is determined according to the U.S. National Formulary Monographs preparation.

10.0 g of a sample is digested with 50 mL of 0.5 N hydrochloric acid at 70 °C for 15 minutes, and filtered. The concentration of leachable arsenic and lead in the solution are determined by ICP emission spectroscopy. The maximal allowable value for leachable arsenic is 10 mg/kg and 10 mg/kg for leachable lead.

## 4.10 Limit of Non-siliceous Substances

Non-siliceous substances are determined according to the U.S. Pharmacopoeia | National Formulary Monographs for purified siliceous earth.

The amount of non-siliceous substances in a purified siliceous earth sample of 200 mg determined by weighing the residue of the sample after reaction with hydrofluoric acid. $SiO_2 + HF \rightarrow SiF_4(g) \rightarrow +H_2O(g) \rightarrow$ after evaporation to dryness, the weight of residue represents the amount of non-siliceous substances. The NF requirements for purified siliceous earth are as follows: Limit of non-siliceous substances: not more than 50 mg or 25 %.

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Publication No. SL-5714-e
Status 05|2021