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Microbial Enumeration, Microsart® @filter, Microbiological Quality Control, Pharma QC, Filtration of Viscous Samples

# Filtration of Viscous Samples for Microbiological Quality Control

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## Abstract

Microbial enumeration through membrane filtration poses challenges when dealing with viscous and oily substances. Pharmacopoeial guidelines recommend altering the temperature of the sample or diluting it in a suitable diluent. This study aimed to examine the impact of temperature and dilution on the filtration rate of viscous oils. The selected material composition of the Microsart® @filter units allows dilution of the sample in isopropyl myristate, facilitating fast filtrations even at room temperature.

## Introduction

The detection and quantification of microorganisms in aqueous solutions is an essential component of quality control in biotechnological and pharmaceutical applications. This includes activities like bioburden testing and microbial limit testing, among others. Membrane filtration has been established as the method of choice for microbiological testing of liquids. Due to their high viscosity, oily solutions, ointments, and creams pose challenges for membrane filtration. In compliance with USP <61><sup>1</sup>, Ph. Eur. 2.6.12.<sup>2</sup>, or JP 4.05<sup>3</sup> viscous products may be diluted as required with a suitable sterile diluent which has been shown to have no antimicrobial activity, such as isopropyl myristate (IPM). Additionally, the temperature of the sample can be raised to 40 °C, or in exceptional cases not more than 45 °C. This study aimed to investigate the time-saving efficacy achieved by elevating the temperature of the sample compared to the dilution in IPM.

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## Methods

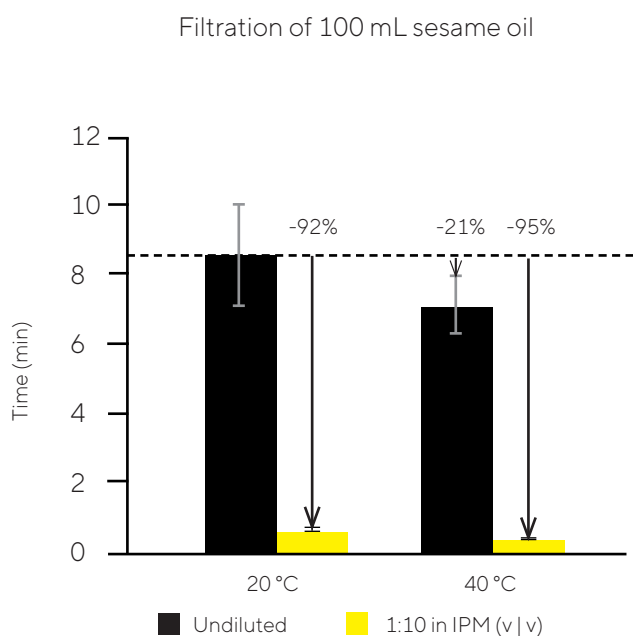
To determine the filtration rates of Microsart® @filter high-flow units with viscous substances, sesame oil and castor oil were tested as shown in Table 1. In addition to the undiluted samples, dilutions in IPM (1:10; v | v) were prepared. All samples were brought to the desired temperature in a water bath. The temperature was monitored with a calibrated thermometer (as listed in Table 2). Three replicates were performed per condition.

**Table 1:** Dynamic viscosities of selected substances at 20 °C<sup>4</sup>. The exact viscosity might vary dependent on the composition of the oil.

Substance	Viscosity at 20 °C (mPa*s)
Water	1
Sesame oil	65
Castor oil	1000

## Results

An average filtration time of 8.74 minutes was determined when 100 mL of undiluted sesame oil were filtered. Increasing the sample temperature from 20 °C to 40 °C reduced the filtration time by 21% as seen in Figure 1. The dilution of sesame oil 1:10 in IPM (v | v) resulted in a 92% reduction in filtration time at 20 °C and a 95% reduction at 40 °C.

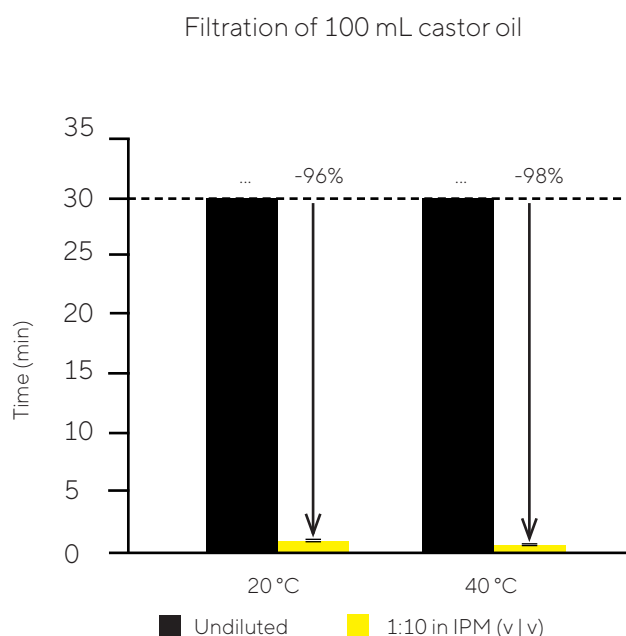


**Figure 1:** Filtration times of undiluted seame oil compared to sesame oil diluted 1:10 in IPM (v | v) at 20 °C or 40 °C.

**Table 2:** Hardware, solutions and consumables used in the study.

Product	Order No.
Microsart® @filter 100, 0.45 µm high-flow	16D01--10-H6--BL
1 branch Microsart® Manifold	168M1-MS
VWR Tracable® Thermometer	620-2091
Castor oil	4702.1
Sesame oil	85067-1L
Isopropyl myristate	143712.1212

As shown in Figure 2, the filtration of 100 mL undiluted castor oil proved to be problematic due to its high viscosity. As acceptable filtration times of less than 30 minutes could not be achieved at either 20 °C or 40 °C, the filtrations were stopped prematurely. In contrast, the dilution of the castor oil 1:10 in IPM (v | v) resulted in a reduction in filtration time of 96% at 20 °C and 98% at 40 °C, respectively.



**Figure 2:** Filtration times of undiluted castor oil compared to castor oil diluted 1:10 in IPM (v | v) at 20 °C or 40 °C. Filtrations for undiluted castor oil were prematurely stopped after 30 minutes.

## Conclusion

Bioburden testing by membrane filtration can be challenging for viscous and oily substances. For samples with a high viscosity, such as castor oil, acceptable filtration times cannot be achieved even if the sample temperature is increased. This study demonstrated that dilution of the sample in IPM features the greatest influence on the filtration time of viscous oils. IPM is the diluent of choice recommended by various pharmacopoeias, e.g. USP <61>, Ph. Eur. 2.6.12., or JP 4.05. Even at 20 °C, filtrations of diluted samples are feasible in less than one minute. Filtrations performed at room temperature also save on time that would otherwise be needed to raise the temperature of the sample. This reduces additional stress on the microorganisms, facilitating reliable microbial enumeration. The chemical compatibility of the Microsart® filter units to IPM enables acceptable filtration times, allowing a high throughput of viscous samples as shown in Table 3.

**Table 3:** Calculated filtrations per hours based on the net filtration time needed for one sample.

Sample	Undiluted at 20 °C	Diluted at 40 °C
Castor oil	<2 samples	63 samples
Sesame oil	6 samples	85 samples

## References

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