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Comparison of Eppendorf Tubes® 5.0 mL to conical 15 mL tubes with a focus on releasable UV-absorbing substances (leachables)

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Abstract

Substances which are released from plastic consumables (leachables) may have an influence on laboratory experiments. When they absorb light in the UV range they can easily be detected by heating water in reaction vessels, followed by photometric analysis. Such substances, for example, can easily falsify DNA measurements. The present Application Note describes experimental comparison of Eppendorf Tubes 5.0 mL and conical 15 mL vessels according to this method. It can be shown that a significant amount of leachables is released from the different 15 mL vessels. In contrast, for Eppendorf Tubes (and a glass control vessel) these extinctions are so low that photometric detection methods are not compromised.

Introduction

The topic of "leachables" (substances leaching from plastics) has been in the news for some years, and it is especially significant in the fields of food packaging and beverage containers [1, 2]. In 2008 a publication in Science brought to light the problems associated with plastic consumables in laboratories. In this and other publications the influence of different additives which may leach from plastic containers and pipette tips on enzyme activity was demonstrated by means of specific enzyme assays [3, 4, 5, 6].
Even standard laboratory methods such as photometric detection of nucleic acids and proteins can be falsified by leachables. Using a simple test system which includes heating of water in reaction vessels (with standard laboratory methods), followed by an absorbance scan in the UV range, it could be demonstrated that UV-absorbent substances leach from single use products [7]. In this publication, as well as in the Eppendorf Application Note 235 [8] it was further shown that water samples thus obtained from Eppendorf reaction vessels did not have significant adverse effects on photometric analyses.
The experiments in the present Application Note were conducted in accordance with the test described above [7, 8]. The aim of this study was the comparison of the Eppendorf Tubes 5.0 mL with conical 15 mL vessels from different manufacturers with regards to leaching of UV-absorbing substances.

Materials and methods

Three Eppendorf Tubes 5.0 mL and three conical 15 mL vessels of four different manufacturers (B, C, F, V) were filled with 1 mL water (for molecular biology) each and incubated for 30 min at 90 °C* in the Thermomixer® comfort, while mixing occurred at 600 rpm. Similar conditions are found in many protocols for sample preparation, such as sample lysis. Following incubation, samples were allowed to cool down for 10 min. The samples were scanned across the range between 220 nm to 340 nm in UVettes, using the Eppendorf BioSpectrometer®. The averages and standard deviations were determined from the three respective replicates. The extinctions may be considered proportional to the amount of substances leaching from the vessels. Non-incubated water was used as the blank, while water which had been incubated in a glass container served as the control. The dsDNA concentration which could theoretically be derived from the measured values was calculated from the extinction at 260 nm using the factor 50 µg/mL per unit of extinction.

Results and discussion

Figure 1 shows the absorbance spectra of water obtained from the different vessels in which it had been incubated for 30 min at 90 °C while being mixed at 600 rpm. In addition, the insert lists the dsDNA concentrations theoretically derived from the extinctions measured at 260 nm, which may therefore be misinterpreted as DNA. A glass control was used since no significant leaching of UV-absorbent material is observed under the present experimental conditions [7].
As expected, the glass vessel produces the lowest values. The absorbance spectrum of water obtained from Eppendorf vessels is only slightly higher than that of the control. The extinction value at 260 nm translates to a theoretical dsDNA concentration of 0.35 µg/mL for the glass control, and to 0.81 µg/mL for the Eppendorf Tubes. These values are below the recommended limit of quantification for the BioSpectrometer (sample extinction values of 0.02–0.03 or dsDNA concentration of 1.0–1.5 µg/mL, respectively [9]). Since other influencing factors such as particles, turbidity or bubbles inside the solution significantly compromise the results at very low extinctions, these data are considered not meaningful with respect to the leaching of substances.

In contrast, significant absorbance spectra are recognizable in all water samples which were heated in the conical 15 mL tubes. The absorbance values obtained at 260 nm translate to theoretical dsDNA concentrations of 2.31 µg/mL to 5.01 µg/mL. It had been shown previously that standard laboratory methods during which sample temperature is elevated (incubation, PCR, centrifugation, sonication) lead to leaching of UV-absorbing substances from the containers [7, 8]. This may yield falsely elevated results during photometric analyses of molecules such as nucleic acids and proteins which are primarily conducted at 260 nm–280 nm. In the case of samples of low concentration, these errors may compromise subsequent experiments.
Additional problems may be presented by the variability of these errors. Figure 2 shows an excerpt of the range between 230 and 280 nm of the same absorbance scan, with the standard deviations overlaid. The values obtained with water from the 15 mL conical vessels show considerable variation, indicating that the substances contained in the vessel material leach at different rates. Thus, the error is not a constant factor but is also subject to large variation.

* Temperature range of Eppendorf Tubes 5.0 mL is -86 °C to 80 °C.
For incubation at 90 °C the Tube Clip 5.0 mL must be used to prevent tubes from opening.

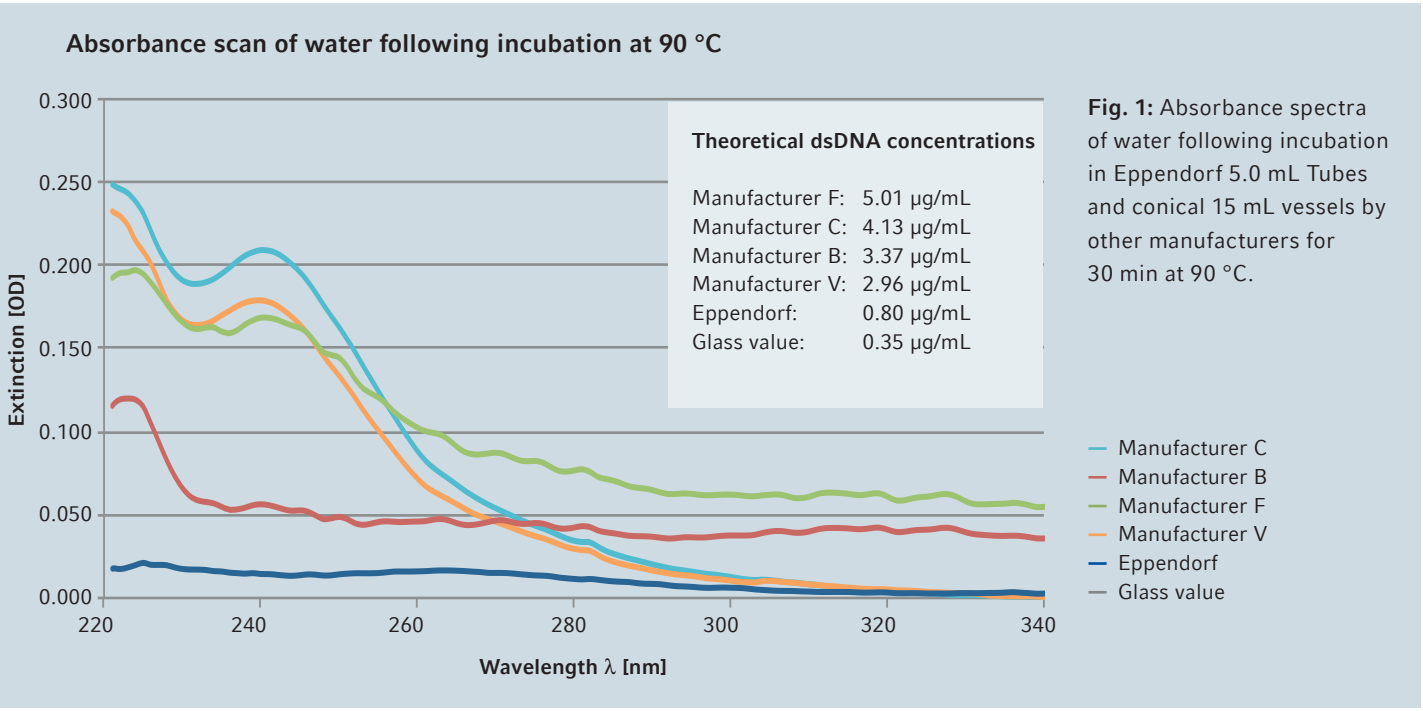

Fig. 1: Absorbance spectra of water following incubation in Eppendorf 5.0 mL Tubes and conical 15 mL vessels by other manufacturers for 30 min at 90 °C.

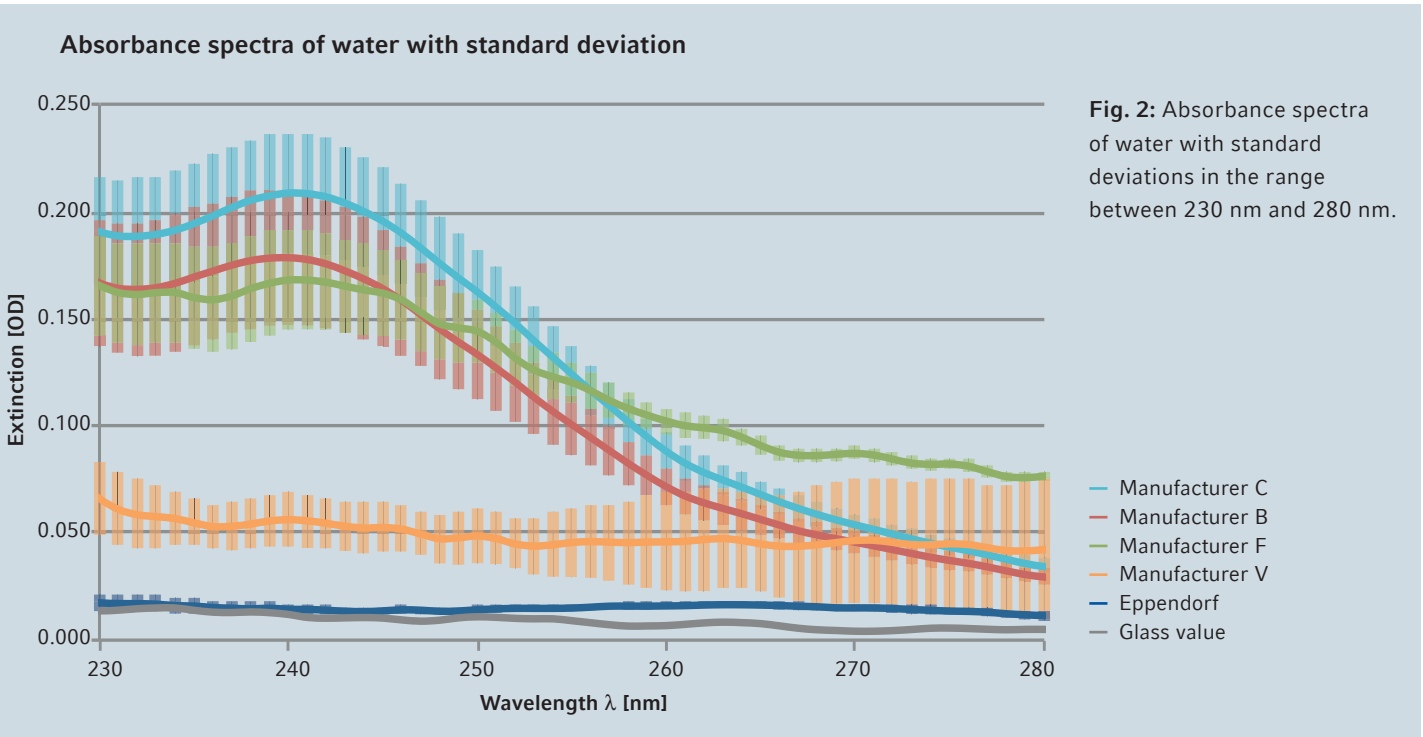

Fig. 2: Absorbance spectra of water with standard deviations in the range between 230 nm and 280 nm.

Conclusion

The data presented in this Application Note show that under the influence of heat, UV-absorbing substances leach from the tested conical 15 mL vessels. These leachables can compromise or falsify downstream analyses such as DNA quantification. The new Eppendorf Tube 5.0 mL yields an extinction which is only slightly above that of the control and thus represents a very good alternative for a large volume tube when minimizing the influence of leachables in sensitive methods is crucial. Owing to the certified omission of problematic additives such as lubricants, biocides or plasticizers in the raw material as well as during the production process, these vessels are very well suited for sensitive analyses. This advantage was previously also demonstrated for smaller tube formats provided by Eppendorf [6, 7, 8].

Literature

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[8] Application Note 235: The influence of UV-absorbing substances released from plastic containers (leachables) on photometric analyses (www.eppendorf.com)
[9] Operating manual Eppendorf BioSpectrometer (www.eppendorf.com)

Ordering Information

Description	Order no. international	Order no. North America
Eppendorf Tubes® 5.0 mL, Eppendorf Quality, 200 tubes	0030 119.401	0030119401
Eppendorf Tubes® 5.0 mL, PCR clean, 200 tubes	0030 119.460	0030119460
Eppendorf Tubes® 5.0 mL, Sterile, 200 tubes	0030 119.487	0030119487
Eppendorf Tubes® 5.0 mL, Eppendorf Biopur®, 50 tubes (individually wrapped)	0030 119.479	0030119479
Eppendorf Protein LoBind Tubes 5.0 mL, PCR clean, 100 tubes	0030 108.302	0030108302
Eppendorf DNA LoBind Tubes 5.0 mL, PCR clean, 200 tubes	0030 108.310	0030108310
Starter Pack Eppendorf Tubes® 5.0 mL, PCR clean, 400 tubes, 2 racks (16 spaces), white, 8 universal adapters for rotors with bore for 15 mL conical tubes	0030 119.380	0030119380
Tube Clip 5.0 mL, 10 pcs., secures lid for boiling	0030 119.509	0030119509
UVette®, Original Eppendorf UV-transparent plastic cuvette, individually wrapped, PCR clean, certified, 80 pcs	0030 106.300	952010051
UVette® routine pack, Eppendorf Quality purity, re-sealable box, 200 pcs.	0030 106.318	952010069
Eppendorf BioSpectrometer® basic	6135 000.009	6135000017

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