Applications

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Technical Report

The influence of UV absorbing substances released from plastic containers (leachables) on photometric analyses

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Abstract

Plastic consumables, which are routinely used in the laboratory, can release substances which may subsequently compromise experiments, so called leachables. This was demonstrated in a number of recent publications. In accordance with a published method it was to be investigated herein whether following a routine application, the heating of samples, UV-absorbing components were detectable in the incubated water. Absorbance scans as well as photometric measurements demonstrated that water samples from 1.5 mL tubes and 0.2 mL PCR tubes by various manufacturers showed elevated extinction values in the UV range. Samples from comparable Eppendorf Tubes did not show higher values, i.e. photometric analyses of proteins and nucleic acids were not compromised in contrast to the competitors' vessels tested (Figure 1).

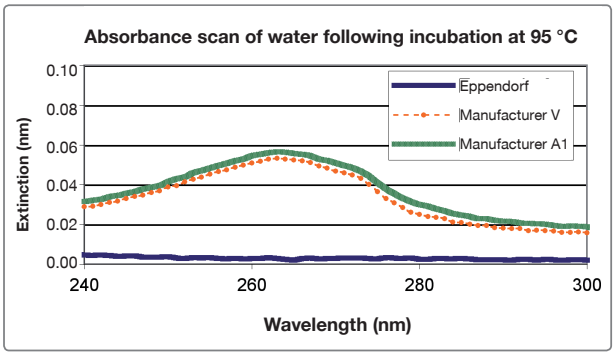

Figure 1: Absorbance spectrum of water in the range from 240 to 300 nm following incubation at 95°C for 30 min (see Figure 2 a).

Introduction

It has been known for some time that plastic products such as food packaging and beverage bottles can leach chemical substances that lead to contamination of the contents [1, 2]. This phenomenon also plays an important role in the laboratory, as plastic consumables are routinely used for sample storage and experiments. A 2008 *Science* publication and other papers described that slip agents such as oleamide and erucamide, as well as biocides, which were washed from vessels and tips, are able to show activity in specific enzyme assays [3, 4, 5]. As a consequence, false positive or false negative results are produced, which cannot be analyzed, thus leading to increased consumption of time and financial resources.

Even routine applications have been shown to be compromised [6]. UV absorbing substances from plastic containers are leached into the sample by laboratory applications requiring temperatures of 37 °C or above (incubation steps, PCR, centrifugation, ultrasound). Since these substances absorb light in the same range as the absorbance maxima of nucleic acids and proteins, they can interfere with photometric detection reactions, thus providing a source of error with adverse effects on downstream applications.

Therefore, the use of high quality consumables, which contain the least amount of leachable additives, is recommended.
Eppendorf Tubes are manufactured from highly pure polypropylene. The materials used comply with the standards of the US Food and Drug Administration (FDA) for food storage (21CFR part 177.1520 Olefin Polymers; 21CFR part 178.2010 Antioxidants and Stabilizers for Polymers for food containers). It is further certified that the materials contain neither plasticizers nor lubricants or biocides, nor that these are included during production.

For the purpose of this Technical Report, experiments were performed in accordance with the publication by Lewis *et al.* [6]. This publication had described that the Eppendorf vessels tested achieved noticeably better results than competitors, but no data were shown. Hence, Eppendorf Safe-Lock Tubes 1.5 mL and Eppendorf 0.2 mL PCR tubes were here tested alongside comparable containers by other manufacturers. Following incubation of water at different temperatures, absorbance scans and measurements in the UV range were performed in order to test whether components were leached which could compromise subsequent photometric analyses. Extinction can be estimated to be proportional to the amount of leached substances.

Materials and Methods

Three Eppendorf Safe-Lock Tubes 1.5 mL and comparable tubes by two competitors (A1 and V) were filled with 1 mL water (molecular biology grade) and incubated under the following conditions:
a) 95 °C for 30 min, b) 70 °C for 30 min, c) 37 °C for 24 h.
0.2 mL PCR tubes by Eppendorf and two other manufacturers (A and T) were filled with 150 µL water (molecular biology grade) each. One half of the containers was subjected to a PCR protocol in a thermocycler (30 PCR cycles: 95 °C - 30 s / 60 °C - 30 s / 72 °C - 2 min), while the remaining vessels were incubated at room temperature (RT). Each sample was subjected to a scan across the range of wavelengths between 190 nm and 400 nm using the spectrophotometer Cary 100 (Varian) and the values for the groups of samples were determined. Non-incubated water was used to set the blank value.
In an additional series of measurements in the BioPhotometer plus, 1 mL water (molecular biology grade) was incubated at 95 °C for 30 minutes in each of three Eppendorf Safe-Lock Tubes and tubes by three competitors (A1, A2 and V). Extinction values at 260 nm and 280 nm were determined, the mean values of the replicates and the SD were calculated. Furthermore, the factor 50 µg/mL was used to determine the theoretical amounts of dsDNA from the measured extinction values.

Results and Discussion

Figure 2 demonstrates that water which was incubated in Eppendorf Tubes shows no significant extinction in the relevant range of wavelengths. The samples taken from the tubes by other manufacturers show a distinct temperature-dependent absorption profile. The highest values were measured at 95 °C (Figure 2 a). At 70 °C (Figure 2 b) and 37 °C (Figure 2 c) these values were lower. Comparable results were obtained for the PCR tubes: the water taken from competitors' tubes treated in the thermocycler yielded higher extinction values than the water incubated at room temperature (Figure 2 d). According to these data, it appears that substances which absorb light in the UV range are released from the competitors' containers tested herein. The maximum values are observed slightly above 190 nm. In addition, water from the 1.5 mL tubes showed absorption signals in the range of 210 - 220 nm as well as 260 nm (Figures 2 a-c). It is exactly this set of wavelengths which plays an important role during photometric detection and quantification of biomolecules. For example, proteins may be measured directly at 205 nm or at 280 nm, whereas nucleic acids are measured at 260 nm.

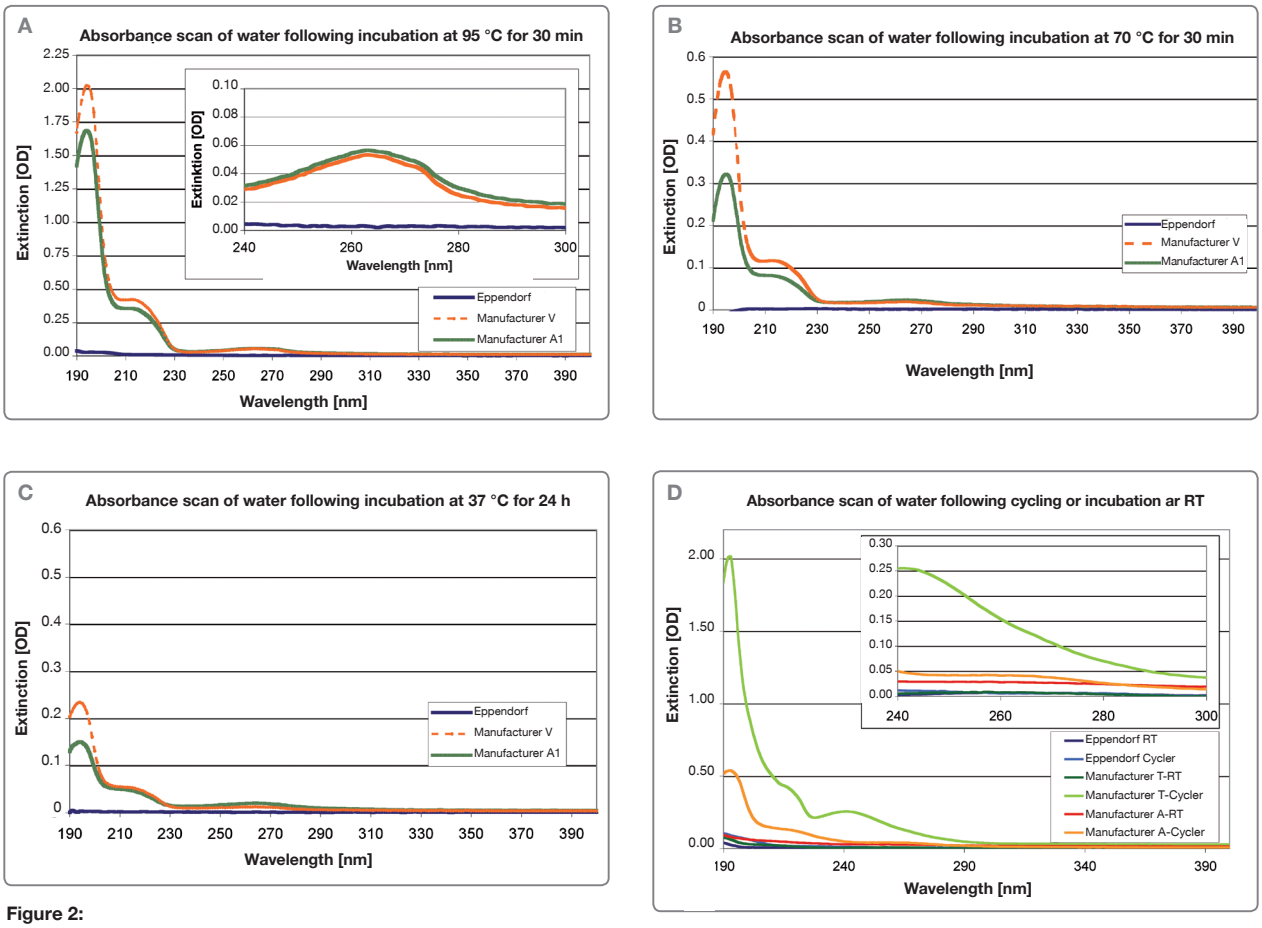

Figure 2:
Absorbance spectrum of water following incubation in different tubes.
a) Incubation of 1.5 mL tubes at 95 °C for 30 min
b) Incubation of 1.5 mL tubes at 70 °C for 30 min
c) Incubation of 1.5 mL tubes at 37 °C for 24 h
d) Incubation of 0.2 mL PCR tubes in the cycler and at room temperature

One consequence of leached UV absorbing components is illustrated in Figure 3: DNA measurements can be negatively affected. Here, extinction values of water incubated at 95 °C in a further experiment are shown at 260 nm and 280 nm. In addition, the theoretical amount of dsDNA, which would result from this value, is shown (values above the bars in Figure 3). The value for the samples obtained from the Eppendorf Safe-Lock Tubes was below the detection level (LOD = limit of detection), whereas the water from the other tested vessels yielded values above the limit of quantification (LOQ). Therefore, elevated results for DNA concentration would be obtained during DNA quantification. When the actual amount of DNA present is lower than the measured value, a negative impact on downstream applications may ensue.

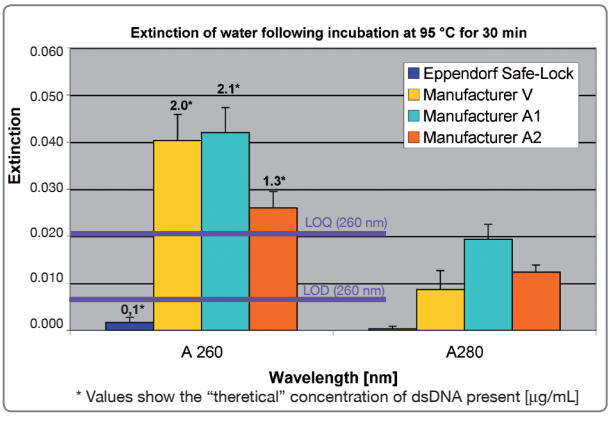

Figure 3: Extinction of water at 260 nm and 280 nm following incubation in 1.5 mL tubes at 95 °C for 30 min.

* The values displayed represent the "theoretical" concentration of dsDNA [µg/mL] calculated from the extinction values. The resulting LOD (limit of detection) was determined at 0.006 E = 0.3 µg/mL; the LOQ (limit of quantification) was determined at 0.021 E = 1.0 µg/mL.

Conclusion

Photometric analyses verified that UV absorbing substances are released from plastic vessels made by certain manufacturers, and that they interfere with the detection of biomolecules such as nucleic acids and proteins. Samples treated in Eppendorf Tubes did not show significantly compromised values. Here, as in a number of published investigations based on sensitive cell-based assays, differences between plastic consumables by different suppliers became evident. By omitting lubricants and other additives during production, Eppendorf Tubes are very well suited for sensitive detection methods in the laboratory.

References

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Ordering information

Description	Order no. international	Order no. North America
Eppendorf Safe-Lock Tube™ 0.5 mL	per 500 pcs. 0030 121.023	per 500 pcs. 022363611
Eppendorf Safe-Lock Tube™ 1.5 mL	per 1000 pcs. 0030 120.086	per 500 pcs. 022363204
Eppendorf Safe-Lock Tube™ 2.0 mL	per 1000 pcs. 0030 120.094	per 500 pcs. 022363352
0.2 mL PCR Tubes	per 1000 pcs. 0030 124.332	per 1000 pcs. 951010006
0.2 mL PCR Tubes, 8-tube strip	pack of 120 (960 tubes) 0030 124.359	pack of 120 (960 tubes) 951010022

Description		
Eppendorf Biophotometer® plus, 230 V/ 50 - 60 Hz	6132 000.008	952000006

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