



Tech Note

SpecPlate

Flattening the Curve: How Liquid Meniscus Affects Absorbance Measurements in Microplates

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In the context of microplate spectrophotometry, the presence of liquid menisci has been observed to induce substantial deviations in the measurement of absorption. In this study, solutions with and without Tween 20 were analyzed using standard plate formats and the SpecPlate with a fixed measurement path to investigate this effect.

Introduction

According to the Beer-Lambert law, absorbance is proportional to concentration and optical path length. In a standard cuvette the path length is fixed (typically 1 cm), but in microplate wells the liquid path length varies with volume and well geometry. Moreover, the formation of liquid meniscus and its shape further influence the path length. As a result, the absorbance in microplates cannot be interpreted for complete accuracy without knowing the true path length of each well.

The shape of the liquid meniscus depends on surface tension, solution polarity, and well material. Different solutions (even at the same volume) form different menisci, leading to varying effective path lengths. In fact, it was demonstrated that an analyte's concentration can influence meniscus curvature: e.g. at higher nucleic acid concentrations the meniscus effect shortens the path length by up to ~15%. [1] The inclination of the meniscus can cause a loss of accuracy in small-volume samples, and some solutions even develop or change their meniscus shape over time. The extent of this effect depends on the microplate brand and the composition of the solution. [2]

PHABIOOC's SpecPlate is a 96-well-format plate with closed, stepped measurement chambers that define a fixed path length (Figure 1). With a sample volume of 36 μL per chamber, the SpecPlate provides a known pathlength and eliminates the influence of surface menisci (and pipetting inaccuracies) on absorbance. This design allows direct UV/Vis-measurements with higher precision.

In this technical note, we investigate the meniscus effect on absorbance by measuring Orange G dye solution at 492 nm in various microplates, with and without surfactant. We compared a standard 96-well plate (150 μL volume), a 96-well half-area plate (80 μL), a 384-well plate (40 μL), and SpecPlate (36 μL). The addition of Tween 20 alters the meniscus, and the effect of the measured absorbance was investigated.

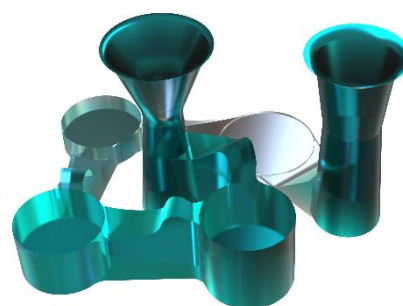


Figure 1 SpecPlate measurement structure

Material

- SpecPlate (PHABIOOC, 400100)
- Standard 96-well microplates, 96-well half-area microplates, 384-well microplates (UV-Star®, Greiner Bio-One)
- Tecan Spark® microplate reader
- Tween 20
- Orange G
- Ultrapure water

Methods

Sample Preparation

A stock solution of Orange G (160 mg/L) was prepared in deionized water. Serial dilutions were generated to obtain a range of concentrations (5, 20, 40, 60, 80, 100 mg/L). To evaluate the influence of

surface-active agents, each dilution was prepared both with and without the addition of 0.005% Tween 20, using a 1% Tween 20 stock solution. The samples containing Tween 20 were prepared in a way that ensured identical Orange G concentrations as in the corresponding samples without surfactant. All solutions were freshly prepared prior to measurement.

Microplates and Measurement Setup

Measurements were performed using a Tecan Spark microplate reader. All experiments were conducted under identical settings, except for the geometry parameters specific to each plate type. Standard microplate formats and the SpecPlate were tested. The solutions and additional water blanks have been measured at 492 nm. The sample volumes were 150 μL for the 96-well plate, 80 μL for the 96-well half-area plate, 40 μL for the 384-well plate, and 36 μL for the SpecPlate. Each condition was measured in eight replicates to ensure reproducibility.

Data Analysis

Raw absorbance data were first corrected by subtracting the corresponding blank values. For each concentration, the mean and standard deviation were calculated from eight replicate measurements. In the case of the SpecPlate, this evaluation was performed individually for each chamber height.

Calibration curves were generated for samples with and without the addition of Tween 20, and linear regression was applied to determine slope and intercept values. To visualize the impact of the

surfactant, plots comparing measured absorbance values with the corresponding Tween-free reference were created. The relative deviation between samples with and without Tween 20 was calculated for each concentration.

To quantify the overall influence of Tween 20, the relative difference between the slopes of the respective calibration curves was used.

Results & Discussion

Results and Discussion

The influence of Tween 20 on UV/Vis-measurements was evaluated based on two complementary analyses: The relative differences in the slopes of the calibration curves obtained with and without surfactant, and the mean relative deviations of the measured absorbance values.

At a Tween 20 concentration of 0.005 %, conventional microplates exhibited a significant response to the presence of surfactant. The relative slope differences ranged from -6.6 % (96 well half area) to -11.6 % (384 well plate). In contrast, the SpecPlate exhibited only minimal effects across all chamber heights. The relative slope differences remained below 2 %, with values of +1.9 %, +1.7 %, +0.8 %, and +1.1 % for the 100 μm , 700 μm , 1400 μm , and 2000 μm chambers, respectively (average is 1.35 % across all measuring chamber heights, see figure 2).



Figure 2 Relative slope difference of the calibration curves with and without Tween 20 in the respective plates

The mean deviations of the absorbance readings reached 6.1 %, 3.8 %, and 5.4 % for the 96 well, 96 half area, and 384 well plates, respectively. These findings indicate that surface-active additives such as Tween 20 can significantly affect optical pathlength and measurement accuracy in open-well systems, most likely due to changes in surface tension and meniscus curvature. The mean deviation of absorbance values for the SpecPlate averaged across all chamber heights was just 1.0 %. These results lie within normal experimental variation and confirm that the closed measurement chambers of the SpecPlate effectively prevent meniscus-related artifacts and maintain a consistent optical pathlength, independent of surfactant presence.

A comparison of the absorbance values obtained with and without Tween 20 clearly illustrates this effect. When plotting the measured values with surfactant (actual) against those without surfactant (target), a distinct shift of the data points becomes evident. This shift directly translates into incorrect concentration estimates when standard plates are used for quantitative analysis. As a case in point, this effect was illustrated in Figure 3 using data from the

96-well plate and in Figure 4 using data from the 2000 μm chamber of the SpecPlate.

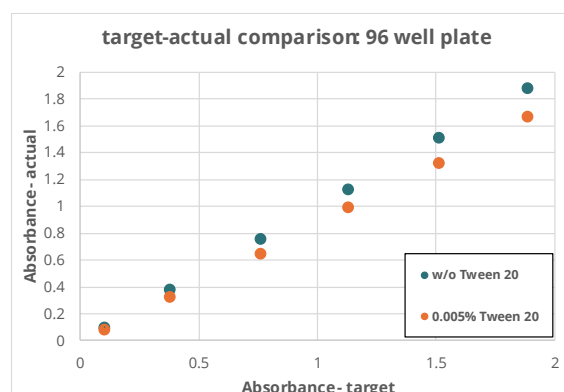


Figure 3 Comparison of measured values with surfactant (actual) with measured values without surfactant (target) for the 96-well plate.

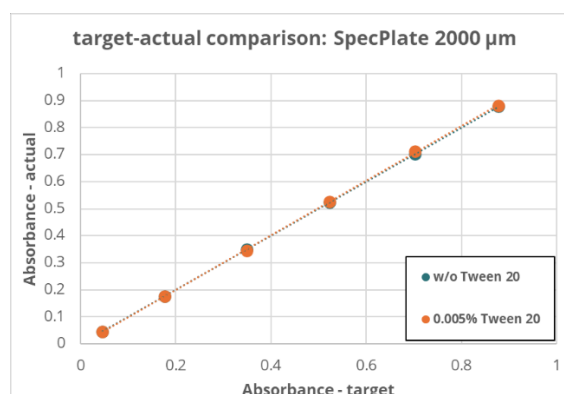


Figure 4 Comparison of measured values with surfactant (actual) with measured values without surfactant (target) for the 2000 μm measuring chamber of the SpecPlate.

It should also be noted that in this study, all Tween-containing samples were prepared to ensure identical surfactant concentrations across wells, resulting in uniformly shaped menisci. In real-world assays, however, sample compositions typically vary between wells, leading to differences in surface tension and thus to further variability in meniscus formation. Consequently, the influence on concentration determination in practical

measurements is expected to be even more pronounced.

Conclusion

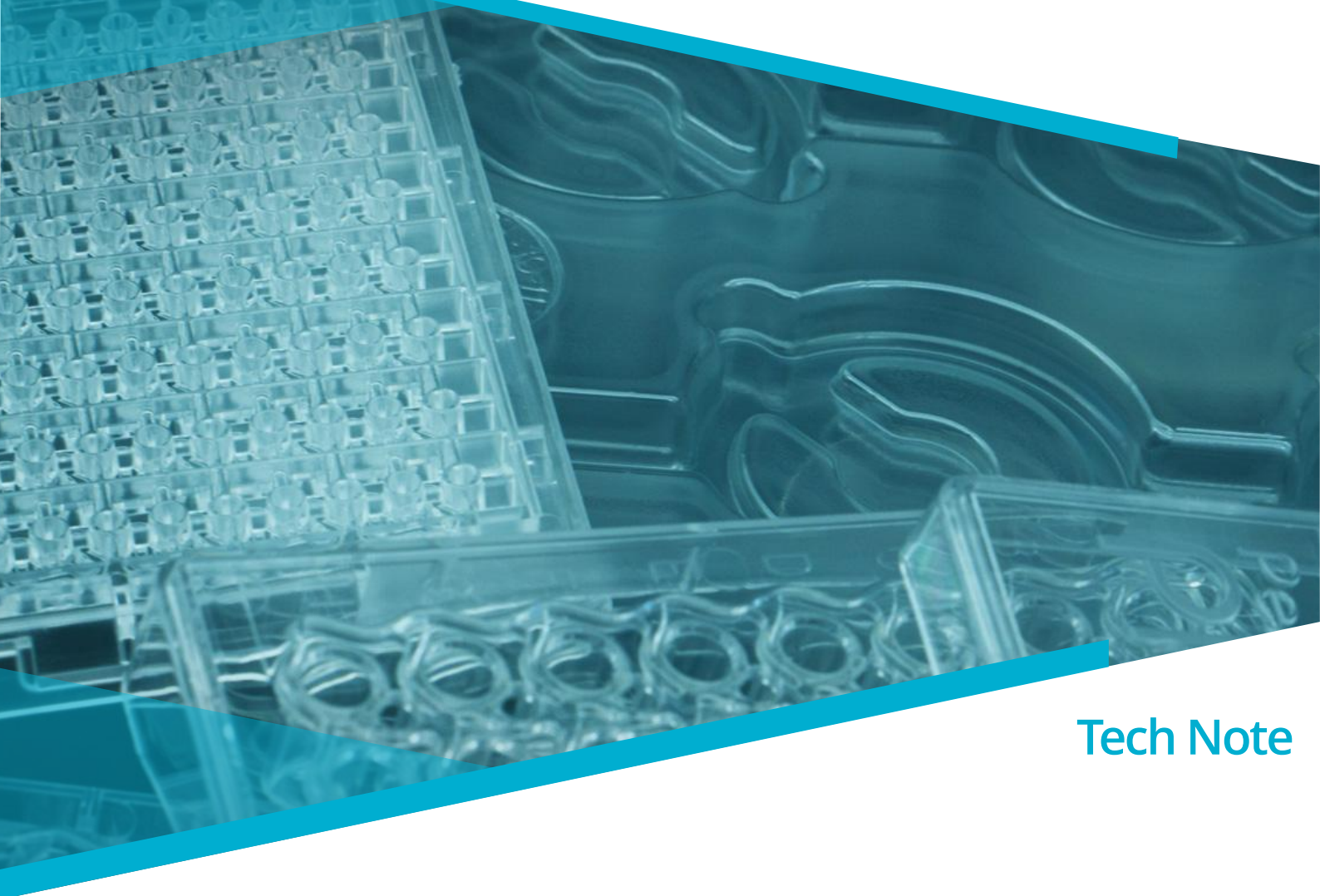
Overall, the results demonstrate that the formation of liquid menisci can substantially affect absorbance readings in standard microplate formats, leading to deviations in calculated concentrations even under controlled conditions. In contrast, the SpecPlate's closed-chamber design with defined optical path lengths inherently eliminates such sources of error, providing consistent and geometry-independent measurements without the need for mathematical correction.

While pathlength correction algorithms implemented in some plate readers can partially compensate for geometric inconsistencies, these methods rely on additional wavelength measurements based on water absorption bands and are limited to aqueous samples.

Across all tested conditions, the SpecPlate maintained highly reproducible absorbance data, even in the presence of surface-active agents such as Tween 20. This robustness underscores its suitability for a wide range of biochemical and pharmaceutical assays, where precision and comparability are essential. By removing meniscus-related variability, the SpecPlate not only improves data reliability but also simplifies experimental workflows, offering a practical and accurate alternative to conventional open-well plate measurements.

[1] <https://documents.thermofisher.com/TFS-Assets/LCD/Application-Notes/AN-SkanIT-Microplate-Based-Pathlength-Correction-Technical-Note-EN.pdf>

[2] <https://medicine.uky.edu/sites/default/files/inline-files/Microplate.pdf#:~:text=%E2%80%A2%20The%20inclination%20of%20the,of%20accuracy%20in%20some%20solutions>



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