APPLICATION NOTE

UV/Vis Spectroscopy

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Accurate RNA Quantification using microvolume cuvettes and the LAMBDA 365+ UV/Vis spectrometer

Introduction

UV/Vis spectrometers are highly versatile instruments employed in a wide variety of applications. When used with standard 10x10 mm pathlength cuvettes, these spectrophotometers require solution volumes of around 2 mL to perform the absorbance analysis. This could hinder high throughput analysis especially for biomolecules-based assays involving proteins or nucleic acids where the sample volumes could be limited. The development of microvolume cuvettes that can be easily fitted in UV/Vis spectrometers allows to overcome such limitation and employ ultra low sample volumes while maintaining the desired accuracy. In this application, the LAMBDA® 365+ UV/Vis spectrometer was used in combination with Nano Stick and TrayCell™ microvolume cuvettes to analyze RNA sample and evaluate the reproducibility of the absorption measurements. Once this could be confirmed, accurate calibration curves were constructed to determine RNA unknown concentrations.[1] The method could be easily adjusted and applied to quantify proteins.[2]

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Materials & Methods

RNA, MS2 (800 µg/mL in 10 mM Tris-HCl, 1 mM EDTA, pH 7.0) was purchased from Sigma-Aldrich and diluted in TE buffer to prepare 80 µg/mL RNA stock solution. This solution was pipetted multiple times on the sample wells of the TrayCell (sample volume 0.7-10 µL) and Nano Stick (sample volume 2 µL) (figure 1) to collect multiple RNA absorption spectra and evaluate the reproducibility of the measurement for each microvolume cuvette. The TrayCell was equipped with 1 mm pathlength cap (x10 factor), while the Nano Stick had a pathlength of 0.5 mm (x20 factor). The multiple RNA absorption spectra were collected in the LAMBDA 365+ spectrometer by placing each microvolume cuvette in the sample cell holder without any additional alignment. The LAMBDA 365+ is a dual beam spectrophotometer and for this type of absorbance acquisition, the reference cell holder was kept empty. Between every measurement the microvolume cuvette was simply cleaned with paper foil before pipetting the next sample solution.

The stock solution was then diluted to prepare the standard working solutions (2.7 – 80 µg/mL) which were used to construct the calibration curve for each microvolume cuvette. The absorption values recorded at 260 nm were obtained from the spectra and subtracted by the baseline at 320 nm directly by the UV WinLab Software. Two independent 80 µg/mL RNA stock solutions were prepared separately to repeat the creation of two independent calibration curves and to validate the method for both Nano Stick and TrayCell. The setting parameters used for the calibration curves and the multiple scans analysis are reported in table 1.

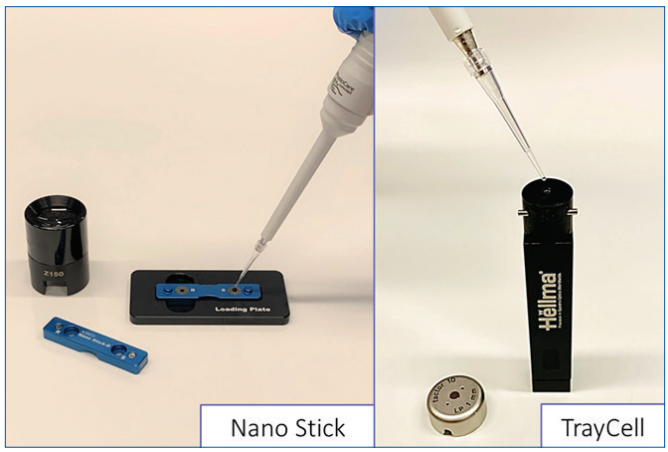

Figure 1: Solution preparation on the sample wells of the Nano Stick (left) and TrayCell (right). The Nano Stick kit comprises a bubble checker to verify that bubbles are not formed after placing the top cover.

Table 1: Scan Setting and Calibration Setting parameters used for the absorption spectra replicates and calibration curves.

Scan Setting		Calibration Setting	
Ordinate Mode	Absorbance	Component	Analyte (µg/mL)
Slit	2 nm	Type of Curve	Linear
Wavelength Range	230 – 330 nm	Ordinate Mode	Height (260 nm)
Scan Speed	480 nm/min	Baseline Correction	Single Point (320 nm)

Results

Multiple absorption spectra of the RNA solution (80 µg/mL) were collected using the Nano Stick (figure 2) and the TrayCell (figure 3) to evaluate the reproducibility of the absorbance measurements. The replicates shown in figure 4 represent the absorbance values obtained at 260 nm, subtracted by the baseline at 320 nm and multiplied by the correction factor based on the light pathlength (x10 for the TrayCell and x20 for the Nano Stick). In the case of the TrayCell, the A260 was 2.25 ± 0.03 and the RSD (relative standard deviation) was equal to 1.4%, while for the Nano Stick the A260 was 2.23 ± 0.02 and the RSD was 0.9% indicating the high precision and reproducibility of the readings.

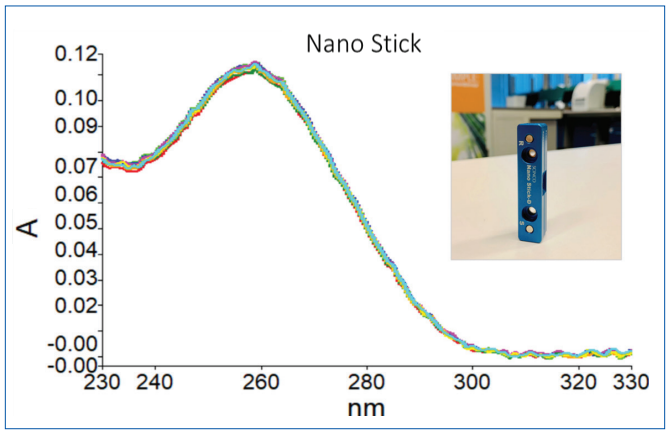

Figure 2: Absorption spectra replicates of RNA solution (80 µg/mL) collected using the Nano Stick with 0.5 mm pathlength.

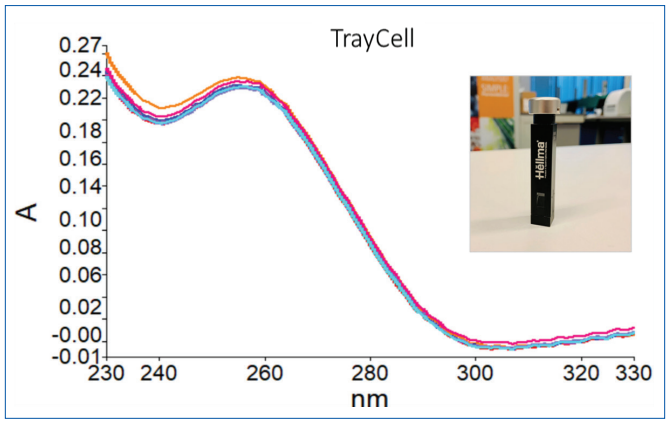

Figure 3: Absorption spectra replicates of RNA solution (80 µg/mL) collected using the TrayCell equipped with 1 mm pathlength cap.

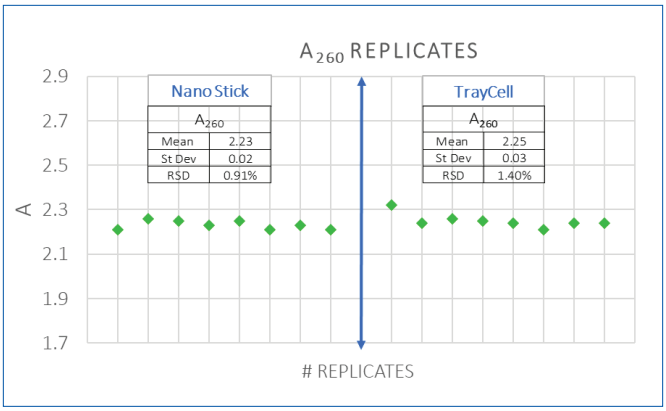

Figure 4: Replicates of the RNA (80 µg/mL) absorbance obtained at 260 nm corrected by the baseline at 320 nm and the light pathlength factor (x20 Nano Stick, x10 for TrayCell) for each microvolume cuvette.

Based on the reproducibility shown by the acquisition of multiple readings of these microvolume cuvettes, calibration curves were constructed for both TrayCell and Nano Stick to provide a tool for the analysts that want to quantify RNA unknown concentration using ultra low volumes of liquids. The results are reported in figure 5 and figure 7. The calibration parameters were readily available in the UV WinLab software at the end of the analysis (see reports in figure 6 and figure 8). For each microvolume cuvette, two independent solutions were used to create two independent calibration curves to validate the accuracy of the method. In all cases, the correlation coefficients obtained for each curve were higher than 0.99 indicating that the linear regression model was appropriate to fit the data.

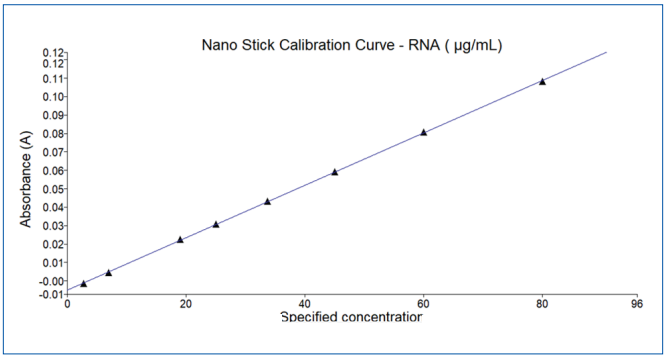

Figure 5: Plot of the absorbance collected at 260 nm and subtracted by the baseline at 320 nm against the corresponding RNA standard concentrations (2.7 – 80 µg/mL). The measurements were carried out using the Nano Stick.

```
Component Name:              RNA
Component Units:             µg/mL

Calibration:                 Calibration Curve – Linear (y=a1x+a0)
Ordinate Mode:               Height
Baseline Correction: Single Point

Settings (nm):               Position:260.00  Base1:320.00

Force through Zero:  No

Calibration Coefficients:
                                  a0 = –0.004828
                                  a1 = 0.001422

Specified Correlation Coefficient: 0.980000
Calculated Correlation Coefficient:  0.999949

StandardID     Specified    Calculated    Residual    Ordinate
-------------------------------------------------------------
RNA_Te_80000    80.0000      79.5982      0.4018      0.1084
RNA_2           60.0000      60.2533     -0.2533      0.0809
RNA_3           45.0000      45.1564     -0.1564      0.0594
RNA_4           33.7500      33.9194     -0.1694      0.0434
RNA_5           25.0000      25.0778     -0.0778      0.0308
RNA_6           19.0000      19.2677     -0.2677      0.0226
RNA_7           6.9000       6.5594      0.3406       0.0045
RNA_8           2.7000       2.5178      0.1822      -0.0012
```

Figure 6: Calibration curve parameters obtained using the Nano Stick.

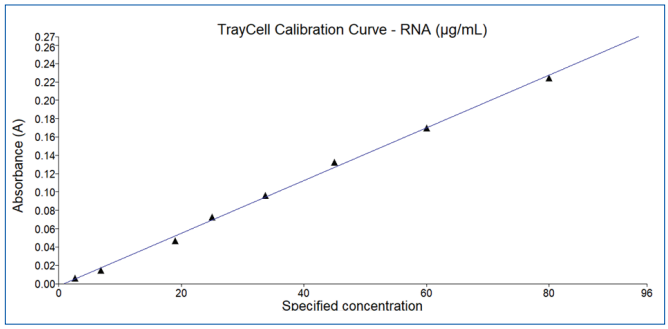

Figure 7: Plot of the absorbance collected at 260 nm and subtracted by the baseline at 320 nm against the corresponding RNA standard concentrations (2.7 – 80 µg/mL). The measurements were carried out using the TrayCell.

```
Component Name:                RNA
Component Units:               µg/mL

Calibration:                   Calibration Curve – Linear (y=a1x+a0)
Ordinate Mode:                 Height
Baseline Correction:  Single Point

Settings (nm):                 Position:260.00  Base1:320.00

Force through Zero:    No

Calibration Coefficients:
                                        a0 = –0.002141
                                        a1 = 0.002874

Specified Correlation Coefficient:      0.980000
Calculated Correlation Coefficient:     0.998843

-----------------------------------------------------------
StandardID    Specified    Calculated    Residual    Ordinate
-----------------------------------------------------------
RNA_1          80.0000      78.8653      1.1347      0.2245
RNA_2          60.0000      59.8942      0.1058      0.1700
RNA_3          45.0000      46.9497     -1.9497      0.1328
RNA_4          33.7500      34.3703     -0.6203      0.0966
RNA_5          25.0000      26.2048     -1.2048      0.0732
RNA_6          19.0000      17.0922      1.9078      0.0470
RNA_7          6.9000       6.0126       0.8874     0.0151
RNA_8          2.7000       2.9609      -0.2609     0.0064
```

Figure 8: Calibration curve parameters obtained using the TrayCell.

Conclusion

In this work, LAMBDA 365+ demonstrated to be a simple yet reliable UV/Vis spectrometer even in combination with micro volume cuvettes such as Nano Stick and TrayCell™. Without any additional alignment, each micro cuvette could be placed in the single cell holder of the spectrophotometer and highly accurate absorption measurements of RNA solutions could be collected. Absorbance replicates for 80 µg/mL RNA solution showed RSD of 0.91% and 1.40% for Nano Stick and TrayCell™, respectively indicating the high degree of reproducibility achieved. The correlation coefficients obtained for the calibration curves showed values above 0.99 for both Nano Stick and TrayCell™. These results confirmed the great flexibility of the LAMBDA 365+ spectrophotometer which, among the extensive range of applications available, also offers an ideal solution for those analysts who need to quantify ultra low volumes of RNA samples (proteins can also be quantified adjusting the method), just by switching the standard cuvette with a microvolume cuvette. This solution allows to overcome the need of a dedicated microvolume UV/Vis spectrometer, while maintaining the accuracy offered by the highly versatile LAMBDA 365+ spectrometer.

References

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2. Edelhoch, H., 1967. Spectroscopic determination of tryptophan and tyrosine in proteins. Biochemistry, 6(7), pp.1948-1954.

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