Performance Studies on the Transmitted Light Drop Analyser

M. O’Neill¹, N.D. McMillan¹, S.R.P. Smith², S. Riedel¹, K. Arthure¹ and M. Morris³

¹ Drop Technology, Tallaght Business Park, Whitestown, Dublin 24, Ireland
² Physics Centre, Essex University, Wivenhoe Park, Colchester CO4 3SQ, UK
³ Spectrecology, 460 Boulder Falls Lane, Jasper, GA 30143, USA

E-mail: martina.oneill@droptechnology.com

Abstract. The testing of a new microvolume spectrometer instrument, the Transmitted Light Drop Analyser (TLDA) is based on drop physics. The advantages, benefits and features of this spectroscopic accessory are elucidated for the first time. The performance of the instrument with regard to drop evaporation, carry-over, calibration standards, photometric accuracy and photometric reproducibility are presented. The paper ends with a brief discussion on the practical significance of the test programme taken as a whole.

KEYWORDS: TLDA; sessile-drop; advantages; features; benefits; evaporation; carry-over.

1. Introduction

The Transmitted Light Drop Analyser (TLDA) provides an elegantly simple ‘drop physics’ approach to UV-visible spectrophotometry as can be seen by Figure 1. The parameters varied in the modelling were vertical position z of the input fibre above the top of the quartz base; (ii) horizontal position x of the input fibre away from the (vertical) centre line of the system; (iii) drop volume V. This model and preliminary testing of the TLDA is described by McMillan et al [1]. The ray-tracing computer model is based on the Laplace model of the sessile drop and properly describes in a quantitative way the physics of the TLDA optical system. This model provides the tool for engineering the optimized system and ensuring the Spectroscopic signal has the best signal-to-noise.

Figure 1. Optical arrangement for sessile drop.
The design of the TLDA seeks to provide ultra-accurate, repeatable measurements on microvolume samples. The TLDA can work as an instrument accessory as shown with a Agilent Cary 50 spectrophotometer in Plate 1 or as part of a collection of components as shown in Plate 2. When working with the Cary50 unit it operates with the Cary external source for this market leading spectrometer or the unit can be adapted to work with any existing UV-visible spectrophotometer with SMA fibre ports. When part of the latter compact system measures it can operate for example with an Ocean USB 4000 spectrometer inside the housing, or alternatively with two fibres to be used with any standard UV-visible spectrophotometer with SMA fibre plug-in source and detector access.

The TLDA, micro-volume spectrophotometer, is a patented ‘real drop physics’ technology that allows for sample volumes as small as 1 µL. Because of this mastery of drop physics it can offer unequalled quantitative performance. The TLDA offers good quantitation for a range of analytical science applications. It has many advantages over other spectrophotometric hardware. Direct, easy measurements can be made in a few seconds – just pipette & wipe it has improved sample handling as the TLDA employs only the drophead surfaces, two optical surfaces are used in some other commercial instruments. The sample placed on this single drophead surface is presented to the source as a discrete and pristine sessile drop. Full spectral output from the spectrometer can be obtained with the Drop Technology TLDA software or working simply and directly with the standard software of the relevant fiber spectrophotometer. Measures of DNA, RNA and Protein concentrations and sample purity can be obtained. Pre-configured methods for common applications such as Nucleic Acid, Protein A280, Bradford, BCA, Biuret, Lowry and Pierce 660nm re incorporated into the TLDA software. The TLDA has an impressive concentration dynamic range without dilutions as drop volumes vary the average pathlength and thereby optimising the concentration range that can be measured. Regular and very simple calibration checks using CRM (certified reference material) standards are provided for those who are concerned about accuracy and minimising measurement error. A full set of Drop Technology accessories including special 1mm optically polished reference cuvettes for absolute calibration of pathlength and absorbance measurement on microvolume samples are available. No instrument adjustments are required and accurate average pathlengths are determined by the pipette setting of the drop sample volume and of course there is low-cost operation – no plates or other consumables.

The TLDA has important features that are worth briefly outlining. The rotating sample loading operation uniquely and importantly can be used with equal facility by either right or left handed users; the top of the instrument rotates in opposite directions. One important practical issue is that the TLDA operates with low cost pipettes with proven unparalleled dispense volume accuracy and surprisingly deliver outstanding measurement quantitation. The TLDA has minimal maintenance and set-up requirements given its simple optical configurations and the drop samples can be viewed directly by
the operator after they are placed on the drophead. It provides considerable time saving in sample preparation and the customised work station for positioning of components and ensuring efficient work practice.

There are a number of benefits associated with the TLDA not least the fact that the accessory can be employed with many user’s own existing hardware and software. The multi-functioning of this drop instrument/accessory provides access to a variety of analytical techniques. The TLDA can be used in a wide-range of applications areas e.g. bio, pharma, medical, chemical, environmental, food and engineering laboratories. Drop Technology Windows based software can be designed for your specialist applications and includes Custom Methods and data export capabilities to Excel. In addition, the TLDA has some important features.

2. Experimental
2.1 Evaporation

The potential evaporation of a drop put on a substrate is a recurrent issue met by all the experimenters who measure sessile drops. Therefore, a study was conducted to investigate the evaporation effect of small volume drops within the TLDA system. The TLDA evaporation ring creates a high humidity closed system where evaporation from the liquid surface in the reservoir occurs and saturates the air space, in preference to loss from the drop.

A reference water drop was placed on the measurement plinth. The absorbance over a twenty minute time scale was recorded. A constant zero absorbance would be expected if no evaporation occurred. Three repeat measurements were carried out in both cases of (a) not filling the evaporation ring with liquid and (b) filling the reservoir. The new drop in each case was saved as the reference drop. The absorbance was measured at 250nm as this would be considered the worse case scenario as the signal slightly drifts with time due to the solarisation of the fibres and also the grating performs least effectively at this wavelength.

Figure 2. Evaporation rate study for a 3µl water drop.
The graph shows that provided the measurement is completed within the first three minutes, no significant evaporation occurs. However, even over a twenty minute period, the results show that by filling the reservoir with liquid reduces the evaporation effect to acceptable levels.

2.2 Photometric Testing
Drop Technology supplies two certified Reference Materials (DTRM-2SG & DTRM-4SG) for validation of the absorbance scale of your TLDA in a screw-top vial format. These CRMs are certified for both peak wavelength and Absorbance values in a calibrated 1mm pathlength cuvette, together with appropriate expanded uncertainty budgets \((k=2)\). Certification is in compliance with ISO Guide 34 & ISO/IEC 17025 protocols. The approach to the calibration of photometric accuracy and reproducibility is illustrated by the statistics of the experimental studies. The wavelength reproducibility and accuracy can also be obtained with these standards.

![Figure 3. Spectra of the calibration standards](image)

The absorbance of the standards should be measured at the three peak wavelengths specified on the certification. In the example shown below, these wavelengths are 257.5nm, 415.8nm and 629.5nm. The absorbance scale is verified by using the certified pathlength (supplied with each TLDA plinth) and applying the Beer-Lambert law to the certified reference values. The spectra of the two reference standards are shown in Figure 3 along with the data in Table 1. These pathlength ‘corrected’ values are then compared to the actual measured values.

The TLDA software carries out this test under the calibration tab. It requests 10 measurements to be made and determines whether the calibration has passed or failed. Regular repeated use of these CRMs will not only provide ‘Evidence of Control’ but also the raw data for additional Quality Assurance (Q.A.) procedures such as Control Charts, etc.

2.3 Carry-over Study
Two tests were run to demonstrate the lack of sample carryover. A high concentration sample was measured followed by measurements of water samples or a higher concentration sample. The tests indicated no significant presence of sample carryover by simply cleaning with a lab tissue in between sample loading. The DNA absorbance was read at 260nm and was converted to its equivalent concentration value. Table 2 shows the concentration values obtained for the five repeat readings and Figure 4 shows the repeat DNA spectra.
The absorbance of the potassium dichromate solutions was recorded at 350nm. Table 3 shows the concentration values obtained for the ten repeat readings and Figure 5 shows the spectra.

| Sample ID | Concentration [ng/µl] |
|-----------|-----------------------|
| Water     | 0.0                   |
| Water     | 0.8                   |
| Water     | 0.4                   |
| Water     | 0.4                   |
| Water     | 0.8                   |
| Sample A  | 539.7                 |
| Sample A  | 540.5                 |
| Sample A  | 539.3                 |
| Sample A  | 542.2                 |
| Sample A  | 540.5                 |
| Water     | 0.8                   |
| Water     | 0.4                   |
| Water     | 0.4                   |
| Water     | 0.0                   |
| Water     | 0.0                   |

The spectra of the UV standards show insignificant carry over as sample2 is the same after running Sample1 in between.

Table 3. Absorbance values of the UV standards.

| K₂Cr₂O₇ Concentrations | Sample2 | Sample1 | Sample2 |
|------------------------|---------|---------|---------|
| 1.275                  | 0.127   | 1.225   |
| 1.233                  | 0.152   | 1.275   |
| 1.309                  | 0.177   | 1.182   |
| 1.284                  | 0.186   | 1.309   |
| 1.292                  | 0.152   | 1.292   |
| 1.242                  | 0.144   | 1.309   |
| 1.368                  | 0.118   | 1.275   |
| 1.242                  | 0.127   | 1.258   |
| 1.225                  | 0.118   | 1.267   |
| 1.292                  | 0.152   | 1.258   |
| Average                | 1.276   | 0.145   | 1.265   |
| Std. Dev.              | 0.044   | 0.023   | 0.039   |

The spectra of the UV standards show insignificant carry over as sample2 is the same after running Sample1 in between.

2.4 The Development of Microclimate Control of Drop Evaporation

One of the authors in a test programme [2] found problems with the evaporation of drops causing noticeable variation in the signal over times frames of minutes. To obviate this problem a circular reservoir was fitted to the drophead which can be seen in Plate 3. The drophead is in fact removable and the reservoir with drophead can be popped out of the TLDA using the Drop Technology tool and a new drophead put in its place. The photograph shows the procedure of drophead removal. The reservoir generates a microclimate around the drophead and reduces evaporation to a negligible amount.
3. Conclusions
This study demonstrates very clearly the value of product beta-testing. The effects of evaporation of the microdroplets were not in any way significant in Ireland. Consequently, the instrument tested well in Dublin. However, the important testing conducted in Jasper, USA showed that drop evaporation was a practical limitation to the performance of the TLDA when operated in dry atmospheric conditions such as found in desert regions.

The evaporation ring was a simple practical answer and then shown by testing to get over this problem. This plastic ring is one that has the added virtue of allowing dropheads to be simply removed as it easily clicks in or out. A special purpose tool has been designed to make the removal a trivial operation. New TLDA drophead are delivered in packaging that in fact acts as a tool for the insertion of the new drophead and avoids any contact by fingers of the measurement surface.

The testing on carry-over and the photometric testing show very satisfactory results when compared to published results of other microvolume instruments.

The study here reports some of the beta-testing trails undertaken to prepare for the launch of this product as it transfers from research into development and onto a full commercial entity. A golden rule in engineering is to over test to guard against product recall. The presentation of this material will no doubt be useful to those with a very specific interest in microvolume spectroscopy but also perhaps to those who can see the general lessons to be drawn from the experience of the authors.

References
[1] McMillan N.D, O’Neill M, Smith S.R.P, Hammond J, Riedel S, Arthure K and Smith S 2009 Reliable TLDA-microvolume UV spectroscopy with applications in chemistry and biosciences for microlitre analysis and rapid pipette calibration J. Opt. A: Pure Appl. Opt. pp 11-22 doi:10.1088/1464-4258/11/5/054016.

[2] Morris M 2010 Private Testing Report of TLDA