Testing by photometric measurement and camera study of theoretical prediction of microvolume universal sessile dropshape
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Abstract. The approach to the theory of sessile dropshapes held on a cylindrical drophead is discussed. It reveals an ‘undifferentiable’ universal micro-dropshape for volumes below 3µL. Camera studies demonstrate the veracity of this prediction exploited in the design of a new microvolume spectrometer. The mean pathlength of light injected through a microvolume sessile drop has been determined both from the model and from experiment. Drop volumes determine accurately the mean pathlength and with this Beer’s law relationship is experimentally confirmed. The Transmitted Light Drop Analyser uses this universal ‘natural cuvette’ to deliver both high-performance UV spectra and absorbance measurements at discrete wavelengths.

1. Introduction
The Transmitted Light Drop Analyser (TLDA) has been developed by the authors based on serious attention to drop physics including developing a model of the optics to optimize the performance of the instrument[1]. This theoretical study was the extension of a major piece of work developing drop spectroscopy for pendant drops[2] that extended from the first 1992 patents. These studies place drop spectroscopy on a firm theoretical foundation. This present study is undertaken to prove theoretical facts that are of some practical importance in it shows that all microvolume drops supported on a cylindrical drophead have the same shape irrespective of the properties of the liquid. Given this fact, it is an obvious step to suggest these universal liquid entities could be a ‘natural cuvette’ for spectroscopy.

2. Experimental
2.1 The Laplace Dropshape Model and Camera Testing
The modelling of drop shapes is done using a numerical integration of the Laplace-Young equation, which describes the shape of a sessile liquid drop in equilibrium under surface tension and gravitational forces. The drop has radius \( r_0 \) at its base, where it sits on the drophead. At a point Q on its surface (radius r, height z above the bottom of the drop), there is a pressure difference \( \Delta p \) between the inside and the outside of the drop.

The pressure forces are balanced by surface tension forces given by the classic Young-Laplace equation:

\[
\Delta p = p(\text{inside}) - p(\text{outside}) = \gamma \left[ \frac{1}{R_{\perp}} + \frac{1}{R_{\parallel}} \right] \tag{1}
\]

where \( R_{\perp} \) and \( R_{\parallel} \) are respectively the radii of curvature out of plane and in plane at Q, and \( \gamma \) is the surface tension.

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The derivation of the equations used in the drop shape model is well known. The equations that are important here as they are the ones used in the computer model using the two characteristic dimensionless parameters $\beta$ and $X_0$, defined by

$$\beta = \frac{\rho g r_0^2}{2\gamma} \quad X_0 = \frac{r_0}{R_0} \quad (2)$$

$\beta$ is characteristic of the liquid that constitutes the drop, and is the same for all drops formed with the same liquid and the same orifice, whilst the parameter $X_0$ (the inverse relative radius of curvature at the apex of the drop) changes with drop size. $R_0$ is the radius of curvature at the apex of the drop.

The computer model for drop shape is implemented using MatLAB and Runge-Kutta methods that are well known. Figure 1 shows the result for a 3 µL drop of radius $r_0 = 1$ mm. It is obvious from (2) that $\beta$ will be controlled by the $r_0^2$ term. For small drops it is found that despite density and surface tension variations, the value of $\beta$ becomes small regardless of the value of the physical quantities and all drops have consequently indistinguishable shapes. A simple physical way of viewing this matter is the surface tension forces become much larger than the gravitational forces and constrain all drop types to have measurably indistinguishable shapes. The drops here are attached to a drophead but if they were free of this surface they would all be spherical.

![Figure 1. Semi-drop shape for a 3 µl drop of radius $r_0 = 1$ mm.](image)

**2.2 Pathlength Studies and Beer’s Law Relationship of Drop Spectroscopy**

The ray-tracing model developed for the TLDA is quantitatively accurate and can be used to predict the pathlength of rays refracted through the drop sample. The most important application for this type of technology is probably DNA analysis and the experimental work has as a consequence been done here on this type of fluid.

It does not follow directly that if drop shapes are indistinguishable the mean pathlength through a drop is the same, because the refractive index variations introduce differences in ray paths even if drop shapes are identical. The model suggests this is a perturbation and indeed mean pathlengths are almost indistinguishable. Experimentally this hypothesis was tested using a range of potassium dichromate solutions which were measured on the TLDA and a conventional UV/Visible Spectrophotometer (1 cm
Good agreement is shown between the experimental pathlength results from the TLDA and the physical model.

Camera images of water, the TE buffer and three DNA solutions of different concentrations were recorded. The four repeat drop measurements of each solution were shown to overlap in drop profile. Therefore, one sample drop from each solution was taken and overlaid using the Layers function in Photoshop to confirm the profiles were the same for small volume drops. A summary of the results are shown visually in Figure 2.

Figure 2(a). Comparison of the drop profile of a 3 μL water drop from a camera image and the physical model.

Figure 2(b). Shows the profiles obtained from camera images of water, buffer and DNA drops. This was to determine whether the different small volume liquids had the same shape and hence the same pathlength.

Figure 2(c). Overlaying of the profile images for the different samples in (b).

The drop profiles of water, TE buffer and 3 DNA drops of different concentration are shown in Figure 2(b). These drop images show that the drop shape and size are indistinguishable for water and DNA samples of such small volume drops (3 μL). This is not much of a theoretical challenge in tacking extremes of physical constants, but indeed water and ethanol drops would also be indistinguishable. Obviously, the choice of sample in these studies have been undertaken specifically to support the DNA assay work.
involved with the mean pathlength. This was fully described in a paper by McMillan et al [2]. The two most important findings of this study were that for drop spectroscopy there is a simple analytical Beer’s Law relationship confirmed experimentally by potassium dichromate standards, the results of this study are presented in Figure 4.

![Potassium Dichromate - 3ul drops](image)

**Figure 4.** Results showing Beer’s law relationship for TLDA with Starna standards and results from replicate readings showing $3\sigma$–error bars.

Secondly, the pathlength variation seen in Figure 5 is known theoretically and has been confirmed experimentally. The scope of this study is obviously too large here to present fully. A large number of measurable quantities beyond those seen in this figure have however been predicted and successfully confirmed.

![Dependence of transmission, mean pathlength and variance in pathlength ($T$, $P$ and $\sigma_p$) on $V$ for a large radius (2 mm) input fibre.](image)

**Figure 5.** Dependence of transmission, mean pathlength and variance in pathlength ($T$, $P$ and $\sigma_p$) on $V$ for a large radius (2 mm) input fibre.
The mean pathlength shows a very small variation with position of the input fibre. If the fibre has a diameter of greater than 2mm (modeling results shown here in Figure 5) then it has been discovered that no variability is found, although in practice it is very difficult to obtain such multimode fibers. Using 1mm or 600 micron fibres still however gives a very acceptable performance for the TLDA.

3. Conclusions
The cuvettes for the TLDA are designed by nature and microdrops are of a precise reproducible shape for any given volume. The pathlengths are known from modelling and experimentally determined. Errors nevertheless occur in the TLDA pathlength derived from errors in pipette volumes. The study has provided insights into important physical aspects of the TLDA design and given some insights into the thinking that led to its invention. The study has combined modelling, photonic testing and camera studies to demonstrate the universal (liquid independent) sample shape of a chosen sample volume. The TLDA uses microvolume drops that allow mean pathlength to be determined by pipetted drop volume. The dynamic range of the TLDA is consequently much greater than on a traditional UV-visible spectrophotometer perhaps 0-50 A-units. Positional invariance of fibre position can be obtained if a large diameter source fibre (above 2mm) is used but very good instrument performance can be obtained with fibres above 500 microns.

References
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