Researches towards establishing procedures and protocols for testing instrumental accuracy and reproducibility of UV-visible microvolume drop spectroscopy instrumentation

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Abstract. The Reference Materials (DTRM-2SG & DTRM-4SG) for validation of the absorbance scale for a Transmitted Light Drop Analyser (TLDA) have been developed specifically for microvolume spectroscopy. The approach to the calibration of photometric accuracy and reproducibility is briefly illustrated. These materials can be used also for wavelength checks. The study addresses however a wider range of issues for microvolume spectroscopy quality assurance. This study concludes with a general discussion of this quality programme on the important practical and philosophical issues needed for drop science quality systems.

1. Introduction

Reference Materials (RMs) for microvolume spectroscopy need to be liquids, or conceivably solids that could be dissolved up to make a reference solution. The first and most important issue in making proposals for RMs is the requirements concerning health and safety standards for the handling of these materials. The RMs have to be chemically safe in all regards including: environmental and health effect including many aspects. The list includes physicochemical characterisation; properties of the materials; identification of any explosion risks; hazard information like acute and chronic toxicity, toxicokinetics, ecotoxicity, bioaccumulation, etc.; safety with regards to cell and tissue penetration, potential circulatory effects, mutagenicity and genotoxicity and many other such issues. Given the absence of established external quality assessment schemes for such a new technique as drop spectroscopy, the aim is here to advance the discussion on RMs for microvolume spectroscopy. Obviously, the drop spectroscopy RM must have very accurate and reproducible drop spectra that can be obtained in a simple and well-established measurement procedure, and must deliver property values suitable for its intended use, up to its expiration date. The property value can therefore be used as a reference value for inter-comparison purposes.

In summary the following criteria should be considered in assessing the fitness of a RM are:
(i) they should be chemically safe.
(ii) their property values should be stable for an acceptable time period, under realistic conditions of storage, transport and use.
(iii) they should be sufficiently homogeneous that the property value(s) measured on one portion of the batch shall apply to any other portion within acceptable limits of uncertainty.
(iv) be manufactured in a well-defined and regulated manner.
(v) provide good inter-laboratory correlation.
(vi) generate reliable test data from automatic, semi-automatic and manual equipment.
(vii) the tensiographic property values of the RM should have been established with a precision and accuracy sufficient to meet the needs of the end users and should include spectroscopic, photometric and volume standards.

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The solution is happily at hand for the UV-visible region of drop spectroscopy as there exists a full gamut of reference materials in this field of spectroscopy which is the most established of conventional spectroscopy disciplines. These materials are available commercially from a number of suppliers but the authors have worked here with Starna Scientific in the development of Reference Materials (DTRM-2SG & DTRM-4SG) [1]. Well-established techniques can be readily adapted for drop spectroscopy and thereby be validated for these calibrations. There are of course numerous existing patents, procedures, methods and assays in existence for UV-visible which actually need to be updated as the theory of ‘true’ drop science and physics requires a new theoretical basis for this field of spectroscopy. These photometric reference materials have been employed in this present study to demonstrate that the technique is accurate and precise, and provide the essential check on instrument performance on a regular basis to ensure that it is within satisfactory parameters i.e. “under control”, and to allow for corrective action to be taken when found to be outside these limits. Their whole range of certified reference materials for absorbance/transmission, wavelength, resolution/bandwidth and stray light could be generally adapted to drop science measurement systems. It is possible that given the use of such materials applications of drop spectroscopy could be approved for use in GLP or ISO 9000 controlled environments, and in due course be accredited to ISO/IEC 17025. Therefore, using the above statement, it is conceivable that drop spectroscopic CRMs with a defined traceable path to national or internationally recognised primary materials or procedures could be made available. By routinely verifying the performance of an instrument it is possible to ensure consistent quality that meets expected performance demands, and drop spectroscopy will have to face up to the need to provide not only RMs but in due course CRMs, as more and more market sector laboratories come under enforced regulatory control. For example, it is a technique that will shortly have to face the most demanding field with respect to CRMs, namely forensic science [2] and this gives an excellent if ambitious guide to considerations for the adoption of RMs for drop science.

2. Experimental
2.1 TLDA Testing with RMs
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 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. These pathlength ‘corrected’ values are then compared to the actual measured values. The TLDA software organises the execution of this test procedure through the data recording under the calibration tab. It requests 10 measurements to be made and determines whether the calibration has passed or failed.

2.2 Thoughts on Quality Programme for Drop Science
Regular repeated use of RMs, and or CRMs, will not only provide ‘Evidence of Control’ but also the raw data for additional Quality Assurance (Q.A.) procedures such as Control Charts, Calibration Test Records, etc. The work undertaken by the authors is a start towards investigating some of these issues with respect to microvolume spectroscopy. It is important here to keep in mind issues related to Good Laboratory Practice (GLP) and in particular to understand the requirements for regulatory overview dealt with by Burgess and Knowles[3]. The first volume of the Burgess and Knowles publication dealt with the crucial issue of cell cleanliness in detail, which as a consequence was only dealt with in a cursory fashion in the second volume [4]. This issue is of course of absolute importance to microvolume spectroscopy and here the conditioning of the quartz drop optics with absorbing lanolin to assist in drops adhering to the plinths has been recommended for some assays and raises serious issues regarding drophead cleanliness. Indeed, the recent development of microvolume spectroscopy raises many new issues of ‘Quality Systems’ in this area of analytical measurements. The performance characteristics and quality are of course central now to good laboratory procedures [5]. This present work here seeks to begin discussion on some new facets of microvolume quality standards, accreditation, methodologies and protocols that will advance quality systems for these new areas of spectroscopic work for drop physics techniques.
The first check beyond the obvious standard RM or CRM procedures for photometric accuracy and reproducibility is to provide a validation to determine the pathlength for microvolume drop spectroscopy. This determination required a reference instrument with proper known performance characteristics. When working with ‘drop physics’ methods a development of standard Beer’s law methods is necessary in considering these RM issues as shown by recent work [6]. The basis for these ‘true drop physics’ techniques is a modified Beer’s law developed on the basis of a ray-tracing Laplace-Young model of the drop. The absorbance/pathlength data from the Specord spectrophotometer used here was selected for the present work because of clear metrological considerations. Importantly, the SPEKOL® systems operating in the range of 190-1100 nm has been referenced against diverse quality standards such as Ph.Eur., USP, TGA and ASTM. The demand of analysis reliability of course requires a regular validation and given microvolume spectroscopy is a new and developing area, it is recognised immediately that it will be necessary to begin defining appropriate quality procedures for microvolume spectroscopy. Starna Scientific have developed recently manufactured a 1 mm pathlength cell. This special cell has been designed to provide a reference quartz cuvette with a pathlength appropriate to microvolume measurements. As with all such Starna cells, these 1mm cuvettes are polished to interferometric precise dimensions. Reference data from the 1cm and 1mm Starna cuvettes was then used to estimate the pathlength for a 3μl drop in the TLDA system by comparative measurements with an RM.

The testing involves applying the modified Beer’s law. The absorbance measure against the pathlength (A/ℓ) should have a constant and indeed predictable value for a RM. For RM the molar absorptivity has a well established value and the pathlength should be constant in the reference cuvette. From this modified Beer’s law it can be seen that an individual sample, should deliver an equal εc (Beers law) no matter what pathlength is used. For microvolume spectroscopy of course the pathlength is an average of various ray paths through the drop and will in the first instance depend on the microdrop sample volume. Comparative measurement between the reference and microvolume measurements should deliver experimentally the TLDA pathlength that agrees with that predicted theoretically from the Laplace-Young model. The value obtained for the path length we have defined as the ‘effective pathlength’ (ℓeff). The results are shown below to illustrate the thinking involved in developing our quality assurance procedures.
The same measurements were repeated on two other TLDA instruments (TLDA2 & TLDA3). This comparative approach is very useful and indeed important in properly determining the experimental "effective pathlength" of the TLDA. Such a series of comparative intra-instrumental measurements are indeed vital in clarifying these quality issues. This TLDA instrument was developed by the authors and detailed in Drop Technology [7]. It may be useful therefore to provide a brief discussion of the work involved in validating this new instrument as part of a more thorough going series of measurements and tests aimed at establishing top-down and bottom-up methods. The Excel spreadsheet from Dr Martina O’Neill who conducted these tests is shown to visually present the procedures to used here to check the predicted theoretical Laplace-Young model values. This is of course a top-down approach from quantitative theoretical values to experiment. The familiar top down RM and CRM traceable measurement issues are well known, but we are here clearly working also with a completely new range of bottom–up issues. Explicit use research knowledge of the instrument’s manufacture should allow extrapolation so as to confirm that all outcomes, that have not specifically individually checked, will still be met. In short, the new ‘drop physics’ demands the TLDA developers to re-evaluate every issue once again and not to simply assume that because these matters have been resolved for traditional Beer’s law instruments that these issues have been laid to rest. Such an assumption would be very dangerous given a new theoretical foundation has been defined for ‘drop physics’. The results presented here are meant to highlight new practical issues and begin this debate. This discussion is meant to underline the importance of developing these new quality approaches which deserve some greater attention than space allows here.

### 2.3 Theoretical Issues on Drop Science

Collimation of light is in non-laser systems really an approximation that in reality cannot seriously be stood over as an absolute achievable reality. When working with cuvettes, there is the vexed matter of multiple reflections from the four cuvette surfaces that further complicate the ‘collimation’ situation.
What is seen either in the spectroscopy associated with either pendant or sessile drop physics is that serious consideration must be given to what is meant by pathlength. This issue was theoretically addressed by McMillan, Smith et al [8] for pendant drops and then later specifically for the sessile drops of the TLDA. There is theoretical integral pathlength defined by \( \ell_1 = \int \rho(P(\ell)) \, d\ell \) computed from the integral of a series of rays passing through the drop of length \( \ell \), each path of which has a probability of \( P(\ell) \), and it is to be remembered the sum of these probabilities for all ray-traced paths is unity. Thus, the probability that the pathlength lies in the range \( \ell \) to \( \ell + d\ell \) be \( P(\ell) \, d\ell \), and normalized such that \( \int P(\ell) \, d\ell = 1 \). Above we have explained we can experimentally determine the effective pathlength \( \ell_{\text{eff}} \). It should be noted that \( \ell_1 \neq \ell_{\text{eff}} \). The idea of an rms pathlength \( \ell_2 \) which is defined from \( \ell_2^2 = \int \ell^2 \rho(P(\ell)) \, d\ell \) is a useful quantity where the rms pathlength is determined as the integral of the square of these paths. The variance in pathlength is defined mathematically as usual as \( \Delta\ell^2 = \ell_2^2 - \ell_1^2 \). If there is a truly collimated light source the \( \ell_1 = \ell_2 \), but this is obviously not the case in a microvolume drop spectrophotometer. It is found that Beer’s law applies in pendant and sessile drops only as an approximation and thus \( A_{\text{Drop}} = \alpha \ell_1 \). For what would be the theoretically and most exacting situations \( A_{\text{Drop}} = \alpha \ell_1 - k \varepsilon \ell_1^2 \Delta\ell^2 \) for the pendant drop where \( \varepsilon \) is the molar absorptivity. A similar first order approximation is required in the TLDA. A small correction applies to situations were we have deviating from the linear Beer’s law relationship. In summary, the effective pathlength is determined experimentally and can be compared with theoretical values derived from the ray tracing model of drops whose complex dropshapes are derived from a Laplace-Young predicted shape. Summarizing, drop physics will require a great deal more rigorous thought than has been required in traditional UV-visible spectroscopy with regards to the thinking connecting theory and practice in ‘drop physics’ based spectroscopy.

3. Conclusions
This are many recent developments pushing forward the analysis small liquid volumes, e.g. <3 µL, due to the increasing demand to preserve in non-destructive testing method of what are precious samples and especially in the biotechnology/pharmaceutical markets. The challenge of making reproducible measurements has of course been recognised by all the commercial leaders in the field. The theoretical and practical task of delivering such systems perhaps however falls to those developing ‘real drop physics’ such as commercialized in the TLDA. In theory, such ‘drop physics instruments’ should provide improved accuracy and reproducibility coupled with the ability to measure micro volumes in a user friendly piece of equipment. The corollary here is these instruments raise a whole raft of new quality issues. This paper has raised some initial thoughts and discussion on procedures for ‘drop physics’ quality requirements and it is clear that this demands attention to both proper definitions of pathlength and measurement protocols.

The user wants simplicity and increased productivity and if you consider our next generation of users as the young people now entering laboratories it is clear that they all live and breathe technology. They will like all new generations welcome the new demands of the technology. Advances in sample handling, associated with micro volume measurements are a good example of an important area of advance in the oldest laboratory technology, namely UV-visible. As applications and demand expand for lower volume sample measurements, new advances approaches are appearing in the field of micro measurements. Of course rapid advances in microelectronics resulting in the development of new light sources, such as high intensity LEDs covering the UV to the NIR region of the spectrum, will contribute to not only the advance of this area of spectroscopy, but also to an opening up of new combinations of spectroscopic instrumentation. Lab-on-a-chip will see new developments in
conventional spectrophotometers and help open the door to lower cost, handheld UV–visible measurement systems. Handheld and disposable instruments and measurement systems will be common. What is obvious that in these new developments there must be greater attention to standards and procedures of calibration and importantly traceability. The demand for UV–visible instruments will continue to increase as it is an inexpensive and non-destructive technique for measuring liquid and solid materials. The work here should open out the discussion of fundamental quality issues for drop physics and a new application field of analytical science.

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