Analytical Biochemistry 510 (2016) 52–55

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Technical note

A low volume 3D-printed temperature-controllable cuvette for UV visible spectroscopy

Jelena Pisaruka, Marcus K. Dymond*

Division of Chemistry, School of Pharmacy and Biological Sciences, University of Brighton, Huxley Building, Lewes Rd, Brighton, BN2 4GL, UK

ARTICLE INFO

Article history:
Received 23 May 2016
Received in revised form
1 July 2016
Accepted 15 July 2016
Available online 19 July 2016

Keywords:
3D-printing
Critical micelle concentration
UV visible spectroscopy
Thermochromic spectra
Water-heated cuvette

ABSTRACT

We report the fabrication of a 3D-printed water-heated cuvette that fits into a standard UV visible spectrophotometer. Full 3D-printable designs are provided and 3D-printing conditions have been optimised to provide options to print the cuvette in either acrylonitrile butadiene styrene or polylactic acid polymers, extending the range of solvents that are compatible with the design. We demonstrate the efficacy of the cuvette by determining the critical micelle concentration of sodium dodecyl sulphate at 40 °C, the molar extinction coefficients of cobalt nitrate and *ds*DNA and by reproducing the thermochromic UV visible spectrum of a mixture of cobalt chloride, water and propan-2-ol.

In recent years there has been a rapid expansion in the number and quality of commercially available, affordable, fused deposition modelling (FDM) 3D-printers. These FDM 3D-printers allow end users to design, test and construct bespoke 3D-fabricated plastic prototypes targeted to their own individual applications [1]. Researchers in the chemical and biomedical sciences have made bespoke integrated reactionware [2–7], DNA adhesives [8], inserts for cuvettes [9] or X-ray absorption spectroscopy [10] that enable spectroelectrochemistry to be performed, surgical models and synthetic organs [11] and microfluidic pumps [12]. However, whilst there are a significant number of recent research success stories demonstrating the potential applications of 3D-printers, a number of key challenges remain. In particular the additive manufacturing process of FDM printing has a tendency to create small gaps between successive extruded layers, meaning 3D-prints are not always air or watertight. This is a particular challenge for FDM 3D-printing in milli or microfluidic applications where the pressure in the device is increased. Strategies for solving this leakage problem differ, one approach is to construct devices with increased wall thicknesses, typically 4 mm [3], although this does impose a lower limit on the size of device that can be constructed. Alternatively, recent work [13] has shown that many of these printing imperfections in ABS prints can be removed with acetone and that 3D-prints treated in this way, post production, have potential uses in fluid handling on a variety of scales.

Here we report our recent success using FDM 3D-printing to develop an inexpensive water heated UV visible cuvette made from ABS or PLA, which fits into a standard UV visible spectrometer.

UV visible spectroscopy was performed on a dual beam Shimadzu Corporation UV-2410PC spectrometer equipped with a single monochromator. BRAND® disposable polystyrene cuvettes (Sigma–Aldrich UK) were used for control studies. Cobalt nitrate, sodium dodecyl sulphate (SDS) and *ds*DNA from salmon sperm were purchased from Sigma–Aldrich UK. Cobalt chloride was purchased from ACROS organics UK and acetone, propan-2-ol and methylene blue were purchased from Fisher Scientific UK. A REFCO (−1 to 3 Bar; class 1.6) pressure gauge and OMEGA® OM-EL-USB-TC thermocouple USB datalogger were used to measure pressure and temperature respectively. 3D-printing was performed on a Makerbot Replicator 2X 3D printer (Makerbot Industries), 1.75 mm diameter ABS and PLA filaments were manufactured by Eliphilament and Makerbot Industries respectively. Prior to printing the build platform was covered with ScotchBlue™ adhesive tape for optimal adherence of the 3D-print. 3D-prints were designed in Autodesk123 and exported as STL files into Makerbot Desktop for slicing. The final optimised 3D printing settings for ABS and PLA are summarised in Tables S1 and S2 and fully printable designs are included as supporting information.

---

* Corresponding author.
E-mail address: M.Dymond@brighton.ac.uk (M.K. Dymond).

http://dx.doi.org/10.1016/j.ab.2016.07.019
0003-2697/

The UV visible spectrometer that we used was configured to accept standard $12 \times 12 \times 45$ mm (width × depth × height) cuvettes; the incident beam centre was 15 mm from the bottom of the cuvette, and the 1 mm polystyrene walls leave a sample path length of 10 mm. Therefore our water heated cuvette needed the following design criteria to function effectively.

1. Sample path length of 10 mm
2. Sample centre 15 mm above the cuvette base.
3. Complete design no bigger than 12×12 mm (width × depth) and around 45 mm in height
4. Watertight

CAD designs of our 3D-printed water heated cuvette are shown in Fig. 1A; we solved a number of problems before coming up with the final working cuvette. The most significant challenge encountered was leaking, either from the water-jacket chamber or the sample chamber. The origin of these leaks was two-fold, firstly small defects between layers resulting from the FDM printing process. Secondly, at printed vertices in the horizontal and vertical planes, sagging of the horizontal printed surface resulted in small voids, which leak. As noted, small defects can be fused using organic solvents [13], however bigger defects at vertices require carefully designed 3D-prints. A key design feature of our cuvette, which prevented sagging in the horizontal plane, was the inclusion of an inverted pyramid structure beneath the sample chamber, as noted in Fig. 1B. The minimum and maximum sample volumes of the cuvette are 0.24 and 0.68 ml respectively.

Freshly printed ABS cuvettes were immersed in acetone (*circa* 20 °C) and freshly printed PLA cuvettes were immersed in chloroform (*circa* 20 °C) for 8 s to fuse defects between the printed layers. Excess solvent was removed and the cuvettes were dried in a fume hood for 2 h before optically transparent plastic, cut from disposable polystyrene cuvettes, was glued to the faces of the printed cuvette, Fig. 1A. To make the sample chamber watertight a slurry of ABS in acetone or PLA in chloroform was used as the glue. This provides an optical quality plastic, which cannot be 3D-printed. For studies utilising *ds*DNA we extended the working wavelength range of the cuvette by gluing on quartz slides using Gorilla™ super glue adhesive. This particular product was chosen due to it retaining strength at 100 °C. After drying, cuvettes were pressure-tested to approximately 200 kPa by connecting the water input to a plastic syringe and sealing the output. Only cuvettes that passed this pressure test were used in subsequent studies, where they were connected to a recirculating water bath and fitted into the UV visible spectrometer. It should be noted that 8 s immersion in organic solvent was optimal and less than 1 in 5 cuvettes failed the pressure test. However it is likely that ambient temperature and the initial resolution of the 3D print will affect success rates.

To ascertain how long samples would need to be equilibrated for during measurements, we characterised the heating rate of the cuvette. This was achieved by attaching the thermocouple probe the internal walls of the cuvette sample chamber. From an ambient water temperature of 17 °C up to a maximum working temperature of 65 °C, the glass transition temperature of PLA, the water bath heated linearly at a rate of $0.033 \pm 0.001\,^{\circ}\mathrm{Cs}^{-1}$. Over the same temperature range the cuvette connected to the water bath heated linearly at $0.031 \pm 0.001\,^{\circ}\mathrm{Cs}^{-1}$ indicating no significant lag between the water bath and cuvette heating. As a precaution samples were left to equilibrate at a constant temperature for 5 min prior to measurement of their absorbance.

Using both disposable polystyrene cuvettes and our 3D-printed cuvette we determined the value of the molar extinction coefficient (ε) for $Co(NO_3)_2$, at 510 nm, to be 480 ± 10 $\mathrm{m^2mol^{-1}}$ in water, indicating that the 3D-printed cuvette makes measurements as accurately as disposable plastic cuvettes. Fig. S1A shows a plot of absorbance versus concentration for solutions of $Co(NO_3)_2$ determined in the 3D printed cuvette at room temperature.

As a further test we determined ε, at 260 nm, of *ds*DNA from salmon sperm in the 3D-printed cuvette (with quartz covers glued to the sample chamber) at room temperature and compared it to the value we calculated using quartz cuvettes. Both sets of cuvettes gave molar extinction coefficients of *ds*DNA of 0.025 ± 0.0005 $(\mu\mathrm{g/ml})^{-1}$ cm^{-1}. The ratio of absorbance at 260 and 280 ($A_{260/280}$), a measure of the protein contamination in DNA, was 1.81 ± 0.01 for both cuvette systems. Indicating that the 3D-printed cuvette with quartz covers also gives reproducible results when compared to the data obtained in quartz cuvettes. Fig. S2A shows a plot of DNA absorbance at 260 nm versus concentration determined in the 3D-printed cuvette and Fig. S2B shows the UV visible spectral plots of DNA from 200 to 400 nm at a range of concentrations obtained in the 3D-printed cuvette.

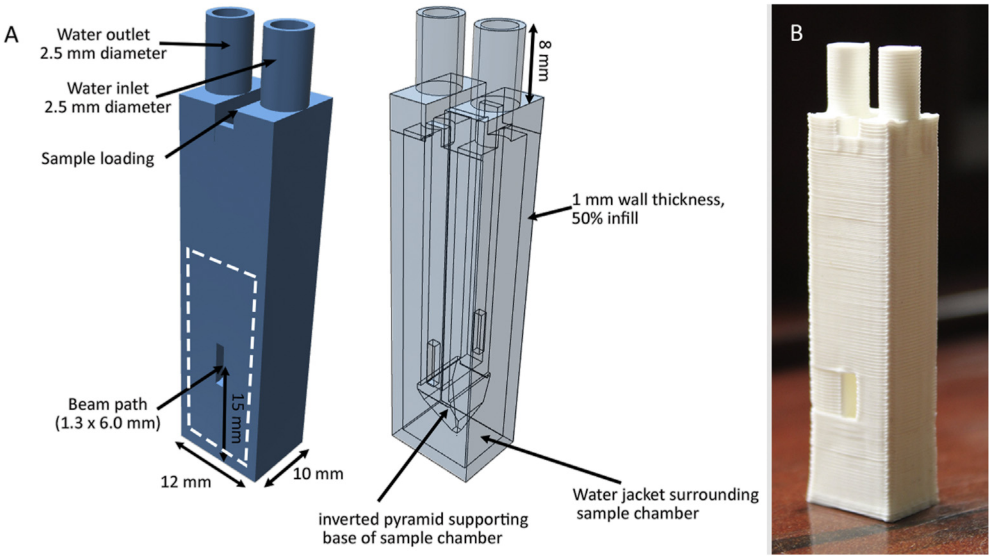

Fig. 1. A) 3D-printed water heated cuvette, showing key dimensions and design features. The white dashed box indicates the approximate location of where optically transparent polystyrene, cut from polystyrene cuvettes was glued. Fig. 1B shows a photograph of final cuvette printed in PLA.