Configurable 3D-Printed Millifluidic and Microfluidic ‘Lab on a Chip’ Reactionware Devices

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1 General Experimental Remarks

All chemical reagents and solvents were purchased from Sigma Aldrich and used without further purification.

**Design Software:** The 3D-printed labware used in this work was designed on the freely distributed 3D CAD software Autodesk123D (http://www.123dapp.com/) although any 3D modelling / CAD software with the ability to export models in a .STL file format would suffice for this, and there are a number of suitable alternative free / open source candidates available on the internet. The device designs were exported as .STL files (available from the authors), which was then interpreted by Bits from Bytes Axon 2 software which produces a 3D printer instruction file (.bfb file) which was subsequently transferred to the 3dTouch™ 3D printer. The printing was conducted in a layer-by-layer fashion by the 3dTouch™ printer, and the devices were printed using polypropylene (PP), and were subsequently fitted with standard PTFE 1/16” OD tubing and connections.

**Device Setups:** All solutions were pumped by means of C-3000 syringe pumps from Tricontinent equipped with 1 mL syringes. An in-house developed Labview application was employed to program the pumps to deliver the desired flow-rates and to control the UV-Vis and IR spectroscopy.

**UV-Vis spectroscopy:** UV-Vis spectra were acquired with a DH-2000 light source and a flow cell FIA-Z-SMA 905 (10 mm pathlength) from Ocean Optics, connected by fiber optics to a AvaSpec 2048 from Avantes. Spectra were collected every 1-2 seconds employing a customized program and processed employing an in-house developed program with Labview.

**IR spectroscopy:** IR spectra were collected employing a Nicolet IS-5 from Thermo Scientific and a ZnSe Golden Gate ATR from Specac equipped with a flow cell. The resolution was set at 4 cm⁻¹ and 16-80 scans were recorded.

**DLS:** DLS spectra were recorded with a NanoZS from Malvern Scientific employing disposable cuvettes 24 hours after collection of samples.

**Mass Spectrometry:** The spectra were recorded using a JEOL JMS 700 (FAB / EI / CI). The observed ca. m/z values are listed.

**Optical Microscopy:** Optical microscopy was performed on a Keyence VHX-600 (Gen II) digital microscope with a 20-200x lens. 3D Channel profiles were calculated by varying the focal length of the microscope in increments of 5 microns and using the Keyence digital microscope software to compile the resulting images into a 3D plot of the channel cross-section.

**NMR Spectroscopy:** All NMR data were recorded on a Bruker Advance 400 MHz, in deuterated MeOH from Goss Scientific, at T = 300 K. All chemical shifts are given in ppm. The peaks are denoted s = singlet and m = multiplet.
2 Organic syntheses

2.1 Imine Synthesis

A 1 M methanolic solution of benzaldehyde (inlet B) was mixed with a 1 M methanolic solution of benzylamine (inlet A) in R1 at the same flow rate (see Figure S2). The total flow rates ranged from 10 to 200 μL min⁻¹. The outlet of the reactor was connected to the flow-cell in a Golden Gate ATR-IR machine. The products were analysed by flow ATR-IR and standard ¹H NMR. ¹H NMR: (400 MHz, CDCl₃) (δ, ppm) 8.32 (s, 1H), 7.72–7.70 (m, 2H), 7.35–7.33 (m, 3H), 7.27–7.24 (m, 4H), 7.20–7.17 (m, 1H), 4.75 (s, 2H). IR: 1704 cm⁻¹ peak from C=O moiety disappeared, 1644 cm⁻¹ peak from C=N-C moiety appeared.

2.2 Secondary amine synthesis

A 1 M solution of benzaldehyde in MeOH was pumped through inlet B into R2 at 2.5-5 μL min⁻¹ and mixed with a 1 M solution of benzylamine in MeOH introduced through inlet A at the same flow rate, allowing sufficient residence time to synthesise the corresponding imine. A reducing agent, namely cyanoborohydride (1 M), was introduced through inlet C at the same flow rate to produce the corresponding amine (see Figure S3). The samples were characterised by flow ATR-IR and MS spectroscopy. MS: (EI⁺) calculated for C₁₄H₁₅N (M⁺) m/z 197.12, found 197.09. IR: 1704 cm⁻¹ peak from C=O moiety disappeared, 1644 cm⁻¹ peak from C=N-C moiety disappeared (see Figure S4).
2.3 Tertiary amine synthesis

A mixture of benzaldehyde (1 M) and cyanoborohydride (1 M) (1:1) (v:v), in methanol, was introduced into R2 through inlet B at 5 μL min⁻¹. Simultaneously, a 1 M solution of benzylamine was pumped through inlet A at 2.5 μL min⁻¹. Allyl bromide (0.25 M) was employed as alkylating agent through inlet C at 7.5 μL min⁻¹. The samples were analysed by flow ATR-IR and MS spectroscopy. The results were a mixture of secondary and tertiary amines containing dibenzylamine (1), tribenzylamine (3) and the product corresponding to the alkylation of dibenzylamine allyl-dibenzyl-amine (2) (see Figure S5). MS: (CI⁺) calculated for C_{14}H_{15}N (1), C_{17}H_{19}N (2) and C_{21}H_{21}N (3) [M+H]⁺ m/z 198.13 (1), 238.16 (2), 288.18 (3), found 198 (1), 238 (2), 288 (3).
Figure S5: An overview of how R2 was used for an alkylation process. The reducing agent does not react with the aldehyde; hence the alkylolation step can take place without the introduction a fourth inlet.

3 Inorganic syntheses

3.1 \{Mo_{36}\} Synthesis

The synthesis of the \{Mo_{36}\} cluster ([Mo_{36}O_{112}(H_2O)_{16}]^{8-}) was successfully conducted in both R1 and R2. A 0.625 M aqueous solution of Na$_2$MoO$_4$·2H$_2$O was pumped through inlet A of R1 at 50 μL min$^{-1}$, while HCl (1 M) was pumped at the same flow rate through inlet B of R1. The product was characterised by UV-Vis spectroscopy and DLS analysis. **UV-Vis:** $\lambda_{\text{max}} = 360$ nm (see Figure S6). **DLS:** Particles of average hydrodynamic diameter of 1.5 nm were observed (see Figure S8 (left)).

Figure S6: Time dependent UV-Vis spectra corresponding to the synthesis of \{Mo_{36}\}.
3.2 \{\text{Mo}_{154}\} Synthesis (Molybdenum blue)

A 0.625 M aqueous solution of Na$_2$MoO$_4$ was introduced into R2 through inlet A. At the same time, a 1 M solution of HCl was introduced through inlet B. After mixing in the reaction channel, a third solution containing hydrazine (0.12 M) was introduced through inlet C. All solutions were pumped at the same flow rates. The total flow rates were 18.25, 37.5 and 75 \(\mu\text{L min}^{-1}\), respectively. The product was characterised by UV-Vis spectroscopy and DLS analysis. **UV-Vis:** Main \(\lambda_{\text{max}} = 750\ \text{nm}\) (some \{\text{Mo}_{36}\} with \(\lambda_{\text{max}} = 360\ \text{nm}\) in also present). See Figure S6. **DLS:** Particles of average hydrodynamic diameter of 3.6 nm were observed (see Figure S8 (right)).

![Figure S7](image)

Figure S7: Time dependent UV-Vis spectra corresponding to the synthesis of \{\text{Mo}_{154}\} from \{\text{Mo}_{36}\}. The pH of the final solutions were between 0.4 and 1.9, which is \{\text{Mo}_{154}\}, \{\text{Mo}_{150}\} or \{\text{Mo}_{148}\}, or a mixture, depending on the accurate pH value.

![Figure S8](image)

Figure S8: The hydrodynamic diameters observed from the DLS analysis; (left) \{\text{Mo}_{36}\}, and (right) \{\text{Mo}_{154}\}.
3.3 Synthesis of \{\text{Mo}_{154}\} using R3

During the printing of R3 the print process was paused and solid sodium molybdate (Na$_2$MoO$_4$·2H$_2$O, 300 mg, 1.24 mmol) was added to “silo A” and hydrazine dihydrochloride (NH$_2$NH$_2$·2HCl, 20 mg, 0.19 mmol) was added to “silo B”, before the print process was restarted and the “silos” closed up (see Figure S9). A pH 1 solution of HCl was pumped at 25 μL min$^{-1}$ consecutively through both chambers. The formation of \{\text{Mo}_{154}\} was observed in the outlet stream by off-line UV-Vis and DLS analysis.

![Figure S9](image)

Figure S9: (top) An overview of the inlet, silos A and B, and the outlet. (bottom) The bottom of silo B and the outlet have turned blue indicating the presence of molybdenum blue.

4 The synthesis of gold nanoparticles

R1 was employed in this synthesis. A (1:1) mixture of aqueous solutions of HAuCl$_4$ (0.2 mM) and sodium citrate (2 mM) was pumped through inlet A at 50-60 μL min$^{-1}$, while an aqueous solution of sodium borohydride (10 mM) was pumped through inlet B at the same flow rate. The synthesis was followed by flow UV-Vis spectroscopy. The absorbance intensity of the gold nanoparticles decreased over time probably due to the deposition of gold on the reactor walls (see Figure S10). **UV-Vis:** λ$_{\text{max}}$ = 537 nm. **DLS:** Particles of average hydrodynamic diameter of 10.1 nm were observed.
Figure S10: Time dependant UV-Vis data showing how the intensity of the absorption maxima of the gold nanoparticles decreased over time.