COPPER COMPLEXATION OF MACROCYCLIC MOLECULES: TOWARDS ON-CHIP RADIOMETALLIC LABELLING OF PET RADIOTRACERS

Mark D. Tarn, Bingyuan Lu, Rachel Smith, Benjamin P. Burke, Stephen J. Archibald and Nicole Pamme*

Department of Chemistry, The University of Hull, Cottingham Road, Hull, HU6 7RX, UK

ABSTRACT

We present the use of microfluidic devices for copper(II) ion complexation of cyclen and cyclam macrocycles. On-chip monitoring of the complexation reactions was achieved via absorbance detection. This work opens the door for the on-chip synthesis of new ^{64}Cu based radiotracers that are important for positron emission tomography (PET).

KEYWORDS: Positron Emission Tomography, Macrocycles, Cyclens, Cyclams, Copper complexation

INTRODUCTION

Microfluidic devices have shown great promise for the production of PET radiotracers [1], but have generally been limited to the incorporation of short-lived isotopes such as ^{18}F and ^{11}C into small molecules. However, due to their short half-lives, these radiolabels are often unsuitable for the monitoring of processes that occur over longer periods of time, such as those involving large biomolecules like proteins, peptides, antibodies, and even nanoparticles. Furthermore, their conjugation to such biomolecules for *in vivo* monitoring is often not suitable given the synthetic routes available and the timeframes available to accomplish them before the radioisotope decays too much. The use of radiometals, such as ^{64}Cu ($t_{1/2}$ = 12.7 h) and ^{68}Ga ($t_{1/2}$ = 68 min), has garnered a great deal of interest for PET imaging due to the relative ease with which they can be complexed with chelating molecules such as macrocycles, that can in turn be conjugated to suitable biomolecules. The long half-life of ^{64}Cu makes it particularly suitable to the monitoring of long-term biochemical processes within the body, whilst also allowing a great deal of leeway in terms of the types of syntheses that can be performed [2-4]. At present, very little work has been undertaken on microfluidic radiometal labelling, with only one group having explored its potential via the ^{64}Cu labelling of a cyclen-based macrocycle (DOTA) conjugated to a peptide [5]. Here, we explore the on-chip labelling of cyclens and, for the first time, cyclams with non-radioactive Cu^{2+} (Fig. 1), towards the later use of radioactive ^{64}Cu incorporation for PET imaging, and employ an absorbance detection setup for the online monitoring of product formation.

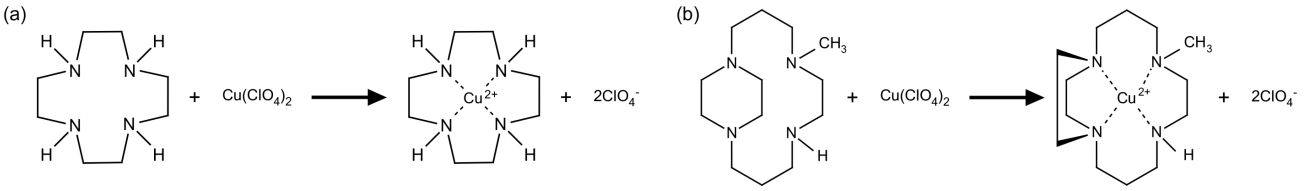

Figure 1: (a) Complexation of cyclen with Cu^{2+} from copper(II) perchlorate. (b) Complexation of a cyclam derivative with Cu^{2+} from copper(II) perchlorate.

EXPERIMENTAL

Two glass microfluidic chips were employed for the formation of copper(II) cyclen (Fig. 2a) and copper(II) cyclam (Fig. 2b) products, respectively. The chips each featured two inlet holes, with copper(II) perchlorate solution introduced via one inlet and aqueous cyclen or cyclam pumped into the other. The reaction section of both chips was identical, with the design consisting of a 50 μm deep serpentine channel that allowed diffusive mixing of the Cu^{2+} ions and the macrocycles as they flowed through the device. Online reaction monitoring was achieved via the use of an absorbance setup, which consisted of the microfluidic device being aligned between two optical fibres, one providing illumination from a light source (HL-2000, Ocean Optics) and the other being used for collection of the light once it had passed through the chip (Fig. 2c). This light collection fibre was coupled to a spectrometer (USB2000, Ocean Optics) for measurement of the absorbance signal. In the case of the cyclen reaction , detection was performed through the serpentine channel itself, giving a pathlength of 50 μm (Fig. 2a). The cyclam reaction was performed on an improved microfluidic device that consisted of three layers. The top layer contained the serpentine reaction channel , while the middle layer featured a 3 mm long drill hole that acted as the pathlength, and a T-shaped channel on the bottom layer allowed removal of the product from the detection region. In both the cyclen and cyclam reactions, initial tests involved pumping concentration standards of the respective Cu^{2+} complexed products through the devices in order to calibrate the systems, before investigating the effect of flow rate on the on-chip formation of the products.

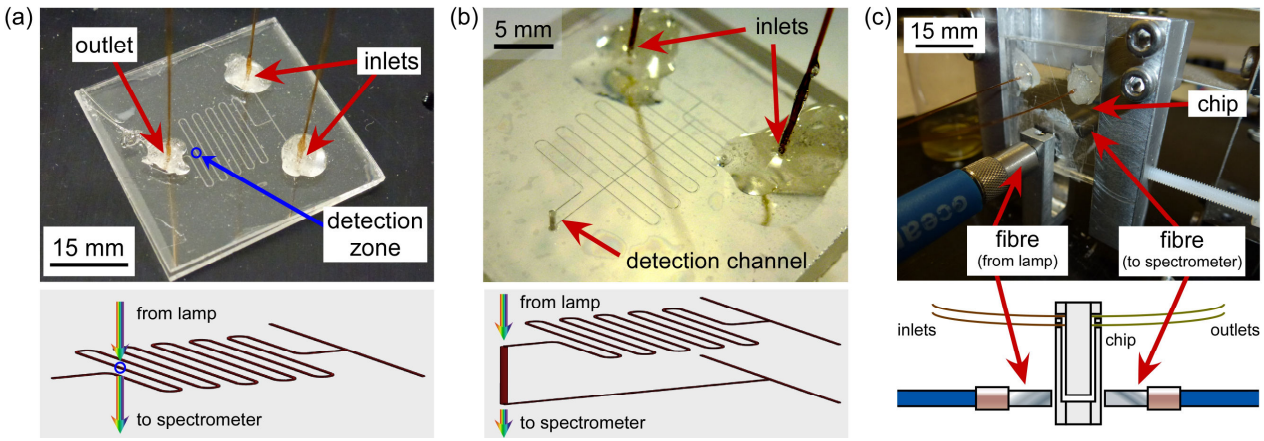

Figure 2: Photograph of the microfluidic device used to produce copper(II) cyclen, with a schematic (below) showing the 50 μm pathlength used for absorbance detection. (b) Photograph of the three-layered device used for the complexation of a cyclam derivative with Cu^{2+}, which featured a 3 mm pathlength drilled through the middle layer. (c) The setup employed for absorbance detection, utilizing optical fibres for illumination and collection of the light once it had passed through the pathlength of the chip.

RESULTS AND DISCUSSION

When the $Cu(ClO_4)_2$ and macrocycle solutions were mixed together the Cu^{2+} ions were coordinated within the macrocycles, and both the cyclen and the cyclam formed dark purple complexes. These purple species were easily identified compared to the translucent blue of the $Cu(ClO_4)_2$ solution and the pale yellow of the cyclen and cyclam solutions, thereby allowing on-chip absorbance detection. A range of copper(II) cyclen standards, with concentrations from 63 to 254 mM were pumped through the serpentine device and the spectra recorded (Fig. 3a), from which a calibration curve of absorbance versus concentration at 598 nm was constructed (Fig. 3b). The limit of detection (LOD) was 28 mM of copper(II) cyclen. When the $Cu(ClO_4)_2$ and cyclen solutions were pumped through the chip (Fig. 2a), the concentration of the product was monitored online while the flow rate was varied between 50 and 400 μL h^{-1} (Fig. 3c). As the flow rate was reduced the mixing times became longer, resulting in an increase in signal at 598 nm that indicated higher yields of product.

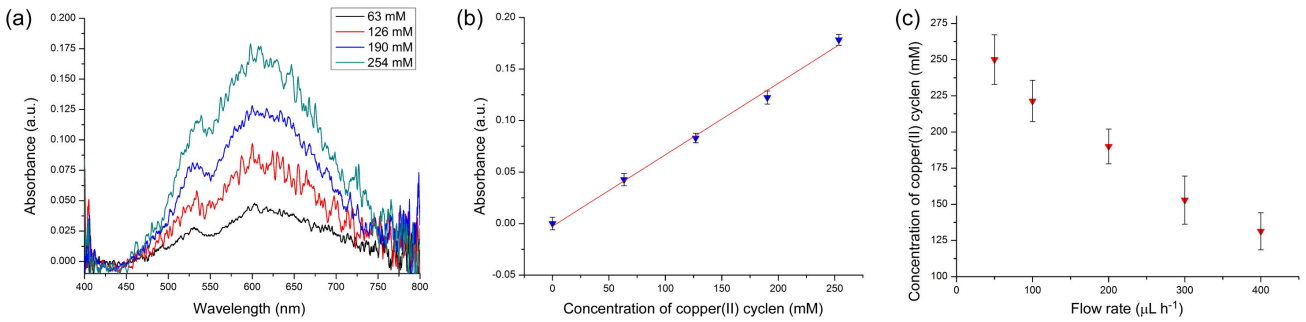

Figure 3: Complexation of cyclen with Cu^{2+}. (a) On-chip spectra of copper(II) cyclen standards from 63 to 254 mM. (b) Calibration graph of absorbance against product concentration at 598 nm. The LOD was 28 mM. (c) The effect of flow rate on the amount of product formed during the on-chip reaction. The concentration of copper(II) cyclen increased with decreasing flow rate due to the longer mixing times in the channel.

When the on-chip production of copper(II) cyclam was attempted using the two-layer device, crystals formed in the channel and blocked the device, even at concentrations near to the limit of detection. Therefore, a lower limit of detection of the setup was desired such that reduced concentrations of reagents could be employed in order to avoid crystal formation. To this end, the three-layer device (Fig. 2b) was constructed that featured a 3 mm detection pathlength as opposed to the 50 μm pathlength of the first device. With this, far lower product concentrations could be detected, as illustrated by the spectra in Fig. 4a, and the calibration graph of absorbance versus concentration at 528 nm (Fig. 4b) showed an LOD of 85 μM using this improved setup. When 600 μM solutions of cyclam and $Cu(ClO_4)_2$ were pumped

through the device, only a thin line of crystals were formed at the initial interface between the two reagents, and the reaction could now be monitored online. The flow rates of the reagents through the device were varied from 0.32 to 400 μL h^{-1}, with results showing far higher levels of copper(II) cyclam coordination at the lower flow rates.

Thus, the on-chip complexation of cyclens and cyclams with copper(II) ions was demonstrated, and online absorbance detection was used to monitor the product formation. Therefore, this system shows potential for the coordination of these macrocyclic molecules with radiometals towards their use as PET radiotracers.

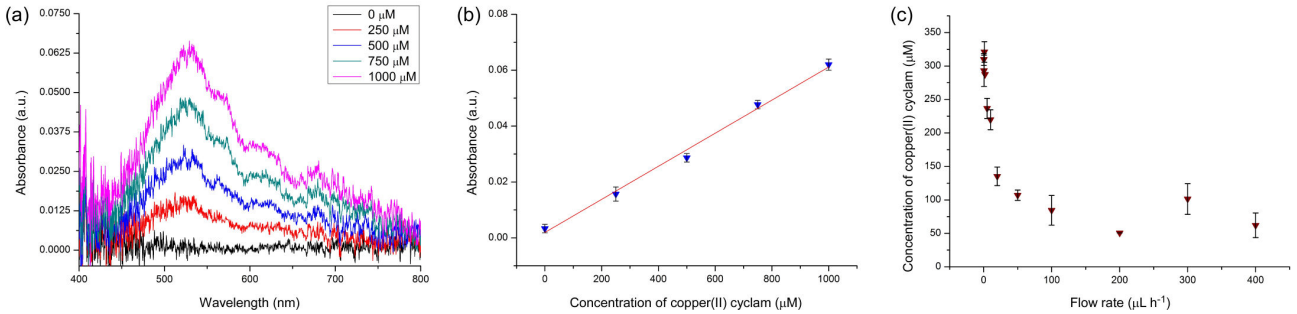

Figure 4: Complexation of cyclam with Cu^{2+}. (a) On-chip spectra of copper(II) cyclam standards from 250 to 1000 μM. (b) Calibration graph of absorbance against product concentration at 528 nm. The LOD was 85 μM. (c) The effect of flow rate on the formation of copper(II) cyclam. Lower flow rates yielded far higher concentrations of product due to the increased mixing times.

CONCLUSION

We have investigated on-chip copper(II) ion coordination interaction with cyclen and cyclam macrocycles, with real-time reaction monitoring achieved via absorbance detection. The next step will involve the incorporation of radioactive ^{64}Cu into the macrocycles for their potential use as PET imaging agents, which will also include the binding of these chelating ligands to target biomolecules. Further work will also involve ^{68}Ga radiolabelling of the molecules.

ACKNOWLEDGEMENTS

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CONTACT

*Nicole Pamme, Tel: +44 1482 465027; n.pamme@hull.ac.uk