



POINT-OF-CARE PORTABLE INSTRUMENT FOR THE DETECTION OF GENETIC TARGETS

Bishwojit Konsam¹ (bk33@hw.ac.uk)

Professor Marc Desmulliez¹, Professor Antonio Martinez-Murcia²

¹Smart Systems Group, ISSS, EPS, Heriot Watt University, Edinburgh, UK; ²Genetic PCR Solutions, Orihuela, Spain

INTRODUCTION

Infectious diseases & POC Device

Mesoporous Gated System (MGS)

- 3 of the top 10 cause of death in 2016 was infectious diseases with over 5.6 million people killed globally¹.
- For low-income African countries, 56% of total deaths were associated with infectious diseases or nutritional conditions².
- In developed countries, foodborne diseases, pathogen outbreak, and sexually transmitted disease (STD) are still a real threat³.
- Early detection of the pathogen is the key to prevent pathogen outbreak and recovery from the infection.
- The current standard detection method is still based on the complex, expensive and time-consuming traditional culturing methods. An accurate, sensitive, and portable point of care (POC) diagnostic device could address early diagnosis and pathogen outbreak.
- The aim of the research is to design, manufacture, and characterize the performance of a mesoporous gated system for a POC device based on genetic detection.

A mesoporous gated system (MGS) consists of:

- > A mesoporous support to enclose fluorophores (MS)
- > A gate material released in the presence of pathogen

A caged indicator from the target ensuring release of the gate (CI)



EXPERIMENTAL RESULTS & DISCUSSION

Fabrication of MGS

Method: The mesoporous substrate (MCM 41) was synthesized by the Stöber sol-gel process⁴.

SEM Image



2.45 µm 4.82 mm

500 nm

	XRD PLOT	
<u>,</u> ≩ 450000 _∓ (100)		
S 400000 - A		
300000 - / /		



- > MCM 41 was successfully synthesized.
- Surfactant was extracted by acidic alcohol process.



- The optimum extraction solvent was found to be 20 ml HCl in 200 ml of ethanol per gram of as-synthesized MCM 41 incubated for 15 hr at 60°C.
- BET surface area, BJH pore volume and pore diameter were 309 m².g⁻¹, 0.610 cm³.g⁻¹, and 1.89 nm.
- (100), (110), (200), and (210) Braggs peaks were observed in XRD spectrum.
- Increasing the quantity of reaction catalyst reduces aggregation of nanoparticles.



- The 2θ angle (100) peak was observed at 2.06 and the corresponding d spacing is calculated as 4.27 nm.
- MCM 41 surface was successfully modified with APTES.
- The average fluorescent dye loading was calculated as 0.735 mmoles per gram and of MCM 41 by UV-Vis adsorption method.



- > The target for the gate release experiment is Salmonella spp.
- > The gate experiment was performed in a microplate reader.
- > 96 well plate black, fluorescent microplate was used for the experiment.
- The fluorescent measurement was performed with the mesoporous substrate suspended in the sample.

Gate Release Experiment



- The cage indicator (CI) release is negligible in the absence of target oligonucleotide.
- In contrast, the CI is released in the presence of target oligonucleotide.
- The CI release achieves 50% intensity in approximately 10 min.
- The intensity reduces gradually after 150 min, a period well beyond the reading of the diagnostic obtained by the POC device.

The limit of detection is 100 femtomoles.

Summary & Future Work

The PhD thesis achieved the following:

- > MCM 41 was successfully synthesized and characterized.
- Mesoporous Gated System was synthesized and characterized.
- In the gate released experiment, CI released is only observed in the presence of target material.
- More experiments need to be performed to fully characterize and optimize the gate release experiment.
- A cost-effective fluorophore detection system is currently under development.



Promoting further and higher education

References

- 1. WHO, "Global Health Estimates 2016: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2016. Geneva," 2018.
- 2. WHO, "World Health Organisation." [Online]. Available: https://www.who.int/.

3. M. Foudeh, T. Fatanat Didar, T. Veres, and M. Tabrizian, "Microfluidic designs and techniques using lab-on-a-chip devices for pathogen detection for point-of-care diagnostics," Lab Chip, vol. 12, no. 18, pp. 3249–3266, 2012.

4. E. Climent et al., "Controlled Delivery Using Oligonucleotide-Capped Mesoporous Silica Nanoparticles," Angew. Chemie Int. Ed., vol. 49, no. 40, pp. 7281–7283, 2010.