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# Preparation and surface functionalisation of poly(styrene maleimide) nanoparticles for bacterial detection

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## INTRODUCTION

The detection of bacteria in water is essential for the prevention of water-borne disease outbreaks. Conventionally, culturing methods are used to detect bacteria in water, whereby the number of bacteria present in a sample is multiplied to a detectable level. The state-of-the-art method currently being employed by affluent municipalities in South Africa is the Colilert® test, requiring a minimum incubation period of 18 hours. Authorities are thus limited to delayed responses in cases of contamination.

There thus exists a definite need for a device, material or technique that can detect a very low concentration of pathogens in water samples rapidly, ideally in less than an hour.

Nanoparticles are extensively utilised in many fields of study. Due to the difference in size between a nanoparticle (<100 nm) and a bacterium (2 µm in length), many fluorescent antibody functionalised particles will theoretically cluster around a single bacterium, leading to amplification of the signal generated by the organism(s), and thus making rapid detection possible. This effect has been demonstrated with dye-doped silica nanoparticles[(1)].

## AIM

The aim of this project is to develop poly(styrene-maleimide) (SMI) nanoparticles (NPs) encapsulated with fluorescent dye molecules for use in rapid detection of *E.coli*.

## APPROACH

SMI nanoparticles encapsulated with a red laser dye (Exalite 613) were manufactured under high temperature and pressure via the imidisation of poly(styrene-*co*-maleic anhydride)[(2)] (**Table 1** and **Figure 1**).

Table1: Reagents and reaction conditions used to manufacture SMI nanoparticles with and without dye

| Reagents | | Reaction conditions | |
|---|---|---|---|
| Poly(styrene maleic anhydride) | 20 g | Pressure | 5 bar |
| Ammonium solution | 5 ml | Temperature | 170 °C |
| Water | 100 ml | Stirring speed | 1 000 rpm |
| Exalite 613 dye (when applicable) | 5 g | Residence time | 6 hours |

The particles were reacted successively with an excess of formaldehyde and an excess of ethylene diamine to introduce amine groups to the particle surfaces for attachment of fluorescent markers and antibodies.

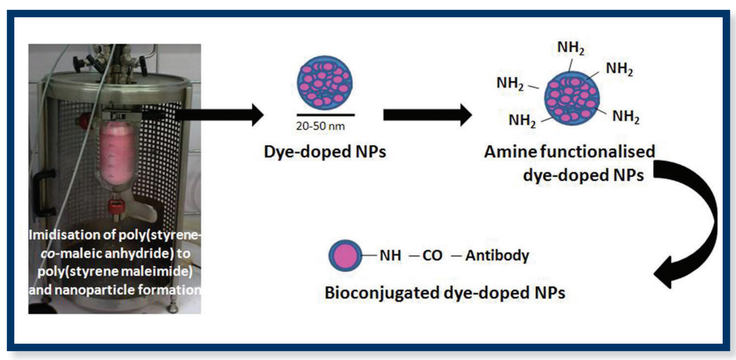


Figure 1: Process diagram of proposed development method of nanoparticles for bacteria detection

Particle characterisation was performed with transmission electron microscopy (TEM), and Attenuated Total Reflection-Fourier Transform Infrared (spectroscopy) (ATR-FTIR).

## RESULTS

The SMI nanoparticles were freeze dried and characterised via the characteristic peaks for SMI (**Figure 2**).

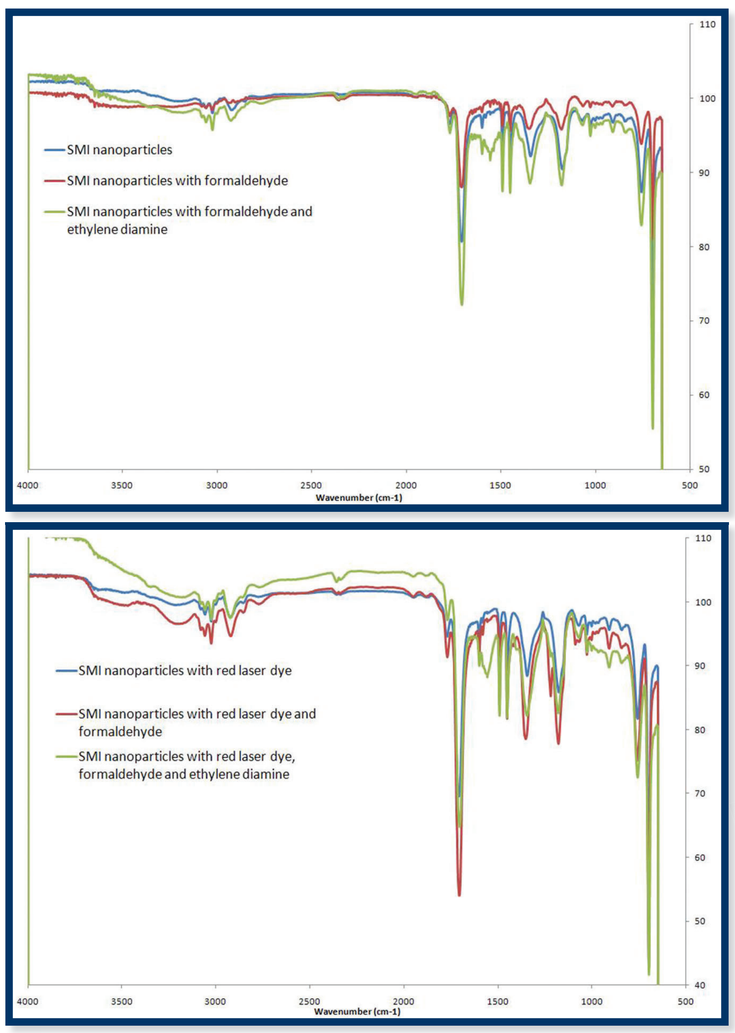


Figure 2: ATR-FTIR spectra of different surface functionalisation steps for nanoparticles without dye encapsulated (top) and with dye encapsulated (bottom)

The appearance of a characteristic peak at 1 550 $cm^{-1}$ after reaction with ethylene diamine indicates the presence of N-H bonds on the particle surfaces.

TEM images (**Figure 3**) showed:

- Relatively monodisperse spherical nanoparticles with an average size of 20 to 50 nm
- Particle size and shape was unaffected by the surface functionalisation process or by encapsulation of dye.

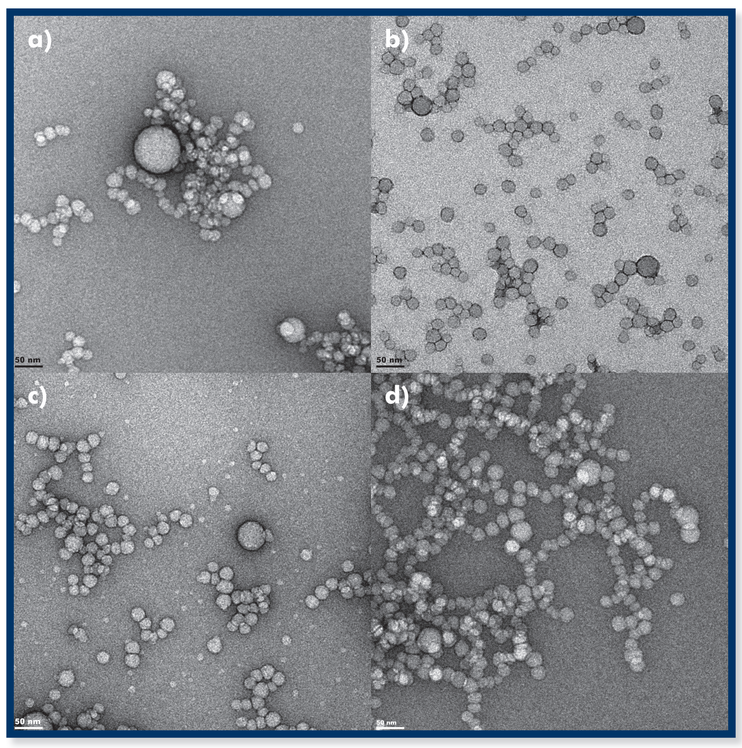


Figure 3: TEM images of nanoparticles without dye before (a) and after surface functionalisation with ethylene diamine (b), and nanoparticles with dye encapsulated before (c) and after (d) reaction with formaldehyde

***The intelligent management of South Africa's scarce water resources is a key focus area for the CSIR. This work aims to develop a rapid method for the detection of microbial contamination in water to assist authorities in the prevention of water-borne disease outbreaks.***

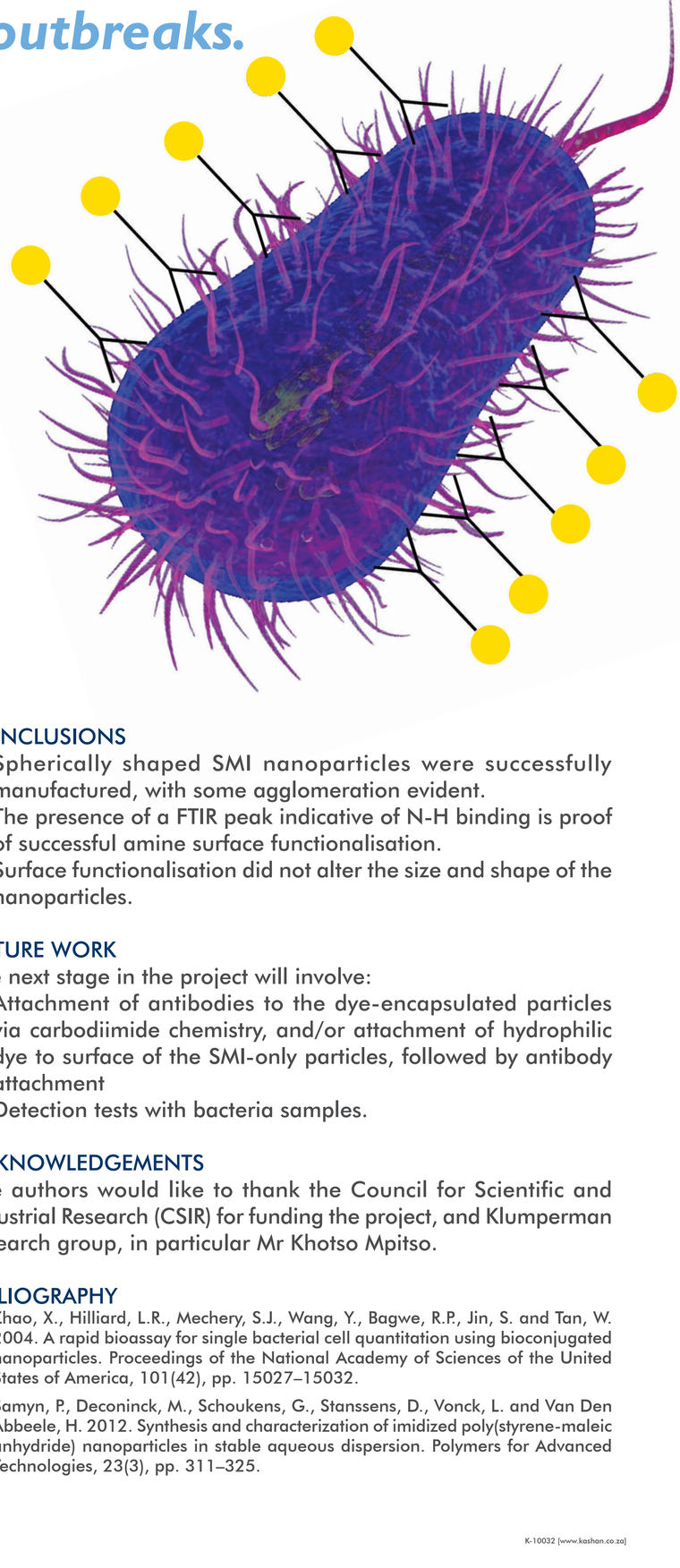

Image courtesy of http://pilotspoint.net/; adapted by Ilse Wepener

## CONCLUSIONS

- Spherically shaped SMI nanoparticles were successfully manufactured, with some agglomeration evident.
- The presence of a FTIR peak indicative of N-H binding is proof of successful amine surface functionalisation.
- Surface functionalisation did not alter the size and shape of the nanoparticles.

## FUTURE WORK

The next stage in the project will involve:

- Attachment of antibodies to the dye-encapsulated particles via carbodiimide chemistry, and/or attachment of hydrophilic dye to surface of the SMI-only particles, followed by antibody attachment
- Detection tests with bacteria samples.

## ACKNOWLEDGEMENTS

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