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**Hosted by;**

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## 1. Aim of this trip:

Initially, the goal for this visit was to visit and learn fluorescent biosensing using the enzyme N-acetyltransferase (NAT) coupled to the fluorescent probe 8-anilino-1-napthalene sulphonic acid for the detection of TB drugs. On my arrival here I was introduced to a more interesting topic(s) which I found to be also relevant to the research work I do at SensorLab. The topic(s) was 'The detection of TB bacterium using magnetic nanoparticles' and 'Following the growth and death patterns TB and E.coli bacterium using microfluicic systems'. Further details of the research work will be discussed below. Secondly, the goal of this trip was to also establish a long lasting collaboration between the above mentioned laboratory and SensorLab with particular emphasis on the TB research work done in both laboratories.

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## 2. The actual work done:

I had the privilege of learning house to design and develop microfluidic systems. This technique took a period of two weeks to complete and to ensure that they were suitable and stable for the desired experiments. I did not do the work which I initially intended to do but I was introduced to a very interesting subject which is the detection of Mycobacterium Tuberculosis using microfluidic systems connected to Precision Impedance analyzers for analyses through two electrodes. This subject was very interesting to me particularly because I had previously developed imunosensors for the detection of Tuberculosis antigens. I was also pleasantly surprised at the delicacy of the procedures involved in this type of work but I was ready to learn so that I can transfer new skills when I arrive back home. The in-house development of the microfluidic systems was also of great interest to me.

## 2.1 The following experiments were achieved.

### 2.1.1 Experiment 1:

One comprised of the decontaminated bacterium alone and the growth of the bacterium was studied over 5 hours. For analysis, 20 μL of the bacterium was inserted into the cassette and the impedance reading was carried out. Five of the same sample was taken and the readings were taken at each hour. The result was a directly proportional relationship between time and the growth of the bacterium.

The second experiment comprised of the decontaminated bacterium as well as the antibiotic ampicillin to study the killing of the bacterium over 5 hours. For analysis, 20 μL of the bacterium was inserted into the cassette and the impedance reading was carried out. Five of the same sample was taken and the readings were taken at each hour.

The result was the inverse relationship between time and the killing of the bacterium.

Due to the sensitivity of the work; these experiments were done several times to ensure stability and repeatability.

### 2.1.2 Implementation of this work at SensorLab.

The foundation of the research work at SensorLab is Electrochemistry therefore to replicate this type of work would require the presence of a conducting material whose electrochemistry would have to followed relative to the growth of death of the bacterium of interest.

In a conventional three electrode system immersed in pH 7.4 phosphate buffer, the bacterium (alone and with ampicillin) would be studied over 5 hours by adding 20 μL to the buffer solution and either growth or the death of the bacterium would be studies amperometrically and by electrochemical impedance spectroscopy (EIS).

The next step would involve a conducting polymer such as polyaniline, poly(8-anilino-1-napthalene sulphonic acid) or polypyrrole that would to be attached onto a working electrode. Thereafter the experiment would have to follow as indicated above.

### 2.1.3 Experiment 2:

The second part of the work involved the detection of the bacteria Mycobacterium smegmatis using magnetic nanoparticles. The aim was to attach the bacteria onto modified magnetic nanoparticles in a fast way such that an infected patient can have their results in a shorter period of time as opposed to currently available methods of detecting these bacteria.

The first part of his study involved the preparation of sub-cultures from the mother culture of M. smegmatis to allow the bacteria to grow in the growth supplement Middlebrook OADC. This part was completed before I arrived. The bacterium was also decontaminated using 2 % NALC solution. UV spectroscopy was used to determine the amount of bacteria before subjection of the magnetic nanoparticles. The conventional plating method was also carried out in order to determine the exact number of bacteria present.

Artificial sputum was the created using 7H10 Agar base and Middlebrook OADC Both and one egg (MEDIA). Thereafter, the bacteria and the MEDIA were all added to the magnetic nanoparticles in a tube and mixing was allowed manually for 5 minutes. Magnets were then placed around the tube to separate the nanoparticles containing the bacteria from the rest of the solution. The supernatant was removed and plated to see whether there were any bacteria left behind. The magnetic nanoparticles with the bacterium were re-suspended in the MEDIA and plated to see how many bacteria were attached onto the nanoparticles.

The results of the plates were available after 4 to 6 days. Once again this work had to be repeated for comparison reasons.

### 2.1.4 Implementation of this work at SensorLab.

At SensorLab work on TB antigen detection has been done for a while now where electrochemical impedance spectroscopy (EIS) was used. In this research work immunosensors were developed on electrode surfaces and antibodies were attached which affinities for the antigens of interests. The EIS analysis to establish the detection usually takes 1 to 5 hours. To shorten this time period, magnetic nanoparticles can be used attached to the antibodies of interest and then subject these complexes to the antigens of interest. Magnetics would obvious be used to separate the bound nanoparticles and further analyses could proceed to establish whether or not the antigens were

bound to the antibody. Amperometry and EIS could be used to analyze the nanoparticles alone, magnetic nanoparticles with antibody (complex) and finally that complex with antigen to see how the conductivity changes. As an additional tool, UV spectroscopy and fluorescence could also be used to see how the absorbance intensities are affected before and after antigen-antibody interaction.

**2.2 Remarks**

Due to the nature of the work which I was introduced too, I really felt that I should have requested for a longer stay than 6 weeks. Although I was able to learn new skills and start some of the work while there, it was not enough time to effectively see the progression of the work before leaving. It was very refreshing to be mentored by PhD students while doing this work. My Chemistry background was of great assistance in the understanding of aspects about their work but they also contributed a lot to the understanding of my work.

While at Mizzou, I also had the opportunity to sit in one of their 3D modeling evening lectures. I was very impressed with quality of the materials taught and the relaxed nature of the atmosphere in the lecture. It was a great experience and I took some idea to use to improve my lectures. Not only was this trip an opportunity for me to improve my research skills, it was also an opportunity to improve my teaching skills and it also awarded me a networking opportunity where I privileged to meet and form partnerships with the following individuals. The next step is obviously to source for funds so that strengthen these partnerships. The following are the individuals I met and had fruitful discussions with; Dr Sheila Grant, Dr Shramik Sengupta, Prof Gangopadhyay Shubra, Dr Heather Hunt and Dr Barizuddins. Additionally, I also met Prof Evangelyn Alocilja from Michigan State University who collaborates with Dr Sengupta and we are currently setting up an agreement for her send us her magnetic nanoparticles to be applied in our sensors and to study their electroactivity.

Personally, this was one of my most productive academic visits. Mizzou is a very welcoming campus and the environment is ideal for visitors because everything is in close proximity to the campus. I encourage and motivate the programme to continue funding African researchers to travel to work class laboratories at the University of Missouri to improve their academic strengths.