CHAPTER 4 RESULTS AND DISCUSSIONS

4.1 MWCNTs/AuNPs Nanocomposite

Preparation of MWCNTs/AuNPs nanocomposites was conducted based on Zang and co-worker [66]. In this experiment, sodium citrate and sodium borohydride were used as reducing agent. Reducing agent will reduce Au^{3+} ions to Au^{0} to synthesize AuNPs. MWCNTs were mixed with reducing agent (sodium citrate or sodium borohydride) to form reducing agent shell on the surface of MWCNTs. When $HAuCl_4$ solution was added, $HAuCl_4$ molecule were reduced when they meet reducing agent at MWCNTs surface. One molecule of Au^{3+} is first reduced to a Au^{0} atom, and then the others reduced Au^{0} will aggregate to this atom and assemble a gold nanoparticle [62]. MWCNTs from two different companies were used in this experiment, Cheap Tube Inc, USA and Ted Pella Inc, USA. The schematic representation of fabrication of MWCNTs/AuNPs nanocomposites and the TEM images of AuNPs on the MWCNTs surface using different reducing agent are shown in Figure 4.1 and 4.2, respectively.

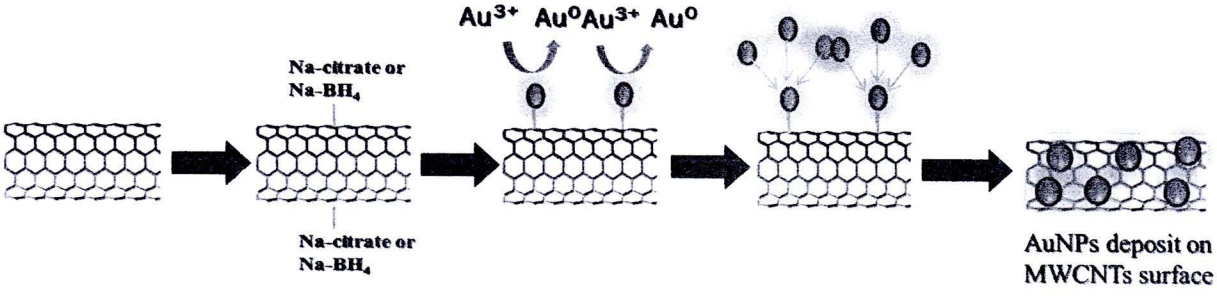


Figure 4.1 Fabrication of MWCNTs/AuNPs nanocomposite [66].

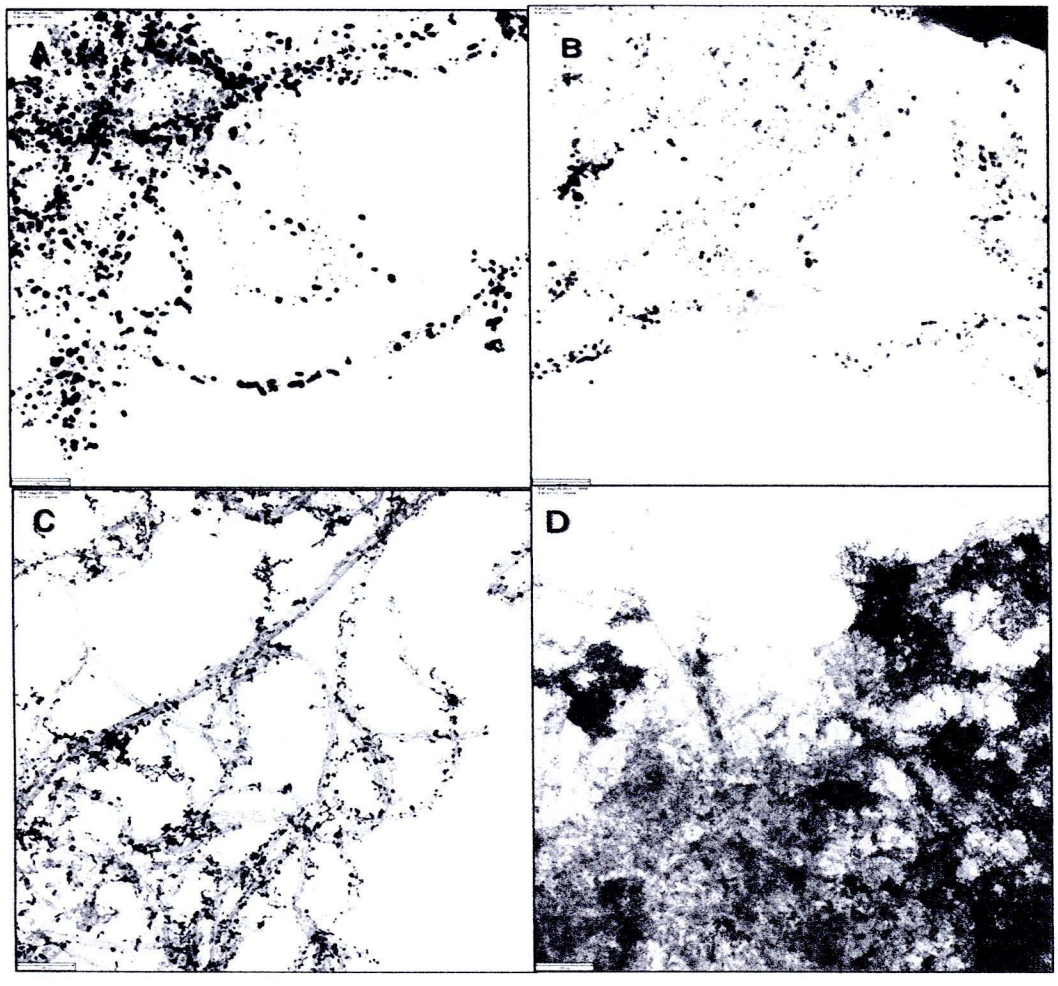


Figure 4.2 TEM images of MWCNTs/AuNPs nanocomposites using different reducing agents. MWCNTs/AuNPs-citrate using MWCNTs from Cheap tube Inc. (A) and Ted Pella Inc (B) MWCNTs/AuNPs-BH_4 using MWCNTs from Cheap Tube Inc. (C) and Ted Pella Inc. (D). Scale bare 200 nm.

As can be seen in the Figure 4.2 by using citrate as a reducing agent, AuNPs could be produced as a spherical shape and have a uniform structure on the MWCNTs wall. The AuNPs had a diameter of 17.3 ± 3.24 nm. However, when using BH_4 as a reducing agent, a tubular shape of AuNPs was observed, with a network-like structure on the MWCNTs from Ted Pella Inc. MWCNTs from Cheap Tube Inc. has a diameter of 10-20 nm, whereas Ted Pella Inc. has a diameter of 4-12 nm. Eventhough MWCNTs from both companies were functionalized with the carboxylic group, MWCNTs from Cheap Tube Inc. gave more consistant AuNPs formation than MWCNTs from Ted Pella Inc.

4.2 MWCNTs/AuNPs Based Immunoassay

To improve the sensitivity *S. enterica* serovar Typhimurium detection based on MWCNTs/AuNPs nanocomposites, optimization conditions such as concentrations of capture antibody, concentrations of MWCNTs/AuNPs, types of gold nanoparticle, types of MWCNTs and blocking buffers were studied. The schematic representation of immunoassay on nitrocellulose membrane is shown in Figure 4.3. Firstly capture antibody was immobilized on nitrocellulose membrane surface. Then, antigen that have specific recognition with capture antibody was added. After that MWCNTs/AuNPs nanocomposite were immobilized with secondary antibody was added forming sandwich antibody antigen. Silver enhancer was added, AuNPs will catalyze silver ion become silver metal then silver metal will deposited on AuNPs surface causing the gold nanoparticle become bigger and the spot can be seen by naked eyes or flatbed scanner.

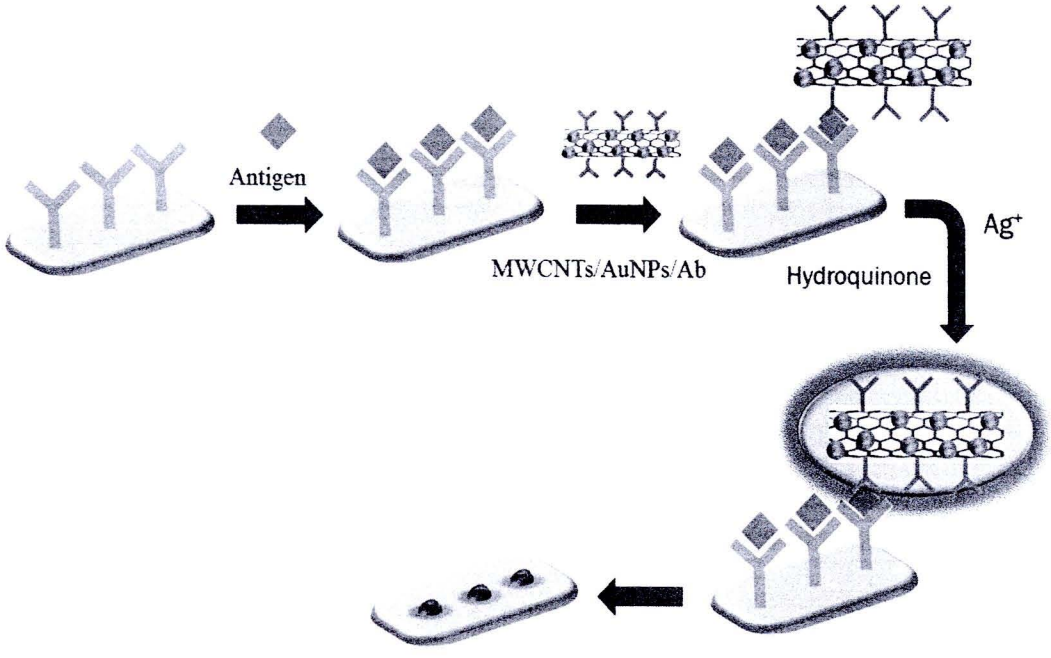


Figure 4.3 Schematic diagram of sensitivity enhancement of *S. enterica* serovar Typhimurium detection based on MWCNTs/AuNPs nanocomposites as a label and silver enhancer for signal amplification

4.2.1 Optimization of Capture Antibody Concentration

To detect *S. enterica* serovar Typhimurium on nitrocellulose membrane, concentrations of capture antibody were optimized to obtain high sensitivity. Three different concentrations of capture antibody (1, 0.5 and 0.25 mg/mL) were evaluated. All capture antibodies were diluted in 10 mM PBS pH 7.4 + 40% glycerol. Glycerol could normalize spot size, decrease drying artifacts and increase antibody spotting density [78]. Different types of MWCNTs/AuNPs were used, i.e. MWCNTs/AuNPs-citrate and MWCNTS/AuNPs-BH_4 with the concentration of 1.5 mg/mL.

Electrostatic linkage between antibody probe and MWCNTs/AuNPs was chosen in this study. The concentration of *S. enterica* serovar Typhimurium was 10^8 cfu/ml.

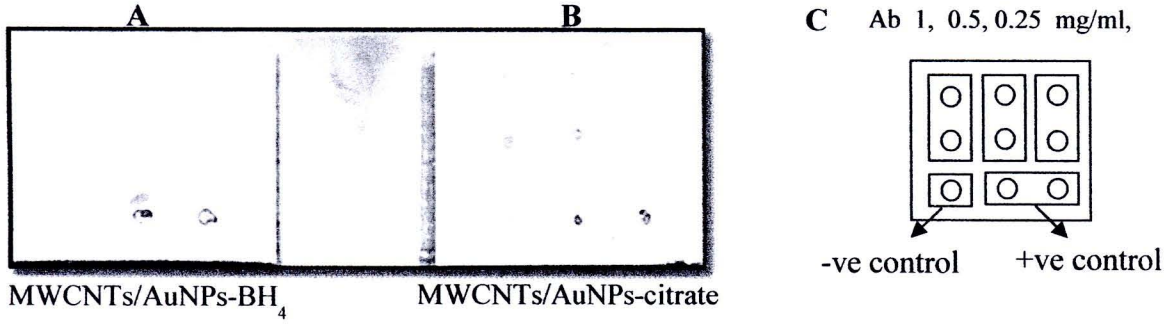


Figure 4.4 Capture probe optimization on the nitrocellulose membrane: MWCNTs/AuNPs-BH_4 (A) and MWCNTs/AuNPs-citrate (B) were used as labels. Pattern of spotting (C). Positive controls is MWCNTs/AuNPs, and negative control is 10 mM PBS pH 7.4 + glycerol (spotting buffer).

The proposed sandwich model is similar to the common sandwich type ELISA, different only in the materials for protein labeling (enzyme is replaced by MWCNTs/AuNPs) and signal amplification process. Physical adsorption was employed to attach antibody on nitrocellulose membrane. For the *S. enterica* serovar Typhimurium detection (Figure 4.4), it was found that the highest concentration of capture antibody (1mg/mL) resulted in the highest signal compare with the others and MWCNTs/AuNPs-citrate gave higher signal than MWCNTs/AuNPs-BH_4. This could be due to the amount of AuNPs-citrate on the surface of MWCNTs is higher than AuNPs BH_4 (Figure 4.2). Accordingly, the capture antibody of 1 mg/mL and MWCNTs/AuNPs-citrate were chosen for the next experiment to ensure successful assay development.

4.2.2 Optimization of Blocking Reagents and MWCNTs Length

Suitable blocking solution will enhance assay performance by increasing signal to noise ratio. BSA is usually used as blocking solutions in immunoassay. In this study 2% BSA was selected for antibody array. The blocking solution prevents non-specific bonding between other material and capture antibody and the interaction between antigen and surface of nitrocellulose membrane [79].

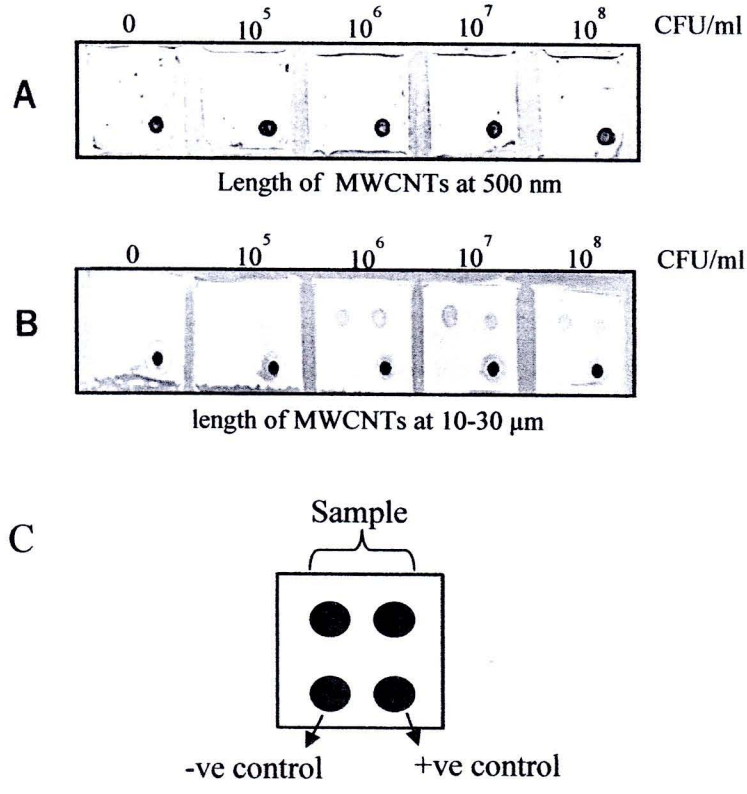


Figure 4.5 Antibody array development on the nitrocellulose membrane. Antibody array using MWCNTs/AuNPs-citrate with length of CNT at 500 nm (A) and 10-30 μm (B) with 2% BSA as blocking solution evaluated. Pattern of spotting (C).

It is known that the length of MWCNTs determines signal intensity of antibody array. In this study, different lengthes of MWCNTs from Cheap Tubes Inc were used i.e. 500 nm and 10-30 μM. Concentrations of MWCNTs/AuNPs was 1.5 mg/mL and antibody was attached on MWCNTs/AuNPs via electrostatic interaction. Different concentrations of *S. enterica* serovar Typhimurium were employed from 10^5-10^8 CFU/mL, adding no antigen was used as a blank to evaluate specificity of the test. As can be seen in Figure 4.5, longer MWCNTs resulted in increasing of the signal intensity. This could be due to the higher number of attached AuNPs with the increasing MWCNTs length. Hence, the MWCNTs with the length 10-30 μm was chosen for the entire experiments.

4.2.3 Optimization Blocking Buffer and MWCNTs/AuNPs/Ab Concentration

Based on the results from previous experiment, in this experiment MWCNTs with the length of 10-30 μm was used. Various concentrations of MWCNTs/AuNPs-citrate and MWCNTs/AuNPs-BH_4 were compared to obtain high signal intensity. The concentrations of MWCNTs/AuNPs were 0.1, 0.25, and 0.5 mg/mL. Two blocking buffers i.e. 2% BSA and 3% skim milk were compared and used to lower background signal on nitrocellulose membrane.

As can be seen in Figure 4.6, the highest concentration of MWCNTs/AuNPs (0.5 mg/mL) resulted in the highest signal as well as background intensity. In contrast the lowest concentration of MWCNTs/AuNPs (0.1 mg/mL) gave the lowest signal intensity and background. However, for MWCNTs/AuNPs at concentration of 0.25 mg/mL the spot intensity was high while giving low background signal. Hence, concentration of MWCNTs/AuNPs at 0.25 mg/mL was chosen for subsequent experiments. In addition, different blocking agents were evaluated to obtain high signal-to-noise ratio. As can be seen in the Figure 4.6, nitrocellulose membrane blocked with 2% BSA gave higher signal intensity than that blocked with 3% skim milk. Accordingly, BSA was chosen as a blocking agent for further experiment. It also can be noticed that MWCNTs/AuNPs-citrate gave higher signal intensity than MWCNTs/AuNPs-BH_4. This could be due to the amount of AuNPs-citrate on the surface of MWCNTs is higher than AuNPs-BH_4. Figure 4.7 shows the TEM images of MWCNTs/AuNPs-citrate and MWCNTs/AuNPs-BH_4 after silver enhancement.

Silver enhancement, also called autometallography, is a process that silver is deposited on the gold surface. AuNPs on MWCNTs surface acts as a catalyst to reduce silver ion to silver metal in the presence of silver enhancer solution. Silver ion reduced by hydroquinone to silver metal and AuNPs catalyze that reaction and silver metal can deposite on AuNPs surface then make AuNPs become bigger. In the presence of silver enhancer solution, the color of the spot become darker within 5 min and make the spot could be observed with naked eyes [80].

For the higher amount of AuNPs the gray level values reached after 5 min, indicating that silver ions were reduced to silver metal by catalysis. From the TEM images (Figure 4.7) MWCNTs/AuNPs-citrate have higher amount of AuNPs on the surface of MWCNTs than MWCNTs/AuNPs-BH_4 and therefore more silver ions can be deposited on AuNPs. The AuNPs had diameter of 37.84 ± 8.91 nm after silver enhancement for 5 min. When using AuNPs-BH_4, the amount of AuNPs attached on MWCNTs was low which led to lower consumption of the silver ions in the reaction solution. Accordingly, silver ions reduced to silver metal decreased and resulted in less intensity of the spot when compared to the AuNPs-citrate [31].

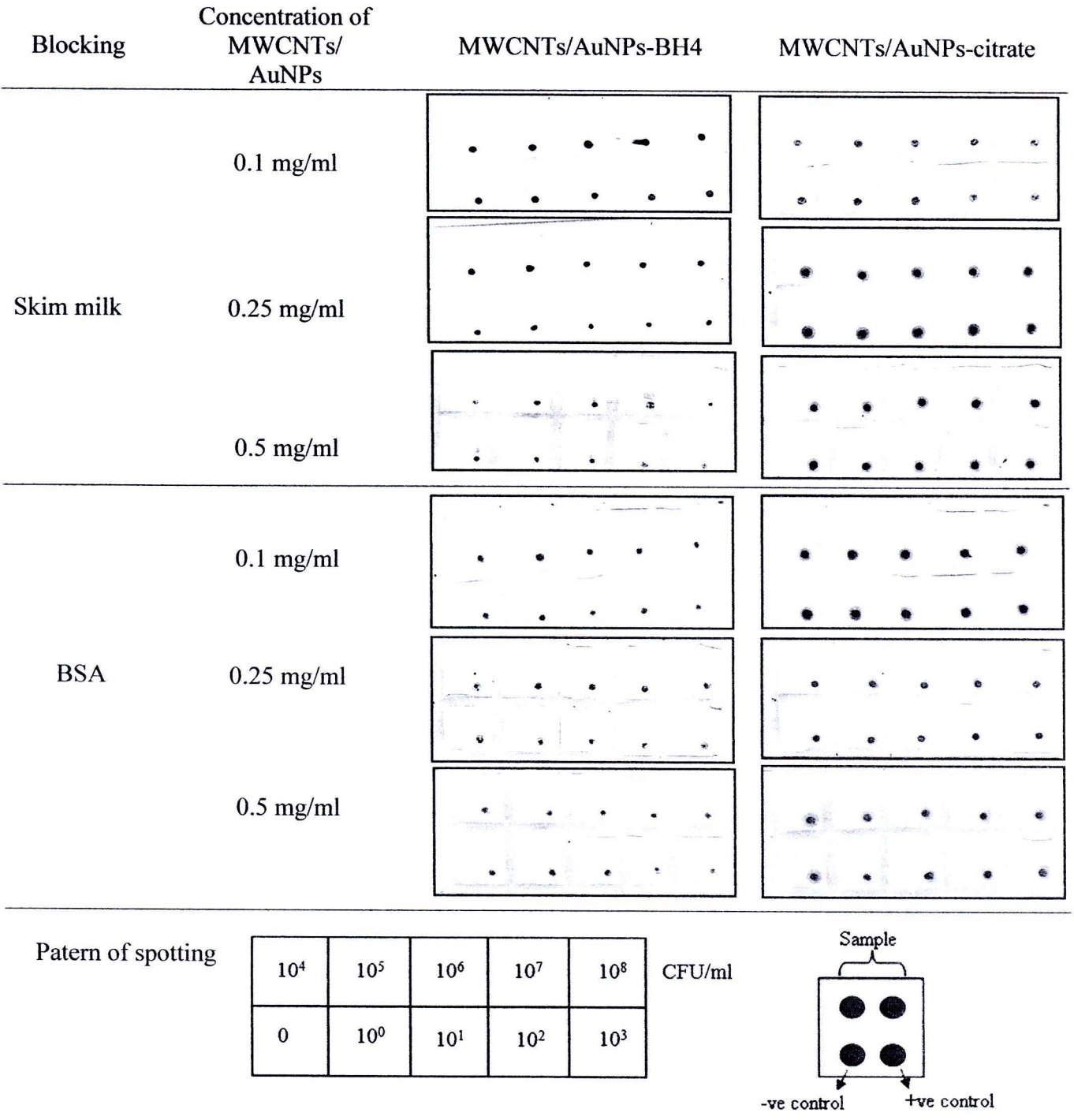


Figure 4.6 Optimization of blocking buffers, MWCNTs/AuNPs concentrations and types of MWCNTs/AuNPs

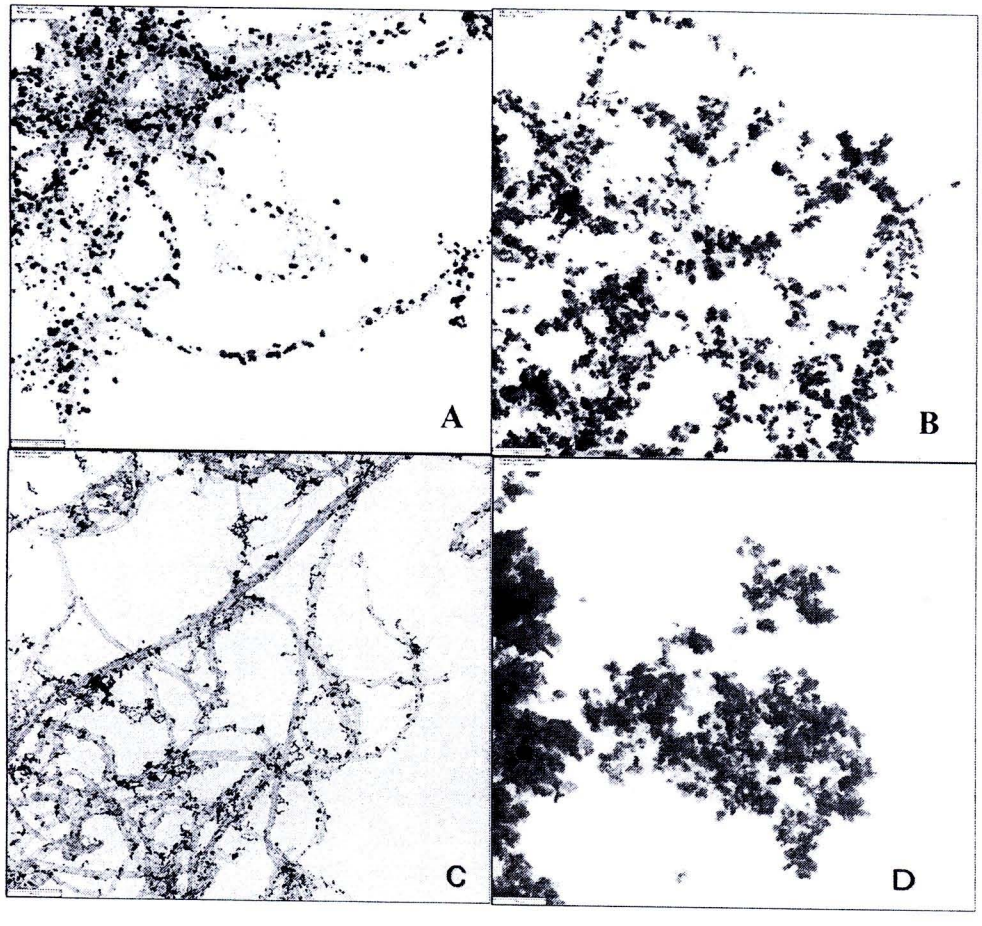


Figure 4.7 TEM images of MWCNTs/AuNPs nanocomposites in different reducing agents before and after adding silver enhancer for 5 min. MWCNTs/AuNPs-citrate before (A) and after adding silver enhancer for 5 min (B). MWCNTs/AuNPs-BH_4 before (C) and after adding silver enhancer for 5 min (D). Scale bare 200 nm.

From the experiment, eventhough the spot intensity was observed by naked eyes overall signal was low and found similar to the negative control. To overcome this problem, we changed from electrostatic binding between antibody and MWCNTs/AuNPs to covalent bonding to ensure stronger interaction and therefore signal intensity.

4.3 Determination Assay Sensitivity

In this study, we employed MWCNTs/AuNPs-citrate to further evaluate the assay. The antibody was linked to MWCNTs/AuNPs nanocomposite through covalent bonding. We found that the signal intensity of each concentration of *S. enterica* serovar Typhimurium employed was similar to the negative control (Figure 4.8). This could be due to the fact that capture antibody was immobilized on the nitrocellulose membrane using physical adsorption which may affect orientation of antibody towards its antigen. In addition, physical adsorption may not strong enough to hold antibody to nitrocellulose membrane, and therefore causing antibody to be

removed from the membrane during washing steps. To solve this problem the nitrocellulose membrane was covered with protein A and streptavidin to facilitate its orientation and thus help increasing affinity binding of capture antibody to nitrocellulose membrane.

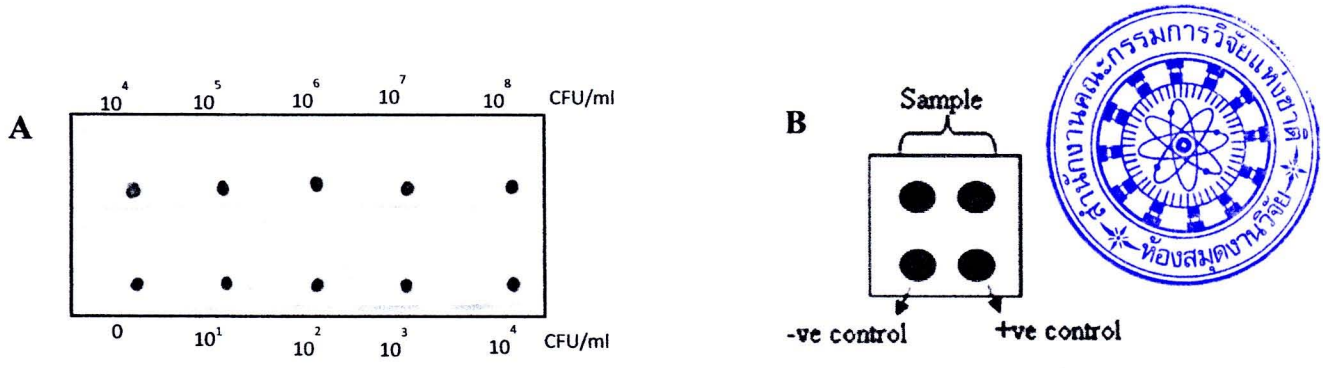


Figure 4.8 Antibody array using MWCNTs/AuNPs and antibody was linked to MWCNTs/AuNPs nanocomposite through covalent bonding (A) and pattern of spotting (B).

4.4 Optimization Condition for Immobilizing Capture Antibody

Protein A is a protein that originally found in the cell wall of *Staphylococcus aureus.* It has found used in biochemical research because of its ability to bind with immunoglobulins. It binds proteins from many of mammalian species, most notably IgGs. It binds with the Fc region of immunoglobulins through interaction with the heavy chain [81]. On the other hand, streptavidin is a protein isolated from actinobacterium *Streptomyces avidinii* that have high affinity for biotin. The binding of streptavidin and biotin has long been regarded as the strongest, noncovalent, biological interaction [82]. Therefore both protein A and streptavidin were used to cover nitrocellulose membrane which is expected to capture antibody that may result in increasing the signal intensity. The preliminary experiment was carried on by coating the membrane with protein A and streptavidin at 1 mg/mL (in 10 mM PBS pH 7.4). Capture antibody and biotinylated antibody were allowed to react with protein A and streptavidin respectively before heat killed *S. enterica* serovar Typhimurium cells at 10^8 CFU/ml was added to the membrane (Figure 4.9). PBS was used as a negative control. In addition, both 2% BSA and 3% skim milk were employed as blocking agents. It was found that there was no signal observed in the presence of heat killed *S. enterica* serovar Typhimurium cells in both protein A and streptavidin coated nitrocellulose membrane (Figure 4.10). Protein A and streptavidin may not strong enough to attach on the nitrocellulose membrane by physical adsorption therefore the protein A and streptavidin could be removed from membrane during washing steps. It this case the membrane that blocked with 2% BSA resulted in higher background than that blocked with 3% skim milk.

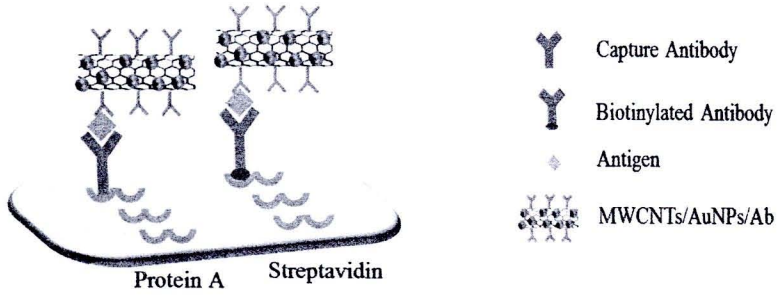


Figure 4.9 Schematic diagram of immobilizing capture antibody by covering nitrocellulose membrane with protein A and streptavidin.

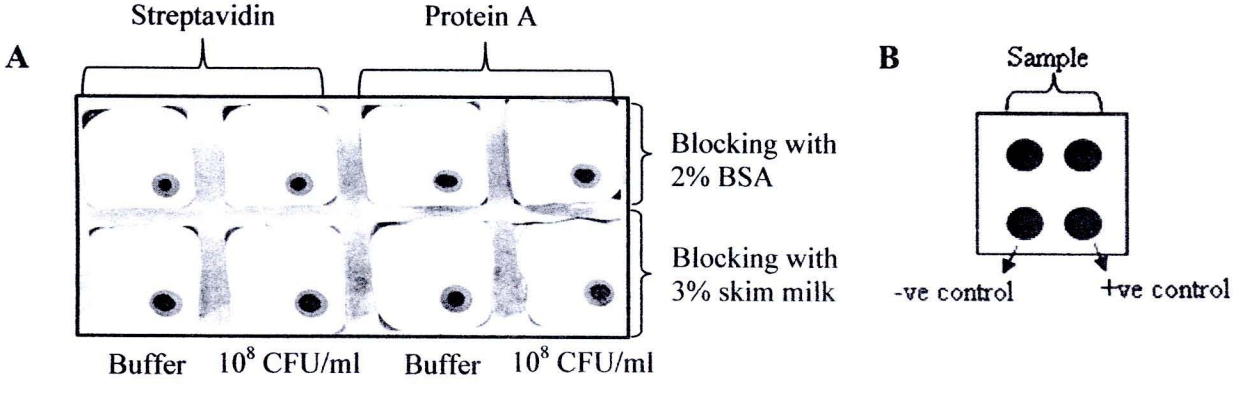


Figure 4.10. Optimization condition for *S. enterica* serovar Typhimurium. Nitrocellulose membrane was covered with streptavidin and protein A (A) and pattern of spotting (B).

From the overall experiment although MWCNTs/AuNPs-citrate were found to give higher signal intensity than MWCNTs/AuNPs-BH_4, they were unable to use to detect *S. enterica* serovar Typhimurium on the antibody array platform because different concentrations of *S. enterica* serovar Typhimurium employed gave the signal intensity similar to the negative control. Therefore, a new strategy employing magnetic bead together with MWCNTs/AuNPs nanocomposite was introduced for possible immuno detetion and signal amplification.

4.5 Study of Magnetic Beads Together with MWCNTs/AuNPs for Immunoassay on Nitrocellulose Membrane

In this study streptavidin-coated magnetic particles were used to immobilize biotinylated antibody and separate MWCNTs/AuNPs/Ab-Ag complex from the unbound MWCNTs/AuNPs/Ab and supernatant. The whole immuno complex was then spotted on nitrocellulose membrane and the silver enhancer was applied to develop the signal, that could be observed by naked eyes. The diagram of immunoassay complex is shown in Figure 4.11.

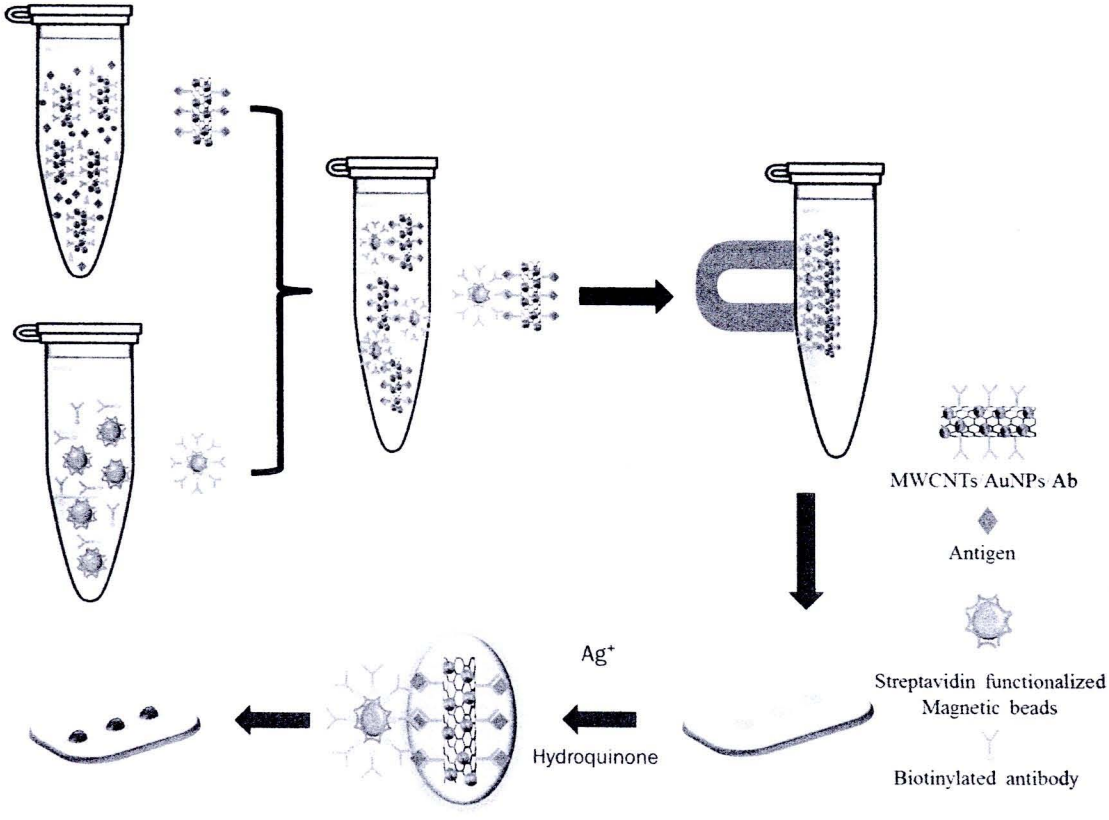


Figure 4.11 Schematic representation of *S. enterica* serovar Typhimurium detection based on MWCNTs/AuNPs nanocomposites together with streptavidin functionalized magnetic beads/biotinylated antibody and signal amplification by silver enhancer.

4.6 Optimized Concentration of MWCNTs/AuNPs and Magnetic Beads

A preliminary experiment to obtain the appropriate concentration of magnetic beads and MWCNT/AuNPs on the nitrocellulose membrane was investigated. Various concentrations of magnetic beads and MWCNTs/AuNPs were dropped and dried on nitrocellulose membrane and the signal developed using silver enhancer for 5 min. The concentrations of magnetic beads are 1, 0.1, 0.01, 0.001, 0.0001 and 0.00001 mg/mL whereas the concentrations of MWCNTs/AuNPs are 3, 2, 1, 0.5 and 0.1 mg/mL. It was observed that after silver amplification step, the intensity of spot increased with the increasing concentrations of MWCNTs/AuNPs. On the other hand, there was no signal from MBs indicating that the MBs did not involve in the signal amplification step

and therefore the signal was truly come from the MWCNTs/AuNPs itself. However, MWCNTs/AuNPs at 0.1 mg/mL was selected for the next experiment because it gave compromise signal intensity that could avoid the background signal due to non-specific binding.

A preliminary study to investigate the ability of MBs/Ab-biotin and MWCNTs/AuNPs/Ab complex to detect *S. enterica* serovar Typhimurium was performed on nitrocellulose membrane. Heat-killed *S. enterica* serovar Typhimurium cells at 10^8 CFU/mL were incubated with MWCNTs/AuNPs/Ab for 1 h and PBST buffer was used a blank. After the reaction, MBs/Ab-biotin and MWCNTs/AuNPs/Ab complex were resuspended and spotted onto nitrocellulose membrane. As can be seen in Figure 4.12 (C), the signal intensity in the presence of heat-killed *S. enterica* serovar Typhimurium cells is much higher than in the buffer. This result suggest that the MBs/Ab-biotin and MWCNTs/AuNPs/Ab complex was formed. In addition, the spot intensity of negative control was found similar to that of MBs, indicating that there was no MWCNTs/AuNPs attach on the magnetic beads in the absence of antigen.

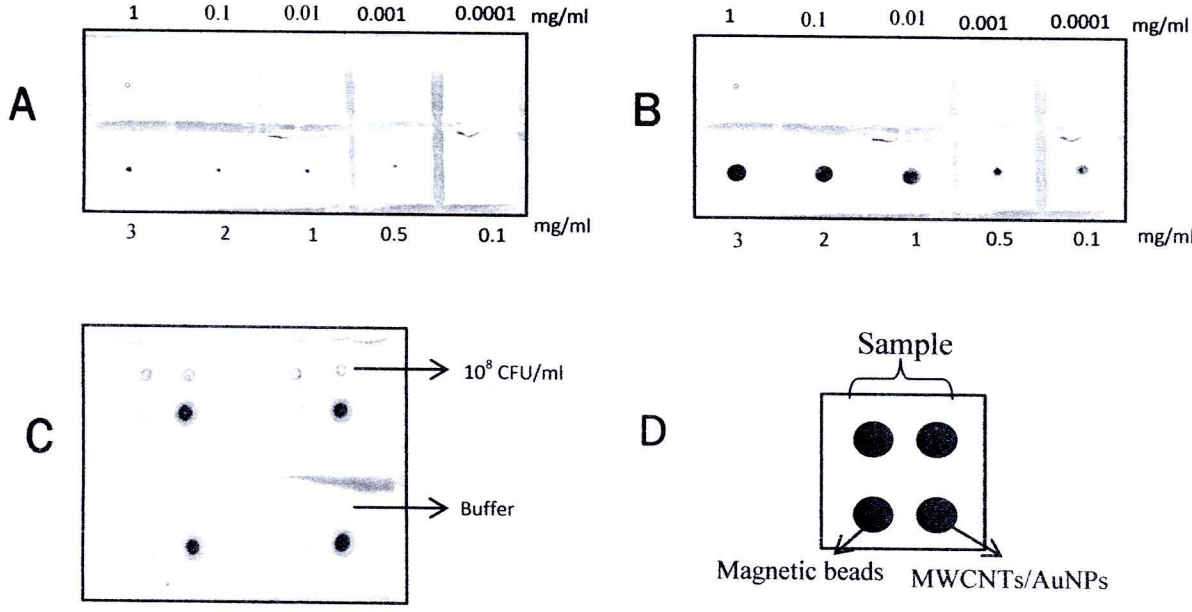


Figure 4.12 Preliminary study of MBs/Ab-biotin and MWCNTs/AuNPs/Ab complex. Drop and dry of various concentrations of magnetic beads and MWCNTs/AuNPs before (A) and after (B) silver enhancement. (C) Immunoassay of MBs/Ab-biotin and MWCNTs/AuNPs/Ab complex. (D) Pattern of spotting.

Scanning electron microscope (SEM) was utilized to characterize the particle shape, size and distribution of streptavidin functionalized magnetic beads. The reaction of 0.1 mg/mL MBs/Ab-biotin together with 0.1 mg/mL MWCNTs/AuNPs and 10^8 CFU/mL of heat killed *S. enterica* serovar Typhimurium had been selected for SEM characterization. The SEM images are shown in Figure. 4.13. The original shape of streptavidin functionalized magnetic beads before linked with MWCNTs/AuNPs are shown in Figure 4.13 (A). Figure 4.13 (B) shows the magnetic beads link with MWCNTs/AuNPs after washing process to remove all unbound MWCNTs/AuNPs. It can be seen the AuNPs really attach on MWCNTs surface that will be react with silver solution and reduce the silver ion to silver metal that can amplify the signal intensity.

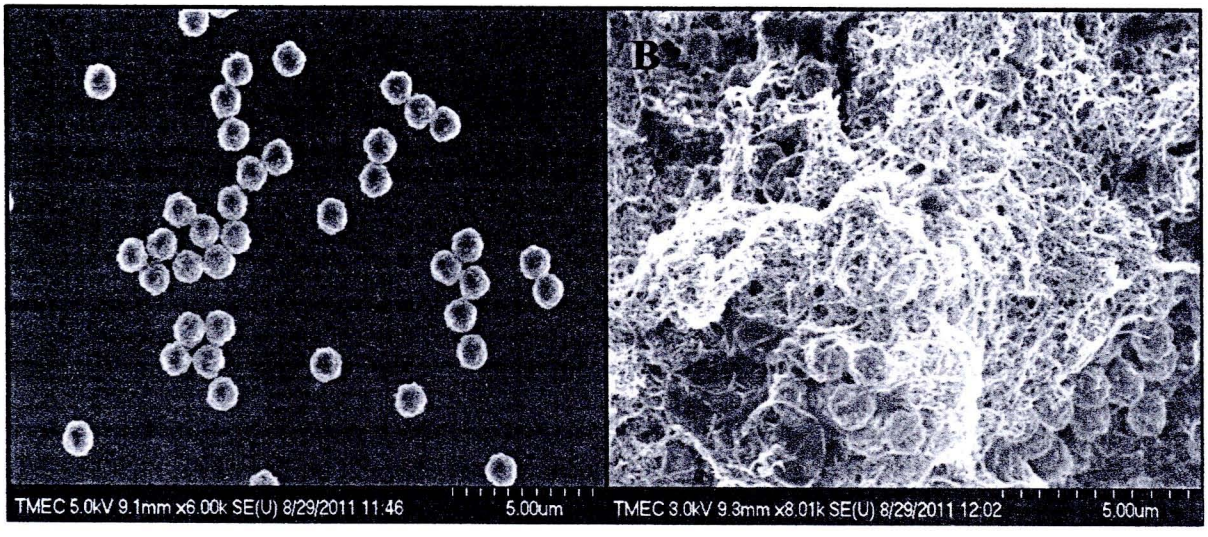


Figure. 4.13 SEM images of 0.1 mg/mL MWCNTs/AuNPs nanocomposites together with 0.1 mg/mL streptavidin functionalized magnetic beads. (A) Streptavidin functionalized magnetic beads and (B) complex of streptavidin functionalized MBs/Ab-biotin and MWCNTs/AuNPs.

4.7 Process Optimization and Limit of Detection Determination

In order to achieve high selectivity and sensitivity of the assay, a blocking step was included in the experiment. In this case, three different conditions were applied and compared for sensitivity i.e. no blocking, blocking with 1% BSA and 2% BSA. At the same time, heat-killed *S. enterica* serovar Typhimurium ranging from 0 to 10^8 CFU/mL were employed to evaluate the sensitivity of the test.

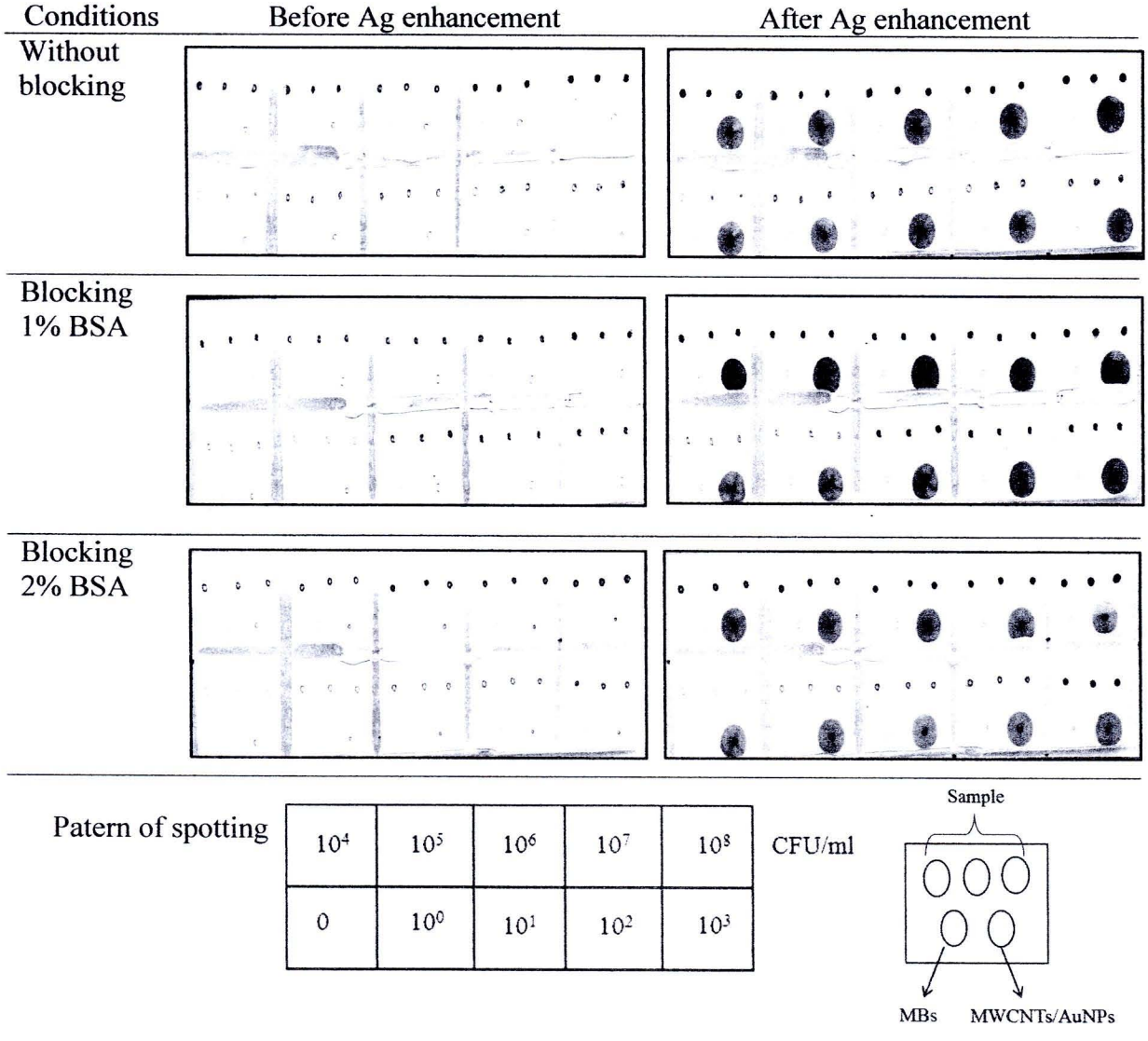


Figure 4.14 Sensitivity comparison of *S. enterica* serovar Typhimurium detection using different types of blocking buffer

As can be seen in Figure 4.14, the signal obtained were affected by the blocking buffers used. The limit of detection was determined from each blocking condition employed and was shown in Figure 4.15.

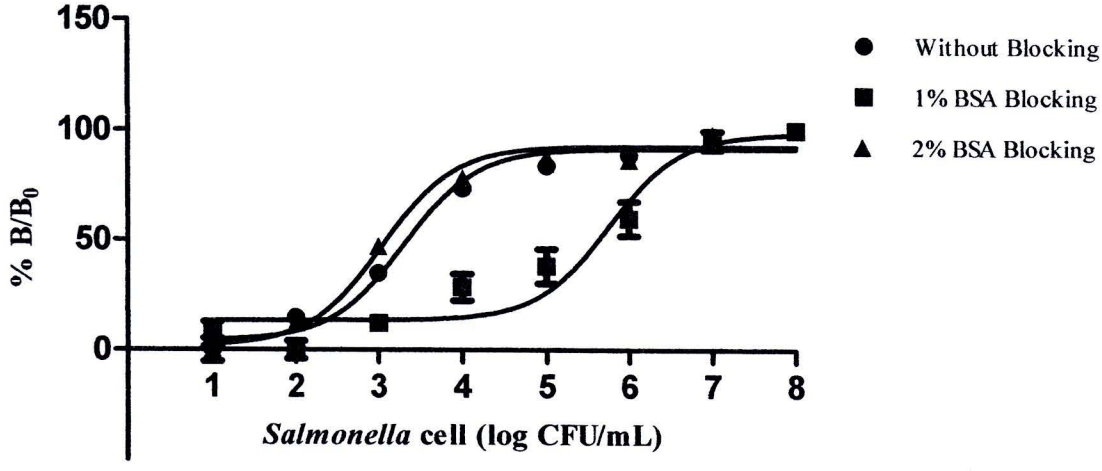


Figure 4.15 Concentrations profile detection of *S. enterica* serovar Typhimurium using MBs/Ab-biotin and MWCNTs/AuNPs/Ab complex with different blocking buffers, concentrations were varied from 10^1 - 10^8 CFU/mL. $\%B/B_0$ is percentage of binding in the presence (B) and the absence (B_0) of *S. enterica* serovar Typhimurium.

Figure 4.15 shows, the result of immunoassay MBs/Ab-biotin and MWCNTs/AuNPs/Ab complex using three different blocking buffers. The correlation coefficient (r^2) for immunoassay without blocking, 1% BSA and 2% BSA blocking were 0.97, 0.90, and 0.98, respectively. The limit of detection was calculated from mean value of blank plus 3SD and was determined by fitting to dose-response curve equation. Detection limit of immunoassay without blocking, 1% BSA and 2% BSA blocking were 18 CFU/mL, 2.11 x 10^5 CFU/mL and 42 CFU/mL, respectively. Eventhough the immuno reaction with no blocking gave the highest sensitivity, 2% BSA also attributed to low limit of detection. Accordingly, blocking MWCNTs/AuNPs with 2% BSA and no blocking were chosen for the next experiment because that gave similar values of limit of detection.

4.8 Antibody Array of *S. enterica* serovar Typhimurium Detection in Real Sample

To evaluate the feasibility of MBs/Ab-biotin and MWCNTs/AuNPs/Ab complex to detect bacteria in food samples, heat-killed *S. enterica* serovar Typhimurium cells ranging from 10^0 to 10^7 CFU/mL were spiked into commercial UHT milk (plain and low fat milk). As can be seen in figure 4.16, the plain and low fat milk without and with 2% BSA blocking gave the different signal intensity that can affect the limit of detection *S. enterica* serovar Typhimurium in milk sample.

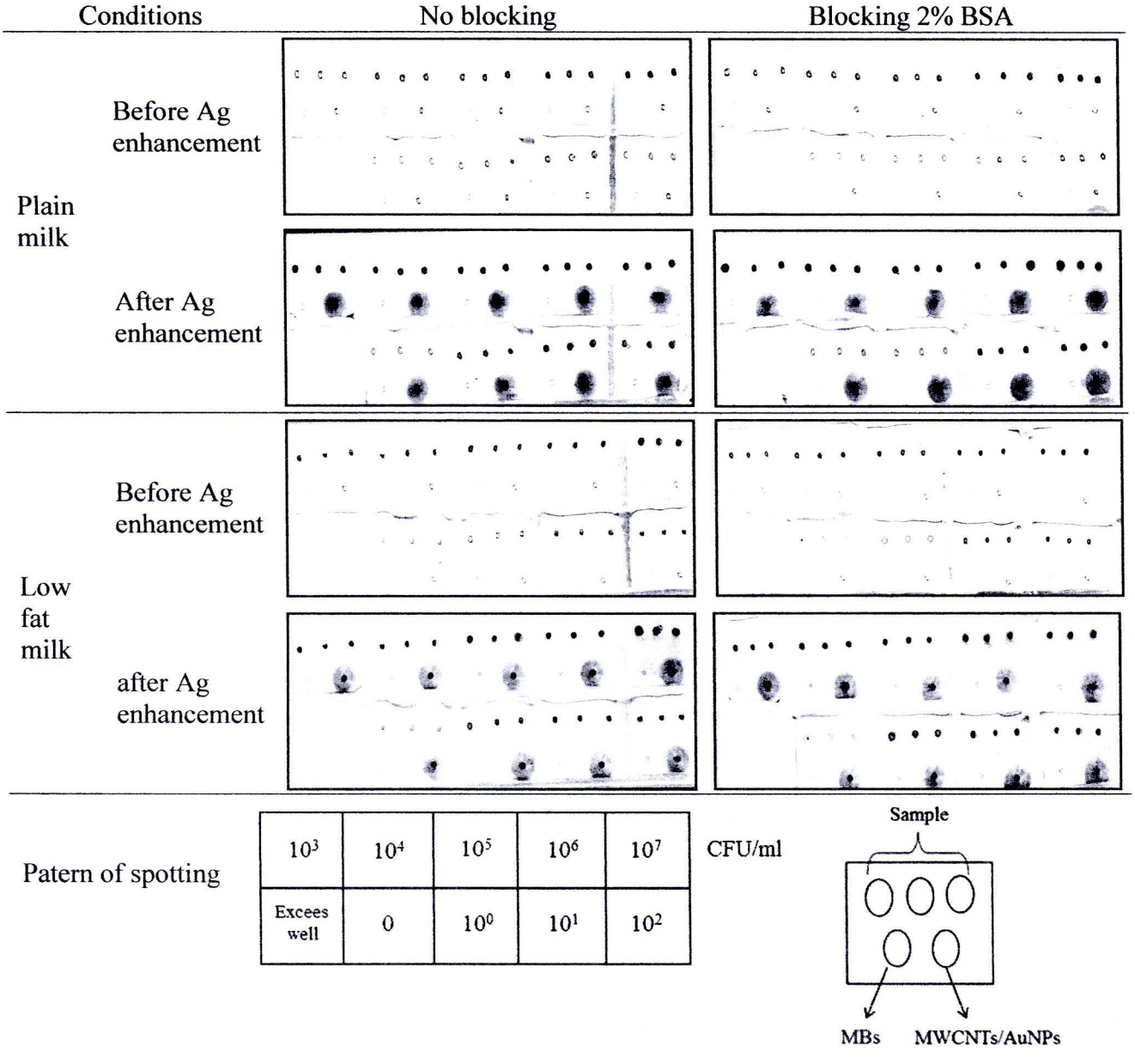


Figure 4.16 Sensitivity comparison of *S. enterica* serovar Typhimurium detection in plain and low fat mik.

The immunoassay to detect *S. enterica* serovar Typhimurium in real samples i.e. plain and low fat milk were investigated. Figure 4.17 and 4.18 show detection range of *S. enterica* serovar Typhimurium in real sample with 2% BSA and without blocking. The correlation coefficient (r^2) for immunoassay in real samples in the range of 10^1-10^7 CFU/mL was found to be 0.92 and 0.96 in plain and low fat milk for non blocking and 0.93, 0.90 for plain and low fat milk for 2% BSA blocking. The limit of detection was calculated from mean value of blank plus 3SD and was determined by fitting to a dose-response curve equation. The limit of detection of *S. enterica*

serovar Typhimurium in plain and low fat milk without blocking were found to be 1,330 CFU/mL and 1,995 CFU/mL, respectively. On the other hand, plain and low fat milk with 2% BSA blocking showed limits of detection of 10,914 CFU/mL and 134 CFU/mL, respectively. In the system where there was no blocking agent used, both samples gave similar limit of detection. However, this system with 2% BSA blocking, low fat milk showed greater limit of detection than the plain milk. The greater signal intensity in low fat milk may be due to fat content in milk, that affects the assay performance [83].

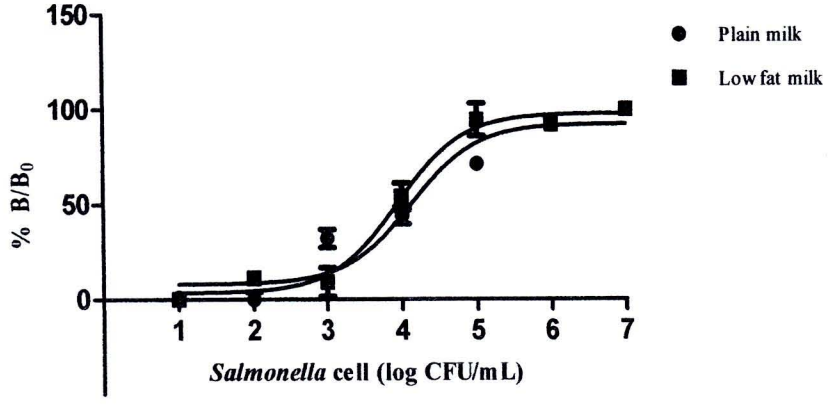


Figure 4.17 Concentrations profile of *S. enterica* serovar Typhimurium using MBs/Ab-biotin and MWCNTs/AuNPs/Ab complex in plain and low fat milk without blocking. Concentrations of *S. enterica* serovar Typhimurium were varied from 10^1-10^7 CFU/mL. %B/B_0 is percentage of binding in the presence (B) and the absence (B_0) of *S. enterica* serovar Typhimurium.

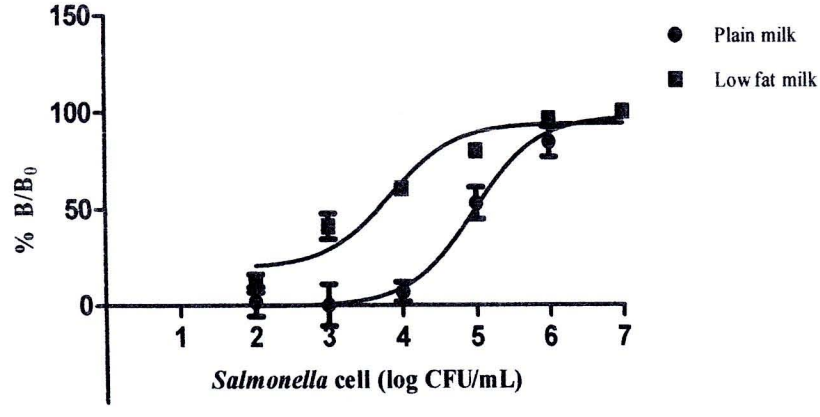


Figure 4.18 Concentrations profile of *S. enterica* serovar Typhimurium using MBs/Ab-biotin and MWCNTs/AuNPs/Ab complex in plain and low fat milk with 2% BSA blocking. Concentrations of *S. enterica* serovar Typhimurium were varied from 10^2-10^7 CFU/mL. %B/B_0 is percentage of binding in the presence (B) and the absence (B_0) of *S. enterica* serovar Typhimurium.

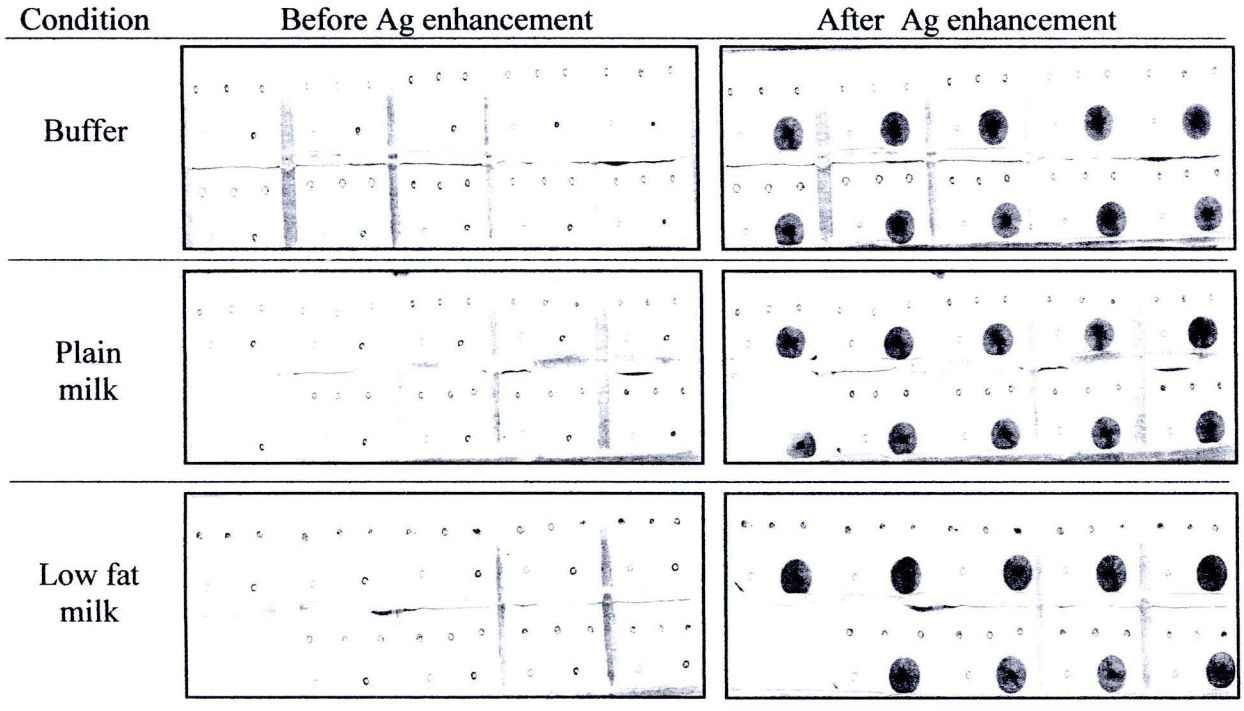
Table 4.1 show performance of previous immunoassay platforms using magnetic beads. It can be seen that limit of detection achieved here is lower than the previous work.

Table 4.1 Comparison of detection limit using magnetic beads based assay.

Method	Microorganism	LOD	Assay time	Ref.
Combining immunomagnetic separation and ELISA	*S. typhimurium*, *S. virchow*, *S. enteritidis*, *S. give*, *S. ealing* and *S. arizonae*	10^5-10^6 CFU/mL	3 h	[84]
Combining immunomagnetic separation and QCM	*S. typhimurium*	10^2 cells/ml	5 h	[85]
Automated immunomagnetic separation (IMS) and enzyme immunoassay (EIA)	*S. typhimurium*, *S. enteritidis*, *S. agona*, *S. vellore*, *S. kentucky, etc.*	10^4–10^6 CFU/mL	48 h	[86]
Electrochemical Magneto-immunosensing	*Salmonella spp.*	2.7 CFU in 25 g of sample	8 h	[22]
Magneto immuno-scanometric detection	*S. enterica* serovar Typhimurium	18 CFU/mL	2.5 h	This work

4.9 Selectivity of the Assay Toward *Escherichia coli*

The selectivity of binding of antigen on antibody depends on the selection of the antibody probe. Then, the most important analytical step is selection of antibody probe. The selectivity study was performed by using heat killed *Escherichia coli*. In order to evaluate the selectivity of the immunoassay using MBs/Ab-biotin and MWCNTs/AuNPs/Ab complex, buffer and commercial UHT milk (plain and low fat milk) were used to test. Various concentrations of heat killed *E.coli* ranging from 1-10^7 CFU/mL were spiked into buffer, plain and low fat milk.



Patern of spotting

10^3	10^4	10^5	10^6	10^7
Excees well	0	10^0	10^1	10^2

CFU/ml

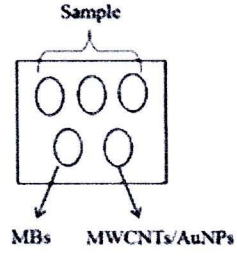


Figure 4.19 Selectivity comparison of *S. enterica* serovar Typhimurium.

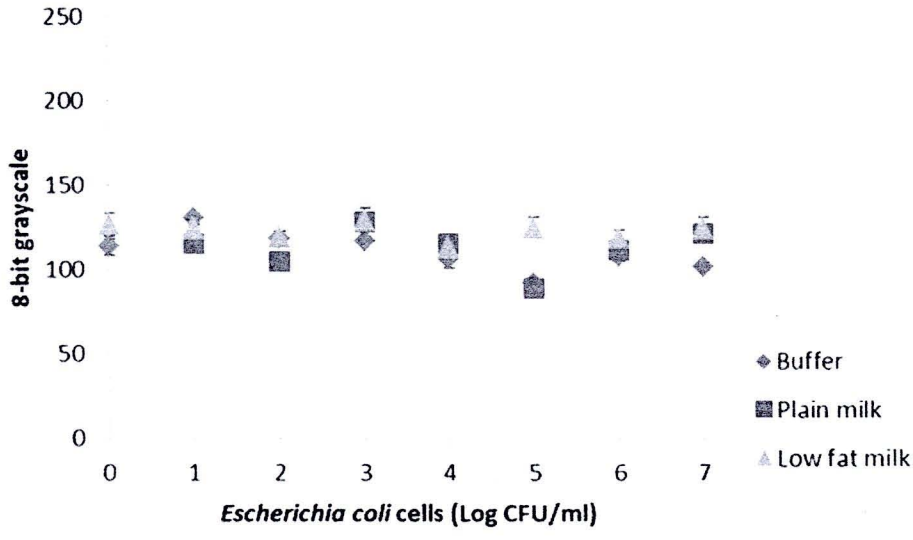


Figure 4.20 Selectivity immunoassay system using MBs/Ab-biotin and MWCNTs/AuNPs/Ab complex in buffer and real sample, concentration of *E.coli* were varied from 1-10^7 CFU/mL.

As observed in Figure 4.19 and 4.20, the signal intensity of each concentration of *E.coli* were found similar to the negative control (0 CFU/ml) in all sample matrix tested. This indicate that the MBs/Ab-biotin and MWCNTs/AuNPs/Ab complex could effectively used to detect *S. enterica* serovar Typhimurium with high selectivity.