IMMUNOASSAY BASED ON CARBON NANOTUBES/GOLD NANOPARTICLES NANOCOMPOSITE FOR *Salmonella enterica* serovar Typhimurium DETECTION

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (BIOTECHNOLOGY)

SCHOOL OF BIORESOURCES AND TECHNOLOGY
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Abstract

Salmonella enterica serovar Typhimurium is one of the most harmful pathogenic bacteria that leads to outbreak related with foods. Effective, precise, fast, selective and sensitive assay are therefore needed. To improve sensitivity of *S. enterica* serovar Typhimurium detection, multiwalled carbon nanotubes (MWCNTs) and gold nanoparticles (AuNPs) were combined and used to increase the signal intensity. In this study, immunoassay for *S. enterica* serovar Typhimurium was developed using a scanometric detection system. MWCNT/AuNP nanocomposite was fabricated by directly assembling of Au^{3+} to MWCNTs and allowing the growth of AuNPs on the surface MWCNTs during synthesis process. The MWCNTs/AuNPs nanocomposite was then attached to anti-*S. typhimurium* antibody to be used as a detecting molecule. For the immunoassay system, streptavidin functionalized magnetic beads was used together with MWCNTs/AuNPs and nitrocellulose membrane were used as a solid support. Several parameters such as blocking reagents, sensitivity and selectivity were optimized. Blocking the MWCNTs/AuNPs with 2% BSA and without blocking treatment resulted in high signal intensity. The highest sensitivity was obtained from the reaction without blocking treatment and 2% BSA at 18 and 42 CFU/mL, respectively, at 5 minute silver enhancement. By using MBs/Ab-biotin and MWCNTs/AuNPs complex, when applied to real sample gave low limit of detection as low as 10,914 CFU/mL in plain milk and 134 CFU/mL in low fat milk with 2% BSA blocking. This immunoassay using MBs/Ab-biotin and MWCNTs/AuNPs complex provided high selectivity toward *S. enterica* serovar Typhimurium as it could not detect heat killed *E.coli* cells in buffer, plain milk and low fat milk.

Keyword: *Salmonella enterica* serovar Typhimurium/ immunoassay/ MWCNTs/AuNPs nanocomposite/ scanometric detection.

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Ab	=	Antibody
Ab-biotin	=	Biotinylatedantibody
AuNPs	=	Gold nanoparticles
BSA	=	Bovine serum albumin
CFU/mL	=	Colony form unit per mililitre
CNTs	=	Carbon nanotubes
EDC	=	1-ethyl-3- (3-dimethylaminopropyl)-carbodiimide
ELISA	=	Enzyme link immunosorbent assay
$HAuCl_4$	=	Tetracloroauric acid
LOD	=	Limit of detection
LPS	=	Lipopolisacharide
mAb	=	Monoclonal antibody
MBs	=	Magnetic beads
mg/mL	=	Miligram per mililitre
MWCNTs	=	Multiwalled carbon nanotubes
MWCNTs/AuNPs	=	Multiwalled carbon nanotubes and gold nanoparticles nanocomposites
MWCNTs-COOH	=	Carboxylic acid functionalized multiwalled carbon nanotubes
$NaBH_4$	=	Sodium borohydride
ng/mL	=	Nanogram per mililitre
NHS	=	*N*-hydroxysuccinimide
PBST	=	Phosphat buffer saline tween
pAb	=	Polyclonal antibody
PCR	=	Polymerase chain reaction
RT	=	Room Temperature
SEM	=	Scanning electron microscopy
SWCNTs	=	Singlewall carbon nanotubes
TEM	=	Transmision electron microscopy