

**UNIVERSITI TEKNOLOGI MARA**

**ULTRASENSITIVE  
ELECTROCHEMICAL  
IMMUNOSENSING OF PORCINE  
SERUM ALBUMIN USING A  
NANOPOROUS ALUMINA  
MILLIROD AND A  
CARBON/GOLD NANOHYBRID**

**ABDUL HADI BIN MAHMUD**

Thesis submitted in fulfilment  
of the requirements for the degree of  
**Doctor of Philosophy**  
(Science)

**Faculty of Applied Sciences**

**December 2025**

## ABSTRACT

Pork adulteration is a pervasive global issue with significant implications for public health, economic stability, and cultural sensitivities. The methods currently employed to detect pork adulteration are often intricate, time-consuming, and financially burdensome, necessitating the development of more efficient solutions. This research addresses these challenges by introducing an innovative electrochemical immunosensor platform designed specifically for the detection of porcine serum albumin (PSA), a reliable biomarker for pork adulteration in raw meats. The proposed platform integrates a nanoporous alumina (NPA) millirod as the biorecognition surface and a self-standing nanohybrid composed of multi-walled carbon nanotubes, polyelectrolytes, and gold nanoparticles (MWCNT/PE/AuNP) as an enhanced signaling probe. The NPA millirod fabrication was optimized at 80 V for 6 h to achieve ideal pore structure for antibody immobilization, while surface functionalization with 2% (v/v) APTES for 4 h ensured stable bioconjugation. Optimization of the MWCNT/PE/AuNP nanohybrid focused on maximizing AuNP adsorption by tailoring the polyelectrolyte (PE) layer configuration via a layer-by-layer assembly. The optimal configuration was identified as a triple-layer structure (PAH–PSS–PAH), which significantly enhanced AuNP adsorption through electrostatic and coordination interactions, with no further improvement beyond this configuration due to steric hindrance and binding site saturation. For electrochemical quantification of AuNPs, differential pulse anodic stripping voltammetry (DPASV) parameters were optimized. The highest signal response and reproducibility were achieved at an electrodeposition potential of  $-0.55$  V, a deposition time of 390 s, and a scan rate of 0.05 V/s. These parameters enabled sensitive detection of dissolved AuNPs at  $+0.68$  V. Furthermore, the MWCNT/PE/AuNP signaling probe demonstrated outstanding stability under refrigerated conditions ( $4$  °C) for 30 days, maintaining electrochemical performance without degradation. The nanohybrid also showed excellent dispersion in deionized water for over a month, overcoming the sedimentation limitations observed in pristine MWCNTs. Under these optimized conditions, the immunosensor achieved a detection limit of 0.5 pg/mL and a linear response range of  $0-1 \times 10^3$  pg/mL for purified PSA. It also exhibited high specificity, with no cross-reactivity to non-target animal meats. Importantly, the sensor successfully detected pork adulteration as low as 0.01% in beef matrices. These results establish the immunosensor as a robust, sensitive, and selective tool for meat authentication, supporting improved food safety, consumer protection, and regulatory compliance.

## ACKNOWLEDGEMENT

First and foremost, I offer my deepest gratitude to Allah for granting me the opportunity to embark on the journey of pursuing my PhD and for guiding me through its many challenges to successful completion. To my late mother,

, I dedicate this work. Her unwavering love and support continue to be a beacon of inspiration for me every single day. Her memory fuels my determination and serves as a constant reminder of the sacrifices made for my pursuit of knowledge.

I also wish to express my heartfelt appreciation to my father, Mahmud bin Hamid, whose continuous prayers, guidance, and encouragement have been a pillar of strength throughout my life. His perseverance and wisdom have deeply influenced my character and academic journey.

My deepest love and gratitude go to my wife, Nurazrin Ezrina binti Abdul Mubdi, whose unwavering faith in me and steadfast support have been the cornerstone of my PhD journey. Her sacrifices, both big and small, have allowed me to focus on my research with confidence and determination. Without her unwavering presence by my side, this milestone would not have been attainable.

To my beloved daughter, Nur Hayfa Eryna, who came into our lives during the final stages of this journey, you are a blessing and a source of renewed strength. Your arrival has filled our hearts with immense joy and given new meaning to every effort I have made along the way.

My sincere thanks go to my supervisor, Dr. Low Kim Fatt, whose exceptional mentorship, guidance, and unwavering belief in my potential have been instrumental throughout this endeavor. His expertise and encouragement have propelled me forward, even in the face of challenges, and I am deeply grateful for his unwavering support. I am equally indebted to my co-supervisor, Prof. Dr. Abdul Mutalib, whose continuous support from the inception of my postgraduate studies until the completion of this thesis has been invaluable. His guidance, wisdom, and encouragement have been a constant source of strength and motivation.

I would also like to express my gratitude to my co-supervisors, Assoc. Prof. Dr. Noor Fitrah Abu Bakar and Dr. Rizana Yusoff, for their invaluable assistance and support throughout this journey. Their expertise and insights have enriched my research and contributed significantly to its success. To my colleagues and lab mates, I extend my sincere thanks for their collaboration, camaraderie, and unwavering support throughout this journey. Their friendship, encouragement, and shared experiences have made this journey more meaningful and rewarding.

As I conclude this chapter of my academic journey, I pray that Allah blesses each of these individuals abundantly and eases their future endeavors. Their collective support and encouragement have been instrumental in shaping this thesis, and for that, I am profoundly grateful.

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# CHAPTER 1

## INTRODUCTION

### 1.1 Background of Study

Meat plays a vital role in the global food supply as a primary source of protein and essential nutrients [1]. However, the safety and authenticity of meat products are increasingly challenged by food adulteration practices, particularly the substitution or inclusion of undeclared animal species like pork in higher-value meats such as beef or lamb [2], [3]. Pork adulteration is primarily motivated by economic gains due to its lower cost and similar texture and color, enabling fraudulent substitution without easy detection. Beyond ethical concerns, this practice poses serious implications for consumers with pork allergies and for those who adhere to dietary restrictions rooted in religious beliefs. Individuals with pork allergies may experience harmful immune reactions triggered by porcine proteins, including gastrointestinal symptoms, urticaria, and anaphylaxis. Additionally, pork is prohibited in religious practices such as Islam and Judaism, which together represent nearly one-third of the global population [4]. The ability to reliably detect pork adulteration in raw meat is therefore critical to ensuring food safety, consumer protection, and religious compliance.

Among the various pork-derived proteins, porcine serum albumin (PSA) has emerged as a reliable biomarker for pork detection. PSA is a 66 kDa protein abundant in pork serum, with a well-defined and highly stable structure, including nine disulfide-bonded loops and a negative charge at physiological pH. Its structural specificity and abundance in pork make PSA a suitable target for analytical techniques aimed at identifying pork adulteration in raw meats.

Conventional methods used to detect pork adulteration include high-performance liquid chromatography (HPLC), enzyme-linked immunosorbent assay (ELISA), Fourier transform infrared spectroscopy (FTIR), and polymerase chain reaction (PCR). While effective, these techniques typically require costly equipment, time-consuming protocols, and skilled personnel, which limits their accessibility for routine screening. Simpler alternatives, such as lateral-flow dipstick assays, have been developed to facilitate rapid on-site testing. However, these methods are often limited