NOVEL GREEN NANOBIO-BASED REAGENT FOR RAPID VISUALISATION OF LATENT FINGERPRINTS ON NON-POROUS SUBSTRATES IMMERSED IN A NATURAL OUTDOOR POND

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ABSTRACT

Candida rugosa lipase (CRL) has been gaining attention in various bioindustries. Despite being commonly reported to have high affinity towards lipids, its potential utilisation for visualising latent fingerprints for forensic purposes remains in its nascent stage. Considering the evidential values of fingerprints during underwater criminal investigations and the need to visualise them using a user- and environmentally-friendly reagent, development of a novel, green, and rapid nanobiobased reagent (NBR) was deemed beneficial. Additional to the limited availability of fingerprint biotechnological reagents utilising CRL, they appeared lengthy with at least three different solutions, non-optimised and did not comply with the prevailing guidelines for fingerprint reagent development. Therefore, this research was aimed at developing a novel, green and optimised NBR for rapid visualisation of fingerprints on wet non-porous substrates, in compliance with the guidelines. This research involved characterisations of the NBR, Response Surface Methodology (RSM) optimisation, stability and sensitivity assays, as well as field assessment of the performance of NBR in a natural outdoor pond for up to four weeks of immersion. While characterisation of NBR using Attenuated Total Reflectance-Fourier Transform Infrared spectroscopy (ATR-FTIR) and Field Emission-Scanning Electron Microscope (FESEM) confirmed the successful attachment of CRL onto the nanosupports, Scanning Electron Microscope (SEM) analysis provided detailed representation of specific attachments of the NBR onto the lipid-lined fingerprint ridges. Semi-quantitative chromatographic analysis confirmed the presence of nhexadecanoic and octadecanoic acids in wet fingerprints immersed in stagnant tap water for 30 days under laboratory-controlled settings. Subsequently, bioinformatics studies supported the presence of hydrogen bonds between the residue of CRL and commonly reported lipids found in fingerprint constituents. It was found that the optimum conditions for preparing the NBR with University of Canberra (UC) comparative scale of 0 were; 100 mg of CRL, 75 mg of acid functionalised multiwalled carbon nanotubes and 5 h of immobilisation interval. Both stability and sensitivity assays revealed that the NBR was able to visualise wet fingerprints even after four weeks of storage as well as up to six-split depletion series with modified-Centre for Applied Science and Technology (m-CAST) absolute scale of 4 and UC comparative scale of 0 particularly on glass slides, respectively. Field assessment revealed better quality of NBR-visualised groomed split fingerprints, particularly on glass slides at four weeks of immersions with UC comparative and m-CAST absolute scales of +1 and 2, respectively when compared to that of Small Particle Reagent (SPR). Furthermore, greenness assessment of NBR revealed the Eco-Scale points of 76, indicating 'excellent green analysis'. Hence, the potential of NBR as the future stateof-the-art green fingerprint visualisation technology is empirically supported.

ABSTRAK

Lipase Candida rugosa (CRL) telah mendapat perhatian dalam pelbagai bioindustri. Meskipun sering dilaporkan mempunyai afiniti yang tinggi terhadap lipid, potensi penggunaannya untuk memvisualkan cap jari untuk tujuan forensik masih berada di peringkat awal. Memandangkan nilai bukti cap jari semasa penyiasatan jenayah dalam air dan keperluan untuk memvisualkannya menggunakan reagen yang mesra pengguna dan alam sekitar, pembangunan reagen baharu yang berasaskan nanobio (NBR), hijau dan pantas adalah bermanfaat. Sebagai tambahan kepada keterbatasan kebolehdapatan reagen bioteknologi cap jari menggunakan CRL, ia dilihat memakan masa dengan sekurang-kurangnya tiga larutan yang berbeza, tidak optimum dan tidak mematuhi garis panduan lazim untuk pembangunan reagen cap jari. Justeru, penyelidikan ini bertujuan untuk membangunkan NBR baharu, hijau dan yang optimum untuk pemvisualan cap jari yang pantas pada permukaan basah tidakberliang dengan mematuhi garis panduan. Kajian ini melibatkan pencirian NBR, pengoptimuman perkaedahan respon permukaan (RSM), ujian kestabilan dan kepekaan serta penilaian lapangan terhadap prestasi NBR di sebuah kolam semula jadi sehingga empat minggu rendaman. Sementara perincian NBR menggunakan spektroskopi transformasi inframerah Fourier pantulan keseluruhan dikecilkan (ATR-FTIR) dan mikroskop imbasan elektron pancaran medan (FESEM) mengesahkan penempelan CRL ke atas sokongan nano, analisis mikroskop imbasan elektron (SEM) pula memberikan gambaran terperinci mengenai penempelan khusus NBR di atas rabung cap jari yang dilapisi lipid. Analisis kromatografi semi-kuantitatif mengesahkan kehadiran asid n-heksadekanoik dan oktadekanoik pada cap jari basah yang terendam selama 30 hari dalam air paip yang bertakung di bawah tetapan makmal yang terkawal. Seterusnya, kajian bioinformatik menyokong kehadiran ikatan hidrogen di antara residu CRL dan lipid yang biasa dijumpai dalam komposisi cap jari. Kajian mendapati bahawa kondisi optimum untuk menyediakan NBR dengan skala perbandingan University of Canberra (UC) 0 adalah; 100 mg CRL, 75 mg nanotiub karbon multidinding berfungsi asid dan 5 jam tempoh masa immobilisasi. Kedua-dua ujian kestabilan dan kepekaan masing-masing menunjukkan bahawa NBR dapat memvisualkan cap jari basah walaupun selepas empat minggu penyimpanan dan mampu memvisualkan sehingga enam siri pisahan susutan dengan skala mutlak Pusat Sains Gunaan dan Teknologi yang diubah suai (m-CAST) 4 dan skala perbandingan UC 0 terutamanya di atas slaid kaca. Penilaian lapangan menunjukkan kualiti cap jari pisah NBR yang lebih baik daripada reagen partikel kecil (SPR) terutamanya di atas slaid kaca selepas empat minggu rendaman dengan skala perbandingan UC +1 dan skala mutlak m-CAST 2. Tambahan pula, penilaian kehijauan NBR menujukkan 76 mata skala-eko, menggambarkan 'analisis hijau yang sangat baik'. Oleh yang demikian, potensi NBR sebagai teknologi pemvisualan cap jari hijau yang terkehadapan adalah disokong secara empirikal.

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ridges and smudged area.

LIST OF ABBREVIATIONS

ATR-FTIR	-	Attenuated total reflectance – Fourier transform infrared spectroscopy
ACE-V	-	Analysis, comparison, evaluation and verification
ANOVA	-	Analysis of variance
BBD	-	Box-Behnken design
BIOFIS	-	Biometric Fingerprint Identification System
CAST	-	Centre for Applied Science and Technology
CRL	-	Candida rugosa lipase
CRL-MWCNTs	-	Candida rugosa lipase – multi walled carbon nanotubes
DNA	-	Deoxyribonucleic acid
DO	-	Dissolved oxygen
FBI	-	Federal Bureau of Investigation
F-MWCNTs	-	Acid functionalised multi walled carbon nanotubes
FESEM	-	Field emission scanning electron microscopy
FLS	-	Forensic light source
GCMSD	-	Gas chromatography mass selective detector
IAFIS	-	Integrated Automated Fingerprint Identification System
IDENT1	-	Integrated identification service
IFRG	-	International Fingerprint Research Group
III	-	Interstate Identification Index
m-CAST	-	Modified-Centre for Applied Science and Technology
MMD	-	Multi metal deposition
MALDI MSP	-	Matrix assisted laser desorption ionisation mass spectrometry profiling
MoS ₂	-	Molybdenum disulfide

MWCNTs	-	Multi walled carbon nanotubes
NBR	-	Nanobio-based reagent
OVAT	-	One-variable-at-a-time
PD	-	Physical developer
PDRM-MAFIS	-	Polis Diraja Malaysia – Malaysian Automated Fingerprint Identification System
RMP	-	Royal Malaysian Police
RMSD	-	Root mean square deviation
RSM	-	Response surface methodology
RUVIS	-	Reflected ultraviolet imaging system
SEM	-	Scanning electron microscopy
SMD	-	Single metal deposition
SPR	-	Small particle reagent
TIC	-	Total ion chromatogram
TiO ₂	-	Titanium dioxide
UC	-	University of Canberra
UTM	-	Universiti Teknologi Malaysia

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indicating its sensitivity at visualising aged fingerprints.

CHAPTER 1

INTRODUCTION

1.1 Background of the research

The admissibility of fingerprints in the court of law has always been on the grounds of its uniqueness, permanency and systematic classification of general ridge patterns, leading to its global acceptance as forensic evidence (Saferstein, 2018). Importantly, the evidential value of fingerprints has outpaced DNA, practically solving more criminal cases than that of DNA (Innes, 2008). Fingerprints can be classified into patent, plastic and latent. While patent fingerprints are prints that are on transferable wet-coloured media (e.g. greasy and bloody prints), plastic fingerprints refer to the impressions of friction ridge skin that are left on soft, malleable media such as wax and soap. On the other hand, latent fingerprints can be problematic and challenging to the forensic investigators due to its hidden nature and hence, requiring the use of suitable fingerprint visualisation methods (Saferstein, 2018). The selection of the visualisation method is surface-dependent i.e. porosity and wetness (Kent, 2013; Houck and Siegel, 2015; Saferstein, 2018). In addition, the successful visualisation of latent fingerprints relies on the different physical and/or chemical reactions of the respective reagents with the available water- (e.g. amino acids) and non-water-soluble (e.g. fatty acids) constituents of fingerprints (Houck and Siegel, 2015; Kasper, 2016a).

It is pertinent to quote here that 'criminals often seek a watery repository for weapons and other evidence of wrongdoing' disposal and 'forensic evidence is not necessarily lost when it has been immersed in water' (Becker, 2013a). Therefore, retrieving potential underwater forensic evidence such as fingerprints is an integral element in criminal investigations, particularly in establishing the identity of the perpetrators. In instances whereby, the water-soluble amino acids might have been washed off or dissolved by water, the use of small particle reagent (SPR) and physical developer (PD), that presumably capitalise on the non-water-soluble constituents of fingerprints, have been recommended for non-porous and porous substrates, respectively (Houck and Siegel, 2015). Interestingly, while the application of SPR and PD for visualising latent fingerprints on wet substrates has been a commonplace in forensic investigations, review of the literature does not reveal any explicit/concrete physical and/or chemical explanations on the interactions of both SPR and PD with the non-water-soluble constituents of fingerprints (Azman *et al.*, 2019a). In fact, the classic presumption that PD reacts with the lipid constituents of fingerprints has been challenged by de la Hunty *et al.* (2015a,b). The challenge was based on the fact that fingerprint constituents using various organic solvents, disputing that PD is specifically targeting the lipids (de la Hunty *et al.*, 2015a). When tested on the eccrine sweat, de la Hunty *et al.* (2015b) postulated on other constituents in the eccrine secretions that protect the lipids from solubilisation by aqueous washes. Therefore, it can be construed that while PD can be useful, suitable explanations on the chemical interactions that enable them to visualise latent fingerprints remain puzzling.

Nonetheless, despite the several attempts to clarify the chemical explanations for PD at visualising latent fingerprints reported in literature (de la Hunty *et al.*, 2015a,b), the same remains unavailable for the SPR. It is pertinent to indicate here that typical SPR is a suspension of fine particles of either titanium dioxide (TiO₂) or molybdenum disulfide (MoS₂) in a surfactant (Ramotowski, 2013), as contrasting agents. Because numerous medical (Wang *et al.*, 2015; Hashem *et al.*, 2020) and environmental studies (Reid, 2002; Norgate *et al.*, 2007) advocated on the toxicity of these two chemical compounds, their exposures towards human and environment must be reduced, and suitable environmentally benign alternative reagent must be developed. In this context, except for the articles published from this present research, review of literature revealed limited studies (Azman *et al.*, 2018; Puspanadan, 2018) focusing on developing an environmentally friendly *Candida rugosa* lipase (CRL) nanoconjugates reagent or other lipases for visualising latent fingerprints on wet, non-porous substrates.

Lipases (triacylglycerol acyl hydrolases EC 3.1.1.3) as biocatalysts have been gaining considerable popularity among various bio-industries (Izrael Živković *et al.*,

2015), owing to its broad specificity (Brahmachari, 2017). They are widely involved in various chemical reactions such as hydrolysis (Anand and Weatherley, 2018; Lu et al., 2018; Urrutia et al., 2018) and synthesis of esters (Abd Manan et al., 2018; Kim et al., 2019a). Among many others, CRL continues to be the popular choice of versatile biocatalyst (Mohamad et al., 2015a; Mohamad et al., 2015b). Since free lipases are often reported as unstable with low catalytic activity, particularly under harsh conditions (Mohammadi et al., 2020), immobilisation of CRL on various supports is commonly observed (Cavalcanti et al., 2018; Onoja et al., 2018; Zare et al., 2018). Besides, immobilisation of CRL specifically onto acid-functionalised multiwalled carbon nanotubes (F-MWCNTs) (CRL-MWCNTs) confers improved activity, stability (Che Marzuki et al., 2015a) and mechanical strength of the biocatalyst, as well as resistant towards premature unravelling (Mohamad et al., 2015a). Interestingly, despite lipases being commonly reported to have high affinity towards lipids (Liu and Kokare, 2017), their potential utilisation for visualising wet latent fingerprints for forensic purposes remains scarce. Hence, this noteworthy aspect of developing a novel and green nanobio-based reagent (NBR) (CRL-MWCNTs) for visualising latent fingerprints on wet, non-porous substrates, the focal point of this research, merits scientific and forensic applications.

1.2 Problem statement of the research

It has been indicated that the use of recreational waterways has been increasing, leading to high incidences of accidents, drownings, violent crimes as well as homicides, and subsequently for disposing evidence (Becker, 2013a). For example, a knife believed to have been used in alleged double murders was discovered in River Stout in Canterbury, United Kingdom (KentOnline, 2016). Similarly, a rubber-tapping knife and a handcuff believed to have been used in a murder were recovered by the Royal Malaysian Police (RMP) Marine Operations Force in Sungai Kanchong, Selangor (TheStar Online, 2010). Acknowledging the soaring trend of criminal cases involving aquatic environment particularly as secondary crime scenes, police subaquatic forensic investigation units have been established in many countries (Becker, 2013b), including Malaysia. Leaving alone that latent fingerprints on

immersed weapons/substrates may be disturbed during underwater crime scene investigations (Becker, 2013a) or even by aquatic fauna, water itself may dissolve its amino acid constituents (Appell *et al.*, 2018).

Because lipids are insoluble in water, the use of SPR that arguably reacts with the lipid constituents of fingerprints on wet, non-porous substrates has been routinely recommended (Kasper, 2016b), although the real physical and/or chemical interactions remain unknown. Since SPR has been prevailingly used for on-site crime scene investigations, and because it contains either TiO₂ or MoS₂ (as contrasting agent), prolonged exposure towards these toxic chemicals may prove detrimental for human and the environment. Although inadequate evidence is available, the International Agency for Research on Cancer (IARC) (2010) recommended that TiO₂ is possibly carcinogenic to humans based on the empirical data in animal studies . For instance, rats exposed to the concentration of as low as 10 mg/m³ via inhalation for 6 hours per day (5 days per week) for two years had increased incidences of lung tumours than those of controls. This is possibly due to the excessive production of intracellular reactive oxygen species (Gao et al., 2015). As for MoS₂, its chronic exposure may cause chronic respiratory effects, irritations of eyes, nose, and skin (Centers for Disease Control and Prevention, 2011). For example, the combination of molybdenum with other environmental stressors in lakes and creeks has resulted in substantial decline in the population of juvenile kokanee salmon (Reid, 2002). Therefore, it can be construed that the continuous use of SPR that involves rinsing with water may lead to not only the chronic build-up of TiO₂ and MoS₂ in the aquatic environments, but also detrimental effects on humans. Despite the current trends of utilising the green chemistry principle for various industrial and experimental applications, such aspect for fingerprint visualisation technology remains in its very nascent stage. Having said that, it is therefore paramount to explore a greener alternative for visualising latent fingerprints on wet, non-porous substrates.

Despite the successful initial attempt to visualise latent fingerprints on wet, non-porous substrates using the novel safranin-tinted CRL nanoconjugates reagent (Azman *et al.*, 2018), the method is (1) time-consuming (about nine mins) because it requires the use of three different solutions. Moreover, that study utilised (2) groomed

fingerprints obtained from a single female donor alone for the field assessment (3) on one type of substrate (knife) only. Therefore, the procedure used for testing the safranin-tinted CRL nanoconjugates reagent that they reported did not comply with the prevailing guidelines prescribed by the International Fingerprint Research Group (IFRG) (2014), possibly limiting its acceptance by the forensic fingerprint community. Hence, this present research was aimed at shortening the time of visualisation by using a single optimised novel solution i.e. CRL-MWCNTs (NBR). Since the optimum working pH for CRL-MWCNTs is 7 (Che Marzuki et al., 2015a; Mohamad et al., 2015b), while the pH in natural waterways can range between 5 - 8 (Jovanelly *et al.*, 2015; Ewaid et al., 2018; Wu et al., 2018), optimisation of the novel NBR using Response Surface Methodology (RSM) proves necessary to ensure its usability for forensic practical caseworks involving varying aquatic environments. The fact that this research also focused at characterising the NBR and elucidating its possible interactions with the non-water-soluble constituents of fingerprint, such information would substantially fill the gap in the body of knowledge, leading to further endeavours to improve the formulations. In addition, its stability (at chilled and sultry storage conditions) and sensitivity (visualising increasingly weaker fingerprints up to six split-depletion series) assays were investigated too. The conceptual framework of this research is provided in Figure 1.1.

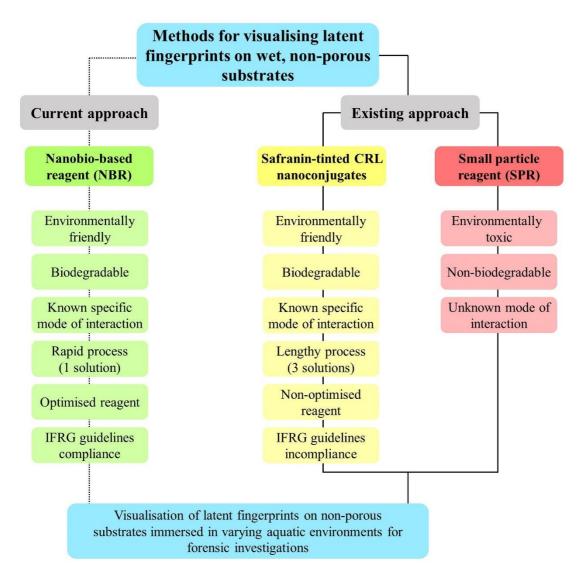


Figure 1.1 The conceptual framework of this present research. Dotted and completed arrows indicated the current nanobiotechnological as well as the existing approaches for visualising latent fingerprints on non-porous substrates immersed in varying aquatic environments, respectively.

1.3 Objectives and hypothesis of the research

1.3.1 Objectives

This research was therefore aimed to:

- 1. Propose and support the interactions between the non-water-soluble constituents of fingerprints with that of the novel NBR.
- 2. Optimise and assess the robustness (i.e. stability and sensitivity assays) of NBR using non-porous substrates immersed in stagnant tap water under laboratory-controlled settings.
- 3. Assess the quality of visualised fingerprints on non-porous substrates immersed in a natural outdoor pond using the novel optimised NBR with that of SPR.

1.3.2 Hypothesis

It was hypothesised that the quality of visualised fingerprints on non-porous substrates immersed at the different periods of immersion in a natural outdoor pond using the novel optimised NBR would be comparable with that of SPR.

1.4 Scope of the research

This research was conducted in three stages. Stage 1 involved characterisation of the NBR using Attenuated Total Reflectance – Fourier Transform Infrared spectroscopy (ATR-FTIR), Field Emission Scanning Electron Microscopy (FESEM), Scanning Electron Microscopy (SEM), Gas Chromatography Mass Selective Detector (GCMSD) and bioinformatics analysis (molecular docking and molecular dynamics). A groomed fingerprint refers to an impression that is loaded with sebaceous constituents obtained after the donor deliberately touches the nose, face and hair (IFRG, 2014) . On the other hand, natural fingerprint refers to an impression that devoid of such touches, containing limited amount of sebaceous constituents.

Stage 2 involved the optimisation of the NBR on groomed split fingerprints immersed for two consecutive weeks in stagnant tap water under laboratory controlledsettings. The relevant parameters (i.e. amount of CRL, amount of F-MWCNTs and immobilisation interval) were optimised using RSM with Box-Behnken design (BBD). Then, the optimised formulation suggested by the RSM was empirically validated using groomed split fingerprints deposited on glass slides immersed in stagnant tap water under laboratory-controlled settings for another two weeks. The optimised formulation of NBR as suggested by RSM was then investigated for its stability at two different common storage conditions viz. chilled (fridge: $2 - 4^{\circ}$ C) and sultry (car: $23 - 4^{\circ}$ C) 45°C) to mimic the normal storage conditions at laboratory (Che Marzuki et al., 2015a; Mohamad et al., 2015b; Azman et al., 2018), as well as for the scene of crime investigating officers. In addition, Stage 2 entailed the use of six split-depletion series - successive impressions of the fingerprint - in evaluating the sensitivity of the optimised NBR. The quality of visualised fingerprints was evaluated against the appropriate scales i.e. University of Canberra (UC) comparative (McLaren et al., 2010) (for RSM optimisation and sensitivity assay) and modified Centre for Applied Science and Technology (m-CAST) absolute scales (Bandey and Gibson, 2006) (for stability assay), as suggested by the IFRG (2014).

Stage 3 involved the forensic assessment of groomed and natural split fingerprints immersed in a natural outdoor pond for a duration of two and four consecutive weeks. The fingerprints were then visualised using both the novel optimised NBR and prevailingly-used SPR. Subsequently, the quality of visualised fingerprints was compared against the UC comparative (McLaren *et al.*, 2010) and m-CAST absolute scales (Bandey and Gibson, 2006). Additionally, the greenness of the novel NBR was also assessed using the prevailing analytical Eco scale suggested by Gałuszka *et al.* (2012).

1.5 Significance and novelty of the research

This research was aimed at developing a novel, green and optimised NBR for rapid visualisation of latent fingerprints on wet, non-porous substrates with comparable performance to SPR (if not better). Because suitable explanations pertaining to the physical and/or chemical interactions between the varying fingerprint visualisation reagents with that of fingerprint constituents remain lacking, specific attempt to investigate such matter for the novel NBR appears relevant. While the development of this novel NBR may provide a relatively greener option for visualising latent fingerprints on wet, non-porous substrates, information on its interactions may serve as a stepping stone for further improvements in the forensic fingerprint technology. Remarkably, this research too would concurrently reduce the adverse and harmful effects of toxic chemical build-ups like TiO₂ and MoS₂ (from SPR) in the drainage, and thus effectuating a safer, relatively greener ecosystem while being mindful of the health of the forensic scientists/investigators at the scenes of crime.

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