UNIVERSITI PUTRA MALAYSIA

PHYSICOCHEMICAL AND CYTOTOXIC PROPERTIES OF MIXED PALM OIL-DERIVED CATANIONIC SYSTEMS

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FBSB 2021 10

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

November 2020

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

PHYSICOCHEMICAL AND CYTOTOXIC PROPERTIES OF MIXED PALM OIL-DERIVED CATANIONIC SYSTEMS

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November 2020

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Versatility of surfactants marked its importance in industrial applications. Carboxylic acid derived from oleochemicals is one of the anionic surfactants. In order to diversify its applications, creating new compound with various functionality such as thermal behaviour and surface activity from the existing carboxylic acid is essential. Catanionic surfactant, the new class of surfactant, is attracting much interest due to its superior physicochemical properties. Some catanionic systems even show anti-cancer activities which is making a breakthrough for cancer treatment. The current study aimed to produce catanionic surfactants systems having palm oil-derived materials such as carboxylic acid and to explore some of their physicochemical properties (thermal behaviour and surface activities) and cytotoxicity. Catanionic surfactants systems having different degrees of chain length asymmetry were prepared from cationic quaternary ammonium surfactants (alkyl chain lengths of 12- to 18-carbon) and anionic palm oil-derived carboxylic acids (alkyl chain lengths of 8- to 18-carbon), respectively. The characteristics of neat catanionic surfactants using Fourier Transform Infrared (FTIR) and X-ray diffraction (XRD) confirmed the formation of catanionic surfactants with crystalline structures. The produced catanionic surfactants displayed greater thermal stability with 10% of weight loss at 125.15°C to 216.29°C and up to six thermal phase transition as compared with the parent surfactants, respectively. Similarly, the total change in enthalpy (ΔH) and entropy (ΔS) of catanionic surfactants were higher than their parent surfactants, in the range of 44.32 kJ mol^{-1} to 157.15 kJ mol^{-1} and 119.07 J K^{-1} mol^{-1} to 409.68 J K^{-1} mol^{-1}, showing higher thermal stability and changes of molecular motion in the formation of more disordered phase when subjected to heat. Noticeably, the total ΔH and ΔS were closely related with the degree of chain length asymmetry in catanionic surfactant. Surface properties of aqueous catanionic systems were investigated via surface tension measurement. The critical aggregation concentration (CAC) obtained for catanionic systems indicated better self-aggregation capability and ranged from 0.0004 mM to 2.130 mM, at least 70% lower than cationic parent surfactants. The feature was also supported by higher surface excess concentration (Γ_{max}) of catanionic systems from 1.26 × 10^{-6} mol m^{-2} to 3.82 × 10^{-6} mol m^{-2} and lower minimum area per molecule (A_{min}) ranging from 43 Å^2 to 131 Å^2 that was induced by an effective area reduction of

oppositely charged headgroups. Other than that, cytotoxicity of catanionic surfactants and their parent surfactants were tested on both normal fibroblast 3T3 and breast cancer MDA-MB-231 cell lines. Cytotoxicity of catanionic surfactants and parent surfactants was found to increase with their alkyl chain length. The $C_{18}TA_{18}$ possessed highest cytotoxicity with half maximal inhibitory concentration (IC_{50}) of 5.9 µM ± 0.3 µM on normal 3T3 cell line and 4.0 µM ± 0.1 µM on cancerous MDA-MB-231 cell line. Incorporation of anti-cancer agent into $C_{18}TA_{18}$ was found to exert lower cytotoxicity on 3T3 cell line than the treatment with $C_{18}TA_{18}$ alone, the cytotoxicity was reduced by 1.8-fold to 4.6-fold. In conclusion, palm oil-derived catanionic surfactants exhibited enhanced physicochemical and anti-cancer activities, with $C_{18}TA_{18}$ incorporated with anti-cancer agents exhibited potential anti-cancer activities.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PENCIRIAN FIZIKOKIMIA DAN KESITOTOKSIKAN PENCAMPURAN SISTEM KATANIONIK BERASASKAN SAWIT

Oleh

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Surfaktan yang serbaguna merupakan bahan yang sangat penting dalam aplikasi industri. Asid karbosilik yang diperolehi dari oleokimia ialah salah satu surfaktan anionik. Bagi mempelbagaikan aplikasinya, pembentukan sebatian baru dengan pelbagai fungsi seperti prestasi haba dan aktiviti permukaan daripada asid karbosilik yang sedia ada adalah diperlukan. Katanionik surfaktan merupakan kelas surfaktan baharu telah menarik perhatian para penyelidik disebabkan oleh keunikan prestasi haba dan aktiviti permukaan. Malah, segelintir sistem katanionik menunjukkan ciri-ciri anti-kanser yang membawa kemajuan untuk rawatan kanser. Kajian ini bertujuan untuk menghasilkan sistem surfaktan katanionik yang mengandungi bahan berasaskan sawit seperti asid karbosilik dan menerokai beberapa jenis ciri-ciri fizikokimia (prestasi haba dan aktiviti permukaan) dan kesitotoksikan. Sistem surfaktan katanionik yang mempunyai tahap asimetri yang berbeza telah disediakan daripada kationik surfaktan ammonium quaterik (rantaian alkil 12- hingga 18-karbon) dan anionik asid karbosilik berasaskan sawit (rantaian alkil 8- hingga 18-karbon) masing-masing. Pencirian surfaktan katanionik pejal menggunakan inframerah fourier transformasi (FTIR) dan difraksi sinar-X (XRD) telah mengesahkan pembentukan surfaktan katanionik yang berstruktur kristal. Surfaktan katanionik memaparkan kestabilan haba yang lebih tinggi daripada surfaktan induk dengan pengurangan berat 10% pada suhu antara 125.15°C dan 216.29°C serta mempunyai sehingga enam peralihan fasa haba. Dalam perbandingan dengan surfaktan induk, perubahan jumlah entalpi (ΔH) dan entropi (ΔS) surfaktan katanionik juga telah dipertingkatkan, iaitu dalam lingkungan 44.32 kJ mol^{-1} hingga 157.15 kJ mol^{-1} dan 119.07 J K^{-1} mol^{-1} hingga 409.68 J K^{-1} mol^{-1}. Ini juga menunjukkan peningkatan kestabilan haba dan perubahan pergerakan molekul dalam pembentukan fasa yang tidak teratur semasa rawatan haba. Perubahan jumlah ΔH dan ΔS dipengaruhi oleh tahap asimetri surfaktan katanionik. Penilaian ciri-ciri aktiviti permukaan sistem katanionik dilakukan melalui analisis ketegangan permukaan. Kepekatan kritikal pengumpulan (CAC) bagi sistem katanionik telah memaparkan keupayaan penggumpalan diri yang lebih baik dengan sekurang-kurangnya 70% lebih rendah daripada surfaktan induk kationik, antara 0.0004 mM dan 2.130 mM. Data kepekatan berlebihan permukaan (Γ_{max}) dan keluasan minimum bagi setiap molekul (A_{min}) sistem katanionik juga menyokong

hasilan CAC di mana Γ_{max} adalah antara 1.26×10^{-6} mol m^{-2} dan 3.82×10^{-6} mol m^{-2} manakala A_{min} adalah dari 43 $Å^2$ hingga 131 $Å^2$. Kumpulan anionik dan kationik beratur dengan rapat untuk mendorong pengurangan keluasan dengan lebih berkesan. Selain itu, kesitotoksikan surfaktan katanionik dan surfaktan induk diuji pada sel fibroblas normal 3T3 dan sel kanser payudara MDA-MB-231. Kesitotoksikan surfaktan katanionik dan surfaktan induk didapati meningkat dengan peningkatan rantaian alkil. $C_{18}TA_{18}$ merupakan surfaktan katanionik yang mempunyai kesitotoksikan tertinggi dengan kepekatan perencatan maksima separuh (IC_{50}) 5.9 µM ± 0.3 µM pada sel normal 3T3 dan 4.0 µM ± 0.1 µM pada sel kanser MDA-MB-231. Pengabungan agen anti-kanser dalam $C_{18}TA_{18}$ didapati memberi kesitotoksikan yang lebih rendah pada sel 3T3 daripada $C_{18}TA_{18}$, kesitotoksikan dikurangkan sebanyak 1.8 hingga 4.5 kali ganda. Kesimpulannya, surfaktan katanionik berasaskan sawit memaparkan ciri-ciri fizikokimia dan aktiviti anti-kanser yang telah dipertingkatkan di mana $C_{18}TA_{18}$ yang digabungkan dengan agen anti-kanser mempunyai potensi aktiviti anti-kanser.

ACKNOWLEDGEMENTS

This thesis and research project could only be done successfully due to the contributions of many parties throughout the project. First and foremost, I express my sincerest gratitude to my main supervisor, Associate Professor Dr. Phang Lai Yee and co-supervisors, Dr. Lim Wen Huei, Associate Professor Dr. Noorjahan Banu Mohamed Alitheen and Professor Dr. Chuah Cheng Hock for their continuous support, encouragement and excellent guidance throughout the project.

My utmost appreciation also goes to my awesome laboratory seniors and friends, Dr. Voon Phooi Tee, Ms. Ng Yen Teng, Ms. Lee Sin Tien, Ms. Thio Cui Wen, Ms. Nabiha Iran, Ms. Maliya Azilah, Ms. Irmaliayana Norisam and Ms. Saw Mei Huey for all their help, motivation, sharing and caring throughout the study. Additionally, I have benefited from the valuable advice and guidance from Ms. Koh May Zie, Ms. Liyana Yusof and Dr. Noraini regarding cell culture study techniques. Without their support, my experimental work would have been at a standstill. A special thanks to the Animal Tissue Culture Laboratory for the well-equipped cell culture facilities required during this study. I would also like to acknowledge the Malaysian Palm Oil Board that offered me a scholarship and technical support for this study. Without their support and funding, this project could not have reached its goal.

Last but not least, I would like to express my warmest and hearties thanks to my loving family and wonderful friends who were there giving me unconditional love and endless support throughout it all. It is my pleasure to convey my appreciation and deepest thanks to those who contributed to my project. Thank you.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

A_{min}	Minimum area per molecule
CAC	Critical aggregation concentration
CMC	Critical micelle concentration
C_8	Octanoic acid
C_{10}	Decanoic acid
C_{12}	Dodecanoic acid
C_{14}	Tetradecanoic acid
C_{16}	Hexadecenoic acid
C_{18}	Octadecanoic acid
DNA	Deoxyribonucleic acid
DSC	Differential scanning calorimetry
FTIR	Fourier Transform Infrared
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
IC_{50}	Half maximal inhibitory concentration
MES	2-(N-morpholino)ethanesulfonic acid
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
rpm	Revolutions per minute
SDS	Sodium dodecylsulfate
TA_{16}	Hexadecyltrimethylammonium bromide
TAC_m	Alkyltrimethylammonium chloride, where m = 12, 14, 16 and 18
TA_m	Alkyltrimethylammonium bromide, where m = 12, 14, 16 and 18
TGA	Thermal gravimetric analysis
TRF	Tocotrienol rich fraction
USA	United States of America

XRD	X-ray diffraction
Π_{CAC}	Surface pressure at CAC
Γ_{max}	Surface excess concentration
ΔH	Change in enthalpy
ΔS	Change in entropy

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Oleochemicals are chemicals derived directly or indirectly from natural oils or animal fats by different oil breaking processes. The basic oleochemicals produced are carboxylic acids, glycerine, fatty alcohols, fatty methyl esters and fatty amines. Plant-based oleochemicals present as abundant, cheap, biodegradable and renewable resources for sustainable development (Salimon et al., 2012). Increasing demand of biodegradable or sustainable products to replace petrochemicals has driven the global oleochemical markets. Oleochemicals are commonly involved in surfactants, personal care, food additives, agrochemical and biodiesel applications. The global market of surfactants has experienced steady growth in the past years and is forecasted to grow by 5.4% annually from 2018 to 2025 (Shasttri and Sumant, 2018).

Surfactants are versatile products with various industrial applications such as oil recovery, detergents, cosmetics, automobiles, paints, dyestuffs, fibers, plastics, agrochemicals, pharmaceuticals. Nowadays, applications of surfactants have extended to high technology areas including biotechnology, nanotechnology, electronic printing, microelectronics and magnetic recording. For industrial applications, especially in cleaning and detergency, surfactants are often commercialized in the form of mixtures. Surfactants in single pure form is not required because surfactant mixtures are much more economical and efficient for cleaning and detergency purpose. Researchers studied on mixed surfactant systems and discovered their interesting surface activity (Wang et al., 2019; Tomašić and Mihelj, 2017; Murphy and Taggart, 2002). Catanionic surfactants, a relatively new class of surfactants produced from the mixing of two oppositely charged ionic surfactants at equimolar ratio are firstly introduced by Jokela and co-workers in 1987 (Jokela et al., 1987). The two charged headgroup of catanionic surfactant are held together by strong electrostatic interactions. Due to this strong ionic pairing of oppositely charged surfactants, catanionic surfactants that formed were similar to those double-chain surfactants (Dew et al., 2008; Šegota and Težak, 2006; Kaler et al., 1992).

Catanionic surfactants give extraordinary thermal and surface behaviour. Catanionic surfactants usually form liquid crystal phase upon heat treatment due to possible conformational and positional disorder of the alkyl chains and two-dimensional disordering of the surfactant headgroup layers. Liquid crystals are widely applied in smart devices, sensors, video screens, semiconductor quantum dots and solar cells (Chen et al., 2017; Kumar and Kumar, 2017; Lagerwall and Scalia, 2012; Mirzaei et al., 2012). Catanionic surfactants provide a future prospect in application of liquid crystal. Other than that, aqueous catanionic surfactants also give better surface activity and display rich self-aggregation phase behaviour. One of the most interesting features is the ability of catanionic surfactants to form aggregates at lower concentration compared with their individual surfactants, giving a lower critical aggregation concentration (CAC) or critical micelles concentration (CMC) values (Bryant et al., 2019; Jiang et al., 2014b; Pucci et al., 2014; Wong et al., 2012; Silva et al., 2007), indicating higher surfactant adsorption efficiency and effectiveness.

Two major factors contribute to different thermal and surface behaviour of catanionic surfactants from molecular structure aspect are electrostatic interactions of hydrophilic headgroup (Marques et al., 2006; Silva and Marques, 2005; Vill et al., 2000; Salkar et al., 1998) and hydrophobic interactions between alkyl chain (Tomašić and Mihelj, 2017; Mihelj et al., 2014b; Matos et al., 2013; Marques et al., 2003). In other words, rich phase behaviour containing aggregates of particular shape and size is the product of fine balance between electrostatic interaction and packing properties. Hence, catanionic surfactants with desirable properties and structure can be tailored made by modifying the nature of the components to give different hydrogen bonding networks, electrostatic effect, surfactant molecular size and geometry.

Malaysia is the largest palm oil producer and exporter in the world after Indonesia (Kushairi et al., 2018; Oosterveer, 2015). Undoubtedly, the economy of Malaysia, mainly driven by the palm oil industry as well as the oleochemical industry, which have contributed 20% to the global capacity as one of the major producers (Kushairi et al., 2019). Carboxylic acids are one of the major basic oleochemical produced from palm oil. It has vast diversity of applications but at the same time facing huge competition from other oleochemicals and vegetable oils. As such, creating a new chemical compound from the existing basic oleochemical to fine chemical that have different functionality is vital. Catanionic systems derived from carboxylic acid is one of the possible new compounds that exhibited various functionalities such as thermal behaviour, surface activity and anti-cancer activities.

So far, involvement of palm oil-derived carboxylic acids and quaternary ammonium surfactants in catanionic surfactants is scarcely reported and has not been investigated extensively. Therefore, studies on palm oil-derived catanionic systems are possible to produce value-added products from basic oleochemicals and further promotes the growth of oleochemical industry. This study was carried out to determine the physicochemical and *in vitro* cytotoxic properties of palm oil-derived catanionic systems with different degree of chain length asymmetry.

1.2 Objectives

This study aimed to produce new palm oil-derived catanionic systems with different degree of chain length asymmetry and investigate their physicochemical and cytotoxic properties. The specific objectives are:

1. To produce and study the thermal properties of neat palm oil-derived catanionic surfactants with different degree of chain length asymmetry;
2. To evaluate the surface properties of palm oil-derived catanionic systems with different degree of chain length asymmetry in aqueous and
3. To determine the cytotoxicity of palm oil-derived catanionic surfactants and combination effect with different weight ratios of anti-cancer agents on the breast cancer cell line MDA-MB-231.

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