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SELF-ASSEMBLIES BEHAVIOUR OF PALM OIL-BASED GALACTOSIDE

(Sifat Swapenyusunan Galaktosida Berasaskan Minyak Sawit)

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Abstract

Analyses of palm kernel oil-based galactosides self-assemblies are important to determine their applications in pharmaceutical products. The palm kernel oil-based galactosides (GalPKO) are synthesized by adopting Fischer glycosylation method with minor modification. A birefringent fan-shaped texture was observed upon cooling using optical polarizing microscope (OPM) indicates formation of a smectic phase. The galactosides form isotropic phase completely at 137 °C. Water contact penetration showed that GalPKO does not form other lyotropic system except hydrated smectic phase. Based on its lyotropic behavior, critical aggregation concentration (CAC) is determined at value 0.11 mM indicating low solubility of the galactosides in water. Based on finding, galactoside can form a special class of liquid crystal mesophases and give a variety interesting application.

Keywords: galactoside, thermotropic, lyotropic, liquid crystal, mesophase

Abstrak

Analisa swapenyusunan galaktosida berasaskan minyak kernel sawit penting bagi menentukan aplikasinya dalam produk farmaseutikal. Galaktosida berasaskan minyak kernel sawit (GalPKO) disintesis menggunakan kaedah Fischer glikosilasi yang diubahsuai. Tekstur birefringen berbentuk seperti kipas diperhatikan menggunakan mikroskop polarisasi optik (OPM) semasa proses penyejukan sampel, menunjukkan pembentukan fasa smektik. Galaktosida membentuk fasa isotropik sepenuhnya pada 137 °C. Penyerapan air menunjukkan bahawa GalPKO hanya membentuk sistem liatropik fasa smektik terhidrat. Berdasarkan sifat liotropik itu, kepekatan gumpalan kritikal (CAC) ditentukan pada 0.11 mM menunjukkan keterlarutan galaktosida dalam air yang memuaskan. Hasil penyelidikan menunjukkan galatoksida ini bakal membentuk satu kelas khas fasameso cecair hablur serta kemungkinan dalam pelbagai aplikasi menarik.

Kata kunci: galaktosida, termotropik, liotropik, cecair hablur, fasameso

Introduction

Glycolipids or glycosides being one of important component in membrane exhibit liquid crystals behavior of both thermotropic and lyotropic properties because of its amphiphilic structure [1]. Interestingly, glycolipids form a special class of liquid crystal mesogens capable of giving variety of interesting applications [2]. Glycolipids are produced by connecting carbohydrates with lipids through covalent bond [3]. They consist of a sugar head group and an alkyl chain attached to an anomeric carbon *via* the glycosidic bond. The head group could be a monosaccharide or several sugar units whereas the attached alkyl chain could be a single or a branched chain [4]. Commonly, glycosylation takes place when hydrogen of OH group C1 of the sugar is being replaced by a hydrophobic chain [5]. Galactoside is one type of glycoside which consists of galactose head group. For this work, the galactosides are made from reduced palm kernel oil (PKO) fatty acids. PKO was selected as the alkyl chain

since it composed of mostly saturated fatty acids. Although, there are no data on this galactosides toxicity and biocompatibility, they are assumed to be non-toxic and biocompatible since these glycosides are made from food materials [6].

Currently, there are more than 300 natural glycolipids have been isolated. For some cases, structural features of glycolipids were modified by additional functional groups such as phosphates, sulfates, glycerol, etc. to fulfill certain applications [7]. However, to obtain natural glycolipids in large quantities is a problem because they are difficult to extract in sufficient quantities. Moreover, purification for biophysical experiments and total synthesis of these lipids often proves challenging [8]. In order to overcome these problems, researchers are more encouraged to develop synthetic glycolipids [9]. Current awareness that prefers environmental friendly technology leads to high demand for biodegradable materials that can be synthesized from renewable resources also low in cost. Some studies have shown that these synthetic glycolipids structurally mimicking natural glycosides which be used to replace the natural glycolipids in certain biological functions [7, 10].

Synthetic glycosides are prepared through enzymatic synthesis, chemical processes, or a combination of both methods [11]. The common glycosylation strategy is using 'Fisher glycosidation' [12]. In this paper, galactoside was synthesized *via* modified version of this method which is described in details at methodology section. The objectives of this research are to investigate the thermotropic and lyotropic properties of palm kernel oil based galactoside in order to determine the types of self -assembly formation. This research only focuses on glycosides which contains of galactose as sugar head and PKO alcohol as their tail. Thermotropic properties are determined by studying phase transitions stimulated thermally using calorimetric and microscopic techniques. Lyotropic behavior is commonly observed in amphiphilic molecules where the molecules have at least two different solubility capacities towards a solvent (usually water) within itself [13]. The lyotropic properties are determined using microscopic technique and surface tension measurements to obtain the critical aggregation concentration (CAC).

Materials and Methods

Materials

Palm kernel oil (PKO) obtained from Golden Jomalina Food Industries Sdn. Bhd. (Malaysia) was reduced to PKO alcohol by using lithium aluminum hydride. D-galactose and boron trifluoride (BF_3) required for glycosides syntheses were purchased from Sigma Aldrich (USA).

Synthesis of galactoside

The glycolipids were synthesized using glycosylation procedure by Hashim et al. with minor modifications [14]. Synthesis method consists of three major steps, i.e. protection and activation of the sugars (acetylation), glycosylation and deprotection of the sugars (deacetylation). The alcohol mixtures were produced from the reduction of palm kernel oil (PKO) using a general reduction method [15]. Acetylation is a protection strategy for selective glycosylation at galactose anomeric carbon as well as for activation was prepared by a known method [16].

For glycosylation, peracetylated sugar (15 mmol) and the PKO alcohol (17 mmol) were dissolved in 60 ml dichloromethane and stirred in a closed apparatus at room temperature. Boron trifluoride (18.2 mmol) that act as catalyst was slowly injected into the solution and the glycosylation reaction took 24 hours to complete for a 2:1 ratio of alpha and beta anomeric mixture. After that, the reaction was stopped by neutralizing the solution with saturated sodium bicarbonate solution and the organic layer was washed two times with water. Dichloromethane was evaporated and acetonitrile was added later to the product. Hexane extractions were done a few times to completely remove the excess alcohol before organic solvent was evaporated.

Finally, deacetylation (removal protection group) step was performed by adding a catalytic amount of sodium methoxide to induce a basic medium into a methanolic solution of peracetylated glycosides (50 ml). The solution was stirred overnight. After the reaction was completed, methanol was added by Amberlite-IR resin to neutralize the excess sodium methoxide. Then methanol was evaporated and product was purified from unreacted sugar with n-butanol and water extractions. N-butanol was evaporated and the product was dried in a vacuum oven at 50 ºC for 48 hours before will be characterized by Nuclear Magnetic Resonance (NMR) spectra NMR.

Nuclear magnetic resonance analysis
Structure elucidation of the synthesized galactosides and its starting materials were determined through NMR technique using Varian NMR Systems spectrometer at 500MHz. NMR samples were prepared in methanol-d4 (CD_3OD) solutions and recorded at room temperature. 1H NMR: δ = 5.35 (m, 0.3H, CH), 5.15 (m, 1H, H-1"), 4.81 (d, αH-1, J = 3.6 Hz), 4.22 (d, βH-1, J = 8.0 Hz), 3.40–3.90 (m, bulk sugar signals), 2.02–2.07 (m, 0.6H, CH 1.62–1.67 (m, 2H, CH 1.31 (m, 20H, CH 0.92 (t, 3H, CH

Optical polarizing microscope analysis
The instrument used in the investigation is an Olympus BX52 optical polarizing microscope equipped with a Mettler FP82 Hotstage (heating stage). Sample preparation for OPM measurement for thermotropic and lyotropic study was same. A thin layer of sample was placed sandwiched between slip cover and the glass slide. The sample was heated from room temperature to °C-140 ºC. The end temperature depends on the clearing point of the sample, therefore every sample has a different end temperature. For thermotropic study, phase transition temperatures were recorded at 5°C/min for both heating and cooling rate. Mesophases exhibited by samples were identified based on the texture. The melting and clearing temperatures were confirmed by differential scanning calorimetry (DSC).

For lyotropic study, a sample was heated until its melting point then cooled to room temperature. Then solvent was introduced at the edge of covered slide. The solvent slowly moved towards the sample in a capillary action surrounding the sample before any contact occurred. The lyotropic phase was observed by OPM and identified based on texture and time. The images were captured for every 5-10 minutes. Water was used as the solvent in this investigation due to the application task of the material as surfactant. All experiments were performed at room temperature.

Differential scanning calorimetric analysis
Samples were measured based on a heating-cooling-heating cycle. The temperature range applied was based on data obtained from OPM studies. Samples, weighing around 4-8 mg, were placed in standard aluminum pans (crucibles) of 40µl size and sealed tightly. Then, heated to isotropic state, cooled down to the initial temperature (25 °C) and followed by second heating to isotropic temperature. A heating and cooling rate of 10°C/min and 5°C/min was applied respectively.

Critical aggregation concentrations measurements
CAC was determined through surface tension measurements of galactoside solutions using Du Nuoy ring method [17]. A series of solutions of various concentrations were prepared from stock solution containing 10 mg of palm kernel oil based galactoside in 250 ml of deionised water. 10ml series of concentration is then derived from the stock solution was measured.

Results and Discussion

Synthesis
GalPKO (Figure 1) for this study was prepared by 24-hour glycosylation process to produce an α dominant mixture. Glycosylation time determines the amount of α and β anomers produced since each anomer has different stability factor; α anomer is thermodynamically favored whereas the latter is more kinetically favored. For this work, a slightly α dominant mixture (2:1 α/β ratio) was selected because it showed to be a better topical drug carrier in terms of providing flexibility to vesicle membrane which increase drug delivery [18]. GalPKO contains a mixture of 48% Dodecyl galactosides ($GalC_{12}$), 16% Oleyl galactosides ($GalC_{18:1}$) and other saturated and unsaturated chains galactosides of α and β configurations based on the fatty acids content of palm kernel oil.

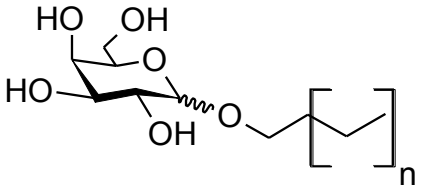

Figure 1. Structure of palm oil galactosides (GalPKO). Sample consists of stereoisomers of various alkyl chains mixture

Thermotropic study

GalPKO, like its pure components forms smectic A phase above ambient temperature [19]. It displays a bâtonnet texture immediately after temperature went below its clearing temperature, 140 °C (observed by OPM) (Figure 2(a)). However, this texture disappeared as temperature decreased to room temperature which at this point, only an isotropic with birefringence defects was observed (Figure 2 (b)). The observed birefringence defects are the shape of Maltese cross.

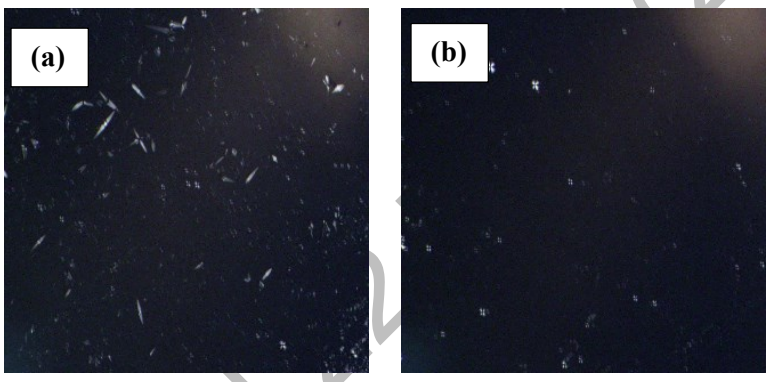

Figure 2. OPM images captured upon cooling at 137 ºC showing a bâtonnet texture immediately after isotropic point (a) and at 30 ºC where a Maltese cross texture was observed (b). All textures were observed under magnification of x10

Table 1 shows the transition temperatures of GalPKO based on DSC measurements. From four cycles of heating and cooling, the clearing temperatures are consistently observed at 137 °C. Generally, clearing temperature of a liquid crystal compound is when an isotropic phase or more commonly known as liquid phase is formed from a liquid crystalline phase. When comparing clearing temperature of GalPKO and its pure components, $GalC_{12}$ and $GalC_{18:1}$, the transition temperature is considerably low. $Gal\beta C_{12}$ and $Gal\beta C_{18:1}$ are reported to have isotropic temperatures of 166 °C and 145 °C, respectively [19]. Presence of unsaturated glycosides would lower transition temperature because double bond prevents close packing assemblies due to bending alkyl chain [20]. Thus, we could say that 16% of $GalC_{18:1}$ in GalPKO mixture attributes to lowering the clearing temperature of the mixture. However, to further explain low clearing temperature of GalPKO is caused by stereochemistry factor would be objectionable. Pertaining to a previous work, α anomer of galatosides was reported to be more stable than its β counterpart with difference in transition temperatures to be almost 16 °C, which is significantly higher compared to glucosides anomers [19]. Thus, it is conjectured the low clearing point of GalPKO is due to synergistic effect of the components.

Table 1. Thermotropic transition temperature and enthalpies based on DSC measurements

	First Heating	Second Heating	First Cooling	Second Cooling
T_{iso} range (°C)	133 – 149	131 – 147	127 – 139	125 – 139
T_{iso} peak (°C)	138	137	137	137
ΔH_{iso} (kJ mol^{-1})	-5.58	-3.64	3.04	3.43

From DSC thermogram, only one phase transition (smectic → isotropic) was observed for monoalkylated GalPKO (Figure 3) while pure $GalC_{12}$ and $GalC_{18:1}$, showed two transitions: crystal → smectic and smectic → isotropic. The absence of crystal to smectic phase transition also could be attributed to synergistic effect which probably diminished intermolecular interactions to form a more ordered crystal phase. The enthalpy change for the transition from smectic liquid crystal to liquid (ΔH_{iso}) is substantially larger compared to pure components (the difference is approximately 2 kJ mol^{-1}) showing that more energy is required to break molecular interactions in the mixture than in pure compounds [19]. Enthalpies of isotropic clearing are consistent for three cycles of heating and cooling except for the first heating which shows a larger enthalpy value. This may be due to a small amount of crystals formed during storage. It is a common practice to neglect any observation from first sample heating. It is confirmed no degradation towards the sample as observed by the microscope.

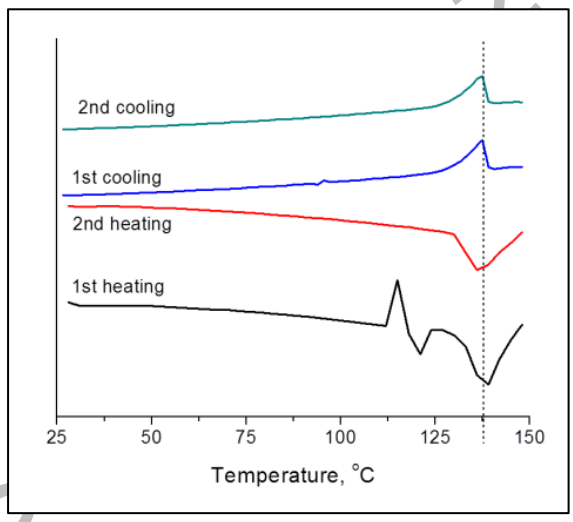

Figure 3. DSC curves obtained at heating and cooling rate of 10 °C/min and 5 °C/min of GalPKO

Lyotropic study

Lyotropic properties of GalPKO were determined using contact penetration method. This method provides a qualitative phase diagram with increasing water concentration. To acquire concentrations at phase boundaries, a quantitative method is required though is not within the scope of this work. Figure 4 shows water penetration of GalPKO at room temperature. When water penetrated dry GalPKO sample, a probable lamellar phase hydration was observed considering lack of birefringence at the penetrated region. At higher water content, GalPKO slightly dissolved as micellar solution. On contrary, similar observation was not observed in pure galactoside components as they only exhibited myelin figures [19]. These observations further support the conjecture of synergistic effect in GalPKO.

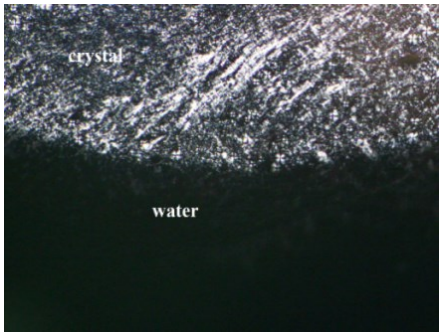

Figure 4. Contact penetration of water into GalPKO at room temperature under x10 magnification

Critical aggregation concentration (CAC) measurements grant quantitative values towards the improved water solubility of GalPKO. Typically, low CAC value indicates high hydrophobicity of an amphiphile [21]. Table 2 shows surface tension data of GalPKO and pure galactosides. Comparing GalPKO and these galactosides it is easy to notice that GalPKO has a higher CAC and capable of reducing surface tension of water by ca. 3 mNm^{-1} and ca. 7.5 mNm^{-1} more than $GalC_{18:1}$ and $GalC_{14}$ respectively.

Table 2. Surface tension data of GalPKO and pure galactosides

Compound	**CAC (mM)**	**γ_{CAC} (mNm^{-1})**
GalPKO	0.110	28.0
$GalC_{14}$*	0.020	35.5
$GalC_{18:1}$*	0.023	31.0

*pure galactosides data was taken from Garofalakis [22]

Conclusion

GalPKO, a galactosides mixture produced from glycosylation of galactose sugar with fatty alcohols from palm kernel oil has been studied with respect to its thermotropic and lyotropic behavior. The palm kernel oil-based galactoside was found to have different behavior compared to its pure galactoside components. It is believed that a synergistic effect is an important determinant of changes in GalPKO liquid crystalline properties.

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