MONOGLYCERIDES FROM 9,10-DIHYDROXYSTEARIC ACID FOR THE COSMETIC INDUSTRY

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ABSTRACT

Monoglycerides of dihydroxystearic acid (MGDHSA) were synthesized using an acid catalyst. Factors that may affect the esterification reaction such as reaction time and temperature were studied. Esterification of dihydroxystearic acid with excess glycerol by an acid catalyst at 150°C for 4 hr gave up to 40% yield of MGDHSA. The reaction product containing approximately 45% MGDHSA was found to be non-irritant to the skin, non-toxic to the aquatic environment and readily biodegradable. The toxicity value of MGDHSA was found to be more than 100 mg litre^{-1} while its biodegradability reached the pass level of 60% in 20 days. This compound is compatible with a wide variety of oils. MGDHSA seem to have better emulsifying properties in an oil-in-water (O/W) system with high water content compared to glyceryl monostearate (GMS) and glyceryl monohydroxystearate (MGHSA).

Keywords: dihydroxystearic acid, esterification, monoglycerides, emulsion.

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INTRODUCTION

Monoglycerides are the most polar component of simple lipids. They have detergent-like properties, hence, they easily form micelles in aqueous solutions. Their ability to form stable emulsions make monoglycerides suitable as internal and external lubricants in fibre and textile technologies. In metal processing, monoglycerides of long-chain fatty acids are used as emulsifying agents or lubricants. The plastics industry also utilizes monoglycerides of long-chain fatty acids as internal lubricants. Monoglycerides being non-ionic surfactants provide a wide range of applications as emulsifiers in the food, cosmetic and pharmaceutical industries (Elfman-Borjesson and Harrod, 1999; Eychenne and Moulougui, 1999).

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Monoglycerides have been produced by reacting a variety of substrates with glycerol. Depending on the starting substrate, monoglycerides may be prepared by direct esterification of fatty acids and glycerol, transesterification of triglycerides with glycerol, or hydrolysis of triglycerides or fats (Peyrou *et al*., 1996; Moulougui *et al*., 1998). Monoglycerides with interesting properties can be obtained by reacting glycerol, an abundant by-product of the oleochemical processing industry in Malaysia, with fatty acids which have different functional groups. In this study, monoglycerides from 9,10-dihydroxystearic acid was synthesized (Awang *et al*., 2004) and the properties of the resultant product were evaluated. This work was part of a programme to prepare new derivatives of domestically available fatty acids for various applications such as detergents, cosmetic/personal care products and lubricants.

MATERIALS AND METHODS

Materials

The 9,10-dihydroxystearic acid (DHSA) was prepared in the laboratory (Awang *et al*., 1998).

Glycerol 99% was purchased from an oleochemical company in Malaysia and *p*-toluene sulphonic acid (*p*-TSA) was obtained from Fluka (Switzerland). Sodium hydrogen sulphate ($NaHSO_4$) and sulphuric acid (H_2SO_4) were purchased from Merck (Darmstadt, Germany). All other reagents were of analytical grade and were used as received.

Experimental Procedure

The experiments were carried out in a 250-ml three-necked round-bottom flask equipped with a magnetic stirrer, a thermometer, an inert gas inlet tube and a condenser. Glycerol (0.6 mol), DHSA (0.1 mol) and the catalyst were placed in the flask and heated to 150°C. An oil bath was used to maintain a constant temperature. The reaction mixture was stirred continuously in a dry nitrogen atmosphere, for 4-6 hr. After this time, the hot mixture was quantitatively transferred into a separatory funnel and the mixture was allowed to separate into two layers. The bottom layer was separated from the top layer. The separation had to be carried out when the mixture was still hot in order to prevent the top layer from solidifying. The progress of the reaction was monitored by analysing the amount of unreacted DHSA in the reaction mixture by a titrimetric method.

Analytical Method

The products obtained were tested for different physical and chemical properties. Acid value, hydroxyl value and saponification value were determined by standard procedures (AOCS Official Methods): acid value, Te 1a-64; hydroxyl value, Cd 13-60; saponification value, TI 1a-64 [AOCS, 1995]).

Gas chromatography (GC) analysis was carried out using a Hewlett-Packard HP-6860A *plus* GC (Palo Alto, CA). The samples were silylated using *N,O*-bis-(trimethylsilyl) trifluoroacetamide before being injected into the gas chromatograph. The trimethylsilyl (TMS) derivatives of the samples were separated on a non-polar column HT-5 (Hewlett-Packard, 30 m x 0.25 mm x 0.25 μm) with helium as the carrier gas. The oven was programmed to hold at 120°C for 1 min followed by ramping from 120°C to 300°C at a rate of 6°C min^{-1}. The final temperature was held at 300°C for 6 min. The injector and flame ionization detector were set at 340°C.

Dermal Irritancy Test

A dermal irritancy test of monoglycerides of dihydroxystearic acid (MGDHSA) was conducted using the Irritection Assay System, which consists of a test kit, instrumentation and computer system. Samples were weighed at four different concentrations – 50, 75, 100 and 125 μl – and placed into the membrane discs. Reagent and blanking buffer (1250 μl) were added into a 24-well assay plate. The membrane discs containing the various concentrations of MGDHSA samples were inserted into the corresponding blank and test sample wells of the plate. The assay plate was then incubated at 25°C for 24 hr. Then, the membrane discs were removed from the assay plate and 250 μl of reagent and blanking buffer were transferred into a 96-well reading plate. This plate was inserted into the MRX Microplate Reader.

Biodegradation and Toxicity Test

The biodegradability and ecotoxicity tests were carried out using the OECD 301D closed bottle test method and the OECD 302 fish acute toxicity test. For the biodegradability test, a solution of the test substance in mineral medium was inoculated with a relatively small number of microorganisms and kept in completely full, closed bottles in the dark, at constant temperature. Biodegradation over 28-day period was followed by analysis of dissolved oxygen. The amount of oxygen taken up by the microbial population during biodegradation of the test substance, corrected for uptake by the blank inoculum run in parallel, was expressed as a percentage of ThOD (theoretical oxygen demand). The dissolved oxygen of the samples was measured every four days to allow for the construction of a biodegradation curve.

For the toxicity test, the fish (*Tilapia nilotica*) were exposed to various concentrations of the test substance for 96 hr. Mortality was recorded at 24, 48, 72 and 96 hr, and the concentration that killed 50% of the fish (LC_{50}) was determined.

Compatibility of MGDHSA with Cosmetic Oils

MGDHSA (10% w/w) in various types of oils were heated until the MGDHSA were dissolved. The oils used were medium chain triglycerides (MCT), octyl dodecanol, isopropyl myristate (IPM), isopropyl palmitate (IPP), mineral oil, cyclomethicone and castor oil. The appearance of the mixture after mixing at room temperature and after overnight storage was observed.

Emulsification Properties

The emulsions were prepared with different phase ratios: 80/20; 70/30; 60/40 and 50/50 (water: oil). Each sample (2% w/w) was added to an oil phase. The water phase was heated in a jacketed vessel to 70°C and the oil phase was heated in a beaker at 70°C. The oil phase was added to the water phase and mixed using a homogenizer at 4000 rpm for 3 min at 70°C. The solution was then cooled to room temperature by slow mixing using a

stirrer to obtain an emulsion. After a stable emulsion was formed, the emulsion was placed in a 25-ml measuring cylinder. The emulsion was placed at room temperature and in an incubator at 45°C. The degree of separation and the time taken to separate into two layers were determined every day for one week. The emulsifying power was calculated based on the degree of separation:

% separation = (top volume, ml)/(total volume, ml) x 100%

RESULTS AND DISCUSSION

Formation of MGDHSA was confirmed by GC analysis. The identification of the product mixture was based on a comparison of retention times (Rt) of the peaks with glyceryl monostearate (GMS) and impure glyceryl monohydroxystearate (MGHSA). The presence of an extra hydroxyl groups in MGDHSA caused the Rt for the MGDHSA peak to be shifted slightly to 16.83 min compared to Rt for the GMS peak at 14.49 min and MGHSA at 16.08 min. The chromatogram also indicated that the reaction products were not only monoglycerides but also isomeric and diastereoisomeric diglycerides of DHSA, which appeared at 30.1 min and 30.62 min (Awang *et al*., 2004). The composition of MGDHSA increased with increasing reaction temperature from 100°C to 180°C, as well as with reaction time (*Figure 1*).

Properties of the starting materials and the crude products, such as acid value, hydroxyl value, saponification value and melting point, are shown in *Table 1*. The results show that the acid value decreased from the reactant to the product indicating the formation of an ester. The saponification value was also decreased which may be due to the increase in molecular weight of the product. The hydroxyl value of the product was lower than that of glycerol but slightly higher than DHSA.

TABLE 1. PROPERTIES OF STARTING MATERIALS AND PRODUCT*

Parameter	Glycerol	DHSA	Product
Acid value, mg KOH g^{-1}	ND	178.7	53.7
Hydroxyl value, mg KOH g^{-1}	1 612.4	317.2	322.7
Saponification value, mg KOH g^{-1}	0.0	183.9	141.2
Melting point, °C	LRT	90.6-91.3[a]	67.5-69.1

Notes: *The reaction was carried out at 150°C for 4 hr using 0.5% *p*-TSA as the catalyst.
LRT: liquid at room temperature.
ND: not determined.
[a] Theoretical value: 94°C-95°C.

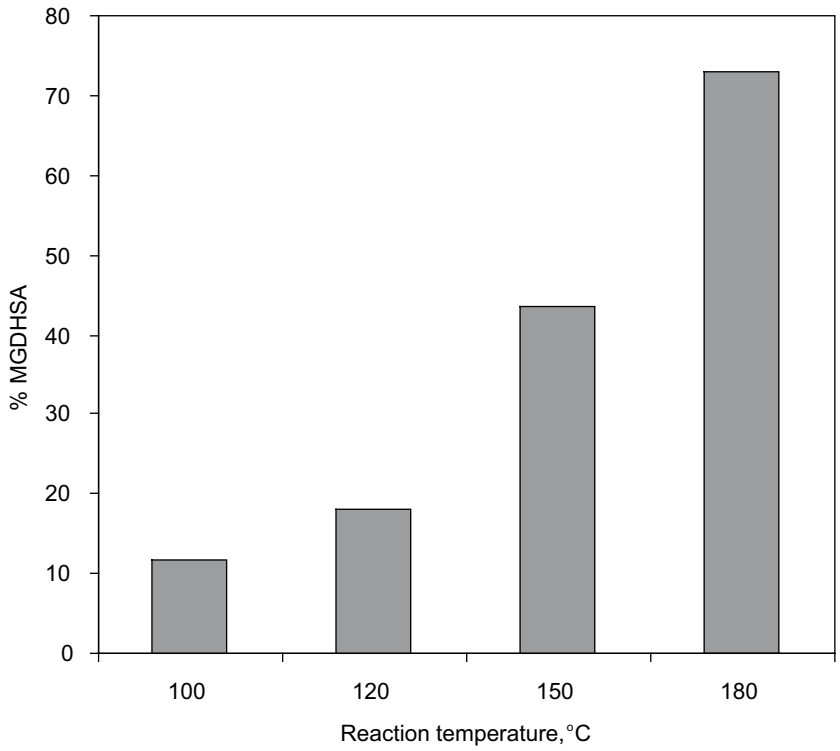

Figure 1. Effect of reaction temperature on the composition of monoglycerides of dihydroxystearic acid (MGDHSA).

Dermal Irritancy Properties

Raw materials used for cosmetics should be non-irritant because they are in contact with the skin for a long period of time. There are various methods to test skin irritation – *in vivo* as well as *in vitro*. The dermal irritancy of MGDHSA was evaluated *in vitro* using the Dermal Irritection Assay system. It was found that the MGDHSA produced were non-irritant to the skin, with the Human Irritancy Equivalent (HIE) score obtained being less than 0.9 at all the concentrations tested (*Table* 2).

Biodegradation and Toxicity

The biodegradability of MGDHSA was evaluated using the closed bottle test method as described in the Materials and Methods section. *Figure 2* shows the time course of the biodegradation of the MGDHSA based on their biochemical oxygen demand (BOD) and theoretical oxygen demand. MGDHSA degraded by more than 60% in 20 days, which is considered readily biodegradable. This compound was non-toxic to the aquatic environment for which the toxicity value was more than 100 mg litre^{-1}.

Compatibility of MGDHSA in Cosmetic Oils

MGDHSA were dissolved in various types of cosmetic oils by heating (*Table 3*). The blend of MGDHSA (10% w/w) and 90% medium chain triglycerides dissolved at 74°C. The system gave a light yellow liquid, but lumps and crystalline aggregates were formed after overnight storage at room temperature. The blends of MGDHSA with other cosmetic oils such as mineral oil, IPP, IPM and octyl dodecanol readily dissolved at temperatures ranging from 52°C to 76°C. The viscosity of the blends was slightly higher than the viscosity of the cosmetic oils.

TABLE 2. IRRITANCY TEST OF MONOGLYCERIDES OF DIHYDROXYSTEARIC ACID (MGDHSA) AT VARIOUS CONCENTRATIONS

Concentration, µl	Irritancy score	Irritancy classification
50	0.12	Non-irritant
75	0.19	Non-irritant
100	0.18	Non-irritant
125	0.31	Non-irritant

TABLE 3. COMPATIBILITY OF MONOGLYCERIDES OF DIHYDROXYSTEARIC ACID (MGDHSA) (10%) WITH VARIOUS COSMETIC OILS

Cosmetic oil	Temperature (°C)	Appearance at room temperature
Medium chain triglycerides	74	Translucent
Octyl dodecanol	52	Clear
Isopropyl myristate	58	Clear
Isopropyl palmitate	56	Clear
Mineral oil	76	Separation
Cyclomethicone	76	Sediment
Castor oil	52	Clear

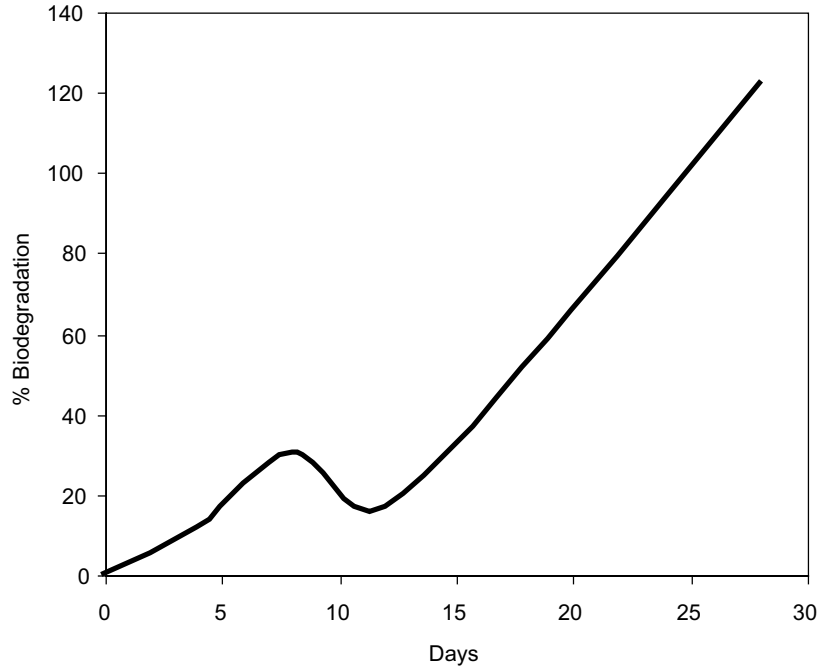

Figure 2. Biodegradation curve of monoglycerides of dihydroxystearic acid (MGDHSA).

Emulsification Properties

Table 4 shows the effect of MGDHSA concentration on the emulsion stability at room temperature and at 45°C. The system containing more than 1% (w/w) of MGDHSA in MCT was stable at room temperature, whereas upon storage at 45°C for one week the systems containing 2% (w/w) and above were more stable. This study suggests that the stability of the emulsion increased with increasing MGDHSA concentration from 1% to 5% (w/w). The stability of the oil/water emulsion systems at various phase ratios was also evaluated. The system containing 80% (w/w) water and 20% (w/w) oil was most stable without any stabilizer/co-emulsifier (*Figure 3*).

Emulsions using MGDHSA as an emulsifier showed higher stability in the system with higher water content compared to the emulsion system containing MGHSA and GMS as an emulsifier. There was an increasing trend in the percent separation for emulsions containing MGDHSA with an increase in the oil phase, whereas emulsion systems of MGHSA and GMS had a decreasing trend in percent separation (*Table 5*). These observations might be due to the presence of the two hydroxyl groups attached to the hydrophobic part in the MGDHSA structure.

TABLE 4. STABILITY OF OIL/WATER EMULSION SYSTEMS AT VARIOUS CONCENTRATIONS OF MGDHSA AFTER SEVEN DAYS' STORAGE

MGDHSA (%)	% separation	
	Room temperature	45°C
1	20	32
2	0	4
3	0	0
5	0	0

Notes: % separation = (top layer/total volume) x 100.
MGDHSA = monoglycerides of dihydroxystearic acid.

TABLE 5. STABILITY OF OIL/WATER EMULSION SYSTEMS OF MONOGLYCERIDES OF DIHYDROXYSTEARIC ACID (MGDHSA), GLYCERYL MONOHYDROXY STEARATE (MGHSA) AND GLYCERYL MONOSTEARATE (GMS) AT VARIOUS PHASE RATIOS AFTER SEVEN DAYS' STORAGE AT ROOM TEMPERATURE

Phase ratio (water:MCT)	% separation		
	MGDHSA	MGHSA	GMS
80:20	0	60	60
70:30	0	36	50
60:40	2	22	40
50:50	6	6	30

Notes: Homogenizer speed = 4000 rpm, emulsifier= 2% weight, mixing time = 3 min.
MCT = medium chain triglycerides.

CONCLUSION

Monoglycerides of DHSA were successfully synthesized. MGDHSA can be used as a replacement for GMs having better properties as an emulsifier in

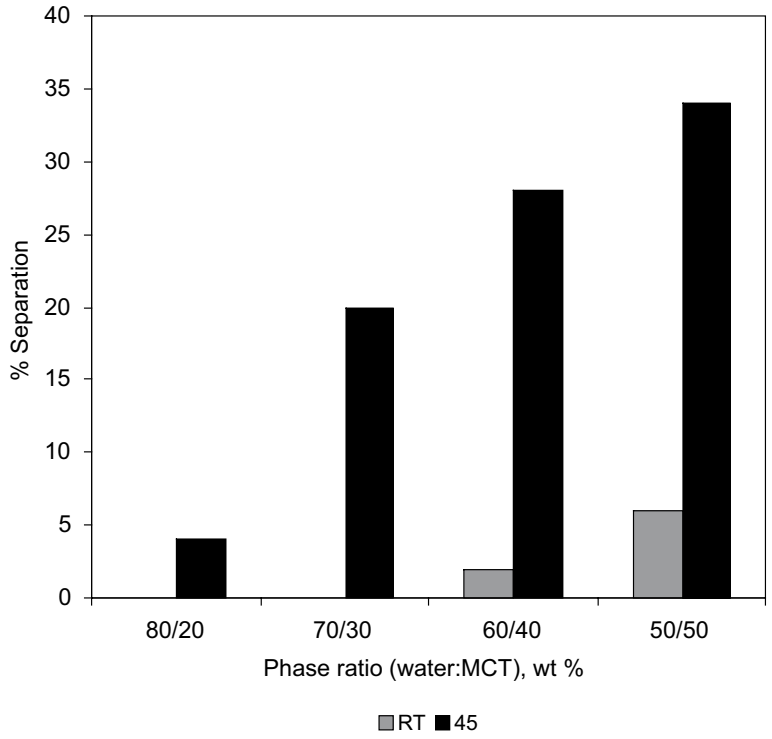

Figure 3. Stability of an oil/water emulsion system of monoglycerides of dihydroxystearic acid (MGDHSA) at various phase ratios after seven days' storage at room temperature (RT) and at 45°C.

the oil/water emulsion system. This compound was found to be non-irritant to the skin and non-toxic to the aquatic environment.

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