Kinetics of enzymatic esterification of oleic acid and decanol for wax ester and evaluation of its physico-chemical properties

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ABSTRACT

Decyl oleate is an important skin conditioning agent derived from the esterification of oleic acid and decanol. It is widely used in many cosmetic products as it has a composition similar to that of natural skin lipids. This study presents the kinetics of wax ester synthesis from oleic acid and decanol in the presence of Novozym 435 enzyme. The effects of various parameters such as reaction time, enzyme concentration, reaction temperature, and oleic acid–decanol mole ratio were investigated. The optimal conditions were found to be 1:1 oleic acid–decanol mole ratio, 2.5% enzyme concentration, 45 °C temperature and 60 min reaction time. A second order reversible reaction kinetic model was proposed to describe this reaction and a good agreement was observed between the experimental data and the model values. The effect of temperature on the forward reaction rate constant and equilibrium constant were determined by fitting them to the Arrhenius and van't Hoff equations respectively. The activation energy for forward reaction and the heat of reaction were found to be 16.24 kcal/mol and 16.63 kcal/mol respectively. The physico-chemical properties of the product were also evaluated and compared with jojoba oil.

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1. Introduction

Wax esters are long chain esters which belong to an important class of fine chemicals that are widely used as base materials in pharmaceuticals, cosmetics, lubricants, paints, wood coatings and perfumery products [1–3]. Wax esters are biodegradable, non-toxic and are prepared from renewable sources such as vegetable oils which make them industrially important chemicals. Specialty liquid wax esters such as jojoba and sperm whale oil have wide industrial applications as premium lubricants, parting agents, anti foaming agents and in cosmetics.

The product of interest in this study, *i.e.* decyl oleate, is synthesized from decanol and oleic acid, a naturally occurring fatty acid. Decyl oleate has good lubrication properties and possesses low viscosity. It is widely used in moisturizers, anti-aging treatments, sunscreens, eye shadows and hand, foot and eye creams. It is also recommended for makeup and lipsticks without the drawback of unsaturation [4]. It has a composition similar to that of natural skin lipids and has

special properties of re-fatting and good solvency for lipophilic active ingredients [5].

Several researchers worked with different types of enzymes for the synthesis of various wax esters. Garcia et al. [6] optimized the conditions of enzymatic synthesis of myristyl myristate ester by factorial design and analysis of experiments using a fungal lipase from *Candida antarctica*. Parameter estimation for kinetic model was carried out by Garcia et al. [7,8] by numerical calculation algorithms. The validity of the proposed models was quantified by ANOVA. Kinetic models for the lipase-catalyzed esterification in a biphasic organic–aqueous system has been proposed by Oliveira et al. [9] for ethyl oleate, and Shintre et al. [10] and Radzi et al. [11] for oleyl oleate.

Hadzir et al. [12] screened five immobilized lipases for the alcoholysis reaction of triolein and oleyl alcohol. Similar product was synthesized by Kapucu et al. [13] using Novozym 435 and optimized by adopting four variable central composite rotatable design. The maximum oleyl oleate concentration predicted by the model (737 g/l) agreed well with the experimental value (734 g/l). *Candida rugosa* lipase (CRL) was used in a solvent less esterification reaction to yield 12 wax esters by Guncheva and Zhiryakova [14]. Within 10 h, they reported complete conversion at 50 °C when immobilized PEG_{2000}-activated *Candida rugosa* lipase was added to the reaction mixture.

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Woodcock et al. [15] synthesized nine alkyl esters employing Novozym 435 in a pressure driven, packed-bed, miniaturized, continuous flow reactor. Gunawan and Suhendra [16] used lipozim for palm kernel oil and oleyl alcohol. Gunawan et al. [17] carried out alcoholysis of palm oil and oleyl alcohol using *Rhizomucor miehei* (Lipozyme IM). Cetyl oleate was synthesized using immobilized lipase from *Candida* sp. 99–125 [18] and the optimum conditions reported for 98% conversion were 40 °C, 1:0.9 acid–alcohol molar ratio, lipase dosage of 10% of oleic acid and reaction time of 8 h.

Kuo et al. [19] employed response surface methodology (RSM) and 5-level-4-factor central composite rotatable design (CCRD) to evaluate the effect of reaction parameters on the biocatalytic preparation of cetyl octanoate using two commercial immobilized lipases, *i.e.* Lipozyme RMIM (*Rhizomucor miehei*) and Novozym 435 (*Candida antarctica*). Similar methodology was adopted by Sellami et al. [20] for the preparation of different wax esters.

In the present study, enzymatic synthesis of decyl oleate was carried out for the first time. The optimization of process parameters for the preparation of wax esters from oleic acid and decanol has been made along with the kinetic modeling of the reaction. The properties of the decyl oleate have also been measured and these data are very useful in the preparation of personal care products and emollients.

2. Materials and methods

2.1. Materials

Analytical grade decanol (99.5%) was procured from Sd. Fine Chem. Ltd., Mumbai. Oleic acid used in the study was 99% pure and was prepared in the laboratory from enriched high oleic sunflower oil methyl esters. Hexane used was of HPLC grade obtained from Merck (Darmstadt, Germany) and was used as such. Novozym 435 was obtained from Novozymes A/S (Bagsvaerd, Denmark) and consisted of *Candida antarctica* lipase B immobilized on macroporous acrylic resin.

2.2. Experimental procedure

Oleic acid and decanol were taken in 1:1 mole ratio (*i.e.* 0.01773 moles) in a 100 ml stirred vessel. 10 ml hexane along with 2.5% enzyme and 10% molecular sieves (3 Å) were added to the reactor. The reaction mixture was maintained at constant temperature of 45 °C and kept under continuous stirring for 60 min. This study was carried out by comparing two sets of reactions, *i.e.* reaction with enzyme and without enzyme (taking without enzyme reaction as control reaction). The samples were drawn at various time intervals for each set of reactions, and were filtered, desolventized, dried and analyzed by titration for the evaluation of unreacted oleic acid in the reaction mixture using automatic titrator. Conversion of ester was calculated as

Conversion of wax ester

$$= \frac{\left[\begin{array}{l} \text{Volume of NaOH used (without enzyme)} - \\ \text{Volume of NaOH used (with enzyme)} \end{array}\right]}{\text{Volume of NaOH used (without enzyme)}}$$

Different sets of experiments were carried out for the generation of data under different operating conditions to arrive at optimum process conditions. The first set of experiments was carried out at different enzyme concentrations ranging from 0 to 10% based on total weight by keeping fixed molar ratio of oleic acid–decanol at 1:1, at a temperature of 45 °C. The second set of experiments was carried out at different molar ratios of oleic acid–decanol of 1:0.5, 1:1, 1:1.5 and 1:2 at 45 °C temperature and 2.5% enzyme concentration. The third set of experiments was carried out at different temperatures ranging from 35 °C to 50 °C with oleic acid–decanol mole ratio of 1:1 using 2.5% enzyme concentration.

2.3. Statistical analysis

The data were analyzed by a paired Student's *t*-test to evaluate the level of statistical significance.

2.4. Analytical methods

The fatty acid composition of the final decyl oleate was analyzed using Gas Chromatograph Agilent 6890 series equipped with flame ionization detector. The stationary phase used was a capillary column, DB-225 MS (i.d. 0.25 mm, length 30 m). The oven temperature was programmed as follows: 160 °C for 2 min and raised to 230 °C at 5 °C/min and held at this temperature for 20 min. The carrier gas used was nitrogen with a flow rate of 1 ml/min. The injector and detector temperatures were maintained at 230 °C and 270 °C respectively. The area percentage was recorded using HP Chem Station Data System.

2.5. Evaluation of physico-chemical properties

The physico-chemical properties of decyl oleate such as acid value, iodine value and saponification value were determined according to the standard AOCS methods Cd 3d-63, Cd 1-25 and Cd 3-25 respectively. Flash point, specific gravity and pour point were determined as per ASTM D 93, D 4052 and D 97 methods respectively. Contact angle measurement was carried out using a contact angle Goniometer (Model G10, Kruss, Germany). Refractive index measurement was carried out using Refractometer (RFM 870, Belingham Stanley Ltd.).

3. Kinetic model

For the present reaction system, a kinetic model with second-order for both forward and backward reactions was proposed [21,22]. The reaction mechanism for the kinetic model involving the reversible reaction is as follows:

$$A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C + D \tag{1}$$

The reaction rate equation is expressed as:

$$r_A = -\frac{dC_A}{dt} = k_1 C_A C_B - k_2 C_C C_D \tag{2}$$

where C_A, C_B, C_C and C_D denote the concentrations of acid, alcohol, ester and water formed during reaction respectively. k_1 and k_2 are kinetic rate constants for the forward and backward reactions respectively.

As $C_A = C_{Ao}(1 - X)$ (where X is the conversion of acid and C_{Ao} is the initial concentration of acid), $C_B = C_{Bo} - X C_{Ao}$ (where C_{Bo} is the initial concentration of alcohol) and $C_C = C_D = C_{Ao} - C_A = C_{Bo} - C_B = C_{Ao}X$, substituting these in Eq. (2), we get,

$$\frac{dX}{dt} = k_1(1 - X)(C_{Bo} - X C_{Ao}) - k_2 C_{Ao} X^2 \tag{3}$$

At equilibrium, $\frac{dX}{dt} = 0$ and $X = X_E$, and from Eq. (3), we get,

$$\text{Equilibrium constant}, K = \frac{k_1}{k_2} = \frac{C_{Ao} X_E^2}{(1 - X_E)(C_{Bo} - X_E C_{Ao})} \tag{4}$$

$$k_2 = \frac{k_1(1 - X_E)(C_{Bo} - X_E C_{Ao})}{C_{Ao} X_E^2} \tag{5}$$

By substituting k_2 in Eq. (3) and rearranging the terms, we get,

$$\frac{dX}{dt} = \frac{k_1}{X_E^2} \left[(X_E C_{Ao} + X_E C_{Bo} - C_{Bo})X^2 - X_E^2(C_{Ao} + C_{Bo})X + X_E^2 C_{Bo} \right] \tag{6}$$