Enzymatic Synthesis and Evaluation of Surface Active Properties of Di-Basic Acid Esters of Poly-ethylene Glycols

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Abstract: The present work has synthesized two polyethylene glycol (PEG) esters with two di-basic acids by bioesterification process and has investigated the interface properties like interfacial tension, surface excess concentration, minimum surface area per mole and as well as the Gibbs adsorption and thermodynamic behavior of micelle formation for the PEG-400 esters of the two acids. The esterification reaction was carried out by agitating the reactants in different molar ratios with *Mucor miehei* (RMIM) lipase enzyme at 70-75°C for 7 hours and 2 mm of Hg pressure. The conversion of PEG to esters ranged from 40-64% depending on the molecular size of di-basic acids.

Key words: esterification, polyethylene glycol, di-basic, surface property, interfacial tension, thermodynamic property

1 Introduction

Fatty acid derivatives (1) like sugar esters, glycol esters, alkanol amides, imidazolines, mono and di-glycerides, amine ethoxylates, ester sulphonates etc., have commercial uses. In fact, they have wide range of applications (2-12) as cosmetic and food emulsifiers, and from fiber finishes to lubricating components. These fatty acid esters can be prepared by chemical and enzymatic methodologies. It is true that the enzymatic processes are superior to other conventional processes (13-14).

For esterification reactions lipase has a wide range of applications (15-16). Low molecular weight esters (17-18) e. g. geranyl acetate, benzyl propionate have also been prepared enzymatically for flavour and aroma quality.

By esterification reactions surface active agents can be prepared by using enzyme (19) from fatty acids. These special type of surfactants have wide range of

applications from cosmetic to detergents, from food emulsions to oil digging (2-5). Widely used surfactants are of two types - anionic and non-ionic. Non-ionic surfactants have many advantages over anionics (20) and they are gaining much more importance now-a-days. Recently enzymatically prepared glycol esters are the most growing non-ionic surfactants. Ducret *et al.* prepared sugars and sugar alcohol esters from fatty acids (21).

Polyethylene glycol esters of monobasic acids have been prepared and examined to have wide range of application in surfactant field as emulsifiers in cosmetics and pharmaceutical industries, as lubricants, cohesion agents for fiber finishes, softeners in textile industry, binding agents in ceramic industry and so on (22-28). Ghosh and Bhattacharyya synthesized (29) the PEG esters of hydroxy fatty acid by enzyme technology process and measured the surface active property of the esters. There is, however no information about the preparation of di-basic acid esters of polyethylene gly-

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cols and their surface active properties.

The present study reports on the preparation of some di-basic acid esters of a polyethylene glycol molecule by enzymatic esterification process using a lipase like lipozyme (*Mucor miehei*) and examination of some surface active properties and molecular compositions.

2 Experimental Procedure

2.1 Materials

The two di-basic acids - adipic acid ($\text{HOOC}-(\text{CH}_2)_4-\text{COOH}$, melting point is 152°C) and sebacic acid ($\text{HOOC}-(\text{CH}_2)_8-\text{COOH}$, melting point $131-134.5^\circ\text{C}$) are obtained from Loba Chemie Industrial company, P. B. No. 6136, Bombay 400005 and E. Merck (India) Ltd, Bombay-400018, respectively

Polyethylene glycols of varying molecular weights, viz. 400 and 600 were all purchased from E. Merck (India) Ltd., Bombay-400018

The lipase *Mucor miehei*, lipase also referred to as Lipozyme IM-20 currently changed to Lipozyme RMIM, immobilized on resin was a gift of Novo industries, A/S Copenhagen, Denmark.

All Chemicals are reagent grade and supplied by S. D. fine Chemicals Ltd. CHCl_3 used for the separation process was purchased from S. D. fine Chemicals Ltd.

2.2 Methods

The di-basic acid was mixed with a polyethylene glycol in 1:1 molar ratio and the mixture was warmed with rigorous stirring to achieve homogeneity of the reactants. The enzyme *Mucor miehei* (RMIM) was added to the mixture at 10% (on the basis of total charge, w/w) level and the temperature was kept at $70-75^\circ\text{C}$. Stirring was allowed to continue with the help of Teflon coated magnetic bar. The reaction was carried at 2 mm Hg pressure. The course of reaction was followed by determining acid values of the products periodically till the value reached a steady state. The product was then subjected to the isolation process.

A sufficient amount of chloroform was added to the product mixture followed by warming at 50°C for few minutes. The chloroform solution was next filtered to remove both enzyme and unreacted di-basic acid. The chloroform solution was treated with distilled water to remove the polyethylene glycol, if any. The desired ester product was collected from chloroform solution by removing the chloroform under vacuum (2 mm Hg

pressure). The acid value and saponification value of chloroform soluble portion was measured to identify the desired product.

2.3 Separation Flow Chart

PEG 400/600 + Sebacic acid or Adipic Acid (1:1) + 10% Enzyme

2.4 Measurement of Surface Property

The interfacial tension (chloroform/water) vs molar concentration of different esters was measured at 27°C by well known drop weight method (30) based on the equation

$$\gamma_{1/11} = (\rho_1 - \rho_{11})vg/2\pi rf$$

where γ = interfacial tension at the liquid 1 and 11 interface, v = volume of the drop of the liquid, g = gravitational acceleration, r = radius of the capillary (< 1 mm), f = correction factor. The measurement was done for 10 drops in all cases. To eliminate the evaporation loss a closed system was adopted. From the experimental values of volume corresponding to 10 drops, volume per drop (v) and $r/v^{1/3}$ were calculated. The correction factor corresponding to $r/v^{1/3}$ was finally determined from the tabulated values of ref.(30). From the break of interfacial tension vs concentration curves the CMC values of the samples were determined. The apparatus was calibrated with glass distilled water

The emulsification property of the ester products was tested by using different types of oil. The emulsion was prepared by dissolving the surfactant in the required amount of oil and the emulsion was formed by slow addition of the water phase to the oil phase and mixing intensely with a magnetic stirrer. The emulsions were allowed to settle at a constant temperature with out any further shaking. The emulsion height was measured after 24h. The emulsification index (E_{24}) was calculated

by determining the percentage volume occupied by the emulsion phase after 24h.

2.5 Infrared(IR) Spectroscopy of the Esters

The functional group determination of different PEG

esters was done by IR spectroscopy. The instrument was a Perkin-Elmer IR spectrophotometer with a data processor (model 1330). The samples were taken on a KBr plate in a holder. The IR spectrum was recorded in the region of 4000 - 400 cm^{-1} .

Table 1 Enzymatic Esterification Reaction between Polyethylene Glycols of Different Molecular weight and Sebacic Acid with Different Mol Ratio with Time in Hour.

Enzyme : RMIM (10% of total charge)

Temperature : 70-75°C

Time : 7 Hours

Pressure : 2 mm Hg

PEG400:Sebacic Acid	Percent Conversion with Time in Hour						
	1	2	3	4	5	6	7
2:1	6.8	14.2	19.6	36.2	40.3	46.4	47.1
1:1	27.7	32.3	35.7	41.6	52.3	63.2	63.9
PEG600:Sebacic acid	Percent Conversion with Time in Hour						
	1	2	3	4	5	6	7
2:1	3.4	10.1	15.8	18.3	22.7	24.6	30.5
1:1	14.8	20.4	25.8	30.6	35.0	44.5	48.1

$$\text{Percent Conversion} = \frac{(\text{Total Equivalent di-basic Acid} - \text{equivalent di-basic acid in the Product}) \times 100}{\text{Total Equivalent di-basic Acid}}$$

Table 2 Enzymatic Esterification Reaction between Polyethylene Glycols of Different Molecular Weight and Adipic Acid with Different Mol Ratio with Time in Hour.

Enzyme : RMIM (10% of total charge)

Temperature : 70-75°C

Time : 7 Hours

Pressure : 2 mm Hg

PEG400:Adipic Acid	Percent Conversion with Time in Hour						
	1	2	3	4	5	6	7
2:1	8.3	10	13.4	18.3	20.0	28.3	38.8
1:1	10	19.2	25.3	32.8	40	45	49.1
PEG600:Adipic acid	Percent Conversion with Time in Hour						
	1	2	3	4	5	6	7
2:1	5	8	10.3	15.2	18.3	19	20
1:1	9.5	12.4	17.3	20.3	28.3	30.3	35.1

$$\text{Percent Conversion} = \frac{(\text{Total Equivalent di-basic Acid} - \text{equivalent di-basic acid in the Product}) \times 100}{\text{Total Equivalent di-basic Acid}}$$

3 Results and Discussion

Di-basic acid esters of polyethylene glycols of different molecular weight could be prepared by enzyme catalyzed esterification reaction. It can be seen from the drop of acid values as determined by the AOCS method (31) that *Mucor-miehei* lipase (RMIM) acts as an excellent catalyst for esterification.

The percent converse of dibasic acids to esters depends on the size of polyethylene glycol as well as on the nature of di-basic acids as shown in the **Table 1 & 2** and **Fig. 1** and **2** when di-basic acids both adipic and sebacic and polyethylene glycol are used in 1:1 molar proportion. As the size of the polyethylene glycol

(PEG) increases, the percent conversion decreases to some extent. This is due to inhibition of enzyme activity during the reaction due to the increase in the molecular size of the PEG.

It is also clear that sebacic acid reacts more easily with PEG-400 and PEG-600 than adipic acid at 70-75°C. Sebacic acid can form a homogeneous mixture which is essential to carry out the reactions.

For each di-basic acid ester it is observed that the interfacial tension decreases with increase in the percentage concentration (**Table 3**) of the solution although after a certain concentration the rate of decrease is very small or negligible. This indicates that the di-basic acid esters of polyethylene glycol can

Fig. 1 Percentage of Conversion of PEG400 with Sebacic Acid and Adipic Acid with 1:1 Mole Ratio.

Fig. 2 Percentage of Conversion of PEG600 with Sebacic Acid and Adipic Acid with 1:1 Mole Ratio.

Table 3 Measurement of Interfacial Tension at Different Concentration of Ester Solution.

Sample	Concentration of chloroform solution						
	0.01%	0.05%	0.1%	0.5%	1%	2.5%	5%
S1	24.1	23.1	22.0	20.9	18.9	17.2	16.0
A1	23.5	22	21	17.9	16.8	15.2	13.6

Table 4 Measurement of Different Surface Properties and Thermodynamic Property of Ester Solution.

Sample	CMC Mols/L	γ_{CMC} mN/m	Γ_{max} Mol/cm ²	A_{min} (Å ²)	$-\Delta G_{\text{MIC}}^0$ KJ/mol
S1	10×10^{-5}	21.2	10.1×10^{-11}	164.3	22.9
A1	3.13×10^{-5}	20.9	7.7×10^{-11}	231.9	25.8

Chloroform/water interfacial tension is 33.5 mN/m at 27°C

S1 = Ester of PEG400 and Sebacic Acid

A1 = Ester of PEG400 and Adipic acid

behave as distinct surfactants. The data on the different surface active properties are included in **Table 4**. CMC values are taken as the molar concentration at the intersection (**Fig. 3**) of the two linear portions of the curve of γ vs. $\log C$ above and below the discontinuity. It is evident that the increase in the chain length of the hydrophobic group has a pronounced effect on the CMC values. In case of glucamide series a sharp decrease in the CMC value is seen with increasing alkyl chain length (32). In the present case the ester molecule contains a free OH- group and a carboxylic acid (-COOH-) group. The CMC value of sebacic acid ester is higher (10×10^{-5}) than that of adipic acid (3.16×10^{-5}) presumably due to the only difference in the carbon chain length between molecular structure of the two esters. The observation noted in the case of di-basic acid esters of the present study differs from the corresponding poly ethylene glycol esters of monobasic fatty acid where the CMC values show an opposite trend (33). The effectiveness of the interfacial tension reduction is the interfacial tension value at CMC (γ_{CMC}). The value of γ_{CMC} of sebacic acid ester of PEG-400 is 21.1 mN/m and that of adipic acid ester is 20.9 mN/m. Hence it can be concluded that with increase in the size of the di-basic acid the value of effectiveness increases and the surface active agent becomes less effective. The surface excess concentration (Γ_{max}) in mol/cm² and minimum area/ molecule (A^2) at the interface of CHCl₃/H₂O were calculated from equation 1 and 2 (32).

$$\Gamma_{\text{max}} = -\frac{1}{n \cdot 2.303RT} (\partial\gamma/\partial\log C)_{\text{max, T}} \quad (1)$$

S1 = Ester of PEG400 and Sebacic Acid, A1 = Ester of PEG400 and Adipic Acid

Fig. 3 Plot of Interfacial Tension vs log of Molar Concentration of S1 and A1(Ester of PEG-400 and Sebacic Acid and Adipic Acid (1:1)).

$$A_{\text{min}} = 10^{16} / N\Gamma_{\text{max}} \quad (2)$$

The Γ_{max} value of sebacic acid ester is higher than adipic acid ester. This observation is explained by considering the chain chain interaction at the interface with increase in chain length from C₆ (adipic) to C₁₀ (sebacic). Consequently the reverse order is observed for A_{min} values for the two di-basic acid esters.

The thermodynamic property like standard free energy of micellization (ΔG_{MIC}^0) is calculated from the equation

$$\Delta G_{\text{MIC}}^0 = RT \ln \text{CMC} / \omega$$

Taking $\omega = 1$ ΔG_{MIC}^0 of sebacic acid ester of PEG-400 is 22.9 KJ/mol and that of adipic acid ester is 25.8 KJ/mol. The ΔG_{MIC}^0 increases with the decreasing size of di-basic acids.

The IR data (**Fig. 4** and **Fig. 5**) indicate the formation of di-basic acid esters. Ester formation was also confirmed from the acid value of the product. The spectral data of -OH- group ($\sim 3000\text{cm}^{-1}$), -COOR- group

Fig. 4 IR Spectrum of S1 (Sebacic Acid Ester of PEG400).

Fig. 5 IR Spectrum of A1 (Adipic Acid Ester of PEG400).

($\sim 1735\text{cm}^{-1}$) and $-\text{COOH}$ group ($\sim 1650\text{--}1550\text{cm}^{-1}$) at their respective wave lengths represent the formation of the polyethylene ester of the two dibasic acids containing two polar functional groups.

PEG ester of adipic and sebacic acids have distinct surface active properties and they may find new uses as surfactant.

4 Conclusion

The PEG esters of dibasic acids with distinct interface properties and thermodynamic behavior could be synthesized by bioesterification process using *Mucor miehei* (RMIM) lipase as a catalyst.

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References

1. H. MAAG, Fatty Acids Derivative: Important Surfactants for Household, Domestic and Industrial Purpose, *J. Am. Chem. Soc.*, Vol. **61**, 259 (1984).
2. A. MEFFERT, H. KGaA and Zree-FETTCHEMIE, Technical Use of Fatty Acid Esters, *J. Am. Oil Chem. Soc.*, Vol. **61**, 255-258 (1984).
3. Lion Corp., *Jpn. Kokai Tokkyo Koho*, JP 57156410 (1982).
4. Lion Corp., *Jpn. Kokai Tokkyo Koho*, 8171021 (1981).
5. T. ONOZAKI, *J. SCCJ*, Vol. **15**, 38 (1981).
6. K. HOFFMAN, *Seifen Oele fett Wachse*, Vol. **104**, 4 (1978).
7. T.H. KUNZMANN, *Seifen Oele fett Wachse*, Vol. **98**, 563 (1972).
8. M.G. DENAVARRE, *The Chemistry and Manufacture of Cosmetics*, 2nd edn., Vol. **11**, Van Nostrand, Princeton, NJ, p. 84 (1962).
9. O.M. MESNIK, *USSR patent*, 588274 (1978).
10. K.F. SCHIERMAYER, *U. S. Patent*, 2, 913,407 (1959).
11. M. YAMAMOTO, *Jpn Patent*, 7,313,737 (1973).
12. W. POSTMANN, *Text. Res. J.*, Vol. **50**, 444 (1980).
13. P.E. SONNET, Lipase Selectivity, *J. Am. Oil Chem. Soc.*, Vol. **65**, 900 (1988).
14. N.N. GANDHI, S.B. SAWANT and J.B. JOSHI, Studies on the Lipozyme-Catalysed Synthesis of Butyl Laurate, *Biotechnol. Bioeng.*, Vol. **46**, 1 (1995).
15. R.G.JR. BISTLINE, A. BILYK and S.H. FEAIRHELLER, Lipase Catalysed Formation of Fatty Amides, *J. Am. Oil Chem. Soc.*, Vol. **68**, 95 (1991).
16. S.E. GODTFREDSEN, K. INGVOSEN, B. YDE and O. ANDERSON, *Enzymes as Catalyst in Organic Synthesis* (M.P. SCHNEIDER, ed.), Reidel Publishing Co., Philadelphia, p. 77 (1986).
17. F.W. WELSH, R.E. WILLIAMS and K.H. DAWSON, Lipase Mediated Synthesis of Low Molecular Weight Flavor Esters, *J. Food Sci.*, Vol. **55**, 1679 (1990).
18. N.N. GANDHI, S.B. SAWANT and J.B. JOSHI, Specificity of a Lipase in Ester Synthesis: Effect of Alcohol, *Biotechnol. Progr.*, Vol. **11**, 282 (1995).
19. A. DUCRET, M. GIROCUX, M. TRANI and R. LORTIE, Characterization of Enzymatically Prepared Biosurfactant, *J. Am. Oil Chem. Soc.*, Vol. **73**, 109 (1996).
20. D. MYERS, *Surfactant Science and Technology*, 2nd edn., VCH publishers Inc., New York, p. 66 (1992).
21. A. DUCRET, M. GIROCUX, M. TRANI and R. LORTIE, Enzymatic Production of Biosurfactants from Sugars or Sugar Alcohols and Fatty Acids in Organic Media under Reduced Pressure, *Biotechnol. Bioeng.*, Vol. **48**, 214 (1995).
22. M. BOURDET, *Conger. Mondial Detergence Prod. Tensio-Paris*, Vol. **2**, p. 735 (1954).
23. G. ALS and N. KROG, *J. Am. Oil Chem. Soc.*, Vol. **67**, 73 (1990).
24. L. MARCOU, *Rev. Fr. Crops Gras*, Vol. **23**, 459 (1976).
25. B.C. CARVER, *DOS 2* 556, 142 (1976).
26. R.A. RECK, *Fatty Acids in Industry* (R.W. JONSON and E. FRITZ), M. DEKKER, New York.
27. D.R. ENDERSON and A.J. FRISQUE, *DOS 2*, 154,081 (1970).
28. R. MAEDLAR, *et al.*, *Ger (East)* 71863 (1968).
29. M. GHOSH and D.K. BHATTACHARYYA, Enzymatic Preparation of Polyethylene Glycol (PEG) Esters of Castor Oil Fatty Acids and Their Surface Active Properties, *J. Surfactant Detergent*, Vol. **1**, No.4 (1998).
30. D. HARKINS and F.E. BROWN, Determination of Surface Tension (Free Surface Energy), and the Weight of Falling Drops. The Surface Tension of Water and Benzene by the Capillary Height Method, *J. Am. Chem. Soc.*, **XLI**, 499-524 (1919).
31. AOCS methods for the measurement of Acid Value.
32. YUN-PENG, M.J. ROSEN, P.K. VINSON and S.W. MORRALL, Surface Properties of N-Alkanoyl-N-Methyl Glucamines and Related Materials, *J. Surfactant Detergent*, Vol. **2**, No3, July (1999).
33. Ph. D. Thesis (Tech) of Suparna Basu, Calcutta University, 'Studies on Lipase Catalysed Process Technologies in Making Certain Fatty Acid Derivatives for Better Utilization', (1998).