International Journal of Emerging Engineering Research and Technology
Volume 2, Issue 7, October 2014, PP 279-282
ISSN 2349-4395 (Print) & ISSN 2349-4409 (Online)

Enzyme Esterification Route to Prepare Glyceryl Tributyrate

Ramnath V. Patil[1], Satish R. Inamdar[1] , Rajnish Kumar[2]

[1]Chemical Engineering Department, Vishwakarma Institute of Technology, Bibwewadi, Pune
[2]Chemical Engineering and Process Engineering Division, National Chemical Laboratory, Pune
satish.inamdar@rediffmail.com

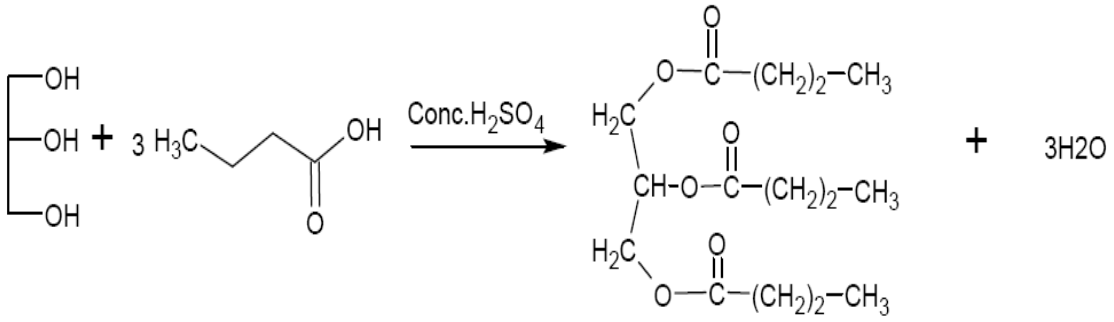
Abstract: *In the present work, we investigate how conversion and yield for an unknown process route to prepare tributyrin can be optimized. The experimental optimization has been done by applying the chemometric methods. The yield and conversion are experimentally obtained for various catalyst-solvent pairs. The parameters used in experimental study are molar ratio, stirring speed, catalyst, solvent, reaction temperature and reaction run time. Overall conversion up to 97.46% of ester and 2.53% butyric acid and impurity 0.04 % are detected by GCMS. The significance of water is that the formation of water inhibits the esterification reaction as it favors reversal. Immobilized enzyme lipozymes, Novozyme, Bakers yeast were employed as catalysts for the esterification reaction. Amongst the enzymes tried as options, novozyme was found to be better than the rest two enzymes.*

1. INTRODUCTION

In the present article we have studied the experimental optimization of enzyme esterification reaction to prepare glyceryl tributyrate. We have not found any references in the open literature on the kinetics and reaction synthesis of glyceryl tributyrate. Therefore, it forms a motivation for carrying out research for this product. The glyceryl tributyrate is used in preparing flavors, cosmetics, and fragrances in the perfumes [1-4]. Clearly, a new process route, as the esterification reaction, can be investigated to study new process technology and its economic potential.

In this work, we will focus on esterification route to prepare glyceryl tributyrate. The reaction synthesis was perceived as a chemical balance equation and in terms of the molecular structures the reaction can be represented as,

Glycerol + Butyric acid → Glyceryl tributyrate + Water



Since there is no literature data available on kinetics, rate expressions, and thermodynamic conditions for the catalytic reaction to occur, our primary objective is to study the reaction synthesis as a problem of experimental optimization. We will apply the chemometric methods for experiment design and forms motivation for this study. The catalysts selected were many (Novozyme, concentrated sulfuric acid, lypozyme in immobilized form, Amberlite-15) and in this note we present experimental results for enzymatic esterification alone. The rest of the experimental data will be published elsewhere. The factors selected for experimental optimization were molar ratio (glycerol moles: butyric acid moles), temperature of reaction, stirring rate in RPM, solvent selected for a catalyst considered

2. EXPERIMENTAL PROCEDURE

The esterification reaction can be carried out as a chemical synthesis for preparing the glyceryl tributyrate. First the experiments were carried out using a rotary shaker reaction unit as rupture of catalyst particles can be eliminated. Later from initial results obtained for this reaction synthesis, further investigation was done using a stirred laboratory batch reactor unit. While carrying the reaction synthesis, we ensured that the system in reactor reached to a desired reaction temperature and then was maintained at this temperature value within ± 1°C variation. The experimental setup is shown in figure 1. The batch reaction time required for the addition of reactants and for the reactor to attain thermal equilibrium was ~20 min. The low boiling impurities were removed at the start and later the acid was removed to an extent of more than 90%.Thus, the rest of product i.e. ester in rotary flask was collected as residue. With several washes given, the purity increased up to 97%.Different types of catalysts and solvents were used in recording the maximum conversion from the reaction. However, the observations from experimental optimization only for lypozome catalyst are presented in this note. The effects of parameters such as molar ratio, temperature, solvent, catalyst weight were studied. It may be noted that the water content in the reaction was determined by the Karl-Fischer analyzer.

Fig1. *Setup of experimental batch reactor*

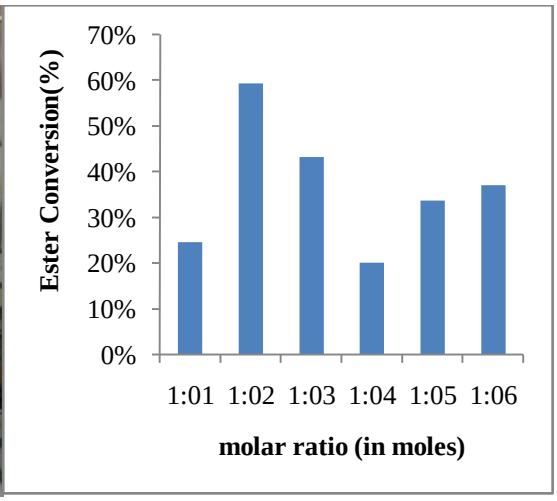


Fig2. *Different molar ratios vs. ester conversion*

Table1. *Application of Chemometrics [5]. Experimental optimization for synthesis of glyceryl tributyrate*

a) Factors for Experiment Design

Parameter	High Value (1)	Low Value (-1)
RPM	1000	500
Molar Ratio	1:5	1:3
Temperature (^oC)	120	100
Time (hr)	4	2

b) Design Matrix

Sl. No.	RPM	Molar Ratio	Temperature (^oC)	Time (hr)	Conversion (%)
1	1	-1	-1	1	36.6%
2	1	1	-1	-1	33.3%
3	1	1	1	-1	38.55%
4	-1	1	1	1	38%
5	1	-1	1	1	25.23%
6	-1	1	-1	1	33.5%
7	-1	-1	1	-1	19.21%
8	-1	-1	-1	-1	30.1%

* *Catalyst used is lypozome*

c) Response Curve Computation Showing Optimum Condition

Figure 2 shows batch reactor output for various molar ratios (glycerol: butyric acid) and percent conversion to ester product. The work presented essentially discusses the experimental optimization

procedure used as a factorial design. The theory and design matrix preparation for a two level factorial experiment was done as explained in a textbook on Chemometrics [5] and the results were tabulated in Table 1. The data fitting for response curve i.e. response in terms of conversion from reaction as a function of factors stirring RPM, molar ratio, temperature of reaction and reaction run time are recorded in Table 1. The optimum conditions can now be determined.

We consider an equation that can be used to fit the data in Table 1 to compute the coefficients in response curve expression. This equation is given as

$$y = a_0 + a_1 x_1 + a_2 x_2 + a_3 x_3 + a_4 x_4 + a_5 x_1^2 + a_6 x_2^2 + a_7 x_3^2 + a_8 x_4^2 + a_{12} x_1 x_2 + a_{13} x_1 x_3 + a_{14} x_1 x_4 + a_{23} x_2 x_3 + a_{24} x_2 x_4 + a_{34} x_3 x_4$$

Where quadratic terms with dissimilar variables represent interaction terms. The computed response curve coefficients will produce a hypersurface, but the results cannot be plotted as dimensionality is more than three. So we generate values using a symbolic manipulator MATHEMATICA 9.0.1 (of Wolfram Research) and find the supremum of the conversion values. The results of numerical computations (data fitting giving coefficients in above response curve equation) are given here.

$a_0 = 0.385103$; $a_1 = 0.000185853$; $a_2 = -0.0310788$; $a_3 = -0.00218766$; $a_4 = 0.0503224$;
$a_5 = \rightarrow -3.44742 \times 10^{-8}$; $a_6 = -0.0283379$; $a_7 = -0.0000682777$; $a_8 = -0.000452049$;
$a_{12} = -0.0000869563$; $a_{13} = 1.35 \times 10^{-7}$; $a_{14} = 0.0000602558$; $a_{23} = 0.00400125$;
$a_{24} = -0.020604$; $a_{34} = -0.00015375$;

To obtain the supremum value from above response curve equations the low and high values in above experiments were varied in a limited range and supremum if single conversion value was obtained; and using this maximum value the corresponding values of variables (factors in experiment design) were identified. The supremum value found was y=0.386995 and optimum numerical values of factors in experiment design were obtained as { $x_1 = 500.$, $x_2 = 4.$, $x_3 = 100.$, $x_4 = 2$}.

Mass balance calculations (Conversion to glyceryl tributyrate)

The calculation procedure to find conversion from reaction is provided for the sake of clarity being given to results presented for the experiments done and optimization procedure followed to obtain needed results.

Glycerol	+	Butyric acid	⟷	Glyceryl tributyrate	+	Water
51.88 gm	+	248.12 gm		144.35 gm	+	25.81 gm

From output obtained by gas chromatograph (GC) analysis, we find that85 % moles are converted from the feed. The material balance calculations are shown in Table 2.

Table2. *Summary of calculations for experimental run conducted.*

Reactant – Glycerol (0.563 moles in feed; 0.08445 moles unreacted).
Reactant – Butyric acid (2.8195 moles in feed; 1.3855 moles unreacted).
Product – Glyceryl tributyrate (0.478 moles formed).
Product – Water (1.434 moles formed).
Conversion of glycerol is 85% by mole.

3. CONCLUSIONS

In this work, for the first time the kinetics of the catalytic reaction via enzymatic esterification route has been studied to react glycerol and butyric acid to prepare an important ester, glyceryl tributyrate, also known as tributyrin. The results of experimental optimization have been presented using a factorial design method.

REFERENCES

[1] M. Berger, K. Laumen and M. P. Schneider . Enzymatic Esterification of Glycerol I. Lipase-Catalyzed Synthesis of Regioisomerically Pure 1,3-sn-Diacylglycerols, Journal of A.C.S., 69, (10) (October 1992).

[2] E. Battistel, C. Calapriceb, E. Gualdic, E. Rebescod, E. Maria Usaie , Co-production of butyrate methyl ester and triacetyl glycerol from tributyrin and methyl acetate, Applied Catalysis A: General, 394, pp.149–157, (2011).

[3] T. Tittabut and W. Trakarnpruk, Mg-Al metal oxides for transesterification of glyceryl tributyrate and palm oil , Journal of A.C.S., (2008).

[4] Y. Xi, R.J. Davis, Influence of water on the activity and stability of activated Mg–Al hydrotalcites for the transesterification of tributyrin with methanol, Journal of Catalysis, 254, pp. 190–197, (2008).

[5] R. G. Brereton, Chemometrics. Data Analysis for the Laboratory and Chemical Plant, Wiley (2006).