Chemical Science Review and Letters ISSN 2278-6783

Research Article

Productivity Study for Enzymatic Synthesis of Diesters of Sebacic Acid

Sarbani Ganguly and Sumit Nandi*

Department of Chemistry, Narula Institute of Technology, Agarpara, Kolkata 700109, West Bengal, India.

Abstract

Sebacic acid diesters have wide range of applications for the synthesis of basic oleo chemicals and oleo chemical derivatives. Specifically diesters of sebacic acid with the alcohols like isoamyl and isobutyl alcohol are important in this aspect. The present work aimed at preparation of these diesters in the presence of lipases from a variety of microbial origin. We have screened four commercial lipases e.g. 1, 3 specific RM-IM lipase (Source: *Rhizomucor miehei*), 1, 3 specific TL IM lipase (Source: *Thermomyces lanuginosus*), non specific NS40013 lipase (Source: *Candida Antarctica*) and non specific NS 435 lipase (Source: *Candida Antarctica*) for their ability to catalyze the synthesis of diesters with different alcohols. Different reaction parameters like lipozyme concentrations, temperature and substrate concentration were studied for this purpose by varying the mole ratio of acid and alcohol from 1:1 to 1:16. Rate of conversion increased with the increase in acid: alcohol molar ratio up to a certain level. Our results show that the maximum catalyzing action was observed for the enzyme NS 435 towards esterification reaction and due to higher productivity, this enzyme was successfully recycled for 10 times without any appreciable change in percent conversion which proves the admirable commercial viability of the process.

Correspondence
Sumit Nandi,
Email: sumitnandi5@gmail.com,
Ph.-09830548335/09804469759.

Key words: Sebacic acid, Enzyme, Diesters, Lipase.

Introduction

Synthesis of different dibasic acid esters with enzyme catalyst attracts a new dimension of work for the researchers and academicians in the field of chemical, biological and medicinal applications. Production of diesters of sebacic acids is quite relevant in this aspect as these diesters have extensive commercial applications for preparation of synthetic lubrication oil, cold resistant plasticizer, mixed with durable phthalate plasticizers, manufacture of foundry core, additives, diluents, fixatives, fragrance characteristics enhancer and perfume stabilizer. Recent years have witnessed the increased use of biocatalysts in organic media, with new theoretical implications and practical applications [1-3]. Biotechnology involving lipase catalysed reactions (specific or non-specific) in the processing of fats and oils, synthesis of basic oleo chemicals and oleo chemical derivatives are well documented in recent years. When a lipase is immobilised on a water insoluble carrier, its stability is increased compared with an original lipase. Okumura *et al.* [4] synthesized esters of oleic acid with various alcohols by using microbial lipases of various origin like *Aspergillus niger*, *Rhizopus delemer*, *Geotrichum candidum* and *Penilillium cyclopium*. Langrand *et al.* [5] screened thirteen commercial lipase preparations for their ability to catalyse the formation of flavour esters (isoamyl or geranyl acetate, propionate and butyrate). Recent interest has developed in using immobilized lipase for the preparation of dicarboxylic acid esters, which has tremendous industrial applications in various fields.

The lipase catalysed esterification reaction of dicarboxylic acids with diols has been studied extensively and are used for the production of biodegradable polymers and surfactants [6-8]. Villeneuve *et al.* [9] worked on the synthesis of polyfunctional glycerol esters of dicarboxylic acids for use as pre polymeric synthons. Gryglewicz *et al.* [10-11] worked on synthesis of various dibasic acid esters of varied chemical structures. These esters were tested in

terms of their suitability as additives of fully synthetic engine oils and lubricating oil. Shiseido Co. Ltd., Japan [12] has developed a method for improving the quality of the fragrance of perfumes by incorporating at least one perfume controlling agents in the perfumes. Aliphatic dibasic diesters of general formula $R_1OCOR_2COOR_3$ where R_1 and R_3 are the same or different and represent saturated branched chain alkyl groups containing 4 to 5 carbon atoms, and R_2 represents straight chain alkylene group containing 4-6 carbon atoms have been found to be colourless, odourless, tasteless low viscosity liquids. When at least one of these compounds is added to a perfume, the fragrance characteristics and stability of the perfume is greatly increased.

The present work aimed at preparation of diesters of sebacic acid with isoamyl and isobutyl alcohol. Four commercial lipases RM-IM, TL IM, NS40013 and NS 435 were screened for their ability to catalyze the esterification reactions of which NS 435 was found to be most efficient. Different reaction parameters i.e. lipozyme concentrations, temperature and substrate concentration were studied. The study reveals that the enzyme could be successfully recycled for more than 10 times which proves the commercial viability of the process.

Materials & methods

Materials

The immobilized lipases were used; Lipozyme RM-IM (Source: *Rhizomucor miehei*)1,3 specific lipase, TL IM (Source: *Thermomyces lanuginosus*) 1,3 specific lipase, NS40013 random lipase, NS 435 (Source: *Candida Antarctica*), all these were donated by M/s. Novozymes South Asian Pvt. Ltd., Bangalore, India, for research purpose. Sebacic acid, isoamyl alcohol, isobutyl alcohol, petroleum ether, hydrochloric acid, sodium hydroxide, ethanol, methanol and all solvents used were obtained from E. Merk (India) Ltd., Mumbai, India.

Methods

Lipase catalyzed esterification reaction

The esterification reactions were carried out in a round-bottomed flask fitted with an air condenser, containing sebacic acid (0.2g, 1.0 mM), with different alcohols like isoamyl and isobutyl alcohol under different reaction conditions e.g variation of enzymes, enzyme concentration, acid alcohol ratio and temperature. Reactions were stopped by adding 20 mL ethanol and the excess free fatty acid was neutralized with 0.1M NaOH. The percentage conversion was calculated from the amount of acid consumed in the reaction.

Isolation of mono and di-ester

After esterification reaction, the product mixture contained unreacted dicarboxylic acids, diester, monoester and the unreacted alcohol. After neutralization with 0.1(M) NaOH, the reaction mixture was extracted thrice with petroleum ether to remove all the diesters produced. The mixture was then acidified with 1(N) HCl and extracted thrice with petroleum ether to recover the monoesters formed. The extracts were evaporated to dryness and monoester and diester formed were estimated gravimetrically. Monoesters and diesters were also confirmed by thin layer chromatography (TLC).

Estimation through Gas-Chromatographic Analysis

Purity of isolated monoester and diesters were also confirmed by GLC analysis. Isolated monoester was converted to mono methyl ester of corresponding alcohols and analysed on a Hewlett Packard-HP 5890A Gas Chromatograph (Carrier gas-N_2, Flow rate-30 ml/min., oven temperature was programmed from 100°C to 190°C at 5°/min, Inj. Temp-230°C, Detector-FID, Temp. -240°C). Column:- 10% DEGS, 6’ × 1/8” i.d. supported on chromosorb WHP.

Results & discussions

The bio esterification reaction of sebacic acid with two different alcohols namely isoamyl and isobutyl alcohol had been studied extensively. **Table-1** shows the percent (%) conversion of isoamyl and isobutyl esters of sebacic acid at

different reaction time (hrs) under the catalyzing action of different enzymes. All the enzymes (RM IM, TL IM, NS 435 & NS40013) used here are 10% of the substrate concentration at 45^0C maintaining a ratio of acid: alcohol 1:4. Di isopropyl ether was used as solvent in our study. It has been observed from **Table-1** that conversion of ester is maximum within 1 hour when NS 435 is used. This enzyme gives maximum esterification product after three hours compared to other enzymes. Thus due to higher catalytic activity, enzymes NS 435 was selected for further studies and the time of esterification reaction was chosen for 1 hour only.

Table 1 Percent conversion of esters (10% lipase, 45^0C, Molar ratio acid : alcohol 1:4)

Enzymes	Alcohol	1	2	3	4	5
RM-IM	Isoamyl alcohol	52.3± 0.7	69.0± 0.034	86.0± 0.011	87.0± 0.168	87.5± 0.180
	Isobutyl alcohol	48.5± 0.3	63.0± 0.045	74.3±0.090	75.0±0.189	77.0±0.090
TL-IM	Isoamyl alcohol	54.2±0.110	67.3±0.055	86.0±0.142	86.8±0.213	89.0±0.178
	Isobutyl alcohol	42.3±0.039	61.8±0.035	75.4±0.124	80.1±0.054	80.3±0.148
NS40013	Isoamyl alcohol	53.1±0.115	70.4±0.144	89.0±0.123	89.2±0.114	89.5±0.187
	Isobutyl alcohol	49.7±0.108	65.3±0.123	82.0±0.145	82.2±0.198	83.1±0.135
NS 435	Isoamyl alcohol	86.0±0.171	88.0±0.086	90.1±0.065	88.2±0.079	87.5±0.156
	Isobutyl alcohol	87.4±0.076	88.5±0.058	90.3±0.090	90.0±0.079	89.7±0.132

Values are reported as mean ± s.d., where n=3

Percent esterification depends on the concentration of enzyme, as more enzymes mean more active binding sites and so the rate of reaction would be higher. But after complete binding of the active sites of the enzymes with substrate, increasing the concentration of enzyme does not increase the conversion of desired product as the concentration of substrate is fixed. It is evident from **Table 2** that increasing enzyme concentration does not always increase the conversion. 10% enzyme concentration is the ideal in this case. So the present study deals with this concentration of enzyme. Overall productivity increased from 60±0.161% to 86±0.171% for isoamyl sebacate and 57±0.121% to 87.4±0.076% for isobutyl sebacate on increasing the lipozyme concentration from 2.5% to 10%. The reactions were also studied with 20% of enzyme concentration but use of enzyme in excess of 10% does not reflect any significant change in percent conversion.

Table 2 Effect of enzyme concentration on the esterification reaction of sebacic acid with alcohols (1 hour, NS 435, 45^0C)

Substrates	2.5%	5%	7.5%	10%	20%
Sebacic acid + Isoamyl alcohol	60.0±0.161	71.8±0.045	79.1±0.090	86.0±0.171	86.5±0.057
Sebacic acid + Isobutyl alcohol	57.0±0.121	69.4±0.132	78.7±0.076	87.4±0.076	88.7±0.032

Values are reported as mean ± s.d., where n=3

Table 3 Effect of temperature on the esterification reaction of sebacic acid with alcohols (10% NS 435, 1hr)

Substrates	40°C	45°C	50°C
Sebacic acid + Isoamyl alcohol	78.5±0.146	86.0±0.171	86.5±0.453
Sebacic acid + Isobutyl alcohol	79.9±0.163	87.4±0.076	89.2±0.038

Values are reported as mean ± s.d., where n=3

Every enzyme has a thermal stability range beyond which it will be deactivated. In our present study, we analyze the activity of enzyme at different temperatures as shown in **Table 3**. Esterification reaction were carried out from 40 ± 1ºC to 50± 1ºC and were found that with the increase in reaction temperature, there was an increase in the conversion

rate of sebacic acid to esters. Percent conversion of sebacic acid increased from 78.5±0.146 to 86.5±0.453% and 79.9±0.163 to 89.2±0.038% for isoamyl and isobutyl alcohol respectively on increasing the temperature from 40^0C to 50^0C. But the enhancement of temperature from 45^0C to 50^0C does not increase the percent conversion notably. So, we have chosen the temperature 45^0C as an optimum temperature for this purpose.

Table 4 Effect of substrate concentration on the esterification reaction of sebacic acid with alcohols (1hr, 45^0C, 10% NS 435)

Molar ratio (Acid : Alcohol)	Isoamyl alcohol	Isobutyl alcohol
1:1	50.1±0.163	48.1±0.023
1:2	67.4±0.144	70.3±0.126
1:3	80.1±0.079	82.3±0.155
1:4	86.0±0.171	87.4±0.076
1:8	86.5±0.231	88.1±0.218
1:16	85.3±0.132	84.5±0.191

Values are reported as mean ± s.d., where n=3

Table 4 shows the percent conversion of sebacic acid to the esters of isoamyl and isobutyl alcohols on variation of the alcohol concentration during 1 hour of reaction period. When the acid: alcohol ratio was kept at 1:1 molar ratio, the percent conversion was nearly 50% for both the alcohols but the rate of conversion was increased with the increase in acid: alcohol molar ratio from 1:1 to 1:8 for all the substrate pairs. Actually, 86.0±0.171% and 87.4±0.076% conversion were achieved for isoamyl and isobutyl alcohol respectively with a ratio of 1:4 for 1 hour. On further increasing the ratio, percent conversion does not increase but there is a possibility of an inhibition reaction in most of the cases. Therefore, we maintain a ratio of 1:4 for acid and alcohol as an ideal ratio.

Use of enzyme in chemical reactions can be cost effective only when it is recycled. In our research work, lipase NS 435 was recycled 10 times for esterification reaction with new batch and it has been observed from **Table 5** that after one hour of operation, enzyme is still active. Slight drop in conversion later is probably due to the presence of excess water in the immobilized lipase produced from esterification reactions. So from this study, it can be predicted that these enzymatic esterification reactions with recycling may be implemented in industrial scale also.

Table 5 Percent conversion of sebacic acid to esters during recycling of enzyme (1 hr, 10% lipase, 45^0C)

No of recycling	Isoamyl alcohol	Isobutyl alcohol	No of recycling	Isoamyl alcohol	Isobutyl alcohol
1	86.0±0.171	87.4±0.076	6	84.3±0.087	87.5±0.145
2	85.2±0.165	88.0±0.143	7	84.0±0.167	87.2±0.142
3	84.7±0.188	87.5±0.057	8	84.1±0.231	87.0±0.054
4	84.7±0.142	87.6±0.145	9	83.1±0.146	85.6±0.180
5	84.7±0.133	87.6±0.214	10	82.4±0.175	85.0±0.045

Values are reported as mean ± s.d., where n=3

For all the reactions studied here, the synthesized products were a mixture of di and monoester of their respective acid and alcohol. The end product isoamyl sebasate and isobutyl sebasate contained 93±0.817% and 97±0.731% diester and 7±0.031% and 3±0.073% monoester respectively as evidenced from **Table 6.** The results showed that mainly diesters were produced in the enzymatic esterification reaction which is an advantage of using enzyme as catalyst for selective product.

Table 6 Composition of esters of sebacic acid with different alcohols (Temperature-45^{0}C, NS435-10%, Acid: Alcohol:: 1:4, time 3 hrs)

Alcohols	Diester	Monoester
Isoamyl alcohol	93±0.817%	7±0.031%
Isobutyl alcohol	97±0.731%	3±0.073%

Values are reported as mean ± s.d., where n=3

Conclusion

The present study investigated the productivity study of enzymatic preparation of diesters of sebacic acid with two different alcohols, isoamyl and isobutyl alcohol using four different enzymes. Among the four enzymes used in our study, NS 435 is the most effective catalyst for esterification purpose at certain reaction conditions. Due to absence of by product, isolation of enzyme is also straightforward. Maximum amount of diester is produced in our study with a little amount of monoester which is advantageous of enzymatic esterification. Recycling of enzyme can be successfully done in our study and we recycle ten times in our study which ultimately reduces the process cost. Therefore, enzymatic esterification process can be adopted in bench scale to obtain maximum productivity and in this regard, it is advantageous for the preparation of raw materials for the basic oleo chemicals. Our study also helps future researchers in better understanding for the synthesis of dibasic acid esters using different alcohols and different enzymes at a particular reaction conditions with recycling of enzymes in different chemical and biochemical field.

Acknowledgements

We are indebted to Late Dr. Santinath Ghosh, University College of Science & Technology, 92, A.P.C. Road, Kolkata, India under whose able guidance this work has been executed.

References

[1] G. Carrea, *Trends in Biotechnology*, **1984**, 2, 102.
[2] C. Ratledge, *FETE SEIfen Austrichmittel*, **1984**, 10, 379.
[3] A. Klibanov, *Chem Tech*, **1986**, 6, 354.
[4] S. Okumura, M. Iwai, Y. Tsujisaka, *Biochim Biophys Acta*, **1979**, 575, 156.
[5] G. Langrand, C. Triantaphylides, J Baratti, *Biotechnology Letters*, **1986**, 10, 594.
[6] F. Binns, S. Roberts, A. Taylor, C. Williams, *Chem. Soc. Perkin Trans*, **1993**, 1, 899.
[7] Z. Wang, K. Hiltunen, P. Orava, J. Seppala, Y. Linko, *Macro. Sci. Appl. Chem*, **1996**, A33, 599.
[8] Y. Linko, Z. Wang, J. Seppala, *Biotechnology*, **1995**, 40, 133.
[9] P. Villeneuve, T. Foglia, T. Mangos, A. Nunez, *J. Am. Oil. Chem. Soc.*, **1998**, 75, 1545.
[10] S. Gryglewicz, M. Stankiewicz, F. Oko, I. Surawska, *Tribology International*, **2006**, 39, 560.
[11] S. Gryglewicz, F. Oko, *Industrial Lubrication and Tribology*, **2005**, 57, 128.
[12] W. Evers, H. Heinsohn, E. Shuster, F. Schmitt, *α-Oxy(Oxo) Sulphides in Perfume*, U.S. Patents, **1978**, 4,094,824.

Publication History
Received 11th Sep 2013
Revised 20th Dec 2013
Accepted 25th Dec 2013
Online 05th Jan 2014