CHEMOENZYMATIC, CONVENIENT SCALABLE TEMPERATURE SYNTHESIS AND ENTHALPY CALCULATIONS OF TRIETHYL GLYCOL POLY ORTHO ESTER

Madhuresh Kumar Sethi*[1], Sanjay Mahajan[1], Bhairaiah Mara[1], Rajakrishna Yerramalla[1] and Lakshminarayana Vemula[1]

[1*]R and D Tianish Laboratories Private Limited, (100% Subsidary of Matrix Pharma Corp), Plot No.34A Part, Survey No.81 and 84, Anrich Industrial Estate, Bollaram, Jinnaram Mandal, Sangareddy (District) 502325, Telangana, India.

ABSTRACT

Tri (ethylene glycol) poly(orthoester) (TEG-POE) (Formula-1) can serve as an excipient of many pharmaceutically active compounds. Utilizing immobilized lipase as a biocatalyst, a novel enzymatic method for TEG-POE synthesis has been developed. This method involves concise process of the polymerization using Lipase (Addzyme) at convenient room temperature against the existing very high temperature chemical conversion. This efficient method provides green and environmentally friendly industrial oriented scalable process of TEG-POE in comparison with chemical processes which contains very high temperature conditions. In addition, the enthalpy calculations were derived and compared between chemical reaction and enzymatic reaction. Green chemistry principles are promoted by the synthesis cost-effectiveness and global accessibility, which is facilitated by the use of readily available and affordable lipase.

KEYWORDS

Tri (ethylene glycol) poly(orthoester) (TEG-POE), Enzymatic Conversion, Lipase (Addzyme), Green chemistry and Enthalpy calculations.

Author for Correspondence:

Madhuresh Kumar Sethi,
R and D, Tianish Laboratories Pvt. Ltd (100% subsidiary of Matrix Pharma Corp), Plot No.34 A Part, Survey No.81 and 84, ANRICH Industrial Estate, Bollaram, Jinnaram, Sangareddy, Telangana, India.

Email: madhuresh.sethi@tianishpharma.com

INTRODUCTION

Triethylene Glycol Polyorthoester is used as an excipient. The techniques outlined in U.S. Pat. Nos. 4,549,010 and 5,968,543 are used to prepare the polyorthoesters. Triethylene Glycol Polyorthoester having the compound of the formula I. The current invention relates to a process for the Triethylene glycol poly(glycolide) preparation and further conversion to Triethylene Glycol Polyorthoester.

Formula I

Polyorthoesters can be created by polyadding a diketene acetate and diol or by transesterifying orthoesters with diols. The general structure for polyorthoester is – [–R–O–C(R_1, OR_2)–O–R_3–]$_n$– .Polyorthoesters (POE-IV) have a considerable capacity to produce bioerodible pharmaceutical products and outstanding control over the rate of erosion. Polyorthoester polymers represent a family of bioerodible polymers for controlled drug delivery. The hydrophobicity of the orthoester functional groups hinders water penetration in combination with fast hydrolytic degradation. These properties result in surface erosion rather than a bulk degradation. Four generations of POE are known from literature, with different applications for drug-delivery purposes. Polymers are typically used for injectable depot dosage forms, which require a comparably low weight average polymer molecular weight (Mw) and preferably semi-solid material properties. POE used in a commercial drug formulation is triethylene glycol (TEG) in combination with triethylene glycol-glycolide (TEG-GA) containing POE IV (TEG-POE IV). Brand name of the drug product is Sustol® for controlled release of Granisetron. The Mw of POE IV utilized in this product is reported approximately 6 kDa. Glycolide building blocks incorporated into the polymer structure play a key role in increasing the acidity to control the degradation rate. Typical degradation time for the sustained release application is approximately 7 days. In US Patent 5968543, the demonstrated zero-order drug release profile of low Mw TEG-POE IV renders this polymer applicable for drug delivery systems, especially for injectable depot dosage forms.

Bio catalysis accelerates chemical reactions using natural materials like enzymes or cells[1]. The catalysis of hundreds of reactions, such as the fermentation of alcohol and the breakdown of milk proteins to produce cheese, depends critically on enzymes[2]. The structure and functional activities of enzymes have been better understood thanks to recent developments in science, which has increased the enzymes' stability, activity, sustainability[3] and substrate selectivity. There are currently one hundred distinct biocatalytic processes in use in the food, pharmaceutical, chemical and agro-based industries.

Particularly those lipases[4] that are primarily active against substrates that are insoluble in water, like triglycerides made of long-carbon chain fatty acids, have significant potential for application in industry. To obtain the best performance for these substrates, however, engineered variants must typically be built. It has been reported that lipases can be made to have better properties through protein engineering methods, such as by swapping the lid domain or introducing particular mutations in the esterases or lipases cap domain. Here, we enhanced the lipase activity of a "lipase associated with the Actinoalloteichusgenus (WP_075743487.1, or Lip MRD), which was obtained from the Marine Metagenomics MarRef Database. The enhancement was made possible by site-directed mutagenesis and the replacement of Rhizopus delemar lipase's (formerly R. oryzae; UniProt accession number, I1BGQ3) lid domain (FRGTNSFRSAITDIVF) with that of the former. The findings showed that redesigned mutants exhibit increased activity against larger triglycerides, including coconut oil, olive oil, palm oil, and glyceryl tridecanoate and tridodecanoate. The lipase activity increase which has been also attained with lid swapping seems to be attributed to residue W89 (LipMRD numbering). This study results support the notion that the amino acid composition of the lid domains plays a vital role in determining the lipases substrate specificity. However, more research may be necessary to determine whether lid domain swapping among lipases can be generalized or whether specific mutations in the lid domain" can enhance activity of lipase. Addzyme exhibits good conversion and high enantiopurity for the conversion among several commercial lipases that have been screened.

The heat of reaction, also known as the enthalpy of reaction, refers to the change in enthalpy[5] of a chemical reaction which had been occurs under constant pressure. It measures energy produced or released per mole during a reaction using thermodynamics. Enthalpy, derived from pressure, volume and internal energy[6], is a state function. It

was created as a unit of measurement to calculate energy changes in systems when it was difficult to measure ΔU, or internal energy change, by measuring heat and work exchanged. See the enthalpy section for more information on measuring enthalpy change at constant pressure.

ΔH or ΔH° is used to specify the temperature along with the "pressure of the heat of reaction ΔH. The ΔH° can be positive or negative. The units for ΔH° are kilo Joules / mole, or kj/mol.

ΔH or - ΔH

Δ = depicts enthalpy change; (ΔH -ΔH)

A positive value" depicts a heat-requiring endothermic reaction or higher enthalpy.

Negative values indicate exothermic reactions or reactants with higher enthalpy.

$^\circ$ = indicates a standard enthalpy change at a set pressure/temperature.

= indicates that the enthalpy of reaction is this change.

Akin to ΔH, ΔH° represents the standard heat of reaction or enthalpy of a reaction. On the other hand, ΔH° occurs under "standard" conditions, which imply that the reaction occurs at the temperature of 25°C and 1atm.

Measuring ΔH under standard conditions allows for a correlation between values since all ΔH° values occur under the same conditions.

MATERIAL AND METHODS
Reagents and Chemicals
All of the reagents along with solvents utilized in the experimental section of this study were purchased commercially, unless otherwise noted.
Methodology
Condensing Triethylene glycol formula III with glycolide of formula II in an organic solvent in the presence of an enzyme to get Triethylene glycol poly (glycolide) formula IV.

EXAMPLES
3, 9-Divinyl-2, 4, 8, 10-tetraoxaspiro[5,5] undecane or DVTOSU (200g, 1.0mole) in the presence of Ethylene diamine (600ml) and potassium tertiary butoxide (317g, 3.0mole) by refluxing the temperature at 100±5°C for 24hrs. converts to 3, 9-

Diethylidine - 2, 4, 8, 10-tetraoxaspiro [5, 5] undecane or DETOSU. After that reaction mass cool to room temperature and quenched in ice-cold water and extracted with n-hexane (3 liters) and washed with water twice (2 x 1liters). Organic layer dried over anhydrous Na_2SO_4 and anhydrous $MgSO_4$ and distilled-off under vacuum completely to get crude oily mass (187.3g). Obtained crude oily mass purified using n-Hexane (800ml) and TEA (4ml) at -25±5°C twice. Finally collect the pure compound by HVD to get 3, 9-Diethylidine-2, 4, 8, 10-tetraoxaspiro [5, 5] undecane or DETOSU (135g) with purity 97.90% and monomer impurity-1.45%.

Stage-II: Preparation of Triethylene glycol poly (glycolide) (TGP-II)
Triethylene glycol (31g) condensed with glycolide (25g) for 25-30hrs the temperature at 175±5°C to get Triethylene glycol poly (glycolide) (53.2g).
Triethylene glycol (10g) condensed with glycolide (8.0g) in THF in the presence of addzyme-015 (1.0g, 10%) (Note: Addzyme-015 (1g) stir for 20 minutes at ~ 50°C in 2-Methyl THF (10ml). Filter and wash with 2-Methyl THF (10ml) and degas for 30 minutes at ~50°C under vacuum) for 60 hrs at 25-50°C. Filter the addzyme-015 and wash with THF (20ml). Finally distilled-off solvent under vacuum completely followed by degassing for 2 hrs at 50±5°C to get Triethylene glycol poly (glycolide) (17.6g).

Stage-III: Preparation of Triethylene Glycol Polyorthoester (TGP-III)
In a flask charge DETOSU or TGP stage-I (15g) and Triethylene glycol (8.4g) and THF (20ml). Stir for clear solution. Add Triethylene glycol poly (glycolide) or TGP stage-II dissolved in THF (10ml) within 0-5 minutes. (Note: During addition THF will condense). Then allow reaction mass itself to cool to RT and stir at RT for 2 hrs. Finally filter through 0.22 or 0.45µ and distill-off THF under vacuum to get Triethylene Glycol Polyorthoester or TGP-III (27.3g) with GPC molecular weight ~5000-7000 Daltons and poly dispersity index-1.5-2.0.

Heat of Reaction calculation
Bond Energy values

Bond	D(KJ/mole)	Bond	D(KJ/mole)	Bond	D(KJ/mole)
H-H	432	C-C	346	Si-Si	222
H-B	389	C=C	602	Si-N	355
H-C	411	C≡C	835	Si-O	452
H-Si	318	C-Si	318	Si-S	293
H-Ge	288	C-Ge	238	Si-F	565
H-Sn	251	C-Sn	192	Si-Cl	381
H-N	386	C-Pb	130	Si-Br	310
H-P	322	C-N	305	Si-I	234
H-As	247	C=N	615	Ge-Ge	188
H-O	459	C≡N	887	Ge-N	257
H-S	363	C-P	264	Ge-F	470
H-Se	276	C-O	358	Ge-Cl	349
H-Te	238	C=O	799	Ge-Br	276
H-F	565	C≡O	1072	Ge-I	212
H-Cl	428	C-B	356	Sn-F	414
H-Br	362	C-S	272	Sn-Cl	323
H-I	295	C=S	573	Sn-Br	273
		C-F	485	Sn-I	205
		C-Cl	327	Pb-F	331
		C-Br	285	Pb-Cl	243
		C-I	213	Pb-Br	201
				Pb-I	142

Bond	D(KJ/mol)	Bond	D(KJ/mol)
B-B	293	F-F	155
B-O	536	Cl-Cl	240
B-F	613	Br-Br	190
B-Cl	456	I-I	148
B-Br	377	At-At	116
		I-O	201
		I-F	273
		I-Cl	208
		I-Br	175

Bond Energy values

Bond	D(KJ/mole)	Bond	D(KJ/mole)	Bond	D(KJ/mole)
O-O	142	N-N	167	Kr-F (KrF$_2$)	50
O=O	494	N=N	418	Xe-O	84
O-F	190	N≡N	942	Xe-F	130"
S=O	522	N-O	201		
S-S (S$_8$)	226	N=O	607		
S=S	425	N-F	283		
S-F	284	N-Cl	313		
S-Cl	255	P-P	201		
Se-Se	172	P-O	335		
Se=Se	272	P=O	544		
		P=S	335		
		P-F	490		
		P-Cl	326		
		P-Br	264		
		P-I	184		
		As-As	146		
		As-O	301		
		As-F	484		
		As-Cl	322		
		As-Br	458		
		As-I	200		
		Sb-Sb	121		
		Sb-F	440		
		Sb-Cl (SbCl$_5$)	248		
		Sb-Cl (SbCl$_3$)	315		

HEAT OF REACTION CALCULATION

$\Delta H°rxn = \Sigma \ \Delta H°f \ (products) \ minus \ \Sigma \ \Delta H°f \ (reactants)$

	Example				
Glycolide					
	C=O	2	799	1598	KJ/mole
	C-C	2	346	692	KJ/mole
	C-H	4	411	1644	KJ/mole
	C-O	2	358	716	KJ/mole
				4650	KJ/mole
Triethylene glycol	"C-H	12	411	4932	KJ/mole
	O-H	2	459	918	KJ/mole
	C-O	2	358	716	KJ/mole
	C-C	3	346	1038	KJ/mole
				7402	KJ/mole"
	$\Sigma \ \Delta H°f \ (reactants)$			12254	KJ/mole
Triethylene glycol poly (glycolide)	C-H	16	411	6576	KJ/mole
	C-C	5	346	1730	KJ/mole
	C-O	7	358	2506	KJ/mole
	C=O	2	799	1598	KJ/mole
	O-H	2	459	918	KJ/mole
	$\Sigma \ \Delta H°f \ (products)$			13328	KJ/mole
	$\Delta H°rxn \ of \ stage \ (TGP-II)$			1074	KJ/mole
	No of Moles			0.8615	moles
	$\Delta H°rxn \ of \ stage \ (TGP-II)$			925.25	KJ
	KSM Weight			100	Gm
	Mol. Weight of KSM			116.07	
	Non exothermic				

Note: Heat of reaction is + ve, the reaction is endothermic in nature and energy is required for completion of reaction.
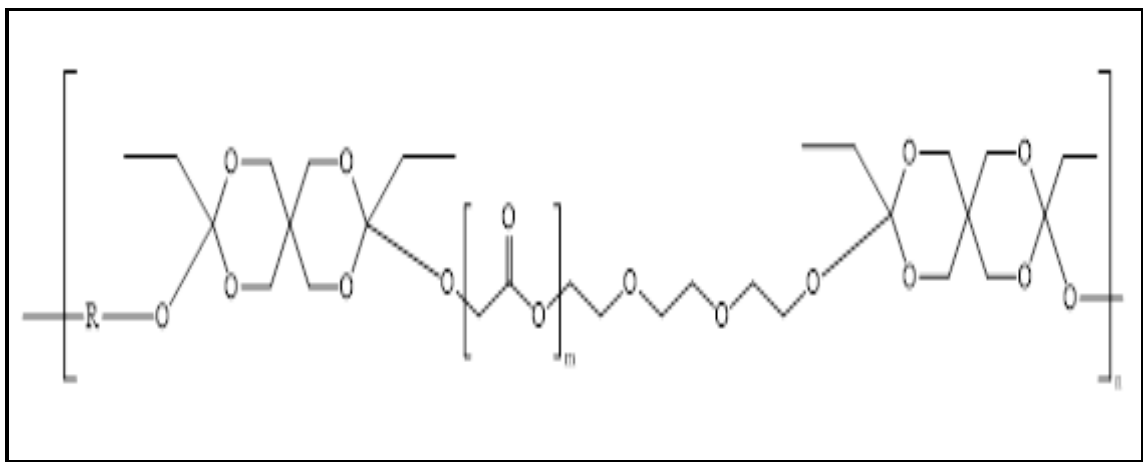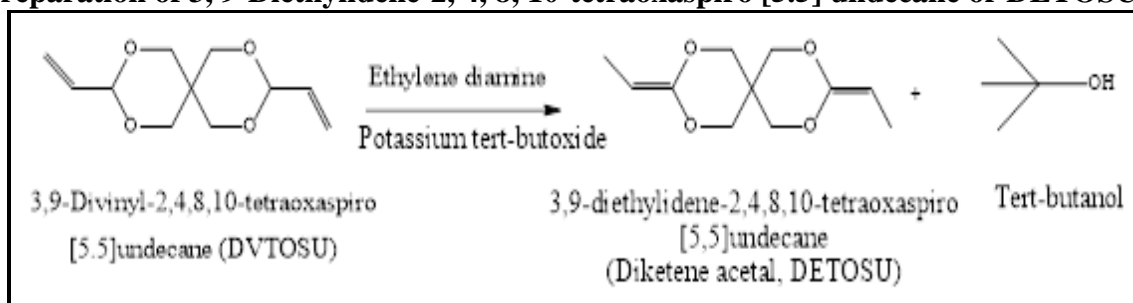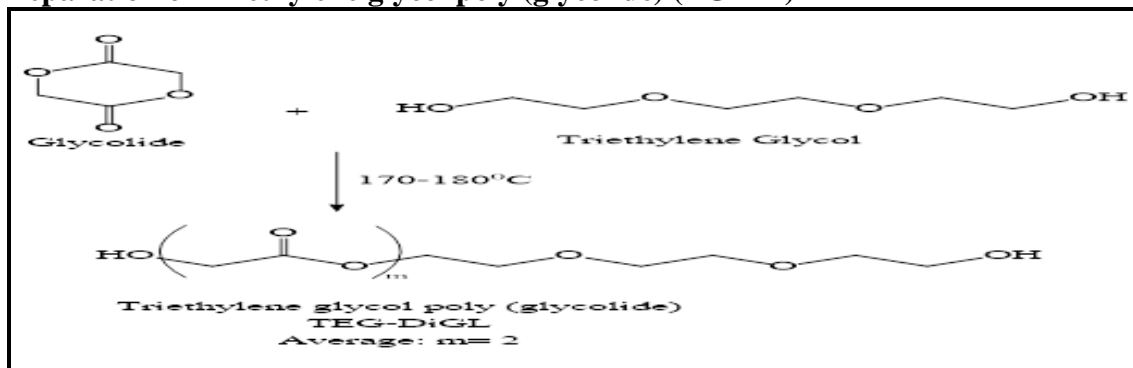

Formula I
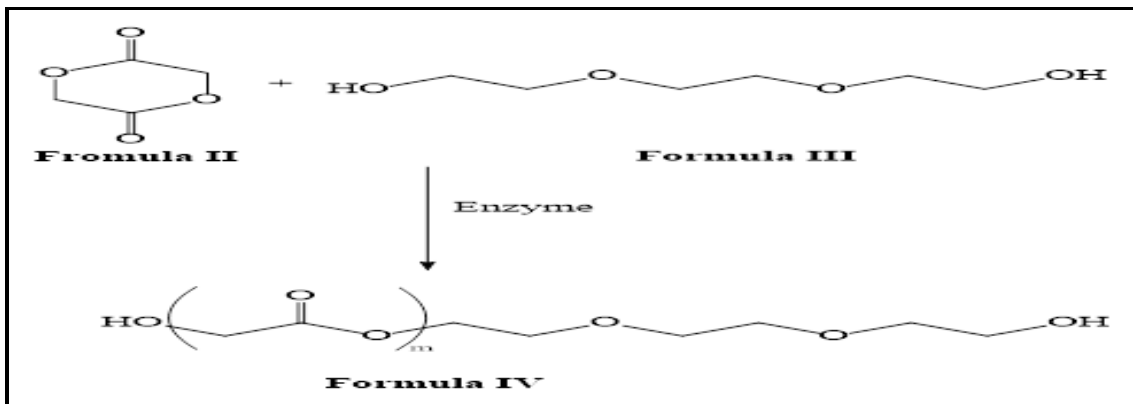
Isolating the Triethylene glycol poly (glycolide) formula IV Wherein m is 2
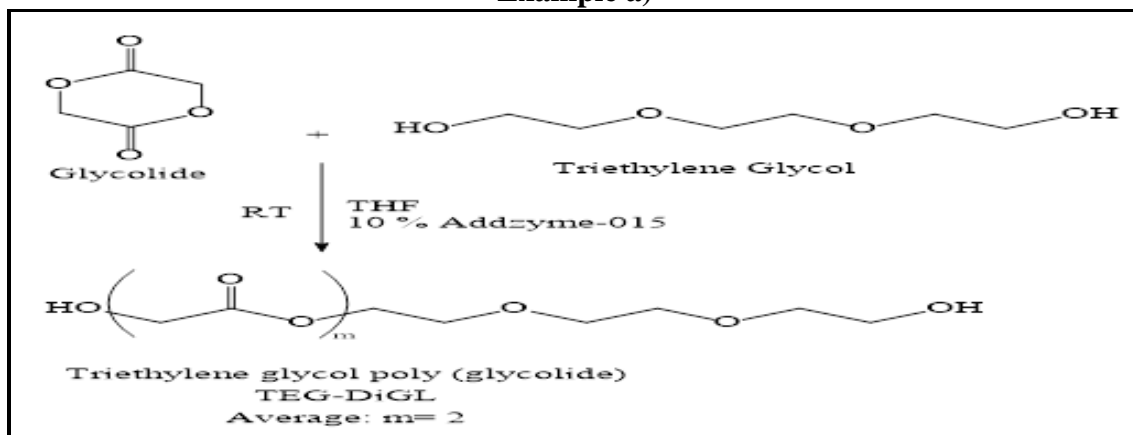**Stage-I: Preparation of 3, 9-Diethylidene-2, 4, 8, 10-tetraoxaspiro [5.5] undecane or DETOSU (TGP-I)**

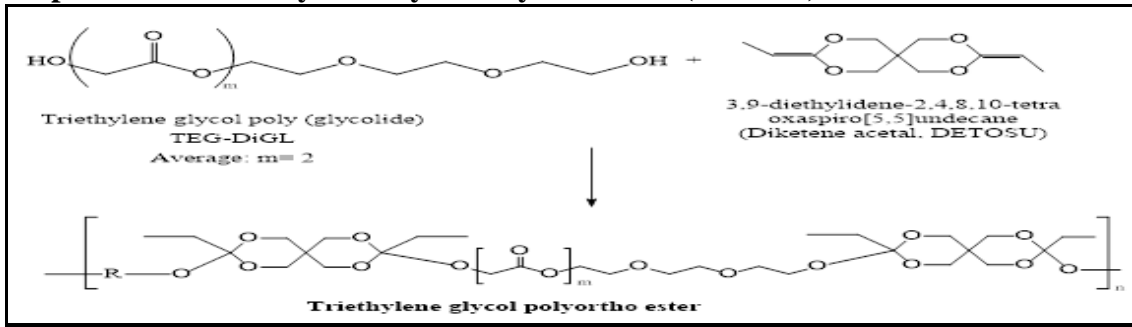**Stage-II: Preparation of Triethylene glycol poly (glycolide) (TGP-II)**
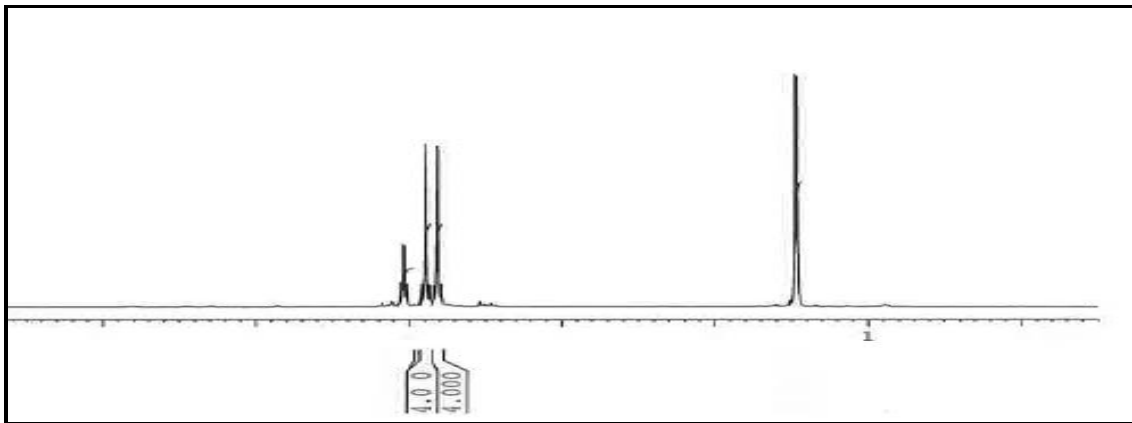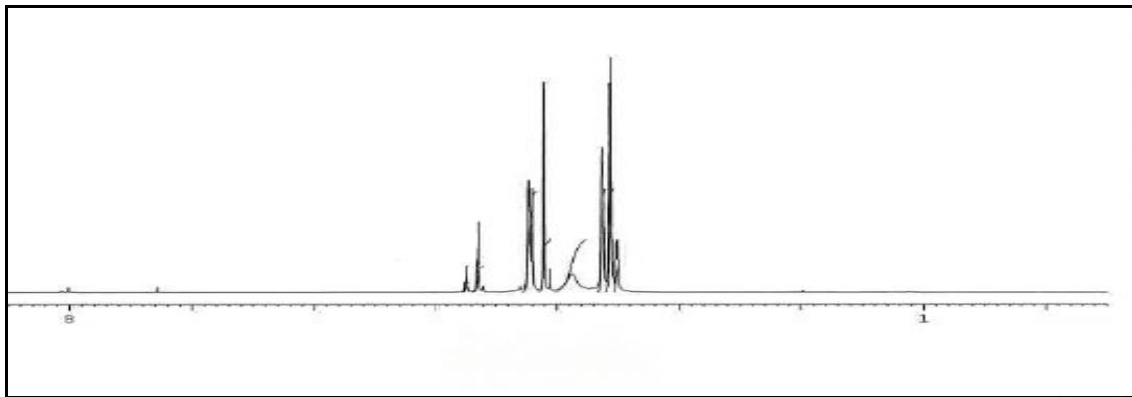

Example a)

Example b)

Stage-III: Preparation of Triethylene Glycol Polyorthoester (TGP-III)
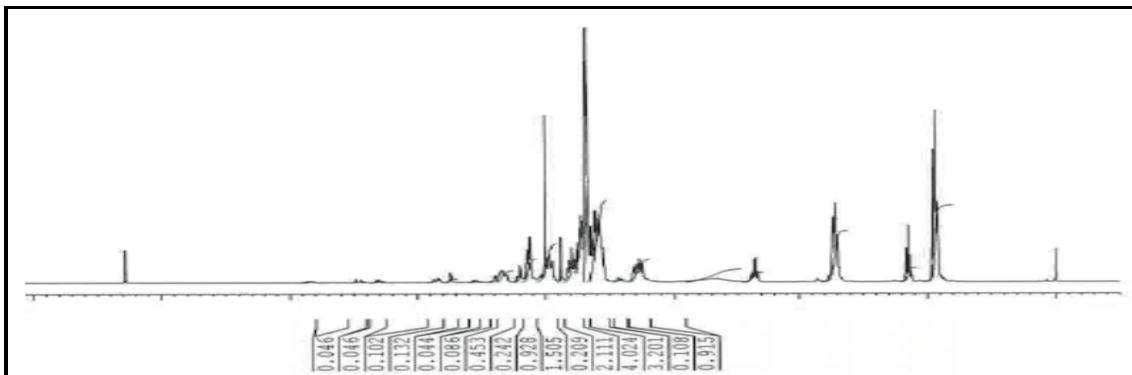

RESULTS AND DISCUSSION

NMR of TGP-I

NMR of TGP-II

NMR of TGP_III

CONCLUSION

By using bio catalysis technique and Lipase enzyme we have prepared the Tri (ethylene glycol) poly(orthoester) in environmentally friendly conditions. NMR of all intermediates and Tri (ethylene glycol) poly(orthoester) are confirming the structures. Enthalpy calculations suggests that reaction was suitable for scale up for commercial activities.

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CONFLICT OF INTEREST

None.

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