How to Cite:
Verma, V. S., Jain, H. K., Badwaik, H. K. R., Alexander, A., & Ajazuddin. (2022). Statistical optimization of oxidative derivatization of polyethylene glycol to polyethylene carboxylate using custom design approach. *International Journal of Health Sciences*, *6*(S3), 7086–7097. https://doi.org/10.53730/ijhs.v6nS3.7659

Statistical optimization of oxidative derivatization of polyethylene glycol to polyethylene carboxylate using custom design approach

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***Abstract*---**Reaction optimization has been a tedious task for the research chemist for very long and many approaches has been employed to achieve the best reaction condition resulting in highest yield. As an answer to this problem the statistical optimization approach has better chances of providing most acceptable solution to it. Through, our present work we have found that among other statistical approaches the custom design approach is the best

methodology to be adopted for optimizing any synthetic reaction. The oxidative transformation of polyethylene glycol into polyethylene carboxylate by the use of TEMPO, NaClO and KBr; has been optimized by identifying the key factors affecting the yield and exact ratios of them to achieve highest possible yield. Here, we have employed custom design approach to further limit the experimental runs and yet find a better possible combination of reagents and conditions to get highest yield with maximum purity. This method has reduced the number of runs to only 16, which was 1024 in case of the traditional OVAT approach and in full factorial approach it was 64 runs. Thus, custom design method of DoE has been satisfactorily utilized for optimizing the oxidative derivatization of polyethylene glycol to polyethylene carboxylate.

***Keywords*---**Polyethylene glycol carboxylates, oxidation reaction, Statistical optimization, Synthesis, Polymer derivatization.

Introduction

Synthesis of molecules plays important role in the field of pharmaceutical chemistry research. Development of novel method for synthesis has been the core area of research and the acceptability of any process depends on the yield obtained. For enhancing the yield in any synthetic process, the optimisation of that process is very essential. In traditional approach practically alterations of all the parameters having role/influence in obtaining the yield is monitored simultaneously, thus leading to finalization of optimized condition of synthesis. This approach requires a huge amount of inventory, capital and long period of study, thereby increasing the cost of production and manpower (Ibrahim et. al., 2020; Uy & Telford, 2009).

Design of Experiment (DOE)

The 'Design of Experiments' technique (Figure 1) is a statistical technique for reaction optimization that enables the simultaneous adjustment of several aspects in order to screen 'reaction space' for a certain procedure. Notably, this allows a large array of reaction conditions to be evaluated in a limited number of tests. While process chemists in a variety of companies and engineering academics utilize this approach often, it is rarely employed in academic chemistry. This seems to be regardless of the fact that optimizing specific reactions takes a long time in every research endeavour aimed at developing novel synthetic methods. One compelling cause for this is a considerable 'energy barrier' in academics due to the absence of experience mostly in application of this technology. Through use of DoE in reaction optimization in work that has been carried out in partnership with industry partners is a particularly common anomaly (Churro et. al., 2021; Jones & Sall, 2011; Zmirli et. al., 2022).

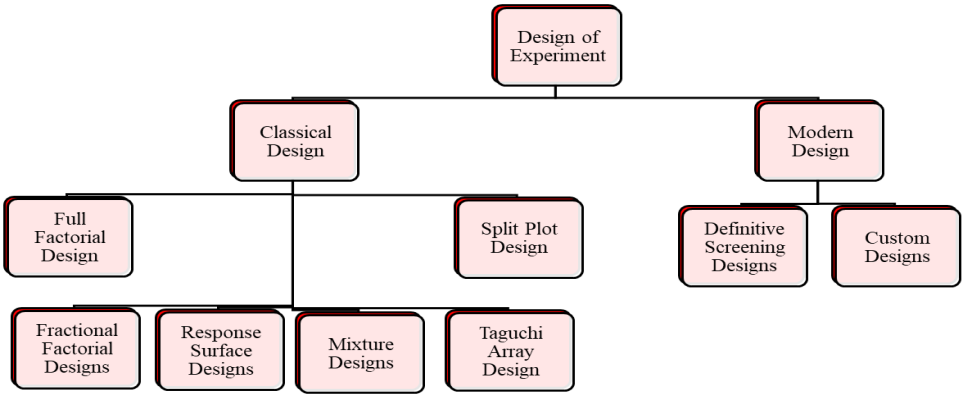


Figure 1. Flow diagram for several forms of Design of Experiment (DoE).

Statistical Optimization

Statistical Techniques is used by DOE to make the model of the experiment and utilizes each of the variables and also consider various interactions between the variables themselves and studies about the possible effect on the yield of the product. The design of experiment (DOE) cross verifies each individual screening performed in the DOE and is the integral feature of the DoE optimization software (Murray et. al., 2016; Uy & Telford, 2009).

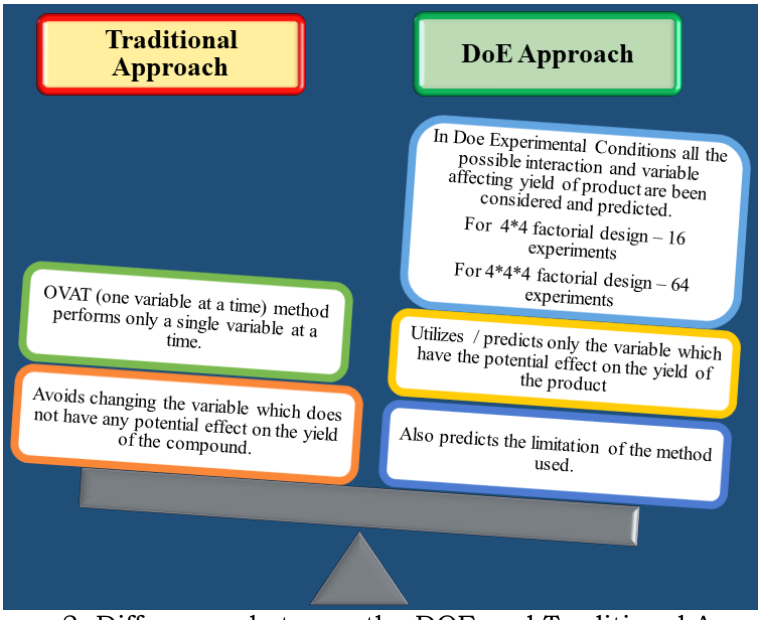


Figure 2. Differences between the DOE and Traditional Approaches

According to a review in *Pharmaceutical Statistics*, JMP is often used as a graphical front-end for a SAS system, which performs the statistical analysis and tabulations. It has much benefits in comparison to traditional approaches for

optimisation of any process (Figure 2). JMP Genomics, used for analysing and visualizing genomics data, requires a SAS component to operate and can access SAS/Genetics and SAS/STAT procedures or invoke SAS macros. JMP Clinical, used for analysing clinical trial data, can package SAS code within the JSL scripting language and convert SAS code to JMP.JMP software is partly focused on exploratory data analysis and visualization. It is designed for users to investigate data to learn something unexpected, as opposed to confirming a hypothesis. JMP links statistical data to graphics representing them, so users can drill down or up to explore the data and various visual representations of it. Its primary applications are for designed experiments and analysing statistical data from industrial processes (Araki et. al., 2005; Jones & Sall, 2011; Uy & Telford, 2009). Here in this work, we are proposing the use of custom design approach of a DOE (design of Experiment) using JMP statistical software for optimising the reaction condition for synthesis of polyethylene carboxylate from polyethylene glycol via oxidation reaction catalysed by TEMPO, Sodium hypochlorite and potassium bromide (Li et. al., 2005).

Materials and Methods

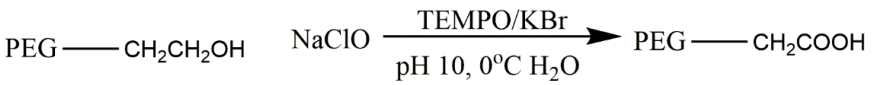
Materials

TEMPO (2,2,6,6-teramethyl–1-piperidineoxyl), Sodium Hypochlorite (NaClO), Potassium Bromide (KBr), Polyethylene Glycol (PEG) (1500), Hydrochloric Acid (HCl), Sodium Hydroxide (NaOH), Ethanol (CH_3CH_2OH) and Dichloro Methane (DCM) (CH_2Cl_2). All the chemicals were of analytical grade and were obtained from Sigma Aldrich (USA) and all the solvents were of laboratory grade reagent obtained from Loba chemie (Mumbai, India).

Synthesis of PEG carboxylate

Synthesis of polyethylene carboxylate from polyethylene glycol followed reaction and its mechanism (Li et. al., 2005) as shown in figure 1.

REACTION:



MECHANISM:

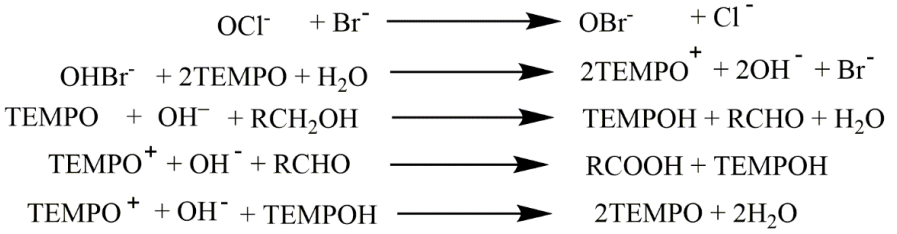


Figure 3. Principal reaction and mechanism of reaction, involved in oxidation of PEG-to-PEG carboxylates

TEMPO (1-5 mmol×10^{-3}), PEG (1500) (7.5 gm), and KBr (0.1-0.3 mmol) were dissolved in water (70 ml). By adding aqueous 4M HCl to sodium hypochlorite solution (strength of solution is 8%, where 1-4 mmol of NaClO was dissolved in 1 mmol of ethanol), the pH was set to 10. Both solutions were blended at the same time after being chilled to the necessary temperature (0^{o} C) in an ice bath. By adding 0.5M NaOH and using a pH-stat, the temperature was kept constant during the process, and the pH was kept at pH 10. Excess hypochlorite was quenched with ethanol (5 ml) and the pH was set to 3 with 4 M HCl after the reaction was continued for 5 hours.

Reaction Optimization

In the traditional OVAT (one variable at a time) approach for the drug discovery. Firstly, the reaction scheme is developed and in the preceding step of the discovery the various concentration levels of the variables are identified and the best condition is then obtained using various possible concentration and then the optimum condition giving the best yield is the reproduced and repeated at least 3 times for the ascertaining the yield.

By using the DoE approach

Utilizing DoE experimental conditions help us identify and use of the variables that have potential effect on the reaction condition and also identifies the possible interactions between the variables itself. In the custom design approach for the model making, we use only the variable that could have potential effect on the reaction condition and then optimized the various conditions of the experiment to give the condition which yield the greatest amount of the product and with highest purity (Murray et. al., 2016; Li et. al., 2005).

Spectral Analysis and Characterization

FT IR analysis (4000-450 1/cm) of all the molecules was done by Agilent Cary 630 FTIR (USA). Proton (^{1}H) and Carbon (^{13}C) NMR spectral analyses were performed through Bruker AvIII HD-300 [FT NMR] (USA). MALDI-TOFMS analysis was done using AB Sciex 4800 plus MALDI TOF-TOF Analyzer (USA).

Results & Discussion

Reaction optimization

In academics there is trial and error approach for the synthesis and yielding the best optimized condition considering about all the variables in the reaction scheme but while using the DOE approach the variable which actually affects the reaction scheme are considered and the concentration which yields the maximum product. The different variables in the optimization of the reaction scheme for the synthesis of the PEG-Carboxylates are TEMPO, NaClO, KBr, Temperature, pH. As per the OVAT method there will be large number of experimental runs, which would be time consuming as well as great loss of chemicals would also be there. Now, at this situation the DOE optimization software comes in handy and leading

us to use the JMP Statistical software for the reaction condition optimization to attain the maximum product yield.

Reaction optimization using DOE

If there are 5 variables with 4 levels each then there are 1024 possible experimental conditions (4*4*4*4*4 factorial design) and thus if we perform 1024 experiments then it would be a tedious scheme to perform and there is great loss of the chemicals and the manpower involved for the reaction scheme. And after that we get the best condition and then we check the repeatability and reproducibility of the best condition that is predicted from the experiment (Murray et. al., 2016; Li et. al., 2005). In this condition the temperature and pH had been kept at pH 10 and temperature 0° C by performing the experiment in the laboratory. These conditions had been kept constant in our experimental design as by performing this experiment we saw that they don't have much effect on the desired result. While using the JMP software by employing full factorial design, there are in all 64 runs (Table 1) obtained by constraining the variables to 3 in numbers (namely, TEMPO, NaClO, and KBr). These factors have been obtained and selected on the basis of their significance after analysis as per the Table 2.

Table 1
Possible number of combinations of various factors for experimental runs in Full Factorial approach

Sr. No.	Pattern	TEMPO	NaClO	KBr		Sr. No.	Pattern	TEMPO	NaClO	KBr
1	111	0	1	0		17	211	1	1	0
2	112	0	1	0.1		18	212	1	1	0.1
3	113	0	1	0.2		19	213	1	1	0.2
4	114	0	1	0.3		20	214	1	1	0.3
5	121	0	2	0		21	221	1	2	0
6	122	0	2	0.1		22	222	1	2	0.1
7	123	0	2	0.2		23	223	1	2	0.2
8	124	0	2	0.3		24	224	1	2	0.3
9	131	0	3	0		25	231	1	3	0
10	132	0	3	0.1		26	232	1	3	0.1
11	133	0	3	0.2		27	233	1	3	0.2
12	134	0	3	0.3		28	234	1	3	0.3
13	141	0	4	0		29	241	1	4	0
14	142	0	4	0.1		30	242	1	4	0.1
15	143	0	4	0.2		31	243	1	4	0.2
16	144	0	4	0.3		32	244	1	4	0.3
Sr. No.	Pattern	TEMPO	NaClO	KBr		Sr. No.	Pattern	TEMPO	NaClO	KBr
33	311	2	1	0		49	411	5	1	0
34	312	2	1	0.1		50	412	5	1	0.1
35	313	2	1	0.2		51	413	5	1	0.2
36	314	2	1	0.3		52	414	5	1	0.3

37	321	2	2	0		53	421	5	2	0
38	322	2	2	0.1		54	422	5	2	0.1
39	323	2	2	0.2		55	423	5	2	0.2
40	324	2	2	0.3		56	424	5	2	0.3
41	331	2	3	0		57	431	5	3	0
42	332	2	3	0.1		58	432	5	3	0.1
43	333	2	3	0.2		59	433	5	3	0.2
44	334	2	3	0.3		60	434	5	3	0.3
45	341	2	4	0		61	441	5	4	0
46	342	2	4	0.1		62	442	5	4	0.1
47	343	2	4	0.2		63	443	5	4	0.2
48	344	2	4	0.3		64	444	5	4	0.3

Table 2
Full Factorial designing parameters estimated 4*4*4 factors

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	41.96625	4.04706	10.37	<.0001*
TEMPO[5]	19.91875	7.009714	2.84	0.0295*
TEMPO[1.25]	-32.98625	7.009714	-4.71	0.0033*
TEMPO[2.5]	5.60875	7.009714	0.80	0.4541
NaClO[1]	-26.73125	7.009714	-3.81	0.0088*
NaClO[2]	-4.92375	7.009714	-0.70	0.5087
NaClO[3]	22.92625	7.009714	3.27	0.0170*
KBr[0]	4.48125	7.009714	0.64	0.5463
KBr[0.1]	-20.29875	7.009714	-2.90	0.0275*
KBr[0.2]	-4.84625	7.009714	-0.69	0.5152
KBr[0.3]	-22.29875	7.009714	-3.18	0.0295*

Custom design Analysis

Table 3
Different conditions for the oxidation of PEG 1500 have an effect on the Carboxylated PEG yield

Entry	TEMPO/mmol x 10^{-3}	NaClO/mmol	KBr/mmol	YIELD
1	1.25	1	0.3	3.65
2	1.25	2	0	0.8
3	1.25	3	0.1	26.67

4	1.25	4	0.2	4.8
5	2.5	1	0	20.17
6	2.5	2	0.3	75.47
7	2.5	3	0.2	72.98
8	2.5	4	0.1	21.68
9	3.75	1	0.2	16.9
10	3.75	2	0.1	18.1
11	3.75	3	0.3	78.9
12	3.75	4	0	83.8
13	5	1	0.1	20.22
14	5	2	0.2	53.8
15	5	3	0	81.02
16	5	4	0.3	92.5

Custom design distribution graph

According to the custom design – distribution approach graphs the maximum yield derived during the experimental synthesis of the PEG – Carboxylates is 92.5% and the best conditions which provide this yield is TEMPO = 5: NaClO = 4: KBr = 0.3 (5:4:0.3) (Table 3). While using the DOE approach for the optimization of the reaction conditions the conditions best optimized statistically is TEMPO = 5, NaClO = 3, and KBr = 0.3 and when we performed experiment using these conditions then obtained yield is 98.78% (Table 4).

Table 4
Comparison of different conditions for the oxidation of PEG to Carboxylated PEG as per respective sources

TEMPO/mmol $\times 10^{-3}$	NaClO/mmol	KBr/mmol	Temp./˚C	pH	Yield (%)	Sources
2	4	0.1	0	11	92.6	(8)
2.5	4	0.1	0	10	21.68	8 (Table 3)
5	3	0.3	0	10	105.475	DoE optimized
5	3	0.3	0	10	98.78	Practically

Thus, we can say that there has been a significant increase in the yield when the concentration of the TEMPO = 5: NaClO = 4: KBr = 0.3 (5:4:0.3) according to the statistical approach design and when that is practically performed approximately similar result was obtained i.e 98.78%.

Graphical analysis of factor effects

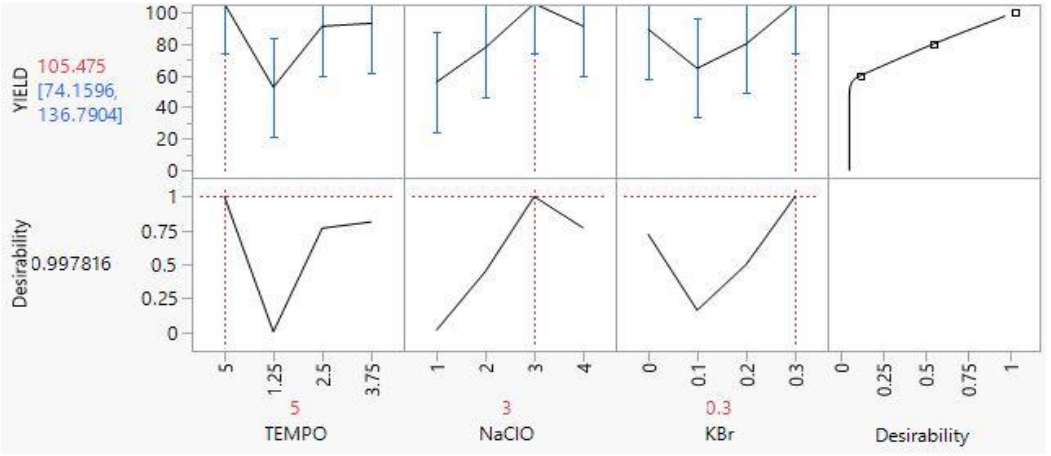


Figure 4. PEG oxidation yield profiles with expected values of factors

The profiles for the estimated response impact of the factors on the "Yield of oxidation of PEG" are shown in the figure above (Figure 4). The factors impacting PEG Output oxidation include TEMPO, NaClO, and KBr, while pH and temperature were maintained fixed because they have a minor effect on the yield of Oxidized PEG Carboxylates. Whenever the amount of a few of the factors (variable) rises from a low to a high quantity as the reaction advances, the parameter seems to have a beneficial impact on the reaction. As can be shown in fig., TEMPO, NaClO, and KBr all have a favourable impact on the reaction.

Estimation of the coefficients and statistical analysis of the effects of the variables under consideration

Table 5 & 6 summarises the model's parameter values, their relevance, and their influence on the model's performance. As indicated in the table, the p-value and F-value are associated to each coefficient.

Table of parameter values for effect testing

The F-values (Table 5) have been used to determine the validity of the correlations within each variable at a meaningful level greater than or equal to 95 percent, and indeed the p-values are established as the minimal significance threshold, culminating in the rejection of the null hypothesis Ho (Ho: the factor is not influential). The outcome (effect parameters in Table 6) reveals that indeed the different factors like TEMPO, NaClO, and KBr, have a substantial impact on the reaction (yield). As a result, the variables that were recognised one of most influence on the reaction.

Table 5
Effect tests data

SOURCE	Nparm	DF	Sum of Squares	F Ratio	Prob > F
TEMPO	3	3	6287.7613	7.9979	0.0161*
NaClO	3	3	5362.4282	6.8209	0.0232*
KBr	3	3	3530.3902	4.4906	0.0561

Table 6:
Estimates of parameters

Term	Estimate	Std error	t ratio	Prob>[t]
Intercept	41.96625	4.04706	10.37	<.0001*
TEMPO [5]	19.91875	7.009714	2.84	0.0295*
TEMPO [1.25]	-32.98625	7.009714	-4.71	0.0033*
TEMPO [2.5]	5.60875	7.009714	0.80	0.4541
NaClO [1]	-26.73125	7.009714	-3.81	0.0088*
NaClO [2]	-4.92375	7.009714	-0.70	0.5087
NaClO [3]	22.92625	7.009714	3.27	0.0170*
KBr [0]	4.48125	7.009714	0.64	0.5463
KBr [0.1]	-20.29875	7.009714	-2.90	0.0275*
KBr [0.2]	-4.84625	7.009714	-0.69	0.5152
KBr [0.3]	-22.29875	7.009714	-3.18	0.0295*

The best optimized condition with maximum yield, reproducibility and repeatability was found to be TEMPO: NaClO: KBr (5: 3: 0.3) molar ratio with yield of 105.475% and this condition was performed in the laboratory to verify the optimized conditions then it is found that the obtained yield in the laboratory was found to be 98.78% (Table 4).

Spectral Analysis

Structural transformation of polyethylene glycol 1500 to Polyethylene carboxylate was obtained from spectral analysis (viz. FTIR, Proton NMR, C13 NMR and Mass Spectra) of the obtained product after synthesis. FTIR spectra clearly showed the change in peaks of -OH (alcoholic) at 3411.19 to peak of -OH (carboxylic) at 3433.99; along with the peaks of ether and ethylene bond at respective ranges as shown in table 7. Retention of peaks in proton NMR and Carbon NMR; for ether and ethylene hydrogen and carbon respectively confirms the retention of structure of Polyethylene glycol's polymeric backbone. Mass spectra also confirms the change in molecular mass, which further confirms the statement; that, structural morphology of parent chain was not disturbed during this chemical transformation of functional group from hydroxyl group at the terminal to carboxylic group. All the spectral observations have been tabulated in Table 7.

Table 7
Spectral data for PEG and PEG carboxylate derivatives

Spectral Analysis	Molecule Name	Peaks Details (in cm^{-1})
FTIR	*PEG 1500*	3411.19(-OH), 1098.21(-C-O-C-), 2879.64(-CH_2-CH_2-)
	PEC 1500	3433.99(-OH of -COOH), 1732.91(>C=O of –COOH), 1100.91(-C-O-C-), 2908.64(-CH_2-CH_2-)
H^1 NMR	*PEG 1500*	3.6254-3.7231(-C-O-C-), 1.2011-1.3274 (-CH_2-CH_2-)
	PEC 1500	3.4973-3.6011(-C-O-C-), 1.0967-1.2451 (-CH_2-CH_2-)
C^{13} NMR	*PEG 1500*	18.7993-31.7825(-CH_2-CH_2-), 62.8745- 78.6987(-C-O-C-)
	PEC 1500	23.6478-30.3214(-CH_2-CH_2-) 60.2587-78.6532(-C-O-C-)
Mass	*PEG 1500 ($C_{68}H_{138}O_{35}$)*	1513.77(Base Peak), 1517.91 (Mass Peak).
	PEC 1500 ($C_{68}H_{134}O_{37}$)	1541.78 (Base Peak), 1545.59 (Mass Peak).

Conclusion

This work has satisfactorily justified the use of custom design method for the optimisation of derivatisation of polyethylene glycol. This approach also led us to identify the parameters having significant effect in the desired reaction processes, which help us in limiting the test runs. The custom design has led us to the best possible reaction condition to yield polyethylene glycol carboxylate derivatives with maximum yield and of highest purity. This method has reduced the number of runs to only 16, which was 1024 in case of the traditional OVAT (one variable at a time) approach and in full factorial approach it was 64 runs. Thus, custom design method of DoE has been satisfactorily utilized for optimizing the oxidative derivatization of polyethylene glycol to polyethylene carboxylate.

The custom design method can be used to optimise various other reactions employed in the research and production in industries. This will successfully minimize the use of inventory and thereby will be quite effective in cost cutting in the process. Thus, this could be beneficial for both the manufacturers and consumers. Because the cost cutting will lead to lessened cost of production resulting in more profit margin for the manufacturers, which will lead them to make product available in low price to the consumers. The use of similar approach has been prevailing in the industries, but in academics such application is limited due to lack of information. Thus, this work may prevail a leading path for the researchers to apply this knowledge in effectively optimising their reaction in their respective research areas.

Acknowledgment

The authors are thankful to Perpetual Association of Professional Innovations (PAPI) for providing facilities for carrying out the research work.

Conflict of Interest

The authors declare no conflict of interest.

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