www.sphinxsai.com

ChemTech

International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN : 0974-4290
Vol.6, No.4, pp 2469-2474, July-Aug 2014

# Application of Artificial Neural Network for Multivariate Optimization in the Lipase-Catalyzed Esterification Reaction of BetulinicAcid with Phthalic Anhydride

**Mansour Ghaffari-Moghaddam[1], Faujan Bin H. Ahmad[2,*] and Nur Hana BintiFaujan[3]**

**[1]Department of Chemistry, Faculty of Science, University of Zabol, Zabol, Iran.**
**[2]Natural Product Laboratory, Faculty of Applied Sciences, UniversitiTeknologi Mara, 40450 Shah Alam, Malaysia.**
**[3]Department of Chemistry, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.**

***Corres.author: faujan@salam.uitm.edu.my**

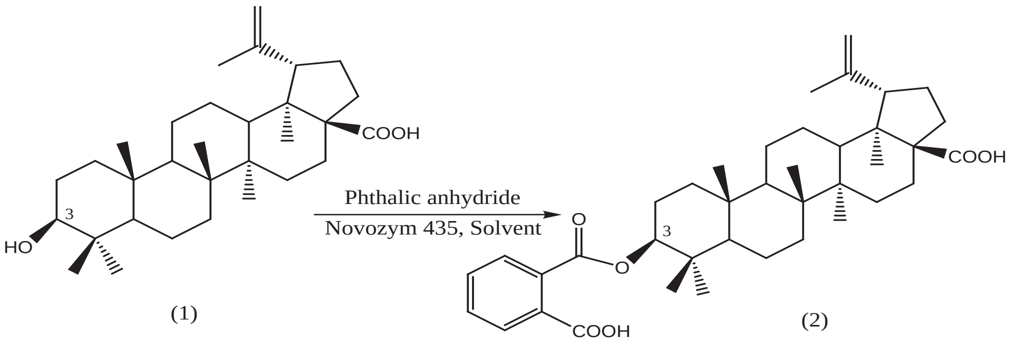
**Abstract:** In this study, an artificial neural network (ANN) trained with backpropagation algorithm based on the quick propagation (QP) algorithm was applied to optimize the reaction conditions in the enzymatic synthesis of betulinic acid ester. The inputparameters of the model were reaction time, reactiontemperature, enzyme amount and substrate molar ratiowhile the percentage isolated yield of ester was the output. Using the ANN analysis, the optimum conditions to obtain the highest yield were 148.3 mg enzyme, reaction temperature of 53.1°C, reaction time of 20.3 hours and betulinic acid to phthalic anhydride molar ratio of 1:1.24. The predicted and actual yields were 64.9 and 64.3%, respectively.
**Key Words:** Artificial neural network, Optimization, Esterification, Lipase, Betulinic acid.

## Introduction

Betulinic acid (1) is a natural product which can be isolated from the outer bark of various Betula species[1,2]. It shows several pharmacological activities including inhibition of human immunodeficiency virus (HIV), antibacterial, antimalarial, antiinflammatory, anthelmintic, antioxidant and anticancer properties[3]. This compound is regarded by the scientific community as an accessible and valuable bioactive natural product[4]. The introduction of the polar groups, such as phthalates at C-3 position of betulinic acid, is an interesting way to increase the hydrosolubility and anticancer activity of betulinic acid[5].

Lipases usually catalyze hydrolytic reactions. However, when employed in organic solvents (low water environment), they can perform the reverse reaction, namely, esterification just as efficiently[6]. Such biocatalysts present many advantages over chemical catalysts: their specificity, regioselectivity and enantioselectivity allow them to catalyze reactions under mild conditions of temperature and pressure, with lower side products and waste treatments costs[7].

Artificial neural network (ANN) is a highly simplified model of the structure of a biological network[8]. The fundamental processing element of artificial neural network is an artificial neuron (or simply a neuron). A biological neuron receives inputs from other sources, combines them, generally performs a non-linear operation on the result and then outputs the final result[9]. The ability of the artificial neural networks, to recognize and reproduce the cause-effect relationships through training for the multiple input-output systems makes them efficient to represent even the most complex systems[10].Employing neural network models would lead to savingtime and cost by predicting the results of the reactions so that the most promising conditions can then be verified[11]. Recently, ANN has been shown to be a powerful tool for the optimization of multivariate parameters in a great variety of areas, such as in enzymatic synthesis[8,11-15], fermentation processes[10,16,17]and in pharmaceutical studies[18-20]. The main purpose of this study is to optimize the reaction parameters of lipase-catalyzed esterification of betulinic acid (**Fig. 1)** for obtaining the highest yield of the isolated ester using artificial neural network.




(1) Betulinic acid

(2) 3-*O*-phthalyl- betulinic acid

**Fig.1:** Enzymatic Reaction of betulinic acid with phthalic anhydride using Novozym 435 as a biocatalyst

## Material and Methods

### Materials

Immobilized enzyme (triacylglycerol hydrolase, EC 3.1.1.3; Novozym 435, 10000 PLU/g), *Candida antarctica*lipase, supported on a macroporous acrylic resin with a water content of 3% (w/w), was purchased from Novo Nordisk A/S (Bagsvaerd, Denmark). Chloroform and *n*-hexane (Fisher Chemical, Loughborough, UK) were used as the organic solvents. Betulinic acid was isolated from the Malaysian *Callistemon speciosus* according to the procedure described by Ahmad et al[21]. Phthalic anhydride was purchased from Acros Organics (Geel, Belgium). Ethyl acetate, Celite®545, $Na_2SO_4$, $K_2CO_3$, and HCl were obtained from Merck (Darmstadt, Germany). All the chemicals were of the analytical reagent grade.

### Enzymatic reaction

To a magnetically stirred solution of betulinic acid (25 mg, 0.0547 mmol), $K_2CO_3$ (6 mg), Celite®545 (170 mg), different amounts of enzyme (50-250 mg), chloroform (10 ml) and hexane (10 ml) was added phthalic anhydride with difference molar ratio (betulinic acid /phthalic anhydride; 0.2-1). The reaction mixture was magnetically stirred (150 rpm) at different reaction temperatures (40-60°C) and reaction times (8-24 h) as shown in Table1. Each reaction was repeated in triplicate and the results represented the mean values of three independent experiments. The control experiments were performed in the absence of enzyme. As a result, no chemical acyl transfer reaction was detected. Qualitative analysis of the reaction mixtures was made by thin layer chromatography (TLC) on silica gel plates eluted with system *n*-hexane/ethyl acetate (9:1, v/v). The plates were visualized under UV lamp and/or iodine vapor. Under these conditions, 3-*O*-phthalyl-betulinic acid (2) had an $R_f$valueof 0.9. The quantitative analysis of samples was carried out according to the procedure described by Kvasnicaet al[5]. At the pre-determined time intervals, the flasks were taken and the enzyme was removed by filtration and washed with chloroform twice. The filtrate was evaporated to dryness and ethyl acetate was then added and washed with aqueous solution of HCl and twice with water. The organic layer was dried over $Na_2SO_4$ and concentrated under reduced pressure. The residue was chromatographed with gradient on silica gel 60 (n-hexane/ethyl acetate, 9:1– 5:1, v/v). The ester fractions were combined and weighed after the evaporation of the solvents. The percentage of the isolated yield of ester (%Yield) is defined as:

$$\%Yield = \frac{mmol\ \ isolated\ \ betulinic\ \ acid\ \ ester}{mmol\ \ initial\ \ betulinic\ \ acid} \times 100 \qquad (1)$$

The characterization of the product was made by recording the $^1H$ and $^{13}C$-NMR spectra of the compound on a Varian Unity Inova 500 NMR spectrometer operating at 26°C and this matched the data available in the literature[5].

### ANN description

The experimental data used for ANN design are presented in Table 1. The experimental data were randomly divided into two data sets using the option available in the software: 21 of data sets were used as training and four data sets were used as testing.A multi-layer perceptron (MLP) based feed-forward ANN, which makes use of the back-propagation learning algorithm, was applied for modeling the enzymatic reaction. The network consists of an input layer, one hidden layer and an output layer. The inputs for the network include reaction time, reaction temperature, enzyme amount and substrate molar ratio; output is the percentage of the isolated yield of ester. A commercial ANN software, known as NeuralPower version 2.5 was applied throughout the present study. This software has been used by several researchers[12-14,18,22-24].

**Table 1:** Experimental values, actual and model predicated of isolated yield on the enzymatic reaction

| **Run No.** | **Time (h)** | **Temperature (°C)** | **Enzyme Amount (mg)** | **Molar ratio[1]** | **Isolated Yield (%)** | |
|---|---|---|---|---|---|---|
| | | | | | **Actual** | **predicted** |
| Training Data | | | | | | |
| 1 | 8 | 50 | 150 | 0.6 | 33.3 | 33.28 |
| 2 | 24 | 50 | 150 | 0.6 | 58.8 | 58.85 |
| 3 | 16 | 40 | 150 | 0.6 | 31.1 | 31.11 |
| 4 | 16 | 50 | 50 | 0.6 | 39.8 | 39.78 |
| 5 | 16 | 50 | 250 | 0.6 | 43.1 | 43.15 |
| 6 | 16 | 50 | 150 | 0.2 | 29.5 | 29.51 |
| 7 | 12 | 45 | 100 | 0.4 | 20.2 | 20.24 |
| 8 | 20 | 45 | 100 | 0.4 | 36.5 | 36.49 |
| 9 | 20 | 55 | 100 | 0.4 | 47.4 | 47.39 |
| 10 | 12 | 45 | 200 | 0.4 | 27.6 | 27.57 |
| 11 | 20 | 45 | 200 | 0.4 | 43.2 | 43.15 |
| 12 | 12 | 45 | 100 | 0.8 | 35.6 | 35.58 |
| 13 | 20 | 45 | 100 | 0.8 | 49.1 | 49.11 |
| 14 | 12 | 55 | 100 | 0.8 | 55.2 | 55.22 |
| 15 | 12 | 45 | 200 | 0.8 | 40.8 | 40.81 |
| 16 | 20 | 45 | 200 | 0.8 | 58.6 | 58.54 |
| 17 | 12 | 55 | 200 | 0.8 | 52.5 | 52.44 |
| 18 | 20 | 55 | 100 | 0.8 | 62.7 | 62.64 |
| 19 | 16 | 60 | 150 | 0.6 | 53.3 | 53.3 |
| 20 | 16 | 50 | 150 | 1.0 | 58.9 | 58.94 |
| 21 | 16 | 50 | 150 | 0.6 | 54.7 | 54.57 |
| Testing Data | | | | | | |
| 22 | 20 | 55 | 200 | 0.4 | 46.4 | 46.69 |
| 23 | 12 | 55 | 100 | 0.4 | 36.2 | 35.32 |
| 24 | 12 | 55 | 200 | 0.4 | 35.4 | 36.20 |
| 25 | 20 | 55 | 200 | 0.8 | 60.4 | 60.12 |

[1]Molar ratio = mmolbetulinic acid/mmolphthalic anhydride.

## Results and Discussion

### ANN modeling

Four different algorithms, belonging to two different classes, namely gradient descent (in three versions; incremental back propagation, batch back propagation and quick propagation) and Levenberg-Marquardt were used to train the neural networks. Details of the ANN modeling have been published in the previous study[14]. The results showed that the quick propagation (QP) algorithm had a better performance relative to the incremental back propagation (IBP), batch back propagation (BBP), and Levenberg–Marquardt (LM) back propagation algorithms. As shown in Fig. 2, the predicted model using QP algorithm was fitted well to the actual values for training and testing data sates. The ANN architecture using QP algorithm is shown in Fig. 3. The predicted values of the best model for the training and testing sets are presented in Table 1. In Fig. 4, the importance of independent variables in the construction of ANN model has been shown. As it is clear from the Fig.4, the enzyme amount shows higher contribution than the others.

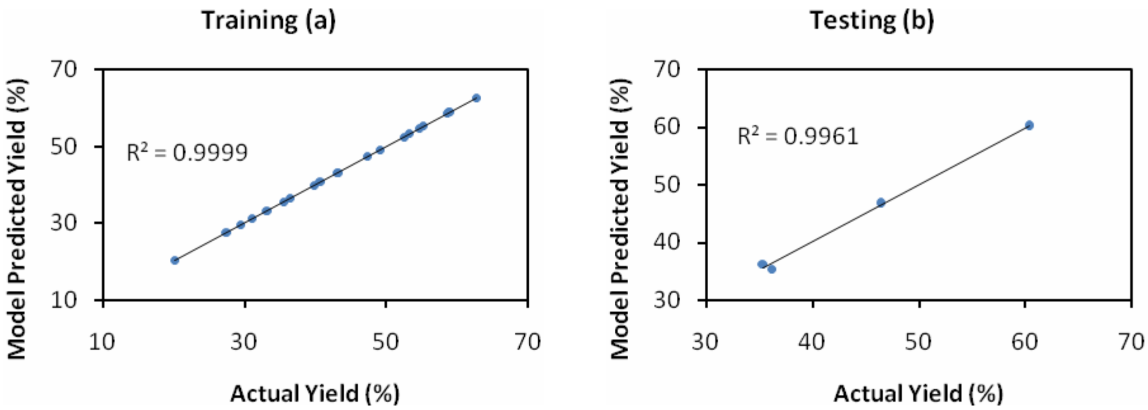



**Fig. 2:** The scatter plots of ANN predicted yield *versus* actual yield for training (a) and testing (b) data set using quick propagation algorithm

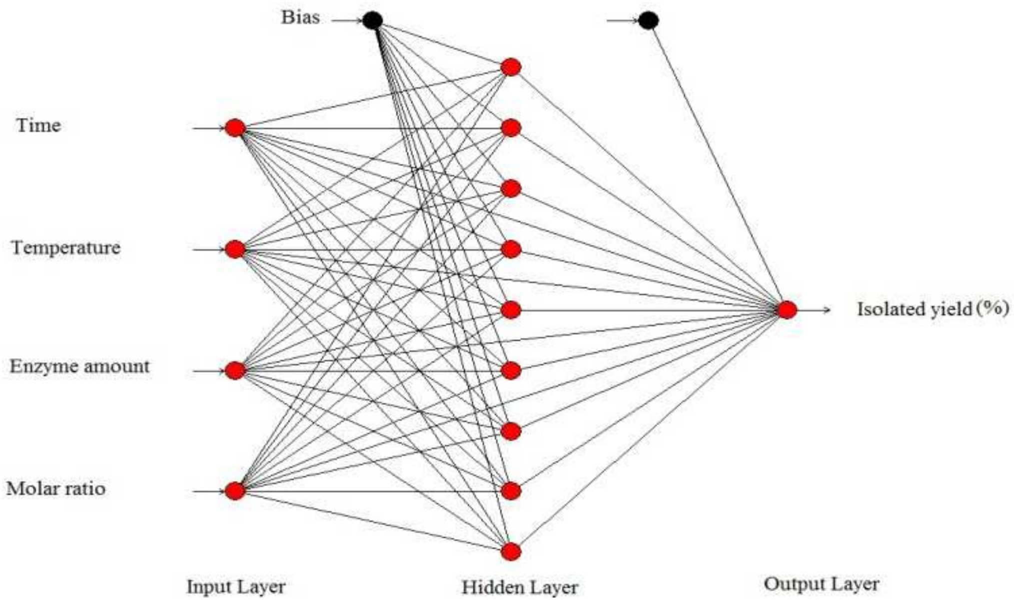



**Fig. 3:** A multilayer feed forward perceptron (MLP) network consisting of four inputs, one hidden layer with nine neurons and one output

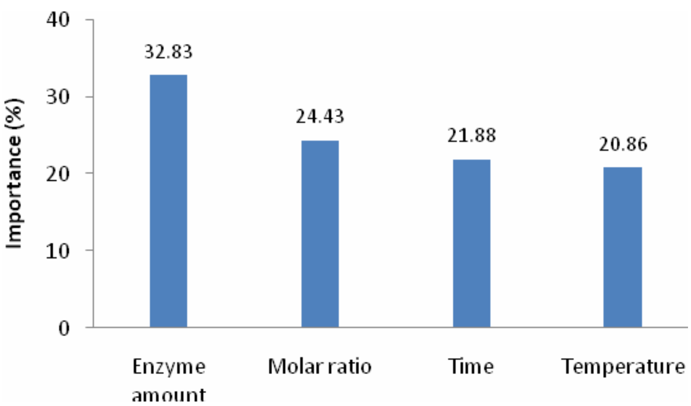



**Fig. 4:** The importance of independent variables in the constructed ANN model

### Optimization of enzymatic reaction

The optimal conditions for the lipase-catalyzed estrification of betulinic acid were predicted as presented in Table 2.The optimum reaction parameters were enzyme amount of 148.3 mg, reaction temperature of 53.1°C, reaction time of 20.3 hrs and betulinic acid to phthalic anhydride molar ratio of 1:1.24. The predicted and actual yields were 64.9 and 64.3%, respectively. A comparison of the predicted and actual values revealed a good correspondence between them, implying that the model derived from ANN analysis could be used to predict the experimental values in the lipase-catalyzed synthesis of betulinic acid ester.

**Table 2:** Optimum conditions derived from the ANN analysis using QP algorithm for enzymatic synthesis of betulinic acid ester

| **Optimal conditions** | | | | **Isolated yield (%)** | |
|---|---|---|---|---|---|
| **Time (h)** | **Temperature (ºC)** | **Enzyme amount (mg)** | **Molar ratio[1]** | **Predicted** | **Actual** |
| 20.3 | 53.1 | 148.3 | 1:1.24 | 64.9 | 64.3 |

[1]Molar ratio = mmolbetulinic acid/mmolphthalic anhydride

## Conclusions

In the present work, an artificial neural network (ANN) was applied tooptimize the enzymatic esterification reaction of betulinic acid andphthalic anhydride. The independent variables, namely time, temperature, enzyme amount and molar ratio were fed as inputs to an artificial neural network while the output of the network was the percentage of isolated yield of ester. A multilayer feed-forward network was trained by the sets of input-output patterns using quick propagation (QP) algorithm. According to the ANN analysis, a maximal yield of ester (64.3%) can be obtained using 148.3 mg enzyme, reaction temperature of 53.1 °C, reaction time at 20.3 hours and betulinic acid to phthalic anhydride mole ratio of 1:1.24. Thus, the optimum conditions for the synthesis of betulinic acid ester can be successfully predicted since the experimental results showed close correlation to the predicted values obtained.

## Acknowledgment

This work was supported by a grant from UniversitiTeknologi Mara (UiTM) (No: 600-RMI/DANA5/ 3/RIF (392/2012)) and is gratefully acknowledged.

## References

1. O'Connel, M.M., Bently, M.D., Campbell, C.S. and Cole, J.W.,Betulin and lupeol in bark from four white-barked birches, Phytochem.,1998,27, 2175-2176.
2. Habiyaremye, I., Stevanovic-Janezic, T., Riedl, B., Garneau, F-X. and Jean, F-I.,Pentacyclictriterpene constituents of yellow birch bark from Quebec, J. Wood Chem. Technol., 2001, 22, 83-91.
3. Yogeeswari, P. and Sriram, D., Betulinic acid and its derivatives: A review on their biological properties, Curr. Med. Chem., 2005, 12, 657-666.
4. Krasutsky, P.A. Birch bark research and development, Nat. Prod. Rep., 2006, 23, 919-942.
5. Kvasnica, M., Sarek, J., Klinotova, E., Dzubak, P. and Hajduch, M., Synthesis of phthalates of betulinic acid and betulin with cytotoxic activity, Bioorg. Med. Chem., 2005, 13, 3447-3454.
6. Krishna, S.H., Sattur, A.P. and Karanth, N. G., Lipase-catalyzed synthesis of isoamylisobutyrate optimization using central composite rotatable design, Process Biochem., 2001, 37, 9–16.
7. Villeneuve, P., Muderhawa, J.M., Graille, J. and Haas, M.J., Customizing lipases for biocatalysis: a survey of chemical, physical and molecular biological approaches, J. Mol. Catal. B, Enzym.,2000, 9, 113–148.
8. Manohar, B. andDivakar, S., An artificial neural network analysis of porcine pancreas lipase catalysed esterification of anthranilic acid with methanol,Process Biochem., 2005, 40, 3372-3376.
9. Pareek, V.K., Brungs, M.P., Adesina, A.A. and Sharma, R., Artificial neural network modeling of a multiphase photodegradation system, J. Photochem. Photobiol., A, Chem., 2002, 149, 139-146.

10. Desai, K.M., Survase, S.A., Saudagar, P.S., Lele, S.S. andSinghal, R.S., Comparison of artificial neural network (ANN) and response surface methodology (RSM) in fermentation media optimization: Case study of fermentative production of scleroglucan, Biochem. Eng. J., 2008, 41, 266-273.
11. Abdul Rahman, M.B., Chaibakhsh, N., Basri, M., Salleh, A.B. and Zaliha, R.N.R.A.R.,Application of artificial neural network for yield prediction of lipase-catalyzed synthesis of dioctyladipate, Appl. Biochem. Biotech.,2009, 158, 722-735.
12. Masoumi, H.R.F., Kassim, A., Basri, M., Abdullah, D.K. and Haron, M.J., Multivariate optimization in the biosynthesis of a triethanolamine (TEA)-based esterquat cationic surfactant using an artificial neural network, Molecules,2011, 16, 5538-5549.
13. GhaffariMoghaddam, M., Ahmad, F.B.H., Basri, M. and Abdul Rahman, M.B., Artificial neural network modeling studies to predict the yield of enzymatic synthesis of betulinic acid ester, Electron. J. Biotechnol., 2010, 13. Available from Internet: http://www.ejbiotechnology.cl/content/vol13 /issue3/ full/9/index.html.
14. Basri, M., Rahman, R.N.Z.R.A., Ebrahimpour, A., Salleh, A.B. Gunawan, E.R., Abdul Rahman, M. B. andRahman, A.,Comparison of estimation capabilities of response surfacemethodology (RSM) with artificial neural network (ANN) in lipase-catalyzed synthesis of palm-based wax ester, BMC Biotechnol., 2007, 7, 53-66.
15. Fernandes, F.A.N., and Rodrigues, S., Optimization of panose production by enzymatic synthesis using neural networks,Process Biochem., 2006, 41, 1090-1096.
16. Dutta, J.R., Dutta, P.K. and Banerjee, R., Optimization of culture parameters for extracellular protease production from a newly isolated Pseudomonas sp. using response surface and artificial neural network models, Process Biochem., 2004, 39, 2193-2198.
17. Polanski, J., Bak, A., Gieleciak, R. and Magdziarz, T., Self-organizing neural networks for modeling robust 3D and 4D QSAR: Application to dihydrofolatereductase inhibitors, Molecules, 2004, 9, 1148-1159.
18. Ghaffari, A., Abdollahi, H., Khoshayand, M.R. and Bozchalooi, I.S., Performance comparison of neural network training algorithms in modeling of bimodal drug delivery, Int. J. Pharm., 2006, 327, 126-138.
19. Plumb, A., Rowe, R., York, P. and Brown, M.,Optimisation of the predictive ability of artificial neural network (ANN) models: A comparison of three ANN programs and four classes of training algorithm, Eur. J. Pharm. Sci., 2005, 25, 395-405.
20. Chaibva, F., Burton, M. and Walker, R.B., Optimization of salbutamol sulfate dissolution from sustained release matrix formulations using an artificial neural network, Pharmaceutics, 2010, 2, 182-198.
21. Ahmad, F., Omar, J. and Ali, A.M., Chemical examination of local plant; Triterpenes from leaves of Malaysian *Callistemonspecious* D.E.,Ultra Science, 1999, 11,357–360.
22. Sanchooli, M. and GhaffariMoghaddam, M., Evaluation of acidity constants of anthraquinone derivatives in methanol/water mixtures using real quantum descriptors, J. Chem. Eng. Jap., 2012, 45, 373-379.
23. GhaffariMoghaddam, M. and Khajeh, M., Comparison of response surface methodology and artificial neural network in predicting the microwave-assisted extraction procedure to determine zinc in fish muscles, Food Nut. Sci., 2011, 2, 803-808.
24. Jain, S.K., Sarkar, A. and Garg, V., Impact of declining trend of flow on harike wetland, India, Water Resour. Manage.,2008, 22, 409-421.


*****