INTERNATIONAL JOURNAL OF COMPUTER ENGINEERING & TECHNOLOGY (IJCET)

ISSN 0976 – 6367(Print)
ISSN 0976 – 6375(Online)
Volume 6, Issue 3, March (2015), pp. 46-52
© IAEME: www.iaeme.com/IJCET.asp
Journal Impact Factor (2015): 8.9958 (Calculated by GISI)
www.jifactor.com

IJCET

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APPLICATION OF ARTIFICIAL NEURAL NETWORK STATISTICAL DESIGN (ANN) IN ENHANCED PRODUCTION OF BIOPHARMACEUTICALS

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ABSTRACT

Biopharmaceuticals are medical drugs produced by biotechnology which includes proteins, nucleic acids and living microorganisms like virus and bacteria. They are used for both therapeutic and diagnostic purposes. Biopharmaceuticals are produced from microbial cells, mammalian cell lines and plant cell culture and moss of plants in bioreactors. The global competition in the biopharmaceutical industry and the increased demand for affordable and effective medicines has shifted the industry's focus on manufacturing efficiency. Therefore process development and design are gaining importance. Artificial Neural Network (ANN) statistical design has been established as a tool for effortless designing and computation. ANN has found applications in fault diagnosis, process identification, property estimation, data smoothing and error filtering, product design and development, optimization, dynamic modeling and control of chemical processes. In this work comparison of ANN and RSM techniques for optimization of important biopharmaceuticals like Gentamycin production, comparison of estimation capabilities of RSM with ANN in lipase catalyzed synthesis of palm-based wax ester which is an excellent wetting agent and optimization of particle size for targeting diseased microvasculature using ANN are explained.

Key Words: 1.Biopharmaceuticals 2.Gentamycin 3.Artificial Neural Network4. Response Surface Methodology and 5. Optimization.

INTRODUCTION

Artificial Neural Networks (ANN) has been established as a tool for effortless computation. It is a Mathematical/computational tool, inspired by the structural and functional properties of the

biological neural network, and consists of a collection of processing units (nodes or neurons) organized into layers and mutually interconnected through connecting links.
In artificial neural networks, there are three distinct types of layers: the input layer, comprising all the input nodes; the hidden layers collecting the processing nodes; and the output layer, comprising all the output nodes. In this work, fully connected networks are used where all the nodes in each layer receive connections from all the nodes in the preceding layer. Information enters the network through the input nodes, is then passed to the sets of hidden layers, and eventually reaches the output nodes.

ANN have been successfully employed in solving problems in areas such as fault diagnosis, process identification, property estimation, data smoothing and error filtering, product design and development, optimization, dynamic modeling and control of chemical processes, for the prediction of vapor-liquid equilibrium(VLE) data and estimation of activity coefficients. ANN hasremarkable ability to derive meaningful information from complicated or imprecise data. It can be used to extract patterns and detect trends, which are too complex to benoticed by other computational technique. Neural networks, inspired by the information processing strategies of the human brain, are proving to be useful in a variety of engineering applications. ANN may be viewed as paralleled computing tools comprising of highly organized processing elements called neurons which control the entire processing system by developing association between objects in response to their environment. The researches have proposed many architectures of the network. Two widely used network for modeling the non-linear problems in engineering systems arethe Back propagation and Radial Basis Function (RBF) networks. Radial basis networks require lesser neurons than the standard feed forward back propagation networks and theycan be trained in a fraction of time.

Response surface methodology (RSM) is an effective statistical technique for developing, improving, and optimizing of complex process, now days commonly applied to three factorial designs giving numbers of input (independent) factors and their corresponding relationship between one or more measured dependent responses. RSM is a collection of statistical and mathematical techniques that can be used to define the relationships between the response and the independent variables. RSM defines the effect of the independent variables, alone or in combination, in the processes.

RSM is widely used for multivariableoptimization studies in several biotechnological processessuch as optimization of media, process conditions, catalyzedreaction conditions, oxidation, production, fermentation, etc.,

OPTIMIZATION OF GENTAMICIN PRODUCTION

Gentamicin is an aminoglycoside antibiotic, and can treat many types of bacterial infections, particularly Gram negative infection. Gentamicin is one of the few heatstable antibiotics that remain active even after autoclaving, which makes it particularly useful in the preparation of certain microbiological growth media. Gentamicin is a basic and water-soluble antibiotic, first invented by Weinstein et al (1963) from soil fungus M*icromonospora purpurea*.

The objectives of this work are to find out the optimum production medium by response surface methodology for the production of gentamicin by *Micromonospora echinospora subs pallida* and to compare the RSM predicted values with ANN predicted values.

Experimental Design and Procedure

Response surface methodology is used in this study. The experimental variables at different levels used for the production of Gentamicin by *Micromonouspora echinosporasubs pallida* using CCD. A total of 50 runs are used to optimize the medium. The average from two replicated values of each run is taken as dependent variables or response or yield (production of gentamicin).

Central composite design (CCD) is used to identify the optimum operating condition in order to obtain maximum gentamicin production (*Y1*) as response. The collection of experiments provides an effective means for optimization through these process variables. Besides, the design permits the estimation of all main and interaction effects. On the other hand, the purpose of the center points is to estimate the pure error and curvature.

RESULT

An attempt was made to compare the ANN with RSM for the optimization of gentamicin production. Input variables and data for the ANN were given. Initially the data was trained by varying the number of neurons in the hidden layer from three to eight. The experimental and predicted values of gentamicin production are given. The correlation coefficient of 1 for training the model and 0.9953 for testing the model was obtained. The predicted output values of RSM and ANN showed that they preformed well and offered stable responses in predicting the combined interactions of the independent variables with respect to the response, yet the ANN based approach was better in fitting to the measured response in comparison to the RSM model.
Thus artificial neural network modeling of gentamicin production is highly justified for the batch production.

Comparison of estimation capabilities of RSM with ANN in lipase-catalyzed synthesis of palm-based wax ester

Wax esters are long chain esters that are derived from fatty acids and alcohols with chain lengths of 12 carbons or more. The compounds have many potential applications due to their excellent wetting behavior at interfaces and a non-greasy feeling when applied on skin surfaces.Wax esters are important ingredients in cosmetic formulations(cleansers, conditioners and moisturizers), pharmaceuticals(as an anti foaming agent in the production ofpenicillin), lubricants, plasticizers and polishes and other chemical industries.

Natural waxes originate from animals, vegetables andminerals. Many of the important commercial waxes containrather high percentages of saturated wax esters, suchas beeswax. Other raw materials for saturated and unsaturated wax esters are sperm whale and jojoba oil. Since the naturally occurring wax esters are expensive and limitedin access, the need to synthesize the compound hasgrown. Wax esters have been synthesized via chemical and enzymatic reactions. Enzymatic synthesis uses lower temperatures than chemical synthesis.Wax esters can be produced by alcoholysis of vegetable oils such as palm oil. Palm oil consists of triacylglycerides, which are a combination of glycerol and different fattyacids. Enzymatic synthesis of wax esters from rapeseed fatty acid methyl ester and lipase-catalyzed alcoholysis of crambe and camelina oil have been reported.

One of the most important stages in a biological processis modeling and optimization to increase the efficiency of the process and. The classical method of optimization involves varying one parameter at a time and keeping the other constant. This technique is not only time-consuming but also does not depict the complete effects of the parameters in the process and ignores the combined interactions between the physicochemical parameters. Response surface methodology (RSM) is an effective statistical technique for developing, improving, and optimizing of complex processes.

Although RSM has so many advantages, and has successfully been applied to study and optimize the enzyme synthesis of flavor ester and biodiesel (fatty acid alkyl ester) and also optimizing enzyme production from microorganisms and it is hard to say that it is applicable to all optimization and modeling studies. Bas¸ and Boyacı reported that the second-order polynomial equation was not suitable in explaining the effects of pH and substrate concentration on the initial reaction rate of the enzymatic reaction. Similar observations were made on the data of some RSM articles. The past decade has seen a host of data analysis tools based on biological phenomena developed into well established modeling techniques, such as artificial intelligence and evolutionary computing. Artificial neural networks (ANNs) are now the most popular artificial learning tool in biotechnology, with applications ranging from pattern recognition in chromatographic spectra and expression profiles, to functional analyses of genomic and proteomic sequences.

In the present investigation, RSM and ANN analysis of enzymatic synthesis of wax esters from palm oil and oleyl alcohol was carried out using a commercial immobilized lipase.

RESULT

This study compared the performance of the RSM and ANN in the estimation of Lipozyme-catalyzed synthesis of wax ester from palm oil and oleyl alcohol. Though both models provided good quality predictions for the four independent variables (reaction time, temperature, amount of enzyme and substrate molar ratio) in terms ofthe percentage yield of wax esters, yet the ANN methodology showed a clear superiority over RSM as a modelling technique for data sets showing nonlinear relationships. As a modeling technique, artificial neural network was better than RSM for both data fitting and estimation capabilities. Regression-based response surface models require the order of the model to be stated (i.e., second, third or fourth order), but unfortunately most of the packed program produced for the application of RSM use second order model equation and then the major drawback of RSM is to fit the data to a second order polynomial, while ANN tends to implicitly match the input vectors to the output vector and indeed ANN is a superior and more accurate modeling technique when compared to the RSM as it represents the nonlinearities in much betterway. On the other hand, neural networks also have the disadvantage of requiring large amounts of training data incomparison with RSM that offers a large amount of information from a small number of experiments. This advantage of RSM is because of its experimental design. To overcome this ANN problem, in present study we used the RSM idea, and then a statistical experimental design CCRD, was employed to reduce the number of experiments. Thus, ANN could be a very powerful and flexible tool for modeling the optimization process.

Optimizing particle size for targeting diseased microvasculature

The use of nanoparticles in the early diagnosis, treatment, and imaging of a number of disorders, such as cancer and cardiovascular disease, is emerging as a powerful tool. Sufficiently small nanoparticles can be administered at the systemic level,transported by blood flow, and reach potentially any site within the macrovascular and microvascular circulation carrying imaging and therapeutic agents. A large variety of nanoparticles have been developed, and exhibit differences in size, shape, surface physicochemical properties, material composition, and deformability.

In cancer treatment and imaging, the maximum nanoparticle diameter has been traditionally limited to 200–300 nm in order to take full advantage of the well knownenhanced permeation and retention effects. Given that tumor vasculature has been shown to be discontinuous, with “fenestrations” a few hundred nanometers in size, sufficiently small nanoparticles would more likely extravasate by crossing the fenestrations passively and accumulating in the tumor interstitium. Within this sizerange, many different nanoparticle types have been proposed including liposomesand polymeric particles, dendrimers with a characteristic size of 4–10 nm, super paramagnetic iron oxide particles for cancer imaging and magnetic hyperthermia,gold nanoshells for photothermal therapy, and nanoporous silica beads for drug delivery and imaging.

However, enhanced permeation and retention-based delivery strategies are recognized to have important limitations, i.e, the fenestration size varies with the type, location, and stage of the disease, and indeed with the patient. Most importantly, vascular fenestrations are cancer-specific and are not found in other diseases directly involving the vascular apparatus, eg, atheroma. Here, we consider a more general nanoparticle delivery strategy based on targeting the diseased vasculature without relying on fenestrations and the enhanced permeation and retention effect. In this case, nanoparticles were designed to recognize and adhere efficiently to the walls of the diseased blood vessels and to resist dislodging hydrodynamic forces.

Mathematical models have been proposed to predict the probability of vascular adhesion as a function of nanoparticlesize, shape, and surface properties, and biophysical conditions at the site of adhesion. By contrast, in this workartificial neural networks in conjunction with flow chamber experiments are proposed as a tool to predict and optimizevascular adhesion of nanoparticles.

Any arbitrary complex function can be approximated by an artificial neural network,but in a non constructive manner. This implies that the number of internal degrees of freedom (ie, number of hidden layers, total number of nodes, node repartition between hidden layers) for the best approximation of an artificial neural network needs to be defined by the user. In this work, artificial neural networks with one and two hidden layers were considered, namely ANN231 (one hidden layer with three nodes) and ANN2321 (two hidden layers with three and two nodes). The experimental data used for training the networks are listed. The values for the mean (μ) and standard deviation (σ) for the experimentally measured number of particles adhering perunit area are listed in another table, as a function of the particle diameter (d) and wall shear rate (S). The network can capture the complexity of the experimental results, with optimal particle diameter (dopt) reducing as S increases. The characteristic errors of the training processes are shown in the root mean squared error for learning and test sets. It should be noted that, for each network, the training process should be stopped when the root mean squared error of the test set reaches a minimum, with a still decreasing learning root mean squared error. However, given that the behavior of the test error for ANN2321 is substantially flat, we have used in this instance an “over-trained” version of it (ie, a very high number of iterations where neither the learning error nor the test errors are changing). Finally, the two proposed networks were used to predict the optimal particle size (dopt) as a function of the shear rate (S). In both cases, dopt reduced with S in agreement with the experiments, but the ANN2321 has smoother behavior which captures the physics of the problem more appropriately.

CONCLUSION

Response surface methodology was applied for the optimization of production medium components for the production of gentamicin. The model developed for CCD had$R2$ values of 0.9286 for gentamicin production. The optimum values obtained by substituting the respective coded values of variables are: 8.9-g/L starch, 3.3-g/L soya bean meal, 0.88 g/LK2HPO4, 4.2 g/L CaCO3 and 0.033 g/L FeSO4. The analysis of the data shows that optimized values of medium components give more production of gentamicin (1020 mg/L) in comparison with the conventional optimization methods. From the results it was also found that the ANN predicted values (R2-0.9953) are closer than the RSM predicted values.

For synthesis of palm based wax ester the coefficient of determination (R2) and absolute average deviation (AAD) values between the actual and estimated responses were determined as 1 and 0.002844 for ANN training Set, 0.994122 and 1.289405 for ANN test set, and 0.999619 and 0.0256 for RSM training set respectively. The predicted optimum condition was: reaction time 7.38 h, temperature 53.9°C, amount of enzyme 0.149 g, and substrate molar ratio 1:3.41. The actual experimental percentageyield was 84.6% at optimum condition, which compared well to the maximum predicted value by ANN (83.9%) and RSM (85.4%). The R2 and AAD were determined as 0.99998696 and1.377 for ANN, and 0.99991515 and 3.131 for RSM respectively.

Foroptimizing particle size for targeting diseased microvasculature the proposed artificial neural networks captured the complexity of the physical problem, exhibiting biphasic behaviour for the *ns* (*d*) relationship, and demonstrating the existence of an optimal particle diameter (*dopt*) for which the number of adhering particles is maximized. The ANN2321offered slightly smaller characteristic errors than ANN231,and predicted more accurately the variation of *dopt* with *S*. This work suggests that the number of long parallel plateflow chamber experiments can be reduced by using artificial neural networks, without compromising the accuracyof the study. This same procedure could be used for in vivo applications leading to a significant reduction in the number of animal experiments.

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