Journal of Pharmaceutical Sciences xxx (2016) 1-7

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutics, Drug Delivery and Pharmaceutical Technology

Application of Artificial Neural Networks in the Design and Optimization of a Nanoparticulate Fingolimod Delivery System Based on Biodegradable Poly(3-Hydroxybutyrate-Co-3-Hydroxyvalerate)

Shadab Shahsavari [1], Leila Rezaie Shirmard [2, 3], Mohsen Amini [4], Farid Abedin Dokoosh [2, 5, *]

[1] Department of Chemical Engineering, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran
[2] Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[3] Department of Pharmaceutics, Faculty of Pharmacy, Ardebil University of Medical Science, Ardebil, Iran
[4] Department of Medicinal Chemistry, Drug Design and Development Research Center, Tehran University of Medical Sciences, Tehran, Iran
[5] Medical Biomaterials Research Center (MBRC), Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 4 March 2016
Revised 1 July 2016
Accepted 26 July 2016

Keywords:
artificial neural network
drug delivery
Fingolimod
poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
response surface methodology
training algorithms

ABSTRACT

Formulation of a nanoparticulate Fingolimod delivery system based on biodegradable poly(3-hydroxybutyrate-co-3-hydroxyvalerate) was optimized according to artificial neural networks (ANNs). Concentration of poly(3-hydroxybutyrate-co-3-hydroxyvalerate), PVA and amount of Fingolimod is considered as the input value, and the particle size, polydispersity index, loading capacity, and entrapment efficacy as output data in experimental design study. *In vitro* release study was carried out for best formulation according to statistical analysis. ANNs are employed to generate the best model to determine the relationships between various values. In order to specify the model with the best accuracy and proficiency for the in vitro release, a multilayer percepteron with different training algorithm has been examined. Three training model formulations including Levenberg-Marquardt (LM), gradient descent, and Bayesian regularization were employed for training the ANN models. It is demonstrated that the predictive ability of each training algorithm is in the order of LM > gradient descent > Bayesian regularization. Also, optimum formulation was achieved by LM training function with 15 hidden layers and 20 neurons. The transfer function of the hidden layer for this formulation and the output layer were tansig and purlin, respectively. Also, the optimization process was developed by minimizing the error among the predicted and observed values of training algorithm (about 0.0341).

Introduction

Fingolimod, 2-amino-2-[2-(4-octylphenyl) ethyl]-1, 3-propanediol] as a novel immunosuppressive modulator, is characterized by a reversible reduction of circulating peripheral blood lymphocyte counts. In experimental allogenic organ transplantation studies, Fingolimod exhibited mild immunosuppression; however, this has been clinically effective only at concentrations 5-fold higher than the dose administered normally and higher steady-state levels will need to be achieved. The current available capsule formulation supports daily administration with dose-proportional pharmacokinetics to achieve active steady-state levels in multiple sclerosis patients at 0.5 mg daily. Furthermore, alternative formulations that enable decreasing lymphocyte count with reduced impact on other non-target tissues will be necessary.[1]

The polymeric nanoparticle (NP)-based drug delivery system is ideal for controlled release drug delivery due to increased solubility of hydrophobic drugs; enhanced half-life of drugs; improved drug efficiency; reduced drug toxicity, and minimum side effects which are associated with multiple drug dosing.[2]

Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) is the most commonly studied polymer of polyhydroxyalkanoates family. It is a biodegradable, non-toxic polyester with a low production cost; PHBV has been intensively investigated as a biomaterial for tissue engineering and an NP-based drug delivery system.[3]

Artificial neural network (ANN) is a training computational method based on simulating the neurological influence of the human brain to design the best proper model.[4] This model works according to non-linear relationships between input factors and

* *Correspondence to:* Farid Abedin Dokoosh (Telephone: +982188009440; Fax: +982188026734).
E-mail address: dorkoosh@tums.ac.ir (F. Abedin Dokoosh).

http://dx.doi.org/10.1016/j.xphs.2016.07.026
0022-3549/

pharmaceutical outputs through data iterative training and optimization of the outcomes to minimize errors.[5]

ANNs have many usages in the field of pharmaceutics; they have been employed for modeling the responses in drug delivery systems and for evaluating the ANN model with multiple linear regression.[6,7] ANNs used as a parallel system consist of interactions between a huge number of simple calculation elements, nominated node or neurons, and fulfill intricate information processing and learn by models.[8] The main important example of this model is the new structure of the data processing system. It is the combination of a large number of highly bound processing origin (neurons) functioning in order to solve specific problems. An ANN is employed for a special application, such as design identification or information assortment, by means of learning action. Learning in biological systems consists of regulations to the synaptic interfaces within the neuron networks.[9]

In this work, based on the suitable properties of PHBV, Fingolimod-loaded PHBV NPs are formulated to control the release of hydrophobic Fingolimod by encapsulating it within hydrophobic PHBV NPs. Moreover, a feed forward, multi-layer perceptron (MLP) type of ANN is discussed for prediction of the mechanism of release of Fingolimod NPs.

Materials and Methods

Materials

PHBV with 3 wt.% PHV and polyvinyl alcohol (PVA) (average molecular weight 30,000-70,000) were purchased from Sigma-Aldrich (St. Louis, MO). Fingolimod was synthesized at the Danish Pharmaceutical Development Company. Analytical grade LiChrosolv acetonitrile was purchased from Merck (Darmstadt, Germany) and other chemicals used for the analytical methods were of analytical grade and purchased from Sigma Chemical Company (St. Louis, MO).

Preparation and Characterization of Nanoparticles

Fingolimod-loaded PHBV NPs were prepared by emulsification and solvent evaporation technique which can be useful for poorly water soluble drugs.

Different formulation variables such as concentration of PHBV and PVA, amount of Fingolimod, and speed of magnetic stirrer (300, 600, 1000 rpm) varied, and the effect on particle size, polydispersity index (PdI), loading capacity, and loading efficacy were considered. Only one parameter was changed at a time in each set of experiments. Briefly, chloroform (1 mL) containing PHBV (14.1 mg) as oil phase with Fingolimod (5 mg) was sonicated for 2 min, and oil phase was added drop wise to 5 mL PVA (as aqueous phase). The probe ultrasonication was continued for 10 min and then the mixture was gently stirred at room temperature for 4 h to allow complete evaporation of the organic solvent. The obtained suspension was centrifuged at 14,000 rpm for 30 min, the NPs were settled down and lyophilized for further studies, and the supernatant was used for determination of loading capacity and loading efficacy and also study of drug released from NPs.

Experimental Design

Further than the information achieved by preliminary studies, which defined the most significant factors, optimum levels of the PHBV concentration (A), PVA concentration (B), and Fingolimod amount (C) that can significantly affect the particle size, PdI, loading, and encapsulation efficiency were analyzed using the response surface methodology. Size and zeta potential of particles were determined using photon correlation spectroscopy (Malvern Instruments, Malvern, UK).

The mathematical relevance between the responses and independent variables were modeled by a second-order polynomial equation.

In order to graphically show the interactions between the variables and the response, three-dimensional (3D) surface plots were used in this study.

In Vitro *Drug Release Study*

The cumulative release of Fingolimod from the PHBV NP in non-biological conditions was done during 30 days by a dialysis bag (cut-off 12 kDa) in phosphate buffered saline (PBS) at pH 7.4 and 37°C (in sink condition).

At specified time intervals (30 min; 1, 2, 4, 8, 12, 24, 48, 72, and 120 h; 1, 2, 3, and 4 weeks), 1 mL of the medium was removed for analysis and fresh PBS of an equal volume was displaced. The *in vitro* release of Fingolimod was measured in triplicate. The samples were analyzed by HPLC method by using C8, 125 × 4.6 mm, 5 μm column, acetonitrile and phosphate buffer pH 3 (45:55) as mobile phase and UV detection of 220 nm.

Prediction and Optimization Functions of ANN

Commercially available software, MATLAB® R2008a (MathWorks, Inc., Natick, MA), was applied to write mathematical code for training and measuring the ANN developed and used for formulation optimization.

MATLAB is a mercantile software developed by MathWorks, Inc. It is a mathematical software package for theoretical and simulating numerical calculation.[10] Backpropagation, an abbreviation for "backward propagation of errors," is a usual method of training ANNs applied in conjunction with an optimization procedure such as gradient descent (GD). The method calculates the gradient of a loss function with respect to all the weights in the network. Also, the multi-layer network is sometimes referred to as a backpropagation network. However, the backpropagation technique that is used to compute gradients and Jacobians in a multi-layer network can also be applied to many different network architectures.[11]

A neural network includes an interconnected group of artificial neurons, working in union to solve particular problems. ANNs, like human, learn by example. The neuron has 2 methods of operations: the training/learning mode and the using/testing mode. In original cases, an ANN is an adjusted system that converts its structure based on external or internal information that goes through the network in the learning phase. Recent neural networks are nonlinear statistical data modeling tools. They are commonly used to model complex relationships among inputs and outputs or to find models in data. MLP learning algorithm is an administered learning algorithm. It is one of the most important progresses in neural networks. This learning algorithm is used in multilayer feed-forward networks including processing elements (neurons) with continuous recognizable activation functions (tan-sigmoid and log-sigmoid). Given a set of training input-output pair, this algorithm prepares a procedure for changing the weights in a back-propagation network to classify an input correctly. The sense for this weight update algorithm is mainly the GD method applied in case of simple perceptron networks with recognizable units. This is a procedure where the error is propagated back to covered unit. The aim of the neural network is to train the net to attain a balance between the net's ability to reply (memorization) and its capability to give reasonable responses to the input that is alike but not identical to one that is applied in training.[12]