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Research Paper

Optimization of controlled release nanoparticle formulation of verapamil hydrochloride using artificial neural networks with genetic algorithm and response surface methodology

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ABSTRACT

This study was performed to optimize the formulation of polymer–lipid hybrid nanoparticles (PLN) for the delivery of an ionic water-soluble drug, verapamil hydrochloride (VRP) and to investigate the roles of formulation factors. Modeling and optimization were conducted based on a spherical central composite design. Three formulation factors, i.e., weight ratio of drug to lipid (X_1), and concentrations of Tween 80 (X_2) and Pluronic F68 (X_3), were chosen as independent variables. Drug loading efficiency (Y_1) and mean particle size (Y_2) of PLN were selected as dependent variables. The predictive performance of artificial neural networks (ANN) and the response surface methodology (RSM) were compared. As ANN was found to exhibit better recognition and generalization capability over RSM, multi-objective optimization of PLN was then conducted based upon the validated ANN models and continuous genetic algorithms (GA). The optimal PLN possess a high drug loading efficiency (92.4%, w/w) and a small mean particle size (~100 nm). The predicted response variables matched well with the observed results. The three formulation factors exhibited different effects on the properties of PLN. ANN in coordination with continuous GA represent an effective and efficient approach to optimize the PLN formulation of VRP with desired properties.

1. Introduction

Verapamil hydrochloride (VRP) is a calcium channel blocker commonly used to treat hypertension, cardiac arrhythmias and angina [5,16]. It has also been used as a P-glycoprotein (P-gp)-inhibitor to enhance chemotherapy of multidrug resistant cancer [7,27,29]. Although VRP is well absorbed through the gastrointestinal tract (⩾90%), its systemic bioavailability (20–35%) is poor and variable due to the extensive first-pass metabolism by cytochrome CYP450 3A4 [31]. Its short elimination half-life (2.8–7.4 h) demands dosing of 3–4 times per day [17]. Hence a sustained release nanoparticle delivery system able to bypass liver metabolism would increase bioavailability and reduce dosing frequency.

As lipids are absorbed mainly through the lymphatic system in the intestines, lipid-based particulate systems offer a promising approach to bypassing first-pass metabolism *via* lymphatic transport thus enhancing drug bioavailability [9,21,35,36,39]. In particular, solid lipid nanoparticles (SLN) [37] have been reported to improve oral bioavailability and optimize plasma profiles of loaded drugs [6,8,30,32,34,52].

Owing to the hydrophobic nature, SLN is not an ideal carrier for sustained release of a highly water-soluble drug like VRP with an aqueous solubility of 83 mg/mL. In order to deliver ionic water-soluble drugs while maintaining the advantages of SLN, our group exploited the characteristic physicochemical properties of polymer counterions and developed a novel polymer–lipid

Abbreviations: PLN, polymer–lipid hybrid nanoparticles; VRP, verapamil hydrochloride; DS, dextran sulfate sodium; DA, dodecanoic acid; VRP-PLN, verapamil hydrochloride loaded polymer–lipid hybrid nanoparticles; ITC, isothermal titration calorimetry; TEM, transmission electron microscopy; CCD, central composite design; ANN, artificial neural networks; RSM, response surface methodology; GA, genetic algorithms; AIC, Akaike's information criterion; DLE, drug loading efficiency; DLC, drug loading capacity; HLB, hydrophile–lipophile balance; DDI water, distilled and deionized water.

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hybrid nanoparticle (PLN) system [51]. This system exhibited sufficient drug loading, lowered initial burst release and subsequently sustained drug release. To further improve the performance of PLN, drug–polymer–lipid interactions, internal structure, drug loading and release mechanisms were investigated [25]. These efforts resulted in a rationally designed formulation with high drug loading capacity and sustained release kinetics [26]. Nevertheless, the variables of PLN formulation (e.g. drug to lipid ratio and surfactant concentrations) need to be further optimized in order to obtain relatively uniform particle size in the range of ~100 nm for effective intestinal lymphatic uptake [12,46] while maintaining maximal drug loading levels.

Our preliminary tests suggested that for PLN, the drug loading capacity and particle size depended on the processing conditions and formulation factors including the weight ratio of drug to lipid, and the amounts and ratio of surfactants (e.g. Pluronic F68 and Tween 80). Owing to multiple variables and their unclear cause-and-effect relationship, we applied factorial experiment design [3,41,42,53] and numerical modeling techniques to optimize the PLN formulation. The central composite design (CCD), a second-order polynomial model [38] was employed, as CCD is very efficient and able to provide much information on the effects of experiment variables and overall experimental error with a minimum number of required runs.

Combining the factorial design with statistical modeling techniques provides a particularly useful way of understanding the underlying structure between the independent and dependent variables. Models, established by the response surface methodology (RSM) or artificial neural networks (ANN) [18], can then be used to predict the responses to the combinations of the independent factors that are not explicitly studied experimentally. Due to the simplicity and ease of deployment, RSM utilizing a quadratic equation to fit the experimental data has been widely used in pharmaceutical research [33,50]. Compared to RSM, ANN are less restrictive and hence suitable for recognizing more complex, multi-dimensional and non-linear patterns [4,19,20,40,45,48]. Since different modeling strategies are adopted in RSM and ANN, and each method has its own inherent advantages and limitations, we elected to compare their modeling performance in the optimization of PLN in this work.

Once the model is established, contour plots are normally constructed to visualize the relationship between independent and dependent variables so as to search the optimal formulation manually. However, three independent variables and two dependent variables are involved in the optimization of PLN in this study. Identifying an optimal formulation of this system using the superimposing contour method is cumbersome. To overcome this difficulty, the equations for each of the dependent variables were incorporated into a single equation, i.e., a generalized distance function [22], which is called a 'cost function' or an objective function in the optimization process. Then a numerical optimizer can be used to locate the values of the independent variables that minimize the cost function. We employed the continuous genetic algorithms (GAs) [11,44,15] to minimize the cost function with respect to the three independent variables. GA is a robust, stochastic, adaptive heuristic searching algorithm. It can avoid the problem of being stuck in local minima which is common to the gradient-based algorithms. Moreover, continuous GA is better than binary GA as it utilizes original experimental data as input and thus can avoid the loss of information which takes place in the transformation of data.

In summary, we used a three-factor spherical second-order CCD to map the underlying pattern between each of the dependent variables (particle size and drug loading of PLN) and independent variables (drug to lipid ratio and concentrations of Pluronic F68 and Tween 80). RSM and ANN were used to model and predict the responses. Continuous GA was utilized to perform the search for a global optimum.

2. Materials and methods

2.1. Materials

Verapamil HCl (pK_a = 8.6, solubility: 83 mg/mL), dodecanoic acid (DA, MP: 44–46 °C), dextran sulfate sodium (DS, MW: 5000 Da) and Tween 80 were purchased from Sigma–Aldrich Canada (Oakville, ON, Canada). Calcium chloride, potassium dihydrogen phosphate and di-sodium hydrogen orthophosphate anhydrous (dibasic) were purchased from Fisher Chemicals (Pittsburgh, PA, USA). Pluronic F68 was a gift from BASF (Mississauga, ON, Canada). Amicon® centrifugal filters (MWCO: 30,000 Da) were acquired from Millipore Inc. (Toronto, ON, Canada). Distilled and deionized (DDI) water was prepared with a Milli-Q water purifier (Milli-Pore, Etobicoke, ON, Canada).

2.2. Preparation of VRP-PLN

PLN were prepared by a modified micro-emulsion method followed by ultrasonication [51]. The process of PLN formation is schematically illustrated in Fig. 3. Briefly, the lipid, DA, was melted using a thermostated water bath at 50 °C. A particular amount of VRP (dependent on the desired drug loading capacity of PLN) was added to the molten lipid. Under constant magnetic stirring with a Corning stirring plate, an aqueous phase was prepared by dissolving specific amounts of Pluronic F68 and Tween 80 in DDI water and heating to the same temperature as the lipid phase. The hot aqueous phase was added to the lipid phase subsequently. A known amount of DS was added slowly to the mixture to obtain an ionic molar ratio of DS to VRP at 1.0. The coarse o/w micro-emulsion was left in the water bath for 20 min under vigorous stirring at ~700 rpm and then sonicated for another 5 min at the same temperature. The PLN nanoparticles were obtained by injecting the final emulsion into a fixed volume of cold water (2–4 °C) under magnetic stirring.

2.3. Measurements of particle size and surface charge

The hydrodynamic diameters and surface charge of PLN were measured by a particle sizer equipped with the function for zeta potential measurements (Nicomp, Model 380 ZLS, Particle Sizing Systems Inc., Santa Barbara, CA, USA). All values were measured in DDI water at a fixed angle of 90° at 25 °C in 10 mm diameter cells with a He/Ne laser light source at 632.8 nm. Each experiment was performed in triplicate.

2.4. Determination of drug loading efficiency

The drug loading efficiency of the system was determined by measuring the concentration of free drug remained in the dispersion medium, compared to the initial drug added. Briefly, 3 mL of undiluted sample of PLN was placed in the sample chamber of a Millipore filter consisted of a membrane with MWCO of 30,000 Da. The unit was centrifuged at 4000 rpm (relative centrifugal force: 3150*g* for 45 min). The PLN containing encapsulated drug–polymer complex remained in the sample chamber and the aqueous phase passed into the recovery chamber through the filter membrane. The aqueous filtrate (i.e., first filtrate from centrifugation) was collected, the recovery chamber was washed three times using DDI water to remove uncapsulated drug, 1 mL of the 3× diluted filtrate was incubated with the same volume of 0.3 M $CaCl_2$ solution for 24 h at room temperature. The concentrated

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