SHORT COMMUNICATIONS

SCREENING OF MOST EFFECTIVE VARIABLES FOR DEVELOPMENT OF TELMISARTAN LIPOSOMES BY TAGUCHI DESIGN

ABSTRACT

The aim of this study was the selection of the most effective variable for the formulation of telmisartan liposomes. Liposomes were prepared by dry film deposition method using a Rota evaporator. The effects of formulation and processing variables on different response variables were studied using standard Taguchi orthogonal array L8 design. The independent variables selected were the amount of lipid, amount of cholesterol, sonication time, speed of Rota evaporator, hydration time, hydration volume and temperature, and the dependent variable selected were particle size (nm) and entrapment efficiency (%). All the batches of liposomes were prepared as per the Taguchi design. The influence of variables analyzed by Pareto ranking showed that the most effective factors for the selected responses were the amount of cholesterol, amount of lipid and sonication time ($P<0.05$).

Keywords: Liposomes, Taguchi design, telmisartan

INTRODUCTION

Telmisartan is a poorly water-soluble BCS class-II drug. It comes under the category of an angiotensin-II receptor blocker and is used as an anti-hypertensive drug. It has very poor solubility, which leads to about 30-40 % bioavailability in the blood stream[1]. Absorption of orally administered telmisartan is slow and incomplete; hence there is a dire need for an alternative oral formulation of telmisartan that can improve its bioavailability[2-3]. Many efforts are being made to increase the bioavailability of drugs, such as SLNs, spray drying techniques, microsphere, solid dispersion techniques, and nano suspension[4-5]. Currently, the liposomal drug delivery system has been increasingly explored to improve the bioavailability and solubility of poorly water-soluble drug[6]. Lipid-based vesicles drug delivery system is extensively useful for effective lymphatic drug delivery[7]. Now a days, DOE experimental design is frequently used for the optimization of formulations. Dr. Genichi Taguchi, a Japanese scientist, developed a new method for the screening of factors at a preliminary level, which is known as orthogonal array design. Taguchi design is enough for the screening of the large number of experimental parameters[8-9]. The liposome formulation will be offering exclusive advantages such as A) improvement in lymphatic circulation which will further increase the absorption and bioavailability, B) protect the drugs from the gastric environment and C) it can prolong the half-life of drugs in blood and can raise their therapeutics index[9].

MATERIALS AND METHODS

Telmisartan was obtained as a gift sample from Alembic Pharmaceuticals Ltd. Vadodara, India. Phospholipid 90 H was gifted by Lipoid Gmb H, Ludwigshafen, Germany. Cholesterol was purchased from S.D. Fine Chem. Ltd., Mumbai. All the other ingredients used were of analytical grade and the solvents used in this experiment were of HPLC grade. Distilled water used throughout the experiment was freshly prepared.

Preparation method of liposomes

Liposomes were prepared by dry film disposition method using a Rota evaporator (ROTA). The accurate amount of lipid mixture consisting of phospholipid 90 H and cholesterol was weighed as mentioned in Table I and this mixture and the drug (20 mg) were dissolved in 20 mL of the solvent mixture containing methanol and chloroform in the ratio of (4:2 V/V) was prepared. This mixture solution was transferred into a 250 mL round bottom flask. The flask was then attached to a Rota evaporator and the organic solvent was evaporated under the pressure at different temperature, and speed. After complete evaporation of the organic solvent, the films were dried overnight. The liposomal suspension was kept overnight to get complete hydration[10].

Particle size and entrapment efficiency determination

Particle size determination was carried out using Zeta sizer (Zan 3690 Malvern). All the samples were kept in

Table I: Preparation of liposomes based on Taguchi screening design

Formulation Code	Amount of Cholesterol (mg)	Amount of Lipid (mg)	Sonication time (min)	Speed of Rota evaporator rpm)	Hydration time (min)	Hydration volume (mL)	Temperature (C°)	Particle Size (nm)	Entrapment efficiency (%)
F1	15.3	45.91	5	40	30	30	50	215.5±0.12	30.57±0.07
F2	15.3	45.91	15	70	50	60	60	319.3±0.34	45.32±0.05
F3	22.3	61.22	5	40	50	30	60	520.8±0.51	81.34±0.27
F4	22.3	61.22	15	70	30	60	50	434.3±0.02	75.63±0.11
F5	22.3	45.91	5	70	50	60	50	202.5±0.15	71.65±0.04
F6	22.3	45.91	15	40	30	30	60	210.4±0.17	38.54±0.37
F7	15.3	61.22	5	70	30	60	60	225.5±0.29	55.01±0.64
F8	15.3	61.22	15	40	50	30	50	226.7±0.47	44.35±0.01

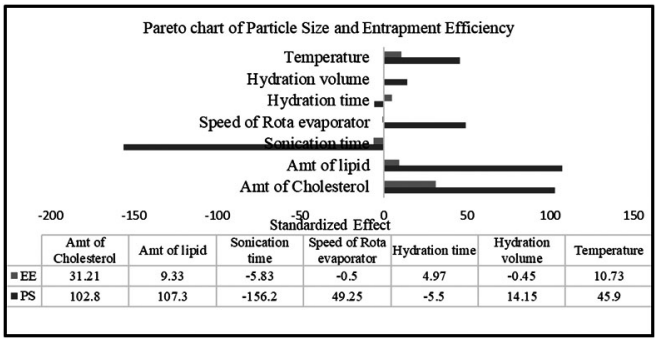

Fig. 1: Pareto chart of the standardized effects of various formulations and process factors on particle size and entrapment efficiency

the refrigerator before determination[11]. The sample was poured into the transparent disposable cuvette.

The entrapment efficiency of liposomal suspension was determined by centrifuging the sample at 4000 rpm for 18 min at 4 °C temperature using Remi cooling centrifuge to separate the free drug from the formulation. The supernatant was again centrifuged at 12000 rpm for 35 min at 4 °C temperature. From the tube, pellets consisting of liposomes were redispersed in distilled water for study. The liposomes consisting of unentrapped free drug were mixed with 10 mL mixture of organic solvent followed by 2 min of sonication. The disrupted liposomes discharged the telmisartan in the solution. The amount of telmisartan was estimated by using UV spectrophotometer at 296 nm[11].

Screening of most effective variables

Several formulations and processing parameters affect the overall quality of the liposomes. Here, Taguchi orthogonal array design was applied in which particle size and entrapment efficiency were investigated as the dependent variables. All run sets shown in Table I describe different combinations of levels to the factors. Every experiment was done in triplicate for accuracy purpose. The effects of different formulation combinations on the particle size and entrapment efficiency were analyzed by using Design Expert software 12[12].

RESULT AND DISCUSSIONS

Effect of the various variable on particle size and entrapment efficiency

The evaluation of liposomal particle size indicated that it was greatly affected by amount of cholesterol. Particle size increases with increased amount of cholesterol, as it improves the distribution into phospholipid bilayers which can lead further to increase the diameter of liposomes. Moreover, increase in particle size was observed with increased amount of lipid. On the other hand, elevation in sonication time brought down the liposomal diameter. The positive effect of speed of Rota evaporator was found to increase the particle size. Number of bilayers is also one of the factors which can affect the particle size. As increase in liposomal hydration time leads to formation of more lipid bilayers, it can result into increasing the vesicular size[12-13].

Entrapment efficiency is also one of the important characteristics which can directly affect the therapeutic effects of the drug. It is directly proportional to the amount of cholesterol, lipid hydration time and temperature. But it is inversely proportional to sonication time and speed of the Rota evaporator. Results shown decrease in entrapment efficiency when sonication time and speed of ROTA evaporator was increased, as the ultrasound waves caused leakage of drug from the vesicles[12-13].

Screening of most effective formulation and process variable

In the present study, eight formulations and process parameters, as mentioned in Table I, were studied at two different levels. Here, L8 orthogonal array design was used to identify the most significant variables for further optimization of the formulation[14]. Pareto charts were constructed by using coefficient values as standardized effect and plotted against different responses (Fig. 1).

This standardized effect identifies the strength of each factor. Higher value describes the greater effect of that specific factor on the response. The positive value shows that the factor favors the response, and a negative value shows an inverse relationship between the response and the factor. The Pareto ranking analyses indicated that the most effective significant factor was the amount of cholesterol (P=0.0347), amount of lipid (P=0.0310), and sonication time (P=0.0113) (where, $P < 0.05$) as compared to other factors. Based on the Taguchi screening design results, further experiment design such as Box-Behnken design and CCD is suggested for generating polynomial equation and optimization of the formulation[15].

CONCLUSION

The application of Taguchi design orthogonal L8 array discovered the effect of formulations and processing variables on the different response variables by applying optimization techniques. This present study showed that the most effective variable in the liposomal formulation of telmisartan was the amount of cholesterol, amount of lipid, and sonication time, which directly affect the response variables for the development of liposomes.

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