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Preparation and evaluation of naringin-loaded polycaprolactone microspheres based oral suspension using Box-Behnken design

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ABSTRACT

The objective of the current investigation was to study the outcome of the concentration of polycaprolactone (PCL), the concentration of polyvinyl alcohol (PVA) and the concentration of hydroxypropyl methylcellulose (HPMC) on the average particle size of microspheres and the drug entrapment efficiency of naringin-loaded polycaprolactone microspheres based oral suspension. Naringin-loaded polycaprolactone microspheres based oral suspension were prepared by solvent evaporation method. Further, in this work optimization of the formulation was done using a three factors, three levels Box-Behnken design (BBD). Drug-loaded microsphere suspension was prepared by employing solvent evaporation technique. Fourier transform infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC) analysis was carried out to determine any incompatibility between the naringin and other components used in the formulations. Polymer alone (PVA concentration) or interaction effects of polymers (PCL-HPMC, PCL-PVA, HPMC-PVA, and PCL in higher order) have a substantial influence on particle size of microspheres. Amount of PVA and HPMC have a significant effect on entrapment efficiency of microspheres. The optimal conditions of PCL concertation were 7.7% w/v; HPMC concentration was 3.2% w/v and PVA concentration was 0.081% w/v. The optimized suspension showed particle size of 24 μm with drug entrapment efficiency 96%. As a result, due to uniform distribution of microspheres, drug entrapment efficiency was achieved in optimized suspension.

1. Introduction

Current progress in the field of phytotherapy has directed to the identification and isolation of therapeutically active phytoconstituents that can be used for improving diseases precisely. However, till now a lesser number of phytodrugs have been commercially advanced to fit customer use [1]. These days with the technological improvement, novel drug delivery systems exposed the entrance to the growth of improving the bioavailability of phytoconstituents as a delivery system. Different novel carriers such as liposomes, microspheres, nanoparticles, transferosomes, ethosomes, lipid-based drug carrier systems etc. have been described for the creation of herbal ingredients into dosage forms [2]. Several bioactive compounds possess antioxidant activity but most of them are shows low oral bioavailability [3]. Therefore, most of the researchers try to find out a successful formulation with the low soluble bioactive compounds so that they can exhibit pharmacological properties with lesser side effects or toxicity. Naringin also suffers from bioavailability problem.

Naringin (4′,5,7-Trihydroxyflavanone 7-rhamnoglucoside), is a well-known flavanone glycoside found in grapes and citrus fruits. Two rhamnose units are link to its aglycone part, naringenin, at the 7-carbon location [4,5]. From different scientific literatures it was found that both naringin and its aglycone portion (naringenin) possess several pharmacological actions such as anti-oxidant [6], anti-ulcer [7], anti-microbial [8], superoxide scavenging [9] and anti-cancer effects [10]. Interaction of the flavonoids with colonic microflora was informed to impact their bioavailability. It was reported in the literature that a substantial quantity of the consumed flavonoids was not absorbed in the small intestine [11]. Naringin is quite soluble in water. The gut microflora breakdowns naringin glycoside into its aglycon naringenin in the intestine which is then absorbed from the gut [12]. The metabolism of naringin when given orally in rats can be described as follows: at first hydrolysis of naringin was takes place by intestinal bacteria to naringenin or by partial hydrolysis of naringin to naringenin glucuronides and then, the metabolites get absorbed and some of the naringenin could combine again with free glucuronides [13].

Fuhr and Kummert (1995) found that when mice fed with high-fat regime, naringin reduced visceral adiposity and dropped plasma lipid amount, perhaps by initiation of AMP kinase [12]. Naringin supplementation decreased the raised plasma lipid concentrations in high-fat-diet-

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fed rats and decreased plasma lipids and cholesterol in high-cholesterol-diet-fed rats [14]. The cardioprotective benefits of naringin supplementation were established by decreased lipid peroxidation, improved antioxidant enzymes, and reduced inflammatory cell and fibrosis in hearts of isoproterenol-treated rats [15]. Naringin along with vitamin C was seen to improve streptozotocin-induced diabetes in rats by bettering insulin amount thus representing its hypoglycemic action too [5,16,17].

Recently, naringin was encapsulated by a novel water-soluble fiber, resistant maltodextrin with spray drying technique was evaluated by Pai et al., (2015). They used the factorial design of experiments in full with 2-levels of factors such as fiber-naringin ratio and spray dryer inlet temperature on the solubility enhancement. They concluded that the solubility improvement takes place with increasing fiber-naringin ratio and spray drying temperature [18]. Xu et al., (2015) prepared naringin-lecithin complex and they found that the antioxidant activity tests of samples in a linoleic acid system showed that the antioxidant activity of naringin in oil could be significantly enhanced by the naringin-lecithin complex [3].

The purpose of this research work is to enhance the therapeutic effectiveness of naringin through the formulation of microsphere-based oral suspensions. Till now no report so far available which shows the systematic approach of optimization of naringin-loaded polycaprolactone microspheres based oral suspension using a three-factor, three-level Box-Behnken Design (BBD). The independent process variables (factors) were the concentration of polycaprolactone (X_1), hydroxypropyl methylcellulose (X_2) and polyvinyl alcohol (X_2). The dependent responses were the average size of microparticles and drug entrapment efficiency in the microspheres based oral suspension. The prepared formulations were further characterized for the physicochemical property which can be modulated in planning a suitable pharmaceutical dosage form. The drug loading, particle size, pH, redispersibility, morphology study, FT-IR and DSC study of all the prepared formulations carried out to establish any incompatibility between the used polymers and other ingredients. Further, the acquired model was tested, and the expected response values and the experiential response values were compared statistically.

2. Materials and methods

2.1. Materials

Naringin ($C_{27}H_{32}O_{14}$; MW 580.4 g/mol) was procured from SRL India. Polycaprolactone (MW 80,000) was obtained from Sigma Aldrich. Chloroform, hydroxypropyl methyl cellulose (HPMC K15M), polyvinyl alcohol, tween 80 and span 20 were purchased from Sigma-Aldrich. The other reagents used in this research work were of analytical grade.

2.2. Statistical experimental design

2.2.1. Experimental design

The traditional method is a time-wasting process because during the formulation progress it is essential to alter one factor at a time [19,20]. The traditional method fails to study the interactions effects among the factors if more than one factor can affect the final formulation properties [19]. Therefore, by using a statistical approach, a three-factor, three-level Box-Behnken design (BBD) (Design-Expert®7 trial version software, Stat-Ease Inc., Minneapolis, USA) was applied for the optimization of oral suspension containing naringin-loaded polycaprolactone microspheres. This Box-Behnken design is suitable for investigative the quadratic response surfaces and also helps to accumulate a second-order polynomial equation. This design assists us to get an optimal formulation with execution a least number of trial runs [21]. The design comprised of simulated center points and other points lying at the midpoints of each edge of the multidimensional cube which will describe the section of attention for assessing the main effects, interactions effects and quadratic effects of the formulation factors [22]. The dependent response variable (Y) of the non-linear quadratic model was clarified via the resulting equation [23]:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 + b_{11}{X_1}^2 + b_{22}{X_2}^2 + b_{33}{X_3}^2$$

where, Y is the dependent response variable related with each factor level combination, b_0 an intercept and b_1-b_{33} are the regression coefficients values. X_1, X_2, and X_3 are the main selected factors, X_1X_2, X_2X_3, and X_1X_3 are the interaction effects and X_1^2, X_2^2 and X_3^2 are the quadratic expressions.

The concentration of polycaprolactone (PCL), hydroxypropyl methylcellulose (HPMC) and polyvinyl alcohol (PVA) was selected as independent factors X_1, X_2 and X_3 respectively. In this design, each independent process variables (factors) has three levels such as high level (+1), medium level (0) and low level (−1) (Table 1). The seventeen experimental runs were generated by Box-Behnken design but actually, fifteen runs were actually carried out by deleting two runs and further proceed to generate contour plot and 3-D plots and then optimization of the suspension was done. Each of the experimental run being conducted three times.

2.2.2. Preparation of naringin-loaded microsphere suspension

Various formulations of drug-loaded microsphere suspension were prepared by utilizing Box–Behnken design employing solvent evaporation method using following polymer polycaprolactone (PCL) (7–10% w/v), HPMC (2–4% w/v) and PVA (2–4% w/v). PCL, span 20 and naringin were dissolved in chloroform at room temperature under stirring. This organic solution was added dropwise in an aqueous phase containing water, HPMC, PVA and Tween 80 under magnetic stirring at room temperature. Microspheres were instantaneously formed by rapid solvent diffusion. Chloroform and a large proportion of water were eliminated at 35 °C to a final suspension volume of 30 mL. The schematic diagram (Fig. 1) was followed.

2.3. Preparation of standard curve of naringin

A standard solution is prepared by dissolving 100 mg of naringin in 100 mL of simulated intestinal fluid without enzyme (pH 6.8). Then from this stock solutions naringin at different concentrations (1 to 30 μg/mL) were prepared by diluting it with simulated intestinal fluid without enzyme (SIF; pH 6.8). Absorbance values of each concentration were noted at λ_{max} 283 nm using a UV-spectrophotometer (Shimadzu, Japan) and a standard calibration curve was made by plotting absorbance value against concentration. The calibration equation (Absorbance = 0.019 * concentration − 0.003) of naringin was generated and it was found to be linear between 1 and 30 μg/mL concentration range, thus it follows Beer-Lambert's Law. The regression value was found to be 0.999.

2.4. Determination of drug entrapment efficiency and particle size analysis

The definite volume of the prepared suspensions was taken and diluted in 100 mL phosphate buffer so that the drug concentration was about 12.5 μg/mL. The samples were stirred using magnetic stirrer at 100 rpm. One milliliter of the sample was withdrawn and was replaced

Table 1
Independent process variables, coded units and their levels used for Box-Behnken design.

Independent process variables	Coded units	Levels		
		−1 (low)	0 (medium)	+1 (high)
PCL concentration (% w/v)	X_1	7	8.5	10
HPMC concentration (% w/v)	X_2	0	2	4
PVA concentration (% w/v)	X_3	0	2	4