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Research paper

Box-Behnken experimental design for preparation and optimization of ciprofloxacin hydrochloride-loaded $CaCO_3$ nanoparticles

Solmaz Maleki Dizaj [a, b], Farzaneh Lotfipour [c], Mohammad Barzegar-Jalali [d], Mohammad-Hossein Zarrintan [d], Khosro Adibkia [d, *]

[a] Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran
[b] Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[d] Drug Applied Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

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ABSTRACT

The purpose of this study was to prepare and optimize ciprofloxacin hydrochloride loaded-$CaCO_3$ nanoparticles, using a chemical precipitation method and the Box-Behnken experimental design methodology (using Minitab 16). The molar ratio of $CaCl_2$:Na_2CO_3 (X_1), the concentration of drug (X_2) and the speed of homogenization (X_3) were selected as independent variables while the particle size and entrapment efficiency were considered as dependent variables. Contour plots and surface plots were used for further understanding of the interaction between the different variables. The results indicated that the speed of homogenization was the main contributing variable for particle size; while, in the case of entrapment efficiency, the drug concentration showed the major role. The optimum values for the molar ratio of $CaCl_2$:Na_2CO_3, the concentration of drug and the speed of homogenization were found to be 1:1 ratio in mole, 2.18 g/dl and 12,000 rpm, respectively, when entrapment efficiency and particle size were predicted as 37.99% and 107.26 nm, respectively. It was concluded that chemical precipitation technique alongside the Box-Behnken experimental design methodology are fast and useful methods for preparation and optimization of drug incorporated calcium carbonate nanoparticles.

1. Introduction

Osteomyelitis, bone infectious disease, is caused by a variety of pathogens [1]. *Staphylococcus aureus* (*S. aureus*) is the organism most commonly isolated from all forms of osteomyelitis. *S. aureus* infects bone cells and provides a reservoir of bacteria [2]. According to researches, targeting of infected bone by antibiotics may be more appropriate for the treatment of chronic bone infection than excluding only the pathogens colonized in the bone matrix [1,3]. However, clinical utilization of antibiotics has shown obvious disadvantages such as; side effects after systemic administration, very low accessibility of the infected zone to blood flow, inducing pathogenic resistance to the antibiotic therapy and irretrievable bone loss [4–6].

Local delivery systems for antibiotics have been suggested since the 1970s [7]. At first, beads composed of poly (methyl methacrylate) were introduced for local delivery of antibiotics to bone cavities [8]. These systems are still used in the treatment of osteomyelitis. Nevertheless, these systems have numerous problems, including: non-biodegradability, bio film formation, need to surgically removal and tendency to show burst release [5]. Another commonly used carrier to deliver antibiotics into bone cavities is calcium sulfate. It was utilized as a carrier for vancomycin and gentamicin, to reduce the risks of infections attributable to the implantation of materials [9–11]. Collagen was also used as an antimicrobial carrier to transport the drug into bone cavities [6,12].

Delivery of antibiotics by novel drug delivery systems such as nanoparticles is one of the successful procedures for efficient control of the physicochemical behavior of a drug, target the drug to the desired site, and thus decrease the side effects [13–16].

* Corresponding author. Faculty of Pharmacy, Tabriz University of Medical Sciences, Golgasht Street, Daneshgah Ave., Tabriz, Iran.
E-mail address: adibkia@tbzmed.ac.ir (K. Adibkia).

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Pharmaceutical nanoparticles are ultrafine colloidal particles with size commonly ranging between 10 and 1000 nm which incorporate the drug(s) and show different properties in comparison with their own original materials [17,18]. Antimicrobial-loaded nanoparticles can enter into the cells via endocytosis and hence, release the drug to eradicate microbe-induced intracellular infections [16]. Calcium carbonate ($CaCO_3$) nanoparticles are the natural choice for antibiotic delivery platforms in bone therapy. $CaCO_3$, the natural mineral component of the bone, is of low cost, accessible, biocompatible, bioresorptive and an osteoconductive material [19–22]. Due to the slow degradation of $CaCO_3$ matrices, these nanoparticles can also be used as sustained release systems, to maintain the drugs in targeted sites for extended times after administration [23–25]. Therefore, $CaCO_3$ nanoparticles could be applied as potential drug carriers in bone related diseases such as osteomyelitis.

There are some methods for the preparation of $CaCO_3$ nanoparticles including emulsion based methods [23], high pressure homogenization [26], chemical precipitation methods [27], decomposition of cockle shells [28], flame synthesis [29], spray drying [30]. Among these methods, precipitation approach compared with other methods provides a facile, simple and rapid way for low cost and size-controlling production, which does not need expensive raw materials and complicated tools [25,31–33]. Not requiring organic solvents also is one of the great advantages of this process [32].

Ciprofloxacin hydrochloride is a synthetic antibiotic which belongs to the second generation of fluoroquinolones [34]. It has been beneficially administrated to eradicate various pathogens that cause common bone infectious diseases such as osteomyelitis [35]. Preparation of a local implant of ciprofloxacin hydrochloride using hydroxyapatite and tricalcium phosphate in combination with poly D,L-lactide (PLA) has been reported in the study of Castro et al. Their findings revealed that the serum concentration of the drug appropriately increased close to the implant site [36]. Dillen et al. prepared ciprofloxacin-loaded nanoparticles with a controlled release manner. Their results showed that the activity of these nanoparticles against *S. aureus* was comparable with an equally concentrated ciprofloxacin solution [37].

Response Surface Methodology (RSM) is a collection of useful statistical techniques for analyzing the effects of several independent variables [38]. The Box-Behnken designs contains fewer design points, and is less expensive compared to central composite designs with the same number of factors. However, they are not appropriate for sequential experiments because of their variable factorial design [39]. Considering the ideal potentials of $CaCO_3$ especially osteophilicity and osteoconductivity properties, as well as the advantages of nanoparticulae based drug delivery systems, the aim of the present investigation was to carry out a statistical study on the preparation of ciprofloxacin hydrochloride-$CaCO_3$ formulations via a chemical precipitation method. For this purpose, a 3-factor, 3-level Box-Behnken design was used to derive a polynomial equation and construct contour plots.

2. Materials and methods

2.1. Materials

Calcium chloride and sodium carbonate were purchased from Merck Company (Darmstadt, Germany) and ciprofloxacin hydrochloride was supplied from Temad Co. (Tehran, Iran).

2.2. Methods

2.2.1. Experimental design

The molar ratio of $CaCl_2$:Na_2CO_3 (X_1), drug concentration (X_2) and the speed of homogenization (X_3) were selected as three independent variables. The coded and un-coded descriptions of these variables are given in Table 1. Fifteen formulations were designed based on the ranges of independent variables (Table 1) by a 3-factor, 3-level Box-Behnken methodology. Details of experimental design for these formulations are shown in Table 2.

2.2.2. Preparation of ciprofloxacin hydrochloride-$CaCO_3$ nanoparticles

All fifteen predicted formulations were prepared via chemical precipitation method [27]. For this purpose, 100 μl aqueous solution of $CaCl_2$ and 100 μl aqueous solution of ciprofloxacin hydrochloride were magnetically stirred at 800 rpm (Heidolph-Germany) for 15 min. Then, the mixture was homogenized (Ultra-Turrax-Germany); and 100 μl aqueous solution of Na_2CO_3 was added drop-wise into the mixture. The homogenization was continued for 15 min (Table 2) until the nanoparticles were precipitated. Subsequently, 5 ml of distilled water was added into the obtained suspension. The suspension of nanoparticles was centrifuged (Eppendorf-Germany) in 12,000 rpm for 5 min to separate the nanoparticles.

2.2.3. Characterization of nanoparticles

2.2.3.1. Particle size. The average diameter of the nanoparticles was measured via Particle Size Analyzer (PSA) technique (Shimadzu (Sald-2101), Japan) at 25 ± 1 °C. Prior to measurements, the nanoparticles were suspended in distilled water. At least three independent samples were taken for each formulation. Mean particle size for all fifteen samples are shown in Table 2.

2.2.4. Entrapment efficiency

A fraction of the drug incorporated into nanoparticles relative to total amount of the drug is defined as entrapment efficiency. After dissolving 100 mg of the prepared ciprofloxacin hydrochloride - $CaCO_3$ nanoparticles in EDTA 0.5 M (pH 7.5), the amount of the drug in the precipitate was measured by UV–visible spectrophotometer (UV1800 Shimadzu, Japan). Then using previously prepared standard curve (linear in the range of 2–32 mcg/ml, $y = 0.0379 \times - 0.0154$, $R^2 = 0.9998$), the percent of entrapment efficiency was calculated according to the following equation:

$$EE\% = (\text{drug content}/\text{total drug added}) \times 100$$

where; EE% represents the entrapment efficiency percent. Each entrapment efficiency data was reported as an average of three calculations (Table 2).

2.2.5. Effect of the variables

After preparation and characterization of all fifteen samples, the particle size and entrapment efficiency values of each sample were added into the design environment of software. To study the effect of three independent variables (X_1, X_2 and X_3) on the characteristics of drug loaded-$CaCO_3$ nanoparticles (particle size and entrapment

Table 1
Factors setting in the design.

Factors/codes	−1	0	1
Molar ratio of $CaCl_2$: Na_2Co_3 (X_1)	1:1	3:1.5	5:2
Drug concentration (X_2); g/dL	1	2	3
Speed of homogenization (X_3); rpm	2000	7000	12,000