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Ciprofloxacin Hydrochloride Encapsulated into PLGA Nanoparticles for Drug Delivery Application: Fractional Factorial Design

Tajudeen Adebileje[1,2*], Sikiru Adebileje[3], P. O. Aye[2]

[1]Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences-International Campus (TUMS-IC), Tehran, Iran
[2]Department of Mathematical Sciences, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria
[3]Department of Medical Physics and Biomedical Engineering, Tehran University of Medical Sciences-International Campus (TUMS-IC), Tehran, Iran
Email: *tajudeenayodele@gmail.com, *tajudeenadebileje@gmail.com, *t-ayodeleadebileje@razi.tums.ac.ir

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Abstract

Over several decades, poly (lactic-co-glycolic acid) (PLGA) have been widely used as Micro- and Nano-carriers of therapeutic agents for drug delivery applications. However, encapsulation process of therapeutic agents into PLGA Nanoparticles (NPs) necessitates a defined step to understand the effects and interactions of parameters involved in production process. In pharmaceutics formulations, compared to one factor at a time (OFAT) approach, statistical design of experiments (DOE) supersedes OFAT approach due to limited number of experiments required to investigate effects and interactions of a process parameters. The major objectives of the present study were to: 1) prepare and understand the effect of selected formulation parameters on particles size and drug recovery of PLGA NPs encapsulating Ciprofloxacin Hydrochloride (Cip-HCl) using a fractional factorial design (FFD) as a DOE approach; 2) understand the *in-vitro* release of Cip-HCl from PLGA NPs. Cip-HCl loaded PLGA were prepared by $W_1/O/W_2$ double emulsion solvent evaporation (DESE) method using poly-vinyl alcohol as a stabilizer. The Sizes of NPs were within 202 nm to 530 nm and percentage Cip-HCl recovered from dried NPs were within 1.7% w/w to 15.7% w/w. Increasing concentrations of PLGA and Cip-HCl was observed to increase NPs size. Increasing PVA concentration was observed to either reduce or increase NPs size. Increasing PLGA concentration was observed to increase the amount of Cip-HCl recovered. Within 1 - 24 hours, optimized formulations shows a controlled release of Cip-HCl from PLGA NPs.

Subject Areas

Medicinal Chemistry, Pharmacology

Keywords

Ciprofloxacin Hydrochloride, PLGA Nanoparticles, Drug Recovery, Double Emulsion Solvent Evaporation, Fractional Factorial Design

1. Introduction

Recently, the branch of nanomedicine that involves encapsulation of active therapeutic agents into micro and nano-particulate delivery system overcomes certain hurdles, such as protection of therapeutic agents against *in-vivo* chemical and enzymatic degradation, reduce administered dosage and toxic effects, improved efficacy and controlled release of therapeutic agents [1]. Aside delivery systems using particles, carriers such as nanobubbles can encapsulate either therapeutic agents or NPs within and unto the bubble structure, and these carriers are known to perform both diagnostic and therapeutic functions in cancer therapy [2]. Nanoparticles (NPs) encapsulating therapeutic agents are able to deliver therapeutic agents to less accessible sites when compared with other dosage form and consequently increases the bioavailability of the therapeutic agents due to their high surface area to volume ratio [3]. Therapeutic agents such as ciprofloxacin hydrochloride (Cip-HCl) which is an antibiotic and a second generation of fluoroquinolone demonstrate a broad spectrum of activity that consequently results in the death of bacterial infections [4]. Implantable ciprofloxacin formulations have been shown to be effective in the treatment of skin and bones infection [5], and have been put forward for management and prevention of endophthalmitis [6] [7].

Nanomaterials such as natural and synthetic polymers can be fabricated into micro- and NPs so as to encapsulate and deliver therapeutic agents or target damaged cells via different route of administration. PLGA as a synthetic polymer have been used in detailed degree for both encapsulation and delivery of therapeutic agents. PLGA particles encapsulating therapeutic agents are commonly prepared by nanoprecipitation (solvent diffusion or solvent displacement), solvent evaporation (either single or double emulsion), salting out, spray drying and solvent extraction techniques [8]. Among these methods, $W_1/O/W_2$ double emulsion solvent evaporation methods are used to encapsulation hydrophilic therapeutic agents. However, obtaining the desired particles properties in terms of size and drug content of particles depends on both the composition (amount) of material used and the technical parameters (such as sonication and stirring parameters) for production.

Cip-HCl as a hydrophilic agent is sparingly soluble from 10 - 33 mg/ml specifically at 10 mg/ml in aqueous acidic solution [9] [10]. Cip-HCl could be encapsulated into PLGA matrix using double emulsion solvent evaporation method. A study prepared positively charged ciprofloxacin-loaded NPs by water-in-oil-in-water (w/o/w) double emulsion using Eudragit® RS100 and RL100

as non-biodegradable polymers and PLGA as a biodegradable polymer [11]. However, the production of PLGA NPs encapsulating Cip-HCl requires designed procedure, so as to understand the effects and interactions of its production parameters.

Design of experiments (DOE) such as full factorial, central composite, Box-Behnken Design (BBD) and fractional factorial design (FFD) are some useful approach that are widely used in pharmaceutical development to understand the effect of process parameters affecting experimental outcome. Considering the multiple formulation parameters (both technical and composition parameters) in double emulsion solvent evaporation method. While keeping some technical parameters fixed, no work so far as detailed the effects of composition parameters affecting the particles size of PLGA encapsulating Cip-HCl, and precisely on the percentage of Cip-HCl recovered from PLGA NPs using a FFD. Using BBD as a response surface methodology based on DOE, the effect of selected process parameters of PLGA NPs encapsulating bovine serum albumin was recently investigated [12].

In this study, FFD was used to plan and analyze experiments of PLGA NPs encapsulating Cip-HCl. NPs were prepared by double emulsion solvent evaporation method and we attempt to investigate the effect of composition parameters on particles size of PLGA NPs encapsulating Cip-HCl and the percentage drug recovered (DR) from PLGA NPs. Technical experimental parameters of double emulsions were kept constants, concentrations of PLGA, Cip-HCl and PVA were selected to be the three composition parameters affecting particles size and percentage DR. Furthermore, we investigated the *in-vitro* release of Cip-HCl loaded PLGA NPs from validated and optimized formulations derived from FFD.

2. Materials and Methods

2.1. Materials

High purity medical grade PLGA (50:50 with molecular weight 50 kDa) was purchased from Shenzhen Esun Industrial Co., LTD (Shenzhen, China). Polyvinyl alcohol (PVA) was purchased from VAM & P.VAL Co. Ltd (Tokyo, JAPAN). Cip-HCl was purchased from Sigma-Aldrich Inc. (St. Louis, MO). Chloroform (molecular mass = 119.3 g/mole, spec. density: 1.48 g/cm^3) was purchased from Dr. Mojallali chemical complex Co (Tehran, Iran).

2.2. Design of Experiments

In experiments, factors are defined specifications at a given extent or levels e.g. reaction time, concentration, temperature, pH, etc. that affects the outcome of a procedure. The classically applied univariate one-factor at-a-time (OFAT) approach for experimental validation has disadvantages, such as the interactions between factors of an experimental outcome are not taken into consideration. Compared to OFAT approach, statistical DOE can be used to study interactions

between experimental process parameters [13]. Full factorial DOE, is a branch of DOE, the general rule to determine the total number of experiments follows the formula L^K (where K is the numbers of factors at L levels of each factor to study). Therefore, factorial design with 2 factors at 2 levels of each factor requires $L^K = 2^2 = 4$ experiments, factorial design with 2 factors at 3 levels of each factor requires 3^2 experiments, and that of 3 factors at 3 levels of each factor requires $3^3 = 27$ experiments. However, disadvantages in full factorial DOE is that the numbers of experiments increases as K and L increases.

A fractional factorial DOE is defined as a fraction of a full factorial design, which follows a general rule of L^{K-p} where p is the fraction of original factorial design. The subset or fraction of full factorial design is chosen so as to report information about most relevant features of the problem studied. Therefore, a fraction of 4 factors at 3 levels of each factors of factorial experiments generates $3^{4-1} = 27$ experiments instead of 81 factorial experiments, also a fraction of 3 factors at 3 levels of each factors of factorial experiments generates $3^{3-1} = 9$ experiments instead of 27 factorial experiments.

2.3. Experimental Formulation

Water-in-Oil-in-Water ($W_1/O/W_2$) double emulsion solvent evaporation method was designed for encapsulation of Cip-HCl loaded into PLGA NPs. While setting the volume of inner aqueous phase (W_1) to 1 ml, volume of organic phase (O) to 4 ml, volume of external aqueous phase (W_2) containing PVA to 25 ml. Technical parameters such as, first emulsion sonication process at 100 W for 60 sec, second emulsion sonication process at 100 W for 120 sec, organic solvent (chloroform) evaporation rate at 1000 rpm for 4 hours at room temperature, NPs collection during centrifugation at 12,000 rpm for 50 minutes were also kept constant.

Concentrations of Cip-HCl dissolved in inner aqueous phase, PLGA dissolved in organic phase (chloroform) and PVA at the external aqueous phase (distilled water) were suggested to be the 3 independent preparation parameters affecting particles size and percentage DR. Considering 3 different level of each composition parameters (see Table 1), 3^3 FFD was designed using STATISTICA™

Table 1. Concentration levels of composition parameters considered for experimental design.

	Factors	Composition parameters	Low level	Centre level	High level
1	X_1 (%w/v)	Concentration of PLGA in organic phase (Chloroform)	1.0	1.5	2.0
2	X_2 (%w/v)	Concentration of Cip-Hcl in inner aqueous phase (Distilled water)	0.5	1.0	1.5
3	X_3 (%w/v)	Concentration of PVA in external aqueous phase (Distilled water)	0.1	0.2	0.3

Ver.12.0 software package (Stat Soft Inc., USA) to generate 9 experiments (see Table 2). PLGA NPs were prepared, the particles size and percentage DR were also determined. Statistical analysis on both particles size and DR was carried out using two-way linear-linear interactions on composition parameters.

A regression equation was obtained from statistical analysis for the prediction of both particles size and percentage DR. To validate the regression equations obtained from FFD, Two (2) additional experiments were carried out after the predicting the values of NPs size and percentage the regression equations. Finally, *in-vitro* release of Cip-HCl from PLGA NPs was then performed on the two optimized formulations.

2.4. Preparation of PLGA Nanoparticles Encapsulating Cip-Hcl

Table 2 demonstrates parameters considered for 11 experiments of Cip-Hcl prepared $W_1/O/W_2$ double emulsion solvent evaporation method. Briefly, first emulsion (W_1/O) was obtained by dissolving Cip-HCl into 1 ml of distilled water and was emulsified with 4 ml organic phase (chloroform) containing PLGA using a probe sonicator, (Development of Ultrasound Technology (Tehran, Iran)). The W_1/O emulsion was dispersed into 25 ml of distilled water containing PVA to obtain a $W_1/O/W_2$ double emulsion (Table 1 and Table 2). The $W_1/O/W_2$ double emulsion were sonicated and organic solvents were allowed to evaporate under magnetic stirrer (1000 rpm) for 4 hours at room temperature. Samples were centrifuged and the pellets were washed twice using Model 5810R, Eppendorf centrifuge (Hamburg, Germany). Subsequently, the pellets obtained were freeze-dried and stored for analysis. Freeze-drying process were performed at

Table 2. Experimental design with designed composition parameters including experimental observed and predicted outcomes (NPs size and percentage drug recovered).

Standard run	Composition parameters			Experimental observed			Predictions	
	PLGA (%w/v)	Cip-HCl (%w/v)	PVA (%w/v)	Size (nm)	DR (%w/w)	DL (%w/w)	Size (nm)	DR (%w/w)
1	1.0	0.5	0.1	443	5.85	1.125	443.00	5.85
2	1.0	1.0	0.3	235	3.70	1.490	237.33	4.89
3	1.0	1.5	0.2	302	1.70	0.990	299.67	0.51
4	1.5	0.5	0.3	372	15.70	1.455	369.67	14.51
5	1.5	1.0	0.2	384	4.28	1.425	384.00	4.28
6	1.5	1.5	0.1	436	2.96	1.155	438.33	4.15
7	2.0	0.5	0.2	347	8.86	1.055	349.33	10.05
8	2.0	1.0	0.1	358	5.28	1.055	355.67	4.09
9	2.0	1.5	0.3	353	3.21	1.120	353.00	3.21
Validation and optimization experiments								
1 V	1	0.2	0.1	530	7.83	1.618	558.84	10.01
2 V	1	0.5	0.4	202	8.49	1.301	215.00	19.38

DL: Drug Loading; DR: Drug Recovered.

0.401 mbar and −50˚C for 48 hours using TELSTAR technologies S.L, Lyo-Quest-55 (Terrassa, Switzerland).

2.5. Particles Size Analysis

10 mg of lyophilized particles were dispersed into 5 ml of distilled water and stirred gently for 15 minutes on a magnetic stirrer, the particles size were obtained by dynamic light scattering (NPs Size Analyzer, Model: SOS I, K-ONE (Seoul, South Korea)), the median hydrodynamic diameter (d50) of particles obtained by the instrument was considered as the NPs size (Table 2).

2.6. Quantification of Drug Content

20 mg of lyophilized particles were dispersed into 5 ml chloroform and then 10 ml distilled water was added to extract Cip-HCl. The mixture was stirred on a magnetic stirrer for 3 hours to break down the polymer chains and subsequently evaporate the chloroform. The samples were centrifuge at 4000 rpm for 15 minute to separate aqueous solution containing Cip-HCl. Using a standard curve of known Cip-HCl concentrations, separated Cip-HCl solution were quantified by UV-spectrophotometer, CECIL CE 7250 (Cambridge, United Kingdom) at a wavelengths of 279 nm. The drug content was then transformed to percentage drug loading (Equation (1)) and percentage DR (Equation (2)).

$$\text{Drug loading } \% = \frac{\text{mass of drug in NPs}(\text{mg})}{\text{mass of NPs recovered}(\text{mg})} * 100\% \quad (1)$$

$$\text{Drug Recovery } \% = \frac{\text{mass of drug in NPs}(\text{mg})}{\text{mass of drug initially used}(\text{mg})} * 100\% \quad (2)$$

2.7. Drug Release

40 mg of freeze-dried PLGA particles obtained from additional optimized experiments (experiments 1 V and 2 V) were dispersed individually in 10 mL of PBS (pH 7.4), and were rotated on a Rotary mixer, Fan azmar gostar (Tehran, Iran) at 20 rpm and room temperature. Samples (2 mL) were withdrawn for centrifugation for 20 min at 10,000 rpm and 5˚C, and the supernatant were analyzed for drug content by UV spectroscopy at a wavelength of 279 nm. The withdrawn volume were subsequently replaced with fresh release medium (PBS) between 1 - 24 hours.

3. Results

Results were discussed in terms of particles size, recovered Cip-HCl from PLGA NPs and the release of Cip-HCl from PLGA NPs. The drug loading is defined as the recovered amount of Cip-HCl in the NPs obtained after the freeze-drying process of pellets obtained from centrifugation (Equation (1)). The DR is defined as the amount of Cip-HCl recovered from NPs related to the amount of drugs initially used (Equation (2)).

Experimental Optimization and Validation Steps

The results in Table 3 demonstrates the effect estimates obtained from two way linear to linear interactions of experimental formulation parameters on NPs size and DR. Quadratic effect of PLGA, linear effect of PVA and the interaction between PLGA and Cip-HCl shows to be statistically significant on NPs size due to their *p*-value below 0.05. The effects of process parameters on percentage DR were observed to be statistically insignificant due to the *p*-value above 0.05. Figure 1 and Figure 2 shows the Pareto chart which represents the standardized effect estimates ranked in order of significances of process parameters affecting NPs size and percentage DR respectively.

Table 3. Effect estimates on NPs size and percentage drug recovered.

Factor	Nanoparticles size MSR = 32.6667 R^2 = 0.999			Drug recovered MSR = 8.5443 R^2 = 0. 941		
	Effect	Std.Err.	*p*-value	Effect	Std.Err.	*p*-value
Mean/Interc.	358.89	1.91	0.0034	5.73	0.97	0.1073
PLGA (L)	26.00	4.67	0.1131	2.03	2.39	0.5508
PLGA (Q)	57.67	4.04	0.0445	2.88	2.39	0.3963
Cip-HCl (L)	−23.67	4.67	0.1239	−7.51	2.39	0.1958
Cip-HCl (Q)	−49.83	4.04	0.0515	−1.96	2.07	0.5169
PVA (L)	−92.33	4.67	0.0321	2.84	2.39	0.4449
PVA Q)	29.83	5.72	0.1205	−3.09	2.92	0.4823
PLGA (L) * Cip-HCl (L)	103.33	8.08	0.0497	−3.84	4.13	0.5234

MSR: Mean Square Residual; L: linear effect of composition parameters; Q: Quadratic effect of composition parameters; PLGA (L) * Cip-HCl (L): Linear interaction between PLGA and Cip-HCl; Std.Err: Standard error.

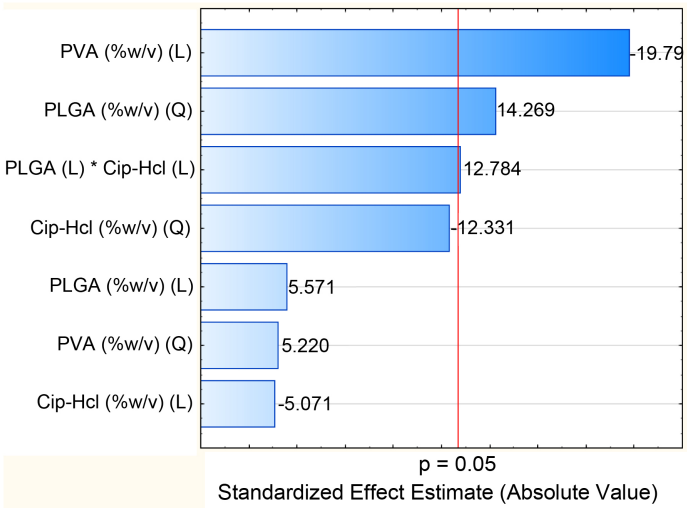

Figure 1. Pareto chart demonstrating standardized effects of composition parameters on NPs size ranked in their order of significances. L: linear effect of composition parameters; Q: Quadratic effect of composition parameters; PLGA (L) * Cip-HCl (L): Linear interaction between PLGA and Cip-HCl.

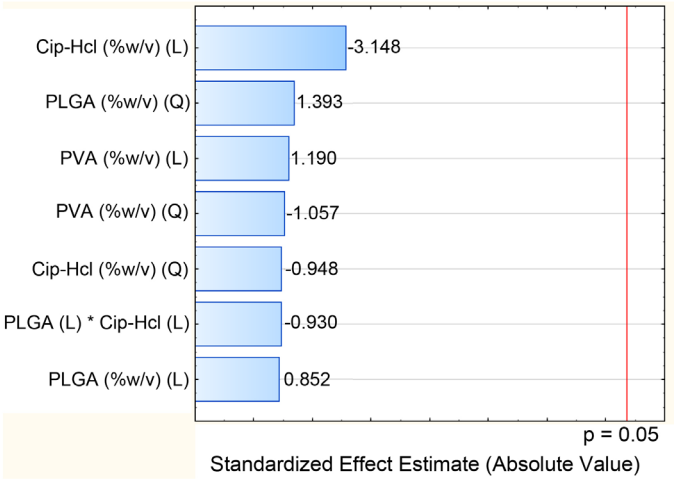

Figure 2. Pareto chart demonstrating the standardized effect of composition parameters on percentage drug recovered ranked in their order of significances. L: linear effect of composition parameters; Q: Quadratic effect of composition parameters; PLGA (L) * Cip-HCl (L): Linear interaction between PLGA and Cip-HCl.

Equations (3) and (4) represent a model obtained from the regression analysis on FFD for the prediction of NPs size and percentage DR, with X_1, X_2 and X_3 representing Concentrations of PLGA, Cip-HCl and PVA respectively. A good correlation was established between predicted and observed value as indicated by $R^2 = 0.999$ on NPs size and $R^2 = 0.941$ on DR. Mean square residual errors obtained in this model for the predictions of NPs size and percentage DR were 32.6666 and 8.544267 respectively, corresponding to standard error of 5.72 and 2.92 for both NPs and percentage DR $\left(\text{Standard error} = \sqrt{(\text{MS Residual})}\right)$.

At a desirability values (Concentrations of PLGA = 2 %w/v, Cip-HCl = 1.5 %w/v, and PVA = 0.3 %w/v), response surface curve at a two dimensional space of each parameters studied were generated to study their effects on NPs and percentage DR. Additional experiments (1 V and 2 V) were predicted from regression Equation (3) and Equation (4) prior to particles preparation. The optimized formulations 1 V and 2 V were observed to show similar results to predicted values on NPs size and minimal deviation on percentage DR.

Equation (3): Particle size

$$Y = 332 + 511.33X_1 - 230.67X_1^2 - 732.33X_2 + 199.33X_2^2 + 732X_3 - 2983X_3^2 + 206.67X_1 * X_2 \quad (3)$$

Equation (4): Drug recovered

$$Y = -11.34 + 44.27X_1 - 11.52X_1^2 - 11.67X_2 + 7.84X_2^2 - 109.4X_3 + 309X_3^2 - 7.68X_1 * X_2 \quad (4)$$

4. Discussions

4.1. Effect of Process Parameters on NPs Size

The particles size obtained were in the range between 202 nm to 530 nm which

seems to be well suited for a drug delivery of Cip-HCl in ophthalmic application in which the particles size should not exceed 10 μm so as to avoid scratching [14]. Figures 3-5 shows that increasing simultaneously the concentrations of PLGA and Cip-HCl increases NPs size. The effect of PVA in Table 3 demonstrates that increasing the concentration of PVA either reduce (linear effect) or increase (Quadratic effect) NPs size.

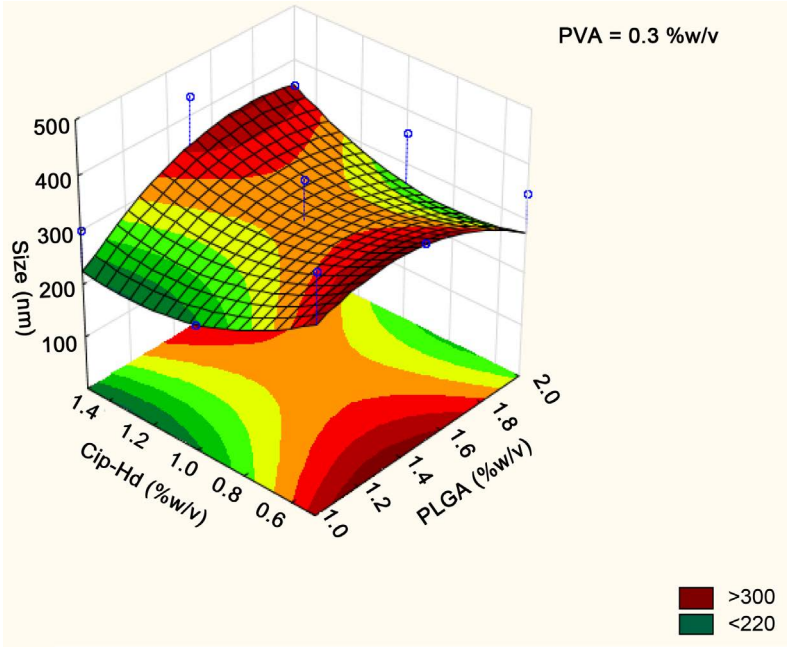

Figure 3. Response surface curve showing the interaction between PLGA and Cip-HCl on particles size when PVA concentration is fixed at 0.3 %w/v.

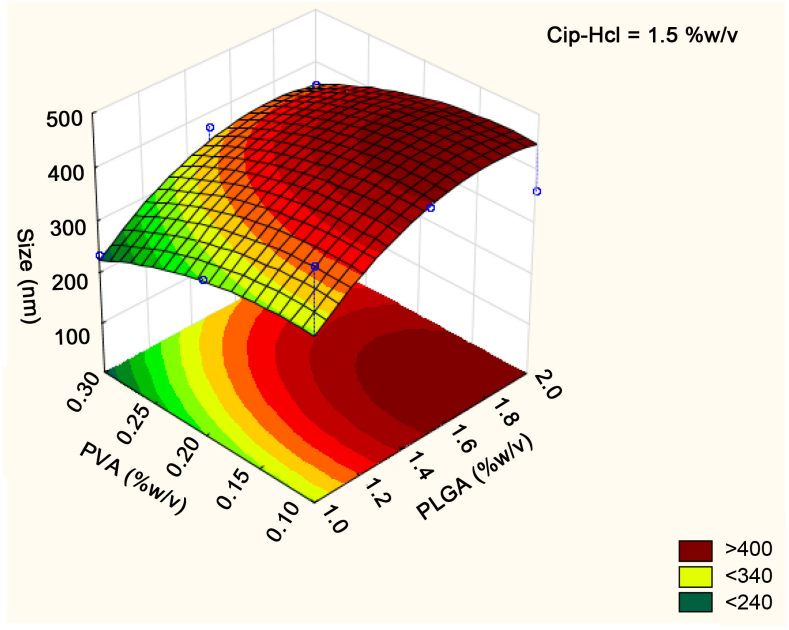

Figure 4. Response surface curve showing the interaction between PLGA and PVA on particles size when Cip-HCl concentration is fixed at 1.5 %w/v.

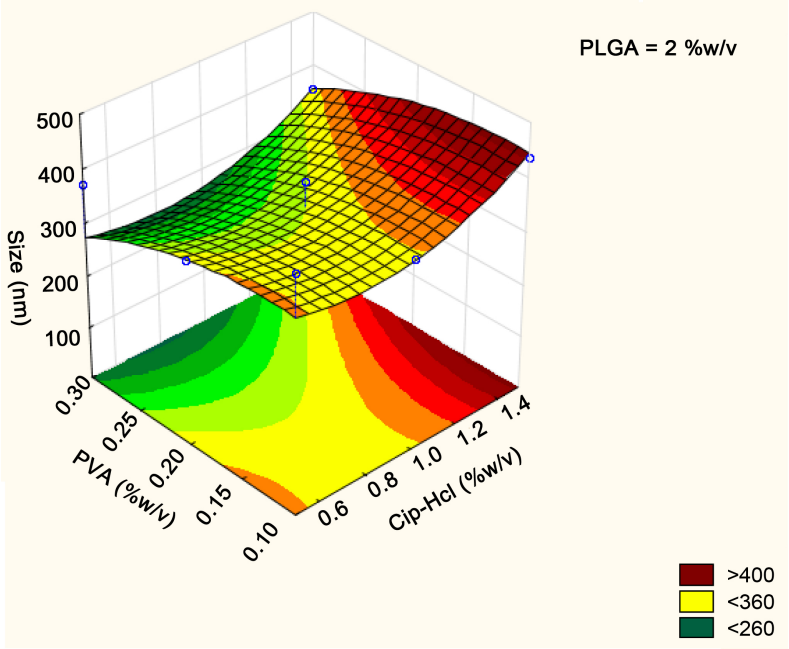

Figure 5. Response surface curve showing the interaction between Cip-HCl and PVA on particles size when PLGA concentration is fixed at 2 %w/v.

In similar work, Increasing PLGA concentration had been observed to increase the mean diameter of NPs [15]. Either an increase or reduction in the size of PLGA NPs has also been observed while increasing the concentration of PVA [16]. In recent studies, the size of PLGA NPs have been observed to be dependent on the viscosity of emulsion during preparation, such that, low viscosity emulsions results to small particles size and high viscosity emulsions result to higher particles size [17] [18].

Increase in the content of PLGA, Cip-HCl and PVA can leads to increment in viscosity of emulsion droplets and therefore leads to an increase in the size of PLGA NPs encapsulating Cip-HCl. However, linear effect of PVA in **Figure 1** shows that increment in PVA concentration leads to reduction in NPs size and was observed to be the most significant effect affecting the particles size. While the effect of PVA concentration that leads to increment in NPs was observed to have a non-significant and negligible effect on NPs size (Quadratic effect of PVA). Therefore, the effect at which the concentration of PVA either reduce or increase the NPs is suggested to be attributed to stability and viscosity effect of the formulations.

4.2. Effect of Process Parameters on Cip-HCl Recovery

The percentage of Cip-HCl obtained from NPs after freeze drying process were in the range of 1.7 - 15.7, while the percentage drug loading reported were in the range of 0.99 - 1.49 (data not analyzed using statistical software). Increasing PLGA concentration was observed to increase the percentage DR after centrifugation. The increase in the amount of PVA was observed to either reduce or in-

crease the percentage DR of NPs while the amount of Cip-HCl recovered from PLGA NPs was reduced at high concentration of Cip-Hcl. The Pareto chart of DR and Figure 6 shows that the amount of Cip-HCl recovered increases while increasing the concentration of PLGA. However, Statistical analysis of effect estimates of composition parameters on percentage DR proves to be non-significant. A possible explanation could be attributed to excess PVA, non-encapsulated drug and some possible NPs removed during centrifugation/washing process, which might lead to lower percentage DR, even at high concentration of Cip-HCl. since the centrifugation parameters are responsible for the collection of particles at a certain range [19] [20].

In recent study, Low drug loading (1.9%) of PLGA particles encapsulating ciprofloxacin prepared by W/O/W double emulsion solvent evaporation have also been reported by Dillen *et al.* [21].

Despite the level of insignificance, and centrifugation parameters, the effect of obtaining high percentage of drug in NPs can be attributed to the first emulsion process, such that, high concentration of PLGA at the organic phase can be able to encapsulate more Cip-HCl during the emulsification process. A justification for the low amount of experimental Cip-HCl loaded into PLGA NPs here in this study is suggested to be attributed to two possible unclear effects. The first effect is related to the concentrations of both PLGA and PVA, such that, an increase in their concentrations is equivalent to additional materials being introduced to each formulation which tends to reduce amount of Cip-Hcl recovered. The second effect might be attributed to when Cip-Hcl concentration is high, some Cip-Hcl might diffuse from first emulsion to the external aqueous phase of

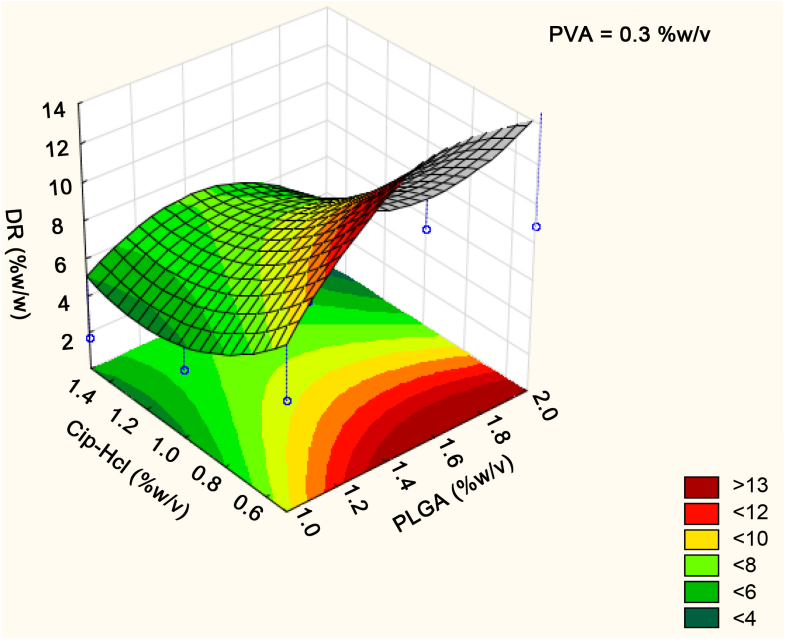

Figure 6. Responds surface curve demonstrating the interaction between PLGA and Cip-HCl on percentage drug recovered when PVA concentration is fixed at 0.3 %w/v.

double emulsion due to drug solubility in water. Nevertheless, it should be noted that percentage of Cip-HCl recovered can be attributed to the amount of NPs collected during the process of centrifugation.

4.3. Drug Release

In the present study, **Figure 7** shows that the optimized formulations (1 V and 2 V) followed a similar prolonged release of Cip-HCl from PLGA NPs, approximately 41% - 45% of Cip-HCl was released slowly without a burst release during 24 h from the PLGA NPs.

In recent studies, a slow release pattern of drugs has been observed from PLGA NPs [18] [22]. It has also been suggested that PVA as a stabilizer affects the release of drugs from polymeric particles [23]. Also another study suggests that due to larger surface area of NPs, PVA molecules on particles surface reduces the amount of drug released [24].

Although the majority of PVA, non-encapsulated drug and possibly some NPs were removed during centrifugation and washing steps. Here, we suggest two possible effects of controlled release of Cip-HCl from PLGA NPs. The first effect can be as a result of less Cip-HCl on NPs surface, therefore erosion of the polymer chains or degradation of PLGA matrix might leads to controlled release of Cip-HCl from PLGA NPs. The second effect might be attributed to PVA attached to NPs surface which might cause a slow release of Cip-HCl.

5. Conclusion

In this study, PLGA NPs encapsulating ciprofloxacin hydrochloride were prepared by water-oil-water ($W_1/O/W_2$) double emulsion solvent evaporation method. Technical parameters (volume of solvents used, stirring speed sonication and centrifugation parameters for NPs collection) of double emulsion were kept

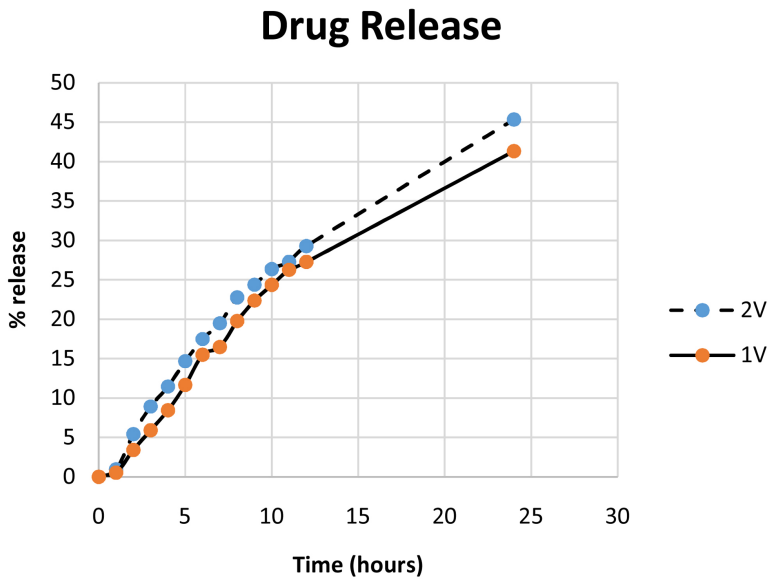

Figure 7. *In-Vitro* release of Cip-HCl from PLGA NPs.

constant. The effects of composition parameters on NPs size and percentage drug recovery of PLGA NPs encapsulating ciprofloxacin hydrochloride were investigated by using a FFD. Composition parameters considered were concentrations of Ciprofloxacin Hydrochloride dissolved in inner aqueous phase (W_1), PLGA dissolved in chloroform phase (O), and PVA at external aqueous phase (W_2). Increasing the concentrations of composition parameters was observed to increase the NPs size, high concentration of PVA was also observed to reduce particles size. Increasing the concentration of PLGA was observed to increase percentage DR. Optimum formulations were obtained, equations to predict both particles sizes and percentage drug recovery of PLGA NPs were also derived. Furthermore, optimum formulations showed a prolonged release of ciprofloxacin hydrochloride from PLGA NPs within 1 - 24 hours. However, during experiments, centrifugation as a technical parameter was observed to affect the amount of drug recovered due to low amount of NPs collected. Conclusively, this experimental approach provides an optimum condition to produce PLGA NPs encapsulating ciprofloxacin hydrochloride for drug delivery applications.

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Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, International Campus, Tehran University of Medical Sciences, Iran.

Conflict of Interests

The authors declare that there is no conflict of interest.

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