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Original Article

Formulation and physicochemical characterization of azithromycin-loaded cubosomes

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Abstract

Background and purpose: Azithromycin (AZ) is a macrolide antibiotic that is soluble in saliva pH; its bitter taste can be well sensed, decreasing the ability of the patient to get the drug. Thus, handling such a bitter taste is challenging in developing the oral formulation. A wide range of methods has been applied to tackle this problem. Cubosomes are considered nanoparticles forming cubic three-dimensional structures with a taste-masking effect. This research aimed to apply cubosomes to mask AZ's bitter taste.
Experimental approach: Cubosomes which contained AZ were obtained by applying the film hydration method. Design expert software (version 11) was then employed for optimizing cubosomes that contained the drug. The encapsulation efficiency, particle size as well as polydispersity index of drug-loaded cubosomes were then subjected to evaluation. Assessment of particle morphology was done through SEM. The antimicrobial qualities of AZ-loaded cubosomes were then assessed by utilizing the disc diffusion method. Then, the taste masking study was carried out by referring to human volunteers.
Finding/Results: AZ-loaded cubosomes were spherical in terms of shape and in the 166-272 nm range, with a polydispersity index of 0.17-0.33 and encapsulation efficiency of 80-92%. The results related to the microbial culture revealed that the antimicrobial qualities related to AZ-loaded cubosomes were like those of AZ. The results obtained by taste evaluation also revealed that the cubosomes could well mask the drug's bitter taste.
Conclusion and implications: These findings, thus, revealed that while the antimicrobial impact of AZ is not under the influence of loading in cubosomes, its taste could be well improved.

Keywords: Azithromycin; Cubosomes; Oral delivery; Taste masking.

INTRODUCTION

Azithromycin (AZ) is known as a macrolide antibiotic that is commonly consumed by the pediatric population for diseases such as otitis media, acute bacterial sinus, tonsillitis, pharyngitis, pneumonia, skin infections, and bronchitis (1). AZ is soluble at saliva pH; however, it is practically insoluble in water, causing the drug to solubilize in the mouth and exposure to taste buds, thus feeling bitter (2). Further, the potential of AZ to decompose in an acidic medium is high (3). A serious problem in the completion of the treatment, however, in sensitive patients as well as children is the obnoxious bitter taste, as a large number of people could not tolerate the drugs' bitter taste and may vomit out, leading to mental stress, grimace, and suboptimal therapeutic value (4). Taste masking can be considered a serious problem for scientists in the pre-formulation as well as final formulation of the liquid oral dosage form (5).

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A wide range of approaches have been applied to tackle this problem; these include encapsulation, granulation, micronization, ion exchange, spheronization, taste suppressants, flavors, and sweeteners (6). Among these standard techniques, the most frequent and at the same time, simple process for a pediatric formulation's taste masking is adding sweeteners and flavor; however, it is not successful for bitter drugs like AZ (7). Nanocarrier systems have recently received the attention of researchers owing to their effectiveness for taste masking purposes. Cubosomes can be considered attractive lyotropic liquid crystals, given the amphiphilic lipids' spontaneous self-assembly in aqueous environments. Characterization of them is done by a three-dimensional cubic inner structure, which is composed of a highly twisted-lipid bilayer as well as two non-intersecting water channels. Cubosomes are made of biocompatible lipids such as glyceryl monooleate (GMO) which could be dispersed in water by applying surfactants such as tween 80 or pluronic. Given their structure, they provide more attractive characteristics when compared to liposomes such as high stability and mechanical rigidity (8). Cubosomes have been applied as an oral drug delivery system to enhance the poorly water-soluble drug's oral bioavailability, like simvastatin (9), cyclosporine A (10), 20 (S)-protopanaxadiol (11), ibuprofen (12), ubidecarenone (13), and tamoxifen citrate (14). Cubosomes have considerable internal and external surface area; thus, they are ideal from a theoretical point of view for drug encapsulation. Encapsulation of drug can be done in the cubosomes water channels or lipid bilayers, thus resulting in isolation from taste buds; this makes them plausible for taste-masking. In addition, the lyotropic liquid crystals' oral liquid form, particularly concerning pediatric medicine, is easier for swallowing and adjusting the dosage. Previous research conducted by Fan *et al.* (15) has substantiated cubosomes taste-making potential as a delivery system for cefpodoxime. The present research study, thus, aimed to develop AZ-loaded cubosomes having a good taste-masking property using the film hydration method. The effects of sonication time as a process variable, as well as formulation variables such as amounts of GMO, pluronic F127 (PF127), and polyvinyl alcohol (PVA) % on different physicochemical properties of cubosomes were evaluated using fractional factorial design. Then, *in vitro* antimicrobial activity of optimized AZ-loaded cubosomes were compared concerning free AZ. The incorporation of AZ into cubosomes has been recently reported by Zheng *et al.* (16) for the treatment of periodontitis in which cubosomes were prepared with melt emulsification and sonication method but the effect of formulation and processing variables on particle characteristics was not examined.

MATERIALS AND METHODS

Materials

AZ powder and AZ suspension (100 mg/5 mL) were bought from Farabi Pharmaceutical Company, Isfahan, Iran. PVA was gotten from Merck, Germany. PF 127 and GMO were received as a gift from Pasaddak Co (Tehran, Iran). Mueller Hinton agar was purchased from Merck, Germany. Dialysis membrane was procured from Sigma, USA. All other chemicals and reagents applied in this research study were in analytical grade.

Experimental design

Our preliminary experiments as well as other studies revealed that the amounts of GMO, PF127, and PVA%, as well as sonication time, impacted the cubosomes physicochemical characteristics. The impact of such factors at two levels was investigated by applying the fractional factorial design. Studied variables as well their levels, as applied in the preparation of AZ-loaded cubosomes, are displayed in Table 1. Runs that were suggested by Design Expert 11 (version 11, USA) Software are represented in Table 2. Dependent parameters were particle size, encapsulation efficiency (EE) %, polydispersity index (PDI), and release efficiency during 2 h in an acidic medium (RE_{2h}%). All experiments were conducted in triplicate. For the determination of each factor's statistical significance, as well as the evaluation of the impact of the independent variables on responses, the use was made of the Design Expert software.

Table1. Variables used in the Design Expert experimental design.

Variables	Symbols	levels	
		I	II
Glyceryl monooleate (mg)	A	100	200
Pluronic F127 (mg)	B	5	10
Sonication time (min)	C	2	4
Polyvinyl alcohol (w/v%)	D	0	2.5%
Particle size	Y_1	Minimize	
Polydispersity index	Y_2	Minimize	
Release efficiency (%) at pH 1.2	Y_3	Minimize	
Encapsulation efficiency (%)	Y_4	Maximize	

Table 2. Composition of different studied azithromycin-loaded cubosomes.

Formulations	Glyceryl monooleate (mg)	Pluronic F127 (mg)	Sonication time (min)	Polyvinyl alcohol (w/v%)
$G_{100}F_{10}S_4P_{2.5}$	100	10	4	2.5
$G_{200}F_{10}S_4P_0$	200	10	4	0
$G_{100}F_{10}S_4P_0$	100	10	4	0
$G_{100}F_5S_2P_{2.5}$	100	5	2	2.5
$G_{100}F_{10}S_2P_{2.5}$	100	10	2	2.5
$G_{200}F_5S_4P_{2.5}$	200	5	4	2.5
$G_{100}F_5S_4P_0$	100	5	4	0
$G_{100}F_5S_2P_0$	100	5	2	0
$G_{200}F_5S_4P_0$	200	5	4	0
$G_{200}F_{10}S_2P_0$	200	10	2	0
$G_{200}F_5S_2P_{2.5}$	200	5	2	2.5
$G_{200}F_{10}S_2P_{2.5}$	200	10	2	2.5

Preparation of AZ-loaded cubosomes

AZ-loaded cubosomes were prepared by applying the film hydration method, according to a previous report, through some minimal modifications (17). Accordingly, 20 mg AZ, diverse amounts of GMO (100-200 mg), and PF127 (5-10 mg), as reported in Table 2, were dissolved in 2 mL chloroform; then organic solvent was evaporated under reduced pressure using a rotary evaporator (Heidolph, Germany), till a thin film of GMO and PF127 was formed. Then, the thin film was hydrated by utilizing 10 mL deionized water that contained 0-2.5%w/v PVA at 70 ± 2 °C. The dispersions were subsequently sonicated by applying a probe sonicator (Bandelin electronic, Germany) for 2-4 min; the pulse was turned off for a period of 2 s and with 2 s intervals. Cubosomes dispersions were kept under stirring conditions for a period of 2 h and then cooled to room temperature; after that, they were stored in glass vials at room temperature for more analyses.

Determination of particle size and PDI of AZ-loaded cubosomes

Determination of particle size, as well as PDI of AZ-loaded cubosomes, was done by applying a zeta sizer (PCS, Zeta sizer 3000, Malvern, UK). Dilution of all formulations was done 20 times with deionized water prior to analysis. Each test was conducted three times.

EE determination

EE% of different formulations was determined by an indirect method. Accordingly, 2 mL of each cubosome formulations were subjected to centrifuging (Sigma 3K30, Germany) by applying a microcentrifuge filter tube (Amicon Ultra, Ireland, cut off 10 kDa) at 14,000 rpm for a period of 10 min; after that, an equal volume of 25 N sulfuric acid was added to the filtrate that contained free drug (18). The obtained mixture was then kept at the temperature of 50 °C for 15 min; determination of absorbance was done by applying the ultraviolet (UV) spectrophotometer (ShimadzuVR, Japan) at 482 nm. Plain cubosomes which were without AZ were then applied as blank samples. The EE% related to AZ in cubosomes was estimated by applying the equation below:

$$EE\% = \left(\frac{\text{total amount of drug added} - \text{free drug}}{\text{total amount of drug added}}\right) \times 100 \quad (1)$$

Investigating the optimized formulation's morphology by scanning electron microscopy

Scanning electron microscopy (SEM; HITACHI S-4160, Japan) was applied to determine the optimized formulation's morphology. Accordingly, one drop of AZ-loaded cubosomes was inserted into the stub. It was subsequently coated under vacuum condition with gold through examination under SEM.

In vitro release of AZ from cubosomes

One mL of each of the formulations was filled in the dialysis bag (with the molecular weight cut-off of 12,000 Da); the bag was then immersed in some glass tubes that contained 20 mL of HCl medium (pH 1.2) at 37 °C for 2 h. At a predetermined time, 1 mL of each of the media was removed and then refreshed with new ones. To determine the released drug, an equal volume of 25 N sulfuric acid was added to the samples; then the mixture was kept at 50 °C for 15 min; determination of absorbance was then carried out by applying the UV spectrophotometer at 478 nm. The RE% parameter was applied for the comparison of the release profile in the acidic medium; it was estimated by applying the equation below:

$$RE\% = \frac{\int_0^t y.dt}{y100.t} \times 100 \quad (2)$$

where y is the released percent at time t.

Freeze-drying of cubosomes

Fifty mL of optimized AZ-loaded cubosomes (equivalent to 100 mg AZ) were subjected to a freeze dryer by applying sucrose to serve as a cryoprotectant in the 1% concentration. The mentioned samples were then frozen by putting the glass vials in a freezer at -20 °C for 24 h; they were then transferred to a freeze dryer (Christ, Alpha 2-4 LD plus, Germany). Sublimation took 48 h at the temperature of -40 °C and pressure of 0.001 bar. Following freeze-drying, the rehydration of the samples was done with 5 mL of water; particle size and PDI were then evaluated as described earlier.

Antibacterial activity measurement

Antibacterial activities related to free AZ and AZ-loaded cubosomes were assessed by applying the agar well diffusion method against *Streptococcus pneumonia* (ATCC 49619) (19). The bacterial suspension (10^4 CFU/mL) was inoculated into Müller Hinton agar medium plate. Six-mm wells were then created on Mueller Hinton agar plates by applying gel puncture. Each of the formulations at concentrations ranging from 25 to 200 µg/mL was poured into the wells and incubation was done for 48 h at 37 °C. The inhibition zones were determined by measuring the bacterial growth inhibition zone's diameter through a ruler. Each assay was carried out in triplicate.

Taste masking evaluation

The taste masking study of freeze-dried AZ-loaded cubosomes after reconstitution in 5 mL water was carried out by applying six human volunteers (20). that were in the age range of 20-33. Before conducting the studies related to taste masking evaluation, volunteers signed an informed consent form in relation to their participation. The ethical approval was received from the ethics committee of Isfahan University of Medical Sciences (Ethics Vo. IR.MUI.RESEARCH.REC.1399.576).
Participants got 5 mL of each sample which was equal to 100 mg AZ. The samples were then kept in the mouth for a period of 15 s and subsequently discarded. Then, the participants were asked to assess the sample's bitterness and give their feedback in a scoring pattern as followed; 1: undetectable bitterness, 2: slightly bitter, 3: moderately bitter, 4; bitter, or 5; very bitter. Prior to the evaluation of the second sample, the participants drank water as some palate cleanser. In this research study, a comparison of the marketed AZ suspension to the optimized formulation was made.

RESULTS

Preparation and characterization of AZ-loaded cubosomes

In the present research study, diverse formulations of AZ-loaded cubosomes were prepared by applying the film hydration method; basic characteristics of as-prepared formulations, which included particle size, EE%, PD, and RE%, were determined as can be seen in Table 3. The analysis of the obtained responses was done by applying the Design Expert software.

Table 3. Physicochemical characteristics of different azithromycin-loaded cubosomes.

Formulations	Particle size (nm)	Polydispersity index	Encapsulation efficiency (%)	Release efficiency at pH 1.2 (%)
$G_{100}F_{10}S_4P_{2.5}$	173.86 ± 10.61	0.21 ± 0.01	90.5 ± 1.02	35.7 ± 5.12
$G_{200}F_{10}S_4P_0$	214.06 ± 10.94	0.25 ± 0.01	82.57 ± 2.56	31.29 ± 4.38
$G_{100}F_{10}S_4P_0$	192.66 ± 10.82	0.23 ± 0.002	88.67 ± 0.81	37.44 ± 5.91
$G_{100}F_5S_2P_{2.5}$	193.6 ± 5.01	0.23 ± 0.007	92.23 ± 7.03	26.16 ± 2.37
$G_{100}F_{10}S_2P_{2.5}$	182.9 ± 2.65	0.24 ± 0.01	91.41 ± 3.41	35.46 ± 6.65
$G_{200}F_5S_4P_{2.5}$	224.36 ± 6.44	0.23 ± 0.01	86.45 ± 6.5	23.18 ± 4.3
$G_{100}F_5S_4P_0$	265.86 ± 5.07	0.29 ± 0.006	90.4 ± 4.12	26.4 ± 5.09
$G_{100}F_5S_2P_0$	262.23 ± 10.08	0.33 ± 0.01	90.6 ± 8.34	25.75 ± 4.71
$G_{200}F_5S_4P_0$	272.43 ± 1.65	0.26 ± 0.002	81.9 ± 6.74	21.36 ± 4.01
$G_{200}F_{10}S_2P_0$	258.33 ± 16.07	0.30 ± 0.005	80.84 ± 2.02	24.87 ± 4.98
$G_{200}F_5S_2P_{2.5}$	166.56 ± 1.26	0.17 ± 0.005	85.81 ± 4.35	21.96 ± 4.19
$G_{200}F_{10}S_2P_{2.5}$	191.6 ± 13.62	0.26 ± 0.03	82.06 ± 9.03	20.79 ± 4.52

Particle size

The particle size of AZ-loaded cubosomes was from 166 to 272 nm; their PDI ranged from 0.17 to 0.33. PDI is an indicator of dispersion homogeneity that is obtained from the square root of standard deviation/mean diameter. It varies from 0 to 1 and any PDI which is greater than 0.5 reflects the inhomogeneity of formulation (21). Equation (3) was applied for the determination of the possible impact of each factor's given level on particle size.

$$\text{Particle size} = +218.52 - 14.65X_2 - 25.79X_4 + 5.81X_1X_2 + 2.37X_1X_3 - 3.42X_1X_4 - 14.94X_2X_3 + 3.30X_2X_4 + 8.56X_3X_4 - 2.61X_1X_2X_3 \quad (3)$$

In this equation, X_1 refers to GMO amount, X_2 stands for PF127 amount, X_3 denotes sonication time, and X_4 is PVA concentration. The positive sign for the coefficient of each of the factors as well as their interaction in the polynomial equation provided the indication of the synergistic effect on the response, whereas the negative sign reflected an antagonistic relationship. According to the analysis, by applying the Design Expert Software, the cubosomes size was affected by PF127 as well as the amount of PVA ($P < 0.05$). However, a rise in the amount of GMO led to no significant change in the cubosomes' particle size.

EE% determination

As can be seen in Table 3, the AZ-loaded cubosomes EE values were in the range of 80-92%. The formulation parameters' effect on EE% can be understood by the equation below:

$$EE\% = +87.32 - 3.82X_1 - 0.70X_2 + 0.16X_3 + 1.15X_4 + 0.07X_1X_2 + 0.435X_1X_3 + 0.2888X1X4 - 0.4150X_2X_4 + 0.4413X_3X_4 + 0.666X_1X_2X_3 \quad (4)$$

Data analysis revealed that the amount of GMO and PF127 had a significant effect on EE%. The impact of the amount of GMO and PF127 on EE% is displayed in Fig. 3B.

In vitro drug release from cubosomes

The *in vitro* release profiles related to AZ from cubosomes and AZ suspension against time at pH 1.2 is depicted in Fig. 1. RE% is a parameter applied to compare the drug-release rate of diverse formulations. The obtained results are displayed in Table 3. When RE% is increased, the drug release rate will be faster. The possible impact of formulation parameters on RE% can be understood by the equation represented below:

$$RE_{2h}\% = 41.13 - 4.52X_1 + 5.85X_2 + 0.86X_3 - 1.64X_4 - 3.15X_1X_2 + 1.18X_1X_3 + 1.39X_2X_3 + 1.29X_1X_2X_3 \quad (5)$$

The results obtained in our research study revealed that GMO amount, PF127 amount, sonication time, and the combined impact of GMO and PF127 amounts could be regarded as the main parameters influencing RE% ($P < 0.05$). The impact of the amount of GMO, PF127 and sonication time on RE% is displayed in Fig. 3C,3D.

Optimization

The computer optimization process by Design Expert Software and a desirability function determined the effect of the levels of independent variables on the responses. Constraints including particle size, PDI, and RE% in the acidic medium were $166 \leq Y_1 \leq 272$ nm, $0.17 \leq Y_2 \leq 0.33$, and $20.79 \leq Y_3 \leq 37.44$, respectively, with the target set at a minimum, according to the results obtained in this research study. EE constraints consisted of $80.84 \leq Y_4 \leq 92.23\%$, with the goal set at the obtained data maximum.

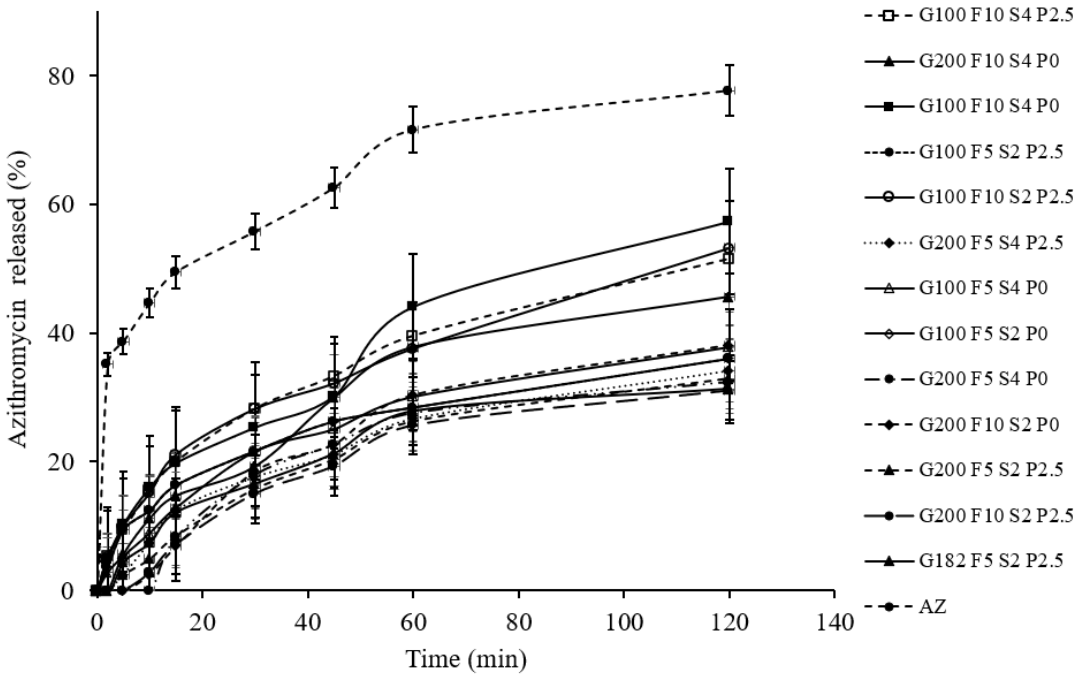

Fig. 1. Release profiles of different formulations at pH 1.2.

Table 4. Comparative levels of predicted and observed responses for the optimized formulation.

Response	Particle size (nm)	Polydispersity index	Encapsulation efficiency (%)	Release efficiency at pH 1.2 (%)
Actual values	190.3 ± 1.25	0.23 ± 0.003	92.5 ± 7.18	22.73 ± 4.02
Predicted	171.38	0.185	86.9	22.75
Absolute error (%)	11.03	27.7	6.4	0.08

Table 5. Comparison of physicochemical properties of the optimized formulation after and before freeze-drying.

Formulation	Particle size (nm)		Polydispersity index		Dispersion time (s)
	BF	AF	BF	AF	AF
Optimized	190.3 ± 1.25	240.86 ± 2.75	0.23 ± 0.003	0.31 ± 0.003	35

BF, Before freeze-drying; AF, after freeze-drying.

The optimized formulation which was selected with 80% desirability was prepared by applying 182 mg GMO, 5 mg PF127, 2.5 w/v% PVA and 2 min sonication time. Table 4 shows the predicted and observed response levels for the optimized formulation. The selected formulation's morphology, as evaluated through SEM, is presented in Fig. 2. As can be seen, the prepared cubosomes were nano-sized particles cubic in shape, with uniform size, good dispersion, and acceptable separation from each other.

Freeze-dried products characterization

Freeze-drying can be regarded as a common process for guaranteeing high stability, storage ease, and formulation handling. Given the accelerated degradation observed in polymers and lipids which are applied in nanoparticle formulation, freeze-drying can be considered a kind of viable option for the purpose of long-term stability. Table 5 represents the characteristics of the freeze-dried cubosomes formulation when the concentration of sucrose was 1% w/v. The particle size and PDI of the cubosomes' lyophilized formulation following re-dispersion were found to be larger, as compared to those recorded prior to lyophilization, which was owing to the small population formation of aggregated particles. The dry cubosomes powder's reconstitution time was about 35 s.

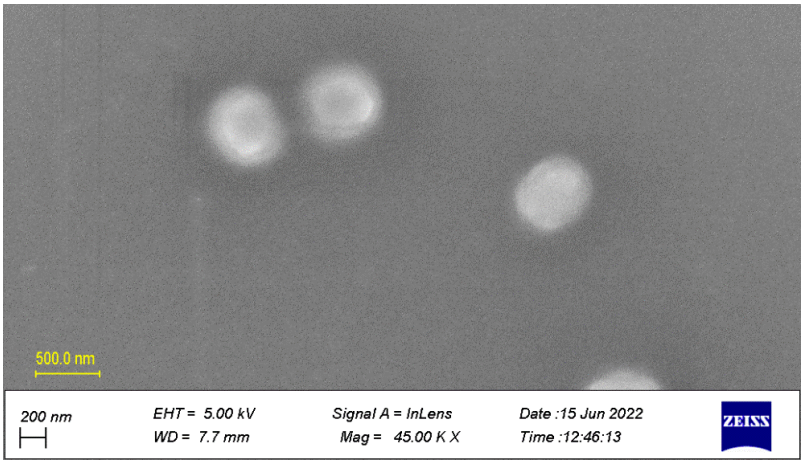

Fig. 2. Scanning electron microscopy morphology of azithromycin-loaded optimized cubosomes.

Table 6. The diameter of the area does not allow bacteria to grow on the culture medium.

Concentrations (µg/mL)	Inhibition zone of azithromycin-loaded cubosomes (mm)	Inhibition zone of free azithromycin
200	18.33 ± 0.57	17 ± 1.73
100	14.66 ± 1.52	14.66 ± 2.08
50	10.33 ± 0.57	11.33 ± 2.30
25	6	5.33 ± 5.03

Volunteers taste testing

Evaluation of the AZ and optimized AZ-loaded cubosomes' taste masking efficiency was done *in vivo* in human volunteers. The scores which were given to AZ and optimized AZ-loaded cubosomes by the participants were 4.66 ± 0.51 and 1.33 ± 0.52, respectively, indicating cubosomes' good taste masking effect.

Antibacterial activity measurement

Evaluation of the antibacterial activities related to free AZ and AZ-loaded cubosomes was done against *Streptococcus pneumonia* by applying the well diffusion method. As shown in Table 6, the antibacterial activity which was expressed in the zone of inhibition raised as the drugs concentration was increased regardless of the treatment type applied. In addition, in spite of the slow-release behavior of AZ-loaded cubosomes, in comparison with AZ, AZ-loaded cubosomes displayed an inhibitory effect similar to that of AZ.

DISCUSSION

The impact of the amount of PF127 and PVA on particle size is displayed in Fig. 3A. As represented, particle size was reduced with the rise of the PVA and PF127 concentration ($P < 0.05$). This was in agreement with what was found by Aboud *et al*. (22). It could be ascribed to the PF127 and PVA's ability as a surfactant to reduce surface tension. This caused a decrease in the cubosomes ' surface energy; which in turn, would lead to preventing particle aggregation. The surfactant's amount plays the main role in the process of emulsification and in the protection of droplets from aggregation. It was gathered at the produced droplets' W/O interfacial area. The surfactant's greater gathering at the W/O interface with the higher concentration resulted in producing smaller particles and protecting them against agglomeration (3). The amount of the drug that could be entrapped in the nanocarrier depends on the drug's physicochemical characteristics and the applied preparation process (23). Given AZ's low solubility in the external aqueous phase, the EE% values for all prepared cubosomes were high and varied from 80% to 92%. The results of analysis displayed that the rise of the GMO and PF127 level reduced EE% (Fig. 3B). The decrease of EEs with the rise of the PF127 concentration could be because PF127 surfactant properties raised the drug's solubility in the environment, thus leading to drug’s leakage to release medium. Results could be well matched to a previous report (24). While particle size is constant, the rise of the GMO level decreased EE% (Fig. 3B),

which could be attributed to the competition occurring between the drug and GMO in cubosomes, thus excluding the drug from the nanoparticles and decreasing the EEs. As indicated in Fig. 1, cubosomes decreased the drug release at acidic pH when compared with the AZ suspension, which could be ascribed to the protective impact of cubosomes that would happen due to the lipophilic drug's dissolution in the hydrophobic matrix, slowing its release into the aqueous medium. It can be beneficial since AZ can have a high decomposition potential in an acidic medium (25). RE% is a parameter which is used to compare different formulations' drug release rates. The results analysis revealed that raising GMO and reducing PF127 led to decreasing the drug release at the pH value of 1.2 (Fig.3 C). The apparently retarded release with raising the amount of GMO showed the enhanced affinity of AZ with the liquid crystalline matrices (26). The RE reduction with increasing concentration of PF127 in the aqueous phase (Fig. 3C) could be due to the increased fraction of PF127 that formed a stable network on the cubosomes surface. PF127 is a copolymer of poly propylene oxide and poly ethylene oxide. While cubosomes are formulated, hydrophobic poly propylene oxide part of PF127 penetrates into cubosomes and binds to the particle surface resulting in reduced release rate (27). Further, the rise of the sonication time enhanced the release rate (Fig. 3D). The score which was given to AZ and optimized AZ-loaded cubosomes by the participants also signified cubosomes' good taste masking. Although, AZ-loaded cubosomes had slow-release behavior, they showed similar antibacterial activity compared with free AZ which could be due to the composition of cubosomal particles with the presence of GMO as a penetration enhancer in addition to its bioadhesive (28). These properties facilitate a possible adhesion and/or fusion to the bacterial cell membrane and consequently improve the drug permeability into bacterial cells. In another research study conducted by Nasr *et al*. the incorporation of gatifloxacin into cubosomal particles resulted in a significant reduction in minimal inhibitory concentration (28).

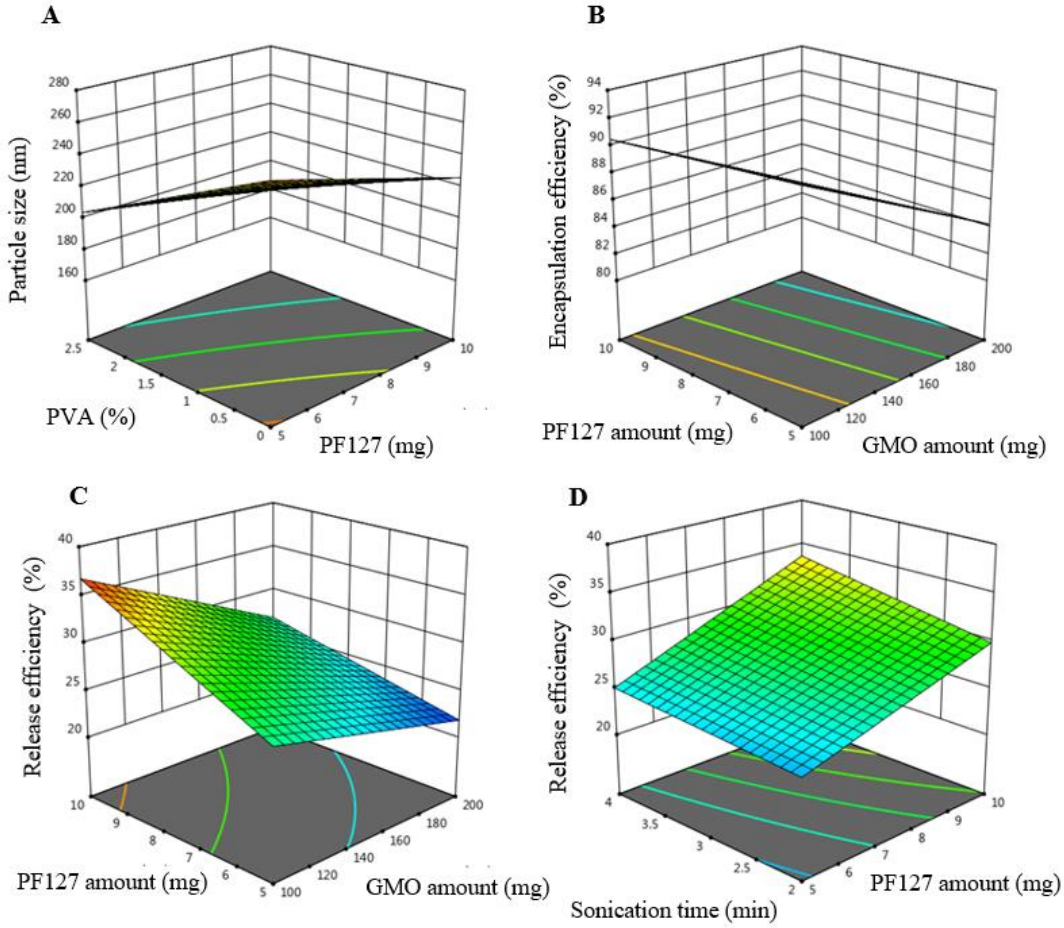

Fig. 3. Influence of different levels of studied parameters on (A) particle size, (B) encapsulation efficiency%, and (C and D) release efficiency% at pH 1.2. PF 127, Pluronic F127; GMO, glyceryl monooleate.

CONCLUSION

AZ-loaded cubosomes were successfully prepared in this study. The optimized formulation which was prepared by applying 182 mg GMO, 5 mg PF127, 2 min sonication time, and 2.5 w/v% PVA had a good morphology, particle size, EE%, and RE% of AZ-loaded cubosomes in the acidic medium. The *in vivo* evaluation also showed that the drug bitterness intensity was well alleviated following the AZ incorporation into cubosomes. The results obtained through the experiments showed the value for the pharmaceutical industries which would deal with bitter drugs for the improvement of patients' compliance, thus leading to effective pharmacotherapy.

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Conflict of interest statement

The authors declared no conflicts of interest in this study.

Authors' contribution

H. Zaker contributed to the investigation and writing the manuscript; S. Taymouri contributed to the conceptualization, methodology, analysis, writing, editing of the article, and supervising the study; A. Mostafavi contributed to the conceptualization, methodology, and supervising of the study. The final version of the manuscript was approved by all authors.

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