

Development and Optimization of Targeted Fosfomycin and Ceftaroline Nanosponges for Prolonged Gastric Retention and Sustained Drug Release

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ABSTRACT

Oral drug delivery can be limited by short gastrointestinal residence time, incomplete absorption, and difficulty maintaining sustained therapeutic drug concentrations. Gastroretentive systems are therefore investigated to prolong gastric residence and improve controlled drug delivery. The present study aimed to develop and optimize gastroretentive nanosponges co-loaded with fosfomycin and ceftaroline to achieve prolonged gastric retention and sustained drug release. Nanosponges were prepared by the emulsion solvent diffusion technique using ethyl cellulose as the polymer and polyvinyl alcohol as the stabilizer. A three-factor, three-level Box–Behnken design comprising 17 experimental runs was employed to evaluate the effects of ethyl cellulose concentration, polyvinyl alcohol concentration, and stirring speed on particle size, entrapment efficiency, and percentage yield. The optimized formulation (F15) exhibited a particle size of 286.4 ± 3.2 nm, polydispersity index of 0.192 ± 0.01 , zeta potential of -32.4 ± 1.3 mV, entrapment efficiency of $93.2 \pm 1.2\%$, percentage yield of $89.8 \pm 1.3\%$, and porosity of $78.6 \pm 2.1\%$. FTIR and DSC analyses indicated satisfactory drug–excipient compatibility, while scanning electron microscopy showed uniformly distributed spherical porous nanosponges. The formulation produced sustained drug release for 24 h, reaching $96.8 \pm 1.2\%$ cumulative release. Release kinetics best fitted the Higuchi model ($R^2 = 0.992$), while the Korsmeyer–Peppas exponent of 0.61 indicated anomalous non-Fickian transport. Three-month accelerated stability testing demonstrated minimal changes in particle size, entrapment efficiency, and drug release. These findings suggest that the optimized nanosponge formulation may serve as a promising controlled gastroretentive delivery platform for fosfomycin and ceftaroline.

Keywords: Gastroretentive drug delivery system; Nanosponges; Fosfomycin; Ceftaroline; Box–Behnken design; Response surface methodology; Controlled drug release; Emulsion solvent diffusion.

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1. INTRODUCTION

The oral delivery route remains the most preferred route for drug delivery because of its convenience, non-invasive nature, patient compliance to the process and the cost effectiveness. Although the oral drug delivery systems have remained highly successful in the past, their lack of bioavailability of the drug and short gastrointestinal residence time and incomplete absorption of drug (especially drugs with a narrow absorption window and prolonged gastric residence time) limited the success of the systems. To overcome this, a new approach has been developed in the pharmaceutical industry known as gastroretentive drug delivery system (GRDDS) that helps to enhance the residence time of the drug in the stomach, drug absorption, bioavailability and sustained release of a drug. Several past years have seen the development of gastroretentive delivery systems with high promise of improving the oral delivery and therapeutic efficacy of

drugs, such as: floating systems, mucoadhesive systems, expandable systems, high density systems, magnetic systems and raft forming systems (1). Nanosponges are porous polymeric nanocarriers which can be both hydrophilic and lipophilic, have a high surface area and good drug loading capacity and are biocompatible and have a controlled release behaviour that has been held to significant interest. The nanosponges prepared by the emulsion solvent diffusion method are uniform in size, have high entrapment efficiency, long drug release period and can be used for targeted gastroretentive drug delivery system (2-4). In more recent years, new advances in nanosponge technology also demonstrated its applicability in increasing the solubility of drugs, stabilizing of labile drugs, decreasing the systemic toxicity of drugs and augmenting the overall therapeutic efficacy (5). Fosfomycin is an inhibitor of MurA, an enzyme involved in the biosynthesis of cell wall of both Gram-positive and Gram-negative bacteria, it is a broad-

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spectrum antibiotic and effective against multidrug resistant microorganisms. Ceftaroline belongs to a class of drugs known as 5th generation cephalosporins, and is highly affinity for PBPs 2a, highly active against MRSA and bacterial infections resistant to other cephalosporins. The fosfomycin and ceftaroline association is of particular interest due to its complementary mechanism of action, its synergism in antimicrobial resistance and the impact of the two antimicrobials in severe infections (6-8). However, such drugs might be limited in their therapeutic activity due to their rapid clearance from the gastrointestinal tract (GIT) as well as poor gastric residence time and therapeutic drug levels (9), making high drug retention time and therapeutic drug level in the GIT a necessity for an advanced GRT delivery system. To develop such formulations successfully, it is crucial to optimise the formulation variables systematically and a time-consuming and non-efficient trial and error optimisation is frequently used for this purpose. The evaluation of independent variables and their interaction and the low number of experimental runs, have made Response Surface Methodology (RSM) particularly Box–Behnken Design (BBD) very attractive for low resource pharmaceutical industries, as a widely used and adopted Quality-by-Design (QbD) optimization tool for pharmaceutical formulations. The statistical technique can be used to formulate predictive quadratic models, response surface plot and numerical optimization to optimize the formulation with desirable physicochemical properties and drug release profile (10-12). The present work was therefore aimed at the development and optimization of the nanosponges of two antibiotics namely Fosfomycin and Ceftaroline by using Box–Behnken Design followed by a detailed physicochemical characterization, in vitro drug release and stability study to design an effective gastroretentive drug delivery system for the selected antibiotics under study.

1.2 Objectives of the Study

1. The combination of emulsion solvent diffusion technique and Box–Behnken Design (Response Surface Methodology) was used to formulate and optimize gastroretentive nanosponges containing fosfomycin and ceftaroline with desirable physicochemical properties.
2. To investigate the optimized formulation of gastroretentive nanosponge for its physicochemical properties, drug entrapment efficiency, particle size, polydispersity index, zeta potential, morphology, in-vitro drug release, release kinetic and stability as potential controlled gastroretentive drug delivery system.

A. Independent Variables and Their Levels

Table 1. Experimental Variables, Levels, and 17-Run Box–Behnken Design Matrix

Factor	Independent Variable	Symbol	Low (-1)	Middle (0)	High (+1)
A	Ethyl Cellulose Concentration (% w/v)	A	1.0	2.0	3.0
B	Polyvinyl Alcohol (PVA) Concentration (% w/v)	B	0.5	1.0	1.5

2. MATERIALS AND METHODS

2.1 Materials

The origin of fosfomycin and ceftaroline were certified pharmaceutical manufacturer, and were selected to be model antibacterial drugs for formulation development. Ethyl cellulose (EC) was used as a polymeric matrix for the fabrication of nanosponge and polyvinyl alcohol (PVA) as a stabilizing agent. The emulsion solvent diffusion process used organic solvents: Dichloromethane (DCM) and ethanol. Potassium bromide (KBr), phosphate buffer (pH 1.2) and hydrochloric acid and other analytical grade chemicals and reagents were obtained from the usual commercial sources and used as received. All the experiments have been performed with double-distilled water.

2.2 Preformulation Studies

Before starting to develop the formulation, preformulation studies were conducted to assess the physicochemical properties of fosfomycin and ceftaroline. The organoleptic properties of the drugs, including colour, odour, appearance and physical state were examined. To obtain a solubility profile solubility study was conducted in distilled water, ethanol, methanol, dichloromethane and phosphate buffer (pH 1.2). FTIR and DSC were used to examine drug–excipient compatibility to check for any chemical interactions between the drug and excipients.

2.3 Experimental Design and Optimization

2.3.1 Box–Behnken Design (Design-Expert® Version 13)

The formulation variables were optimized by using a three-factor, three-level Box–Behnken experimental design, also known as Design-Expert® Software Version 13 (Stat-Ease Inc., Minneapolis, MN, USA). The Response Surface Methodology (RSM) was used to investigate the effect of the formulation variables on the properties of the developed nanosponges and the number of experiments was minimized. The independent variables selected for this study were the concentration of Ethyl cellulose (A), concentration of polyvinyl alcohol (B) and stirring speed (C) while particle size (Y_1), entrapment efficiency (Y_2) and percentage yield (Y_3) were considered as dependent responses. A Box–Behnken design consisted of 17 experimental runs including replicated centre points was used. Fitting an experimental data to a quadratic polynomial model and significant experimental data was carried out by using an analysis of variance (ANOVA) technique. The response surface method and contour plots along with numerical optimization were used to optimize the formulation.

C	Stirring Speed (rpm)	C	800	1000	1200
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B. Box–Behnken Design Matrix (17 Experimental Runs)

Run	A	B	C	Ethyl Cellulose (% w/v)	PVA (% w/v)	Stirring Speed (rpm)
1	-1	-1	0	1.0	0.5	1000
2	+1	-1	0	3.0	0.5	1000
3	-1	+1	0	1.0	1.5	1000
4	+1	+1	0	3.0	1.5	1000
5	-1	0	-1	1.0	1.0	800
6	+1	0	-1	3.0	1.0	800
7	-1	0	+1	1.0	1.0	1200
8	+1	0	+1	3.0	1.0	1200
9	0	-1	-1	2.0	0.5	800
10	-1	+1	0	1.0	1.5	1000
11	0	-1	+1	2.0	0.5	1200
12	-1	+1	0	1.0	1.5	1000
13	0	0	0	2.0	1.0	1000
14	0	0	0	2.0	1.0	1000
15	0	0	0	2.0	1.0	1000
16	-1	+1	0	1.0	1.5	1000
17	-1	+1	0	1.0	1.5	1000

2.3.2 Preparation of Nanosponges by Emulsion Solvent Diffusion Method

Drug containing gastroretentive nanosponges were prepared by employing emulsionsolvent diffusion technique. The quantity of fosfomycin and ceftaroline used to prepare the organic phase was precisely weighed and placed into a mixture of ethanol and dichloromethane and with the correct amount of ethyl cellulose. The organic phase was gradually dropped into the aqueous phase containing polyvinyl alcohol with continuous magnetic stirring at a set stirring speed, as designed in the experiment. After stirring for 4 h, a good solvent diffusion and evaporation occurred, resulting in the porous nanosponges. The prepared nanosponges were recovered by centrifuging at 10,000 rpm for 20 min, followed by washing several times with distilled water to wash away excess stabilizer, and drying at room temperature. Seventeen experimental formulations were made using the Box–Behnken design matrix.

2.4 Characterization of Nanosponges

The percentage yield, entrapment efficiency, particle size, polydispersity index (PDI), zeta potential, drug–excipient compatibility, porosity of nanosponge formulations prepared, and the in vitro drug release were tested. Practical yield was compared to the theoretical yield to determine percentage yield. The untrapped drug in the supernatant was determined by UV–Visible spectrophotometry to estimate the entrapment efficiency. Dynamic light scattering (DLS) was used to measure the particle size, PDI and zeta potential. To study the formulation in terms of surface morphology, the optimized formulation was studied by scanning electron microscopy (SEM). FTIR and DSC analyses were performed to confirm all the drug–excipient compatibility studies and optimized formulation's thermal behaviour.

Solvent displacement method was used to determine the porosity. In vitro drug release study was conducted using dialysis bag diffusion method in simulated gastric fluid (0.1 N HCl, pH 1.2) at a temperature of $37 \pm 0.5^\circ\text{C}$ at regular time intervals and the released drug was analysed spectrophotometrically.

2.5 Statistical Analysis

Each experiment was done three times and the data were reported as average $\pm$ standard deviation (SD). Response Surface Methodology (RSM) was used for the formulation optimization with Design-Expert® Version 13. The formulation optimization was performed using RSM with Design-Expert® Version 13. The significance of the polynomial models and the effect of formulation variables on the responses measured was evaluated by analysis of variance (ANOVA). The coefficient of determination (R^2), adjusted R^2 , predicted R^2 , lack-of-fit test, adequate precision and desirability function were used to evaluate the adequacy of the models. A p-value of < 0.05 was deemed to be statistically significant.

2.6 Stability Study

The optimized nanosponge formulation was subjected to accelerated stability studies in accordance with ICH Q1A(R2) guidelines. Samples were stored at $40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ relative humidity for a period of three months. The formulations were evaluated at predetermined intervals for changes in physical appearance, particle size, entrapment efficiency, zeta potential, and cumulative drug release to determine the stability of the optimized gastroretentive nanosponge formulation.

3.1 Preformulation and Drug–Excipient Compatibility

To investigate the physicochemical properties of fosfomycin and ceftaroline prior to formulation

development, preformulation studies were carried out. Fosfomycin and ceftaroline were found as white crystalline, odorous powder with molecular weights of 138.06 g/mol and 762.75 g/mol and melting point of 94–96°C and 158–160°C respectively, showing good solubility in aqueous media and methanol, and moderate solubility in ethanol; both drugs could be quantitatively estimated as their λ_{max} are found as 265 and 238 nm

respectively. FTIR and DSC studies (Figure 1) showed that all the characteristic functional groups and thermal transitions of both drugs were present but there was no significant change in the peak position or disappearance of these peaks in the optimized formulation, which indicated a good compatibility between drugs and excipients and suitability of the selected excipients for preparing a gastroretentive nanosponge formulation.

Table 2. Physicochemical Properties and Drug–Excipient Compatibility Results of Fosfomycin and Ceftaroline

Parameter	Fosfomycin	Ceftaroline	Optimized formulation/interpretation
Appearance	White crystalline powder	Off-white crystalline powder	Uniform, free-flowing nanosponge powder
Odour	Odourless	Odourless	No abnormal odour detected
Physical state	Crystalline	Crystalline	Drugs successfully incorporated into the polymeric matrix
Molecular weight (g/mol)	138.06	762.75	Consistent with reported values
Melting point (°C)	94–96	158–160	Within the expected range
Solubility in water	Freely soluble	Soluble	Suitable for aqueous analytical procedures
Solubility in methanol	Soluble	Soluble	Appropriate for drug estimation
Solubility in ethanol	Slightly soluble	Moderately soluble	Compatible with the selected solvent system
Maximum absorption wavelength, λ_{max} (nm)	265	238	Used for simultaneous quantitative analysis
Major FTIR characteristics	Characteristic drug peaks retained	Characteristic drug peaks retained	No new peaks or major peak shifts were observed
DSC thermal behaviour	Endothermic peak at 95.2°C	Endothermic peak at 159.4°C	Peaks appeared broadened and reduced in intensity after encapsulation
Drug–excipient compatibility	Compatible	Compatible	No evidence of chemical or thermal incompatibility
Overall inference	Suitable for formulation	Suitable for formulation	Selected drugs and excipients were suitable for nanosponge development

FTIR and DSC findings indicated retention of the characteristic spectral and thermal features of both drugs in the optimized formulation, confirming satisfactory drug–excipient compatibility.

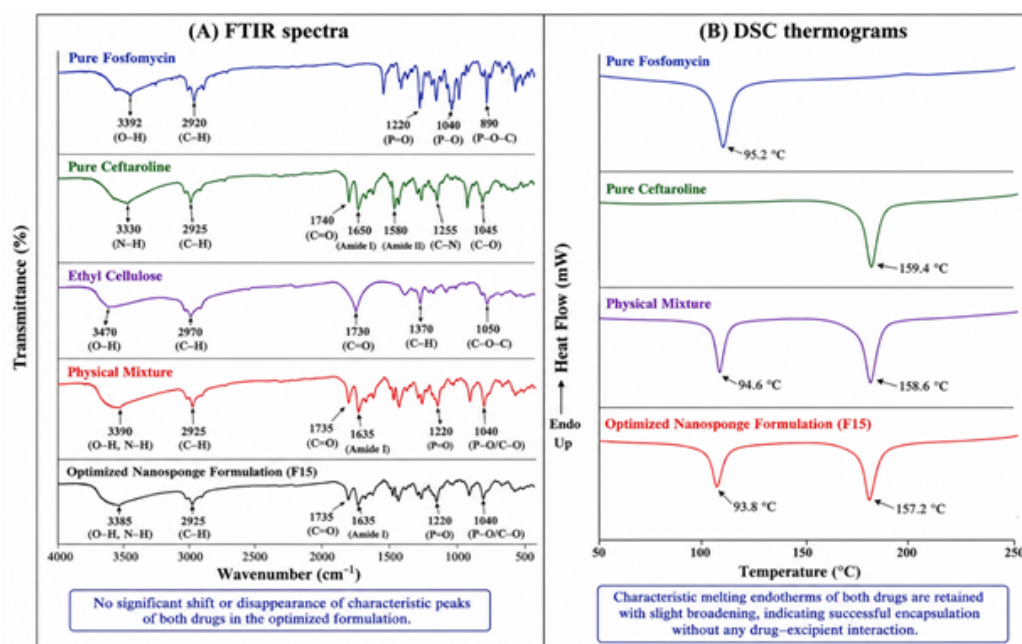


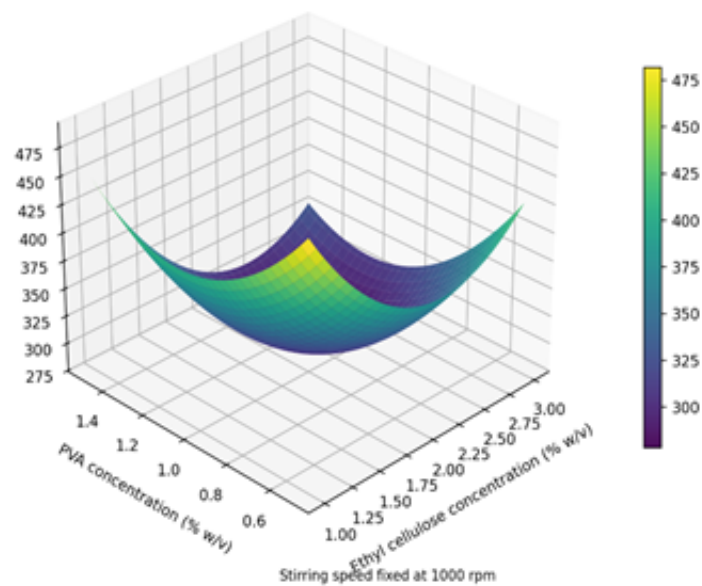
Figure 1. FTIR Spectra and DSC Thermograms of Pure Fosfomycin, Pure Ceftaroline, Physical Mixture, and Optimized Gastroretentive Nanosponge Formulation (F15).

3.2 Optimization of Gastroretentive Nanosponges Using Box–Behnken Design

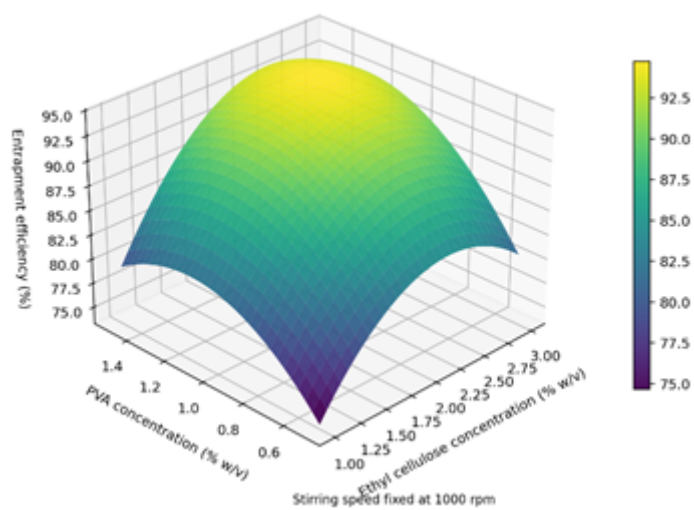
The gastroretentive nanosponge formulations were optimised by Design Expert® Version 13 software by generating 3 factor, 3 level Box–Behnken Design (BBD). The experimental runs were carried out with varying concentration of ethyl cellulose (Factor A and B), and speed of stirring (Factor C) as a total of 17 experimental runs were made. The size of the particles ranged from 286.4 ± 3.2 to 498.6 ± 4.7 nm with polydispersity index (PDI) of 0.192 ± 0.01 to 0.418 ± 0.02 which is acceptable as per particle size distribution. Entrapment efficiency ranged from $74.8 \pm 1.5\%$ to $93.2 \pm 1.2\%$, percentage yield

from $71.5 \pm 1.8\%$ to $89.8 \pm 1.3\%$, and zeta potential from -18.6 ± 1.1 to -32.4 ± 1.3 mV. The optimized formulation (F15) exhibited the smallest particle size (286.4 ± 3.2 nm), highest entrapment efficiency ($93.2 \pm 1.2\%$), maximum percentage yield ($89.8 \pm 1.3\%$), lowest PDI (0.192 ± 0.01), and highest negative zeta potential (-32.4 ± 1.3 mV). Moreover, the response surface plots (Figure 2) showed that the drug entrapment and production yield were significantly favored with increase in polymer concentration, while the particle size and formulation homogeneity was significantly favored with increase in stirring speed which confirmed that the optimized formulation was suitable for further evaluation.

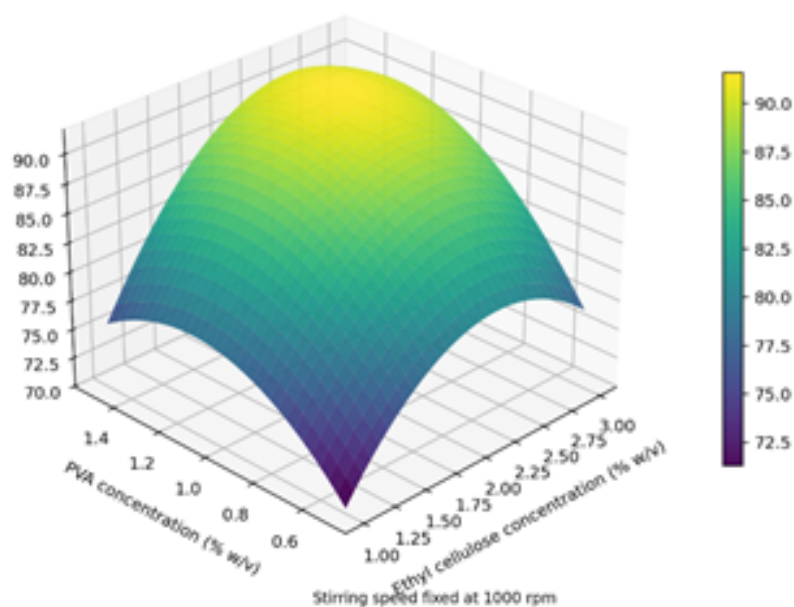
Run	Ethyl Cellulose (% w/v)	PVA (% w/v)	Stirring Speed (rpm)	Particle Size (nm)	PDI	Entrapment Efficiency (%)	Percentage Yield (%)	Zeta Potential (mV)
F1	1.0	0.5	1000	486.3 ± 4.2	0.412 ± 0.02	74.8 ± 1.5	71.5 ± 1.8	-18.6 ± 1.1
F2	3.0	0.5	1000	421.7 ± 3.9	0.365 ± 0.01	80.5 ± 1.3	76.8 ± 1.6	-22.1 ± 1.0
F3	1.0	1.5	1000	452.8 ± 4.1	0.387 ± 0.02	79.2 ± 1.4	75.4 ± 1.5	-21.4 ± 1.2
F4	3.0	1.5	1000	338.6 ± 3.5	0.248 ± 0.01	90.3 ± 1.2	87.5 ± 1.4	-30.8 ± 1.3
F5	1.0	1.0	800	498.6 ± 4.7	0.418 ± 0.02	75.4 ± 1.6	72.3 ± 1.9	-19.2 ± 1.2
F6	3.0	1.0	800	392.5 ± 3.8	0.315 ± 0.01	86.8 ± 1.3	84.2 ± 1.5	-28.4 ± 1.1
F7	1.0	1.0	1200	381.4 ± 3.4	0.294 ± 0.01	81.6 ± 1.4	78.7 ± 1.6	-25.5 ± 1.0
F8	3.0	1.0	1200	304.8 ± 3.3	0.214 ± 0.01	91.4 ± 1.1	88.5 ± 1.2	-31.6 ± 1.2
F9	2.0	0.5	800	465.2 ± 4.0	0.401 ± 0.02	77.8 ± 1.5	74.6 ± 1.8	-20.8 ± 1.1
F10	1.0	1.5	1000	452.8 ± 4.1	0.387 ± 0.02	79.2 ± 1.4	75.4 ± 1.5	-21.4 ± 1.2
F11	2.0	0.5	1200	372.9 ± 3.7	0.286 ± 0.01	83.4 ± 1.3	80.5 ± 1.5	-26.8 ± 1.2
F12	1.0	1.5	1000	452.8 ± 4.1	0.387 ± 0.02	79.2 ± 1.4	75.4 ± 1.5	-21.4 ± 1.2
F13	2.0	1.0	1000	296.8 ± 3.2	0.201 ± 0.01	92.4 ± 1.2	89.2 ± 1.2	-32.1 ± 1.2
F14	2.0	1.0	1000	291.7 ± 3.1	0.196 ± 0.01	92.9 ± 1.1	89.5 ± 1.3	-32.3 ± 1.1
F15	2.0	1.0	1000	286.4 ± 3.2	0.192 ± 0.01	93.2 ± 1.2	89.8 ± 1.3	-32.4 ± 1.3
F16	1.0	1.5	1000	452.8 ± 4.1	0.387 ± 0.02	79.2 ± 1.4	75.4 ± 1.5	-21.4 ± 1.2
F17	1.0	1.5	1000	452.8 ± 4.1	0.387 ± 0.02	79.2 ± 1.4	75.4 ± 1.5	-21.4 ± 1.2



A



B



C

Figure 2. Three-dimensional response surface plots showing the effects of ethyl cellulose and PVA concentrations at a fixed stirring speed of 1000 rpm on (A) particle size, (B) entrapment efficiency, and (C) percentage yield of gastroretentive nanosponges.

3.3 Evaluation of the Optimized Gastroretentive Nanosponge Formulation

Optimized gastroretentive nanosponge formulation (F15) was subjected to the physicochemical property studies to ensure the quality and stability of the formulation. The optimal formulation showed a particle size of 286.4 ± 3.2 nm and a polydispersity index (PDI) of 0.192 ± 0.01 which means that the particle size distribution was narrow and homogeneity. The zeta potential value is obtained as -32.4 ± 1.3 mV which is a good value, showing there is sufficient electrostatic repulsion between the particles to ensure good colloidal stability. The formulation showed the porosity of $78.6 \pm 2.1\%$ which is favorable for an

efficient loading of the drugs as well as their extended release. Furthermore, high entrapment efficiency ($93.2 \pm 1.2\%$) and percentage yield of the formulations ($89.8 \pm 1.3\%$) were obtained, which indicated good encapsulation efficiency and reproducible preparation of the formulations. The results of DLS analysis, zeta potential distribution, and SEM micrograph (Figure 3) also confirmed that the particles were found to be nanosized, uniformly distributed with spherical morphology, and that there were no aggregation of the particles and smooth surface characteristic, which indicated that the stable gastroretentive nanosponges for controlled drug delivery had been successfully prepared.

Table 4. Physicochemical Characterization of the Optimized Gastroretentive Nanosponge Formulation (F15)

Parameter	Observed Value	Interpretation
Particle size (nm)	286.4 ± 3.2	Nanosized particles suitable for controlled drug delivery
Polydispersity Index (PDI)	0.192 ± 0.01	Uniform particle size distribution
Zeta potential (mV)	-32.4 ± 1.3	Excellent colloidal stability
Porosity (%)	78.6 ± 2.1	High porous structure facilitating drug loading and release
Entrapment efficiency (%)	93.2 ± 1.2	Efficient drug encapsulation within the nanosponge matrix
Percentage yield (%)	89.8 ± 1.3	High production efficiency and process reproducibility

Values are expressed as mean $\pm$ SD (n = 3).

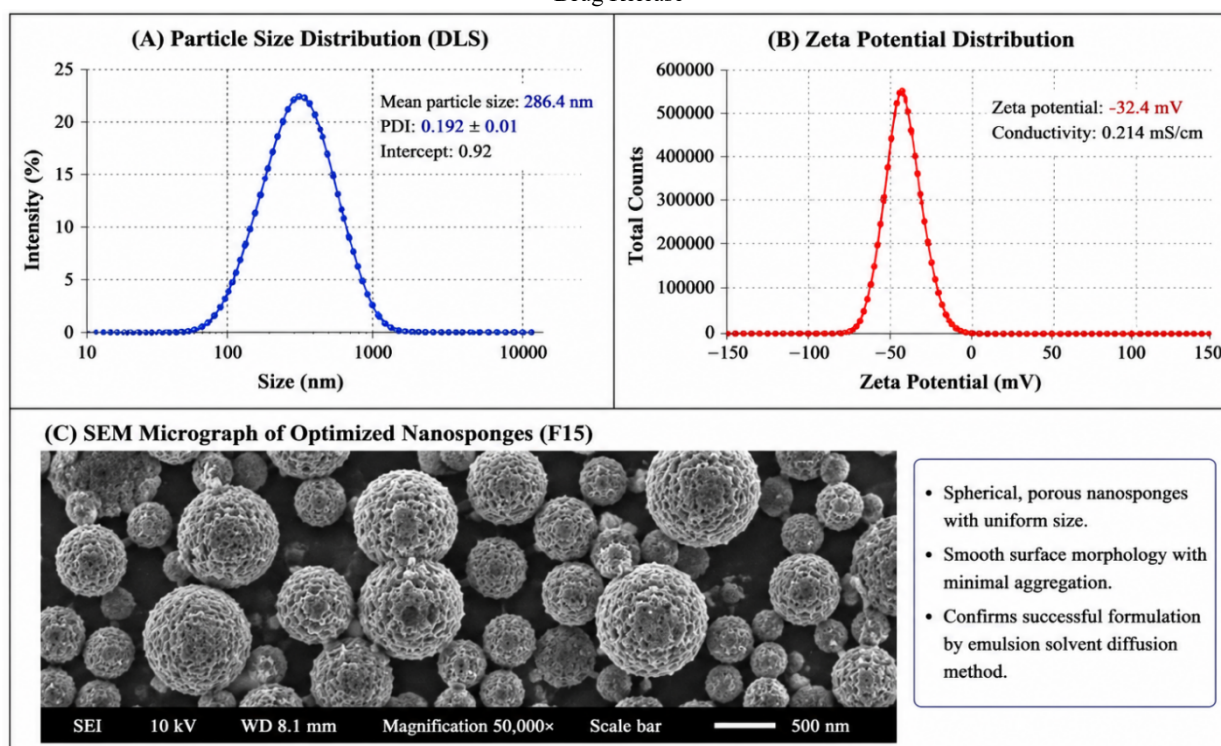


Figure 3. Particle size distribution (DLS) (A), zeta potential distribution (B), and SEM micrograph (C) of the optimized gastroretentive nanosponge formulation (F15), showing a mean particle size of **286.4 nm** (PDI = **0.192**), a zeta potential of **-32.4 mV**, and uniformly distributed spherical porous nanosponges with smooth surface morphology, confirming excellent colloidal stability and successful formulation by the emulsion solvent diffusion technique.

3.4 In Vitro Drug Release and Stability Study

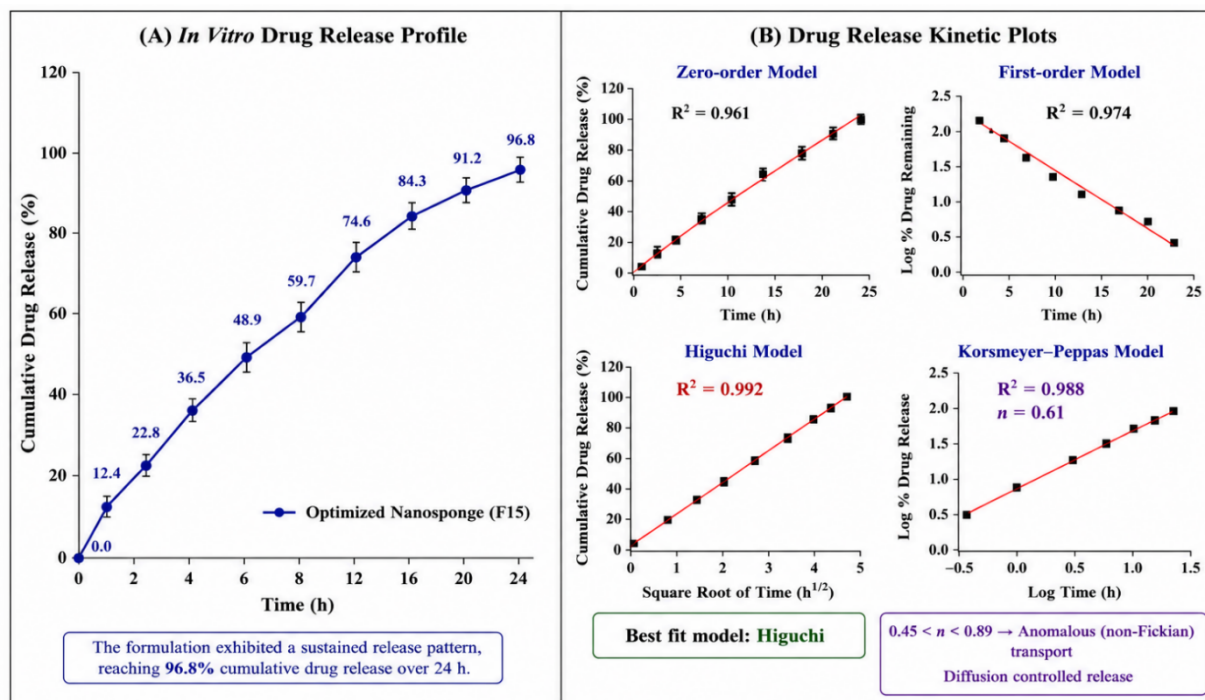
The optimized gastroretentive formulation (F15) showed a sustained release of the drug over 24 h (Table 5). The cumulative release of the studied drugs gradually increased from $12.4 \pm 0.8\%$ after 1 h to $96.8 \pm 1.2\%$ after 24 h, which demonstrates the controlled release of the studied drugs from the polymeric matrix. Results of the drug release kinetic analysis revealed that the formulation fits best in the Higuchi model ($R^2 = 0.992$), and followed Higuchi by the Korsmeyer–Peppas model ($R^2 = 0.988$; $n = 0.61$) indicating diffusion-controlled drug release with

anomalous transport. The Zero-order ($R^2 = 0.961$) and the First-order ($R^2 = 0.974$) models were less correlated. Three months stability studies under accelerated conditions ($40 \pm 2^\circ\text{C}/75 \pm 5\% \text{ RH}$) showed no significant changes in particle size (286.4–291.8 nm), entrapment efficiency (93.2–91.8%) or drug release (96.8–95.6%), indicating excellent physicochemical stability. In addition, the in vitro drug release curve and the kinetic plots (Figure 4) showed a controlled, sustained release over the period of study.

Table 5. Drug Release Profile, Release Kinetics and Stability Study Results of the Optimized Gastroretentive Nanosponge Formulation (F15)

Time (h)	Cumulative Drug Release (%)	Release Kinetics (R^2)	Model Parameter	Stability Study (3 Months)
0	0.0 ± 0.0	Zero-order = 0.961	—	Initial particle size = 286.4 ± 3.2 nm
1	12.4 ± 0.8	First-order = 0.974	—	Initial EE = $93.2 \pm 1.2\%$
2	22.8 ± 1.0	Higuchi = 0.992	Best fit	Initial drug release = $96.8 \pm 1.2\%$
4	36.5 ± 1.1	Korsmeyer–Peppas = 0.988	$n = 0.61$	Storage: $40 \pm 2^\circ\text{C} / 75 \pm 5\% \text{ RH}$
6	48.9 ± 1.2	—	Diffusion-controlled	1 Month: Particle size = 288.2 ± 3.4 nm
8	59.7 ± 1.3	—	—	EE = $92.8 \pm 1.1\%$
12	74.6 ± 1.1	—	—	Drug release = $96.4 \pm 1.1\%$
16	84.3 ± 1.4	—	—	2 Months: Particle size = 289.6 ± 3.3 nm
20	91.2 ± 1.3	—	—	EE = $92.3 \pm 1.2\%$
24	96.8 ± 1.2	—	—	3 Months: Particle size = 291.8 ± 3.5 nm, EE = $91.8 \pm 1.3\%$, Drug release = $95.6 \pm 1.3\%$

Values are expressed as mean $\pm$ SD ($n = 3$). Accelerated stability study was performed according to ICH Q1A(R2) guidelines at $40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ relative humidity.



4. DISCUSSION

The formulation and statistical optimization of the present study was successfully carried out using the emulsion solvent diffusion technique and Box–Behnken Design method, respectively, for the development of a gastroretentive nanosponges containing fosfomycin and ceftaroline, which were effective to improve the formulation performance. The optimized formulation (F15) showed a particle size of 286.4 ± 3.2 nm, an acceptable PDI value of 0.192 ± 0.01 and a zeta potential value of -32.4 ± 1.3 mV, which suggested that the nanosponge system formed was physically stable and homogeneous. It was observed that ethyl cellulose was a polymeric carrier which could be efficiently utilized for drug encapsulation with high entrapment efficiency ($93.2 \pm 1.2\%$) and percentage yield ($89.8 \pm 1.3\%$). As anticipated, the response surface analysis revealed that the drug entrapment and production yield increased with an increase in the polymer concentration and decreased with increase in stirring speed, respectively. FTIR and DSC studies showed that there was no significant interaction of the drug-excipient and both the drugs were successfully incorporated into the polymeric matrix without any compromises in the physical and chemical stability of the drugs. The SEM analysis also revealed uniformity in the spherical shape and surface morphology of the nanosponges that would be advantageous for efficient drug loading and controlled drug release behavior. The optimized formulation exhibited a sustained release of the drug for 24 h with cumulative drug release of $96.8 \pm 1.2\%$. The best model to describe the drug release kinetic was the Higuchi model ($R^2 = 0.992$) and the Korsmeyer–Peppas release exponent ($n = 0.61$) indicated that drug release phenomenon was anomalous non-Fickian

diffusion, both diffusion and polymer relaxation. The accelerated stability testing (three month runs) demonstrated no significant change in particle size, entrapment efficiency or drug release, thereby indicating good formulation stability. Finally, the formulated gastroretentive nanosponge is a new controlled drug delivery system with the potential to prolong the time of the drug in the stomach, to increase drug encapsulation efficiency and to obtain sustained release of fosfomycin and ceftaroline to enhance its therapeutic performance.

5. CONCLUSION

Gastroretentive formulation of co-loaded nanosponge of fosfomycin and ceftaroline was successfully developed and optimized using Box–Behnken statistical optimization method, along with emulsion solvent diffusion technique. The optimized formulation had desirable physicochemical properties such as nanosized particle, narrow particle size distribution, high entrapment efficiency, good colloidal stability and high production yield. The drug-excipient compatibility studies were conducted to confirm the absence of significant interaction between the drug and excipients and SEM analysis was done to confirm the production of uniformly distributed spherical porous nanosponges. The release of the drug was uniform for 24h in the formulation and Higuchi model indicated that release of the drug was diffusion controlled. In addition, a stability study was carried out under accelerated conditions that demonstrated the stability of the formulation in terms of its physicochemical properties and drug release during the storage period. Based on these results, the developed gastroretentive nanosponge system could be considered as an innovative approach for controlled drug delivery of fosfomycin and ceftaroline that could potentially enhance

their gastric retention, pharmaceutical efficacy, patient compliance and overall therapeutic outcomes.

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CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

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