Research Article

Formulation, Optimization and Evaluation of Biopharmaceutics Classification System (BCS) Class-IV drug Nanocoacervates

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ABSTRACT

The present study was aimed to formulate, optimize and evaluate the Tetrabenazine nanocoacervates. Tetrabenazine is a BCS Class-IV drug with low solubility and low permeability, hence nanocoacervates were prepared to improve its solubility and permeability. The Tetrabenazine nanocoacervates are prepared by coacervation method using chitosan as polymer. The formulation was optimized by applying 2^4 Full factorial design of DoE. Molarity of NaOH, Chitosan concentration, sonication time, stirring speed were considered as independent factors and encapsulation efficiency and % drug diffusional release were considered as dependent factors. Total 16 formulations were prepared and characterized for encapsulation efficiency, particle size, zeta potential, poly dispersity index, SEM and *in-vitro* drug diffusional release and drug release kinetics. The results of evaluation showed good encapsulation efficiency, optimum zeta potential, nanometric particle size and extended drug diffusional release profile. FTIR and DSC studies revealed no interaction between drug and excipients. SEM studies revealed scaly morphology of Tetrabenazine nanocoacervates. The optimized formulation`s encapsulation efficiency and % drug diffusional release was found to be 78.02% and 98.131% for an extended period of 12 hrs with zero order release kinetics and super case-II transport mechanism. Hence Tetrabenazine nanocoacervates are promising vehicles for effective drug delivery in the treatment of Huntington's disease.

Keywords: Tetrabenazine, Nanocoacervates, Chitosan, Full Factorial Design, Zero order release kinetics.

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INTRODUCTION

Biopharmaceutics Classification System (BCS) Class-IV drugs exhibit many characteristics that are problematic for effective oral and peroral delivery. Low aqueous solubility, poor permeability, unpredictable and poor absorption, inter and intra-subject variability and major positive food effects leading to low and variable bioavailability are some of the associated issues. Formulation and development of an efficacious delivery system for BCS Class-IV drugs are herculean tasks for any formulator. Lipid-based delivery systems, polymer-based nanocarriers, crystal engineering (nanocrystals and co-crystals), liquisolid technology, self-emulsifying solid dispersions are some of the techniques used. Polymer-based nanoparticle drug delivery system is proven to guarantee secure and effective delivery of active compounds and to boost bioavailability. As a result, drug delivery via nanocoacervates (NCs) has been effective in recent years and the improved pharmacodynamic and pharmacokinetic profiling of a drug has been recorded in several ongoing studies[1,2].

Coacervation is one of the most easily applied techniques for the production of nanocarriers. The coacervation technique underlies the creation of the coacervate phase by polyelectrolyte mixture and its deposition of active material. The method can be called simple coacervation and complex coacervation, depending on the number of polymer types used. For the coacervation process, electrostatic attraction between oppositely charged molecules is the common driving force. Hydrogen bonding and hydrophobic interactions have an inverse effect on complex coacervation, in addition to electrostatic interactions between biopolymers of opposite charges. As surface charges increase, the efficiency of nanoencapsulation is enhanced. The coacervation technique can obtain nanocarriers varying between 100 and 600 nm. The most widely used wall materials are gelatin, acacia gum, and chitosan[3].

Tetrabenazine (TBZ) is a dopamine-depleting agent. It is the only US FDA approved drug for the treatment of chorea coupled with Huntington's disease. TBZ is a BCS Class-IV drug which has low solubility, low permeability and due to a high first-pass metabolism, the systemic bioavailability is low[4], hence to evade all these problems TBZ NCs were prepared using chitosan as polymer. NCs have an ability to enhance oral bioavailability BCS class-IV drugs, it also eliminates pre-systemic metabolism gastrointestinal degradation and permeability related issues.

Full factorial design is an experimental design, which uses dimensional factor space at the corner of the design space. A full factorial design contains all possible combinations of a set of factors. This is the most fool proof design approach. Factorial experiments with two-level factors are used widely because they are easy to design, efficient to

run, straight forward to analyze, and full of information. Two-level full factorial designs are the most powerful screening designs, they allow to estimate main effects of input factors and their interactions on output responses[5]. The current research work is aimed to formulate nanocoacervates of Tetrabenazine by using chitosan as polymer and optimize the nanocoacervates formulation by 2^4 full factorial design to warrant safe and efficient delivery of active compounds and enhanced bioavailability.

MATERIALS AND METHODS

Tetrabenazine was procured as gift sample from Lupin Limited, Pune. Chitosan was purchased from Fine Chem. Industries, Mumbai. Acetic acid, Sodium hydroxide and all other chemicals used were of analytical grade.

Drug- Excipient compatibility studies

FTIR studies

FTIR Spectroscopy was done to identify the functional groups present and interaction between drug and excipients. FTIR spectra were developed for pure drug and formulations by preparing potassium bromide disks. Taking few mg of Tetrabenazine drug or formulation with potassium bromide, the disk was prepared by compression of 10 tons pressure for 5 min. Finally, disk was placed in a holder of FTIR machine and a spectrum was recorded from 4000cm^{-1} to 500cm^{-1} band width.

DSC studies

DSC is a thermoanalytical technique gives an insight into the melting behaviour of substance. Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. The pure drug and formulation samples were subjected to DSC Studies using Perkin Elmer pyres 4 series DSC equipment (Massachusetts USA) samples were sealed in 40µl aluminum pans an identical empty pan was used as a reference, all samples were scanned at 5°C/min with a 20ml/min nitrogen purge from 20 - 320°C.

Formulation of TBZ Nanocoacervates

Nanocoacervates batches were formulated according to the Full factorial design (FFD) to study the effect of 4 critical process parameters (CPP) i.e., Molarity of NaOH, Chitosan concentration, Sonication time, Stirring speed on the critical quality attributes (CQA) i.e., encapsulation efficiency & % drug release. A 2^4 FFD was used for the optimization procedure. The minimum and maximum specifications of the processing variables at randomized 2 levels are entered in to the software (Design Expert, Version 12.0.12.0, Stat-Ease) to obtain 16 formulation designs as illustrated in tables no 1 & 2.

Table 1: Design summary of Formulation variables

Factor	Name	Units	Type	Coded Low Minimum	Coded High Maximum
A	Molarity of NaOH	M	Numeric	-1 ↔ 0.50	+1 ↔ 2.50
B	Chitosan Concentration	mg/ml	Numeric	-1 ↔ 1.00	+1 ↔ 5.00
C	Sonication Time	min	Numeric	-1 ↔ 10.00	+1 ↔ 30.00
D	Stirring Speed	rpm	Numeric	-1 ↔ 1000.00	+1 ↔ 3000.00

Table 2: Formulation table obtained by using Full Factorial design

Formulation code	Molarity of NaOH (M)	Chitosan Concentration (mg/ml)	Sonication Time (min)	Stirring Speed (rpm)
F1	0.5	5	10	3000
F2	2.5	5	30	1000
F3	0.5	1	10	1000
F4	2.5	1	10	3000
F5	2.5	5	30	3000
F6	0.5	5	30	1000
F7	0.5	1	30	3000
F8	0.5	1	10	3000
F9	2.5	1	30	3000
F10	0.5	1	30	1000
F11	2.5	1	30	1000
F12	2.5	5	10	1000
F13	0.5	5	10	1000
F14	0.5	5	30	3000
F15	2.5	1	10	1000
F16	2.5	5	10	3000

Method of Preparation

Preparation of Tetrabenazine nanocoacervates

Chitosan solution (1-5 mg/ml) was dissolved in 1% v/v glacial acetic acid, sonicated for 10-30 mins and stirred overnight continuously at 1000-3000 rpm. Thereafter, TBZ (6mg/ml) was added in NaOH solution of different molar concentrations (0.5M, 2.5M) then it was sprayed in chitosan solution with the help of a sprayer, under continuous stirring, forming coacervates droplets in nanometric size range. Lastly separation and purification of particles was done by centrifugation, followed by successive washing of coacervates solution with buffer or distilled water[1].

Figure 1: TBZ nanocoacervates preparation by coacervation method

Evaluation of TBZ Nanocoacervates

Determination of Drug Encapsulation efficiency

The encapsulation efficiency (EE) of TBZ in the formulated coacervates system was determined by estimating the free drug available in the supernatant. The supernatant was analyzed at 280 nm and the encapsulation efficiency (EE) was calculated using the following equation.

$$\text{Encapsulation efficiency (\%)} = \frac{CS_D - CSS_D}{CS_D} \times 100$$

Where, CS_D = Total loaded drug in chitosan solution and CSS_D = drug in supernatant determined by UV-Visible Spectrophotometer at 280 nm[1].

Particle Size, Polydispersity Index & Zeta Potential

The mean particle diameter and Poly dispersity index (PDI) were determined with the aid of Malvern Zeta sizer nano ZS-V2.0 at 25°C after appropriate dilutions in Milli Q water filtered through 0.22µm poly vinylidene difluoride filters. Size measurement was carried out using DTS-5.10 clear disposable sizing cuvettes with measurement position set at 4.65mm. Zeta potential is an important parameter to evaluate and establish an optimum condition for stability of colloids or disperse systems. It is also determined by the above mention equipment before the measurements, the samples were diluted and probe sonicated to avoid possible interference during the measurements means of results. Measurements were carried out at 25°C using water as a dispersant (refractive index :1.330) in a clear disposable zeta cell.

Surface Morphology by SEM

The morphology of the nanocoacervates was investigated by scanning electron microscopy (Carl Zeiss- Supra 55) at an accelerating voltage of 10 kV. The sample was fixed on a SEM- stub using double- sided adhesive tape and conductive carbon paint is applied along the edges of coverslips and the sample is allowed to air dry. The samples were coated with thin layer of gold under vacuum using Sputter Coater vacuum coater (JEOL, JFC 1600, Auto fine Coater) to minimize electrostatic charging. The images as different magnification were captured.

In-vitro drug diffusion studies

The *in- vitro* diffusion analysis was done to compare the pattern of drug release from TBZ NCs through the dialysis membrane in modified Franz diffusion cell. The activated dialysis membrane was mounted between the donor and receiver compartment. The donor compartment was filled with the TBZ NCs equivalent to 25 mg drug and receiver compartment with phosphate buffer pH 6.8 and kept on continuous stirring for 12 hours. The diffused samples were collected after every 1,2,4,6,8,10,12 hrs. of time interval from the outlet port of receiver compartment and were compensated with equal volume of fresh Phosphate buffer to maintain the equilibrium state. Then absorbances of test samples were taken at 280nm[1].

Statistical Analysis

Analysis of variance test (ANOVA) was applied to determine whether the results obtained from the experiment were significant or not. A probability level of $p < 0.05$ was considered to be significant.

Prediction of optimized formulation

Using the design expert software, the obtained data for each response were analyzed and after optimization of multiple responses, the optimized TBZ NCs formulation was predicted, prepared and evaluated for all responses.

In-vitro drug release kinetics

The drug release kinetics and mechanism of drug release of TBZ nanocoacervates were determined by fitting *in-vitro* release data in to various models such as zero order, first order, and Higuchi equations, Korsmeyer- Peppas model.

RESULTS AND DISCUSSION

Drug-Excipient compatibility studies

FTIR studies

FTIR spectrum of TBZ and its formulation is depicted in Fig.no.2, it showed-CH stretching at 2919.52 cm^{-1}, C=O

stretching at 1697.96 cm^{-1}, C-O stretching at 1007.91 cm^{-1}, C-N stretching at 860.25cm^{-1}, N-H rocking at 641.40cm^{-1}. The TBZ NCs formulation showed no major shifting of any functional peaks of drug. Hence it was indicated that there was no interaction between drug and used excipients.

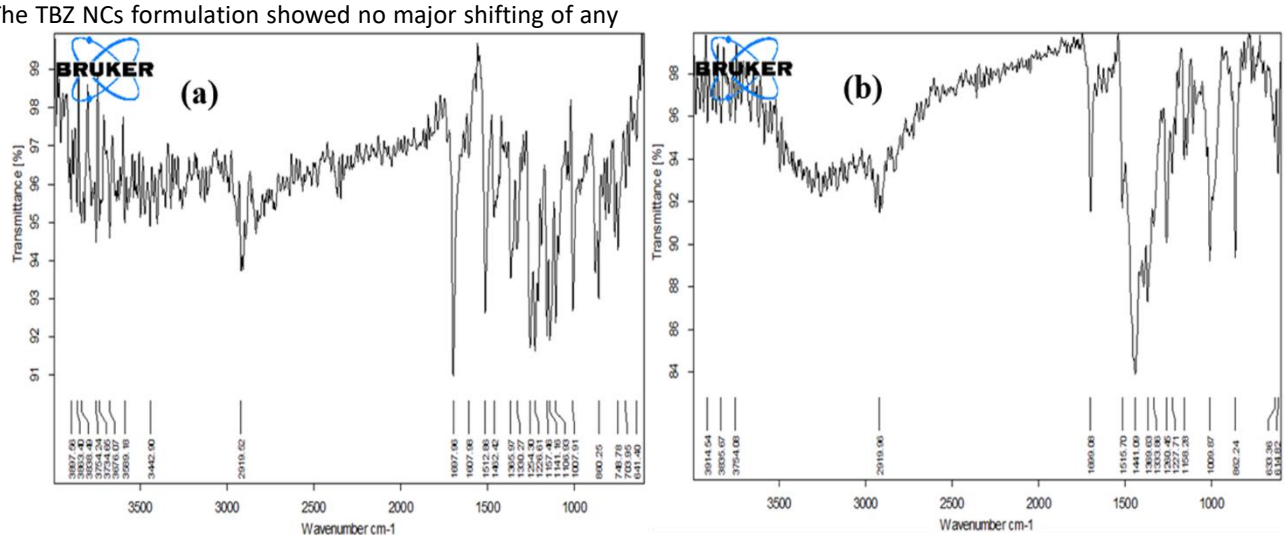


Figure 2: FTIR spectrum of (a) TBZ (b) TBZ Formulation

DSC studies

DSC thermogram of pure drug TBZ and formulation are shown in Fig no.3. In DSC study of pure drug, a peak observed at 132.07°C indicates the drugs melting point. In DSC thermogram of TBZ Formulation two distinctive separative peaks were observed at 107.56^{0}C and 126.82^{0}C corresponding to polymer and drug respectively. This indicates chitosan and Tetrabenazine are compatible with each other.

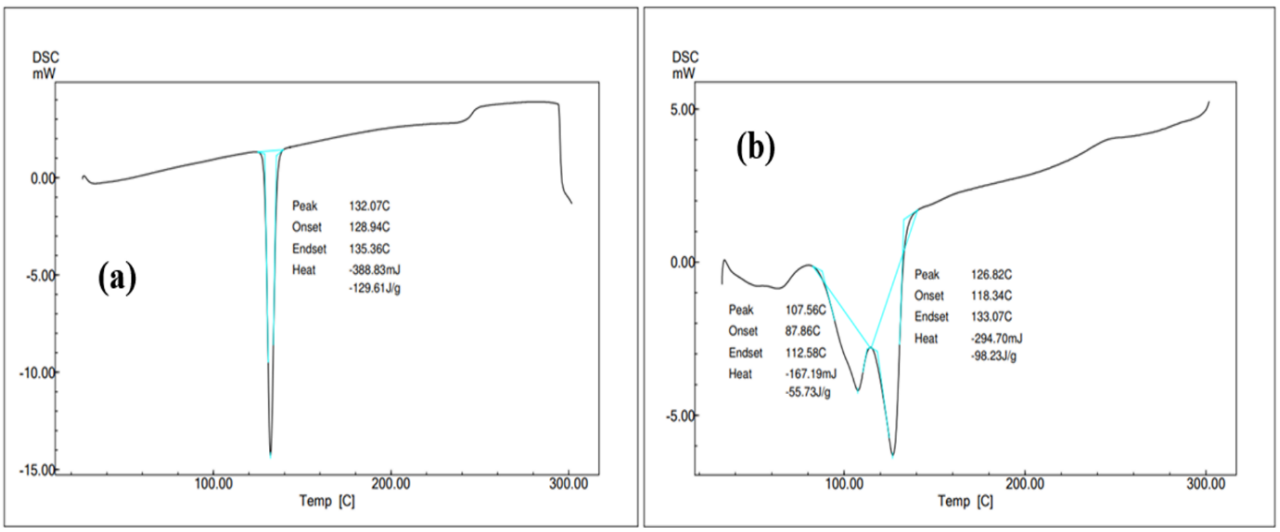


Figure 3: DSC thermograms of (a) TBZ (b) TBZ Formulation

Physicochemical characteristics of TBZ NCs

The formulated nanocoacervates are evaluated for parameters like encapsulation efficiency, particle size, zeta potential and PDI. Here all the formulations are prepared with different molarity of NaOH, chitosan concentration, sonication time, stirring speed. These changes show different encapsulation efficiency, particle size, zeta potential and PDI. From encapsulation efficiency results it has been observed that drug encapsulation increased with increase in molarity of NaOH, Chitosan concentration, sonication time, and by decreasing stirring speed. Encapsulation efficiency of nanocoacervates ranged from 63.6% to 86.8%. The particle size of TBZ nanocoacervates were in the range of 163.8 nm to 3518 nm. Results indicate that particle size of nanocoacervates increased with increase in chitosan concentration, particle size reduced with increase in sonication time.

The PDI is a measure of the heterogeneity of a sample based on size. Polydispersity can occur due to size distribution in a sample or agglomeration or aggregation of the sample. The PDI values of TBZ nanocoacervates are in range of 0.012 to 1.00. Zeta Potential analysis is a technique for determining the surface charge of nanocoacervates in solution. The magnitude of the zeta potential is predictive of the colloidal stability. Zeta potential of prepared nanocoacervates is in the range of +5.07 to -43.4 mV as tabulated in table no 3.

Table 3: Evaluation results of TBZ NCs

Formulation code	Encapsulation efficiency* (%)	Particle size (nm)	PDI	Zetapotential (mV)
F1	75.91±0.15	2285.7	0.658	-27.1
F2	86.8±0.24	658.2	1.00	5.07
F3	65.97±0.33	393.3	1.00	-10.1
F4	75.53±0.45	216.38	0.262	-29.5
F5	84.23±0.56	556.2	0.790	-26.1
F6	79.7±0.65	685.3	0.688	-17.8
F7	67.8±0.74	177.3	0.75	-35.4
F8	63.6±0.82	235.4	1.00	-8.95
F9	73.49±0.92	163.8	0.84	-23.58
F10	69.36±0.88	204.4	0.568	-38.97
F11	74.95±0.75	184.8	0.055	-14.5
F12	83.68±0.69	3240.58	0.676	-7.44
F13	76.59±0.49	3518	0.012	-4.53
F14	78.62±0.37	602.75	0.854	-43.4
F15	72.82±0.85	342.6	1.00	-7.91
F16	80.71±0.54	1022	0.585	-41.54

***All the values are calculated as Mean, ± S.D, n=3**

***In-vitro* drug diffusion studies of TBZ NCs**

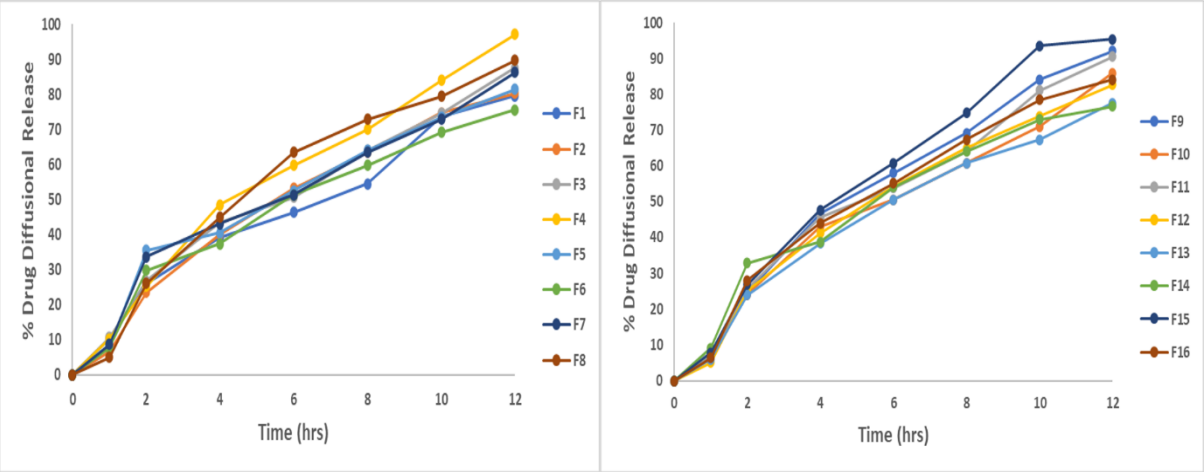


Figure 4: *In-vitro* drug diffusion studies of all formulations from F1-F16

In- vitro drug diffusional release studies of pure drug TBZ and TBZ nanocoacervates were compared to study the permeability through the dialysis membrane. It was observed that TBZ pure drug gave 22.897% drug diffusional release upto 12 hrs., whereas TBZ nanocoacervates exhibited sustained drug diffusional release pattern upto 12 hrs., % drug diffusional release ranged from 75.701% to 97.196%. The reason behind less % drug diffusional release of pure drug is due to its low solubility and low permeability nature. The nanocoacervates exhibited significant increase in % drug diffusional release which can be attributed to their increased solubility and permeability after being modified as polymer based nanocarriers. The % drug diffusional release from nanocoacervates was affected by various parameters. % drug diffusional release in nanocoacervates increased with increase in Molarity of NaOH and by decreasing the chitosan concentration, sonication time.

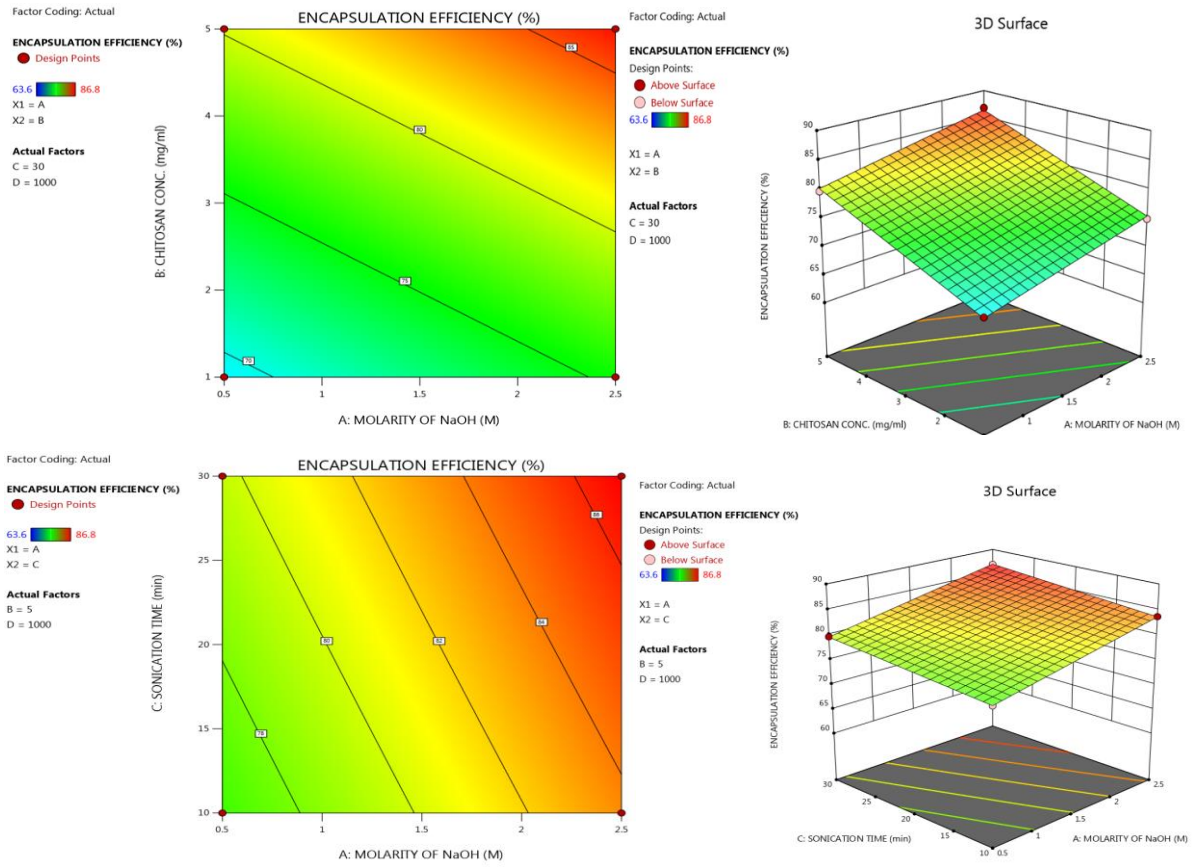
Statistical analysis

ANOVA with multiple regression analysis of the responses (R1, R2) using Design Expert software were implemented for statistical analysis of FFD formulations. The estimated factors effects with p-values on the responses were presented in table 4. The effects of these factors on the responses were displayed in contour and 3D response surface plots (Fig no 5 & 6).

Table 4: ANOVA Table for Response-1 & 2

	% Encapsulation Efficiency		% Drug Diffusional Release	
Source	**F-value**	**p-value**	**F-value**	**p-value**
Model	179.89	< 0.0001	162.64	< 0.0001
A-Molarity of NaOH	401.98	< 0.0001	310.36	< 0.0001
B-Chitosan Concentration	1254.27	< 0.0001	1182.13	< 0.0001
C-Sonication time	103.02	0.0002	96.61	0.0002
D-Stirring speed	36.58	0.0018	20.81	0.0060
AB	0.0345	0.8600	6.17	0.0555
AC	0.4601	0.5277	3.86	0.1065
AD	2.11	0.2058	0.0672	0.8058
BC	0.0079	0.9327	4.47	0.0881
BD	0.0235	0.8841	0.0124	0.9155
CD	0.4384	0.5372	1.88	0.2284
R^2	0.9972		0.9969	
Adeq Precision	43.2144		40.0348	

The statistical analysis of Response-1 (% EE) & Response-2 (% DR) are illustrated in table no 4. The **Model F-value** of Response-1 & 2 are 179.89 &162.64 respectively, its **P-values is** less than 0.0500 implies the model is significant. For both responses A, B, C, D are significant model terms. Higher proximity of R^2 value towards 1, highlights the model strength, R^2 value for designed model system was recorded as 0.9972 and 0.9969 confirming the higher interdependence of the model parameters. **Adequate Precision** ratio greater than 4 is desirable. The obtained ratio of 43.214 & 40.034 indicates these models can be used to navigate the design space.



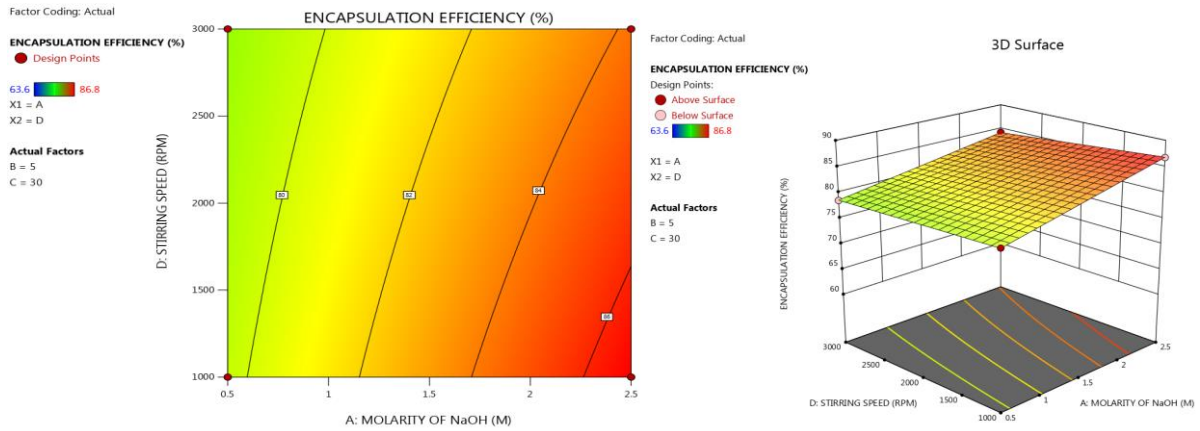


Figure 5: Contour and 3D surface plots of RESPONSE-1 (%EE)

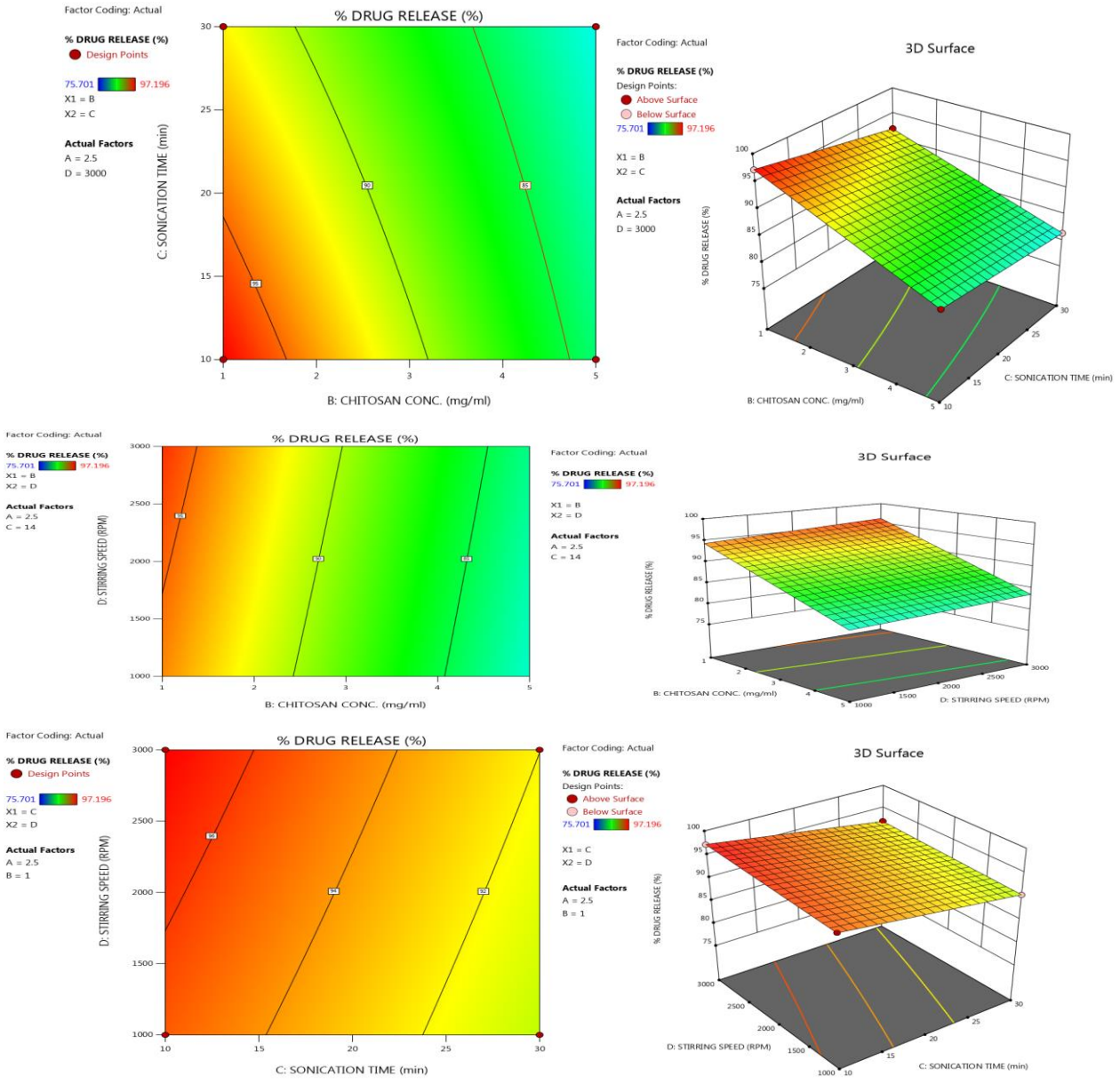


Figure 6: Contour and 3D surface plots of RESPONSE-2 (% DR)

Mathematical modelling of experimental data

Depending on the analysis of the observed values of the responses; a mathematical model for each response was generated and presented in the form of equations.

Quadratic equation for Encapsulation efficiency

% Encapsulation efficiency (R1) = 75.30 +3.10*A +5.48*B +1.57*C -0.9362*D -0.0288*AB -0.1050*AC -0.2250*AD -0.0138*BC +0.0237*BD +0.1025*CD

(A=Molarity of NaOH, B= Chitosan concentration, C=Sonication time, D = Stirring speed)

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. In the above equation Factor, A, B, C have + ve sign. This indicates as the Molarity of NaOH, Chitosan concentration, Sonication time increases, Encapsulation efficiency increases. The factor D (Stirring speed) has -ve sign indicating as Stirring speed decreases, Encapsulation efficiency increases. The interaction effects of factors AB, AC, AD and BC have - ve sign indicating antagonistic effect on Encapsulation efficiency. The interaction effects of factors BD and CD have + ve sign indicating synergistic effect on Encapsulation efficiency.

Quadratic equation for % Drug Diffusional Release

% Drug Diffusional Release (R2) = 85.18 +2.77*A -5.41*B - 1.55C +0.7184*D - 0.3913*AB -0.3096*AC +0.0408*AD +0.3331*BC - 0.0176*BD - 0.2161*CD

(A=Molarity of NaOH, B= Chitosan concentration, C=Sonication time, D = Stirring speed)

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. In the above equation factors, A, D have + ve sign, this indicates as the Molarity of NaOH, Stirring speed increases, Drug Diffusional Release increases. The factor B, C have -ve sign indicating as Chitosan concentration, Sonication time decreases, Drug Diffusional Release increases. The interaction effects of factors AB, AC, BD, CD have -ve sign indicating antagonistic effect on Drug Diffusional Release. The interaction effects of factors AD, BC have + ve sign indicating synergistic effect on drug diffusional release.

Optimization of TBZ NCs

The resultant experimental data of all prepared formulations F1 to F16 were used to develop an optimized TBZ NCs with maximum % Encapsulation efficiency and % Drug Diffusional release by using FFD in design expert software. The suggested optimized formulation has Molarity of NaOH 2.5 M, Chitosan concentration 1 mg/ml, Sonication time 10 mins, Stirring speed 3000 rpm. To validate these values, the optimized TBZ NCs formulation was prepared and evaluated. The observed responses of this formulation were 78.02% encapsulation efficiency, particle size of 221.1 nm, PDI of 0.214, -26.7mV zeta potential and 98.131% drug diffusional release for 12hours as illustrated in Fig no 7 & 8. These observed values are in a close agreement with the predicted values. This proved the feasibility of the optimization procedure using FFD in developing a new TBZ NCs formulation with controlled release.

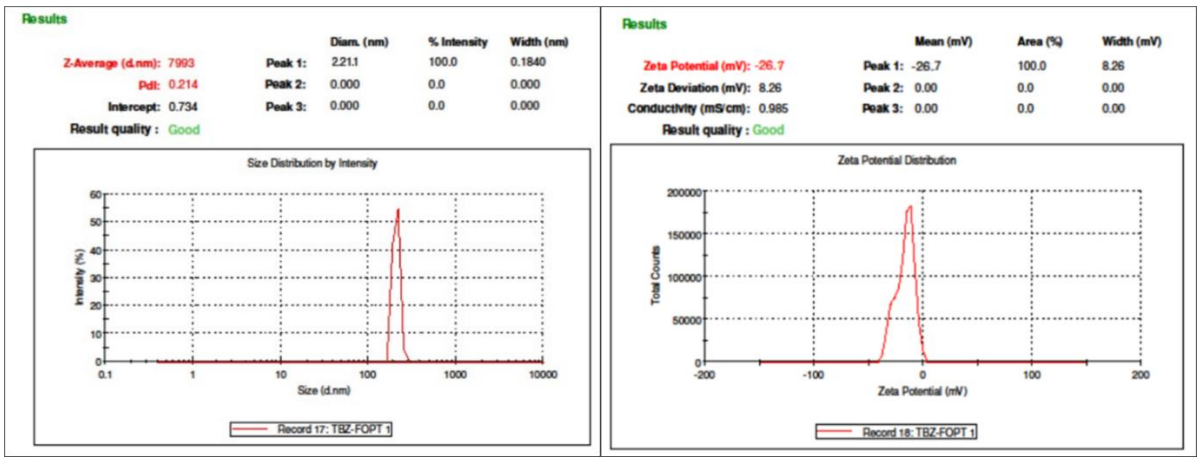


Figure 7: Particle size, PDI & Zeta potential of Optimized formulation

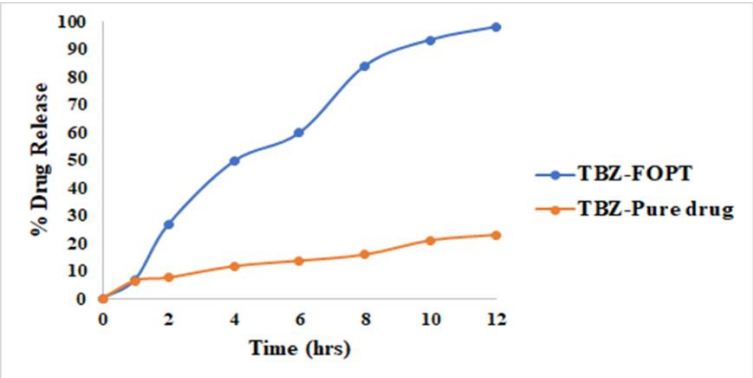


Figure 8: *In-vitro* drug diffusion studies of Optimized formulation

Surface morphology by SEM

The morphology of TBZ NCs was observed and the results were shown in Fig no.9. The results displayed scaly nanocoacervates with average particle size of 221.1 nm.

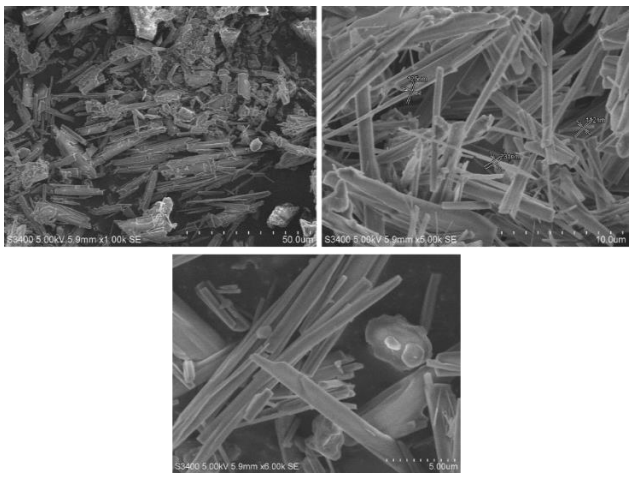


Figure 9: SEM studies of TBZ Optimized formulation

***In-vitro* drug release kinetics**

The *in-vitro* drug release kinetics of optimized formulation followed zero order (r^2=0.9606). The obtained value of diffusion exponent (n) is 1.1956, indicating that the release behaviour was Super case-II transport.

CONCLUSION

TBZ NCs were successfully optimized using 2^4 Full factorial design, which gave maximum encapsulation efficiency and high drug diffusional release for extended period of 12 hrs. The release of TBZ was found to follow zero order kinetics with super case -II transport behaviour. On comparing the drug diffusional release profile of pure drug and optimized nanocoacervates it can be concluded that this method may prove to be a suitable potential option for effective delivery of BCS Class-IV drugs.

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