IJBPAS, February, 2026, 15(2): 734-746

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

FORMULATION AND EVALUATION OF TOLVAPTAN LOADED SOLID LIPID NANOPARTICLES

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Received 15th Oct. 2024; Revised 5th Dec. 2024; Accepted 5th Feb. 2025; Available online 1st Feb. 2026

https://doi.org/10.31032/IJBPAS/2026/15.2.9837

ABSTRACT

This study aims to improve the oral bioavailability and solubility of Tolvaptan by creating and optimizing solid lipid nanoparticles (SLNs). "Tolvaptan SLNs were produced via solvent evaporation and heat homogenization. Fifteen formulations were developed employing experimental design, and their in vitro drug release, zeta potential, particle size, entrapment efficiency, and drug content were evaluated. With an in-vitro drug release of 92.1%, a particle size of 349 nm, a zeta potential of -25.9 mV, and an entrapment efficiency of 1.93%, the H2 formulation exhibited the best possible qualities, according to the results. An entrapment efficacy of 82.49%, a particle size of 403nm, a zeta potential of -28.5mV, and an in-vitro drug release of 85.7% were some of the prominent properties of the S2 formulation that utilised solvent evaporation. Two formulations were compared, and the results indicated that the H2 one was closer to the predicted values. The optimised formulation was lyophilized and contained in hard gelatin capsules. It had a 95.1% w/v drug content and a 93.8% w/v drug release at 8 hours after first-order kinetics. Tolvaptan capsules were shown to be stable in stability testing that lasted for three months. Since solid lipid nanoparticles provide enhanced drug entrapment efficiency and regulated drug release, our work shows that they might be a good delivery strategy for Tolvaptan.

Keywords: solid lipid nanoparticles, Tolvaptan, design of experiments, hot homogenisation, particle size, zetapotential

INTRODUCTION

Solid lipid nanoparticles (SLNs) are a form of nanoparticle medication delivery that has piqued the curiosity of the nanotechnology and pharmaceutical industries. An attractive alternative to traditional carriers, their colloidal system improves drug stability, allows for controlled release, and increases solubility (Baek and Cho, 2017) **[1]**. A specific vasopressin V2 receptor antagonist, tolvaptan increases sodium levels in hyponatremia and decreases the formation of kidney cysts in ADPKD. This research aims to develop a formulation that overcomes the issue of inadequate bioavailability.

MATERIALS AND METHODOLOGY

Evaluation of Lipid Solubility profile of Tolvaptan

Glyceryl monostearate, palmitic acid, stearic acid, compritol ATO888, and a number of other lipids were studied for their solubility tests. The drug concentration was measured in a known volume of each lipid at a temperature 5^0C higher than the lipids' melting points. The drug's most water-soluble lipid was chosen **[2, 3]**. The lipid and surfactant exhibiting maximum solubility were selected and formulations were prepared by two methods namely hot homogenization and solvent evaporation techniques. Particle size and invitro drug release were the dependent variables, whereas lipid and surfactant concentrations were the independent variables in a two-level factorial design.

Formulation design depicted by design expert sort software

Name of the factor	Units	Type	Subtype	Minimum	Maximum	Coded low	Coded high	Mean	StdDev.
Lipid Conc	mg	Numeric	Continuous	50.00	250.00	+1-50.00	+1-150.00	124.49	74.27
Surfactant conc	mg	Numeric	Continuous	50.00	250.00	+1-50.00	+1-150.00	123.57	77.38

Preparation of Tolvaptan loaded solid lipid nanoparticles by Hot Homogenization technique

The design of studies determined the combination of lipid and surfactant concentrations, such as stearic acid and poloxamer 188, which were used to generate different formulations **(Figure 1)**. Melted lipid containing 10 mg of Tolvaptan was heated to 5°C higher than the lipid's melting point. Added to this melted lipid was 50 mg of span 60, a lipophilic surfactant. To melt the lipid, a mixture of water and a hydrophilic surfactant (poloxamer 188) was heated to the same temperature. Then, the liquid was gently stirred to include the melted lipid. After 30 minutes, it was homogenised at 5000 rpm. After the coarse oil-in-water emulsion was generated, it was ultrasonicated to make the particles smaller.

After letting it settle, we strained out the solid lipid nanoparticles and transferred the supernatant to a new container **[4, 5] (Table 1)**.

Preparation of Tolvaptan loaded Solid Lipid Nanoparticles by Solvent evaporation Technique

Lipid and surfactant concentrations, such as stearic acid and poloxamer 188, were varied to create various formulations. To the lipid melt, the drug was added in a methanol solution. Rotating evaporation was used to thoroughly evaporate the organic solvent combination at 70°C. At the same temperature, the lipid layer containing the drug was put into an aqueous solution that also included surfactant. The mixture was homogenised at 700 rpm for three hours. The solid lipid nanoparticles were obtained by filtering off the liquid that was above the silt after the mixture had settled **[5, 6]**. **(Table 2)**.

Determination of calibration of drug

The drug solution in 10 ml of methanol was scanned using a UV spectrophotometer within the 200–400nm range. The λ max was noted and compared with that reported in literature **[7]**.

Table 1: Formulation table of Tolvaptan by Hot homogenization method

Formulation no	Tolvaptan(mg)	Stearic acid(mg)	Polaxamer(mg)	Span(mg)
H1	10	50	50	50
H2	10	250	250	50
H3	10	250	50	50
H4	10	50	250	50
H5	10	150	50	50
H6	10	150	150	50
H7	10	250	250	50
H8	10	50	150	50
H9	10	56.292	50	50
H10	10	56.292	50	50
H11	10	103.5	53.5	50
H12	10	100	150	50
H13	10	100	150	50
H14	10	150	100	50
H15	10	100	100	50

Table 2: Formulation table of Tolvaptan by solvent evaporation method

Formulation no	Tolvaptan(mg)	Stearic acid(mg)	Polaxamer(mg)	Span(mg)
S1	10	50	50	50
S2	10	250	250	50
S3	10	250	50	50
S4	10	50	250	50
S5	10	150	50	50
S6	10	150	150	50
S7	10	250	250	50
S8	10	50	150	50
S9	10	56.292	50	50
S10	10	56.292	50	50
S11	10	103.5	53.5	50
S12	10	100	150	50
S13	10	100	150	50
S14	10	150	100	50
S15	10	100	100	50

Characterization and evaluation of solid lipid nanoparticles

A colour and homogeneity check was performed on all of the solid lipid nanoparticles that were created".

Drug content and entrapment efficiency

Bath sonication was used to thoroughly mix 1 ml of the generated solution with the SLN dispersion, then the absorbance measured at 274 nm. The entrapment efficiency is done by subjecting the formulation at 17500rpm for 45min, following an appropriate dilution with phosphate buffer pH7.4, the absorbance was measured at 274 nm.

Measurement of particle size and zeta potential

The dispersion's average solid lipid nanoparticle diameter was measured using a Malvern Mastersizer and zeta potential was measured by Malvern zeta potential unit, with prior addition of 10ml of dispersion medium (double-distilled water) was added to one drop of each selected formulation **[8, 9]**.

Surface morphology

The Surface morphology of nanoparticles was determined by using scanning electron microscopy (SEM), dried emulsions are sprinkled onto sample holders coated with various conductive metals **[10]**.

In vitro- drug release studies

The dialysis membrane was used to measure the in-vitro drug release of Tolvaptan from various SLN dispersions. A predetermined volume of SLN sample was placed into it and kept in a pH7.4 buffered dissolving medium at 37°C ± 0.5°C at 50 rpm and its contents were connected to the paddle. 5 mL aliquots were taken and replenished with fresh buffer at 30 minutes, 1 hour, 2 hours, 4 hours, 5 hours, 6 hours, 7 hours, and 8 hours. The absorbance was determined **[11, 12]**.

Freeze Drying of Tolvaptan Solid Lipid Nanoparticles Dispersion

A lyophilizer was used to freeze-dry 30 millilitres of the optimised solid lipid nanoparticles dispersion. A free-flowing powder was obtained by freezing the samples at -70°C for an hour and then allowing them to dry for about 5-6 hours.

Formulation of Oral Solid Dosage Form of Tolvaptan-SLNs

A combination of aerosol (10%) and the optimised freeze-dried Tolvaptan solid lipid nanoparticles was placed within a hard gelatin capsule **[13, 14]**.

Evaluation of the optimized Tolvaptan capsules

Drug content and invitro dissolution studies

Drug content was determined using UV Spectrophotometer set at 274 nm. The drug release of Tolvaptan in its pure drug, marketed, and Tolvaptan-SLNs capsule forms was studied in 900 ml of phosphate buffer (pH 7.4) using a USP apparatus II at 37°C±0.5°C and 50 rpm with a rotating paddle. A syringe filter (0.22 nm) was used

to remove 5 ml of samples**,** which were then tested at 274 nm using a UV spectrophotometer for up to 8 hours. [15] The optimised Tolvaptan formulation underwent the ICH-mandated accelerated stability test over a three-month duration with a storage environment of 40°C±2°C/75% RH±5%RH. The formulation's invitro drug release parameters, including particle size and entrapment effectiveness, were studied for a duration of 0 to 3 months **[16, 17]**.

RESULTS AND DISCUSSIONS

CONSTRUCTION OF CALIBRATION CURVE FOR TOLVAPTAN

Determination of absorption maximum (λ max): The maximum absorption wavelength (λ max) of Tolvaptan was measured at 274 nm by use of a UV-Visible spectrophotometer. The R^2 value was found to be 0.9993 **(Figure 1)**.

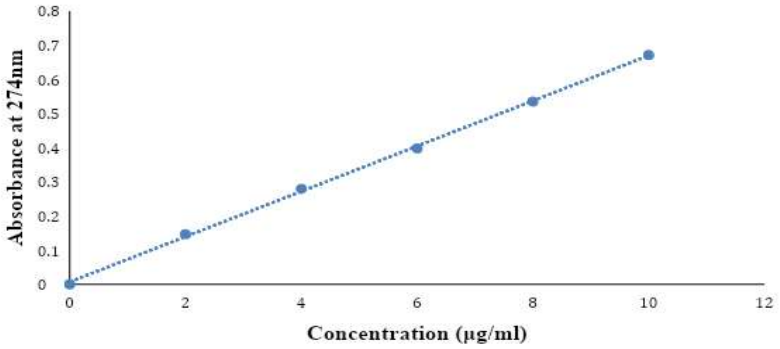

Figure 1: Standard plot of Tolvatptan

Evaluation of lipid solubility profile of Tolvaptan

Selection of lipid was done on the basis of absence of toxicity, absence of risk of degradation and its solubility in lipid since the higher the solvent capacity is, the higher will be the drug loading potential. Out of different lipids used, Tolvaptan showed high solubility in stearic acid and glyceryl monostearate at temperature 5°C above its melting point and insoluble in campritol and palmitic acid. Maximum solubility was observed in stearic acid therefore, stearic acid was used for further study.

Evaluation parameters for Hot Homogenization technique and solvent evaporation techniques

All of the formulations from H1 to H15 had a homogeneous appearance, a smooth consistency, and a milky white colour.

Estimation of Drug Content and entrapment efficiency

Using a UV-Spectrophotometer, the drug content was found to range from 70.92%w/v to 95.21%w/v for H1-H15 formulations, and from 69.99%w/v to 93.33%w/v for S1-S15 formulations. Among the H1-H15 formulation, percentage entrapment

efficiency was maximum for H2 and H7 formulation whereas among S1-S15 maximum entrapment efficiency was observed for S2 formulation (78.49%). According to the findings, the entrapment efficiency improved as the surfactant content rose. This could be because the drug becomes more soluble in the lipid as the surfactant concentration rises. The chosen model may be used to traverse the design space, since the ANOVA 0.05 showed that the model is significant and the appropriate precision is larger than 4(5.72) **(Table 3)**.

Table 3: ANOVA performed by software for response 1(entrapment efficiency); Response 1: Entrappment efficiency

Source	Sum of squares	df	Mean square	F-value	p-value	
Block	354.46	1	354.46			
Model	756.99	3	252.33	3.90	0.048	significant
A-Lipid conc	204.59	1	204.59	3.16	0.1091	
B-Surfactant con	46.92	1	46.92	0.7252	0.4165	
AB	168.47	1	168.47	2.60	0.1411	
Curvature	0.0853	1	0.0853	0.0013	0.9718	
Residual	582.32	9	64.70			
Lack of fit	582.3	6	97.05			
Pure Error	0.0000	3	0.0000			
Cor Total	1693.85	14				

St.Dev.	8.04	R^2	0.5652
Mean	74.79	Adjusted R^2	0.4203
C.V.%	10.76	Predicted R^2	NA
		Adeq precision	5.7247

Determination of Particle size

Particle sizes ranging from 349 nm to 990 nm were observed for several formulations (H1-H15; S1-S15). H2 formulation was observed to have minimum particle size (349nm) **(Table 4 and Figure 2)**. These findings are derived from the analysis of variance (ANOVA) on the response particle size. The model is statistically significant, since the p-value is less than 0.005. One way to test signal-to-noise ratio is using Adequate Precision. With a ratio of 11.643, the model is able to travel the design space, indicating a sufficient signal **(Table 4)**.

Table 4: ANOVA performed by software for response 2 (particle size) Response 2: Particle size

Source	Sum of squares	df	Mean square	F-value	p-value	
Block	5.693E-07	1	5.693E-07			
Model	2.44E-06	3	8.146E-07	20.16	0.0002	significant
A-Lipid conc	1.430E-07	1	1.430E-7	3.54	0.0927	
B-Surfactant con0.7868	1.128E-08	1	1.128E-08	0.279	0.6100	
AB	8.437E-07	1	8.437E-07	20.88	0.0013	
Curvature	3.138E-09	1	3.138E-09	0.0776	0.7868	
Residual	3.638E-07	9	4.042E-08			
Lack of fit	3.638E-07	6	6.063E-08			
Pure Error	0.00000	3	0.0000			
Cor Total	3.380E-06	14				

St.Dev.	0.0002	R^2	0.8704
Mean	0.0017	Adjusted R^2	0.8273
C.V.%	11.78	Predicted R^2	NA
		Adeq precision	11.64

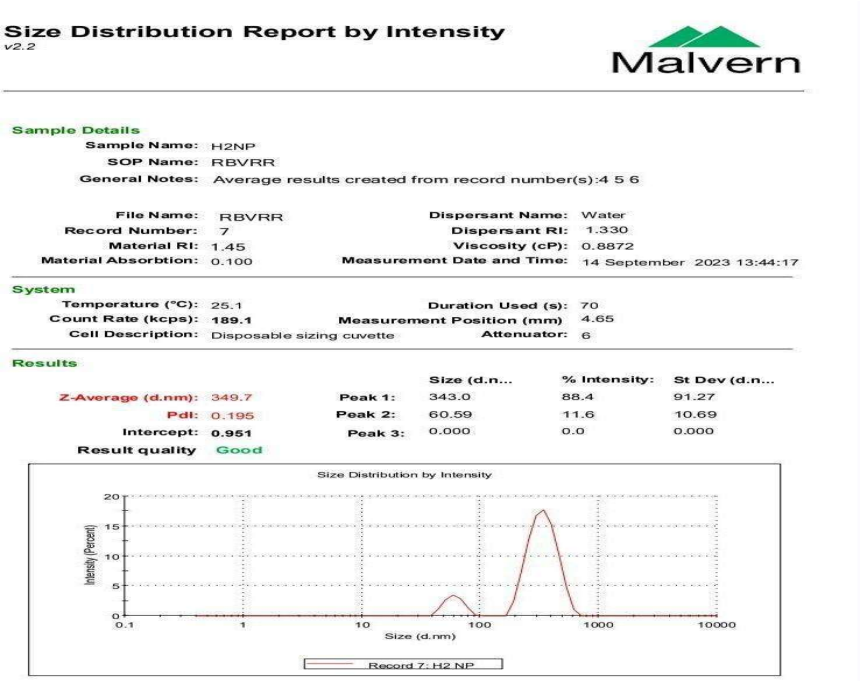

Figure 2: Particle Size report of H2 formulation of SLNs

Determination of Zeta potential

The zeta potential can be determined by using phase analysis light scattering. The Zeta potential is measured by the Malvern zeta potential apparatus, Zeta potential of all formulations determined by both the methods was found in range of -21.6mV to -50.4mV indicating the stability of all formulations during storage **Table 5 and Figure 3**.

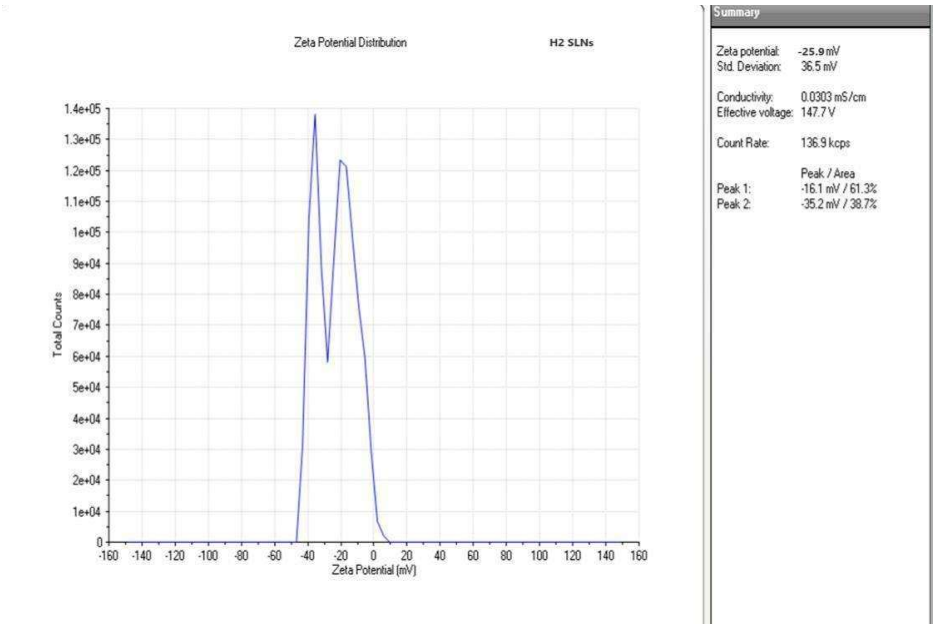

Figure 3: Zeta Potential report of H2 formulation of SLNs
Each value is average of three separate determinations ±SD n=3

Table 5: Different evaluation tests of all the formulations

Formulation	Drug content (%)	Entrapment efficiency (%)	Particle size(nm)	Zeta potential(mv)
H1	82.15±0.002	63.45±0.001	750	-40.1
H2	95.21±0.001	89.43±0.002	349	-25.9
H3	82.0±0.002	70.73±0.005	584	-42.5
H4	83.21±0.009	66.73±0.001	715	-38.4
H5	80.11±0.001	60.40±0.003	660	-30.9
H6	86.0±0.002	75.47±0.001	590	-30.2
H7	95.21±0.001	89.43±0.002	349	-25.9
H8	80.45±0.005	76.12±0.002	581	-22.6
H9	83.78±0.003	59.02±0.009	618	-32.7
H10	83.78±0.003	59.02±0.009	618	-32.7
H11	85.10±0.003	67.10±0.004	608	-49.1
H12	89.11±0.001	55.51±0.005	754	-40.7
H13	89.11±0.001	55.51±0.005	754	-40.7
H14	73.22±0.002	61.85±0.001	708	-35.1
H15	70.92±0.001	59.11±0.001	650	-30.0

Formulations	Drug content (%)	Entrapment Efficiency (%)	Particle size (nm)	Zeta potential mV
S1	80.09±0.001	67.23±0.008	770	-41.4
S2	93.1±±0.002	82.49±0.001	403	-31.5
S3	82.55±0.002	69.99±0.001	585	-29.0
S4	85.11±0.005	76.01±0.003	718	-39.9
S5	79.21±0.007	63.0±0.001	640	-40.5
S6	90.33±0.002	78.49±0.005	430	-28.5
S7	93.1±0.009	82.49±0.001	403	-31.5
S8	86.11±0.008	75.22±0.003	590	-21.6
S9	84.37±0.002	70.11±0.007	990	-50.4
S10	84.37±0.002	70.11±0.007	990	-50.4
S11	82.01±0.004	65.19±0.005	650	-37.8
S12	86.22±0.005	62.06±0.004	840	-45.3
S13	86.22±0.005	62.06±0.004	840	-45.3
S14	70.05±0.001	63.66±0.001	710	-37.1
S15	69.99±0.003	54.55±0.001	620	-34.7

Determination of Surface morphology by scanning electron microscope

The particle size of the H2 formulation was determined by means of a scanning electron microscopy (SEM) study **(Figure 4)**.

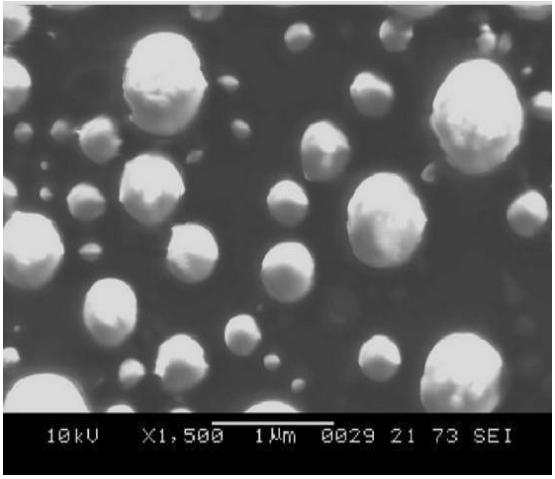

Figure 4: SEM image of H2 formulation

In-vitro drug release

All of the formulations, including H1–H15 and S1–S15, had drug release percentages ranging from 65.0% to 92.1%. H2 formulation appears to prolong the release of drug till 8hr of time relative to other formulations. Better release was obtained in the formulation with 1:1 ratio of lipid and surfactant. Poloxamer 188 having low molecular weight with low viscosity and high HLB enhanced the release **(Figure 5)**.

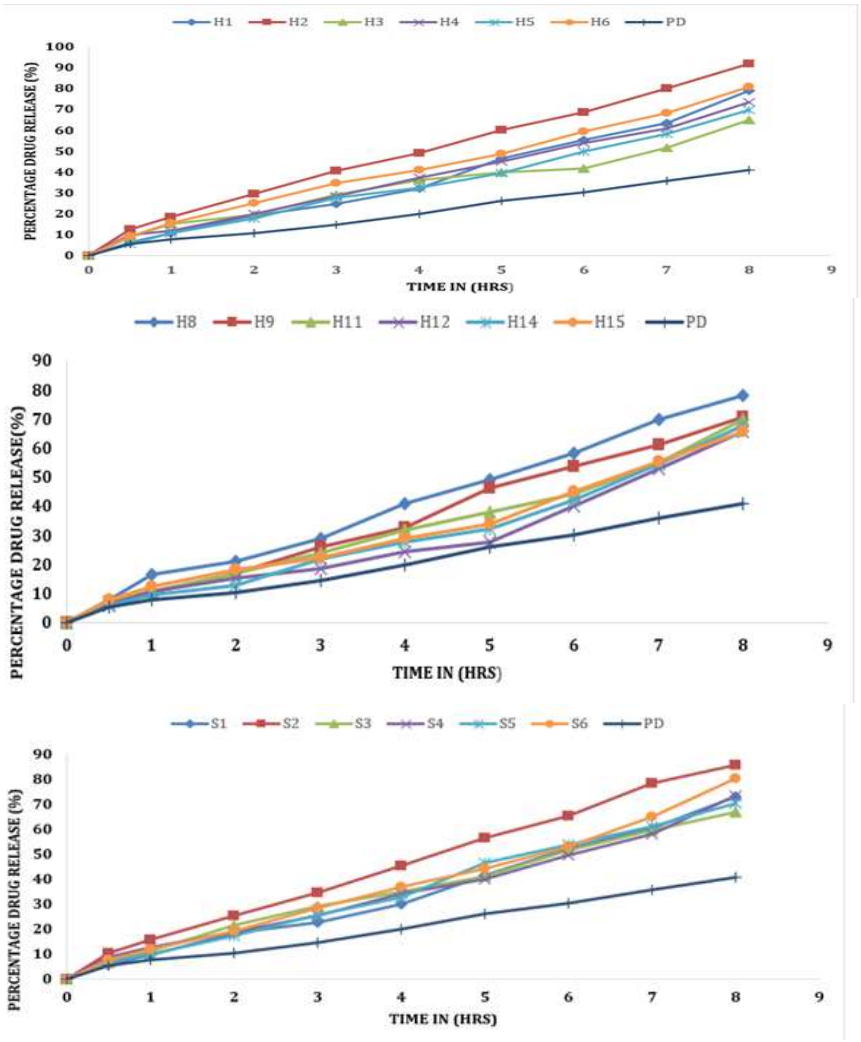

Figure 5: Invitro drug release of all the formulations

Optimised formulation suggested by design expert

An optimised formulation was created based on the solutions of the experimental design, with the responses being given relative importance. An attractiveness index (DI) is used to identify each possible option. **Figure 6, Table 6** show that each response was given an equal weight. The experimental values correspond with the expected mean of 89.83% entrapment efficiency and 367.38 nm particle size for Run 2, as indicated by the correlation coefficient of 1.

Table 6: Confirmation results of the modelled response

Run 2 response	Predicted mean	Predicted median	Observed	Std Dev	n	SE Pred	95%PI Low	95% PI high
Entrappment efficiency	**89.88**	**89.88**	**92.43**	**8.04**	**1**	**9.9940**	**67.27**	**112.491**
Particle size	**367.386**	**365.414**	**349**	**26.989**	**1**	**NA**	**302.877**	**460.496**

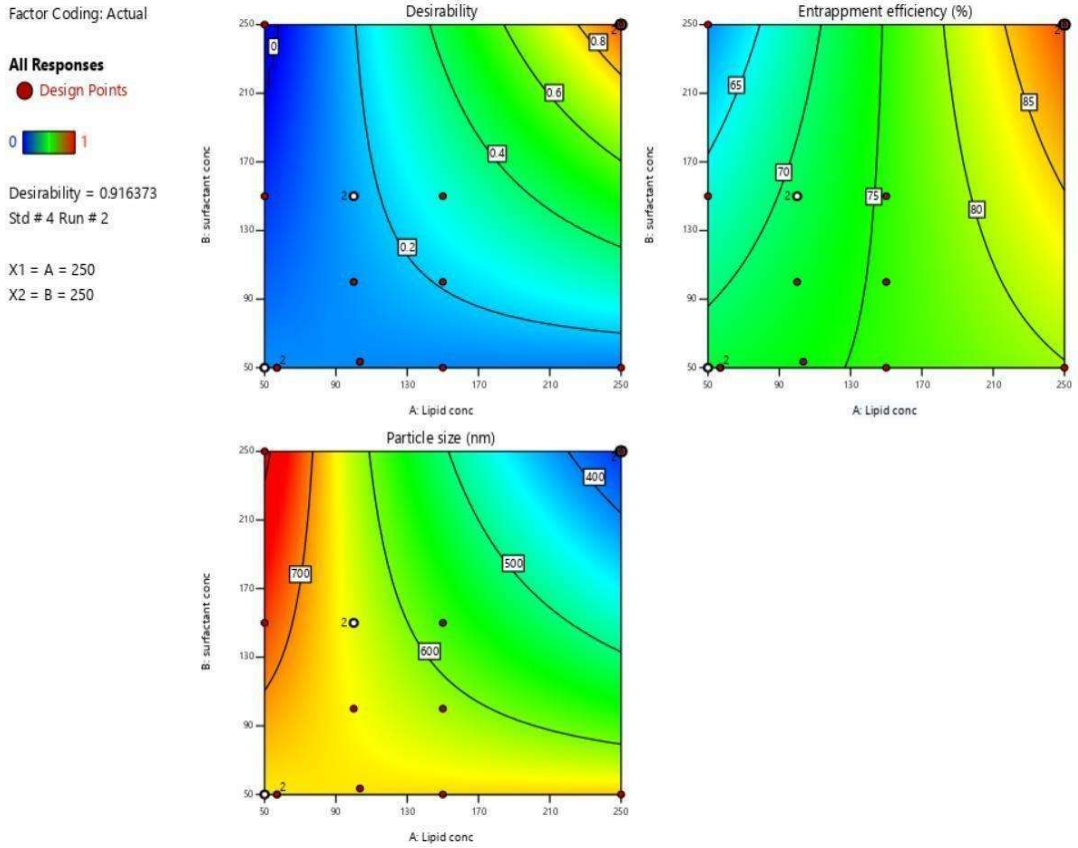

Figure 6: contour plot for all responses

Formulation H2 had the best combination of all 15 parameters (zeta potential, largest drug content, smallest particle size, and highest entrapment efficiency) out of the 15 tested formulations (H1–H15). Formulation S2 also showed the best drug content, entrapment efficiency, and prolonged drug release out of the fifteen formulations (S1–S15).When comparing the two formulations, H2 showed optimized characteristics. As a result, Formulation H2 was chosen to move forward in the development of the final product. In order to create an oral solid dosage form, the H2 formulation was freeze-dried in a lyophylizer. A free-flowing powder was obtained by freezing the dispersion at -70°C for an hour and then allowing it to dry for about 5-6 hours.

In-vitro drug release profile of optimised formulation

Table 7 displays the in-vitro dissolving profile of Tolvaptan-SLN capsules, including the optimised formulation, pure

drug, and marketed version. As a result, the Marketed formulation demonstrated 95.45% drug release after 4 hours, whereas Tolvaptan-SLN demonstrated 93.8% drug release after 8 hours. Because a larger particle size increases the diffusional channel for the drug's release, a smaller particle size is required to achieve the same percentage of drug release, suggesting an inverse link between the two variables. Testing the formulations' in-vitro drug release performance with different amounts of lipid material and surfactant showed that the release was controlled by both the surfactant concentration and the lipid material concentration **[18]**. Formulation follows first-order release and non-fickian diffusion, a three-month stability study was conducted on the particle size of the capsule containing the solid dispersion under the following conditions: 40°C±2°C/ 75%RH±5% RH. There is no significant change in the characteristics indicating colloidal stability and this might be attributed due to protection by the surfactant and lipid used providing excellent hindrance providing particle aggregation.

Table 7: Comparision studies of invitro drug release of optimized formulation with pure drug and marketed formulation

Sl.no	Time(hrs)	Cumulative drug release of Tolvaptan SLN	Pure drug	Marketed formulation
	0.5	12.5±0.03	5.5±0.04	22.1±0.07
	1	22.5±0.06	7.8±0.02	42.6±0.01
	2	32.9±0.01	10.5±0.01	62.8±0.04
	3	46.1±0.04	14.7±0.02	81.8±0.06
	4	52.8±0.02	19.9±0.02	95.4±0.02
	5	63.9±0.02	26.1±0.05	
	6	77.4±0.03	30.3±0.03	
	7	82.2±0.01	38.8±0.01	
	8	93.8±0.01	41.0±0.02	

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