Formulation and Evaluation of oil Based PLGA Hybrid Nanocarrier for Highly Lipophilic Drug

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ABSTRACT

Objectives:

The study's objective was to create and assess a biocompatible PLGA-oil hybrid for high loading and regulated distribution of lipophilic drugs utilizing the solvent evaporation method. PLGA nanoparticles and synthetic/vegetable oils are combined in this formulation to improve stability, adaptability, size homogeneity, drug loading, and therapeutic effectiveness. A unique approach to drug delivery systems is represented by hybrid nanoparticles that combine PLGA with either synthetic or vegetable oils.

Methods:

Tizanidine hydrochloride was chosen as a model drug for this study. A novel oil-based PLGA hybrid was developed using the solvent evaporation method, varying the ratios of Olive oil concentration and homogenization speed. The formulation was optimized using Design Expert software, and the stability of the top three optimized batches was evaluated.

Results:

A novel oil-based PLGA hybrid has been successfully formulated. Its particle size falls within the Nano range, and the zeta potential indicates excellent stability. Notably, the exhibits enhanced saturation solubility and drug content compared to the pure drug. Further characterization using FT-IR, DSC, and scanning electron microscopy confirms its structure. The in vitro release profile over 24 hours demonstrates that the optimized formulation achieves superior control over drug release and stability." **Conclusions:**

According to the release studies, adding oil to the polymeric matrix may result in nanostructures that stabilize drugs and allow them to be released more gradually.

keywords:

Hybrid polymeric nanocarriers, PLGA, lipophilic drug, vegetable oil.

INTRODUCTION

Hybrid nanoparticles enable customized drug delivery by combining diverse carrier properties. A polymer-vegetable oil nanostructure is developed for drugs, leveraging oil's dual role as a matrix component and bioactive agent. Oil dispersion increases the matrix's amorphous character.

This design merges PLGA nanoparticles with oils to enhance stability, adaptability, uniformity, and therapeutic impact. [1]

Hybrid nanoparticles combining PLGA with synthetic or vegetable oils represent a novel approach in drug delivery systems. The synergy between PLGA nanoparticles and oils enhances stability, versatility, and particle uniformity. This combination also facilitates efficient drug encapsulation and taps into the therapeutic potential of vegetable oils. This emerging trend aims to enhance the pharmaceutical and therapeutic effectiveness of highly drugs through improved PLGA-oil nanostructures. PLGA-based nanocarriers are among the most adaptable systems in personalized Nanomedicine suitable platform for theranostic applications is provided by combining PLGA nanoparticles with grapheme derivatives. To guarantee drug stability, safety, and therapeutic efficacy, designing successful hybrid carriers requires a careful examination of the physicochemical characteristics of each component as well as an understanding of targeting and release processes. [2]

The ability of biodegradable polymers to release medications in a targeted and regulated manner has made them indispensable in drug delivery systems. A well-researched synthetic biodegradable polymer, polylactic-co-glycolic acid (PLGA) is renowned for its advantageous qualities and "smart polymer" traits, which react to environmental cues. PLGA-based systems have been widely reported to treat or diagnose a variety of illnesses and ailments. [3]

Vegetable oils have been a crucial component in various formulations such as emulsions, Nano-emulsions, and certain semisolid dosage forms. However, their use is limited in many pharmaceutical applications due to issues like incompatibility, stickiness, greasiness, and the potential for rancidity. To address these challenges, vegetable oils can be modified to include cross- linkable functionalities, making them more suitable for pharmaceutical use. Vegetable oils are abundant, cost-

effective, and possess unique functional groups that confer biodegradable, renewable, and eco-friendly properties. [4, 5]

Hybrid natural fiber polymer composites are gaining prominence due to their sustainable and economical nature. Combining different fibers, such as natural and synthetic or various natural fibers within a single polymer matrix, can significantly enhance the composite's mechanical properties compared to using a single type of fiber. [6]

Novel drug delivery systems represent an innovative approach to enhancing drug efficacy and longevity. Novel drug delivery systems aim to optimize therapy by modifying the drug's structure or incorporating it into a carrier. The main goals of these systems are to minimize drug degradation, reduce adverse effects, and improve solubility and bioavailability. [7, 8]

With advancements in technology, novel drug delivery systems (NDDS) now also encompass herbal formulations, aiming to improve stability, bioavailability, and protection from degradation while reducing toxicity. [9]

With an emphasis on enhancing the medicinal and pharmacological qualities of medications, the proposed study attempts to create oil-based hybrid nanocarriers as a novel drug delivery mechanism. The suitability of these hybrid nanocarriers for use in medicinal applications will be evaluated.

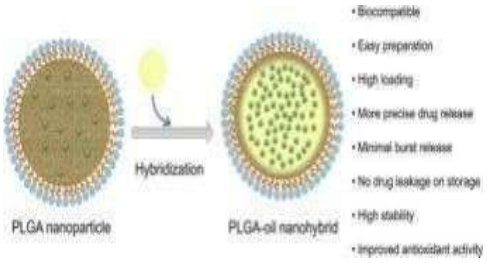


Fig:1. PLGA-oil Nano hybrid

MATERIALS AND METHODS-

Materials: Mumbai's Evonic India PVT LTD donated PLGA [POLY(D,L-LACTIDE-CO-

GLYCOLIDE)] 75:25 (RESOMER RG 757S). We bought the Tizanidine hydrochloride from Sichuan Credit Pharmaceutical Co., Ltd. in China. The supplier of Poloxamer P 188 was Researchable Fine Chem. Industries in Mumbai. Mumbai-based Deoleo India PVT LTD provided the olive oil. Thomas Bakers (Chemicals) PVT LTD in Mumbai supplied the potassium dihydrogen phosphate and sodium hydroxide, while Merck Life Sciences PVT LTD in Mumbai supplied the acetone and methanol, the other solvents. Without any additional purification, the components are used immediately upon receipt.

Methods:

1. Preliminary Analysis of Tizanidine Hydrochloride:

A. A standard solution of Tizanidine Hydrochloride was prepared by dissolving 100 mg in 100 ml of phosphate buffer (pH 6.4). After diluting 0.1 ml of this solution to 100 ml, a 1 µg/ml concentration was achieved. The solution's UV-Visible spectrum was then scanned between 190-400 nm to identify the optimal wavelength for analysis[10]

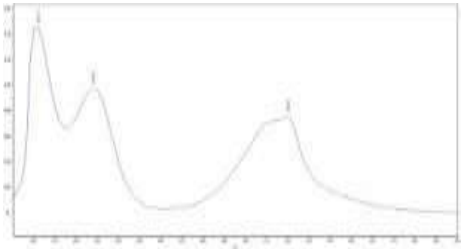


Fig:2. Absorption Spectra of Tizanidine Hydrochloride

In Figure: 2 Tizanidine Hydrochloride showed absorption maxima at 202, 228 and 320 nm. The lambda max chosen for analysis of Tizanidine was 228 nm. [10]

B. Construction of Calibration curve: Calibration curve was constructed using two diluents as below;

1. Calibration curve for saturation solubility – Methanol : Water (50:50) (Table:1)

2. Calibration curve for dissolution study – Phosphate buffer pH 6.4(Table:2)

In Figure 3 & 4 Concentration (µg/ml) was plotted against absorbance to create the calibration curve. For the dissolving research, the concentration range was 0-1.0 µg/ml, and for saturation solubility, it was 0-1.25 µg/ml. [11]

Calibration curve in Methanol: Water (50:50)	
Conc. (uGu/ml)	**Absorbance**
0.00	0.000
0.25	0.205
0.50	0.419
0.75	0.584
1.00	0.789
1.25	0.998

Table: 1. Calibration curve in methanol andwater water

Calibration curve in Phosphate buffer pH 6.4	
Conc. (uGu/ml)	**Absorbance**
0.00	0.000
0.20	0.184
0.40	0.352
0.60	0.529
0.80	0.726
1.00	0.918

Table: 2. Calibration curve in Phosphate buffer pH 6.4

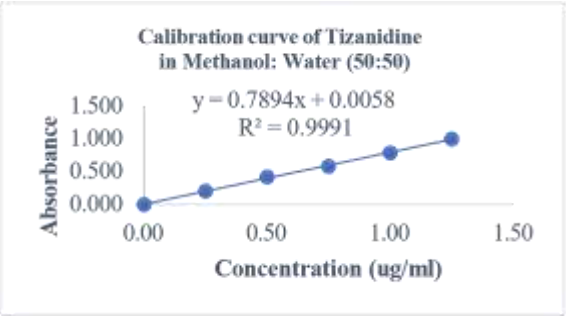


Fig: 3. Calibration curve in methanol and water

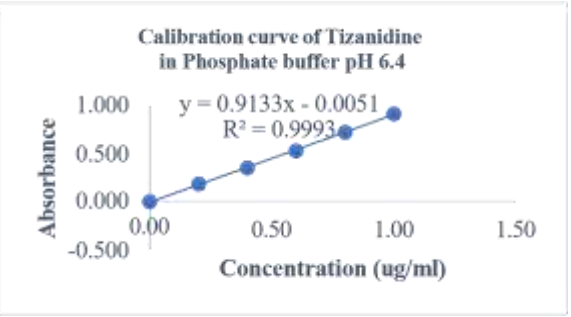


Fig: 4. Calibration curve in Phosphate buffer pH 6.4

C. Solubility of Tizanidine Hydrochloride in different oils: 5 ml of various oils (Castor oil, Olive oil, Sesame oil, Peanut oil, Capryol 90, Isopropyl Myristate, and Light Liquid Paraffin) were mixed with an excess of Tizanidine using a mechanical mixer overnight. The following day, the samples were centrifuged for 20 minutes at 2500 rpm. After collecting and suitably diluting the supernatant layer

with a 50:50 methanol: water ratio, the absorbance was measured and is displayed in Table 3. The concentration in milligrams per milliliter was computed using the regression coefficient derived from the calibration curve. (Figure: 5). [12]

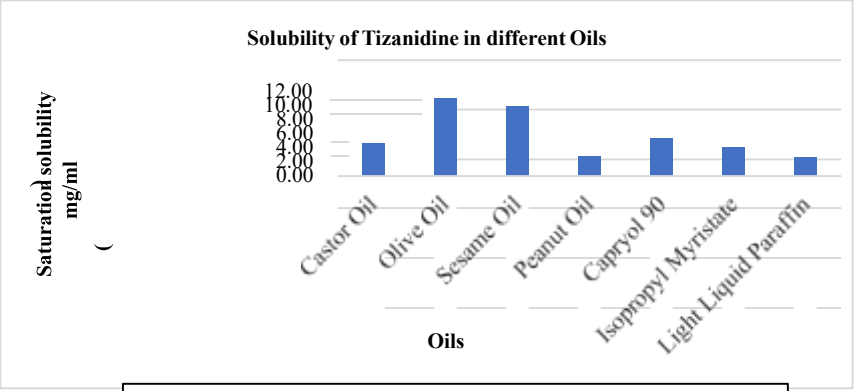


Fig: 5. Solubility of Tizanidine Hydrochloride in different oils

Sr. No.	Sample	Absorbance	Conc. (ug/ml)	Dilution	Actual Conc. (ug/ml)	Actual Conc. (mg/ml)
1	Castor Oil	0.377	0.47	10000	4703.81	4.70
2	Olive Oil	0.882	1.11	10000	11095.31	11.10
3	Sesame Oil	0.798	1.00	10000	10032.16	10.03
4	Peanut Oil	0.225	0.28	10000	2780.03	2.78
5	Capryol 90	0.425	0.53	10000	5311.31	5.31
6	Isopropyl Myristate	0.336	0.42	10000	4184.89	4.18
7	Light Liquid Paraffin	0.213	0.26	10000	2628.15	2.63

Table: 3. Solubility of Tizanidine Hydrochloride in different oils

Design of Experiment for optimization of nanoparticle formulation:

Central composite Design:

Sr. No	Independent Variable	Unit	Low	High
1	Oil Concentration	mg	1 !0	200
2	Homogenization Speed	rpm	5000	1000 0

Table: 4. Central composite Design

Response:

Sr. No.	Parameter	Unit
1	Particle Size	nm

Table: 5. Response

Actual Design:

	Factor 1	Factor 2	Response 1
Run	A:Oil Conc.	B:Homogenization Speed	Particle Size
	Mg	RPM	nm
1	160	5000	152.8
2	160	7500	140.2
3	120	10000	178.4
4	200	5000	178.5
5	200	7500	164.6
6	160	10000	132.7
7	200	10000	156.3
8	120	7500	187.9
9	120	5000	209.6

Table: 6. Actual Design

Formulation Table:

Sr No.	Ingredients	NCB-1	NCB-2	NCB-3	NCB-4	NCB-5	NCB-6	NCB-7	NCB-8	NCB-9
1	Tizanidine Hydrochloride (mg)	80	80	80	80	80	80	80	80	80
2	Olive oil (mg)	160	160	120	200	200	160	200	120	120
3	PLGA (mg)	200	200	200	200	200	200	200	200	200
4	Acetone (ml)	15	15	15	15	15	15	15	15	15
5	0.5% P188 Solution (ml)	100	100	100	100	100	100	100	100	100
Homogenization speed (rpm)		5000	7500	10000	5000	7500	10000	10000	7500	5000
Homogenization Time (min)		30	30	30	30	30	30	30	30	30

Table:7.Formulationcomposition of hybrid Nano carrier

Nano carriers were prepared by Emulsification-solvent evaporation method Figure: 6. initially dissolve drug and oil in organic solvent (Acetone), then dissolve PLGA in it. Add this solution to 0.5% Poloxamer 188 solution drop wise under constant stirring. Homogenize for 30 min using ice bath. Stir overnight to remove all the organic solvent. Filter and dry the Nano carriers for Further experiment (Table: 4, 5, 6 & 7). [13]

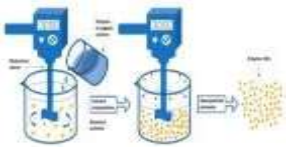


Fig: 6. The emulsification-solvent evaporation technique is depicted schematically.

Evaluations:

1. **Particle Size, PDI and Zeta Potential:** Particle size analysis was conducted using a Horiba SZ-100 at 25°C. To ensure consistency, disposable sizing cuvettes were utilized, maintaining a constant refractive index, viscosity, and dielectric constant across all samples. Dynamic light scattering (DLS) was employed to determine particle size. Following dilution with deionized water, samples were analyzed using the instrument[14]
2. **Thermo gravimetric analysis:** Differential scanning calorimetric was used to examine the drug's physical condition inside the Nano carriers (DSC Mettler-Totala, Switzerland). Using DSC, thermo grams were acquired. About 1 mg of the sample was used for this, and it was enclosed in an aluminum pan and allowed to equilibrate for 10 minutes at 25°C. By heating at a rate of 10 °C per minute while being exposed to an environment of nitrogen (20 ml/min), the analysis was carried out between 25 and 300 °C. Enthalpy (m/w) vs. temperature (°C) was plotted to record the DSC thermo grams [15]
3. **FT-IR:** Fourier transform infrared (FT-IR) spectra were acquired (Bruker) to ascertain the chemical makeup of the surface layer. After being thoroughly dried, the study's samples were turned into pellets using the KBr disc process. Tizanidine standard spectra were used for the spectral analysis and comparison. [16]
4. **Scanning electron microscopy:** SEM imaging was performed using a Quanta Inspect F50. Samples were prepared by drying and coating, and then examined at different

magnifications using a field emission gun operating at 20 kV... [17].

5. **Drug release studies:** To ascertain the drug release properties of Nano carriers, the dialysis bag method was employed. Nano carriers were loaded into a pre-swelled dialysis bag with 2 mg of medicine in 1 ml of mili-Q water. They were then immersed in 200 ml of phosphate buffer (pH 6.4) in a covered beaker. The beaker was placed over a magnetic stirrer at 37 ± 2°C and 100 rpm. As long as the sink condition was maintained, 1 ml samples were obtained at predetermined intervals. The samples were diluted using the appropriate amount of dissolving media prior to analysis using a UV visible spectrophotometer [17]
6. **Drug Content: Drug Content by UV:** The UV Visible Spectrophotometer was used to determine the drug's content. Using methanol as a blank, absorbance was measured at 228 nm after the standard and Nano carrier samples were dissolved in methanol to create solutions with a concentration of 1 µg/ml. [18] The percent drug content was estimated by using following formula:

$$\% \, Drugcontent = \frac{Cu}{Cs} \times 100$$

Where,
Cu = Concentration of Tizanidine in samples, µg/ml

Cs = Concentration of Tizanidine in Standard, µg/ml**.** [18]

Drug Content by HPLC [19]: (as per IP 2018, Volume III, Page No. 3385)

The drug content of the drug was determined by using HPLC. [20]

Preparation of test solution: Weigh and powder Nano carrier containing about 20 mg of Tizanidine, disperse in 50 ml of Phosphate buffer pH 6.6 and dilute to 100 ml with Acetonitrile and filter.

Preparation of Standard solution: Weigh 10 mg of Tizanidine Hydrochloride, dissolve in 25 ml of Phosphate buffer pH 6.6 and dilute to 50 ml with Acetonitrile.

Chromatographic conditions: Column: Luna C18 (2) (250 x 4.6mm, 5µ), Mobile Phase: a mixture of 80 volumes of phosphate buffer pH 6.6 and 20 volumes of acetonitrile

Detection rate: 1 milliliter per minute: 320 nm, Injection volume: 20µl

$$\%\ Drugcontent = \frac{SampleArea}{StandardArea} \times \frac{Cu}{Cs} \times purityofstandard$$

Where, Cu = Concentration of Tizanidine in samples, µg/ml, Cs = Concentration of Tizanidine in Standard, µg/ml

7. **Saturation Solubility:** Excess amount of Tizanidine pure drug and samples were added to 5 ml of water and kept for mixing overnight using mechanical shaker and next day samples were centrifuged at 2500 rpm for 20 minutes. The supernatant layer was collected and diluted appropriately using Methanol: Water (50:50) and absorbance was measured. Using the regression coefficient obtained from calibration curve the concentration in mg/ml was calculated. [21]
8. **Stability Study:** The Stability study of optimized batch was conducted at 40℃ and 75% relative humidity for 1 month and after the stability interval completes the optimized batch was evaluated for all the evaluations including particle size, drug content, FT-IR, thermo gravimetric analysis and drug release study. [22]

RESULTS AND DISCUSSION

Evaluation of nanoparticles by DOE

Particle size: The particle size of obtained nanoparticles was ranged from 132.7 nm to 209.6 nm. The ANOVA (Analysis of Variance) for 2FI model was used to analyses the particle size of the

prepared nanoparticles. The p-value and R2 value was found to be 0.0006 and 0.9507 respectively, showing the model is significant.

DOE Results for particle size:

The significance of the model is indicated by its F-value of 143.06. The likelihood that an F-value this large may be caused by noise is only 0.09%. Model terms are considered significant when the P-value is less than 0.0500. A, B, and A2 are important model terms in this instance. The model terms are not significant if the values are higher than 0.1000. Model reduction could make your model better if it has a lot of unnecessary terms (apart from those needed to maintain hierarchy).

Fit Statistics :(Table: 8)

Std. Dev.	2.57	**R-sq.**	0.9958
Mean	166.78	**Adjusted R-sq.**	0.9889
C.V. %	1.54	**Predicted R-sq.**	0.9507
		Adel Precision	36.6631

Table: 8. Fit Statistics

The predicted R^- sq. value of 0.9507 closely aligns with the adjusted R^2 value of 0.9889 as the difference between them is less than 02 indicating a good level of agreement [23]

Final Equation in Terms of Coded Factors (Table: 9)

Particle Size	=
+139.36	
-12.75	A
-12.25	B
+2.25	AB
+37.32	A^2
+3.82	B^2

Table: 9. Final Equation in Terms of Coded Factors

The coded factor equation enables response predictions for specified factor levels, with high and low levels defaulting to +1 and -1, respectively. This coded equation is particularly useful for assessing the relative influence of each factor by comparing the coefficients of the factors.

Final Equation in Terms of Actual Factors (Table: 10)

Particle Size	=
+885.52222	
-7.95083	Oil Conc.
-0.017660	Homogenization Speed
+0.000022	Oil Conc. * Homogenization Speed
+0.023323	Oil Conc.^{2}
+6.10667E-07	Homogenization Speed^2

Table: 10. Final Equation in Terms of Actual Factors

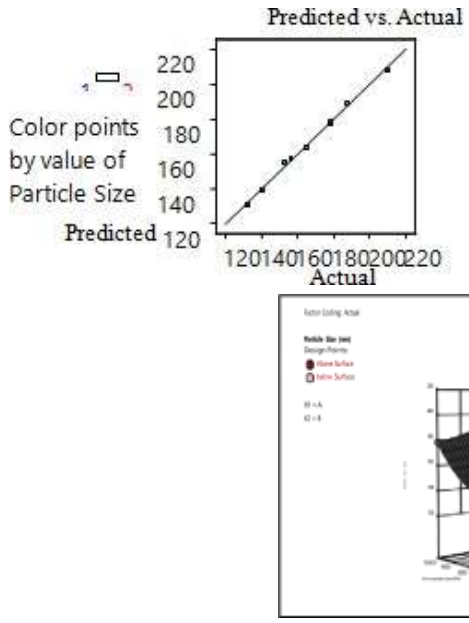
The equation, expressed in act ual factor terms, facilitates response pred iction for specified factor levels. Factor levels must be entered in their original units. However, this equation is not suitable for comparing the relative effects of each factor, as coefficient scaling is unit-dependent and the intercept is offset from the design space center. [23]

REPORT (Table: 11)

Run Order	Actual Value	Predicted Value	Residual	Levera ge ge	Internally Studentized Residuals	Externally Studentized Residuals	Cook's Distance	Influence on Fitted Value DFFITS	Standard Order
1	152.80	155.42	-2.62	0.556	-1.533	-2.688	0.489	-3.005(1)	7
2	140.20	139.36	0.8444	0.556	0.494	0.420	0.051	0.470	9
3	178.40	178.74	-0.3389	0.806	-0.299	-0.248	0.062	-0.505	3
4	178.50	177.74	0.7611	0.806	0.673	0.596	0.312	1.213	2
5	164.60	163.92	0.6778	0.556	0.396	0.332	0.033	0.372	6
6	132.70	130.92	1.78	0.556	1.039	1.061	0.225	1.186	8
7	156.30	157.74	-1.44	0.806	-1.272	-1.529	1.117(1)	-3.113(1)	4
8	187.90	189.42	-1.52	0.556	-0.890	-0.847	0.165	-0.947	5
9	209.60	207.74	1.86	0.806	1.645	4.284	1.868(1)	8.720(1)	1

Table: 11. REPORT

ACUTAL VS PREDICTED (Figure: 7) CONTOUR PLOT (Figure: 8) 3D SURFACE PLOT (Figure: 9)



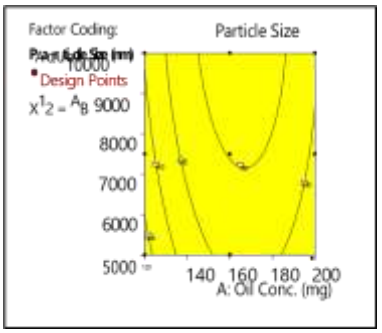


Fig: 7. Predicted vs. Actual value

Fig: 9.Surface Plot

1. **Particle size, PDI and Zeta potential** (Table: 12): The particle size for prepared nanoparticles of optimized batches was 178.4 nm, 136.3 nm and 156.3 nm, as determined by dynamic light scattering (DLS) (Figure: 10, 11, 12, 13). The zeta potential of the optimized batches particles was -23.1 mV, -29.8 mV and -22.3 mV, indicating good stability of the dispersion due to sufficient electrostatic repulsion between particles. The polydispersity index (PDI) of optimized batches was measured at 0.468, 0247 and 0.684, reflecting a relatively narrow size distribution and uniformity in particle size (Figure: 14, 15, 16, and17).

Formulation No.	Formulation Code	Particle size (nm)	PDI	Zeta potential (mV)
	1	TZN 3369.0 nm	2.057	-15.2mV
	2	NCB-3 178.4 nm	0.468	-23.1 mV
	3	NCB-6 136.3 nm	0.247	-29.8 mV

4	NCB-7	156.3 nm	0.684	-22.3 mV

Table: 12.Particle size, Polydispersity index and Zeta potential of Tizanidine Hydrochloride and optimized batches.

Fig: 10. Particle size for TZN

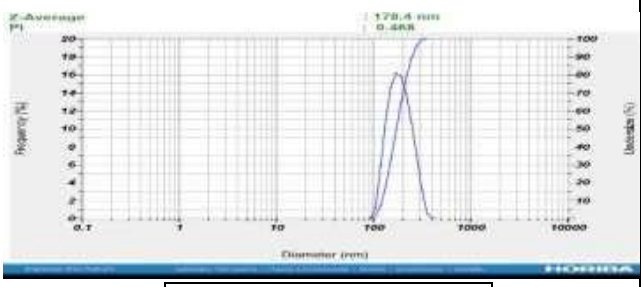


Fig: 11. Particle size for NCB-3

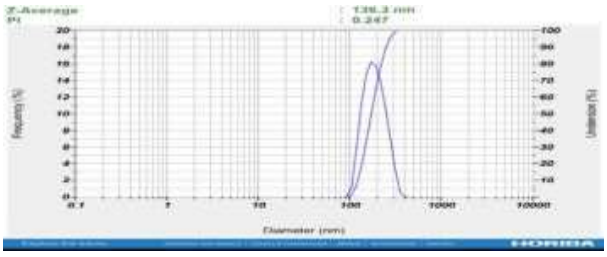


Fig: 12. Particle size for NCB-6

Fig:13.Particle size for NCB-7

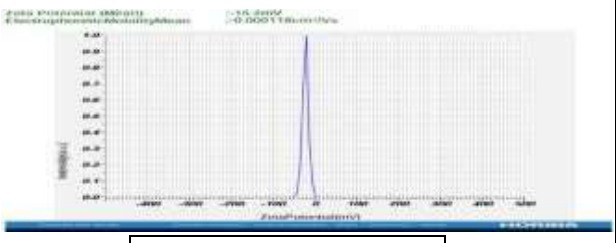


Fig: 14. Zeta Potential for TZN

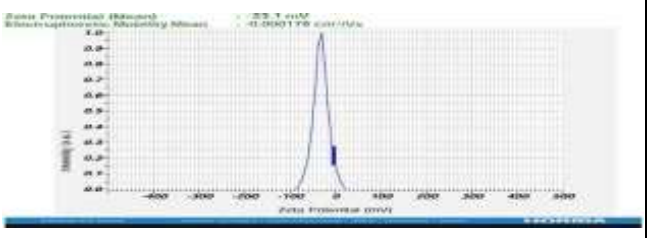


Fig: 15. Zeta Potential for NCB-3

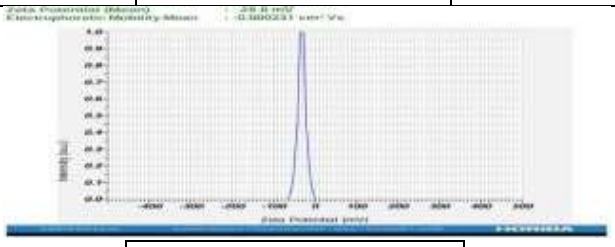


Fig: 16. Zeta Potential for NCB-6

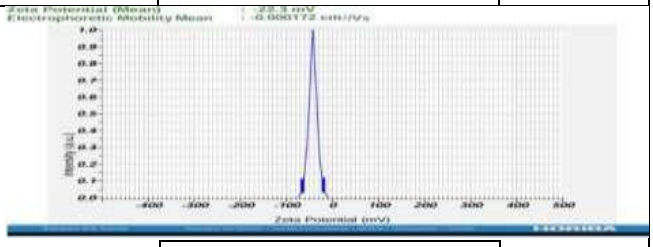


Fig: 17. Zeta Potential for NCB-7

2. **Thermo gravimetric Analysis:** Thermal analysis of pure drug tizanidine chloride and its optimized batches was conducted using DSC. The pure drug Tizanidine Hydrochloride exhibited a peak at 288.44 ^{O} C. In the Optimized batch, the peak was observed at 257.62^{O} C, 256.47^{O} C, 256.43^{O} C respectively (Figure: 18, 19, 20, 21, and 22).

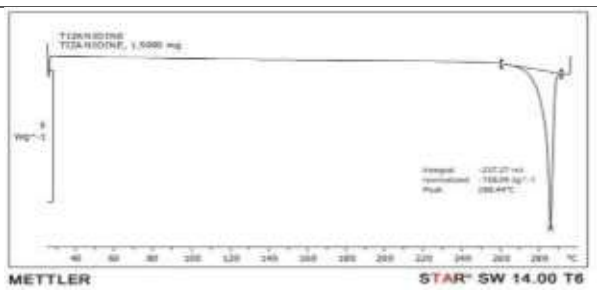


Fig: 18. DSC for TZN

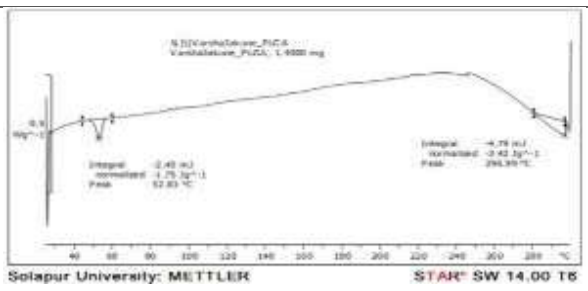


Fig: 19. DSC for PLGA

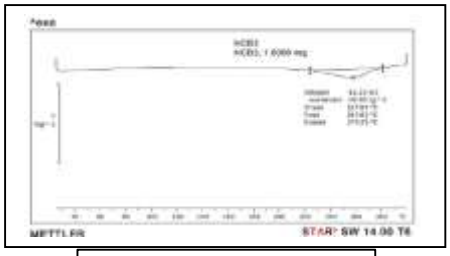


Fig: 20. DSC for NCB-3

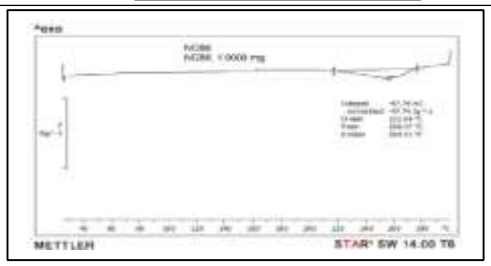


Fig: 21. DSC for NCB-6

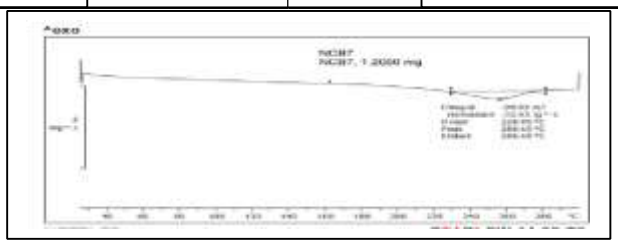


Fig: 22. DSC for NCB-7

3. **FT-IR:** The FTIR Spectrum of Pure TZN HCL exhibited characteristic stretching vibrations at 3241.88 cm-1 (N-H), 3076.93 cm-1C-H, 1647.52 cm-1 C=N. Occurrence of all these peaks in the physical mixture of the polymers and pure drug to the conclusion that no drug- excipient interaction occurs on using the components together. The demonstrates the compatibility of Pure drug with PLGA Polymer(Figure:23,24,25,26,27,28)

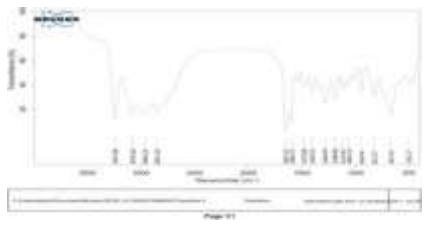


Fig: 23. FTIR of TZN HCL

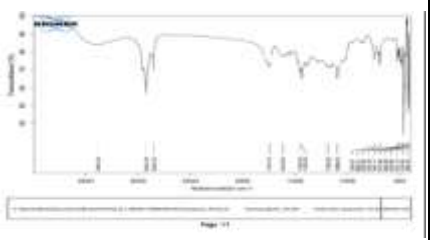


Fig: 24. FTIR of PLGA

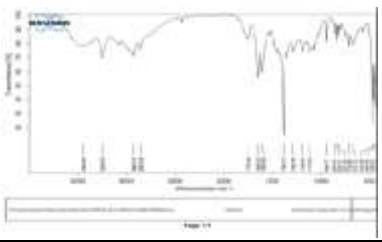


Fig: 25. FTIR of Mixture

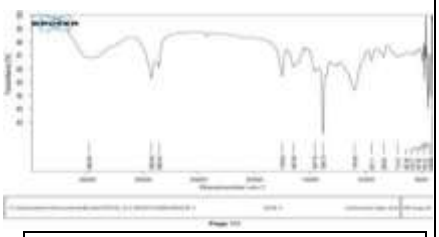


Fig: 26. FTIR of NCB -3

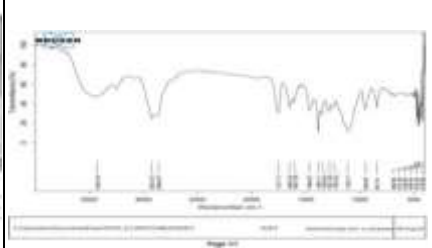


Fig: 27. FTIR of NCB-6

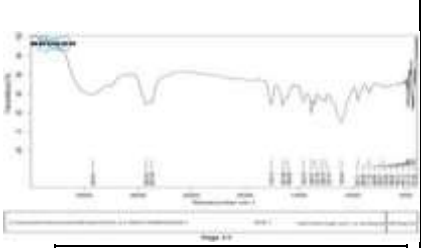


Fig: 28. FTIR of NCB-7

4. Scanning electron microscopy:

The surface morphology and shape of the drug Tizanidine Hydrochloride and the optimized nanoparticle formulation were examined using scanning electron microscopy (SEM). The SEM image of pure Tizanidine Hydrochloride, shown in Figure, illustrates its crystalline structure, while Figure depicts the optimized formulation, highlighting its amorphous and smooth surface characteristics(Figure:29,30,31,32).

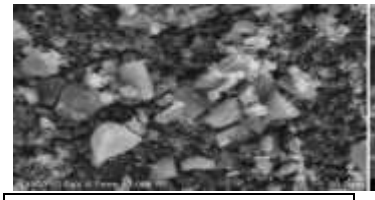


Fig: 29. SEM for TZN HCL

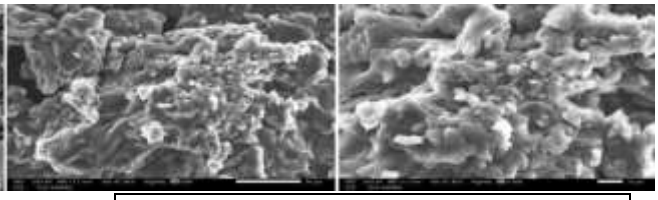


Fig: 30.SEM for NCB-3

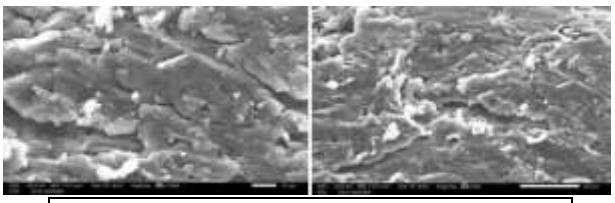


Fig: 31. SEM for NCB-6

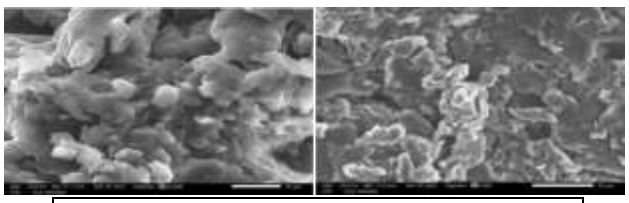


Fig: 32. SEM for NCB-7

5. Drug Content: The drug content of all 9 formulations was evaluated. From the Table 14 three formulationsNCB-3, NCB-6 and NCB-7 showed maximum drug content 98.47 %, 99.13 % and 99.72 % respectively compared to other formulations s (Table: 14).

HPLC (Table: 13) (Figure: 33, 34, 35)

Sample ID	Wt. (mg)	diluted to (ml)	Conc. (mg/ml)	Area	% Purity	% Assay

Standard	10.1	50	0.202	6823406	99.4	-
Batch F6	110.2	100	0.2004	6711254	-	98.55

Table: 13. Drug content of optimized batch

Batches	Drug Content (%)
NCB-1	85.79
NCB-2	92.48
NCB-3	98.47
NCB-4	89.44
NCB-5	93.67
NCB-6	99.13
NCB-7	99.72
NCB-8	94.82
NCB-9	95.38

Table: 14. Drug content of optimized batch.

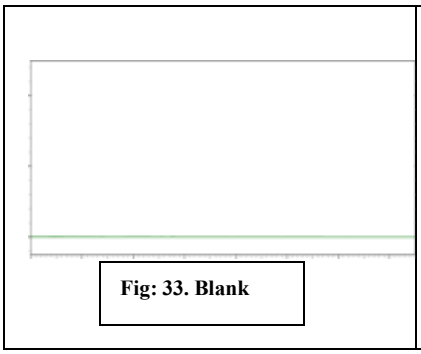


Fig: 33. Blank

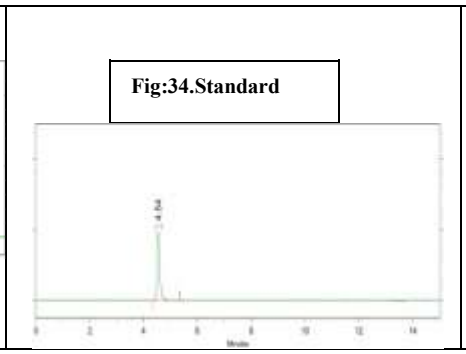


Fig:34.Standard

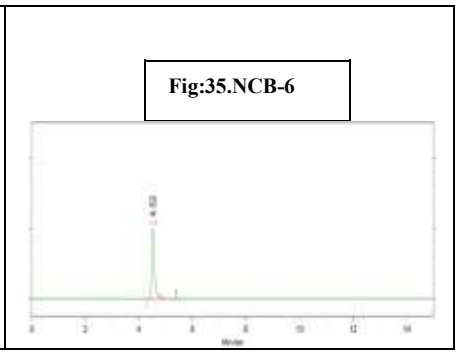


Fig:35.NCB-6

6. **Drug release studies:** From Figure No.36, In vitro drug release study for Pure drug Tizanidine Hydrochloride was revealed that 39.38% and In vitro drug release studies from formulations NCB-3, NCB-6, and NCB-7 revealed that formulation NCB-6 exhibited the highest drug release at 94.27 % after 24 hours, outperforming the other formulations(Table:15).

Time (hrs.)	Pure Drug	NCB-3	N
0	0.00	0.00	
0.5	0.47	0.98	
1	0.98	4.31	
2	1.95	11.88	
3	2.45	18.76	
4	4.87	26.21	
8	13.23	51.35	
12	21.36	62.37	
18	29.73	71.84	
24	39.38	77.29	

Table: 15. In vitro study for Tizanidine hydrochloride and optimized batches

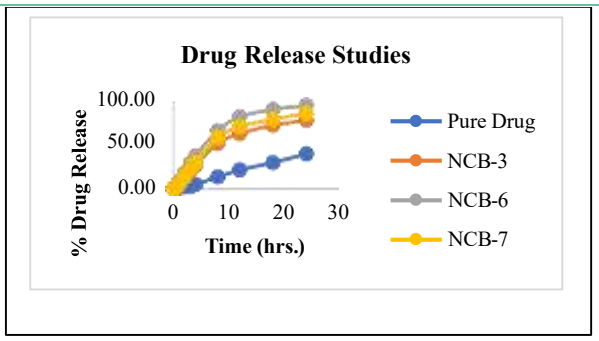


Fig: 36. In vitro study for Tizanidine hydrochloride and optimized batches

7. **Saturation Solubility:** From Figure: 37The Saturation solubility of Pure drug Tizanidine Hydrochloride and optimized formulations was evaluated. From the Table pure drug Tizanidine

Hydrochloride showed 21.51% and Saturation solubility from formulations NCB-3, NCB-6, and NCB-7 showed that formulation NCB-6 exhibited the highest saturation solubility 43.64 mg/ml Compared to other formulations (Table: 16, 17).

Calibration curve in Methanol: Water (50:50)

Conc. (up/ml)	Absorba
0.00	0.000
0.25	0.205
0.50	0.419
0.75	0.584
1.00	0.789
1.25	0.998

Table: 16. Calibration curve in Methanol:

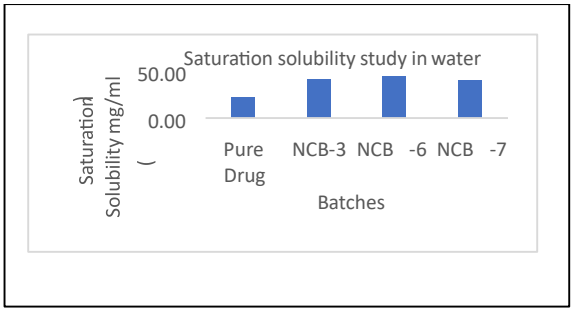


Fig: 37. Solubility study for Tizanidine hydrochloride and optimized batches

Batches	Absorbance	Conc. (ug/ml)	Dilution Factor	Actual concentration (ug/ml)	Solubility (mg/ml)
Pure drug	0.3452	0.4301	50000	21506.65	21.51

NCB-3	0.6519	0.8183	50000	40915.30	40.92
NCB-6	0.6950	0.8729	50000	43642.76	43.64
NCB-7	0.6203	0.7783	50000	38915.58	38.92

Table: 17. Solubility study for Tizanidine hydrochloride and optimized batches

8. **Stability Study:** From Figure: 38,39,40,41,42The Stability study of optimized batch was conducted at 40℃ and 75% relative humidity for 1 month and after the stability interval completes the optimized batch was evaluated the particle size result was 132.7nm to 136.3 nm. The drug content by UV result was 99.13 % to 98.71 %. The % drug release for 24 hours result was 94.27 % to 93.25 %. The FTIR study and DSC study result was complies with standard. The DSC study result was 256.47ºC to 262.26ºC (Table:18,19,20,21)

Sr. No.	Parameter	0 Day	1 month
1	Drug Content by UV	99.13%	98.71%
2	% Drug release (@ 24 hours)	94.27%	93.25%
3	Particle Size	132.7 nm	136.3 nm
4	FTIR	Complies with Standard	Complies with Standard
5	DSC	256.47^0C	262.26^0C
6	SEM	Amorphous and smooth surface	Amorphous and smooth surface

Table: 18. Stability study for Tizanidine hydrochloride and optimized batches

1. **Drug Content by UV:**

Conc. (ug/ml)	Absorbance
0.00	0.000
0.25	0.205
0.50	0.419
0.75	0.584
1.00	0.789
1.25	0.998

Table: 19. Calibration curve in methanol

Batches	Absorbance	Conc. (ug/ml)	Dilution Factor	Actual Conc. of Sample (ug/ml)	Conc. of Standard (ug/ml)	Drug Content (%)
NCB-6	0.7886	0.9913	1	0.9913	1	99.13
NCB-6 1M	0.7853	0.9871	1	0.9871	1	98.71

Table: 20. Drug Content study of Optimized batch after 1 month

2. % Drug Release:

Time (hrs.)	NCB-6	NCB-6 1M
0	0.00	0.00
0.5	3.56	3.42
1	8.31	7.66
2	17.81	16.21
3	28.37	25.64
4	37.29	35.73
8	65.62	63.49
12	81.31	80.45
18	89.71	88.25
24	94.27	93.25

Table: 21. % Drug Release study of Optimized batch after 1 month

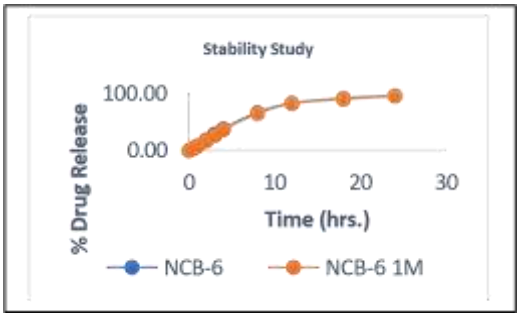


Fig: 38. % Drug Release study for Tizanidine hydrochloride and optimized batches

3. Particle size

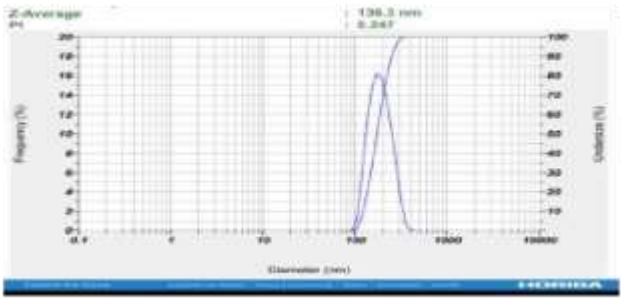


Fig: 39. Particle size for optimized batch

4. FT-IR

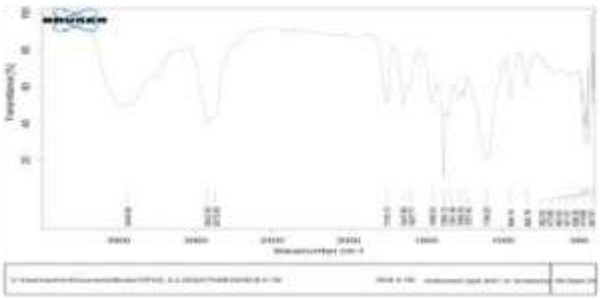


Fig: 40. FT-IR for optimized batch

5. Thermo gravimetric Analysis

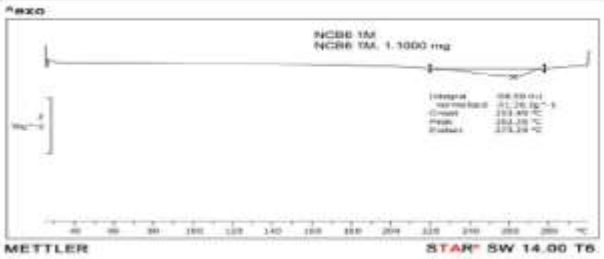


Fig: 41. DSC for optimized batch

6. SEM

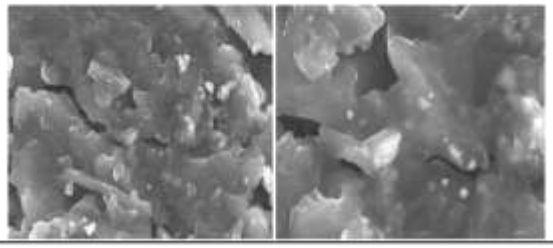


Fig: 42. SEM for optimized batch

Conclusion:

This is an oil-based PLGA hybrid Nano carrier with a particle size in the Nano range. The zeta potential of the oil-based PLGA hybrid Nano carrier indicates good stability. The hybrid exhibits enhanced properties, including higher saturation solubility and drug content compared to the pure drug. This was characterized using FT-IR, DSC, and SEM techniques. An in vitro release study was conducted over a period of 24 hours. The formulation demonstrated improved drug release and stability. Oil incorporation in the polymeric matrix stabilizes drugs and enables sustained release.

Future Direction:

Develop oral formulations and conduct biological studies to assess efficacy and safety

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Conflict of interest: Regarding this inquiry, the writers have no conflicts of interest.

References:

1. Whitman J, Stan R, Cecoltan S, Chifiriuc MC, Iovu H. Hybrid nanocarriers based on PLGA-vegetable oil: A novel approach for high drug delivery. Journal of Drug Delivery Science and Technology. 2018 Aug 1; 46:162-72.
2. Whitman J, Biru EI, Stan R, Iovu H. Review of hybrid PLGA nanoparticles: Future of smart drug delivery and theranostics medicine. Materials & Design. 2020 Aug 1; 193:108805.
3. Kapoor DN, Bhatia A, Kaur R, Sharma R, Kaur G, Dhawan S. PLGA: a unique polymer for drug delivery. Therapeutic delivery. 2015 Jan 1; 6(1):41-58.
4. Bharadwaj A, Shirkol A, Kamath S. Preparation of Bio based Polymers from Epoxide Soyabean Oil. Research journal of chemical and environmental sciences 2016; 4 [4S]; 43-46.
5. Islam MR, Beg MD, Jamari SS. Development of vegetable-oil-based polymers. Journal of applied polymer science. 2014 Sep 15; 131(18).
6. Shukla N, Devnani GL. A review on mechanical properties of hybrid natural fiber polymer composites. Materials Today: Proceedings. 2021 Jan 1; 45:4702-5.
7. Bhagwat RR, Vaidhya IS. Novel drug delivery systems: an overview. International Journal of Pharmaceutical sciences and research. 2013 Mar 1; 4(3):970.
8. Bandawane A, Saudagar R. A review on novel drug delivery system: a recent trend. Journal of Drug Delivery and Therapeutics. 2019 May 15; 9(3):517-21.
9. Patil S, Mhaiskar A, Mundhada D. A review on novel drug delivery system: a recent trend. International Journal of Current Pharmaceutical & Clinical Research. 2016; 6(2):89-93.
10. Pavithra K, Bhagawati ST, Manjunath K. Development and evaluation of Tizanidine hydrochloride loaded solid lipid nanoparticles. Asian J. Pharm. Clin. Res. 2019; 12:152-8.
11. SJ G, Deshpande MM, MP M, Sawant SD. Spectrophotometric method development and validation for estimation of Tizanidine and Aceclofenac in Bulk Drug & Tablet formulation.
12. Fadhel AY, Rajab NA. Tizanidine Nano emulsion: Formulation and in-vitro Characterization. Journal of Pharmaceutical Negative Results. 2022 Sep 16; 13(3):572-81.

13. Whitman J, Stan R, Ghebaur A, Cecoltan S, Vasile E, Iovu H. Novel PEG-modified hybrid PLGA-vegetable oils nanostructured carriers for improving performances of indomethacin delivery. Polymers. 2018 May 24; 10(6):579.

14. Gupta, Ritu & Bajpai, Meenakshi. (2016). Formulation optimization of Tizanidine hydrochloride nanoparticles using 32 factorial designs. International Journal of Pharma and Bio Sciences. 7. P161-P173.

15. Gawali Chhaya Hiraman1 * and Junagade M. S. FORMULATION AND EVALUATION OF NANOSPONGES LOADED HYDROGEL OF TIZANIDINE HYDROCHLORIDE World Journal of Pharmaceutical researchvol 8, Issue 8, 2019.

16. Jineshbhai Pandwala, Dhaval Madat, Tushar Patel, Nayan ratnakarformulation Development and Evaluation of Sustained Release Matrix Pellets of Tizanidine Hydrochloride Journal of Pharmaceutical Science and Bioscientific Research. 2016 6(3):461-468

17. Sinha S, Thapa S, Singh S, Dutt R, Verma R, Pandey P, Mittal V, Rahman MH, Kaushik D. Development of biocompatible nanoparticles of Tizanidine hydrochloride in or dispersible films: In vitro characterization, ex vivo permeation, and cytotoxic study on carcinoma cells. Current Drug Delivery. 2022 Dec 1; 19(10):1061-72.

18. Mohsen AM, El-Hashemy HA, Salama A, Darwish AB. Formulation of Tizanidine hydrochloride–loaded provesicular system for improved oral delivery and therapeutic activity employing a 23 full factorial design. Drug Delivery and Translational Research. 2023 Feb; 13(2):580-92.

19. Mahadik KR, Paradkar AR, Agrawal H, Kaul N. Stability-indicating HPTLC determination of Tizanidine hydrochloride in bulk drug and pharmaceutical formulations. Journal of pharmaceutical and biomedical analysis. 2003 Nov 24; 33(4):545-52...

20. IP 2018, Volume III, Page No. 3385

21. Bhalani DV, Nutan B, Kumar A, Singh Chandel AK. Bioavailability enhancement techniques for poorly aqueous soluble drugs and therapeutics. Biomedicines. 2022 Aug 23; 10(9):2055.

22. Ritu G, Meenakshi B. Preparation and physicochemical characterization of Tizanidine hydrochloride nanoparticles. Journal of Pharmaceutical Research. 2013 Mar 1;12(1):15-22.

23. Gupta ritu and bajpai meenakshi formulation optimization of Tizanidine hydrochloride nanoparticles using 32 factorial designs, InternationalJournal of Pharma Biosciences Volume 7

Issue 1, 2016 (January - March), Pages:161-173