STM JOURNALS

Journal of
Polymer & Composites

ISSN: 2321-2810 (Online)
ISSN: 2321-8525 (Print)
Volume 12, Issue 6, 2024
DOI (Journal): 10.37591/JoPC

http://engineeringjournals.stmjournals.in/index.php/JoPC/index

Research JoPC

Formulation and Development of Lamivudine Nanoemulsion by Using Emulsifying Polymers and Its Evaluation

Sakshi D. Dandgawal[1,*], Dhananjay M. Patil[1], Deepak D. Sonawane[1], Yogesh P. Sharma[1]

Abstract

Nanoemulsion is a novel approach to medicine delivery that helps to increase the bioavailability of drugs that aren't particularly soluble in water. In this research work, nanoemulsion of Lamivudine was formulated by using polymers such as SPAN 80, TWEEN 80 and Whey protein concentrate with the help of Ultrasonication technology. The formulation was optimized. It was evaluated by assessing pH, viscosity, turbidity, in vitro franz diffusion study, particle size distribution, zeta potential and stability over three months. The main objectives of the research work are preformulation studies of the drug, polymer studies, creation and evaluation of drug – loaded nanoemulsion formulation, optimization of formulation batches, stability studies as per ICH guidelines and in vitro evaluation of the prepared formulations.As Tween 80 and SPAN 80 are non- ionic, biodegradable polymers, they were found to be good excipients of choice for the formulation. Formulation F6 is an optimized batch of nanoemulsions used to administer the white, solid crystalline medication lamivudine. The pH of Formulation F6 was 5.7, its viscosity was 3.4 cp, and its turbidity was 1.72. Over the course of the three-month stability trial period, a high percentage of drug release (97%) was sustained, as evidenced by in vitro diffusion experiments. Significant effects of Tween 80 and Span 80 on pH and drug release, respectively, were shown by the ANOVA analysis. Formulation F6 is the best nanoemulsion batch for lamivudine administration as it performed better overall in all areas, including stability, drug release, and physical characteristics.

Keywords: Polymer, tween 80, span 80, whey protein concentrate, lamivudine nanoemulsion.

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Received Date: July 03, 2024
Accepted Date: July 29, 2024
Published Date: September 02, 2024

Citation: Sakshi D. Dandgawal, Dhananjay M. Patil, Deepak D. Sonawane, Yogesh P. Sharma. Formulation and development of Lamivudine Nanoemulsion by using Emulsifying Polymers and It's Evaluation. Journal of Polymer & Composites. 2024; 12(6): 22–35p.

INTRODUCTION

A nanoemulsion is an isotropically translucent or opaque mixture of two liquid phases that are immiscible, such as oil and water, sustained by the emulsifier molecules' interfacial coating. It's an isotropic combination of medication, water, polymers such as surfactant (Smix) with oil [1]. A common non-ionic emulsifying polymer used to foods, medications, and cosmetics is Tween 80. The molecular formula is $C_{64}H_{124}O_{26}$. It is very soluble in water & soluble in methanol, ethanol. It is one of the important polymers for the formulation.[2] Based on sorbitol, a sugar alcohol, and oleic acid, a naturally occurring fatty acid, Span 80 is a biodegradable polymer. When combined with its ethoxylated derivative, Tween 80, this sorbitan ester is extremely efficient in forming oil in water emulsions [3].

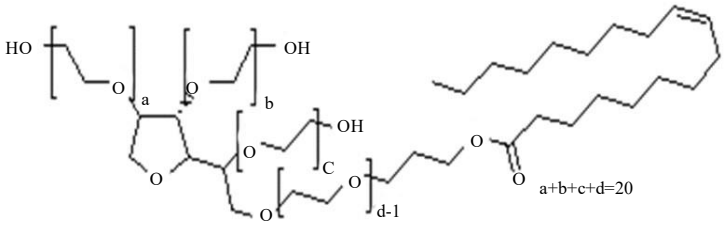

Figure 1. Structure of Tween 80[2].

The structure of the polymer Tween 80 is given in Figure. 1 [2].

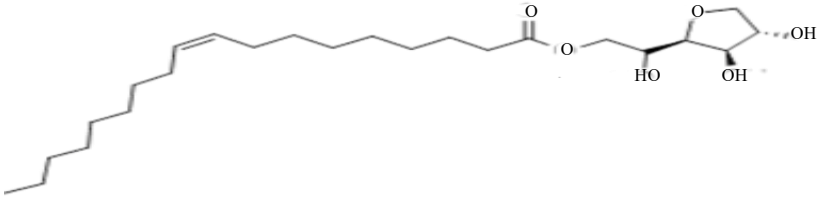

Figure 2. Structure of Span 80 [3].

The structure of the polymer Span 80 is given in Figure. 2[3].

Ultrasonication

The technique of ultrasonic emulsification is highly effective in lowering droplet size. This technique uses sonotrodes known as Sonicator probes to supply energy. It might have quartz crystal with piezoelectric properties, which change size in response to alternating electrical power.[4] When the Sonicator's tip makes contact with a liquid medium, cavitation and mechanical vibration can happen. Vapor cavities in liquid undergo cavitation and collapse. Therefore, emulsion can be immediately produced by ultrasonic. This technique is mostly used in laboratories to produce emulsion droplets as small as 0.2 mm. High surfactant concentrations are necessary for the low-energy creation of nanoemulsions, which can be harmful. Researchers employ the ultrasonic emulsification method because it is effective, affordable, and produces smaller particle sizes while using less emulsifier. By modifying the process parameters, ultrasonication may also be used to modify the stability, particle dimensions, polydispersity index (PDI), and zeta potential, and other properties of nanoemulsions. Through cavitation, it produces intense turbulence and micro-jets that split huge droplets into smaller ones. RSM, or response surface methodology, is a mathematical and statistical approach which examines a connection between one or more response variables and multiple independent factors by employing quadratic polynomial models. Although simpler, univariate models require a lot of time and do not take into account the influence of relationships between independent variables in optimization.[5]. One multivariate optimization technique for quadratic response surface models is box-Behnken design (BBD). Time and money are saved by the straightforward and effective experimental design [6].

MATERIALS AND METHOD

Chemicals & Apparatus

1. *Chemicals used for nanoemulsion*: Lamivudine, Tween 80, Span 80, Sunflower oil
2. *Instruments:* Ultraviolet spectroscopy, Magnetic Stirrer, Ultrasonicator, Electronic weighing balance, Digital pH Meter, Zeta Sizer.

Method For Making of W1/O/W2 Nanoemulsions

Utilizing a modified two-step emulsification procedure, W/O/W emulsions were created.[6] By the use of a magnetic stirrer, the hydrophilic surfactant polymer Tween-80 (0.5-2%, w/w) C and hydrophilic lamivudine (5, 10, 20, 30, 40%, w/w) were combined to create the innermost water phase (W1). The mixture was then left for five minutes. Sunflower oil (Merck, Mumbai) was taken as the oil phase (O) containing oil soluble emulsifier polymer Span-80 (2-4%, w/w) (Sigma Aldrich). The oil phase was prepared by heating to 50-60ºc and then the oil soluble emulsifier was added and mixed by using magnetic stirrer for 5 min. WPC-70 (1%, weight by weight) was used to prepare the outer aqueous phase (W2), which was then mixed for five minutes under mild circumstances using a magnetic stirrer.

Preparation of the nanoemulsion using an ultrasonic device that consists of a disruptor horn stepped down to a half-inch diameter at the tip, a power source, and a sonic convertor. To achieve the desired ultrasonic output, the temperature, pulser, automated timer, manual amplitude adjustment, and power supply of the sonifier were all adjusted. Every sample was set up on a wooden block with the Sonifier's probe tip 1.2 cm above the beaker's interior bottom. It was kept in an ice bath to preserve the temperature. The probe was cleaned with distilled water and dried in between each sample as soon as it was sonified.[7].

EXPERIMENTAL WORK:

- **Preformulation Studies:**
  - *Appearance:* Visually observed
  - *Colour:* A little quantity of drug was observed in a well-lit environment after being wrapped in butter paper.
  - *Odour:* Very small quantity of drug was used to get odour.

ASSESSMENT CRITERION FOR THE NANOEMULSION SYSTEM:

- *Turbidity:* By employing a Spectrophotometer (SPECORD-200, Analytik Jena) the turbidity of the nanoemulsions was determined at 600 nm.[8]
- *Analysis of drop size*: Droplet size analysis of nanoemulsion was measured by a diffusion method utilizing the light-scattering particle size analyser (Nano ZS90, Malvern Pvt. Ltd. USA). Additionally, correlation spectroscopy, which examines variations in light scattering brought on by Brownian motion, is used to quantify it. Photon correlation spectroscopy (PCS) and Transmission electron microscopy (TEM) and were also used to analyze the droplet size of NE [9].
- *Determination of viscosity:* A nanoemulsion system needs the ideal viscosity. A Brookfield-type rotational viscometer was used to measure the thickness of the nanoemulsion at different temperatures and shear rates.[8]
- *Drug content:* The formulation's chemical content was assessed using UV spectrophotometric and HPLC techniques. For UV, 100 milliliters of solvent were employed to disintegrate the ten milligram analogous to the medicinally laden nanoemulsion (drug having optimum solubility of that solvent). Grab One milliliter of this pre-made arrangement then mild it using 10 ml of solvent (drug-loaded nanoemulsion-free). Additionally, the drug molecule's stated Lamda max was used to compute the drug content.[10]
- *pH:* The pH of the NE framework was measured with a pH meter. (PH system, Indian Systronic 362 μ).[10]
- *Zeta potential:* Zeta potential was used to measure the potential. The composition was examined utilizing Zetasizer. Following a 100-fold dilution with water that has been double distilled. (Malvern Pvt. Ltd., USA; Nano ZS90) An essential property of nanocarriers is their zeta potential. It provides information on the charge of the particles and their propensity to aggregate or remain discrete in a formulation.[11]
- *Percentage transmittance:* With the help of a UV spectrophotometer, using spectrophotometry, the optimum nanoemulsion formulations' % transmittance was determined at the specific Lamda max of the drug molecule. The following formula can be used to determine absorbance from percent transmittance (%T):[12]

Absorbance = 2 – log(%T)

- *Stability:* By using a centrifuge and thermal stress studies After a day, the initial stability of the prepared nanoemulsion was evaluated. Stability was assessed using droplet size analysis and macroscopic emulsion observation. These studies sought to find a stable, low-surfactant formulation with stable physicochemical properties and a droplet size comparable to that of a nanoemulsion. Three preparations of the chosen nanoemulsion were made, and three different temperatures were maintained for the samples: 25 ± 2°C, 40 ± 2°C, and 5 ± 2°C. Testing was run seven, fifteen, thirty, sixty, and ninety days following preparation. Electrical conductivity, pH level, and droplet size were measured during the analysis.[12]

RESULT AND DISCUSSION

Preformulation Study

Physical characterization of Lamivudine

a. Organoleptic properties:

- *Appearance:* Solid crystalline
- *Colour:* White

b. Solubility

It is soluble in water.

Table 1. Absorbance in Water [13].

Sr. No	Concentration	Absorbance
1	10	0.234
2	20	0.452
3	30	0.688
4	40	0.868
5	50	1.106

Table 1 shows the lamivudine absorption in water [13].

c. *Melting point determination:* The melting point was reported to be 160-162 °C

d. The λmax determination:

LAMIVUDINE

Determination of Wavelength of Maximum Absorption

Using water as a blank, the standard solutions containing 1 µg/ml of lamivudine were scanned in the UV range of 200–400 nm. 271 nm is discovered to be the wavelength of greatest absorption.

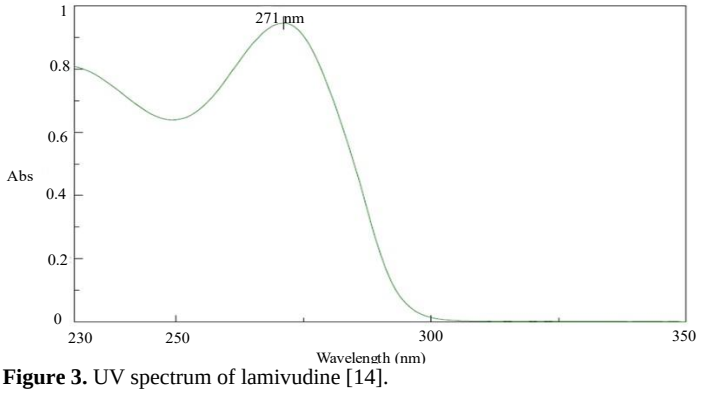

Figure 3. UV spectrum of lamivudine [14].

Figure 3. displayed the λmax of Lamivudine i.e at 271 nm.

Determination of Linearity and Range

From Figure. 4 it was discovered that the regression coefficient for the medication lamivudine in distilled water was within the linearity range and quite close to one. It has been determined that the standard concentration approach, which complies with Beer's law, is appropriate for determining drug entrapment and drug release studies [14].

Table 2. Calibration curve observation of Lamivudine [14].

Concentration(mg/ml)	Absorbance
0	0
1	0.1042
2	0.2011
3	0.3042
4	0.3943
5	0.4947
6	0.6

Absorbance of drug at different concentrations has been shown in Table 2.[14].

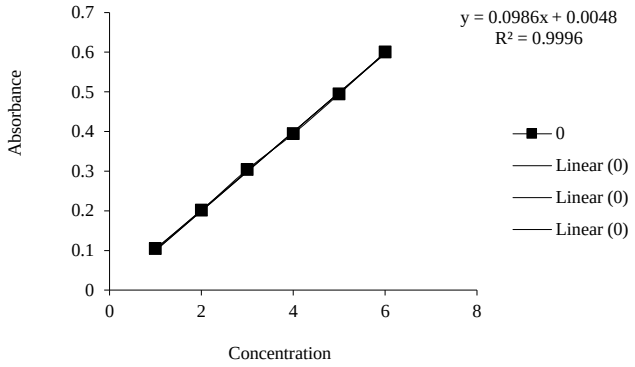

Figure 4. Calibration curve of Lamivudine [14].

e. *FTIR spectroscopy:* Figure 5 shows the FT-IR of Lamivudine. Absorption peaks are shown in table no. 3. These peaks are specific to specific functional group. The functional groups in the Lamivudine structure match the functional groups displayed by the IR spectra. This finding led to the conclusion that the sample medicine, lamivudine, was pure.

Characterization of Nanoemulsions

A multitude of methods can be combined to describe the structural and physical characteristics of nanoemulsions. For instance, a spectrophotometer, pH meter, and rheometer are used to measure the macroscopic aspects of turbidity, viscosity/viscoelasticity, and pH, respectively.

2. pH

The optimization of F6's pH level highlights the importance of pH control in formulation processes, potentially influencing factors such as stability, efficacy, and compatibility.

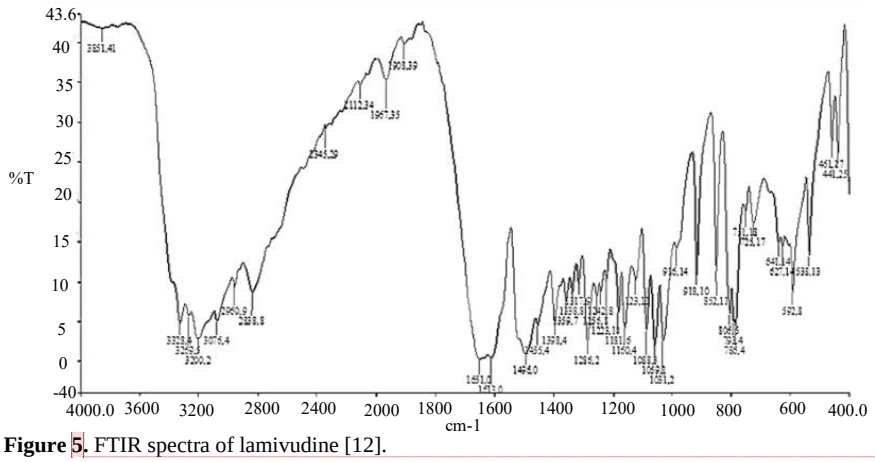

Figure 5. FTIR spectra of lamivudine [12].

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Table 3. Spectrum interpretation of Lamivudine [12].

Functional group	Wavenumber (cm^1)
N-H stretch	3076.4
Alkyl C-H Stretch	2838.8
C=O stretch	1651.6
C-H bend (para)	852.17

Table 4. pH [10].

Formulation code	pH
F1	6.9
F2	5.8
F3	5.4
F4	4.9
F5	6.4
F6	5.7
F7	5.9
F8	4.5
F9	4.8

The pH for all the nine batches were determined. F6 shown the optimum pH as per table 4[10]

ANOVA FOR THE LINEAR FRAMEWORK

Table 5 indicates The factor code is encoded. Square sums are of Type III, PartialF – value of model is 6.37(Significant) [10].

Table 5. Response 1: pH [10].

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3.84	3	1.28	6.37	0.0368	significant
A-Tween 80	1.71	1	1.71	8.51	0.0331	
B-Span 80	2.10	1	2.10	10.45	0.0231	
C-WPC-70	0.0313	1	0.0313	0.1555	0.7096	
Residual	1.01	5	0.2010			
Cor Total	4.85	8				

Table 6. Fit Statistics [10].

Std. Dev.	**0.4484**	**R^2**	**0.7927**
Mean	5.59	Adjusted R^2	0.6683
C.V. %	8.02	Predicted R^2	0.5386
		Adeq Precision	6.9419

There is less than 0.2 discrepancy between the Adjusted R^2 of 0.6683 and the Predicted R^2 of 0.5386, indicating a satisfactory agreement as per table 6[10]

Table 7. Complete Equation with Coded Factors [10].

pH	=
+5.59	
-0.4625	A
-0.5125	B
-0.0625	C

By comparing the factor coefficients, the equation that is encoded helps to determine the factors' respective impacts shows table 7.[10]

Table 8. Final Equation in Terms of Actual Factors [10].

pH	=
+8.08472	
-0.616667	Tween 80
-0.512500	Span 80
2541-0.250000	WPC-70

Table 8. indicates the final equation.[10].

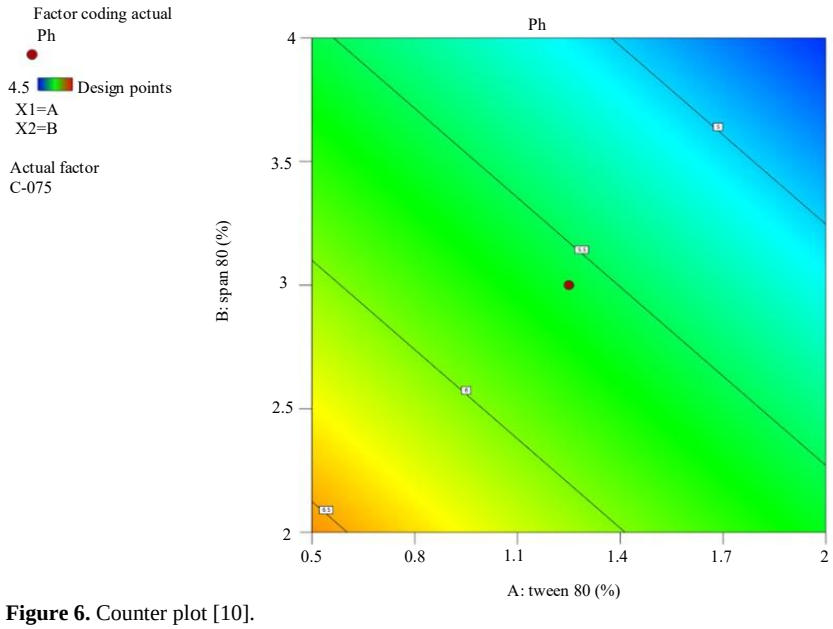

Figure 6. Counter plot [10].

The counter plot for pH has been shown in Figure 6 [10].

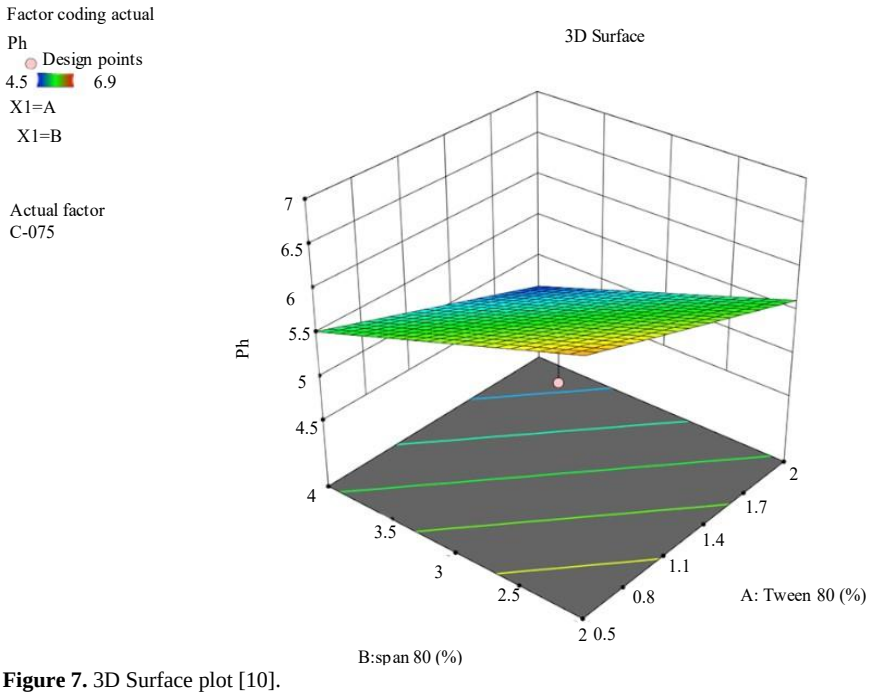

Figure 7. 3D Surface plot [10].

3D surface plot for pH has been shown in Figure. 7 [10].

VISCOSITY (CP)

Data from Table 9 presents a range of viscosity values across different formulation codes, with measurements varying from 3.4 cp to 6.5 cp. notably, formulation F6 stands out with a significantly lower viscosity of 3.4 cp, identified as an optimized batch. This lower viscosity in F6 could suggest intentional adjustments aimed at enhancing specific characteristics or performance attributes. While the other formulations exhibit relatively consistent viscosity levels, the optimized nature of F6 prompts further inquiry into the rationale behind this optimization [8].

Table 9. Viscosity [8].

Formulation code	Viscosity (cp)
F1	4.4
F2	6.5
F3	4.9
F4	5.4
F5	6.2
F6	3.4
F7	5.2
F8	4.5
F9	5.6

TURBIDITY

The optimum turbidity was found to be 1.72 as shown in Table 10 [8].

Table 10. Turbidity [8].

Formulation code	Turbidity
F1	3.14
F2	2.47
F3	3.46
F4	2.46
F5	2.13
F6	1.72
F7	1.87
F8	2.67
F9	1.97

IN VITRO FRANZ DIFFUSION STUDY

F6 represents an optimized batch, as its values consistently outperform those of other metrics across various time intervals. From the onset, F6 exhibits higher values compared to the rest, indicating superior performance and efficiency in the optimization process. This trend persists throughout the observation period, further solidifying the notion that F6 represents an optimized batch. Therefore, the conclusion drawn is that the F6 batch is indeed optimized, consistently demonstrating superior performance compared to other batches [15].

Table 11. Drug release profile [15].

Time min (hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
15	12.34	10.44	15.67	13.56	10.87	17.86	14.58	15.79	16.87
30	20.06	23.46	29.46	28.88	30.45	31.05	28.77	29.86	25.34
1	35.44	38.77	41.46	45.23	45.67	49.77	39.97	35.46	32.54
2	49.78	47.16	59.94	58.47	61.47	65.83	43.48	49.97	48.77
5	62.44	55.46	68.99	63.87	78.53	78.85	62.35	53.56	62.35
6	75.46	62.36	75.66	79.97	85.63	86.33	78.99	75.58	76.89
8	80.47	85.44	79.98	81.46	89.66	92.56	85.32	82.36	83.56
12	87.16	92.46	80.13	82.46	90.14	97	88	84.13	85.34

In vitro diffusion study reults have been displayed in Table 11. F6 shows sustained release effect.[15].

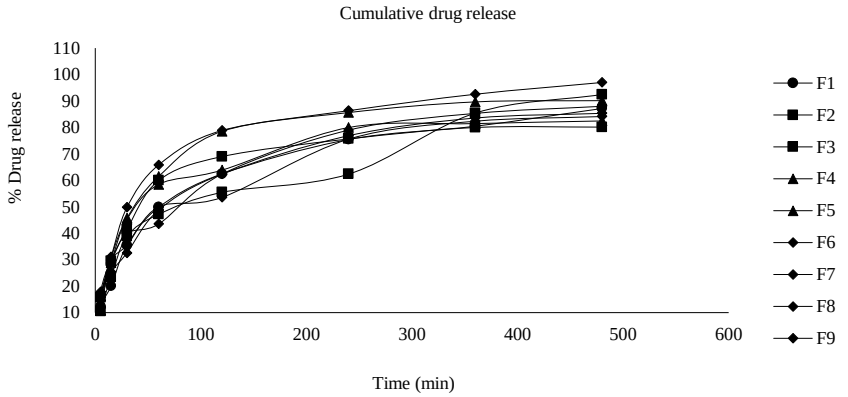

Figure 8. Cumulative drug release of F1-F9 batch [15].

From Figure. 8, Batch F6 shows better % release of drug. It shows good performance.[15].

PARTICLE SIZE DISTRIBUTION AND ZETA POTENTIAL OF THE NANOEMULSIONS

Table 12 indicates the particle size and zeta potential of two batches. [11]

Table 12. Particle size and zeta potential of W1/O/W2 lamivudine nanoemulsions [11].

Lamivudine (10%) W1/O/W2	F6	F2
Size (nm)	124.3 ± 69.1	113.1±70.7
Zeta potential (mV)	-25.0	-110.3

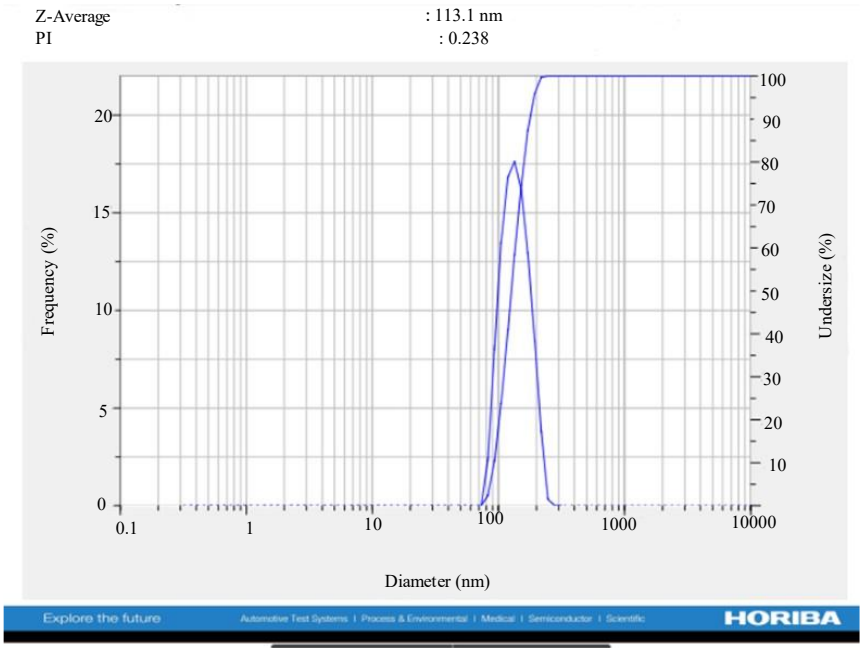

Figure 9. Particle size of lamivudine W1/O/W2 nanoemulsions of F6 batch [11].

F6 batch has particle size of 124.3 ± 69.1 nm according to Figure. 9 [11]

STABILITY STUDY

The stability study conducted on formulation F6, identified as the optimized batch, reveals consistent pH levels throughout the observation period of three months, with readings maintaining at 5.7 as shown by Table 13This stability suggests robust formulation integrity regarding pH maintenance over time. Furthermore, the drug release percentage remains consistently high, with minimal variation, indicating sustained performance. The stability and consistent drug release percentages underscore the effectiveness of the optimization process for formulation F6, affirming its suitability for the intended application [12].

F6 batch has zeta potential of -25.0 mv according to Figure. 10 [11].

F2 batch has particle size of 113.1 ± 70.7 nm as shown in Figure. 11 [11]

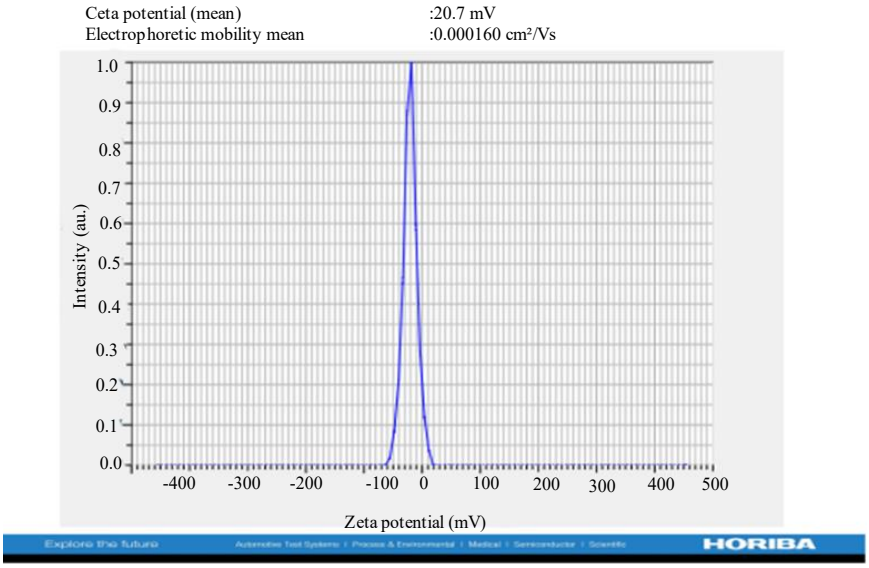

Figure 10. Zeta potential of lamivudine W1/O/W2 nanoemulsions of F6 batch [11].

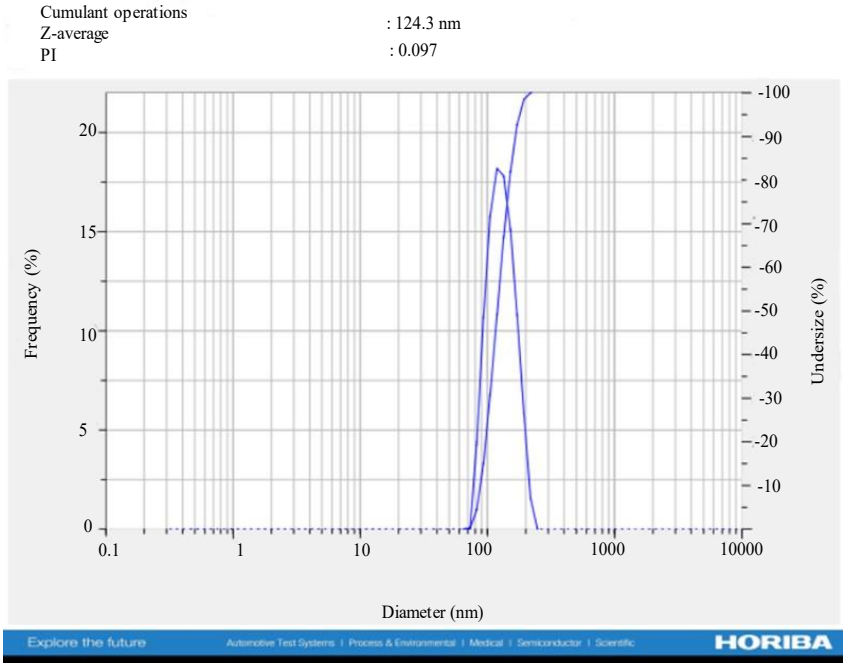

Figure 11. Particle size of lamivudine W1/O/W2 nanoemulsions of F2 batch [11].

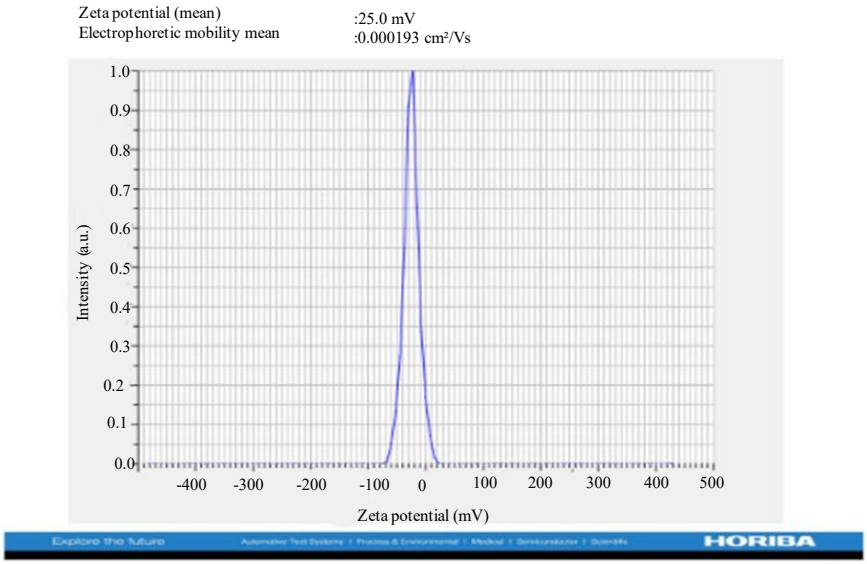

Figure 12. Zeta potential of lamivudine W1/O/W2 nanoemulsions of F2 batch [11].

F2 batch has zeta potential of -110.3 mv according to Figure. 12 [11].

Table 13. Stability study (F6) [12].

Formulation	Test/Parameter	Stability study			
		Initial	***First month***	***Two month***	***Third month***
F6	Ph	5.7	5.6	5.7	5.7
	Drug release (%)	97	96.7	97	97

CONCLUSION

Tween 80 and SPAN 80 were determined to be excellent excipients of choice for the formulation since they are non-ionic, biodegradable polymers.Formulation F6 represents the optimized nanoemulsions batch for the delivery of Lamivudine, a solid crystalline drug with white colour. The Preformulation study involved the physical characterization of Lamivudine, including organoleptic properties, solubility, melting point determination, UV spectrum analysis, and FTIR spectroscopy. The λmax determination revealed a maximum absorption wavelength of 271 nm, and the calibration curve demonstrated linearity, indicating suitability for drug entrapment and release studies.

For the W1/O/W2 nanoemulsions, the selection of materials for the inner (W1) phase involved the use of high HLB hydrophilic surfactant Tween 80 to enhance stability. Sunflower oil with Span 80 as an oil-soluble emulsifier was chosen for the oil (O) phase, while whey protein concentrate (WPC-70) was introduced into the outer aqueous phase (W2) to improve stability.

Characterization of nanoemulsions involved assessing pH, viscosity, turbidity, in vitro Franz diffusion study, particle size distribution, zeta potential, and stability over three months. Formulation F6 exhibited a pH of 5.7, viscosity of 3.4 cp, and turbidity of 1.72. In vitro diffusion studies demonstrated consistent and sustained drug release, with a high percentage of drug release (97%) maintained over the three-month stability study period.

The ANOVA analysis indicated significant effects of factors such as Tween 80 and Span 80 on pH and drug release, respectively. The optimized formulation equation in terms of actual factors was provided, aiding in predicting responses for given levels of each factor.

Overall, formulation F6 demonstrated superior performance across various parameters, including stability, drug release, and physical properties, making it the optimized nanoemulsion batch for the delivery of Lamivudine.

Conflict of Interest

The authors have no conflicts of interest regarding this investigation.

Acknowledgement

The writers are very appreciative of SSS'S Divine College of Pharmacy, Satana, Nashik for the ongoing inspiration, assistance, and direction for such action as well as for offering the facilities necessary to complete the assigned assignment.

REFERENCES

1. Kumar SH, Singh V. Nanoemulsification-a novel targeted drug delivery tool. Journal of Drug Delivery and Therapeutics. 2012 Jul 15;2(4).
2. Nielsen CK, Kjems J, Mygind T, Snabe T, Meyer RL. Effects of Tween 80 on growth and biofilm formation in laboratory media. Frontiers in microbiology. 2016 Nov 22;7:1878.
3. Bowers RR, Temkin AM, Guillette LJ, Baatz JE, Spyropoulos DD. The commonly used nonionic surfactant Span 80 has RXRα transactivation activity, which likely increases the obesogenic potential of oil dispersants and food emulsifiers. General and comparative endocrinology. 2016 Nov 1;238:61-8.
4. Ahmad N, Alam MA, Ahmad FJ, Sarafroz M, Ansari K, Sharma S, Amir M. Ultrasonication techniques used for the preparation of novel Eugenol-Nanoemulsion in the treatment of wounds healings and anti-inflammatory. Journal of drug delivery science and technology. 2018 Aug 1;46:461-73.
5. Ahire K, Gorle AP, Maharashtra I. An overview on methods of preparation and characterization of nanoemulsion. World J of Pharmacy and Phar Sci. 2021 Jun 1;10(8):897-907.
6. Huang X, Tu R, Song H, Dong K, Geng F, Chen L, Huang Q, Wu Y. Gelatin-EGCG-high methoxyl pectin ternary complex stabilized W1/O/W2 double emulsions loaded with vitamin C: Formation, structure, stability, in vitro gastrointestinal digestion. International journal of biological macromolecules. 2022 Sep 1;216:891-905.
7. Qian C, McClements DJ. Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: Factors affecting particle size. Food hydrocolloids. 2011 Jul 1;25(5):1000-8.
8. Patel RP, Joshi JR. An overview on nanoemulsion: a novel approach. International Journal of Pharmaceutical Sciences and Research. 2012 Dec 1;3(12):4640.
9. Shaikh NM, Swamy SV, NARSING NS, Kulkarni KB. Formulation and evaluation of nanoemulsion for topical application. Journal of Drug Delivery and Therapeutics. 2019 Aug 15;9(4-s):370-5.
10. Prasetyo BE, Maruhawa SM, Laila L. Formulation and physical evaluation of castor oil based nanoemulsion for diclofenac sodium delivery system. Research Journal of Pharmacy and Technology. 2018;11(9):3861-5.
11. Kotta S, Khan AW, Ansari SH, Sharma RK, Ali J. Anti HIV nanoemulsion formulation: optimization and in vitro–in vivo evaluation. International Journal of Pharmaceutics. 2014 Feb 28;462(1-2):129-34.
12. Selvam RP, Kulkarni PK. Design and evaluation of self nanoemulsifying systems for poorly water soluble HIV drug. J PharmaSciTech. 2014;4(1):23-8
13. Jozwiakowski MJ, Nguyen NA, Sisco JM, Spancake CW. Solubility behavior of lamivudine crystal forms in recrystallization solvents. Journal of pharmaceutical sciences. 1996 Feb 1;85(2):193-9.

14. Haque MA, Mounika K, Bakshi V. Development and Validation of UV Spectrophotometric Method for Estimation of Lamivudine in Bulk and Tablet Dosage Form.
15. Chime AS, Ezegbe CA, Iyanyi CJ, Onunkwo CG, Ezegbe AG, Okafor NP. Formulation, In-Vitro and In-Vivo Evaluation of Lamivudine-Loaded Solid Lipid Nanoparticles (SLNs). Mathews Journal of Pharmaceutical Science. 2024 Jun 13;8(1):1-8.

