International Journal of Enhanced Research in Science, Technology & Engineering
ISSN: 2319-7463, Vol. 11 Issue 3, March-2022, Impact Factor: 7.957

Selection and Optimisation of Suspending Agent in Lamotrigine Oral Suspension: Physical and Chemical Stability

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ABSTRACT

Background: Suspensions are coarse dispersions defined as a biphasic liquid dosage form of medicaments in which insoluble solid particles are suspended uniformly in a liquid. Disperse phase is also known as internal phase and continuous phase is also known as external phase.Lamotrigine is a broad spectrum anticonvulsant drug that has been used for mono- or adjunct- therapy in adults and children.

Objectives: The aim of this study was to prepare suspension of lamotrigine by using different suspending agents, select and optimise its concentration. After selection of suitable suspending agent with optimised concentration, further aim was to determine the effects of that suspending agent on physicochemical properties of lamotrigine suspension formulation.

Materials and Methods: To formulate the lamotrigine suspension with various suspending agents obtained from natural as well as synthetic sources includingveegum HV, cekol 700P, cekol 2000P, avicel RC 591, carbopol 974P, PVP K90, sodium alginate and HPMC E4 premium. After incorporation of suspending agents and other suitable excipients, the suspension formulations were observed for sedimentation and ease of re-dispersibility.The selected suspension was further evaluated for different physicochemical parameters such as pH,viscosity, drug and preservatives assay contents, related substances and drug dissolution profile.Stability study was conducted on selected formulation as per ICH guidelines.

Results: Theresults collectively presented in this paper indicate the suspension prepared with different suspending agents were failed in physical properties whereas ones prepared with carbopol 974P shows good physical and chemical properties with 97.60% of assay of lamotrigine and 100.38% & 100.01% of assay for methylparaben and propylparaben, respectively. Related substances are found well within predetermined criteria. The *in-vitro* study of lamotrigine suspension against commercially available lamictal tablet shows more than 85% release within 15mins. Prepared suspension demonstrated an acceptable drug chemical stability remained above 90% at accelerated condition for period of six months.

Conclusions: The suitability of carbopol 974P as a pharmaceutical suspending agent in formulation of lamotrigine oral suspension, and this is confirmed by its physicochemical properties. The suspension is re-dispersible and readily pourable.Thus, this newer formulation of lamotrigine could have potential to treat the epilepsy in children and geriatric patients.

Keywords: Epilepsy, Re-dispersibility, Stability, Suspension

INTRODUCTION

Lamotrigine works by inhibiting the voltage-dependent sodium channels. It stabilises the neurons, which produce glutamate and aspartate, resulting in their decreased release. Lamotrigine was originally brought to market by GlaxoSmithKline, trademarked as Lamictal; it is also available in generic form under many brand names worldwide. It was first marketed in the United Kingdom in 1991, and approved for use in the United States in 1994. It is on the World Health Organization's List of Essential Medicines. It is sold under the brand name of LaMICtal, LaMICtal CD, LaMICtal XR as a tablet which is administered 2-3 times per day. [1,2] Although, tablets are very

simple to manufacture and handle, oral liquid are always preferred for paediatric, geriatric even dysphagic patients, due to their ease of administration and patient compliance, even at the time of epileptic attack.

Suspensions are pharmaceutically stabledispersions of a finely divided solid in a liquidvehicle, usually an aqueous solution. TheUSP defines suspensions as "finely divided, undissolved drugs dispersed in liquidvehicles" and can be ready-to-use.[3] One other aspect needs to be clarified at thispoint and that is the difference between asuspension and a colloid.Both can bedispersions of a solid in aliquid, however,the particle size of a suspension is such thatsedimentation occurs due to the force ofgravity, while the particle size of a colloid issmall enough so that thermal energy orBrownian motion is sufficient to keep theparticles uniformly dispersed and preventsedimentation.Suspensions are basically used to prepare liquid preparations of drugs that cannot be prepared as solutions; either the drug is not soluble or cannot be solubilized by cosolvents, surfactantsetc.[4]

Suspensions are classified as flocculated and deflocculated suspension. In flocculated suspension the loose structure of the rapidly sedimenting flocs tends to preserve in the sediment, which contains an appreciable amount of entrapped liquid. The volume of final sediment is thus relatively large and is easily re-dispersed by agitation. Most stable pharmaceutical suspensions are flocculated. The suspension appear somehow unsight (unappealing) due to its rapid sedimentation and it presents an obvious clear supernatant. This can be minimised by enlarging the volume of the sediments by the use of flocculating agents.

A deflocculated suspension is one in which the electrical repulsive forces between particles exceeds the attractive forces, the particles are kept apart as individuals affected only by the suspending vehicle. Even when brought together by random motion, they resist collision due to the high surface tension. In deflocculated suspension, individual particles are settling, so rate of sedimentation is slow which prevents entrapping of liquid medium which makes it difficult to re-disperse by agitation. This phenomenon called as cracking.In deflocculated suspension, smaller particles settle slowly and therefore remaining supernatant liquid so supernatant appears cloudy and has a pleasing granular appearance whereby in flocculated suspension, even the smallest particles are involved in flocs, so the supernatant does not appear cloudy.

A good suspension should have well developed thixotropy. At rest the solution is sufficient viscous to prevent sedimentation and thus aggregation or caking of the particles. When agitation is applied the viscosity is reduced and provide good flow characteristic from the mouth of bottle.Suspending agents are added to a suspension to enhance viscosity of continuous phaseby remaining as suspended particles in liquid for a long time and thereby, retardsedimentation.Most suspending agents perform two functions i.e. besides acting as a suspending agent they also imparts viscosity to the solution. Suspending agents form film around particle and decrease interparticle attraction. There are many materials that fall into this classification and include cellulosederivatives, clays, natural gums, synthetic polymers and a few miscellaneous materials.Most suspending agents are either neutral or negatively charged. [4,5]

MATERIALS AND METHODS

Lamotrigine was purchased from Aurobindo (Hyderabad, India). All suspending agents used during formulation development of lamotrigine suspension are shown in table 1.

Table 1:Suspending agents used in the formulation development of lamotrigine suspension

S.No	*Name of suspending agent*	*Company*
1	Veegum HV	Vanderbilt Minerals
2	Cekol 700P	C P Kelco
3	Cekol 2000P	C P Kelco
4	Avicel RC 591	DuPont
5	Carbopol 974P	Corel pharma chem
6	PVP K90	BASF
7	Protanal LFR5/60 (Sodium alginate)	FMC
8	HPMC E4 Premium	Colorcon

Preparation of Suspensions

Take weighed quantity of preservatives along with antifoaming agent. Disperse suspending agents in to it. Separately dispersed 0.5 gram of lamotrigine in glycerol to get homogenous dispersion. This dispersion added to previously prepared dispersion and mix using silverson mixer. Add sweetener and flavour. Adjust the pH of suspension between 6.3 to 7.3 using 1N solution of sodium hydroxide. The suspension was then transferred to 100ml measuring cylinder. The ingredients quantities are shown in table 2.

Table 2: Preparation of Lamotrigine suspension with different suspending agents

S. no.	*Ingredients*	*F1*	*F2*	*F3*	*F4*	*F5*	*F6*	*F7*	*F8*
1.	Lamotrigine	0.50 g	0.50 g	0.50 g	0.50 g	0.50 g	0.50 g	0.50 g	0.50 g
2.	Methyl paraben	0.18 g	0.18 g	0.18 g	0.18 g	0.18 g	0.18 g	0.18 g	0.18 g
3.	Propyl paraben	0.02 g	0.02 g	0.02 g	0.02 g	0.02 g	0.02 g	0.02 g	0.02 g
4.	Veegum HV	0.25 g	-	-	-	-	-	-	-
5.	Cekol 700P	-	0.25 g	-	-	-	-	-	-
6.	Cekol 2000P	-	-	0.25 g	-	-	-	-	-
7.	Avicel RC 591	-	-	-	0.25 g	-	-	-	-
8.	Povidone K90	-	-	-	-	0.25 g	-	-	-
9.	Sodium Alginate	-	-	-	-	-	0.25 g	-	-
10.	HPMC E4M remium	-	-	-	-	-	-	0.25 g	-
11.	Carbomer 974P	-	-	-	-	-	-	-	0.25 g
12.	Glycerol	5.00 g	5.00 g	5.00 g	5.00 g	5.00 g	5.00 g	5.00 g	5.00 g
13.	Sodium saccharin	0.07 g	0.07 g	0.07 g	0.07 g	0.07 g	0.07 g	0.07 g	0.07 g
14.	Simethicone PD30	0.05 g	0.05 g	0.05 g	0.05 g	0.05 g	0.05 g	0.05 g	0.05 g
15.	Strawberry flavor	0.10 g	0.10 g	0.10 g	0.10 g	0.10 g	0.10 g	0.10 g	0.10 g

(Note: 1N Sodium hydroxide solution was added to adjust the pH of suspension between 6.3 to 7.3andpurified water was used as a vehicle upto 100ml)

Optimization study of Suspending agent

It is important to enhance the physical stability of oral suspension by use of controlled particle size, wetting and suspending agents. Eight formulations with formulation code of F1, F2, F3, F4, F5, F6, F7 and F8 was formulated as shown in table 2using various suspending agents i.e.,veegum HV, cekol 700P, cekol 2000P, avicel RC 591, povidone K90, sodium alginate, HPMC E4M premium and carbopol 974P.

Evaluation of optimized lamotrigine suspension

Determination of Re-dispersibility *[6]*

Allow a fixed volume of the suspension was kept in a type III amber glass bottle with child resistant cap being examined to settle, undisturbed, for 24 hours. Shake the container for 30 seconds and accurately remove one dose (usually 5 to 10 mL) at a depth of 1 cm below the meniscus. Shake the container again for 10 seconds and remove another dose. Repeat this procedure until 10 doses of the suspension have been removed. Assay the 10 doses individually. The formulation complies with the test if the content of each individual dose is between 85% and 115% of the average content. The preparation fails to comply with the test if the content of more than one individual dose is outside these limits or if the content of one individual dose is outside the limits of 75% to 125% of the average content. Report average content of formulation.

Determination of pH *[7]*

The pH monitoring is an important aspect of study, because non-buffered vehicles may experiencechanges in their pH values through time.The lamotrigine suspension was well shaken for 20 seconds prior to measurement. Measurement of pH was carried out with aid of pH meter.

Rheological assessment

Viscosity measurements of the suspension was carried out at room temperature on a Brookfield viscometer CAP 2000+ using spindle. Allow 10 minutes for the cone to come to equilibrium temperature with the plate. At the end of the temperature stabilization period, disperse approximately 67µl of suspension in such a way that not enough or too much suspension is outside the spindle. Lower the handle gently. Allow the cone, plate and suspension to equilibrate to the temperature control setting. Press the run key and allow it to run for 60 seconds. Record the viscometer reading of suspension formulation. Repeat the procedure twice more. Report the mean of three readings.

Assay determination

The proposed in-house method has been developed for determination of assay contentsofLamotrigine, Methylparaben and PropylparabeninLamotrigine oral suspension. The same has been validated as per the current ICH(Q2 Rl) guidelines. Separately inject single injections of Blank, five replicate of standard solution andtwo replicate of sample solution in to liquid chromatography and record thechromatograms. Calculate the % Assay of Lamotrigine, Methylparaben and Propylparabenin percentage of label claimusing peak area obtained with standard solution and sample solution as equation (1), (2) and (3) respectively.

Chromatographic conditions

Instrument : High performance liquid chromatography equipped with pump and

		PDA/UV detector (Agilent LC 1260 or equivalent)
Column	:	BDS Hypersil C-18, (150mm x 4.6mm) 5µm
Column oven temp.	:	30°C
Sampler temp.	:	25°C
Flow rate	:	1.5 ml/min
Detection wavelength	:	270 nm
Injection volume	:	20 µl
Run time	:	15 minutes
Needle wash	:	Diluent
Mode	:	Gradient

Calculation for Lamotrigine

$$\%\text{Assay of Lamotrigine} = \frac{Au}{As} \times \frac{Ws}{25} \times \frac{5}{50} \times \frac{100}{Wu} \times \frac{25}{10} \times \frac{P}{LC} \times Wt/ml \quad \ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Where,
Au =average peak area of analyte obtained from sample solution
As = average peak area of analyte obtained from standard solution
Ws = weight of analyte standard (mg)
Wu = weight of sample (gm)
Wt/ml = density of sample (gm)
LC = label claim (mg/ml)
P = %purity of analyte working standard

Calculation for Methylparaben

$$\%\text{Assay of Methylparaben} = \frac{Au}{As} \times \frac{Ws}{25} \times \frac{2}{50} \times \frac{100}{Wu} \times \frac{25}{10} \times \frac{P}{LC} \times Wt/ml \quad \ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Calculation for Propylparaben

$$\%\text{Assay of Propylparaben} = \frac{Au}{As} \times \frac{Ws}{100} \times \frac{1}{50} \times \frac{100}{Wu} \times \frac{25}{10} \times \frac{P}{LC} \times Wt/ml \quad \ldots\ldots\ldots\ldots\ldots\ldots(3)$$

Determination of Related substances
Separately inject blank, single injection of system suitability, six replicate standardsolutions, single injections of sample solution and placebo solution into liquidchromatography and record the chromatograms. Calculate the impurities of lamotrigine in percentage of label claim using peak area obtained with standardsolution and sample solution by given equations of (4), (5) and (6) for impurity A, unknown impurity and total impurities respectively.

Chromatographic conditions

Instrument	:	High performance liquid chromatography equipped with pump and PDA/UV detector (Agilent LC 1260 or equivalent)
Column	:	Inertsil ODS3V C-18 (250mm x 4.6mm) 5µm
Column oven temp.	:	25°C
Sampler temp.	:	25°C
Flow rate	:	1.0 ml/min
Detection wavelength	:	270nm
Injection volume	:	20µl
Run time	:	45 minutes
Needle wash	:	Diluent
Mode	:	Gradient

Calculations for Lamotrigine Impurity A

$$\%\text{Impurity A} = \frac{Au}{As} \times \frac{Ws}{100} \times \frac{5}{100} \times \frac{1}{50} \times \frac{25}{Wu} \times \frac{10}{5} \times \frac{P}{100} \times \frac{1}{RRF} \times \frac{wt/ml}{LC} \times 100 \quad \ldots\ldots\ldots\ldots(4)$$

Where,
Au = peak area of lamotrigine impurity A obtained from sample solution
As = average peak area obtained from standard solution
Ws= weight of working standard (mg)
Wu= weight of sample (gm)

Wt/ml= density of sample
LC= label claim (mg)
P = %purity of working standard
RRF= relative response factor

Calculations for unknown impurity

$$\%\text{Impurity} = \frac{Au}{As} \times \frac{Ws}{100} \times \frac{5}{100} \times \frac{1}{50} \times \frac{25}{WT} \times \frac{10}{5} \times \frac{P}{100} \times \frac{wt/ml}{LC} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

Where,
Au = unknown peak area obtained from sample solution
As = average peak area obtained from standard solution
Ws= weight of working standard (mg)
W_T= weight of sample (gm)
Wt/ml= density of sample (gm)
LC= label claim (mg)
P = % purity of working standard

Calculations for Total impurity
% Total Impurity = Sum of all known + unknown impurities…… (6)

In-vitro dissolution study
The proposed in-house method adopted for dissolution of lamotrigine from lamotrigine oral suspension has been validated as per the current ICHguideline (Q2 R1). Separately inject single injections of sample solution and placebo solution into liquidchromatography and record the chromatograms. Disregard the peaks due to blankand placebo from test chromatogram. Calculate the release of lamotrigine inpercentage of label claim using peak area obtained with standard solution andsample solution by using following equation (7).

Chromatographic conditions

Instrument	:	High performance liquid chromatography equipped with pump and PDA/UV detector (Agilent LC 1260 or equivalent)
Column	:	BDS Hypersil C-18, (150mm x 4.6mm) 5µm
Column oven temp.	:	30°C
Sampler temp.	:	25°C
Flow rate	:	1.5 ml/min
Detectionwavelength	:	270nm
Injection volume	:	20µl
Run time	:	15 minutes
Needle wash	:	Diluent
Mode	:	Gradient

$$\%\text{ Drug release} = \frac{At}{As} \times \frac{Ws}{50} \times \frac{5}{100} \times \frac{900}{Wu} \times \frac{25}{10} \times \frac{P}{LC} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

Where,
At = average peak area of analyte obtained from sample solution
As = average peak area of analyte obtained from standard solution
Ws = weight of analyte working standard (mg)
Wu = weight taken for the sample
LC = Label claim (mg/ml)
P = % purity of analyte working standard

Table3: Dissolution parametersfor lamotrigine in lamotrigine oral suspension

Media	0.1N HCI
Media volume	900 ml
Agitation rpm	50 rpm
Apparatus	Paddle
Bath temperature	37°C±1°C
Time point	60 minutes
Samplewithdrawal volume	10 ml
Replenish volume	10 ml

Stability studies

The shelf life of the product was fixed by performing stability studies for the prepared formulation. Accelerated stability study was conducted for the prepared formulation by storing the containers at 40±2ºC temperature and 75±5%RH and studied for six months.

RESULTS AND DISCUSSION

Optimization study of suspending agent

Eight different suspending agents i.e.veegum HV, cekol 700P, cekol 2000P, avicel RC 591, povidone K90, sodium alginate, HPMC E4M premium and carbopol 974P, which are suitable for most pharmaceutical applications, were examined. Table4 shows observations of all formulations prepared by using various suspending agents. During this optimization study of suspending agents, we have observed rapid appearance of a large supernatant and the caking tendencies of suspension prepared by using different suspending agents except carbopol 974P. This suggests that, suspension prepared with carbopol 974P is suitable as suspending agent in lamotrigine oral suspension. Further, this suspension was assessed for physical and chemical analysis.

Table4: Observations of prepared formulation batches of lamotrigine oral suspensions

Formulation	*Sedimentation*	*Re-dispersibility*	*Remark*
F1	NA	NA	Cracking
F2	+	-	Caking after 2 days
F3	+	-	Caking along with precipitation
F4	+	+	Drug was not release completely
F5	+	-	Cake formation
F6	+	+	Crystal growth after 15 days at RT
F7	+	-	More viscous
F8	+	+	Homogenous suspension

(NA-Not applicable, RT-Room temperature, "+" indicates "Yes","–" indicates "No")

Evaluation of selected F8 lamotrigine suspension

The suspension prepared by using carbopol 974P (*F8*) was found to be best when compared to other formulations in all aspects. The bulk visual appearance of the lamotrigine suspension was found to be homogenous and re-dispersible. The pH value of the suspension found well in specification range. Viscosity of suspension was found to be 69 cp using brookfield viscometer. The assay value of lamotrigine and preservatives of suspension was found within range that complies with the desired specification.Formulation exhibits good re-dispersibility on mild shaking. The *in vitro* release profile of lamotrigine from lamotrigine oral suspension were evaluated against commercially available marketed formulation. From obtained graph, it was concluded that release of reference product-lamictal Tablets and test product-lamotrigine oral suspension shows complete release profile. The % RSD observed for both the profiles were found within the limits which shows that both the profiles were comparable. Multimedia dissolution were performed in three different media- 0.1N Hydrochloric acid, 4.5 pH acetate buffer, 6.8 pH phosphate buffer. The reference product and test product formulation released more than 85% within 15mins as per obtained graphs shown in figure 1, figure 2 and figure 3for 0.1N HCl, 4.5 pH acetate buffer, 6.8 pH phosphate buffer, respectively.The result summarylamotrigine suspension is shown in table 5.

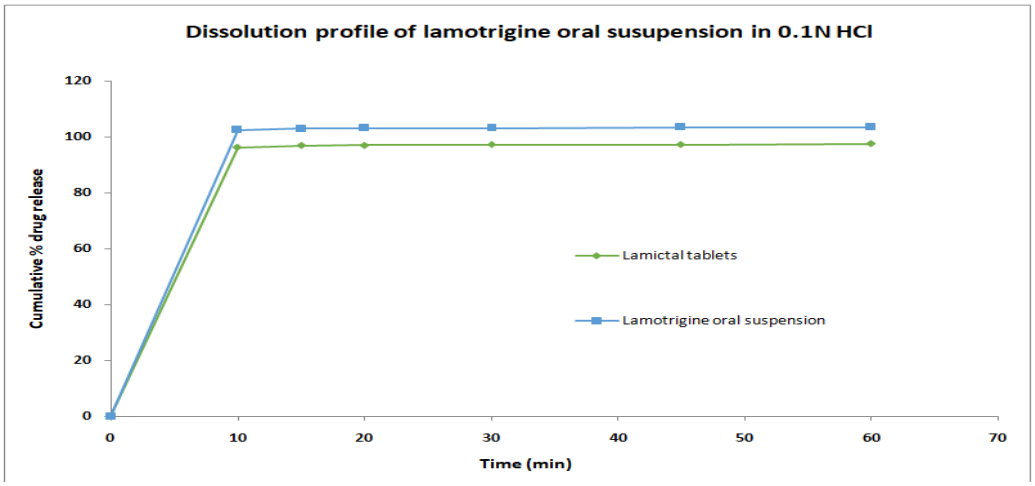

Figure 1: Comparative in-vitro dissolution profiles of marketed product-Lamictal Tablets and test product-Lamotrigine oral suspension in release media (0.1N HCl)

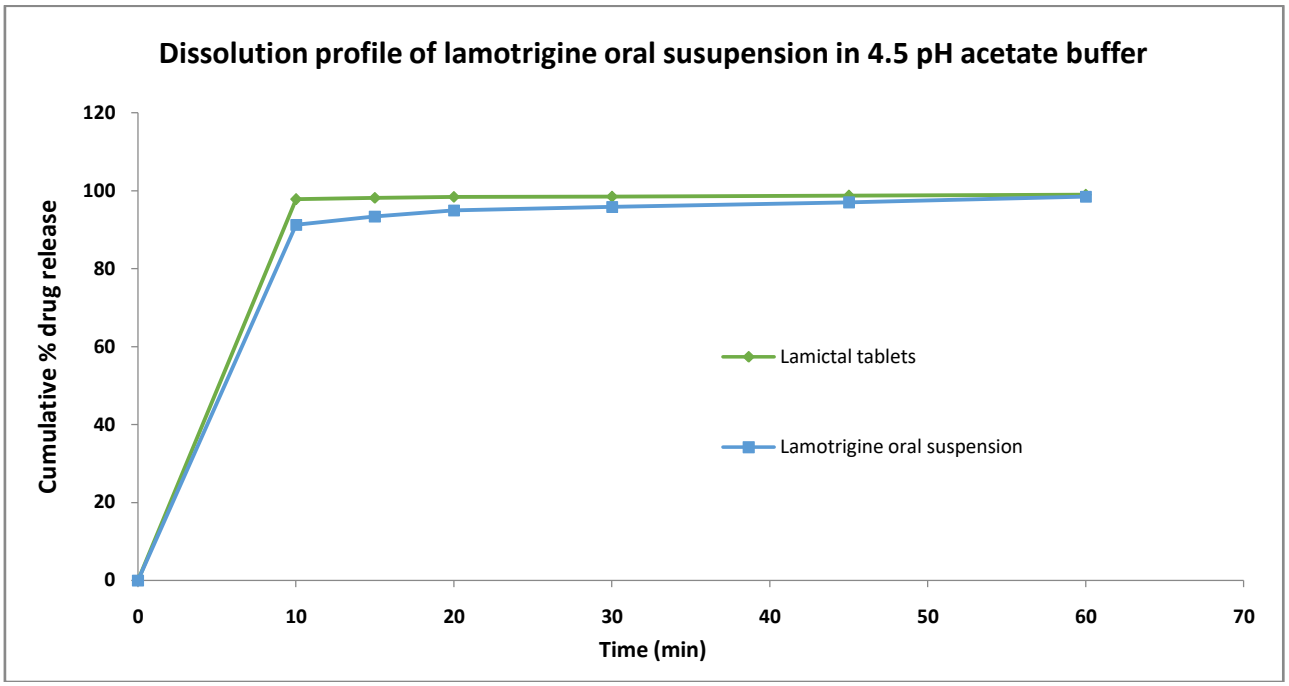

Figure 2: Comparative in-vitro dissolution profiles of marketedproduct-Lamictal Tablets and test product-Lamotrigine oral suspension in 4.5 pH acetate buffer

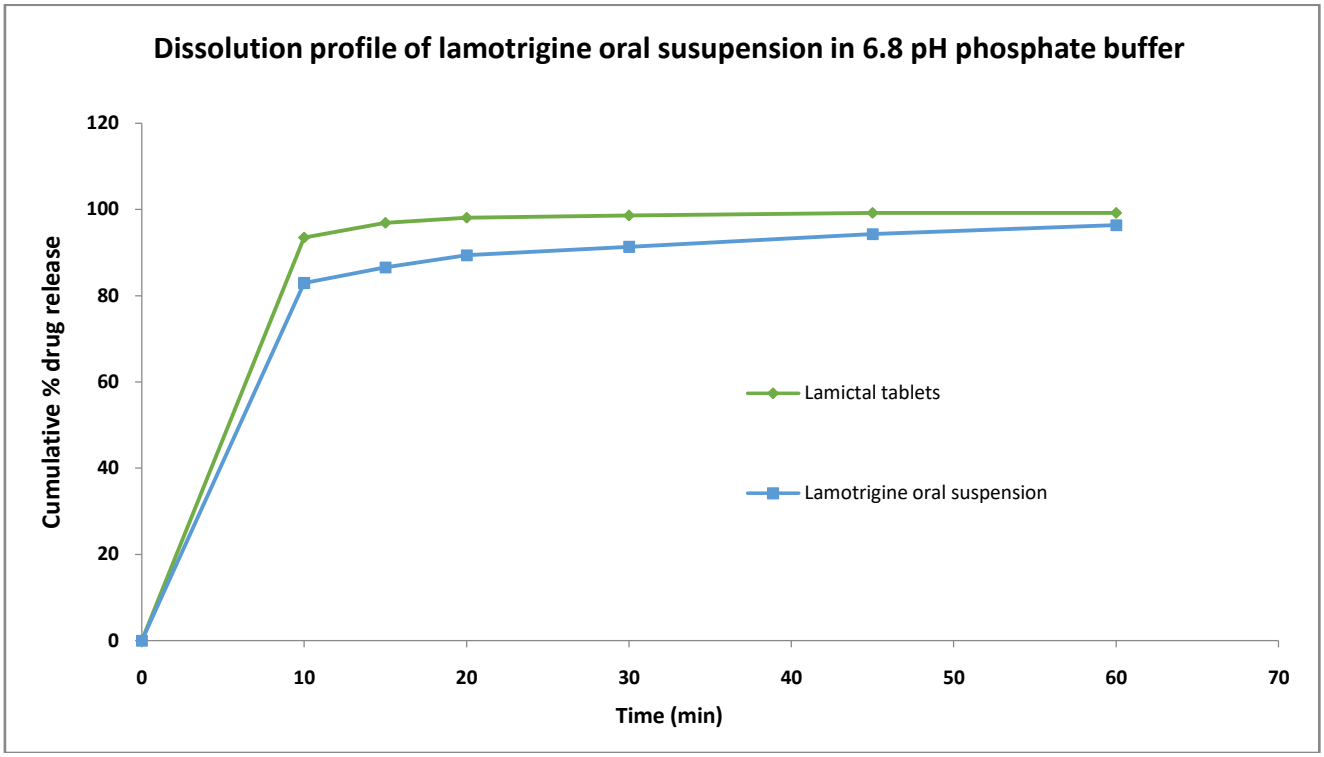

Figure 3: Comparative in-vitro dissolution profiles of marketedproduct-Lamictal Tablets and test product-Lamotrigine oral suspension in 6.8 pH phosphate buffer

Table 5: Summary of Physicochemical analysis ofoptimized lamotrigine suspension (*formulation-F8*)

S.no.	*Parameters*	*Results*
1	**Description**	Off-white coloured homogenous suspension
2	**Re-dispersibility**	98.62
3	**pH**	6.80

4	**Viscosity**	69 cp
5	**Assay**	
	Lamotrigine	97.60 %
	MHB	100.38 %
	PHB	100.01 %
6	**Related substances (Impurity)**	
	Impurity A	0.018 %
	Unknown impurity	Not detected
	Total impurity	0.018 %

Stability Studies

The stability study was conducted for the selected formulation F8 as per ICH guidelines for a period of 6 months. The stability results are shown in table 6.From the results it was found that the selected formulation F8 was found to be stable. There was no significant change from initial readings to final results after 6 months of stability studies.

Table 6: Stability study report of the selected formulation F8

S.no.	***Parameters***	**After 1 month**	**After 3 months**	**After 6months**
1	**Description**	Off-white colored homogenous suspension	Off-white colored homogenous suspension	Off-white colored homogenous suspension
2	**Degree of re-dispersibility**	Readily re-dispersible	Readily re-dispersible	Readily re-dispersible
3	**pH**	6.80	6.78	6.67
4	**Viscosity**	69 cp	--	62 cp
5	**Assay**			
	Lamotrigine	97.60 %	99.09 %	98.49%
	MHB	100.38 %	99.85 %	91.27%
	PHB	100.01 %	99.63 %	97.00 %
6	**Related substances (Impurity)**			
	Impurity A	0.018 %	0.015 %	0.604 %
	Unknown impurity	Not detected	Not detected	0.076 %
	Total impurity	0.018 %	0.015 %	0.707 %

CONCLUSION

Among the formulated suspensions F8 showed good re-dispersibility and sedimentation compared to other formulated suspensions. The results obtained from physicochemical properties of lamotrigine suspension it was concluded that carbopol 974P is best suitable suspending agent. From the stability studies it was confirmed that the prepared formulation F8 was found to be stable. So the formulation F8 containing carbopol 974P 0.25% is effective in all the aspects. Lamotrigine was homogeneously distributed in newly invented suspension dosage formand this guaranteed the correct dose when administered to children and geriatric patients. The formulationsenable pharmacists to administer a variable dose, which adapts to epileptic patient, and gives the possibility of treatment when swallowing lamictal tablets is not possible.

Declaration of Conflict of Interests

The author(s) declared no potential conflicts of interest with respectto the research, authorship, and/or publication of this article.

ACKNOWLEDGEMENT

The authors are very thankful to the *Orbit Pharmaceuticals, Ahmedabad*for providing excellent professional support to complete this research work successfully.

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