International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X; Impact Factor: RJIF 5.22
www.pharmacyjournal.in
Volume 1; Issue 4; September 2016; Page No. 38-45

Preformulation studies intended for targeted lamotrigine polymeric nanosuspension

[1] Hussein Othman Ammar, [2] Mahmoud Mohammad Ghorab, [3] Azza Ahmed Mahmoud, [4] Iman Muhammad Higazy

[1, 3, 4] Department of Pharmaceutical Technology, National Research Centre, Cairo, Egypt
[1, 3] Department of Pharmaceutics and Pharmaceutical Technology, Future University, Egypt
[2] Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Egypt

Abstract
The purpose of this study is to screen various preformulation studies in early stages of dosage form development, in order to reach a preliminary optimized group of lamotrigine (LTG) loaded polymeric nanoparticles, with certain characteristics that would permit brain targeting through parenteral administration. Various preformulation studies were carried out including UV absorbance studies, whose data were a guide to LTG solubility in various solvents, FTIR studies, where interaction between LTG and any other preparation components was studied, investigating the appropriate cryoprotectant, "cloud point titration technique" that indicated the adequate solvent ratio in both binary and tertiary systems, and finally transmission electron microscopy to screen all preparations. LTG was most soluble in acetone whereas polymers had higher solubility in dichloromethane. Pluronic®F68 was the most effective cryoprotectant producing dried re-suspendable stable formulations. Transmission electron microscopy indicated the success of preformulation studies in achieving spherical LTG-loaded stable nano formulations whose particle size is less than 200nm, which can pass the blood brain barrier without being stuck through the reticulo-endothelial system.

Keywords: preformulation, brain targeting, lamotrigine, biodegradable polymer, nanoparticles

1. Introduction
Preformulation involves application of biopharmaceutical principles to physicochemical parameters of drug substance with the goal of designing optimum drug delivery system in support of promising experimental formulations [1]. Its core objective lies in development of stable, effective and safe dosage form, since it generates useful information to the formulator to design an optimum drug delivery system [2]. Lamotrigine (LTG), is a well-tolerated anticonvulsant drug, approved for treatment of focal and generalized epilepsy in the elderly and in patients with epilepsies plus depressive disorders [3]. It undergoes extensive metabolism, and is mainly eliminated *via* the kidney [4]; where 94% of the orally administered dose is eliminated in urine and 2% in feces. Unchanged LTG accounts for 10% of the products detectable in urine [5].

Polymeric nanoparticles (PNPs) have been extensively studied as promising drug delivery systems due to their controlled and sustained drug release properties, subcellular size, and biocompatibility with tissue and cells, which enable them to penetrate capillaries, and be taken up by cells. Thereby, increasing drug accumulation at target sites [6]. Inspite of the increased research work on PNPs, there emerges safety concerns; represented in cytotoxicity of by-products and scalability. Hence, biodegradable polymers were positioned as the safer and healthier option in manufacturing PNPs [7]. Poly (dl- lactide); PLA, poly-ε-caprolactone; PCL, and their copolymers, have generated tremendous interest because of their excellent biocompatibility, biodegradability, mechanical strength and most importantly, they have been approved by the FDA for drug delivery [8]. Poly (dl-lactide-co-ε-caprolactone), PLCL; is a biodegradable copolymer of PLA and PCL, possessing good mechanical properties, and its degradation rate can be tuned by varying the ratio of the constituent polymers [9]. Thereby; PLCL is considered a promising biomaterial in pharmaceutical dosage form design and development.

In this study, preformulation studies would be used to reach an optimum LTG loaded PNPs formulation, intended for sustained parenteral administration. Since LTG is a CNS acting drug, thereby, particle size would be the main challenge in achieving our goal, where particles should be less than 200 nm in order to pass through the blood brain barrier without being subjected to the reticulo-endothelial system.

2. Materials and Methods
2.1 Materials
Lamotrigine was kindly donated by Delta Pharma, Egypt, Pluronic®F127, Pluronic®F68, polyvinyl alcohol (low molecular weight: 13000- 23000 Da), dichloromethane (DCM) were purchased from Sigma Chemical Company, St. Louis, USA, Polyvinyl alcohol (high molecular weight: 1,15,000 Da) was purchased from Loba Chemie, Mumbai, India, Poly-ε-(dl-lactide-co-caprolactone); PLCL (polylactide: polycaprolactone; 80: 20), IV 0.79, PLCL (25: 75), IV 0.79 were purchased from Lactel Bioabsorbables, USA, Ethanol absolute HPLC grade was purchased from Fisher scientific, UK, Acetone for HPLC, ethylacetate (HPLC grade Mwt. 88.10 Da),
potassium dihydrogen orthophosphate, were purchased from Sisco Research Laboratories, Mumbai, India, Sodium phosphate dibasic heptahydrate was purchased from Riedel-de Haën, Germany.

2.2 Methods

2.2.1 Assessment of calibration curves for LTG through analytical UV studies

Standard stock solutions of LTG were prepared and scanned spectrophotometrically; over the range of 200–400nm with double beam spectrophotometer (Shimadzu UV spectrophotometer, 240 j/PC, Japan), against the respective blank, to determine wave length of maximum absorbance (λ_{max}). Specificity [10] and linearity[11] were assessed at respective absorbance values of λ_{max}, for each medium.

2.2.2 Determination of thermodynamic solubility

Shake-flask method of Higuchi and Connors (1965) was used. Samples were shaken on a rotary shaker at 37ºC until equilibrium. The two phases are then separated by filtration [11]. Amount of solute in supernatant is then determined using UV spectrophotometric analysis at the corresponding λ_{max} of each medium.

2.2.3 Compatibility study using Fourier transform infrared (FTIR)

LTG was mixed with each of the components at an appropriate ratio; equivalent to that used in formulation process. Each mixture was stored in an open glass bottle at 40°C, 75% relative humidity (RH) for one week. FTIR spectroscopy, Shimadzu, Model 8400, Japan, was used to study the compatibility of pure drug and other preparation composites, by KBr pellet method; and scanned from 4000 to 400 cm^{-1} [12].

2.2.4 Formulation of LTG polymeric nanosuspension

LTG polymeric nanosuspension formulations were prepared by spontaneous emulsification solvent diffusion method, where an amount of polymer weighing 125 mg was dissolved in 25 ml of organic solvent together with 25 mg of LTG (polymer: drug ratio of 5: 1). The drug-polymeric solution was then drop wisely poured into 50 ml of emulsifier aqueous phase, while agitating by a magnetic stirrer at 15,000 rpm (Wisestir® digital hotplate magnetic stirrer MSH-30D, PMI-Labortechnik Gmbh, Germany) [13].

2.2.5 Formula purification by solvent-evaporation method

The dispersion formed was transferred into a suitable bulb shaped glass flask to be condensed to 25 ml through evaporation under reduced pressure (Rotavapor, Heidolph VV 2000, Germany), at 60°C for about 30 min. It was then ultra-centrifuged at 24,000 rpm for 30 min at -4°C, and the precipitate was then washed with 25 ml phosphate buffer (pH 7.4). This process was repeated three times to remove any residuals [14].

2.2.6 Freeze-thaw test for selection of suitable cryoprotectant

Nanoparticle dispersion was freezed for 12 h. Exactly 5 ml of surfactant were added drop-wisely to the finally LTG loaded formulation, to be then homogenized on magnetic stirrer for less than 1 min at 500 rpm, and subsequently poured into a petri dish and put into freezer overnight, followed by thawing at 28ºC. Size and size distribution before and after freeze–thawing were measured by Zetasizer Nano ZS (Malvern Instruments, UK). Cryoprotection factor (CF) was calculated according to the following equation: $CF = S_f/S_i$, where, Si is the nanoparticle size after addition of the protectant and S_f is the nanoparticle size after freeze-thawing. Protection system was considered efficient when its CF values are lower than 2 [15].

2.2.7 Lyophilization process

The prepared nanosuspension was freeze-dried in vacuum at -60°C and pressure of 0.3 mbar to obtain powdered NPs. Main drying was performed for 8.5 hr, and final drying for 30 min, where freeze-drying was carried out in a non-aseptic area[18], using Lyophilizer, FD-81, Eyela, Japan. The freeze-dried samples were then stored at 8°C before analysis.

2.2.8 Optimization of PNPs by changing biodegradable polymer weight ratio

Two sub-types of PLCL; having the same intrinsic viscosity and molecular weight, but varying poly (d,l-lactide): poly-ε-caprolactone ratios; 80: 20 and 25: 75, respectively, were used.

2.2.9 Optimization of PNPs by changing solvent system composition

Based on solubility test, various solvent systems were used as follows: (a) Mono solvent system: organic phase is single solvent, (b) Binary system: Cloud point titration: 125 mg polymer was dissolved in a specified volume of organic solvent, then 2 ml were poured into a 20 ml glass test tube. Resultant solution was titrated with the other organic solvent, usually alcohol, to cloud point, where polymer precipitate. Alcohol percentage at cloud point, CL_{alc}, was determined as index representing affinity between polymer and solvent[15]. For surfactants; 2 ml of surfactant solution were placed in 20 ml glass test tube and titrated with the chosen solvent mixture. The volume percentage of solvent mixture at the cloud point, CL_{binary}, was determined as the index representing affinity between surfactant and solvent mixture [14]. (c) Tertiary system: A mixture of polymer and LTG were added into DCM-binary solvent mixture; where DCM: binary system mixture was 1: 9 [16]. The mixture was vortexed (Paramix II vortex, Germany) for dissolution, then slowly poured into aqueous surfactant solution using a high speed homogenizer (Heidolph Silent Crusher Homogeniser) at 24,000 rpm for 5 min or magnetic stirrer at speed 15000 for 10 min.

2.2.10 Optimization of PNPs by changing surfactant type and concentration

Four emulsifiers were used: HPVA (13,000-23,000 Da), and LPVA (115,000 Da), Pluronic®F68 and Pluronic®F127. Various concentrations of emulsifiers were used: 0.5, 1, 2 and 4 %w/v for PVAs, and 1.25 and 2.5%w/v Pluronics®, and examined while keeping aqueous: organic phase ratio constant (2: 1), along all experiments.

2.2.11 Transmission electron microscopy (TEM)

Morphology of various PNPs were examined by TEM; operated at 120 kV (JEOL-JEM-1230, Japan) using computer program named AMT Image Capture Engine V601, USA. Particles were stained with phosphotungestic acid (2%w/v) and placed on copper grids with Formvar films for viewing.

3. Results

3.1 Assessment of calibration curves for LTG through analytical UV studies

Various standard curves of LTG were assessed at corresponding λ_{max}, listed in table (1), and method specificity was demonstrated through standard deviation, where λ_{max} for each solvent, proved to be specific, ($p \geq 0.05$). In addition; coefficient of determination (R^2) was calculated, as reference to degree of linearity; tested by plotting data and looking for curvature, indicating a positive correlation showing an intercept in the regression model [17]. However, PLCL (80: 20) and (25: 75), did not show any λ_{max} when preparing their standard solutions in variable solvents.

Table 1: Maximum absorbance wavelength (λ_{max}) and linearity factor values for LTG

Organic solvents	Maximum absorbance wavelength (λ_{max} ± SD)	R^2 ± SD	|r|
Acetone	333.4 ± 0.01	0.997 ± 0.002	0.998
Ethanol	306.2 ± 0.03	0.998 ± 0.001	0.999
Methanol	274.6 ± 0.13	0.992 ± 0.004	0.996
Acetonitrile	299.2 ± 0.10	0.998 ± 0.001	0.999
DCM	268.4 ± 0.16	0.992 ± 0.003	0.996
Phosphate buffer pH 7.4	306.5 ± 0.01	0.999 ± 0.001	0.999

3.2 Determination of thermodynamic solubility

Minimum required solubility (MRS), is used to evaluate solubility [18] of LTG, PLCL (80: 20) and (25: 75). Both; time and amount required for polymer solution to reach saturation against a fixed amount of solvent, were used as comparative factor for qualitative solubility of polymer. Results shown in table (2), revealed that both polymers were highly soluble in DCM > acetone > acetonitrile > ethanol > methanol. Results also indicate that LTG was most soluble in acetone > methanol > ethanol > acetonitrile > DCM.

Table 2. Solubility data of biodegradable polymers and LTG in various solvents

Solvent	Time for saturation (hr ± SD)		Mean MRS (mg/ml ± SD)		
	PLCL (80: 20)	PLCL (25: 75)	PLCL (80: 20)	PLCL (25: 75)	LTG
Ethanol	24 ± 6.5	24 ± 3.50	5.0 ± 0.82	7.5 ± 1.04	32.51 ± 4.08
Acetone	36 ± 4.3	36 ± 5.25	25.0 ± 3.33	22.5 ± 2.97	117.09 ± 20.71
Acetonitrile	3 ± 0.5	3 ± 0.25	15.0 ± 2.98	17.5 ± 2.15	18.77 ± 2.77
Methanol	6 ± 1.2	6 ± 1.50	2.5 ± 0.20	2.5 ± 0.01	85.05 ± 11.40
DCM	72 ± 8.5	72 ± 6.00	50.0 ± 6.13	60.0 ± 4.71	8.40 ± 0.30

3.3 Compatibility study using Fourier transform infrared (FTIR)

All readings obtained are within ranges reported in previous studies [19]. Briefly; LTG molecule has 23 atoms and possesses C_1 symmetry configuration. FTIR spectra of HPVA and LPVA are consistent with literature [20]. Differences are reflected in total peak number, insignificant, ($p \geq 0.05$) shift of peak, and peak intensity. Two stretching bands appear in LPVA FTIR instead of one as in HPVA spectrum [21]. Similarities in molecular structure of Pluronic®F68 and Pluronic®F127, were reflected through FTIR absorption bands, where relatively broad peaks prevail due to their large molecular size and partially amorphous nature [22]. FTIR spectra of PLCL (80: 20) and (25: 75), were all compatible with data reported in literature [23]. Randomly chosen physical mixtures were classified into: (A) PVA physical mixtures were analyzed for interaction by FTIR, which indicated an overlapping of several peaks from various functional groups have led to insignificant shifting, ($p \geq 0.05$), in some peaks. Overall, no interaction is detected since no new peaks appeared, or other old peaks disappeared either. (B) Pluronic® physical mixtures had FTIR data that expressed no inter-composition interaction.

3.4. Formulation of LTG polymeric nanosuspension

Table 3: Polymeric LTG loaded nanosuspension preparations

Preparation code	Composition[h]		
	Surfactant		125 mg of biodegradable polymer type
	Type	Concentration (%w/v)	
F1	HPVA	0.5	PLCL (80PLA: 20PCL)
F2		4	
F3		0.5	PLCL (25PLA: 75PCL)
F4		4	
F5	LPVA	0.5	PLCL (80PLA: 20PCL)
F6		4	
F7		0.5	PLCL (25PLA: 75PCL)
F8		4	
F9	Pluronic®F68	1.25	PLCL (80PLA: 20PCL)
F10		2.5	
F11		1.25	PLCL (25PLA: 75PCL)
F12		2.5	

F13	Pluronic® F127	1.25	PLCL (80PLA: 20PCL)
F14		2.5	
F15		1.25	PLCL (25PLA: 75PCL)
F16		2.5	

(h) Each formulation contained 25 mg LTG

3.5 Freeze-thaw test for selection of suitable cryoprotectant

Cryoprotectant effect of 5%w/v LPVA presented in table (4) show that all systems were efficiently protected, with CF< 2. There is significant difference ($p< 0.05$), in CF of (F2< F1), (F4< F3), (F6< F5), (F8< F7), (F10< F9), (F12< F11), (F14< F13), and finally (F16< F15). As per PS and PDI changes; PS significantly increased ($p< 0.05$) in all PVA-containing PNPs as surfactant, while there was no significant PS change, ($p\geq 0.05$), in Pluronic®-containing PNPs as surfactant. This would indicate that Pluronics® might be improving the cryoprotectant effect of 5%w/v LPVA.

Table 4: Cryoprotectant activity of 5%w/v low molecular weight polyvinyl alcohol

Preparation symbol	PS before freeze-thawing (Mean ± SD)	PS after freeze-thawing (Mean ± SD)	PDI before freeze-thawing (Mean ± SD)	PDI after freeze-thawing (Mean ± SD)	CF
F1	96.730 ± 11.610	152.300 ± 24.210	0.180 ± 0.002	0.276 ± 0.036	1.574
F2	261.800 ± 34.112	372.150 ± 39.870	0.274 ± 0.011	0.325 ± 0.023	1.422
F3	111.500 ± 17.461	165.500 ± 23.116	0.144 ± 0.014	0.177 ± 0.013	1.484
F4	310.700 ± 41.880	425.300 ± 49.002	0.146 ± 0.009	0.196 ± 0.021	1.240
F5	212.400 ± 42.100	321.100 ± 31.922	0.304 ± 0.025	0.362 ± 0.021	1.403
F6	265.800 ± 29.673	341.410 ± 29.542	0.213 ± 0.017	0.279 ± 0.030	1.284
F7	102.100 ± 13.562	152.400 ± 15.340	0.118 ± 0.009	0.160 ± 0.018	1.492
F8	140.000 ± 9.207	185.300 ± 13.452	0.145 ± 0.027	0.207 ± 0.013	1.323
F9	116.300 ± 20.012	204.400 ± 33.477	0.207 ± 0.013	0.236 ± 0.021	1.758
F10	94.550 ± 11.431	122.900 ± 14.111	0.185 ± 0.007	0.200 ± 0.016	1.406
F11	119.800 ± 21.094	155.300 ± 23.001	0.146 ± 0.004	0.172 ± 0.012	1.296
F12	74.920 ± 9.068	87.070 ± 10.720	0.158 ± 0.031	0.169 ± 0.003	1.162
F13	91.690 ± 18.001	112.100 ± 23.340	0.149 ± 0.010	0.162 ± 0.005	1.223
F14	78.680 ± 19.010	93.300 ± 15.972	0.124 ± 0.005	0.136 ± 0.012	1.186
F15	94.800 ± 8.973	111.100 ± 8.002	0.109 ± 0.017	0.121 ± 0.011	1.171
F16	92.870 ± 17.560	105.900 ± 13.681	0.108 ± 0.005	0.117 ± 0.020	1.140

3.6 Effect of type and concentration of cryoprotectant

Optimization of both type and concentration of cryoprotectant involves PNPs that showed significantly, ($p< 0.05$), lower CF values, compared to their homologues: F2, F4, F6, F8, F10, F12, F14, and F16. 5%w/v HPVA, Pluronic®F127, and Pluronic®F68 were tested, with CF values presented and compared in table (5).

Table 5: Cryoprotectant activity of 5%w/v of several surfactants

Preparation symbol	CF values ± SD		
	HPVA	Pluronic®F127	Pluronic®F68
F2	1.643 ± 0.011	1.188 ± 0.053	1.142 ± 0.021
F4	1.890 ± 0.009	1.182 ± 0.039	1.104 ± 0.019
F6	1.497 ± 0.017	1.213 ± 0.073	1.132 ± 0.029
F8	1.867 ± 0.027	1.285 ± 0.029	1.086 ± 0.017
F10	1.589 ± 0.007	1.133 ± 0.038	1.059± 0.022
F12	1.482 ± 0.031	1.108 ± 0.090	1.062± 0.009
F14	1.628 ± 0.005	1.119 ± 0.057	1.082± 0.011
F16	1.885 ± 0.005	1.081 ± 0.041	1.029± 0.019

All surfactants, resulted in CF<2, assuring their cryoprotectant efficiency through CF values below "2". Cryoprotectant effect of all surfactants was compared, through their CF. Pluronic®F68 is the most effective cryoprotectant; showing minimum CF, while maximum CF were resulted from HPVA, with significant difference ($p< 0.05$). Pluronic®F68 was thereby, used as model cryoprotectant for further investigations, as shown in table (6). Results revealed that 10, 15 and 20%w/v Pluronic®F68 have anti-aggregation effect, reflected in CF approximately approaching value of "1", and insignificant PS and PDI changes. CF values at 5%w/v significantly ($p< 0.05$) varied from 20%w/v, while no significant differences ($p\geq 0.05$) were noticed between CF at 15 and 20% w/v.

Table 6: Cryoprotectant activity of increasing concentrations of Pluronic®F68

Preparation symbol	Cryoprotectant Factor (CF) on adding Pluronic®F68			
	5%w/v	10%w/v	15%w/v	20%w/v
F2	1.142	1.103	1.070	1.052
F4	1.104	1.062	1.043	1.028
F6	1.132	1.084	1.051	1.036
F8	1.086	1.048	1.034	1.021
F10	1.059	1.026	1.012	1.008
F12	1.062	1.038	1.020	1.013
F14	1.082	1.042	1.028	1.017
F16	1.029	1.011	1.002	1.002

3.7 Optimization of PNPs

Various parameters have been changed, by combining several solvent systems, surfactant types and concentrations, and polymers.

Table 7: Cloud point of ethanol in acetone- polymer solution

Polymer type	$CL_{alc\ (\%)}$	Acetone: ethanol ratio
PLCL (80PLA: 20PCL)	60	1: 1.5
PLCL (25PLA: 75PCL)	63	1: 1.7

Table 8: Cloud point between surfactant solution and drug-polymer solvent system

Surfactant type	Surfactant concentration (%w/v)	Solvent system: Surfactant solution			CL_{binary} (%)		
		Mono	Binary	Tertiary	Mono	Binary	Tertiary
HPVA	0.5	1: 1.5	1: 1.6	1: 1.7	60	62	63
	1	1: 1.6	1: 1.6	1: 1.8	62	62	64
	2	1: 1.8	1: 1.7	1: 1.9	64	63	66
	4	1: 2	1: 2	1: 2	67	67	67
LPVA	0.5	1: 1.2	1: 1.3	1: 1.3	55	57	57
	1	1: 1.4	1: 1.5	1: 1.4	58	60	58
	2	1: 1.7	1: 1.8	1: 1.16	63	64	62
	4	1: 2	1: 2	1: 2	67	67	67
Pluronic®F68	1	1: 1.5	1: 1.2	1: 1.6	60	55	62
	2	1: 1.8	1: 1.5	1: 1.9	64	60	66
Pluronic®F127	1	1: 1.3	1: 1.2	1: 1.4	57	55	58
	2	1: 1.6	1: 1.4	1: 1.7	62	58	63

Alcohol percentage in polymer solution at cloud point, CL_{alc}, was recorded in table (7), as index of polymer affinity to solvent. As for surfactants; volume ratio of (solvent mixture: surfactant solution) at cloud point, CL_{binary}, was determined in table (8), as index of surfactant affinity to solvent mixture.

3.8 Transmission Electron Microscopy (TEM)

In figure (1), effect of varying solvent system is studied through [LTG/HPVA/PLCL (80: 20)] PNPs; where results indicated that solvent system has no effect on either morphology or PS, with all PNPs showing PS less than 200nm, which is optimal for passing BBB without getting caught by the RES. Figure (2) shows the effect of varying surfactant concentration and molecular weight on morphology and PS through investigating [LTG/HPVA/PLCL (25: 75)] and [LTG/LPVA/PLCL (25: 75)] formulations. No considerable change was noticed.

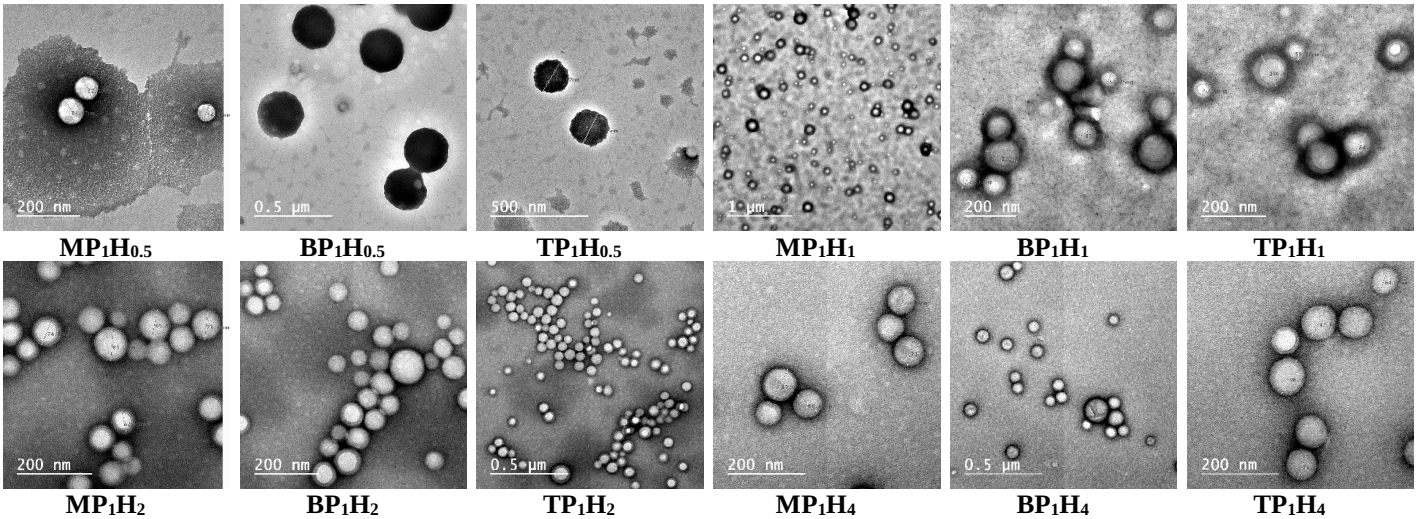

Fig 1: TEM of [LTG/HPVA/PLCL (80: 20)] polymeric nanoparticles

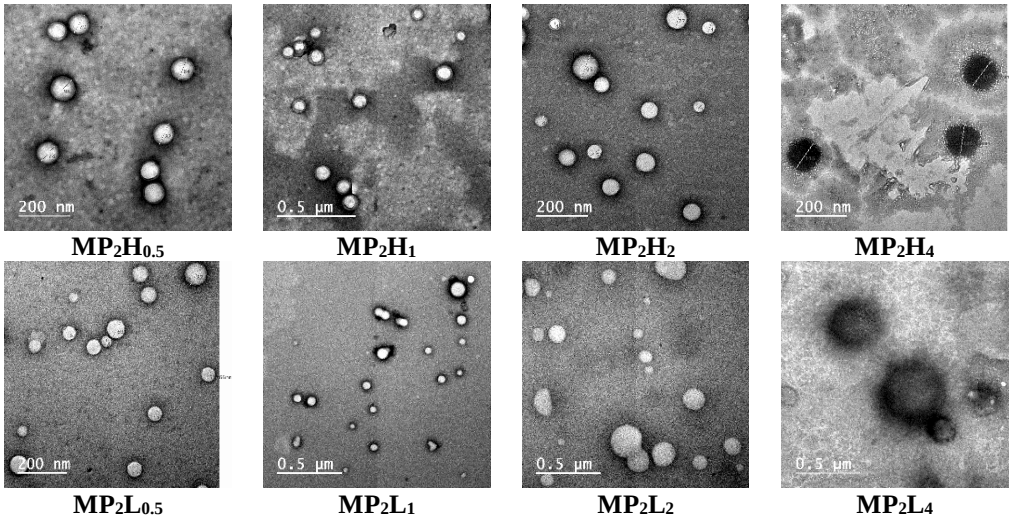

Fig 2: TEM of [LTG/HPVA/PLCL (25: 75)] and [LTG/LPVA/PLCL (25: 75)]

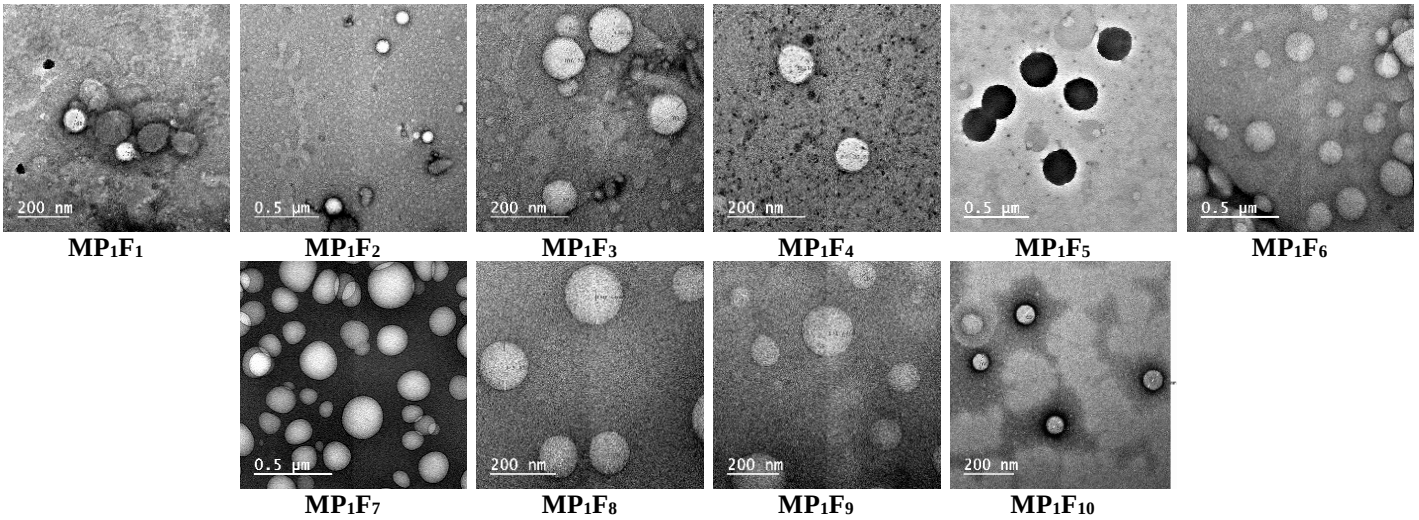

Fig 3: TEM of [LTG/Pluronic®/PLCL (80: 20)] polymeric nanoparticles

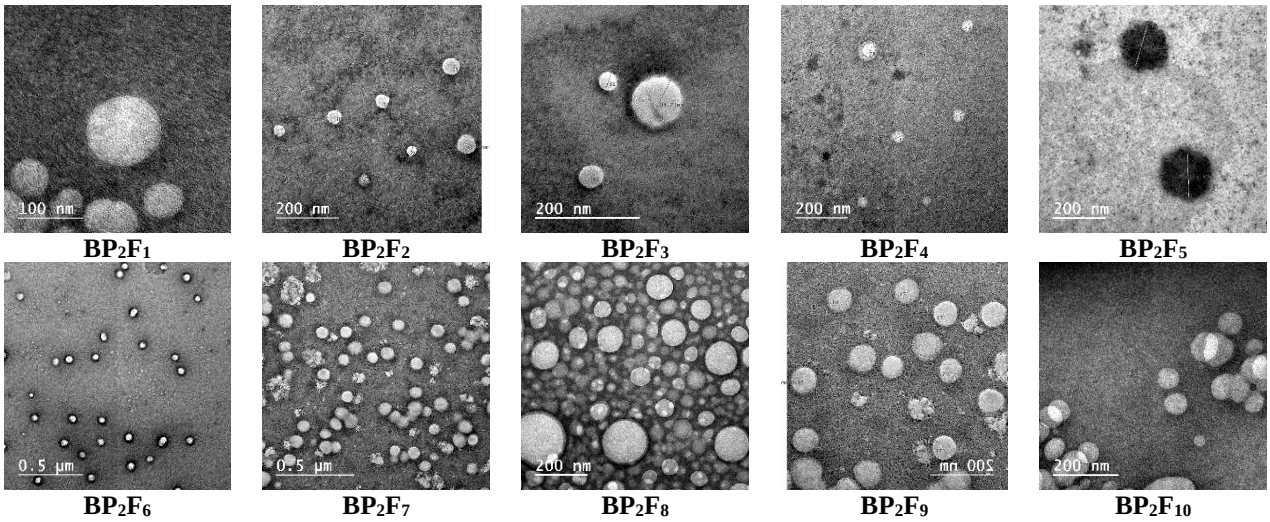

Fig 4: TEM of [LTG/Pluronic®/PLCL (25: 75)] polymeric nanoparticles

Figures (3) and (4), showing TEM of Pluronic® based PNPs, illustrating effect of changing polymer and surfactant type, as well as (polymer: surfactant) ratio. Results reveal no effect of changing polymer or surfactant type or even (polymer: surfactant) ratios, since all particles appeared to be spherical in shape with size lying below 200nm.

4. Discussion

4.1 Assessment of calibration curves for lamotrigine

All R^2 values calculated are compatible with the "ideal R^2 model" interpretation. Also, values of |r| were all > 0.7, indicating a strong correlation between the two variables[17]; drug concentration and absorbance (as reflection of solubility). R^2 values generally ranged between 0.992 and 0.999 (± SD). These results reflect a strong linear component of the presented models.

4.2 Determination of thermodynamic solubility

Acetone would be solvent of choice for mono-solvent system, as it solubilizes both drug and polymers. But, single solvent would not promise optimum solubilization, so, cosolvent was suggested. Since, cosolvency power is stronger for water-miscible alcohols, because; as explained by Ladaa *et al*. as carbon chain length of alcohols increases, their cosolvency power increases, thereafter; ethanol would be preferred to methanol, as it enhances solubility of hydrophobic drugs as LTG, as most of cosolvents contain hydrogen bond donor and/or acceptor groups with little part of hydrocarbon regions, in addition to interfering with water's self-association and decreasing water's ability to pull out non-polar hydrophobic components [24]. In addition, DCM shows maximum solubility to polymers, and minimum solubility to LTG, thereby; it might be used as secondary cosolvent in combination with alcohol.

4.3 Compatibility study using Fourier transform infrared (FTIR)

IR bands of PVA are quite broad and overlapped in "600 to 1500" cm^{-1} region, due to: (1) half PVA bulk consists of crystallites embedded in glassy matrix, where difference of symmetry between free and unfree PVA molecules contributes to two sets of frequencies [25]. (2) slight shift between equivalent frequencies in these two phases contributes to overlapping. (3) PVA possesses an "intermediate" region other than crystalline and amorphous phases. Thus; these regions show a mixed symmetry due to random placement of OH groups along the chain, resulting in relatively broad and intense OH stretching band, due to polymeric association of free and bonded OH vibrations [26]. From previous results, no interaction between drug and excipients was noticed, since no new peaks appeared, neither were there any disappearing peaks. All functional groups, although showing variable wavelengths within different physical mixtures; but they all appeared within their registered ranges in literature.

4.4 Freeze-thaw test for selection of suitable cryoprotectant

Freeze-dried suspensions usually show strong tendency to agglomerate after drying, due to phase separation during freezing into ice and cryoconcentrated solution. In case of PNPs, cryoconcentrated phase contains NPs, free surfactants, buffers and unloaded drug [27]. Crystallization of ice may exercise mechanical stress on PNPs leading to their destabilization; resulting in an aggregate final formulation that cannot be reconstituted for administration. Thereby, cryoprotectants must be added to increase stability upon storage [28]. Freeze–thaw study is short and quick compared to freeze drying and hence used as pre-test for screening of cryoprotectant that would be used [29]. Results preliminarily suggest that: as total content of surfactant increases, the whole system becomes more protected against aggregation. Unlike conventional cryoprotectants that inhibit freezing by interacting with water, ice blockers as PVA, act by direct molecular recognition of ice nucleators [30]. This selective attraction to ice growth surfaces permits PVA to exert significant effects while present at very low molecular weights and concentrations [31], thus, protect systems against aggregation without additional toxicity.

4.5 Effect of type and concentration of cryoprotectant

Pluronic®F68 is the most effective cryoprotectant; showing minimum CF. Pluronics® are reputed as one of the most effective stabilizers. Steric stabilizers, as Pluronics® act by steric hindrance and shielding particles' surface against freezing stresses, thus decreasing hydrogen bonding between particles [32], preventing their agglomeration. Concentration-dependent cryoprotection was observed through increase in PS at lower cryoprotectant concentrations. Increase in PDI was also noticed, due to cryoprotectant micelles formation[40].

5. Conclusion

Various preformulation studies were carried out starting by UV absorbance studies, whose data were a guide to LTG solubility in various solvents. Acetone was chosen as the mono solvent to solubilize both LTG and polymers, while ethanol was the cosolvent of choice, and finally DCM was the subsidiary cosolvent in a tri-solvent system. Pluronic®F68 was the most suitable cryoprotectant capable of producing dried re-suspendable stable preparations, with no significant increase in either particles size or PDI. Finally, TEM was an evidence of the success of preformulation studies in achieving spherical LTG-loaded stable PNPs formulations whose particle size is less than 200nm to assure their intended parenteral administration for sustained central anti-epileptic effect.

6. References

1. Raja SRP, Chandrasekhara RG. Spatio temporal release of lamotrigine by buoyant gastroretentive drug delivery Development and evaluation. Int J Pharm Pharm Sci. 2014; 6(4):604-610.
2. Vilegave K, Vidyasagar G, Chandankar P. Preformulation studies of pharmaceutical new drug molecule and products: An overview. American Journal of Pharmacy and Health Research. 2013; 1(3):1- 20.
3. Castel-Branco MM, Almeida AM, Falcao AC, Macedo TA, Caramona MM, Lopez FG. Lamotrigine analysis in blood and brain by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl. 2001; 755(1-2):119-127.
4. Stefan H, Feuerstein TJ. Novel anticonvulsant drugs. J Pharmacol & Therapeutics. 2007; 113(1):165-183.
5. Product Information Lamictal®, lamotrigine tablets. Glaxo Wellcome Co., Research Triangle Park, NC, 2001.
6. Arias JL, Ruiz MA, López-viota M, Delgado AV. Poly (alkylcyanoacrylate) colloidal particles as vehicles for antitumour drug delivery: a comparative study. Colloids Surf B Biointerfaces. 2008; 62(1):64-70.
7. Handy RD, Shaw BJ. Toxic effects of nanoparticles and

nanomaterials: implications for public health, risk assessment and the public perception of nanotechnology. Health, Risk and Society. 2007; 9(2):125-144.
8. Koleske J. Blends containing poly (ε-caprolactone) and related polymers. In: Paul D, Newman S, editors. Polymer blends. New York: Academic Press Inc. 1978, 369-389.
9. Yang F, Murugan R, Wang S, Ramakrishna S. Electrospinning of nano/ micro scale poly(L-lactic acid) aligned fibers and their potential in neural tissue engineering. Biomaterials. 2005; 26(15):2603-2610.
10. Higuchi T, Connors KA. Phase-solubility techniques. Advances in Analytical Chemistry and Instrumentation. 1965; 4:117-212.
11. Kawakami A, Miyoshi K, Ida Y. Impact of the Amount of Excess Solids on Apparent Solubility. Pharmaceutical Research. 2005; 22:1537-1543.
12. Amrutkar PP, Patil SP, Todarwal AN, Wagh MA, Kothawade PD, Surawase RK. Design and evaluation of taste masked chewable dispersible tablet of lamotrigine by melt granulation. Int J Drug Delivery. 2010; 2(2):183-191.
13. Murakami H, Kobayashi M, Takeuchi H, Kawashima Y. Preparation of poly (DL-lactide-co-glycolide) nanoparticles by modified spontaneous emulsification solvent diffusion method. Int. J Pharm. 1999; (187):143-152.
14. Jiang XJ, Zhou CS, Tang KW. Preparation of PLA and PLGA nanoparticles by binary organic solvent diffusion method. J Cent. South Univ. Technol. 2003; (10):202-206.
15. Stupar P, Pavlović V, Nunić J, Cundrič S, Filipič M, Stevanović M. Development of lyophilized spherical particles of poly (epsilon-caprolactone) and examination of their morphology, cytocompatibility and influence on the formation of reactive oxygen species. Journal of Drug Delivery Science and Technology. 2014; (24):191-197.
16. Abazinge M, Jackson T, Yang Q, Owusu-Ababio G. *In vitro* and *in vivo* characterization of biodegradable enoxacin microspheres. Eur. J Pharm. Biopharm. 2000; (49):191-194.
17. Rovine MJ, Von Eye A. A 14th way to look at a correlation coefficient: Correlation as the proportion of matches. The American Statistician. 1997; (51):42-46.
18. Hsu HW, Cheng YJ, Chen LK, Wang YP, Lin CJ, Lee CN, *et al*. Differential analgesic effect of tenoxicam on the wound pain and uterine cramping pain after cesarean section. Clin. J Pain. 2003; (19):55-58.
19. Ramya T, Ramkumaar GR, Gunasekaran S. Structural and Qualitative Analysis of Lamotrigine. Int J Neurorehabilitation Eng. 2014; 1(4):135-138.
20. Jayasckara R, Marding I, Bowater I, Christic GBY, Lonergan GT. Preparation, surface modification and characterization of solution cast starch PVA blended films. Polymer Testing. 2004; (23):17- 27.
21. Shehap AM. Thermal and Spectroscopic Studies of Polyvinyl Alcohol/Sodium Carboxy Methyl Cellulose Blends. Egypt J Solids. 2008; (31):75-91.
22. Vippagunta SR, Maul KA, Tallavajhala S, Grant DJW. Solid-state characterization of nifedipine solid dispersions. Int J Pharm. 2002; (236):111-123.
23. Coates JP. Interpretation of infrared spectra, a practical approach. Encyclopedia of Analytical Chemistry: Meyers RA (Ed.). 2000; 10815-10837.
24. Millard JW, Alvarez-Nunez FA, Yalkowsky SH. Solubilization by cosolvents: Establishing useful constants for the log-linear model. Int J Pharm. 2002; (245):153-166.
25. Bunn CW. Crystal Structure of Polyvinyl Alcohol. Nature. 1948; (161):929-930.
26. Sakellariou P, Hassan A, Rowe RC. Phase separation and polymer interactions in aqueous poly (vinyl alcohol)/hydroxypropyl methylcellulose blends. Polymer. 1993; (34):1240-1248.
27. Abdelwahed W, Degobert G, Fessi H. Investigation of nanocapsules stabilization by amorphous excipients during freeze-drying and storage. Eur J Pharm Biopharm. 2006; 63:87-94.
28. Abdelwahed W, Degobert G, Stainmesse S, Fessi H. Freeze-drying of nanoparticles: Formulation, process and storage considerations. Adv Drug Delivery Reviews. 2006; 58:1688-1713.
29. Schwarz C, Mehnert W. Freeze-drying of drug-free and drug-loaded solid lipid nanoparticles (SLN). Int J Pharm. 1997; (157):171-179.
30. Taylor MJ, Song YC, Brockbank KGM, Vitri FI cation in tissue preservation: new developments, In: Fuller BJ, Lane N, Benson EE (Eds.), Life in the Frozen State, CRC Press, Boca Raton FL. 2004, 604-641.
31. Wowk B, Fahy GM. Inhibition of bacterial ice nucleation by polyglycerol polymers. Cryobiology. 2002; (44):14-23.
32. Donini C, Robinson DN, Colombo P, Giordano F, Peppas NA. Preparation of poly (Methacrylic acid-g-poly (Ethylene Glycol) nanospheres from methacrylic monomers for pharmaceutical applications. Int J Pharm. 2002; (245):83-91.