Research Article

Investigation of Formulation Variables Affecting the Properties of Lamotrigine Nanosuspension Prepared by Using High Pressure Homogenizer Using Factorial Design

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ABSTRACT

Lamotrigine undergoes extensive hepatic metabolism upon oral administration and its absorption is affected in presence of food.This study was aimed to prepare a nanosuspension using high pressure homogenizer and to study the variables affecting properties of lamotrigine nanosuspension using factorial design. A 2^3 factorial design was used and the variables used for factorial design were stabiliser concentration,polymer concentration and bar pressure. The PSD data of Batch no LMG 02 is most promising as compared with other batches with a particle size of 232.2 nm d(10),457.1 nm d (50),879.4 nm (90) and polydispersity index of 0.269. The saturation solubility was with 3.2 ± 0.2 mg/ml higher than the one of the raw material which has 0.17 mg/ml. Lyophilization was performed to dry LMG aqueous nanosuspension into powder (nanocrystals). Batch no. 2 was chosen for further investigation because it showed highest physical stability.Redispersibility study showed that the suspension was easily redispersible. X-ray diffraction patterns were visualized in diffractograms. All XRD patterns showed the crystallinity of lyophilized LMG nanocrystals with the different stabilizers. In addition, the diffractogram showed slight reduction in peak intensities for LMG nanocrystal formulations. According to these results, all nanocrystals were still in the same crystalline state as the raw material.

Keywords: Lamotrigine, High pressure homogenizer,factorial design.

INTRODUCTION

An increasing number of newly developed drugs are poorly soluble; in many cases drugs are poorly soluble in both aqueous and organic media excluding the traditional approaches of overcoming such solubility and bioavailability factor. An alternative and promising approach is the solubility enhancement by using wet milling (high pressure homogenization technique-HPH) to overcome these problems. The major advantages of this technology are its general applicability to most drugs and its simplicity.In addition, poorly water soluble drugs are specially challenging, as they cannot achieve dissolution and therefore they have a very difficult pass through the dissolving fluid to contact the absorbing mucosa. If the dissolution process of the drug molecule is slow, due to the physicochemical properties of the drug molecules or formulation factors, then dissolution may be the rate-limiting step in absorption and will influence drug bioavailability.(1) This is the case of BCS class II drugs, e.g.Lamotrigine (LMG). Lamotrigine (LMG), an antiepileptic drug of Phenyltriazine class, is used for the treatment of partial seizures and those associated with the Lennox-Gastant syndrome. It is a basic drug (pKa 5.3) with an intrinsic solubility of 0.17 mg/ml and undergoes extensive hepatic metabolism upon oral administration. Also, its absorption is affected in the presence of food. Existing formulations of LMG provide immediate release with tmax ranging from 1.4 to 4.8 hrs and results into a release profile with various peaks and troughs.Lamotrigine (LMG) undergoes extensive hepatic metabolism upon oral administration and its absorption is affected in the presence of food.(9) Therefore, it was proposed to enhance the solubility of LMG by using wet milling (high pressure homogenization technique-HPH), which is further incorporated with extended release matrix core which would provide the plasma concentrations within the therapeutic window over a longer period of time.

MATERIAL AND METHODS

MATERIAL

Lamotrigine (LMG),Hpromellose (HPMC E-5) were obtained as gift samples from Lupin Pharmaceuticals Ltd, India. Sodium lauryl sulphate (SLS) was procured from Sigma Aldrich, India. All other chemicals were of analytical grade and used without any further purification.

METHODS

Preparation of the nanosuspension by using wet milling technique i.e. High pressure homoginization

A formulation screening was carried out to identify the most suitable formulation for further processing. Drugs were dispersed in water and stabilizers (SLS and HPMC E-5) were added. Then a pre-suspension was prepared by high speed homoginization using an D-15 (Miccra, Germany) at 11600 rpm for 10 minutes. A Panda 2000 (GEA Hiro Soavi, Germany) was used for the HPH. The suspension was processed using homogenization cycles of 1000 and 1500 bar. Dispersed drugs as microparticles were passed through a continuous Panda 2000 at room temperature applying the variable pressure profile described above. At certain cycles,samples were withdrawn for particle size analyses. Profiles of the particle size reduction as function of cycle number were plotted to illustrate the homogenization effectiveness.(4,6).Fig 1 shows high pressure homogenizer of Panda 2000 make (GEA Hiro Soavi, Germany).

Kinetic solubility measurement of LMG-Nano crystals

Solubility studies were performed with a shaker at 25ºC which allowed an accuracy in temperature of ±0.01ºC. Excess drug was added in 40 ml capped vials, then sonicated in a water bath for 2 min. Vials were sealed to avoid changes due to evaporation and shaken for 24 hrs. in a thermostated storage at 25 ± 0.01ºC. After the equilibrium was reached, suspensions were filtered through Sartorius® 0.1 µm filters. An aliquot from each vial was withdrawn by 1ml syringe and absorption was measured by UV spectrophotometer at 270 nm to evaluate the amount of drug dissolved. The sample volume taken was not replaced by new solvent. Dilution was intentionally avoided, to prevent any possible interference with the chemical equilibrium, particularly considering the presence of colloidal particles. Experiments were carried out in triplicate, solubility data were averaged[1].

Dissolution studies of LMG-Nanocrystals

The dissolution rate of Lamotrigine (Pure drug) and LMG-Nano crystals was measured by adding dose equivalent to twenty five mg in a size “1” capsule to 900 ml 0.1 N HCl. Five ml samples were withdrawn after 5, 10.15,20 min and replaced each time with 5 ml fresh 0.1 N HCl. Solutions were immediately filtered through 0.45 membrane filter, diluted and concentration of Lamotrigine was determined spectrophotometrically at 270 nm. The dissolution conditions are as follows

1. Appratus :- USP type I
2. RPM :- 100
3. Volume: - 900 ml.
4. Medium: - 0.1 N HCl.

Determination of drug content of LMG-Nano crystals by HPLC method

Drug concentrations were determined by high performance liquid chromatography (HPLC). A modified method has been developed for determination drug content in a LMG-Nanocrystals. The chromatographic system equipped with a pump and U.V. detector. The analytical columns (Intersil ODS 3V, 1.50 x 4.6) and specific mobile phases were used.[12]

Particle size determination

Particle size distributions of the nanosuspensions were examined by laser-diffractometry (Beckman Coulter-Delsa™nano, Germany)[14]. The LD data obtained were evaluated using the volume distribution diameters (d) 10%, 50%, 90%. The diameter values 10% to 99%indicate the percentage of particles possessing a diameter equal or lower than the given size value.

Preparation of dried LMG-Nanocrystal

Lyophilization is the process of freeze-drying a composition to remove excess water. The process involves sublimation of the frozen water, usually under reduced pressure conditions. Freeze-drying works by freezing the material and then reducing the surrounding pressure and adding enough heat to allow the frozen water in the material to sublimate directly from the solid phase to gas. Nanosuspension was frozen in rotary vacuum evaporator at -78.5˚C using dry ice,then this powder was added to lyophilizer (Virtis,model-2SEL).The temperature was maintained at -30˚C and the vacuum of 100 metric tons.Fig.2 shows LMG nanosuspension and LMG lyophillized nanocrystal powder.

Re-dispersability and Phase separation study of LMG Nanocrystal

The re-dispersability of LMG nanosuspensions stored in glass bottles was determined by tilting the bottle up and down by hand until the sediment was dispersed in the aqueous phase uniformly. The number of times tilted was noted and rated as fast, medium and low. Phase separation was determined visually in all formulations during long-term storage.

Powder x-ray diffraction (PXRD) of LMG-Nanocrystals

A powder X-ray diffractometer (wide angle scattering-WAXD, Philips, Amedo, the Netherlands) was used for diffraction studies. PXRD studies were performed on the samples by exposing them to CuKα radiation (40 kV, 25 mA) and scanned from 10^{o} to 30^{o}.[12]

RESULTS AND DISCUSSION

Preparation of Lamotrigine (LMG) nanosuspensions

Nanosuspension on a lab scale is typically produced by using a Panda 2000 in the discontinous version (GEA Hiro Soavi, Germany). The aim of the research is to obtain a product of good quality. This means homogeneity of the nanosuspensions with a very small amount of microparticles and to re-disperse easily into a medium after the drying process for oral administration. With high pressure homogenization it is possible to obtain a uniform product with a very low fraction of particles in the micrometer range, or even with all particles in the nanometer range. To avoid effects such as anaphylactic shock or other allergic reactions because of presence of surfactants, it was important to reduce the amount of stabilizer without losing a stable suspension. To achieve this screening of formulations design of experiment was performed by using factorial design with different concentration of surfactants, polymers commonly used as stabilizer and different bar pressure.2^{3} factorial design was applied. The factorial batches and results were tabulated in a below table.

Table 1: Control variables with their levels used in experimental design

Variables	Levels (-1)	Level (+1)
Stabilizer Concentration	0.3%	0.6%
Polymer Concentration	0.6%	1.0%
Pressure Bar in HPH	1000	1500

Table 2: Factorial batches for LMG-Nanosuspension by HPH

Batch No.	Pressure (bar)	Stabilizer Concentration (%)	Polymer Concentration (%)
LMG 04	1500	0.6	0.6
LMG 03	1000	0.6	0.6
LMG 01	1000	0.3	0.6
LMG 07	1000	0.6	1.0
LMG 02	1500	0.3	0.6
LMG 08	1500	0.6	1.0
LMG 05	1000	0.3	1.0
LMG 06	1500	0.3	1.0

Table 3: PSD and Polydispersability index data of different factorial batches for LMG-Nanosuspension by HPH

Batch No.	No. Of cycles in HPH	Particle size distribution (nm)			Polydispersability Index
LMG 01		D10	D50	D90	
	12	470.1	1548.2	5859.3	0.359
	24	392.1	1191.6	3838.1	0.330
	36	280.1	1004.5	4021.9	0.375
	48	279.1	858.2	2803	0.336
	60	268.2	730.9	2052.7	0.257
LMG 02	12	330.4	963.1	2980.2	0.315
	24	238.7	676.6	2030	0.316
	36	230.2	627.5	1788.2	0.281
	48	219.5	622.9	1838	0.300
	60	232.2	457.1	879.4	0.269
LMG 03	12	558.9	1966.9	7371.7	0.383
	24	313.3	1086.5	3961.8	0.372
	36	263.1	744.5	2255	0.294
	48	250.3	689.1	1989.6	0.304
	60	214.4	637.2	1955.2	0.318
LMG 04	12	268.6	815.7	2588.4	0.337
	24	223.6	626	1823.3	0.296
	36	229.4	615.7	1753.4	0.279
	48	215.8	555.6	1492.1	0.253
	60	193.3	554.8	1685.6	0.281
LMG 05	12	523.2	1588.9	5155.9	0.328
	24	436.5	1258.5	3822.4	0.288
	36	425	839.7	1869	0.328
	48	351	1057.6	3393.1	0.322
	60	331.5	960.9	2948.9	0.271
LMG 06	12	463.5	1512.5	5199.5	0.354
	24	375.6	1111	3475.7	0.299
	36	334.1	1140.2	2556.6	0.349
	48	318.3	855.3	2404.2	0.259
	60	309.5	842.7	2400.9	0.265
LMG 07	12	449	1645.2	5981.3	0.349
	24	399.9	1127.4	3356.5	0.261
	36	367.7	1095	3441.4	0.316
	48	436.5	900.7	1757.3	0.271
	60	335.9	990.5	3064	0.298
LMG 08	12	412.7	1287.3	4432	0.358
	24	360.8	1115	3535.4	0.341
	36	300.1	913.3	2948.4	0.316
	48	305.1	817.5	2291.9	0.254
	60	285.9	787.9	2283.7	0.298

Table 4: PSD and polydispersability index data of different factorial batches for LMG-Nanosuspension by HPH after 30 days

Batch No.	No. Of cycles in HPH	Particle size distribution (nm)			Polydispersability Index
LMG 01		D10	D50	D90	
	60	291.3	875.9	2794.7	0.326
LMG 02	60	209.8	637.5	1029	0.328
LMG 03	60	254.4	737.2	1985.2	0.323
LMG 04	60	192.7	592.8	1904.1	0.310
LMG 05	60	450.1	1476.8	5172.8	0.370
LMG 06	60	384	810.3	1966.5	0.354
LMG 07	60	436.1	825.1	1642.2	0.196
LMG 08	60	445.1	951.2	2100.1	0.203

From the above data it can be concluded that, the particle size decreases with an increasing number of cycles and increasing homogenization pressure. To obtain maximum physical stability (avoidance of Ostwald ripening) the nanosuspension should be as homogenous as possible, that means reduction of the number of micrometer particles .distribution of the raw material (microcrystals) before homogenization. The volume size distribution of the raw material revealed 10% (d10%) of the particles were below 600 nm, but 90% (d90%) of the particles were below 3000 nm. The PSD data of Batch

no LMG 02 is most promising as compared with other batches.Typically, after sixty homogenization cycles the d90% of the particles was below 3 µm. The volume size distribution d50% of the nanosuspensions by laser diffractometry (LD) were below 1 µm, It means all formulations have fulfilled requirements of a nanosuspension. Polydispersity index (PI) values for all nanosuspensions were below 0.5 which clearly indicate that all the formulation were homogeneous. All formulations after 60 cycles of the LMG nanosuspensions have shown sufficient physical stability for long-term storage at room temperature, increase in size were almost indetectable over a monitoring period of 30 days expect batch no. LMG 05 which may due high concentration of Hypromellose.

Lyophilization/ Freeze-drying

LMG nanosuspension should be converted into powder. Lyophilization was performed to dry LMG aqueous nanosuspension into powder (nanocrystals).As discussed before, batch no. 2 was chosen for further investigation because it showed highest physical stability. After 24 hours, lyophilized LMG nanocrystals were collected from the lyophillizer. Lyophilized LMG nanocrystals were further investigated with respect to redispersability, crystalline state, saturation solubility and dissolution velocity. In many processes of lyophilization, cryoprotectant is usually added to the nanosuspension to protect nanoparticles or nanosuspension from freeze damage (damage due to ice formation) and to reduce particle size growth during lyophilization. In this study mannitol was added as cryoprotectant for avoiding nanocrystals aggregation/agglomeration. Surprisingly, it was found that without cryoprotectant, lyophilized LMG nanocrystals can be re-dispersed properly in water.

Drug content of LMG Nanocrystals

It was observed that 132.43 mg blend of LMG nanocrystal contain 25 mg Lamotrigine.

Redispersability and phase separation

The aqueous nanosuspensions can be post processed for dispersability as dry powder for solid dosage forms such as tablets, capsules, pellets,effervescents or lyophilized material for injectable products. These dried powders from nanocrystals are designed to re-disperse into nanometer-sized particles when placed in water or an alternate water-based environment. Ideally the same size and size distribution should be obtained as before drying. The ability of the dried nanocrystals to re-disperse into non aggregated/non agglomerated nanoparticulate dispersion is critical to the development of a solid dosage form that maintains the benefits of this enabling drug delivery technology. The aim of the transformation of an aqueous nanosuspension to dried nanocrystals is to process them to solid dosage forms. The choice of drying technology is a critical in obtaining an optimal final dried nanocrystal product. Dried powders of nanocrystals are designed to re-disperse into nanometer-sized particles. Therefore, this study must focus on whether the dried powder is properly formulated and able to be re-dispersed completely prior to formulating it astablets, capsules, pellets and effervescent tablets (solid dosage forms). Lyophilized LMG nanocrystals stabilized with 0.3% of SLS and 0.6% HPMC E-5 could be redispersed completely into aqueous dispersion. Upon the addition of water the lyophilized LMG nanocrystals could be easily re-dispersed without aggregates or agglomerates. The particle size distribution of the re-dispersed LMG nanocrystals is not so much different to the original aqueous LMG nanosuspension. The particle size (D90) of the original LMG nanosuspension was 879 nm with a polydispersity index (PI) of 0.269 nm. The PCS size average and polydispersity index (PI) of re-dispersed LMG were of 906 nm and of 0.278 nm. The data are identical to the original LMG nanosuspension. These results confirm that the transform process of the LMG aqueous nanosuspensions to lyophilized LMG nanocrystals (dry powder) using a freeze dryer definitely did not or just little influenced the particle size (e.g. growth by agglomeration).

PXRD study

X-ray diffraction is used to study the atomic and molecular structure of crystalline substances such as drugs and excipients. The sample, a single crystal or powder, is exposed to x-rays at various angles; the diffraction patterns produced are then compared with reference standards for identification. X-rays diffraction patterns (diffractograms) can be used to confirm the crystalline nature of a sample. Therefore, this information is used to verify whether the substances are amorphous, partially amorphous crystalline or fully crystalline as well as the polymorphic form being present. To confirm the crystalline state of the dried LMG nanocrystals, x-ray diffraction was performed using lyophilized LMG nanocrystals (batch no. LMG 02) stabilized with the various stabilizers and physical

mixture of the same. X-ray diffraction patterns were visualized in diffractograms. All XRD patterns can be seen in **Fig. No 3 and Fig. No 4,** proving the crystallinity of lyophilized LMG nanocrystals with the different stabilizers. In addition, the diffractogram showed slight reduction in peak intensities for LMG nanocrystal formulations. According to these results, all nanocrystals were still in the same crystalline state as the raw material. Applying energy by being homogenized and subjecting them to the drying processes did not transform LMG nanocrystals to be fully or partially amorphous. Likewise the different stabilizers did not influence the crystallinity of the LMG nanocrystals. In general, more crystalline substances are physically more stable compared to amorphous forms. Therefore, lyophilized LMG nanocrystals will be physico-chemically stable during the storage time. In addition, better physicochemical properties such as the observed enhanced solubility and dissolution velocity can be attributed to the particle size reduction and not to alterations in crystalline state.

Kinetic solubility measurement of LMG-Nano crystals

It has to be differentiated between kinetic and thermodynamic solubility. The thermodynamic solubility is the concentration in the solute in equilibrium with a normally sized powder, this condition being physically stable. The kinetic solubility, e.g. achieved by size reduction or from amorphous powders, is higher than the thermodynamic solubility but physically not long–term stable. With regard to the thermodynamic solubility, it is a supersaturated solution with higher energy, transforming to a lower energy level and lower concentration by precipitation of crystals, finally reducing the concentration of the kinetic solubility. The kinetic saturation solubility of LMG nanocrystals was investigated over 24 hrs. The results of this testing showed that the solubility of the LMG nanocrystals in water was increased at 25ºC. The saturation solubility was with 3.2 ± 0.2 mg/ml higher than the one of the raw material which has 0.17 mg/ml.

Dissolution Study

Dissolution studies of the lyophilized LMG nanocrystals clearly showed this advantageous phenomenon. Dissolution velocities of the dried LMG nanocrystals were distinctly superior compared to the raw material. Lyophilized LMG nanocrystals dissolved 41% within 5 minutes where as pure API dissolved 13% in buffer at pH 1.2. In case of the class II drugs of the biopharmaceutics classification system (BCS), the increase in dissolution velocity is most important because it is the rate limiting step for oral absorption.

CONCLUSION

High pressure homogenization (HPH) can be employed to produce aqueous drug nanosuspensions that are stable up to 30 days. Aqueous nanosuspension can be converted to dry drug nanocrystals by a lyophilisation process with addition of cryoprotectant. Dried drug nanocrystals offer superior physicochemical properties. The very fine particles of the dried nanocrystals re-disperse completely in water. This characteristic is critical in improving the kinetic solubility and the dissolution behavior of drugs, especially in tablet dosage forms.

Fig. 1: High pressure homogenizer of Panda 2000 make (GEA Hiro Soavi, Germany).

Fig. 2: LMG nanosuspension and LMG lyophillized nanocrystal powder

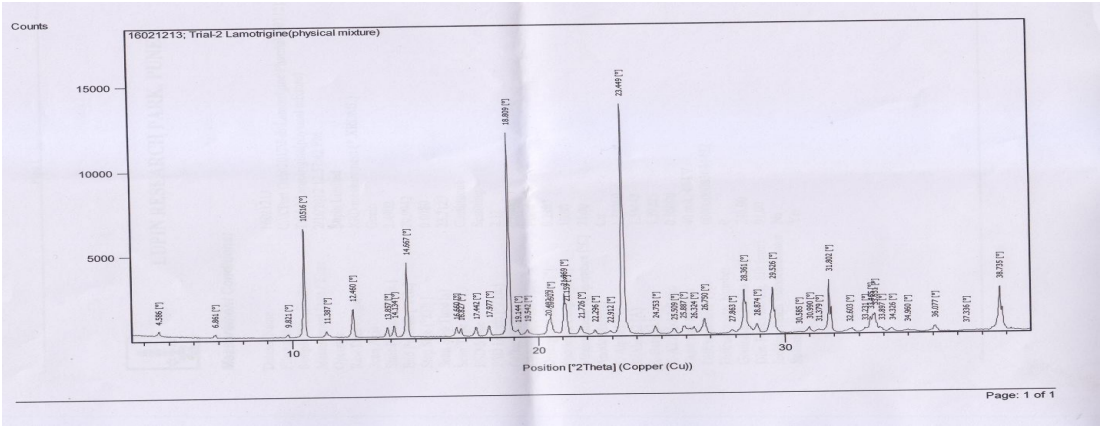

Fig. 3: PXRD of Lamotrigine (Trial 2 physical mixture)

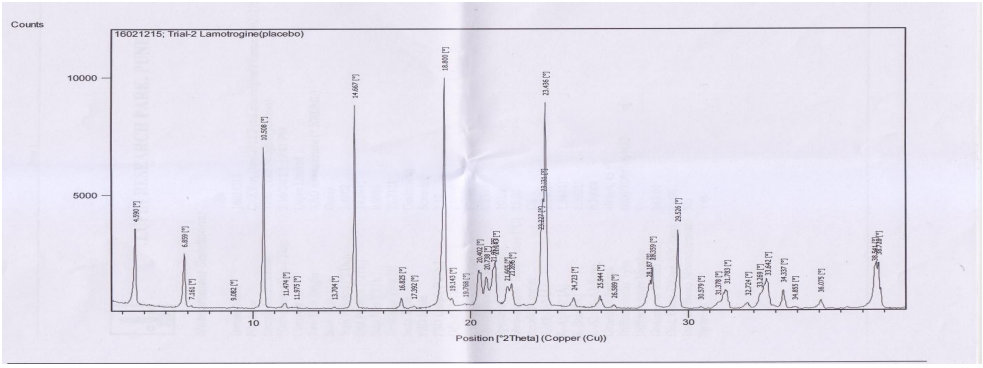

Fig. 4: PXRD of Lamotrigine (placebo)

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