J Incl Phenom Macrocycl Chem (2009) 65:263–268
DOI 10.1007/s10847-009-9577-y

ORIGINAL ARTICLE

Inclusion complexes of lamotrigine and hydroxy propyl β-cyclodextrin: solid state characterization and dissolution studies

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Received: 23 January 2009 / Accepted: 20 March 2009 / Published online: 2 April 2009

Abstract Lamotrigine (LMN) is an antiepileptic drug, with poor aqueous solubility, which might lead to erratic bioavailability. The objective of the present work was to improve the dissolution characteristics of the LMN using Hydroxy propyl β-cyclodextrin (HP β-CD), which might offer reliable bioavailability. The phase solubility profile was classified as A_L-type, revealing 1:1 stoichiometric complexation, with a stability constant (*Ks*) of 573 M^{-1}. Binary systems of LMN and HP β-CD were prepared in different molar ratios (1:1, 1:2, 1:3 and 1:4) by kneading method. The binary systems were characterized by Fourier Transform Infrared (FT-IR) Spectroscopy, Differential Scanning Calorimetry (DSC) and Powder X-ray Diffraction Analysis (PXRD). Results revealed that in the kneaded products the entire drug was entrapped inside the HP β-CD cavity and reduction in drug crystallinity also took place, which may be responsible for improved dissolution characteristics as compared to that of the pure drug as depicted from the dissolution studies.

Keywords Lamotrigine · Hydroxy propyl β-cyclodextrin · Inclusion complexes · Solid state characterization · Dissolution enhancement

Introduction

The formulation of poorly water soluble drugs for oral delivery is a challenging task for the formulation scientists [1]. According to Biopharmaceutical Classification System, for drugs having poor solubility and highly permeability (Class II), the rate of oral absorption is often limited by the dissolution of the drug in the gastrointestinal tract [2] and improving the dissolution characteristics for such drugs might offer improved bioavailability. There are numerous techniques for improving the dissolution characteristics of the drugs namely preparation of solid dispersion [3, 4], use of surfactants [5], micronization [6], use of polymorphs [7], self emulsifying formulations [8], inclusion complexation with cyclodextrins [9] etc.

Cyclodextrins form an important group of pharmaceutical excipients. They are cyclic oligosaccharides composed of a-1,4-linked D-glucopyranose units. The most common of these ring-shaped molecules are α, β and γ-CDs formed by six, seven, and eight glucose units, respectively. Cyclodextrins have lipophillic inner cavity and hydrophilic outer region which enables the lipophillic drug to form noncovalent inclusion complex [10]. Cyclodextrins (CDs), especially HP β-CD, is widely used in the pharmaceutical field owing to their high aqueous solubility and ability to stabilize drug molecules [11, 12].

LMN [6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine] is an antiepileptic agent shown to be effective in adjunctive treatment for refractory partial seizures and generalized seizures [13]. It works by inhibiting voltage dependent sodium channels, resulting in decreased release of the excitatory neurotransmitters glutamate and aspartate [14]. It has poor aqueous solubility (0.17 mg mL^{-1} at 25 °C) [15] which could be the rate limiting step for its efficient absorption and thus various researchers have tried to improve the solubility and dissolution rate of LMN to improve its therapeutic efficacy [16].

Thus, in light of this the present investigation was carried out to improve dissolution characteristics of LMN by inclusion complexation with HP β-CD. The inclusion

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complex of LMN with HP β-CD was prepared by kneading technique. Solid state characterization was done by Fourier Transform Spectroscopy (FT-IR), Differential Scanning Calorimetry (DSC) and Powder X-ray Diffraction Analysis (PXRD). The dissolution properties of the binary systems were studied and compared with pure LMN.

Materials and methods

Materials

LMN was kindly gifted by Rantus Pharma Pvt. Ltd. (Hyderabad, India). HP β-CD was a generous gift sample from Gangwal Chemicals Pvt. Ltd. (Mumbai, India). All the reagents used were of analytical grade. Double distilled water was used throughout the work.

Methods

Phase solubility studies of LMN with HP β-CD

Phase solubility studies were carried out in distilled water according to method described by Higuchi and Connors [17]. Constant amount of LMN (50 mg) that exceeded its solubility was added to 25 mL of aqueous solutions of HP β-CD in various molar concentrations (2–20 mM). Then the suspensions were shaken on the rotary shaker at 25 °C for 3 days. The samples were filtered, diluted and the concentration of LMN was determined spectrophotometrically at 306 nm. The apparent 1:1 stability constant was calculated from the phase solubility graph using the following equation,

$$Ks = \frac{\text{Slope}}{So(1 - \text{slope})} \quad (1)$$

Where, So is the solubility of LMN in absence of HP β-CD.Gibbs free energy of transfer (ΔG_{tr}°) of LMN from pure water to aqueous solution of HP β-CD was calculated using the equation,

$$\triangle G_{tr}^{0} = -2.303RT \log\left(\frac{So}{Ss}\right) \quad (2)$$

Where, So/Ss is the ratio of the molar solubility of LMN in aqueous solution of HP β-CD to that of the pure water.

Preparation of solid binary systems

Preparation of physical mixtures of LMN and HP β-CD The physical mixtures of LMN and HP β-CD were prepared by mixing of individual components in the mortar in different molar ratios of 1:1, 1:2, 1:3 and 1:4 of LMN:HP β-CD.

Preparation of kneaded products of LMN and HP β-CD LMN and HP β-CD were accurately weighed and placed in the mortar. Homogeneous paste was prepared with the aid of 1:1 v/v ethanol/water mixture. The paste was kneaded for 15 min and then dried in the oven for 24 h at 40 °C. Then the kneaded binary systems were pulverized and stored in desiccators until further use.

Determination of drug content

Drug content was determined by dissolving accurately weighed quantity of binary system of LMN and HP β-CD in 1:9 ethanol:water system. Then the solution was appropriately diluted and concentration was measured spectrophotometrically at 306 nm.

Characterization studies

Fourier transform infrared (FT-IR) spectroscopic studies Moisture free powdered samples of LMN, HP β-CD, their physical mixture, and inclusion complex with HP β-CD were characterized using a FT-IR spectrometer (Thermoscientific, U.S.A) by potassium bromide (KBr) pellet method. The scanning range was between 4,000 and 450 cm^{-1}. An average of twenty scans is reported.

Differential scanning calorimetry (DSC) analysis DSC spectra of samples were recorded using DSC (Shimadzu 60, Japan). The samples (6–7 mg) were accurately weighed in crimped aluminum pans and heated from 50 to 300 °C, at a scanning rate of 10 °C min^{-1} under air flow (100 mL min^{-1}).

Powder X-ray diffraction (PXRD) analysis The PXRD patterns of pure LMN and all binary systems of LMN with HP β-CD were recorded using X-ray diffractometer (X-pro Pan analytical, Phillips, Mumbai, India) with a copper tube anode over the interval 5–70° $2\theta^{-1}$. The operation data were as follows: generator tension (voltage) 40 kV; generator current 30 mA; scanning speed 2° min^{-1}.

Dissolution studies Dissolution studies of LMN, physical mixture and kneaded binary systems were carried out in the dissolution rate test apparatus USP Type I (Electrolab dissolution tester USP, TDT 06P, Mumbai, India) using 900 mL 0.1 N HCl at 37 ± 0.5 °C at 50 RPM [18]. Twenty-five milligram of drug or formulations containing equivalent amount of drug were placed in the basket. Five ml samples were taken at different time intervals: 5, 10, 15, 20, 25, 30, 45, 60 and 90 min and were replaced by the fresh media. The samples were then filtered and concentration was determined spectrophotometrically at 266 nm.

Results and discussion

Phase solubility studies of LMN with HP β-CD

The phase solubility graph for the complex formation between LMN and HP β-CD is shown in Fig. 1. The plot showed that the drug solubility increased with increase in the concentration of HP β-CD. According to Higuchi and Connors, the phase solubility profile can be considered as A_L (linear) type. The slope calculated was 0.254 which is less than 1, thus 1:1 stoichiometry was suggested [17]. The value of the stability constant was found to be 573 M^{-1}. The stability constant between the range of 100 and 1,000 M^{-1} is considered as an ideal value, smaller value indicate weak interaction between drug and cyclodextrin, while larger value indicate incomplete drug release from the inclusion complex [19]. The line equation from the linear regression analysis for the system was $y = 0.254x + 0.593$. The obtained values of $\Delta G_{tr}°$ are shown in Table 1. The negative values of calculated Gibbs free energy transfer indicated the spontaneous solubilization of LMN in aqueous solution of HP β-CD.

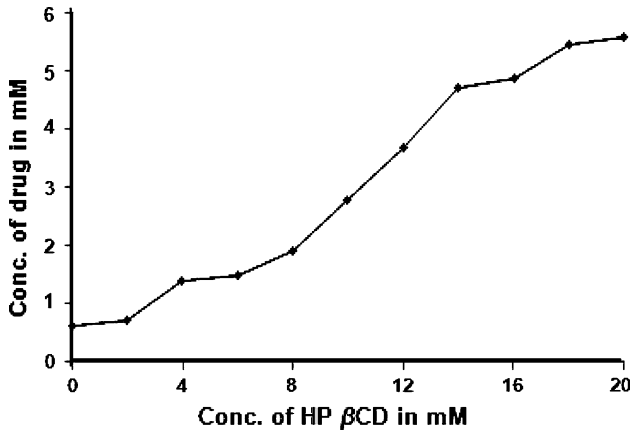

Fig. 1 Phase solubility diagram of LMN with HP β-CD

Table 1 Gibbs free energy of transfer (ΔGtr°) for solubilization process of LMN in aqueous solutions of HP β-CD at 25 °C

Concentration of HP β-CD (mM)	$\Delta G_{tr}°$ (KJ/mol) at 25 °C
2	−0.44
4	−2.09
6	−2.28
8	−2.88
10	−3.81
12	−4.53
14	−5.13
16	−5.21
18	−5.49
20	−5.56

Percentage drug content

Drug content of inclusion complexes was between 94.82 and 97.58%, which is in good agreement to theoretical drug content.

Characterization studies

Fourier transform infrared (FT-IR) spectroscopic Studies

FT-IR spectra of LMN, HP β-CD, physical mixture (1:1) and inclusion complexes are shown in the Fig. 2. The IR spectrum of LMN (Fig. 2a) is characterized by principal absorption peaks at 3,447 cm^{-1} (N–H aromatic); 3,208 cm^{-1} (C–H aromatic); 1,619 cm^{-1} (C = N); 1,319 cm^{-1} (C–N); 1,556 cm^{-1} (C = C aromatic); 1,053 cm^{-1} (C–Cl); 738 cm^{-1} (o substituted benzene); 747, 795 and 959 cm^{-1} (m substituted benzene). The IR spectrum of HP β-CD (Fig. 2b) shows prominent peaks at 3,414 cm^{-1} (O–H stretching vibrations); 2,928 cm^{-1} (C–H stretching vibrations); 1,647 cm^{-1} (H–O–H bending); 1,034 and 1,083 cm^{-1} (C–H, C–O stretching vibrations). The IR

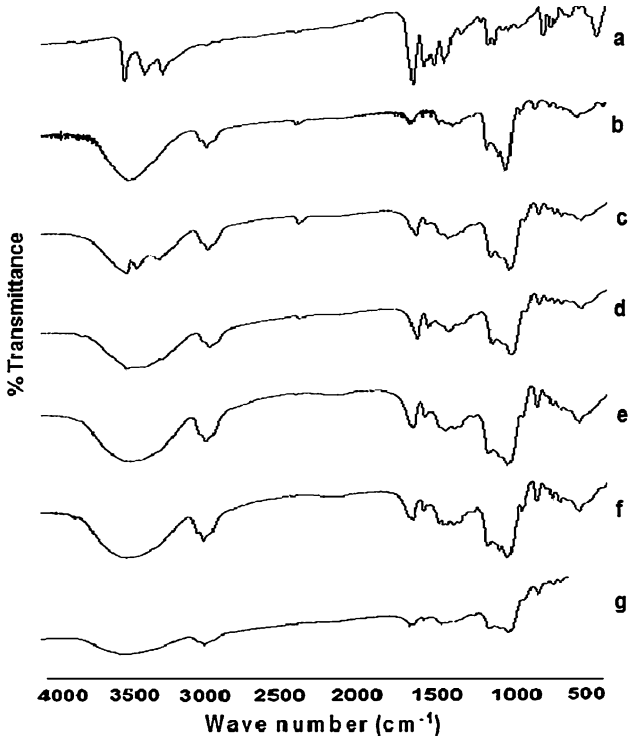

Fig. 2 FTIR spectra of single components and binary systems of LMN and HP β-CD. (a) LMN, (b) HP β-CD, (c) PM 1:1, (d) KN 1:1, (e) KN 1:2, (f) KN 1:3, (g) KN 1:4, *PM* physical mixture, *KN* kneaded binary system

spectra of physical mixture (1:1) (Fig. 2c) shows a shift of 3,208 to 2,927 cm^{-1} (C–H aromatic) and 1,619 to 1,623 cm^{-1} (C = N), with decrease in peak intensities. While in inclusion complexes (Fig. 2d–g) the peak intensities were smoothened and disappeared as the ratio of the drug to polymer increased, indicating strong physical interaction between LMN and HP β-CD [16].

Differential scanning calorimetry (DSC) analysis

Thermal Analysis has been reported as a method for characterization of cyclodextrin complexes [20]. DSC study provides the information regarding the inclusion complexation of LMN and HP β-CD. Figure 3 illustrates the DSC spectra of LMN, HP β-CD, physical mixture (1:1) and kneaded system (1:1). DSC thermogram of pure LMN (Fig. 3a) showed an endothermic peak at 219.6 °C, representing the melting point of crystalline LMN. The DSC spectra of HP β-CD (Fig. 3b), showed an endothermic peak at 80.59 °C, indicating loss of water content [21]. In DSC spectra of physical mixture (Fig. 3c), the melting peak of drug was decreased and also slightly shifted to 201.44 °C. For the kneaded binary system (1:1) (Fig. 3d), the characteristic melting peak of crystalline LMN was lost, indicating that the crystalline drug was converted to amorphous state and entrapped inside the cyclodextrin cavity. For the physical mixture and inclusion complex the peak of HP β-CD was shifted from 80.59 to 77.0 °C indicating physical interaction. For inclusion complexes the peak was shifted from 80.59 to 77.9 °C showing the same thermal behavior.

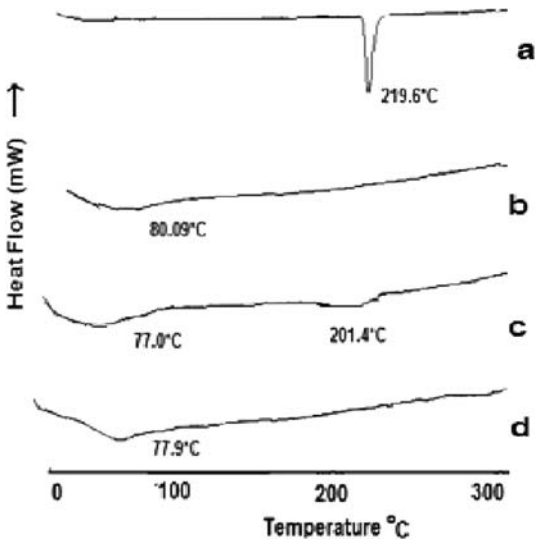

Fig. 3 DSC spectra of single components and binary systems. (a) LMN, (b) HP β-CD, (c) PM 1:1, (d) KN 1:1, *PM* physical mixture, *KN* kneaded system

Powder X-ray diffraction (PXRD) analysis

Figure 4 illustrates the PXRD spectra of LMN, HP β-CD, physical mixture (1:1) and inclusion complexes. The PXRD pattern of LMN (Fig. 4a) showed sharp and intense peaks indicating its crystalline nature. LMN showed major peak at 2θ values of 5.05, 5.59, 12.45, 26.75 and 29.41. Due to amorphousness of HP β-CD (Fig. 4b), no sharp peaks were observed in the spectra of HP β-CD. In physical mixture (Fig. 4c) the crystalline peaks are somewhat decreased. While in inclusion complexes (Fig. 4d–g) the peaks are almost smoothened and are of decreased intensity, indicating the decreased crystallinity of the drug.

Dissolution studies

The dissolution profile for LMN and kneaded binary systems are shown in Fig. 5. The release profiles were expressed as percentage of drug release (vs.) time. There was not much difference among the dissolution profile of all the four kneaded binary systems, similar results were reported by earlier researchers [19]. Table 2 shows the percentage drug dissolved in 15 min (DP_{15}) for LMN and binary systems. For all the kneaded systems more than 90% drug was released within 15 min, where percentage drug

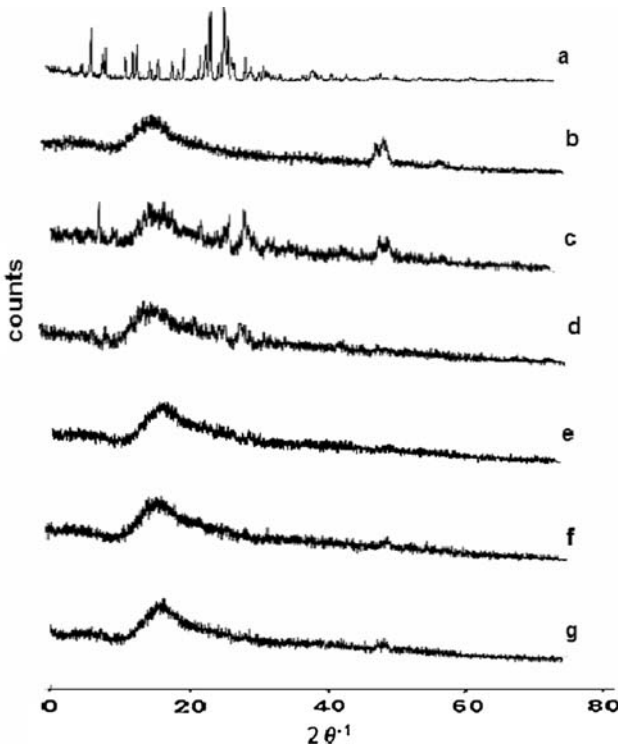

Fig. 4 PXRD spectra of single components and binary systems of LMN and HP β-CD. (a) LMN, (b) HP β-CD, (c) PM 1:1, (d) KN 1:1, (e) KN 1:2, (f) KN 1:3, (g) KN 1:4, *PM* physical mixture, *KN* kneaded binary system

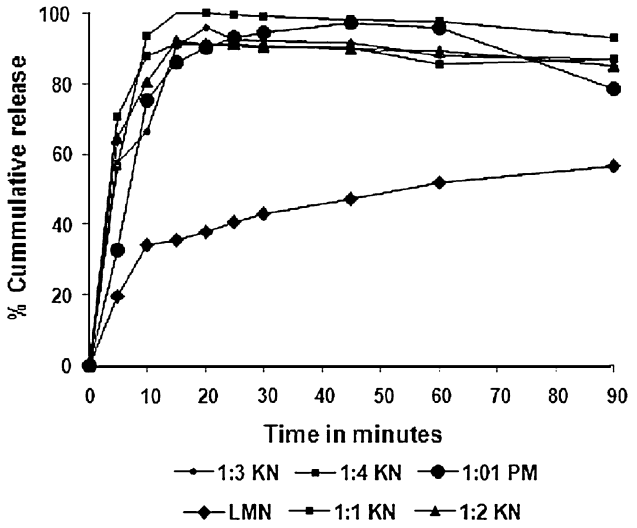

Fig. 5 Dissolution curves of LMN, kneaded binary systems (KN) and physical mixture (PM)

Table 2 Percentage drug dissolved in 15 min for LMN from different samples in 0.1 N HCl

Sample	DP_{15} (percentage of drug dissolved in 15 min)
LMN	35.90
1:1 PM	86.09
1:1 KN	100.02
1:2 PM	84.02
1:2 KN	92.17
1:3 PM	73.53
1:3 KN	90.88
1:4 PM	70.54
1:4 KN	91.20

release of pure drug in 15 min is 35.90. From the graph it could be concluded that the dissolution rate of all binary systems was ameliorated as compared to that of pure drug. This behavior may be due to inclusion complexation and/or decrease in the drug crystallinity [22].

Conclusion

The inclusion complex of LMN and HP β-CD was prepared successfully by kneading technique. Dissolution rate of the drug was improved as compared to that of pure drug. FTIR study revealed that there was a strong physical interaction between LMN and HP β-CD, which is further supported by the results of DSC and PXRD studies indicating the entrapment of LMN in the HP β-CD cavity and decrease in the drug crystallinity, which might be the basis for the marked improvement in the dissolution characteristics of LMN. Thus, dissolution characteristics of LMN could be improved by complexation with HP β-CD.

Acknowledgment Authors would like to thank Rantus Pharma Pvt. Ltd., Gangwal Chemicals Pvt. Ltd. for providing LMN and HP β-CD respectively and Department of Physics, Saurashtra University, Rajkot for conducting FTIR and PXRD study of the samples complimentarily.

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