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Research Article

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Hydroxypropyl-β-cyclodextrin/levodopa complex: An experimental study

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ABSTRACT

The possible formation of a host-guest complex between levodopa and HP-β-CD was studied accordingly. A simple phase-solubility study was conducted based on the method proposed by Higuchi and Connors. The complexation constant (K_c) calculated was 30.53 M^{-1}. An improved solubilty of levodopa was achieved as a result of complex formation with HP-β-CD. From the ^{1}H-NMR data, upfield shifts in all HP-β-CD protons were observed. In particular, we found that Δδ H3 > Δδ H5 and this finding suggests that partial inclusion has been formed between levodopa and HP-β-CD. SEM images revealed distinct morphological images between the starting materials and the complex. Our data were all in great agreement which evidenced the formation of HP-β-CD/levodopa.

Keywords: Levodopa, cyclodextrin, complex

INTRODUCTION

Cyclodextrins (CDs) have been extensively studied in many research areas due their unique capability to form complexes with a wide range of molecules [1,3]. Naturally, CDs are crsytalline, homogenous and non hygroscopic substances where the outer layer is relatively hydrophilic and the inner cavity was found to be hydrophobic [1,7]. Through host-guest reaction, complexations with CDs can be achieved. These stable non-covalent complexes offer an improved physicochemical characteristics of the unmanipulated drugs including possibility for increased solubility for poorly soluble drugs, solution stability as well as controlled-release of the drugs [1,8].

Levodopa (LD) is an effective treatment for symptomatic relief of Parkison's disease (PD). LD or 3,4-dihydroxy-L-phenylalanine, the naturally-occuring amino acid serves best as dopamine precursor helps in managing (or even preventing) motor complications in PD patient. LD is taken up by facilitated, saturable uptake process localized in the proximal upper gastrointestinal tract. Its extensive presystemic metabolism in the proximal small intestine, a very short half life due to rapid metabolism as well as poor solubility enable only a small fraction of LD to finally reach the brain since most of it is taken up by skeletal muscle, liver and kidney [2,6]. Reformulation of LD into the controlled-release form may therefore provide longer half-life and more stable plasma levels while an enhanced solubility form of LD may offer faster and more reliable absorption which would be beneficial for patients with "no-on" or "delayed-on" phenomenon. The aim of this study is to investigate the possible formation of complex between levodopa and HP-β-CD.

EXPERIMENTAL SECTION

Materials

Levodopa was purchased from the United States Pharmacopoeia (Rockville, MD, USA). HP-β-CD, pottasium bromide (KBR) were obtained from Sigma-Aldrich (USA). Water used throughout the study was double-deionized.

Complex preparation

HP-β-CD and levodopa were dissolved in a 50 mL volumetric flask filled with deionized water. The mixture was stirred for at least 48 hours at room temperature. The resulting solution was filtered using 0.45 μm Nylon membrane filter to remove insoluble particles (if any). The solution was then frozen at 20 ^{0}C for another 24 hours and further lyophilozed over a period of 36 hours using a freeze-dryer (Thermo Scientific, USA).

Phase solubility study

Excess amounts of levodopa were added to 25 mL volumetric flasks containing an increasing amounts of HP-β-CD (1, 2, 4, 6 and 8 mM). The mixtures were shaken for 48 hours at room temperature until homogeneity and equilibrium were reached. Then, the mixture were filtered through a 0.45 μm Nylon membrane filter and the dissolved concentration of levodopa were measured by using a Lambda 35 UV/Vis systems, Perkin-Elmer. Experiments were done in triplicate.

Nuclear Magnetic Resonance (NMR)

NMR spectra were each recorded at 27 ^{0}C on a Bruker DRX 400-AVANCE spectrometer operating at 400 MHz equipped with a 5 mm inverse probe with z-gradient coil. ^{1}H-NMR experiment was performed to confirm the protons assignments of free cyclodextrin and levodopa and its inclusion compound.

Scanning electron microscopy (SEM)

The morphology images of free HPβCD, levodopa as well as their complex were captured using an electron micropscope (LEO Supro 50 VP FESEM, Carl Ziess, Germany). Prior to data collection, samples were fixed on a brass stub using double-sided tape and were then gold sputtercoated to render them electrically conductive.

RESULTS AND DISCUSSION

Figure 1 shows the phase solubility study of levodopa in HP-β-CD. An enhanced solubility of levodopa was observed upon addition of increasing concentrations of HP-β-CD. Based on the Higuchi and Connors concept, this system can be defined as an A_L complex type as the relationship between substrate (levodopa) solubility and ligand (HP-β-CD) concentration was found to be linear. The complexation constant (K_c) was 30.53 M^{-1}. The calculation was performed according to the Higuchi and Connors equation [4].

K_c = slope/[S_o](1-slope)

where, S_o represents the concentration of levodopa with no addition of cyclodextrin. The realtively low K_c value obtained may be due to two factors: (a) HP-β-CD is highly soluble in water and thus lowering the driving force to form a more stable complex with levodopa and (b) the size of levodopa may not fit well within the HP-β-CD cavity. The size of the guest molecules must be compatible enough to fit into the CD cavity in which the geometrical factors are decisive in determining the kind of guest molecules that can penetrate into the CD cavity [5]. Table 1 reveals the ^{1}H-NMR assignments of free HP-β-CD and the complex. NMR spectroscopy can be considered as the most complete and useful analytical technique that provides a wealth information on the interaction between CDs and guest compounds. It allows the elucidation of the structure of the complex (i.e., the orientation of the guest molecule inside the host cavity) in solutions. From the table, upfield shifts of the proton signals were observed. This phenomenon should be expected as a consequence of penetration of guest molecule into the CD cavity. From our data, Δδ H3 > Δδ H5, this finding suggests that partial inclusion complex has been formed [3]. These observations were also reported in the literature for different systems. De Sousa et al. (2008) [5] used NMR experiments to prove the interaction between fluoxetine (FLU) with ß-CD in solution where they observed strong modifications in the proton chemical shifts between both free FLU and ß-CD and ßCD/FLU as a result of changes in the electronic density upon complexation which evidence the formation of interactions between FLU and ß-CD in solution . SEM images (fig. 2) taken at the magnification of 500X also provide distinct morphological images of the free starting materials and the complex which may also suggest the formation of HP-β-CD/levodopa in the solid state.

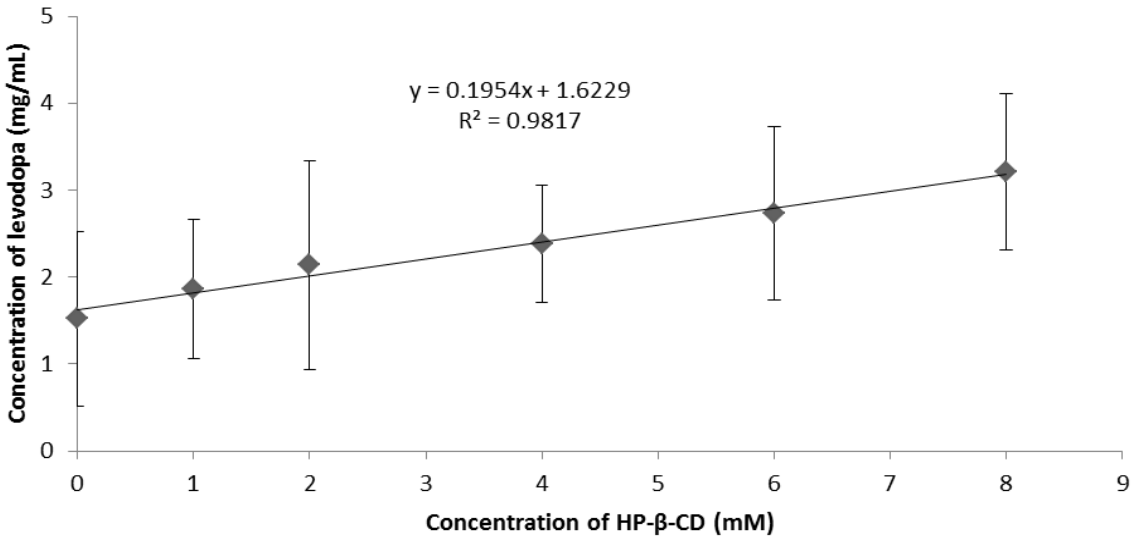

Fig. 1 Phase solubility study of levodopa in HP-β-CD. Values are mean ± SEM from triplicate measurements
Values represent mean ± SD from triplicate measurements.

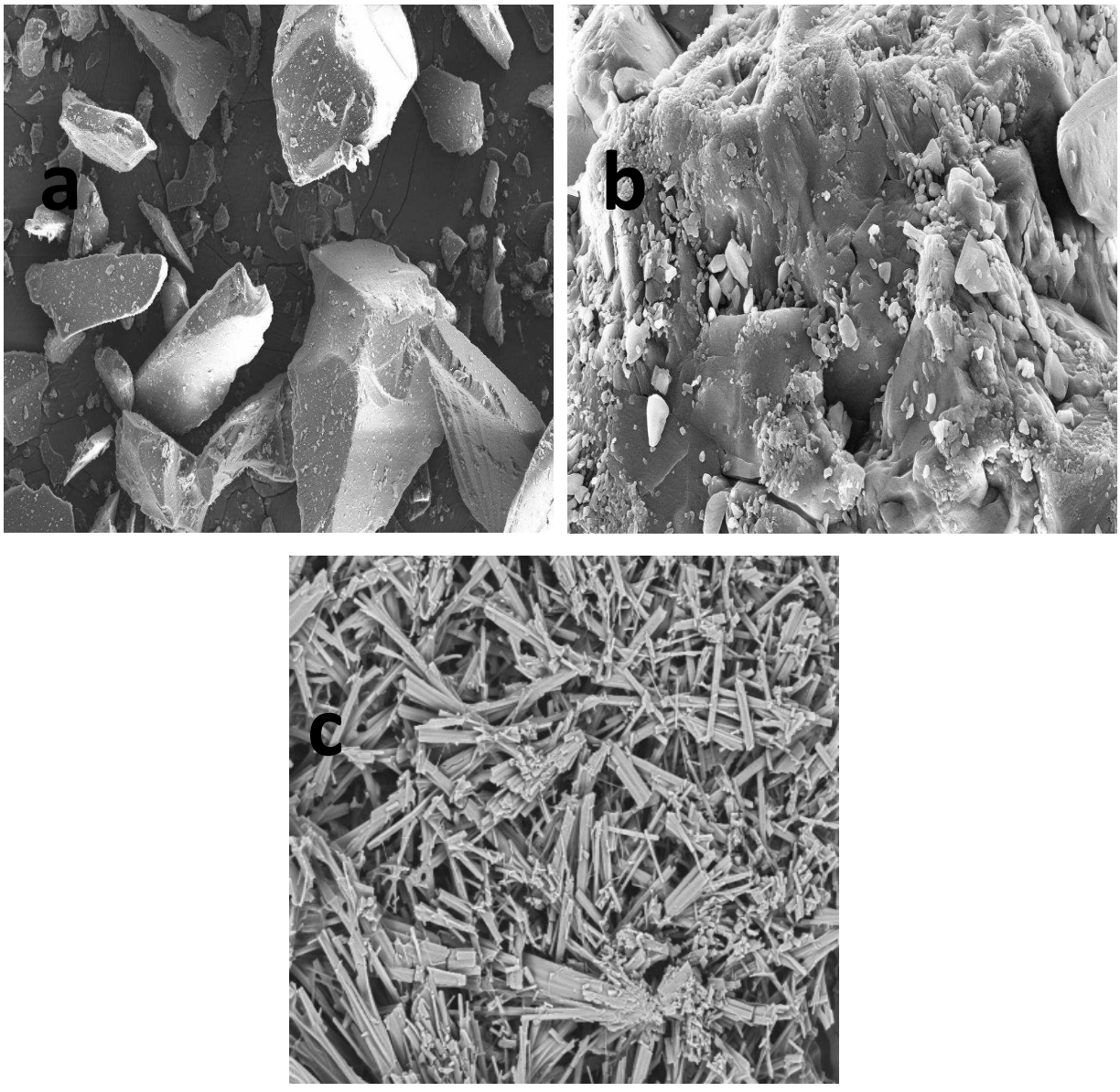

Fig. 2 SEM images captured the magnification of 500X of a) free HP-βCD, b) free levodopa and complex

Table 1 ^{1}H-NMR assignments of free HP-β-CD and the complex

1H	Chemical shifts		
	δ (HP-βCD) / ppm	δ complex / ppm	Δδ
	5.2546	5.2622	-0.0076
	3.5997	3.6023	-0.0026
	4.0244	4.1475	-0.1231
	3.5210	3.5236	-0.0026
	3.7230	3.8370	-0.1140
	3.8701	3.8712	-0.0011

Negative values indicates upfield shift

CONCLUSION

The results obtained from different techniques used throughtout this study were in the same line suggesting the formation of HP-βCD and its complex with levodopa. The capability of HP-β-CD to improve the solubility of levodopa may alter its bioavailability properties which may eventually improve its pharmaceutical potentials.

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