Synthesis and Characterization studies of Cisplatin/Hydroxypropyl-β-Cyclodextrin Complex

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Summary

Solubility and the dissolution rates of poorly water soluble drugs is an important aspect of formulation and development. Cisplatin is a drug with small molecular weight (Mol. Wt. 300.05) and is inherently associated with lack of tumor selectivity and short blood circulation time, which cause various toxic side effects. The purpose of the present study was to prepare a physically and chemically stable Cisplatin conjugate to increase the drug solubility, to improve its dissolution rate and to examine the possibility for reduced toxicity. Therefore, we prepared Cisplatin/HP-β-CD complexes and investigated the stabilizing effect of HP-β-CD on Cisplatin. Solid inclusion complexes of Cisplatin/HP-β-CD were prepared in 1:1 and 1:2 molar ratios by freeze-drying method. Complex formation was evaluated by comparing the infrared (FT-IR) spectra of the solid complexes with a simple physical mixture containing the same amount of Cisplatin. FT-IR experiments provided data indicating that the amino groups of Cisplatin was involved in the inclusion process. Differential scanning calorimetry (DSC) indicated stronger drug amorphization and entrapment in HP-β-CD. Phase solubility study was used to evaluate the complexation in solution at 25 °C. The phase solubility studies indicated the formation of Cisplatin/HP-β-CD inclusion complexes at a 1:1M ratio in solution. The solubility and dissolution rate of Cisplatin were significantly improved by complexation with HP-β-CD. The complexes formed were quite stable.

Key Words: Cisplatin, Hydroxypropyl-β-cyclodextrin, Cyclodextrin inclusion complex, Phase solubility, Stability constant, Freeze-drying.

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Introduction

Cis-Diaminedichloroplatinum (II) (Cisplatin) is a major antineoplastic drug widely used in the treatment of several malignancies[1]. It has been widely used because of its potent cytotoxic effects upon a variety of tumor types including testicular, ovarian, and cervical carcinoma[2]. We chose to study Cisplatin for several reasons. The toxicity[3], antitumor activity[4] and pharmacokinetics have been studied in detail[5]. Solubility of poorly water soluble drugs is an important aspect of formulation and development. The purpose of the present study was to increase the drug solubility and to prepare a physically and chemically stable Cisplatin complex and to examine the possibility for reduced toxicity. Therefore, we prepared a Cisplatin/HP-β-CD complex and investigated the stabilizing effect of HP-β-CD on the physicochemical properties of Cisplatin.

Cyclodextrins (CDs) are groups of cyclic oligosaccharides which have been shown to improve physicochemical properties of many drugs through formation of inclusion complexes. CDs are cyclic oligosaccharides composed of several D-glucose units linked by a-(1, 4) bonds. This cyclic configuration provides a hydrophobic internal cavity and gives the CDs a truncated cone shape. Many hydroxyl groups are situated on the edges of the ring which make the CDs both lipophilic and soluble in water. As a result, CDs are able to form inclusion complexes with a wide variety of hydrophobic compounds, and thus change the physical–chemical properties of the guest molecules. Cyclodextrin complexation has been thoroughly investigated for bettering the unfavorable biopharmaceutical properties of drugs, such as poor solubility and/or stability[6]. In particular, HP- β -CD is most commonly employed because of lower toxicity compared to parent CDs[7] and also due to their higher water – solubility and a better biocompatibility. As the first approved CD derivatives by FDA, HP-β-CDs have widely applications in food, agriculture and the pharmaceutical field[8]. The large and very hydrophilic HP-β-CD molecules do not penetrate the biological membranes and thus act as true carriers and penetration enhancers by assuring constant high concentration of dissolved drug at the membrane surface[9].

Materials and Methods

Materials

Cisplatin was obtained as gift sample from Cipla, India. HP-β-CD was purchased from HiMedia, India, HPLC grade water from Qualigens Fine chemicals, India. All solvents and chemicals were used of analytical grade. All products and chemicals were used as received from the manufacturers.

Preparation of Cisplatin - Hydroxypropyl-β-Cyclodextrin complexes

An inclusion complex was prepared by freeze drying a solution of Cisplatin and Hydroxypropyl-β-Cyclodextrin in different molar ratios (molar ratio of 1:1and 1:2). Cisplatin and HP-β-CD were dissolved in HPLC grade water and was sonicated in an ultrasonic bath for 1 h at 25.0 ± 0.1 °C and filtered through a 0.22 μM filter. The filtrate was frozen at –40 °C and then freeze-dried using MoDULYOD-230 freeze drier (ThermoElectron Corporation, Milford, MA, USA) at −60 °C for 24 h to obtain a white amorphous powder[10]. A physical mixture was prepared by mixing freeze-dried Cisplatin and HP-β-CD together using a glass mortar. The Cisplatin and HP-β-CD complex was characterized by DSC and FT-IR methods.

Phase solubility studies

Solubility diagrams were obtained according to Higuchi and Connors[11]. Excess amounts of Cisplatin (25 mg) were added to 5 ml of Isotonic NaCl solution pH 7.2, Phosphate buffer pH 2.5 and water containing HP-β-CD (0 - 17 mM) in a series of 50 ml stoppered conical flasks. The suspensions formed were sonicated in an ultrasonic bath for 1 h and were shaken for 72 h at room temperature (28 ± 0.5 °C) on a rotary flask shaker (RS-24 BL, Remi, India). After equilibration, an aliquot was filtered through 0.45 mm polyvinylidene difluoride membranes (Millipore), the equilibrium pH of each solution was measured (pH Analyzer LI 614, Elico, India), suitably diluted and analyzed by UV spectrophotometry (PharmaSpec UV – 1700, Shimadzu Corporation, Japan). The apparent stability constant ($K_{1:1}$) of the HP-β-CD complex was determined as a function of the added ligand concentration ($[CD]_{tot}$)[12]. Since the phase solubility diagrams were of A_L-type and assuming a 1:1 complex, the apparent stability (or formation) constant $K_{1:1}$ was calculated using the slope from the linear regression analysis of the phase solubility isotherm using the following equation:

$$K_{1:1} = \frac{\text{slope}}{S_o\,(1 - \text{slope})}$$

Where S_o is the solubility of the pure drug. The determinations were performed in triplicate. Each test was repeated three times (coefficient of variation C.V. < 3%).

Characterization of cisplatin/HP-β-CD complex by fourier-transform infrared spectroscopy (FT-IR)

Potassium Bromide disks of inclusion complex, physical mixture and pure substances were analyzed by a FT-IR spectrometer (FT-IR-8400S, Shimadzu Corporation, Japan). The data was obtained in the range of 400–4000 cm^{-1} for each sample. The FT-IR spectra of binary complexes were compared with their physical mixtures, and with pure Cisplatin and HP-β-CD.

Characterization of Cisplatin/HP-β-CD complex by Differential Scanning Calorimetry (DSC)

Thermal analysis was performed using a differential scanning calorimeter (DSC60, Shimadzu Corporation, Japan). Thermograms of the different samples (inclusion complex, physical mixture and pure substances) were obtained from a DSC equipped with a thermal analysis data system. Weighted samples (10 mg) were contained in holed aluminum pans and scanned at a rate of 10 °C/min, between −35 °C and 250 °C, using nitrogen as a purging gas.

Dissolution rate study

In vitro dissolution studies of Cisplatin and its inclusion complexes were carried out using USP paddle method by dispersed powder technique. Samples equivalent to 10 mg of Cisplatin was added to 900 ml of distilled water (pH 7.4) containing 0.25% w/v sodium lauryl sulphate at 37 ± 0.5 °C and stirred at 50 rpm. The dissolution rate was studied using the DISSO 2000 (LabIndia Instruments, India). Sodium lauryl sulfate was added to the dissolution fluid to maintain sink conditions. An aliquot of 5 ml was withdrawn through a nylon filter disc (0.45 μ) at different time intervals. The withdrawn volume was replenished immediately with the same volume of the dissolution medium (maintained at 37 ± 0.5 °C) in order to keep the total volume constant. The filtered samples were suitably diluted and assayed spectrophotometrically at 280 nm after derivatization. The mean of at least three determinations was used to calculate the drug release.

Effect of HP-β-CD on the stability of Cisplatin

The Cisplatin in 0.9% w/w Sodium Chloride solution, pH 7.2 and Cisplatin/HP-β-CD complex were stored in tightly closed glass vials 24 hours at room temperature (25 °C). Drug content was determined by HPLC[15]. Samples, taken at set intervals, were filtered through a 0.22 µm filter (Millipore India Ltd.) and analyzed by HPLC (following appropriate dilution). To determine the stability of the freeze dried product, the Cisplatin concentration was determined by HPLC after storing the freeze dried material for 3 and 6 months in sealed vials at 4 °C.

Results and discussion

Phase solubility studies

Phase solubility profile of Cisplatin with HP-β-CD in Isotonic NaCl solution (pH 7.2), phosphate buffer (pH 2.5) and water are shown in Figure 1. The diagram obtained can be classified as A_L type according to Higuchi and Connors[11] (Higuchi T. and Connors K., 1965). This indicates that the aqueous solubility of Cisplatin increases linearly as function of HP-β-CD, indicating the formation of 1:1 drug-cyclodextrin soluble complex. Stability constants for the complex calculated from the slope of the initial straight portion of the solubility diagram was 55.76, 40.07 and 106.83 M^{-1} respectively (Table 1). Cisplatin is a moderately weak acidic drug with a pKa of 6.56 for its monohydrated form[13] and mainly exists in its ionized form in pH 7.2 leading to lower effectiveness on the interaction between the drug and the carrier. Cisplatin exists mainly in its unionized form at pH 2.5 contributing to greater efficiency in interaction between HP-β-CD. There was a 3.8, 3.3 and 5.8 fold increase in solubility of Cisplatin in isotonic sodium chloride solution, phosphate buffer and water respectively. The phase solubility of Cisplatin showed greatest $K_{1:1}$ values and highest aqueous solubilities (Table 1). The reason behind the decrease in the complex stability constant in the presence of buffer salts could be associated with formation of hydrogen bonds between the hydroxyl groups of cyclodextrin leading to salt formation and steric hindrance for the drug to fit into the cavity of the HP-β-CD. Cyclodextrins are known to form inclusion complex in a host-guest fashion where central hydrophobic cavity of cyclodextrin acts as a host for whole drug or part of it. The inclusion process is thought to be driven primarily by expulsion of enthalpy-rich water molecules from hydrophobic cavity, which cannot satisfy hydrogen-bonding potential of water molecules. However some researchers hypothesize role of hydrophobic forces such as Van der Waals forces in formation of complex[14].

Infrared studies

Differences between the FT-IR spectra of the inclusion complexes, physical mixture and pure materials indicate the interaction between Cisplatin and HP-β-CD (Figure. 2). In case of inclusion complexes, the characteristic amine stretching peak (3400 to 3200 cm^{-1}) of Cisplatin was found to be attenuated. The asymmetric amine bending (1600-1500 cm^{-1}), the symmetric amine bending (1300-1200 cm^{-1}) and hydroxyl stretching (3600-3000 $cm^{-1)}$ moved to a lower wavelength in inclusion complexes. In the FT-IR spectra of the physical mixture there are no changes since they are the superposition of the spectra of the single components. Where as in the FT-IR spectrum of the inclusion complexes absence characteristic bands of Cisplatin was observed. According to these changes, we might suggest that amino groups of the drug are involved in the inclusion process.

Table 1. Cisplatin Solubility and apparent 1:1 stability constants

Solvent	HP-β-CD (mM/l)	Solubility (mg/ml)	Type of curve	Stability Constant $K_{1:1}$ (M^{-1})
Isotonic NaCl pH 7.2	0	0.98	A_L	55.76
	1	1.47		
	2	1.74		
	4	2.01		
	8	2.60		
	12	3.23		
	15	3.59		
	17	3.73		
Buffer NaOH–KH_2PO_4 pH 2.5	0	0.99	A_L	40.07
	1	1.16		
	2	1.27		
	4	1.54		
	8	1.96		
	12	2.46		
	15	3.07		
	17	3.23		
Water	0	0.99	A_L	106.83
	1	1.30		
	2	1.83		
	4	2.33		
	8	3.44		
	12	4.46		
	15	5.50		
	17	5.72		

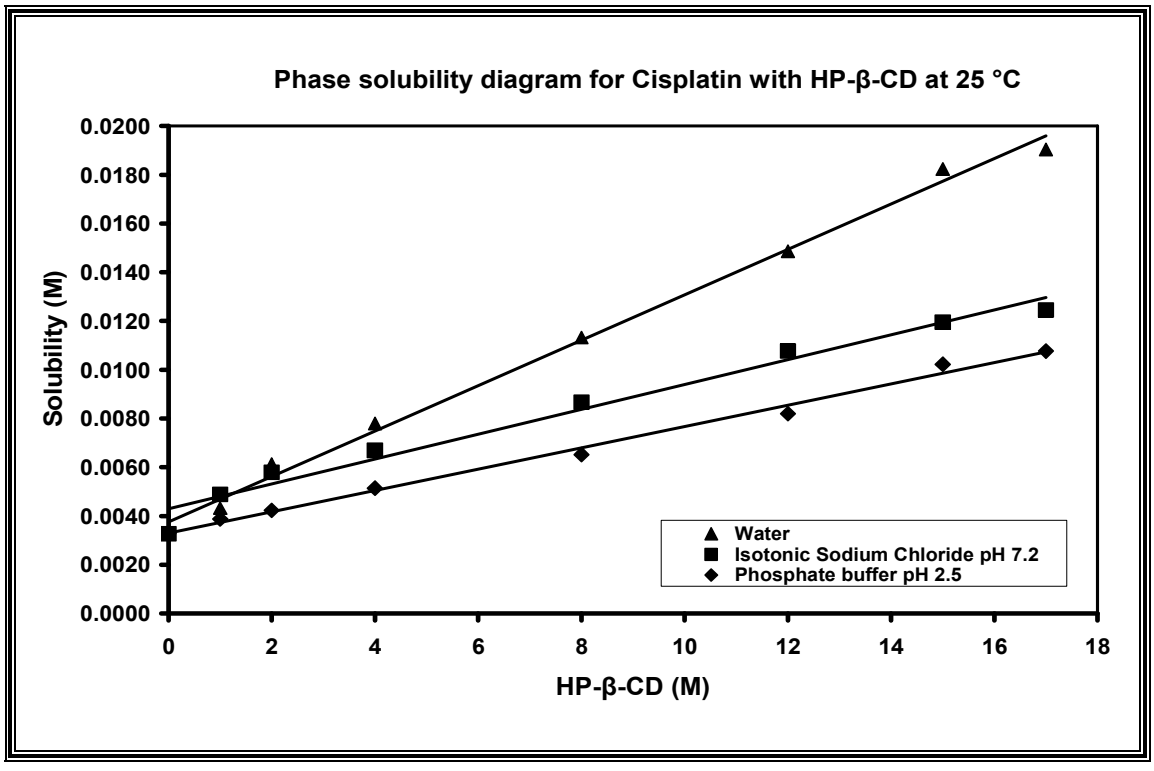

Figure 1. Phase Solubility diagrams for Cisplatin with HP-β-CD at 25 °C. Each point represents the mean of three determinations.

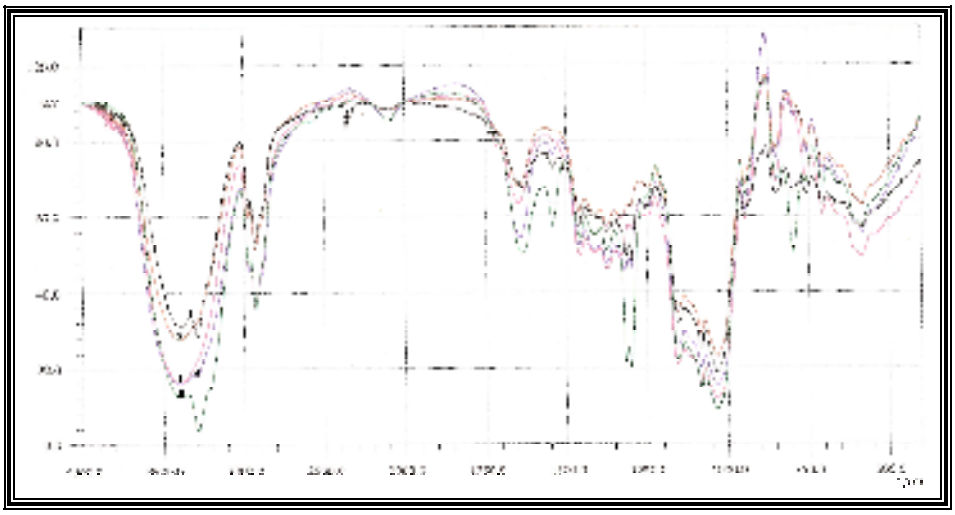

Figure 2. FT-IR spectra of Physical mixture (1), and 1:2 Cisplatin- HP-β-CD complex (2), HP-β-CD (3), 1:1 Cisplatin- HP-β-CD complex (4) and Cisplatin (5).

Differential scanning calorimetry studies

Differential scanning calorimetry studies (DSC) was used to identify the complexes in the freeze dried powder. In contrast to a physical mixture of Cisplatin and HP-β-CD, no endothermal melting peak of Cisplatin (at 120 °C) was observed in the freeze dried material, indicating that inclusion complexes have been formed (Figure 3). The inclusion complexes showed a broad endothermic peak at about 80 °C. The physical mixture thermogram was nearly identical to that of pure Cisplatin and HP-β-CD. The disappearance of the endothermic peak of Cisplatin and HP-β-CD and appearance of other endothermic peaks, may indicate the occurrence of an inclusion complex between Cisplatin and HP-β-CD.

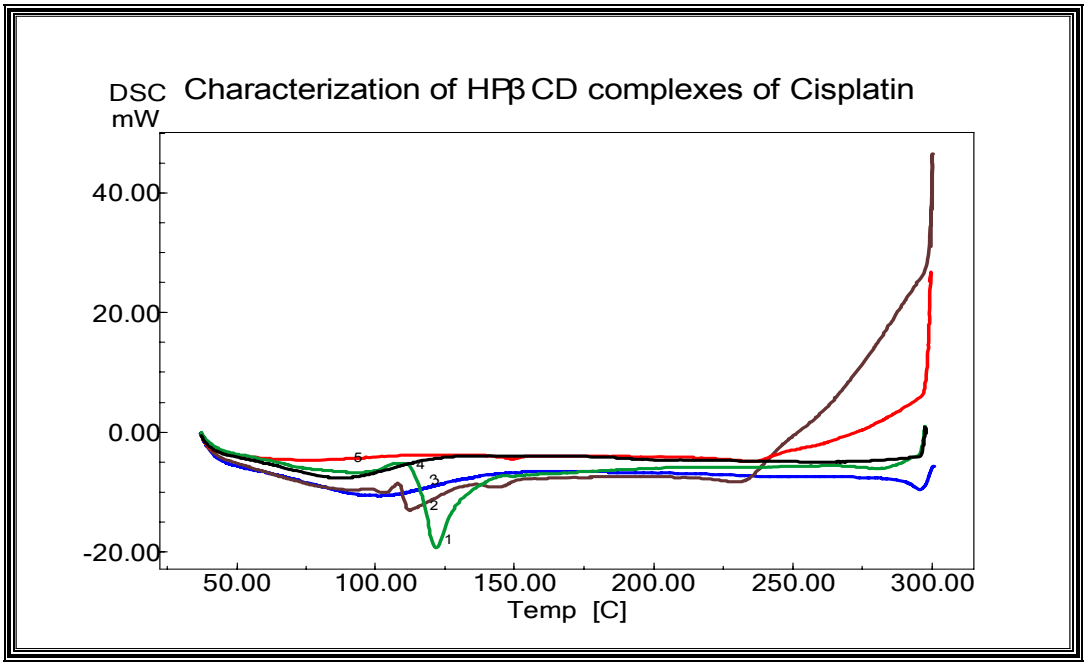

Figure 3. DSC thermogram of Cisplatin(1); Physical mixture(2); 1:1 Cisplatin- HP-β-CD complex(3); HP-β-CD(4) and 1:2 Cisplatin- HP-β-CD complex(5)

Dissolution rate study

The dissolution rate of Cisplatin alone and its inclusion complexes were studied in water containing 0.25% sodium lauryl sulfate. Sodium lauryl sulfate was included in the dissolution medium to maintain sink conditions. The dissolution profiles of various complexes are shown in Figure 4. The dissolution of Cisplatin was rapid and higher from both the complexes when compared with pure drug and physical mixture. The complexes exhibited higher rates of dissolution and dissolution efficiency values than Cisplatin, indicating rapid and higher dissolution of Cisplatin from its HP-β-CD complexes.

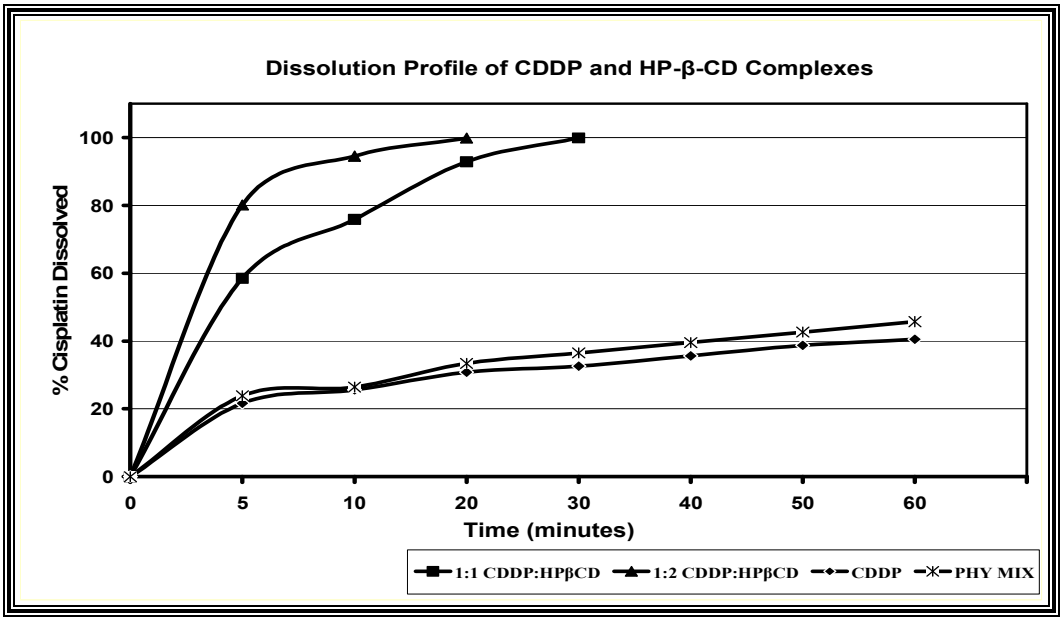

Figure 4. Dissolution Profile of Cisplatin and and 1:1 Cisplatin:HP-β-CD and 1:2 Cisplatin: HP-β-CD

Effect of HP-β-CD on the stability of Cisplatin

To study the effect of HP-β-CD on the stability of Cisplatin, Cisplatin and its HP-β-CD complexes (1:1 and 1:2 molar ratios) were stored dissolved and stored in 0.9% NaCl solution, pH 7.2, stored at 25 °C. Stability is expressed as the concentration of pure drug Cisplatin present at initial time of the experiment to the concentration of pure drug remaining at the end of the study i.e., 24 hours. The data is as shown in Table 3.4. 99.63 % and 98.95 % of Cisplatin remained intact at the end of 24 hours in 1:1 and 1:2 Cisplatin: HP-β-CD complexes respectively as compared to the pure drug (97.24%). Cisplatin is unstable in pure water. In aqueous solutions Cisplatin decomposes due to reversible substitution of water for chloride. Its stability is enhanced in sodium chloride solutions because of excess of chloride ions available. A solution in 0.9% injection has been reported to lose 3% of the drug in less than one hour and to remain stable at this equilibrium value for 24 hours at room temperature and the results obtained is in accordance with the reported values. Precipitation upon dilution can be a major problem when drugs with a poor aqueous solubility are given intravenously via an infusion set. Throughout all experiments precipitation upon dilution did not occur. The stability of freeze dried product stored at 4 °C is shown in figure 5. The results indicated very minute or no change in the Cisplatin content at the end of the 6 month study confirming that the pure drug and the complexes were highly stable figure 6.

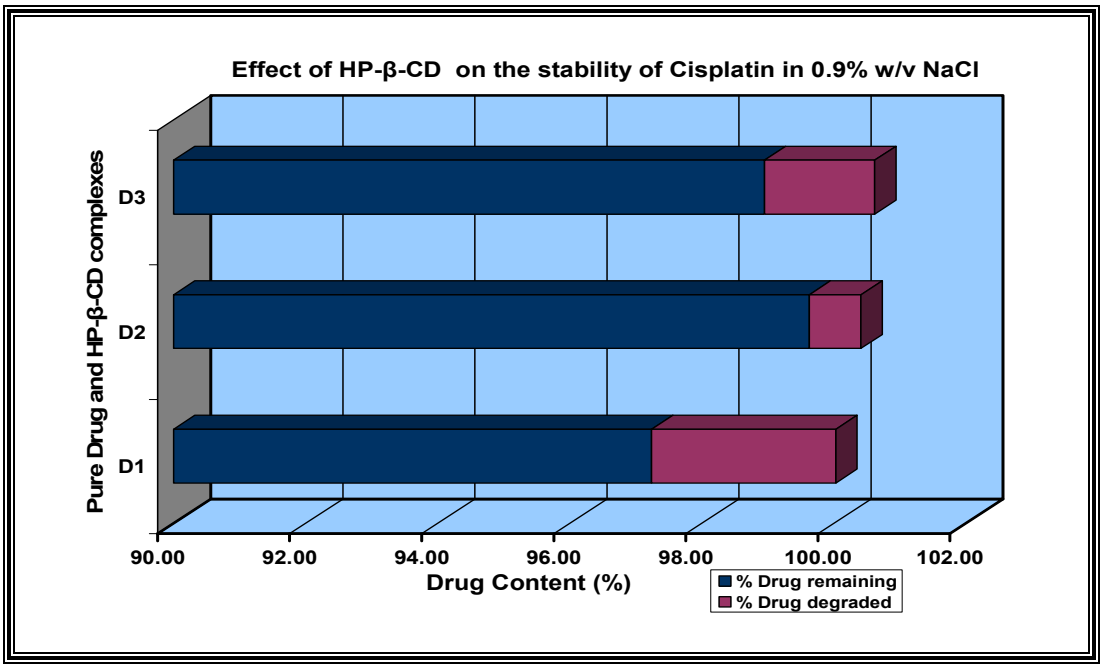

Figure 5. Effect of HP-β-CD on the stability of Cisplatin

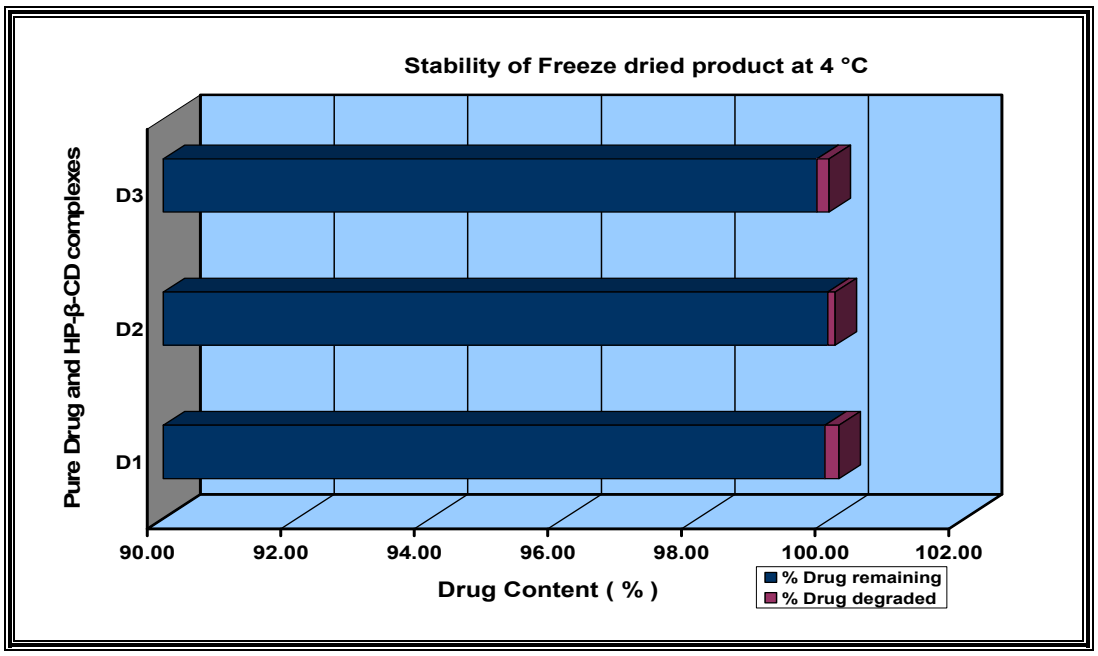

Figure 6. Stability of Freeze dried products at 4 °C

Conclusions

In the present study we prepared Ciplatin/HP-β-CD complexes by freeze drying method. Cisplatin interacts with HP-β-CD and forms a complex. Phase solubility, FT-IR and DSC results suggest the formation of a stable 1:1 stoichiometric complex of Cisplatin: HP-β-CD. The complexes showed greatest $K_{1:1}$ values and highest aqueous solubilities. The complexes had improved solubility and exhibited rapid and higher rates of dissolution enhancing the availability of Cisplatin at the site of action. Complexation of Cisplatin with HP-β-CD significantly enhanced the stability of the drug. These results suggest that the Cisplatin/ HP-β-CD complex represents an effective novel formulation permitting to enhance the entrapment of drugs with low aqueous solubility.

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References

1. Loehrer, P.J., Einhorn L.H. Drugs five years later. Cisplatin, Ann. Intern. Med. 1984;100:704–713.
2. Lehman, M., Thomas, G. Is concurrent chemotherapy and radiotherapy the new standard of care for locally advanced cervical cancer? Int. J. Gynecol. Cancer 2001;11:87– 89.
3. Wagstaff, A.J., Ward, A., Benfield, P. and Heel R.C. A preliminary review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy in the treatment of cancer, Drugs. 1989;37:162-190.
4. Araki, H., Tani T., Kodama M. Antitumor effect of Cisplatin incorporated into polylactic acid microcapsules, Artif. Organs, 1999;23:161–168.
5. Wiltshaw E. Ovarian trials at Royal Marsden, Cancer Treat Rev. 1985;12:67-71.
6. Del Valle, E. M. M. Cyclodextrins and their uses: A review. Process Biochemistry 2004;39:1033–1046.
7. Rajewski, R.A., Stella, V.J. Pharmaceutical applications of cyclodextrins. 2. In vivo drug delivery. J. Pharm. Sci 1996;85:1142–1169.
8. Hedges, R. A. Industrial applications of cyclodextrins. Chem Rev, 1998;5:2035–2044
9. Szejtli J., 1988. Cyclodextrin Technology, Kluwer Academic Publishers, Boston, Dordrecht and The Netherlands 1988;39:188-192.
10. Alcaro S., Ventura C.A., Paolino D., Battaglia D., Ortuso F., Cattel L., Puglisi G., Fresta M. Preparation, characterization, molecular modeling and in vitro activity of paclitaxel–cyclodextrin complexes, Bioorg. Med. Chem. Lett. 2002;12: 1637–1641.
11. Higuchi T., Connors K. Phase solubility techniques, in: C. Reilly (Ed.), Advances in Analytical Chemistry and Instrumentation, Wiley/Interscience, New York 1965:117-212.
12. Fromming K.H., Szejtli J. (Eds.). Cyclodextrin in Pharmacy, Kluwer Academic Publisher, Dordrecht 1994.
13. Andersson, A., Hedenmalm, H., Elfsson, B., Ehrsson, H. Determination of the acidic dissociation constant for cis – diammineaquachloroplatinum(II) ion. A hydrolysis product of Cisplatin. J Pharm Sci. 1994;83:859-862.
14. Connors K. A. The stability of Cyclodextrin complexes in solution, Chem Rev 1997;97:1325-58.
15. Lopez-Flores A, R. Jurado, P. Garcia-Lopez. A high-performance liquid chromatographic assay for determination of Cisplatin in plasma, cancer cell, and tumor samples, Journal of Pharmacological and Toxicological Methods 2005;52:366 – 372.