Journal of Inclusion Phenomena and Macrocyclic Chemistry **44:** 251–256, 2002.

Complexation of Vinpocetine with Cyclodextrins in the Presence or Absence of Polymers. Binary and Ternary Complexes Preparation and Characterization

LAURA RIBEIRO* and FRANCISCO VEIGA
Laboratório de Tecnologia Farmacêutica, Faculdade de Farmácia, Universidade de Coimbra, 3000 Coimbra, Portugal

(Received: 7 May 2002; in final form: 1 October 2002)

Key words: complexation, cyclodextrins, vinpocetine water-soluble polymers

Abstract

Inclusion complexation between β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD), water-soluble polymers (PVP and HPMC) and vinpocetine was studied in aqueous solution and in the solid state. Phase solubility studies were used to evaluate the complexation in aqueous solution at room temperature. Stability constants (K_c) of binary and ternary complexes were determined spectrophotometrically. Differential scanning calorimetry (DSC) was used to characterize kneaded, co-evaporated and lyophilised binary and ternary systems. The K_c values obtained were 70.14 M^{-1} and 35.01 M^{-1} for vinpocetine-β-CD and vinpocetine-HP-β-CD and increased in a range of 17% to 94% by addition of water-soluble polymers. Some preliminary evidences of inclusion complexation were obtained from DSC suggesting that co-evaporated and lyophilised binary and ternary systems were truly inclusion complexes.

Introduction

Cyclodextrins (CDs) are typical host molecules known for forming inclusion complexes with many guest molecules including lipophilic drugs, affecting their physical and chemical properties, such as solubility and stability [1]. Unfortunately, the efficiency complexation of drugs by CDs is rather low and consequently a significant amount of CDs is needed to solubilise a small quantity of drug. Besides, many vehicles additives commonly used in drug formulation such as non-ionic surfactants, buffer salts, preservatives and organic solvents contribute even more for the reduced efficiency complexation [2].

For those reasons associated with toxicological considerations, formulation bulk and production costs it is important to use as little CDs as possible in pharmaceutical preparations. Thus, it is therefore imperative to develop methods that can be applied to enhance the complexation efficiency of CDs, such as the addition of small amounts of water-soluble polymers to aqueous CD solution, along with heating [3].

The aim of this work was to examine the potential of β-CD and HP-β-CD in association with water-soluble polymers as solubilizing agents for vinpocetine, a poorly water-soluble base-type drug with significant peripheral vasodilator effect [4] using the phase solubility study technique and characterize the complexes formed by DSC.

* Author for correspondence: E-mail: laurear6@hotmail.com

Materials and methods

Chemicals

β-CD (Lot 341001; MW 1135) and HP-β-CD (Lot 955477D; TDS 6.3; MW 1300) were a gift from Roquette Vinpocetine (Lot CV/VP010701; MW 350.46) was purchased from Covex (Madrid, Spain). Polyvinylpyrrolidone K30 (PVP), HPMC 4000 cps and tartaric acid were supplied by Sigma Chemical Co. (St. Louis, USA).

Phase solubility studies

Phase solubility studies in deionised water at room temperature (22 ± 1 °C) were carried out for both binary and ternary systems according to the method of Higuchi and Connors [5]. Excess amounts of vinpocetine base were weighted into 20 ml glass flasks to which were added 10 ml of aqueous solutions containing increasing amounts of β-CD (0.001–0.015 M or 0.001–0.028 M in the presence of polymers) or HP-β-CD (0.001–0.08 M), with or without a fixed polymer concentration of 0.25% (W/V) PVP and 0.1% (W/V) HPMC.

In binary systems, glass containers were sealed and mechanically stirred until reaching equilibrium (about 72 hours). In the case of ternary systems, glass containers were sealed and heated in an autoclave at 120 °C for 20 minutes and then allowed to equilibrate for 72 hours.

All suspensions were filtered through a 0.45 μm membrane filter (Millipore) and analysed spectrophotometrically (UV-1603, Shimadzu) at 316 nm for drug content. To nullify the absorbance due to the presence of cyclodextrins and polymers, the apparatus were calibrated with the corres-

ponding blank every assay. Three replicates have been made for each experiment.

The apparent stability constants (K_c) were estimated from the straight line of the phase solubility diagrams according to the following equation of Higuchi and Connors:

$$K_c = \text{slope/intercept}\,(1 - \text{slope})$$

Preparation of vinpocetine binary and ternary solid systems

Solid systems were prepared with equimolar ratio of vinpocetine and cyclodextrins using three distinct methods: kneading, co-evaporation and freeze-drying.

Physical binary and ternary mixtures

Physical mixtures of vinpocetine with cyclodextrins (β-CD and HP-β-CD) were prepared by previously sieving each component (75–100 μm) and subsequently by mixing them in a ceramic mortar for 5 minutes.

For ternary physical mixtures were added 15% (W/W) of PVP and 6% (W/W) of HPMC. They were also prepared, following the same procedure, physical mixtures with equimolar amounts of tartaric acid:vinpocetine from binary and ternary physical mixtures previously described.

All mixing procedures were performed adopting the geometric method.

Kneaded binary and ternary

Kneaded systems were prepared from physical binary and ternary mixtures by adding a small volume of 17% (W/V) tartaric acid solution equivalent to the 1:1 molar ratio vinpocetine:tartaric acid. After wetting the physical mixtures in a ceramic mortar, the resultant systems were vigorously kneaded with a pestle for 30 minutes to produce a homogeneous dispersion. Once homogeneous slurry was obtained, samples where dried at 40 °C for 48 hours.

Co-evaporated binary systems

0.571 mmol of cyclodextrin (β-CD or HP-β-CD) was dissolved in 50 ml of deionised water and 0.571 mmol of vinpocetine in 10 ml of 1.5% (W/V) tartaric acid solution. The two solutions were subjected to stirring (100 rpm) at 60 °C for three hours and the obtained clear solution was evaporated under vacuum at 50 °C in a rotatory evaporator (Heidolph, Laborota). The solid residue was further dried at 40 °C for 48 hours.

Co-evaporated ternary systems

0.571 mmol of cyclodextrin (β-CD or HP-β-CD) was dissolved in 50 ml of a 0.25% (W/V) of PVP solution or 0.1% (W/V) of a HPMC solution and 0.571 mmol of vinpocetine in 10 ml of 1.5% (W/V) tartaric acid solution. The two solutions were mixed and subjected to sonication in an ultrasound water-bath for 15 minutes and then heated in an autoclave (Uniclave 88) at 120 °C for 20 minutes. The resultant clear solution was allowed to equilibrate at room temperature for 72 hours and then evaporated under vacuum at 50 °C in a rotatory evaporator (Heidolph, Laborota). The solid residue was further dried at 40 °C for 48 hours.

Lyophilised binary systems

0.571 mmol of cyclodextrin (β-CD or HP-β-CD) was dissolved in 50 ml of deionised water and 0.571 mmol of vinpocetine in 10 ml of 1.5% (W/V) tartaric acid solution. The two solutions were sonicated for 15 minutes in an ultrasound water-bath and mixed for 2 at 50 °C. Finally, the resultant clear solution was frozen by immersion in an ethanol bath (Shell Freezer, Labconco, Freezone® model 79490) at −50 °C. After that, frozen solutions were lyophilised in a freeze-dryer (Lyph-lock 6 apparatus, Labconco) for three days.

Lyophilised ternary systems

0.571 mmol of cyclodextrin (β-CD or HP-β-CD) was dissolved in 50 ml of a 0.25% (W/V) of PVP solution or 0.1% (W/V) of a HPMC solution and 0.571 mmol of vinpocetine in 10 ml of 1.5% (W/V) tartaric acid solution. The two solutions were mixed and subjected to sonication for 15 minutes in an ultrasound water-bath and then heated in an autoclave (Uniclave 88) at 120 °C for 20 minutes. The resultant clear solution was allowed to equilibrate at room temperature for 72 hours and subsequently frozen by immersion in an ethanol bath (Shell Freezer,Labconco, Freezone® model 79490) at −50 °C. Finally, frozen solutions were submitted to lyophilization in a freeze-dryer (Lyph-lock 6 apparatus, Labconco) for three days. All resultant dried systems were crushed and sieved, and fractions smaller than 100 μm were collected for further studies.

Differential scanning calorimetry (DSC)

The DSC curves of pure materials, binary and ternary systems were recorded on a Shimadzu DSC-50 System with a DSC equipped with a computerized data station TA-50WS/PC. The thermal behaviour was studied by heating all accurately weighted samples (1 mg of vinpocetine or its equivalent) in a sealed aluminium pan, using an empty pan sealed as reference, over the temperature range of 30–400 °C, at a rate of 10 °C/minute and under a nitrogen flow of 20 cm^3/minute.

Indium (99.98%, m.p. 156.65 °C, Aldrich®, Milwauukee, USA) was used as standard for calibrating the temperature. Reproducibility was checked by running the sample in triplicate.

Results

Phase solubility studies

The influence of CD concentration in the presence or absence of water-soluble polymers on the solubility of vinpocetine was studied using the method of Higuchi and Connors. This is based on monitoring changes in the solubility of a substrate by the addition of complexing agents assayed, in this case, spectrophotometrically at 316 nm.

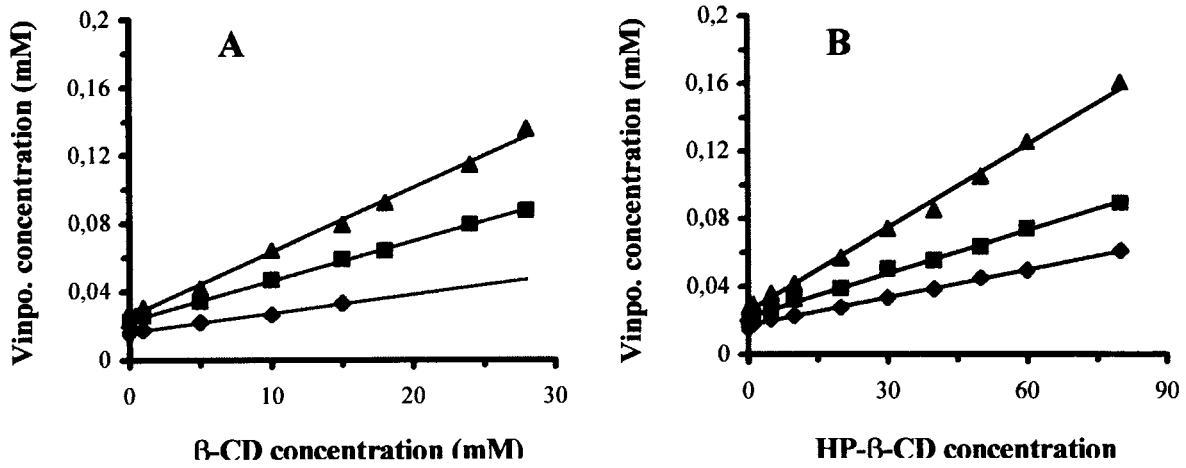

Figure 1. Phase solubility diagrams for vinpocetine at room temperature in the presence of β-CD (A) and HP-β-CD (B) without water-soluble polymers (♦) and with 0.25% (W/V) PVP (■) or 0.1% (W/V) HPMC (▲). Each point is the mean of (±SD) of three determinations.

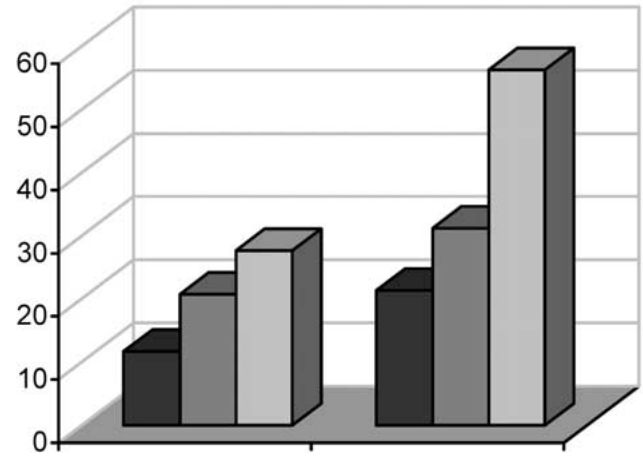

Figure 2. Vinpocetine solubility by complexation in 16 mM aqueous solution of β-CD and in 80 mM aqueous solution of HP-β-CD (♦) without polymers and in presence of 0.25% (W/V) PVP (■) or 0.1% (W/V) HPMC ( ).

The phase-solubility studies diagrams obtained with both CDs (β-CD and HP-β-CD) with and without water-soluble polymers (PVP and HPMC) are shown in Figure 1. They displayed A_L type (Higuchi and Connors) equilibrium phase solubility diagrams for Vinpocetine:β-CD and Vinpocetine:HP-β-CD binary and ternary systems, showing that vinpocetine solubility increase linearly as a function of CD concentration and that soluble complexes were formed without occurrence of precipitation in the range of CD concentration used.

The slope values were in all diagrams less than one suggesting the formation of 1:1 stoichiometry complexes in solution and allowing to the calculation of the apparent stability constants (K_c) of the drug:CD complexes as from the Higuchi and Connors equation. The values of the apparent stability constants of vinpocetine:CD binary and ternary complexes are collected in Table 1.

As can be seen, addition of water-soluble polymers to the CD solution did not change the type of phase-solubility diagrams obtained for binary systems and always resulted in a K_c increase that varied from a minimum of 17% to a maximum of 94%, depending on the CD and water-soluble polymer considered. The observed enhancement of K_c upon addition of the polymers shows that the polymers are able to interact with drug-CD binary complexes [6]. The solubilizing effect of CDs was increased in the presence of both 0.25% (W/V) PVP and 0.1% (W/V) HPMC being particularly this one remarkably effective in increasing the solubilizing effect of β-CD and HP-β-CD. Consequently, a synergistic effect in vinpocetine solubility was observed in the presence of CDs and those water-soluble polymers after heating in an autoclave at 120 °C for 20 minutes. Moreover, the presence of polymers changed the β-CD concentration range making possible an increase on vinpocetine solubility under a wider range of β-CD concentration (0–28 mM). The vinpocetine solubility enhancement after complexation with CDs and polymers is represented in Figure 2.

Interestingly, it was also demonstrated that vinpocetine:β-CD binary and ternary systems were more stable than those with HP-β-CD, probably because the subsistent hydroxypropyl groups of HP-β-CD hamper the inclusion of guest molecules into CD cavity via steric hindrance [7].

Differential scanning calorimetry

Differential scanning calorimetry (DSC) represents a first choice analytical tool for an accurate physicochemical characterization of drug-CD systems in the solid state and is commonly used as a routine method for a rapid preliminary qualitative investigation of the thermal behaviour of the single components, their physical mixtures and the inclusion compound candidate prepared according to a variety of standard procedures (e.g., kneading, co-evaporation and freeze-drying). The purpose consists of providing evidence that differences between the physical mixtures and the putative inclusion complex exist [8].

The DSC profiles of pure components and of the respective binary and ternary systems prepared by different methods in the melting range of the drug and dehydration of the carrier are shown in Figures 3 and 4. The thermal curve of pure vinpocetine was typical of a crystalline anhydrous

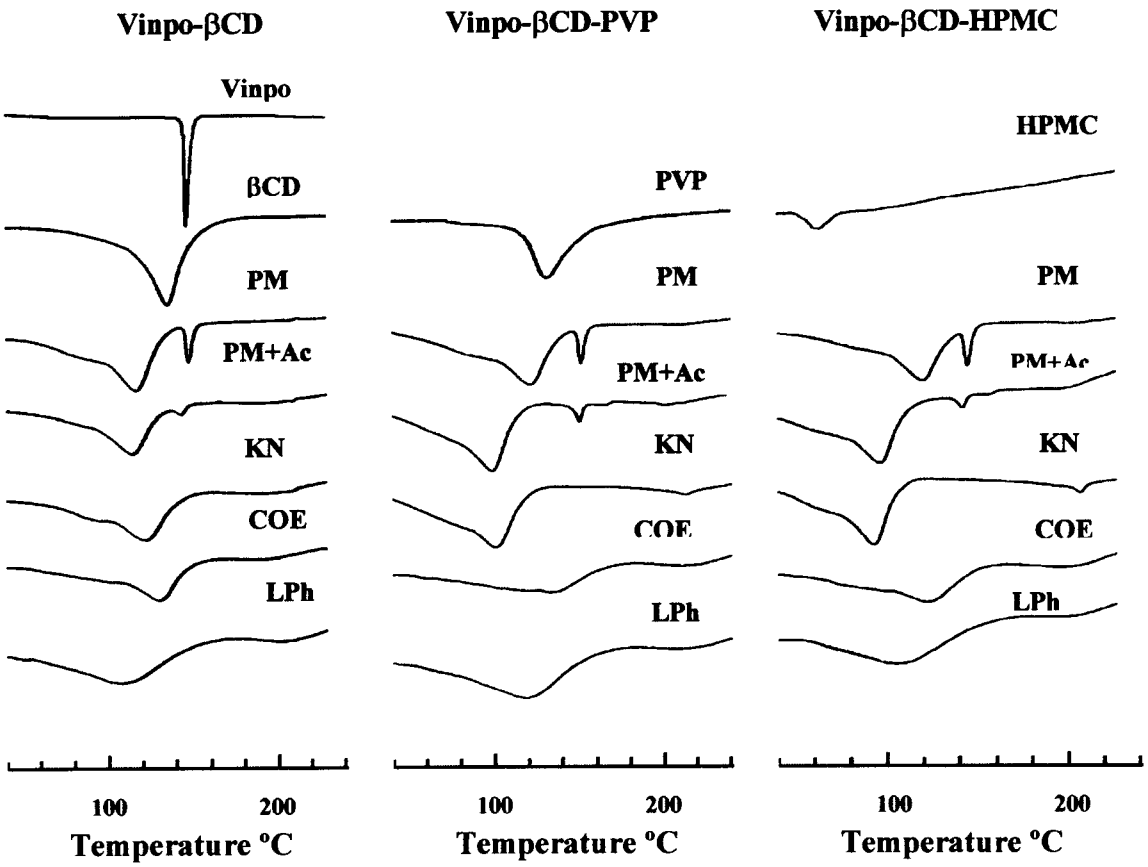

Figure 3. Differential scanning calorimetry curves of vinpocetine (Vinpo), β-cyclodextrin (β-CD), water-soluble polymers (PVP and HPMC), binary and ternary systems containing them: physical mixtures (PM), physical mixtures with tartaric acid in equimolar amount (PM + Ac), kneaded (KN), co-evaporated (COE) and lyophilised (LpH) products.

Table 1. Values of K_c for binary and ternary complexes; K_{TS}/K_{BS} is the ratio of K_c for binary and ternary complexes

Polymer	β-CD		HP-β-CD	
	K_c (M^{-1})	K_{TS}/K_{BS}	K_c (M^{-1})	K_{TS}/K_{BS}
No polymer	70.14	–	35.01	–
0.25% (W/V) PVP	102.00	1.45	41.10	1.17
0.1% (W/V) HPMC	136.41	1.94	60.60	1.73

substance with a sharp endothermic peak at 149.3 ± 0.6 °C corresponding to the melting point of the drug. The DSC curve of β-CD showed the liberation of crystal water as an endothermal effect peaked at about 135 °C, whereas broader endotherms were associated with water loss from amorphous HP-β-CD, PVP and HPMC.

The comparison of DSC curves from binary systems with those belonging to ternary systems did not result in significant differences. Both characteristics peaks of vinpocetine (drug melting) and CDs (water loss) were clearly distinguishable in binary and ternary physical mixtures, being the DSC thermograms of those the superposition of the individual components. Concerning the binary and ternary physical mixtures with addition of tartaric acid, the thermal characteristic peak of vinpocetine was shifted to lower temperatures at around 146.0–147.9 °C and its intensity was reduced. Those little changes relative to the peak of pure vinpocetine may suggest a weak interaction between the components of the physical mixtures with tartaric acid during the mixing or heating for DSC scanning [9–10].

Oppositely, the complete disappearance of the drug endothermal effect was observed in all binary and ternary systems obtained by kneading, co-evaporation and lyophilization. This phenomenon, thought not unequivocally attributable to inclusion complex formation is however undoubtedly indicative of a stronger interaction in the solid state [11–12] between vinpocetine, CDs, tartaric acid and water-soluble polymers when those are manipulated by the procedures previously described.

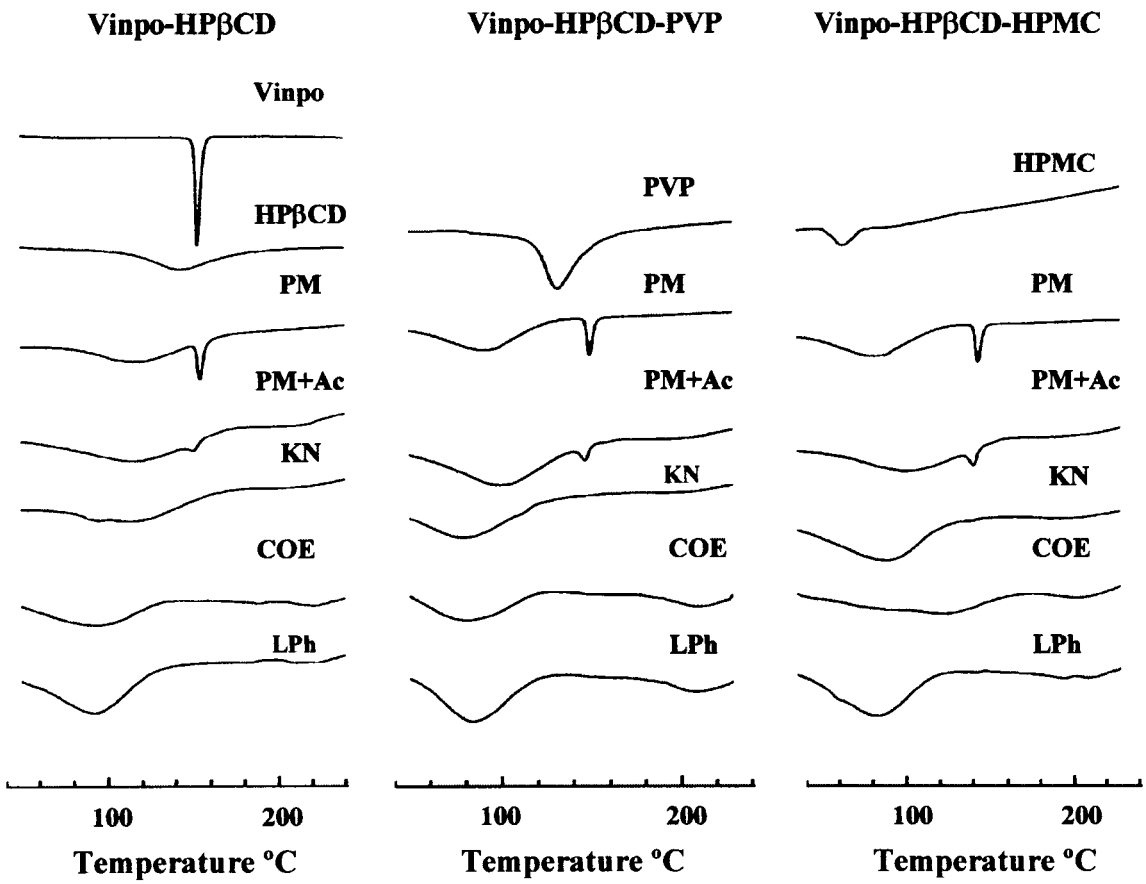

Figure 4. Differential scanning calorimetry curves of vinpocetine (Vinpo), β-cyclodextrin (β-CD), water-soluble polymers (PVP and HPMC), binary and ternary systems containing them: physical mixtures (PM), physical mixtures with tartaric acid in equimolar amount (PM + Ac), kneaded (KN), co-evaporated (COE) and lyophilised (LpH) products.

Conclusions

As reported previously by other authors [3, 13], water-soluble polymers increase the solubilizing effect of cyclodextrins, in aqueous solutions, on various hydrophobic drugs by increasing K_c of the drug-CD complexes. This increase enhances the complexation efficiency and thus less cyclodextrin is needed to solubilise a given amount of drug when polymers are present in the aqueous media.

Specifically in this work, the addition of polymers to cyclodextrin solutions made the slope of the solubility isotherms emphasized and in agreement with previous reports [14–16] the resultant ternary systems showed higher stability constants than the binary vinpocetine-CDs complexes, which account for the enhancement on vinpocetine solubility. Furthermore, the total solubility of β-CD was enhanced by addition of small amounts of polymers by increase in both β-CD intrinsic solubility and in aqueous solubility of the resulting complexes. There, the polymers not only increase the βCD intrinsic solubility but also enhanced its complexation efficiency [6]. By the fact of the absolute disappearance of vinpocetine endothermal effect in kneaded, co-evaporated and lyophilised binary and ternary products, solid-state characterization by DSC suggests that they are strong evidences for these products being true inclusion compounds.

These observations and other experimental data, as yet not published, indicate that the polymers participate directly in the complexation under the formation of drug-CD-polymer complexes.

References

1. K.H. Frömming and J. Szejtli: in K.H. Frömming and J. Szejtli (eds.), *Cyclodextrins Inclusion Complexes (Cyclodextrin in Pharmacy)*, Vol. 5, Kluwer Academic Publishers, Dordrecht, pp. 45–81 (1994).
2. T. Loftsson, M. Másson and J.F. Sigurjónsdóttir: *S.T.P. Pharma Sciences* **9**, 237 (1999).
3. T. Loftsson: *Pharmazie* **53**, 733 (1998).
4. P. Bönöczk, B. Gulyás, V. Adam-Vizi, A. Nemes, Kárpáti, B. Kiss, M. Kapás, C. Szántay, I. Koncz, T. Zelles and A. Vas: *Brain Research Bulletin* **53**, 245 (2000).
5. T. Higuchi and K.A. Connors: in C.N. Reill (ed.), *Phase-Solubility Techniques (Advances in Analytical Chemistry and Instrumentation*, Wiley-Intersciences, New York, pp. 117–212 (1965).
6. T. Loftsson and H. Fridriksdóttir: *International Journal of Pharmaceutics* **163**, 115 (1998).
7. A. Yoshida, M. Yamamoto, T. Irie, F. Hirayama and K. Uekama: *Chemical and Pharmaceutical Bulletin 37*, 1059 (1989).
8. F. Giordano, C. Novak and J.R. Moyano: *Thermochimica Acta* **380**, 123 (2001).
9. P. Mura, M.T. Faucci and G.P. Bettinetti: *European Journal of Pharmaceutical Sciences* **13**, 187 (2001).
10. G. Dollo, P. Le Corre, M. Chollet, F. Chevanne, M. Bertault, J.L. Burgot and R. Le Verge: *Journal of Pharmaceutical Sciences* **88**, 889 (1999).

11. G.P. Bettinetti, A. Gazzaniga, P. Mura, F. Giordano and M. Setti: *Drug Development and Industrial Pharmacy* **18**, 39 (1992).
12. P. Mura, M.T. Faucci, P.L. Parrini, S. Furlanetto and S. Pinzauti: *International Journal of Pharmaceutics* **179**, 117 (1998).
13. J. Savolainen, K. Jarvinen, H. Taipale, P. Jarho, T. Loftsson and T. Jarvinen: *Pharmaceutical Research* **15**, 1696 (1998).
14. B. Cappello, C. Carmignani, M. Iervolino, M.I. La Rotonda and M.F. Saettone: *International Journal of Pharmaceutics* **213**, 75 (2001).
15. H. Fridriksdóttir, T. Loftsson and E. Stefánsson: *Journal of Controlled Release* **44**, 95 (1997).
16. H. Fridriksdóttir, T. Loftsson, J.A. Gudmundsson, G.J. Bjarnason, M. Kjeld and T. Thorsteinsson: *Pharmazie* **51**, 39 (1996).