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Characterization of Sesquiterpene Polygodial-Beta Cyclodextrin Inclusion Complex

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Abstract

Polygodial is a sesquiterpene drimane isolated from the genus *Drimys*, which exhibits anti-asthmatic, anti-allergic, anti-inflammatory and antinociceptive effects. We have prepared a polygodial-β-cyclodextrin inclusion complex for further pharmacological studies. The inclusion complex was synthesized by co-precipitation and analyzed by Thermogravimetric Analysis, showing a decrease in the number of water molecules of hydration in relation to the native β-cyclodextrin. Differential Thermogravimetric Analysis indicated a peak corresponding to the evaporation of polygodial. With Differential Scanning Calorimetry, the melting peak to polygodial was not observed, however, there was an increase in the energy of vaporization of the water molecules in relation to native β-cyclodextrin. Using a Scanning Electron Microscopy a clear difference in the morphology of crystals of the inclusion complex and native β-cyclodextrin could be seen. The association constant between polygodial and β-cyclodextrin, measured by UV spectroscopy was 1,006 M^{-1} at 37 °C, pH 7.0 and ionic strength 0.2 M, following stoichiometry 1:1.

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides known for their ability to form inclusion complexes with many lipophilic drugs, thereby changing their physico-pharmaceutical properties. Inclusion complexes have a lower solubility in water than native cyclodextrin, and can dissociate easily to release the guest molecule. They are used in the formulation of oral and topically-applied drugs to improve both physical and chemical stability and bioavailability and to reduce the incidence of side effects [1, 2].

A number of experimental techniques have been employed in the study of interactions between cyclodextrins and guest molecules, such as fluorescence spectroscopy [3], UV-visible spectroscopy [4, 5], solubility method [6–8], thermal [7–10] and X-ray analysis [8–10].

The UV-visible spectroscopy technique for guests that have a chromophoric group can be suitable for the study of inclusion complexes in aqueous phase. The absorbance of guests is modified by complexation; thus, the association constant can be obtained. This is particularly important, since only stability constants between 200–10,000 seem to be suitable for pharmaceutical utilization. Very labile complexes lead to premature drug release and very stable complexes lead to retarded or incomplete drug release in the organism [11].

Another technique applicable to the characterization of inclusion complexes in solid state, is the thermal analysis, i.e., Thermogravimetric Analysis and Differential Scanning Calorimetry. Tian and co-workers applied this analysis and showed that β-cyclodextrin-cinnamic aldehyde and β-cyclodextrin-cinnamyl alcohol can form stable inclusion complexes improving the physical stability of the guests. In addition, they determined the composition of these inclusion complexes and the mechanism of decomposition as the temperature increased [12, 13].

Polygodial is a sesquiterpene drimane isolated from some *Drimys* species, Figure 1, which has demonstrated, particularly in previous studies conducted by our research group, interesting pharmacological effects, such as anti-asthmatic, anti-allergic, anti-inflammatory and antinociceptive [14–16]. It is considered a new lead for drug development because it targets a variety of pathways involved in pain perception and inflammation [17]. The interactions between cyclodextrin and this drug could provide an inclusion complex with improved pharmacological effects and stability. This work describes the characterization of polygodial-β-cyclodextrin inclusion complexes for further pharmacological studies.

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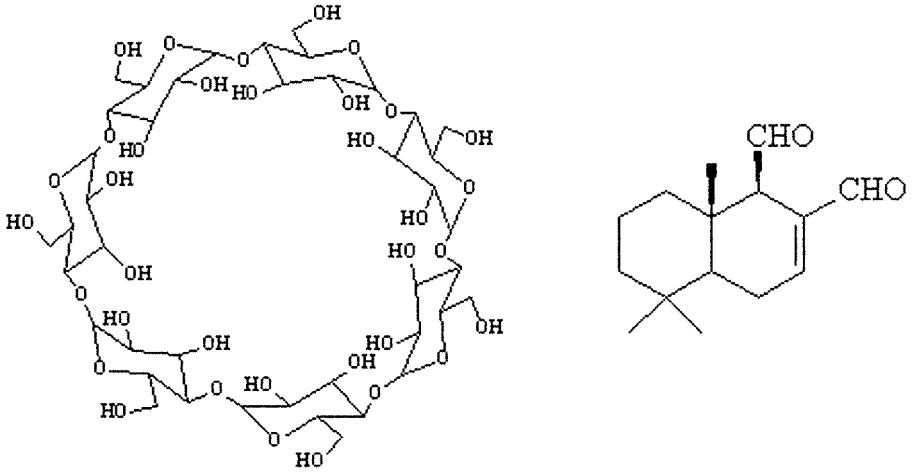

Figure 1. Structures of β-CD and polygodial.

Experimental

Reagents and solutions

β-Cyclodextrin was obtained from Cerestar USA, Inc. Polygodial was previously isolated from the $CHCl_3$ extract of *Drimys brasiliensis* barks [18]. All other reagents and solvents were of analytical grade. Freshly prepared distilled water was used throughout the study.

Synthesis of inclusion complex

The inclusion complex was prepared using the methodology reported by Claudy and co-workers [19]. Polygodial (3.75×10^{-4} mols) was dissolved in 25 mL of n-hexane. A solution of β-cyclodextrin (3.75×10^{-4} mols) was prepared in 25 mL of water. The solutions were then allowed to react for 4 h at 50 °C. A white microcrystalline powder was formed, the solid was filtered off and washed with water and hexane and dried under vacuum for 24 h and stored at room temperature.

Instrumentation and measurement

The inclusion complex was analyzed using the following techniques: Ultraviolet spectroscopy (UV), Thermogravimetric Analysis (TG), Differential Scanning Calorimetry (DSC), Scanning Electron Microscopy (SEM).

The UV analysis was carried out in a Hitachi U-2000 Spectrometer at 247 nm, 37 °C, optical pathway (b) = 1.0 cm, buffer phosphate 0.01 M to pH 7.0, ionic strength 0.2 M (KCl) and [polygodial] = 6.62×10^{-5} M (acetonitrile:water = 1.00% (v/v)), measuring the solution absorbance against a reference containing cyclodextrin at the same total concentration $[CD]_t$ (see Equation (2)).

Thermogravimetric Analyses (TG) were performed in a Shimadzu TGA50 at a heating rate of 5 deg min^{-1} under flowing nitrogen, 50 mL min^{-1}. Sample mass was about 12 mg. The Differential Thermogravimetric Analyses (DTG) were carried out deriving TG data.

Differential Scanning Calorimetry (DSC) was carried out in a Shimadzu DSC50 at a heating rate of 5 deg min^{-1} under nitrogen dynamic atmosphere, 50 mL min^{-1}. Sample mass ranged from 6 to 9 mg.

Scanning Electron Microscopy (SEM) was carried out in a Philips XL30 instrument using samples dispersed in carbon and attached to aluminum discs. To improve conductivity, samples were gold coated.

Results and discussion

Determination of β-cyclodextrin-polygodial association constant (K_{CDS})

UV spectroscopy was utilized to determine the association constant. It considers the system as a single 1:1 complex CDS (Equation (1)). The system considers Equation (2) as the binding isotherm [20, 21];

$$S + \mathrm{CD} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [\mathrm{CDS}], \tag{1}$$

$$K_{\mathrm{CDS}} = k_1/k_{-1},$$

$$\frac{\Delta A}{b} = \frac{[S]_t.K_{\mathrm{CDS}}.\Delta\epsilon_{\mathrm{CDS}}.[\mathrm{CD}]}{(1 + K_{\mathrm{CDS}}.[\mathrm{CD}])}, \tag{2}$$

which can be rearranged. Its inverse yields the Scott's equation [20] (Equation (3));

$$\frac{b.[\mathrm{CD}]}{\Delta A} = \frac{[\mathrm{CD}]}{([S]_t.\Delta\epsilon_{\mathrm{CDS}})} + \frac{1}{(s_t.K_{\mathrm{CDS}}.\Delta\epsilon_{\mathrm{CDS}})}, \tag{3}$$

where b represents the optical pathway; $[S]_t$ is the total concentration of substrate, and $\Delta\epsilon_{\mathrm{CDS}}$ is the molar absorptivity difference ($\Delta\epsilon_{\mathrm{CDS}} = \epsilon_{\mathrm{CDS}} - \epsilon_S - \epsilon_{\mathrm{CD}}$, using a reference of cyclodextrin at the same total concentration $[CD]_t$ then $\Delta\epsilon_{\mathrm{CDS}} = \epsilon_{\mathrm{CDS}} - \epsilon_S$). It was assumed that $[CD]_t \gg [CDS]$.

The Scott equation correlates the spectroscopic properties of polygodial (substrate – S) with increasing concentration of cyclodextrin. From this equation it is possible to

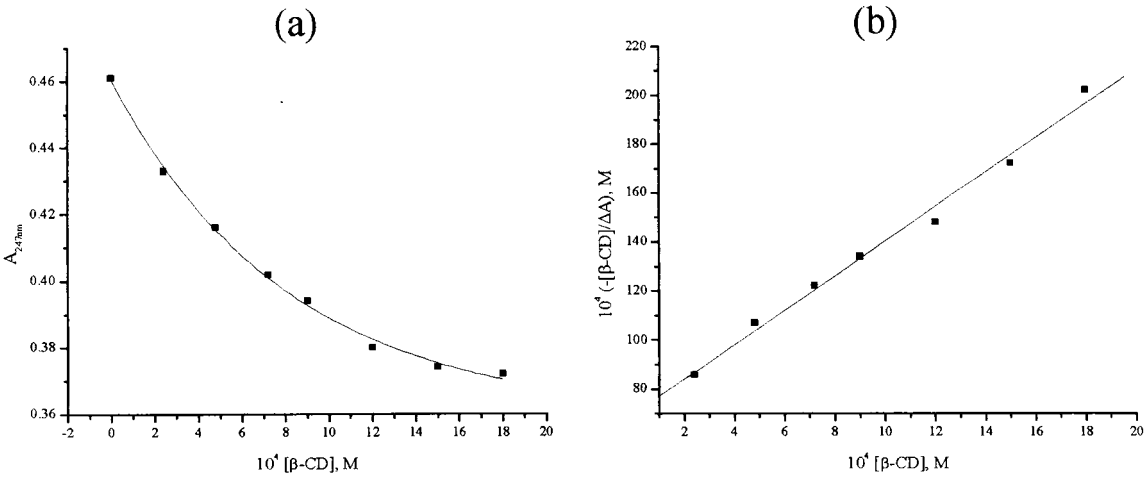

Figure 2. (a) Absorbance of polygodial with increasing concentration of cyclodextrin; (b) Data from "a" treated with Scott's equation. [Polygodial] = 6.62 $\times$ 10^{-5} M. Ionic strength 0.2 M (KCl), pH 7.0 ([Phosphate] = 0.01 M) and 37 °C.

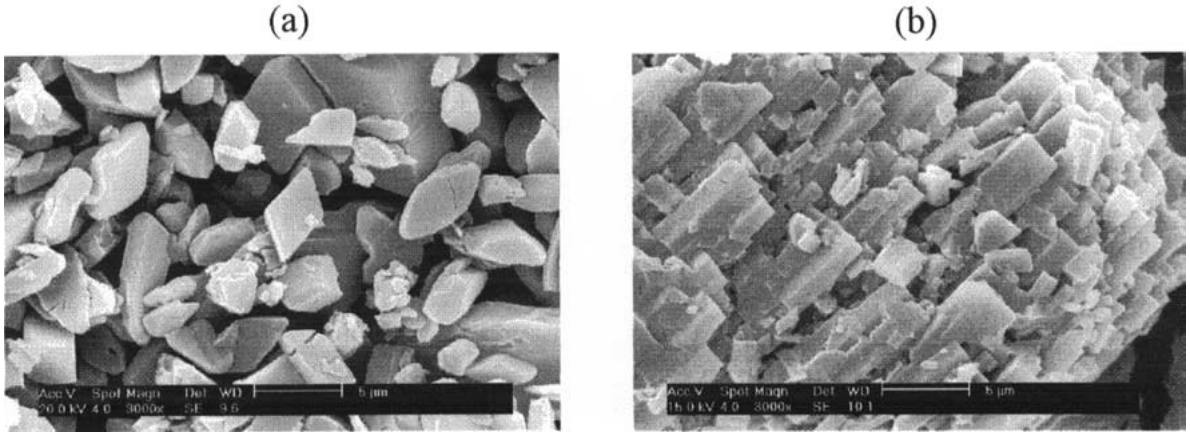

Figure 3. Electron images of the (a) inclusion complex between polygodial and β-CD and (b) native β-CD.

obtain the association constant (K_{CDS}) from $(\text{slope})_{eq.3}/(y\text{-intercept})_{eq.3}$ and $\Delta\epsilon_{CDS}$ from $1/[S]_0.(\text{slope})_{eq.3}$. This constant ($K_{CDS}$) is 1,006 M^{-1} ($\Delta\epsilon_{CDS}$ = 2147), and follows stoichiometry 1:1, as shown in Figure (2b).

Scanning Electron Microscopy (SEM)

A clear difference in the morphology of the crystal forms of the inclusion complex and β-cyclodextrin was observed. Both have the appearance of rhomb-shaped crystals, showing clear differences in sizes and angles of the faces (Figure 3).

Thermogravimetric analysis

TG and DTG plots indicated that thermal degradation of the inclusion complex occurred in three stages (Figures 4 and 5).

The first one occurred at 32–157 °C, and corresponded to the loss of water molecules of the inclusion complex. The DTG curve (Figure 4) demonstrates that this dehydration occurred in different stages, indicating that there were water molecules with distinct binding forces in relation to the native β-cyclodextrin. Therefore, different energies were required for dehydration. The area of two peaks, at 67 and 93 °C, corresponded to 4.2%, equivalent to three water molecules. The TG plot (Figure 5) shows a great difference in the number of water molecules lost from the native β-cyclodextrin compared to the inclusion complex, 12.9% equivalent to 9.3 water molecules. Steiner and Koellner have found that the number of water molecules decreases from 12.3 to 9.4 as the relative humidity changes from 100% to 15% at room temperature [22].

The second stage begins at 275 °C and continues up to 312 °C, and corresponded to vaporization of polygodial from the complex, 10.0% equivalent to 0.6 polygodial (PG) molecules.

The last process corresponds to thermal degradation of β-cyclodextrin, which begins at 311 °C.

The composition of the inclusion complex was determined by TG as β-CD.0.6PG.3H_2O. The degradation of the inclusion complex occurred as follows:

$$\beta\text{-CD-0.6PG.3H}_2\text{O} \rightarrow \beta\text{-CD-0.6PG} + 3\text{H}_2\text{O},$$

$$\beta\text{-CD-0.6PG} \rightarrow \beta\text{-CD} + 0.6\text{PG},$$

$$\beta\text{-CD} \rightarrow \text{Thermal degradation.}$$

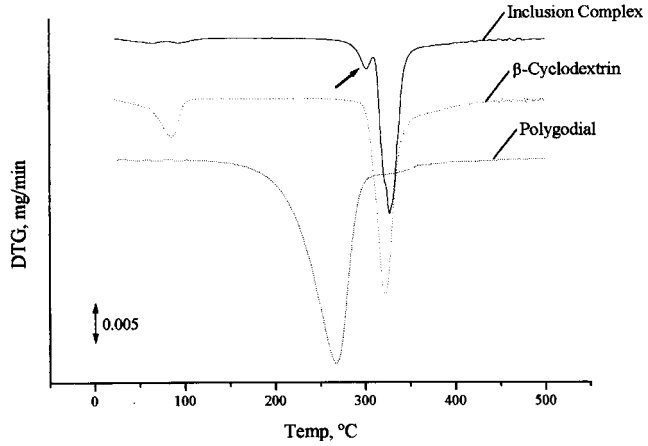

Figure 4. DTG analysis of pure polygodial, native β-CD and inclusion complex between polygodial and β-CD. The arrow indicates vaporization of polygodial from the inclusion complex. Heating rate of 5 deg min^{-1} under flowing nitrogen, 50 mL min^{-1}.

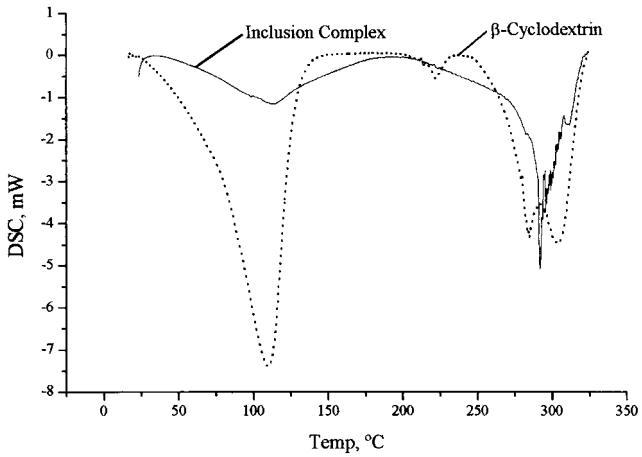

Figure 6. DSC analysis of native β-CD and inclusion complex between polygodial and β-CD. Heating rate of 5 deg min^{-1} under flowing nitrogen, 50 mL min^{-1}.

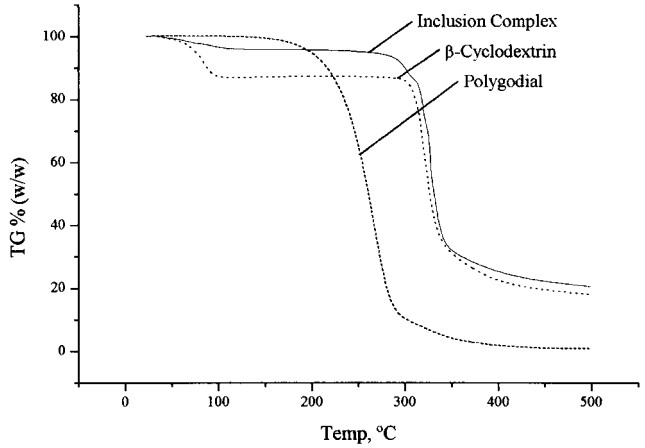

Figure 5. TG from pure polygodial, native β-CD and inclusion complex between polygodial and β-CD. Heating rate of 5 deg min^{-1} under flowing nitrogen, 50 mL min^{-1}.

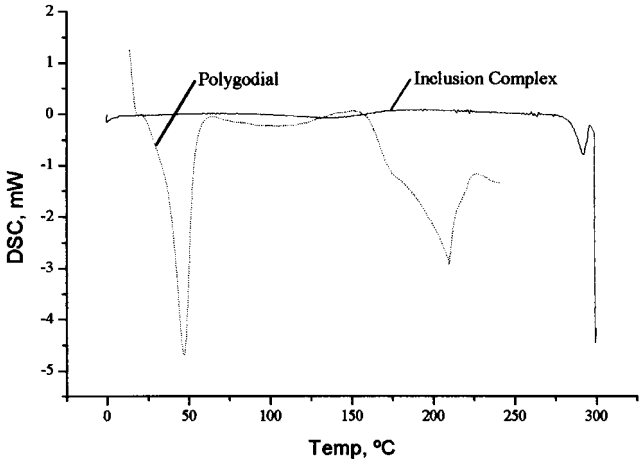

Figure 7. DSC analysis of pure polygodial and inclusion complex between polygodial and dehydrated β-CD. Heating rate of 5 deg min^{-1} under flowing nitrogen, 50 mL min^{-1}.

Differential scanning calorimeter (DSC)

DSC analysis of native β-cyclodextrin shows an endothermic peak at 25–153 °C, with an area equivalent to a dehydration enthalpy of 7.49 kJ/mol_{water} (9.31 water molecules) or 0.804 kJ/mol H_2O for each water molecule. For the inclusion complex, this process occurred at 38–190 °C, with an area equivalent to a dehydration enthalpy of 2.57 kJ/mol_{water} (3.00 water molecules) or 0.849 kJ/mol H_2O to water molecules. This shows that fewer water molecules of hydration exist in the inclusion complex than in the native β-CD; however, there is more energy involved in the dehydration of inclusion complex, as can be observed in Figure 6.

In general, the inclusion into the cyclodextrin cavity causes changes in microscopic properties of the guest such as melting point, boiling point, etc. In this case, the most important fact is that the melting peak of polygodial is not observed, because guest–guest interactions were completely replaced by guest-host interactions [23]. Thus, the DSC plot (Figure 6) shows that the inclusion of polygodial was complete. However, the melting peak of polygodial is positioned in the same region of dehydration. In order to prove this fact more clearly, the dehydration of the inclusion complex was carried out on a sample previously heated to 130 °C, then cooled, and heated again for the analysis to be performed. The melting peak of polygodial that occurs at 20–66 °C was not observed, demonstrating that inclusion was complete (Figure 7).

Conclusion

The results showed that the inclusion complex polygodial-β-cyclodextrin was obtained. DSC thermal analysis revealed the absence of an endothermic peak for the melting point of polygodial. TG showed that the stoichiometry for the complex was β-CD.0.6polygodial.$3H_2O$. In addition, SEM images showed that the inclusion obtained by this technique had crystalline appearance. The K_{CDS} of 1,006 M^{-1} shows that inclusion complex is sufficiently stable for oral administration.

Acknowledgments

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References

1. D. Duchêne, C. Vaution, and F. Glomot: *Drug Dev. Ind. Pharm.* **12**, 2193 (1986).
2. J. Szejtli: *Drug Invest.* Suppl. 4, 11 (1990).
3. T.C. Werner and I.M. Warner: *J. Incl. Phenom. Mol. Recognit. Chem.* **18**, 385 (1994).
4. C.A. Georgiou, S.G. Skoulika, and M.G. Polissiou: *J. Incl. Phenom. Mol. Recognit. Chem.* **34**, 85 (1999).
5. M.M. Meier, M.T.B. Luiz, P.J. Farmer, and B. Szpoganicz: *J. Incl. Phenom. Mol. Recognit. Chem.* **40**, 291 (2001).
6. T. Shinoda, S. Kagatani, A. Maeda, Y. Konno, H. Hashimoto, K. Hara, K. Fujita, and T. Sonobe: *Drug Dev. Ind. Pharm.* **25**, 1185 (1999).
7. G. Dollo, P. Le Corre, M. Chollet, F. Chevanne, M. Bertault, J.-L. Burgot, and R. Le Verge: *J. Pharm. Sci.* **88**, 889 (1999).
8. A. Miro, F. Quaglia, A. Calignano, F. Barbato, B. Cappello, and M.I. La Rotonda: *S. T. P. Pharma Sci.* **10**, 157 (2000).
9. M. Fodor, C. Novák, R. Rákosa, K. Tomor, G. Pokol, and S. Gál: *J. Thermal Anal.* **48**, 515 (1997).
10. A. Fini, M.J. Fenández-Hervás, M.A. Holgado, L. Rodriguez, N. Passerini, and O. Caputo: *J. Pharm. Sci.* **86**, 1303 (1997).
11. J.L. Atwood, J.E. Davies, and D.D. Macnicol: *Inclusion Compounds*, vol. 3, Academic Press, London (1984), p. 350.
12. S.-Z. Yu, X.-T. Li, J.-H. Li, J.-Y Wang, and S.-J. Tian: *J. Thermal Anal.* **49**, 1517 (1997).
13. J.-H. Li, N. Zhang, X.-T. Li, J.-Y Wang, and S.-J. Tian: *J. Thermal Anal.* **49**, 1527 (1997).
14. V. Cechinel Filho, V. Schlemper, A.R.S. Santos, T.R. Pinheiro, R.A. Yunes, G.L. Mendes, J.B. Calixto, and F. Delle Monache: *J. Ethnopharmacol.* **62**, 223 (1998).
15. E. André, A. Malheiros, V. Cechinel Filho, R.A. Yunes and J. B. Calixto: *European J. Pharmacol.* **386**, 47 (1999).
16. M. El-Sayah, V. Cechinel Filho, R. A. Yunes, A. Malheiros, and J.B. Calixto: *J. Cardiov. Pharmacol.* **35**, 670 (2000).
17. O. Sterner and A. Szallasi: *Trends in Pharmacol.* **20**, 459 (1999).
18. A. Malheiros, V. Cechinel Filho, C.B. Schmitt, A.R.S. Santos, C. Scheidt, J.B. Calixto, F. Delle Monache, and R.A. Yunes: *Phytochemistry* **57**, 103 (2001).
19. P. Claudy, J.M. Létoffé, P. Germain, J.P. Bastide, A. Bayol, S. Blasquez, R.C. Rao, and B. Gonzalez: *J. Thermal Anal.* **37**, 2497 (1991).
20. K.A. Connors: *Binding Constants*, John Wiley & Sons, New York (1987).
21. J. Szejtli: *Cyclodextrin Technology*, Kluwer Academic Publishers, Dordrecht (1988).
22. T. Steiner and G. Koellner: *J. Am. Chem. Soc.* **116**, 5122 (1994).
23. D. Duchene: *Cyclodextrins and Their Industrial Uses*, Editions de Santé, Paris (1987), p. 157.