International Journal of Pharmaceutics, 86 (1992) 263–265

IJP 02923

Microcalorimetric study of the interactions of aspartame with β-cyclodextrin and hydroxypropyl-β-cyclodextrin: The anomalous heat of dilution of the latter

D. Moelands, N.A. Karnik, R.J. Prankerd, K.B. Sloan, H.W. Stone and J.H. Perrin

Department of Medicinal Chemistry, College of Pharmacy, University of Florida, J. Hillis Miller Health Center, Gainesville, FL 32610 (USA)

(Received 24 February 1992)
(Modified version received 8 May 1992)
(Accepted 1 June 1992)

Key words: Hydroxypropyl-β-cyclodextrin; β-Cyclodextrin; Aspartame; Binding constant; Heat of dilution; High solubility

Summary

Hydroxypropyl-β-cyclodextrin has been shown to interact less strongly with aspartame than does β-cyclodextrin. Solutions of hydroxypropyl-β-cyclodextrin in phosphate buffers exhibit a large concentration-dependent apparent heat of dilution, presumably as a result of the loss of extensive intermolecular attraction during dilution, which accounts for its great solubility in water.

During investigations of the stabilization of aspartame by cyclodextrins it became of interest to determine the equilibrium constant for the 1:1 interaction between these molecules under well defined conditions of pH and temperature. The constants were expected to be low, and as the determination of the heat of reaction was also desirable, flow microcalorimetry seemed to be the technique of choice. The measurements were made as previously described (Hardee et al., 1978), except that the solutions were introduced into the mixing cell by two LKB HPLC 2150 pumps (Pharmacia, Bromma, Sweden). Measurements were made at 25°C in a 0.15 M phosphate buffer of pH 4.0. The aspartame was obtained from the Nutrasweet Company (Deerfield, IL) and the cyclodextrins from American Maize Products (Hammond, IN). For the determination of the equilibrium constant between aspartame and β-cyclodextrin, the comparatively low solubility of the cyclodextrin meant that aspartame had to be used as the titrant. A small linear correction was necessary for the heat of dilution of aspartame, but none was necessary for the heat of dilution of β-cyclodextrin. The binding parameters were estimated using MINSQ version 2 (Micromath Scientific Software, Salt Lake City, UT). The average equilibrium constant for the β-cyclodextrin-aspartame interaction from four

Correspondence to: J.H. Perrin, Department of Medicinal Chemistry, College of Pharmacy, University of Florida, J. Hillis Miller Health Center, Gainesville, FL 32610, U.S.A.

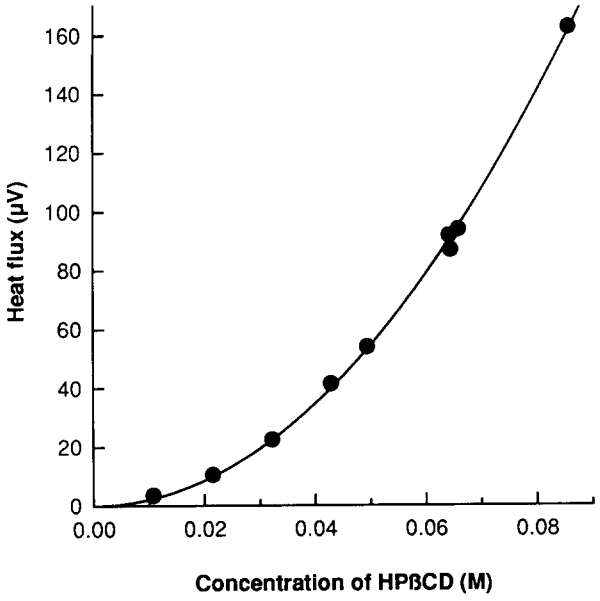

Fig. 1. Dilution of hydroxypropyl-β-cyclodextrin in 0.15 M phosphate pH 4.0 at 25°C with an equal volume of buffer at a total flow rate of 30 ml h^{-1}.

independent titrations was 128 M^{-1} (S.D. 22) and the heat of reaction was −11 700 (S.D. 700) J mol^{-1}. This binding constant compares with values (from NMR data) of 90 M^{-1} in D_2O (Takahashi et al., 1986) and of 211 M^{-1}, apparently in DMSO (Maheswaran and Divikar, 1991). Temperatures were not reported for either NMR study.

Hydroxypropyl-β-cyclodextrin is a mixture of components; as a result its apparent water solubility is greater than that of β-cyclodextrin. The higher water solubility of the mixture (31–33% substituted with hydroxypropyl groups) allowed the concentration of the host to be varied in the titrations. A plot of observed heat flux against hydroxypropyl-β-cyclodextrin concentration at a fixed aspartame concentration did not have the shape typically associated with a 1 : 1 equilibrium. Dilution of the substituted β-cyclodextrin by an equal volume of phosphate buffer showed a large exothermic and concentration-dependent heat flux as shown in Fig. 1. This marked deviation from ideality and the consequent heat evolved on dilution allows an explanation for the very high solubility of hydroxypropyl-β-cyclodextrin. In an ideal mixture, the total solubility is the sum of the solubilities of the individual components. The limited literature (Rao et al., 1990) on the solubilities of the individual components of the commercial hydroxypropyl-β-cyclodextrin clearly shows that the solubility is in great excess of that expected from ideality. Estimation of the water necessary to hydrogen-bond to all the ether linkages and hydroxyl groups in the 33% substituted hydroxypropyl-β-cyclodextrin indicates that there is insufficient water in a 50% w/v solution to satisfy all of them. It is of interest to note that water sorption experiments (Yoshida et al., 1988) indicate that solid β-cyclodextrin is paradoxically more hygroscopic than solid hydroxypropyl-β-cyclodextrin, given the higher aqueous solubility of the latter. These observations, taken together with the heat of dilution data, make it possible to postulate that the high solubility of hydroxypropyl-β-cyclodextrin is due to strong interactions between non-hydrated groupings, whereas with β-cyclodextrin, where hydration is more complete, the hydration minimizes the intermolecular interaction. Using MINSQ version 2, the observed heat flux for the dilution of a given concentration of hydroxypropyl-β-cyclodextrin by an equal volume of buffer was found to obey the following equation:

$$\text{observed heat flux} = A(\text{concentration})^n$$

where $A = 23\,400$ (S.D. 3190) and $n = 2.02$ (S.D. 0.04). t-Tests based on these standard deviations show that the coefficients are statistically significant with $p < 0.001$ for n and $p < 0.01$ for A. Table 1 shows the value of A and statistical parameters when n is fixed at 1, 2 or 3. Clearly, the data is best satisfied when $n = 2$. Differentiation of the equation for heat flux enables an

TABLE 1

Statistical data for the dilution of hydroxypropyl-β-cyclodextrin

n	A	S.D.	t	r^2
1	1435.9	129.05	11.13	0.939
2	21968	172.45	127.4	0.9995
3	293200	18465	15.88	0.969

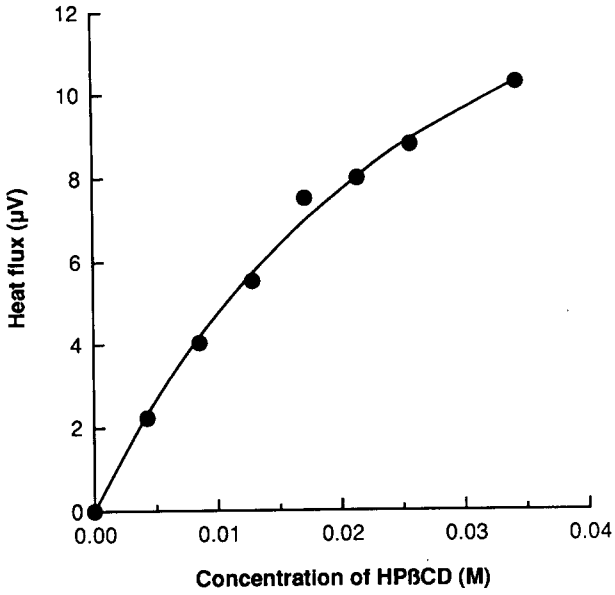

Fig. 2. Interaction of hydroxypropyl-β-cyclodextrin with aspartame (conditions as in Fig. 1) with the aspartame concentration fixed at 8.573×10^{-3} M. Heat fluxes were corrected for the apparent heat of dilution of hydroxypropyl-β-cyclodextrin from Fig. 1.

apparent heat of dilution of -9800 J mol^{-1} for a 0.1 M solution of the derivatized cyclodextrin to be estimated. When the appropriate corrections are made for the heats of dilution to the titration data, a curve typically associated with 1:1 binding is obtained (Fig. 2), giving a binding constant of 46 M^{-1} (S.D. 9) and a very low exothermic heat of reaction of 2200 (S.D. 200) J mol^{-1}. This low binding constant and small heat of reaction mean that these measurements are made near to the limits of the LKB flow microcalorimeter. It is also of interest to note that the affinity of the non-substituted cyclodextrin for aspartame is significantly greater than that of the substituted cyclodextrin.

References

Hardee, G.E., Otagiri, M. and Perrin, J.H., Microcalorimetric investigations of pharmaceutical complexes. *Acta Pharm. Suecica*, 15 (1978) 188–199.

Takahashi, S., Suzuki, E. and Nagashima, N., NMR study on inclusion complexes of L-phenylalanine and aspartame with cyclodextrin in aqueous solutions. *Bull. Chem. Soc. Jap.*, 99 (1986) 1129–1132.

Maheswaran, M.M. and Divikar, S., Studies on the structure of the inclusion compound of β-cyclodextrin with aspartame. *Ind. J. Chem.*, 30A (1991)30–34.

Rao, C.T., Fales, H.M. and Pitha, J., Pharmaceutical usefulness of hydroxypropylcyclodextrins E pluribus unum is an essential feature. *Pharm. Res.*, 7 (1990) 612–615.

Yoshida, A., Arima, H., Uekama, K. and Pitha, J., Pharmaceutical evaluation of hydroxyalkyl ethers of β-cyclodextrins. *Int. J. Pharm.*, 46 (1988) 217–222.