International Journal of Pharmaceutics, 88 (1992) 189–199

IJP 02914

Degradation of aspartame in acidic aqueous media and its stabilization by complexation with cyclodextrins or modified cyclodextrins

R.J. Prankerd [a], H.W. Stone [a], K.B. Sloan [b] and J.H. Perrin [b]

[a] *Department of Pharmaceutics and* [b] *Department of Medicinal Chemistry, College of Pharmacy, University of Florida, J Hillis Miller Health Center, Gainesville, FL 32610 (USA)*

(Received 30 March 1992)
(Modified version received 26 May 1992)
(Accepted 27 May 1992)

Key words: Aspartame; Degradation; Self-aminolysis; Diketopiperazine; Hydrolysis; Complexation; Cyclodextrin; Modified cyclodextrins; Stabilization; Half-life

Summary

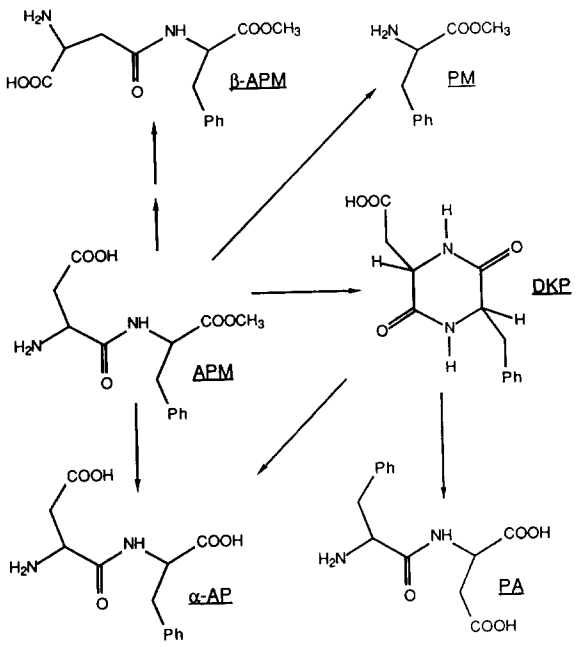
The degradation of aspartame was studied under a range of conditions (pH, 2.0–4.0; ionic strength, 0.05–0 50 M; temperature, 4–50°C) The loss of aspartame and formation of its degradation products were followed by a stability-indicating isocratic high-performance liquid chromatographic method that was able to separate aspartame from all known primary and some secondary reaction products. The ability of three unmodified cyclodextrins (α-, β- and γ-cyclodextrins) and four modified cyclodextrins (2,6-dimethyl-, 2,3,6-trimethyl-, 2-hydroxyethyl- and 2-hydroxypropyl-β-cyclodextrins) to lower the overall degradation rate was studied. The most effective agent was unmodified β-cyclodextrin. The stability enhancement of 2-hydroxypropyl-β-cyclodextrin was better for a sample with a lower average extent of substitution (19 2% of available hydroxyl groups substituted) compared to a sample with a higher extent of substitution (30–33% substituted), although neither was as good as β-cyclodextrin Stabilization by complexation with β-cyclodextrin was found to be more effective at lower temperatures and at higher ionic strengths. At low temperatures (4°C), the distribution of reaction products was different from that at higher temperatures.

Introduction

Aspartame (α-L-aspartyl-L-phenylalanine, methyl ester; APM) degrades in aqueous solution by several pathways. These have been reported to be first order in aspartame (Gaines and Bada, 1988). The half-life of aspartame at 25°C is 260 days at pH 4.3 (the pH of maximum stability), 12 days at pH 1.0 and 1 day at pH 7 (Mazur, 1976; Anon, 1987). Scheme 1 summarizes the most likely degradation products.

The most important pathway under neutral and acidic conditions (pH > 2) is intramolecular self-aminolysis, leading to formation of the cyclic dilactam 3-methylenecarboxyl-6-benzyl-2,5-diketopiperazine (Gaines and Bada, 1988). Ring-opening of the 2,5-diketopiperazine (DKP), then

Correspondence to: R.J. Prankerd, Department of Pharmacy, University of Queensland, St. Lucia, Queensland 4072, Australia



Scheme 1

hydrolysis to the isomeric dipeptides L-phenylalanyl-α-L-aspartic acid (PA) and α-L-aspartyl-L-phenylalanine (α-AP) occurs in acidic solutions. Under more acidic conditions (pH ≤ 2), the predominant reaction product is α-AP, presumably from acid-catalyzed hydrolysis of the methyl ester. Other degradation pathways for aspartame include hydrolysis of the peptide bond to L-phenylalanine methyl ester (PM) and isomerization to β-L-aspartyl-L-phenylalanine methyl ester (β-APM). The isomerization reaction has been reported (for other peptides) to occur through a succinimide intermediate (Kirsch et al., 1989; Bhatt et al., 1990; Patel and Borchardt, 1990). All primary reaction products are further hydrolyzed, eventually forming aspartic acid, phenylalanine and methanol. The routes of degradation are pH-dependent (Gaines and Bada, 1988), with a pH of maximum stability near 4.3, for overall loss of APM.

Phenylalanine has been reported to form a complex with α-cyclodextrin (α-CD), presumably by inclusion of the aromatic group into the cyclodextrin cavity, but not with β- or γ-cyclodextrins (β- or γ-CD) (Matsuyama et al., 1987). NMR studies have been used to show that APM complexes with both α- and β-cyclodextrin ($K_{assoc} = 90\ M^{-1}$). These indicated that interaction of the peptide amide N-H region with the CD assists stabilization of the complex (Takahashi et al., 1986). This was suggested by small changes in chemical shifts (^1H and ^{15}N) for the amide N-H group, which indicated that this group was close enough to the C2 or C3 hydroxyl groups to form hydrogen bonds. It has been recently suggested that the APM-β-CD complex (in DMSO solution) is also stabilized by interaction of the aspartyl β-carboxyl group with the CD (Maheswaran and Divakar, 1991). In preliminary studies, complexation with a cyclodextrin was reported to stabilize hydrolytically susceptible molecules such as APM (Ojima, 1983; Brewster et al., 1991).

Degradation of aspartame by intramolecular self-aminolysis requires that the molecule adopt a restricted cyclic conformation. Also, hydrolysis of APM to PM or to α-AP requires that the amide or ester carbonyl groups be accessible. The cyclodextrin-induced conformational restraint on the motions of the groups in aspartame indicated by the above studies suggests that decomposition of aspartame in aqueous solution could be inhibited to some extent by complexation. The present study is intended to demonstrate stabilization of aspartame in liquid preparations under conditions of temperature, pH and ionic strength which are typical of its uses. This approach suggests an equilibrium-controlled reservoir which would liberate free aspartame to replace material lost by degradation. It is implicit in this approach that increased total concentrations of aspartame would be required in formulated liquid products. HPLC was used to quantify aspartame and to separate it from some of its degradation products.

Materials and Methods

Materials

Aspartame (α-L-aspartyl-L-phenylalanine methyl ester, APM), 3-methylenecarboxyl-6-benzyl-2,5-diketopiperazine (DKP), α-L-aspartyl-L-phenylalanine (α-AP), L-phenylalanine methyl es-

ter (PM), β-L-aspartyl-L-phenylalanine methyl ester (β-APM), and L-phenylalanine (L-PHE) were used as supplied by the NutraSweet Co. (Deerfield, IL). α-Cyclodextrin (lot 130; Mol. Wt 972.9; Advanced Separation Technologies, Inc.) (α-CD), β-cyclodextrin (lot 127; Mol. Wt 1135.0; Advanced Separation Technologies, Inc.) (β-CD), γ-cyclodextrin (lot 132; Mol. Wt 1297.1; Advanced Separation Technologies, Inc.) (γ-CD), 2,6-dimethyl-β-cyclodextrin (lot 17-1; Mol. Wt 1331.3; gift from Professor H. Ueda) (DM-β-CD), 2,3,6-trimethyl-β-cyclodextrin (lot 3; Mol. Wt 1429.5; gift from Professor H. Ueda) (TM-β-CD), 2-hydroxyethyl-β-cyclodextrin (lot RR 6; average Mol. Wt 1750; average degree of substitution, 62–67%; American Maize Products Co., Hammond. IN) (HE-β-CD) and 2-hydroxypropyl-β-cyclodextrin (lot RR10, average Mol. Wt 1513, average degree of substitution, 31%; lot RR11A, average Mol. Wt 1542, average degree of substitution, 33%; lot EN90-1, average Mol. Wt 1366, average degree of substitution, 19%; lot EN90-4, average Mol. Wt 1370, average degree of substitution, 19%; American Maize Products Co., Hammond, IN) (HP-β-CD) were used as received; average degree of substitution estimates were from the FAB mass spectrometric method and were provided by American Maize Products. Solvents were Fisher HPLC grade and other chemicals were ACS reagent grade or better.

HPLC

Isocratic HPLC was performed with a Spectroflow 400 solvent pump (Kratos Analytical Instruments, Ramsey, NJ), 20 μl loop injector (Model 7125, Rheodyne, Cotati, CA), octylsilyl (C8) reversed-phase column (4.6 mm × 15 cm, 5 μm, nominally 13 500 plates) (Keystone Scientific, Inc., State College, PA) and a Spectroflow 757 variable-wavelength ultraviolet absorption detector (Kratos Analytical Instruments, Ramsey, NJ) (λ_{anal}, 210 nm). The mobile phase (flow rate, 1.2 ml/min) was 12.5% v/v acetonitrile and 87.5% v/v phosphate buffer (0.18 M sodium dihydrogen phosphate, adjusted to pH 2.1 with phosphoric acid), containing 0.020 M sodium heptanesulfonate and 0.114 M KCl. Data acquisition was performed by a Model 3392A integrator (Hewlett-Packard, Corvallis, OR). Instrument response was checked with standards of aspartame and each degradation product; it remained unchanged throughout the study. The following retention volumes were recorded: APM, 23 ml; DKP, 5.0 ml; PA, 7.9 ml; α-AP, 9.5 ml; PM, 15 ml; β-APM, 18 ml; L-PHE, 6.1 ml. In addition, an unidentified small peak was observed with retention volume 30–35 ml. Retention volumes slowly decreased with time, probably from column deterioration due to the low mobile phase pH. To accommodate this deterioration, the acetonitrile content was progressively decreased until it reached 11.0% v/v, at which point the column was replaced.

Reaction kinetics

Kinetic experiments were performed in stoppered 50 or 100 ml volumetric flasks maintained at constant temperature (25.0, 30.0, 35.0, 40.0 or 50.0 ± 0.1°C) in a large water bath controlled by a thermostat (Thermomix II, B. Braun, Melsungen, Germany) or in a laboratory refrigerator (4.0 ± 1.0°C). Reaction solutions were prepared at the following pH values: 2.0 (unbuffered phosphoric acid or phosphate buffer), 2.8–3.3 (phosphate buffer or citrate buffer) and 4.0 (acetate buffer or citrate buffer). The pH was checked before and after kinetic runs with a Model 611 Digital pH Meter (Orion Research Inc., Cambridge, MA), which was calibrated daily with pH 2.0, 4.0 or 7.0 standard solutions (Fisher) at the temperature of the kinetic measurements. Reaction solution buffers (0.05 or 0.15 M in the buffer component) were prepared in glass-distilled water and adjusted to the correct pH. Except in the case of unbuffered phosphoric acid, or where ionic strength was the experimental variable, ionic strengths were adjusted with KCl where necessary.

Reaction solutions were prepared by dissolving APM (50 mg) in the appropriate buffer (100 ml, prewarmed to the temperature of measurement), the volume adjusted to the mark, and then a zero-time aliquot was injected into the HPLC. A portion of the reaction solution was then used to dissolve a weighed amount of the desired cyclodextrin (usually a 5-fold molar excess in a 50

ml flask), the volume was adjusted to the mark, then a zero-time aliquot was injected. This ensured that both of the reaction solutions contained identical initial concentrations of APM (1.70 mM). Alternate injections of the uncomplexed and complexed reaction solutions were then made periodically. Pseudo-first order rate constants (k) and half-lives (t_{50}) were calculated from the slopes of linear semi-log plots of APM peak area as a function of time. Stabilization was evaluated by the percentage increase in t_{50} with a CD present, compared to that without the CD. Systems containing CDs which demonstrated little or no stabilization were followed for one half-life, while those which did exhibit stabilization were studied for two to three half-lives where possible.

Results and Discussion

Chromatography of APM reaction products

A typical chromatogram of a partly degraded solution of aspartame is shown in Fig. 1. PA and α-AP could only be separated with a mobile phase pH near 2. PA was tentatively identified in chromatograms by its (expected) formation from DKP. Hydrolysis of authentic DKP (pH 2) initially gave two peaks of almost equal area. One of these co-eluted with α-AP, while the other was taken to be PA, according to the expected hydrolysis pathways for DKP. In hydrolyses of APM, the peak for α-AP was always about 10-fold larger than that of PA, suggesting that most of the α-AP resulted from primary hydrolysis of the methyl ester, rather than secondary ring opening of DKP (Scheme 1). A small unidentified peak with retention volume 30–35 ml was more apparent in more concentrated solutions.

Stabilization with cyclodextrins

In agreement with previous reports (Mazur, 1976; Anon, 1987), pseudo-first order rate constants for hydrolysis of APM increased as pH was lowered from 4.0 to 2.0. Increased concentrations of all buffer species (especially phosphate) appeared to catalyze the loss of APM. No corrections have been made for these buffer catalytic effects.

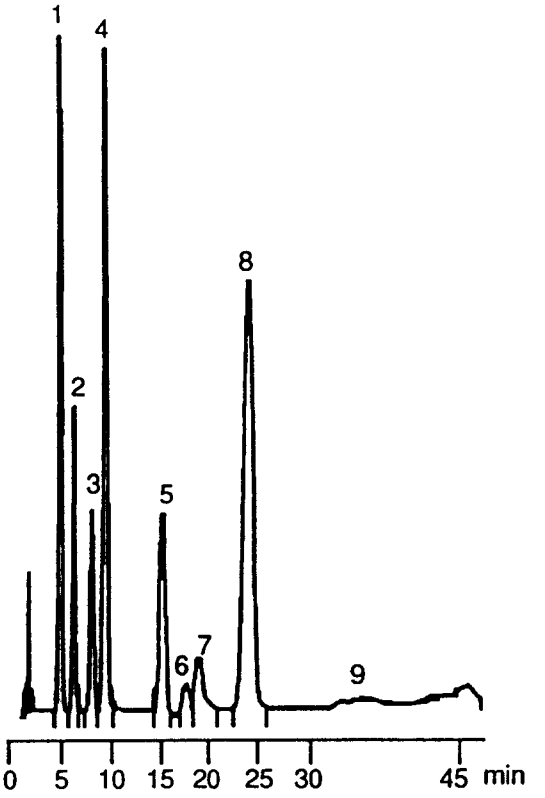


Fig. 1. Chromatogram of APM after incubation in unbuffered phosphoric acid (pH 2.0, 40.0°C) for 792 h. Peak identification: 1, DKP; 2, L-PHE; 3, PA; 4, α-AP; 5, PM; 6, unidentified; 7, β-APM; 8, APM; 9, unidentified.

Preliminary experiments with α-, β- and γ-cyclodextrins indicated that a CD : APM ratio of 1 : 1 demonstrated little or no stabilization, but a ratio of 5 : 1 did for those CDs that were effective. This result is not surprising, given the low reported equilibrium complexation constant of 128 M^{-1} for β-CD at 25°C (Moelands et al., 1992). Thus, a CD : APM ratio of 5 : 1 was used in all subsequent experiments. This ratio required the use of β-CD concentrations that approached its limit of saturation at the lowest temperature of the study. α-CD did not appear to stabilize APM at all (40°C, pH 2.0), while γ-CD was less effective than β-CD. The seven-unit β-cyclodextrin cavity appeared to be optimal for complexation of APM. 2,6-Dimethyl-β-cyclodextrin

TABLE 1

Rate constants and half-lives for degradation of APM with or without β-cyclodextrin

Agent	T (°C)	pH	Buffer [a]	$k(\times 10^4)$ (h^{-1})	t_{50} (days)	% increase in t_{50}
None	4.0	2.0	0 05 M P	0.748 [b]	386	
β-CD	4.0	2.0	0.05 M P	0.546 [b]	529	37
None	25.0	2 0	0.15 M P	10.2 [b]	28.4	
β-CD	25 0	2.0	0 15 M P	7.30 [b]	39.6	40
None	30.0	2.0	0.15 M P	13.7 [b]	21.0	
β-CD	30.0	2.0	0.15 M P	10.0 [b]	28.9	38
None	35.0	3 0	0.05 M P (I = 0.05)	10.5	27.6	
β-CD	35.0	3.0	0.05 M P (I = 0.05)	8.59	33.6	22
None	35.0	3.0	0.05 M P	6.80	42.5	
β-CD	35.0	3.0	0.05 M P	5.48	52.7	24
None	35.0	3.0	0.05 M P (I = 0.50)	6.97	41.4	
β-CD	35.0	3.0	0.05 M P (I = 0 50)	5.28	54.7	32
None	35.0	3.0	0.05 M C (I = 0.063)	6.84	42.2	
β-CD	35.0	3.0	0.05 M C (I = 0.063)	5.63	51.3	22
None	35.0	3 0	0.05 M C (I = 0.063)	6.71	43.0	
β-CD	35.0	3.0	0.05 M C (I = 0.063)	5.38	53.7	25
None	35.0	4.0	0.05 M C (I = 0.084)	3.49	82.8	
β-CD	35.0	4.0	0.05 M C (I = 0 084)	2.61	111	34
None	35.0	2.0	0 05 M P	15.7	18.5	
HP-β-CD [c]	35.0	2.0	0.05 M P	12.4	23.4	27
None	40.0	2.0	H_3PO_4 [d]	15.0	19.3	
α-CD (1 · 1)	40.0	2.0	H_3PO_4	14.9	19.4	0.3
None	40.0	2 0	H_3PO_4	15.0	19.2	
β-CD (1 : 1)	40.0	2.0	H_3PO_4	14.0	20.6	7 3
None	40.0	2.0	H_3PO_4	14.9	19.5	
γ-CD (1 : 1)	40.0	2.0	H_3PO_4	14 4	20.0	3.0
None	40.0	2.0	H_3PO_4	15.3	18.9	
β-CD	40.0	2.0	H_3PO_4	12.0	24.2	28
None	40.0	2.0	H_3PO_4	15.5	18.6	
DM-β-CD	40.0	2 0	H_3PO_4	11.7	25.6	32
None	40.0	2 0	H_3PO_4	15.0	19.2	
TM-β-CD	40.0	2.0	H_3PO_4	14.8	19.5	1.4
None	40 0	2.0	H_3PO_4	15.7	18.4	
HP-β-CD	40.0	2.0	H_3PO_4	12.0	24.1	31
None	40.0	2.0	0.15 M P	52.4	5.51	
HE-β-CD	40.0	2.0	0.15 M P	43.2	6.69	22
None	40.0	2.0	0.15 M P	52.4	5.51	
None	40.0	2.0	0.05 M P	14.0	20.6	
None	40.0	2.0	0.05 M P	14.2	20.3	
None	40 0	2.0	0.05 M P	14.2	20.4	
None	40.0	3.0	0.15 M P	16.0	18 1	
β-CD	40.0	3.0	0.15 M P	12.3	23 5	30

(continued overleaf)

TABLE 1 (continued)

Agent	T (°C)	pH	Buffers [a]	$k(\times 10^4)$ (h^{-1})	t_{50} (days)	% increase in t_{50}
None	40.0	3.0	0.15 M C (I = 0.190)	19.0	15.2	
β-CD	40.0	3.0	0.15 M C (I = 0.190)	17 7	16.3	7
None	40.0	3.0	0 15 M C (I = 0.190)	19 7	14.7	
β-CD	40.0	3.0	0.15 M C (I = 0 190)	17.5	16 5	13
None	40.0	4 0	0.15 M A	13.1	22 0	
β-CD	40.0	4 0	0.15 M A	10.6	27 1	23
None	40 0	4.0	0.05 M A	5.80	49 8	
None	40 0	4.0	0.15 M A	13.4	21 6	
β-CD	40 0	4 0	0.15 M A	10.5	27 4	27
None	40.0	4 0	0.05 M C (I = 0.084)	5 71	50.6	
β-CD	40 0	4 0	0.05 M C (I = 0.084)	4 50	64.2	27
None	40 0	4.0	0.15 M C (I = 0 343)	13.8	21 0	
β-CD	40.0	4 0	0.15 M C (I = 0.343)	11 2	25 8	23
None	40.0	4.0	0 15 M C (I = 0.343)	14.8	19 5	
β-CD	40.0	4.0	0.15 M C (I = 0 343)	12 8	22.6	16
None	40.0	4.0	0 15 M A	12.7	22.8	
DM-β-CD	40.0	4 0	0 15 M A	10.4	27 7	21
None	40.0	4.0	0.15 M A	13 6	21 3	
HP-β-CD	40 0	4 0	0 15 M A	11.6	25 0	17
None	50 0	2.0	0.05 M P	71.8	4.02	
β-CD	50.0	2.0	0 05 M P	58 6	4.93	23
None	50.0	3.0	0.15 M P	62 5	4 62	
β-CD	50.0	3.0	0.15 M P	47 9	6 03	31
None	50.0	3.3	0.05 M C (I = 0.063)	25.3	11.4	
β-CD	50 0	3.3	0.05 M C (I = 0.063)	21.1	13.7	20
None	50.0	4.0	0.05 M C (I = 0.084)	13.8	20.9	
β-CD	50.0	4.0	0.05 M C (I = 0.084)	11 6	24.8	19
None	50.0	2.0	0.05 M P	60.2	4.79	
HP-β-CD [c]	50.0	2.0	0.05 M P	54.0	5.35	12
None	50.0	2.0	0.05 M P	70.0	4.12	
HP-β-CD [e]	50.0	2.0	0.05 M P	60.8	4.75	15
None	50.0	2.0	0.05 M P	70.4	4 10	
HP-β-CD [f]	50.0	2.0	0.05 M P	58 6	4.93	20
None	50.0	2 0	0 05 M P	70.8	4.08	
HP-β-CD [g]	50.0	2.0	0.05 M P	59.9	4.82	18

[a] Buffers: P, phosphate; C, citrate; A, acetate; I, 0.15 M unless stated.
[b] Based on rate data for less than one half-life.
[c] Batch RR10, average degree of substitution, 31%, Amaizo.
[d] H_3PO_4: no buffer used, but the pH was adjusted to 2.0 by the addition of phosphoric acid.
[e] Batch RR11A, average degree of substitution, 33%, Amaizo.
[f] Batch EN90-1, average degree of substitution, 19%, Amaizo.
[g] Batch EN90-4, average degree of substitution, 19%, Amaizo.

and 2-hydroxypropyl-β-cyclodextrin appeared to be of similar effectiveness, compared to β-cyclodextrin (40°C, pH 2.0), while 2,3,6-trimethyl-β-cyclodextrin had no stabilizing effects at all. The lack of stabilizing effects for TM-β-CD is likely to be due to steric hindrance to complexation by the methyl groups. It has been previously reported that TM-β-CD is distorted in shape, due to steric effects of the methyls (Imai et al., 1988), although this distortion did not completely prevent complexation of flurbiprofen. Most of the remaining work was performed with β-cyclodextrin or 2-hydroxypropyl-β-cyclodextrin. Results of all kinetic runs are reported in Table 1. Although the data in the Table are the results of unreplicated experiments, the reproducibility can be gauged from a comparison of results where no complexing agent was used, e.g., the first seven entries of Table 1 at $T = 40$°C and no complexing agent, for which the rate constant was $(1.52 \pm 0.05) \times 10^{-3}$ h^{-1}.

Studies near room temperature (25.0–30.0°C) were frustrated by persistent mold growth in reaction solutions a few days after the commencement of each run. The results obtained at these temperatures are from runs which were followed for less than one half-life.

The effect of temperature on stabilization by complexation with β-CD is clearly seen in Fig. 2. There is a good inverse relationship between the percent increase in t_{50} and temperature (°C) over the temperature range 25–50°C. Data for reactions at 4°C were not compared with those at higher temperatures, as the ionic strengths were different. However, the formation of α-AP (the principal degradation product at pH 2) at 4°C was considerably reduced when β-CD was present (Fig. 3a), but the reduction was less when HP-β-CD was present. The temperature-dependent increase in stabilization probably results from an equilibrium complexation constant that increases in magnitude at lower temperatures. Some support for this hypothesis is provided by a recent flow microcalorimetric study of the binding of APM to β-CD (Moelands et al., 1992) and an NMR study (Takahashi et al., 1986). The microcalorimetric study found a value of 128 M^{-1} (S.D. 22) for the association constant in water at 25°C, while the NMR work (temperature unspecified, but typically 35–40°C) reported a value of 90 M^{-1}. The calorimetric enthalpy of complexation for β-CD ($-11\,700 \pm 700$ J mol^{-1}) was much more exothermic than for HP-β-CD (-2200 ± 200 J mol^{-1}). Substitution of the enthalpies for complex formation into the integrated van't Hoff equation indicates that the association constants for both complexing agents increase at lower temperatures. Temperature-dependent complexation constants have been previously reported for β-cyclodextrin complexes with dantrolene and with two of its analogs (Jansen et al., 1990). A similar dependence of APM stabilization on temperature is seen for HP-β-CD in phosphate buffer (pH 2.0). Under these conditions, the percent increase in t_{50} declined from 27% (35°C) to 13 ± 2% (50°C).

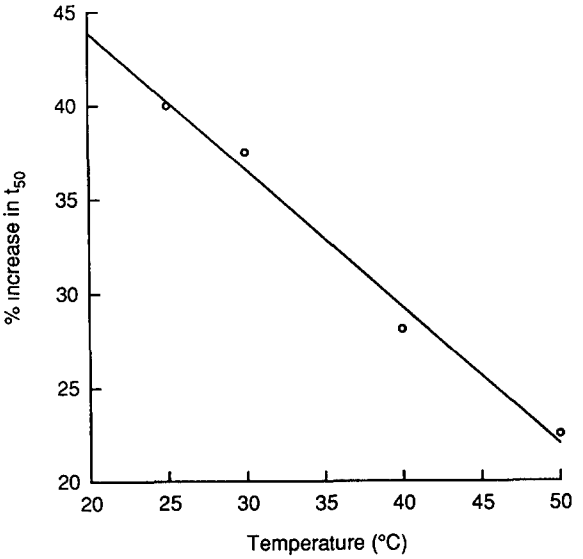
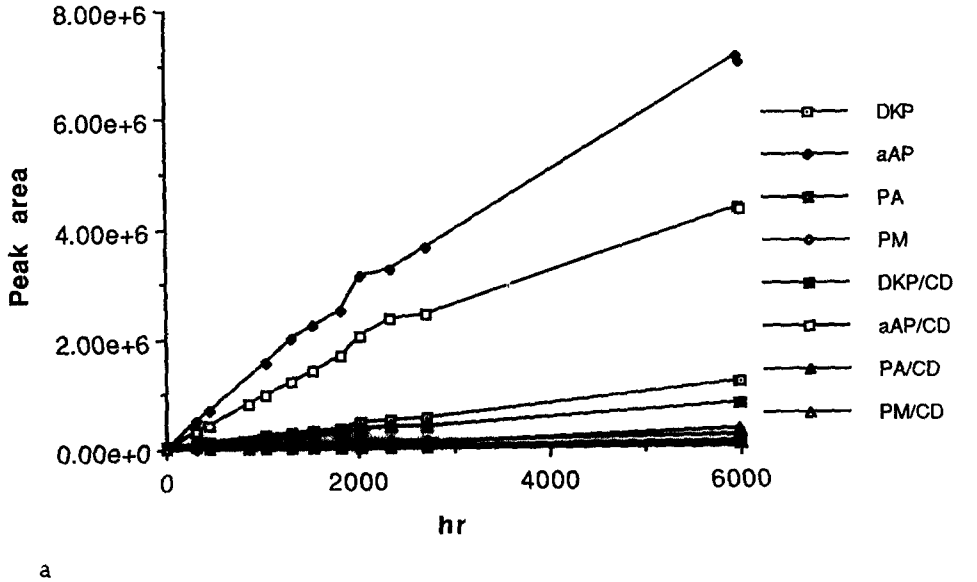


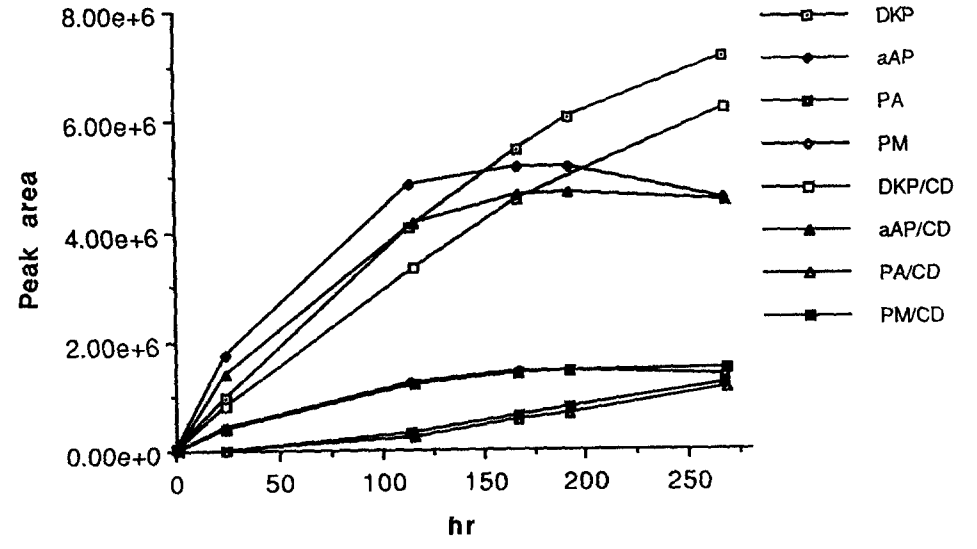
Fig. 2. Plot of stabilization of aspartame (% increase in t_{50}) by β-cyclodextrin ($I = 0.15$ M; pH 2.0) in phosphate buffer as a function of reaction temperature (°C).

Another feature of the kinetic runs at 4°C (pH 2.0) was the marked decrease in formation of DKP, both with and without the CD. At higher temperatures and the same pH, DKP (formed by intramolecular aminolysis) was present in similar amounts to α-AP (formed by ester hydrolysis), but at 4°C, it was only present to a similar extent to the minor products (Fig. 3a and b). Presumably, the intramolecular self-aminolysis reaction

pathway has a greater enthalpy of activation than the ester hydrolysis pathway. At 4°C and pH 2.0, the major degradation product was α-AP. This product can result from direct hydrolysis of the methyl ester, as well as from ring-opening of DKP. As DKP formation is much reduced at this



a



b

Fig. 3. Formation of reaction products (in terms of peak area) as a function of time at (a) 4°C and (b) 50°C. Plots show the effect of presence (/CD) or absence of β-CD on product formation.

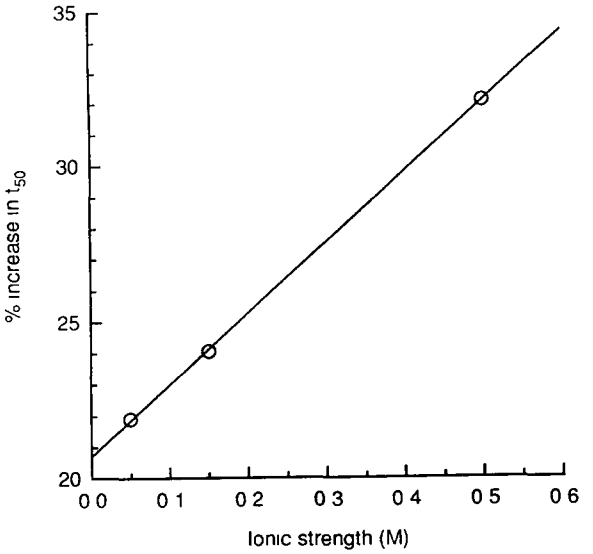


Fig. 4. Plot of stabilization of aspartame (% increase in t_{50}) by β-cyclodextrin (35°C; pH 3.0) in phosphate buffer as a function of ionic strength.

temperature, the bulk of the α-AP should result directly from ester hydrolysis. Fig. 3a and b shows that PA formation is much less than for α-AP (about one-tenth), at both 50 and 4°C, hence methyl ester hydrolysis appears to be a more important degradation pathway at all temperatures.

Fig. 4 indicates that there is a linear relationship between stabilization (as percent increase in half-life) and the ionic strength of the medium. Increased stabilization by β-cyclodextrin appears to result at higher ionic strengths. This could be explained in terms of the mechanism by which cyclodextrins form complexes with guest molecules. The interior of the cyclodextrin cavity contains water molecules in a rather hydrophobic environment (Szejtli et al., 1980). The driving force for complexation of an organic molecule will involve interactions: (i) between the host and the guest; and (ii) between water molecules released from the cavity of the host, water molecules released on dehydration of the guest (from both hydrophobic and dipolar hydration) and the bulk water. This is described by Eqn 1, which is a more exact representation of the equilibrium association constant, K_{assoc}

$$K_{assoc} = \frac{a_{CD-APM(H_2O)_w} \cdot a^x_{H_2O}}{a_{CD(H_2O)_y} \cdot a_{APM(H_2O)_z} \cdot a^w_{H_2O}} \quad (1)$$

The thermodynamic activities $a_{CD-APM(H_2O)_w}$, $a_{CD(H_2O)_y}$ and $a_{APM(H_2O)_z}$ are for the complexing species, and $y + z = x$, where x is the number of water molecules released to the bulk phase ($a^x_{H_2O}$) on formation of one molecule of the complex and w denotes the number of bulk water molecules ($a^w_{H_2O}$) needed to hydrate the complex. This type of complexation is driven by a favorable change in entropy, due to the increase in the number of bulk water molecules ($x > w$), as well as by an exothermic enthalpy change (Matsuyama et al., 1987; Eftink et al., 1989). An increased concentration of ionic species in the medium will reduce the thermodynamic activity of the bulk water molecules (by hydration of the ionic species). The reduction in activity can then be offset (to some extent) by liberation of more water molecules through increased formation of the host-guest complex.

As APM is an amphoteric dipeptide with acidic and basic macroionization constants, it can be expected that the association constant is pH dependent. No explicit studies were performed on this point. Some degree of pH-dependent stabilization can be seen by comparing results obtained at 35°C in citrate buffers at pH 3.0 (increase in t_{50}, 22–25%) and 4.0 (increase in t_{50}, 34%). Conversely, no significant stabilization differences are seen on comparing results for citrate buffer at 50°C at pH 3.3 or 4.0. However, these results are not strictly comparable, as they were obtained at different ionic strengths.

The lots of 2-hydroxypropyl-β-cyclodextrin used in this study were mixtures of many components, each with a variable number of hydroxypropyl groups per cyclodextrin molecule. β-CD has 21 hydroxyl groups which may be substituted by hydroxypropyl, although it should be recognized that hydroxypropylation maintains the total number of hydroxyl groups at 21. Substitution can therefore take place on the hydroxypropyl group

itself, leading to oligomerization. The effect of differences in the average degree of substitution (given as a percentage of the 21 available sites) for several lots of 2-hydroxypropyl-β-cyclodextrin could be seen from data at 50°C (Table 1, last four entries). The more highly substituted lots (31–33% substituted) were less efficient at increasing half-life (average increase, $13 \pm 2\%$) compared to less highly substituted lots (19% substituted), for which the percent increase in half-life was $19 \pm 1\%$. Under similar conditions, β-cyclodextrin gave an increase in half-life of 22.5%.

The differences in these results show that HP-β-CD does not stabilize APM as well as β-CD, and suggest that the hydroxypropyl groups may interfere to some extent with the host-guest complexation phenomenon. This interpretation is supported by the almost total lack of stabilization seen with TM-β-CD, which is exhaustively methylated. These results are also supported by a recent solution calorimetric study (Moelands et al., 1992), in which complexation of APM by β-CD at 25°C in aqueous solution was found to have an equilibrium constant of 128 M^{-1} (S.D., 22 M^{-1}), while the same constant for HP-β-CD (under identical conditions) had a value of 46 M^{-1} (S.D., 9 M^{-1}). These data indicate that the affinity of aspartame for β-CD is substantially greater than that for HP-β-CD. The calorimetric study used the same batches of 31–33% substituted HP-β-CD as those in the present study. Other techniques, such as circular dichroism or NMR relaxation could also be used to further investigate these observations.

The kinetic results in this study and the calorimetric data (Moelands et al., 1992) together suggest that hydroxypropylation, while increasing cyclodextrin solubility when an amorphous mixture of components is present (Rao et al., 1990), reduces complexation, especially when the extent of substitution is higher rather than lower. Other literature reports, to the effect that lower degrees of substitution have higher complexing ability (Müller and Brauns, 1986; Yoshida et al., 1988), are in agreement with the present results. Although HP-β-CD mixtures seem to be not as good as β-CD at protecting APM from degradation, their far greater total aqueous solubility (compared to β-CD) has made them worthy of further study. The greater solubility is due to the fact that present syntheses of 2-hydroxypropyl-β-cyclodextrin result in amorphous mixtures with numerous isomeric and oligomeric components which are presumed to have solubilities which are independent of each other (Rao et al., 1990). A very recent study (Rao et al., 1992) using 2-hydroxypropyl-β-cyclodextrins with a wide range for the average degree of substitution reported that, for phenolphthalein, the complexation constant varied substantially (10-fold). At very low degrees of substitution, the complexation constant was higher than that for β-cyclodextrin, and then began to decrease. The samples with the highest complexation constants (~2-fold higher) had aqueous solubilities which were less than those of β-cyclodextrin itself.

Acknowledgments

This study was funded by the NutraSweet Co., Deerfield, IL. The authors are grateful for support and constructive criticism from Dr H.J. Lambert.

References

Anon, *Technical Bulletin*, The NutraSweet Co. (1987).

Bhatt, N., Patel, K. and Borchardt, R.T., Chemical pathways of peptide degradation. I Deamidation of adrenocorticotropic hormone. *Pharm. Res.*, 7 (1990) 593–599.

Brewster, M.E., Loftsson, T., Baldvinsdóttir, J. and Bodor, N., Stabilization of aspartame by cyclodextrins. *Int J Pharm*, 75 (1991) R5–R8.

Eftink, M., Andy, M.L., Bystrom, K., Perlmutter, H D. and Kristol, D., Cyclodextrin inclusion complexes: Studies of the variation in the size of aliphatic guests. *J. Am Chem Soc.*, 111 (1989) 6765–6772.

Gaines, S.M. and Bada, J L., Aspartame decomposition and epimerization in the diketopiperazine and dipeptide products as a function of pH and temperature. *J. Org. Chem*, 53 (1988) 2757–2764.

Imai, T., Otagiri, M., Saito, H. and Uekama, K., Inclusion mode of flurbiprofen with β-cyclodextrin and heptakis(2,3,6-*O*-trimethyl)-β-cyclodextrin, and improvements of some pharmaceutical properties of flurbiprofen by complexation. *Chem. Pharm. Bull.*, 36 (1988) 354–359

Jansen, A.C.A , Hılbers, H.W., Nı, X R , Van Helden, S.P., and Janssen, L.H.M., Some physıcal-chemical properties of dantrolene and two of ıts analogues. *Int J Pharm.*, 75 (1990) 193–199.

Kırsch, L.E., Molloy, R.M., Debono, M., Baker, P. and Farid, K.Z., Kinetıcs of the aspartyl transpeptidatıon of daptomycın, a novel lipopeptide antıbıotıc *Pharm. Res.*, 6 (1989) 387–393

Maheswaran, M.M. and Divakar, S., Studıes on the structure of the ınclusıon compound of β-cyclodextrin wıth aspartame. *Indıan J Chem.*, 30A (1991) 30–34

Matsuyama, K., El-Gızawy, S. and Perrın, J.H., Thermodynamics of bındıng of aromatic amino acıds to α-, β- and γ-cyclodextrıns *Drug Dev Ind Pharm* , 13 (1987) 2687–2691

Mazur, R H., Aspartame – a sweet surprıse *J Tox Envıron Hlth*, 2 (1976) 243–249

Moelands, D , Karnık, N.A., Prankerd, R.J., Stone, H W., Sloan, K B and Perrın, J H., Mıcrocalorımetric study of the ınteractıons of aspartame wıth β-cyclodextrın and hydroxypropyl-β-cyclodextrin The anomalous heat of dılutıon of the latter. *Int J Pharm.* 86 (1992) 263–265

Muller, B W. and Brauns, U , Hydroxypropyl-β-cyclodextrın derıvatıves, ınfluence of the average degree of substitution on complexıng abılıty and surface activity *J Pharm. Scı.*, 75 (1986) 571–572.

Ojıma, T , *European Patent* 0 097 950 B1 (1983)

Patel, K. and Borchardt, R.T., Chemical pathways of peptıde degradation. II. Kınetics of deamidation of an asparagınyl residue ın a model hexapeptıde. *Pharm Res.*, 7 (1990) 703–711.

Rao, C.T., Fales, H.M. and Pitha, J., Pharmaceutıcal usefulness of hydroxypropylcyclodextrins· "E pluribus unum" ıs an essentıal feature. *Pharm Res* , 7 (1990) 612–615.

Rao, C.T., Pıtha, J , Lındberg, B and Lindberg, J , Dıstrıbutıon of substituents in *O*-(2-hydroxypropyl) derıvatıves of cyclomalto-olıgosaccharides (cyclodextrıns)· ınfluence of ıncreasing substıtutıon, of the base used ın the preparation, and of macrocyclic sıze. *Carbohydr Res.*, 223 (1992) 99–107.

Szejtlı, J., Bolla-Pusztaı, E , Szabo, P and Ferenczy, T , Enhancement of stabılity and bıological effect of cholecalciferol by β-cyclodextrın complexation *Pharmazıe*, 12 (1980) 779–787

Takahashı, S., Suzukı, E and Nagashıma, N , NMR study on inclusıon complexes of L-phenylalanine and aspartame wıth cyclodextrıns ın aqueous solutıon. *Bull. Chem Soc Jap.*, 59 (1986) 1129–1132.

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