Stability of Aspartame in Water:Organic Solvent Mixtures with Different Dielectric Constants

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Abstract \Box In order to examine the influence of solvent composition on the stability of aspartame (*N*- α -L-aspartyl-L-phenylalanine-1-methyl ester) in solution (5 mg/mL), the degradation of aspartame was carried out in water:methanol, water:ethanol, and water:glycerine mixtures with dielectric constant values of 45, 55, and 65, respectively. The rate of disappearance of aspartame was measured by a sensitive HPLC assay. The degradation rate of aspartame increased as the dielectric constant of the solvent mixture decreased in all three solvents systems. For example, at 60 °C, the degradation rate constant swere 4.1, 5.9, and 8.4 × 10⁻³ h⁻¹ at dielectric constant of 65, 55, and 45, respectively. From these results, it can be concluded that the stability of aspartame in aqueous solutions cannot be enhanced by the replacement of water by solvents of lower dielectric constant.

The hydrolytic, or solvolytic, decomposition of liquid pharmaceuticals is not a new problem. In many instances, the usual remedies of inhibiting, or avoiding, solvolysis include judicious maintenance of pH, careful selection of a buffer system and buffer concentration, complexation, use of a less soluble form of the drug, use of surfactants, modification of the chemical structure, and partial or total replacement of water with a solvent of lower dielectric constant. The stability of many drugs in solution, including barbiturates, diazepam, and aspirin, have been significantly increased by the partial or total replacement of water by propylene glycol, ethanol, polyethylene glycol, or glycerine.¹⁻³ However, some drugs, such as chloramphenicol, show an increased rate of decomposition as the proportion of propylene glycol increases.⁴ The change in the stability of these drugs in these solvents must be caused by the change in some property of the medium itself. From a chemical kinetics viewpoint, dielectric constant is recognized as one of the fundamental properties which influence the solvolytic reaction rate.⁵

This investigation is a study of the effect of solvent composition on the decomposition of aspartame $(N-\alpha$ -L-aspartyl-L-phenylalanine-1-methyl ester) in water: alcohol solutions. The stability of aspartame in solution has been shown to be a function of time, temperature, and pH.^{6–8} In solution, aspartame decomposes via a series of reactions that include ester and peptide hydrolysis and cyclization to diketopiperazine (5-benzyl-3,6-dioxo-2-piperazineacetic acid).⁶

Experimental Section

Materials—Drugs and Reagents—Aspartame, N- α -L-aspartylphenylalanine, and L-phenylalanine were obtained from Sigma Chemical Company (St. Louis, MO), diketopiperazine was from U.S.P.C., Inc. (Rockville, MD), and acetonitrile, HPLC grade, was from Mallindcrodt. All other chemicals were reagent grade.

Methods and Equipment—A Waters HPLC system (Waters Associates, Milford, MA) equipped with a multiple wavelength detector (model 450, Waters Associates Inc.) and a model 3390A recorder integrator (Hewlett Packard Company) was used. The stationary phase was a μ Bondapak Phenyl (Waters Associates Inc.)

Analytical Method-A reversed-phase HPLC method was used to

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follow the disappearance of aspartame in the samples with time. The mobile phase was 5% (v/v) acetonitrile in 0.01 M potassium hydrogen phosphate (pH 2.5). A UV detector set at 215 nm was used with guaifenesin as the internal standard. The flow rate was 1.5 mL/min. Chart speed was 1 cm/min, and the temperature was ambient.

Preparation of Samples for Stability—Three solvent systems, water:ethanol, water:methanol, and water:glycerine, and three storage temperatures, 40, 50, and 60 °C, were used in this study. At each storage temperature, mixtures of these solvent systems were prepared with dielectric constant values of 45, 55, and 65 respectively. Equation 1 was used to calculate the fraction of the organic solvent in these mixtures:

$$f_{\rm org} = \frac{D_{\rm mix} - D_{\rm water}}{D_{\rm org} - D_{\rm water}} \tag{1}$$

where $f_{\rm org}$ is fraction of the organic solvent, $D_{\rm mix}$ is dielectric constant of the solvent mixture, $D_{\rm water}$ is dielectric constant of water, and $D_{\rm org}$ is dielectric constant of the organic solvent. Because dielectric constants change with temperature, the values for ethanol, methanol, and glycerol used in this study are shown in Table I. At each temperature, three samples were prepared for each solvent system using the solvent mixtures with dielectric constant values of 45, 55, and 65. Each sample was prepared in duplicate so there were 18 samples at each of the storage temperatures used.

Aspartame was dissolved in an aqueous solution of malic acid and the calculated amount of the organic solvent was added. The apparent pH of the sample was then adjusted by the dropwise addition of a 1 M solution of NaOH. Sodium chloride was used to adjust the ionic strength. The composition of all the samples was the same except for the alcohol concentration. Aspartame concentration was 5 mg/mL, malic acid buffer concentration was 0.05 M, apparent pH was 4.5 (±0.1), and ionic strength was 0.26. The samples (final volume 25 mL) were put in water baths set at 40, 50, and 60 °C (±1 °C). Samples were allowed 30 min to equilibrate, then the initial aliquots (1 mL) were withdrawn. Other aliquots were taken at predetermined intervals until the concentration of aspartame remaining in the solution was one-half of the initial concentration or less. Residual aspartame, in each sample, was assayed at least six times in order to determine the degradation rate constant.

Table I—Dielectric Constants of Solvents at Different Temperatures

Solvent	Temperature, °C	Dielectric Constant
Ethanol	40	22.20
	50	20.87
	60	19.55
Methanol	40	29.03
	50	27.44
	60	25.97
Glycerine	40	37.30
	50	35.53
	60	33.82
Water	40	73.12
	50	69.85
	60	66.62

Experimental Procedure for the pH-Rate Profile-In this experiment, only the water:ethanol solvent system was used. The solvents used were as follows: water, where the dielectric constant at 60 °C is 66.6 (Table I); water:ethanol (24.7%), where the dielectric constant at 60 °C is 55; and water:ethanol (45.9%), where the dielectric constant at 60 °C is 45 (Table II). Duplicate samples of aspartame (5 mg/mL) were prepared at pH 2.5, 3.5, 4.5, 5.5, and 6.5 for each of these three solvents. The apparent pHs of samples were adjusted as described in the preceding section. Ionic strength was also adjusted to 0.26 with NaCl. The samples were equilibrated in a waterbath at 60 °C for 30 minutes. Initial aliquots (1 mL) were then withdrawn, and other aliquots were taken at predetermined intervals until the concentration of aspartame remaining in the solution was one-half of the initial concentration or less. Residual aspartame, in each sample, was assayed at least six times in order to determine the degradation rate constant. The experiment was repeated.

Results

Tables III and IV and Figure 1 summarize the results of this study. The decomposition of aspartame in these solvent systems followed pseudo-first-order kinetics. The plot of the logarithm of the concentration of aspartame remaining in the sample versus time (in hours) was linear, with negative slope. The slope of this plot is the rate constant of the degradation of aspartame. The rate constants presented in Table III are the mean and SD of the rate constants determined for four samples.

Discussion

The kinetic data in this study were examined by the following equation:¹

$$\ln k_0 = \ln k_i - \frac{z_a z_b e^2}{DkTr}$$
(2)

where k_0 is a reaction rate at zero ionic strength, k_i is the limiting rate at infinite dielectric constant and zero ionic strength, $z_a z_b$ are valencies of the two ions, and D, k, T, e, and r are, respectively, dielectric constant, Boltzmann constant, absolute temperature, charge of ion, and distance to which two ions must approach in order to react. According to eq 2, reaction rate between like-sign ions decreases as dielectric constant decreases, and reaction rate between unlike-sign ions increases as dielectric constant decreases. The data in Table III show that at all three temperatures used (40, 50, and 60 °C), the rate constant of aspartame decomposition decreased as the dielectric constant increased. These results suggest that the observed reaction rate is significantly influenced by the reaction between ions of unlike signs, such as the reaction between the ammonium ion of aspartame and the hydroxide ion. The relationship between the natural logarithm of the rate constant of the degradation of aspartame and

Table II—Chemical Composition of the Hydro-Organic Mixtures at Dielectric Constant Values of 65, 55, and 45

Solvent System	Dielectric	Percent of Organic Solvent (v/v)		
•	Constant	60 °C	50 °C	40 °C
Water:ethanol	65	3.4	9.9	15.9
	55	24.7	30.3	35.6
	45	45.9	50.7	55.2
Water:methanol	65	4.0	11.4	18.4
	55	28.6	35.0	41.1
	45	53.2	58.6	63.8
Water:glycerine	65	4.9	14.1	22.7
5,	55	35.4	43.3	50.6
	45	65.9	72.4	78.5

Table III—Stability of Aspartame in Solvent Systems with Different Dielectric Constants

Temperature,	Dielectric Constant	Mean Rate Constant ($-k \times 10^{-3}$), h ^{-1a}		
°C		Water:EtOH	Water:MeOH	Water:Glycerine
60	65	4.36	4.10	4.81
		(0.57)	(0.33)	(0.67)
	55	5.55	5.93	5.74
		(0.39)	(0.08)	(0.47)
	45	8.63	8.84	7.96
		(0.51)	(0.81)	(0.71)
50	65	1.66	1.73	1.67
		(0.15)	(0.17)	(0.16)
	55	2.40	2.52	2.23
		(0.52)	(0.45)	(0.33)
	45	3.85	4.45	2.89
		(0.35)	(1.12)	(0.71)
40	65	0.82	0.82	0.74
		(0.06)	(0. 08)	(0.20)
	55	1.25	1.34	1.00
		(0.12)	(0.39)	(0.25)
	45	2.17	2.08	1.41
		(0.04)	(0.37)	(0.01)

^a Standard deviation is given in parentheses (n = 4).

 Table IV—Activation Energy of the Decomposition of Aspartame in Water:Alcohol Solutions

Solvent System	Dielectric Constant	Activation Energy, kcal/mol
Water:ethanol	65	17.412
	55	15.542
	45	14.664
Water:methanol	65	16.796
	55	15.501
	45	15.133
Water:glycerine	65	19.514
•••	55	18.053
	45	18.032

the reciprocal of the dielectric constant of the solvent mixtures is linear with a positive slope. The slope of this line, S, can be used to calculate r, in the following equation:¹

$$S = \frac{d \ln k_0}{d(1/D)} = -\left(\frac{z_a z_b}{kT}\right) \left(\frac{1}{r}\right)$$
(3)

The order of magnitude of r should be that of the radius of a molecule (i.e., 10^{-8} cm). If the experimentally obtained r is in this order, it can be stated that the change of rate constant to be tested is attributed to the change of dielectric constant and that there is no specific solvent effect on the reaction rate.⁵ The values of r calculated from S were 4.32×10^{-8} , 4.05×10^{-8} , and 6.29×10^{-8} cm in water:ethanol, water:methanol, and water:glycerine mixtures, respectively. These values are in the range of the theoretical requirements.

Another test with respect to the effect of dielectric constant is the difference in activation energy observed when solvents of different dielectric constants are used. For a reaction between like-sign ions, a larger activation energy is necessary in a medium of lower dielectric constant because repulsion between ions increases as the dielectric constant decreases. The difference in activation energy (dE_c) at dielectric constant D_1 and D_2 is represented as follows:⁵

$$dE_{c} = -329.7 \frac{z_{a} z_{b}}{D_{1} D_{2} r} (dD)$$
(4)



Figure 1—The pH–rate profile for the degradation of aspartame at 60 °C in (\bigcirc) water, (\blacksquare) a 24.7% ethanol:water mixture, and (\blacktriangle) a 45.9% ethanol:water mixture.

where dD is the difference of two dielectric constants. Equation 4 demonstrates that the difference in activation energy of the reaction between like-sign ions must increase as the dielectric constant decreases. The activation energies calculated from the Arrhenius plots are shown in Table IV. It is observed that activation energy as well as dE_c increase as the dielectric constant of the medium increases. This observation also supports the suggestion that the reaction studied was between unlike-sign ions.

The monohydric alcohols, methanol and ethanol, were used in order to examine whether the molecular size of the alcohol had an effect on the rate of aspartame degradation. The results showed that the molecular size of the alcohol did not have an effect on the rate of degradation of aspartame. The rate constants of degradation of aspartame in water:methanol mixtures were the same, within experimental error, as the rate constants in water:ethanol mixtures. Methanol was used only for this experimental purpose; it has no practical application as a pharmaceutical solvent because of its toxicity. A more useful pharmaceutical solvent to include in this study would have been propylene glycol; however, information needed to calculate the dielectric constants of mixtures of propylene glycol with water was not available. Dielectric constants of solvent systems used in this study were calculated from tables prepared by Akerlof.⁹

Figure 1 shows the pH-rate profiles of aspartame using water, water:24.7% ethanol, and water:45.9% ethanol as solvents (samples were stored at 60 °C). It can be seen that the increase in the degradation rate constant, in the pH range of 3.5 to 4.8 which accompanied the addition of ethanol to the aqueous solution of aspartame, was not caused by a pH change. The pH-rate plots of aspartame in water:ethanol mixtures were not significantly shifted either to the right or to the left of the pH-rate plot of aspartame in water. In addition, the shape of these three plots are similar.

Therefore, it can be concluded that the increase in the rate of aspartame decomposition which occurs with the addition of ethanol, methanol, or glycerine to aqueous solutions of aspartame may be attributed to a decrease of the dielectric constant of the medium. The stability of aspartame in aqueous solution cannot be enhanced by the replacement of water by solvents of lower dielectric constants.

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