DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 8(6), 857-868 (1982)

DIFFERENTIAL SCANNING CALORIMETRY OF AMPICILLIN-ASPARTAME MIXTURE

Hamed H. El-Shattawy , Dane O. Kildsig and Garnet E. Peck

Department of Pharmaceutics, Division of Pharmacy, Faculty of Medicine, Al-Azhar University, Nasr-City, Cairo, Egypt.
Department of Industrial and Physical Pharmacy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907, U.S.A.

ABSTRACT

The possible interaction of anhydrous and trihydrate ampicillin with aspartame, in the solid state, was investigated by comparing the thermal behavior of physical mixtures of the respective original components in different molar ratios, using differential scanning calorimetry. Aspartame was found to form complexes with anhydrous ampicillin and ampicillin trihydrate. These complexes were found to be dependent on the molar ratios of the mixture components. One of the complexes between anhydrous and trihydrate ampicillin and aspartame, as determined from the enthalpy change of the DSC transitions of the mixtures, was found to have a 2:1 molar ratio.

Correspondence should be addressed to Hamed H.El-Shattawy.

INTRODUCTION

Ampicillin and its sodium salt were reported to be inactivated by dextrose[1-5], glycerol or propylene glycol[6]. The degradation of 6-aminopenicillanic acid was found by Moss and Cole[7] to be accelerated in the presence of dextrose, maltose or lactose. Schneider and de Weck[8] found a reaction between benzylpenicillin and a number of carbohydrates, including reducing sugars nonreducing sugars, dextran and simple glycols.

Hem et al.[9] studied the formation of 1:1 molar compelxes between sucrose and a number of penicillins including anhydrous ampicillin. The present authors[10] utilized DSC to confirm the formation of 1:1, 2:3 and 1:3 molar complexes between anhydrous and trihydrate ampicillin and anhydrous dextrose.

El-Shattawy[11] reported that anhydrous ampicillin was found to be incompatible with sorbitol and Di-Pac, and appears to form complexes with mannitol, granular mannitol and Brownex sugar after their melting transitions.

Aspartame, a new intense sweetener, is N-L-aspartyl-L-phenylalanine-1-methyl ester. The presence of both the free, unsubstituted amino and one carboxyl group of aspartic acid, as well as the distance between them and the absolute configuration of the asymmetric carbon, are completely critical for sweetness[12]. The sweetener was found to fall off rapidly with increasing size of the ester radical[12].

In this study the authors investigate the possible interaction of anhydrous and trihydrate ampicillin with aspartame in the solid state. This is acheived by comparing the thermal behavior, using DSC, of physical mixtures of the respective original components in different molar ratios.

EXPERIMENTAL

Materials

The following materials were used: anhydrous ampicillin (Wyeth), ampicillin trihydrate (Bristol) and aspartame (G.D. Searle and Co.).

Preparation of Physical Mixtures

Physical mixtures of anhydrous ampicillin and aspartame were prepared by mixing then using a mortar and pestle in the following molar ratios: 1:0.17, 1:0.20, 1:0.25, 1:0.33, 1:0.50, 1:1.00, 1:1.50, 1:2.00, 1:2.50, 1:2.75, 1:3.00, 1:4.00, 1:5.00, 1:6.00, 1:7.00 and 1:9.00. Physical mixtures of ampicillin trihydrate and aspartame were prepared in the following molar ratios: 1:0.17, 1:0.20, 1:0.25, 1:0.33, 1:0.50, 1:1.00, 1:2.00, 1:3.00, 1:4.00, 1:5.00 and 1:6.00.

Differential Scanning Calorimetry

Samples (5 mg) were weighed after being finely powdered and encapsulated in flat-bottomed aluminum pans with crimped-on lids. The samples were heated in an atmosphere of nitrogen and thermograms were obtained on a Perkin-Elmer DSC-1B Differential Scanning Calorimeter. Thermograms were obtained by heating at a constant heating rate of 10^{o}C per minutes, a constant range setting of 8 mcal per second and recorded at a constant chart speed of one inch per minute. The individual substances and the physical mixtures of ampicillin, anhydrous and trihydrate, and aspartame were heated over the temperature range 30 to 280^{o}C.

The area under the differential scanning calorimetric heating curve was measured using a K & E planimeter and the heat of transition was then calculated as described previously[13]. At least two replicates were made for each DSC thermogram.

RESULTS AND DISCUSSION

DSC thermograms of anhydrous ampicillin exhibit no transition when scanned over the temperature range of 30 to 214°C[10]. At 214°C anhydrous ampicillin decomposed. Therefore, DSC thermograms of physical mixtures of anhydrous ampicillin with aspartame will reflect the characteristic features of the latter if no interaction occurred.

In a previous investigation[13], aspartame has been shown by DSC to have two endothermic peaks. The first one, with a transition temperature range from 167-190°C and with a maximum peak of transition at 185°C, represents the loss of the methyl ester and conversion to the dipeptide, aspartylphenylalanine. The second peak, with a transition temperature range from 234-254°C and with a maximum peak of transition at 240°C, represents the conversion to diketopiperazine (DKP). At 266°C the DKP decomposed.

Figure 1 illustrates the DSC thermograms of anhydrous ampicillin and aspartame, separately and in physical mixtures, while Figure 3 illustrates the enthalpy change of the physical mixtures as a function of composition. The data for Figure 3 is shown in Table 1. DSC thermograms of 1:0.17, 1:0.20, 1:0.25 and 1:0.33 molar ratios of anhydrous ampicillin-aspartame physical mixtures showed a broadened endothermic peak with an average transition temperature range from 145-180°C and with an average maximum peak of transition at 165°C. This peak corresponds to the first peak of aspartame with a shift to lower temperatures from that of pure aspartame. The decomposition temperatures of these mixtures were found to be 198, 196, 192 and 191°C, respectively, i. e., the mixtures decomposed at temperatures which are markedly lower than those of the pure respective original

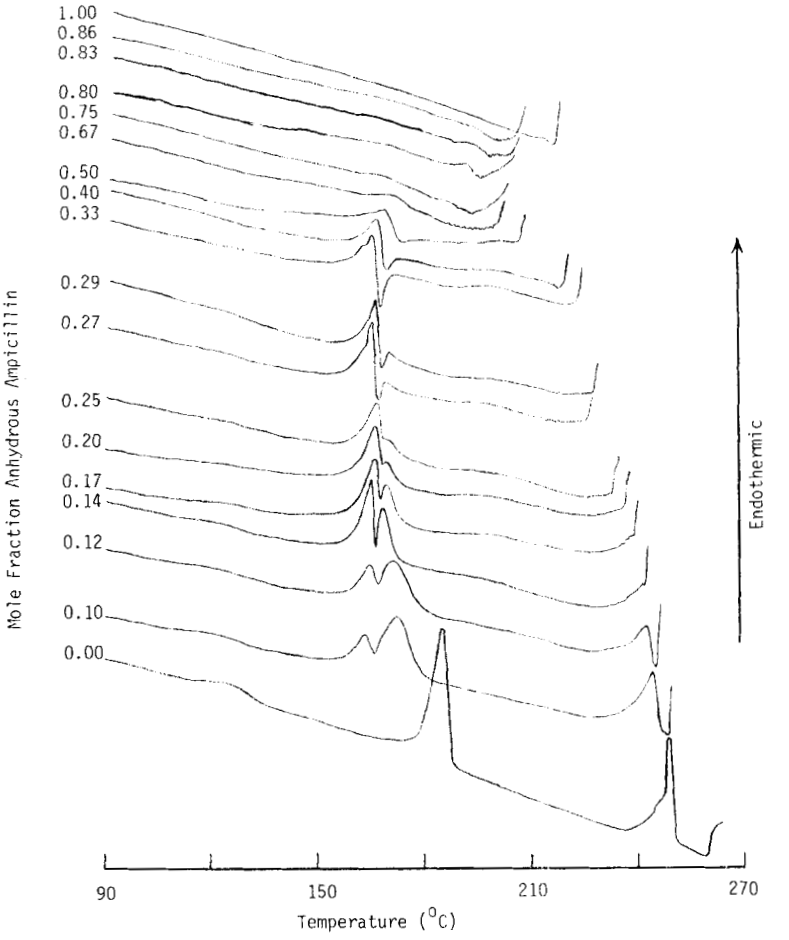

FIGURE 1

DSC thermograms of anhydrous ampicillin and aspartame separately and in physical mixtures.

components with the decomposition temperature decreasing as the concentration of aspartame in the mixture increased. The enthalpy change of these mixtures were found to be 34 to 42 percent less than the predicted values calculated from the exact percentage contribution of aspartame to the total enthalpy change of the mixture.

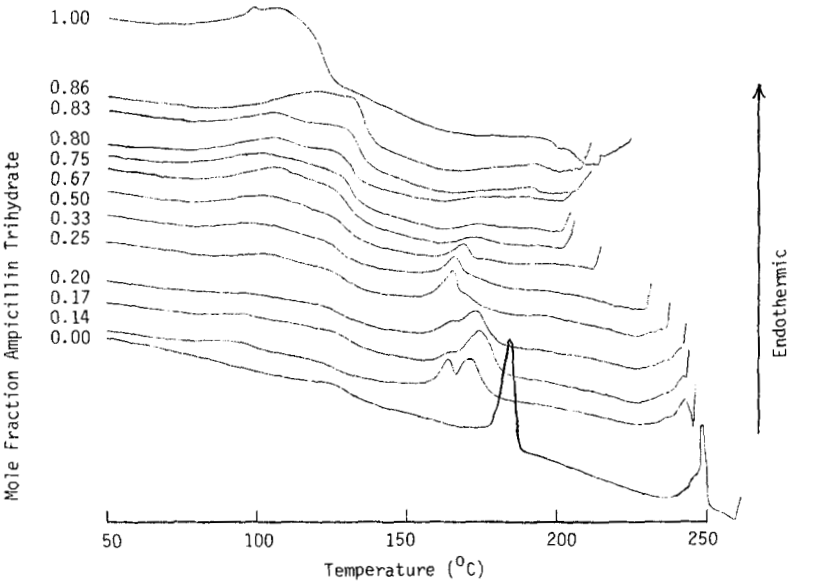

FIGURE 2

DSC thermograms of ampicillin trihydrate and aspartame separately and in physical mixtures.

This decrease in the enthalpy change indicates interaction between anhydrous ampicillin and aspartame in the solid state under the experimental conditions.

A 1:0.5 molar ratio of anhydrous ampicillin-aspartame physical mixture showed the same peak as in the case of the previously mentioned physical mixtures but with a transition temperature range from 140-180°C and with a maximum peak of transition at 170°C. At this molar ratio, the decomposition temperature of 186°C was the minimum observed for all the physical mixtures investigated. The immediate decomposition of the anhydrous ampicillin mixture after the transition and at a temperature markedly lower than those of the pure respective original components is in agreement with previous

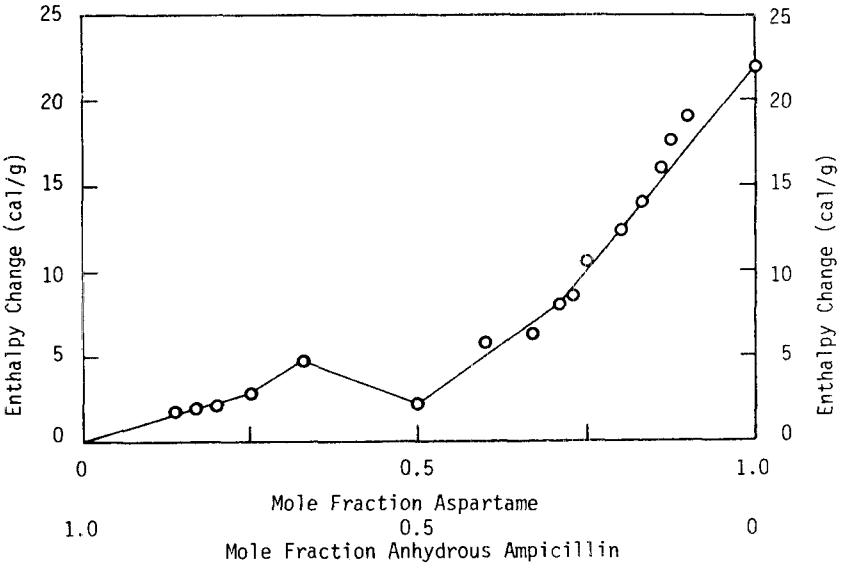

FIGURE 3

Enthalpy change of anhydrous ampicillin-aspartame physical mixtures as a function of composition.

conclusion[10] that complexed ampicillin decomposes at markedly lower temperatures than uncomplexed ampicillin. This finding is also in agreement with the conclusion of Hem et al.[9] in that the complexed penicillin degrades 5-6 times as fast as the uncomplexed penicillin and results in an increased overall rate of degradation.

When the enthalpy change of the physical mixtures was plotted against the mole fraction of the components (Figure 3), the enthalpy change was found to pass through a maximum corresponding to 1:0.5 (2:1) molar ratio of anhydrous ampicillin-aspartame physical mixture. Since enthalpy change is an additive property, this maximum represents an optimum complexation ratio according to the method of continous variation for complexation analysis[14].

TABLE 1
Enthalpy Change of Ampicillin-Aspartmame Mixtures as a Function of Composition

Anhydrous Ampicillin-Aspartame Molar Ratio (mole/mole)	Mole Fraction		Enthalpy Change Cal/g	Ampicillin *Trihydrate*-Aspartame Molar Ratio (mole/mole)	Mole Fraction		Enthalpy Change, Cal/g
	Anhydrous Ampicillin	Aspartame			Ampicillin *Trihydrate*	Aspartame	
1 : 0.00	1.00	0.00	——	1 : 0.00	1.00	0.00	——*
1 : 0.17	0.86	0.14	1.80	1 : 0.17	0.86	0.14	0.40
1 : 0.20	0.83	0.17	1.92	1 : 0.20	0.83	0.17	0.60
1 : 0.25	0.80	0.20	2.20	1 : 0.25	0.80	0.20	0.89
1 : 0.33	0.75	0.25	2.88	1 : 0.33	0.75	0.25	1.66
1 : 0.50	0.67	0.33	4.73	1 : 0.50	0.67	0.33	2.45
1 : 1.00	0.50	0.50	2.19	1 : 1.00	0.50	0.50	1.99
1 : 1.50	0.40	0.60	5.79				
1 : 2.00	0.33	0.67	6.28	1 : 2.00	0.33	0.67	6.01
1 : 2.50	0.29	0.71	8.05				
1 : 2.75	0.27	0.73	8.52				
1 : 3.00	0.25	0.75	10.61	1 : 3.00	0.25	0.75	9.93
1 : 4.00	0.20	0.80	12.46	1 : 4.00	0.20	0.80	14.14
1 : 5.00	0.17	0.83	15.06	1 : 5.00	0.17	0.83	16.28
1 : 6.00	0.14	0.86	15.95	1 : 6.00	0.14	0.86	16.44
1 : 7.00	0.12	0.88	17.63				
1 : 9.00	0.10	0.90	19.04				
0 : 1.00	0.00	1.00	21.91	0 : 1.00	0.00	1.00	21.91

* Ampicillin trihydrate exhibits no transition after the endothermic peak indicative of the water of crystallization loss.

DSC thermograms of 1:1, 1:1.5, 1:2, 1:2.5 and 1:2.75 molar ratios of anhydrous ampicillin-aspartame physical mixtures showed an endothermic peak with an average transition temperature range from 144-166^{o}C and with an average maximum peak of transition at 165^{o}C. The area, and hence enthalpy change, of this peak was found to increase as the concentration of aspartame in the mixture increased. This may be due to a changing stoichiometry for the complex, reflecting an increasing amount of aspartame in the complex. The down curve of this endothermic peak continued below the scanning base line to form a small exotherm with an average transition temperature range from 166-169^{o}C and with an average maximum peak of transition at 168^{o}C. Since the first peak of aspartame represents the loss of the methyl ester, the small exothermic peak may be attributed to a complex rearrangement involving the resulting free carboxyl group. The area of this exotherm increases to a maximum reflecting a new complex stoichiometry at 1:2 molar ratio of anhydrous ampicillin to aspartame; the exotherm then diminishes as a small endothermic peak, representing uncomplexed aspartame, appears immediately after the up curve of the exotherm. The decomposition temperatures of these mixtures were found to increase as the concentration of aspartame in the mixture increased reflecting the relative thermal stability of the rearranged complexes.

DSC thermograms of 1:3, 1:4, 1:5 and 1:6 molar ratios of anhydrous ampicillin-aspartame physical mixtures showed a double peaked transition with an average transition temperature range from 145-180^{o}C, an average maximum first peak of transition at 165^{o}C and with an average maximum second peak of transition at 169^{o}C. The areas of the two peaks representing this double peaked transition

were found to increase as the concentration of aspartame in the mixtures increased. While this was expected for the second peak as it represents uncomplexed aspartame, the thermal behavior of the first peak, representing complexed aspartame, may be attributed again to a change in complex stoichiometry reflecting an increasing amount of aspartame in the complex. The stoichiometry of the complex is probably undefined being an undetermined interaction of aspartame with ampicillin and perhaps ampicillin degradation products. At a 1:6 molar ratio of anhydrous ampicillin to aspartame, new maximum complex formation appears to be achieved. The decomposition temperatures of these mixtures were also found to increase as the contration of aspartame in the mixture increased.

DSC thermograms of 1:7 and 1:9 molar ratios of anhydrous ampicillin-aspartame physical mixtures showed a double peaked transition with an average transition temperature range from 146-185^{o}C, an average maximum first peak of transition at 164^{o}C and with an average maximum second peak of transition at 172^{o}C. The area of the first peak, representing complexed aspartame, decreased as the concentration of aspartame in the mixtures increased, reflecting the decreasing amount of ampicillin present. As would be expected, the area of the second peak and the final peak of aspartame, representing DKP formation, increased as the concentration of aspartame in the mixture increased. The decomposition temperatures of these mixtures were shifted to higher temperatures as aspartame concentration in the mixture increased.

In a previous investigation[10], ampicillin trihydrate was shown by DSC to have a broadened endothermic peak with a transition temperature range from 70-133^{o}C representing the loss of the water of crystallization. After this peak no transition was traced until 203^{o}C where

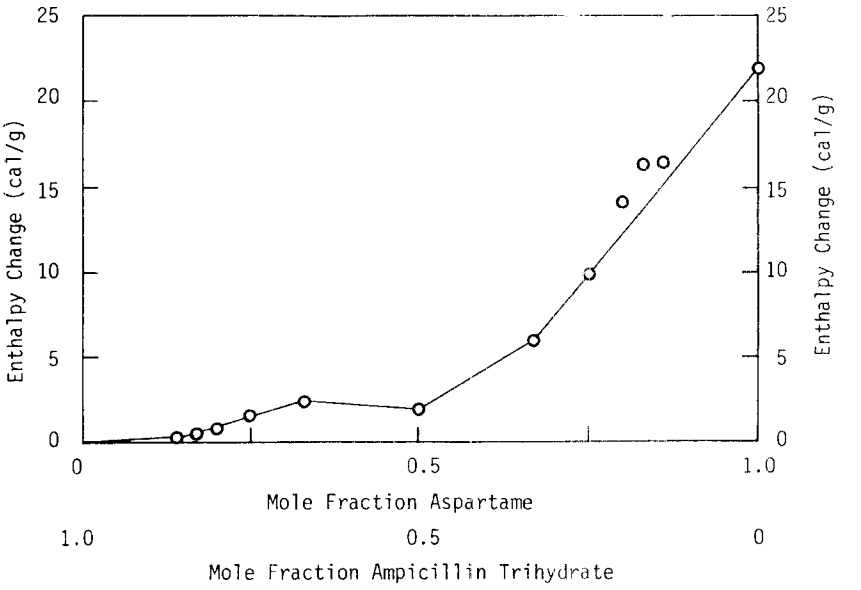

FIGURE 4

Enthalpy change of ampicillin trihydrate-aspartame physical mixtures as a function of composition.

ampicillin trihydrate decomposed. Therefore, DSC thermograms of physical mixtures of ampicillin trihydrate with aspartame will reflect the characteristic features of the thermograms of each component if no interaction occurred.

Figure 2 illustrates the DSC thermograms of ampicillin trihydrate and aspartame, separately and in physical mixtures, while Figure 4 illustrates the enthalpy change of the physical mixtures as a function of composition. The data for Figure 4 is presented in Table 1. It was found that ampicillin trihydrate-aspartame physical mixtures exhibit more or less the same thermal behavior as in the case of the anhydrous ampicillin-aspartame physical mixtures, except that no exothermic peak was traced at a 1:2 molar ratio. This

may be due to the effect of the water of crystallization of ampicillin trihydrate that is released before the complex transition. The enthalpy change of the ampicillin trihydrate-aspartame physical mixtures was found to be lower than the predicted values and, for the most part, lower than the values obtained for the anhydrous ampicillin-aspartame physical mixtures of the same molar composition. The enthalpy change of ampicillin trihydrate-aspartame physical mixtures was found to pass through a maximum, corresponding to 1:0.5 (2:1) molar ratio, when plotted against the mole fraction of the components (Figure 4).

REFERENCES

1. A. Wade and J.E.F. Reynolds, eds., "Extra Pharmacopeia-Martindale," 27th ed., The Pharmaceutical Press, London, 1977, p.1076.

2. B.B. Riley, J. Hosp. Pharm., 28, 228 (1970).

3. B. Lynn, ibid, 29, 183 (1971).

4. J. Ashwin and B. Lynn, Pharm. J., i, 487 (1975).

5. J. Jacobs, I. Nathan, E. Superstine and T. Sacks, Drug Intell. & Clin. Pharm., 4, 204 (1970).

6. B. Lynn, Pharm. J., i, 115 (1966).

7. M.O. Moss and M. Cole, Biochem. J., 92, 643 (1964).

8. C.H. Schneider and A.L. de Weck, Immunochemistry, 4, 331 (1967).

9. S.L. Hem, E.J. Russo, S.M. Bahal and R.S. Levi, J. Pharm. Sci., 62:2, 267 (1973).

10. H.H. El-Shattawy, D.O. Kildsig and G.E. Peck, Drug Dev. and Industrial Pharm., submitted for publication.

11. H.H. El-Shattawy, Drug Dev. and Industrial Pharm., submitted for publication.

12. R.H. Mazur, A.H. Goldkamp, P.A. James and J.M. Schlatter, J. Med. Chem., 13:6, 1217 (1970).

13. H.H. El-Shattawy, G.E. Peck and D.O. Kildsig, Drug Dev. and Industrial Pharm., in press.

14. A.N. Martin, J. Swarbrik and A. Commarta, eds., "Physical Pharmacy," 2nd ed., Lea & Febiger, Philadelphia, 1970, p. 336.