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DIGESTIVE MULTIENZYME PREFORMULATION STABILITY SCREENING USING DIFFERENTIAL SCANNING CALORIMETRY

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ABSTRACT

Differential scanning calorimetry was used for assessing the compatibility in the solid state of pepsin-diastase, pepsin-pancreatin, pancreatin-diastase and pepsin-diastase-pancreatin mixtures using uncoated and Eudragit coated enzymes. The enthalpy change (cal/g) of the resulting thermogram transitions indicated pepsin-diastase and pancreatin-diastase mixtures to be compatible, and pepsin-pancreatin and pepsin-diastase-pancreatin mixtures to be incompatible when uncoated enzymes were used while compatible when coated enzymes with Eudragit were used.

INTRODUCTION

El-Shattawy et al. previously used differential scanning calorimetry (DSC) as a screening technique for assessing the compatibility of aspartame[1,2], anhydrous ampicillin[3], cephalexin[4] and erythromycin[5] with some of the direct compression excipients. The compatibilities of anhydrous ampicillin, ampicillin trihydrate and cephalexin with anhydrous dextrose and with aspartame were also investigated by the same authors[6-8]. In

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this investigation, the author used ISC in preformulation stability studies involving pepsin, diastase and pancreatin.

Enzyme inactivation in aqueous systems has been studied extensively with regard to protein structures and biochemical functions[9-11] . Pepsin solutions are reduced in proteolytic activity by agitation and storage, particularly at or above normal room temperatures[12]. The proteolytic activity of pepsin is also destroyed by pancreatic enzymes in neutral solution[12].

Enzyme stability in the solid state is complicated and is readily affected by factors such as temperature, humidity, and co-existing substances[13] . Sugiura et al.[13] described the application of the Weibull distribution function to solid-state enzyme inactivation in a study involving microbial lipases, microbial α-amylase, pancreatin and diastase.

Graf and Sakr[14] studied the effect of direct compression excipients on the characteristics of Stomach Extract tablets produced under standardized manufacturing conditions. The same authors[15] reported that there are many problems involved in the manufacture of satisfactory pancreatic enzymes oral solid dosage forms due to the very complex nature of the enzymes and their great liability to quickly loose activity during manufacture, on storage and in the gastro-intestinal tract before reaching their specific site of action. Graf et al.[15-17] studied the effects of formulation factors on the stability and bioavailability of pancreatic enzymes tablets manufactured by the direct compression technique.

Although many commercial preparations containing mixed digestive enzymes, no information is available in the pharmaceutical literature about the problem of enzyme-enzyme interaction, in the solid state. In this study the author investigated the compatibility in the solid state of pepsin-diastase, pepsin-pancreatin, pancreatin-diastase and pepsin-diastase-pancreatin mixtures using

uncoated and coated enzymes. This is achieved by comparing the DSC thermograms of the individual enzymes in the mixture and finally on the mixture itself. Although it cannot be conclusively stated that an interaction incompatibility will occur during storage at room temperature[18], DSC can distinguish between enzyme mixtures unlikely to cause a problem and those that may cause trouble and thereby a more rational approach to early formulation designs can be established.

EXPERIMENTAL

Materials

The following materials were used: pepsin, diastase (supplied by Arab Drug Company), pancreatin (supplied by Memphis Chemical Company), Eudragit E and Eudragit S (Rohm and Haas Co., Inc.). All other chemicals were USP or analytical grade.

Manufacture of Enzyme Granules

The enzyme powder was massed with chloroform for pepsin, 95% ethanol for diastase and 90% isopropanol for pancreatin. The mass was forced through an 800 μ sieve and the granules were dried in a vacuum desiccator. The obtained irregular granules were rounded by placing in an ERWEKA coating pan (type UG) for 15 minutes at constant speed. The granules were then passed through an 800 μ sieve and retained over a 630 μ sieve using an ERWEKA screen analyzer (type VT). These retained granules were collected for direct coating.

Coating of the Granules

Eudragit E in chloroform, Eudragit E in 2:3 acetone-isopropanol mixture and Eudragit S in 1:1 acetone-isopropanol mixture were used for the coating of pepsin, diastase and pancreatin, respectively.

The rounded granules(200g) were sprayed in an ERWEKA coating pan with a solution of 10 g Eudragit in 500 ml of the respective organic solvent, by means of an air-pressure

spray gun. The total amount of the polymer solution was applied in 10 ml portions over a period of 2-3 hours. The spraying was stopped at occasional intervals, and the contents of the pan were dried with cold air. Finally the coated granules were dried overnight in a vacuum desiccator.

Differential Scanning Calorimetry

Samples (3-9 mg) were weighed 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°C per minute, a constant range setting of 8 mcal per second and recorded at a constant chart speed of one inch per minute. The individual enzymes and the mixtures thereof were heated over the temperature range 30 to 300°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[1]. At least two replicates made for each DSC thermogram.

RESULTS AND DISCUSSION

The DSC thermogram of pepsin (Trace 1 of Figure 1) showed two endothermic peaks, the first one with a transition temperature range from 134-147°C and with a maximum peak of transition at 140°C, the second one with a transition temperature range from 188-222°C and with a maximum peak of transition at 208°C. The DSC thermogram of coated pepsin (Trace 2 of Figure 1) showed the same two endothermic peaks with a slight shift to higher temperatures as regard the transition temperature range (134-150° and 190-228°C) and the maximum peak of transition (143°C and 210°C) of the two transitions, respectively.

Uncoated and coated diastase exhibit no transition when scanned over the temperature range of 30 to 300°C.

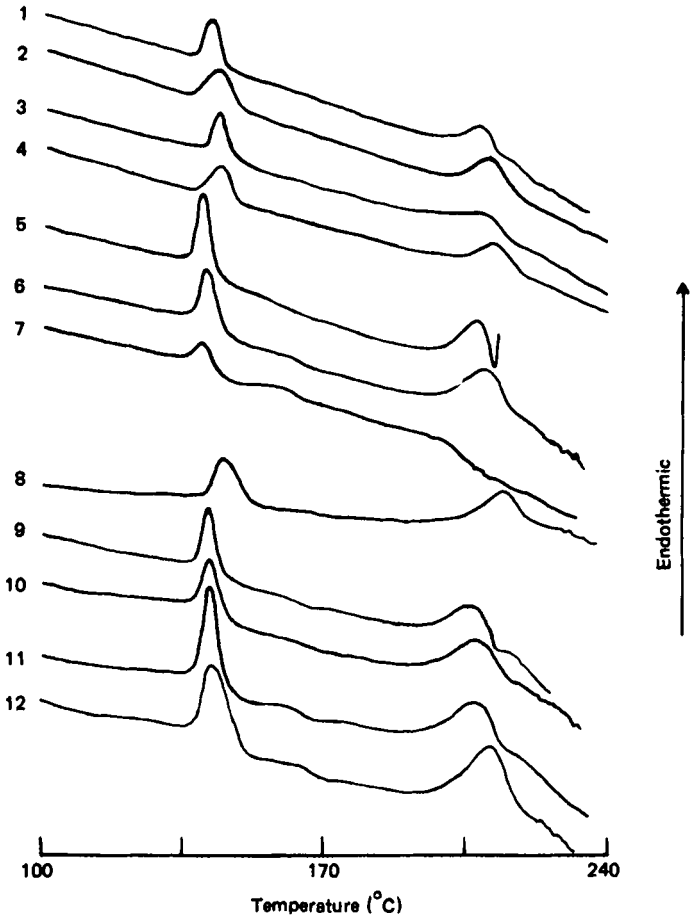

Figure 1: Thermograms of the investigated enzymes and mixtures thereof. Key: (1) uncoated pepsin, 3 mg; (2) coated pepsin, 3 mg; (3) uncoated 1:1 pepsin-diastase mixture, 6 mg; (4) coated 1:1 pepsin-diastase mixture, 6 mg; (5) uncoated pancreatin, 3 mg; (6) coated pancreatin, 3 mg; (7) uncoated 1:1 pepsin-pancreatin mixture, 3 mg; (8) coated 1:1 pepsin-pancreatin mixture, 3 mg; (9) uncoated 1:1 pancreatin-diastase mixture, 6 mg; (10) coated 1:1 pancreatin-diastase mixture, 6 mg; (11) uncoated 1:1:1 pepsin-diastase-pancreatin mixture, 9 mg; (12) coated 1:1:1 pepsin-diastase-pancreatin mixture, 9 mg.

Therefore, DSC thermograms of pepsin-diastase mixtures will reflect the characteristic features of the thermograms of pepsin if no interaction occurred. This is indeed the case as seen in Traces 3 and 4 of Figure 1. Some change in peak shape and height-to-width ratio was expected because of possible differences in the mixture

sample geometry[19]. The enthalpy change (cal/g) of pepsin-diastase, uncoated and coated enzyme mixtures was found to be close to the predicted values calculated from the exact percentage contribution of pepsin to the total enthalpy change of the mixtures (Table 1) indicating no interaction between pepsin and diastase in the solid state under the experimental conditions.

The DSC thermogram of pancreatin (Trace 5 of Figure 1) showed two endothermic peaks, the first one with a transition temperature range from 128-145°C and with a maximum peak of transition at 138°C, the second one with a transition temperature range from 191-213°C and with a maximum peak of transition at 207°C. The DSC thermogram of coated pancreatin (Trace 6 of Figure 1) showed the same two endothermic peaks with a slight shift to higher temperatures as regard the transition temperature range (128-147° and 191-219°C) and the maximum peak of transition (140°C and 208°C) of the two transitions, respectively.

The DSC thermogram of pepsin-pancreatin uncoated enzyme mixture (Trace 7 of Figure 1) showed three endothermic peaks. The first one with a transition temperature range from 129-149°C and with a maximum peak of transition at 140°C corresponding to the first peaks of pepsin and pancreatin. The second peak with a transition temperature range from 150-166°C and with a maximum peak of transition at 160°C is a new transition. The third peak with a transition temperature range from 180-213°C and with a maximum peak of transition at 200°C corresponding to the second peaks of pepsin and pancreatin. The enthalpy change (cal/g) of the first peak was found to be 62.23% the predicted value, calculated from the exact percentage contribution of pepsin and pancreatin to the total enthalpy change of the mixture, while the enthalpy change of the transition corresponding to the second peaks of pepsin and pancreatin was found to be 49.87% the predicted value (Table 1) indicating interaction between pepsin and pancreatin in the solid state under the experimental condi-

Table 1: Enthalpy Change of Uncoated and Coated Enzyme Mixtures

DSC Thermogram of:	Enthalpy Change*, Cal/g					
	First Transition			Second Transition		
	Actual	Predicted**	%	Actual	Predicted**	%
Pepsin-Diastase (1:1 uncoated mixture)	4.90	5.25	93.33	10.49	10.11	103.76
Pepsin-Diastase (1:1 coated mixture)	5.70	5.25	108.57	10.46	10.11	103.46
Pepsin-Pancreatin (1:1 uncoated mixture	8.09	13.00	62.23	10.18	20.41	49.87
Pepsin-Pancreatin (1:1 coated mixture)	13.01	13.00	100.08	20.09	20.41	98.43
Pancreatin-Diastase (1:1 uncoated mixture)	7.41	7.75	95.61	9.46	10.30	91.84
Pancreatin-Diastase (1:1 coated mixture)	7.73	7.75	99.74	10.26	10.30	99.61
Pepsin-Diastase-Pancreatin (1:1:1 uncoated mixture)	7.42	8.66	85.68	8.60	13.61	63.19
Pepsin-Diastase-Pancreatin (1:1:1 coated mixture)	8.76	8.66	101.15	13.61	13.61	100.00

* The enthalpy changes (cal/g) for the coated and uncoated enzymes were found to be the same when calculations were normalized to compare the data on an equivalent weight basis and were found to be 10.50 and 20.22 cal/g for pepsin's first and second transitions, respectively, 15.49 and 20.60 cal/g for pancreatin's first and second transitions, respectively, and no enthalpy change for diastase as it exhibits no transition.

** Predicted values are calculated from the exact percentage contribution of each ingredients to the total enthalpy change of the mixture.

tions. This finding is in agreement with literature[12] that the proteolytic activity of pepsin is destroyed by pancreatic enzymes in neutral solution.

The DSC thermogram of pepsin-pancreatin coated enzyme mixture (Trace 8 of Figure 1) showed also three endothermic peaks as in the case of the uncoated enzyme mixture, but the transition temperature range and the maximum peak of transition shifted to higher temperature and the two main peaks corresponding to pepsin and pancreatin are more distinct. The enthalpy change (cal/g) of these two peaks was found to be close to the predicted values (Table 1) indicating no interaction between pepsin coated with Eudragit E, and pancreatin coated with Eudragit S in the solid state under the experimental conditions.

Traces 9 and 10 of Figure 1 are the thermograms of pancreatin-diastase, uncoated and coated enzyme mixtures, respectively. The enthalpy change (cal/g) of pancreatin-diastase, uncoated and coated enzyme mixtures was found to be close to the predicted values calculated from the exact percentage contribution of pancreatin to the total enthalpy change of the mixture (Table 1) indicating no interaction between pancreatin and diastase in the solid state under the experimental conditions.

Traces 11 and 12 of Figure 1 are the thermograms of pepsin-diastase-pancreatin, uncoated and coated enzyme mixtures, respectively. The enthalpy change (cal/g) of the two main peaks of the uncoated enzyme mixture was found to be 85.68 and 63.19%, respectively, the predicted values while those of the coated enzyme mixture was found to be 101.15 and 100%, respectively (Table 1) reflecting the stability of the investigated coated enzymes mixture.

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