Preparation and Evaluation of Enteric Coated Pancreatin Tablets

S. C. BASAK*, P. SAMPATH KUMAR, R. MANAVALAN AND K.A. NARENDRANATH[1]
Institute of Pharmaceutical Technology, Annamalai University, Annamalainagar-608 002.
T.T.K. Pharma Ltd, Pallavaram, Chennai-600 043.

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Enteric coated pancreatin tablets were prepared with five different enteric polymers. These coated tablets were blister packaged using polyvinyl chloride/aluminium foil (PVC/Al-foil) and polyvinylidene chloride/aluminium foil (PVdC/Al-foil). The tablets were evaluated on the basis of their disintegration time, loss on drying and enzyme activities periodically during a period of 90 d. All tablets but one stored at 45° and 75% RH (relative humidity) failed to meet disintegration test IP. Tablets coated with cellulose acetate phthalate (CAP) and wincoat polymers failed disintegration test IP even at room temperature. All tablet formulations stored at 45° and 75% RH failed loss on drying test IP. It was observed that the activities of pancreatic enzymes decreased on storage, but the loss in activity of individual enzymes were found to be different. Acrycoat L100 with PVdC packaging had shown promising results.

Pancreatin is one of the most important pharmaceutical enzymes having protease, amylase and lipase activities used for replacement therapy in pancreatin insufficiency resulting from cystic fibrosis, pancreatitis and pancreatectomy. In such cases pancreatin is given to patients so as to digest starch, fat and protein[1,2]. Substantial amount of enzyme activity is destroyed by the acid and peptic activity in the stomach[3]. The enteric coated pancreatin may prevent the flux of pancreatin at the acid pH of stomach and enhances the effectiveness of the enzymes[1,4,5].

Attempts have been made to develop enteric coated pancreatin tablets that not only can prevent destruction of enzyme in stomach but also prevent loss of enzyme activity during shelf life and improve therapeutic efficacy. The study was undertaken to develop enteric coated pancreatin tablets including packaging specifications. The disintegration time, loss on drying and enzyme activities at different storage conditions were evaluated to discriminate among formulations developed.

Pancreatin granules were obtained from Advanced Biochem Ltd., Bangalore. Cellulose acetate phthalate was procured from Estman, USA. Wincoat-Wt-N-1003, a ready mix CAP was purchased from Tamhane coatings, Thane. Hydroxy propyl methyl cellulose phthalate-HP-55 was obtained from Shin-Etsu, Japan, while Acrycoat L100, a methacrylic acid copolymer was purchased from Corel Pharma Chem Ltd. Polyvinyl chloride (PVC), polyvinylidene chloride (PVdC) and aluminium (0.02 mm thickness) foils were of commercial grade. The thickness of PVdC foil and PVC foil used were 0.28 mm and 0.25 mm, respectively. All other materials used throughout the study were of reagent grade.

The pancreatin granules were directly compressed into tablets on a 16-station single rotary cadmach machine using 9.5 mm standard concave punches. The tablets were compressed into an average weight of 225 mg. The composition of enteric coating solution is shown in Table 1. There were five groups: I (CAP), II (WINCOAT), III (HPMCP), IV (HPMCP) and V (ACRYCOAT). In III and IV the percent of plasticizer as varied. Group III had 0.67% w/w of diethyl phthalate as plasticizer that equals to 15% of polymer. On the other hand, 20% plasticizer was used in group IV. The composition with-respect to polymer concentration was selected based on trial coating. The concentration of polymer used in each group, specified in Table 1, was developed as a optimal concentration for coating under experimental conditions described below. However wincoat, a ready mix CAP

*For correspondence:
E-mail: scbasak@vsnl.net

TABLE 1: COMPOSITION OF COATING MIXTURE.

Materials	Composition (percentage W/W)				
	I(CAP)	II(Wincoat)	III(HPMCP)	IV(HPMCP)	V(Acrycoat)
CAP	5.7	-	-	-	-
Wincoat-wt-N1003	-	7.0	-	-	-
HPMCP	-	-	4.5	4.5	-
Acrycoat LIOO	-	-	-	-	7.3
n-Butylphthalate	1.5	-	-	-	2.9
Diethylphthalate	-	-	0.67	0.89	3.3
Titanium dioxide	2.4	-	2.1	2.1	-
Solvent mixture	90.4	93.0	92.73	92.51	86.50

III Group HPMCP contains 0.67% w/w of plasticizer, IV Group HPMCP contains 0.89% w/w of plasticizer.

contains equivalent 5.7% w/w of CAP. The coating suspension of each group was sprayed continuously at 10 g/min rate over the tablet bed (pre-warmed to 40°) loaded in 9.4 inches diameter coating pan using 1 mm fluid nozzle spray gun. The rpm of the coating pan, the automizer's air pressure and the drying temperature were maintained at 30, 3 to 3.5 kgf/cm^2 and 55 to 60° respectively. The tablets were coated to weight gain of 7±1% by weight of uncoated tablets and to get uniform thickness of coating. The five different enteric coated pancreatin tablets were blister packaged using two packaging namely PVC/Al-foil and PVdC/Al-foil. The formula code of ten groups were represented as: 1) CAP-PVdC 2) CAP-PVC 3) WC-PVdC 4) WC-PVC 5) HPMCP-15%P-PVdC 6) HPMCP-15%P-PVC 7) HPMCP-20%P-PVdC 8) HPMCP-20%P-PVC 9) AC-PVdC and 10) AC-PVC.

The blister packed samples were stored at ambient temperature (temperature in the working area) 25 to 29°, at 35±2° in an incubator and at 45° with 75% RH. The storage conditions are selected to approximate distribution conditions of finished product. Samples were withdrawn at 0,15, 30, 45 and 90 d and were evaluated for loss on drying, disintegration time and enzyme activities.

The loss on drying was studied by drying 4 pancreatin tablets at 60° for 4 h in vacuum using a pre-dried (60° for 30 min) petridish. The sample was kept in a desiccator to attain room temperature. The percentage loss was calculated. The disintegration test was carried out in the IP disintegration apparatus[6]. Stimulated gastric fluid (pH 1.2) was used as immersion liquid for the first 2 h of the test. At the end of 2 h the immersion liquid was replaced by phosphate buffer of pH 6.8.

The amylase, lipase and protease activities were evaluated periodically using sample size of 10 tablets. The percentage enzyme activities remained on 15, 30, 45 and 90 d were calculated taking the activity 100% on initial day. A volumetric method based on the rate of hydrolysis of starch by the enzyme was used to estimate amylase activity. A modification of the principle of BP method[7] was utilized for the estimation. Twenty five millilitres starch substrate solution (1% W/V in distilled water), 10 ml phosphate buffer (pH 6.8) and 1 ml 0.2 M sodium chloride solution were taken in iodine flask and the temperature was maintained at 25°. One milliliter of the sample (about 100 mg of pancreatin enzyme in 250 ml of phosphate buffer solution) was added and shaken occasionally for 15 min. After 15 min, 10 ml of 0.1 N iodine solution was added, followed by immediate addition of 2 ml 0.1 N hydrochloric acid, 20 ml of distilled water and 45 ml 0.1 N sodium hydroxide solution. The resultant solution was allowed to stand for 15 min in dark. Finally after 15 min, 4 ml of 20% V/V sulphuric acid was added and titrated with 0.1 N sodium thiosulphate solution. The titration was repeated as blank (where hydrochloric acid was added to the substrate at zero point).

The amylase activity (FIP units) per g was calculated as:

$$\text{Amylase activity} = \frac{\text{(Blank-Test) x normality of sodium thiosulphate x 5x250x1000}}{\text{1 - 0.03 (Blank - Test) x normality of sodium thiosulphate x Weight taken}}$$

The estimation of lipase activity is based on the measurement of the rate at which it is capable of bringing about hydrolysis of olive oil and the acid liberated by the lipase in a given time is titrated with standard alkali. Ten millilitres olive oil substrate emulsion (10% v/v) buffered with 8 ml 0.005M tris-(hydroxymethyl)-amino methane solution was taken in a beaker and mixed with 2.0 ml of 8% w/v solution of sodium taurocholate. Then it was diluted to total volume of 30 ml with distilled water and maintained at 37±1°. The pH was finally adjusted to 9.05 with 0.01 N sodium hydroxide. One millilitre of the sample (about 100 mg of pancreatin enzyme in 250 ml of distilled cold water) was added and 0.01 N sodium hydroxide was added drop wise to maintain the pH 9.05. The volume of 0.01 N sodium hydroxide added was noted every minute up to 10 min and the average volume consumed per minute was calculated. The lipase activity (FIP units) per g was calculated as:

$$\text{Lipase activity} = \frac{\text{ml of 0.01 N sodium hydroxide used permin x 250 x 100 x normality of Sodium hydroxide x 1000}}{\text{Weight of pancreatin lipase taken}}$$

A volumetric method based on the rate of hydrolysis of casein by the enzyme was used to estimate protease activity. A slightly modified method, reported by Evers and Smiths was utilized for the estimation. Four grams of purified casein was dissolved in 90 ml of distilled water containing 3 ml of IN sodium hydroxide, pH was adjusted to 8.7 and was made up to 100 ml. Fifteen milliliters of the casein solution was diluted to 45 ml with distilled water. The solution was warmed to 55° and 10 ml unfiltered pancreatin enzyme solution (0.5 g powdered pancreatin in 250 ml water) was added. This was kept in incubator at 55° for 20 min and then cooled rapidly to room temperature. A blank solution was prepared in a manner similar to that of test except, that previously boiled and cooled unfiltered enzyme solution was used. A few drops of 0.1% phenolphthalein and 10 ml of formaldehyde solutions were added to both liquids and titrated against 0.1 N sodium hydroxide solution. The protease activity per gram was calculated as:

$$\text{Protease activity} = \frac{\text{(Test - Blank) x 250 x strength of 0.1 N sodium hydroxide x 1000}}{\text{Weight taken x 10 ml}}$$

Table 2 gives the formulations that failed the requirements of the disintegration test IP for enteric coated tablets. CAP and wincoat enteric coated tablets (both PVC and PVdC packaged) stored for 45 d at room temperature failed the test in phosphate buffer (pH 6.8). The same stored for 30 d at 35° also failed to comply time limit in phosphate buffer

TABLE 2: RESULTS OF DISINTEGRATION TEST IP ON VARIOUS ENTERIC COATED PANCREATIN TABLETS FOLLOWING STORAGE.

Formula	Ambient Temp.		35°		45° and 75% RH	
Code	pH 1.2	pH 6.8	PH 1.2	pH 6.8	pH 1.2	pH 6.8
CAP-PVdC	P	F45	P	F30	F15	-
CAP-PVC	P	F45	P	F30	F15	-
WC-PVdC	P	F45	P	F30	F15	-
WC-PVC	P	F45	P	F30	F15	-
HPMCP-15%P-PVdC	P	P	P	P	F 30	-
HPMCP-15%P-PVC	P	P	P	P	F30	-
HPMCP-20%P-PVdC	P	P	P	P	F 30	-
I-IPMCP-20%P-PVC	P	P	P	P	F 30	-
AC-P VdC	P	P	P	P	P	P
AC-PVC	P	P	P	P	F90	-

P-Doesn't disintegrate in pH 1.2 for 2 h. F 90, F 45, F 30, F 15 indicates failure of test on storage after 90, 45, 30,15 d respectively. "-" indicate the test was not run as it failed in acidic medium itself.

whereas stored at 45° and 75% RH for 15 d failed to comply with requirement in acidic medium (pH 1.2). The tablets coated with HPMCP failed to comply disintegration test IP at the end of 30 d when stored at 45° and 75% RH. These failures to comply disintegration of tablets coated with CAP and HPMCP may be due to alteration of film properties to high humidity and temperature. The tablets coated with acrycoat L100 and PVdC packaging met all the requirements up to 90 d. The tablets coated with acrycoat L100 packed with PVdC/Al-foil did not disintegrate in pH 1.2 compared to same tablets packed with PVC/Al-foil, the reason may be that the PVdC laminated on PVC film might have given added protection of the coating on storage.

The percentage loss on drying both at room temperature and 35° of all the formulations ranged from 4.40±0.86 to 4.92±0.04 and were within IP limit. The periodical evaluation showed no significant change in the loss on drying for the above two temperature conditions. However all the formulations stored at 45° and 75% RH failed the requirements of loss on drying test IP (the limit is not more than 5.0%). The loss on drying at 45° and 75% RH of different formulations is presented in Table 3. The percent loss on drying gradually increased (as days of storage) beyond the IP limit. PVdC/Al-foil packaging was found to be superior in protecting moisture pick up by tablets compared to PVC/Al-foil packaging probably of its less water vapour transmission rate. The tablets coated with acrycoat L100 and PVdC packaging (formula code AC-PVdC) were selected for detailed stability studies. The stability studies revealed that appreciable loss in percent enzyme activity occurred for lipase and amylase. As shown in Table 4, amylase activity decreased significantly at elevated storage conditions as compared to other two enzyme activities.

In conclusion, Acrycoat with PVdC packaging is found

TABLE 3: LOSS ON DRYING AT 45° AND 75% RH.

Formula Code	Percent loss on drying				
	Initial	15 d	30 d	45 d	90 d
CAP-PVdC	4.80±0.15	5.43±0.21	5.79±0.03	6.28±0.59	6.48±0.16
CAP-PVC	4.80±0.46	5.41±0.24	5.93±0.21	6.63±0.43	7.30±0.35
WC-PVdC	4.82±0.24	5.28±0.62	5.81±0.49	6.33±0.71	6.52±0.71
WC-PVC	4.82±0.23	5.48±0.38	5.87±0.60	6.53±0.39	7.31±0.14
HPMCP-15%P-PVdC	4.78±0.75	5.33±0.11	5.71±0.61	6.06±0.57	6.71±0.68
HPMCP-15%P-PVC	4.78±0.34	5.41±0.13	5.87±0.39	6.71±0.18	7.61±0.05
HPMCP-20%P-PVdC	4.59±0.21	5.01±0.32	5.33±0.58	5.86±0.76	6.46±0.20
HPMCP-20%P-PVC	4.59±0.27	5.70±0.78	6.53±0.38	7.01±0.13	7.11±0.55
AC-PVdC	4.80±0.21	4.98±0.28	5.11±0.15	5.73±0.14	6.31±0.73
AC-PVC	4.80±0.50	5.71±0.38	6.03±0.62	6.41±0.25	6.78±0.19

Note-Each value represents mean±SD (n=3).

TABLE 4: STABILITY STUDIES ON THE FORMULATION AC-PVDC AT THE END OF 90 DAYS.

Enzyme activities	Initial values	Storage conditions		
		Ambient temp.	35°	45° and 75% RH
Percentage protease activity remaining	100(1.10)	95.98(1.93)	94.74(1.89)	90.56(1.26)
Percentage lipase activity remaining	100(1.21)	89.39(1.55)	87.86(1.65)	85.60(1.79)
Percentage amylase activity remaining	100(1.17)	83.37(2.16)	76.39(2.21)	73.31(2.01)

Each value represents a mean of three readings. The SD (n=3) values are given in parenthesis.

to be significantly superior in meeting the disintegration test at all elevated storage conditions. PVdC blister packaging is found to be superior in protecting moisture pick up by the tablets. Moreover failure of all tablets stored at 45° and 75% RH to meet loss on drying test IP emphasizes the requirement of storage of pancreatin tablets in controlled humidity. In view of appreciable loss of enzyme activities on storage it is concluded that loss of enzyme activities should be taken into account in calculating percentage overage to be added for desired shelf life. Pancreatin tablets coated with acrycoat and blister packaged with PVdC (formula code AC-PVdC) was found to be better in all aspects compared to other formulations.

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Synthesis and Antimicrobial Activity of Some New Isoxazolines and 1,5-Benzothiazepines

G. R. SUBBANWAD, M. A. BASEER AND Y. B. VIBHUTE*

P. G. Department of Chemistry, Yeshwant College, Nanded-431 602.

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New six isoxazolines and five 1,5-benzothiazepines are synthesised from l-(substituted phenyl)-3-(2-methoxy-l-naphthyd-2-propen-l-ones. Their structural assignments are based on spectral data (IR and PMR) and elemental analysis. All these compounds have been screened for antimicrobial activity. The compounds with a methyl or chloro and methyl as well as chloro group on the aromatic ring showed good antimicrobial activity.

Nitrogen containing heterocyclic compounds like isoxazolines, nitrogen and sulphur containing heterocyclic compounds like benzothiazepines have received considerable attention in recent years due to their wide range of physiological activities. A number of isoxazole derivatives have been found to possess potential antibacterial[1] antitubercular[2], antifungal[3] and antidiabetic[4] activity. Anilidoisoxazolines synthesised by Zarif and Yammi[5] were found to possess remarkable bactericidal activity against some gram positive and gram negative bacteria. Mittal and Singhal[6] have reported antibacterial and antifungal activity in 3-methyl-4-(4'-bromo-2'-methyl benzene azo)-5-isoxazoline. Benzothiazepines such as diltiazen[7] or thiazesim[8] are constantly used as antidepressant, coronary vasodilator and antiangina agents. Levai[9] has reviewed the syntheses of four known groups of optically active 1,5-benzothiazepines. The references included show the most important biological activities like CNS, cardiotonic, histamine H_2 antagonistic and antiulcer. Bioassay screening of some substituted 1,5-benzothiazepines show mild analgesic and anticonvulsant activity[10]. These reports prompted us to synthesize new isoxazoline and benzothiazepine derivatives and evaluate their antimicrobial activity.

Melting points were determined in open capillaries and are uncorrected. The structures of the compounds were sup-

*For correspondence: