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INTERACTION STUDY BETWEEN PEFLOXACIN MESILATE AND SOME DILUENTS USING DSC SUPPORTED WITH ISOTHERMAL METHOD

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The present investigation was carried out to screen compatibility of some diluents with pefloxacin mesilate using differential scanning calorimetry (DSC), isothermal stability studies, along with stability studies in liquid state and to assign relative ranking to diluents. Compatibility was predicted with MCC101, MCC102, DCP anhydrous, Emcompress, while melting endotherm of drug was lost in admixtures of dextrose anhydrous, Pearlitol, Lactopress spray dried, Lactochem fine powder and Lycatab indicating possibility of interaction. Enthalpy changes were used for relative ranking of diluents.

Keywords: *compatibility, diluents, DSC, interaction, pefloxacin mesilate, stability studies*

Introduction

The formulation of a drug substance involves it being blended with a combination of different excipients to maximize the products ability to administer the dosage effectively [1]. It is necessary that the excipients or the additives used in the drug formulation are chemically compatible with the drug [2]. Although excipients have traditionally been considered inert, it is now well accepted that some carry the potential for untoward effects. The effect may be due to the excipient itself or to a residue from the starting material or the process of manufacture, which may bring about significant changes in physical properties of drug [3].

Utilizing thermal methods like differential scanning calorimetry, it is possible to obtain significant data rapidly, and the method has been suggested for routine screening of potential drug excipient interaction at the preformulatory level [4].

Of a series of reports that suggest the use of thermal analysis for rapid prediction of chemical interactions between drugs and formulation adjuvants, very few studies have confirmed thermal interactions with routine stability testing involving chemical analysis [5–10].

In present work compatibility of pefloxacin mesilate (PM) with some directly compressible diluents was investigated using DSC. PM is a broad-spectrum antibacterial 4-quinolones, corresponding to 1-ethyl-6-fluoro-7 (-4 methyl-1-piperazinyl)-4 oxo-1, 4-dihydro quinoline-3-carboxylic acid, having good penetration of the central nervous system [11]. The results of the DSC studies were supported with isothermal stability studies (IST) carried out at ambient conditions and at 50°C, for six months. Influence of diluents on stability of drug in liquid state, along with changes in microenvironmental pH was also determined. After subjecting the data to statistical analysis the diluents were ranked in decreasing order of stability. A correlation between the results of DSC studies with data obtained from IST studies was attempted.

Experimental

Materials

Pefloxacin mesilate was obtained as gift sample from Wockhardt Ltd., Aurangabad, India. Microcrystalline cellulose pH101, and pH102 (Chemsfield, Nagpur, India), dicalcium phosphate anhydrous (Merck Ltd., Mumbai, India), Emcompress (JRS, USA) Pearlitol, Neosorb, Lycatab (Roquette Inc., America), dextrose anhydrous (Cipla Ltd, Mumbai, India) Lactopress spray dried (SD), Lactochem fine powder (FP) (Borculo Domo Ingredients, The Netherlands) were received as generous gift samples. All other chemicals and reagents used were of A.R. grade. Water used in all experiments was deionised and distilled.

Methods

Preparation of solid binary system for isothermal stability studies–kneading method

For isothermal stress studies, PM (#100 mesh) with each selected diluent (#100 mesh) in 1:1 ratio was blended in mortar pestle for 10 min at room tempera-

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ture. The admixture was transferred to petri dish and wetted with 95% ethanol until a paste was obtained, mixed further for 10 min to ensure complete and uniform wetting and finally dried in vaccum oven (Micro instruments, Delhi, India) at 50°C for 1 h at 500 PSI. These admixtures were removed after drying, mildly grounded and divided into two parts for further studies. A control sample of pure drug without (C) and with solvent (R) was also prepared in similar manner.

Storage and analysis of samples

The dried admixtures were stored in 2 sets of amber colored bottles, with tight fitting screw caps and sealed with adhesive tape. One set of admix was kept at ambient conditions ~RT (30±2°C), while the other set was stored in oven (Spectrum, India) with temperature adjusted at 50±2°C. For blends stored at ambient conditions samples were withdrawn from the bottles at 0, 15, 30, 45, 60, 90, 135 and 180 days. Similarly samples were withdrawn from bottles stored at 50°C, at 0, 8, 15, 22, 30, 45, 60, 90, 135 and 180 days and analyzed spectrophotometrically. The blends were also examined for any unusual color change. All the analysis was performed in duplicate.

The preparation of sample for analysis involved weighing of 20 mg admix (10 mg in case of pure drug), diluting the same with 100 mM HCl to obtain 10 mcg mL^{-1} concentration and analyzing against blank, at 277.5 nm. Drug content was determined from the calibration curve prepared within the same range of 2–25 mcg mL^{-1}. The method was found to be linear with studied range (R^2=0.999).

For content analysis and data acquisition UV-visible spectrophotometer (UV-pharmaspec from Shimadzu, Japan) provided with UV probe 2.01 version software, having matched quartz cell, and automatic wavelength accuracy of 10 nm, was used.

Stability studies in liquid state

For stability of drug in liquid state excess of diluent (1 g) was added to 100 mL of solution of PM having strength of 10 mcg mL^{-1} in 100 mM HCl (pH 1.4). Series of such solutions with other diluents were also prepared in iodine flask and kept in water bath shaker (Remi Equipments, Nagpur, India) at speed of 50 RPM for 72 h. The temperature of the water bath was maintained at 37±2°C. Aliquots from these flasks were withdrawn at regular interval, filtered (Whatman filter paper no.2) and analyzed for changes in absorbance (λ_{max} 277.5 nm) and changes in pH (μ pH system, Systronics, India). Solution of pure drug was also prepared (control) and subjected to analogous condition.

Differential scanning calorimetry

All measurements were carried out on an indium calibrated DSC Q10 V9.4 build 287 (TA instruments, USA) provided with refrigerated cooling system. Data were analyzed using the universal analysis 2000 software (TA instruments, USA). Individual samples (drug and diluents #100 mesh) as well as freshly prepared admixtures of drug and selected diluents (2–4 mg) were weighed directly on DSC aluminium pan and scanned between 30–350°C at a heating rate of 20°C min^{-1} in an atmosphere of dry nitrogen (50 mL min^{-1} flow rate). Sample pan were not crimped, instead lids were gently placed on the pan after weighing of sample. An empty pan with lid kept in similar manner was used as reference. Mettler AB-264 balance (Mettler, Switzerland) was used for weighing of samples.

Results and discussion

Isothermal stability studies

In the isothermal stability studies, PM was found to be comparatively more stable at ambient conditions than at 50°C, however no significant degradation was observed in drug content of admixtures of drug with any of the diluents used, till the completion of the study. The blends remain physically stable and no caking, liquefication, discoloration odour or gas formation was observed during storage except in case of DCP-anhyd. The blends of PM with DCP-anhyd. developed yellow coloration under both condition within one week of storage. Drug content analysis of blends of drug with DCP-anhyd. did not revealed any degradation. Fluoroquinolones in general, are known to interact with metal ions like Mg^{2+}, Ca^{2+}, Al^{3+}, Fe^{3+}, Bi^{+} etc. to form metal ion complexes [12]. The discoloration of blends of DCP-anhyd. with PM can be attributed to such interaction. Interestingly no such interaction was observed in case of Emcompress, which also contains calcium. Since degradation kinetics, and the ability of two components to react can depend on a number of factors, mechanism based stability predictions for drug product are exception and keeping this in view, the goal of the compatibility screening in this report was to assign a relative risk level to each diluent. Table 1 list the ranking of diluents in terms of % drug remaining and first order decomposition rate constant at the end of the study at both RT and 50°C.

Stability in liquid state

In liquid state, stability of drug in solution depends on its molecular environment and more specifically pH;

Table 1 Influence of diluents on relative stability of pefloxacin mesilate after six months of storage at 50 and 30°C (rT) and quantification of first order decomposition rate constant

Drug/diluent	50°C					rT~30°C				
	Relative stability		K·10^{-5}	*r	K Ranking	Relative stability		K·10^{-5}	#r	K Ranking
	DR/%	R				DR/%	R			
PM (C)	97.2710	C	6.125	0.9670‡	C	97.9670	C	4.705	0.9551	C
PM (R)	100.1140	R	6.417	0.9595	R	99.5278	R	5.802	0.9737	R
Pearlitol	100.3940	1	5.279	0.9365	1	100.5658	4	4.731	0.9311	3
Lycatab	100.3664	2	5.314	0.8706	2	100.4656	5	5.112	0.9923	6
Dex-anhyd.	100.2620	3	5.571	0.9098	3	100.6594	2	4.805	0.9629	4
Emcompress	100.0730	4	5.853	0.8972	4	100.2779	7	5.567	0.9721	7
LactochemFP	100.0120	5	6.317	0.9464	5	100.3960	6	5.054	0.9316	5
MCC102	99.9726	6	7.025	0.9556	9	100.8948	1	4.129	0.9701	1
LactopressSD	99.8858	7	6.640	0.9246	6	100.5714	3	4.724	0.9556	2
DCP-anhyd.	99.7583	8	7.056	0.9553	10	100.1150	8	6.063	0.9810	9
MCC101	99.7567	9	6.869	0.9280	7	100.0712	9	5.917	0.9685	8
Neosorb	99.7084	10	7.003	0.9413	8	99.7180	10	6.562	0.9202	10

(C): Control (PM) under same storage conditions (treated as 100% at the end of six months), (R): Reference (PM prepared by kneading method) under same storage condition, *r limiting (50°C): correlation coefficient at n=10 and P<0.05 except in ‡where P<0.5, #r limiting (30°C): correlation coefficient at n=8 and P<0.5, DR: drug remaining at the end of six months, R: ranking of diluents based on relative stability

the increase or decrease in which may produce physical or chemical changes in the system [13].

In case of PM no significant absorption, adsorption, degradation or complexation was observed in presence of any diluent. In fact the changes in pH were observed immediately after addition of diluents, after which the pH of the system remained unchanged during the entire study (Table 2). In case of Emcompress and DCP-anhyd. the pH changed from 1.4 to 3.2 and 1.4 to 3.6 respectively. In rest of the systems the change was of not more than 0.2 to 0.6 units. In all the binary solution, no major change in absorbance was noted and drug content was within 95% limit.

Table 2 Stability of pefloxacin mesilate in presence of diluents in liquid state. (Effect on pH and drug content)

Drug/diluent	pH		Drug content/%	
	0 h	72 h	0 h	72 h
PM (C)	1.42	1.42	100.00	99.76
MCC101	1.68	1.66	97.97	97.17
MCC102	1.70	1.72	98.58	98.17
DCP-anhyd.	3.60	3.80	96.64	95.71
Emcompress	3.20	3.40	98.93	98.19
Neosorb	1.50	1.52	97.55	96.79
Dex-anhyd.	1.54	1.56	99.40	99.10
Pearlitol	1.72	1.72	99.93	98.74
LactopressSD	1.74	1.72	96.80	96.19
LactochemFP	1.68	1.68	97.94	97.83
Lycatab	1.62	1.64	99.05	98.97

Differential scanning calorimetry

The DSC curve of PM (Fig. 1) showed an initial dehydration endotherm with onset at 30°C and an enthalpy value of 220.1 J g^{-1}, which was followed by a small shallow endotherm and a sharp melting endotherm at 219.4 and 296°C respectively.

The curves of MCC101 and MCC102 (Fig. 2) revealed an initial dehydration peak due to loss of adsorbed water with maximum peak transition at approximately 75–78°C and a broad endothermic peak with onset at 314°C. In the DSC profiles of the admixture of PM with MCC101 and MCC102 (Fig. 3), the thermal features of drug namely the two endothermic peaks were observed, although with a shift of approximately 5°C in first peak and a shift of 20°C in second peak, to lower temperature. From thermodynamic point of view the Gibbs energy of solid phase at melting temperature, G_{sol} (T), is equal to Gibbs energy of liquid phase at melting temperature, G_{liq} (T). Below melting temperature, G_{sol} is higher than G_{liq} and above melting temperature G_{liq} is higher than G_{sol}. Incorporation of diluent to PM in equal ratio lowered the Gibbs energy below that of the pure PM liquid phase and culminated in decrease of melting point. [14].

The curve of DCP-anhyd. revealed no transition in the scanned region of 30–350°C. The thermal scan of Emcompress showed two peaks in the scanned region, the initial peak corresponding to loss of water of crystallization and the melting peak. The curve of admixture of DCP-anhyd. and emcompress with PM re-

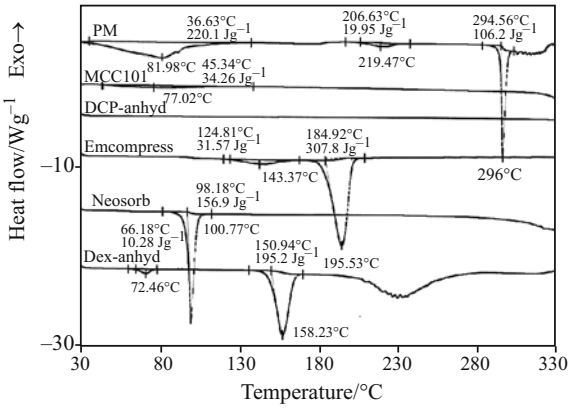

Fig. 1 DSC curves of pefloxacin mesilate and diluents

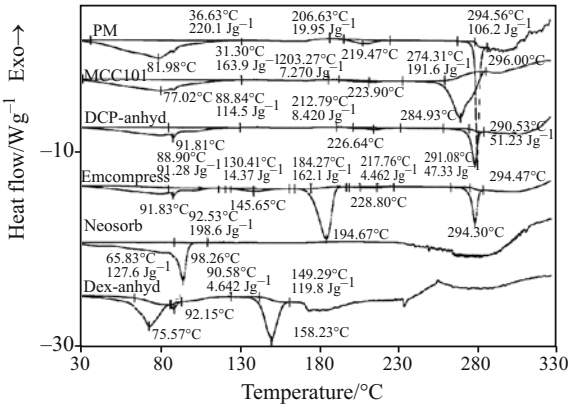

Fig. 3 DSC curves of pefloxacin mesilate and its (1:1 mass/mass) binary mixtures with diluents

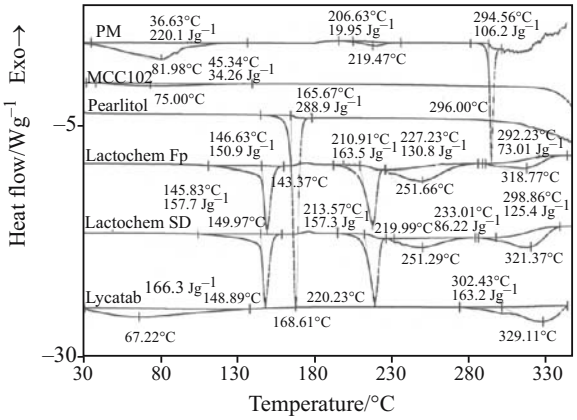

Fig. 2 DSC curves of pefloxacin mesilate and diluents

cords the thermal characteristic features of both drug and diluent. The enthalpy changes of the admixtures were approximately 0.5% (DCP-anhyd.) and 7.46% (Emcompress) of the expected ones, suggesting no interaction between the drug and diluents.

The curve of Neosorb and Pearlitol revealed a single sharp melting endotherm at 100 and 168°C respectively. In thermal curves of binary mixture of Neosorb with PM melting endotherm of Neosorb was fused with dehydration peak of drug, (retained at 98°C), followed by decomposition exotherm with onset at 240°C. Similarly in the curve of admixture of PM with Pearlitol, an initial dehydration peak found in curve of drug and melting peak of Pearlitol at 161.9°C was present followed by the decomposition curve.

The DSC scan of dextrose anhydrous (dex-anhyd.) records an initial peak due to water loss and a sharp melting endotherm at 158.2°C. DSC curve of the admixture of dex-anhyd. with PM showed a broad endothermic peak corresponding to adsorbed water loss, and melting peak of dex-anhyd. at 158°C. The peak of drug was not retained in the admixture of dex-anhyd. with PM.

Neosorb is known to undergo vitrification immediately after fusion [15]. The entrapment of drug in the vitrified matrix of Neosorb might be the probable reason for loss of characteristic melting features of drug. In the admixtures of Neosorb, Pearlitol and dex-anhyd. with PM, the diluent melts and subsequently decomposed prior to the melting of drug, which resulted in the reaction of drug with decomposition products of these diluents, making prediction of compatibility difficult [16].

The DSC scan of Lactochem FP and Lactopress SD showed an endothermic transition starting at 145°C relative to loss of water of crystallization and subsequent changing into anhydrous form with melting transition at 220°C. DSC trace of admixture of Lactochem FP with PM (Fig. 4) retained the dehydration peaks of both drug and Lactochem FP at 80°C due to loss of adsorbed water and at 148°C due to loss of bound water. The melting peak of Lactochem FP at 220°C was shifted to lower temperature at 207°C, while the fusion endotherm of drug was lost. Similar features were observed in admixture of PM with Lactopress SD.

In the DSC endotherm of Lycatab a broad dehydration endotherm along with fusion endotherm with onset at 273°C was observed. The thermal curve of binary mixture reveals an initial dehydration endotherm, shallow endothermic characteristic peak of drug at 222°C and melting peak of Lycatab (diffused in nature) at 252°C. A shift of 20°C to lower temperature was observed in melting endotherm of Lycatab. The enthalpy change of admixture was approximately 78% of the expected enthalpy, and suggested the existence of physical interaction between drug and diluent. However this should be interpreted cautiously, since mixing of two powders intimately and subjecting them to accelerated temperature could result in physical interaction without changing the chemical nature of both the compounds [17]. Thermal parameters and enthalpy values of drug diluent and drug-diluent admixtures is listed in Table 3.

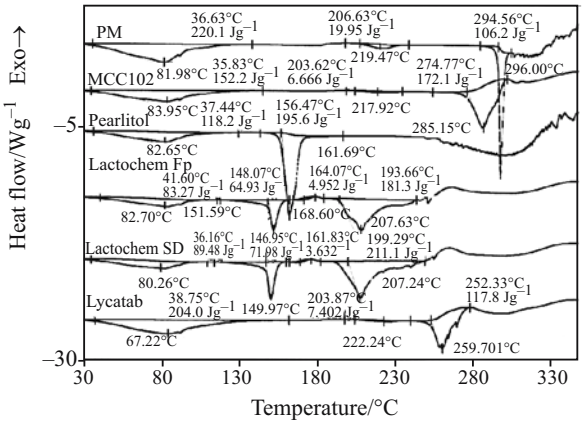

Fig. 4 DSC curves of pefloxacin mesilate and its (1:1 mass/mass) binary mixtures with diluents

Table 3 Thermal parameters of binary mixtures of pefloxacin mesilate with diluents

Diluent	ΔH_{cal}[a]/ J g^{-1}	ΔH_{obsv}/ J g^{-1}	Change in enthalpy of admixture/%
MCC101	190.25	362.77	47.55+
MCC102	195.77	330.96	40.84+
DCP-anhyd.	173.12	174.15	0.59+
Emcompress	342.80	319.54	7.46–
Neosorb	251.57	198.60	26.67–
Dex-anhyd.	275.86	252.04	9.45–
Pearlitol	318.07	313.80	1.36–
LactopressSD	373.73	376.19	0.65+
LactochemFP	395.72	334.45	18.31–
Lycatab	256.27	329.20	22.15+

a enthalpy values calculated from the 1:1 w/w percentage contribution of each ingredient
\+ gain in enthalpy
– loss in enthalpy

Conclusions

Pefloxacin mesilate is a broad spectrum fluoroquinolone antibiotic. In compatibility screening of PM with some diluents, DSC results revealed compatibility of PM with MCC101, MCC102, DCP-anhyd., and Emcompress, as peak of PM was retained in binary mixtures of all these diluents with drug. In rest of the diluents studied i.e. Neosorb, Emcompress, dex-anhyd., Pearlitol, Lactopress SD, Lactochem FP, and Lycatab, the characteristic melting endothermic peak of PM was lost. In spite of this, the IST studies revealed no such degradation of drug in presence of the diluents under both storage conditions, reaffirming the fact that reactions observed at elevated temperatures in DSC are not neccessarily relevant at ambient conditions [18]. From the enthalpy calculations, % change in enthalpy of admixtures in MCC101, MCC102, was comparatively more than in all other admixtures, irrespective of the fact that, drug was found compatible with these two diluents on the basis of preservation of melting endotherm of drug. In case of binary mixtures of dex-anhyd., Pearlitol, and Lactochem FP with PM, although the melting endotherm of drug was not retained, the enthalpy changes in admixtures were within 10% limits of the calculated enthalpy, thereby ruling out the probability of interaction which was quite in accordance with the results of IST studies. Overall IST studies complimented very well with DSC results in assigning a ranking order to various diluents used in compatibility screening. Although no significant statistical correlation could be derived between degradation rate constants obtained from IST results and changes in enthalpy of the system, yet enthalpy changes, in particular proved to be important thermal parameter in ranking of diluents, and could be safely used for prediction of any untoward reactions with drug leading to incompatibility in future.

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