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Research Article

Influence of Microenvironment pH, Humidity, and Temperature on the Stability of Polymorphic and Amorphous Forms of Clopidogrel Bisulfate

Dhara K. Raijada,[1] Saranjit Singh,[1,3] and Arvind K. Bansal[2]

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Abstract. The effect of microenvironment pH, humidity, and temperature was evaluated on the stability of polymorphic and amorphous forms of clopidogrel bisulfate, when present alone or in combinations. Oxalic acid and sodium carbonate were used as solid stressors to create acidic and alkaline pH, respectively. The samples without and with stressors were subjected for 3 months to (1) 0% RH, 25% RH, 75% RH, and 85% RH at 40°C and also to (2) 60°C, 80°C, and 100°C at 0% RH. In case of solid samples without stressors, the mixture of polymorphic and amorphous forms showed more degradation than the individual forms above critical relative humidity (85% RH). Similar higher degradation was observed between 75% RH and 85% RH in case of acid-stressed samples. In alkaline microenvironment, all the samples showed identical decomposition attributed to conversion of bisulfate salt to free base. Thermal studies indicated that polymorphic forms of clopidogrel bisulfate and also its glassy amorphous form were highly resistant to temperature, whereas the rubbery state of the drug degraded significantly at temperatures of ≥80°C.

KEY WORDS: clopidogrel bisulfate; humidity; microenvironment pH; solid-state stability of polymorphs; temperature.

INTRODUCTION

Drugs can exist in amorphous, pseudo-polymorphic, and polymorphic solid-state forms. These may interconvert to one another under various influencing factors (1). Therefore, solid-state reactions are usually explored as a part of preformulation workup, e.g., the effect of temperature and moisture on the amorphous-to-crystalline transformation of stavudine was investigated recently by Strydom *et al.* (2).

Clopidogrel bisulfate, an antiplatelet drug, is commercially available in polymorphic forms I or II. Its amorphous form has also been patented (3,4). In our previous study (5), we observed a typical difference in the drug's stability in solution and in the solid state. While only one degradation product was formed in solution, almost eight were detected in the solid state. The amorphous and two polymorphic forms also differed with respect to rate of decomposition.

The present study was carried out to understand the effect of microenvironment pH, humidity, and temperature on the phase transformation and differential degradation of polymorphs I and II and the amorphous form of clopidogrel bisulfate.

[1] Department of Pharmaceutical Analysis, National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, S.A.S. Nagar, 160 062, Mohali, Punjab, India.
[2] Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, S.A.S. Nagar, 160 062, Mohali, Punjab, India.
[3] To whom correspondence should be addressed. (e-mail: ssingh@niper.ac.in)

EXPERIMENTAL

Materials

Pure polymorphs I and II of clopidogrel bisulfate were obtained as gratis samples from Ind-Swift Laboratories Ltd. (SAS Nagar, Punjab, India). The amorphous form was generated in-house by solvent evaporation method by dissolving polymorph II in ethanol (5). HPLC grade acetonitrile was purchased from J.T. Baker (Phillipsburg NJ, USA). Buffer salts and all other chemicals were of analytical reagent grade. Ultra pure water was obtained from ELGA (Bucks, England) water purification system.

Equipment

HPLC analyses were performed on a 1200 series LC system from Agilent Technologies (Waldbronn, Germany). Chromatographic separations were achieved on a C-8 column (250×4.6 mm i.d., particle size 5 μm, Supelco Discovery Inc., Bellefonte, PA, USA).

Powder X-ray diffraction (PXRD) patterns were recorded at room temperature using D8 diffractometer (Bruker, Karlsruhe, Germany) emitting Cu Kα radiation (λ=1.54060 Å) obtained at 20 mA and 35 kV passing through nickel filter with divergent (0.5°) and receiving (1 mm) slits. The diffractometer was also equipped with a 2θ-compensating slit, which was calibrated with a silicon pellet. Data were processed using DIFFRAC^{plus} EVA (Version 9.0) diffraction software. DSC analysis was performed using 821^{e} instrument

(Mettler Toledo, Schwerzenbach, Switzerland) operating under Star^e software (version 5.1).

Solid-state stability studies at different RHs (25%, 75%, and 85%) were carried out in stability chamber (KBF 720, WTC Binder, Tuttlingen, Germany). The studies at 0% RH were carried out in glass desiccators containing phosphorus pentoxide, which were kept in ovens set at 40°C, 60°C, 80°C, and 100°C. pH/Ion analyzer (MA 235, Mettler Toledo, Schwerzenbach, Switzerland) was used to adjust and check the pH of buffers and solutions. Other equipment used were sonicator (3210, Branson Ultrasonics Corporation, Connecticut, USA), analytical balance (Mettler Toledo, Schwerzenbach, Switzerland), auto pipettes (Eppendorf, Hamburg, Germany), and rotavapor (Buchi Rotavapor R-114, Switzerland).

Critical relative humidities (CRH) of different solid forms were determined using a Q5000SA sorption analyzer (TA instruments, DE, USA).

Characterization of Solid Forms

The solid forms of clopidogrel bisulfate were characterized by DSC and PXRD. For DSC analysis, ~3-mg samples were placed in aluminum pans having pierced lids to allow escape of volatiles. A heating rate of 5°C/min was employed over a temperature range of 40°C to 240°C with continuous nitrogen purging at a flow rate of 80 mL/min for polymorphs I and II. The heating rate for the amorphous form was 20°C/min. The temperature axis and the cell constant were calibrated using indium. For PXRD analysis, the samples were directly kept on zero background sample holder. The scans were run from 3° to 40° 2θ, increasing at a step size of 0.05° with a counting time of 5 s for each step.

Sorption–Desorption Studies

Gravimetric vapor sorption studies were carried out for the determination of CRH of various solid forms and to understand their hygroscopicity behavior. For the same, 30 mg of each solid form was weighed in the sample pan and exposed to predetermined RH, using nitrogen as a carrier gas. The experiment was performed under isothermal condition at 25°C. The humidity was varied in four cycles of sorption–desorption, *viz.*, 25% → 0% (Desorption 1), 0% → 95% (Sorption 1), 95% → 0% (Desorption 2), and 0% → 95% (Sorption 2). The sample weight was recorded at the ramping rate of 5% RH throughout. The humidity was changed to the next step when the change of weight reached ±0.01 mg or after 30 min, whichever was later. Data were collected and exported to an Excel spreadsheet for graphing.

Study of the Influence of Microenvironment pH, Humidity, and Temperature

The two polymorphs (I/II) of clopidogrel bisulfate, the amorphous form, and their combinations [polymorphs I + II (P12); polymorph I + amorphous (P1Am); polymorph II + amorphous (P2Am), and polymorphs I + II + amorphous (P12Am)] were accurately weighed in equal proportions in 15 mL glass vials. Thus, the total amount was 10, 20, and 30 mg for one, two, and three form combinations, respectively. Another two sets of each were generated, where acidic and alkaline microenvironment was created by the addition of oxalic acid and sodium carbonate, respectively (5).

The samples with and without stressors were exposed for 3 months to the following humidity and temperature conditions: (1) 85% RH, 75% RH, 25% RH, and 0% RH at 40°C and (2) 0% RH at 40°C, 60°C, 80°C, and 100°C. The study under various humidities was carried out to understand the distinguishing effect of water adsorbed on the solid surface and in the bulk of the solid in case of all the three forms of the drug. The selection of humidities was based on CRH, determined from sorption studies on the three drug forms, as described above. The data at 0% RH from temperature effect study were considered as control in this case. The main objectives of the differential temperature study at 0% RH were to: (1) observe the effect of temperature alone on the stability of the three solid forms of the drug, (2) provide understanding of the mechanism of decomposition, i.e., whether the solid-state reactions were mediated through moisture only or could take place in the absence of moisture at higher temperatures, and (3) establish the mechanism of phase transformation, whether the same was solution mediated or a solid–solid transformation.

On withdrawal, the exposed samples were subjected to HPLC and PXRD analyses to determine percent drug remaining and phase transformation (if any), respectively.

Determination of the Extent of Decomposition

HPLC studies were carried out by adding to each sample a small quantity of solvent comprising of ACN/water (50:50). The vials were sonicated for 3–4 min in an ultrasonic bath. The volume was made up with 50:50 ACN/water to final drug concentration of 1 mg/mL. The rest of the method was the same, as reported earlier (5). In each condition, the percent drug remaining was calculated to compare the extent of decomposition among various solid forms.

Evaluation of the Phase Transformation

The samples were subjected to investigation of phase transformation by PXRD, employing the same conditions used for the solid-state characterization, as discussed in the previous section. PXRD evaluation was preferred over DSC as enantiotropic polymorphs could transform during the heating run.

RESULTS AND DISCUSSION

Characterization of Different Solid Forms of Clopidogrel Bisulfate

Polymorphs I and II of clopidogrel bisulfate showed enantiotropic relationship in DSC (6), as shown in Fig. 1a and b. Both of them showed melting endotherms at 172–175–177°C (onset–peak–endset), whereas polymorph I showed additional endotherm at 183–184–186°C (onset–peak–endset). This clearly indicated that polymorph I (higher melting form) was thermodynamically less stable and got converted to polymorph II (low melting form) during DSC run. The

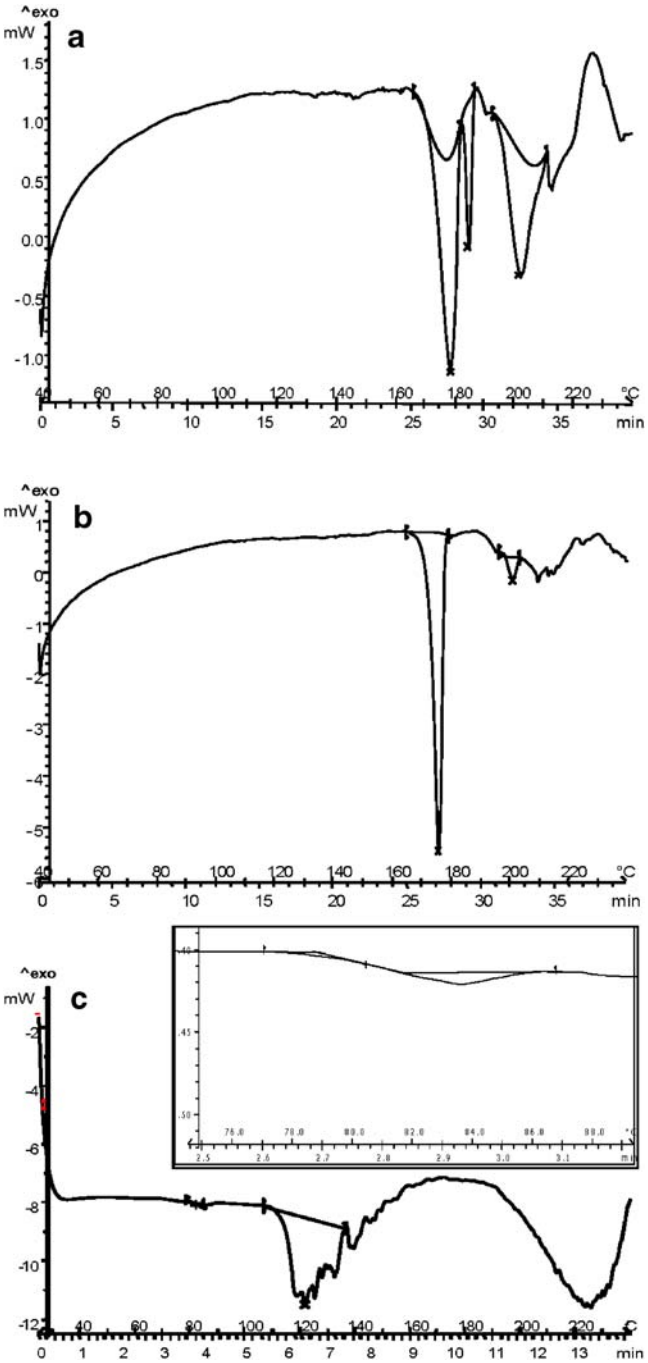

Fig. 1. DSC curves of polymorph I (**a**), polymorph II (**b**), and amorphous form generated by solvent evaporation method (**c**). (*Inset* in **c** shows glass transition event)

amorphous form generated by solvent evaporation method was also characterized by DSC. As evident in Fig. 1c (inset), its glass transition temperature was observed between 78.86°C and 80.36°C (onset–midpoint).

Figure 2 depicts PXRD patterns of the three solid forms. Polymorph I had characteristic peaks at 9.2° and 14.4° 2θ, while polymorph II showed characteristic peaks at 8.91° and 12.42° 2θ. These patterns matched the ones reported earlier (6). The PXRD pattern of amorphous form was observed without intense focused reflections and was featureless, except for a halo with a maximum centered around 35° 2θ.

Sorption–Desorption Studies on Polymorphic and Amorphous Forms

The sorption–desorption isotherms of polymorphs I and II and amorphous form of the drug are shown in Fig. 3. The water sorption by both the polymorphs was in steps (Fig. 3a and b), representing multilayer adsorption phenomena (7). The CRH was shown to be ~80% RH for both the polymorphs. Polymorph I gained ~0.4% moisture until 80% RH and the same increased to 2.5% till 95% RH. The corresponding values for polymorph II were ~0.2% and 0.9% until 80% RH and 95% RH, respectively. Thus, polymorph I was slightly more hygroscopic than polymorph II. In contrast to the polymorphic forms, amorphous form absorbed moisture linearly, indicating its higher hygroscopicity. There was a slight inflection at ~45% RH, which could be attributed to glass transition RH (8). It suggested adsorption of water molecules below 45% RH and absorption above this RH. The sorption isotherm did not reveal any indication of recrystallization, as there was no mass loss during the sorption cycle (8).

A further comparison of sorption–desorption profiles in Fig. 3 revealed that desorption and second sorption cycles in case of polymorphs I and II had similar pattern to that of the first sorption cycle. However, this was not the case with the amorphous form (Fig. 3c). It did not loose water completely during desorption cycle, meaning that it was deliquescent by nature. The same was also observed physically.

Solid-State Phase Transformations among the Investigated Forms

Extensive PXRD studies were carried out to understand the solid-state phase transformations among the three investigated forms of the drug. It was shown that all the solid forms and their combinations, without and with stressors, were stable to phase transformation on storage at 40°C and humidities of 0% RH and 25% RH. However, changes were observed at 40°C/75% RH and 40°C/85% RH. The same are summarized in Table I.

Polymorph I alone was transformed to polymorph II after storage at 40°C/75% RH as well as 40°C/85% RH, while

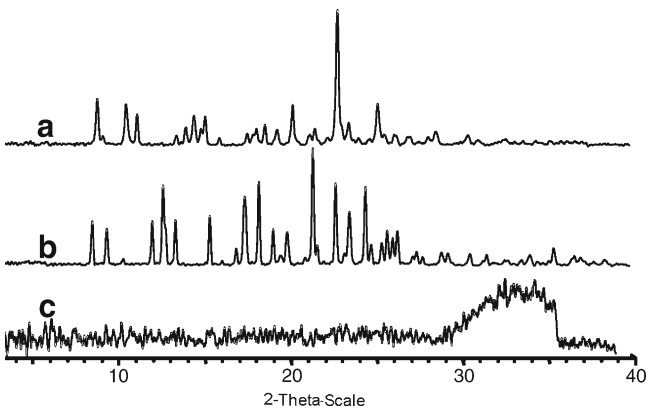

Fig. 2. PXRD patterns of polymorph I (**a**), polymorph II (**b**), and amorphous form (**c**) of clopidogrel bisulfate

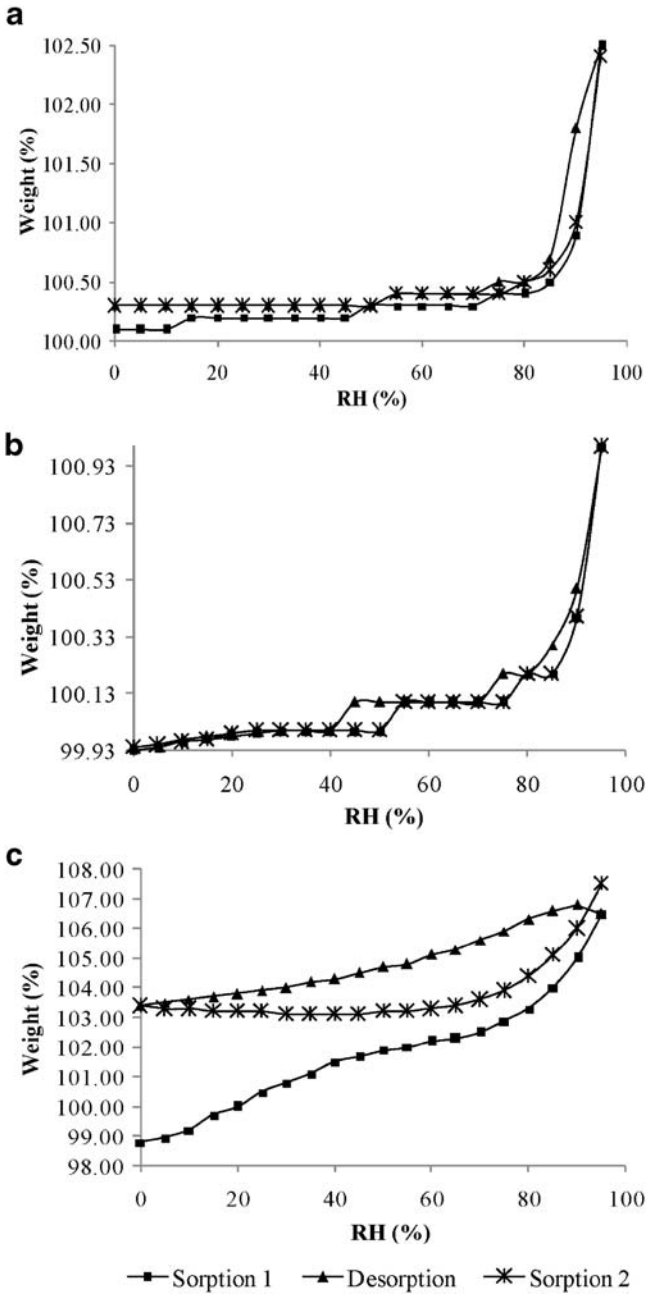

Fig. 3. Sorption isotherms of polymorph I (**a**), polymorph II (**b**), and amorphous form (**c**)

polymorph II and amorphous form were stable. The mixture of polymorphs I and II completely transformed to polymorph II at both high RH conditions. Among the mixtures containing polymorphic form(s) along with amorphous form (P1Am, P2Am, and P12Am), P1Am and P12Am were transformed to P2Am at 40°C/75% RH. P2Am itself was stable to phase transformation at this condition. However, all the mixtures completely lost crystallinity (shown by the halo pattern in PXRD) at 40°C/85% RH.

In case of single-drug forms (P1, P2, Am) with acid stressor, the phase transformation behavior was similar to that of the samples without stressors. The same was also the situation with combinations of the solid forms with acid stressor, which were stored at both 40°C/75% RH and 40°C/85% RH. The phase transformation behavior was again similar to that of the combinations without stressors at 40°C/85% RH.

Table I. Phase Transformation Behaviour of Various Solid Forms with and without Acid and Alkali Stress after 3 Months

	Solid form present in samples stored at 40°C		
	75%RH		85%RH
Samples	Without stress	Acid stress	Without and with acid stress
P1	P2	P2	P2
P2	P2	P2	P2
Am	Am	Am	Am
P1Am	P2Am	Loss of crystallinity[a]	Loss of crystallinity[a]
P2Am	P2Am	Loss of crystallinity[a]	Loss of crystallinity[a]
P12	P2	P2	P2
P12Am	P2Am	Loss of crystallinity[a]	Loss of crystallinity[a]

Under alkali stress condition, all the solid forms and their combinations were converted to free base leading to breakage of crystal structure

[a] Confirmed by the halo pattern in PXRD

Under alkaline stress conditions, the behavior was same for all the three solid forms, whether stored alone or in combinations at 40°C under high humidity of 75% RH and 85% RH. In all the samples, there was an evident physical change—an initial crystal collapse was followed by conversion to an oily paste. It indicated formation of clopidogrel base, which is oily in nature (9). The physical observations were supported by PXRD spectra, which were devoid of any peaks owing to crystalline structure. The phenomenon was similar to that reported for a maleate salt of an anti-Parkinsonian drug (10).

The temperature effect studies at 0% RH and 40°C, 60°C, 80°C, and 100°C revealed no phase transformation of the two polymorphs, either stored alone or in combinations. This confirmed that all the phase transformations occurring at higher RH were moisture mediated and not the solid–solid transformations. At 80°C and 100°C, the amorphous form converted from glassy to rubbery state; whereas there was no such transformation at lower temperatures of 40°C and 60°C. This was correlated with the glass transition temperature of the amorphous form, which was established to be less than 80°C, but higher than 60°C as discussed under the section of characterization of different solid forms of clopidogrel bisulfate. The conversion of glassy solid amorphous form to rubbery paste on storage at temperatures of ≥80°C was also observed physically.

Differential Degradation of the Three Forms and Their Combinations

Figure 4 shows the extent of decomposition of the three individual solid forms at 40°C and 0% RH, 25% RH, 75% RH and 85% RH. It is evident that all the three forms were completely stable at 0% RH, both without and with stressors. In samples without acid or alkali stress (Fig. 4a), the three solid forms were stable even at 40°C/25% RH, while increasing loss of drug occurred at 40°C/75% RH and 40°C/

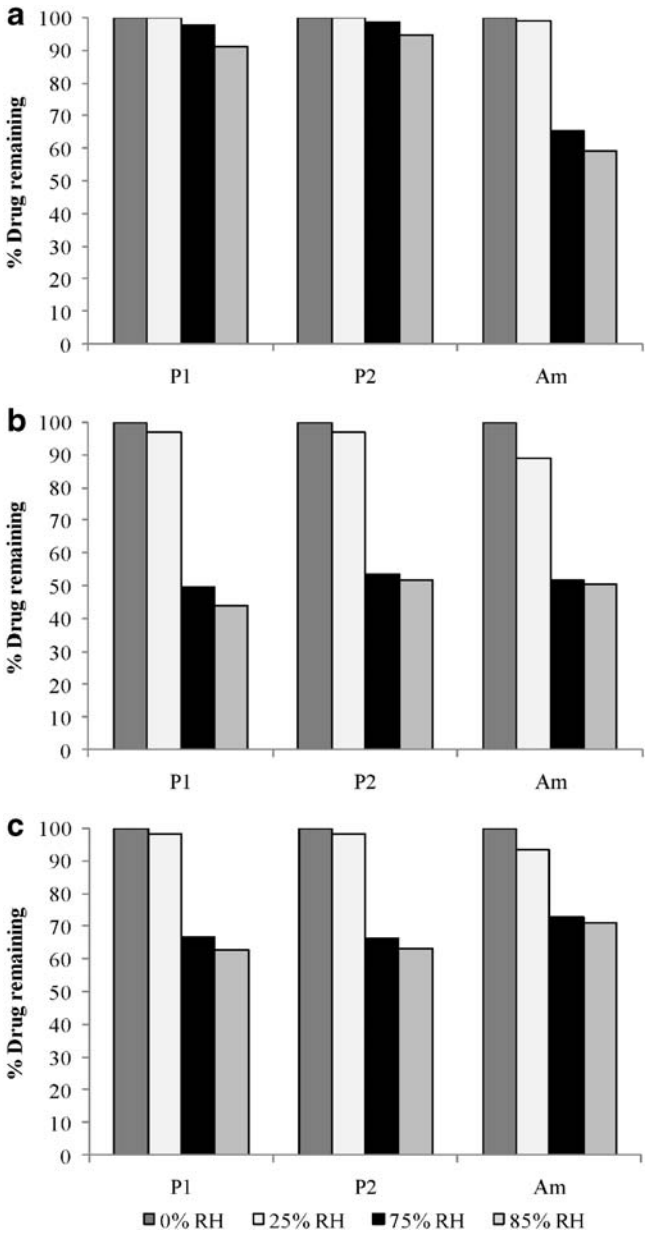

Fig. 4. Extent of degradation for the three solid forms at 40°C and various RHs (0% RH, 25% RH, 75% RH, and 85% RH) for 3 months under unstressed (**a**), acid (**b**), and alkali (**c**) conditions

85% RH. The same was much higher for the amorphous form. In case of P1 and P2, only a small increasing degradation was seen with increase in humidity in the region of CRH. However, the loss of drug was between 30% and 40% for the amorphous form, which was justified considering that once the glass transition RH was crossed, water got absorbed in the bulk of amorphous solid, instead of adsorption on the surface (8). This was even supported by observation of deliquescence in amorphous samples. Under acidic (Fig. 4b) and alkaline (Fig. 4c) stress conditions, small degradation (2-10%) occurred at 25% RH for all the three forms, while a significant loss up to 60% happened with acid stressor at 75% RH and 85% RH. The corresponding loss was lesser (maximum ~40%) at alkaline pH. This difference could be attributed to higher hygroscopicity of oxalic acid than sodium carbonate.

The bar diagrams highlighting the comparative degradation of drug among the single and combined solid forms at 40°C/75% RH and 40°C/85% RH, without and with stressors, are shown in Fig. 5. Here, only higher humidity conditions were chosen, because of significant degradation under these for single forms, as discussed above (Fig. 4). As shown in Fig. 5a and a′, the combination of polymorphs I and II (P12) did not show higher drug degradation than individual polymorphs in the absence of stressors at both the RH. As expected, the amorphous form showed more decomposition at 75% RH (~38%) than combinations (~30%) containing it (P1Am, P2Am, and P12Am). However, an opposite behavior was observed at 85% RH, where pure amorphous form was more stable (~40% drug loss) than the mixtures containing polymorphic and amorphous forms (~55% drug loss). This could be explained by physical conversion, in particular, of amorphous sample at 85% RH to a hard brown gel, which probably acted as a barrier to water penetration and hence further decomposition. Overall, the mixtures showed higher decomposition at 85% RH than 75% RH, which was due to their transformation to a high mobility system (a high energetic transition state during transformation of crystalline to amorphous state) above CRH (85% RH).

A similar behavior was observed for samples containing an acidic stressor at both 75% RH and 85% RH (Fig. 5b and b′), where drug loss in case of P1Am, P2Am, and P12Am (60–70%) was found to be more than Am alone (~50%). Under alkaline condition, except the amorphous form that showed slightly lesser decomposition (28%), all other forms showed higher and similar loss (~35%) of drug at both 75% RH and 85% RH. This was correlated to the PXRD results, which revealed conversion of bisulfate salt form in alkali to the free base for all the solid forms and their combinations.

The temperature effect studies at 0% RH and 40°C, 60°C, 80°C, and 100°C revealed no degradation of any form at 40°C and 60°C (Fig. 6). Also, P1 and P2 and their combination P12 were completely stable at all the temperatures. However, the same was not the case with the amorphous form or the combinations P1Am, P2Am, and P12Am. The amorphous form showed extensive degradation at 0% RH/80°C, with stability improving at 100°C. The combinations showed the same profile but were more stable. The behavior was correlated to the transition of glassy amorphous form to rubbery state at temperatures >78°C. The presence of small quantity of moisture entrapped in the rubbery state of amorphous form (shown by >1% loss of moisture in Sorption 1 at 0% RH in Fig. 3c), higher molecular mobility of molecules, and increased entropy of the system were perhaps responsible for enhanced drug decomposition at 80°C (11). The lower instability at 100°C was likely due to decrease in entrapped water at a high temperature.

The incorporation of stressors to create different microenvironments had no significance at 0% RH even for the amorphous form, as moisture was entrapped in rubbery state and not freely available to create differential effect of microenvironment pH. This is supported by a literature report, which suggests that microenvironment pH plays a role only when some water is present to mediate between the two components in the solid system (12).

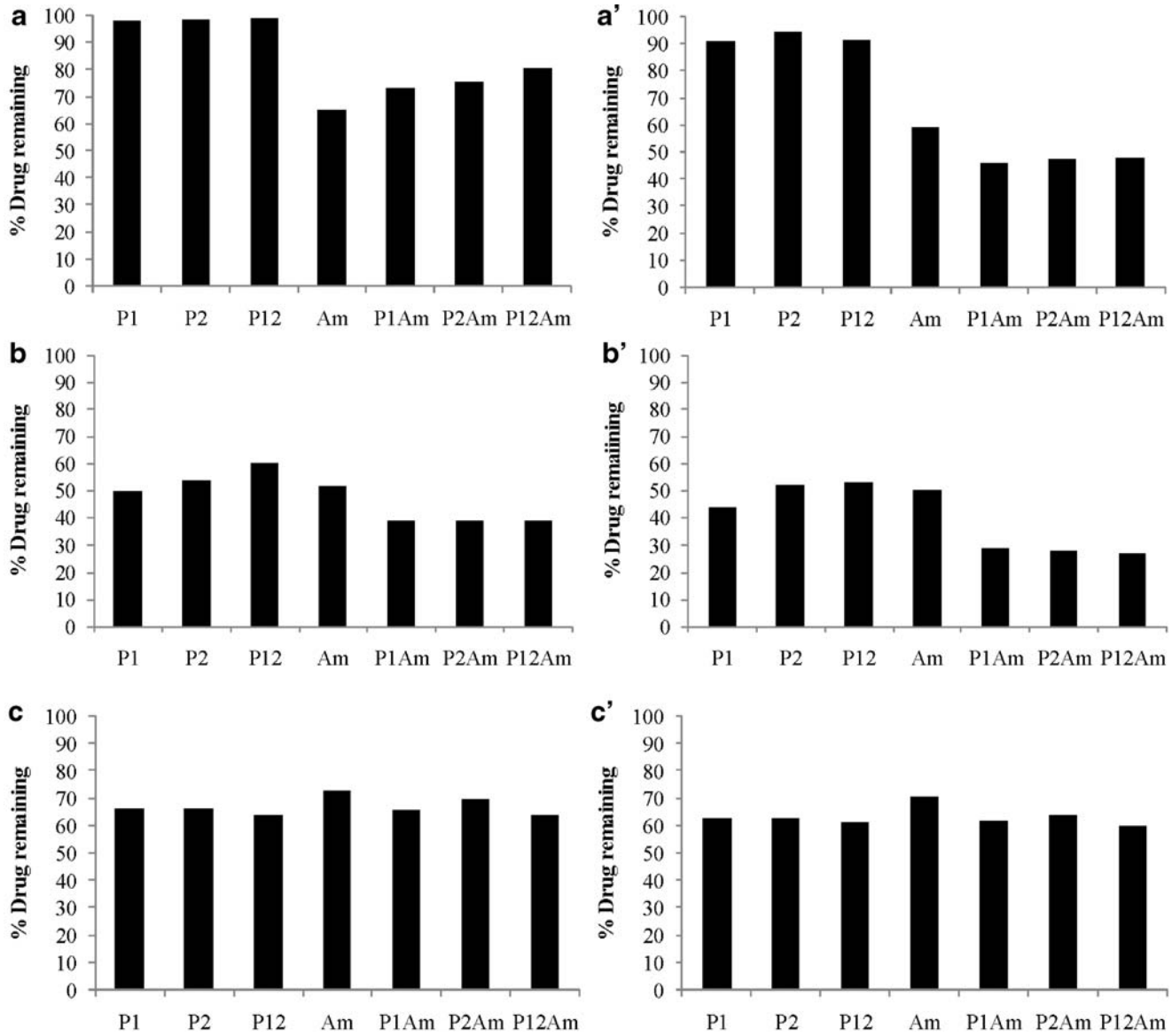

Fig. 5. Extent of degradation after 3 months of storage at 40°C/75% RH (**a**, **b**, **c**) and 40°C/85% RH (**a′**, **b′**, and **c′**). The samples **a** and **a′** contained no stressor, while **b** and **b′** and **c** and **c′** contained acid and alkali stressors, respectively

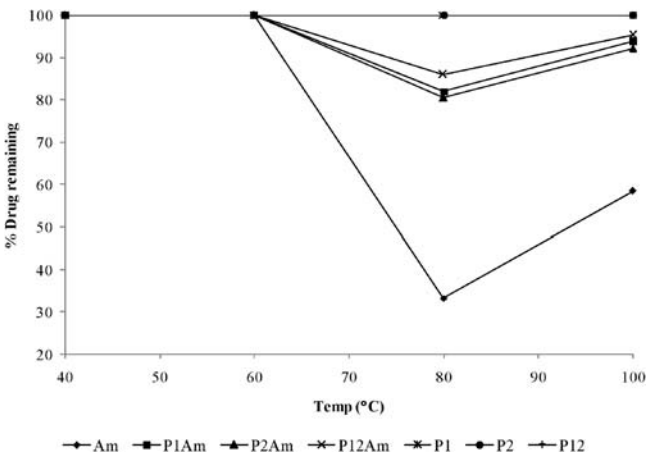

Fig. 6. Extent of degradation for different solid forms and their combinations at 0% RH and temperatures of 40°C, 60°C, 80°C, and 100°C for 3 months

CONCLUSIONS

The significant conclusions drawn from this study are as follows:

1. The study of the effect of microenvironment pH on different solid forms of the drug suggests that excipients with alkaline microenvironment are needed to be avoided, as the drug would be converted to oily free base.
2. As both degradation and phase transformation of the drug differed significantly below and above CRH, it suggests that stability studies on the drug need to be carried out at different RHs, which include points below and above CRH.
3. Similarly, the temperature effect studies at 0% RH indicate that polymorphic forms and glassy amorphous form of clopidogrel bisulfate are highly resistant to temperature, whereas rubbery state of the drug can degrade significantly at higher temperatures.

4. Because polymorph II was found to be stable under all the tested conditions in this study, hence, it should be continued to be preferred over other solid forms for solid dosage formulation development. The use of polymorph I in formulations would require close monitoring of phase transformation during manufacturing and storage. In any case, the polymorphic forms shall be stored preferably well below their CRH.
5. If the selection of amorphous form is favored owing to its advantage of higher bioavailability, it should be assured that there are no residues of crystalline form during the generation of the former. Also, the amorphous form would need manufacture and storage under controlled humidity environment and well below its glass transition temperature. Additionally, products containing amorphous form would be required to be packed in barrier packaging.

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