ORIGINAL ARTICLE

Molecular Complex of Amlodipine Besylate: Pharmaceutical Characterization and Stability Improvisation

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Abstract

The study explains to increase the stability of a drug which is hygroscopic in nature (amlodipine besylate), by the molecular complex technique. The stability enhancement achieved using the cocrystallization method by the formation of a molecular complex. Saccharin as a coformer is formed by molecular complex. The selection of coformer was done based on the Hansen parameter where theoretical value was obtained which gave some coformer values and practically molecular complex study was carried out by the solubility method. No any interaction between amlodipine besylate and saccharin was confirmed by Fourier transform infrared (FTIR) and differential scanning calorimetry (DSC) studies. The stability-enhancing property of cocrystals was carried out by short-term accelerated stability studies. To perform stability analysis, few methods of cocrystallization were carried out. These cocrystals were evaluated by FTIR, DSC, and powder X-ray diffraction studies. It is concluded that the molecular complex of amlodipine besylate with sodium saccharin shows significant stability and highlights the use of cocrystallization in stability enhancement. The miscibility of drug and coformers as predicated by Hansen solubility parameter, can indicate cocrystal preparation. The resultant δ values of drug and coformers are compared and their solid state miscibility is expressed. The possibility of cocrystal formulation by krevelens is $\Delta\delta < 5$ MP and Greenhalgh $\Delta\delta < 7$ MP

Key words: Amlodipine, cocrystallization, coformer, Hansen parameter, hygroscopic, molecular complex, stability

INTRODUCTION

Any functionally important quality feature of a medication product that varies over time falls within the jurisdiction of pharmaceutical scientists and regulators who quantify drug product stability and shelf life. Although in many jurisdictions the maximum shelf life, a regulatory agency will approve a drug product is 5 years, and other products may, if properly maintained and packaged, keep integrity for a decade or more.[1-3]

Amlodipine besylate (AML), (-4R, S)-3-ethyl -5-methyl2-(2-amino ethoxy-methyl) is a strong long-acting Ca channel blocker-4-(2-chlorophenyl)14dihydro6methyl pyridinemonobenzene 3,5-dicarboxylate, sulfonate. Charged calcium channel blocker (CCB) of the dihydropyridine type (DHP) known as HT amlodipine has been used extensively to treat angina and hypertension. Amlodipine has a wide volume of distribution (21 L/kg) and a lengthy half-life (30–58 h). Up to 5–10 times the therapeutic amount can cause toxicity, which develops 30–60 min after intake. It selectively suppresses the proliferation of arterial vascular smooth muscle cells to stop the arteries from becoming increasingly narrower.[4]

The photosensitive and liable to degradation both in solution and in a solid state is shown by amlodipine besylate, resembling

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all members of 1,4-DHP CCBs. The lack of therapeutic effects is due to the light catalyzes causing oxidation to pyridine derivatives, such as amlox (2-[2-aminoethoxy] methyl-4-[2-chlorophenyl]-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl pyridine). Forced degradation studies under thermal stress show amlodipine degrades slowly under heat stress (more so in solution than in the solid state), more quickly under light stress, and even more under acidic, alkaline, and oxidative stress, with alkaline circumstances having the highest levels of degradation.[5]

Solubility is one of the key criteria for pharmacological response because it affects the therapeutic efficiency of drug molecules, which is dependent on bioavailability to attain the required drug concentration in the systemic circulation. Numerous novel medications and their derivatives are now readily available as a result of advanced research and development.[6] More than 40% of lipophilic drug candidates fail to reach the market due to poor bioavailability; however, these medications may exhibit significant pharmacodynamic actions. A high dose of the lipophilic drug is required to reach the market for proper pharmacological action. The aims of the present work were to prepare a molecular complex of amlodipine using four different cocrystallization techniques to evaluate the critical 3D solubilities properties and to obtain information on the degradation pathways during storage of the bulk drug. The degradation product formed on subjecting amlodipine to different conditions. Based on the findings of the forced degradation study, an in-depth understanding of the drug's chemical and physical stability was gained.

Amlodipine was subjected to diverse conditions and a degradation product developed. The drug's chemical and physical stability served as the foundation for the forced degradation study.[7-9] The goal of the study is to produce an amlodipine molecular complex by applying various cocrystallization techniques and improving the cocrystals' stability under various storage conditions.

Hansen solubility parameters (HSPs)

Materials with identical values would be miscible, according to the solubility parameter theory developed by Hildebrand and Scott in 1964 (Hildebrand and Scott). The HSP model, developed in 1967, introduced the idea of breaking down the total cohesive energy into its constituent components, namely, dispersion, polarization, and hydrogen bonding.[9-11] The most effective methods found in Hansen Solubility characteristics are surface wettability, surface-liquid miscibility, polymer mix miscibility, pigment binding capacity, and drug miscibility with excipients/carriers in solid dispersions. HSPs advise using them as a tool in the pre-formulation and formulation development of tablets to forecast the compatibility of therapeutic components. Amlodipine was used as the model active pharmaceutical ingredient in this investigation to determine whether the miscibility of a medication and its coformer components, as predicted by theoretical miscibility tools, could be utilized to forecast the formation of cocrystal.[12,13] Coformers' and amlodipine's HSPs were calculated using group contribution methods. To forecast the miscibility of amlodipine using a coformer, three well-established miscibility tools are used. Thermal techniques and liquid-assisted grinding, which are based on the prediction of miscibility, were used in the laboratory screening for cocrystals. According to Hildebrand *et al.*, the cohesion energy density is the energy of vaporization per unit volume. Hansen determined that total cohesion energy is the sum of dispersion E_D, polar E_p, and hydrogen bond energy E_H.

By dividing both sides of the equation by the molar volume V, we will now have the total HSP or Hildebrand solubility parameter, which is T.

If the T values of the solute and solvent are similar, solubility can be predicted. The most frequently used units for in literatures are (J/m^3) 0.5, MPa 0.5, or (cal/cm^3) 0.5, where one (cal/cm^3) 0.5 is equivalent to 2.0421 MPa 0.5 or (J/m^3) 0.5. According to either direct or indirect measuring of a material's inherent qualities, such as evaporation temperature, viscosity, and solubility in prescribed solvents, calculating methods varied between theoretical and practical ones.

Theoretical screening/prediction of amlodipine for cocrystallization

To determine the solubility parameters for dry solutes, the group contribution method is applied. The currently employed techniques are calculations using Hoys molar attraction constants, Fedors substituent constants, and van Krevelen constants. These techniques were applied in the current investigation to determine the solubility parameter values. The fundamental steps in Fedor's method are to open the rings and create the consequent structure as an open chain compound. The approximation substituent constants are then used.[14,15] The solubility parameter is calculated as the square root of the total sum of substituent constants divided by the total sum of substituent constants in the molar volume after they are summed up. The molar attraction constant to molar volume ratio is expressed using the Hoys method. The solid state miscibility of the medication and conformers is expressed by comparing the resultant values.[16-20] The group contribution approach and the choice of a coformer that is appropriate for the medicine are utilized for theoretical computation. The HSP determines whether drug and coformer are compatible and form the molecular complex with drug and coformer. The Fedors method, Hoys method, and van Krevelens method calculation is based on the attachment of atom or molecules form the structure. These methods are used for theoretical calculation of solubility. The theoretical prediction or possibility of cocrystal formulation by Krevelens $\Delta \leq 5$ MP and Greenhalgh $\Delta \leq 7$ MP.[16-20]

Whether a medicine and coformer are compatible and can form a molecular complex together is determined by the HSP.

Calculations using the Fedors, Hoys, and van Krevelens methods are based on the attachment of atoms or molecules to a structure. These techniques are employed in theoretical solubility calculations. Theoretical cocrystal formulation predictions made by Krevelens and Greenhalgh at $\Delta \leq 5$MP and $\Delta \leq 7$ MP.

MATERIALS AND METHODS

Materials

Amlodipine was obtained as a gift sample from Unique Biological and Chemical Ltd. in Kolhapur, India. All other chemicals, such as saccharine and buffer, as well as solvents, such as ethanol, methanol, acetone, HCl, and Karl-Fischer reagent, were of analytical quality and were purchased from Merck in India and Molychem in Mumbai.

Theoretical prediction of solubility[16,17]

Fedors method/Fedors substituent constants

$$\delta = \sqrt{\frac{\sum \Delta\Delta U}{\sum \Delta V}} \quad (1)$$

Where,
*ΔΔU is constant for energy mixing
**ΔV is constant for molar volume

Hoys method/Hoys molar attractions

According to ([cal cc] 1/2 mol-1) unit

$$\delta = \frac{\sum \text{molarattraction}}{V} \quad (2)$$

van Krevelens Solubility Parameters

The given calculation of solubility parameter and molar volume by van Krevelens method is based on experimental molar volume and measured cm^3 Mol^{-1}

$$\delta d = \sum Fd / V \quad (3)$$

$$\delta p = \sqrt{\sum Fp^2} / V \quad (4)$$

$$\delta h = \sqrt{\sum Uh / V} \quad (5)$$

$$\delta^2 T = \sqrt{\delta d^2 + \delta p^2 + \delta h^2} \quad (6)$$

Preparation of cocrystals

Solvent drop grinding

In the solvent drop grinding process, amlodipine and coformer were weighed in a 1:1 molar ratio and ground together with the addition of 3–4 drops of ethanol. The combination was left at room temperature for 30 min.[21-23]

Slow evaporation method

Amlodipine and the coformer were separately dissolved in ethanol in a 1:1 molar ratio for the slow evaporation method. After stirring, the ingredients were combined and left to sit at room temperature for 48 h. The obtained crystal was collected, put in a secure container, and kept in desiccators for further use.[22]

Anti-solvent addition procedure

Amlodipine and coformer were dissolved in 20 ml of ethanol with moderate agitation before being filtered through Whatman filter paper to eliminate any remaining undissolved substances. The aforementioned solution was then gradually supplemented with distilled water while being constantly stirred to produce cocrystal precipitation. In desiccators, the cocrystals were left to dry overnight.[24]

Slurry method

Amlodipine and coformer were carefully weighed and combined in a mortar at a 1:1 molar ratio until the mixture was homogeneous. 15 ml of water was then added to the mixture to create the slurry sample solution. The cocrystal that had developed was dried for 48 h at 400 C. Desiccators were used to gather and store the solid crystal.

Analysis of molecular complexation by solubility

The molecules are characterized as those in which the majority of the bonding structures, whether they involve atoms or molecules, can be explained by classical valency theories, but one of these bonds is slightly aberrant.[25-28]

Amlodipine stock solution (0.1 M)

The molecular weight amlodipine was used to prepare 0.1 M stock solution.

Saccharine solution

The molecular weight of saccharine is 250.16 weights accurately the required number of sample of saccharine each containing 100 mg.[29,30]

Evaluation of cocrystals of AMB

Saturation solubility of cocrystals

Utilizing methanol and distilled water, research on saturation solubility was conducted. Cocrystals were formed in screw-capped test tubes with a fixed volume of methanol (10 ml) and an excessive amount of amlodipine (10 mg) in each. After treating the resulting suspension at 37°C with 100 rpm in an incubator shaker for 24 h,

samples were removed and filtered using a 0.2 filter. The filtrate was appropriately diluted with ethanol, and a UV visible spectrophotometer was used to observe it at 361 nm.[28]

Drug content

Powdered cocrystals created, equal to 10 mg amlodipine was weighed, dissolved in 100 ml of methanol, filtered using Whatman filter paper, and the volume was adjusted to 100 ml. These samples underwent spectrophotometric analysis at 361 nm.[29]

Moisture content

Using the Veego Digital Karl Fisher Apparatus, the moisture content of produced cocrystals and pure amlodipine was determined. Utilizing methanol with a 0.05% water content of Karl Fischer grade, the device was calibrated. The amount of reagent needed to neutralize the sample was then calculated in terms of % ppm and mg of H_2O present after the addition of 50 mg of sample and titration with the Karl Fisher reagent.[31]

Forced degradation

The accelerated stability study, an experimental design, is used to assess the stability of a product by speeding up the rate of reaction. Amlodipine, accurately weighed at 50 mg, was dissolved in 2 ml of methanol, and the resulting solution was combined with 50 ml of 0.1 N HCl and heated to 40°C. After adding this solution, immediately pipette out 5 ml of the mixture, transfer it to a 10 ml volumetric flask, and then dilute it by 10 ml. Similar steps were now followed, and samples were taken after 10, 20, 30, 40, 50, and 60 min. After diluting every sample, a UV visible spectrophotometer was used to detect the absorbance at 361 nm. The same process was used for 50°C and 60°C.[32,33]

Solid state characterizations of cocrystals of AMB

Fourier-transform infrared spectroscopy (FTIR)

FT-IR (Cary-60 ATR) spectra are utilized to detect any changes in the chemical makeup of amlodipine and its conformers, and spectra were recorded on a Cary-60 ATR. FTIR spectrometer operating in the 4000–400 cm^{-1} range.[34,35]

Differential scanning calorimetry (DSC)

Using a DSC-60A (Shimadzu, Tokyo, Japan) calorimeter, it was determined how amlodipine alone and produced cocrystals behaved thermally. The samples were heated to temperatures ranging from 500 C to 3000 C at a scanning rate of 100 C/min in hermetically sealed aluminium pans with nitrogen flow (30 ml/min).[36]

Powder X-ray diffraction studies (PXRD)

Using a Philips analytical X-ray diffractometer (Model: PW 3710) (Philips, The Netherlands) with a copper target throughout the range of 5–70°, the X-ray diffraction patterns of pure amlodipine and the optimized crystal preparations were captured. The parameters were as follows: acquisition temperature of room temperature; detector scintillation counter detector; sample holder non-rotating holder; voltage 40 kV; current 30 mA; and scanning speed 20/min.[37]

Physical stability of prepared cocrystals

Physical stability of made-up cocrystals: Cocrystals' stability was studied for 3 months at 40°C and 75% relative humidity. A stability chamber (CHM 10S; REMI Instruments Ltd., Thane, Maharashtra, India) was used to hold the crystals for 3 months after they had been placed into cap vials packed with aluminium strips. Samples were taken out, and the presence of drugs was examined.[38-40]

Table 1: Calculation of∂value of amlodipine by F, G, C method

Fragments/Groups	No. of groups	ΔΔU* for each (cal.mol^{-1})	Total ΔΔU	ΔV** for each (m^{-1} mol^{-1})	Total ΔV
-Cl	1	2760	2760	24	24
$-CH_3$	3	1125	3375	33.5	100.5
$-CH_2$	4	11801180	4720	16.1	64.4
-C=	3	1030	3090	13.5	40.5
-CH-	16	820	13120	−1.0	−16
-C-	0	350	0	−19.2	0
-C=O-	2	4150	8300	10.8	21.6
-O-	3	800	2400	3.8	11.4
$-NH_2$	1	3000	3000	19.2	19.2
$-SO_3$	1	4500	45004500	27.6	127.6
-NH	1	2000	20002000	4.5	4.5
Ring closer	2	250	500	16	32
Conjugated bond	7	400	2800	−2.2	−15.4

*ΔΔU is constant for energy mixing. **ΔV is constant for molar volume

Table 2: Theoretical prediction of cocrystal formation by Fedors method

Compound	δ value	Difference δ1–δ2	Δδ	Possibility of cocrystal formation	
				Krevelens Δδ ≤ 5 MP	Greenhalgh Δδ ≤ 7 MP
Amlodipine	12.68 H			-	
Saccharin	13.07 H	12.68–13.07	0.39	Yes	
Sorbitol	23.41	23.41–12.68	10.73	No	

Table 3: Calculation of solubility parameter of amlodipine based on Hoys molar attractions

Fragments/groups	No. of groups	ΔΔU* for each (cal.mol^{-1})	Total ΔΔU	ΔV** for each (m^{-1} mol^{-1})	Total ΔV
-Cl	1	161	161	19.504	19.504
-CH=	0	117.12	0	13.417	0
-CH_2	4	131.5	526	15.553	62.212
-CH_3	3	148.36	445.08	21.548	64.644
C=O	2	262.96	525.92	17.265	34.53
-CH-	16	85.99	1375.84	9.557	152.912
-O-	3	114.98	344.94	6.46	19.38
-C-	0	32.03	0	3.562	0
Six membered ring	2	–23.44	–46.88	0	0
Conjugated bond	7	23.26	162.82	0	0
Ortho	1	9.69	19.38	0	0
Meta	1	6.6	6.6	0	0
Base value	0	0	0	0	0

Table 4: Theoretical prediction of cocrystal formation by Hoys Method

Compound	δ value	Difference δ1- δ2	Δδ	Possibility of cocrystal formation	
				Krevelens δ ≤ 5 MP	Greenhalgh Δδ ≤ 7 MP
Amlodipine	9.78H				
Sucrose	15.31H	15.31–10.03	5.1	Yes	
Saccharin	15.53H	15.53–10.03	5.5	Yes	
Sorbitol	15.13H	17.32–10.03	7.29	No	

RESULTS AND DISCUSSION

Theoretical prediction of solubility

Fedors substitution constants

A method to calculate the solubility parameter without considering the compound's density value, as suggested by Fedors. Two factors support the claim that this approach is superior to Smalls': The method just requires knowledge of the compound's structural formula, and the contributions of a significantly larger number of functional groups have been examined[41] [Table 1]. The following equation is used for directly determining (cf):

$$\delta 2 = \sqrt{\frac{\sum \Delta \Delta U}{\Delta V}} \quad (7)$$

Where, ΔΔU and ΔV are the constant for energy mixing and constant for molar volume for the energy of vaporizations and molar volume, respectively [Table 2]. Calculation of solubility parameter and molar volume of amlodipine based on Fedors substitution constants.

$$\sum = 50565, \ \sum = 314.3$$

Based on Fedors Substitution constants

$$\delta 2 = \sqrt{\frac{\sum \Delta \Delta U}{\Delta V}} = 12.68 \text{ H} \quad (8)$$

Hoys Method

Smalls technique is employed to estimate the SP value for numerous solvents and polymers. The list of constants, however, is not exhaustive. Hoy published more group molar attraction constants that she had calculated by measuring the vapor pressure of numerous different groups[42] [Table 3]. Solubility parameter (δ) is calculated from the following equation:

Table 5: Calculation of solubility parameter and molar volume of Amlodipine by van Krevelens solubility parameter

Fragments/Groups	No. of groups	fd	Total Fd	Fp	Total Fp	Fp2	Uh	Total Uh
-Cl	1	450	450	550	550	302500	400	400
CH2	4	270	1080	0	0	0	0	0
-CH3	3	420	1260	0	0	0	0	0
-C=	3	200	600	0	0	0	0	0
-CH-	16	80	1280	0	0	0	0	0
-C-	0	-70	0	0	0	0	0	0
C=O	2	100	200	410	820	0	0	0
-O-	3	100	300	410	1230	168100	3000	6000
6/5 membered Ring	2	190	380	0	0	672400	3000	6000
			Σ=5060			Σ=974900		Σ=6400

Table 6: Theoretical prediction of cocrystal formation by van Krevelen method

Compound	δ value	Difference δ1–δ2	Δδ	Possibility of cocrystal formation	
				Krevelens Δδ≤5 MP	Greenhalgh Δδ≤7 MP
Amlodipine	6.05H				
Saccharin	2.00H	6.05–2.00	4.05	Yes	
Sorbitol	7.18H	7.71–7.18	1.66	Yes	

$$\frac{cf-density\times\sum Fi}{\text{molecular weight}}$$

Where is the $\sum F$ sum of the group molar attraction constants of the compound. Hoftyzer and van Krevelen published a series of group molar attraction constants similar to small and Hoy [Table 4].

$$\sum = 3142.63 \quad \sum 321.686$$

According to [(cal cc) 1/2 mol-1] unit

$$\delta_{2=}\sum\frac{\text{Molar attraction}}{\text{V2}} = 9.78\ \text{H}$$

van Krevelens method

Van Krevelen derived Fi values for the contributions of atoms, that is, C, H, N, 0, halogens, and constitutional effects [Table 5][42-44] (such as double or tribal bonds). Solubility parameter (δ) can be calculated using the following equation:

$$\delta = \frac{\sum Fi}{Vm} \qquad (9)$$

Where, $\sum = Fi$ is the sum of the atomic contribution and Vm is molar volume [Table 6].

Based on experimental molar volume 216 cm^3 Mol^{-1}

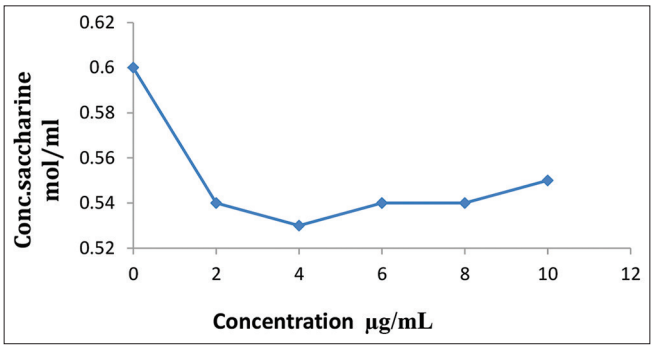

Figure 1: Molecular complex of amlodipine-saccharine cocrystals

$$\delta d = \sum\frac{fd}{v} = \frac{5550}{216} = 25.69\ \text{M.pa}^{1/2}\text{or } 12.84\ \text{cal}^{1/2}\text{cm}^{-3/2}$$

$$\delta p = \sqrt{\sum\frac{fp2}{v}} = \sqrt{\frac{1143000}{216}} = 5.291\ \text{M.pa}^{1/2}\text{or } 2.64\ \text{cal}^{1/2}\text{cm}^{-3/2}$$

$$\delta h = \sqrt{\sum\frac{uh}{v}} = \sqrt{\frac{12400}{216}} = 5.74\ \text{M.pa}^{1/2}\text{or } 2.87\ \text{cal}^{1/2}\text{cm}^{-3/2}$$

$$\delta 2T = \sqrt{\delta d^2 + \delta p^2 + \delta h^2}$$

$$= \sqrt{25.69 + 5.29 + 5.74}$$

$$= \sqrt{36.72}$$

$$= 6.05\ \text{H}$$

Preparation and evaluation of amb cocrystal

Analysis of molecular complexation solubility method[45-47]

Complex compounds are defined as molecules in which the majority of the bonding structures, whether they are atoms or molecules, can be explained by standard theories of valency, but one of these bonds is rather abnormal. Complexes have some characteristics that set them apart from their constituent parts. To confirm complex formation, properties such as solubility, light absorption, conductance, partitioning behavior, and chemical reactivity are examined.

$$\text{Stoichiometric ratio} = \frac{\text{Amlodipine complex}}{\text{Saccharine complex}}$$

Considering the concentration of amlodipine and saccharine entering the complexation during plateau region, B C [Figure 1].

$$\text{Stoichiometric ratio} = \frac{\text{Amlodipine enteringin to complex}}{\text{Saccharine enteringin to complex}}$$

Amlodipine entering into complex = [Amlodipine] at point C – [Amlodipine] at point B = 2 mol/L

Saccharine entering into complex = [Saccharine] total taken – [Saccharine] at point B or C = 0.06 mol/L

$$\text{Ratio} = \frac{\text{Amlodipine complex}}{\text{Saccharine complex}} = 33.33$$

Therefore, donor or accepter = 1:33

Stability constant:

$$\text{Stability constant K} = \frac{\text{Saccharine} - \text{Amlodipine}}{[\text{Amlodipine}]\ [\text{Saccharine}]}$$

Saccharine – Amlodipine complex = $(0.60 \times 10^{-2}) - (0.54 \times 10^{-2}) = 0.06 \times 10^{-2}$ mol/L

Amlodipine complexed = [Saccharine – Amlodipine] = 0.06×10^{-2} mol/L

(Based on equimolar relationship)

[Saccharine] uncomplexed = [Saccharine] at solubility = 0.60×10^{-2} mol/L

Amlodipine = $(2 \times 10^{-2}) - (0.06) = 1.94 \times 10^{-2}$

$$\text{Stability constant K} = \frac{\text{Saccharine} - \text{Amlodipine}}{[\text{Saccharine}]\ [\text{Amlodipine}]} = 5.15 \text{ L/mol}$$

The complex of amlodipine and saccharine is 5.15 L/mol which is equilibrium stability constant [Figure 1]. $\Delta\delta \leq 5$ MP and $\Delta\delta \leq 7$ MP is theoretically and Practically Stability Constant of Saccharine and Amlodipine. By employing saccharine as a coformer, we can predict that amlodipine will form a molecular complex. On a molecular level, cocrystals are miscible systems. Therefore, it is hypothesized that a measure of the component molecules' miscibility in the solid state could foretell the chance of cocrystal formation, which would be helpful in cocrystal screening.

The drug/coformer systems with $\Delta\delta \leq 7$ MP or ≤ 5 MP indicates eutectic/melting point depression explaining that the miscibility predicted by Greenhalgh correlated well with that determined by DSC. The proposed system will be a cocrystal if these two conditions are met. Cocrystals exhibit a decrease in melting point in DSC investigations of amlodipine and coformers, but the difference in values is <5; therefore, the prepared complex can be referred to as cocrystals.

Saturation Solubility Study of amlodipine and amlodipine – coformer cocrystals

Table 7 provides the experimentally measured solubility of amlodipine in methanol solution. When compared to their cocrystals and drug alone, the produced cocrystals with coformer saccharine demonstrated much higher solubility. It is expected that amlodipine will dissolve well in cocrystal form due to a decrease in crystallinity and the development of hydrogen bonds between the drug and conformer.[46] Because the addition of an anti-solvent reduces the solute's solubility in the resulting system or changes the solute through a chemical reaction to produce a substance with much lower solubility, the prepared cocrystal has a higher solubility than amlodipine and other cocrystals.

Table 7: The percentage AMB content in cocrystals using different of preparation

No.	Method of cocrystal preparation	% yield	% amlodipine content in saccharine coformer
F1	Slow evaporation	87	95±0.81
F2	Solvent grinding	90.5	94.11±0.2
F3	Anti-solvent grinding	89	96.60±0.12
F4	Slurry method	92	94.45±0.60

*All values are mean±SD (*n*=3)

Table 8: Moisture content in amlodipine besylate

Parameter	Values
Factor	19.23
KFR reading	1.57
%	28.53640
ppm	2853640
Mg H_2O	8.56092

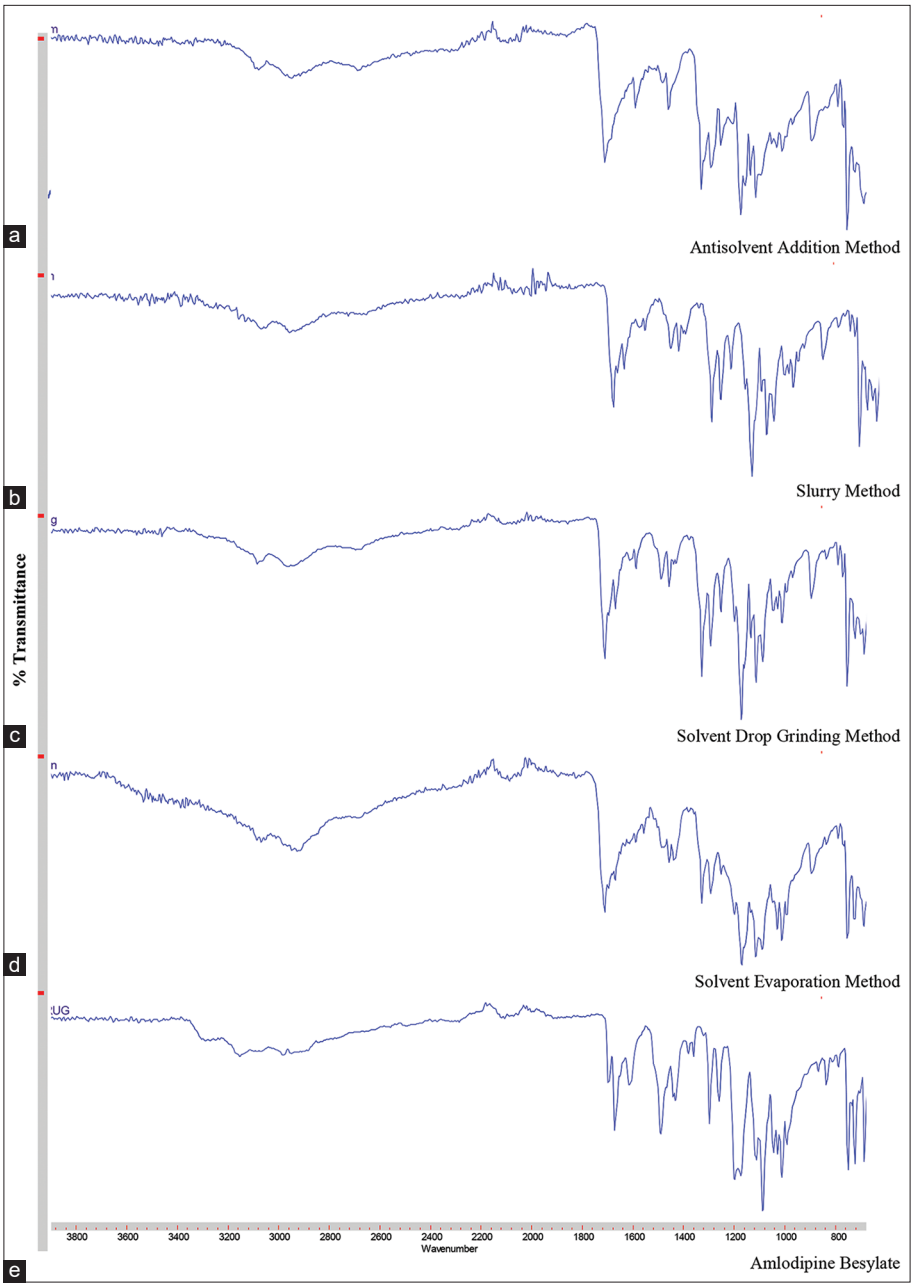

Figure 2: Comparative Fourier transform infrared pattern of amlodipine and co crystals using different methods. (a) Pure amlodipine, (b) slow evaporation, (c) solvent drop grinding, (d) anti-solvent addition method, and (e) slurry method

Table 9: Forced degradation study of amlodipine cocrystals prepared in methanol and water

Method of cocrystal preparation	% drug content in cocrystals with methanol			% drug content in cocrystals with Distilled water		
	Initial (%) drug content	(%) drug content (6 months)		Initial (%) drug content	(%) drug content (6 months)	
		R. T.	45°C		R. T.	45°C
Amlodipine	96.41±1.33	94.15±0.16	69.43±0.58	-	-	-
AAM	96.60±0.12	95.85±0.23	94.66±0.39	89.74±0.31	89.57±0.1	85.47±0.41
SEM	95.00±0.81	94.59±0.64	93.9±0.68	89.21±0.54	87.92±0.86	84.22±0.66
SGM	94.11±0.23	93.36±0.6	92.29±0.6	86.85±0.6	84.38±0.25	82.61±0.59
SM	95.45±0.60	94.33±0.15	94.08±0.61	88.72±0.34	85.54±0.61	83.60±1.64

*All values are mean±SD (*n*=3); AAM: Anti-solvent Grinding method, SEM: Slow evaporation method, SM: Slurry method, SGM: Solvent grinding method

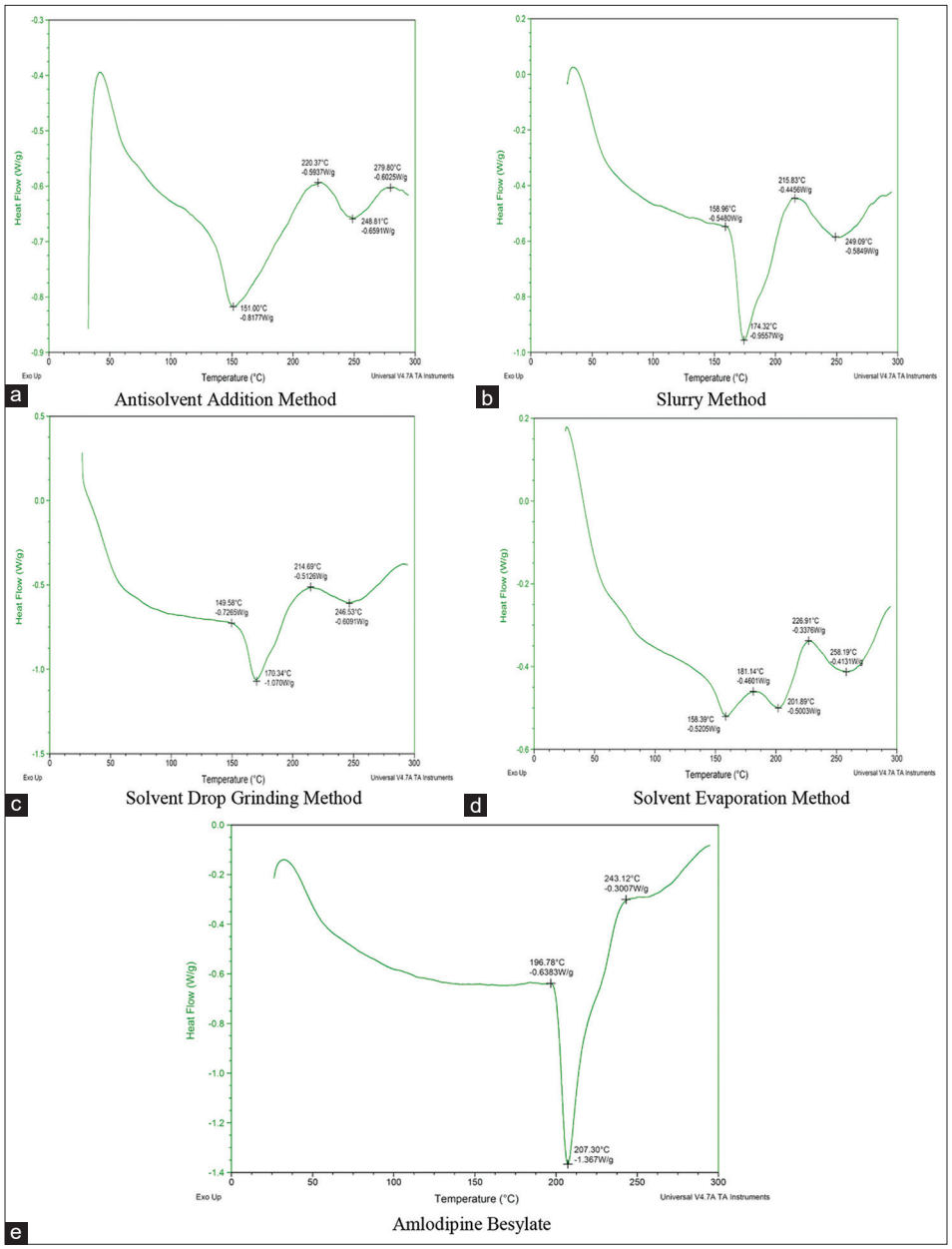

Figure 3: Differential scanning calorimetry thermograms of amlodipine and cocrystals using different methods. (a) Pure amlodipine, (b) slow evaporation, (c) solvent drop grinding, (d) anti-solvent addition method, and (e) slurry method

Drug content

Drug content analysis was done on cocrystals prepared by all methods in triplicate.[47] The amlodipine content in the prepared cocrystals showed in range of 94–95% as mentioned in Table 7.

Moisture content

The presence of moisture in cocrystal prepared by saccharine as a coformer was estimated using Veego Karl Fischer Titration apparatus, this digital apparatus gives reading for content of water/moisture present in the preparation [Table 8].

Compaction, powdered form, and chemical stability due to moisture, a solid dosage form's lubricant sensitivity, dissolving rate, and polymer film permeability are all affected. Microorganism growth is caused by a change in thixotropy in semi-solid dose forms due to moisture content.[44] The solubility property of amlodipine is difficult if water is present in the medicine, and as a result, aqueous stability is diminished due to the presence of moisture in the amlodipine. The presence of moisture in amlodipine besylate also affects aqueous preparations. Table 8 lists the moisture content of cocrystals made with sugar as a conformer.

Forced degradation

When compared to pure amlodipine besylate, a stability study shows that physical mixtures significantly increase the drug's stability. However, stability data for amlodipine cocrystals

show an increase in stability; this could be because the drug's crystal size has grown to take on a crystalline form. The microstructural characteristics of the composites are crucial in the drug dissolving process [Table 9]. By conducting and X-ray photoelectronic spectroscopy investigations, this aspect will be examined. Studies on the stability of physical mixes and cocrystals unequivocally demonstrate that stability rises as the ratio of medication to carrier increases. In addition, a 1:1 ratio was discovered to be the optimum ratio because stability remained steady beyond that point.

Solid state characterizations of amlodipine cocrystals

FTIR spectroscopy

The interaction between amlodipine and the cocrystal formers is explained through FTIR spectroscopy. According to the FTIR data, all significant peaks caused by the drug's functional groups, as well as a few additional peaks, were found in the cocrystals. When manufactured cocrystals of amlodipine were compared to pure medication, the results showed significant differences, showing hydrogen bonding had taken place in the cocrystals [Figure 2].

At 1298 and 1287, the C-N stretch peak is confirmed. Depending on the strength of the connection, any trace of interaction in this situation would be represented by a shift in N-H vibrations. Frequency changes or peak splitting in absorption will be the result of any physicochemical interactions that may occur, such as the formation of hydrogen bonds between the carrier and medication. The cocrystallization of coformer and amlodipine appeared to include a secondary interaction, according to FTIR spectroscopy. The hydroxyl

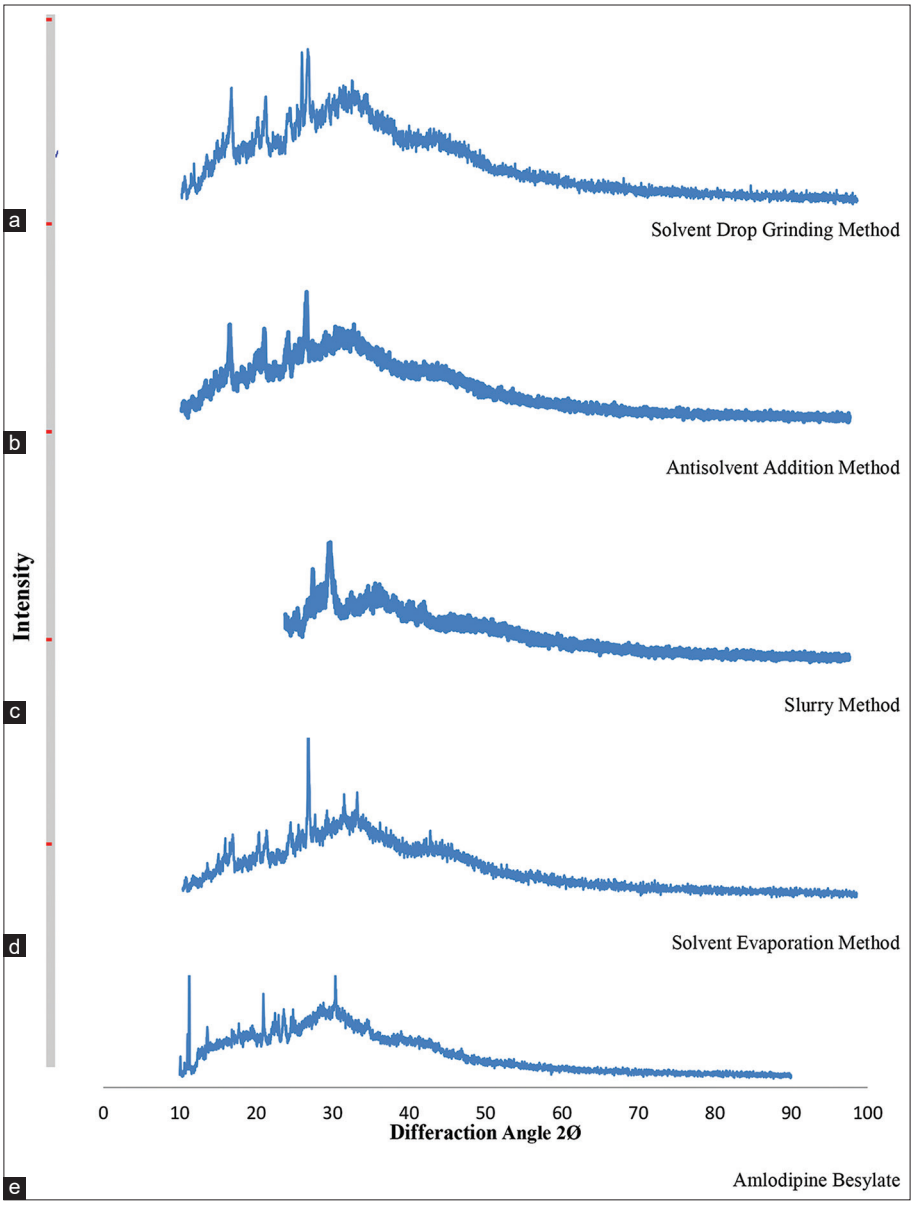

Figure 4: Overlay of comparative powder X-ray diffraction diffractograms of amlodipine and cocrystals using different methods. (a) Pure amlodipine, (b) slow evaporation, (c) solvent drop grinding, (d) anti-solvent addition method, and (e) slurry method

group is where the majority of natural carriers' interactions take place. The positions of all the distinguishing amlodipine besylate peaks matched those in the spectra of anti-solvent grinding method, slow evaporation method (SEM), SDG, and SM. It is evident from FTIR spectra of the region of hydroxyl groups that the intensity of the distinctive peak at 3539/cm altered in accordance with a comparison with SEM suggesting reduction crystal size.[47] The addition spectra of amlodipine – saccharine and amlodipine besylate separately were identical to the spectra of amlodipine cocrystals. This leads to the conclusion that no chemical interaction occurred because the drug's major peak values remained unaltered during cocrystallization. The medication and coformer do not interact chemically, it can be inferred.

DSC

DSC was used to study the molecular dispersion of amlodipine into conformer. Figure 3 compares the DSC curves of cocrystals and pure drug. Solid crystals' complicated structure was discovered using DSC. DSC thermograms for amlodipine and several coformers are obtained. The endothermic peak (Tm) of pure amlodipine, which corresponds to the drug's melting point at 2050 C, was clearly visible. The cocrystals were formed, and endothermic peaks at 214, 151, 201, and 1740 C, respectively, were seen during SA solvent grinding, SA solution cocrystallization, saccharine AG, saccharine slurry conversion, and neat grinding.[8,44] The peak broadening also showed that the majority of the medication is contained in nanocrystalline cocrystals. Due to the medication being reduced to a nanocrystalline state, little change in melting point was seen. Given that the crystallinity has been decreased to a nanocrystalline state, this mechanism is to blame for the stability improvement.[47]

Crystalline state evaluation: PXRD analysis

Figure 4 shows the XRD patterns of the pure drug and cocrystals. The XRD scan of pure amlodipine showed intense peaks of crystallinity at 20.94, 31.08, 22.04, 28.11, 30.18, 26.60, and 28.00 (2θ) with peak intensities of 669, 392, 410, 570, 568, 1500, 2300, and 2800, respectively, indicating its crystalline nature [Figure 4]. By contrasting typical peak heights in the diffraction patterns of the cocrystals with those of reference, crystallinity was ascertained. Isam is the peak height of the sample under inquiry, and Iref is the peak height at the same angle for the reference with the highest intensity, to compute the relative degree of crystallinity (RDC) of amlodipine in cocrystals.[44,45] The freshly produced cocrystals displayed the identical 2, although with weaker intensities, as well as the existence of a new peak for the coformer.

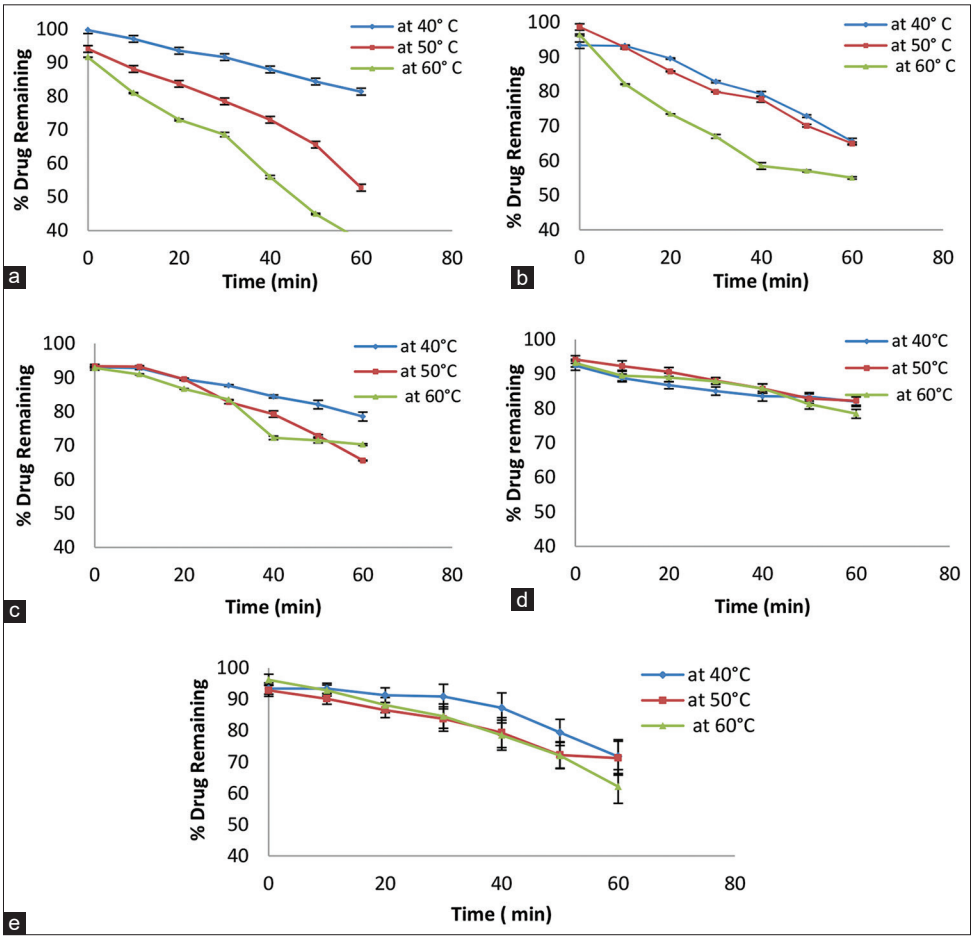

Figure 5: First order kinetics plot of amlodipine and cocrystals using various methods. (a) Pure amlodipine, (b) anti-solvent addition method, (c) slow evaporation, (d) solvent drop grinding, and (e) slurry method.

Physical stability of amlodipine cocrystals

The stability research of cocrystals-containing amlodipine was conducted at 40°C and 75% relative humidity for 3 months to assess stability by accelerating the pace of reaction. In this study, the observed absorbance of amlodipine and cocrystals at various accelerated temperatures was examined. In comparison to cocrystals, amlodipine's concentration rapidly decreases between 500 and 600 C [Figure 5]. Compared to cocrystals at an increased temperature, amlodipine disintegration was quick; nevertheless, drug degradation was delayed. It may be inferred from this data that amlodipine's thermal stability was also increased by employing saccharine as a coformer for cocrystallization. Here, drug degradation occurs over time, although at a slower pace than with pure amlodipine.[45]

CONCLUSION

The stability studies on amlodipine are tested through thermal or chemical denaturation with the goal of calculating the thermodynamic stabilities of their three-dimensional structures. The present study demonstrates that the miscibility of a medication and its coformers, as determined by the HSP, can suggest the formulation of a cocrystal. Amlodipine's HSP was computed using the group contribution method using 20 coformers. Hydrogen bonds in conformers are vividly visible due to their significance in the formation of cocrystals. Hoy's molar attraction constants, van Krevelen's constant, and Fedor's substitution constants were derived and are currently utilized methods. The resultant δ values of drug and coformers are compared and their solid state miscibility is expressed. Possibility of cocrystal formulation by Krevelens is $\Delta\delta < 5$ MP and Greenhalgh $\Delta\delta < 7$ MP. A portion of the molecular complex formulations of amlodipine with coformer have been effectively generated using the experimental cocrystallization method and HSP Approach. According to this study, saccharine is a good coformer candidate for improving the stability of amlodipine. Amlodipine has been changed into a stable crystalline form, according to the results of the FTIR, DSC, and XRD. The molecular complexes of amlodipine and saccharine produce supramolecular systems that may be useful in the development of medicinal formulations. Such molecular complexes alter the amlodipine besylate guest molecule's physical and chemical properties. This study has demonstrated a substantially higher level of protection against ongoing moisture exposure with a lot lower level of degradation than in the solid amlodipine besylate. Cocrystallization developed following hygroscopic amlodipine besylate and molecular compounds with saccharine.

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