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Determination of traces of amorphous carvidilol content in carvedilol drug substance and drug product using modulated differential scanning colorimetry

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ABSTRACT

In the pharmaceutical processes like milling, wet granulation, drying, re-crystallization as well as compaction, there is a possibility of generating disorder in the form of crystal defects or amorphous phase [1]. The amorphous phase generated may need to be quantified using sensitive analytical tools. In the envisaged study, amorphous content in crystalline form of carvedilol drug substance and its formulation was studied by different analytical techniques like Modulated Differential scanning colorimetry (MDSC) and Powder X-Ray diffraction (PXRD). The characteristic glass transition (Tg) of amorphous carvedilol is exhibited at about 35°C. Different concentrations of amorphous content in crystalline drug substance and its formulation have been prepared and analysed. The method was developed based on the fact that the change in the specific heat at the glass transition is linearly proportional to the amorphous content. The linearity of amorphous content was established for drug substance using both MDSC and PXRD. as well as for drug product using MDSC. Superiority of the MDSC has been justified over PXRD in terms of detection of low level of amorphous content in formulation which could be of immense help to the formulator.

Key words: Amorphous phase, Glass transition (Tg), MDSC, Drug product.

INTRODUCION

Amorphous materials do not have long range ordered arrangement. They are physically less stable and tend to de-vitrify into thermodynamically more favored crystalline forms upon storage. Due to instability of amorphous materials, it is difficult to prepare, control and handle during the formulation. The amorphous materials acts as a contaminant, it is often necessary to quantify and determine its concentration in a crystalline sample.

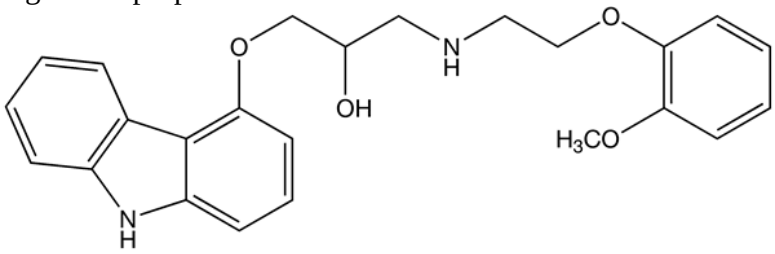
PXRD could quantify amorphous phase in crystalline phase at higher level [2]. This may be due to the interference of well crystalline peaks which suppress the halo background in the diffractogram. Moreover, the density technique is less capable of detecting low level of amorphous content than X-Ray powder diffraction [3].

There are certain limitations by traditional DSC like measurement of heat capacity in a single experiment is not possible. It will give only total heat flow or average heat flow. It can't separate the reversing heat flow & non reversing heat flow from total heat flow and thus relatively lesser sensitive for detection of weak transition. But MDSC can play a very important role in detection of amorphous content in DSC and can also be determined directly from the change of Specific heat capacity (Cp) at glass transition. MDSC can show glass transitions with much increased sensitivity.

The main aim of this study was to demonstrate the applicability of MDSC in the detection of low level amorphous content by comparative evaluation of PXRD.

MATERIALS AND METHODS

Carvedilol (CAR) is the antihypertensive drug with a chemical structure as shown below was used for this investigational purpose.



Carvedilol is a nonselective β –adrenergic blocking agent with α1-blocking activity. It is (±)-1-(Carbazol-4-yloxy)-3-[[2-(o-methoxyphenoxy) ethyl] amino]-2-propanol. Carvedilol is a racemic mixture with the above structure.

3.1 Amorphous sample preparation:

The amorphous sample was prepared by quench cooling the crystalline sample. Carvedilol crystalline form was heated till it melts (130°C) in heating pan and quickly removed the pan and allowed to cool by keeping the pan on the liquid nitrogen Dewar flask. The cooled drug substance was ground and sieved (mesh 60) to get the fine homogeneous powder. Then transfer to the polybag immediately and sealed bag with the nitrogen gas.

3.2 Sample analysis:

Weighed the required quantity of amorphous and crystalline material and blended in different proportions (0-100% w/w) and ground gently to ensure a uniform mix and took 10 mg of blend sample into the aluminum crucible (40 µl), cover with lid and crimped the sample.

Different levels of concentrations have been prepared by spiking the two different components at various levels and plotted a linearity graph.

MATERIALS AND METHODS

4.1 Powder X-Ray Diffraction (PXRD):

The X-ray powder diffraction pattern was recorded on Bruker axs D8 ADVANCE, equipped with Bragg-Brentano θ:θ goniometer having PSD; LynxEye detector. The pattern was recorded at a tube voltage of 40 kV and a tube current of 40 mA, with a step size of 0.008° and time per step of 1.0 sec over an angular range of 3-45 ° 2θ. The sample was grounded gently and filled in a sample holder by top loading method. The sample was exposed to the CuKα radiations (λ= 1.5418 Å).

4.2 Modulated Differential Scanning Calorimetry (MDSC):

The thermal analysis was carried out on DSC, TA Q1000. The thermo gram was recorded from -20°C to 90 °C under the nitrogen flow of 50 mL/min at a heating rate of 3°C/min with a modulation temperature of 1°C per min. Weighed about 2-3 mg sample into aluminum pan and distributed uniformly as a thin layer. The glass transition was recorded as the inflection point up to the step changed base line.

The heat flow was calibrated by enthalpy of indium (28.51J/g) or by the specific heat capacity of Sapphire. The specific heat method used the specific heat of sapphire over a user-defined temperature range. The baseline and sample curves were measured and the calibration was then built automatically. The calibration was checked before running samples by measuring the melting enthalpy of indium by using the same instrumental parameters

RESULTS AND DISCUSSION

Pure crystalline form was characterized by PXRD shown in figure 1. Pure crystalline form was characterized with high intensity characteristic peaks with a stable baseline (using PXRD) and also it does not record any thermal event except melting that may correspond to characterize glass transition (explained in subsequent paragraphs using MDSC).

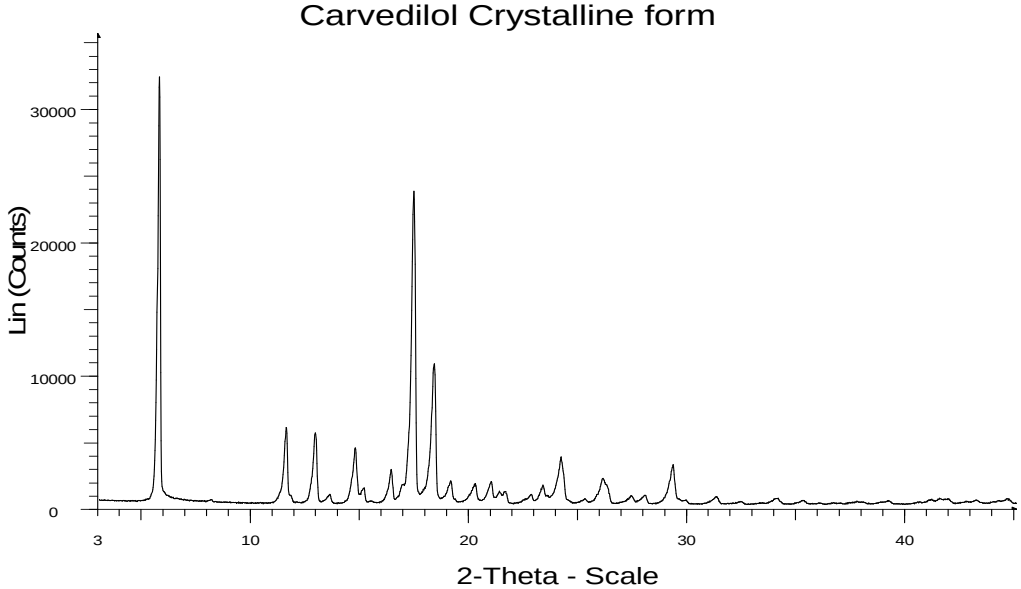


Figure 1: PXRD pattern of crystalline form of Carvedilol

Pure amorphous form was characterized by PXRD and MDSC shown in figure 2 and 3 respectively. Pure amorphous form records a characteristic halo and is free from any type of crystalline peak by PXRD and record a glass transition by MDSC at about 35°C. This is in alignment with the reported glass transition of carvedilol [4]. The crystalline sample however do not record any thermal event that correspond to glass transition suggesting that the sample was free from any amorphous impurity [5]. Also the amorphous form generated was chemically pure (98.9% using HPLC, data not shown).

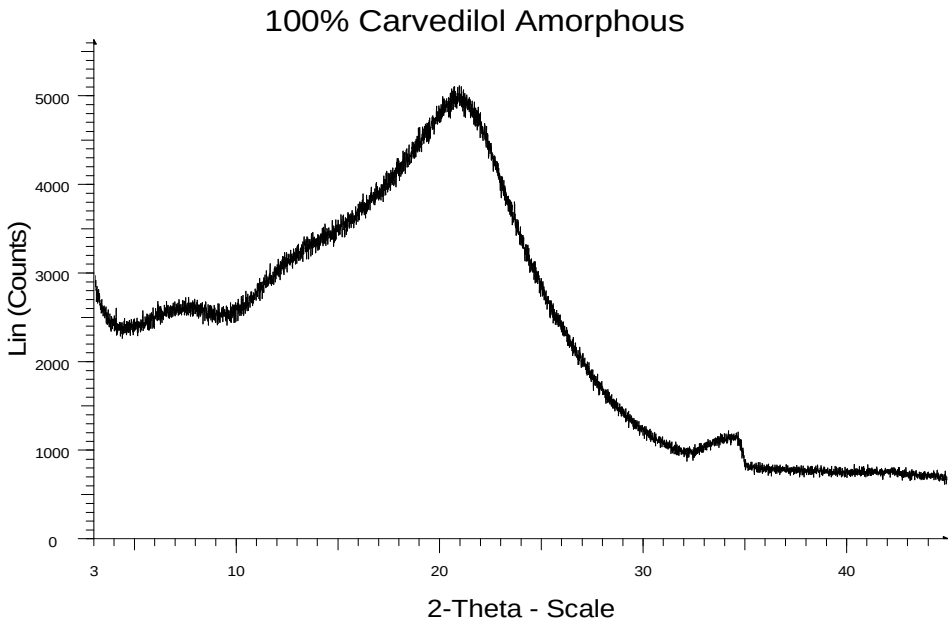
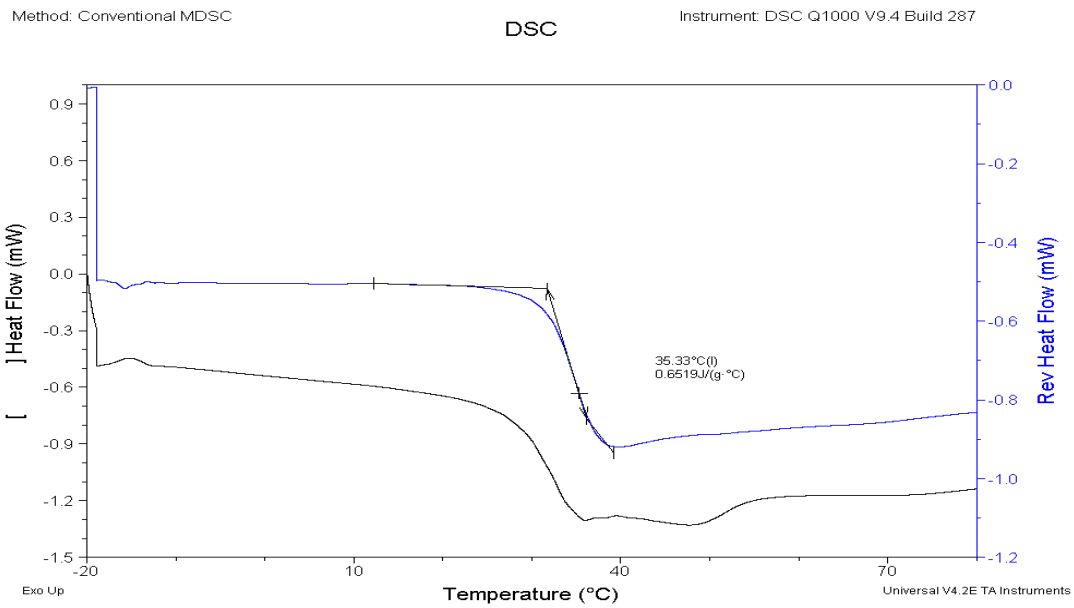
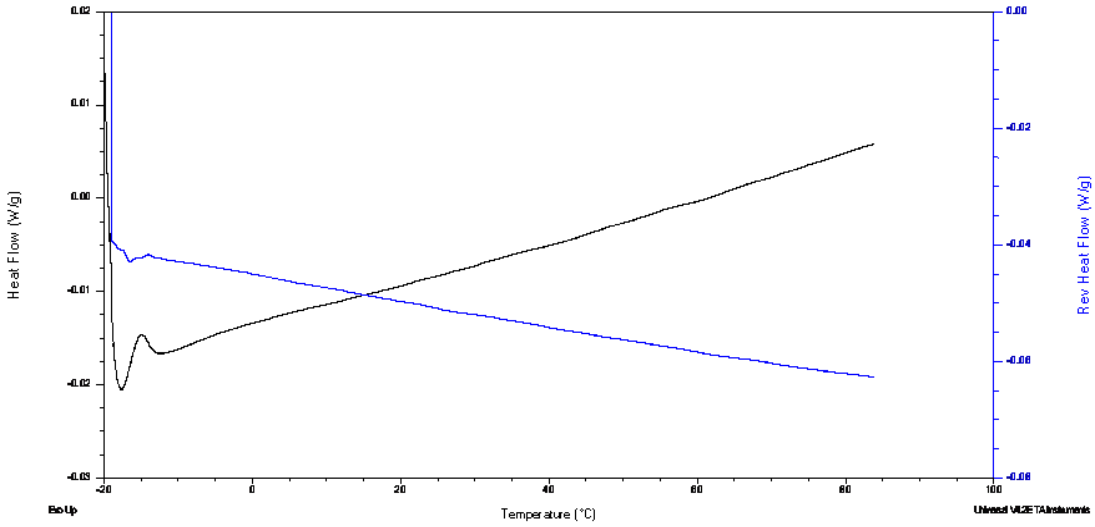


Figure 2: PXRD pattern of amorphous form Carvedilol



(a)



(b)

Figure 3: MDSC thermogram for (a) amorphous and (b) crystalline form

The amorphous content in any crystalline sample can be assessed using PXRD after spiking of amorphous fraction (if the sample is crystalline) or without spiking (if the sample is semi-crystalline or poorly crystalline, and consists of crystalline and amorphous fractions co-existing). For PXRD, the degree of crystallinity (and hence the degree of amorphous content) can be estimated by calculating the total area (Crystalline + Amorphous) and the crystalline area for PXRD using the method of Hermans and Weidinger:

$$Xc = P \times Ic;\ Xa = Q \times Ia$$

$$Ia = (X/Q) - [(P/Q) \times Ic]$$

$$Xcr = Xc/Xc + Xr$$

$$\frac{Ic}{Ic + Q/P \times Ia} \times 100$$

Xc and Xa are crystalline and amorphous fractions respectively.
Ic and Ia are measured crystalline and amorphous intensities respectively.
Sum of crystalline and amorphous fraction

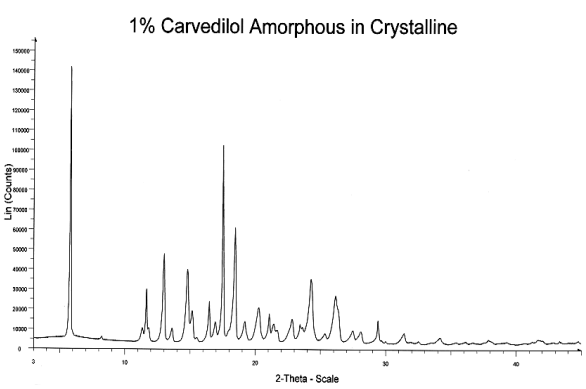
$$X = Xc + Xa.$$

Modulated DSC provides for a separation of heat flow into the reversing and non-reversing components, which can be easily interpreted. The reversing heat flow signal (which is the heat capacity component of the total signal), is extremely useful for measuring glass transition in all types of difficult samples.

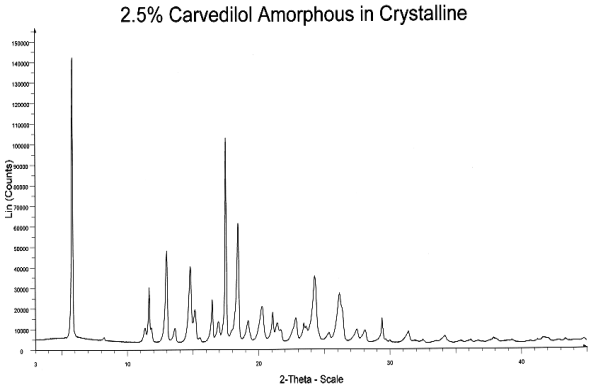
MDSC can play a very important role in detection of amorphous content in DSC and can also be determined directly from the change of specific heat capacity (Cp) at glass transition. MDSC can show glass transitions with much increased sensitivity. The degree of amorphous content in

Carvedilol was determined using both PXRD and MDSC and subsequent paragraphs cover a comparative assessment of relative superiority of one method over the other.

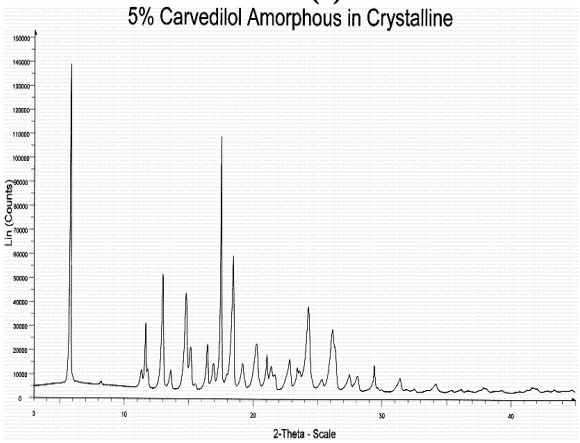
PXRD could quantify amorphous phase in crystalline phase only at higher level. This may be due to the interference of well crystalline peaks which suppress the halo background in the diffractogram at very small levels of amorphous fraction in the sample. In this case, it is difficult to consider the actual hallow due to amorphous content in these samples.



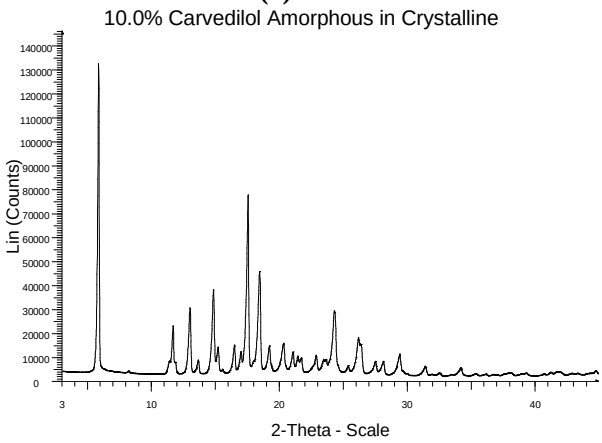
(a)



(b)



(c)



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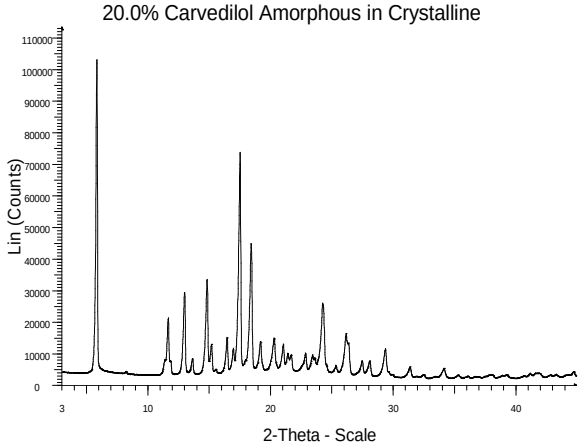

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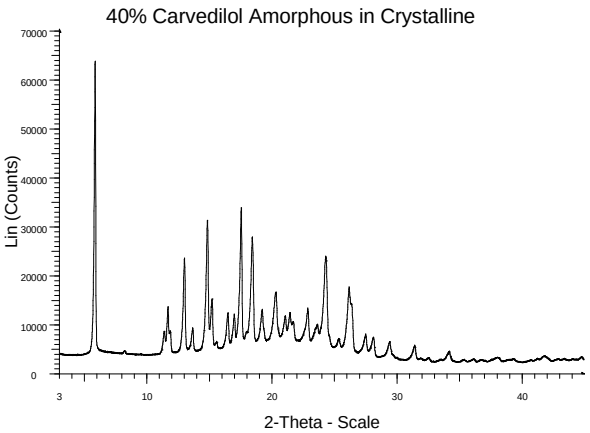

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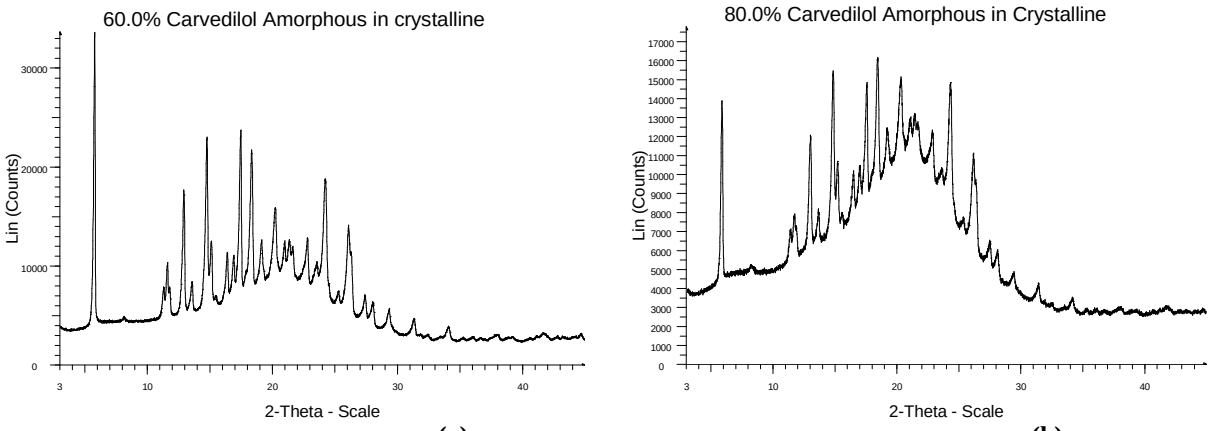
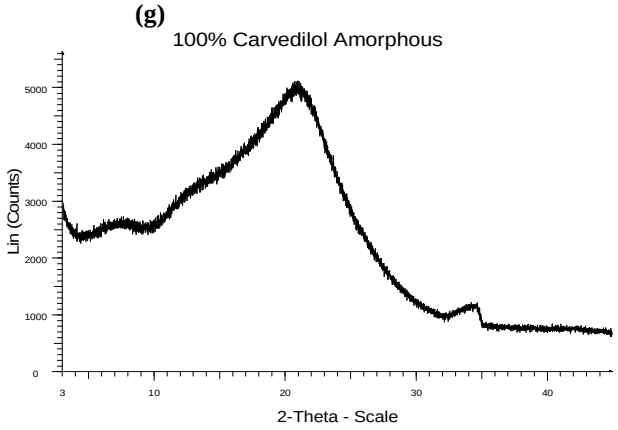


(g) **(h)**



(i)

Figure 4: PXRD of samples spiked with amorphous Carvedilol at (a) 1%, (b) 2.5%,(c) 5%,(d) 10%,(e) 20%,(f) 40%,(g) 60%,(h) 80%,(i) 100% level

Figure 4 and table 1a & 1b shows the different amorphous content ranging from 1 to 100%. The linearity graph was plotted for various concentrations (% w/w) of amorphous in crystalline form against the area under the amorphous halo (Table 1a & 1b). It must be noted here that the linearity at lower level specifically less than 10% of amorphous fraction could not be achieved using PXRD (Figure 5). The linearity however was >0.99 at levels of amorphous content 10% and beyond, clearly demonstrating that PXRD could be a valuable tool to quantify amorphous contents at higher levels of amorphous content (Figure 6).

Table 1a: Regression statistics

Regration statics	**Results**
Amorphous carvedilol spiking concentration (% w/w) (n=9)	1 – 100
R^2	0.98880
Slope	5.56854
Intercept	101.98
t Stat	13.276
P-value	9.89E-07
Lower 95% confidence interval	5.81399
Upper 95% confidence interval	8.25822

Table 1b: Regression statistics

Regration statics	Results
Amorphous carvedilol spiking concentration (% w/w) (n=6)	10 – 100
R^2	0.99550
Slope	6.0127
Intercept	69.91
t Stat	24.41555
P-value	2.15E-06
Lower 95% confidence interval	6.25706
Upper 95% confidence interval	7.72964

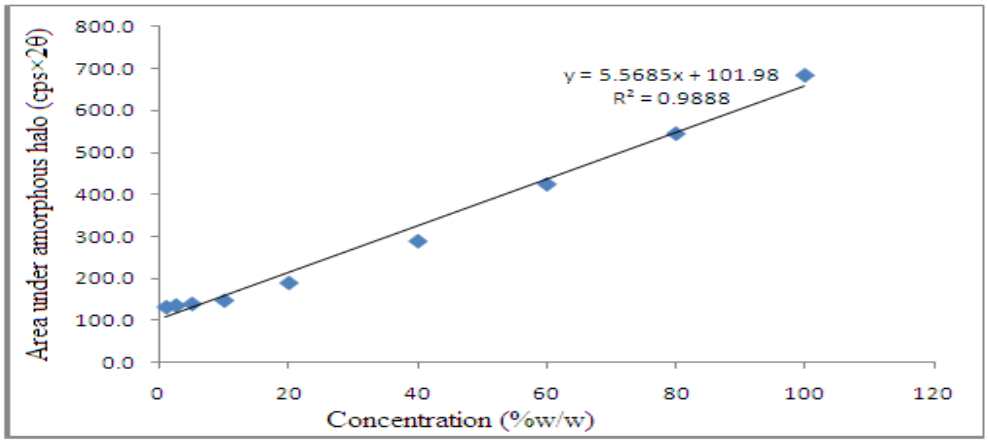


Figure 5: Linearity between the concentrations at various levels (1-100%w/w) Vs Area under amorphous halo (cps×2Θ)

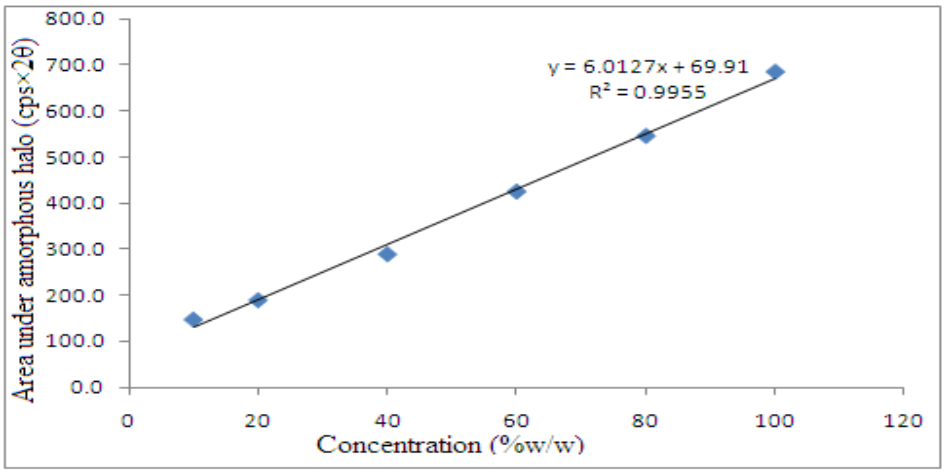


Figure 6: Linearity between the concentrations at various levels (10-100%w/w) Vs Area under amorphous halo (cps×2Θ)

From the above figures 5 and 6, it can be inferred that it is very difficult to see a corrleation of measurable amorphous halo below the 10% level using PXRD with R^2 value of about 0.98 and therefore it is not recomeneded to find out the content of amorphous below this limit. This

suggest that below 10% level of amorphous contaminantion of an otherwise crystalline sample may not get adequately characterized using PXRD.

MDSC on the other hand, could identify low level of amorphous content in crystalline phase due to the sensitivity of glass transition (Tg) event as observed in our study. MDSC could effectively be used for quantification of amorphous fraction at low level (up to 5%) with a correlation coefficient (>0.99) (Fig. 7 and 8, Table 2).

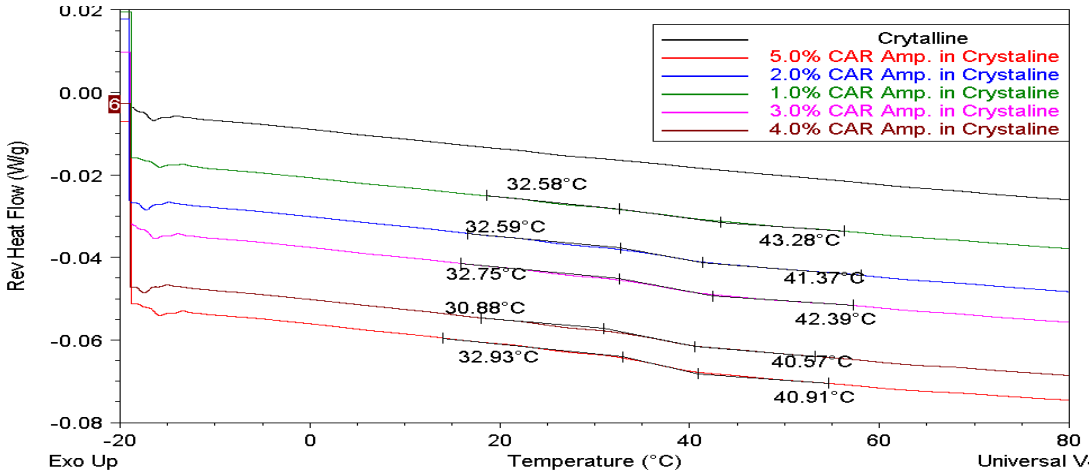


Figure 7: Overlaid thermograms for different concentrations shows the glass transition (Tg)

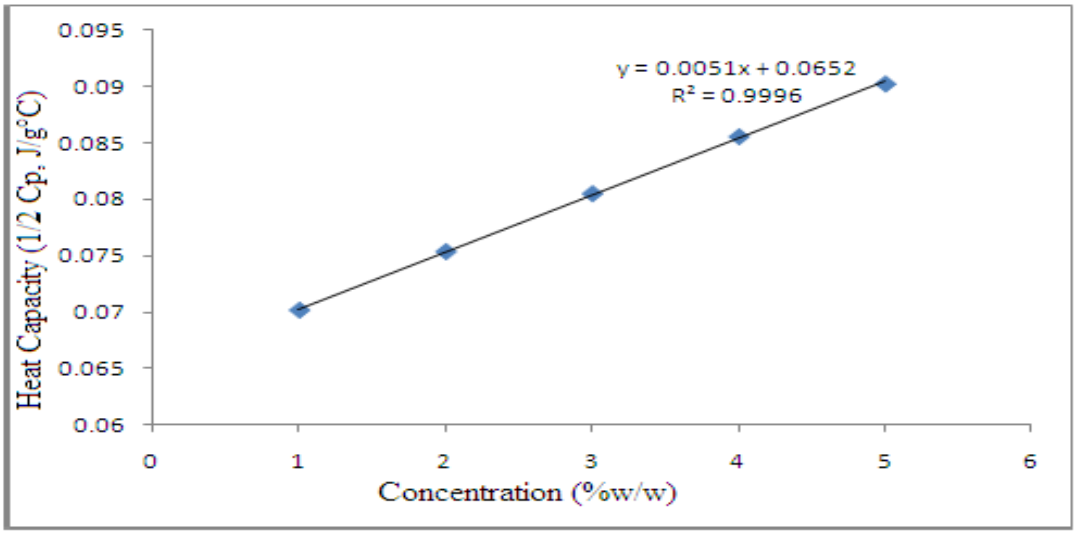


Figure 8: Linearity between the concentrations at various levels Vs Heat capacity at Tg.

Moreover, we also attempted to ensure, if MDSC can be used for quantification of amorphous content at higher level. From the results (Fig. 9 and 10, Table 3), it can be inferred that MDSC is equally useful for amorphous quantification both at low and higher amorphous content in an otherwise crystalline material.

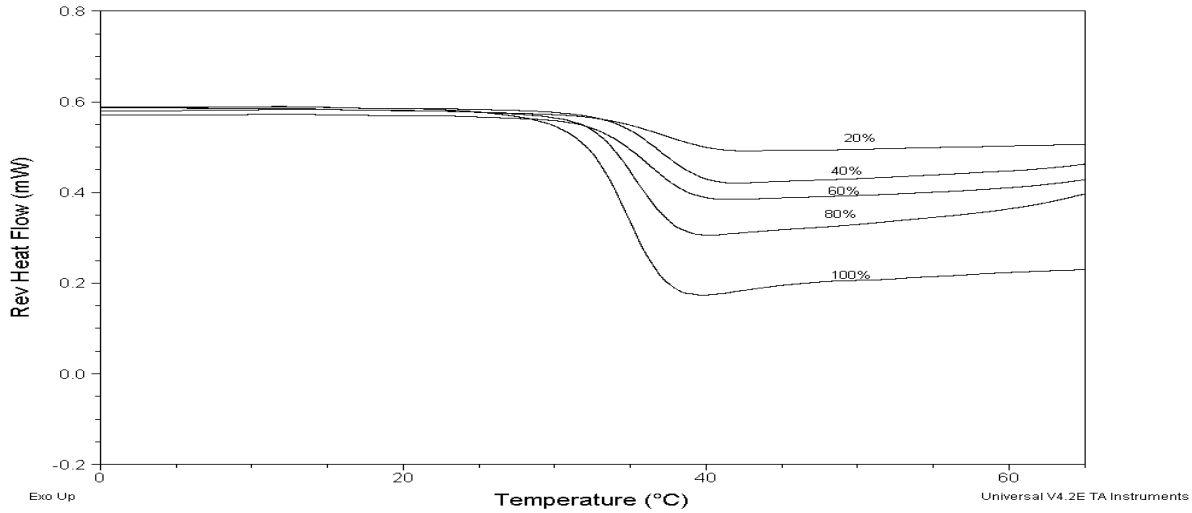


Figure 9: Overlaid thermograms for different concentrations (20% to 100% w/w) shows the glass transition (Tg) as a step change.

Table 2: Regression statistics

Regration statics	**Results**
Amorphous carvedilol spiking concentration (% w/w) (n=5)	1-5
R^2	0.99960
Slope	0.06520
Intercept	0.00505
t Stat	5.4486
P-value	0.00551
Lower 95% confidence interval	0.01120
Upper 95% confidence interval	0.03448

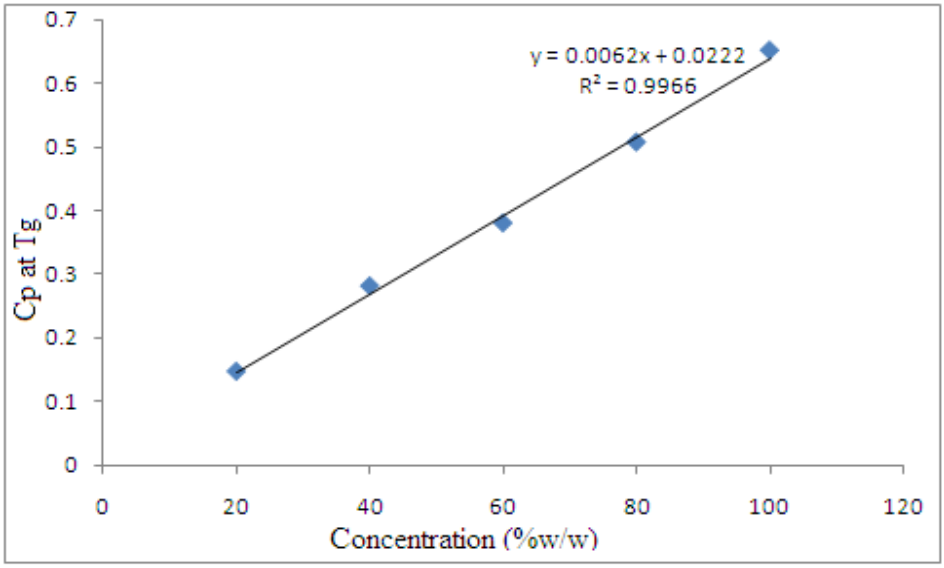


Figure 10: Linearity between the concentrations at various levels Vs Heat capacity at Tg

Table 3: Regression statistics

Regration statics	Results
Amorphous Carvedilol (%w/w) (n=5)	20 - 100
R^2	0.99660
Slope	0.0062
Intercept	0.0022
t Stat	61.63624
P-value	4.15E-07
Lower 95% confidence interval	0.006296
Upper 95% confidence interval	0.006781

We further extended, these findings with a formulation [6] of carvedilol in order to ascertain if the processes used in the formulation manufacturing does not add any surprise. A Carvedilol composition at a highest dose of 25 mg was evaluated for amorphous content using Tg analysis and data is captured in Fig. 11, 12 and Table 4.

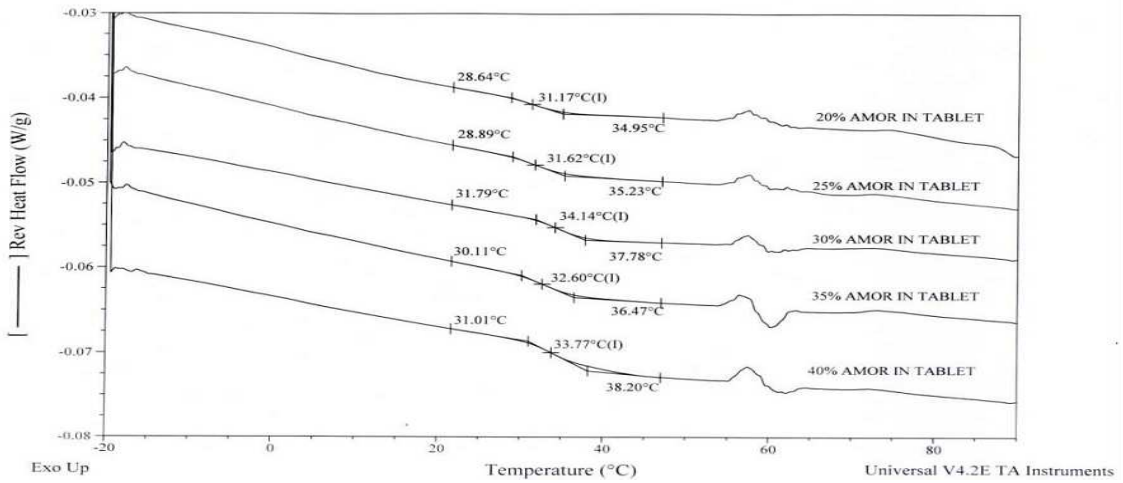


Figure 11: Overlaid thermograms for different concentrations show the glass transition (Tg) in formulations.

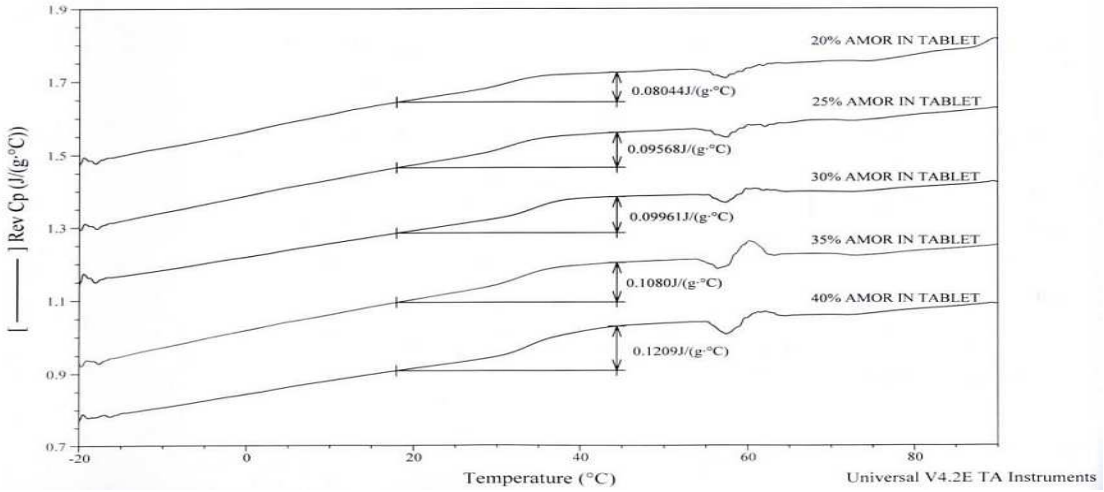


Figure 12: Overlaid thermograms for different concentrations show the glass transition (Cp) in formulations.

Table 4: Regression statistics

Regration statics	Results
Amorphous Carvedilol (%w/w) (n=5)	20 - 40
R^2	0.98918
Slope	0.0019
Intercept	0.045
t Stat	19.11864
P-value	4.41E-05
Lower 95% confidence interval	0.003765
Upper 95% confidence interval	0.003762

These results clearly suggest that irrespective of the sample type (i.e., “as is” API or its formulation), the method based on MDSC, with the help of Tg estimation is quite a useful method of detecting amorphous fraction, where PXRD does not provide adequate information specially at low amorphous content.

CONCLUSION

The usefulness of analytical techniques like powder XRD and modulated DSC was evaluated for the qualitative and quantitative estimation of Carvedilol amorphous content in drug substance and drug product. Based on the above experiments and observations, it can be concluded that to find out the low level content of amorphous in crystalline form of carvedilol, MDSC is one of the best suitable technique.

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