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Research Article

KINETICS OF THERMAL DEGRADATION OF ANTIVIRAL COMPOUND-ACYCLOVIR

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ABSTRACT:

Acyclovir is an antiviral compound used in treatment of Herpes and other viral infections. The thermal degradation kinetics study is carried out and activation energy is calculated using Ozawa method. Activation energies were calculated at various stages of degradation of the drug and the stability of the drug is estimated.

KEYWORDS: Acyclovir, Activation energy, Thermal degradation kinetics, Ozawa method.

1. INTRODUCTION:

Acyclovir, chemically known as acycloguanosine is a guanosine analogue antiviral drug. It is primarily used for the treatment of herpes simplex virus infections and herpes zoster (shingles)[1].

Acyclovir has several synonyms like AC2, Acyclovir, 9-Hydroxyethoxymethylguanine, Acyclovir Sodium, Acycloguanosine and Wellcome-248u. It is marketed under the trade names Avirax, Alti-Acyclovir, Vipral, Virorax, Zovir, Zovirax, Cyclovir, Acivir, Acivirax[3]. Generally acyclovir contains not less than 98.5% and not more than the equivalent of 101.0% of 2 amino-9-[(2-hydroxyethoxy) methyl]-1,9-dihydro-6H-purin-6-one, calculated with reference to anhydrous substance[4]. It has a molecular formula as $C_8H_{11}N_5O_3$ and a molecular mass of 225.046 gram per mole[3].

Figure-1: Acyclovir

Acyclovir is white crystalline powder which is slightly soluble in water (experimental water solubility 1.62 mg/ml at 22 C). It is freely soluble in dimethyl sulphoxide and sparingly soluble in ethanol (96%). It dissolves in dilute solutions of mineral acids and alkali hydroxides[4]. It has a melting point of 256.5 C. It has high distribution rate, 9-33% is protein bound in plasma. Its half life ranges from 2.2 to 20 hours and is excreted renally by glomerular filtration and tubular secretion. Due to its low water solubility, it has poor oral bioavailability (10-20%), hence it is administered intravenously when high concentrations are required. The poor oral bioavailability of acyclovir can be improved by converting it into its ester form known as valacyclovir which has an oral bioavailability of 55%[1].

Acyclovir is the prototype antiviral agent which is activated by the enzyme viralthymidine kinase. The selectivity of acyclovir is due to its affinity for thymidine kinase enzyme encoded by HSV and VZV. Viral thymidine kinase converts acyclovir to acyclovir monophosphate, which in turn converts to the diphosphate by cellular guanylate kinase and finally to the triphosphate by the enzymes phosphoglycerate kinase , phosphoenol pyruvate carboxykinase and pyruvate kinase. Acyclovir triphosphate competitively inhibits viral DNA polymerase and competes with the natural deoxyguanosine triphosphate, for incorporation into viral DNA. Once incorporated, acyclovir triphosphate inhibits DNA synthesis by acting as a chain terminator[3].

Acyclovir can be considered a prodrug, it is administered in inactive (or less active) form and is metabolized into a more active species after administration. Acyclovir formulations are commercially available in the form of tablets (200 mg, 400 mg, 800 mg and 1 gm), a topical cream (5%) ophthalmic ointment (3%)[1], a lyophilized powder which must be reconstituted prior to parenteral administration to a patient[2]. Cream preparations are used primarily for labial Herpes simplex. The ophthalmic ointment preparation is only used for herpes simplex keratitis. When high concentrations of acyclovir are required intravenous injections are used[1]. Apart from treating HSV and VZV infections, acyclovir is also used in the treatment of herpes simplex labialis(cold sores), genital herpes simplex (treatment and prophylaxis), acute chicken pox, herpes simplex encephalitis, acute mucocutaneous HSV infections, herpes simplex blepharitis and Bell's palsy[5].

2. MATERIALS AND METHODS:

The drug used, Acyclovir is purchased from local market. Studies were conducted on TG/DTA, Model 6200 of SIICO, Japan which is coupled with autosampler AST-2 and software EXSTAR-6000. The operation temperature of the machine is from ambient to 1100 °C.

2.1 ACTIVATION ENERGY CALCULATION (OZAWA METHOD)[7-14]

In general, the reaction rate of a specimen according to the law of Arrhenius is expressed by the following equation:

$$\frac{dx}{dt} = A\exp\left(-\frac{\Delta E}{RT}\right). f(x) \quad \text{.....(1)}$$

where,

x = quantity of reaction

t = time

A = frequency factor

Δ E = activation energy

R = gas constant

T = absolute temperature

f (x) = function of x

In thermal analysis, T (temperature) is a function of t (time), and generally, the relation dT/dt = B (constant) holds true, where B is the heating rate (°C/min).

Therefore, if expression (1) above is separated for variables x and t, the following expression can be obtained:

$$\int_{x0}^{x1} \frac{dx}{f(x)} = \int_{t0}^{t1} A \exp\left(-\frac{\Delta E}{RT}\right) dt$$

$$= \frac{A}{B} \int_{T0}^{T1} \exp\left(-\frac{\Delta E}{RT}\right) dT \quad \text{.....(2)}$$

Hence, if F(x) is defined as

$$F(x) \equiv \int \frac{dx}{f(x)}$$

and integration by parts is conducted for the right hand member of expression (2) after changing

variables $-\frac{\Delta E}{RT} \rightarrow y$, expression (2) may be written:

$$F(x_1)\text{-}F(x_0) = \frac{A\Delta E}{BR}\left(P\left[-\frac{\Delta E}{RT}\right] - P\left[-\frac{\Delta E}{RT}\right]\right) \quad \text{.....(3)}$$

Where,

$$P(y) \equiv \frac{e-y}{y} - \int_y^{\infty} \frac{e-y}{y} dy \quad \text{.....(4)}$$

As generally the relation $T_1 > T_0$ holds true, the 2nd term of the right hand equation (4) is usually negligible while the 1st term is not. Hence, the following relation is obtained:

$$\frac{A.\Delta E}{BR} P\left(\frac{\Delta E}{RT_1}\right) = F(X_1)\text{-}F(X_0) \quad \text{.....(5)}$$

If x1 is calculated supposing $x_o=0$, the value of the right hand member of equation (5) above will be a constant, as follows:

$$\frac{A.\Delta E}{BR} P\left(\frac{\Delta E}{RT_1}\right) = const. \quad \text{.....(6)}$$

Hence, when the heating rate B is changed, temperature T_1 whose ratio of reaction is x_1 also changes proportionally, and therefore, the value of the left-hand side of equation (6) does not change as a whole.

With respect to P(y), on the other hand, the following approximate expression is known:

$$\log P(y) = -2.315 - 0.4567(20 < y < 60) \quad \text{.....(7)}$$

From expressions (6) and (7) above,

$$const. = log \frac{A.\Delta E}{BR} P\left(\frac{\Delta E}{RT_1}\right)$$

$$= log \frac{A.\Delta E}{BR} + logP\left(\frac{\Delta E}{RT_1}\right)$$

$$= log \frac{A.\Delta E}{R} - \log B - 2.315 - 0.4567 \frac{\Delta E}{RT_1} \quad \text{.... (8)}$$

If we rearrange expression (8) above for the heating rate B and temperature T_1, it becomes the following equation:

$$\log B = -0.4567 \frac{\Delta E}{R} \cdot \frac{1}{T_1} + \text{Constant} \quad \text{.....(9)}$$

It follows that if several measurements are made at various heating rates, and the relation between $1/T_1$ and log B of each pair of these data is plotted on x-y coordinates, the activation energy of a specimen can be obtained from the slope of a plotted line.

2.2 ACTIVATION ENERGY AND TEMPERATURE DEGRADATION TIME DETAILED CALCULATION METHOD [7-14]

The calculations in the section title can be performed using the TG/DTA Kinetics Analysis Software. The operation method is as follows:

(1)Select sample data

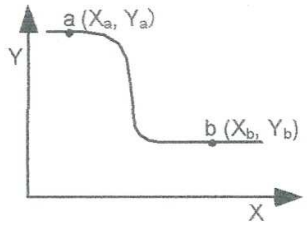
(2) Use the cursor to assign two points a and b on the sample data TG

Save the values in the assigned range (X,Y) into the data area

X = Temperature

Y = TG

Figure:2



(3) Repeat steps (1) and (2) above for each data for which the reaction rate is to be analyzed.

(4) Enter the number of partitions n to divide the a to b range of TG into, and calculate n divisions of TG between a and b; Y_0, Y_1 ...Y_n:

$$Y_{i=}Y_a - \frac{i}{n}(Y_a - Y_b) \quad (i = 0,1,2\ldots.n)$$

(5) Create a set (B_j, Y_i, X_{ij}) for all the data to be used in calculation,

where

j = data No.

B_j = Heating rate of j-th data (°C/min)

Y_i = Y_i calculated in step (4)

X_{ij} = X_i corresponding to Y_i of j-th data

(6) Convert X_{ij} (temperature) to the relevant absolute temperature (°C to K):

$$X_{kij} = X_{ij}+273.15$$

(7) $calculate\ \frac{1000}{X_{kij}}$

$$Z_{ij} = \frac{1000}{X_{kij}}$$

(8) Plot the set (Z_{ij}, log B_j) on x-y coordinates and approximate it linearly to each of

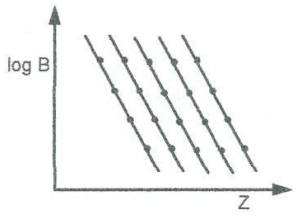
i=1, 2, 3, ... n–1.

Then, based on the following linear expression,

find a value corresponding to Pi, qi:

$$\log B = P_i + q_i \ . Z_i \qquad (\ i=1, 2, 3, \ldots\ n–1\ )$$

Figure: 3



Arrhenius Plot

(9) Calculate the activation energy (ΔE). Each value of ΔE_i for i=1, 2, 3, ... n–1 is calculated with the following expression:

$$\Delta E_i = -\frac{R}{0.4567} \ . q_i \text{ (KJ/mol)}$$

where

R=8.31434 x 10^{-3} (KJ/mol)

(10) Calculate the constant temperature degradation time (τ) as follows:

1) The operator assigns the temperature to be held Tc (°C).

(2) B = $\sqrt{max B_j \,.\, \min B_j}$ (The midpoint of log B is used)

On the basis of:

$$T_i = \frac{q_i}{\log B - P_i}(K)$$

each respective τi to i=1, 2, 3, ... n–1 is calculated by the following expression:

$$\tau i = \frac{\int_{T0}^{T1} \exp\left(-\frac{\Delta E_i}{RT}\right) dt}{B \exp\left[-\frac{\Delta E_i}{R(T_c + 273.15)}\right]}(min)$$

2.3 EXPERIMENTAL PROCEDURE:

The TG/DTA instrument is thoroughly calibrated using calcium oxalate and indium before use. Acyclovir sample is taken and the thermal degradation kinetic studies are conducted by subjecting the sample to controlled heating rates of 2°C/min,5°C/min and 8°C/min under N_2 atmosphere with a flow rate of 60 ml/min. This is to create inert atmosphere in the furnace. The data is recorded using a computer with dedicated software and the thermograms are generated. The thermograms of % weight loss vs. temperature, mass loss vs. temperature, rate of mass loss per minute vs. temperature are generated. The data is subjected to Ozawa method for calculating activation energy of various fractions during degradation. The corresponding graphs are generated and details are recorded and analyzed under the heading of results and discussion.

Temperature ranges are selected as per the degradation pattern obtained in the DTG thermograms for each of the 3 heating rates (2°C/min, 5°C/min and 8°C/min). Depending on the degradation the temperature range for analysis is fixed as 30°C-160°C, 240°C-370°C and 370°C-500°C. Ozawa method is applied, and the activation energy is found for all stages of degradation. The 1st range corresponds to activation energy of loss of water, 2nd range corresponds to activation energy of loss of side chain of the acyclovir, 3rd range corresponds to the loss of remaining drug.

3. RESULTS AND DISCUSSION:

3.1 BOND ENERGIES:

In the acyclovir molecule the bond energies between various atoms are tabulated in Table-1.The structure shows maximum bond energy for keto group (179 K Cal/mole). The minimum is for C-N bond. The side chain of the molecule is linked to the ring structure through a weak C-N bond. It is expected that under constant heating rate the side chain loss is expected to take place at C-N bond. It is observed that the side chain separation takes place approximately between 240°C to 370°C. The theoretical loss in mass due to side chain separation is calculated as 33.3%.

Table-1: Bond energies[6]

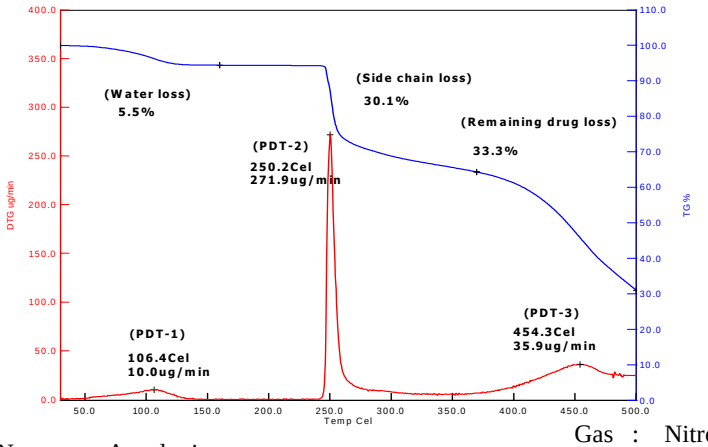
BOND	BOND ENERGY (kCal)	NO OF BONDS
C-C	83	1
C=O	179	1
C-N	73	7
N-H	93	3
C=N	147	2
C=C	146	1
C-H	99	6
C-O	85.5	3
O-H	111	1

3.2 PERCENTAGE WEIGHT LOSS ALONG WITH WATER:-

Table-2 gives the recorded loss of the mass of freewater, side chain and remaining drug till 500°C. It does not constitute to the 100% of drug as some amount of ash is still present after subjecting acyclovir to 500°C. On an average 5.33% of the water loss is observed in the drug samples when subjected to different heating rates (2°C/min, 5°C/min and 8°C/min).

Figure: 4:

Figure-5:



Sample Name: Acyclovir

Sample Weight: 5.378 mg

Reference Name: Aluminium

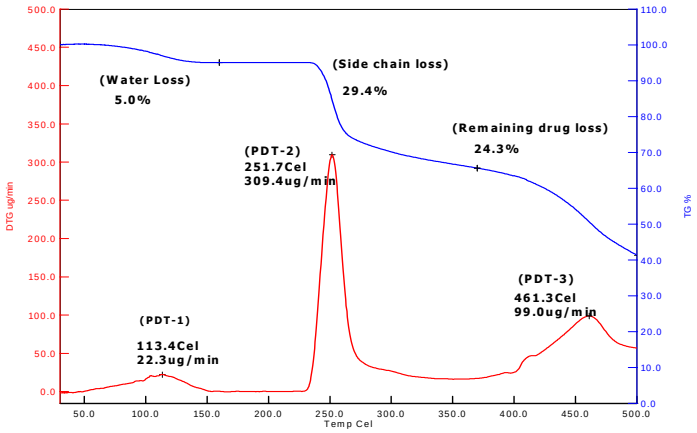
TGA/DTG

Gas : Nitrogen

Pan : Aluminium

Heating rate: 5 °C/min.

Figure-6:



Sample Name: Acyclovir

Sample Weight: 5.095 mg

Reference Name: Aluminium

TGA/DTG

Gas : Nitrogen

Pan : Aluminium

Heating rate: 8 °C/min.

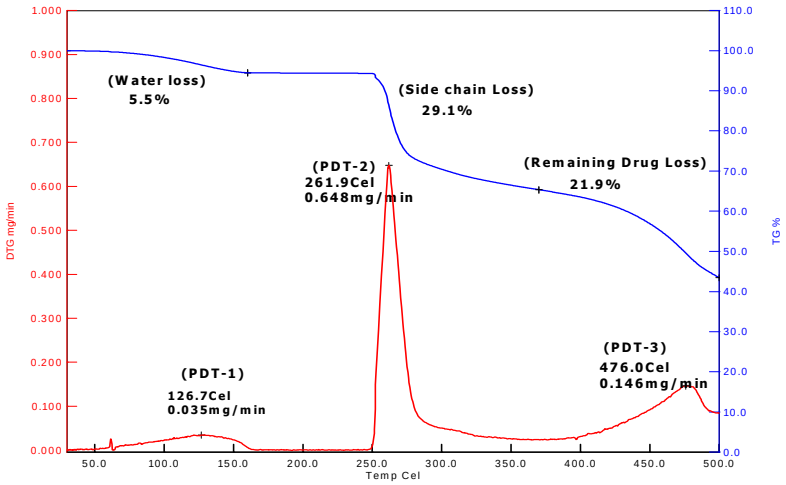


Table -2: **TGA % loss at different stages of degradation.**

HEATING RATE	WATER LOSS	SIDE CHAIN	REMAINING DRUG
2 °C/min	5.5%	30.1%	33.3%
5 ° C/min	5.0%	29.4%	24.3%
8 °C/min	5.5%	29.1%	21.9%

3.3 PERCENTAGE WEIGHT LOSS EXCLUDING WATER:

Table-3 gives the drug weight, water loss, actual side chain and main chain mass loss. The actual mass of the drug is calculated by subtracting mass of water lost and from that the % loss of side chain is calculated and tabulated in Table-3.The actual loss of mass of side chain is found to be 30.63% to 31.85%, which is very close to theoretical value of mass of side chain. This clearly indicates that the second stage loss recorded from 240°C to 370°C is purely due to side chain loss. This indicates that the pure drug is stable upto 240°C at all heating rates.

Table-3:

Heating rate	Drug weight (mg)	Water loss(mg)	Actual side chain loss(mg)	Actual side chain loss (%)	Main chain loss(mg)	Ash left at 500 °C (mg)
2 °C/min	4.01814	0.23386	1.279852	31.851852	1.415916	1.322372
5 °C/min	5.1091	0.2689	1.581132	30.947368	1.306854	2.221114
8 °C/min	4.84025	0.25475	1.482645	30.631579	1.115805	2.2418

3.4 ACTIVATION ENERGY CALCULATION:

Using Ozawa method the activation energy is calculated for all the stages using thermograms of heating rates 2°C/min,5°C/min and 8°C/min. The graphs of log B vs. $\frac{1}{T}$ are drawn and activation energy is calculated as per Ozawa method and results are tabulated.

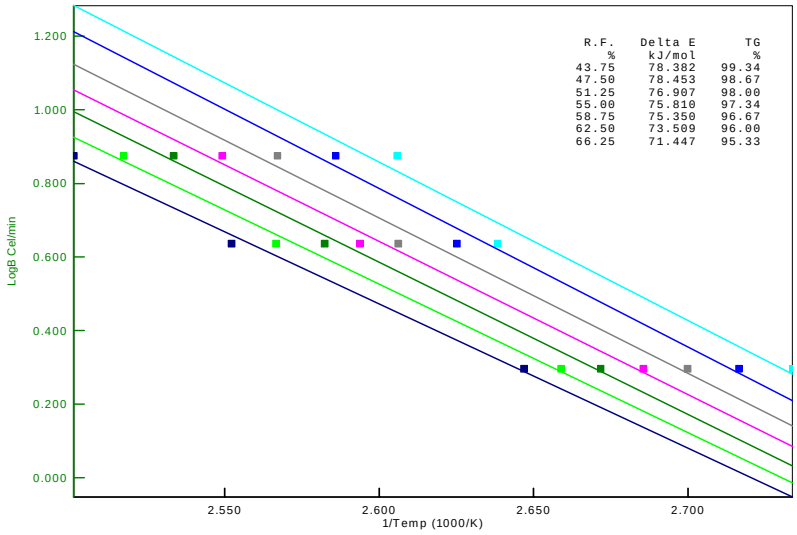
3.4 (a) Activation energy of evaporation of water:

The activation energy of evaporation of water for various reaction fractions (RF) are given in Table-4. The maximum activation energy of 78.382(K J/mol) is recorded for the reaction fraction of 43.75% and minimum of 71.447 K J/mol is recorded for reaction fraction of 66.25%.The average value of 75.694 K J/mol is observed. It is also observed that the absorbed moisture is not interacting with drug molecule which could be supported by TGA thermograms which are parallel to X-axis. This means there is no considerable mass loss of the drug beyond 160°C to 240°C. If the interaction of the drug with absorbed moisture occurs the drug should continuously degrade even after 160°C. This clearly reveals that the absorbed moisture has no effect on drug quality.

Table-4: E_d of evaporation of water.

R.F%	Delta E (kJ/mol)
33.00	187.290
36.00	189.597
39.00	191.535
42.00	191.991
45.00	192.439
48.00	193.807
51.00	192.673
54.00	192.187
57.00	191.626

Figure: 7.



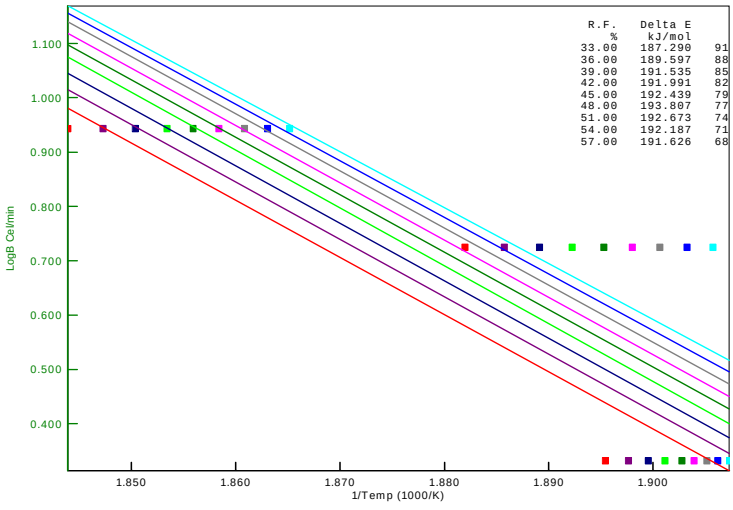
3.4 (b) Activation energy of degradation of side chain:

The activation energy of degradation of side chain for various reaction fractions are given in Table-5.The maximum activation energy of 193.807 KJ/mol is recorded for reaction fraction of 48.00% and minimum of 187.290 KJ/mol is recorded for reaction fraction of 33.00%.The average value of 191.460 KJ/mol is observed. The % weight loss from 240°C to 370°C indicates the second stage of degradation and the mass loss is mainly due to side chain loss, as it breaks first due to comparatively low C-N bond energy than that of others. This fact can be justified as the experimental percentage weight loss of side chain is in close agreement with that of theoretical of acyclovir.

Table-5: **E_d of degradation of side chain.**

R.F%	Delta E (kJ/mol)
43.75	78.382
47.50	78.453
51.25	76.907
55.0	75.810
58.75	75.350
62.50	73.509
66.25	71.447

Figure: 8.



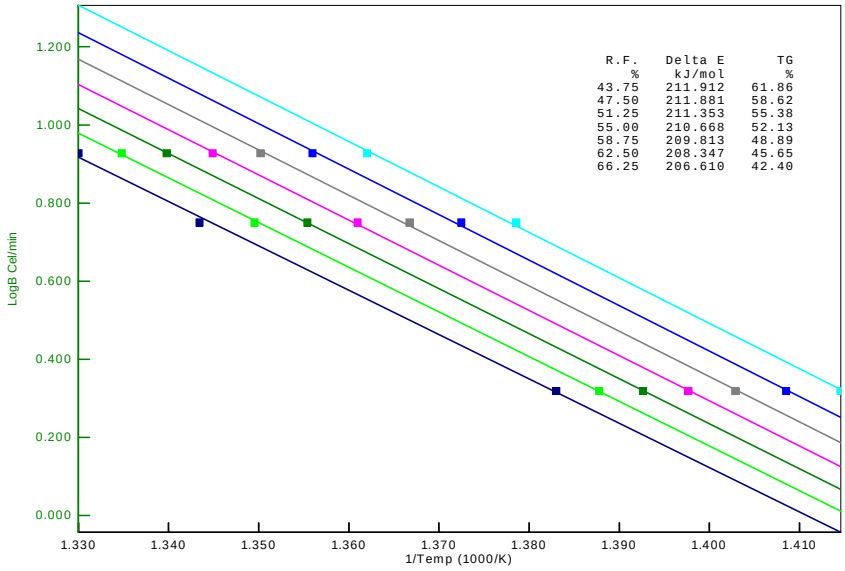
3.4 (c) Activation energy of remaining drug:

The activation energy of remaining drug for various reaction fractions are given in Table-6. The maximum activation energy of 211.912 KJ/mol is recorded for the reaction fraction of 43.75% and minimum of 206.610 KJ/mol is recorded for reaction fraction of 66.25%.The average value of 210.083 KJ/mol is observed and some amount of ash is left out even after subjecting it to 500°C.

Table-6: **E_d of remaining drug.**

R.F%	Delta E (kJ/mol)
43.75	211.912
47.50	211.881
51.25	211.353
55.0	210.668
58.75	209.813
62.50	208.347
66.25	206.610

Figure: 9



3.5 PEAK DEGRADATION TEMPERATURES (PDT):

Peak degradation temperature is the temperature where the fraction under consideration degrades at the highest rate. The peak degradation temperature values of water (PDT_1), side chain (PDT_2) and remaining drug (PDT_3) are recorded in Table-7. The maximum rate of loss of absorbed moisture in the drug is observed between 106.4°C(for heating rate 2°C/min) and 126.7°C(for heating rate 8°C/min).This gives a good picture as to what maximum temperature the drug is subjected to drying before it being used in formulation design. PDT_2 value gives the maximum degradation temperature of side chain. The side chain records a maximum rate of weight loss between 250.2°C (for heating rate of 2°C/min) and 261.9°C (for heating rate 8°C/min). The side chain records an average PDT of 254.6°C. This indicates that the drug if subjected to the average value of 254.6°C will degrade almost completely and drug looses all its properties. The drug is not suitable for use if it is subjected to heating beyond 254.6°C.

Table-7: **Peak degradation temperatures of the 3 peaks.**

HEATING RATE	PDT_1	PDT_2	PDT_3
2 °C/min	106.4 °C	250.2 °C	454.3 °C
5 ° C/min	113.4 °C	251.7 °C	461.3 °C
8 °C/min	126.7 °C	261.9 °C	476.0 °C

4. CONCLUSIONS:

The drug contains on an average of 5.33% moisture. The moisture presence has no impact on either structure or therapeutic action of drug. The stability of drug remains intact despite the presence of moisture. The side chain loss is taking place between 240°C and 370°C. The experimental and theoretical values of side chain loss are close to each other and indicate that the drug is thermally stable up to 240°C. Beyond which the drug loses its properties due to side chain loss. This invention gives an insight about the thermal stability of the drug so, the storage conditions can be determined.

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