Purity，Thermal Decomposition Kinetics and Shelflife of Artemisinin by Thermal Analysis

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Abstract：Thermal decomposing curves of artemisinin at different heating rates were obtained under dynamic conditions in nitrogen atmosphere using non-isothermal differential scanning calorimetry analysis (DSC). Three thermal analysis kinetic methods(Kissinger, Flynn-Wall-Ozawa and Phadnis) were used to speculate the probable mechanism of thermal decomposing reaction and the kinetic parameters while the purity and melting point of artemisinin were obtained by Van′Hoff equation. The shelflife of artemisinin at room temperature was calculated by the kinetic parameters of the thermal decomposition. With the increasing of the heating rate, thermal analysis tempreture of artemisinin rose. The most probable kinetic mechanism of the thermal decomposition was two-dimensional diffusion which corresponded to Jander Equation. In accordance with the datas of atomic charges and bond orders by Gaussian simulation, the mechanism was identical with that artemisinin was decomposed into three compounds at 190 ℃. The shelflife of artemisinin at room temperature was about 3 years based on the apparent activation energy (E_a) and pre-exponential factor (A) of the thermal decomposition.

Key words：artemisinin；DSC；non-isothermal kinetics；Gaussian simulation；shelflife

热分析法研究青蒿素的纯度、热分解动力学及贮存期

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摘　要：利用差示扫描量热技术测得青蒿素在氮气气氛中不同升温速率下的热分析曲线，采用 Van′t Hoff 方程建立回归曲线求得青蒿素的纯度和熔点，使用 Kissinger 法、Flynn-Wall-Ozawa 法和 Phadnis 法等 3 种方法同时进行动力学分析。根据热分解的表观活化能(E_a)和指前因子(A)计算推断青蒿素在室温下的贮存期。研究表明，随着升温速率的提高，青蒿素的热分解温度逐渐升高；青蒿素热分解的机理是二维扩散控制，对应的函数名称是 Jander 方程；经 Gaussian 模拟青蒿素的分子键级和原子电荷数，能够对 190 ℃ 时分解产生 3 个化合物的机理进行验证吻合；根据青蒿素热分解的 E_a 和 A 推断，在室温25 ℃ 下，青蒿素的贮存期为 3 年。

关键词：青蒿素；DSC；非等温动力学；Gaussian 模拟；贮存期

Artemisinin is a sesquiterpene lactone compound extracted from sweet wormwood *Artemisia annua* L, which contains the specific endoperoxide bridge and was developed independently by Chinese scientists [1]. As the only recognized international new anti-malaria substance from China, it is effective against chloroquine-resistant *Plasmodium falciparum* malaria and in patients with cerebral malaria. Furthermore, it was also proved to have a series of biological effects *in vitro* and *in vivo*, such as antiparasitic capacity, treating toxoplasma

gondii infection, resisting arrhythmia, postponing the progress of fibrosis of tissue and organ, relieving asthma, resisting *Trichomonas vaginalis*, diagnosis of lupus nephritis, terminating pregnancy and antifungal properties, etc.[2-4]. Artemisinins have other pharmacological activity such as antitumor, antischistosome, affecting immunity, etc. Larger studies have validated these findings that artemisinins could resist gastric, liver, breast, intestine, ovarian, cervical and prostate cancer[5]. There are numerous studies on its chemical structure, bio-pharmacological activity, extraction and purification methods and detection methods except for its thermal stability and decomposition kinetics. In recent years, the thermal analysis techniques have been widely used in chemical identification, the determination of physical-chemical constants, the investigation of active element of Chinese traditional medicine and the drug quality control[6]. In this work, the thermal characteristics and decomposition mechanism of artemisinin were studied by differential scanning calorimetry analysis and molecular simulation while the non-isothermal kinetic datas were calculated by Kissinger, Flynn-Wall-Ozawa and Phadnis methods.

1 Experimental

1.1 Sample

Artemisinin is standard sample from Xi′an feida bio-tech Co., Ltd.

1.2 Thermal analysis

Thermal analysis was carried out with a DSC 200 F3 Maia analyzer (from Germany-NETZSCH). Before detection, the temperature and sensibility calibrations had been implemented by high purity in, Sn, Zn, Bi and CsCl according to the instruction of the analyzer.

Melting point and purity test：3–5 mg sample was heated to the temperature below the melting point (−10–20 ℃) at the rate of 10 ℃/min from room temperature. Then after constant temperature was kept for 1 min, it was again heated at the rate of 0.5 ℃/min to 160 ℃.

Thermal decomposition test：10 mg samples were heated at the rates of 5, 10, 15 and 20 ℃/min from room temperature to 250 ℃.

The measurements were conducted in a flowing nitrogen atmosphere of 20 mL/min (sweep gas) and 50mL/min(shielding gas). Crucible made of Al was used as the container of samples.

1.3 Calculation for melting point and purity[7-8]

It has been found that temperatures (T_s) in DSC melting curve showed a linear correlation with the inverses of relative peak area ($1/F$), which followed Van′Hoff Equation.

$$T_s = T_0 - \frac{RT_0^2 x}{\Delta H_f} \cdot \frac{1}{F} \tag{1}$$

where：T_0—the melting point of pure substance, K; R—the gas constant, 8.314 J/(mol·K); ΔH_f—the enthalpy of melting, J/mol.

According to the DSC curve by substituting T_s and $1/F$ into Eq. (1), the linear correlation coefficient r, the slope b and the intercept a are obtained by the linear least square method with T_s vs $1/F$. From the value of the slope for the plot, the impurity content x can be obtained. From the value of the intercept, T_0 can be obtained, too.

1.4 Methodology and kinetic analysis

The procedure for data processing of thermal analysis kinetics are as follows.

1.4.1 Multi-heating rate methods Kissinger method[9] and Ozawa method[10], which are multi-heating rate

methods, were used to calculate kinetic datas, respectively. The corresponding equations are shown as Eq. (2) and Eq. (3).

$$\ln \frac{\beta}{T_{\mathrm{p}}^2} = \frac{E_{\mathrm{a}}}{RT_{\mathrm{p}}} + \ln \frac{AR}{E_{\mathrm{a}}} \tag{2}$$

$$\lg \beta = \lg \frac{AE_{\mathrm{a}}}{Rg(\alpha)} - 2.315 - 0.456 \frac{E_{\mathrm{a}}}{RT} \tag{3}$$

where: $g(\alpha)$—the integral mechanism function; α—conversion percentage; T—the absolute temperature, K; T_{p}—the DSC peak absolute temperature, K; A—the pre-exponential factor, min^{-1}; R—the gas constant, 8.314 J/(mol·K); E_{a}—the apparent activation energy, kJ/mol; β—the linear heating rate, ℃/min.

According to the several DSC curves by substituting T_{p}, R and various heating rates β into Eq. (2) and Eq. (3), the linear correlation coefficient r, the slope b and the intercept a are obtained by the linear least square method with $\ln \beta/T_{\mathrm{p}}^2$ vs $1/T_{\mathrm{p}}$ and $\lg \beta$ vs $1/T$. From the value of the slope for the plot, E_{a} can be calculated. From the value of the intercept, A can be calculated, too.

1.4.2　Single heating rate method　Phadnis method[11], which is a single heating rate method, was used to calculate kinetic datas and the corresponding equations is shown as Eq. (4).

$$g(\alpha)f(\alpha) = \frac{RT^2}{E} \frac{\mathrm{d}\alpha}{\mathrm{d}T} \tag{4}$$

where: $f(\alpha)$—the differential mechanism function.

According to one single DSC curve by substituting $g(\alpha) \cdot f(\alpha)$ and $\mathrm{d}\alpha/\mathrm{d}T$ into Eq. (4), the linear correlation coefficient r, the slope b and the intercept a were obtained by the linear least square method with $g(\alpha) \cdot f(\alpha)$ vs $\mathrm{d}\alpha/\mathrm{d}T$. From the value of the slope for the plot, E_{a} can be calculated, as well.

Kinetic parameters can be calculated by differential mechanism function $f(\alpha)$ and integral mechanism function $g(\alpha)$ at the same time. If the linear correlation coefficient r is the best and approaches 1, the corresponding function is the probable mechanism function of a solid phase reaction. The common mechanism functions in nonisothermal reaction kinetics were reported in the literature[12].

1.5　Molecular simulation for bond orders and atomic charges

Chemical structural formula of artemisinin was drawn by ChemBioDraw Ultra 11.0 from ChemBioOffice 2008. It was structural optimized with the minimum energy by ChemBio3D Ultra 11.0. Molecular model was put into Gaussian03(Software of Quantum Chemistry) and its calculation of bond orders and atomic charges was carried out with B3LYP(Density Functional Theory calculation)at a 6-31G level[13-14].
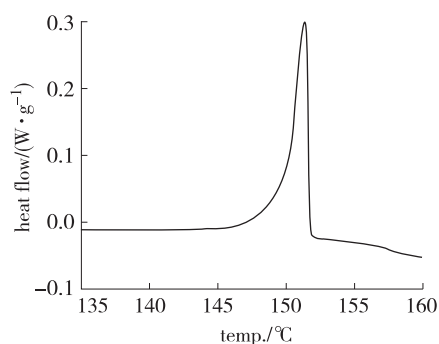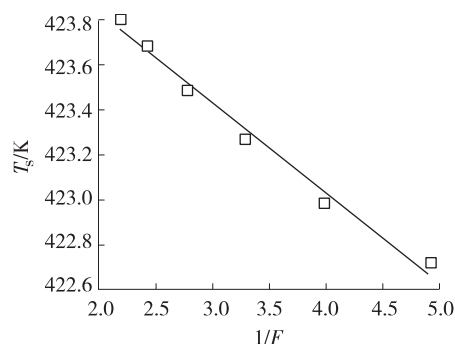
2　Results and Discussion

2.1　Melting point and purity of artemisinin

The DSC curve of the title compound at a heating rate of 0.5 ℃/min in dynamic nitrogen atmosphere is presented in Fig.1. It showed that artemisinin began to melt at 145.15 ℃ and ended at 152.00 ℃ while the DSC peak absolute temperature was 151.30 ℃ and the enthalpy of melting ΔH was 17.11 kJ/mol.

As shown in Table 1 and Fig.2, the linear correlation coefficient r, the slope b and the intercept a were obtained by the linear least square method with T_{s} vs $1/F$. The regression equation was $y = -0.4022x + 424.64$ while the linear correlation coefficient r was 0.9949. From the value of the intercept, the melting point of pure substance T_0 as 424.64 K was obtained. According to the slope $b = (R \cdot T_0^2 \cdot x)/\Delta H_{\mathrm{f}}$, the impurity content of artemisinin x of 0.0046 was calculated. Thus, the purity of the standard was 99.54%.

According to reports in the literatures[15-16], artemisinin was heated at 190 ℃ for 10 min. Three other products, i. e. compound 2 (4%), 3 (12%) and 4 (10%) were isolated and characterized. In this word, according to the datas of atomic charges and bond orders by Gaussian simulation, the mechanism was identical with that artemisinin was decomposed into three compounds at 190 ℃.

Table 2   Atomic charges of artemisinin with hydrogens summed into heavy atoms

atom	atomic charges	atom	atomic charges
C(1)	0.51679	C(11)	0.03032
O(2)	−0.48439	C(12)	0.01929
C(3)	0.54986	C(13)	0.39428
C(4)	0.00896	O(14)	−0.46241
C(5)	0.02291	O(15)	−0.39277
C(6)	0.12883	C(16)	0.03951
C(7)	0.02794	C(18)	0.08785
C(8)	0.01798	O(19)	−0.285
C(9)	0.01372	O(20)	−0.30437
C(10)	0.07071	C(22)	−0.0000170

In rearrangement reaction 01, after cracking of chemical bonds C_{13}—C_{12} and O_{19}—O_{20}, the atom C_{12} was connected with atom O_{20}. There was a carbon-carbon double bond formed between C_{13} and O_{19} so that compound 3 was generated. This could be explained by that atomic charges of C_{13}, O_{19}, O_{20} were high and could easily react while chemical bonds C_{13}—C_{12} and O_{19}—O_{20} were a little lower than most of other bonds.

In rearrangement reaction 02, after cracking of chemical bonds C_{13}—O_{19} and O_{19}—O_{20}, the atom C_{13} was connected with atom O_{20}. There was a hydroxy formed between C_{12} and O_{19} so that compound 4 was generated. Its explaination was that the atomic charges of reacted atom C_{13} was 0.394 28 and very high among all the atomic charges while the chemical bond orders of C_{13}—O_{19} and O_{19}—O_{20} were below 0.94.

In degradation reaction, at first atom C_6 was connected with O_2 and O_{14} after cracking of three chemical bonds C_1—O_2, C_1—O_{14} and C_1—C_6 just because these three chemical bond orders were below 0.96 and atomic charges of C_1, O_2, O_{14} were above 0.45 so that they were very active atoms. Then chemical bonds C_{13}—O_{19}, O_{19}—O_{20} and O_{20}—C_6 cracked owing to the three low chemical bond orders below 0.945 and high atomic charges of C_{13}, O_{19} and O_{20} above 0.28. So in degradation reaction, one molecule of artemisinin lost C_1, O_{19} and O_{20}, which formed one molecule of CO_2.

As shown in Fig. 3 and Table 3, the peak temperatures of DSC rose with the increasing of heating rates. It showed that artemisinin did not crack at the same temperature with the increase. The thermal decomposition was a slow stage and not rapid heat transfer to delay the effective collision to high temperature. Owing to the first stage of melting, thermal decomposition process began at the second stage of DSC curves. Therefore, in this work, the second stage of the thermal characteristics and decomposition mechanism was analyzed.

Table 3   Peak temperatures of DSC curves at different heating rates

heating rate/(℃·min⁻¹)	peak temp. for DSC curves at different heating rates/℃	
	first stage	second stage
5	154.10	211.00
10	155.20	215.30
15	156.10	224.50
20	157.30	226.40

2.3   Thermal decomposition kinetics of artemisinin

2.3.1   Kinetics calculated by multi-heating rate methods   Kinetic datas for the thermal decomposition of the title compound at 5, 10, 15 and 20 ℃/min by the kissinger method are shown in Table 3. By substituting the values of T_p, β in Table 3 into Eq. (2), kinetic parameters were calculated by the kissinger method while

apparent activation energy E_a (kJ/mol), pre-exponential factor ln$A$ and linear correlation coefficient r were 150. 46, 36. 56 and 0. 960 9, respectively.

As shown in Table 4, various decomposition percentage α_i at different temperatures from four heating rates (5, 10, 15 and 20 ℃/min), corresponding temperatures T and heating rates β were obtained from DSC curves.

Table 4　Temperatures for the decomposition by Flynn-Wall-Ozawa method

decomposition percentage (α)	temp. /℃			
	5 min/℃	10 min/℃	15 min/℃	20 min/℃
0. 1	193. 10	199. 14	207. 02	211. 08
0. 2	198. 57	204. 33	212. 57	216. 34
0. 3	201. 99	207. 44	215. 94	219. 40
0. 4	204. 57	209. 68	218. 40	221. 53
0. 5	206. 73	211. 47	220. 40	223. 19
0. 6	208. 66	213. 00	222. 14	224. 56
0. 7	210. 47	214. 36	223. 73	225. 77
0. 8	212. 26	215. 65	225. 28	226. 94
0. 9	214. 13	217. 02	226. 93	228. 44

At the same decomposition percentage α_i, by substituting T and various heating rates β into Eq. (3), the linear correlation coefficient r, the slope b and the intercept a were obtained by the linear least square method with lg β vs 1/T. From the value of the slope for the plot, the values of E_a at different decomposition percentages were calculated and shown in Table 5.

Table 5　Kinetic datas for the decomposition by Flynn-Wall-Ozawa method

decomposition percentage (α)	apparent activation energy (E_a)/(kJ·mol^{-1})	linear correlation coefficient (r)
0. 1	131. 60	0. 9855
0. 2	134. 57	0. 9826
0. 3	137. 45	0. 9796
0. 4	140. 46	0. 9761
0. 5	143. 60	0. 9718
0. 6	146. 79	0. 9663
0. 7	149. 92	0. 9594
0. 8	152. 72	0. 9505
0. 9	153. 68	0. 9412
average	143. 42	—

2. 3. 2　Probable thermal decomposition mechanism by the single heating rate method　　In the present work, both the differential mechanism function $f(\alpha)$ and integral mechanism function $g(\alpha)$ have been applied to study the kinetics of the decomposition processes of artemisinin. The differential and integral equations refered to reference article[12].

In this work, we listed the thermal decomposition datas at $\beta = 10$ ℃/min. With substitution of basic datas in Table 6 and 40 different mechanism functions $f(\alpha)$ and $g(\alpha)$[12], the probable kinetic parameters calculated by the single heating rate methods are shown in Table 7.

Table 6　Thermal decomposition datas by phadnis method ($\beta = 10$ ℃/min)

No.	temp. /K	conversion percentage (α)	rate of conversion (dα/dt)/min^{-1}
1	472. 29	0. 10	0. 1188
2	477. 48	0. 20	0. 2479
3	480. 59	0. 30	0. 3749
4	482. 83	0. 40	0. 4961
5	484. 62	0. 50	0. 6050
6	485. 41	0. 55	0. 6531
7	486. 15	0. 60	0. 6961
8	487. 51	0. 70	0. 7589
9	488. 80	0. 80	0. 7712
10	490. 17	0. 90	0. 6280

Table 7　Linearly dependent kinetic parameters by Phadnis method ($\beta = 10$ ℃/min)

No. of mechanism	apparent activation energy (E_a)/(kJ·mol^{-1})	linear correlation coefficient (r)
2	530.11	0.9777
3	163.66	0.9969
4	654.64	0.9969
5	195.40	0.9766
6	781.59	0.9766
7	608.30	0.9958

2.3.3　Kinetic triplets　The results by Phadnis method was shown in Table 7. Its linear correlation coefficient r was the best and approached 1. As shown in Table 8, the Kinetic triplets calculated by Phadnis, Kissinger and Flynn-Wall-Ozawa methods were compared and found that they were very close to each others.

Table 8　Kinetic datas for the thermal decomposition of artemisinin

mothed	apparent activation energy (E_a)/(kJ·mol^{-1})	pre-exponential factor (ln$A$)	linear correlation coefficient (r)
phadnis	163.66		0.9969
kissinger	150.46	36.56	0.9609
Flynn-Wall-Ozawa	143.42		
average	152.51	36.56	

It can be ascertained that the most probable kinetic mechanisms of the thermal decomposition was two-dimensional diffusion and the corresponding mechanism followed Jander Equation. The differential mechanism function was $f(\alpha) = 4(1-\alpha)^{0.5}[1-(1-\alpha)^{0.5}]^{0.5}$ while the integral mechanism function was $g(\alpha) = [1-(1-\alpha)^{0.5}]^{0.5}$. Order of reaction n was 1/2. The activation energy E_a of this stage was 152.51 kJ/mol and ln$A$ was 36.56.

2.4　Shelflife of artemisinin

On the basis of the apparent activation energy E_a(152.51 kJ/mol) and the pre-exponential factor ln$A$ (36.56 min^{-1}) of the thermal decomposition, the constant of reaction rate k at the certain temperature T_c can be calculated as $k = A\mathrm{e}^{-E_a/RT_c}$. From the value of k, the negative logarithm pk was calculated. It was 10.84.

According to the correlation between the negative logarithm pk of a drug and its shelflife[17], at room temperature(25 ℃) if pk is less than 7.5, its shelflives are 1.52 years. If pk is more than 7.5 and less than 11, its shelflives are 3 years. So it could be infered that the shelflives of artemisinin are 3 years.

3　Conclusions

3.1　There is a apparent effect on the thermal decomposition of artemisinin with the change of heating rate. With the increasing of the heating rate, all of the initial decomposing temperature, maximum decomposing temperature and terminate decomposition temperature rose.

3.2　The purity and melting point of artemisinin were obtained by Van'Hoff equation and non-isothermal differential scanning calorimetry analysis, which shows that the method is preferable with high sensitivity, simple operation and less usage of sample.

3.3　In accordance with the datas of atomic charges and bond orders by Gaussian simulation, the mechanism was identical with which artemisinin was decomposed into three compounds at 190℃. With deducing the equation of decomposition kinetics, three thermal analysis kinetic methods verified each other. The Kinetic triplets calculated by Kissinger, Flynn-Wall-Ozawa and Phadnis methods were compared. It was found that they were very close to each others. Therfore, it is a very reliable result about thermal stability and decomposition kinetics of active constituents in Chinese traditional drugs by multi-heating rate methods and single heating rate methods.

3.4　The shelflives of artemisinin at room temperature are about 3 years. It could be used as evaluation method. It also provides the reference for the related drug administration.

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学 会 园 地

　　1. 中国林学会于 2012 年 12 月 7 日至 8 日在四川省成都市召开了学术工作座谈会。各省级林学会及各分会、专业委员会的分管学术工作的领导 60 多人参加了会议,会上交流了学术工作经验,分析了存在问题,重点讨论了第三届中国林业学术大会的筹备方案和各分会场申请与落实工作。会议最后由中国林学会尹发权副秘书长作总结。

　　2. 林产化学化工分会于 2013 年元月 15 日在南京召开了在宁理事扩大会议。宋湛谦理事长,张宗和副理事长、蒋剑春副理事长等在宁理事及专家 20 多人参加了会议。宋湛谦理事长作了 2012 年分会工作总结和 2013 年工作安排,宋永芳秘书长传达了中国林学会学术工作座谈会情况,会议重点讨论了2013 年工作并对承办第三届全国林业学术大会林化分会场,提出很多建议。会上还讨论了学会换届问题和《林产化学与工业》优秀论文评奖事宜。