

Original Article

# Kinetics of short-time tea infusion using a diffusion model

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This study was to explore the infusion kinetics of the major compounds in tea, caffeine, catechins and gallic acid, using a simple one-leaf diffusion model. The concentrations of caffeine (CA), (-)-epigallocatechin (EGC), (-)-epicatechin (EC), (-)-epigallocatechin gallate (EGCg), (-)-epicatechin gallate (ECg) and gallic acid (GA) in Taiwanese green and black teas manufactured from TTE 12 cultivar were analyzed by HPLC. By fitting the short time infusive concentration, we extrapolated the equilibrium concentration  $C$  from the diffusion equation. This equilibrium concentration was to calculate the first order infusion rate constant ( $k_{obs}$ ) to obtain the activation energy. The results showed that effective delay time was about 15~17 seconds for high temperature infusion (85 and 100 °C) and above 22 seconds for 70 °C steeping. The extractive efficiencies of these compounds from tea leaf were 85.5%~93.8% and 86.6%~98.3% for green and black teas, respectively. The effective activation energies of green tea were 18~27 kJ/mol and black tea were 7~39 kJ/mol. These results were conducted by the molecular weight and the differences in the manufacturing techniques between black and green teas.

**Key words:** Diffusion model, Implicit finite difference method, Infusion kinetics, Effective activation energy

## Introduction

Tea, one of the most popular beverages in the world, is receiving substantial attention for particular health benefits and existence of functional components, such as caffeine, catechins and gallic acid<sup>[1-4]</sup>.

In fresh tea, catechins are the major polyphenol compounds that provide the characteristic bitter and astringent taste of tea and also affect the

color, flavor and taste of manufactured tea<sup>[4,5]</sup>. Four abundant catechins are (-)-epigallocatechin (EGC), (-)-epicatechin (EC) and the gallated catechins, (-)-epigallocatechin gallate (EGCg) and (-)-epicatechin gallate (ECg)<sup>[1]</sup>. These catechins have many functions, including anti-carcinogenicity<sup>[6]</sup>, anti-microbe<sup>[7-9]</sup>, anti-mutagenicity<sup>[10]</sup>, anti-oxygenicity<sup>[11-13]</sup> and anti-tumorigenicity<sup>[14]</sup>. Gallic acid, the most important phenolic acid in tea, has antioxidant properties, antifungal activities and anticarcinogenic effects<sup>[1-3,15]</sup>. Griffiths and Griffiths<sup>[16]</sup> have indicated that caffeine, the major methylxanthine in tea, can stimulate the central nervous system, improve alertness, and mitigate tiredness.

Tea has been consumed for one thousand years by Chinese who usually use an amount of 3 g of tea leaf and 150 mL of water at 70~100 °C to

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brew tea<sup>[17]</sup>. Bag tea steeping is another popular method for tea making in the world because of its convenience. The investigation of dissolved compounds of tea in water has been proceeding for many years. One of the research topics people are interested in is the kinetics of these functional compounds in tea infusion. The infusive kinetics depends on the type of tea, the extracting method, the solvent temperature, and even the structure of the leaf matrix, etc.<sup>[18-21]</sup>. Any future commercial use of teas flavanols as therapeutic drugs would benefit from the knowledge of the extraction behavior of tea into the water.

There are, however, only few reports on the kinetics of tea steeping in long-time infusion and no article exploring the kinetics in short-time infusion data<sup>[19,20,22]</sup>. In the present work, we exploited a simple one-leaf diffusion model to examine the infusive kinetics of caffeine, catechins and gallic acid within four minutes. Black and green bag teas were used for these evaluations and comparisons at three different temperatures. The concentration of compounds,  $U_0$ , contained in the tea was estimated by calibrating the simulation value with those experimental results at the time of four minutes. By solving the diffusion equation with initial condition, we extrapolated the steady-state concentrations  $C_\infty$  to obtain the extraction efficiency  $C_\infty/U_0$ . The results derived from this study may prove useful in the manufacture of instant tea. In addition, the parameters obtained in this work could provide the final concentration and the time to reach steady-state in the processing of commercialized bottled tea to save production cost in the forms of time, energy and raw materials. The same concept could also apply to the similar extractive procedures of natural products in the food industry.

## Materials and experimental and numerical methods

### Chemicals

Caffeine (CA), gallic acid (GA), (-)-epigallocatechin (EGC), (-)-epicatechin (EC) and gallated catechins: (-)-epigallocatechin gallate (EGCg) and (-)-epicatechin gallate (ECg) were

purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile was purchased from Tedia Co. (Fairfield, OH, USA). Acetic acid was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Deionized water was prepared by Milli-Q water purification system (Millipore Co., Bedford, MA, USA) and was degassed under vacuum followed by filtering through a 0.22  $\mu\text{m}$  membrane filter (nylon) prior to use.

### Tea samples

Green tea (non-fermented tea) and black tea (fully fermented tea) were acquired from Wunshan Branch of Taiwan Tea Experiment Station (Taipei, Taiwan). They were manufactured from the same batch of fresh tea leaves of TTE12 species that were originally derived from *Camellia sinensis* var. *sinensis* by hybridization. These teas were ground and sieved into various particle sizes using a set of stainless steel Endecotts sieves. Three grams of the ground tea leaves (the size range chosen was 1~2 mm) were transferred into a tea-bag (nonwoven material) and the side sealed. The final size of the bag was 3 cm by 6 cm.

### Preparation of tea infusion

Eight of the same bag teas were each steeped separately in 150 mL of 70 °C water and taken out 5 ml of solution using pipette after 0.5 to 4 min with 30 sec interval in order. We kept the infusion temperature uniform and sampled the solution carefully without disturbing diffusion process to minimize the convection effect. All of tea infusion was filtrated through a 0.22  $\mu\text{m}$  syringe filter (Millipore Co., Bedford, MA, USA) and they analyzed by HPLC. The same procedures were repeated for temperatures 85 and 100 °C.

### HPLC analysis of tea infusion

The analyses were carried out with a Hitachi L-7100 HPLC pump (Hitachi Instruments Inc., Tokyo, Japan) with a 20  $\mu\text{L}$  injection loop. A Waters 996 photodiode-array detector (Waters Co., Miford, MA, USA) was used to detect CA, catechins and GA. The analytical condition was modified from that developed and used by Goto et al.<sup>[23]</sup>. A LUNA C18 (5  $\mu\text{m}$ , 250  $\times$  4.6 mm) reverse-phase analytical column (4.6 mm i.d.  $\times$  250 mm, 5  $\mu\text{m}$ , Phenomenex Co, CA, USA) kept at 25 °C

with a Colbox column oven (Hipoint Scientific Co., Kaohsiung, Taiwan) was used as the stationary phase. A mobile phase consisted of acetonitrile and 0.9 % acetic acid (prepared with deionized water), 5/95 (v/v), at the first 5 min, 13/87 (v/v) from 6 to 26 min and 71/29 (v/v) from 27 to 40 min. The flow rate was 1 mL/min and the detector was set at 280 nm. Triplicate analyses were conducted and the mean values were determined.

## Diffusion model and computational method

Due to the lack of asymptotic infusive concentration  $C$  of compounds, it was difficult to use the following equation directly to obtain the  $k_{obs}$  values by plotting the logarithm function against time,

$$\ln(C_{\infty}/C_{\infty}-C) = k_{obs}t \quad (1).$$

To analyze the results of short-time data within 4 min, we utilized the diffusion model to simulate the infusive process of such short duration and then extended the infusive time to steady state to acquire concentration  $C_{\infty}$ . Equation (1) can be found in any pharmacokinetics textbook for one-compartment models<sup>[24]</sup> and other related literature<sup>[22]</sup>. A steady state diffusion model developed by Spiro and Jago<sup>[18]</sup> also resulted in the same formula as equation (1). A more detailed consideration was made and the diffusion constants of compounds in leaf matrix and water were introduced therein. Although this diffusion model could explain the experimental result, another parameter was needed to include hindrance effect of tea bag through which the compounds were transported and the time taken before the leaf was completely swollen. The convection mechanism was also not included in our simulation. The diffusion equation in our simulation was

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial x} \left[ D(x) \frac{\partial C}{\partial x} \right] \quad (2),$$

where the diffusion coefficient  $D(x)$  was  $D_1$  within the leaf matrix and  $D_2$  for the compounds diffusing in the water. The initial concentration in the leaf was a function of  $C(x,t=0) = U_0 f(x)$  where  $f(x) = 1$  within the leaf and 0 outside the leaf. The thickness of the tea leaf was set to 1 mm and the

concentration of compounds was assumed to be uniformly distributed in the leaf. To mimic the hindrance of tea bag and the swelling time<sup>[20]</sup>, we parameterized a retarded time  $t_d$  for the diffusion of each compound into the water. The  $t_d$  plays a similar role to that of parameter  $b$  in equation (3),

$$\ln(C_{\infty}/C_{\infty}-C) = k_{obs}t + b \quad (3).$$

The solution of equation (2) was arrived at using the implicit finite difference method which is stable for larger time step<sup>[25]</sup>.  $U_0$  was given arbitrary value initially which could be replaced later by requiring the simulation result to be the same as the experimental data exactly at the time of 4 min. The initial concentration  $U_0$  in the leaf was fitted at 100 °C and then employed in the other two temperature simulations. Adjusting the parameters  $D_1$ ,  $D_2$  and  $t_d$ , we obtained the  $C_{\infty}$  for each compound at different temperatures. Calculation of  $k_{obs}$  and activation energy  $E_a$  is straightforward and can be found in any physical chemistry textbook<sup>[26]</sup>.

## Results and discussion

Although there are many kinds of commercial tea made following different procedures, green tea and black tea are the most widely consumed teas around the world. In this work, our experimental measurements were triplicate averages to fit the infusion kinetics of a time dependent differential equation to obtain the optimized parameters with least square deviation by adjusting the values of parameters. We obtained the optimized parameters by comparing the standard deviation of the experimental data with the numerical simulation results of the diffusion equation which is a function of the kinetic parameters.

### Rate constants

Spiro and Jago<sup>[18]</sup> showed that the rate of infusion of tea is approximately a first order process. Based on this information, we used the diffusion model of only one tea leaf swollen in the water. The computational results of GA, which was obtained by using the procedure described above, are presented in the inset of Fig.1 for three different temperatures. From these parameters and fixed  $U_0$  value, we propagated the equation for up to 90 min

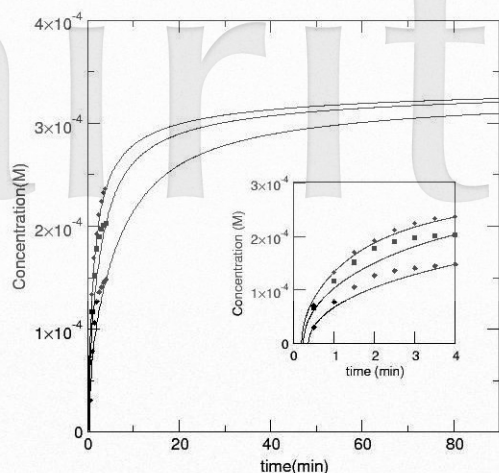


Fig. 1. The dependence of concentration of GA against time. The short-time fitting is shown in the inset. Filled circles are 100 °C infusion, filled squares for 85 °C and filled diamonds are 70 °C infusion, respectively.

and observed the steady state behavior (Fig.1). The fitting procedure of parameters for the other five compounds was the same as that of GA in Fig.1. In our experiment, all the infusive concentrations rose rapidly within the first 10 min of brewing and then reached steady state. By means of the  $C_\infty$  value, the plots of  $\ln(C_\infty/(C_\infty-C))$  vs. time with least square are shown in Fig. 2 for four compounds of green tea at 70, 85 and 100 °C. The data were found to fit

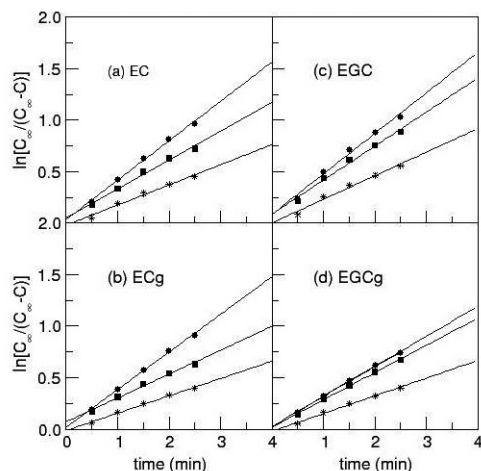


Fig. 2.  $\ln(C_\infty/(C_\infty-C))$  vs. time for (a) EC, (b) ECg, (c) EGC and (d) EGCg of green tea at temperature 100, 85 and 70 °C. Filled circles are 100 °C infusion, filled squares for 85 °C and stars are for the case of 70 °C infusion, respectively.

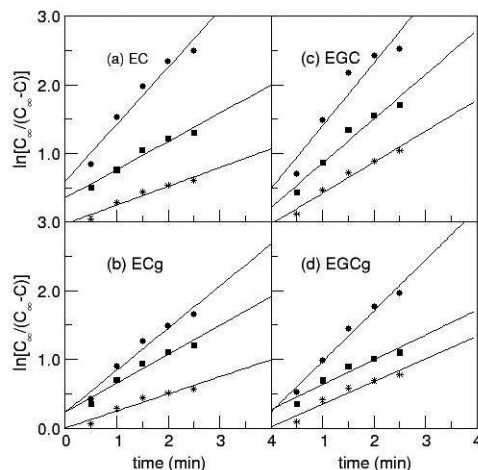


Fig. 3.  $\ln(C_\infty/(C_\infty-C))$  vs. time for (a) EC, (b) ECg, (c) EGC and (d) EGCg of black tea at different temperature 100, 85 and 70 °C. Filled circles are 100 °C infusion, filled squares for 85 °C and stars are for the case of 70 °C infusion, respectively.

very well into the Eq. (3), as can be seen in Fig. 2. Fig. 3 shows the experimental data and the fitted results for black tea. The intercepts,  $b$ , of green tea were smaller than those for the black tea. This may be due to differences in leaf matrix structure and different weight of each compound in composition between the black and green teas. The long-time concentrations,  $C_\infty$  for EC, ECg, EGC and EGCg in green tea were larger than those in black tea, the difference being one or more orders of magnitude. In general, this  $C_\infty$  value is related to the degree of richness originally contained in the tea leaf,  $U_0$ . In contrast, the  $U_0$  of GA and CA in green tea were smaller than those in the black tea (Table 1). The  $U_0$  values in Table 1 indicate that fully fermented tea contained higher levels of GA and CA and much fewer catechins than non-fermented green tea. This was a result of the manufacturing processes used with the TTE12 species. Table 2 shows that EC and EGC had a higher extraction rates ( $C_\infty/U_0$ ) than ECg and EGCg in both green and black

**Table 1.** The concentration of compounds,  $U_0$  (mM), contained in the green and black teas.

Compound	GA	CA	EC	ECg	EGC	EGCg
Green tea	0.346	6.805	0.710	1.376	3.380	4.129
Black tea	0.552	7.450	0.022	0.171	0.063	0.072

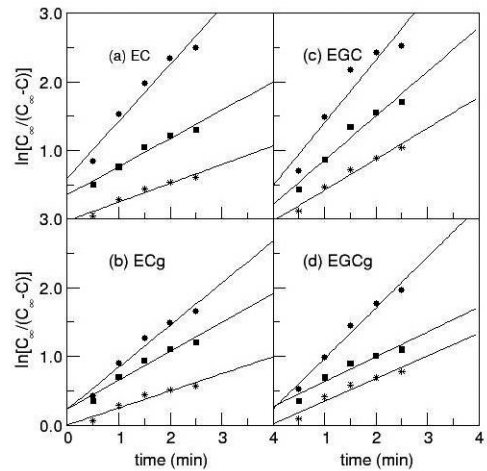
**Table 2.** Extraction rate,  $C_{\infty}/U_0$  for the six compounds of green tea and black tea at temperature 70, 85 and 100 °C, respectively.

Compound	GA	CA	EC	ECg	EGC	EGCg
Green tea						
70 °C	0.893	0.888	0.871	0.855	0.907	0.864
85 °C	0.925	0.923	0.912	0.898	0.933	0.902
100 °C	0.936	0.938	0.938	0.933	0.938	0.915
Black tea						
70 °C	0.896	0.923	0.869	0.866	0.937	0.908
85 °C	0.903	0.940	0.936	0.943	0.960	0.925
100 °C	0.938	0.941	0.983	0.959	0.982	0.972

teas because the large molecular size and weight of ECg and EGCg made it difficult for them to transport through the leaf matrix. However, the extraction rate increased along with temperature. The extraction of GA and CA did not seem to be so sensitive to temperature changes because of small molecular weight resulting in small difference of diffusion coefficients between leaf matrix and water (Table 2). On the other hand, catechins showed notable temperature dependence between 70 and 85 °C. Table 3 show our calculations of the slope in Fig. 2~4 for the rate constants of green and black teas. The rate constants of flavanols of green tea compared with the Japanese green tea have been reported to be 10% larger for EGC or 25% smaller for EC, ECg and EGCg at 70 °C<sup>[19]</sup>. The CA in black tea is reported to be 1.5 times larger than those in black Assam tea<sup>[20]</sup>. As mentioned above, the size and weight effects made EC and EGC rate constants larger than ECg and EGCg's. The rate

**Table 3.** Rate constants  $10^3 k_{obs}(s^{-1})$  and activation energies  $E_a$  of the six compounds in green tea and black tea, respectively.

Compound	GA	CA	EC	ECg	EGC	EGCg
Green tea						
70 °C	3.93	3.47	3.27	2.89	3.82	2.87
85 °C	5.38	5.47	4.63	3.85	5.05	4.42
100 °C	6.57	6.70	6.38	6.08	6.55	4.90
$E_a(kJmol^{-1})$	18.5	23.5	23.8	27.4	19.2	18.9
Black tea						
70 °C	4.85	5.72	4.55	4.15	7.53	5.50
85 °C	4.67	6.80	6.85	7.02	10.75	6.03
100 °C	6.87	6.98	13.82	10.22	15.27	12.28
$E_a(kJmol^{-1})$	12.1	7.2	39.3	32.1	25.0	28.2

**Fig. 4.**  $\ln(C_{\infty}/C_{\infty}-C)$  vs. time for (a) GA in green tea (b) GA in black tea and (c) CA in green tea (d) CA in black tea at different temperature 100, 85 and 70 °C. Filled circles are 100 °C infusion, filled squares for 85 °C and stars are for the case of 70 °C infusion, respectively.

constants of catechins in black tea were higher than they were in green tea, though their amounts in water were less. This might be a concentration effect, as the compound molecules in high concentration solution might hinder the further release of compound particles into the solution and reduce the rate constant.

### Activation energy

The Arrhenius equation

$$\frac{d \ln k_{obs}}{d(1/T)} = -\frac{E_a}{R} \quad (4),$$

where R is the gas constant and T is temperature, was used to determine the effective activation energy  $E_a$  including the swelling time, tea bag hindrance and the barriers of transportation of compounds in the leaf matrix. The least square plots of  $\ln k_{obs}$  against  $1/T$  are shown in Fig. 5 and the resulting effective activation energy  $E_a$  are listed in Table 3 for the six compounds in the green and black teas. The range of  $E_a$  was about 18~27  $kJmol^{-1}$  for green tea and 7~39  $kJmol^{-1}$  for black tea. Jaganyi and Mdletshe<sup>[20]</sup> reported a 43  $kJmol^{-1}$  value of CA in black Assam tea, whereas in our study it was 7.2  $kJmol^{-1}$ . Other studies<sup>[19]</sup> have reported the  $E_a$  values of catechins to 31~50  $kJmol^{-1}$

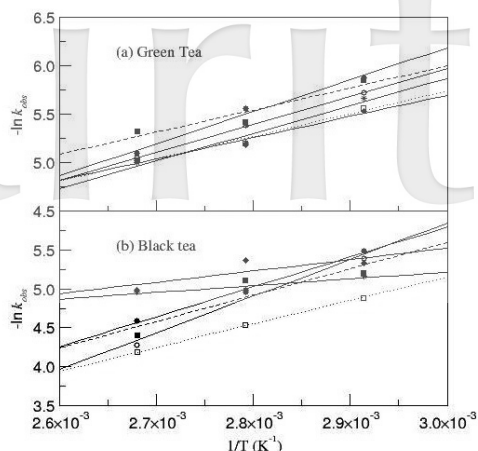


Fig. 5.  $-\ln k_{obs}$  vs.  $1/T$  for flavanols, CA and GA in (a) green tea and (b) black tea. In both cases, open circles are for EC; filled circles for ECg; open squares for EGC; filled squares for EGCg; filled diamonds for GA and stars for CA.

and the CA to be  $58 \text{ kJmol}^{-1}$  in a Japanese green tea (Sen Cha Uji Tsuyu). Gujar, Chattopadhyay, Wagh and Gaikar<sup>[21]</sup> investigated the solvent effect at low temperatures using the diffusion equation similar to ours in equation (2) and found a value of  $30.28 \text{ kJmol}^{-1}$  for EC in Indian green tea, compared to our results of  $23.8 \text{ kJmol}^{-1}$ . Ma, Wang and Liang<sup>[27]</sup> divided the diffusion process into two different stages (fast and slow) to explore the diffusion kinetics of florine and flavanols in green tea. The diffusion kinetics in their paper were similar to our trend and behavior results. It is not easy to compare the activation energy of these compounds in the literature, because the degree of fermentation of the teas they study are different. In addition, chemical compounds of various tea species in the brewed solution interact with each other in a way that makes it difficult to adjust for this interference. However, we can see from the tables that non-gallated catechins have smaller  $E_a$  than the gallated ones due to their lower molecular weight and size in water. Finally, we calculated the fitted diffusion constants and retarded times of six compounds (Table 4). The ratio of diffusion constants between tea leaf and water,  $D_2/D_1$ , was large at low temperatures but it became smaller as temperatures rose, revealing a compound migration tendency from tea matrix into water associated with changes in temperature. GA, CA and EGC had a more

**Table 4.** Ratio of  $D_2/D_1$  and the retarded time  $t_d$  for green tea where  $D_1$  and  $D_2$  are diffusion coefficients in leaf matrix and in water, respectively.

Compound	$D_2/D_1$	Temperature (°C)	Retarded time $t_d$ (sec)
GA	14.5	70	22
	8.6	85	14.5
	3.0	100	12
CA	6.7	70	24
	3.1	85	19
	3.0	100	17
EC	3.3	70	24
	3.2	85	19
	4.4	100	17
ECg	2.9	70	24
	2.1	85	20
	3.1	100	19
EGC	19.4	70	22
	8.7	85	17.5
	3.0	100	15
EGCg	3.6	70	24
	2.5	85	19
	2.9	100	17

notable temperature-dependent mobility than the others (Table 4).

This study has some limitations. There was some simplification in this model. First, we neglected the interaction effects and correlation of different compounds coexisting in the solution. These may be interesting and important for a pharmacodynamic study, and should be considered in the future. In addition, the limited space of tea leaves in the water was omitted by the one-leaf model, so spatial concentration non-uniformity and local concentration saturation was not considered here. A more realistic model taking into account of these factors will be developed in the near future.

## Conclusion

In this paper, we demonstrate the application of a diffusion equation to tea infusion kinetics using short-time data. The characteristics of the compounds in tea influenced their transportation and solubility in water. This diffusion model could potentially be used to determine the

healthful constituents in the human body from a pharmacokinetic point of view.

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# 以擴散模式探討茶葉短時間浸泡溶出的動力學

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本研究以簡單的單葉擴散模式探討茶葉中主要成份：咖啡因、兒茶素與沒食子酸的溶出動力學。我們以高效能液相層析儀（HPLC）定量由台茶12號製成的綠茶與紅茶其溶出的咖啡因（CA），表沒食子兒茶素（EGC），表兒茶素（EC），表沒食子兒茶素沒食子酸酯（EGCg），表兒茶素沒食子酸酯（ECg），與沒食子酸（GA）之濃度，透過短時間濃度的數據擬合，我們可以以外插擴散方程式而得到其平衡濃度。從平衡濃度我們可以進一步計算一階溶出速率常數（ $k_{obs}$ ）與活化能。結果顯示，當高溫浸泡時，等效遲滯時間約15~17秒，低溫時則超過22秒。對綠茶與紅茶的成份萃出率則分別為85.5%~93.8%及86.6%~98.3%。而綠茶與紅茶的等效活化能則分別為8~27 kJ/mol 與 7~39 kJ/mol。此結果主要是因為這些化合物的分子量差異與製茶過程的不同所引起的。

**關鍵詞：**擴散模式、隱性有限差分法、溶出動力學、等效活化能

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