

# A Novel Kinetic Model for Dissolution of Herbal Medicine

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## ABSTRACT

To establish a novel kinetic model for phytochemical constituent dissolution, including comparison of results of open and closed dissolution systems, Buyanghuanwu decoction (BYHWD), a traditional Chinese herbal medicine formula, was selected as our experiment subject. The establishment of a kinetic model was based on the theory of Fick's Rule and Noyes-Whitney equation. By fitting the kinetic parameters of dissolution models, calculating the inherent parameters and the dissolution efficiency in herbal medicine, the results can be used to evaluate phytochemical constituent dissolution. In this study, laetrile, ferulic acid, and paeoniflorin were considered as three marker compounds, which were determined by high-performance liquid chromatography (HPLC). The dissolution processes and results with BYHWD were described and evaluated by these kinetics models. The results showed that the AUC (area under the curve) for open system was 9.21 times higher than the closed system. Decomposition power ( $D_p$ ) in the open system was 1.505 times higher than the closed system, and the calculated transfer power ( $T_p$ ) for the open system was 1.23 times higher than the closed system.

**KEYWORDS:** Dissolution kinetics, mathematical model, Buyanghuanwu decoction (BYHWD), herbal medicine, medicinal botany, traditional Chinese medicine

## INTRODUCTION

With the rising usage of natural medicinal plant products, herbal medicinal treatment is an alternative medication therapy that appears to be irreplaceable. As we know, the dissolution components from processed products of herbal medicine are different than the dissolution of regular medicines or dietary supplements; there is a lack of systematic understanding of the dissolution kinetics of marker compounds in processed products of herbal medicines.

Dissolving effective components from herbal medicine products relies on the dynamic and complicated mass transfer processes, which are influenced by the cytomembrane in botanical cells. Furthermore, the dissolution and diffusion process in herbal medicine chemical constituents is harder to study than solid dosage forms. Many dissolution kinetic models have reported that the determination of technology parameters can be used to develop the orthogonal design, uniform design, or

response surface methodology (1-4). These studies could not take a holistic approach for compound formulation and fail to recognize that the plant membrane structures are contained in these herbal medicines. Compared to other dissolution kinetics models, we have demonstrated that our novel model is more similar to the real dissolution process of herbal medicine. Our model provides a more comprehensive parameter system for large industrial manufacturing.

Open and closed systems have varied the characteristics in dissolution systems of classic herbal products. A closed dissolution system ensures that all the active ingredients maintain contact with the dissolution solvent and cannot encounter new solvent during the dissolution process. The reflux method, impregnation method, and decocting method are closed systems. For an open system, when the fresh solvents are added, there is a constant flow in solution of herbal medicine-containing dissolution medium, which also should be considered in

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this dynamically balanced system. We selected the reflux method and soxhlet method as examples to analyze the characteristics of open and closed dissolution systems, respectively (Fig. 1).

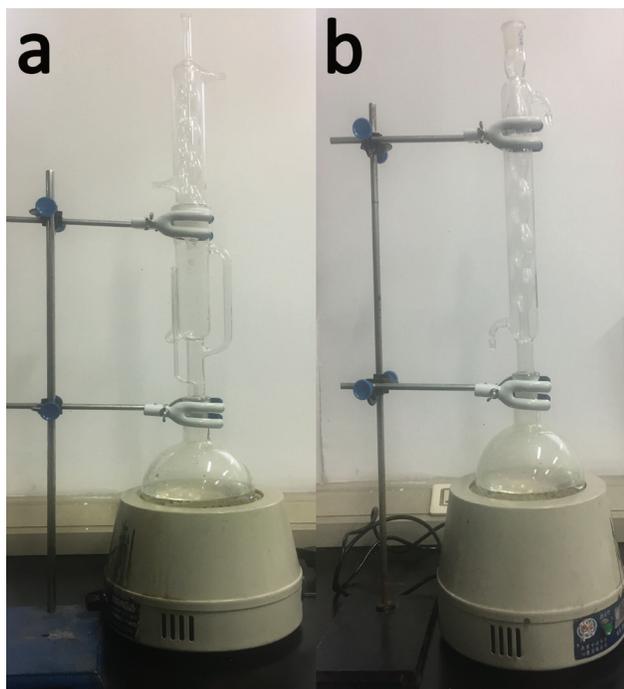


Figure 1. Dissolving experiment for herbal medicine formulation: (a) open system (soxhlet method); (b) closed system (reflux method).

Buyanghuanwu decoction (BYHWD) is a classic herbal medicine formula that has been used in the treatment and prevention of ischemic heart and brain diseases for a long history in China (5). BYHWD is composed of seven kinds of herbal medicine, including *Radix Astragalus mongholicus* (60 g), *Radix Angelica sinensis* (9 g), *Radix Paeoniae rubra* (9 g), *Rhizoma Ligustici chuanxiong* (6 g), *Flos carthami* (9 g), *Semen Persicae* (9 g) and *Dilong* (9 g) (6). Chinese Pharmacopoeia (2015 Edition) is the current legal standard for the quality of herbal medicine, which has guided the quantitative standard and method for each herbal material of BYHWD (7). It has reported that several bioactive aromatic acids, especially ferulic acid, are included in both *danggui* and *chuanxiong* (8–10). Ferulic acid is one of the most abundant water-soluble ingredients and has been identified as the active component of these two herbal medicines. It is usually used to assess the quality of *danggui* and *chuanxiong* in China and has been clinically used to treat angina pectoris and hypertension. Previous investigations have suggested that it could significantly reduce the level of nitrite, improve blood fluidity and oxygen free radicals, lower blood lipids, resist bacteria, and reduce inflammation (11–13). *Chishao* is widely used

to reduce fever, eliminate blood stasis, and activate blood circulation (14). Meanwhile, paeoniflorin is the main bioactive component in *chishao* and the only marker of quality evaluation in Chinese Pharmacopoeia (7). The quantitative determination of paeoniflorin is analyzed by high-performance liquid chromatography (HPLC).

To ensure accuracy of the content in these marker compounds and the stability of detection, hydroxysafflor yellow A, and AST-IV, are not considered as marker compounds due to its structurally unstable when it was detected by HPLC equipment. *Dilong* has been utilized as an animal medicine, which is not suitable for our model (15). For these reasons, laetrile, ferulic acid, and paeoniflorin are regarded as the marker compounds. It can be applied for the dissolution behavior of processed products containing BYHWD and provides a fast quality assessment method in this paper. The chemical structures of these three compounds are shown in Figure 2a.

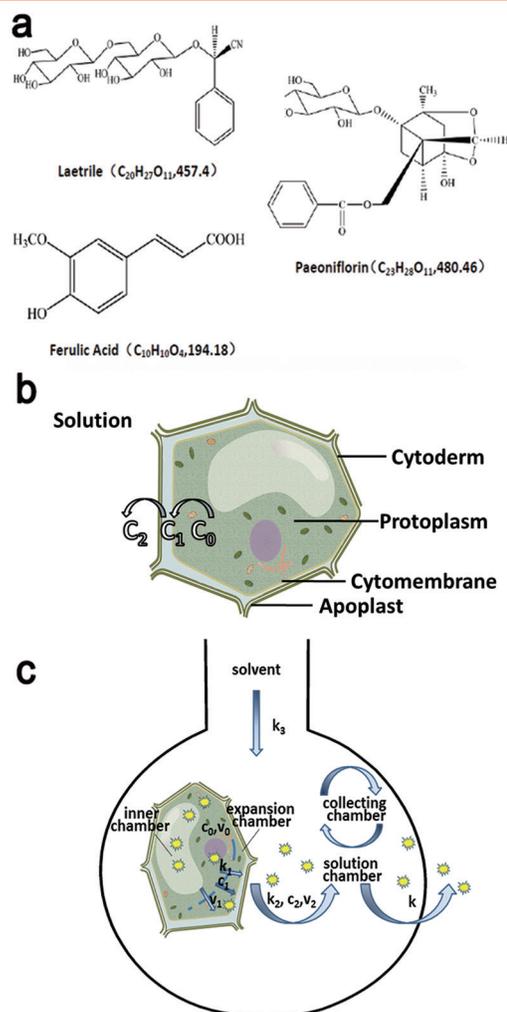


Figure 2. Chemical structures and mass transfer process: (a) chemical structure of laetrile, paeoniflorin, and ferulic acid; (b) dissolution process diagram in botanical tissue; (c) dissolution kinetic processes and relative parameters of marker compounds in detail.

## METHODS

### Dissolution Kinetic Mathematical Modeling and Related Parameters

In some Asian countries, especially in China, people usually process herbal medicine into herbal medicine products and dissolve these powders directly to treat specific diseases. This kind of formulation still includes inactive botanical tissue. As we know, the dissolution process begins with the marker compounds in the protoplasm of herbal products, via moistening and swelling, desorption and dissolution, and diffusion and mass translation processes. The botanical structure exists in three parts: protoplasm, cytomembrane, and apoplast (Fig. 2b). Considering the quantitative transfer process and the principle of material balance simultaneously, herbal medicine dissolution behaviors can be described as three steps (16, 17). The first step can be inferred by as shown in eq 1.1, meaning that deviation to the concentration of marker compounds per unit of time in the protoplasm is equivalent to the markers' concentration flowing out from the protoplasm to cytomembrane, by subtracting the concentration in protoplasm.  $C_0$  is the concentration of the marker compounds ( $\text{mg}\cdot\text{g}^{-1}$ ) in protoplasm,  $t$  is the dissolution time,  $A_1$  is the effective surface area of the cell chamber, and  $V_0$  is the active volume.

The second step in dissolution process is deduced by eq 1.2, meaning that deviation to the concentration of marker compounds per unit of time in cytomembrane room is equivalent to the concentration in cytomembrane added to the inflowing concentration from protoplasm and subtracting the markers' concentration flowing out from the protoplasm to apoplast.  $A_2$  is the effective surface area of apoplast,  $V_1$  is the active volume of apoplast room, and  $C_1$  is the composition concentration.

The final step is the diffusion of the compounds into the solvent bulk ( $S_0$ ), which is usually much slower. It can be regarded as the rate-limiting step for these dissolution systems. In Eq 1.3,  $V_2$  is the liquid volume and  $C_2$  is the composition concentration. Meanwhile, according to the principle of material balance, the maximum dissolvable mass of marker compounds is defined as  $X_0$ , which represents the mass under completely dissolution.  $\rho_2$  and  $\rho_1$  are distribution coefficients which are calculated respectively. The concentration in solution divided by the concentration in apoplast is  $\rho_2$ , and the concentration in apoplast divided by the concentration in protoplasm is  $\rho_1$ . For the closed system, the compound is directly in contact with solvent dissolution, the value of ( $w$ ) was the final weight of marker compounds. For the open system, many thermal unstable chemical compounds under high

temperatures may be decomposed in the collection chamber. Therefore, we deduced to Eq 1.4, where the  $C_3$  expresses the final concentration and the  $V_3$  is the liquid volume in the collection chamber for the open system. The detail of dissolution process is depicted in Figure 2c.

### Solving Equations

Based on the dissolution mass transfer process eqs 1.1–1.3, Eq 1.4 is promoted to build mathematic models for herbal medicine in closed and open systems:

$$\frac{dc_1}{dt} = k_1 A_1 (C_0 - \rho_1 C_1) \quad (1.1)$$

$$\frac{dc_2}{dt} = k_2 A_2 (C_1 - \rho_2 C_2) - K_3 C_2 \quad (1.2)$$

$$C_0 V_0 + C_1 V_1 + C_2 V_2 + V_2 K_3 \int_0^t C_2 dt = w \quad (1.3)$$

$$\frac{dC_3}{dt} = \frac{V_2 K_3 C_2}{V_3} - K_4 C_3 \quad (1.4)$$

where  $k_1$  and  $k_2$  are the mass transfer coefficients in the first and second step respectively,  $k$  is the elimination rate constant of the ingredient in the solution bulk, and  $k_4$  is the decomposition constant in open system.  $\rho_1$  ( $C_0/C_1$ ) and  $\rho_2$  ( $C_1/C_2$ ) are the partition coefficients.

Using a closed system as an example to analyze the model, it is similar to solve the open system. By fitting its properties to an extraction kinetics equation, eqs 1.1–1.3 can be obtained. Due to the complexity of the ternary linear differential equations, these equations cannot be easily solved by direct calculation. For this purpose, we use the Laplace transforms and its inverse transforms, which are common transforms in engineering mathematics to solve equation sets.

$$Lc_1 = k_1 A_1 Lc_0 - k_1 A_1 \rho_1 Lc_1 \quad (2.1)$$

$$Lc_2 = k_2 A_2 Lc_1 - k_2 A_2 \rho_2 Lc_2 - k_3 Lc_2 \quad (2.2)$$

$$Lc_0 V_0 + Lc_1 V_1 + Lc_2 V_2 + \frac{V_2 k_3 \cdot Lc_2}{s} = w \cdot s \quad (2.3)$$

Then, eqs 2.1–2.3 will be iterated, respectively.

$$Lc_2 = \frac{k_1 A_1 k_2 A_2 w_0}{V_0 \cdot L(s)} \quad (3.1)$$

$$\begin{aligned}
L(S) &= s^3 + \frac{(k_1 A_1 V_1 + \rho_1 K_1 A_1 V_0 + k_2 A_2 V_0 \rho_2 + V_0 k) s^2}{V_0} \\
&+ \frac{(k_1 A_1 k_2 A_2 V_2 + k_1 A_1 k_2 A_2 V_1 \rho_2 + k_1 A_1 k_2 A_2 V_0 \rho_1 \rho_2 + k_1 A_1 V_1 k + \rho_1 k_1 A_1 V_0 k) s}{V_0} \\
&+ \frac{k_1 A_1 k_2 A_2 V_2 k}{V_0} \tag{3.2}
\end{aligned}$$

Then, the factors in eq 3.1 are combined into form eq 3.3:

$$LC_2 = \frac{k_1 A_1 k_2 A_2 w_0}{V_0 \cdot L(s)} \tag{3.3}$$

Through the method of undetermined coefficients, we order  $\alpha$ ,  $\beta$ , and  $\pi$  as follows in eqs 4.1–4.3:

$$\alpha + \beta + \pi = Ak'_1 + \rho_2 k'_2 + k \tag{4.1}$$

$$\alpha\beta + \beta\pi + \alpha\pi = k'_1 k'_2 B + Ak'_1 k \tag{4.2}$$

$$\alpha\beta\pi = \frac{k'_1 k'_2 k V_2}{V_0} \tag{4.3}$$

Parameters  $A$ ,  $B$ ,  $k'_1$  and  $k'_2$  are satisfied with eqs 5.1–5.4:

$$A = \frac{V_1}{V_0} + \rho_1 \tag{5.1}$$

$$B = \frac{V_2}{V_0} + \frac{V_1}{V_0} \rho_2 + \rho_1 \rho_2 \tag{5.2}$$

$$k'_1 = k_1 A_1 \tag{5.3}$$

$$k'_2 = k_2 A_2 \tag{5.4}$$

Thus, the relationship between the concentration and dissolution time in a closed system are obtained by eq 6:

$$\begin{aligned}
c_2 &= \frac{k_1 k_2 A_1 A_2 w_0}{V_0 (\beta - \alpha) (\pi - \alpha)} e^{-\alpha t} + \frac{k_1 k_2 A_1 A_2 w_0}{V_0 (\alpha - \beta) (\pi - \beta)} e^{-\beta t} \\
&+ \frac{k_1 k_2 A_1 A_2 w_0}{V_0 (\alpha - \pi) (\beta - \pi)} e^{-\pi t} \tag{6}
\end{aligned}$$

The inverse of the Laplace transform  $C_2$  in eq 6 is the solution of eqs 1.1–1.3. eq 6 can be simplified to eq 7:

$$\begin{aligned}
C_2 &= \frac{k_1 k_2 A_1 A_2 C_0^0}{(\beta_1 - \alpha_1) (\pi_1 - \alpha_1)} e^{-\alpha_1 t} + \frac{k_1 k_2 A_1 A_2 C_0^0}{(\alpha_1 - \beta_1) (\pi_1 - \beta_1)} e^{-\beta_1 t} \\
&+ \frac{k_1 k_2 A_1 A_2 C_0^0}{V_0 (\alpha_1 - \pi_1) (\beta_1 - \pi_1)} e^{-\pi_1 t} \tag{7}
\end{aligned}$$

$$\begin{aligned}
C_2 &= M_1 \exp(-\alpha_1 t) + N_1 \exp(-\beta_1 t) \\
&+ L_1 \exp(-\pi_1 t) \tag{8}
\end{aligned}$$

The relation between the concentration and dissolution time (eq 8) in an open dissolution system can be obtained via the first-order linear differential integral calculus method and Laplace transform rule in a similar way (eqs 9 and 10).

$$\begin{aligned}
C_3 &= \frac{V_2 k_1 k_2 k_3 A_1 A_2 w_0}{V_0 V_3 (\beta_2 - \alpha_2) (\pi_2 - \alpha_2) (k_4 - \alpha_2)} \exp(-\alpha_2 t) \\
&+ \frac{V_2 k_1 k_2 k_3 A_1 A_2 w_0}{V_0 V_3 (\alpha_2 - \beta_2) (\pi_2 - \beta_2) (k_4 - \beta_2)} \exp(-\beta_2 t) \\
&+ \frac{V_2 k_1 k_2 k_3 A_1 A_2 w_0}{V_0 V_3 (\alpha_2 - \pi_2) (\beta_2 - \pi_2) (k_4 - \pi_2)} \exp(-\pi_2 t) \\
&+ \frac{V_2 k_1 k_2 k_3 A_1 A_2 w_0}{V_0 V_3 (\alpha_2 - k_4) (\beta_2 - k_4) (\pi_2 - k_4)} \exp(-k_4 t) \tag{9}
\end{aligned}$$

$$\begin{aligned}
C_3 &= M_2 \exp(-\alpha_2 t) + N_2 \exp(-\beta_2 t) + L_2 \exp(-\pi_2 t) \\
&+ S_2 \exp(-k_4 t) \tag{10}
\end{aligned}$$

For eqs 8 and 10, the relevant parameters ( $M_1$ ,  $N_1$ ,  $L_1$ ,  $\alpha_1$ ,  $\beta_1$ ,  $\pi_1$ ,  $M_2$ ,  $N_2$ ,  $L_2$ ,  $S_2$ ,  $\alpha_2$ ,  $\beta_2$ ,  $\pi_2$ ,  $k_4$ ), and the corresponding exponential polynomial equations are obtained by the simulation and nonlinear multiple regression equations respectively.

### Herbal Medicine Inherent Parameters

Herbal medicine products have their own properties that are attributed to the characteristic value for each medicinal effect. It is known that  $A_1$  (the effective surface area of protoplasm) and  $A_2$  (the effective surface area of apoplast) are difficult to determine using HPLC. However,

we can calculate the combined value with coefficients  $k_1$  and  $k_2$ , presenting as  $k'_1$  and  $k'_2$  in eqs 5.3 and 5.4, respectively.  $W_0$ ,  $C_1$ ,  $C_2$ ,  $C_3$  are determined by HPLC with wavelength at 264 nm;  $V_0$ ,  $V_1$ , and  $V_2$  are measured by density gradient centrifugation, and the value of  $V_3$  is determined by reflux and soxhlet methods for open and closed systems, respectively. Other parameters should be calculated by eqs 11–22.

In reflux systems, herbal medicine characteristic parameters are defined as  $k$ ,  $k'_1$ ,  $k'_2$ ,  $\rho_2$ , and  $\rho_1$ ; dissolution kinetic parameters are defined as  $t$ ,  $M_1$ ,  $N_1$ ,  $L_1$ ,  $\alpha_1$ ,  $\beta_1$ , and  $\pi_1$ . Similarly, in soxhlet systems, herbal medicine characteristic parameters are defined by  $A_1$ ,  $A_2$ ,  $k_1$ ,  $k_2$ ,  $\rho_2$ ,  $\rho_1$ ; dissolution kinetic parameters are defined by  $t$ ,  $M_2$ ,  $N_2$ ,  $L_2$ ,  $S_2$ ,  $\alpha_2$ ,  $\beta_2$ ,  $\pi_2$ , and  $k_4$ . For reflux and soxhlet methods, they have the same evaluation indicators: AUC,  $T_p$ ,  $D_p$ ,  $C_{max}$ , and  $t_{max}$ .

AUC is defined as the area under the curve of marker compounds concentration.

$$AUC_T = \sum_{i=1}^n \left( \frac{M}{\alpha} + \frac{N}{\beta} + \frac{L}{\pi} \right)_i = a_0 \quad (11)$$

$k$  and  $k_4$  are elimination rates of concentration changing from the botanical cell to the solvent in the closed and open systems, respectively.

$$AUC = \int_0^{\infty} c_2 dt = \frac{w_0}{kV_2} \quad (12)$$

$$k = \frac{w_0}{AUC \cdot V_2} \quad (13)$$

The composition concentration is expressed as:

$$AUC = \int_0^{\infty} \frac{c_2 V_2}{w_0} dt = \frac{1}{k} \quad (14)$$

$$k = \frac{1}{AUC} \quad (15)$$

To solve eqs 4.1–4.3, a calculation process parameter  $B$  should be set up.

$$B^2 + [(\alpha + \beta + \pi)k - 2(\alpha\beta + \alpha\pi + \beta\pi)] \frac{V_2 k}{\alpha\beta\pi V_0} B + [(\alpha\beta + \alpha\pi + \beta\pi)(k^2 - (\alpha + \beta + \pi))]k + [(\alpha\beta + \alpha\pi + \beta\pi) - \alpha\beta\pi k] \left( \frac{V_2 k}{\alpha\beta\pi V_0} \right)^2 = 0 \quad (16)$$

The result can be obtained in eq 17:

$$B = \left( \frac{2(\alpha\beta + \alpha\pi + \beta\pi) - (\alpha + \beta + \pi)k}{2} \right) \frac{kV_2}{\alpha\beta\pi V_0} \pm \left( \frac{\sqrt{(\alpha + \beta + \pi)^2 k^2 - 4(\alpha\beta + \alpha\pi + \beta\pi)k^2 + 4\alpha\beta\pi k}}{2} \right) \frac{kV_2}{\alpha\beta\pi V_0} \quad (17)$$

$\rho_1$  can be calculated by using eq 5.2:

$$\rho_1 = \left( B - \frac{V_2}{V_0} - \frac{V_1}{V_0} \rho_2 \right) \frac{1}{\rho_2} \quad (18)$$

Parameter  $A$  can be calculated by using eq 5.1:

$$A = \frac{V_1}{V_0} + \rho_1 \quad (19)$$

It can be obtained by transforming eq 4.3 into eq 20:

$$k'_1 k'_2 = \frac{\alpha\beta\pi V_0}{kV_2} \quad (20)$$

And  $k'_1$  and  $k'_2$  are calculated by using eqs 21 and 22, respectively:

$$k'_1 = \frac{(\alpha\beta + \alpha\pi + \beta\pi) - Bk'_1 k'_2}{Ak} \quad (21)$$

$$k'_2 = (\alpha + \beta + \pi - Ak'_1 - k) \frac{1}{\rho_2} \quad (22)$$

### Dissolution Efficiency Parameters

The time of fully dissolved herbal medicine is  $t_{max}$ ;  $C_2$  converges to the dissolution time as shown in eqs 23 and 24, and  $t_{max}$  can be calculated when the first derivatives of eqs 8 and 10 are zero.

$$\frac{M}{-\alpha} \exp(-\alpha t_{max}) + \frac{N}{-\beta} \exp(-\beta t_{max}) + \frac{L}{-\pi} \exp(-\pi t_{max}) = 0 \quad (23)$$

$$\frac{M}{-\alpha} \exp(-\alpha t_{max}) + \frac{N}{-\beta} \exp(-\beta t_{max}) + \frac{L}{-\pi} \exp(-\pi t_{max}) + \frac{S}{-k_4} \exp(-k_4 t_{max}) = 0 \quad (24)$$

$C_{max}$  is the maximum concentration of the marker compounds dissolution solution.  $C_{max}$  values can be calculated by substituting  $t_{max}$  into eqs 8 and 10.

$T_p$  is transfer power, the proportion of compound in total weight. It can be calculated with eq 25:

$$T_p = \frac{V_2 c_{\max}}{w_0} = \frac{V_2 c_{\max}}{\text{AUC} \cdot KV_2} = \frac{c_{\max}}{\text{AUC} \cdot K} \quad (25)$$

$D_p$  is decomposition power, the proportion of the eliminated amount in the total ingredients weight. It can be calculated with eq 26:

$$D_p = \frac{KV_2 \int_0^{t_{\max}} c_2 dt}{w} = \frac{\int_0^{t_{\max}} c_2 dt}{\text{AUC}} \quad (26)$$

### Model Development for Closed System

The model development for dissolution in closed system can be summarized in following steps:

1. Building mathematic model for component dissolution from botany (eqs 1.1–1.3)
2. Calculating eqs 1.1–1.3 via Laplace transformation
3. Using the method of undetermined coefficients and obtaining the relation of herbal medicine inherent parameters ( $A, k'_1, k'_2, \rho_1, \rho_2, V_0, V_1, V_2$ ) and eqs 5.1–5.4. The value of inherent parameters can be calculated by step 8 and the following steps.
4. Obtaining the relationship between concentration of component and extraction time, as shown in eq 7
5. Simplifying eq 7 by undetermined coefficients ( $M_1, N_1, L_1$ ) and obtaining eq 8
6. Averaging the experiment sample values
7. Using nonlinear multiple regression to obtain the value of  $M_1, N_1, L_1$  and the expression of dissolution for each marker compound
8. Measuring the value of  $t, C_1, C_2, V_0, V_1, V_2$ , and  $\rho_2$
9. Calculating the AUC and  $k$  (eqs 11–15)
10. Calculating the calculation process parameter,  $B$  (eqs 16, 17)
11. Inputting  $B$  and calculating  $A, k'_1, k'_2$ , and  $\rho_1$
12. Calculating  $t_{\max}$  and setting the first derivative of eq 8 as 0 (eq 29)
13. Inputting  $t_{\max}$  to eq 8, obtaining  $C_{\max}$
14. Calculating  $T_p, D_p$  (eqs 25, 26)

### Model Development for Open System

The model development for dissolution in open system can be summarized in following steps:

1. Building mathematic model for component dissolution from botany (eqs 1.1–1.4)
2. Calculating eqs 1.1–1.4 via Laplace transformation
3. Using the method of undetermined coefficients and obtaining the relation of herbal medicine inherent parameters ( $A, V_0, V_1, V_2, k'_1, k'_2, \rho_1, \rho_2$ ) and eqs 5.1–5.4. The value of inherent parameters can be calculated by step 8 and following steps.
4. Obtaining the relationship between concentration of component and extraction time, as shown in eq 9
5. Simplifying eq 9 by undetermined coefficients ( $M_2, N_2, L_2, S_2$ ) and obtaining eq 10
6. Averaging the experiment sample values
7. Using nonlinear multiple regression to obtain the value of  $M_2, N_2, L_2, S_2$ , and the expression of dissolution for each marker compound
8. Measuring the value of  $t, C_1, C_2, C_3, V_0, V_1, V_2, V_3$  and  $\rho_2$
9. Calculating the AUC and  $k$  (eqs 11–15)
10. Calculating the calculation process parameter,  $B$  (eqs 16, 17)
11. Inputting  $B$  and calculating  $A, k'_1, k'_2, \rho_1$
12. Calculating  $t_{\max}$  and setting the first derivative of eq 10 as 0 (eq 24)
13. Inputting  $t_{\max}$  to eq 8, obtaining  $C_{\max}$
14. Calculating  $T_p, D_p$  (eqs 25, 26)

### Materials and Instruments

Analytical grade acetic acid, and HPLC-grade acetonitrile were purchased from Shanghai SSS Reagent Co., Ltd. (Shanghai, China). Water was redistilled, and 1% aqueous acetic acid was used as a dissolution medium. Reference standards of laetrile (110820-200403), ferulic acid (110773-201012), and paeoniflorin (110736-201035) were purchased from the National Institute for Food and Drug Control (China). *Radix Astragalus mongholicus*, *Radix Angelica sinensis*, *Radix Paeoniae rubra*, *Rhizoma Ligustici chuanxiong*, *Flos carthami*, *Semen Persicae*, and *Dilong*

were manufactured by the Pharmacy Department of the Hunan University of Traditional Chinese Medicine (China).

All dissolution tests were performed on a DK-S26 apparatus (Ginghon Technology Co. Ltd., Shanghai, China), which consist of a water bath with the ability to test the dissolution of six samples simultaneously. The quantitative analysis of compounds was performed by using a Waters 2695 HPLC equipped with a Breeze chromatography workstation and a Waters 2487 Dual Absorbance Detector (Waters, Milford, MA, USA). Drugs and reagents were weighed on a Metler Toledo AG 214 balance (Metler, Greifensee, Switzerland).

### Preparation of Tested Substances

The test samples were pulverized according to the Chinese Pharmacopeia (2015 edition). A 55.5-g sample of BYHWD was placed in a round-bottom flask, then triple-distilled water was added to obtain a solid–liquid ratio of 1:5. The reflux and soxhlet system dissolution temperatures were both set at  $100 \pm 0.5$  °C.

Samples were centrifuged at  $5000 \text{ r}\cdot\text{min}^{-1}$  immediately; after that, the supernatant liquid was evaporated and dried at 37 °C under nitrogen stream. The residues were dissolved in 1.0-mL methanol; the samples were then analyzed by HPLC. Parallel trials were performed in triplicate ( $n = 3$ ), and mean values were used for data analysis.

### HPLC Analysis and Validation

HPLC was performed on an Ultimat $\times$ 10-AQ C18 column ( $4.6 \times 250$  mm,  $5.0 \mu\text{m}$ , Welch Materials, USA) at 40 °C with a flow rate of 1.0 mL/min and an injection volume of 20  $\mu\text{L}$ . The wavelength was 264 nm. The mobile phase was included with 1% aqueous acetic acid (mobile phase A) and aqueous acetonitrile (mobile phase B). The mobile

phase gradient program was carried out as follows: 0–10 min, 100%; 10–25 min, 93%; 25–35min, 87.5%; 35–45 min, 82%; 45–55 min, 75%; 55–65 min, 70%; 65–70min, 67.5%; 70–71 min, 63%; 71–80 min, 60%; 80–100 min, 20%; 100–115 min, 0%; 115–120 min, 100%. A short method validation program was performed for HPLC to determine the content of compositions. Linearity, accuracy, precision, stability, recovery, and specificity were assessed.

### Experiment Design

#### Determining the volume and distribution coefficient

Radix paeoniae rubra, Rhizoma ligustici, Semen persicae, and a sample of BYHWD were weighed precisely ( $W_1$ ) and placed in a 500-mL flask. The samples were preheated to 100 °C, and then 500 mL of 100 °C deionized water ( $W_2$ ) was added. A measuring cylinder was used to measure the volume of the leaching water ( $V_2$ ), which was defined as the solution chamber volume. The volume of liquid in the apoplast chamber was determined by centrifugation at different speeds ( $1000\text{--}5000 \text{ r}\cdot\text{min}^{-1}$ ). The centrifugal speed was plotted as the abscissa and the volume of the centrifugal liquid was plotted as the ordinate. The volume of liquid in the apoplast chamber ( $V_1$ ) was determined by the sudden increased volume of centrifugal liquid. Moreover, using eq 27:  $V_1 + V_0 = (W_2 - W_1) / d - V_2$ , where  $d$  is the specific gravity of water at room temperature; we investigated the volume of the cell chamber, to calculate  $V_0$ .

The liquid from the solution chamber and apoplast chamber was diluted and filtered. The concentration of laetrile, ferulic acid, paeoniflorin were determined by HPLC,  $\rho_2$  was calculated by the concentration and the volume. These tests provided the distribution of coefficient ( $\rho_2$ ) in the cell, apoplast, and solution chambers.

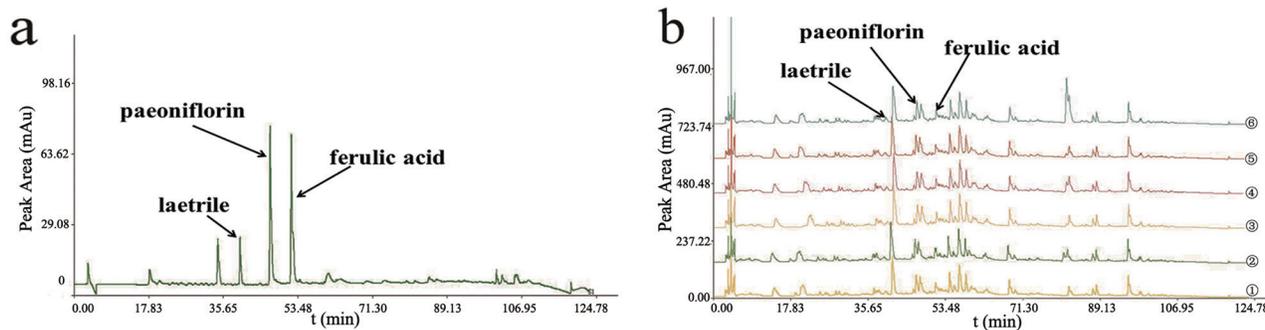


Figure 3. HPLC and determination of content: (a) HPLC for laetrile, paeoniflorin, and ferulic acid standard; (b) HPLC for BYHWD at 0, 3, 6, 9, 12, and 36 h. HPLC: high-performance liquid chromatography; BYHWD: Buyanghuanwu decoction.

## Statistical Analysis

The concentrations of test samples were calculated by linear regression equations. Statistical analysis and dissolution kinetics curve fitting were performed by nonlinear curve parameter estimation in SPSS 20.0 (SPSS Inc. Chicago, IL, USA), which was fitted to the regression equation and multiple correlation coefficients by using the F-Test. The calculated equations for the dissolution parameters of the active ingredients in BYHWD were mentioned above. The level of statistical significance was set as  $p < 0.01$ . Finally, we used experimental data to verify the expression for kinetics and the theoretical parameters.

## RESULTS AND DISCUSSION

### Method Validation

The release data were measured by HPLC equipped with UV detection, which showed a good separation of the three active ingredients peaks from other peaks. The theoretical plates were all greater than 3000, and the retention times of laetrile, paeoniflorin, and ferulic acid were 39.38, 47.17, and 52.33 min, respectively (Fig. 3). The standard calibration curves of these components all exhibited linearity with coefficients ( $r$ ) greater than 0.99. The accuracy of spiked solutions was between 95.01% and 99.83%; intra- and inter-precision was less than 2.98% and 1.96% relative standard deviation (RSD) ( $n = 6$ ), respectively; and 36-hour stability was less than 2.33% RSD ( $n = 6$ ), which was appropriate for the assessment of analytes in dissolution study (Table 1).

Table 1. Precision, Recovery, and Stability of Marker Compounds in BYHWD

Marker Compound	Spiked Concentration	Precision (%RSD)		Recovery (%)	Stability (%RSD for 36 h)
		Intra-run	Inter-run		
Laetrile	0.860	2.65	1.96	96.11	2.33
Paeoniflorin	0.518	1.43	1.77	95.01	2.06
Ferulic acid	0.044	2.98	1.67	99.83	1.57

BYHWD, Buyanghuanwu decoction; RSD: relative standard deviation.

### Herbal Medicine Inherent Parameters

The herbal inherent parameters were calculated and are listed in Table 2 ( $V_0$ ,  $V_1$ ,  $V_2$ , and  $p_2$ ) and Table 3 ( $k$ ,  $\rho_1$ ,  $k_1'$ ,  $k_2'$ ). The results of ANOVA curve fitting and the related kinetic parameters for the three marker compounds and one multi-compound formulation (BYHWD) dissolution kinetics are listed in Tables 3 and 4, respectively. The level of statistical significance was set as  $p < 0.01$  in each experiment group (Table 3). These data indicated that

the three kinds of component dissolution kinetics curves were close to the actual conditions, which can be used to characterize the dissolution kinetics.

### Curve Fitting and Parameter Calculation

We calculated the dissolution kinetic expressions for the three marker compounds using eq 8 and 10 in open and closed systems, respectively, after substitution of the kinetic parameters ( $M_1$ ,  $N_1$ ,  $L_1$ ,  $M_2$ ,  $N_2$ ,  $L_2$ ,  $S_2$ ,  $\alpha_1$ ,  $\beta_1$ ,  $\pi_1$ ,  $\alpha_2$ ,  $\beta_2$ ,  $\pi_2$ ,  $k_4$ ). By inserting these kinetic parameters into eqs 8 and 10, eqs 28–34 can be solved. The calculated dissolution process for the multi-compound formulation (BYHWD) was similar to the single marker compounds. According to the inherent properties of these three marker compounds, the kinetic parameters of the tri-component can be calculated. By using nonlinear multiple regression, the related values and the expression of dissolution for each marker compound can be obtained. AUC, the dissolution degree indicator, was defined as the area under the curve of marker compounds concentration. This concentration of tri-composition can be presented as a superposition of the three single markers concentrations. The multi-compound formulation kinetics dissolution formulas are shown in eqs 31 and 35.

For closed system:

Laetrile:

$$C = 0.493e^{-0.000314t} - 1.76e^{-0.0383t} + 1.27e^{-0.0393t} \quad (28)$$

Paeoniflorin:

$$C = 0.192e^{-0.0000343t} - 0.192e^{-0.0445t} + 0.0001e^{-0.0759t} \quad (29)$$

Ferulic acid:

$$C = 0.696e^{-0.0000784t} - 1.04e^{-0.0199t} + 0.345e^{-0.0199t} \quad (30)$$

Tri-composition:

$$C_T = 0.6451e^{-0.00001t} - 0.6452e^{-0.01672t} - 0.0001e^{-0.7002t} \quad (31)$$

For open system:

Laetrile:

$$C = 609.689e^{-6.603t} + 2.7269e^{-4.944t} - 611.732e^{-0.671t} - 0.684e^{-0.02996t} \quad (32)$$

Table 2. Volumes and Distribution Coefficients in Three Marker Compounds in Open and Closed Dissolution Environments

Dissolution Environment	V <sub>0</sub> (mL)	V <sub>1</sub> (mL)	V <sub>2</sub> (mL)	ρ <sub>2</sub>		
				Laetrile	Paeoniflorin	Ferulic acid
Closed System	66.50	71.00	140.0	0.918	1.322	0.447
Open System	210.07	19.76	90.0	1.326	1.668	1.903

V<sub>0</sub>: volume of cell chamber; V<sub>1</sub>: volume of apoplast chamber; V<sub>2</sub>: volume of solution chamber; ρ<sub>2</sub>: distribution coefficient.

Table 3. Results of Curve Fitting and Related Kinetic Parameters for Dissolution of Three Marker Compounds in Open and Closed Systems

Closed System (Reflux Method)									
	k (min <sup>-1</sup> )	k <sub>1</sub> ' (min <sup>-1</sup> )	k <sub>2</sub> ' (min <sup>-1</sup> )	ρ <sub>1</sub>	AUC (mAu.min)	t <sub>max</sub> (min)	C <sub>max</sub> (%)	T <sub>p</sub> (%)	D <sub>p</sub> (%)
Laetrile	6.40 × 10 <sup>-5</sup>	4.91 × 10 <sup>-4</sup>	0.071	1.393	1560	134.6	46.85	46.85	3.282
Paeoniflorin	1.79 × 10 <sup>-5</sup>	4.53 × 10 <sup>-4</sup>	0.068	5.633	5600	161.2	19.12	19.12	0.475
Ferulic Acid	1.13 × 10 <sup>-5</sup>	1.60 × 10 <sup>-4</sup>	0.081	1.065	8850	279.4	67.83	67.83	1.780
	M <sub>1</sub> (%)	N <sub>1</sub> (%)	L <sub>1</sub> (%)	α <sub>1</sub> (min <sup>-1</sup> )	β <sub>1</sub> (min <sup>-1</sup> )	π <sub>1</sub> (min <sup>-1</sup> )	R <sup>2</sup>	F*	p
Laetrile	0.493	-1.760	1.270	3.12 × 10 <sup>-4</sup>	0.038	0.039	0.884	134.2	< 0.01
Paeoniflorin	0.193	-0.192	1.0 × 10 <sup>-6</sup>	3.43 × 10 <sup>-5</sup>	0.045	0.076	0.761	110	< 0.01
Ferulic Acid	0.696	-1.041	0.345	7.84 × 10 <sup>-5</sup>	0.020	0.019	0.977	457.3	< 0.01
Open System (Soxhlet Method)									
	k (min <sup>-1</sup> )	k <sub>1</sub> ' (min <sup>-1</sup> )	k <sub>2</sub> ' (min <sup>-1</sup> )	ρ <sub>1</sub>	AUC (mAu.min)	t <sub>max</sub> (min)	C <sub>max</sub> (%)	T <sub>p</sub> (%)	D <sub>p</sub> (%)
Laetrile	2.996 × 10 <sup>-2</sup>	2.076	4.256	1.769	23,045.79	266.25	89.55	64.35	8.103
Paeoniflorin	6.74 × 10 <sup>-3</sup>	4.623	10.56	0.745	9464.81	257.64	58.84	24.42	1.368
Ferulic Acid	8.709 × 10 <sup>-3</sup>	3.362	76.15	0.711	8338.57	159.06	59.50	59.50	2.400
	M <sub>2</sub> (%)	N <sub>2</sub> (%)	L <sub>2</sub> (%)	S <sub>2</sub> (%)	α <sub>2</sub> (min <sup>-1</sup> )	β <sub>2</sub> (min <sup>-1</sup> )	π <sub>2</sub> (min <sup>-1</sup> )	R <sup>2</sup>	p
Laetrile	609.7	2.727	-611.7	-0.684	6.603	4.944	0.671	0.988	< 0.01
Paeoniflorin	-0.395	10.70	4.94	-15.25	17.43	5.446	1.318	0.986	< 0.01
Ferulic Acid	-1.593 × 10 <sup>-5</sup>	1.170	0.077	2.011	145.64	2.145	2.011	0.989	< 0.01

Herbal medicine characteristic parameters are defined as k, k<sub>1</sub>', k<sub>2</sub>', ρ<sub>2</sub>, and ρ<sub>1</sub>, where k is the elimination rate constant of the ingredient in the solution bulk and p is the distribution coefficients. Dissolution kinetic parameters are defined by M<sub>1</sub>, N<sub>1</sub>, L<sub>1</sub>, α<sub>1</sub>, β<sub>1</sub>, and π<sub>1</sub> in reflux systems and by M<sub>2</sub>, N<sub>2</sub>, L<sub>2</sub>, S<sub>2</sub>, α<sub>2</sub>, β<sub>2</sub>, and π<sub>2</sub> in soxhlet systems. AUC, area under the curve; T<sub>p</sub>, transfer power; D<sub>p</sub>, decomposition power; \*, F<sub>0.01(110)</sub> = 10.0, F<sub>0.05(110)</sub> = 4.9

Table 4. Kinetic Parameters for a Multi-Compound Formulation (BYHWD) in Open and Closed Dissolution Environments

	α <sub>1</sub> (min <sup>-1</sup> )	β <sub>1</sub> (min <sup>-1</sup> )	π <sub>1</sub> (min <sup>-1</sup> )	M <sub>1</sub> (%)	N <sub>1</sub> (%)	L <sub>1</sub> (%)	k (min <sup>-1</sup> )	R <sup>2</sup>	AUC (mAu.min)
Closed System (Reflux Method)	1.000 × 10 <sup>-5</sup>	0.0167	0.7002	0.6451	-0.6452	0.0001	1.551 × 10 <sup>-5</sup>	0.8844	64,475
	k <sub>1</sub> ' (min <sup>-1</sup> )	k <sub>2</sub> ' (min <sup>-1</sup> )	ρ <sub>1</sub>	ρ <sub>2</sub>	t <sub>max</sub> (min)	C <sub>max</sub> (%)	T <sub>p</sub> (%)	D <sub>p</sub> (%)	p
	3.268 × 10 <sup>-3</sup>	1.097	0.725	0.6481	442.0	64.19	64.185	0.382	< 0.01
	α <sub>2</sub> (min <sup>-1</sup> )	β <sub>2</sub> (min <sup>-1</sup> )	π <sub>2</sub> (min <sup>-1</sup> )	M <sub>2</sub> (%)	N <sub>2</sub> (%)	L <sub>2</sub> (%)	S <sub>2</sub> (%)	k <sub>4</sub> (min <sup>-1</sup> )	AUC (mAu.min)
Open System (Soxhlet Method)	10.36	4.763	1.362	12.65	-1.628	9.369	-20.393	6.915 × 10 <sup>-4</sup>	593,891.35
	k <sub>1</sub> ' (min <sup>-1</sup> )	k <sub>2</sub> ' (min <sup>-1</sup> )	ρ <sub>1</sub>	ρ <sub>2</sub>	t <sub>max</sub> (min)	C <sub>max</sub> (%)	T <sub>p</sub> (%)	D <sub>p</sub> (%)	R <sup>2</sup>
	4.152	6.320	0.739	1.632	355.56	84.19	79.08	0.575	0.988

Temperature: 100 ± 0.5 °C. Herbal medicine characteristic parameters are defined as k, k<sub>1</sub>', k<sub>2</sub>', ρ<sub>2</sub>, and ρ<sub>1</sub>, where k is the elimination rate constant of the ingredient in the solution bulk and p is the distribution coefficient. Dissolution kinetic parameters are defined by M<sub>1</sub>, N<sub>1</sub>, L<sub>1</sub>, α<sub>1</sub>, β<sub>1</sub>, and π<sub>1</sub> in reflux systems and by M<sub>2</sub>, N<sub>2</sub>, L<sub>2</sub>, S<sub>2</sub>, α<sub>2</sub>, β<sub>2</sub>, π<sub>2</sub>, and k<sub>4</sub> in soxhlet systems. AUC, area under the curve; T<sub>p</sub>, transfer power; D<sub>p</sub>, decomposition power; BYHWD, Buyanghuanwu decoction

Paeoniflirin:

$$C = -0.395e^{-17.434t} + 10.704e^{-5.446t} + 4.942e^{-1.318t} + 15.251e^{-0.00674t} \quad (33)$$

Ferulic acid:

$$C = -0.00001593e^{-145.642t} + 1.170e^{-2.145t} + 0.077e^{-2.011t} + 2.011e^{-0.008709t} \quad (34)$$

Tri-composition:

$$C_T = 12.652e^{-10.360t} - 1.628e^{-4.763t} + 9.369e^{-1.362t} - 20.393e^{-0.0006915t} \quad (35)$$

Therefore, the relationship between the concentration and dissolution time of three marker compounds is obtained. Based on above expressions, plotted dissolution kinetics curve of BYHWD are shown in Figure 4. Figure 4a and 4b are the dissolution curves for closed system; Figure 4c and 4d are the dissolution curves for open system.

### Dissolution Test Results

We used a dissolution model to identify the key dissolution indices and efficiency using the soxhlet and

reflux methods. The curves in Figure 4 indicate that the weight of compounds increased with dissolution time in the initial stage of the dissolution of herbal medicine compounds, but when it reached the equilibrium state, the rate of dissolution increased gradually. The reason was that the concentrations of these compounds were dissolved in solution, which reached its saturated solubility.

From Table 4, the AUC of soxhlet method for BYHWD achieved 9.21 times more than the reflux method, 0.80 times shorter  $t_{max}$  than reflux method, and 1.31 times higher concentration ( $C_{max}$ ) than the reflux method. The  $D_p$  value in the open system was 1.505 times higher than the closed system, and the calculated  $T_p$  value for the open system was 1.23 times higher than the closed system. Compared to the closed system, the open system had higher efficacy of dissolution. Meanwhile, the analysis indicated that the real contact area was the main factor to affect the materials transfer process. When the heat crossed the interface between the medical materials and the dissolution solution, the high-temperature environment in the soxhlet method process may cause decomposition of the marker compounds.

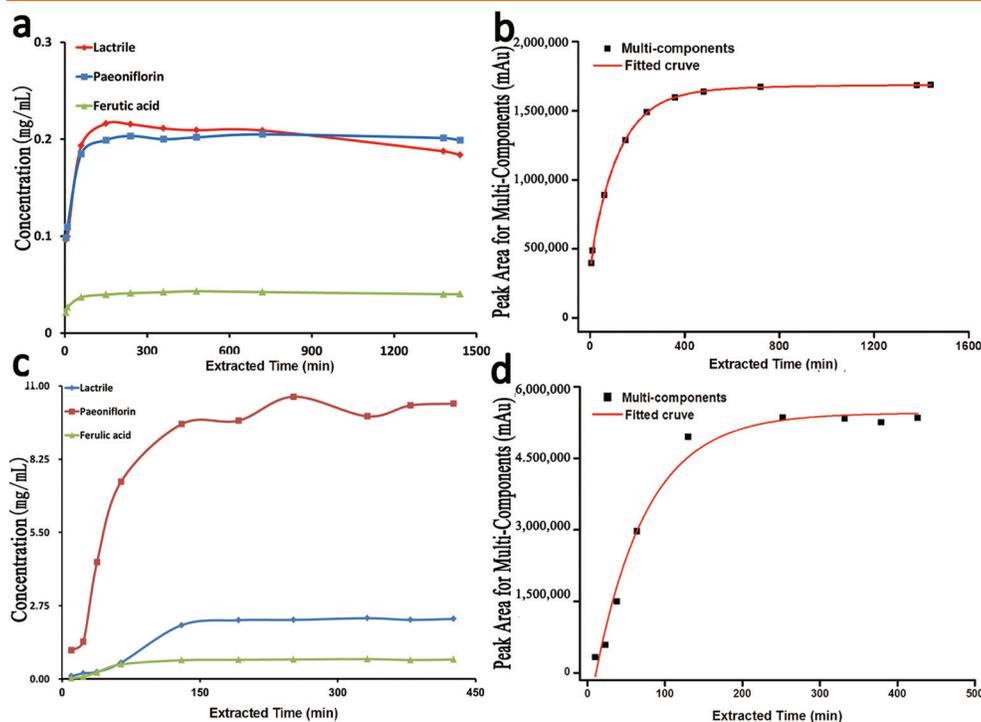


Figure 4. Dissolution concentration curves for laetrile, paeoniflorin, and ferulic acid using a closed system (a, b) and open system (c, d). Values are expressed as the means  $\pm$  SD ( $n = 6$ ).

## CONCLUSIONS

Theoretically, according to this model, the dissolution curve was related to the herbal medicine inherent parameters and dissolution system. We performed the experiment to achieve the inherent parameters of BYHWD and calculated different dissolution method parameters using our kinetic models. After that, quantitative transfer maximum efficiency parameters, including AUC,  $C_{max}$ ,  $t_{max}$ ,  $P$ , and  $D$ , were treated as the dissolution degree indicators. These indicators could help to evaluate the herbal medicine dissolution degree and efficiency.

This methodology was fit for herbal medicine formulations products, especially pulvis, decoction, and vinum. Based on previous experimental work (small scale), we could predict the dissolution trajectory and select an appropriate dissolution method for large scale production. Hence, our model might be an effective tool for investigating the dissolution process of each ingredient in herbal medicine formulations and could help optimize the conditions for dissolution of herbal medicine products.

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## CONFLICT OF INTEREST

The authors disclosed no conflicts of interest related to this article.

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