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Original article

A novel release kinetics evaluation of Chinese compound medicine: Application of the xCELLigence RTCA system to determine the release characteristics of Sedum sarmentosum compound sustained-release pellets

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ABSTRACT

Purpose: To establish a novel release kinetics evaluation method of Chinese compound medicine (Sedum Sarmentosum compound) with xCELLigence Real-Time Cell-based Assay (RTCA) system. *Methods*: Cell lines sensitive to Sedum Sarmentosum compound are screened, and cell index-time (CI-T) graphs and cell index release kinetics models are established based on the cell index (CI) monitored. The methodological studies of precision and repeatability were processed by the cell monitors system. The release profiles of the sustained-release Sedum Sarmentosum compound were determined. Consequently, the sustained-release property was characterized by the kinetic parameters based on the cell-index. *Results*: The accumulative release rate based on cell index of Sedum sarmentosum compound sustained-release preparation was determined and it had a good correlation with time, fitting better with First-order model, Higuchi model and Ritger-Peppas model, and fitting best with Weibull model. It indicated that the release rate is proportional with the diffusion coefficient. *Conclusion*: The new method of cell-index release kinetics may provide a quantitative description for the release of the multi active agents from Traditional Chinese Medicines. The application of xCELLigence RTCA system for evaluating the release kinetics of Chinese compound medicine is feasible.

1. Introduction

In recent years, with the advance and innovation in modernization of Traditional Chinese Medicine (TCM), various studies have performed to achieve the sustained release and prolonged pharmacokinetics of TCM, both *in vitro* and *in vivo* (Xie et al., 2016). Currently, the method used to evaluate the release kinetics is based on the determination of one or several principal ingredients of TCM. However, due to its complicated composition, it is very difficult to obtain the accurate pharmacokinetic parameters for the TCM formulation since Chinese herbal compounds are generally composed of many ingredients that can hardly be indexed by one or several monomer compositions. Though the release kinetics of several monomer compositions could be calculated respectively, each of them are different. It is hard to estimate the release rule of whole TCM formulation. Thus, the prolonged-release preparation for TCM is difficult to design in theory (Chen et al., 2007).

Recently, novel methods and new theories are developed for the evaluation of release kinetics of TCM. For example, Zhang et al. developed a new theory of the chemomic release/dissolution kinetics of TCM, which was employed to evaluate the *in vitro*

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release characteristic of multi-component TCMs (Zhang et al., 2008a, 2008b; Chen et al., 2008a, 2008b; Li et al., 2010). In their study, the release characteristics were shown as a whole which is in line with the overall concept of Chinese medicine. Although this research suggests the potential direction for the kinetic study of TCM, the conclusion is still weak, and more studies are required for the thoroughly evaluation of sustained-release preparation of TCM.

The sustained-release pellets of Sedum sarmentosum compound for hepatitis treatment were composed of Sedi Herba (*Sedum sarmentosum* Bunge), Bupleuri Radix (*Bupleurum chinense* DC), and Glycyrrhizae Radxi Et Rhizoma (*Glycyrrhiza uralensis* Fisch). The holistic drug release characteristics *in vitro* could not be reflected since current evaluation is based on flavonoids, saikosaponin, and glycyrrhizin. There are no integral release kinetics that could be summarized. Therefore, in this manuscript, we adopt a novel xCELLigence Real-Time Cell-based Assay (RTCA) system Yan et al., 2014 to explore the release kinetics of sustained-release formulations of TCMs. The xCELLigence RTCA system is based on continuous monitoring of cellular impedance in real-time, which produces specific time/dose-dependent cell response profiles upon treatment with biologically active compounds. The changes of cellular impedance stimulated by the compounds are defined as Cell Index (CI), which can reflect the concentrations of chemical components aggregations from TCM preparations, including known and unknown. Therefore, the CI also can reflect the holistic drug effect and dynamic release rules of sustained-release preparations of TCMs.

In this research, the LX2 cells were treated with samples at different time points, and then CI-T profiles were obtained by using xCELLigence RTCA, the cumulative release of a sustained release formulation at different time points was calculated, thus the release characteristics *in vitro* of sustained-release formulations were evaluated. Furthermore, the kinetic model *in vitro* was constructed, which is more similar to the environment *in vivo* compared with the conventional release test *in vitro*, and more convenient compared with the pharmacokinetics test *in vivo*. This method provides a new way and thinking for the pharmacokinetics study of sustained-release formulations of TCMs.

2. Materials and methods

2.1. Cell culture

The LO2, HepG2, LX2 cell lines were purchased from American Type Culture Collection (ATCC) and cultured in a humidified incubator at 37 °C with 5% CO_2 in accordance to optimal media and growth conditions specified by ATCC(Yang et al., 2016).

2.2. Screening of cell lines and sample concentrations

2.2.1. The preparation of the samples

The sustained-release pellets of Sedum sarmentosum compound were accurately weighed and then thoroughly ground into a fine powder, and transferred to a dissolution beaker containing 900 mL degassed distilled water. The paddle dissolution method was employed with rotation speed and solution temperature of 100 r min^{-1} and 37 °C for 12 h respectively (Yang et al., 2006). The solution from the dissolution vessel was filtered, concentrated and dried.

2.2.2. Determination of cell index

The samples were accurately weighed respectively, dissolved into PBS, and the final concentrations were 2.0, 1.5, 1.0, 0.78, 0.39, 0.19 mg/ml. The Real-Time Cell Electronic Sensing System (RT-CES) was provided by the ACEA Biosciences, San Diego (Keese et al., 2002; Solly et al., 2004). To obtain CI-T, 5μL of medium was added to 96-well E-plates to obtain background readings followed by the addition of 145 μL of cell suspension. The E-plates containing 5000 cells were allowed to incubate at 37 °C before being placed onto the reader in the incubate or for continuous recording of impedance as reflected by cell index(CI). The cells were allowed to attach and grow for 18–24 h to reach a stable baseline before the addition of the medicine. The cells were continuously monitored to capture the long-term CI-T and the data after adding solution 2.0, 1.5, 1.0, 0.78, 0.39, 0.19 mg/ml.

2.3. Methodology study

2.3.1. Precision

9, 12, 15 mg samples were accurately weighed and dissolved into 1000 μL PBS respectively. The solution concentrations were 9, 12, 15 mg/ml and final concentrations were 0.6, 0.8, 1.0 mg/ml.

2.3.2. Repeatability

The nine parts of 12 mg sample were accurately weighed and dissolved into 1000 μL PBS. The solution concentration was 12 mg/ml and final concentration was 0.8 mg/ml.

2.3. Determination of cumulative release rates

The release rates of the sustained-release pellets of Sedum sarmentosum compound were determined using the second method detailed in Ch.P. (2015), The Fourth Part, General Principles 0931. Distilled water was used as the release medium and the temperature was set at (37 ± 0.1) °C. 10 mL sample solution was collected at 1, 2, 4, 6, 8, 10, 12, 12 h, and 10 mL distilled water was added immediately. The sample solutions were filtered and dried.

The cumulative release of sarmentosum compound sustained-release preparations at each time point was calculated with cell index values. The formula was as follows.

$$Q = (CI_i - CI_{con})/(CI_0 - CI_{con}) \times 100\%$$

Q the cumulative release rate.
CI_i cell index values of sample released at i time point.
CI_{con} cell index values without sample.
CI_0 cell index values of completely released sample.

3. Results

3.1. Screening of cell lines and sample concentration

LO2, HepG2, LX2 were exposed to the solution of 2.0, 1.5, 1.0, 0.78, 0.39, 0.19 mg/ml, and the responses were presented in Figs. 1, 2, and 3, respectively.

In the experiment, our first objective was to choose a proper cell line that is sensitive to the study. LO2, HepG2, and LX2 cell lines were tested and exposed to various concentrations of samples. Then, the CI-T profiles were obtained, respectively (Figs. 1, 2, 3). Fig. 1 showed that CI of LO2 cells varied with agent concentration in the initial stage, the higher concentration, the greater cell index. However, the CI decreased with the concentration increasing after drug action for 6 h, and 20 h later, the cell index-time trend began to reverse. It indicated that the effect of drug on LO_2 cell lines was unstable, suggesting that it may not be used as the model cell line; HepG2 cells had certain concentration-dependent effects within certain limits, but the distinction was not clear, and multiple curves overlapped, indicating unobvious concentration-dependent (Fig. 2); The concentration-dependent effect of LX2 cells was the most obvious, and the curves were clearly distinguished in the different concentrations of the drug. However, at the

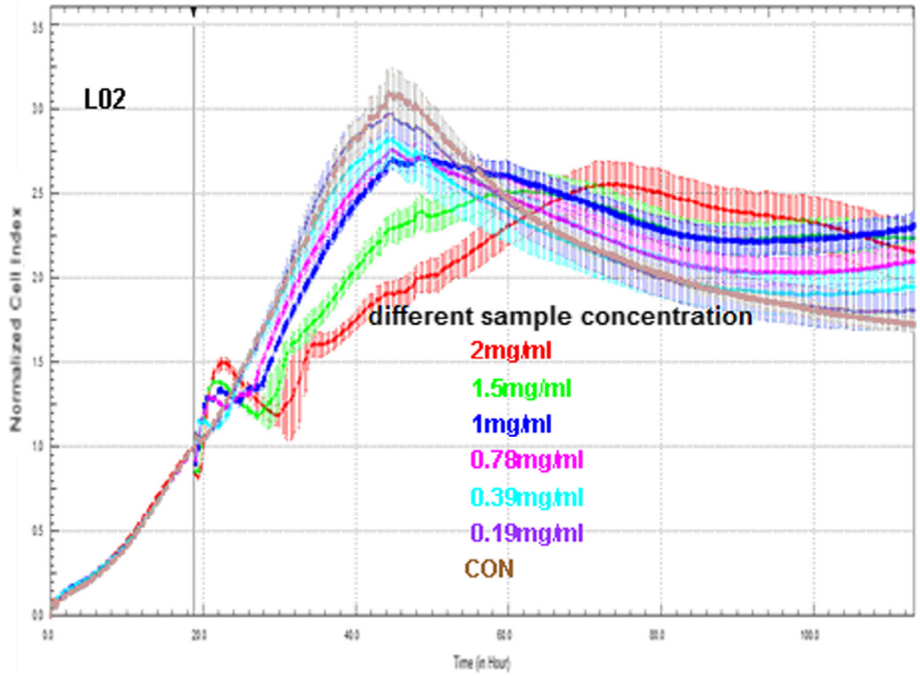

Fig. 1. Effect of different sample concentration on real-time cell analyzer curves generated with LO2 cells.2 mg/ml (red line), 1.5 mg/ml (green line),1.0 mg/ml (blue line), 0.78 mg/ml (pink line),0.39 mg/ml (turquoise line), 0.19 mg/ml (purple line), control (brown line).

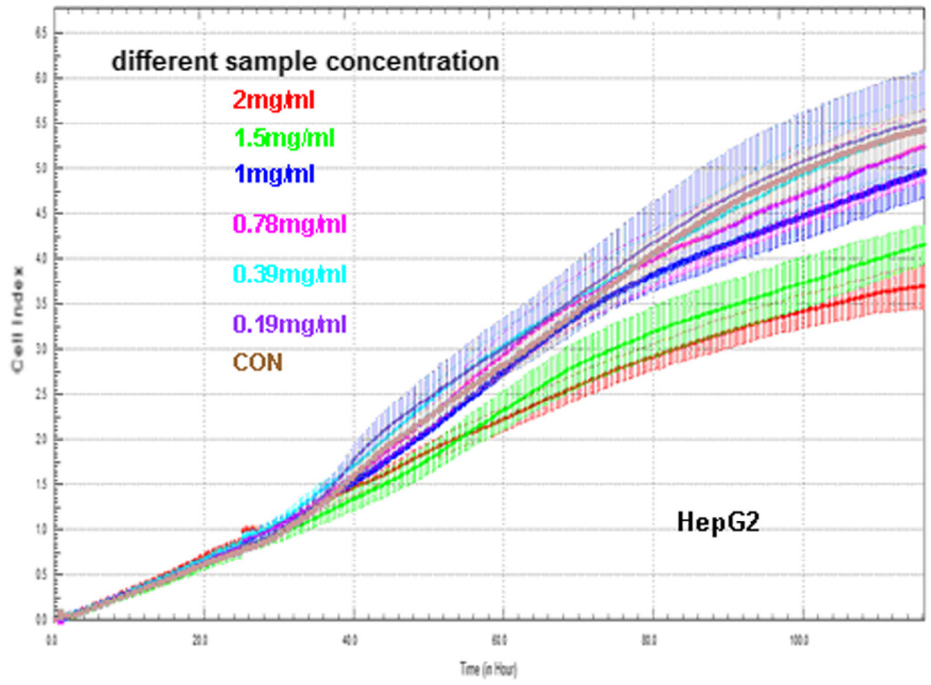

Fig. 2. Effect of different sample concentration on real-time cell analyzer curves generated with HepG2 cells. 2 mg/ml (red line), 1.5 mg/ml (green line), 1.0 mg/ml (blue line), 0.78 mg/ml (pink line),0.39 mg/ml (turquoise line), 0.19 mg/ml (purple line), control (brown line).

concentration of 2 mg mL^{-1}, the cell index tended to zero after a certain time, indicating potential cell death due to its high concentration (Fig. 3). In summary, LX2 cells were selected as the model cell line for studying the release property of sustained-release pellets of Sedum sarmentosum compound, and the optimum concentration was in the range of 0.19–1.5 mg ml^{-1}.

3.2. Methodology study

3.2.1. Precision

The relative standard deviation (RSD) of cell index values generated by the effect of 0.6 mg/ml, 0.8 mg/ml, 1.0 mg/ml solution with LX2 cells were summarized in Tables 1–3.

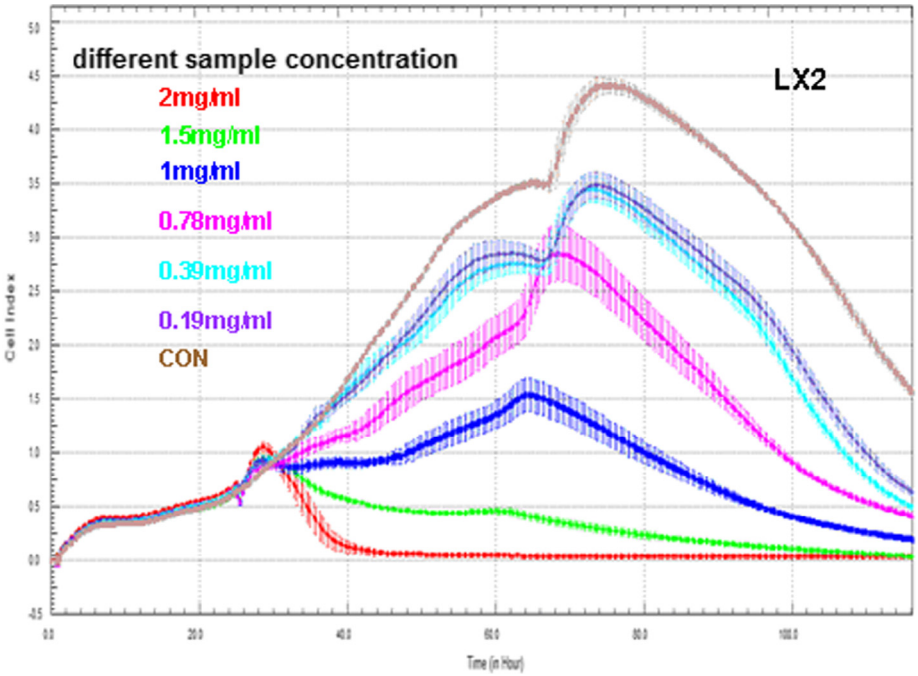

Fig. 3. Effect of different sample concentration on real-time cell analyzer curves generated with LX2 cells. 2 mg/ml (red line), 1.5 mg/ml (green line), 1.0 mg/ml (blue line), 0.78 mg/ml (pink line),0.39 mg/ml (turquoise line), 0.19 mg/ml (purple line), control (brown line).

Table 1
Precision results of 0.6 mg/mL solution.

Time (h)	1.5–10.7	11–26.3	26.3–61.2	61.4–64.2	64.4–67.7	67.9–114.9
RSD (%)	0.3–3	0–0.3	0.3–3	3–4	0.3–3	3–9

Since the required RSD was no more than 3%, the cell index values of 61.4–64.2 h and 67.9–114.9 h were not desirable.

Table 2
Precision results of 0.8 mg/mL solution.

Time (h)	1.5–10.2	10.5–24	24.5–25.3	25.3–59.4	59.7–64.2	64.4–67.4	67.7–114.9
RSD (%)	0.3–3	0–0.3	0.3–3	0–0.3	3–4	0.3–3	3–7

Since the required RSD was no more than 3%, the cell index values of 59.7–64.2 h and 67.7–114.9 h were not desirable.

Table 3
Precision results of 1.0 mg/mL solution.

Time (h)	2–17	17.2–24	24.5–25.3	25.3–28.3	28.5–55.7	55.9–114.9
RSD (%)	0.3–3	0–0.3	0.3–3	0–0.3	0.3–3	3–13

Since the required RSD was no more than 3%, the cell index values of 55.9–114.9 h were not desirable.

3.2.2. Repeatability

The RSD of cell index values (Fig. 4.) generated by the effect of nine samples with LX2 cells were expressed in Table 4.

Based on the data of methodology study, the range of 0–45.0 h was selected as the optimum cell index to calculate the accumulative rate. Furthermore, the cells were exposed to samples after cultivated for 24 h, and 24.0–45.0 h was chosen as the optimum data range.

3.3. Determination of cumulative release rates

The CI-T (Fig. 5) showed the effect of samples released at different time point with LX2 cells. The cell index curves of sample released at 1, 2, 4, 6, 8, 10, 12 h on LX2 cells were presented by ①,②,③,④,⑤,⑥,⑦. The cell index curve of completely released sample was presented by ⑧, the curve of solution without sample was presented by CON. Samples were added in 96-well E-plates, the cells were continuously monitored to capture the long-term (72 h) CI-T (A), the cells were continuously monitored to capture the short-term (2 h) CI-T (B).

Although the trends of the cell index at different times were similar, the distinctions existed. It showed that the effect of sample released at different times with LX2 cells was different. 2 h after adding sample, a gradient cell index was presented, and the cell index value reached a lower value between 24.1–24.2 h. The cell index fell faster before 24.1 h, indicating that the cells were affected obviously by the drug. After that, the index value increased continuously after 24.2 h, indicating that the cell status was less stable. Therefore, 24.1–24.2 h could be considered as a reference to calculate the cumulative release. The results are shown in Table 5 and Fig. 6.

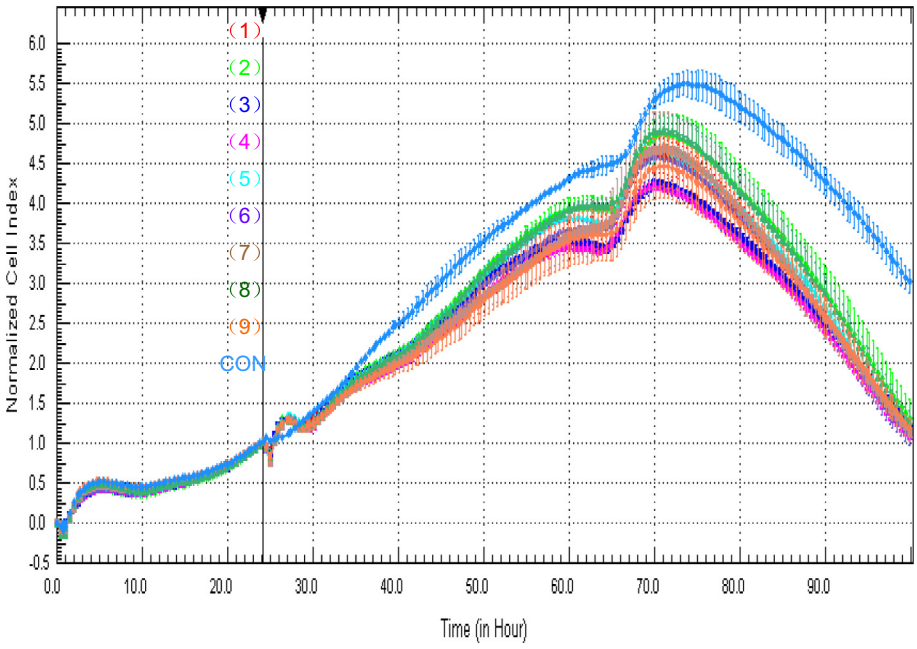

Fig. 4. Effect of nine samples on real-time cell analyzer curves generated with LX2 cells.

Table 4
Results of repeatability.

Time (h)	1.7–18.2	18.5–24.5	24.6–25.7	25.7–45.0	45.3–101.4	101.7–114.4
RSD (%)	0.3–3	0–0.3	0.3–3	0.3–3	3–11	0.3–3

Since the required RSD was no more than 3%, the cell index values of 45.3–101.4 h were not desirable.

The cumulative release rate (Q) in the first time point (1 h) was less than 30%, Q in the second time point (2–4 h) was approximately 50%, Q in the end time point (8–12 h) was more than 75% (totally release considered), up to the specification.

3.4. Fitting with release models

Accumulative release rates based on cell index were fitted with zero-order model, First-order model, Higuchi model, Weibull model and Ritger-Peppas model, respectively. The results are shown in Table 6.

The results showed that the accumulative release rate based on cell index of Sedum sarmentosum compound sustained-release preparations had a good correlation with time, fitting better with First-order model, Higuchi model and Ritger-Peppas model, and fitting best with Weibull model.

From the cell index release model analysis, the release rates of sustained-release pellets of Sedum sarmentosum compound fitted best with Weibull model, indicating that the release rate is proportional with the diffusion coefficient. The release rates also fitted better with Ritger-Peppas model, according to the principle of Ritger-Peppas release, release mechanism was inferred with the slope of the fitting equation: when the slope $\leqq 0.43$, the drug release mechanism is Fickian diffusion (Fickian diffusion); when $0.43 < \text{slope} < 0.89$, the drug release mechanism is non-Fickian diffusion (Anomalous transport); when the slope $\geqq 0.89$, the drug release mechanism is skeleton dissolution. Therefore, the release mechanism of the sustained-release pellets of Sedum sarmentosum compound was diffusion and matrix skeleton dissolution.

4. Discussion

The cumulative release of solid drug at different points of time is usually calculated by the index of one or a several number of components. This explicit and simple method is widely applied in the pharmaceutical research (Zhu et al., 2007; Yang et al., 2015; Zhou et al., 2014; Yang et al., 2014), but the release characteristics of multi-component TCM preparations cannot be accurately described by this way.

As the chemical composition of TCM preparations is complex, even a single Chinese medicine is composed of a lot of chemical ingredients, including known ingredients and unknown ingredients which are both active and efficient. Therefore, a new way to evaluate the release characteristics of TCM preparations in a holistic view is in need. Nevertheless, RTCA technology provides a breakthrough for the difficult, the change of cell lines is real-time monitored by this system, and the information on cell lines of drug action is provided by the high degree of consistency and repeatability dynamic monitoring (Xing et al., 2005; Halai and Cooper, 2012).

In this paper, the new method of cell-index release kinetics of TCM was employed to evaluate the *in vitro* release characteristic of multi-component TCM preparation. The sustained-release pellets of Sedum Sarmentosum compound as the subject in this research, LX2 cell lines sensitive to Sedum Sarmentosum compound were screened, and cell index-time (CI-T) graphs and cell index release kinetics models are established based on the cell index (CI) monitored. Finally, the pharmacokinetic parameters were obtained. The results showed that it was possible to employ an evaluation system incorporating the basic characteristic of the holism of TCM based on cell-index of TCM herbals and complex TCM prescriptions. This new method quantifies TCMs in terms of new scientific connotations cell-based, label-free technologies preclude the need for cellular labeling or over-expression of reporter proteins. Furthermore, the RTCA technology with noninvasive measurement and allowing the performance of kinetic measurement has already shown great potential in many applications, such as investigations of cell adherence, cell spreading, cell shape

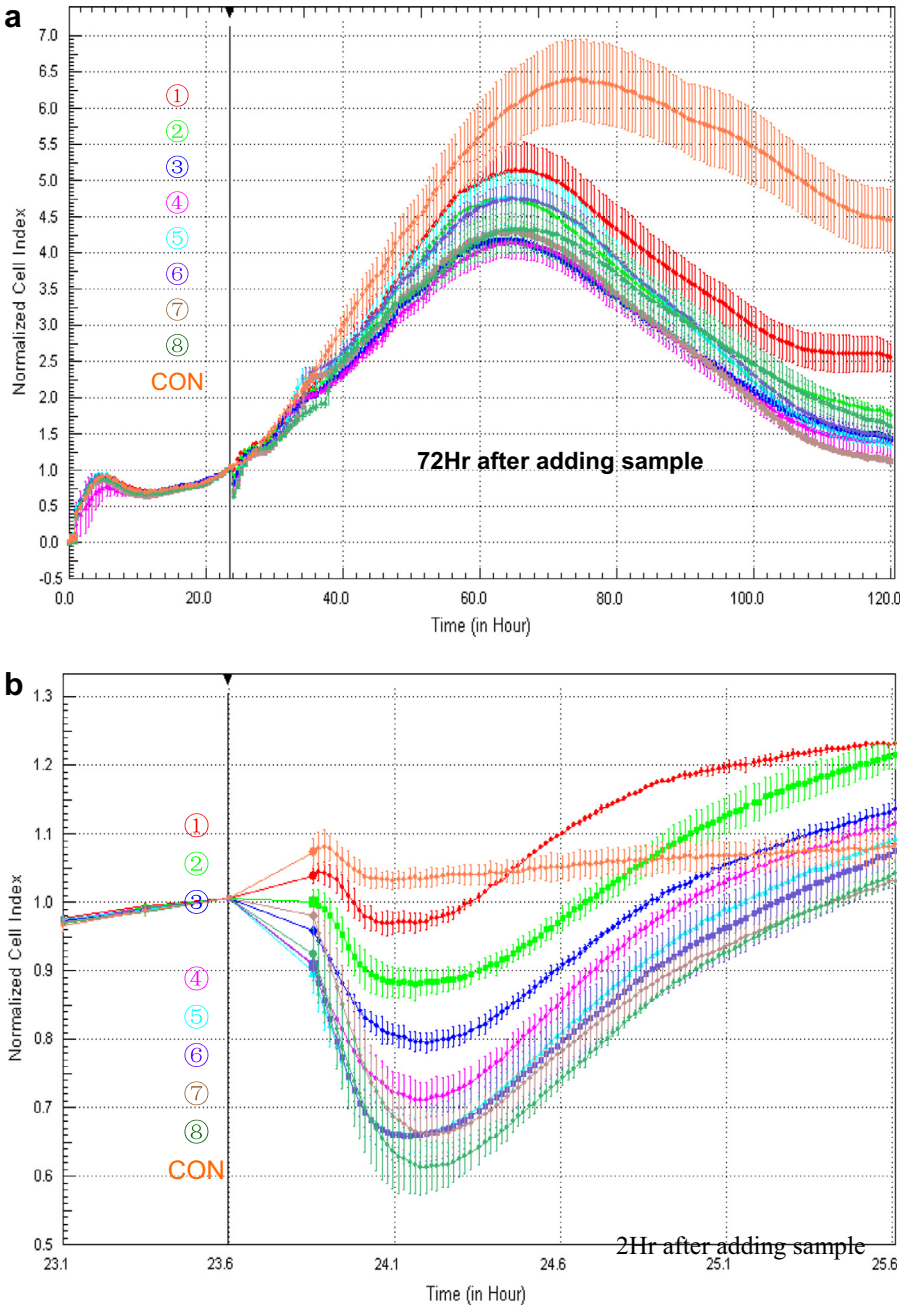

Fig. 5. CI-T generated by the first sarmentosum compound sustained-release preparations.

Table 5
Range of 24.10–24.20 h CI index of solution in different release time point and cumulative release (Q).

t(h)	T(h)								
	24.10	24.12	24.13	24.15	24.17	24.18	24.20	Average	Q (%)
1	0.9615	0.9613	0.933	0.9635	0.9671	0.9672	0.9693	0.9647	15.59
2	0.8769	0.8763	0.8774	0.8789	0.8803	0.8795	0.8809	0.8786	36.35
4	0.7954	0.7924	0.7936	0.7918	0.793	0.7946	0.7963	0.7939	56.78
6	0.762	0.7697	0.769	0.7695	0.7618	0.764	0.7644	0.7615	63.55
8	0.679	0.6787	0.6794	0.6743	0.6745	0.6761	0.6787	0.6772	84.89
10	0.6574	0.6584	0.658	0.6607	0.6617	0.6631	0.6658	0.6607	88.87
12	0.6681	0.6643	0.6619	0.6611	0.6607	0.6606	0.6617	0.6626	88.41
total	0.6186	0.6165	0.6129	0.6124	0.6131	0.6136	0.6149	0.6146	–
con	1.0276	1.0294	1.0263	1.0291	1.0299	1.0308	1.0326	1.0294	–

t: time point of sampling; *T*: time point of cell index detection; *Average*: mean value of cell index between 24.10 h and 24.20 h; *Q*: the cumulative release rate.

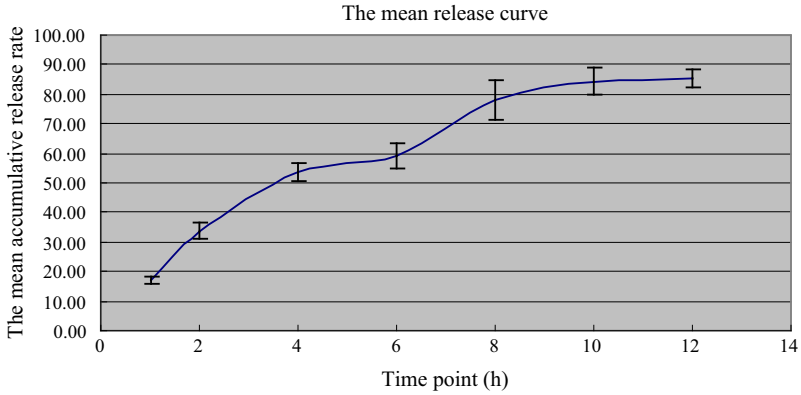

Fig. 6. The mean release curve of the first Sedum sarmentosum compound sustained-release preparations.

Table 6
Fitting results of the release rates.

Releasing model	Equation	r
Zero-order model	Q = 0.0614t + 0.2104	0.9197
First-order model	ln(1-Q) = −0.1658t-0.0598	0.9706
Higuchi model	$Q = 0.2849t^{1/2} - 0.0767$	0.9736
Weibull model	Ln{ln[1/(1−Q)]} = 0.9445lnt − 1.6291	0.9863
Ritger-Peppas model	LnQ = 0.6443Lnt − 1.6438	0.9687

change, cell migration, cell growth inhibition, and cytoprotection, and for safety and efficacy experiments (Halai and Cooper, 2012; Atienzar et al., 2011; Fu et al., 2011). Herein, our objective was to show the utility of RTCA to evaluate the release property of TCM preparations and the reproducibility of the assay in different experiments (Zhang et al., 2014; Yan et al., 2013). Therefore, the study not only is a new idea and method to evaluate TCM sustained-release formulations, but also lay a solid foundation for the research and development of the TCM sustained-release formulations, and it has an important significance for TCM formulations to meet modern clinical needs.

5. Conclusion

The new method of cell-index release kinetics may provide a quantitative description for the release of the multi active agents from traditional Chinese medicines. The application of xCELLigence RTCA system for evaluating the release kinetics of Chinese compound medicine is feasible.

Conflicts of interest

The authors declare that they have no competing interests.

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