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A novel kinetic model to describe the ultra-fast triggered release of thermosensitive liposomal drug delivery systems

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ABSTRACT

Thermosensitive liposomes, as one of the stimuli-responsive drug delivery systems, receive growing attention, due to their ability to generate rapid and massive drug release in the heated area, and marginal release of contents in non-heated parts of the body. This typical triggered release behavior cannot be fitted adequately by most of the current mathematical kinetic models. The aim of this study was to establish the proper kinetic equation to describe the rapid release of drugs from trigger-sensitive drug delivery systems. We summarized all commonly used kinetic models mentioned in the literature and fitted the release data with these models, finding that only the Korsmeyer-Peppas and the Weibull models show acceptable fitting results. To better describe the release from thermosensitive liposomes with a size below 100 nm, we took Laplace pressure as a release-driving force and proposed a new equation that demonstrates improved fitting in liposomes ranging down to a size of 70 nm. Our new kinetic model shows desirable fitting, not only at the optimal temperature but also of releases within the whole release-temperature range, providing a useful kinetic model to describe release profiles of smaller nano-sized stimuli-responsive drug delivery systems.

1. Introduction

Liposomes are the most successful nanosized pharmaceutical drug delivery carriers. Liposomal formulation changes the pharmacokinetics and toxicity of encapsulated drugs, such as increasing circulation time or reducing side-effects, thus improving therapeutic effect [1–5]. Among liposomal formulations, thermosensitive liposomes (TSL) receive growing attention due to the fast release of content in response to changes in temperature, one of the most advanced control methods available and with profound possibilities in the clinic [6–11]. Because of this heat-controlled release feature, TSL can achieve high drug levels locally (i.e. in the tumor) thus obtaining improved tumor cell kill [7,10,12]. This rapid release feature of TSL occur at a temperature range at which the liposomal membrane is going through a phase transition, which causes openings in the membrane to release contents [6]. During the phase transition, manipulating the temperatures can alter the density of gaps in the liposomal membrane, thus controlling the release; which will reach a maximal rate at T_{max} (Fig. 1).

At phase transition TSL exhibit a release pattern containing an initial ultra-fast part and a follow-up slow sustained release part, of which the initial part commonly shows massive release of payload within a matter of seconds or minutes after being triggered by hyperthermia, resulting in a release profile similar to a"Γ" shape curve (Fig. 2).

To better understand drug release profiles and predict in vivo performance, mathematical modeling of drug release can be very helpful. There have been a number of mathematical models to depict drug release kinetics of different formulations (Table 1) [13–15]. Among these kinetic equations, zero-order, first-order and Higuchi models are the most commonly used to describe sustained-controlled release formulations [16]. It is generally believed that drug release from conventional liposomes follow first-order kinetics [17,18]. We studied the release behavior of different thermosensitive liposomal formulations, finding that within the phase transition temperature range, the fastest release was obtained when temperature reaches the T_{max}. Before and after the T_{max}, first-order or Higuchi equation could be used to fit the release of content [6]. However, these equations poorly fit drug release at T_{max}. The ultra-fast release at the targeted sites, i.e. in the tumor, favors target tissues to take up drug rapidly before wash-out occurs. Therefore, studying the ultra-fast release at T_{max} of TSL and its mathematical kinetic model is of important clinical significance. Proper fitting of release kinetics is essential for modeling of drug release and drug accumulation in tumors and will benefit in silico simulation of drug

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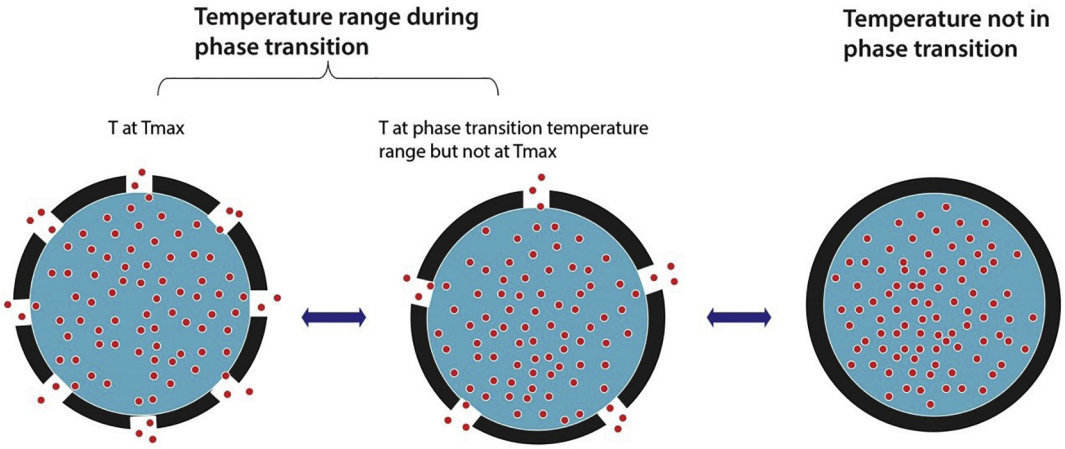

Fig. 1. Illustration of the release of thermosensitive liposomes (TSL) at different temperatures. When the temperature does not reach phase transition, TSL do not show release; while temperature reaches the phase transition range, TSL generate some release, increasing to massive and rapid release when the maximum release temperature (T_{max}) is reached.

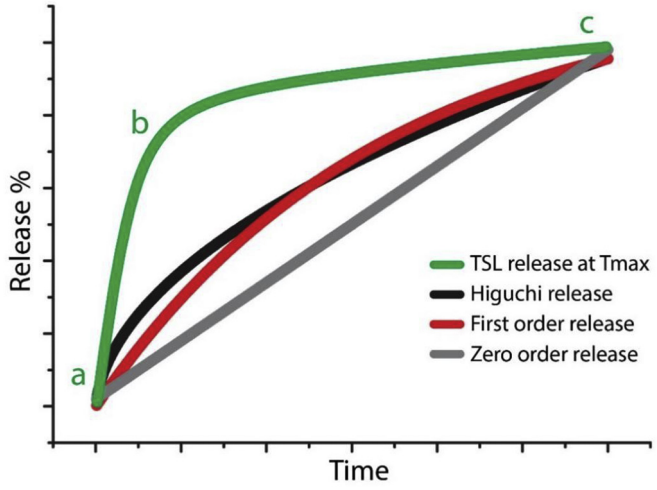

Fig. 2. Depiction of the shape of release curves of zero-order, first-order, Higuchi, compared to thermosensitive liposomes release curve at T_{max}, which shows an ultra-fast release initially (a-b) followed by a relatively slow release (b-c), thus forming a "Γ" shape-like curve.

Table 1
Commonly used kinetic models in pharmaceutical science research.

Release kinetic model	Mathematical equation
Zero order	$Q = Q_0 + k * t$
First order	$Q / Q_0 = 1 - e^{(-k * t)}$
Higuchi	$Q = k * t^{1/2}$
Hixson-Crowell	$Q^{1/3} - Q_0^{1/3} = k * t$
Korsmeyer-Peppas	$Q / Q_0 = k * t^n$
Weibull	$Q / Q_0 = 1\text{-}e^{(-b * t\hat{}a)}$
Baker-Lonsdale	$(1 - (1- Q / Q_0)^{2/3}) * Q / Q_0 = 2/3 * k * t$
Hopfenberg	$Q / Q_\infty = 1 - (1 - k * t)^3$
Gompertz	$Q / Q_0 = e\hat{}(\alpha * e^{(\beta * logt)})$

Q represents the amount of drug released at time t; Q_0 is the initial amount of drug; k is the release constant.

delivery using heat-triggered nano-carriers. To describe the entire drug release profile of thermosensitive liposomes at T_{max}, in this study we investigated the maximal release curves of thermosensitive liposomes with different compositions and sizes, and aimed to search and establish a suitable kinetic equation that describes the heat-triggered fast release from nano-sized drug delivery systems.

2. Methods and materials

2.1. Chemicals and agents

1,2-dipalmitoyl-sn-glycero-3-phosphcholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-PEG_{2000} (DSPE-PEG) were provided by Lipoid (Ludwigshafen, Germany). Fetal calf serum (FCS) was purchased from Sigma Aldrich. Purified carboxyfluorescein (CF) was kindly provided by Dr. Lars Lindner and colleagues. PD-10 columns were obtained from GE Healthcare (UK). Other chemicals were purchased from Sigma Aldrich unless otherwise specified.

2.2. Preparation of liposomes

Thermosensitive liposomes were composed of DPPC/DSPC/DSPE-PEG in a molar ratio of x/(100-x)/5 (x = 100, 80,60,40,20,0, namely TSL 100, TSL 80, TSL 60, TSL 40, TSL 20 and TSL 0) by using the thin lipid film hydration method, followed by heated extrusion[6]. Briefly, 100 μmol of lipids was dissolved in methanol/chloroform (1/9 *v*/v) mixed solvent which was then evaporated at 40 °C, followed by nitrogen flush for 30 min to remove residual solvent. The resulting dried lipid film was hydrated with CF (100 mM, pH 7.4) solutions at 60 °C. Unilamellar vesicles with different diameter were obtained by extrusion through Nuclepore® (Whatman Inc., USA) filters with pore size of 200 nm, 100 nm or 50 nm on a Thermobarrel extruder at 65 °C (Northern Lipids, Canada). Unencapsulated CF was removed with a PD-10 column. Similarly, cholesterol containing TSL prepared by using 20 mol% cholesterol, 80 mol% DSPC and 5 mol% DSPE-PEG; Idarubicin-TSL was formulated with 60 mol% DPPC, 35 mol% DSPC and 5 mol% DSPE-PEG according to our previously reported method [19]. Diameter (*Z*-average) and polydispersity index (PDI) were measured by using Zetasizer Nano-ZS (Malvern Instruments Ltd., UK).

2.3. CF-loaded TSL time- and temperature-dependent release

Twenty μl of 1 mM (lipid) CF-TSL suspension was added to 2 ml 100% FCS in a quartz cuvette at a series of determined temperature for 10 min. Real-time release of CF was detected with a water bath combined spectrofluorimetry (Ex. 493 nm/Em. 517 nm, Ex. slit 5 nm/Em. slit 5 nm) (Hitachi F-4500 Fluorescence Spectrophotometer, Japan). The average fluorescence intensity of the initial 5 s was recorded as I_0 of CF-TSL release, while fluorescence was measured as I_t at 10 min. After 10 min, detergent (10% Triton X-100) was used to disrupt all liposomes to measure maximal CF fluorescence, which was recorded as I_{max}.

Release (%) = $(I_t - I_0) / (I_{max} - I_0) \times 100$ [6].

2.4. Fitting by kinetic equations

The maximal release percentages at phase transition were fitted with the kinetic equations shown in Table 1 by using Origin (7.5 version and 2019 version). The reduced chi-square statistic (Chi [2]/DOF), coefficient of determination (R [2]) and Bayesian Information Criterion (BIC) were recorded, and the release equation with best goodness-of-fit was evaluated based on the values of Chi [2]/DOF and BIC shown by Origin results.

3. Results

3.1. Determination of T_{max} for each TSL formulation through temperature-dependent release

Thermosensitive liposomes with different compositions prepared at different defined sizes (70, 120 and 170 nm) and desired PDI (below 0.1) were used in this study (Supplementary Table 1). Temperature-dependent release assay was performed to reveal the maximal CF release temperature for each TSL formulation. Results show the maximal release temperatures at 40 and 41 ˚C for TSL 100, 42 and 43 ˚C for TSL 80, 44 ˚C for TSL 60, 47 ˚C for TSL 40, 49 and 50 ˚C for TSL 20 and 53 ˚C for TSL 0 (Fig. 3). Dramatically decreased release was observed in TSL with larger diameter, which is consistent with our previous results [6] and others [20,21].

3.2. Release fitting by various commonly used kinetic models

The release data at T_{max} of each formulation of TSL were fitted through these commonly used release kinetic equations (Table 1). Most equations either showed poor determination coefficients or hardly fitted the data. Here we evaluated the non-linear fitting mainly based on reduced chi-square statistic value and Bayesian Information Criterion. Besides, coefficient of determination was also demonstrated as a complementary data for evaluation. It is found that only Korsmeyer-Peppas and Weibull models can describe the release profiles at T_{max} (Fig. 4). Release from liposomes, especially with a size of 170 and 120 nm both Korsmeyer-Peppas and Weibull models demonstrate good fitting (Fig. 4A, D). However, the fitting curves from Korsmeyer-Peppas model were not consistent with the experimental data when we looked at thermosensitive liposomes of 70 nm by showing an significant increase Chi [2]/DOF with an average value over 30 (Fig. 4B) and declined R^2 (Fig. 4C), indicating a poor goodness of fit using the Korsmeyer-Peppas equation on release from smaller sized liposomes. By comparison, the Weibull model demonstrates improved fitting in liposomes at a size of 70 nm by showing Chi [2]/DOF and R^2 values of 6.8 and 0.92 on average, respectively (Fig. 4E, F and Supplementary Table 2). A better fitting selection was also observed based on Bayesian Information Criterion in the Weibull model by showing a smaller BIC value in liposomes with a size of 70 nm compared to the Korsmeyer-Peppas model (Supplementary Table 3).

3.3. Establishment of a new release model

Although using the Weibull equation manifests improved fitting effect compared to the Korsmeyer-Peppas model, with the liposomal size decrease from 170 nm to 70 nm the R^2 values decreased as well, accompanying with increased Chi2/DOF values (Supplementary Table 2). To better fit the release data at T_{max} of TSL with a small size (e.g. below 100 nm), we looked for a more suitable model. We calculated the reciprocal of accumulative release (y) and observed that it decreases dramatically with time (t) during the initial period, followed by a slow decline, which forms a typical inverse proportional function curve (Supplementary Fig. 1), suggesting a inversely proportional relation between time and release at T_{max}:

$$\frac{1}{y} \propto \frac{1}{t} + C$$

where the empirical parameter C is added here to avoid $1/y = 0$ when t goes to infinity.

Based on above expression, we can establish it into an equation as:

$$\frac{1}{y} = B * \left(\frac{1}{t} + C\right) \quad (1)$$

which can be written as:

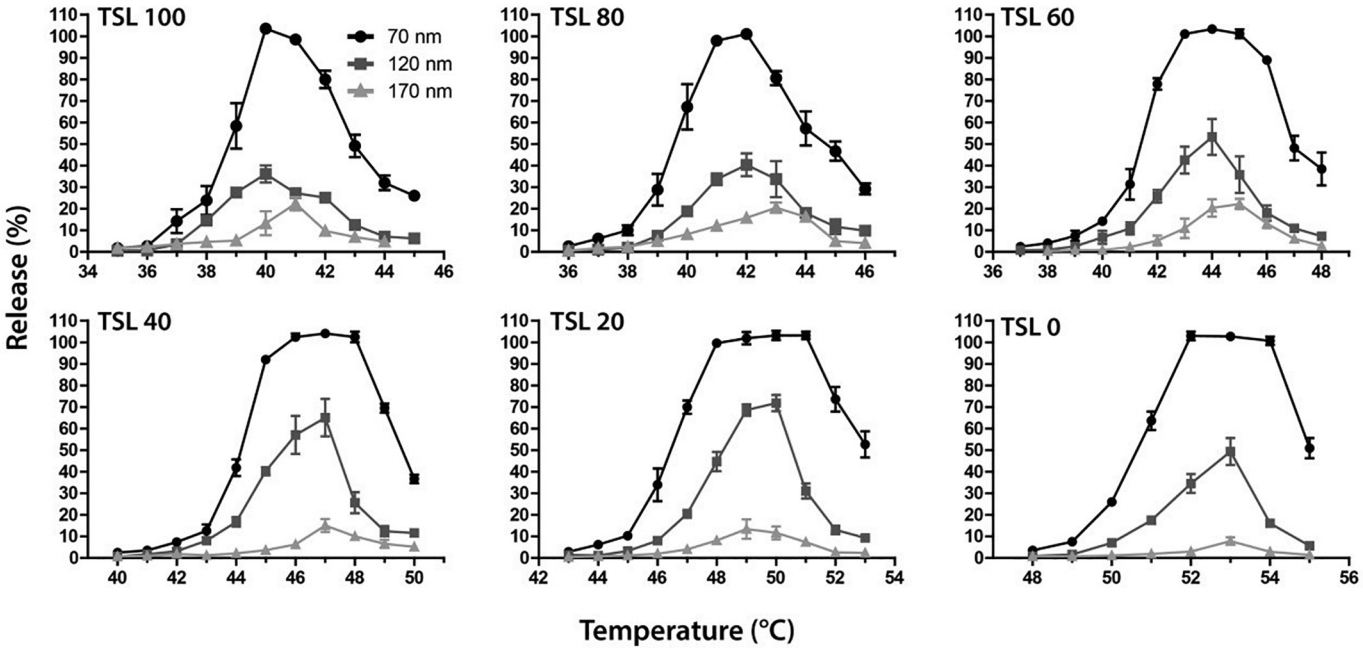

Fig. 3. The maximum release temperature of each TSL formulation is determined by applying temperature-dependent release study in FCS. Mean ± SD are shown of 3 independent experiments.

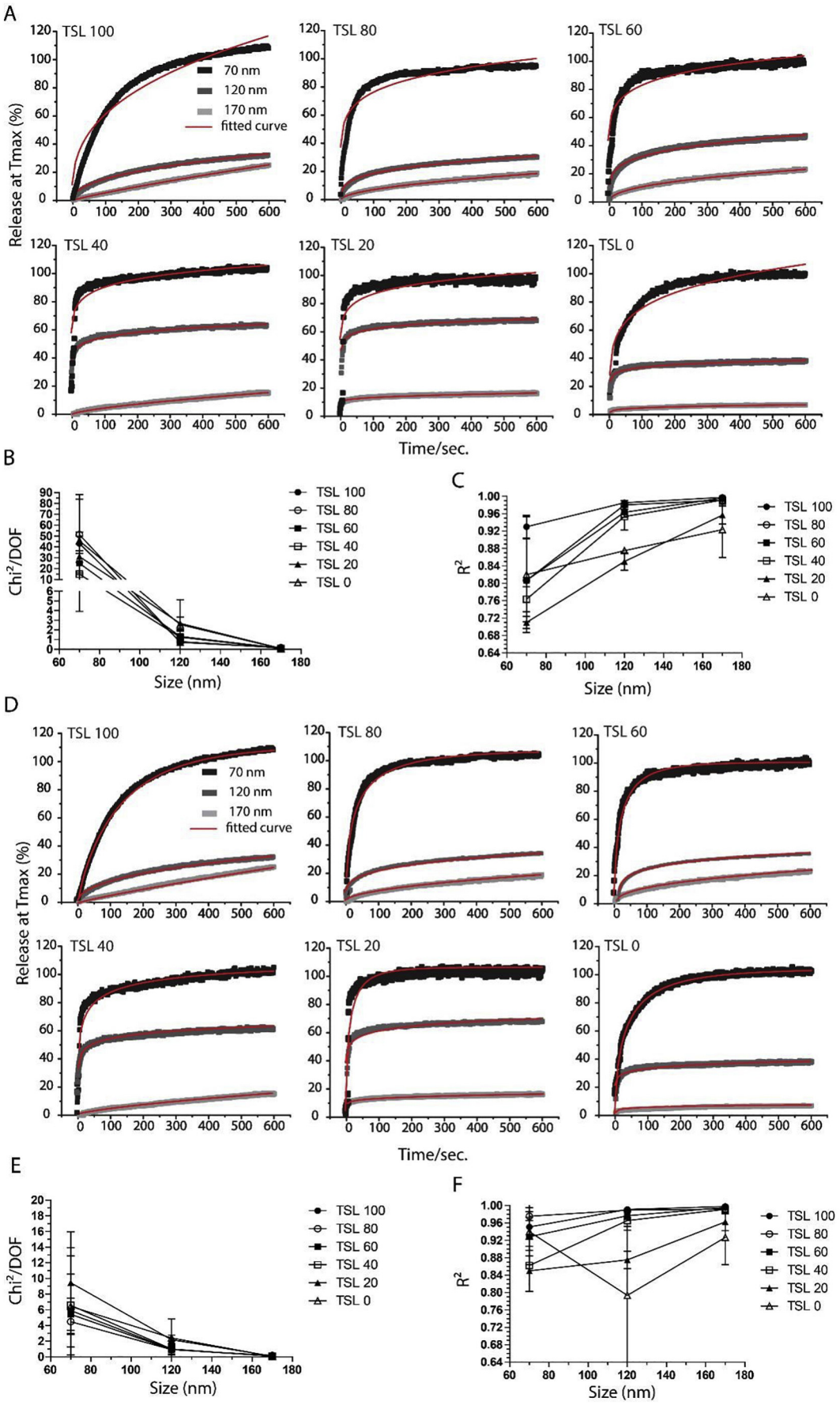

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Fig. 4. Korsmeyer-Peppas (A) and Weibull (D) models are used to fit T_{max} release from thermosensitive liposomes with different sizes, respectively. The Chi2/DOF (B, E) and R^2 (C, F) indicate the low goodness of fit when using the Korsmeyer-Peppas on TSL at 70 nm compared to the Weibull model. Mean ± SD are shown of 3 independent experiments.

$$y = \frac{\frac{1}{B} * t}{1 + C * t} \quad (2)$$

where B is an empirical parameter.

The eq. (2) can be converted to a simple expression and thus we proposed a new empirical release-equation as:

$$\frac{Q}{Q_0} = y = \frac{k * t}{1 + a * t} \quad (3)$$

where k and a are empirical parameters.

3.4. Release fitting by new kinetic model

Applying our proposed eq. (3) to fit the release at T_{max} of six different TSL formulations with 3 different sizes, we obtained the results as shown in Fig. 6A. Comparable fitting effects were observed in thermosensitive liposomes with diameters of 120 and 170 nm with the Korsmeyer-Peppas and Weibull kinetic models. Besides, the new model also shows improved fitting in liposomes with a diameter of 70 nm by showing decreased Chi [2]/DOF below 6 and increased R^2 to 0.95 on average (Fig. 5B, C, Supplementary Table 2). Additionally, Bayesian Information Criterion reveals the goodness-of-fit comparison of release at T_{max} between the Korsmeyer-Peppas, the Weibull and the new equations (Fig. 5D, Supplementary Table 3). Similarly, in large sizes (120 and 170 nm) the fitting by the new model did not appear an improvement, showing fluctuated BIC values compared to the Korsmeyer-Peppas and the Weibull models in these six TSL formulations. While at a size of 70 nm, the new model shows the best goodness-of-fit compared to the other two models by demonstrating the lowest BIC value between TSL 100 to 0 except for TSL 40 (Fig. 5D, Supplementary Table 3), suggesting that our proposed new model may better describe the release in smaller sized liposomes.

3.5. Fitting of CF release with the new model from thermosensitive liposomes at non-T_{max}

Massive and rapid release at T_{max} is a typical release profile of thermosensitive liposomes and important to predict and model drug release and distribution. This new kinetic model demonstrates better fitting results at T_{max} than other established kinetic models. However, in the clinical setting improper or partial heating of the target area (e.g. tumor) is likely to happen. Therefore a fraction of the thermosensitive liposomes will be exposed to suboptimal temperature below, or possibly above, T_{max}. Therefore, to evaluate the applicability of this model on release at non-T_{max}, release profiles at different temperatures were fitted. Our new kinetic model shows comparable excellent fitting at non-maximum release temperatures. TSL with a diameter of 70 nm are depicted as an example here (Fig. 6). Over the whole release temperature range, and for TSL 100 to TSL 0, this new kinetic model presents desirable fitting, showing an average R^2 above 0.9 and Chi [2]/DOF with an average below 15 (Fig. 6A). As a comparison, the Weibull model was used to fit these non-T_{max} releases as well, which does not show obvious improvement on Chi [2]/DOF or R^2 (Supplementary Fig. 2). Besides, Bayesian Information Criterion analysis also indicates that the Weibull model does not give better goodness-of-fit compared to the new model when fitting the release over whole release temperature range (Fig. 6B), indicating a wide applicability of this proposed model for release description.

3.6. Fitting of release with the new model from liposomes with different composition and different drug encapsulate

To evaluate our proposed model applicability on other formulations, we prepared cholesterol containing CF-TSL based on the TSL 0 formulation. The maximal release temperature was determined by performing a temperature-dependent release study (Fig. 7A). Fitting results indicate similar goodness-of-fit between the Korsmeyer-Peppas, the Weibull and the new models by showing comparable reduced chi-square statistic values (Fig. 7C). However, at the maximal release temperature (e.g. 52 °C) the lowest BIC was observed when release was fitted with the new model (Fig. 7E).

Besides CF containing TSL, we also tested idarubicin-TSL which contain precipitated idarubicin [19]. Based on the temperature-dependent release graph (Fig. 7B), a better fitting was observed with the Weibull and the new model, showing a lower value for Chi [2]/DOF (Fig. 7D). Similarly, Bayesian Information Criterion analysis indicates the improved goodness-of-fit of release at T_{max} by using the new model, showing the lowest BIC at 42 to 44 °C in this formulation.

4. Discussion

Released drug can effectively accumulate in target areas when the thermosensitive liposomes produce maximal content release at the diseased site. This maximum release is achieved when TSL are exposed to the maximum release temperature (T_{max}). Hence, studying the release kinetics of thermosensitive liposomes at T_{max} is of importance. Allen and Cleland reported that the initial 70–80% release from conventional liposomes shows a linear relation between the log(% entrapped content in liposome) and time, thereby they proposed that the release from liposomes can be a single exponential process [18]. In our previous work on DPPC-DSPC-based thermosensitive liposomes, we reported that formulation of TSL with 100 to 40% DPPC rather follow the Higuchi release model, and TSL 20 and 0 follow better the first-order release based on release at non-maximum release temperature [6]. However, we observed that this specific "Γ" shape-like release profile at T_{max} of thermosensitive liposomes is poorly fitted by zero-order, Higuchi or first-order models, which is generally assumed to describe liposome release kinetics [17,18,22] (Supplementary Table 2).

In this study, we based on the reduced chi-square statistic value and applied Bayesian Information Criterion, together with the coefficient of determination value to evaluate the fitting effect. We observed that thermosensitive liposomes with relatively large sizes of 120 and 170 nm, fitting with the Korsmeyer-Peppas, the Weibull and the new model all exhibit high coefficient of determination and low reduced chi-square statistic value than the other models listed in Table 1. However, the release from TSL with a size of 70 nm, our new model shows an improved fitting as indicated by the decreased Chi [2]/DOF (average 5.9 ± 1.0) and increased R^2 (average 0.95 ± 0.01) compared to the Korsmeyer-Peppas (Chi [2]/DOF: 35.1 ± 10.0; R [2]: 0.82 ± 0.01) and the Weibull (Chi [2]/DOF: 6.8 ± 0.1; R [2]: 0.92 ± 0.02) models (Supplementary Table 2), together with the BIC values, showing an improved goodness-of-fit when the new model is applied for fitting the release at T_{max} from small sized thermosensitive liposomes.

To evaluate the suitability of our proposed model, liposomes with a different drug encapsulated (e.g. forming a precipitate inside the liposome) or different composition (e.g. apart from lipid also cholesterol was added) were tested as well. The same results was observed: the release from these formulations at maximum temperature show the best goodness-of fit when using the new model compared to the Korsmeyer-Peppas and the Weibull models (Fig. 7). Thus regardless of the

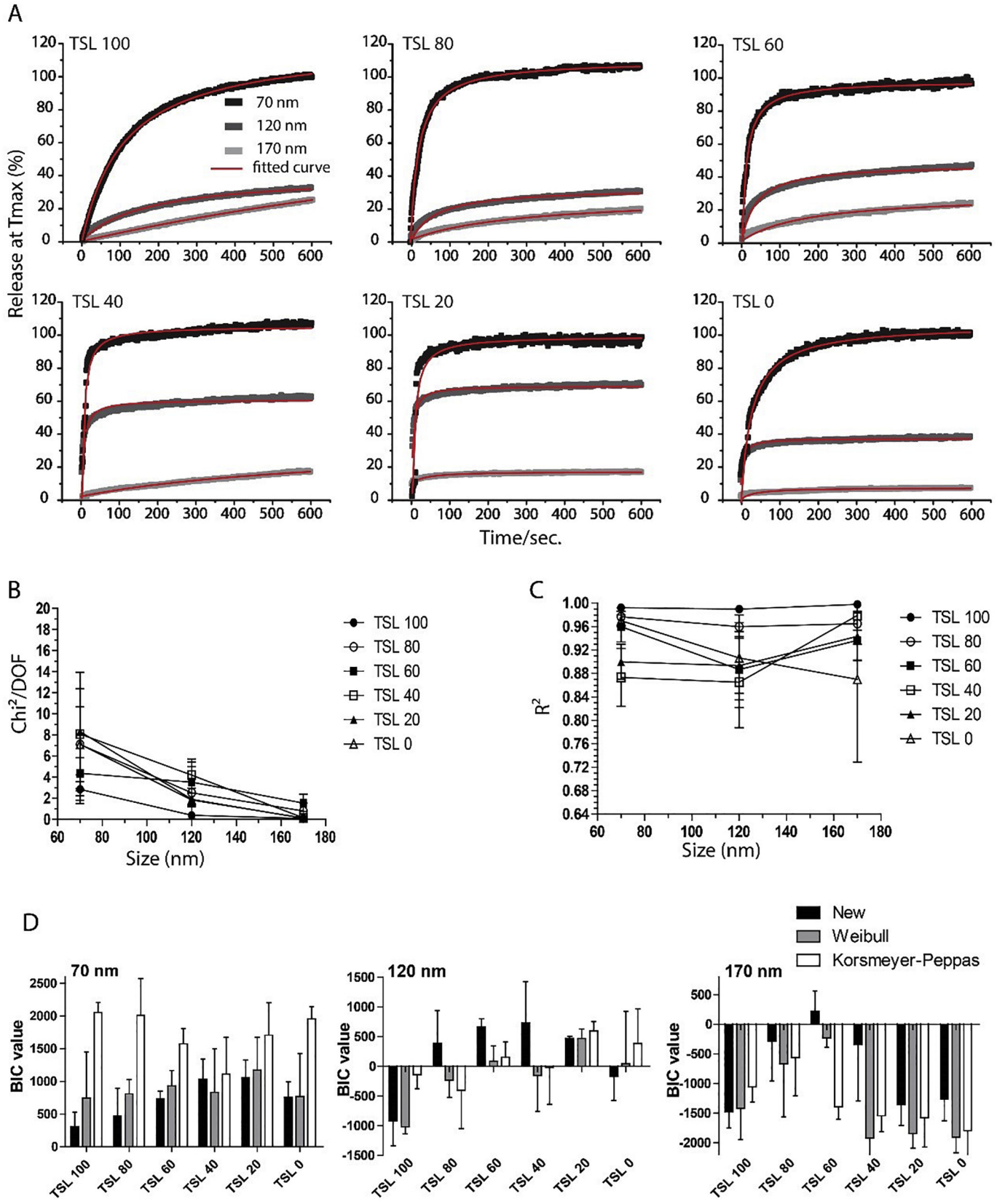

Fig. 5. The new model is used to fit T_{max} release from thermosensitive liposomes with different sizes, respectively (A). The values of Chi2/DOF (B), R^2 (C) and BIC (D) indicate that the new model improves the goodness-of-fit in TSL with a size at 70 nm compared to the Korsmeyer-Peppas model. Mean ± SD (B, C) or Mean + SD (D) are shown of 3 independent experiments.

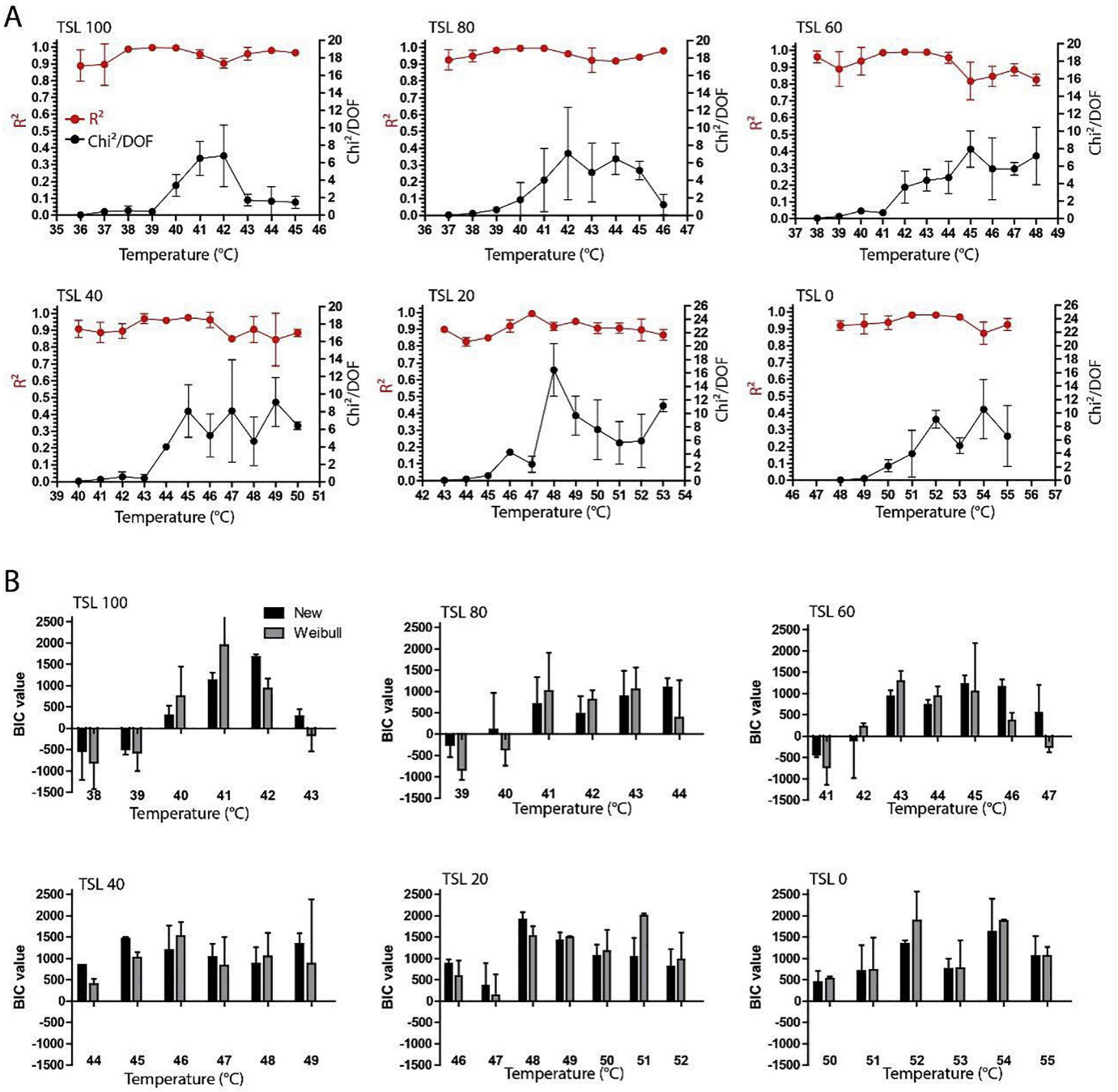

Fig. 6. TSL with a size of 70 nm were selected as examples, and illustrate that the new kinetic equation can fit not only the release at T_{max} but also the release below and above T_{max}, showing that all $R^2$are above 0.9 and Chi [2]/DOF values below 15 (A), with a comparable goodness-of-fit compared to the Weibull model (B). Mean ± SD (A) or Mean + SD (B) are shown of 3 independent experiments.

formulation of TSL or formation of drug precipitates inside TSL, this new kinetic model can be used to fit release from thermosensitive liposomes. In addition to different composition or content, the release at non-T_{max} also indicated comparably excellent fitting results (Figs. 6, 7. Supplementary Fig. 2), which implies that the new model can also be used to fit the release over the whole release temperature range.

Given that the release from TSL in this study shows improved fitting by the new model, it suggests that the release of these thermosensitive liposomes at T_{max}, especially with a size below 100 nm may be not mainly dominated by diffusion [23,24]. New equations may describe better with a non-diffusion-controlled release profile. We think that there are two main driving forces which cause release from thermosensitive liposomes: the Laplace pressure and the chemical potential difference. Under the effect of pressure difference between the inside and the outside of the liposomal spherical surface, the efflux rate of content from "holes" in the liposome membrane at phase transition can be deduced as:

$$\frac{dV}{dt} = \Delta P * \frac{A}{L} \tag{4}$$

where V is the efflux volume, ΔP is Laplace pressure, and A represents the total area of release "holes" in liposome membrane and L is the thickness of liposome membrane. Considering the bilayer spherical surface of liposome, Laplace pressure ΔP can be written as:

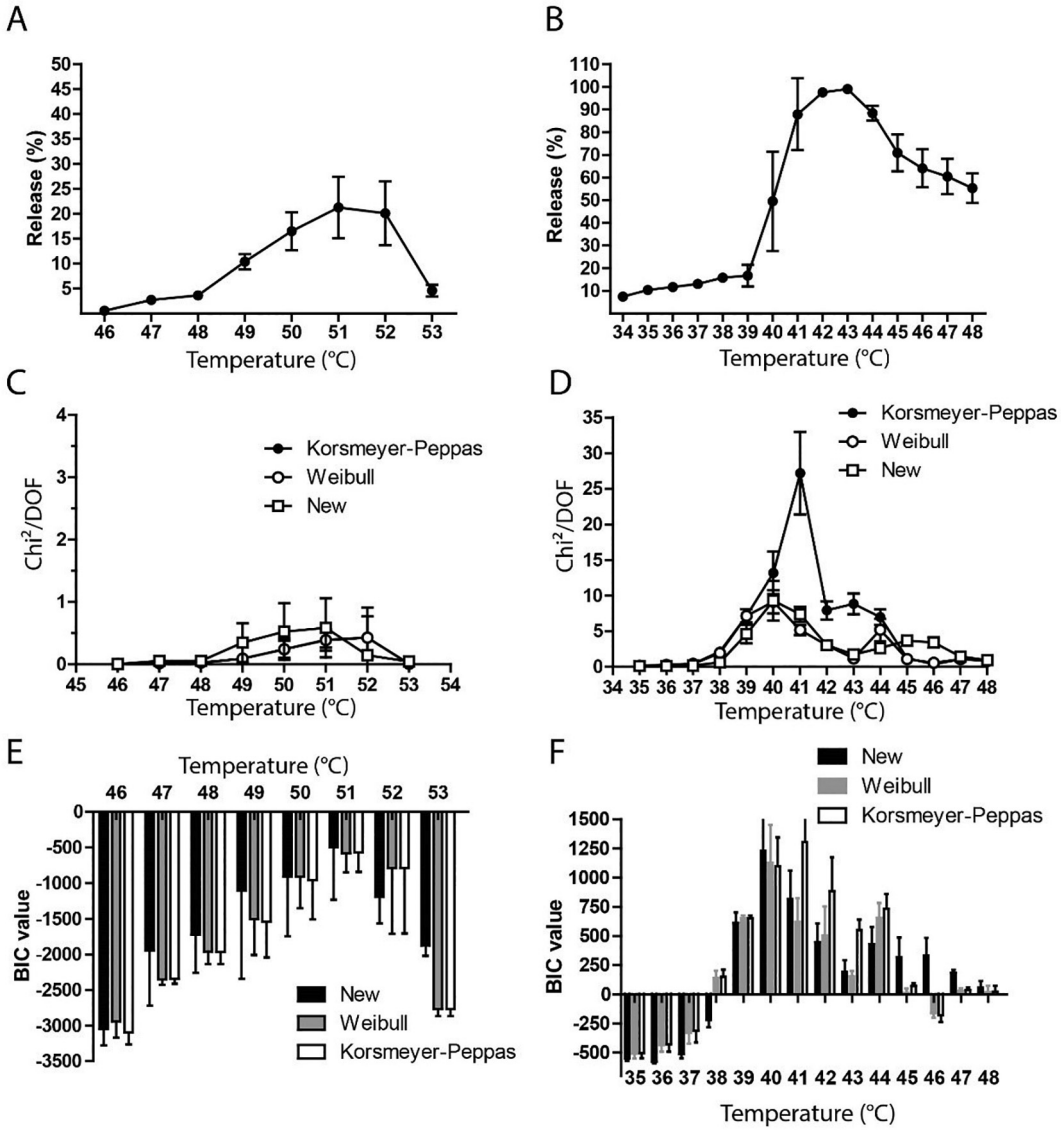

Fig. 7. Evaluation of fitting with the new model of release of content from cholesterol containing TSL (A, C, E) and idarubicin encapsulated TSL (B, D, F). Temperature-dependent release is applied to determine the maximal release temperatures (A, B). Goodness-of-fit is evaluated based on reduced chi-square statistic value (C, D) and BIC value (E, F). Mean ± SD, $N = 3$ (A, C, E); $N = 2$ (B, D, F).

$$\Delta P = 4 * \frac{\sigma}{r} \tag{5}$$

σ is surface tension of liposome and r is liposome radius.

Thus the content efflux rate dn_e/dt can be summarized as:

$$\frac{dn_e}{dt} = C_i * \frac{dV}{dt} = \frac{A}{L} * 4 * \frac{\sigma}{r} * C_i \tag{6}$$

n_e represents the content release in moles, and C_i represents the concentration of content inside liposome.

The other release driving force is from chemical potential difference as a result of the differing concentrations of content inside and outside liposomes. The drug efflux rate can be written as:

$$\frac{dn_e}{dt} = A * \frac{D}{L} * (C_i - C_e) \tag{7}$$

where D is the diffusion coefficient and A is the release area. L is the thickness of liposome membrane, C_i and C_e represent the content concentration in the interior and exterior of liposome, respectively.

We believe that the release of content from liposomes is the result of above two driving forces together. Thus the content release rate equation can be written as:

$$\frac{dn_e}{dt} = \frac{A}{L} * 4 * \frac{\sigma}{r} * C_i + A * \frac{D}{L} * (C_i - C_e) \tag{8}$$

which can be simplified by factoring out the constant parameters (namely L, σ and D in eqs. (5–8)):

$$\frac{dn_e}{dt} = K1 * \frac{A}{r} * C_i + K2 * A * (C_i - C_e) \tag{9}$$

Eq. (9) well describes the features of thermosensitive liposome release. First, over the phase transition temperature range, the release area in the membrane (namely the interfaces between liquid crystal and solid gel phases) increases with temperature until reaching T_{max} (Fig. 1) at which TSL show the maximal release rate, and then reduces [6]. Thus the release profile of thermosensitive liposomes presents a parabola-shape-like curve in the phase transition range (Fig. 3). Secondly, decreasing TSL size increases the Laplace pressure to drive content release from liposomes, thereby increasing the release rate compared to TSL with a larger size. Third, at the initial release at T_{max} the highest intra- and extra-liposome content concentration difference exists ($C_e \approx 0$),

which together with the Laplace pressure promotes an ultra-fast release, leading to an initial sharp release (part a➔b in Fig. 2), in which the pressure difference dominates the release. Where after, the concentration of content inside TSL declines substantially with release ($C_e > > 0$) by showing a relatively slow release afterwards (part b➔c in Fig. 2), in which the concentration difference presents as dominant driving force of release.

Based eq. (9), we can deduct the equation below (details seen supplementary materials):

$$\frac{Q}{Q_0} = k_1 - \frac{k_1}{e^{k_2 * t}} \tag{10}$$

A further degeneration of above equation can be considered [25]:

$$\frac{Q}{Q_0} = k_1 - \frac{k_1}{e^{k_2 * t}} \approx k_1 - \frac{k_1}{1 + k_2 * t} = \frac{k_1 * k_2 * t}{1 + k_2 * t} \tag{11}$$

Eq. (11) is namely equivalent to our proposed empirical kinetic eq. (3). Thus the parameter k and a in eq. (3) are expressed as:

(12)

$$a = k_2 = (\Delta P * V_e + D * (V_i + V_e)) * A/(L * V_i * V_e) \tag{13}$$

where V_e is the volume of dissolution medium and V_i represents the total volume of liposomes.

The value of diffusion coefficient (D) of most molecules is considerably small [26], thus we further simplify above k (eq. 10) to:

$$k = \Delta P * \frac{A}{L * V_i} \tag{14}$$

The unit of parameter k in our new model thus can be achieved as $Pa*m^{-2}$, indicating the increased pressure (namely Laplace pressure) per unit of release area. In another word, at a given temperature a higher k value suggests the higher release rate of the content.

This can be confirmed from experimental data by plotting k against thermosensitive liposome sizes and compositions (Fig. 8). It is observed that at the same temperature for each size (e.g. at T_{max}), almost all k, except TSL 0 at a size of 120 nm, increase with declined size of TSL which leads to a higher release (Fig. 8A, Table 2), indicating that the value of the parameter k is affected by the radius of nano-carrier. In addition to size, different TSL formulations (at the same size) also exhibit an effect on k, showing increased k in the formulation of TSL 40 or 20 in this study (Fig. 8B, Table 2). The increased k in TSL 40 and 20 can be explained by the increased release areas at respective T_{max}, due to the different densities of grain boundary in the liposome membrane described in our previous paper [6]. Together, experimental data indicates that the parameter k indeed is mainly related to thermosensitive liposome radius and composition (that influences the release area), which supports the theoretically deduced eq. (14). However, we did not see a relation of the parameter a between size or composition of TSL (Supplementary Fig. 3, Table 2).

Table 2
Parameter k and a of fitting of CF release at T_{max} by the new kinetic model. Mean ± SD, N = 3.

Size	Liposome	k	a
70 nm	TSL 100	5.10 ± 3.05	0.056 ± 0.037
	TSL 80	5.37 ± 0.31	0.053 ± 0.006
	TSL 60	10.60 ± 1.11	0.110 ± 0.02
	TSL 40	23.10 ± 9.62	0.420 ± 0.303
	TSL 20	26.15 ± 8.27	0.267 ± 0.086
	TSL 0	8.88 ± 4.39	0.089 ± 0.047
120 nm	TSL 100	0.22 ± 0.01	0.005 ± 0
	TSL 80	0.58 ± 0.16	0.013 ± 0.005
	TSL 60	3.92 ± 3.79	0.065 ± 0.050
	TSL 40	9.85 ± 5.50	0.150 ± 0.062
	TSL 20	21.16 ± 1.81	0.340 ± 0.067
	TSL 0	14.67 ± 9.23	0.280 ± 0.115
170 nm	TSL 100	0.06 ± 0.02	0.001 ± 0
	TSL 80	0.14 ± 0.07	0.005 ± 0.003
	TSL 60	0.26 ± 0.11	0.009 ± 0.003
	TSL 40	0.10 ± 0.07	0.232 ± 0.397
	TSL 20	0.14 ± 0.08	0.017 ± 0.012
	TSL 0	0.13 ± 0.12	0.223 ± 0.365

Therefore, our new kinetic model includes pressure-difference as a driving force to model drug release. To our knowledge, most established release kinetic models are based on concentration difference as main driving force (e.g. first-order model), which are not able to well describe the pressure-driven release profiles. Besides, according to the theoretical derivation of this kinetic equation we tried to define the parameters in this new model, of which based on the value of parameter k it is possible to evaluate and compare the release rate of different thermosensitive liposomes. It is reasonable to speculate from eq. (8) that the release area and the Laplace pressure in the nano-carrier dominate content release profile regardless of the formulation composition or the form of content, provided that the drug is encapsulated inside thermosensitive liposomes.

Understanding of the release in vitro will aid to understanding the distribution in the tumor. Our new model can be used to predict the release profile in vivo. For instance, applying hyperthermia on tumor patient, at a given temperature and time we can obtain the potential drug accumulative release with the new model, from which we can further estimate the potential drug accumulation and distribution in the tumor. Combination of our model with modeling of intratumoral drug flow kinetics and uptake by tumor and stromal cells helps to develop formulations, plan treatment and predict outcome.

5. Conclusion

Rapid and substantial release occurs when thermosensitive liposomes are exposed to desirable hyperthermia, presenting a “Γ” shape-

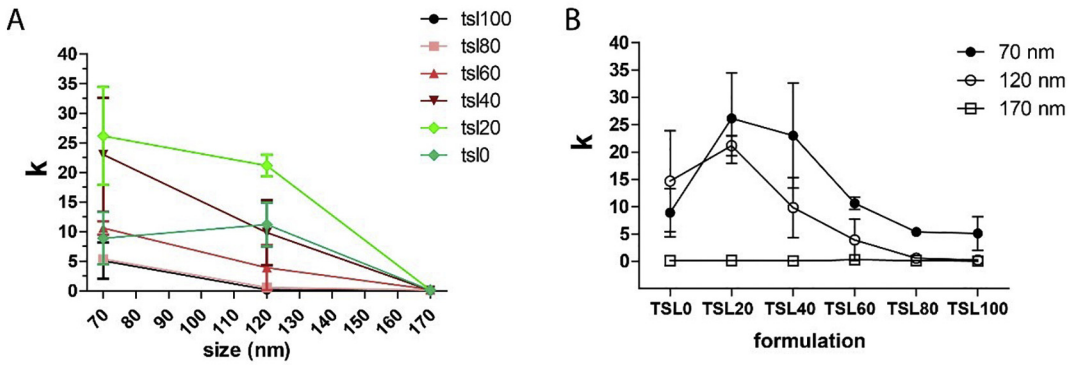

Fig. 8. The values of parameter k, resulting from fitting through the new model, are plotted against different thermosensitive liposome sizes (A) and formulations (B), at maximal release temperatures of CF-TSL. Mean ± SD, $N = 3$.

like release curve, which is the feature of not only thermosensitive liposomes but also other stimuli-responsive fast release drug delivery systems. It is necessary to find a proper mathematical model to describe this typical release behavior at maximum release temperature as this can be used to predict the release kinetics in vivo and model drug delivery for clinical studies. Most release models are established on concentration difference (i.e. diffusion-controlled release), which cannot describe this rapid release from thermosensitive liposomes. Here we take the Laplace pressure as a main release-driving force for release from these small nano-sized vesicles and propose a new and also relatively simple equation to fit the typical release profile at Tm, with which improved fitting of release curves of both large and small liposomes is obtained. However, other extensive drug delivery systems need to be tested for further evaluation of the suitability of this new kinetic model in the following study.

Credit Author Statement

Tao Lu designed the study, performed the experiments, analyzed experiment data and write the manuscript.

Timo L.M. ten Hagen revised the manuscript and helped the data analysis.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jconrel.2020.05.047.

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