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Note

A simulation of oral absorption using classical nucleation theory

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ABSTRACT

The purpose of the present study was to investigate the applicability of the classical nucleation theory (CNT) for oral absorption simulation. The CNT was used to simulate the precipitation of low solubility basic compounds in the fasted state simulated intestinal fluid. The infusion–precipitation experiment data reported by Kostewicz et al. [Kostewicz, E.S., Wunderlich, M., Brauns, U., Becker, R., Bock, T., Dressman, J.B., 2004. Predicting the precipitation of poorly soluble weak bases upon entry in the small intestine. J. Pharm. Pharmacol. 56, 43–51] was used for validation. The surface tension of a drug and a pre-exponential factor were obtained by fitting to the experimental data. The CNT adequately simulated the precipitation characteristics of experimental data such as the increase of the precipitation rate and less sensitivity of maximum concentration by the increase of infusion rate.

1. Introduction

Recently, the number of low solubility compounds has increased in drug discovery and development (Sugano et al., 2007). In the case of a low solubility basic compound, a salt form is often selected as the active pharmaceutical ingredient for oral products. Usually a salt dissolves quickly in the gastrointestinal tract (Serajuddin, 2007). However, free form can precipitated out at neutral pH (Badawy et al., 2006; Sugano et al., 2007). In addition, precipitation can also occur when a solution formulation and a solid dispersion formulation are administered. Recently, computational oral absorption simulation is anticipated to be a powerful tool to improve the efficacy of product development. Therefore, computational simulation of a precipitation process is of great interest. The precipitation of a drug can be characterised by the critical supersaturation concentration and the precipitation rate. However, these cannot be adequately represented by first order kinetics which has been utilised in some commercial software for oral absorption simulation.

The purpose of the present study was to investigate the applicability of the classical nucleation theory (CNT) for precipitation simulation. As the experimental precipitation data, the precipitation data in a biorelevant media reported by Kostewicz et al. (2004) was used.

2. Theory

2.1. Classical nucleation theory

Precipitation can be divided into two processes, nucleation and particle growth. The classical nucleation theory (CNT) is briefly described since there are many articles (He et al., 2006; Lindfors et al., 2008; Liu, 1999; Sugano, 2009). According to the CNT, the primary nucleation rate per volume per time can be expressed as (Lindfors et al., 2008; Liu, 1999):

$$\frac{dN_{nc}}{dt} = f(C_{aq}; \beta, \gamma; S_{aq}, v_m) = \beta D_{mono}(N_A C_{aq})^2 \left[\frac{k_B T}{\gamma}\right]^{1/2} \ln\left(\frac{C_{aq}}{S_{aq}}\right) \times \exp\left(-\frac{16\pi}{3}\left(\frac{\gamma}{k_B T}\right)^3 \left(\frac{v_m}{\ln(C_{aq}/S_{aq})}\right)^2\right) \quad (1)$$

where N_{nc} is the number of nuclei, N_A is Avogadro's number, C_{aq} is the concentration of a free monomer drug in aqueous phase (not in bile micelles), S_{aq} is the solubility of the precipitant in water without bile micelles, k_B is the Boltzman constant, T is the temperature, γ is the surface tension, v_m is the molecular volume, D_{mono} is the diffusion coefficient of a free monomer drug, β is a lump constant of various factors such as the number of the foreign particles for heterogeneous nucleation and the attachment probability of a molecule onto a nuclei. In the present study, the two unknown parameters, γ and β were obtained from curve fitting. C_{aq}/S_{aq} is

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the degree of supersaturation. Nucleation occurs (N_{nc} becomes >1) when the concentration of dissolved molecule exceeded the critical supersaturation concentration (C_{cssc}). C_{cssc} is mainly determined by the surface tension (γ).

The growth of the nuclei (particle) can be expressed by the Nernst Brunner equation as the reciprocal process of dissolution (Lindfors et al., 2008; Sugano, 2009):

$$\frac{dX_{nc,i}}{dt} = 4\pi r_p^2 \frac{D_{eff}}{h}(S_b - C_b) \quad (2)$$

$$r_p = \left(\frac{4}{3\pi}\frac{X_{nc}}{\rho}\right)^{1/3} \quad (3)$$

where $X_{nc,i}$ is the amount of particles (weight or mole) generated during a time period (subscript i was used to represent a particle group generated at a different time point), r_p is the particle radius at time t, D_{eff} is the effective diffusion coefficient in a bile micelle media, ρ is the density, h is the diffusion layer thickness, C_b is the sum of the concentrations of free monomer and the bile micelle bound molecule, and S_b is the solubility in the bile micelle media. $C_{aq}/S_{aq} = C_b/S_b$ since the bile partition coefficient is cancelled out (Glomme et al., 2006). h was calculated as previously reported (basically $h = r_p$, $r_p < 20\,\mu m$) (Sugano, 2008). The initial particle radius is equal to the critical nuclei radius which is given as (Lindfors et al., 2008):

$$r_p^* = \frac{2\gamma v_m}{k_B T \ln(C_m/S_{aq})} \quad (4)$$

2.2. Simulation of in vitro precipitation

The concentration–time profiles were simulated by integrating Eqs. (1)–(3) and compared with the experimental results reported by Kostewicz et al. (2004). Kostewicz et al. (2004) investigated the precipitation of three low solubility basic compounds in biorelevant media. In brief, a basic drug was dissolved in 0.01N HCl (3 mg/mL for dypyridamol) and infused into 500 mL of the fasted state simulated intestinal fluid (pH 6.5 phosphate buffer, taurocholic acid 3 mM, phosphatidylcholine 0.75 mM) (Vertzoni et al., 2004). The infusion rate (zero order) was changed from 0.5 mL/min to 9 mL/min which would cover the gastric emptying rate. In the present study, the experimental data of dipyridamol will be discussed in detail. Computer simulation was performed as previously reported (Sugano, 2009). In brief, the Runge Kutta 4th method was used for numerical integration of Eqs. (1)–(3) with integration interval of 1 min. Generation of particles by nucleation was expressed as generation of ith particle bins. Newly generated nuclei in one integration interval were assigned to one particle bin with a particle radius of critical nuclei (Eq. (4)). The particle growth of each bin was simulated by Eqs. (2) and (3). S_b was set to be the concentration after the termination of infusion (60 μg/mL for dipyridamol). D_{mono} (6.2×10^{-6} cm^2/s for dipyridamol) (Avdeef et al., 2004), D_{eff} (2.3×10^{-6} cm^2/s for dipyridamol), v_m (431 cm^3 mol^{-1} for dipyridamol) and density (1.2 g/cm^3 for dipyridamol) was calculated as previously reported (Cao et al., 2008; Girolami, 1994; Okazaki et al., 2008). The fluid volume change by zero order infusion rate was taken into account for simulation. For comparison purpose, simulation with a first order kinetic was also used for precipitation.

$$\frac{dX_{nc}}{dt} = k_{prec}(S_b - C_b) \quad (5)$$

where k_{prec} is the precipitation constant. k_{prec} was obtained by fitting to the experimental data (least square method).

Goodness of fit (GOF) was calculated as:

$$\text{GOF} = \frac{1}{\text{Data point} - \text{parameter number}} \sum \frac{C_{b,obs} - C_{b,calc}}{C_{b,calc}}$$

where subscript obs and calc indicate observed and calculated data, respectively.

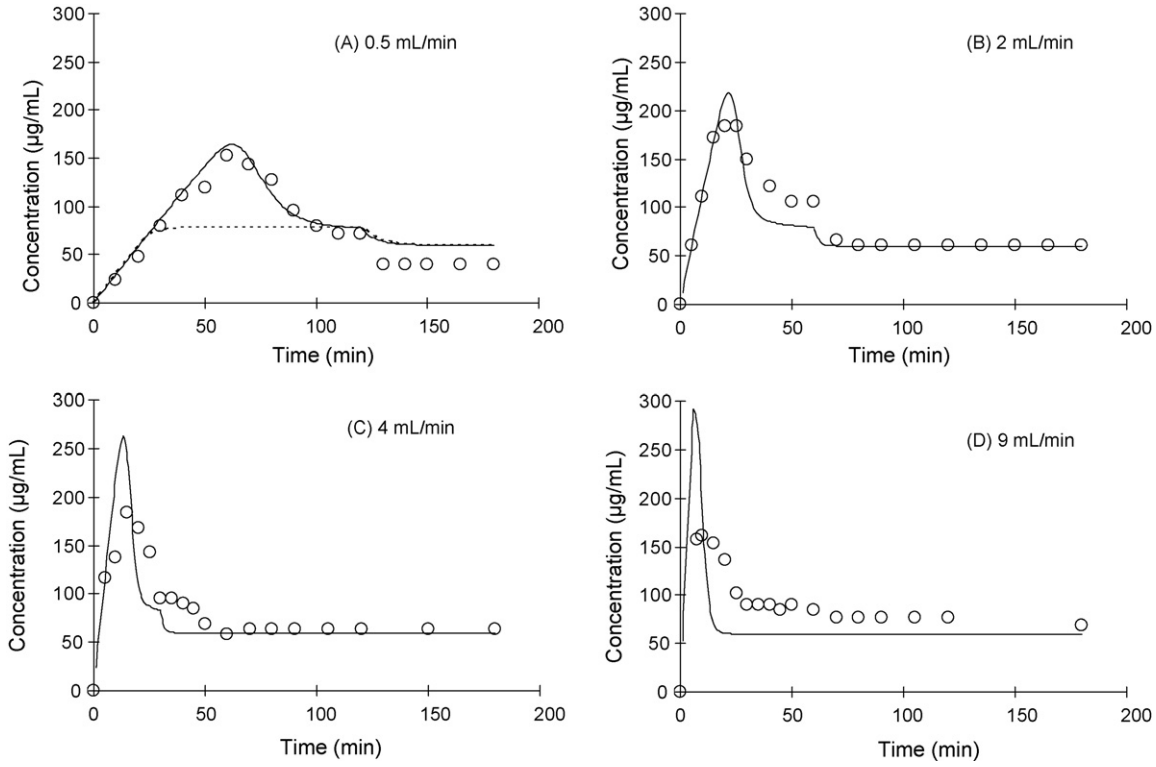

Fig. 1. Experimental and simulated concentration–time profile in the infusion–precipitation system. Dypiridamol of 3 mg/mL was infused into the FaSSIF of 500 mL. The infusion rate was changed from 0.5 mL/min to 9 mL/min. Infusion was stopped at 120 min, 60 min, 30 min and 6.6 min for 0.5 mL/min, 2 mL/min, 4 mL/min, 9 mL/min, respectively. The solid line is the simulation result by applying the CNT. The dotted line in (A) is the simulation result by applying the first order kinetic for precipitation.

Table 1
Observed and simulated key parameters in infusion–precipitation system.

Flow rate (mL/min)		C_{max} (μg/mL)	T_{max} (min)	T_{75}[a] (min)	Particle number	Particle radius[b] (μm)	Goodness of fit
0.5	Observed[c]	152.1 ± 7.5	60	19	–	–	–
	Simulated	164	62	11	0.5×10^8	8.3	3.2
2	Observed	184.2 ± 7.5	20	10	–	–	–
	Simulated	218	22	4	1.9×10^8	6.9	2.7
4	Observed	185.9 ± 5.2	15	8	–	–	–
	Simulated	262	13	3	3.6×10^8	5.5	9.6
9	Observed	160.5 ± 2.7	10	10	–	–	–
	Simulated	291	6	3	3.4×10^8	4.1	23.7

[a] Time to 75% of C_{max} after T_{max}.
[b] The average particle radius after the infusion termination.
[c] Kostewicz et al. (2004).

3. Results and discussion

According to the CNT, C_{cssc} strongly depends on the surface tension, and the precipitation rate strongly depends on the number of nuclei. Therefore, γ and β are the key determinants of precipitation.

γ and β were obtained by visually fitting the simulation curve to the experimental data at the infusion rate of 0.5 mL/min (Fig. 1A). $\beta = 1 \times 10^{-18}$ and $\gamma = 0.0030$ N/m were obtained which correspond to CSSR of 1.7 (GOF = 3.2). By using these parameters, the experimental data of the different infusion rate was simulated (Fig. 1B–D, Table 1).

In the report of Kostewicz et al., it was observed that, as the infusion rate increased, the precipitation rate increased, whereas the maximum concentration (C_{max}) did not increase or increased less proportionally. They suggested that the number of generated nuclei was larger at higher infusion rates. These features were appropriately represented by the CNT as shown in Fig. 1 and Table 1. Similar simulation results were observed for the other two low solubility basic compounds reported by Kostewicz et al. using the same β value with dipyridamol, but different γ values ($\gamma = 0.0033$–0.0042 N/m).

In a certain commercial oral absorption simulation program, a first order kinetic equation (Eq. (5)) is used to represent precipitation. As shown in Fig. 1A (dotted line), the first order kinetic equation could not express the observed concentration–time profile (GOF = 46.8). A plateau concentration was achieved when the infused rate and the precipitation rate balanced, and a supersaturation phenomenon was not appropriately simulated. This result suggested that it is important to incorporate the CNT into the oral absorption simulation.

At higher infusion rates, the CNT slightly overestimated the precipitation rate and C_{max}. A possible explanation for these discrepancies is that, the local concentration at the pouring position could become higher than the average concentration because of insufficient agitation. Therefore, nucleation could predominantly occur at the pouring position.

The γ values obtained in the present study were significantly smaller than the typical γ values between a drug particle and water for low solubility compounds (Lindfors et al., 2008). In addition, the β value is very small compared to the theoretical value of 1 for homogeneous nucleation. These would suggest that the nucleation process is heterogeneous nucleation and the bile micelles might affect the nucleation process.

For *a priori* prediction without obtaining γ and β by fitting process, the precipitation mechanism should be further investigated. However, direct measurement of γ is more resource intensive than an in vitro infusion–precipitation experiment. Therefore, the practical strategy in drug discovery and development would be to obtain γ (and β) by fitting to in vitro infusion–precipitation data, and use these values for in vivo prediction (Carino et al., 2005; Sugano et al., 2007). In addition, C_{cssc} can be obtained by a pH titration method (Box et al., 2006) and 96-well based precipitation method (γ can be back calculated from C_{cssc}) (Bevan and Lloyd, 2000; Dehring et al., 2004; Lindfors et al., 2008).

It is worth noting that the particle number and size of the precipitant, which is required to simulate the re-dissolving of the precipitant in the gastrointestinal tract, can be estimated by the CNT. Simulated particle sizes of the precipitant were also shown in Table 1.

In conclusion, the results of the present study suggested that the CNT can represent some key attributes of the precipitation of a drug in a biorelevant media. Therefore, the CNT can be used to simulate the precipitation in computational oral absorption simulation.

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References

Avdeef, A., Nielsen, P.E., Tsinman, O., 2004. PAMPA—a drug absorption in vitro model: 11. Matching the in vivo unstirred water layer thickness by individual-well stirring in microtitre plates. Eur. J. Pharm. Sci. 22, 365–374.
Badawy, S.I.F., Gray, D.B., Zhao, F., Sun, D., Schuster, A.E., Hussain, M.A., 2006. Formulation of solid dosage forms to overcome gastric pH interaction of the factor Xa inhibitor, BMS-561389. Pharm. Res. 23, 989–996.
Bevan, C.D., Lloyd, R.S., 2000. A high-throughput screening method for the determination of aqueous drug solubility using laser nephelometry in microtiter plates. Anal. Chem. 72, 1781–1787.
Box, K.J., Volgyi, G., Baka, E., Stuart, M., Takacs-Novak, K., Comer, J.E.A., 2006. Equilibrium versus kinetic measurements of aqueous solubility, and the ability of compounds to supersaturate in solution—a validation study. J. Pharm. Sci. 95, 1298–1307.
Cao, X., Leyva, N., Anderson, S.R., Hancock, B.C., 2008. Use of prediction methods to estimate true density of active pharmaceutical ingredients. Int. J. Pharm. 355, 231–237.
Carino, S.R., Sperry, D.C., Hawley, M., 2005. Relative bioavailability estimation of carbamazepine crystal forms using an artificial stomach–duodenum model. J. Pharm. Sci. 95, 116–125.
Dehring, K.A., Workman, H.L., Miller, K.D., Mandagere, A., Poole, S.K., 2004. Automated robotic liquid handling/laser-based nephelometry system for high throughput measurement of kinetic aqueous solubility. J. Pharm. Biomed. Anal. 36, 447–456.
Girolami, G.S., 1994. A simple "back of the envelope" method for estimating the densities and molecular volumes of liquids and solids. J. Chem. Educ. 71, 962–964.
Glomme, A., März, J., Dressman, J., 2006. Predicting the intestinal solubility of poorly soluble drugs. In: Testa, B., Krämer, S., Wunderli-Allenspach, H., Folkers, G. (Eds.), Pharmacokinetic Profiling in Drug Research. Wiley–VCH, Zurich, pp. 259–280.
He, G., Bhamidi, V., Tan, R.B.H., Kenis, P.J.A., Zukoski, C.F., 2006. Determination of critical supersaturation from microdroplet evaporation experiments. Cryst. Growth Des. 6, 1175–1180.
Kostewicz, E.S., Wunderlich, M., Brauns, U., Becker, R., Bock, T., Dressman, J.B., 2004. Predicting the precipitation of poorly soluble weak bases upon entry in the small intestine. J. Pharm. Pharmacol. 56, 43–51.
Lindfors, L., Forssen, S., Westergren, J., Olsson, U., 2008. Nucleation and crystal growth in supersaturated solutions of a model drug. J. Colloid Interface Sci. 325, 404–413.

Liu, X.Y., 1999. A new kinetic model for three-dimensional heterogeneous nucleation. J. Chem. Phys. 111, 1628–1635.
Okazaki, A., Mano, T., Sugano, K., 2008. Theoretical dissolution model of polydisperse drug particles in biorelevant media. J. Pharm. Sci. 97, 1843–1852.
Serajuddin, A.T.M., 2007. Salt formation to improve drug solubility. Adv. Drug Deliv. Rev. 59, 603–616.
Sugano, K., 2008. Theoretical comparison of hydrodynamic diffusion layer models used for dissolution simulation in drug discovery and development. Int. J. Pharm. 363, 73–77.
Sugano, K., 2009. Introduction to computational oral absorption simulation. Expert. Opin. Drug Metab. Toxicol. 5, 259–293.
Sugano, K., Okazaki, A., Sugimoto, S., Tavornvipas, S., Omura, A., Mano, T., 2007. Solubility and dissolution profile assessment in drug discovery. Drug Metab. Pharmacokinet. 22, 225–254.
Vertzoni, M., Fotaki, N., Kostewicz, E., Stippler, E., Leuner, C., Nicolaides, E., Dressman, J., Reppas, C., 2004. Dissolution media simulating the intralumenal composition of the small intestine: physiological issues and practical aspects. J. Pharm. Pharmacol. 56, 453–462.