International Journal of Pharmaceutics 478 (2015) 318–327

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Evaluation of the coat quality of sustained release pellets by individual pellet dissolution methodology

Min Xu, Celine Valeria Liew, Paul Wan Sia Heng *

GEA-NUS Pharmaceutical Processing Research Laboratory, Department of Pharmacy, National University of Singapore, 18 Science Drive, 117543, Singapore

ARTICLE INFO

Article history:
Received 8 July 2014
Received in revised form 13 November 2014
Accepted 26 November 2014
Available online 27 November 2014

Keywords:
Sustained release pellets
Coat quality
Drug release profile
Individual pellet dissolution

ABSTRACT

This study explored the application of 400-DS dissolution apparatus 7 for individual pellet dissolution methodology by a design of experiment approach and compared its capability with that of the USP dissolution apparatus 1 and 2 for differentiating the coat quality of sustained release pellets. Drug loaded pellets were prepared by extrusion–spheronization from powder blends comprising 50%, w/w metformin, 25%, w/w microcrystalline cellulose and 25%, w/w lactose, and then coated with ethyl cellulose to produce sustained release pellets with 8% and 10%, w/w coat weight gains. Various pellet properties were investigated, including cumulative drug release behaviours of ensemble and individual pellets. When USP dissolution apparatus 1 and 2 were used for drug release study of the sustained release pellets prepared, floating and clumping of pellets were observed and confounded the release profiles of the ensemble pellets. Hence, the release profiles obtained did not characterize the actual drug release from individual pellet and the applicability of USP dissolution apparatus 1 and 2 to evaluate the coat quality of sustained release pellets was limited. The cumulative release profile of individual pellet using the 400-DS dissolution apparatus 7 was found to be more precise at distinguishing differences in the applied coat quality. The dip speed and dip interval of the reciprocating holder were critical operational parameters of 400-DS dissolution apparatus 7 that affected the drug release rate of a sustained release pellet during the individual dissolution study. The individual dissolution methodology using the 400-DS dissolution apparatus 7 is a promising technique to evaluate the individual pellet coat quality without the influence of confounding factors such as pellet floating and clumping observed during drug release test with dissolution apparatus 1 and 2, as well as to facilitate the elucidation of the actual drug release mechanism conferred by the applied sustained release coat onto the pellets.

1. Introduction

Coated pellets have extensive applications in the pharmaceutical industry, ranging from aesthetic purposes to therapeutic necessity. Ideally, good quality coated multi-particulates should display an even coverage of coat, with smooth surface and no defects (Hogan, 1995). The uniform thickness and the integrity of the deposited coat are critical to the functionality of sustained release multi-particulates. For instance, coat defects such as blisters, flaking, cracks and pin-holes usually result in premature exposure of the core content to the extracting media. In the case of sustained release pellets, faster than expected drug release could occur when the coat thickness is compromised.

Coat weight gain, which is often expressed as the percentage of coating polymer applied onto the pellets, is popularly used to infer the pellet coat thickness (Ringqvist et al., 2003). However, the weight gain measurement is non-specific and does not consider the physicochemical properties of the pellet coat (Ho et al., 2009). Tristimulus colour assessment has been found to be useful for routine quality control and colour stability study of coated pellets (Turi et al., 1972). For example, spot colour measurement using the tristimulus colorimeter was employed to evaluate the colour coat distribution on pellet surface and to study the influence of process factors on the coating efficiency of the Wurster fluidized bed processor (Chan et al., 2001). However, there are limitations to the application of these colorimetric techniques as they require the pellet samples to be colour coated. In terms of pellets with functional coat for the purpose of taste masking or sustaining drug release, visual observations with light and scanning electron microscopy could be used to measure the coat thickness of individual pellets. However, such microscopy techniques are restricted to the sample surface properties and there are inevitable practical problems such as the difficulty in cross-sectioning spherical pellets (Wesdyk et al., 1990). Confocal laser scanning

* Corresponding author. Tel.: +65 65162930; fax: +65 67752265.
E-mail address: phapaulh@nus.edu.sg (P.W.S. Heng).

http://dx.doi.org/10.1016/j.ijpharm.2014.11.057
0378-5173/

microscopy has been introduced as a non-destructive technique for imaging film-core interface and quantifying the coat quality of individual thinly-coated small pellets by measuring their coating thickness (Depypere et al., 2009). Other analytical techniques used to investigate the physical characteristics of pellet coat include atomic force microscopy (Ringqvist et al., 2003), nuclear magnetic resonance spectroscopy, energy dispersive X-ray imaging (Ensslin et al., 2008), terahertz imaging (Haaser et al., 2013), electron paramagnetic resonance spectroscopy (Ensslin et al., 2009), and fluorescence microscopy (Andersson et al., 2000). Often, certain assumptions are required such as a perfectly spherical pellet when the estimation of pellet coat thickness is made by these measurement techniques. However, in reality, pellets often have less than perfect shapes and may be presented as elongated, elliptical or even rod-like particulates. In addition, although these microscopy and spectroscopy techniques could indicate pellet coat uniformity by measuring the coat thickness, there may not be direct relationship between these physical coat properties and the drug release behaviour of the coated pellets.

For coated pellets designed for sustained release drug delivery, *in vitro* dissolution tests are often carried out using compendial dissolution testers to assure coat quality and release performance (Zahirul and Khan, 1996). However, it has been reported that the cumulative release kinetics observed in a multiple-unit system, being a summation of drug release from all the subunits, does not accurately characterize the basic release mechanism of an individual subunit (Hoffman et al., 1986). Moreover, it was demonstrated that the sustained release behaviour of different pellets from the same coating batch varied to a great extent (Borgquist et al., 2004). Such wide ranging release properties of the individual coated pellets in a dosage form suggested poorly controlled manufacturing processes especially during pellet coating. Thus, manufacturing robustness requires introspection. In pellet coating, data on drug release properties of individual pellets could identify the uniformity of the spray coating process and be used in process optimization. Therefore, a dissolution technique that allows profiling of single pellet drug release would be desirable for the examination of individual dissolution characteristics.

The reciprocating holder dissolution apparatus, designed in compliance with all the compendial requirements of United States Pharmacopeia (USP) apparatus 7, is a small-volume dissolution testing system for non-disintegrating and extended release dosage forms. It was found to be suitable for dissolution studies of slowly releasing actives from medical devices such as subcutaneous implants, drug eluting stents (Crist, 2009) and small transdermal patches (Zhou et al., 2007). This apparatus uses a reciprocating motion to dip the dosage form in a medium at programmed time intervals. Sustained release pellets with water-insoluble polymer coats are able to remain structurally unchanged in aqueous medium for a prolonged period and as the drug content in an individual pellet is rather low, the small-volume dissolution system of apparatus 7 is ideal for determining its release characteristics. Thus, the objective of this study was to explore the application of dissolution apparatus 7 for assessing individual pellet coat quality by a design of experiment (DoE) approach and to compare its findings with that of the USP dissolution apparatus 1 and 2 for the determination of drug release from sustained release pellets.

2. Materials and methods

2.1. Materials

Commercially available α-lactose monohydrate (GranuLac 200, Meggle, Germany) and drug metformin hydrochloride (BP grade, Granules, India) were used to prepare pellets with microcrystalline cellulose (MCC, Avicel PH 101, FMC Biopolymer, Ireland). Acetonitrile (HPLC grade, TEDIA, USA) and acetic acid (analytical grade, Merck, Germany) were used as supplied. The ethyl cellulose coating dispersion (Surelease, Colorcon, USA) was diluted with water from 25%, w/w solids to 15%, w/w solids and stirred for approximately 30 min before use. Unless otherwise mentioned, water used was deionized water (Milli-Q, Millipore Corporation, USA).

2.2. Preparation of sustained release pellets

2.2.1. Preparation of drug loaded pellets by extrusion–spheronization

An appropriate amount of powder (25%, w/w MCC, 25%, w/w lactose and 50%, w/w micronized metformin) was first blended in a double-cone blender (AR 400E, Erweka, Germany) at 10 rpm for 20 min. The blended mixture was transferred into a planetary mixer (Major 250, Kenwood, UK) and moistened for 5 min with 28.8%, v/w deionized water amounting to 80% of the W_{Tmax} (the amount of water corresponding to the maximum mean torque value in the rheological profile) obtained from mixer torque rheometry studies (MTR, Caleva Process Solutions, UK) on the corresponding powder blend. The rheological studies of powder blend and preparation of drug loaded pellets followed the method as described by Sarkar (Sarkar et al., 2013). The resultant wet mass was extruded using a screw speed of 85 rpm through a radial screw extruder (E140, GEA-AF, UK) fitted with a screen of 0.8 mm aperture diameter and thickness to target producing pellets in the size range from 710 to 1000 μm. Extrudates were spheronized (S320, GEA-AF, UK) on a 30 cm cross-hatched frictional base plate rotated at 500 rpm for 5 min. The resultant pellets were dried in a fluid bed dryer (Strea-1, GEA-AF, UK) set with an inlet temperature of 80 °C. The drying process was stopped when the monitored product temperature reached 40 °C. The pellets were then further subjected to oven drying at 60 °C for at least 12 h.

2.2.2. Preparation of sustained release pellets by fluidized bed coating

Drug loaded pellets (710–1000 μm) weighing 250 g were transferred into Wurster fluid bed coater (Strea-1, GEA-AF, UK) and coated with aqueous ethyl cellulose dispersion to produce sustained release pellets with 8% and 10%, w/w coat weight gains (designated, 8% coat and 10% coat batches, respectively). The process parameters used were as follows: inlet air volume, 80 m^3/h; inlet temperature, 60 °C; product temperature, 38 °C; spray rate, 3–5 g/min; and atomization pressure, 1.2 bar. The coating dispersion was delivered by a two-fluid spray nozzle with a nozzle tip of 1.2 mm and set to protrude at 1 mm from the flushed bottom position. After coating, the pellets were oven dried at 60 °C for 12 h. The fines and agglomerates were fractionated using sieves with aperture sizes of 710 μm and 1400 μm, respectively.

2.3. Characterization of sustained release pellets

2.3.1. Size and size distribution

Sustained release pellets with 8% and 10% coats (approximately 50 g) were accurately weighed and sieved through a nest of sieves (Endecotts, UK) with aperture sizes chosen in a $\sqrt{2}$ progression from 710 μm to 90 μm on a sieve shaker (VS1000, Retsch, Germany) vibrated at 1 mm amplitude for 10 min. The relative weights (%, w/w) of pellets in each sieve size fractions were determined and the cumulative percentage weight undersize graph plotted. Median pellet size (D_{50}) was determined from the percentage weight undersize plot. Span value of pellets (SP_{pel}) was calculated according to Eq. (1).

$$SP_{pel} = \frac{D_{90} - D_{10}}{D_{50}} \quad (1)$$

where D_{10},D_{50} and D_{90} are the pellet diameters at the 10, 50, and 90 percentiles of the cumulative percentage weight undersize plot, respectively. SP_{pel} reflects the degree of homogeneity in pellet size distribution. A smaller SP_{pel} value indicates a narrower and tighter size distribution.

Sustained release pellets with 8% and 10% coats were first passed through the 850 μm aperture size sieve and pellets then gently re-sieved using the same sieve. Pellets trapped in the sieve mesh were carefully extricated using a soft brush, for later use in shape, coat thickness measurement, individual pellet weight and drug content analyses and individual pellet dissolution studies.

2.3.2. Shape

Shape analysis of the 850 μm pellets was carried out using an image analyser on a stereomicroscope (SZH, Olympus, Japan). The images of two hundred randomly selected pellets were captured with a digital camera (DSP 3CCD, Sony, Japan) and analysed using an imaging software (Image-Pro Version 6.3, Media Cybernetics, USA). Two shape determinant parameters, roundness and aspect ratio, were calculated by Eqs. (2) and (3), respectively. Roundness values emphasize the sphericity of pellets while pellet elongation is described by the aspect ratio (Bouwman et al., 2004). For a perfect sphere, these two shape parameters would bear the value of unity.

$$\text{Roundness} = \frac{P^2}{4\pi A} \quad (2)$$

$$\text{Aspect ratio} = \frac{l}{b} \quad (3)$$

where P, A and l are the perimeter, area and length of the two-dimensional particle outline, and b is the perpendicular width against l.

2.3.3. Pellet coat thickness measurement by scanning electron microscopy (SEM)

Sustained release pellets with 8% and 10% coats were sliced equatorially using a razor blade. The cut pellets were examined and photomicrographs taken at 80 times magnification using a SEM (JSM-6010LV, JEOL, Japan) with secondary electron imaging and an accelerating voltage of 1.5 kV. The pellet coat thickness measurements were made from the photomicrographs by using the imaging software (InTouchScope Software, JEOL, Japan). Ten random measurements were made for each cut pellet surface and ten cut pellets were sampled for each batch.

2.3.4. Individual pellet weight

A sample of 50 pellets was accurately weighed (AG 135, Mettler Toledo, USA) and the average pellet weight (W_s) was calculated. Triplicated measurements were conducted for sustained release pellets with 8% and 10% coats and results averaged.

2.3.5. Drug content

One 850 μm size pellet was sampled randomly and ground with a mortar and pestle. The resultant powder was transferred into a 25 mL volumetric flask, deionized water added and sonicated for 10 min to completely dissolve the drug. After making up to volume, the solution was filtered using 0.45 μm membrane filter (Regenerated Cellulose, Sartorius Stedim Biotech, Germany) into vials. The vials were then assayed using reversed-phase high performance liquid chromatography (HPLC; SIL-10AD VP, LC-20AT VP, Shimadzu, Japan) with the ODS C_{18} column (150 mm × 4.6 mm, 5 μm; Hypersil, Thermo Scientific, USA) at 40 °C and a diode array detector (SPD-M10A VP, Shimadzu, Japan) set at a wavelength of 233 nm. Mobile phase of acetonitrile, purified water and acetic acid (55:44.75:0.25, v/v/v, respectively) was delivered at flow rate of 0.5 mL/min and an injection volume of 10 μL used. For the sustained release pellets with 8% and 10% coats, 25 pellets from each batch were subjected to the drug content assay and results averaged.

2.4. In vitro *dissolution studies*

2.4.1. Drug release from ensembles of pellets

Dissolution studies were conducted using USP dissolution apparatus 1 and 2 (9ST, Caleva, UK) with 900 mL deionized water maintained at 37 ± 0.5 °C and stirred at 100 rpm. At predetermined time intervals, samples of 5 mL were withdrawn and filtered (0.45 μm). The amount of metformin was determined spectrophotometrically (3101 PC, Shimadzu, Japan) at 233 nm. The final sample was withdrawn after rotating the baskets at 300 rpm for 30 min to ensure complete drug release. Runs were triplicated and results averaged. From the plot of cumulative drug release (%) against time, T10 and T50, the times required for 10% and 50% drug release were determined. Model independent comparison of dissolution profiles was carried out using the difference factor ($f1$) as calculated by Eq. (4). Two dissolution profiles are considered similar when the $f1$ value varies from 0 to 15 (Moore and Flanner, 1996).

The *in vitro* drug release data were fitted to three release kinetics models which are commonly used for sustained release dosage forms, namely zero-order, first-order and Higuchi models, employing, Eqs. (5)–(7), respectively. The best-fit model was identified by evaluating the coefficient of determination (R^2),

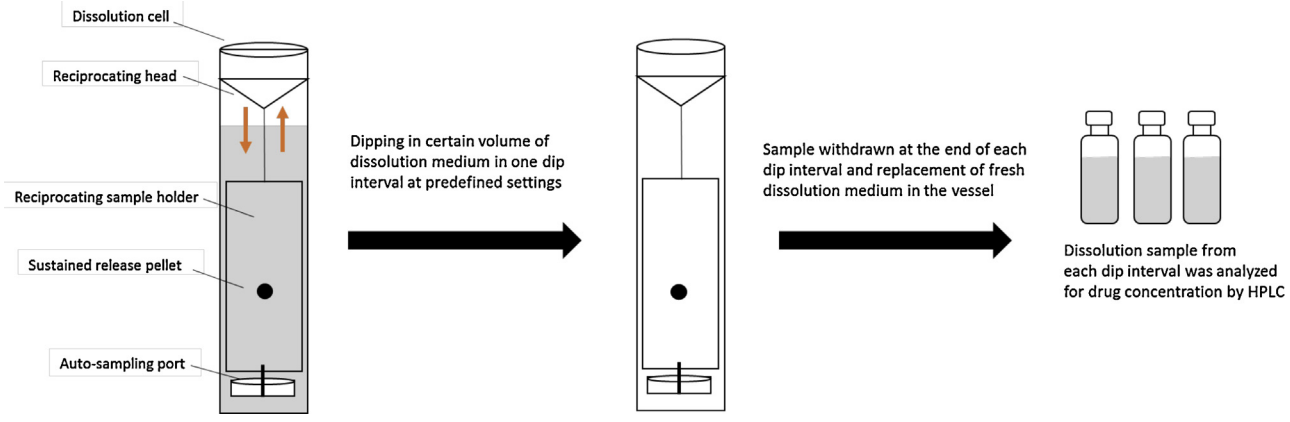

Fig. 1. The schematic diagram of a dissolution set in 400-DS dissolution apparatus 7 and the illustration of one dip cycle during the drug release test.

where the highest R^2 value indicates the best fit.

$$f1 = \frac{\sum_{i=1}^{n}|R_i - T_i|}{\sum_{i=1}^{n}(R_i + T_i)/2} \times 100 \quad (4)$$

$$M_0 - M_t = K_0 t \quad (5)$$

$$\ln\left(\frac{M_0}{M_t}\right) = K_1 t \quad (6)$$

$$M_t = K_h\sqrt{t} \quad (7)$$

where n is the total number of sampling points, R_i and T_i are the mean drug release values for the two profiles at time point i, respectively. M_0 and M_t correspond to the drug amounts at time zero and dissolved at a particular time t, respectively. K_0, K_1 and K_n refer to the release kinetic constants obtained from the linear curves of zero-order, first-order and Higuchi models, respectively.

2.4.2. Drug release from individual pellet

Five coated pellets were randomly selected and subjected to drug release test using the dissolution apparatus 7 (400-DS, Agilent Technologies, USA) in deionized water as dissolution medium maintained at 37 ± 0.5 °C by heating jackets around the dissolution cells. The schematic diagram of a dissolution set in 400-DS dissolution apparatus 7 and the illustration of one dip cycle during the dissolution study is shown in Fig. 1. At the end of each dip interval, 1.5 mL of the medium was withdrawn through the auto-sampling port at the base of the dissolution cell into a HPLC vial on the sample collection tray. An equivalent volume of fresh medium was then introduced by a fluidic module. The amount of drug released in each dip interval was assayed and percentage drug released for one pellet at all dip intervals during the dissolution run were averaged to obtain the percentage drug release in each dip interval for the pellet (R_x) and the standard deviation of percentage drug release (SD_x) was also determined. For a total of five pellet samples in each run, the average percentage drug release in each dip interval (R_{avg}) and the standard deviation of percentage drug release in each dip interval (R_{std}) were calculated by Eqs. (8) and (9):

$$R_{avg} = \frac{1}{5}\sum_{i=1}^{5} R_x \quad (8)$$

$$R_{std} = \frac{1}{5}\sum_{i=1}^{5} (SD_x) \quad (9)$$

Cumulative drug release profiles over a period of 240 min under optimized settings in 400-DS were also plotted for individual sustained release pellets with 8% and 10% coats with a total of 15 pellets for each sample.

2.5. Design of experiment

A three-level, three-factorial Box–Behnken experimental design was used to evaluate the effects of the following design variables: A, dip speed (DPM); B, dip interval (min); C, volume of dissolution medium (mL), on the response variables. The objective of the design was to optimize the operational parameters used for conducting drug release test of individual pellet in the 400-DS dissolution apparatus 7 so that an appropriate amount of drug would be released within each dip interval and the variability would be minimal. The variability in drug release could be characterized by the standard deviation of inter-pellet percentage drug release rates. Hence, R_{avg} and R_{std} were chosen as the response variables in the design. The design variables along with their low, medium and high levels which were decided based on the results of preliminary experiments and together with response variables selected, are shown in Table 1. Based on the settings selected, 17 experiments, including 5 centre points, were generated. These centre points could be used to check for non-linearity and reproducibility of the system. Hence, no further replication of the design was needed. The experiments were conducted in a randomized order to reduce the effects of any possible factors that were not included in the study, particularly effects that were time-dependent. The possible effects of the design variables on the response variables were examined by fitting the responses to a least-order polynomial model using response surface methodology (RSM). Process optimization was then carried out based on the following criteria: (a) R_{avg} should be within 10–15%; (b) R_{std} should be minimal and not exceeding 5%.

2.6. Statistical analysis

The statistical analysis and process optimization were carried out using Design-Expert (Version 8.0.7.1, Stat-Ease Inc., USA). The response surface modelling was performed by a sequential procedure of collecting experimental data from each dissolution study, estimating polynomials and checking model adequacy. Regression analysis was conducted in coded units, where low level was coded as −1 while high level was coded as 1. The level of significance was defined as $p < 0.05$.

Process optimization was conducted by response optimization of Design-Expert software. Once a significant model for a response variable was generated, it formed the design space for this response variable and its desired value range could be input to predict the contributing design variables. Triplicate confirmation runs ($n = 5$) were subsequently conducted using the optimized parameters to check the validity of the models generated by RSM. The responses were averaged and compared with 95% prediction interval of the predicted value.

Table 1
Design variables and response variables for individual dissolution study of sustained release pellets in 400-DS dissolution apparatus 7.

Order		Design variables			Response variables	
Std[a]	Run[a]	A	B	C	R_{avg}	R_{std}
1	1	10	20	9	6.47	4.97
16	2[b]	20	40	9	8.19	3.80
5	3	10	40	6	10.21	7.22
6	4	30	40	6	6.91	5.30
8	5	30	40	12	13.71	8.62
10	6	20	60	6	12.21	5.83
7	7	10	40	12	25.83	13.93
11	8	20	20	12	5.05	2.98
17	9[b]	20	40	9	9.42	6.66
13	10[b]	20	40	9	10.88	7.77
3	11	10	60	9	26.54	14.74
12	12	20	60	12	12.02	4.93
14	13[b]	20	40	9	8.70	3.35
2	14	30	20	9	9.65	7.46
9	15	20	20	6	6.48	4.17
15	16[b]	20	40	9	9.07	5.29
4	17	30	60	9	12.03	6.28

(A) Refers to dip speed (DPM). (B) Refers to dip interval (min). (C) Refers to the volume of dissolution medium (mL).

[a] Std order refers to the original order of the design while run order refers to the exact running order of the experiments after randomization.

[b] Denotes centre points of the Box–Behnken design.

Table 2
Physicochemical properties of sustained release pellets with 8% and 10% coats.

Coat (%)	D_{50} (μm)	SP_{pel}	Aspect ratio	Roundness	W_s (μg)	Drug content (%)	Coat thickness (μm)
8	813 ± 24	0.35 ± 0.01	1.11 ± 0.05	1.13 ± 0.01	516 ± 10	48.7 ± 4.0	13.9 ± 2.2 (15.7%*)
10	849 ± 56	0.40 ± 0.03	1.10 ± 0.05	1.12 ± 0.01	513 ± 8	49.4 ± 3.5	15.7 ± 0.9 (5.9%*)

* The relative standard deviation of pellet coat thickness.

3. Results and discussion

3.1. Physicochemical characteristics of sustained release pellets

The physicochemical characteristics of sustained release pellets with 8% and 10% coats are summarized in Table 2. The theoretical drug loading of 50%, w/w was found to be very close to the assayed content values of 48.7 ± 4.0% and 49.5 ± 3.5% for the pellets with 8% and 10% coats, respectively. The two types of pellets were similar in their median pellet size, span, shape and individual weight. The D_{50} values were found to be between 800–900 μm and the span values were around 0.4, indicating narrow size distributions of the pellets. The roundness and aspect ratio values were close to 1 and average individual pellet weights of the two types of pellets were approximately 500 μg. The average coat thickness of pellets with 8% and 10% coats were 13.9 μm and 15.7 μm, respectively. The examples of cut pellets with 8% and 10% coat are shown in Fig. 2. The coat thickness of these two batches of sustained release pellets differed by less than expected but the relative standard deviation of pellets with 10% coat was found to be much lower (5.9%) than that of pellets with 8% coat (15.7%), indicating more uniform coat formation of pellets with 10% coat.

3.2. Drug release behaviour of ensemble pellets in dissolution apparatus 1 and 2

It has been established that the underlying mechanism of drug release from pellets coated with ethyl cellulose was primarily by drug diffusion through the water insoluble polymeric semi-permeable membrane, irrespective of the type of pellet core and release medium, and the pellet diameter remained relatively constant throughout the 8 h observation period (Muschert et al., 2009). Therefore, in this study, any change in pellet diameter during dissolution was assumed to be minimal. The following mass transport sequence was proposed for such system upon oral administration: water penetration into the system, partial dissolution of the drug and diffusion of dissolved drug out through the film coat into the surrounding bulk fluid. The drug diffusion process is the rate-limiting step during dissolution run. In this study, the pellet core produced by extrusion–spheronization had a high drug load of 50% and the pellet core was physically separated from the bulk medium during dissolution by the release rate controlling ethyl cellulose coat. Therefore, if the initial drug concentration in the core is above the drug solubility, the dissolution behaviour of such system with a spherical shape should ideally follow the zero-order release kinetics. The dissolution rate of the drug could be calculated using Eq. (10), which is derived from Fick's first law of diffusion.

$$\frac{dM}{dt} = \frac{4\pi DKC_S R_0 R_i}{\ln\left(\frac{R_0}{R_i}\right)} \times t \tag{10}$$

where dM is the mass transported in the time interval dt, D is the diffusion coefficient, K is the partition coefficient of the drug within the membrane, C_s is the solubility of the drug in the core, R_i and R_0 are the inner and outer radii of the spherical device, respectively.

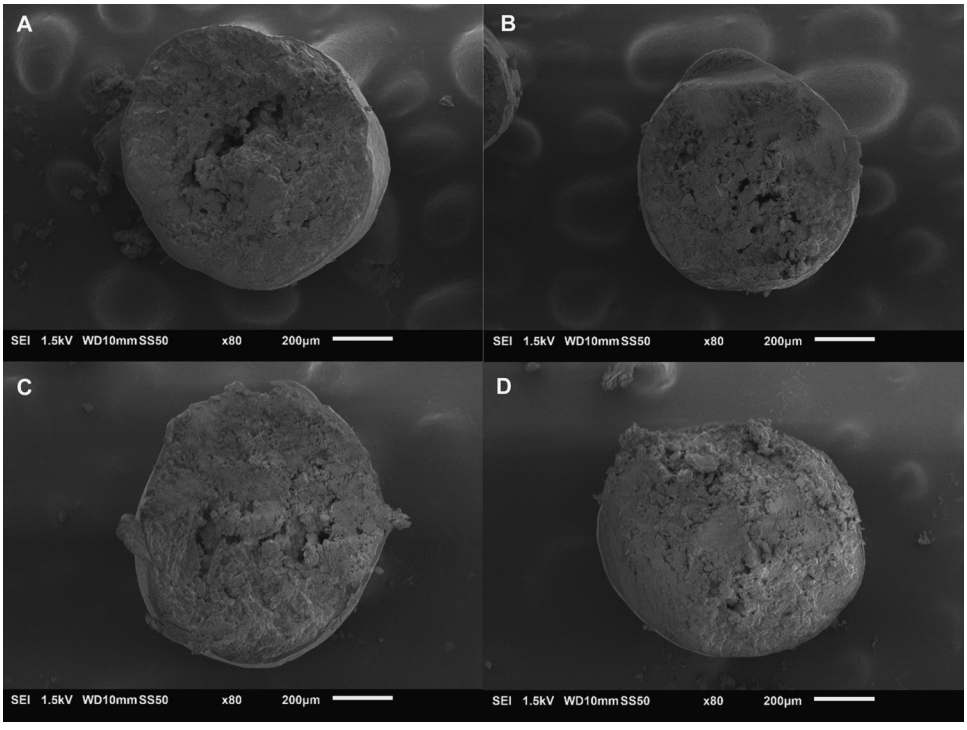

Fig. 2. SEM microphotographs showing the cross-section of sustained release pellets with 8% (A and B) and 10% coat (C and D).

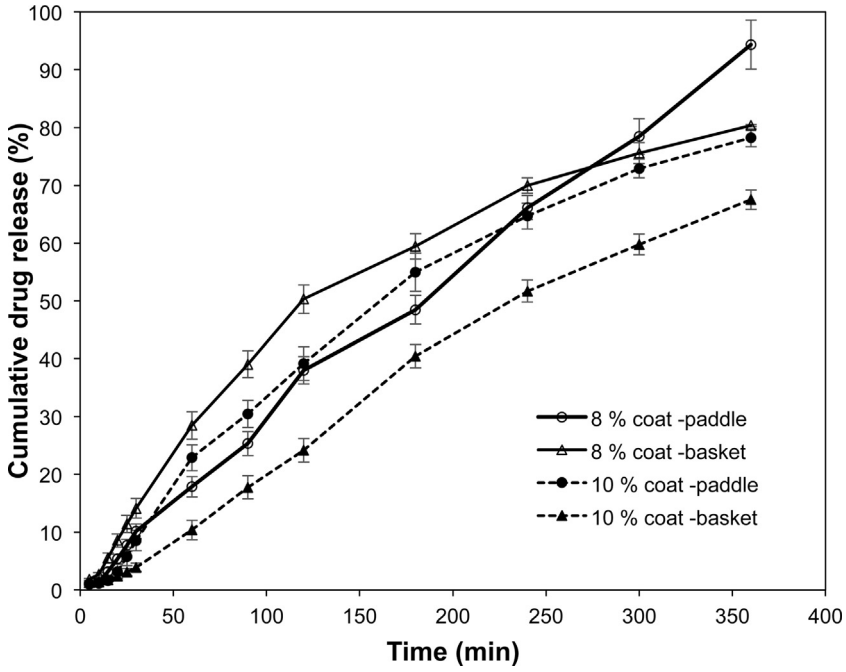

Fig. 3. Cumulative drug release profiles of ensemble of sustained release pellets with 8% and 10% coats using USP dissolution apparatus 1 (basket) and apparatus 2 (paddle).

The cumulative drug release profiles of ensemble pellets with 8% and 10% coats using the dissolution apparatus 1 and 2 are shown in Fig. 3 and the modelled dissolution data are summarized in Table 3. As can be seen, the two types of pellets exhibited different release patterns and rates in the two dissolution apparatus. With the paddle dissolution apparatus, the release profile of pellets with 8% coat was observed to follow the zero-order model ($R^2 = 0.990$) while that of pellets with 10% coat followed closer to the Higuchi model ($R^2 = 0.991$). However, when using the basket dissolution apparatus, the dissolution profile of pellets with 8% coat fitted better to Higuchi model ($R^2 = 0.989$) and that of pellets with 10% coat followed closer to the zero-order release model ($R^2 = 0.990$). More importantly, for both pellet batches with 8% and 10% coats, the values of difference factor $f1$ were above 15, indicating that their drug release profiles obtained from the two dissolution apparatus were significantly different. Therefore, the cumulative release profiles of ensemble pellets seemed to be confounded when USP dissolution apparatus 1 and 2 were applied for differentiating the release properties of the sustained release pellets.

For the paddle dissolution apparatus, pellets with 8% coat were observed to float on the surface of dissolution medium throughout the drug release test. T10 and T50 values from the drug release profile were found to be longer than those obtained using the basket dissolution apparatus, which suggested a slower drug release rate of 8% coat pellets with the paddle apparatus. On the other hand, the pellets with 10% coat were found to float initially and then sink into the dissolution medium during the drug release test with the paddle dissolution apparatus. The shorter T10 and T50 values of the release profile indicated a faster drug release rate than that with the basket dissolution apparatus. Floating of pellets had significantly reduced the effective surface area in contact with the dissolution medium and thus slowed down the drug release rate (Pillay and Fassihi, 1998). Moreover, excessive variability in drug release rate has also been reported due to the highly heterogeneous fluid flow and complex hydrodynamics with the paddle dissolution apparatus (Ameur and Bouzit, 2013). The use of a wire or glass helix sinker, enclosing the dosage unit, to prevent it from floating (Nakamichi et al., 2001) could also significantly impede drug release and lead to an uneven dissolution from the sides of the 'sinked' dosage form (Burgess et al., 2004). Therefore, the cumulative release profile of the ensemble pellets obtained by the paddle method may not truly reflect the actual drug release mechanism of sustained release pellets and may even result in erroneous interpretations of the applied pellet coat properties.

With the basket dissolution apparatus, the pellet floating problem was avoided by entrapping the pellets in the dissolution basket. However, during the dissolution studies, conditions such as the clumping of pellets could occur due to limits imposed by the confined space and low shear force in the basket (D'Arcy et al., 2006). As can be seen in Fig. 3, the cumulative release profile of ensemble pellets with 8% coat appeared to have a two-phase release profile with a more rapid release initial phase followed by a slower release second phase. It was likely that the initial faster release behaviour of the sustained release pellets was the outcome of turbulence among the pellets with propensity to float while

Table 3
Coefficients of determination obtained from ensemble of sustained release pellets with 8% and 10% coats according to three mathematical models and comparison of their dissolution profiles using T10, T50 and f1.

Coat (%)	Dissolution apparatus	Zero-order		Higuchi		First-order		T10 (min)	T50 (min)	$f1$
		R^2	K_0	R^2	K_h	R^2	K_1			
8	Paddle	0.990	0.253	0.982	5.331	0.726	0.005	26.0 ± 3.5	125.6 ± 4.4	20
8	Basket	0.925	0.238	0.989	5.158	0.689	0.004	19.5 ± 3.3	109.0 ± 11.3	
10	Paddle	0.959	0.238	0.991	5.126	0.693	0.005	32.6 ± 2.4	152.8 ± 13.1	30
10	Basket	0.990	0.202	0.967	4.223	0.824	0.005	56.8 ± 6.1	225.4 ± 7.5	

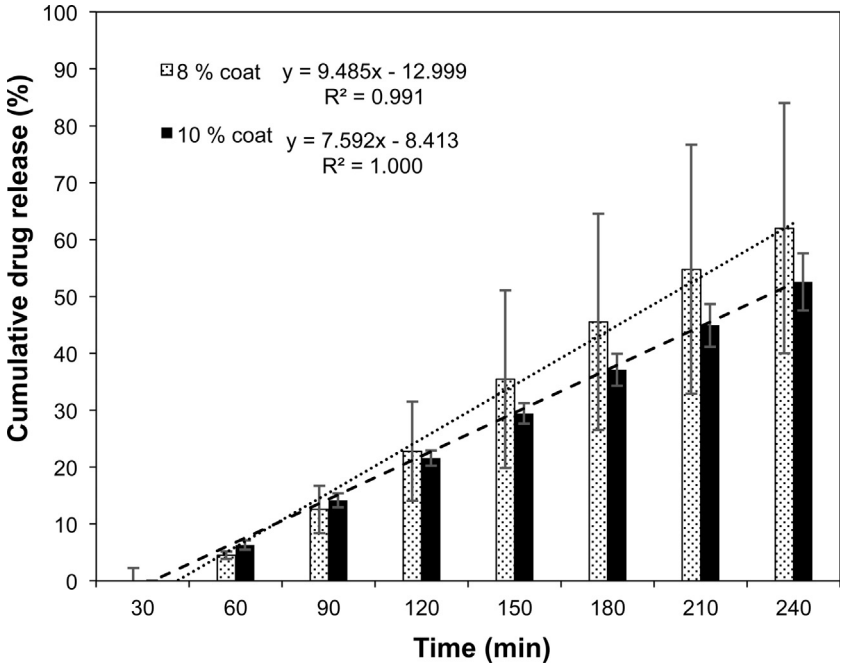

Fig. 4. Cumulative drug release profiles of individual sustained release pellets with 8% and 10% coats and their linear correlation values.

being confined within the dissolution basket. In the second phase, as the tendency for the pellets to float reduced with the imbibed dissolution medium, clustering of the pellets may occur at the bottom of the basket and thereby limited the surface area available for the release of drug. These are possible explanations for the two-phase release behaviour for the pellets with 8% coats when drug release test was carried out using the basket dissolution apparatus. For pellets with 10% coat, the zero-order drug release rate was obtained as possibly, due to their heavier weights with more complete and uniform coating on the pellets, the outcome was less buoyant pellets with much reduced tendency to float. Thus, the pellets with 10% coat were more easily dispersed in the dissolution media and wetted to form the dissolution barrier capable of providing a constant drug diffusion rate. Therefore, in the subsequent studies, the pellets with 10% coat were selected as model pellets for the investigation into the feasibility of individual pellet dissolution using the 400-DS dissolution apparatus 7 by the DoE approach.

Table 4
Estimated response surface coefficients and model significance.

Coefficient	R_{avg}	R_{std}
Intercept	−92.65	8.63
A	8.45	−1.05
B	1.09	0.35
C	9.35	—[a]
AB	−0.07	−0.01
AC	−0.88	—[a]
A^2	−0.18	0.04
A^2B	1.22×10^{-3}	—[a]
A^2C	0.02	—[a]
Significance of the model developed	0.000	0.003
R^2 (%)	98.75	72.02
R^2_{pre} (%)	97.50	62.70
R^2_{adj} (%)	91.46	49.19
Adequate precision[b]	31.27	10.11
Lack of fit significance[c]	0.60	0.45

(A) Refers to dip speed (DPM). (B) Refers to dip interval (min). (C) Refers to the volume of dissolution medium (mL). (—) It is desirable for the model to fit.
[a] The term was excluded from the model at $\alpha = 0.05$ level.
[b] Measures the signal to noise ratio. A ratio greater than 4 is desirable.
[c] Implies the chance that a 'lack of fit' value could occur due to noise. Non-significant lack of fit is good.

3.3. Optimizing operational parameters for the dissolution of individual sustained release pellet in the 400-DS dissolution apparatus 7 by the DoE approach

The results of R_{avg} and R_{std} from a total of 17 experiments in the DoE are shown in Table 1. The results were analysed by ANOVA and RSM to determine the possible main and interaction effects of design variables on the responses. The estimated response surface coefficients and model significance are listed in Table 4.

3.3.1. Influence of design variables on the average percentage drug release in each dip interval

The R_{avg} was found to be between 5.05% and 26.54% (Table 1). A modified cubic model ($p = 0.000$) was successfully developed to describe the relationship between the design variables with R_{avg} and there was insignificant lack of fit of the model ($p = 0.598$). Theoretically, R^2 tells how well a model fits the data, whereas R^2_{pre} reflects how well the model could predict the future data. As the R^2 value always increases when more design variables are added to the model, R^2_{adj} would be a better estimation of the degree of relationship between design variables and responses (Meyers and Montgomery, 2002). As can be seen in Table 4, the R^2_{pre} (97.50%) and R^2_{adj} (91.46%) values indicated that the model developed for R_{avg} fitted the data well and was good in its prediction of R_{avg}. It was also found that R_{avg} was significantly affected by the dip speed ($p = 0.000$) and dip interval ($p = 0.000$). Dip speed interacted with dip interval and volume of dissolution medium but collectively, they showed a negative effect on the R_{avg} ($p < 0.001$). The squared term of dip speed was observed to contribute negatively to the R_{avg} ($p = 0.000$). The squared term of dip speed was shown to interact with dip interval and volume of dissolution medium but contributed positively to the R_{avg} ($p < 0.01$).

These findings suggested that a higher dip speed would increase R_{avg} and there are synergistic effects with other variables. A higher dip speed of the reciprocating holder in the 400-DS dissolution apparatus 7 could contribute to stronger

Table 6
Predicted and actual characteristics of R_{avg} and R_{std} under optimized conditions in 400-DS dissolution apparatus 7.

Actual characteristics from triplicated confirmation runs					Predicted characteristics (SD)	95% PI low	95% PI high
	1	2	3	Mean (SD)			
R_{avg}	9.66	9.62	10.31	9.86 (0.39)	11.19 (0.96)	9.33	13.04
R_{std}	5.85	6.39	5.98	6.07 (0.29)	5.25 (2.01)	2.21	8.29

SD refers to the standard deviation of actual characteristics from triplicated confirmation runs.

hydrodynamic forces in the dissolution medium. The diffusion controlled dissolution process could be explained by the commonly used Nernst–Brunner equation as follows (Siepmann and Siepmann, 2013):

$$\frac{dm}{dt} = \frac{S \times d}{\delta} \times (c_s - c_t) \quad (11)$$

where dm is the amount of drug which dissolves in the time interval dt, S denotes the surface area available for drug diffusion, d is the diffusion coefficient of the drug within the unstirred liquid boundary layer, δ is the thickness of the boundary layer, c_s and c_t are the concentrations of dissolved drug at the pellet surface and in the dissolution medium at time t, respectively.

Nernst–Brunner equation could be used to elaborate the transport of drug molecules through the unstirred liquid boundary layer surrounding a sustained release pellet. During the dissolution study of individual sustained release pellet using a high dip speed, a more efficient agitation of dissolution medium decreased the thickness of unstirred boundary layer δ, through which the drug diffused. The reduced boundary layer thickness consequently resulted in a faster drug diffusion rate, or higher dm/dt. The observed increase in R_{avg} was likely to be due to a more efficient transport of drug molecules between the unstirred boundary layer and the well agitated dissolution medium. The dip interval and volume of dissolution medium could also influence dm/dt by altering c_t, concentration of dissolved drug in the dissolution medium.

3.3.2. Influence of design variables on the standard deviation of average percentage drug release

The R_{std} was found to range from 3.35% to 14.74% (Table 1). A significant quadratic model was successfully developed ($p = 0.003$) to describe the effects of design variables on R_{std}. The model had relatively poor R^2_{pre} (62.70%) and R^2_{adj} (49.19%) values but could still serve to facilitate better understanding of the relationship between design variables and R_{std}. ANOVA revealed that dip speed had a significant negative effect ($p = 0.04$) on R_{std}. The squared term of dip sed ($p = 0.003$) was also observed to be significant, suggesting the relationship between dip speed and R_{std} was non-linear. A reasonably high dip speed generated an unimpeded and constant flow of the dissolution medium, which could reduce the unstirred boundary layer to an optimal thickness around the pellet during the drug release test. However, if the dip speed had been too high, the distribution of shear forces around the unstirred layer would likely be non-uniform. Such uneven shear forces might lead to heterogeneity in the thickness of the unstirred boundary layer, resulting in highly variable, non-reproducible drug release rates (Siepmann and Siepmann, 2013).

The positive coefficient ($p = 0.053$) of dip interval indicated that R_{std} increased when the dip interval was longer, probably due to the accumulation of dissolved drug in the dissolution medium over a longer dip interval. However, if drug concentration was excessively high, the presentation of a sink condition in the dissolution medium could be impeded, resulting in less consistent drug diffusion rate. The dip speed and dip interval were also found to have a synergistically negative effect on R_{std} ($p = 0.018$), which revealed that neither of these two operational parameters in 400-DS dissolution apparatus 7 contributed to the variation in drug release rate of a sustained release pellet independently.

Table 5
Results of numerical optimization with the highest D-value and the optimized conditions chosen for use in individual dissolution by 400-DS dissolution apparatus 7.

Number	A	B	C	R_{avg}	R_{std}	D-value
1	15.53	29.37	11.94	10.32	4.97	1
2	16.23	31.29	11.95	10.06	4.99	1
3	13.26	23.59	11.45	10.70	4.95	1
4	15.77	30.02	11.63	10.06	4.98	1
5	13.35	22.60	11.34	10.10	4.75	1
6	15	30	12	11.19	5.25	0.984

(A) Refers to dip speed (DPM). (B) Refers to dip interval (min). (C) Refers to the volume of dissolution medium (mL).

3.4. Optimization of dissolution parameters in the 400-DS dissolution apparatus 7

As the experimental range of R_{avg} was from 5.05 to 26.54% (Table 1), a medium average percentage drug release was chosen and set at 10–15%, in order to allow for sufficient number of samples in each dissolution test and to avoid too fast drug release. For R_{std}, the target range was set to a minimum of 3–5% to reduce the variability in average drug release in each dip interval. Table 5 shows the results of numerical optimization with highest desirability by the numerical optimization function in Design-Expert statistical software. Desirability value (D-value) is a measure of the accuracy of prediction. The closer the predicted response to the target, the closer the D-value is to one. Although five combinations of dependent variables with the highest D-value (Numbers 1–5) were generated by the software, they were very close to each other and not the best operating parameters from the technical point of view. For instance, it was operationally challenging to set the dip speed of the reciprocating holder to exactly 15.53 DPM and the dissolution volume to be 11.94 mL. Therefore, taking everything into consideration, optimized parameters with a slightly lower D-value of 0.984 were chosen (Number 6) to confirm the validity of the models generated by RSM.

Triplicated confirmation runs of individual dissolution in 400-DS dissolution apparatus 7 under the chosen optimized parameters were conducted. The results are summarized in Table 6 and the mean of R_{avg} and R_{std} were compared to the predicted response. 95% PI was defined as 95% of prediction interval generated from the models developed in the DoE and was used to predict the 95% confidence interval of response variable for the next single observation under a specific setting of design variables. 95% PI could help to assess the response of confirmation experiments where more than one experimental run at the optimized settings of design variables were carried out. If the response of confirmation experiments were within the 95% PI then the confirmation would be considered a success. As can be seen, both the average R_{avg} and R_{std} fell within the 95% PI of predicted responses and their standard deviations were rather low, indicating the drug release profiles of individual sustained release pellets in the 400-DS dissolution apparatus 7 could be accurately predicted by the RSM models developed in this study.

3.5. Comparison of release behaviours between the ensemble pellets and individual pellets

The cumulative release profiles of ensemble pellets were confounded by the conditions of pellets during dissolution studies, such as whether they tended to float on the surface of dissolution medium or underwent dissolution as an aggregated cluster in the dissolution basket. Hence, the ensemble pellets release profile represents both the cumulative release of all the pellets within as well as their potential interactive, like aggregation, or non-interactive, like floating, tendencies. Thus, the ensemble pellets release may not represent the drug release behaviours of individual pellets. The 400-DS dissolution apparatus 7 has a variety of customized reciprocating holders, which are available to accommodate dosage forms of different sizes and shapes. These sample holders could contain the whole dosage form and be agitate according to the programmed agitation rate. Such agitation allows the dosage form to be suspended freely in the dissolution medium. Hence, the confounding effects caused by conditions such as bunching or aggregations of pellets during dissolution studies were minimized. The cumulative release profiles of individual sustained release pellets with 8% and 10% coats are shown in Fig. 4. Linear relationships between cumulative drug release and dissolution time were obtained for sustained release pellets with 8% and 10% coats with $R^2 = 0.991$ and 1.000, respectively. The observed constant drug release rate supported the zero-order release model of sustained release pellets with 10% coat using the basket dissolution apparatus. As the pellet samples chosen for individual dissolution studies were consistent in their diameters (850 μm sieve mesh aperture size), the effects of pellet size and surface area on the dissolution rate were minimized. The higher standard deviation in the individual drug release profiles indicated greater differences in the coat quality of pellets with 8% coat. Similarly, the significantly lower inter-pellet variability in cumulative drug release for sustained release pellets with 10% coat also suggested a more uniform coat formation with more ethyl cellulose polymer deposited onto the pellet surface. This is also corroborated by the coat thickness measurement using SEM, in which the relative standard deviation of coat thickness for pellets with 10% coat was much lower. Therefore, the coat quality of sustained release pellets can be distinguished by the standard deviations of cumulative drug release profiles obtained from individual dissolution methodology using the 400-DS dissolution apparatus 7.

4. Conclusion

Pellet floating and clumping during dissolution studies of sustained release pellets with dissolution apparatus 1 and 2 could confound the release profiles of ensemble pellets and therefore the results did not characterize the actual drug release behaviour of individual sustained release pellets. This study explored the feasibility of the 400-DS dissolution apparatus 7 to distinguish the individual coat quality of sustained release pellets and to elaborate their underlying drug release mechanisms. A systematic DoE approach was used to optimize the operational parameters of the 400-DS dissolution apparatus 7 and mathematical models were successfully developed to describe the relationships between design variables and responses. The dip speed and dip interval of the reciprocating holder were found to be critical parameters that affected the drug release rate of sustained release pellets by changing the hydrodynamics and sink conditions in the dissolution medium.

Individual pellet dissolution study for sustained release pellets in 400-DS dissolution apparatus 7 was conducted using the optimized parameters and subsequently compared with their ensemble release profile. The drug release mechanism of sustained release pellets could be elaborated by the cumulative release profile of individual pellets and the standard deviation of drug release rate was found to be a useful coat quality indicator of the batch of sustained release pellets. The individual pellet dissolution using the 400-DS dissolution apparatus 7 is a promising technique to evaluate pellet coat quality and provide better understanding of the actual drug release mechanism of a batch of sustained release pellets.

Acknowledgements

The authors would like to acknowledge the financial support from GEA-NUS PPRL fund (N-148-000-008-001) and A*STAR SERC Grant No. 102 161 0049 (R-148-000-157-305). Agilent Technologies is also acknowledged with appreciation for the loan of the 400-DS dissolution apparatus 7. Min Xu is a recipient of the National University of Singapore Graduate Research Scholarship.

References

Ameur, H., Bouzit, M., 2013. 3D hydrodynamics and shear rates' variability in the United States pharmacopeia paddle dissolution apparatus. Int. J. Pharm. 452, 42–51.
Andersson, M., Holmquist, B., Lindquist, J., Nilsson, O., Wahlund, K.-G., 2000. Analysis of film coating thickness and surface area of pharmaceutical pellets using fluorescence microscopy and image analysis. J. Pharm. Biomed. Anal. 22, 325–339.
Borgquist, P., Nevsten, P., Nilsson, B., Wallenberg, L.R., Axelsson, A., 2004. Simulation of the release from a multiparticulate system validated by single pellet and dose release experiments. J. Control. Release 97, 453–465.
Bouwman, A.M., Bosma, J.C., Vonk, P., Wesselingh, J.H.A., Frijlink, H.W., 2004. Which shape factor (s) best describe granules? Powder Technol. 146, 66–72.
Burgess, D.J., Crommelin, D.J., Hussain, A.S., Chen, M.-L., 2004. Assuring quality and performance of sustained and controlled release parenterals. Eur. J. Pharm. Sci. 21, 679–690.
Chan, L.W., Chan, W.Y., Heng, P.W.S., 2001. An improved method for the measurement of colour uniformity in pellet coating. Int. J. Pharm. 213, 63–74.
Crist, G.B., 2009. Trends in small-volume dissolution apparatus for low-dose compounds. Dissolution Technol. 16, 19–22.
Depypere, F., Van Oostveldt, P., Pieters, J.G., Dewettinck, K., 2009. Quantification of microparticle coating quality by confocal laser scanning microscopy (CLSM). Eur. J. Pharm. Biopharm. 73, 179–186.
D'Arcy, D.M., Corrigan, O.I., Healy, A.M., 2006. Evaluation of hydrodynamics in the basket dissolution apparatus using computational fluid dynamics – dissolution rate implications. Eur. J. Pharm. Sci. 27, 259–267.
Ensslin, S., Moll, K.P., Metz, H., Otz, M., Mäder, K., 2009. Modulating pH-independent release from coated pellets: effect of coating composition on solubilization processes and drug release. Eur. J. Pharm. Biopharm. 72, 111–118.
Ensslin, S., Moll, K.P., Paulus, K., Mäder, K., 2008. New insight into modified release pellets–internal structure and drug release mechanism. J. Control. Release 128, 149–156.
Haaser, M., Karrout, Y., Velghe, C., Cuppok, Y., Gordon, K.C., Pepper, M., Siepmann, J., Rades, T., Taday, P.F., Strachan, C.J., 2013. Application of terahertz pulsed imaging to analyse film coating characteristics of sustained-release coated pellets. Int. J. Pharm. 457, 521–526.
Hogan, J.E., 1995. Sugar coating. In: Cole, G.C., Hogan, J.E., Aulton, M.E. (Eds.), Pharmaceutical Coating Technology. Taylor and Francis, UK, pp. 53–63.
Hoffman, A., Donbrow, M., Gross, S.T., Benita, S., Bahat, R., 1986. Fundamentals of release mechanism interpretation in multiparticulate systems: determination of substrate release from single microcapsules and relation between individual and ensemble release kinetics. Int. J. Pharm. 29, 195–211.
Ho, L., Cuppok, Y., Muschert, S., Gordon, K.C., Pepper, M., Shen, Y., Siepmann, F., Siepmann, J., Taday, P.F., Rades, T., 2009. Effects of film coating thickness and drug layer uniformity on in vitro drug release from sustained-release coated pellets: a case study using terahertz pulsed imaging. Int. J. Pharm. 382, 151–159.
Meyers, R.H., Montgomery, D.C., 2002. Response Surface Methodology. Wiley, New York, NY.
Muschert, S., Siepmann, F., Leclercq, B., Carlin, B., Siepmann, J., 2009. Prediction of drug release from ethylcellulose coated pellets. J. Control. Release 135, 71–79.
Moore, J.W., Flanner, H.H., 1996. Mathematical comparison of dissolution profiles. Pharm. Technol. 20, 64–74.
Nakamichi, K., Yasuura, H., Fukui, H., Oka, M., Izumi, S., 2001. Evaluation of a floating dosage form of nicardipine hydrochloride and hydroxypropylmethylcellulose acetate succinate prepared using a twin-screw extruder. Int. J. Pharm. 218, 103–112.
Pillay, V., Fassihi, R., 1998. Evaluation and comparison of dissolution data derived from different modified release dosage forms: an alternative method. J. Control. Release 55, 45–55.

Ringqvist, A., Taylor, L.S., Ekelund, K., Ragnarsson, G., Engström, S., Axelsson, A., 2003. Atomic force microscopy analysis and confocal Raman microimaging of coated pellets. Int. J. Pharm. 267, 35–47.
Sarkar, S., Heng, P.W.S., Liew, C.V., 2013. Insights into the functionality of pelletization aid in pelletization by extrusion–spheronization. Pharm. Dev. Technol. 18, 61–72.
Siepmann, J., Siepmann, F., 2013. Mathematical modeling of drug dissolution. Int. J. Pharm. 453, 12–24.
Turi, P., Brusco, D., Maulding, H.V., Tausendfreund, R.A., Michaelis, A.F., 1972. Rapid determination of color stability of tablet formulations. J. Pharm. Sci. 61, 1811–1814.
Wesdyk, R., Joshi, Y.M., Jain, N.B., Morris, K., Newman, A., 1990. The effect of size and mass on the film thickness of beads coated in fluidized bed equipment. Int. J. Pharm. 65, 69–76.
Zahirul, M., Khan, I., 1996. Dissolution testing for sustained or controlled release oral dosage forms and correlation with in vivo data: challenges and opportunities. Int. J. Pharm. 140, 131–143.
Zhou, M.X., Shoudt, D., Calderon, G., Feng, M., 2007. Application of USP Apparatus 7 to in vitro drug release in scopolamine transdermal systems. Dissolution Technol. 14, 25–29.