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Research Article

Application of Quality by Design (QbD) Approach to Ultrasonic Atomization Spray Coating of Drug-Eluting Stents

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Abstract. The drug coating process for coated drug-eluting stents (DES) has been identified as a key source of inter- and intra-batch variability in drug elution rates. Quality-by-design (QbD) principles were applied to gain an understanding of the ultrasonic spray coating process of DES. Statistically based design of experiments (DOE) were used to understand the relationship between ultrasonic atomization spray coating parameters and dependent variables such as coating mass ratio, roughness, drug solid state composite microstructure, and elution kinetics. Defect-free DES coatings composed of 70% 85:15 poly(DL-lactide-co-glycolide) and 30% everolimus were fabricated with a constant coating mass. The drug elution profile was characterized by a mathematical model describing biphasic release kinetics. Model coefficients were analyzed as a DOE response. Changes in ultrasonic coating processing conditions resulted in substantial changes in roughness and elution kinetics. Based on the outcome from the DOE study, a design space was defined in terms of the critical coating process parameters resulting in optimum coating roughness and drug elution. This QbD methodology can be useful to enhance the quality of coated DES.

KEY WORDS: biodegradable polymers; coating; drug-eluting stents; processing; quality by design.

INTRODUCTION

Drug-Eluting Stents

Cardiovascular disease is the number one cause of death in the world today. The underlying cause is most often due to atherosclerosis or a buildup of plaque in the coronary blood vessels resulting in blockage due to narrowing (*i.e.*, stenosis). Percutaneous coronary intervention (PCI), or coronary angioplasty, is commonly used to increase blood flow through the artery. The procedure involves inflating a balloon within the narrowed vessel to open the artery. A stent is often embedded in the artery wall during PCI to keep the vessel open (1,2). Restenosis (re-blockage) rates have been cut from 20% to 25% for bare metal stents to less than 5% by use of stents coated with a drug mixed in polymer that reduces cell proliferation as the drug is slowly released (or eluted) (3,4). Everolimus and sirolimus are commonly used antiproliferative drugs in stent coatings (*e.g.*, XIENCE™ Everolimus Eluting Coronary Stent System by Abbott Laboratories and CYPHER™ Sirolimus-eluting Coronary Stent by Cordis, respectively) that have greatly reduced the need for re-intervention procedures because of restenosis.

Variability in Drug Elution Rates

The drug dose and elution rate are important factors for achieving maximal therapeutic benefit. Since drug-eluting stents (DES) are permanently implanted in patients with life-threatening artery disease, there is a very low tolerance for inadequate drug delivery. As such, inter- and intra-batch variability in drug elution rates is a critical quality control issue for DES (5,6). Unlike traditional drug products, batch sizes for DES manufacturing are small and extensive end-product testing is not feasible. Alternatively, in-process quality controls may be applied based on a good understanding of the stent coating process.

One common method of coating stents (7), and sometimes balloons (8), is by spraying an ultrasonically atomized solution of drug, polymer, and solvent onto the device. Coatings have been applied ultrasonically to medical devices since the 1980s and to stents since 2001 (7,9). By this method, a polymer/drug solution flows onto the surface of a vibrating nozzle causing the liquid to form standing capillary waves.

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Increasing the power of the vibrations increases the height of the capillary waves until a critical amplitude is reached at which small droplets of liquid are ejected from the tops of the waves (10–13). The critical frequency is a constant for a particular nozzle mass and design. These droplets are much smaller compared to droplets generated by other methods previously used by stent manufacturers, such as the air brush method. A focusing gas flowing through the center of the nozzle controls the shape of the mist of droplets forming at the nozzle tip (14) and transports it to the stent, where the droplets impact, flow, and evaporate on the stent.

Variation in this coating process may be the cause of the inter- and intra-batch variability in drug elution rates mentioned above, because an inconsistent coating mass will alter the drug loading on the stent. For spray coating methods such as ultrasonic spray coating, the quality control parameter that determines drug loading is coating mass. Coating mass is impacted by the efficiency of the coating process, which is measured as the "mass ratio," or coating mass divided by the mass of solids sprayed while the stent is being coated. The mass ratio for ultrasonic coating of stents is reported to be in the range of 30%–70% (7,9). The mass ratio indicates how processing changes can impact the amount of spray solidifying on the stent and hence coating mass and drug loading. The ultrasonic coating process may also affect the drug spatial distribution on the stent. For example, coating processes that result in higher coating solution evaporation rates were shown to reduce the time available for the drug phase to separate from the polymer matrix, resulting in less drug at the coating surface and lower drug release (15,16). Higher evaporation rates for ultrasonic spray coating stents occurs when a more volatile spray solution is used or the volume of solution accumulated on the stent surface at any instant during solution spraying is reduced as a result of the selected processing parameters. Roughness was hypothesized to influence elution kinetics and/or coating loss by its effect on surface area or coating thickness changes between peaks and troughs. In this study, we relate variation in coating processing to coating mass, distribution of drug in the coating, roughness and drug release.

Publications discussing the elution kinetics of DES or balloons generally do not provide values for the ultrasonic coating parameters used to fabricate test specimens except in a couple of instances (8,11). Only two studies are available that report any relationship between DES ultrasonic coating parameters and coating properties (17,18) There are no publications reporting relationships between ultrasonic process parameters and elution kinetics. To understand how processing affects coating characteristics and drug release kinetics, we studied the relationships between ultrasonic coating processing, coating mass, drug solid state composite microstructure in the coating, and elution kinetics for stent coatings made of 85:15 poly(DL-lactide-co-glycolide) (PLGA 85:15) (4,19–21) and everolimus (3,22) using the MediCoat stent coating system (11,17,23). These materials and coating apparatus are used by manufacturers to fabricate DES. The purpose of this study was to identify the critical quality attributes influencing inter- and intra-batch variability in drug elution rates for DES (5,6).

Quality-by-Design Methodology

This is the first published report in which quality-by-design (QbD) methodology (24) was used to study ultrasonic spray coating of DES. The term QbD is defined in ICH Q8(R2) (25) as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management." Implementation of this paradigm requires a thorough understanding of the product and its manufacturing process.

The QbD approach was used here to understand quality problems associated with the production of DES and to identify a control strategy that would reduce their prevalence. The coating process was identified to be the main reason for intra- and inter-batch variability, so this was further evaluated by performing a risk analysis using the Ishikawa approach (26) to elucidate relationships between DES materials, ultrasonic coating parameters, coating quality control (QC) criterion (*e.g.*, mass, drug solid state composite microstructure, roughness), and performance metrics (drug elution) (15,27).

The first step in implementing this QbD approach involved determining the quality target product profile (QTPP) of the DES. This is a prospective summary of the quality characteristics that, under ideal circumstances, will be achieved to ensure the desired quality level, taking into account safety and efficacy of the drug product. Next, physical, chemical, biological, and microbiological properties (critical quality attributes (CQAs)) were defined for this particular drug-device combination within an appropriate limit, range, or distribution to ensure the desired product quality (see Appendix). The attributes and process parameters were linked to the CQAs to perform a risk assessment which involved collating all the factors that could influence the quality of DES due to failure of the manufacturing process and controls (*e.g.*, failure of assay tests, variance in the rate of drug elution, physical and chemical instability of the coated DES, and microbiological contamination). The drug elution rate variability was identified as the most common cause of DES drug quality failures, so factors affecting this were evaluated in this study using the Ishikawa approach (26) (Fig. 1). From this, we concluded that the coating properties that most likely will affect drug elution include coating thickness (mass), drug loading, and drug/polymer distribution. Since coating mass and drug loading are generally preset, experiments were focused on studying the impact of variation in coating process conditions on drug elution kinetics, mass ratio, coating roughness, and drug solid state composite microstructure. Using the experimental data, the design space and a control strategy were determined for the ultrasonic spray coating process. Enhanced process understanding gained from implementing this QbD approach can be used for continuous improvement of the product over its life cycle.

MATERIALS AND METHODS

Design of Experiments

Our hypothesis was that the coating properties are determined by the solvent evaporation rate during spraying, which, in turn, is determined by the ultrasonic spray coating process parameters. Therefore, we studied the impact of six ultrasonic coating parameters (coating solution flow rate, nozzle-to-stent spray droplet travel distance, temperature, solvent volatility, shroud gas flow rate, and stent rotation rate during spray coating) that are most likely to result in lower evaporation

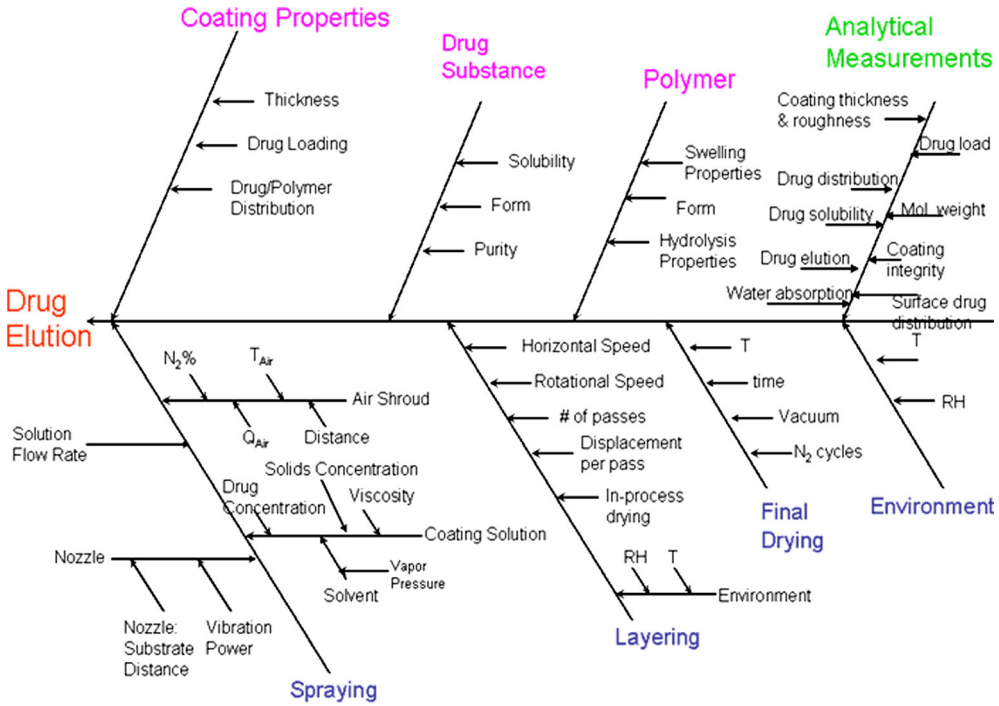

Fig. 1. Ishikawa diagram of factors affecting drug elution

rates and/or greater volume of solution on coated stents. These six factors were identified from the Ishikawa diagram (Fig. 1) that was performed during risk assessment. Preliminary experiments were performed to determine the maximum range of the six chosen ultrasonic coating parameter values that would produce coatings with a constant average coating mass and no defects. Next, two separate statistically based design of experiment (DOE) studies were developed to evaluate the relationships between processing, coating characteristics, and elution kinetics and to define a product quality design space.

A screening design based on a fractional factorial DOE was used to gauge the relative importance of the six ultrasonic coating process variables (DOE 1). The objective was to evaluate the effect of the ultrasonic coating parameters (Table I) on the following performance metrics: mass of the coating on the stent, mass ratio, drug composite microstructure, roughness, and amount of drug released after 30 min and 48 h of soaking. The rationale for selecting these five DOE responses was outlined in the "INTRODUCTION" under the "Variability in Drug Elution Rates" section. For each run, two stents were coated consecutively (one stent for coating structure analysis and the other for drug release experiments). Analysis of DOE 1 data using Minitab statistical software showed that solution flow, % tetrahydrofuran (THF) in the solution, nozzle-stent distance, and temperature were statistically significant with respect to roughness and drug elution after both 30 min and 48 h of soaking. A normal plot for drug elution at 48 h is included in this paper as an example (Fig. 2).

A full factorial DOE (DOE 2) was then carried out using the four statistically significant parameters that were identified from DOE 1. Two parameters, rotation and shroud pressure, were not included in DOE 2 because analysis of DOE 1 data showed they had the least effect on elution and there were no physical reason to anticipate that they would vary significantly. DOE 2 included 17 different methods or combinations of ultrasonic coating parameters to make DES coatings (Table II). The choice of ranges for DOE 2 were adjusted to

Table I. Values of Ultrasonic Coating Parameters for Design of Experiments (DOE) 1 and 2

Parameter	Minimum	Center	Maximum
DOE 1:			
% THF	40	60	100
Solution flow (μL/min)	15	25	35
Nozzle to stent distance (mm)	7.5	12.5	17.5
Temperature (°C)	19	24	29
Rotation (rpm)	60	90	120
Shroud pressure (psi)	2	3	4
DOE 2:			
% THF	40	70	100
Solution flow (μL/min)	25	35	45
Nozzle to stent distance (mm)	7.5	12.5	17.5
Temperature (°C)	19	24	29

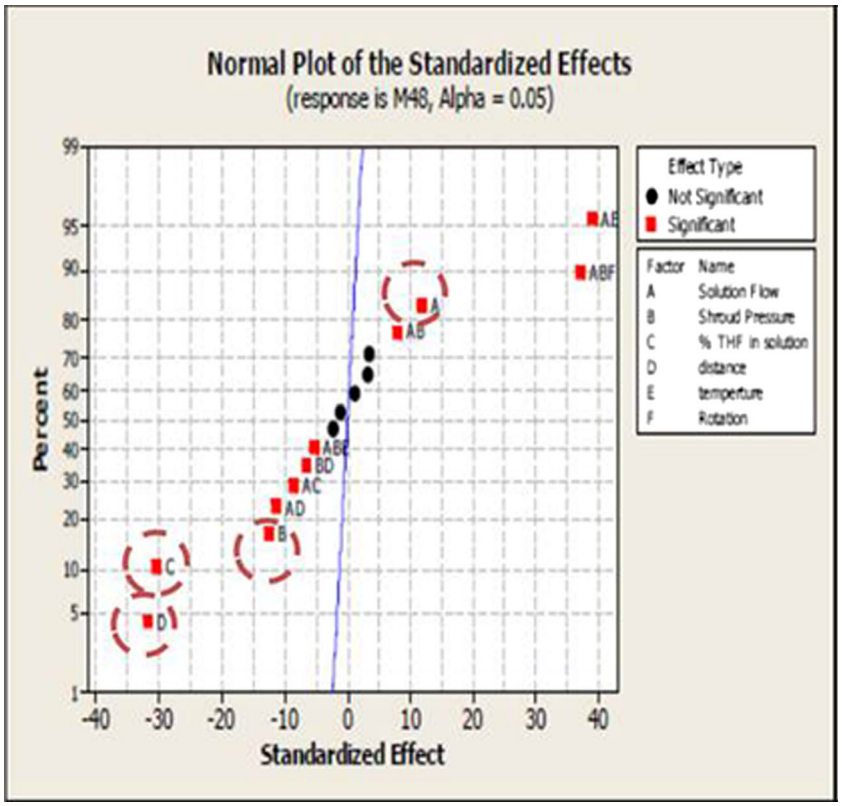

Fig. 2. Normal plot for DOE 1, with the statistically significant terms *circled out*

eliminate variability in mass of coating on the stent, roughness, and coating defects (*i.e.*, to optimize the coating quality). The center point (run #1) was repeated four times, resulting in a total of 20 spray coating runs in DOE 2. Figure 3 shows that the elution curves from the center point replicates are all aligned, demonstrating repeatability of the method. There were two stents coated consecutively per run for a total of 40 coated DES. McGrath *et al.* (28) published a similar multifactorial process-formulation-quality-attributes study for a set of six ultrasonic coating parameters. However, their coating system (methylcellulose coatings sprayed onto microneedles using a Düsen-Schlick ultrasonic coating nozzle) and outputs

Table II. Inputs and Outputs for DOE 2

	Inputs					Outputs			
Run #	Number of passes	Temperature °C	Solution flow rate uL/min	THF in solution %	Nozzle-to-stent distance mm	Mass ratio	A_1	A_2	A_3
1	11	24	35	70	12.5	40	48	7.3	3.8
2	7	19	45	40	7.5	47	56	3.5	2.5
3	14	19	25	40	7.5	45	43	7.2	4.0
4	14	29	25	40	7.5	46	27	7.8	4.7
5	7	29	45	40	7.5	45	42	6.3	4.1
6	10	19	45	40	17.5	37	45	7.5	3.3
7	18	19	25	40	17.5	36	39	7.0	3.6
8	10	29	45	40	17.5	34	38	7.6	4.0
9	18	29	25	40	17.5	36	26	6.8	4.4
10	14	19	25	100	7.5	47	35	4.6	4.6
11	13	29	25	100	7.5	42	39	8.1	5.2
12	7	29	45	100	7.5	44	18	6.3	4.7
13	7	19	45	100	7.5	47	40	4.0	4.4
14	18	19	25	100	17.5	35	52	6.7	3.1
15	11	19	45	100	17.5	37	58	5.1	2.3
16	10	29	45	100	17.5	29	47	7.3	3.6
17	18	29	25	100	17.5	34	37	7.7	3.1

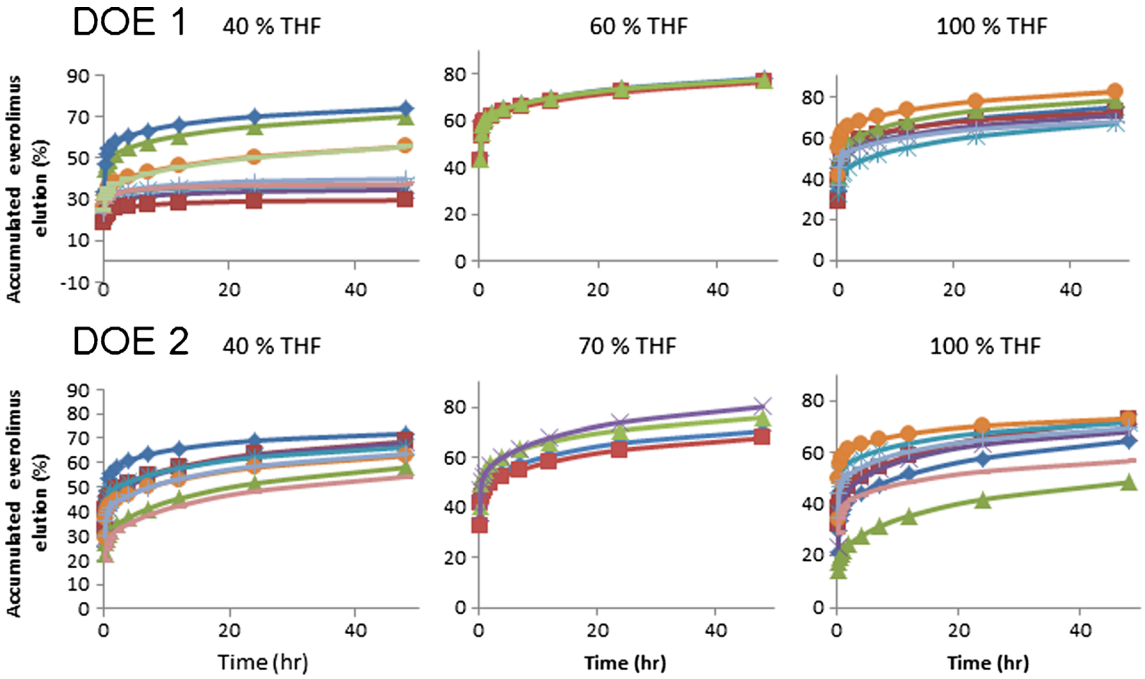

Fig. 3. The elution curves in the center are from nominal coatings (center values in Table I) that were all fabricated using the same processing methods. Elution curves of coatings fabricated using various ultrasonic coating parameters, *i.e.*, temperature, distance, and flow, are plotted together on the *left* (which were all made using 40% THF) and *right* (which were all made using 100% THF)

(coalescence, blemishes, bubbles, thickness, and roughness) were quite different from ours, so their study results are not comparable.

DOE 1 and DOE 2-coated stents were reproducible, defect-free, and had low target mass variation (0.67± 0.06 mg), demonstrating that the processing methods resulted in acceptable coatings. The target mass was in the middle of the range of stent coating masses reported in the literature for the stent size used (29). As such, the DOE could detect the effects of changes in process variables on coating characteristics and drug elution kinetics. Coating thickness was approximately 5.8 μm as measured from a cross section of the coating viewed edge on by scanning electron microscopy (SEM). The mass ratio (R) (also known as coating efficiency) and the coating mass both varied as process parameters changed, especially for the nozzle-stent distance. For example, coating efficiency varied from 35% to 46% as nozzle-stent distance varied from 17.5 to 7.5 mm. However, once R was determined for any given spray method (Table II), the number of layers (N) to be sprayed could be changed while keeping all the other parameters selected for a given run constant to obtain the target coating mass using the following equation:

$$R = M/S = (Mv)/(QCNL), \quad (1)$$

where:

M	Coating mass
$S=QCt$	Solids mass sprayed during the time the spray is over the stent
Q	Coating solution flow
C	Solids fraction in the coating solution
$t=d/v$	Time the spray is over the stent
v	Average translation velocity of the stent under the spray
$d=LN$	Total distance the stent moves in translation
L	Stent length
N	Number of layers sprayed or number of passes under the nozzle

DOE results were analyzed using Minitab Version 15 statistical software package (30). A factor was delineated as statistically significant if there is correlation with a p value of less than 0.05.

Materials

Acetic acid (ACS grade), sodium acetate (ACS grade), THF (sequence grade, without butylated hydroxytoluene (BHT)), and acetone (National Formulary and Food Chemicals Codex grade) were purchased from Fisher Scientific; acetonitrile (high-performance liquid chromatography (HPLC) grade) was purchased from Alfa Aesar, Ward Hill, MA; and Tween 20 (polyethylene glycol sorbitan monolaurate) was purchased from Sigma-Aldrich. Deionized water was prepared using a Barnstead nanopure Diamond™ Ultrapure Water System (Barnstead International, Dubuque, IA). Ester-terminated (nominal) 85:15 poly(DL-lactide-co-glycolide) (PLGA 85:15) (inherent viscosity 0.55–0.75 dL/g in $CHCl_3$) was purchased from Lactel, Birmingham, AL. Everolimus used to fabricate coatings and to develop the chemical analytical techniques was purchased from Sigma-Aldrich, Saint Louis, MO and everolimus used as a reference standard were purchased from LC Laboratories, Inc.,

Woburn, MA. The 316LVM stainless steel stents (27 mm long, about 1.5 mm inner diameter and 1.7 mm outer diameter, surface area 0.273 in. (2)) were purchased from ECHOBIO, LLC, Bainbridge Island, WA.

Stent Cleaning and Mass Measurements

Stents were cleaned ultrasonically for 15 min in THF, followed by ultrasonic cleaning 10 min in methanol. The stents were then dried at 55°C under vacuum (about 5 mmHg). After drying, the stents were examined using a stereomicroscope model SZH-ILLK (Olympus, Tokyo, Japan) at ×10 magnification and the cleaning procedure repeated if there were any signs of contaminants. Before and after ultrasonic coating, stents were individually protected from contact with other surfaces and contaminants during manipulation, transport, weighing, and storage. Sample weights for the preliminary and DOE 1 experiments were collected on a Mettler Toledo XS205 balance (Plainview, NY), accurate to 0.01 mg. A Sartorius balance (model M5E-F, Bohemia, NY, accurate to 0.001 mg) was used for the DOE 2 sample preparation and analysis.

Coating Solution Preparation

Tetrahydrofuran was dried by pouring into a buret with 6–7 in. of alumina on top of 1–2 in. of glass fiber. The THF was then filtered by pushing it through a 0.45-μm hydrophilic PTFE membrane filter with a syringe. This THF was used in all preparations and experiments. A 6% (*w*/*w*) stock polymer solution was made by dissolving PLGA in THF. The solids fraction was measured by casting 250 μL of this solution, allowing it to dry, and then weighting the residue. A stock drug solution was made by pouring THF into a vial containing 10 mg of everolimus. The drug readily dissolved and was transferred to a 25-mL glass vial to which the right proportions of THF, acetone, and PLGA stock solution were added to obtain a coating solution with a solids concentration of 1% (*w*/*v*) (*i.e.*, 0.01 g solids/mL solvent or 1 g solids/100 mL solvent) and a dry coating drug fraction of 30%. The solution was mixed well *via* swirling by hand and then stored at −20°C. The drug and polymer were completely soluble for each of the three solvent fractions. The solution viscosity was constant since viscosity is determined by the solids concentration.

Coating Application

Before coating, the stent was weighed three times and inspected for contaminants and particles. The coating solution was also examined for particles and filtered if any were observed. All inspections were conducted at ×10 magnification using the stereomicroscope, model SZH-ILLK (Olympus, Tokyo, Japan). Coating was performed using a MediCoat Benchtop Stent Coating System (Sono-Tek Corp., Milton, NY) with a MicroMist ultrasonic atomizing nozzle at a frequency of 120 kHz. Coating solution flow was achieved using a 10-mL syringe and syringe pump. Solution flow, stent rotation, and translation were controlled by computer software. This system produces droplets about 10–20 μm in diameter, which are propelled by a nitrogen gas flow at about 0.25 to 0.4 m/s (31) in a direction normal to the axis of the stent. Nitrogen, rather than air, was used because the surface tension of the liquid is lower in nitrogen, resulting in better liquid flow and smoother coatings. Spray coatings were fabricated in a glove box filled with nitrogen to control the temperature, humidity, and rate of solvent vapor exhaust.

Prior to coating, any bubbles in the solution throughout the system were removed and the temperature equilibrated to the set temperature as measured by two thermocouples 10± 5 mm from the stent and recorded by a TC-08 Thermocouple Data Logger and PicoLog software (Pico Technology, St. Neots, UK). After coating, the stent was immediately removed and examined under the stereomicroscope for defects, dust, and other contaminants; then dried under 5-mmHg vacuum at 55±5°C for an hour; and finally, stored in vials in a desiccator under vacuum.

Structure Analysis

Drug-polymer phase separation at the coating surface, which may result in a drug solid state composite microstructure, was evaluated in a representative set of coated stents with an atomic force microscope (AFM) (model MFP 3D, manufactured by Asylum Research, Santa Barbara, CA) in tapping mode with a silicone OMCL-AC240TS probe (spring constant 1–3 N/m). To study coating defects and surface structure, all stents which were not used for drug release experiments were gold sputter-coated using a Denton Vacuum Desk IV Sputter Coater (Moorestown, NJ) and imaged in secondary electron imaging mode using a JEOL SEM model JSM-35 (Jeol, Tokyo, Japan). Typical SEM settings were 10 mm working distance, 30–50 spot size, and 10 kV accelerating voltage. Images were taken at ×33, ×300, ×1000, ×3000, and ×10,000 as necessary to show representative images of the front, back, and side surfaces of the stent struts.

Drug Release Studies

The *in vitro* drug release method was developed based on established procedures that are representative of *in vivo* performance (32). Stents were reciprocated through 10 mL of elution media a distance of 3 cm per cycle at 5 cycles/min at 37°C using a USP 7 apparatus (Varian, Inc., Dissolution Systems, Cary, NC). The elution media consisted of 0.4% Tween 20, 7% acetonitrile in 0.01 M sodium acetate (pH 5.0). Release medium pH was adjusted to 5 with acetic acid and tested with an Accumet Basic AB 15 pH meter from Fisher Scientific. Samples of 1.5 mL release medium were collected at 10, 20, 30, and 45 min and 1, 2, 4, 7, 12, 24, and 48 h, with the entire medium removed and replaced at each time point.

High-Performance Liquid Chromatography

High-performance liquid chromatography was used to quantify everolimus release based on Baldelli's method (33). This consisted of a Waters 2695 separation module and a 2998 photodiode array (PDA) (both from Waters Technologies, Inc., Palo Alto, CA) with a thermostated column compartment set to 60°C. The HPLC column was a Supelcosil C-8 (from Sigma-Aldrich) with a particle size of 5 μm. The mobile phase was an acetonitrile/water mixture (in a ratio of 65:35) with a flow rate of 1 mL/min. The injection volume was

100 μL, and the auto sampler chamber temperature was set to 5°C. Everolimus, its isomer, and BHT could be totally separated by this system. The HPLC method vas validated over the concentration rage from 8 to 4600 ng/mL. Total everolimus was reported as the sum of the main everolimus peak and its isomer because the two species are equivalent. The resolution between everolimus and its isomer was 2.5, the tailing factor (symmetry of a peak) was 1.05, recovery was 99.0%, the relative standard deviation of six replicate injections was 0.06%, the detection limit was 5 ng/mL, and the quantity limitation was 8 ng/mL. Elution samples were directly injected into the HPLC.

RESULTS AND DISCUSSION

Coating Processing Defects

There were two primary processing defects observed in the preliminary experiments that were used to explore how changes in ultrasonic coating parameters affected the drug coating. The first was the formation of fibers, webbing, and excess coating at the stent ends (Fig. 4), which resulted from too much spray solution reaching the stent. Reducing the solution volume on the stent mitigated these defects, but this lead to the second problem of precipitation on the nozzle because of insufficient solution volume to maintain drug and/or polymer solubility on the nozzle tip during droplet generation. The solids that precipitated on the nozzle either deflected the spray away from the stent or nucleated a liquid drop that fell off the nozzle and thus lowered the coating mass if it missed the stent or created a large particle defect if it landed on the stent.

Unfortunately, adjustments in coating parameters which decreased the prevalence of one processing defect caused an increase in the other. A window of ultrasonic coating parameter values (Table I) was experimentally determined that avoided both problems and produced coatings without defects by trading off more solution on the nozzle *versus* less at the stent. After a series of investigations, it was determined that precipitation on the nozzle could be delayed until after the coating was completed by (1) removing contaminants and scratches on the nozzle surface that could cause nucleation by cleaning the nozzle and checking it for damage; (2) eliminating water in the coating system that could cause the polymer to precipitate on the nozzle by drying the solvents; (3) reducing the humidity in the coating environment by filling the glove box and spraying with nitrogen; and (4) maintaining a low solids concentrations in the solution on the nozzle by adjusting the solution flow rate, shroud gas flow rate, ultrasonic power, gas flow conditions, and other coating system parameters that reduced evaporation on the nozzle (34). In addition, the preliminary testing also led to key adjustments to the ultrasonic coating system necessary to achieve a consistent coating mass by keeping the stent at a constant position and environment within the coating chamber, aligning the stent under the spray, correcting off-axis motion of the stent during rotation, and as discussed above, by preventing precipitates and drops on the nozzle.

Mass Ratio

There was an inverse relationship between mass ratio and nozzle-stent distance due to lateral dispersion of droplets in the spray. Analysis of DOE 2 data showed that nozzle-to-stent distance was the only statistically significant term that affected mass ratio (Fig. 5). To maintain a constant coating mass as nozzle-stent distance was varied, the number of coating layers sprayed was adjusted as needed as described in the methods.

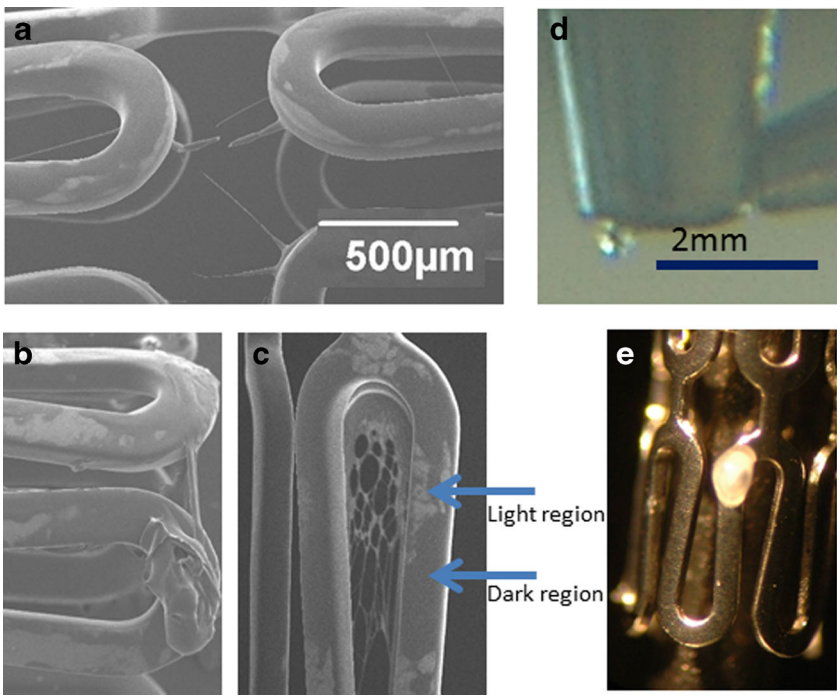

Fig. 4. Coating processing defects: **a** fibers, **b** excess coating at the stent ends, **c** webbing, **d** liquid drop on the nozzle, and **e** large particle defect on the stent

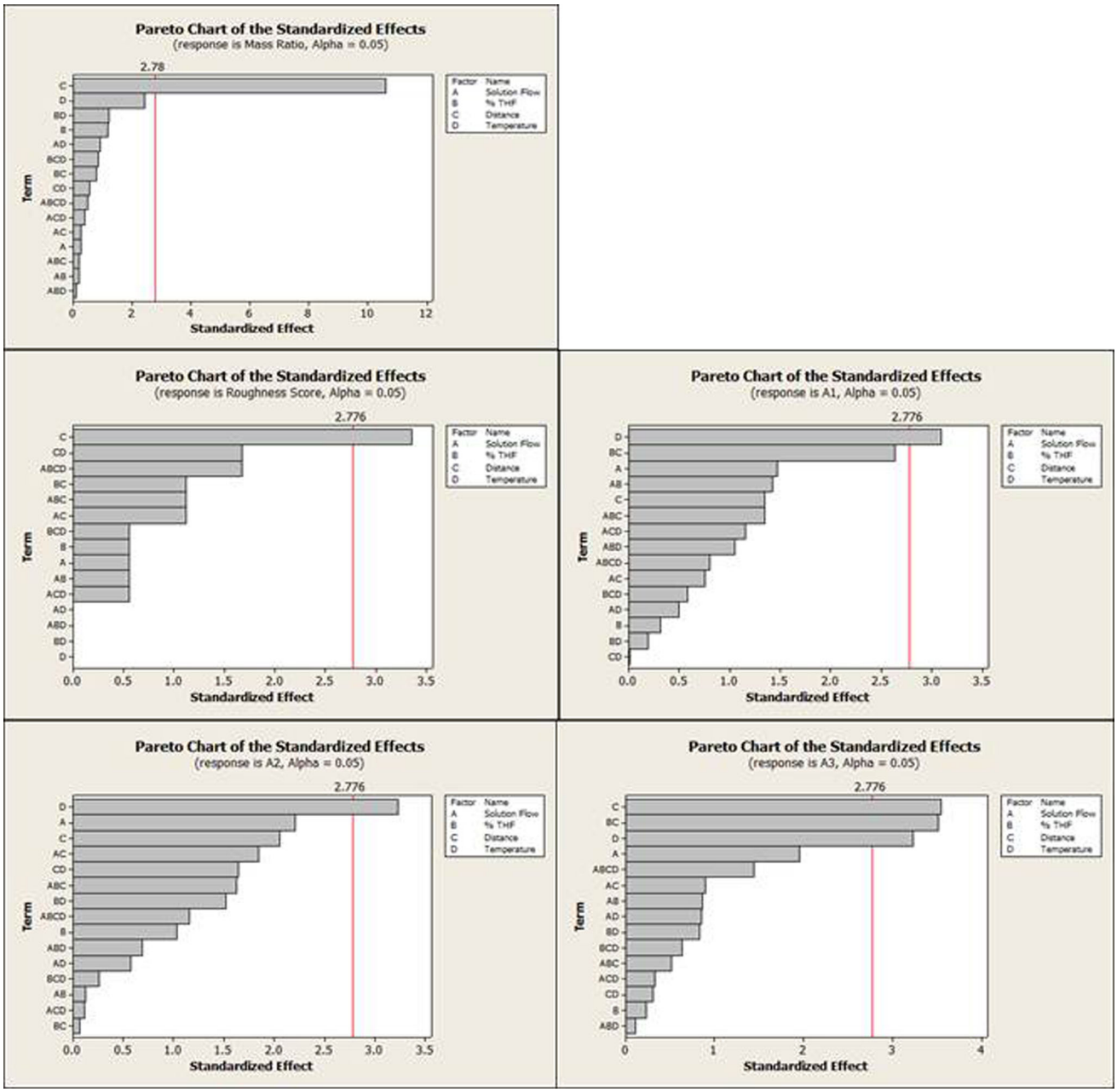

Fig. 5. Pareto plots for DOE 2

We found no other statistically significant correlations between the other processing parameters and mass due to the narrow range of target coating mass values. Wang *et al.* (18) measured correlations between coating thickness, spray solution concentration, and number of layers sprayed, parameters which were constants in our study.

Surface Roughness

Coating roughness was semi-qualitatively graded on a scale from 1 to 4 (Fig. 6). This scale correlated with peak-to-trough measurements made on the SEM micrographs (r^2=0.72). Overall, DOE 2 coatings were less rough (roughness grades were 1 or 2) than DOE 1 coatings (roughness grades 1–4), likely because of variation in only four coating parameters used to make the DOE 2 coatings compared to six for making the DOE 1 coatings. Pareto chart for DOE 2 results (Fig. 5) showed that of all the ultrasonic coating parameters and nozzle-stent distance had a statistically significant impact on roughness. A lower nozzle-to-stent distance tended to result in smoother coatings, perhaps because this led to an even flow of the coating solution on the stent surface. Similar results were reported by Bose *et al.* (35) for polyvinylpyrrolidone ultrasonically sprayed onto a 5×20 mm area, and Shanshan *et al.* (17) also reported that higher flow rates resulted in smoother coatings of PLGA (LA/GA=75/25, Mw 100000) containing 5%, 7%, or 9% PEG plasticizer (to reduce PLGA cracking) and PLGA:sirolimus ratios of 2:1; 3:1; or 4:1. Shanshan also found that changing the solvent (and presumably the evaporation rate) affected roughness, a relationship which we did not observe in our study.

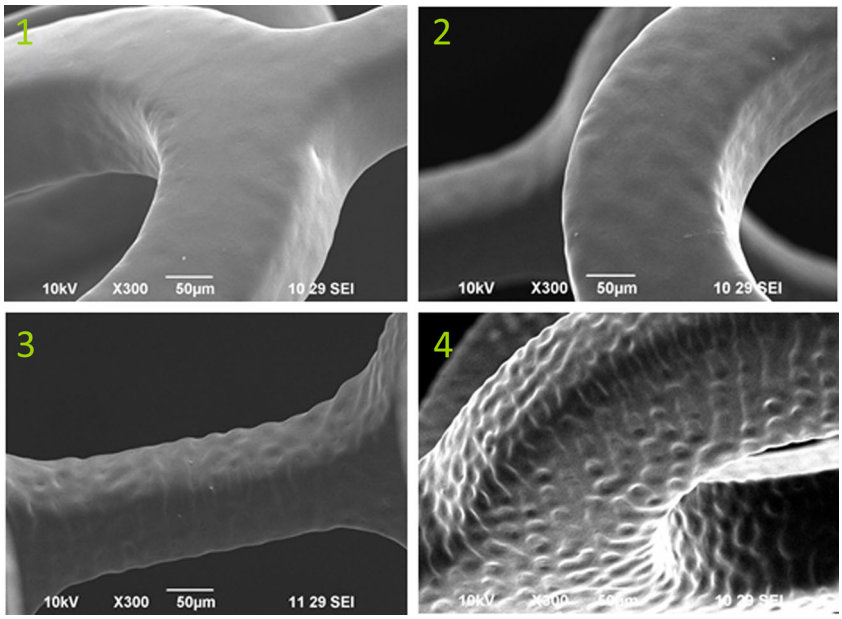

Fig. 6. The roughness of coatings were qualitatively graded on a scale from 1 to 4

Atomic force microscopy

The drug solid state composite microstructure of representative DOE 1 and 2 stent coatings were studied by AFM because prior publications showed that variation in size and distribution of the drug phase can affect drug elution kinetics (15,16,36–38). We observed phase-separated everolimus particles embedded in PLGA coatings ultrasonically sprayed on both flat substrates (Fig. 7a) and on stents (Fig. 7b, c) using approximately the same ultrasonic spraying conditions and coating solution. However, the drug particles in coatings on a flat substrate were 10 to 40 times larger than particles on stents. Also, drug particles on stents were not always resolved by AFM due to their small size and limitations on imaging at this extreme magnification. Drug particles on the substrate were larger than those on the stent because solution droplets on a flat substrate can pool together, causing the solution to evaporate more slowly, allowing more time for the drug phase to separate from the polymer and form larger drug particles (39,40). Apparently, the solution does not accumulate on the narrow 100-µm stent struts, so it evaporates rapidly and there is less time for drug phase separation. Though we did not confirm that drug-rich regions did not form beneath the surface, it is not likely based on the fine dispersion of drug on the surface. This may be confirmed by other techniques in the future (36,37).

Scanning Electron Microscopy

Drug particles on the surfaces of all DOE 1 and 2 stent coatings were not discernible by SEM due to the limited SEM resolution. Likewise, Pan *et al.* (41) did not observe drug particles by SEM on the surface of curcumin-PLGA (LA/GA=85:15, Mn 95; 800) ultrasonically spray-coated stents. However, on the surface of about 20% of the stents, there were unidentified light regions, while there were none on the other 80% of the coated stents. For stents on which light regions occurred, the light regions covered 0% to 10% of the area of the coating on the front side of the strut (Fig. 4c) and 20% to 80% on the back side of the strut (Fig. 7e).

We hypothesize that the light areas were areas of thin coating because they occurred more frequently on the back of the strut, which is most likely thinner. Also, bare metal appears lighter compared to coated areas. However, at high magnification, there was no edge or any sign of a change in thickness between a light and dark region. The light areas were not associated with roughness and there were no trends in elution kinetics associated with the area coverage of the stent by light areas. However, it was observed that a lower stent-nozzle distance or a higher solution flow tended to result in more light areas. These processing parameters cause higher rates of solution to be applied onto the stent, which may cause spatial variation in coating thickness or a higher solution accumulation on the stent, leading to a lower evaporation rate and enrichment of surface drug as discussed above.

Coating Loss During Drug Release Studies

The DES samples were soaked in a Varian USP 7 apparatus to relate changes in process variables and coating characteristics to drug elution kinetics. After 2 days of reciprocation in the drug release medium, holes on the order of 10 µm in diameter were observed by SEM in the coatings (Fig. 7d). The luminal or "back" side of struts facing the interior of the stent which have a thinner coating lost more coating than the thicker coating on the abluminal or front side. The coating loss observations described here were also reported by Kamberi *et al.* (32) under similar conditions.

Based on gravimetric measurements, about a quarter (26 ±6.7%) of the coating mass was lost after 2 days, which was the sum of 20% drug loss plus 6% PLGA loss. This means most of the holes that formed resulted from drug eluting out, which is consistent with the following observations. First, SEM micrographs show that more holes were observed in PLGA coatings with drug compared to PLGA coatings with the same

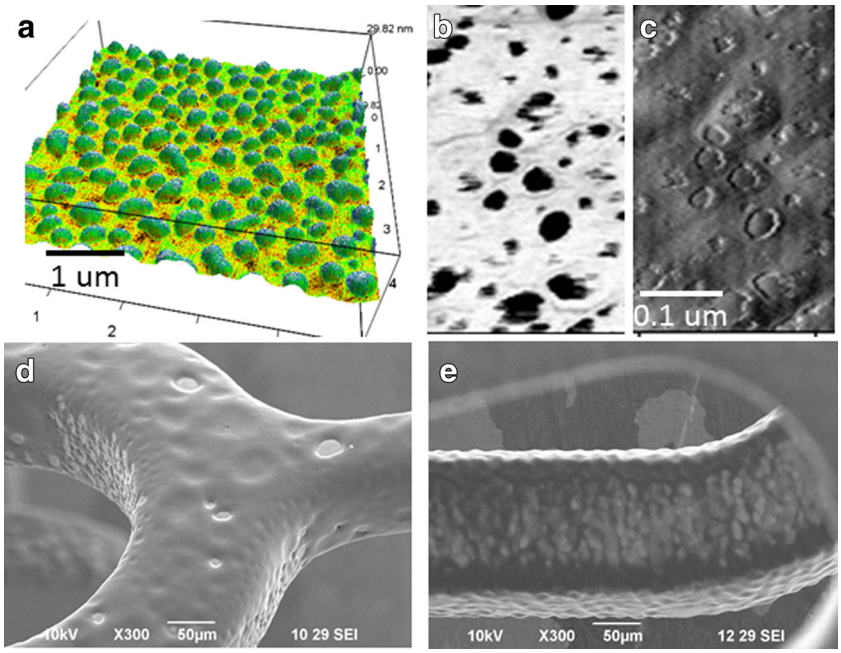

Fig. 7. Coating structures before soaking: **a** a 3D AFM image of an ultrasonically sprayed coating on a metal substrate showing 200–300-nm-diameter phase-separated everolimus particles (*green*) embedded in PLGA (*yellow*). **b** and **c** The AFM images (in phase mode and amplitude mode, respectively) of a DOE 2 coating on a stent showing 5–30-nm-diameter phase-separated everolimus particles (*black* in (**b**)) embedded in PLGA (*white* in (**b**)). **d** A typical SEM micrograph of a stent after reciprocation in 37°C Tween solution for 2 days, showing the front side of a strut had less coating lost than the sides. **e** Light regions on the back side of a strut

mass but without drug. Further, gravimetric measurements demonstrated that the mass of PLGA lost from coatings containing a 30% drug fraction and no drug was about the same (6% and 6.9% of the initial mass of PLGA in the coating was lost, respectively). Finally, though the Tween solution accelerates drug release, it is not expected to significantly affect degradation of the polymer in 48 h based on a study of PLGA 75:25 stent coatings (32) in which the molecular weight (96,000 Da) of the PLGA 75:25 remained unchanged after soaking for 24 h in various media composed of acetonitrile (ACN), Tween 20, and sodium acetate (pH 5.0). In conclusion, the mechanism for the coating loss is primarily drug release since the PLGA has not degraded significantly in 2 days.

Detached particles of coating could have contributed to the coating loss; however, SEM micrographs (Fig. 7d) do not suggest this occurred because the coating shrank into a continuous, interconnected network, without any signs of detachment. Further, isolated patches of coating residue on the back and sides of struts remained attached to the smooth metal substrate despite the loss of 67% of the drug initially present from the solid mixture of 70% PLGA plus 30% everolimus. Presumably, this is possible because water and other chemicals in the elution media swelled and plasticized the PLGA/drug solid mixture.

Drug Elution

As can be seen in the elution curves (Fig. 3), variation in drug released from the nominal coatings, which were all fabricated using the same processing method, was less than the variation for coatings made using the same coating solution composition but different values of three ultrasonic coating parameters (temperature, distance, and flow). Differences in coating mass between samples does not account for this since the mass standard deviation for all DOE 1 and 2 coatings taken together is only 8% of the average mass.

To correlate ultrasonic coating parameters and drug release, the elution curves for the coatings in this study were first parameterized by fitting the elution data to Eq. 2 (42). The equation assumes that the coating structure can be approximated as a composite of two films, *viz*., a pure drug top layer and a sub-surface polymer matrix with drug homogenously embedded throughout. Because release from the top surface is assumed to be fast relative to the time scale of the elution studies, it is modeled based upon a late time approximation of cumulative release from the surface layer, which is represented by the first two terms (A_1 and A_2) in Eq. 2. Most important from a clinical effectiveness standpoint is the A_3 term, which captures the long-term release kinetics resulting from sub-surface drug, assuming it follows Higuchi-type diffusion-limited kinetics: (42)

$$M(t) = A_1\left(1 - e^{-A_2 t}\right) + A_3\sqrt{t}, \tag{2}$$

where M is the cumulative fraction of drug released as a function of time, t, and A_i are the fit parameters. For each set of elution data, the unknown coefficients, A_i, were fit using a standard nonlinear least squares algorithm (leastsq from SciPy (43)). The maximum relative standard error between the model and the actual elution data are about 3%, 17%, and 9% for A_1, A_2, and A_3, respectively; thus, the equation

appears to be a reasonable model for the experimental data in this study. The impact of the coefficients was studied with respect to the processing parameters using Pareto charts (Fig. 5) for the DOE 2 data. The following processing parameters had statistically significant effects on the coefficients in the following order of significance:

A_1 Temperature
A_2 Temperature
A_3 Nozzle-stent distance>the combination of nozzle-stent distance and % THF>temperature

Pareto charts showed that A_1 and A_2 (burst release coefficients) were most affected by temperature, which is related to the rate of solvent drying from the surface of the stent. Pareto charts also showed that A_3 (long-term release coefficient) is significantly impacted by nozzle-to-stent distance. This is consistent with the previous discussion (35) that increasing nozzle-to-stent distance resulted in significantly rougher coatings that impacts drug release.

Ultrasonic Coating Parameter Design Space

An ultrasonic coating parameter design space for the specific drug, polymer, stent, and ultrasonic spray system described here can be constructed based on contour plots of nozzle-stent distance and temperature for roughness, A_1, A_2, and A_3 (Fig. 8). The goal is to minimize roughness, minimize burst release (*i.e.*, lower A_1 and higher A_2), and maximize the long-term release of drug (higher A3). Roughness tended to be less at higher solution flow rates so flow is set to the maximum value of 45 mL/min. Solvent composition did not appear to cause any trends in the elution curves nor demonstrate any statistically significant effects on the elution coefficients. Therefore, % THF is set at the center value of 70%. As shown in Fig. 8, if the contour plots for all four responses are overlaid, the design space for coating operation would be between temperature of 24 and 28°C and the stent-to-nozzle distance being between 8 and 11.

CONCLUSIONS

The current study demonstrates the usefulness of the application of QbD approaches to gain a fundamental understanding of processing factors that affect the performance of drug-eluting stents (DES). Variability in drug elution rate was identified as one of the main causes of quality-related failures in DES. Risk analysis showed that the ultrasonic coating process is an important factor affecting drug elution. The coating process was then further studied using multiple design of experiments. Analysis of the full factorial DOE data showed that nozzle-to-stent distance and temperature are

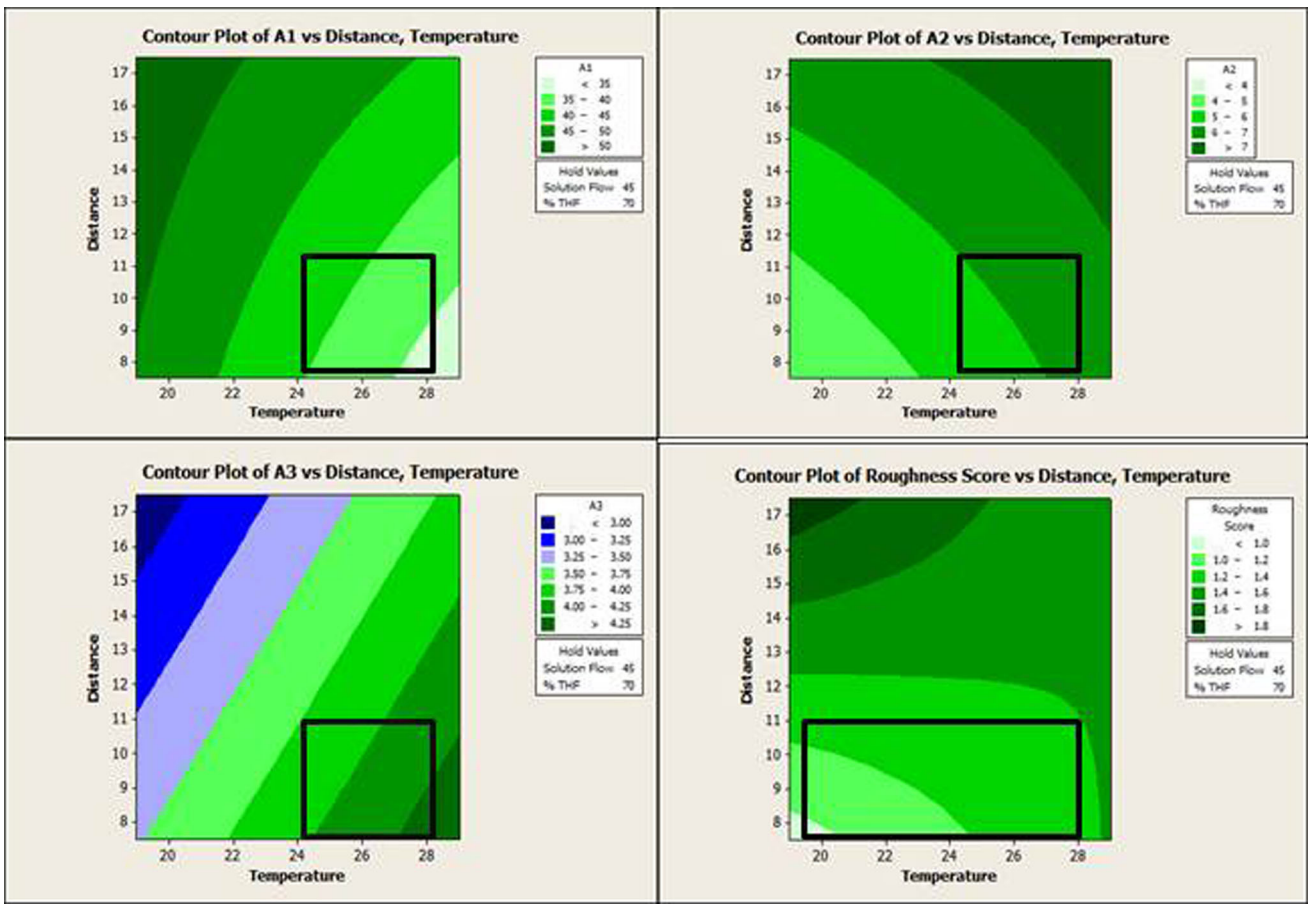

Fig. 8. DOE 2 contour plots of nozzle-stent distance *versus* temperature for roughness and the three elution model coefficients (A_1, A_2, and A_3). The *interior of the rectangles* indicates the region of the nozzle-stent distance *versus* temperature design space that will minimize roughness, minimize burst release, and maximize long-term release of drug

critical parameters affecting the coating roughness and drug elution rate and thus the overall quality of DES. Additionally, a design space was defined on the basis of the DOE data to meet the objective of minimizing coating roughness and burst release as well as maximizing long-term release of the drug. This study showed the feasibility of implementation of the QbD paradigm to develop complex drug device combination products.

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Conflict of interest The views expressed are those of the author(s) and do not reflect the official views of the FDA.

APPENDIX

QTPP and CQA for the drug-device combination under study

QTPP for a DES are as follows:

- Identity
- Potency and strength
- Bioavailability/local activity/bio-performance
- Adequate insertion
- Purity and integrity
- Stability

The corresponding CQA are as follows:

- Identity
- Amount of drug in the coated stent (*i.e.,* assay)
- Content uniformity of coated stent
- Coating uniformity of an individual stent
- Biocompatibility (*e.g.,* degradation of polymer, *in vivo* polymer absorption)
- Drug elution rate
- Stent mechanical properties (includes stiffness and compliance, depends on intended duration inside the body)
- Particulates/extraneous matter
- Degradants/related substances
- Endotoxins
- Residual solvents
- Sterility
- Polymer shelf life
- Device shelf life

The QTPP is related to CQA as follows:

- Potency and strength is related to assay and content uniformity of coated stent.
- Bioavailability/local activity/bio-performance is related to the antiproliferative drug selected (*i.e.*, identity), coating uniformity of an individual stent, biocompatibility (*e.g.*, degradation of polymer, *in-vivo* polymer absorption), and drug elution rate.
- Adequate insertion is related to the stent mechanical properties.
- Purity and integrity is related to the particulates/extraneous matter, degradants/related substances, endotoxins, residual solvents, and sterility.
- Stability is related to the polymer and device shelf life.

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