Sys Rev Pharm 2020;11(11):1947-1952
A multifaceted review journal in the field of pharmacy

Development of an Extended-Release Exenatide Once a Week Depot Formulation

***Jabar A Faraj[1], Shaimaa M. Mohammed[2], Khulood M. Alsaraf [3], Ihab I. Alkhalifa[4], Kadum A. Al Shareffi[1], and K. Burton[5]**

[1]PhD Pharmaceutics / Al-Mustaqbal University College, Babylon, Iraq
[2]PhD Clinical Chemistry / Al-Mustaqbal University College, Babylon, Iraq
[3]PhD Pharmacology and Toxicology / Al-Israa University College, Baghdad, Iraq
[4]PhD Pharmacology and Toxicology / Al-Rasheed University College, Baghdad, Iraq
[5]Evonik Corporation – Birmingham Laboratories, Birmingham, AL 35211, USA

ABSTRACT

Bydureon™, the Exenatide extended-release formulation depot, has been approved in the USA for once weekly treatment for type 2 diabetes mellitus. Exenatide is a synthetic version of exendin-4 with biological properties similar to human glucagon-like peptide-1 (GLP-1) agonist. Evonink Industries at Birmingham - Alabama, USA developed the process based on available reported information to produce Exenatide loaded microparticles that demonstrate a similar in vivo release profile compared to the reference listed drug (RLD). The drug loading, initial burst and other characteristics of the exenatide loaded microparticles are in the range of RLD. Surface characterization by scanning electron microscopy (SEM) of the Evonik produced microparticle visually matches with RLD.

Keywords: Exenatide, Microparticle, Depot, Diabetes

Correspondence:
Jabar A Faraj
PhD Pharmaceutics / Al-Mustaqbal University College, Babylon, Iraq
Email: jabar.Faraj@gmail.com

INTRODUCTION

GLUCAGON-LIKE PEPTIDE-1 (GLP-1) receptor agonists are a class of biologics for the treatment of type 2 diabetes.[1] Including exenatide,[2,3] liraglutide,[4,5] and various agents,[1,6] these drugs mimic the activities of GLP-1, a hormone that is released from specialized intestinal L-cells in response to nutrient ingestion.[1]

Exenatide, the first GLP-1 receptor agonist approved for clinical use, is a synthetic version of the naturally occurring *Heloderma suspectum* peptide exendin-4 and has an amino acid sequence approximately 50% identical to that of human GLP-1. The subcutaneous administration of exenatide twice-daily was shown to reach its peak plasma concentration in approximately 2.0 hours and to be eliminated subsequently with a terminal half-life of about 2.4 h.[7] In randomized clinical trials, the subcutaneous administration of exenatide decreased hemoglobin A1c (HbA1c) levels, and reduced weight in patients with type 2 diabetes.[2,3] In addition, some effects were also noticed such as blood pressure lowering, small decreases in low-density lipoprotein cholesterol, increases in high-density lipoprotein cholesterol, and decreases in fasting triglyceride levels.[8]

Commercial exenatide formulations are currently injected via the subcutaneous route either twice daily for an immediate-release formulation (Byetta®, Eli Lily/Amylin, 2005) or once weekly for extended-release suspensions (Bydureon™, AstraZeneca, 2012) [4]. In order to achieve enhanced therapeutic efficacy for a prolonged period of time and reduce the need for frequent injections, recent studies have been focused on the development of long-acting sustained release drug delivery systems, by entrapping the active ingredient in biodegradable polymer such as D, L-lactic-co-glycolic acid to make microspheres(PLGA-Ms), in order reduce frequency of injections [5].

Water-in-oil-in-water (W/O/W) double emulsion is a commonly used method to prepare protein/peptide loaded PLGA-Ms due to its simple preparation procedure [6]. However, the high initial burst and low encapsulation efficiency limit its application, as the encapsulated drugs could easily migrate to the external water phase during the fabrication process. In addition, the structure and functionality of proteins/peptides may be changed due to exposure to organic solvents [7]. The low encapsulation efficiency and inactivation of drugs may also increase the costs as well as decrease the therapeutic efficacy.

The purpose of this study was to demonstrate the capabilities of Evonik company using its know-how to develop the processes for exenatide loaded microparticles that matches marketed product, Bydureon™. This study demonstrates that typical challenges such as initial burst, particle size distribution, and (*in vivo – in vitro*) profiles have been achieved to match the original product by Bydureon™ successfully.

In this study, three goals were addressed:

- To fabricate four different batches of biodegradable polymer microspheres containing exenatide with a target profile shown in **Table 1.**
- To establish in vitro exenatide and the reference listed drug (Bydureon™, AstraZeneca) release as a function of time,
- To use one set of the in vitro parameters to demonstrate and perform the in vivo study and investigate the serum concentration and release for loaded microspheres using a single injection of PLGA polymer in rat.

Accomplishment of these three goals could provide proof of concept for the feasibility of long-term exenatide loaded microspheres and impetus for further research to transform this novel idea from a development concept to a meaningful intervention in individuals suffering from type 2 diabetes.

Table 1: Quality Target Product Profile

Profile Component	Target
Dosage form	Microsphere suspension in diluent
Route of administration	Subcutaneous injection
Strength	2 mg
Duration	1 week
Formulation	Exenatide-loaded microparticles
	Injection vehicle (liquid diluent) supplied in a prefilled syringe
Polymer	50:50 DL-4A PLGA
PK Profile	Statistically Similar to Bydureon®
Injection Volume	1.5 mL
Container/Closure	3 mL vial
Sterility	Sterile
Storage	2-8°C and protected from light
Shelf Life	42 months

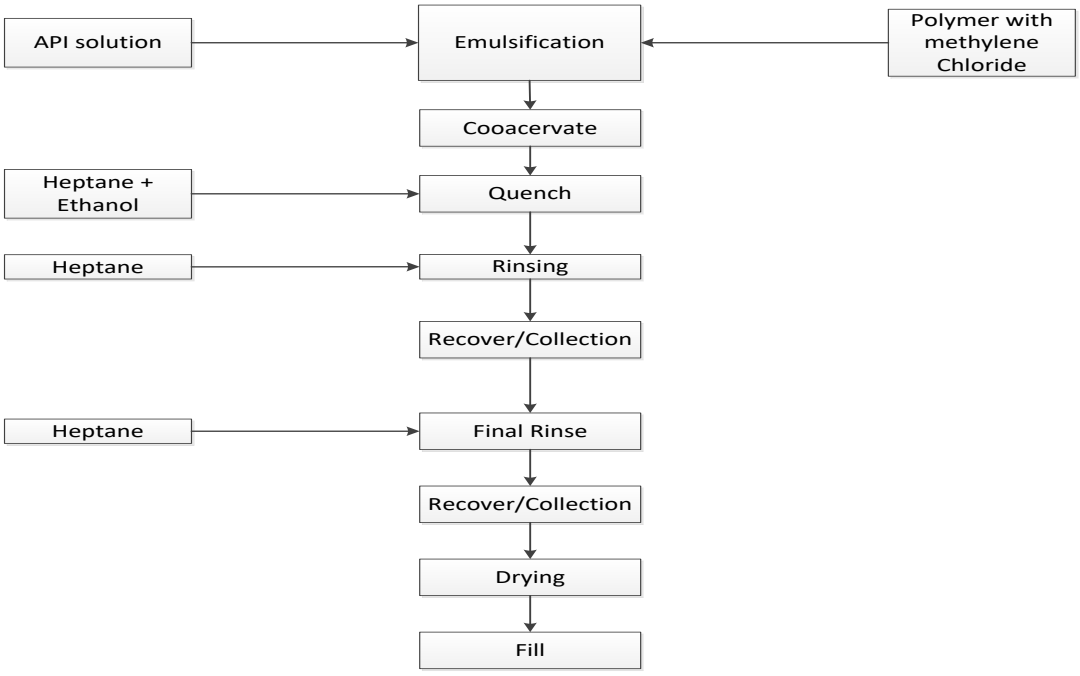
MATERIALS AND METHODS

Microspheres Technology

United States Patents 5468432, 6824822 B2, 7223440 B2, 7456254 B2, 7563871 B2, 7612176, and 8431685 B2 were reviewed to understand the processing technologies that are most likely used in the production of Bydureon® Amylin®. In addition, Australian Public Assessment Report for Exenatide, CMC memo for filing to Center for Drug Evaluation and Research with an application number 022200 Orig1S000, and several publications related to the encapsulation and stability of Exenatide were reviewed to understand and design the generic Exenatide formulation. In summary, the process derived from the literature used to make the Exenatide microspheres, utilizes the phase separation (coacervation) method as shown in **Scheme 1** below.

General Process Description

The peptide Exenatide is emulsified as an aqueous solution in a dilute PLGA, 50:50, 4.5A polymer solution with methylene chloride, which acts as a phase inducer, a chemical that aids in the phase separation, to make a primary emulsion. Silicone oil is then added to this dispersion at a defined rate, preferably within 3 minutes, reducing the solubility of PLGA in its solvent (methylene chloride). The polymer-rich liquid phase (coacervate) encapsulates the dispersed Exenatide particles and the embryonic microspheres are subjected to a hardening and washing step using heptane and ethyl alcohol. The micro particles were produced by phase separation process, collected, followed by downstream processing and drying. Various parameters of the process were modified based on Evonik's know how to achieve the desired product (time used for homogenization process, mixing time during the formation of embryonic microspheres, and temperature for hardening process). Four batches A, B, C, and D were prepared according to the above process with different process parameters.



Scheme 1: Micro particle Process Flow Chart (derived from US patent 7612176)

EXPERIMENTAL PART

In Vitro Studies

Experiment 1: **Exenatide Assay and Microparticles Characterization**

For exenatide assay/purity, samples were analyzed by an HPLC method using an Aeris Peptide XB-C18, 250 2.1 mm, 3.6 µm column, a gradient method (Mobile Phase: 0.1% TFA in water: 0.1% TFA in acetonitrile), column temperature of 60°C, auto- sampler temperature of 5°C, 214 nm wavelength, and 20 µL injection volume.

Particle size measurements were obtained by laser diffraction using a Coulter LS 13320 Particle Size Analyzer. The surface morphology for four feasibility lots and the RLD (Bydureon® injection) were examined by scanning electron microscopy (SEM) (Hitachi SU-8230, Cold Field Emission Gun SEM, University of Minnesota). In addition, residual solvents (heptane, methylene chloride, ethyl alcohol) were accounted using different method of analysis. Karl- Fisher was used to analyze water content in the microspheres and also the content of silicone oil was also checked and documented.

Experiment 2: In vitro release study.

For in vitro release analysis, a known amount of microparticles were weighed into a 2-mL microcentrifuge tube, a known volume of 10 mM sodium acetate buffer, pH 4.5, was added, then the tubes were placed upright in a 37°C ± 2°C shaker bath set at 120 ± 10 RPM. At each time point, the tubes were centrifuged, an aliquot removed and then replaced before returning to the shaker bath. Samples were analyzed using a shorter gradient HPLC method.

In vivo study

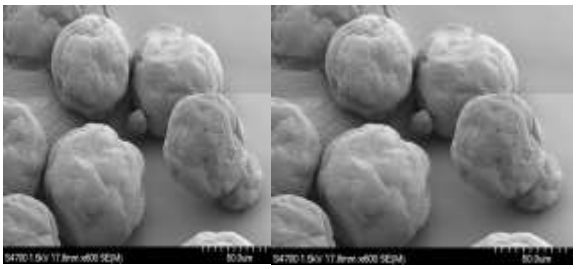
A pharmacokinetic (PK) study in Sprague-Dawley rats was conducted. An injection vehicle formulated by Evonik was used to administer the formulations. **Table 2** shows the PK study design. Blood samples were drawn at the following time points: pre-dose, 0.5, 2, 4, 6, 10, 24, 48, and 96 hours /8, 11, 15, 18, 22, 25, 29, 36, 43, 50, 57 and 64 days. Blood plasma samples were analyzed by ELISA assay.

Table 2. Pharmacokinetic (PK) study design

Treatment Description	Number of Animals	Dose Level (mg/animal)
RLD Lot(Bydureon® Amylin®)	8	1.0
Evonik Lot A	8	1.0
Evonik Lot B	8	1.0
Evonik Lot C	8	1.0
Evonik Lot D	8	1.0

RESULTS AND DISCUSSION

It is known that the surface structure of the microparticle has great impact on the release profile. The results for lot Evonik particles showed a very compact and rigid surface indicating that the drying cycle was effective in annealing the exterior surface microspheres. The appearance and surface of microspheres in the SEM image looked similar to the SEM image of the RLD as seen below in **Figure 1**,



RLD **Evonik**

Figure 1. Surface Characterization by SEM

Multiple lots of the reference product (RLD) and four Evonik lots (A, B, C, and D) were examined to establish the target product profile. Results for the lots examined are shown in **Table 3**. These results demonstrated potential lot-to-lot variability in the drug content, particle size distribution, residual solvents, moisture content, and in vitro release of the reference product. The characterization parameters for RLD were almost very similar to that for the four Evonik's lots.

Table 3: Comparison Characterization Results of RLD lots (Bydureon® Amylin®) to Evonik Pharmacokinetic Studies selected lots.

Lot Number	Drug Content	Purity %	Mean particle Size	24 hr Burst %	IVR Day 33 (Cum %)	Moisture Content	Residual Solvent %		
							EtOH	DCM	Heptane
Bydureon® Lots Range[1]	4.6-4.9	98.7-99.2%	56-66	0.0-0.1	62-73	0.41-0.45	0.35-0.55	0.14-0.21	0.81-1.1

Target Acceptable Range	**3.5-5.5**	**Monitor**	**Pending[1]**	**Not more than 0.5**	**>50**	**Monitor**	**Monitor**	**Monitor**	**Monitor**
Lot A	5.10	80.0	131	0.0	56	1.1	0.5	0.1	0.2
Lot B	5.20	82.0	93	0.0	54	1.1	0.2	0.1	0.3
Lot C	7.70	94.0	77	0.0	50	1.1	0.2	0.3	0.6
Lot D	5.1	93.0	65	0.0	53	1.3	0.2	0.2	0.4

IVR: Denoted for in vitro release.
EtOH: Denoted for ethyl alcohol.
DCM: Denoted for dichloromethane.
[1] Three lots of Bydureon were used for comparison.

Three lots of the reference product (RLD) and two of Evonik lots (A and B) were examined to establish the in vitro release profile. Results for the lots examined are shown in **Figure 2**. The key characteristics of RLD IVR profile include minimum initial burst, lag period of ~3 weeks, and sigmoid release profile during the release phase. At the initial development stage, Evonik produced formulation meet these criteria. Further optimization can be implemented to refine the process.

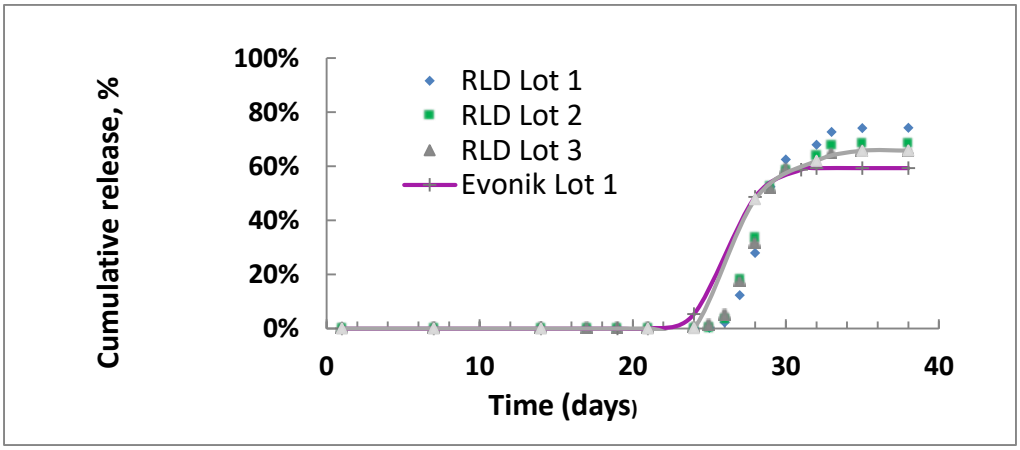


Figure 2: In Vitro Release of Evonik lots versus RLD (Bydureon® Amylin®)

During the development, several lots were produced while varying the process parameters to obtain the desired target product profile. From the promising lots, a subset was chosen for analysis in a PK study (pharmacokinetic study). **Figure 3** and **Figure 4** show the resulting plasma concentration profiles of the reference product lot in comparison to an Evonik-produced lot. Exenatide Pharmacokinetic Parameters Following a Single Subcutaneous Injection Administration of 1 mg/animal Test Formulations or Reference Product to Male Rats are shown in **Table 4**. The ratio of area under curve (AUC) of Evonik formulation compared to RLD is 1.08. This result indicates the in vivo release profile of the Evonik produced formulation is similar to RLD.

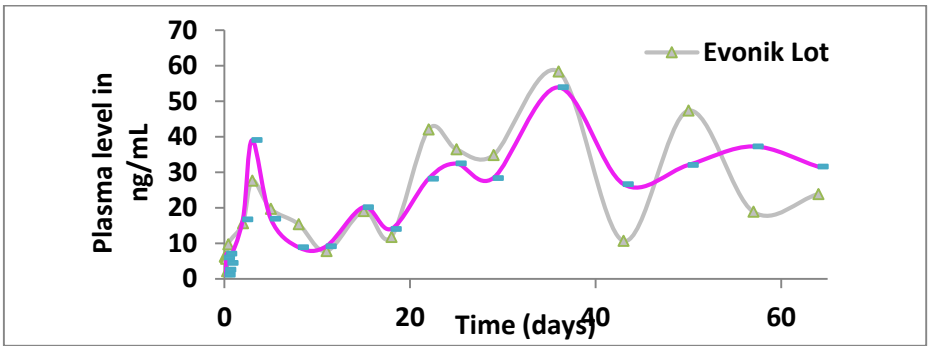


Figure 3: In Vivo Release of Evonik Lot versus RLD (Bydureon® Amylin®)

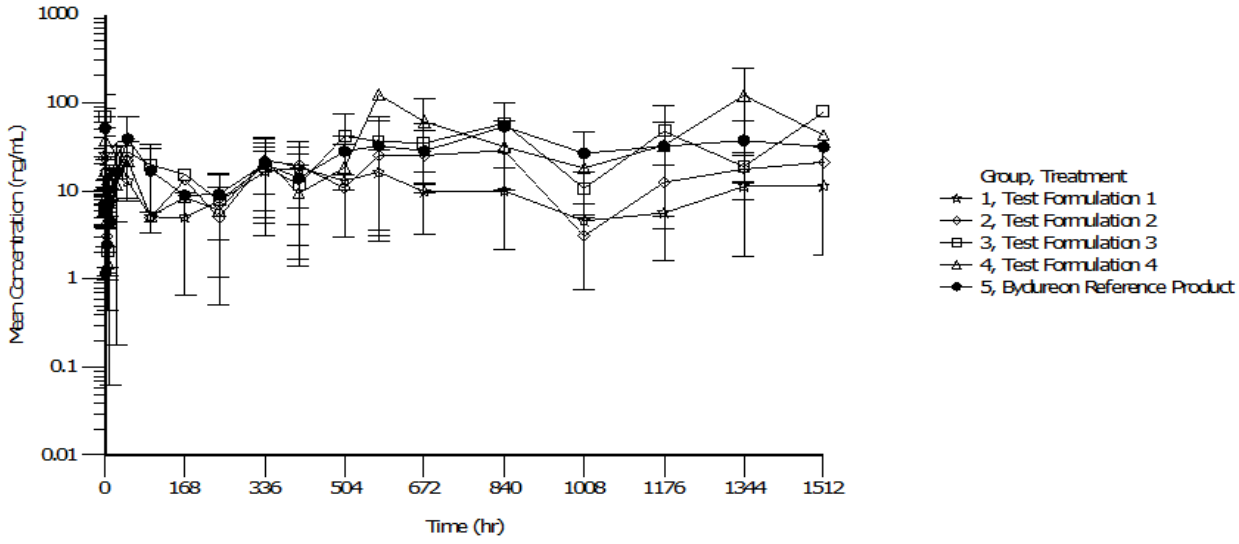


Figure 4: Mean (±SD) Exenatide Plasma Concentration-Time Profiles Following a Single Subcutaneous Injection Administration of 1 mg/animal Test Formulations or Reference Product to Male Rats -Day 1 – Time 0 to 1512 hour (Log:Linear).

Table 4: Mean (±SD) and CV% Exenatide Pharmacokinetic Parameters Following a Single Subcutaneous Injection Administration of 1 mg/animal Test Formulations or Reference Product to Male Rats

Subject Group	Dose (mg)	Treatment Description	Statistic	C_{max} (ng/mL)	T_{max} (hr)[a]	T_{last} (hr)[a]	AUC_{Tlast} (hr*ng/mL)	$AUC_{0\text{-}1512hr}$ (hr*ng/mL)	$AUC_{0\text{-}24hr}$ (hr*ng/mL)	TF:RP[b]	TF:RP[c]
4	1	Test Formulation 4	N	8	8	8	8	8	8	1	1
			Mean	198	NA	1512	62000	63200	216	1.44	1.07
			SD	295	(0 1512)	(1344 1512)	65800	65300	155	NA	NA
			CV%	149	NA	NA	106	103	71.9	NA	NA
4[f]	1	Test Formulation 4	N	7	7	7	7	7	7	1	1
			Mean	95.9	840	1512	39600	41000	199	0.93(	0.98
			SD	70.3	(0-1512)	(1344-1512)	19100	19100	160	NA	NA
			CV%	73.3	NA	NA	48.2	46.7	80.2	NA	NA
5	1	Bydureon Reference Product	N	8	8	8	8	8	8	1	1
			Mean	85.5	48	1512	42800	43800	202	NA	NA
			SD	36.0	(0 840)	(1344 1512)	20100	19800	131	NA	NA
			CV%	42.1	NA	NA	46.9	45.3	65.2	NA	NA

NA - Not applicable
a: Median (minimum - maximum), median value only reported if actual collection interval.
b: TF:RP = $AUC_{0\text{-}1512hr\ Test\ Formulation}/AUC_{0\text{-}1512hr\ Reference\ Product}$
c: TF:RP = $AUC_{0\text{-}24hr\ Test\ Formulation}/AUC_{0\text{-}24hr\ Reference\ Product}$
d: Animal number 608 was excluded from descriptive statistics due to low dose administration on Day 1
e: Animal number 622 was excluded from descriptive statistics due to observed pharmacokinetic inconsistencies.
f: Animal number 628 was excluded from descriptive statistics due to observed pharmacokinetic inconsistencies

CONCLUSION

One of the challenges in developing the parenteral formulation is to control the initial burst that contributes to unwanted side effects. During this stage of the development, Evonik produced exenatide loaded microparticles using its know-how that demonstrates low to no initial burst, drug content, and particle size distribution similar to RLD. Most importantly, in vivo release profile of the Evonik formulation is similar to RLD.

The current process can be optimized and scaled up to produce the commercial product similar to Bydureon™**.**

ACKNOWLEDGEMENT

MPI Research, Mattawan, MI, USA
Pharmaceutical Sciences Research Institute at Samford University, Birmigham, AL, USA
Characterization Facility, University of Minnesota, Minneapolis, MN, USA

REFERENCES

1. Noguchi H.Stem cell applications in diabetes .J Stem Cells.2012; 7: 229-244.
2. Hinton P.S. Role of reduced insulin-stimulated bone blood flow in the pathogenesis of metabolic insulin resistance and diabetic bone fragility Med Hypotheses 2016;93: 81-86.
3. Tonneijck, M.M. Smits, M.H.A. Muskiet, T. Hoekstra, M.H.H. Kramer, A.H.J.Danser, *et al.* Acute renal effects of the GLP-1 receptor agonist exenatide in overweight type 2 diabetes patients: a randomized, double-blind, placebo-controlled trial. Diabetologia.2016;59: 1412-1421
4. Scott L.J. Exenatide extended release: a review of its use in type 2 diabetes mellitus Drugs. 2012; 72:1679-1707
5. Kang J., Wu F., Cai Y., Xu M., He M., Yuan W. Development of recombinant human growth hormone (rhGH) sustained-release microspheres by a low temperature aqueous phase/aqueous phase emulsion method. Eur J Pharm Sci, 2014; 62: 141-147.
6. Lin Q., Cai Y., Yuan M., Ma L., Qiu M., Su J. Development of a 5-fluorouracil-loaded PLGA microsphere delivery system by a solid-in-oil-in-hydrophilic oil (S/O/hO) novel method for the treatment of tumors. Oncol. Rep.2014; 32: 2405-2410.
7. A.S. Hasan, A. Sapin. C. Damgé, P. Leroy, M. Socha, P. Maincent Reduction of the *in vivo* burst release of insulin-loaded microparticles
8. J Drug Deliv Sci Technol.2015;30 : 486-493.
9. Patent No. US7, 612, 176 B2, Nov. 3, 2009.
10. Bydureon TM package insert.