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Development of a Highly Stable, Nonaqueous Glucagon Formulation for Delivery via Infusion Pump Systems

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Abstract

Background: Despite a vigorous research effort, to date, the development of systems that achieve glucagon stability in aqueous formulations (without reconstitution) has failed to produce any clinical candidates. We have developed a novel, nonaqueous glucagon formulation based on a biocompatible pharmaceutical solvent, dimethyl sulfoxide, which demonstrates excellent physical and chemical stability at relatively high concentrations and at high temperatures.

Methods: This article reports the development of a novel, biocompatible, nonaqueous native human glucagon formulation for potential use in subcutaneous infusion pump systems.

Results: Data are presented that demonstrate physical and chemical stability under presumed storage conditions (>2 years at room temperature) as well as "in use" stability and compatibility in an Insulet's OmniPod® infusion pump. Also presented are results of a skin irritation study in a rabbit model and pharmacokinetics/pharmacodynamics data following pump administration of glucagon in a diabetic swine model.

Conclusions: This nonaqueous glucagon formulation is suitable for further clinical development in pump systems.

Keywords
glucagon, hypoglycemia, infusion, nonaqueous

Glucagon is currently approved for the treatment of severe hypoglycemia.[1,2] Because glucagon is not stable in aqueous solutions, these preparations are provided as lyophilized powders for reconstitution. Once reconstituted, these preparations begin to degrade and fibrillate rapidly and must be discarded if not used immediately. While suitable for treatment of severe hypoglycemia, these glucagon preparations are unstable and thus not suitable for development of additional indications including minidosing for mild to moderate hypoglycemia[3] and infusion pump applications. In particular, a stable glucagon suitable for use in infusion pump systems creates an opportunity for treatment of hypoglycemia under different conditions, including as a component of an artificial or "bionic" pancreas system.[4,5]

Despite a vigorous research effort, to date development of aqueous-based glucagon formulations (without reconstitution) has failed to produce any clinical candidates. We have developed a novel, nonaqueous glucagon formulation based on a biocompatible pharmaceutical solvent, dimethyl sulfoxide, which demonstrates excellent physical and chemical stability (up to 2 years at room temperature)[6] at relatively high concentrations. The solubility and stability of this formulation allow for development of a higher concentration formulation (5 mg/ml vs 1 mg/ml for the currently approved glucagon formulations) to facilitate administration from devices such as autoinjectors, pens, pumps, and so on. Furthermore, this glucagon formulation is in clinical development as a treatment for severe hypoglycemia[6] and moderate hypoglycemia and for management of blood glucose in pump applications.

This article presents stability, compatibility, and preclinical data supporting progression of this formulation into clinical development.

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Methods

Glucagon Formulation Prep

Glucagon (Bachem, Bubendorf, Switzerland) was prepared at 5 mg/mL with glycine and trehalose excipients via dissolution into a dimethyl sulfoxide (DMSO) (Gaylord, USP Grade, Tuscaloosa, AL, USA) solution. The solution was filtered through 0.22 mm filter and aliquoted into sterile, 2 mL glass vials, each at 1 mL fill volume.

Pump Stability and Compatibility

Filling OmniPod Pumps for Glucagon Drug Product Stability Studies. A total of 2 mLs of nonaqueous glucagon solution each was injected through the fill ports of 3 separate OmniPod® pumps via transfer syringe; neat DMSO was added to the 4th pump. The devices were automatically primed. Each OmniPod was linked to its respective personal diabetes manager (PDM), which was used to wirelessly program the pump to bolus 300 µL of sample into HPLC autosampler vials through the cannula at predetermined time intervals. Each pump was stored at 37°C (without humidity control) for a total of 6 days. Drug formulation was collected from the pump via "bolus dosing" on the collection days.

Filling OmniPod Pumps for Compatibility Studies. Three OmniPod patch pumps were used to provide triplicate sampling for this study. Each pump was filled through the pump's septum with ~1.1 mL DMSO and stored static at 37°C (without humidity control) for 6 days. For the first 3 time points (T: 0, 1 day, and 3 days) 300 mL were dispensed through each of the pump's integrated needle/cannula system into a vial. Following the T: 3 day time point, the OmniPod was deactivated (PDM programmed to discard the OmniPod) but remained in the 37°C chamber until T: 6 days. At the 6-day time point, the remaining DMSO was withdrawn from the OmniPod pumps through the septum using a DMSO compatible syringe needle and compatible syringe. Sample vials were screw-capped with a DMSO-compatible stopper/cap and stored at 5°C, protected from light. At the end of the sample collection stage, all samples and corresponding blank solutions were tested for leachables by liquid chromatography–mass spectrometry (LC-MS), gas chromatography–mass spectrometry (GC-MS), and inductively coupled plasma–mass spectrometry (ICP-MS).

Analytical Methods Used for Glucagon Drug Product Stability Studies

Visual inspection. Visual inspection was performed for the samples (collected at each time point) under a white light source against a dark background. Digital photographs were acquired.

Absorbance spectrophotometry. Absorbance measurements were collected using a Beckman Coulter DU 800 spectrophotometer. Analyses were performed from 250 to 650 nm to evaluate for both turbidity and any sign of leachables in the individual samples. All samples were analyzed neat with a quartz cuvette.

RP-HPLC. Absorbance at 280 nm was monitored, following a 30 µg injection via an autosampler held at 37°C. Injection was made onto a Thermo Scientific BioBasic-8, 250 × 4 mm column maintained at 25°C and the mobile phase consisted of a combination of (1) 0.1% TFA in milli-q water and (2) 0.1% TFA in acetonitrile, run as a gradient, at flow rate of 1.0 mL/min. RP-HPLC analysis was also used to determine the concentration of glucagon in the study. This was accomplished by generating a 3-point standard curve at each time point from the reference standard at 15, 30, and 45 µg loads. The peak area from each sample was evaluated against the standard curve to extrapolate the glucagon concentration value.

Size exclusion–high performance liquid chromatography (SE-HPLC). Absorbance at 280 nm was monitored, following a 30 µg injection via an autosampler held at 25°C. Injection was made onto a TOSOH TSKGel G2000SWXL, 7.8 × 300 mm column maintained at 35°C and the mobile phase consisted of 3.2 mM HC and 100 mM NaCl in aqueous solution run at a flow rate of 1.0 mL/min.

Analytical Methods Used for Pump Compatibility Studies

LC-MS. Sample aliquots of 100 mL were diluted with 0.4 mL methanol and 0.5 mL water, spiked with internal standard (di-n-butyl phthalate-d), then analyzed using LC-MS. A 50 × 2.1 mm Xterra C18MS, 3.5 mm column was used. The mobile phase was a combination of (1) 1 mM ammonium formate in 95/5 water/ methanol, and (2) 1 mM ammonium formate in 50/50 methanol/ acetonitrile. Gradient was 90:10 (1):(2) held for 1 min, then to 100% (2) in 9 minutes, held for 15 minutes. The flow rate was 0.3 mL/ min and injection volume was 5 µL. The detector was an electrospray version whose needle voltage 6000 V, shield voltage 600 V, capillary voltage 40 V. The drying gas was nitrogen at 200 C and 23 psi. Sample was nebulized at 54 psi and the mass spectrum scanned from 100-1500 amu at 1.0 sec/scan.

GC-MS. An aliquot of each sample was weighed, spiked with internal standards and dissolved in methylene chloride. Each dissolved sample was analyzed by GC-MS. A 30 × 0.32 mm Rxi-5Sil MS, 1 micron film column was used. This column was held at 40°C for 3 minutes initially, and then ramped at 12°C/min to a temperature of 320°C. The injector temperature was 320°C. Mass spectrum was scanned at a rate of 3.32 scans/sec, between 35-475 amu.

ICP-MS. Sample portions of 0.1 g were mixed with 10 g of a solution containing 1% nitric acid, 3% hydrochloric acid, and internal standards. The fully dissolved sample thus prepared was subjected to ICP-MS per an internal protocol at Exova (Sabre Springs, CA).

Pharmacodynamics and Pharmacokinetics of Glucagon in Yorkshire Pigs

This study was conducted at Legacy Research Institute (LRI) in Portland, Oregon. Four Yorkshire pigs were obtained from

an outside source and acclimated in the LRI vivarium for 2 weeks prior to study. Each of the 4 pigs was observed in 6 experiments for a total of 24 experiments. Each experiment consisted of an injection of 1 formulation, either Xeris G-Pump™ (glucagon infusion), 7-days-aged Xeris G-Pump (glucagon infusion), or fresh Novo GlucaGen® and then sampling of blood at various time points over a 2-hour period, as described below, from the vena cava. After an experiment was completed, the pig was returned to the vivarium and not studied again for at least 1-2 weeks to allow for adequate drug washout. The pig then received a second injection of another formulation. This sequence was repeated until the 6 injections were completed so that each individual pig was administered each of the 3 formulations twice. This study design was carried out the same way for each of the 4 pigs, although the formulations were delivered in a separate order for each pig for a total of 24 experiments. As a result, it was possible to obtain pharmacokinetics (PK) data on each of the 3 formulations, in 8 experiments. Pigs were fasted overnight and prepared for experiments the following morning. Anesthesia consisted of isoflurane administered via an endotracheal tube. Multiple hemodynamic, cardiac, and pulmonary parameters were monitored continuously. All procedures were survival studies. A central venous line was placed in the vena cava prior to drug administration. Octreotide (Bedford Laboratories, Bedford, OH) (44 μg/kg) was administered subcutaneously 30 minutes prior to the start of the experiment. Octreotide suppresses the release of insulin and glucagon and allows for complete analysis of the glucagon time course in the absence of the effects of endogenous insulin or glucagon.

The 3 formulations were delivered with an OmniPod. The OmniPod was filled with a test formulation, and then placed on the lower abdomen using an adhesive to ensure no movement of the pod occurred during the 2 hour experiment. The OmniPod was then activated resulting in insertion of a cannula through the skin into the subcutaneous space. The OmniPod activity was then initiated to provide a bolus of drug at a dose of 2 μg/kg. The rate of delivery of nonaqueous glucagon and Novo GlucaGen differed because of the difference in concentration. Xeris glucagon (5 mg/mL) was delivered over a period of approximately 1 minute, whereas the Novo GlucaGen (1 mg/kg) was delivered over a period of approximately 5 minutes. These differences, while minor, would result in a difference in the rate of absorption into the circulation and thus were taken into account and compensated for in the analysis of the glucose time course described below. Blood samples for glucose and glucagon assay were obtained at –10, –5, 0, 5, 10, 15, 20, 30, 45, 60, 120, 180, and 240 minutes from the start of bolus delivery. A very small sample was obtained (less than 1 ml) and blood glucose levels were measured using the Hemocue 201 point-of-care instrument (Angelholm, Sweden).

Glucagon was measured at the Oregon Health and Science University Immunoassay Core Laboratory from plasma samples using a radioimmunoassay kit supplied by Millipore Corp (catalogue # GL-32K) and performed according to the kit procedure. The procedure utilizes I^{125} labeled glucagon in competition with unlabeled glucagon for binding to glucagon antiserum. Multiple concentrations of unlabeled material are added to obtain a standard curve. I^{125} counts per second are obtained from a gamma counter. Plasma samples from the studies were assayed in duplicate and diluted prior to reassay if they exceeded the range of the standard curve. Acceptance criteria were the same as those specified by the kit manufacturer.

Rabbit Cutaneous Irritation Study

This study was conducted by Charles River Laboratories (Spencerville, OH) under good laboratory practices (GLP). The study was conducted on 72 New Zealand White male rabbits, weighing between 2.5 and 3.5 kg, obtained from a Charles River colony. The animal's scapular and middorsal areas were clipped free of hair before the first dose and as often as necessary thereafter to allow for clear visualization of the test site. Care was taken during the clipping procedure to avoid abrasion of the skin and the site of application was delineated. Drug was administered via an OmniPod. Removal and ingestion of the unit were prevented by placing a stockinette over the trunk and test area. Collars were placed on each animal at the time of dosing and remained in place until removal prior to termination. The rabbits were allowed access to water and food at all times. During the study, clinical observations were obtained daily, food consumption recorded, and behavior observed. After a period of acclimation to the OmniPod and sleeve, groups of 12 rabbits received either saline, Xeris glucagon, or vehicle as 3 separate boluses of 30 minutes 8 hours apart per day (3 boluses per day) for either 24 hours or 72 hours at a dose of 0.15 mg/kg or an equal volume of saline or vehicle. This is a 9-fold multiple of the exposure anticipated if 1 mg/day of glucagon were infused into a human subject. The study design is indicated in Table 1.

Following completion of the exposure period, the stockinette and unit were removed from each animal and the corners of the test site were redelineated using a marker. The area where the unit was placed was then rinsed using gauze moistened with deionized water followed by dry gauze. The rabbits were then sacrificed, the skin removed, and a histopathological analysis conducted. For purposes of following the time course of any pathological or histopathological changes, each of the groups shown in Table 1 was subdivided into 4 rabbits as described in Table 2.

Results

Drug Product Stability Studies

Visual Observations. Sample aliquots were retrieved from each OmniPod pump following 0, 1, 3, and 6 days of 37°C

Table 1. Experimental Design for Rabbit Skin Irritation Study.

Group	Test material	Dose level (mg/kg/injection)	Dose volume (mL/kg/injection)	Male animals (n)	
				24 hours	72 hours
1	Saline	0	0.03	12	12
2	Vehicle	0	0.03	12	12
3	Glucagon	0.150	0.03	12	12

Table 2. Histology and Histopathology Schedule.

Group	Number of animals	Scheduled euthanasia day	Necropsy procedures		Histology (preparation of tissue slides)	Histopathology (evaluation of tissue slides by board-certified pathologist)
			Necropsy	Tissue collection		
1	4		X	X	Injection site(s)	Injection site(s)
2	4	2			Injection site(s)	Injection site(s)
3	4				Injection site(s)	Injection site(s)
1	4		X	X	Injection site(s)	Injection site(s)
2	4	4			Injection site(s)	Injection site(s)
3	4				Injection site(s)	Injection site(s)
1	4		X	X	Injection site(s)	Injection site(s)
2	4	8			Injection site(s)	Injection site(s)
3	4				Injection site(s)	Injection site(s)
1	4		X	X	Injection site(s)	Injection site(s)
2	4	10			Injection site(s)	Injection site(s)
3	4				Injection site(s)	Injection site(s)
1	4		X	X	Injection site(s)	Injection site(s)
2	4	15			Injection site(s)	Injection site(s)
3	4				Injection site(s)	Injection site(s)
1	4		X	X	Injection site(s)	Injection site(s)
2	4	17			Injection site(s)	Injection site(s)
3	4				Injection site(s)	Injection site(s)

incubation. No changes in coloration or opacity were detected for the samples stored in the pumps during 37°C incubation.

Absorbance Results. Absorbance analyses were performed on each aliquot from the study to test for turbidity and investigate the presence of any leachables from the OmniPod pumps. Samples were analyzed in a broad spectral range (250-650 nm). Figure 1 illustrates the absorbance results of each sample stored in the pumps for 0, 1, 3, and 6 days at 37°C incubation. No significant changes were observed in the absorbance profiles between 350 and 650 nm for the samples at each time point, thus indicating no turbidity or leachables. Although all the T = 0 samples displayed an absorbance peak near 440 nm, this peak was not present in each of the subsequent time points and is likely an artifact. Also, since samples were analyzed neat, the high concentration of glucagon generated saturated absorbance levels between 250 and 300 nm.

RP-HPLC Results. The glucagon drug product stored in OmniPod pumps were also evaluated by RP-HPLC for chemical modification and the presence of leachables. Figure 2 shows the chromatograms of each sample over 6 days of incubation in OmniPod pumps at 37°C. Each glucagon drug product sample demonstrated a high level of glucagon purity (≥99.4%) throughout the study. Trends in the levels of RP-HPLC main peak purity for the samples stored in OmniPod pumps are shown in Figure 3. These trend graphs illustrate only slight differences in main peak purity for the nonaqueous glucagon samples over 6 days at 37°C. The results obtained for each sample are comparable with the reference standard. Thus, effectively no degradation of glucagon was observed when analyzed after up to 6 days of incubation in the OmniPod at body temperature.

SE-HPLC Results. SE-HPLC analysis was used to monitor for oligomeric and cleavage products for glucagon drug product following storage in OmniPod pumps. The SE-HPLC chromatograms obtained for each sample stored in an OmniPod pump over 6 days at 37°C are illustrated in Figure 4. At the different time points, each glucagon drug product samples displayed high glucagon purity. No fragmentation, aggregation,

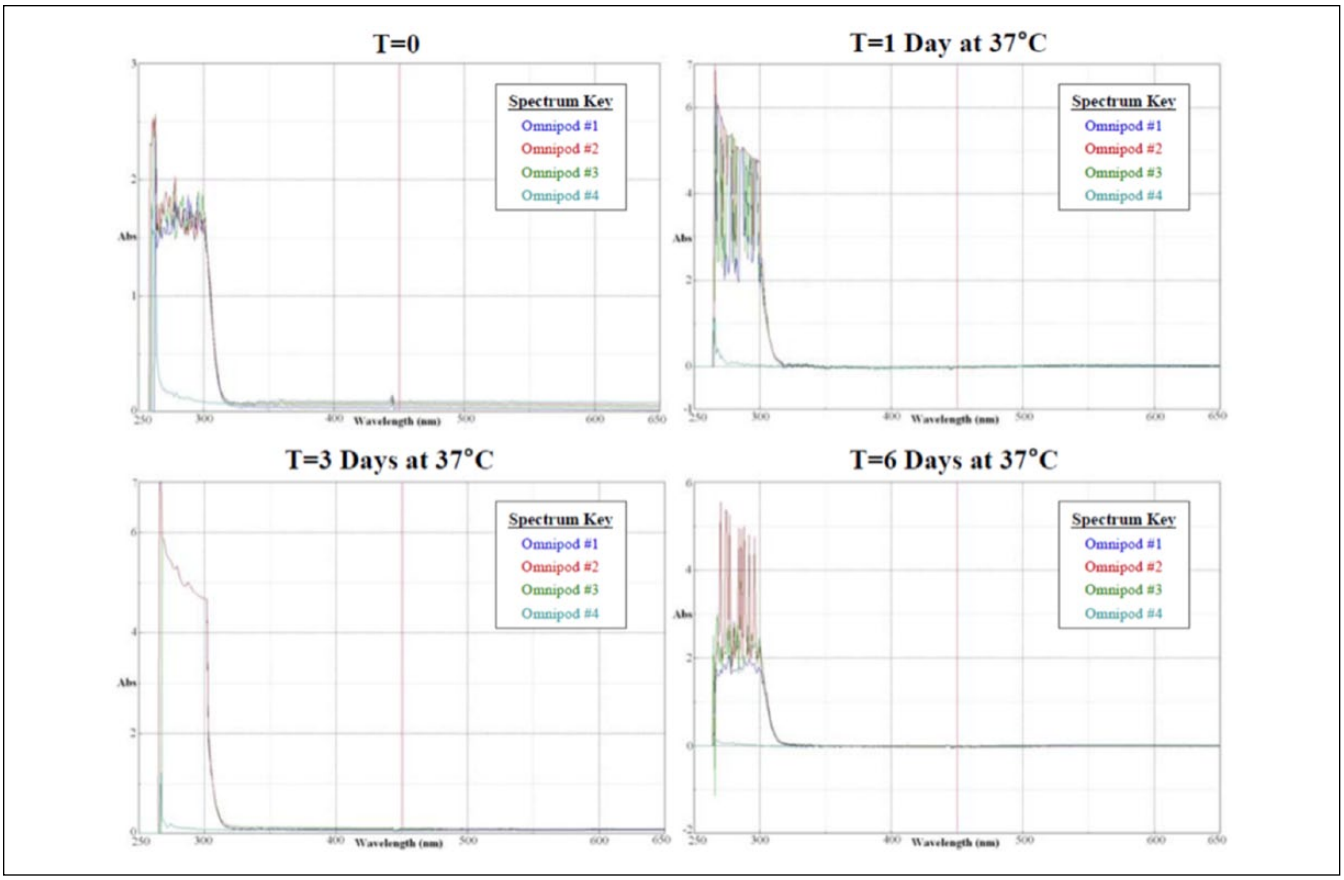


Figure 1. Absorbance scans of sample aliquots removed from the OmniPod pumps after incubation at 37°C for up to 6 days. Samples 1 to 3 are glucagon formulation; sample 4 is a dimethyl sulfoxide (DMSO) blank.

or artifacts were detected for the samples stored in the pumps during 37°C storage. Importantly, no aggregation or higher molecular weight products were observed after incubation at 37°C. Among degradation products, higher molecular weight products are particularly concerning due to the potential for increased immunogenicity.[7]

Compatibility Studies

GC-MS. Intermittent presence of 2 organic semivolatile impurities poststorage in OmniPod pump for ≥1 day was observed. Both impurities, when present, were at ≤50 ppm or lower. These values are far below those required to initiate any toxicological studies in any species.

LC-MS. Intermittent presence of organic impurities poststorage in OmniPod pump for ≥1 day was observed. All of the peaks detected in these samples are consistent with poly-(oxyethylene)-containing compounds. Although a number of lower mass peaks are present in the spectrum of this peak, it is impossible to determine if these are due to fragmentation or simply background, as this peak is close to detection limit and the signal:noise is low. These values are far below those required to initiate any toxicological findings in any species.

ICP-MS. Intermittent presence of several metals was observed. With the exception of sodium and potassium, all detected metals were present at ≤1 ppm levels. Since sodium and potassium occur in the body and the levels of other detected metals were significantly low, these findings are unlikely to present issues relevant to humans exposed to pumped glucagon drug product. These values are far below those required to initiate any toxicological findings in any species.

Pharmacodynamics of Glucagon Preparations

The effects of Xeris nonaqueous glucagon, 7-days-aged nonaqueous glucagon, and Novo GlucaGen are illustrated in Figure 5. The curves shown in Figure 5 incorporate a running average of each value and thus are smoothed using a 10-minute filter. This filter imparts a delay of (filter length/2) = 5 minutes, which was accounted for. In addition, a correction factor of 2.5 minutes was applied to the Novo data to account for the delay in administration time for the Novo GlucaGen

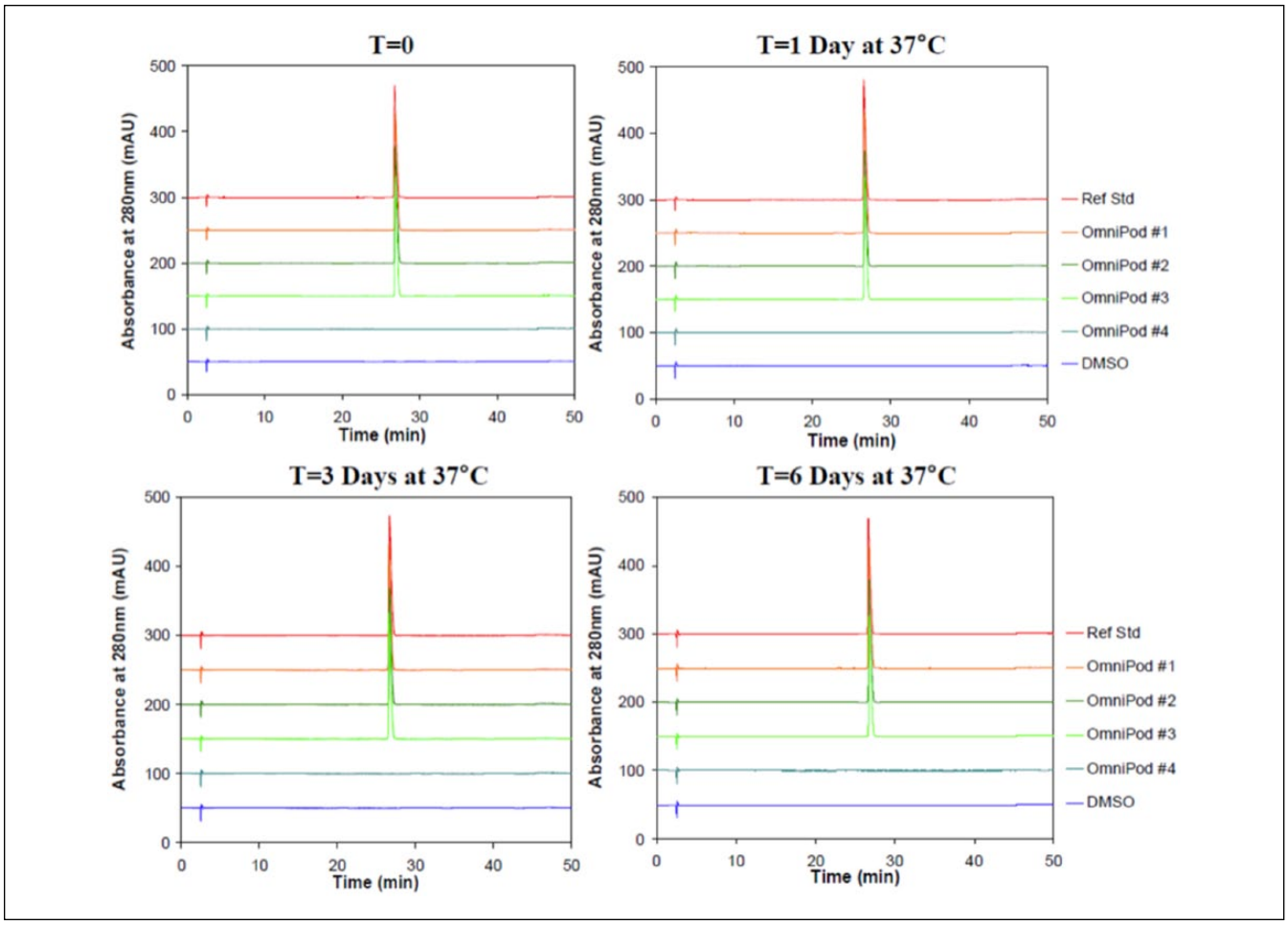


Figure 2. Reverse phase–high performance liquid chromatography (RP-HPLC) chromatograms of samples removed from the OmniPod pumps after incubation at 37°C for up to 6 days. Samples 1 to 3 are glucagon formulation; sample 4 is a dimethyl sulfoxide (DMSO) blank.

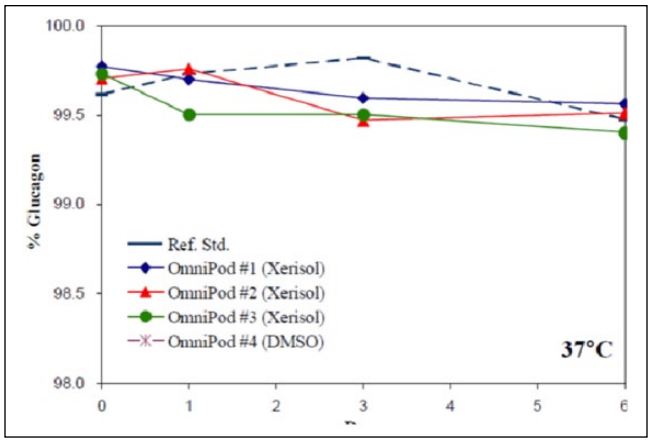


Figure 3. Reverse phase–high performance liquid chromatography (RP-HPLC) trend graph of samples stored in OmniPod pumps at 37°C after incubation at 37°C for up to 6 days. Samples 1 to 3 are glucagon formulation; sample 4 is a dimethyl sulfoxide (DMSO) blank.

as compared to Xeris glucagon. The data were deemed to be nonskewed (normally distributed), and thus a *t* test was used to detect differences among the groups. This analysis indicated that there were no significant differences among the 3 formulations. Importantly, aging Xeris nonaqueous glucagon in a pump for 7 days had no effect on the glycemic response.

Additional confirmatory analysis was conducted in which the absolute rise in glucose, the time to reach 50% of the maximum response, the t_{max}, and the time to decrease 50% from the maximum response were evaluated. The data are shown in Figure 6. The rise to maximal blood glucose (BG), the time to the early (rising) 50% maximum BG, the time to the late (decreasing) BG, and the time to return to baseline (100% max) for all 3 formulations were very similar. Statistical evaluation of these data indicated no differences among the 3 formulations. Overall, the data demonstrate a rapid rise in glucose levels of biologically significant magnitude resulting from fresh and aged nonaqueous glucagon that was not different from Novo GlucaGen.

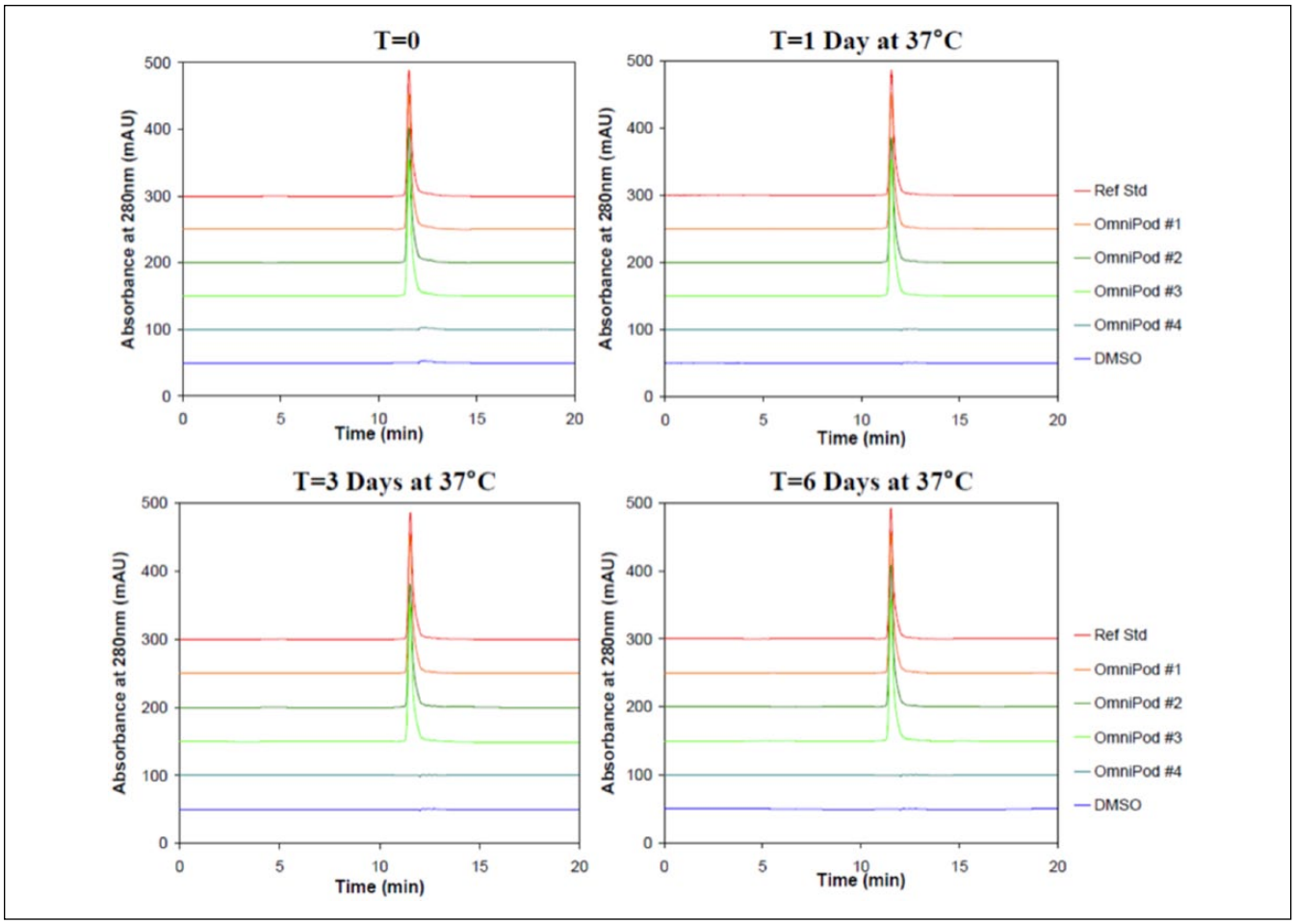


Figure 4. Size exclusion–high performance liquid chromatography (SE-HPLC) chromatograms of samples removed from the OmniPod pumps after incubation at 37°C for up to 6 days. Samples 1 to 3 are glucagon formulation; sample 4 is a dimethyl sulfoxide (DMSO) blank.

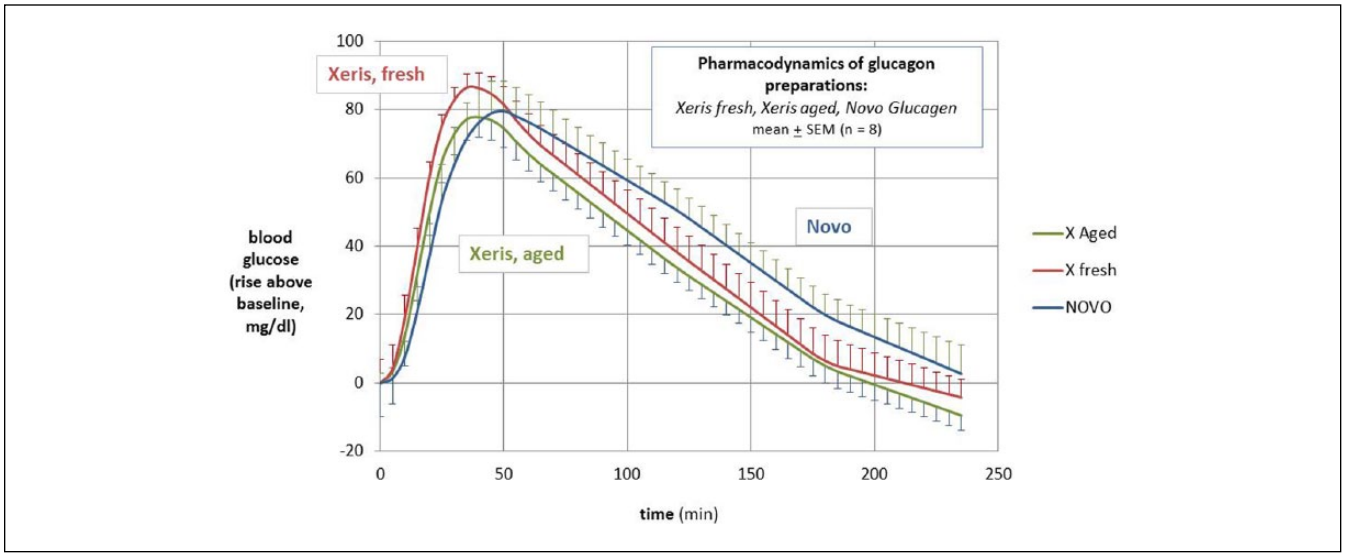
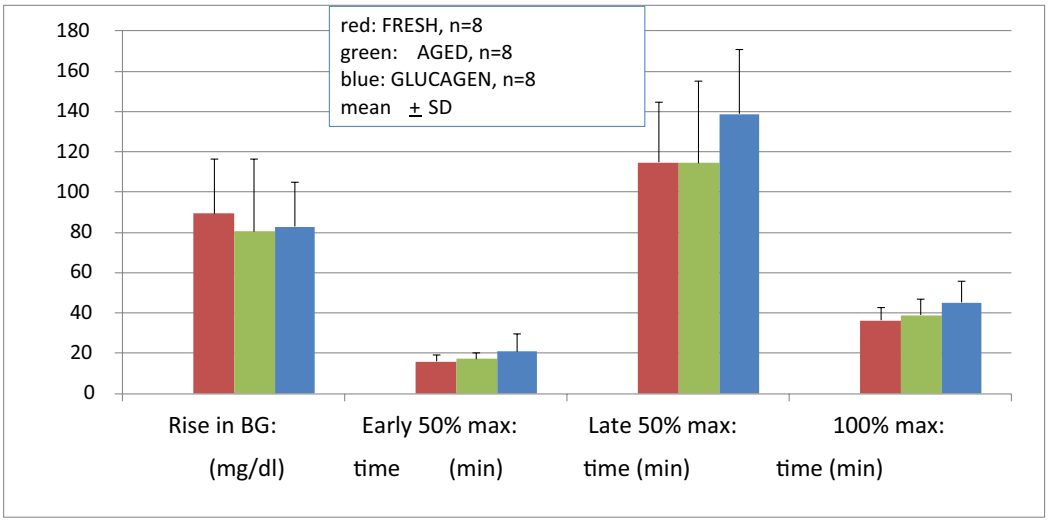


Figure 5. Elevations in blood glucose levels after administration of Xeris glucagon, 7-days-aged Xeris glucagon, and Novo GlucaGen. Blood levels were measured with a glucometer using a small drop of blood obtained from a vena caval line. No differences were observed among the 3 formulations.



	mean	SD
FRESH XERIS		
Rise in BG:	89.4	27.2
Early 50% max:	15.8	3.2
Late 50% max:	114.6	29.9
100% max:	36.3	6.6

	mean	SD
AGED XERIS		
Rise in BG:	80.5	35.7
Early 50% max:	17.2	3.1
Late 50% max:	114.5	40.6
100% max:	38.8	7.9

	mean	SD
GLUCAGEN		
Rise in BG:	82.7	22.5
Early 50% max:	20.7	8.8
Late 50% max:	138.7	32.1
100% max:	45.1	10.7

Figure 6. Kinetics of glucose levels after administration of Xeris glucagon, 7-days-aged Xeris glucagon, and Novo GlucaGen. The data indicate that the rate of rise of glucose, the maximum glucose levels, and the rate of decrease in glucose are not significantly different among the 3 formulations.

Pharmacokinetics of Glucagon Preparations

The time course of glucagon levels are illustrated in Figure 7. The curves shown in Figure 7 incorporate a running average of each value and thus are smoothed using a 10-minute filter. This filter imparts a delay of (filter length/2) = 5 minutes. In addition, a correction factor of approximately 2.5 minutes was applied to the Novo data to account for the delay in administration time for the Novo GlucaGen. The data were deemed to be skewed (not normally distributed) and thus were analyzed and plotted according to the median, the 25th percentile, and the 75th percentile. Because of the nonparametric nature of the data, comparisons were made with the Mann–Whitney *U* test. Additional analyses were conducted in which the absolute rise in glucagon, the area under the curve, the time to reach 50% of the maximum response, the t_{max}, and the time to decrease 50% from the maximum response were evaluated. Figure 7 illustrates the absolute rise in glucose for the 3 formulations. There was no significant difference among the 3 formulations.

Additional analyses were conducted in which the absolute rise in glucagon concentration, the area under the curve, the time to reach 50% of the maximum response, the t_{max}, and the time to decrease 50% from the maximum response were evaluated. Figure 8 illustrates the area under the curve for the first 60 minutes of the experiment. There were no significant differences among the 3 formulations. Evaluation of the other pharmacokinetic parameters also failed to indicate a difference among the 3 formulations.

Limitations of this study include the fact that despite octreotide administration, we cannot guarantee that insulin was fully suppressed during the entire study—after glucagon-induced hyperglycemia, BG did return to baseline levels. In addition, we concede that despite its benefits to inhibit endogenous insulin and glucagon secretion, octreotide may have independent effects on carbohydrate metabolism.[8] A third limitation is that there have been reports that general anesthesia can have direct effects on carbohydrate metabolism, specifically to raise glucose levels.[9,10] Despite these limitations, by the design of the study, all of these potentially confounding effects would equally apply to all formulations, and, for this reason, would not nullify the validity of the conclusions.

Rabbit Cutaneous Skin Irritation Study

No test-article-related clinical signs occurred during the study. Observations of scabs at the injection sites were

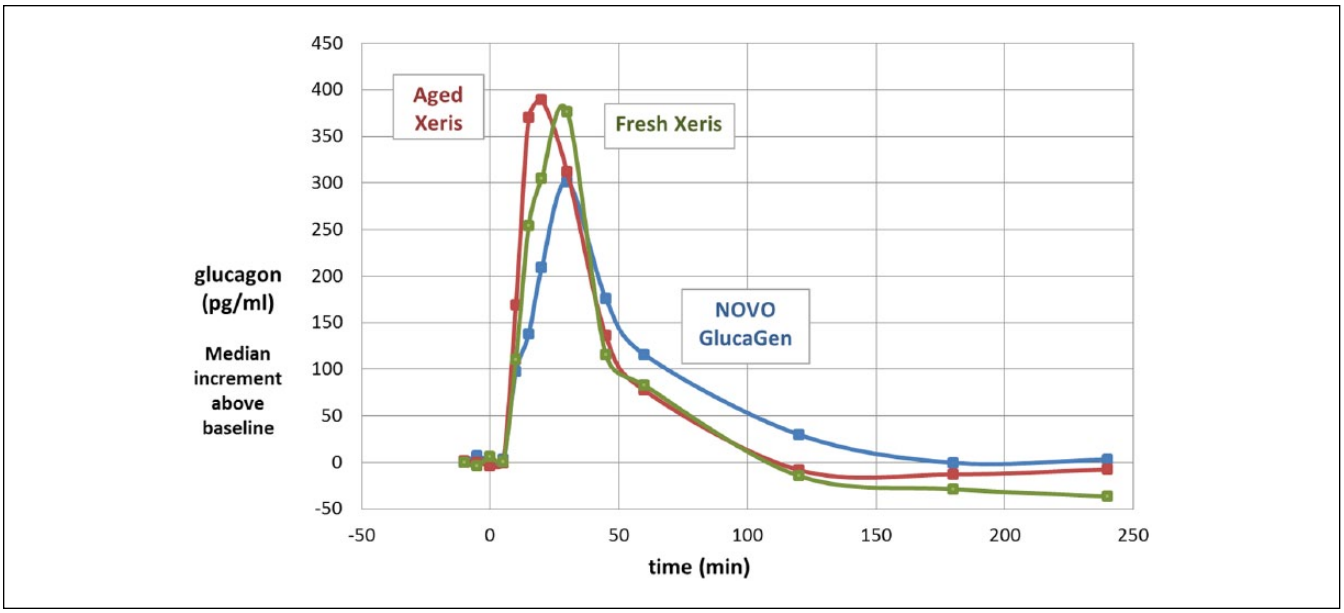


Figure 7. Pharmacokinetics of Xeris glucagon, 7-days-aged Xeris glucagon, and Novo GlucaGen. The curves incorporate a running average of each value and thus are smoothed using a 10-minute filter. This filter imparts a delay of (filter length/2) = 5 minutes. In addition, a correction factor of approximately 2.5 minutes was applied to the Novo data to account for the delay in administration time for the Novo GlucaGen.

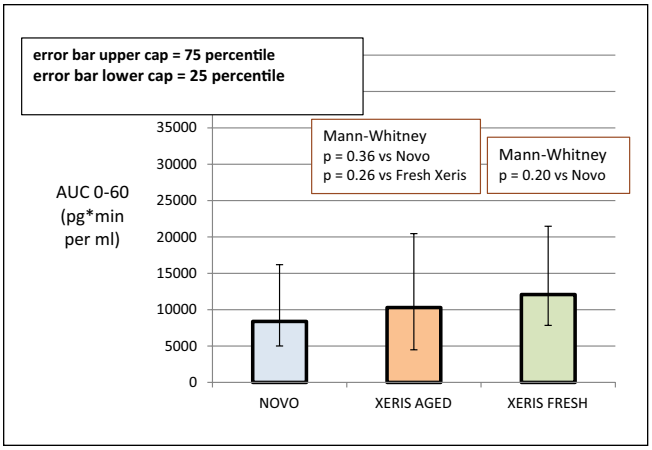


Figure 8. Areas under the curve for the 3 glucagon formulations over the first 60 minutes. No statistical differences among the 3 formulations were detected.

noted across treatments and were associated with the insertion of the catheter, or were a result of the adhesive used to secure the OmniPod to the skin. No adverse dermal changes were noted during the study. Barely perceptible erythema (grade 1) was noted in all 3 dose groups as well as for exposure times of 24 hours and 72 hours. Isolated barely perceptible edema (grade 1) was noted as well. Purple skin was noted across the groups and was considered to be a result of the adhesive used to attach the OmniPod and was not related to the administration of saline, vehicle, or glucagon.

Gross Pathology. There were few test-article-related gross pathological findings in any of the groups over the first 3 days. However, pathological observations and histopathological examination were slightly limited by the occurrence of occlusions of a few cannulae in all groups. Gross pathological findings were observed in all test groups and generally related to bleeding and scabbing at the catheter insertion site. All resolved within the recovery period without treatment.

There were no test-article-related effects on body weight or body weight change during the study. Body weights tended to remain the same, or increase or decrease slightly over the course of the study, which commonly occurs in rabbits with applied stockinettes.

Histopathology—24-Hour Treatment. No significant test-article-related microscopic findings were noted. The microscopic findings observed were considered related to the method of administration and were of similar incidence and severity in the saline control, vehicle control, and glucagon groups and, therefore, were considered unrelated to administration of glucagon. These changes consisted of very minor cell necrosis and mixed cell inflammation. There was evidence of recovery from the dosing procedures as indicated by fewer findings were noted in the saline control, vehicle control, and glucagon-treated animals at terminal euthanasia on day 15 compared to terminal euthanasia on days 2 and 8.

Histopathology—72-Hour Treatment. There were no glucagon-related histopathology findings. However, there did

appear to be a vehicle control effect. The microscopic findings observed were considered related to the method of administration and were of similar incidence and severity in the saline control, vehicle control, and glucagon groups and, therefore, were considered unrelated to administration of glucagon. As observed for the 24-hour treatment, there was evidence of recovery from the dosing procedures as indicated by fewer findings in the saline control, vehicle control, and glucagon-treated animals at terminal euthanasia day 10 compared to terminal euthanasia day 4 and at terminal euthanasia day 17 compared to terminal euthanasia days 4 and 10.

Discussion and Conclusions

Drug stability, pump compatibility, skin irritation, and preclinical PK/pharmacodynamics (PD) studies support initiation of clinical studies with a novel, nonaqueous solution formulation. Glucagon stored in the pumps for up to 6 days shows no measurable drug degradation. Observed leachables are few and well below safety thresholds. Skin irritation studies indicate that the formulation is well tolerated after pump infusion at a high multiple to anticipated human exposures. PK/PD studies in a diabetic swine model indicate that the nonaqueous glucagon formulations have comparable PK performance as well as glycemic response.

Taken together, these results demonstrate that this novel, nonaqueous glucagon formulation is suitable for further clinical development in pump systems.

Abbreviations

AUC, area under the curve; BG, blood glucose; DMSO, dimethyl sulfoxide; GC-MS, gas chromatography–mass spectrometry; GLP, good laboratory practices; ICP-MS, inductively coupled plasma–mass spectrometry; LC-MS, liquid chromatography–mass spectrometry; PD, pharmacodynamics; PDM, personal diabetes manager; PK, pharmacokinetics; RP-HPLC, reverse phase–high performance liquid chromatography; SE-HPLC, size exclusion–high performance liquid chromatography; TFA, trifluoroacetic acid.

Declaration of Conflicting Interests

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