Rapid reconstitution packages (RRPs) for stable storage and delivery of glucagon

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Abstract
Current emergency injectors of glucagon require manual reconstitution, which involves several steps that may lead to dosage errors. Rapid reconstitution packages (RRPs) are new devices, designed using computational fluid dynamics (CFD) to optimize fluid mixing, integrating physical properties of active pharmaceutical ingredients (APIs), excipients and diluents. RRPs improve drug stability for long-term storage and ease of delivery. Device prototypes were manufactured using advanced stereolithography apparatus (SLA) 3D printing technology. Reconstitution of glucagon with RRPs was evaluated by high-performance liquid chromatography (HPLC) and optical spectroscopy methods. Enzyme-linked immunosorbent assays were performed to test in vitro activity. Experimental results showed that RRPs effectively reconstituted glucagon even after exposure to 60 °C for a 24-h period. RRPs exhibited improved performance at maintaining drug stability compared to lyophilized glucagon stored in a standard glass vial under the same temperature conditions. RRPs represent a portable platform for rapid reconstitution of lyophilized drugs, compatible with standard syringes available in any clinical setting. The RRP provides an alternative to manual reconstitution process, especially designed for medical emergencies.

Keywords Reconstitution · Hypoglycemia · Glucagon · Microfluidics · Computational fluid dynamics (CFD) · Stability · Drug delivery · Diabetes · Emergency medicine · Ambulatory settings

Introduction

Glucagon is a peptide hormone produced by the alpha cells of the islets of Langerhans from the pancreas in response to hypoglycemia [1]. Glucagon represents a first-line pharmacological therapy for severe hypoglycemia in emergency settings [2–5]. Every year, up to 10% of children and adults with type 1 diabetes in the USA experience a severe hypoglycemic episode [6]. Patients with type 1 diabetes will experience in average one severe hypoglycemic episode once in a year, and 2–4% of deaths in this population have been attributed to hypoglycemia [7]. Glucagon formulations are available to be administrated intramuscularly (IM) or subcutaneously (SC) by trained caregivers, as opposed to dextrose, which must be administered intravenously by trained health personnel [8].

Glucagon is therefore mostly administered in emergency settings. The pharmacological therapy is designed for one-time use for patients in critical need. In order to avoid instability of the active pharmaceutical ingredient (API) due to hydrolysis, aggregation, precipitation, and photolysis, glucagon is stored in lyophilized form in hermetically sealed glass vials for subsequent on-demand reconstitution and delivery. Emergency kits rely on manual reconstitution, typically requiring shaking of a vial to mix a diluent with the lyophilized API.

There are two commercially available glucagon emergency kits: Elly Lily, USA, and the GlucaGen Hypokit from Novo Nordisk, Denmark [9, 10]. These emergency kits require several time-consuming steps, summarized as follows: removing diluent glass vial seal; wiping diluent vial rubber cover with alcohol; removing the protecting cap from the kit syringe needle; drawing the diluent using syringe; removing drug glass vial seal; wiping drug vial rubber cover with alcohol; filling the drug vial with the diluent using the kit syringe; mixing drug and diluent by shaking until the drug dissolves, a critical

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but inaccurate step requiring visual inspection to observe a homogenous solution; using the syringe to draw the reconstituted solution; administration of the pharmacology therapy, which was defined as a 2-s standard in realistic conditions.

The multistep process associated with reconstitution is therefore not without complications. Manual reconstitution is associated with medical mishaps, including wrong use of diluent and incomplete mixing [11–15]. Such errors were shown to lead to inconsistencies in drug dosage and solubilization, which may include precipitates, overall compromising the pharmacological therapy [16, 17]. In addition, increasing manipulation of the needles during the reconstitution process expose patients and health workers to risk of needle-stick injuries with the potential risk of transmitting blood-borne pathogens [18].

Novel reconstitution modalities have been developed to address such issues. One of the most recent intranasal delivery systems was developed by Locemia Solutions [19–21]. Advantages of intranasal glucagon system include a one-step administration and a needleless delivery. Even though the bioavailability of intranasal glucagon does not match parenteral administration levels, its efficacy has been demonstrated to be acceptable for emergency administration. In spite of such advantages, common nasal diseases, such as cold and rhinitis, may limit glucagon intranasal absorption and bioavailability. It is still unclear the impact of these common clinical conditions on the efficacy of this newly developed delivery modality of glucagon [22–26].

Parenteral delivery routes for glucagon are still preferable and considered the standard ones for care. Therefore, there is an impending need for a parenteral delivery system for glucagon capable of maintaining stability while reducing the number of preparation steps in the administration process.

The rapid reconstitution package (RRP) was developed as a prefilled cartridge solution capable of long-term storage, on-demand reconstitution, and delivery of therapeutic drugs using standard syringes.

The RRP was designed as a cylindrical cartridge to be inserted into a standard syringe and activated when pushed by the syringe plunger, as a one-step reconstitution delivery process. The cartridge is inserted inside of a standard syringe and the process is seamless to the user. The RRP was designed for reconstitution of high molecular weight (MW) APIs characterized by low solubility and relatively large payloads (mg–g range). The first-generation RRP was already demonstrated using tissue plasminogen activator (tPA) [27]. The presented RRP design was optimized for use with glucagon, which requires reconstitution of 1 mg of API in 1 mL diluent for therapeutic use.

Design optimization was performed using numerical methods based on computational fluid dynamics (CFD) to improve the reconstitution process, avoiding dosage errors due to incomplete mixing.

Materials and methods

Device design

The RRP was designed as a cylindrical cartridge, as shown in Fig. 1a. The device operation follows a standard syringe application procedure as RRPs were designed to fit in standard syringes. Figure 1b shows a standard 20/25 mL syringe with the RRP. Figure 2 shows the device assembly and preparation for loading drug and diluent into the RRP chambers before storage in eight schematic steps. In step 8 of Fig. 2, the system is ready to be packed in the manufacturing facility. In this study, glucagon was purchased from Sigma-Aldrich (Cat. No. G2044-25MG), Lilly USA LLC (Cat. No. NDC 0002-8031-01, MS8031), and Bedford Laboratories (GlucaGen. Cat. No. NDC 5390-0004-01).

The RRP operation is straightforward: syringe plunger is removed from the syringe barrel, subsequently, the RRP is placed inside of the syringe barrel, and finally, the syringe plunger is reinserted into the syringe. The RRP is placed inside of the syringe only when is ready to be used. The RRP activation relies on the telescopic operation of an internal plunger, which opens a valve between the hermetically sealed diluent chamber and drug chamber, displacing the diluent into the drug chamber. The drug-diluent product is rapidly mixed, released, and delivered. Activation sequence and fluid flow path are shown in Fig. 3. A safety twisting motion mechanism will be incorporated in future versions to prevent accidental activation.

Numerical modeling

There are three regions defined in the device: drug reconstitution chamber, mixing passage, and particle restriction. In the reconstitution chamber, the drug is dissolved by the diluent, in the mixing passage, the drug solution is mixed, and in the particle prior to exit, the drug solution is forced through a restriction to prevent large particles from exiting the device.

To optimize the RRP design for glucagon, numerical analysis based on CFD was implemented using the finite volume method to model flow and drug behavior during the reconstitution process [28]. Due to geometrical symmetry of the cylindrical device, only a small angular sector was modeled and optimized, limiting the computational load at the expense of neglecting angular flows.

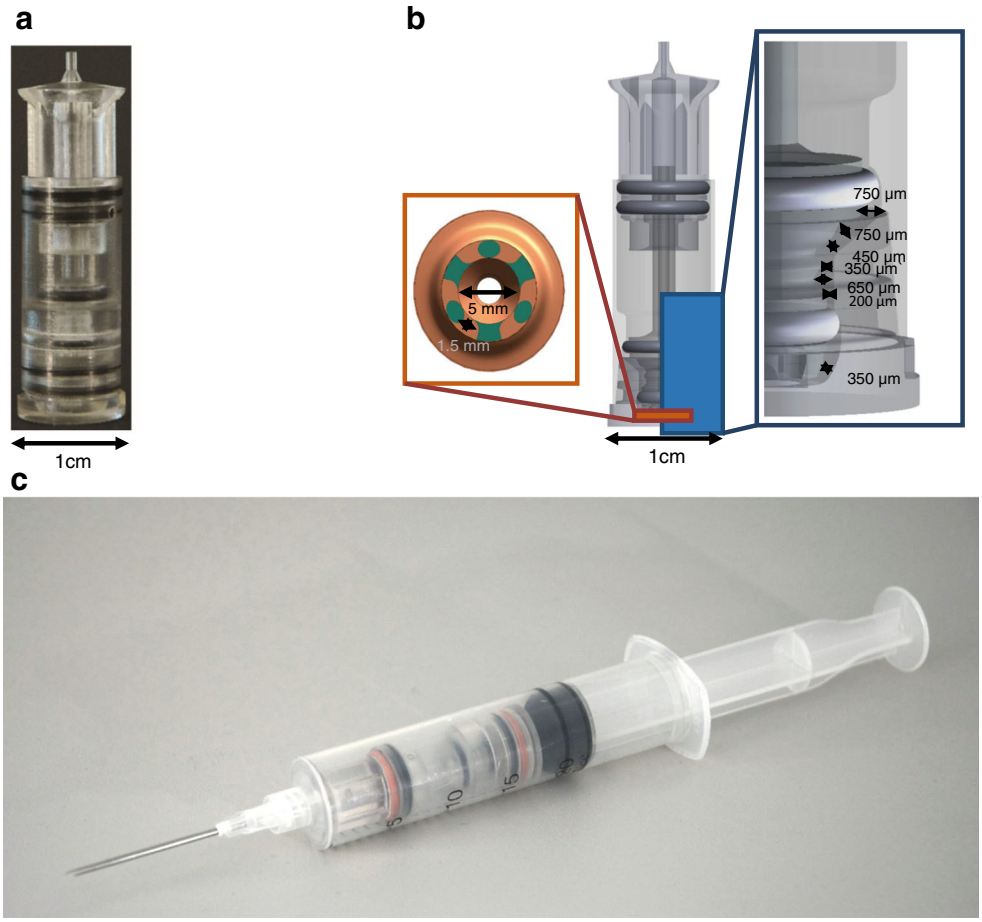

Fig. 1 **a** Actual RRP. **b** RRP inside a syringe

There were two processes described by the numerical simulations: the diluent filling the drug chamber (i) and the drug reconstitution (ii). Two computational models were therefore used to model the mixing process, which were implemented and solved concurrently [29].

Diluent filling the drug chamber process

To address the diluent filling the drug chamber process, a multiphase homogeneous model without surface tension was implemented that captures the injection of the liquid into a

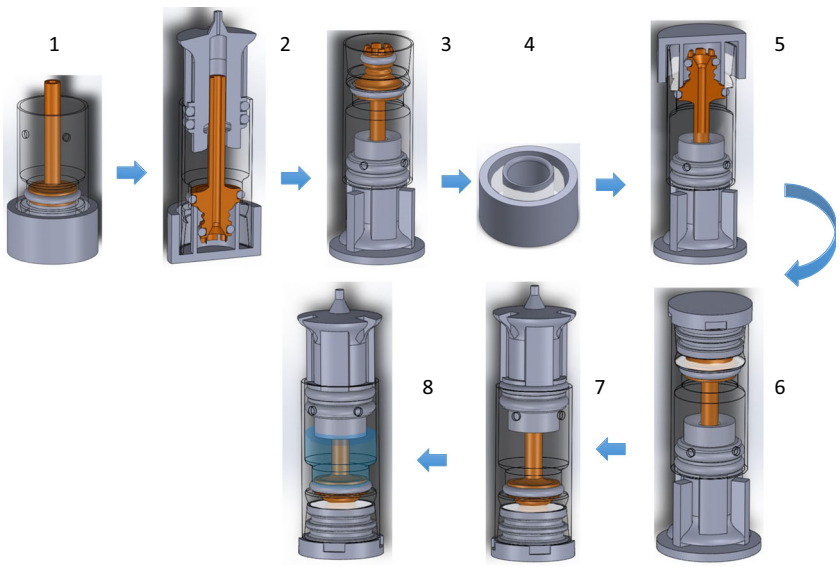

Fig. 2 Step-by-step loading and assembly for RRP

cavity filled with air and drug. For the liquid phase, the random averaged Navier-Stokes equations were used, with the Boussinesq hypothesis to deal with turbulence. A zero-equation model was adopted to determine the eddy viscosity [29]. Gas phase was treated as incompressible because of the low velocities involved. Air density and water density were defined as 1.185 kg/m^3 and 997 kg/m^3, respectively.

Boundary conditions were defined so that for the defined input mass rate, $\dot{m}_{\text{filling}}$, there were no slip conditions at the walls and fixed pressure at the output. The activation time was defined from the time of valve opening to the full activation of the internal plunger. The resulting $\dot{m}_{\text{filling}}$ was thereby adjusted so that device elution rate fit the experimental data, as shown in the results section.

Drug reconstitution process

It was experimentally observed that a fraction of the drug, m_{wall}, was adhered to the walls of the drug chamber in the form of a thin layer, while the remaining fraction, m_{loose}, stayed as loose powder in the chamber. This behavior was driven by two clearly different mass transfer mechanisms that had to be appropriately described: the reconstitution of drug deposited in a thin layer on the walls and the reconstitution of the loose drug.

For the reconstitution of the drug deposited in a thin layer, a drug-eluting source was set at the wall with a fixed release rate, $\dot{m}_{\text{wall release}}$, which remained active as a source until it became fully depleted (all m_{wall} is reconstituted). From the device geometry, the area covered by the thin layer was measured, having a value of 2.408 mm^2.

For the reconstitution of the loose drug, it was modeled as a small mass of saturated diluent-drug drop with a concentration of 20.95 kg/m^3 of the drug in a diluent, placed inside the drug chamber. This saturated diluent-drug drop mixes with the entering diluent during the filling process. The mixing process was modeled as a transported scalar solved simultaneously with the random averaged Navier-Stokes equations for the flow, governed by the convection equation. Glucagon diffusivity was considered negligible due to molecule size.

Finally, by solving the model iteratively, the required parameters, m_{wall}, $\dot{m}_{\text{wall release}}$, and m_{loose}, were determined and adjusted from experiments to better fit output actual concentration patterns.

RRP fabrication

RRP parts were designed using computer-aided design (CAD) [28] and fabricated with stereolithography apparatus (SLA) 3D printing (Project 6000, 3D Systems, Inc.). The selected resin was a UV-sensitive acrylic polymer (VisiJet® Clear, 3D Systems, Inc.). After 3D printing, each part was cleaned, air dried, and UV cured for 45 min. The resulting parts were then assembled into fully functional RRPs. Several design-manufacturing iterations were performed until obtaining the optimal prototype that provided the most accurate design geometry.

Sample preparation

RRPs were initially kept at 20 °C and subsequently exposed to 60 °C for 24 h inside a vacuum drying oven. Testing samples at 60 °C provided a basis to simulate harsh environmental conditions for which an actual glucagon kit may be stored during ambulatory emergency settings. Samples were kept in six different storage conditions for 24 h prior to assay preparation: (1) standard lyophilized glucagon in glass vials kept at 20 °C; (2) reconstituted glucagon solution kept in glass vials

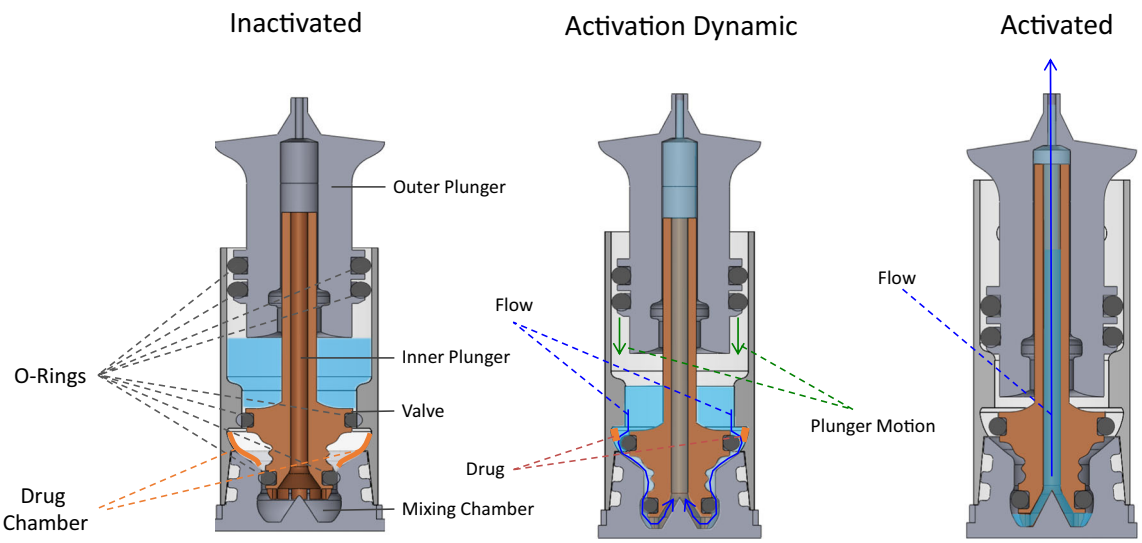

Fig. 3 RRP activation and fluid flow

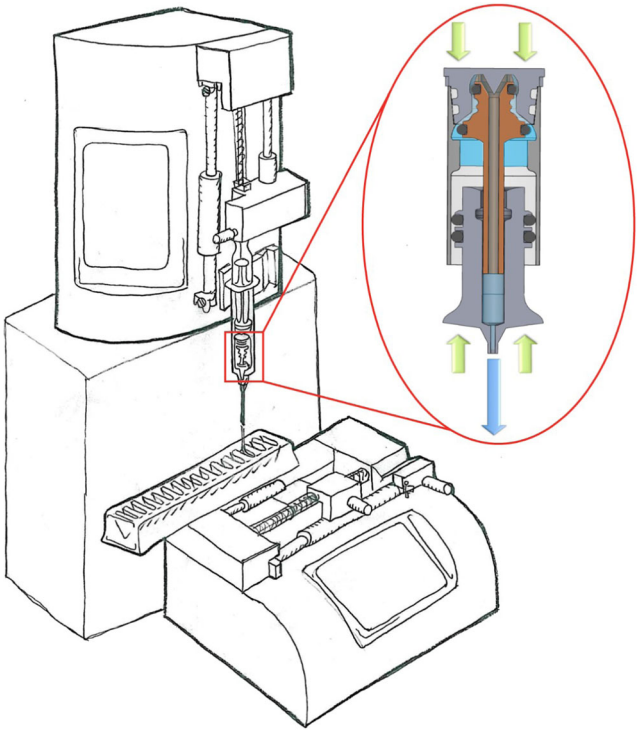

Fig. 4 Experimental setup used for characterization of release profiles

kept at 20 °C; (3) lyophilized glucagon loaded into RRPs and kept at 20 °C; (4) lyophilized glucagon kept in glass vials kept at 60 °C; (5) reconstituted glucagon solution and stored in glass vials kept at 60 °C; and (6) lyophilized glucagon loaded into RRPs kept at 60 °C. After a 24-h period, each sample in lyophilized form, including those stored in the RRP, was reconstituted. All samples used 1 mL Millipore water (39–42 μs/cm, 18 MΩ/cm, 3 ppb) for reconstitution. Subsequent experiments for analytical evaluation of samples relied on enzyme-linked immunosorbent assay (ELISA), ultraviolet-visible (UV-vis) spectroscopy, and high-performance liquid chromatography (HPLC) tests.

Flow output characterization

RRPs were loaded with the homogeneously mixed dry formulation of API and excipient exactly as the formulated therapeutic dosage (1 mg of glucagon and 49 mg of lactose, SIGMA-ALDRICH, Saint Louis, USA) into the drug chamber and 2.5 mL of diluent (12 mg/mL of glycerol, SIGMA-ALDRICH, Saint Louis, USA, water for injection and 0.05 N hydrochloric acid) into the solvent chamber. Each RRP was placed in a 20/25 cc syringe (Excel International, Co., Cat. No. 26280). Syringes were then fixed into a syringe pump (Customized 4X PhD Series, Harvard Apparatus, Inc.) to active the RRPs at the rate of choice. The syringe pump was set to inject a standard 20/25 cc in 2 s, simulating realistic operation by a medical practitioner in clinical conditions. The output elution from each RRP reconstitution experiment was collected using a customized linear actuator to capture samples for subsequent characterization of the concentration profile. The actuator consisted of a collection tray with 12 equal wells such that each well collected a sample from the RRP elution in a time increment of approximately 330 ms. Drug concentration in each well was analyzed using a HPLC monitored with UV spectrometer (model: 8453 UV-Vis, Spectroscopy System, Agilent Technologies, Inc.) at 214- and 280-nm wavelengths. The concentration for each sample per well was measured for multiple trials ($N = 10$). Data from all trials was then compiled and compared against a calibration curve, obtaining the average concentration profile of reconstituted and delivered glucagon. Figure 4 shows the experimental setup.

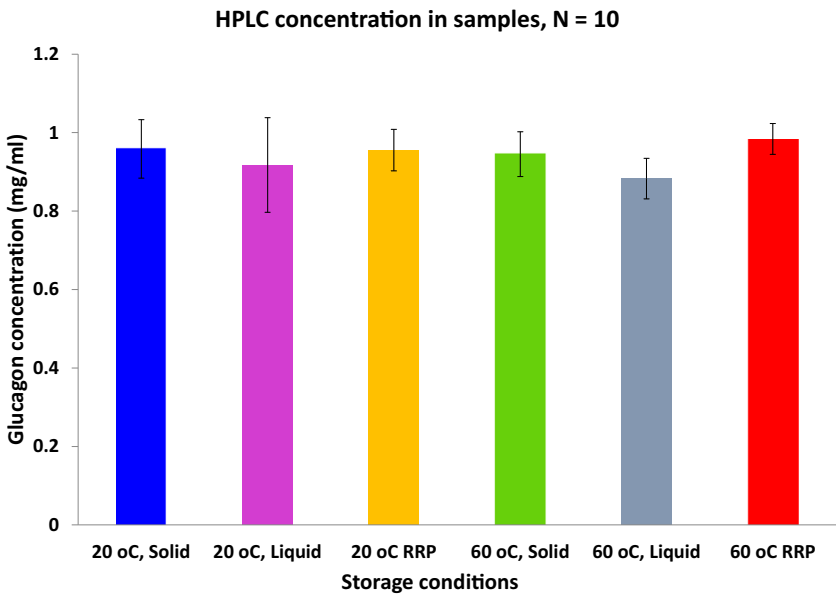

Fig. 5 Reconstituted glucagon concentration per delivery modality for different storage temperature conditions during a 24-h period

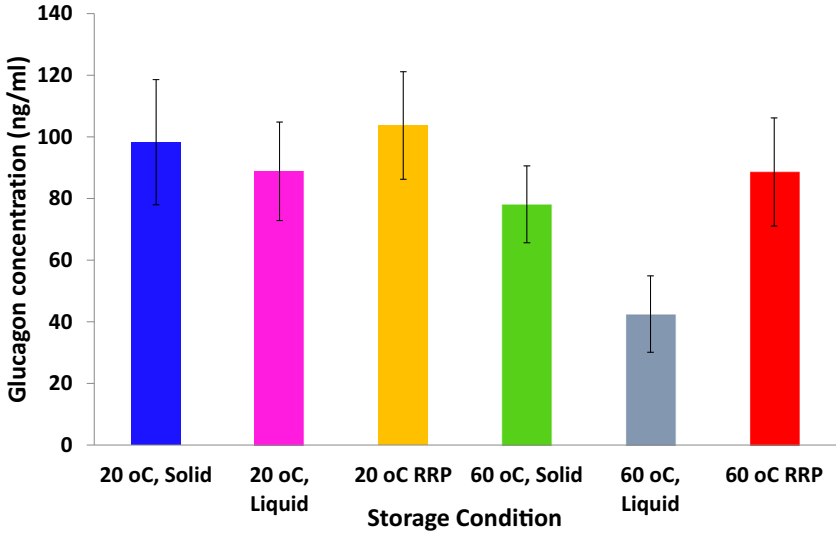

Fig. 6 Comparative API activity of reconstituted glucagon per delivery modality for different storage temperature conditions during a 24-h period. Activity was analyzed using ELISA, $N = 10$

In vitro activity tests

Immunoassays were implemented to characterize and compare the RRP performance with standard reconstitution of glucagon in glass vials at various storage conditions. ELISA was selected for evaluation of in vitro activity of glucagon. Stability tests were conducted within an hour after reconstitution since glucagon was reported as unstable in aqueous solution over a few hours at room temperature (20 °C). ELISA assays were used to test the stability of samples (Sandwich ELISA kit for glucagon, Phoenix Pharmaceutical, Inc.) that were exposed to temperature conditions of 20 °C and 60 °C over a period of 24 h. The samples ($N = 3$, each 50 μL) were placed into each ELISA sample well plate and left to incubate for 2 h at 25 °C. After each wash step, a second antibody horseradish peroxidase (SA-HRP)-conjugated anti-glucagon was added into each sample plate (12 μL). The SA-HRP-conjugated anti-glucagon bound onto the opposing side of the glucagon molecule already bound onto the plate-fixed antibodies were left to incubate for 2 h at 25 °C. After a second wash step, a color-inducing substrate (TMB substrate) was then added into the sample plates (100 μL), which bound onto the SA-HRP to induce a measurable gradient representing the amount of glucagon bound and left it for incubation for 1 h at 25 °C. In order to prevent oversaturation of the antibodies, the samples were diluted to fall within the assay sensitivity range of 100 pg/mL–100 ng/mL. Hydrochloric acid 2 N (100 μL) was added to stop the reaction. After 20 min, optical signal intensity from test plates was measured with a spectrophotometer at a wavelength of 450 nm (PowerWave HT Microplate Spectrophotometer, BioTek Instruments, Inc.). Each sample was normalized against a calibration curve derived from a five-parameter logarithmic fit of the glucagon standards ran concurrently with the samples.

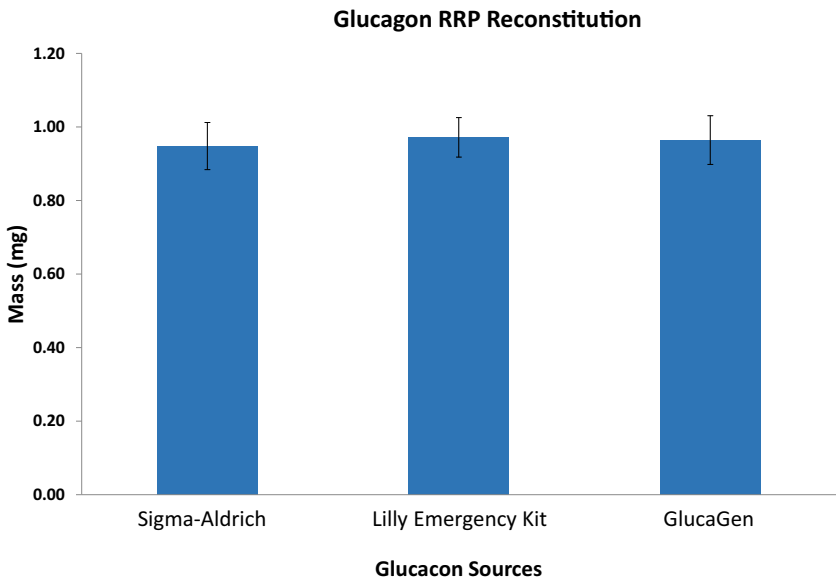

Fig. 7 Glucagon mass from different sources reconstituted with RRP

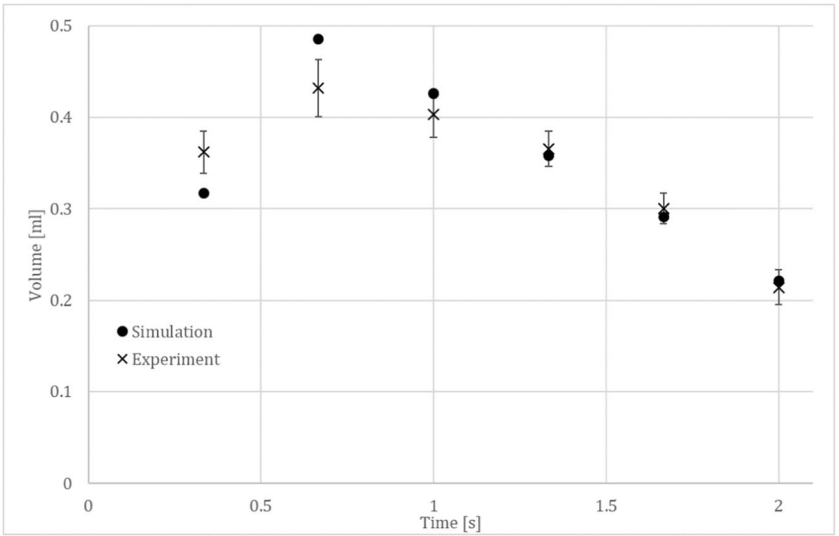

Fig. 8 Comparison of experimental and simulated eluted injection volume

Total output concentration analysis

HPLC analysis was performed to analyze and compare standard reconstitution from APIs and RRPs using concentration from full elution from each method. Each sample was initially loaded into HPLC chamber vials to be analyzed by analytical methods. Assays were performed with HPLC (1100 Model, Agilent Technologies, Inc.) with a reversed-phase column (ZORBAX SB-CN, 4.6 mm × 250 mm, 5 μm SiO_2 particles, columns, Agilent Technologies, Inc.). The mobile phase was a gradient with an initial hold for 0 min at a ratio of 90:10:0.1 water:acetonitrile (ACN):trifluoroacetic acid (TFA), brought to 5:95:0.1 water:ACN:TFA for 2 min and sustained at that ratio over 8 min. Next, the ratio was brought to 90:10:0.1 water:ACN:TFA for 1 min and subsequently sustained for 4 min. Acid medium (TFA, pKa = 0.23) was used to separate glucagon from solution. The flow rate was 1 mL/min and the injection volume was 50 μL. The glucagon presence was detected by UV spectrometry at 214 nm.

Results and discussion

Experimental results

The concentration profiles from the RRPs and manual mixing at different temperature conditions were characterized using a 2-s injection time. The concentration output of all samples analyzed

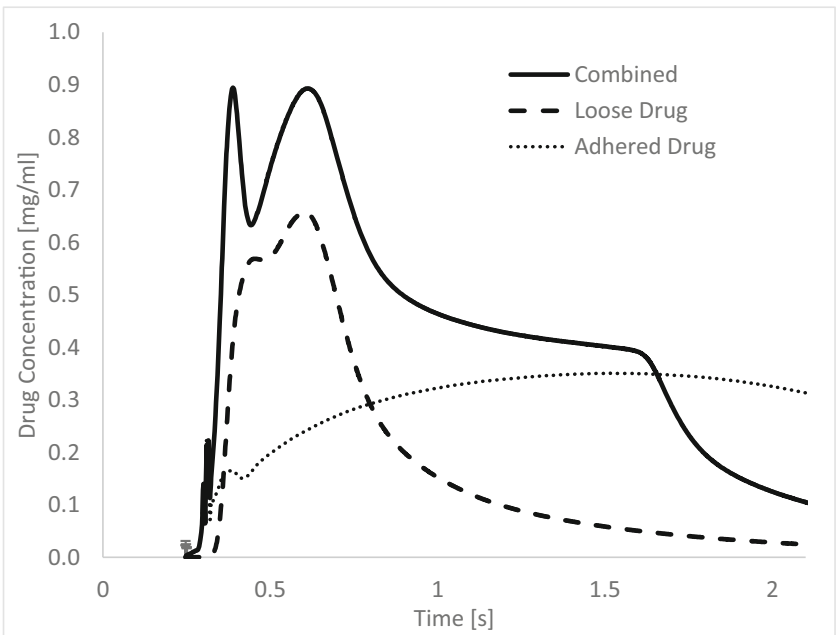

Fig. 9 Simulated concentration profiles showing the following: adhered drug only (dotted line), loose drug eluting from source on the sidewall (dashed line), and combined concentration profiles (solid line)

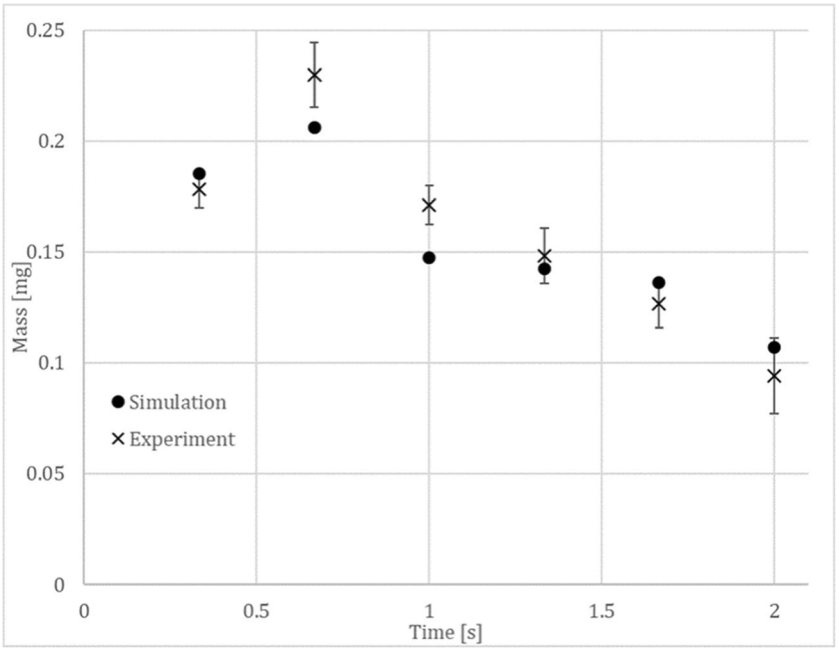

Fig. 10 Comparison of experimental vs simulated eluted glucagon mass

by HPLC ($N = 10$) did not show a significant difference in variability. The concentration profiles for each storage conditions are shown in Fig. 5, plotting the average concentration value and standard deviation per each temperature condition. Preservation of pharmacology activity is shown in Fig. 6, plotting average concentration activity values and standard deviation per temperature condition. Experimental results of ELISA trials were performed to test for an in vitro activity level of glucagon in the samples stored at 20 °C and 60 °C for a 24-h period. High activity levels were observed for samples of glucagon that were in lyophilized form in standard sealed glass vials and RRPs exposed to 20 °C and 60 °C. In addition, reconstituted solution practically showed no activity reduction for the 20 °C exposure. The reconstituted glucagon in glass vials exposed to 60 °C suffered the greatest loss of activity, expressing approximately 30% activity relative to the standard activity average at 20 °C.

RRPs exhibited improved performance at maintaining drug stability compared to lyophilized API stored in a standard glass vial under the same temperature conditions. Experimental results showed that RRPs were able to reconstitute an average of 88.61% of total drug in stable form compared to the manual reconstitution of glucagon from glass vials that rendered an average of 78.07% of the drug in a stable form. Characterization of the RRP concentration profiles was performed using a 2-s injection time at a flow of 150 mL/min which was chosen to emulate a typical injection time as delivered by trained medical personnel. The mass output of all the samples analyzed by HPLC ($N = 10$) showed a consistent profile, as shown in Fig. 7. These results demonstrated that the RRP exhibited a repeatable and robust performance.

Simulation results

An accurate numerical model for the drug chamber diluent filling process was developed by adjusting the time-dependent mass flow rate at the input, $\dot{m}_{\text{filling}}$, to follow actual experimental behavior. Figure 8 shows the volumes measured in each well plotted against simulated ones, once the filling rate was adjusted.

For the drug reconstitution, the proposed model had to be tuned iteratively to determine m_{wall}, $\dot{m}_{\text{wall release}}$, and m_{loose} until a good agreement with experimental results was found. Figure 9 shows the profile of drug-diluent concentration, presented for a case with all the drug mass considered adhered to the walls (i), a case with all the drug considered loose in the chamber (ii), and a combination of both (iii). It is possible to observe that drug adhered to the walls showed an almost constant concentration output, where duration and concentration was controlled by $\dot{m}_{\text{wall release}}$. On the other hand, the loose drug shows high concentration at the beginning followed by a descending slope. The compound combination of these two effects constitutes the device output concentration profile.

Numerical analyses were performed by trial and error for loose drug, adhered drug, and wall release rate to finally determine the combination that minimizes the error in output concentration profiles while accounting for the total mass of drug eluted. Figure 8 shows the volume correlation and Fig. 10 shows the drug mass one. The resulting parameters resulted as follows: $m_{\text{wall}} = 0.801$ mg, area-specific release rate, $\dot{m}_{\text{wall release}} = 1.433$ μg/mm^2-s, and $m_{\text{loose}} = 0.199$ mg.

Conclusions

The rapid reconstitution package (RRP) was tested for use with glucagon using an in silico/experimental analytical method to optimize the drug reconstitution process. RRPs provide a platform for maintaining APIs in stable conditions and facile method for reconstitution and delivery, without the need for trained personnel for administration. Experimental results showed that RRPs provide a robust platform for large payload drugs, making it suitable for the treatment in a number of clinical settings. Experimental results showed that in vitro activity of glucagon in RRP was preserved under harsh environmental conditions. Such results show that RRPs provide a platform for stable storage and reconstitution of pharmaceutical drugs. Future analytical experiments will be required to completely determine long-term shelf life stability using accelerated and long-term stability studies. Other studies will also include pre-clinical tests to assess in vivo biological activity and bioavailability and characterize overall pharmacodynamics, which will ultimately lead to clinical trials for FDA clearance. RRP-glucagon can be manufactured in sterile conditions by lyophilizing the directly the API and excipients in the cartridge within a sterile chamber and subsequently loading the loaded cartridge into the RRP assembly. Additionally, the diluent is the last step prior to fully completing device assembly and packaging. The diluent can be loaded via the use of inlet with automatized injector. To prevent bubbles, outlets will be implemented as shown in Fig. 2. After loading, the device can be sealed. It is also possible to implement the use of gaskets, as opposed to O-rings, to increase structural robustness and therefore obtain a longer shelf life. In addition, the use of stable polymers, such as polycarbonate and cyclic olefin copolymer (COC), can be used for RRP manufacturing as these materials can be molded and provide nearly glass-like properties in terms of inertness, leaching, delamination, etc. [30]. RRPs could therefore provide a simple way to expand the usage of pharmacological therapies in harsh environments as a simple and intituitive method for drug delivery (injection), reduction of medical errors, and reliable reconstitution.

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Compliance with ethical standards

Conflict of interest N. M. Elman works at GearJump Technologies, LLC. S. D'hers, A. N. Abad Vazquez, and P. Gurman declare that they have no conflict of interest.

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