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Influencing factors on gelatin matrix for chlorhexidine delivery

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Abstract

Objective: The objective was to evaluate the influencing factors in the fabrication of gelatin matrix (gelatin chips) for drug delivery. The attributes affecting drug release characteristics of the gelatin products were examined.

Significance: Understanding the attributes that affect drug release from gelatin matrix could provide the knowledge base for the development, manufacturing, and performance evaluation of gelatin-based drug products for sustained drug delivery.

Methods: Chlorhexidine (CHX) was the model drug in the gelatin-product testing. The gelatin products were fabricated by two methods: a single-pot mixing of all the components and a two-step gelatin cross-linking followed by drug loading. Different gelatin types (Type A porcine and Type B bovine), glutaraldehyde (GTA) crosslinking conditions, glycerin concentration, and CHX concentration in drug loading and loading time were used to fabricate the products. The cumulative amounts of CHX release from the gelatin products were determined using *in vitro* release testing (IVRT).

Results: The attributes affecting CHX release from the gelatin products were gelatin type, GTA crosslinking, and CHX loading concentration. The fabrication methods (two-step method of gelatin crosslinking and drug loading by equilibration vs. direct mixing of the components) also affected CHX release. Other attributes such as glycerin and CHX loading time did not show significant effects on drug release under the conditions studied. In addition, the results in the two IVRT methods employed in this study were comparable.

Conclusion: Gelatin products of qualitative (Q1) and quantitative (Q2) differences could lead to different drug release behaviors. Drug release was also affected by the ingredient mixing steps during gelatin chip fabrication.

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Keywords

Gelatin; chlorhexidine; drug release; *in vitro* release testing (IVRT); dissolution study; Q1 and Q2 equivalence

Introduction

Biodegradable sustained release formulations provide the ability to deliver drugs at predetermined rates to achieve the desired drug levels at the target sites. These systems offer advantages over conventional dosage forms including reduced fluctuation of drug concentration in the body to increase drug safety and improve therapy, less frequent drug administration to increase patient compliance, and better drug utilization to reduce costs. Some sustained release systems are biodegradable crosslinked gelatin systems. Gelatin is derived from collagen and can form a polymer matrix to provide controlled drug release for local drug delivery. For example, local administration of antimicrobial drugs using a sustained release system in the periodontal pocket is common for periodontitis and such treatments usually provide sustained local drug release at the site for several days. An example of periodontal products is PerioChip[®]. Other long-acting periodontal drug products are Actisite[®] for tetracycline, Atridox[®] for doxycycline hyclate, and Arestin[®] for minocycline hydrochloride. PerioChip[®] is a long-acting product in the form of a gelatin implant that is placed in the periodontal pocket after scaling and root planing procedures [1]. In addition to being in the form of an implant, gelatin is also used in other drug delivery systems such as gelatin films, fibers, nanoparticles, and microparticles [2–5]. The performance of these drug products (such as drug release rate and duration) is related to the selection of the ingredients and manufacturing methods. The critical attributes in the manufacturing processes for drug release from gelatin products have not been systematically studied.

In vitro release testing (IVRT) provides drug product performance evaluations for formulation design in drug product development and assurance in quality control of product manufacturing [6,7]. For example, IVRT, in combination with other specification tests, is commonly used to monitor batch-to-batch consistency and product quality during manufacturing that can affect the performance of a drug product. IVRT can also provide information on the characteristics of the dosage form to support bioequivalence between test product and reference listed drug (RLD) in FDA approval of generic drug products [8–10]. IVRT has been used to assist formulation development and testing of different dosage forms such as oral, parenteral, and transdermal drug products [6,11,12]. Even when the inactive ingredients and amounts of the inactive ingredients (qualitative Q1 and quantitative Q2, respectively) of the test product are the same as the RLD, the drug release characteristics can be different and affect product performance. For example, differences in the manufacturing process of a local sustained release product can result in differences in the arrangement of drug and excipients in the product, and subsequently, drug release behavior and bioavailability of the product [13]. A reliable IVRT can identify not only Q1 and Q2 differences but also physicochemical (Q3) differences that may arise from the manufacturing process.

Sustained delivery dosage forms are commonly evaluated by IVRT using United States Pharmacopeia (USP) dissolution apparatus [14]. The USP dissolution Apparatus 1 uses a basket. USP Apparatus 2 uses a paddle. USP Apparatus 3 has a reciprocating cylinder. USP Apparatus 4 is a flow-through cell. USP Apparatus 5 (paddle over disk) and Apparatus 6 (cylinder) are based on Apparatus 2 and Apparatus 1, respectively, and USP Apparatus 7 uses a reciprocating holder in a container [15]. An example of local sustained release products is PerioChip® [1], which is a long-acting local periodontal drug product composed of chlorhexidine digluconate (the drug) and hydrolyzed gelatin crosslinked with glutaraldehyde (the biodegradable polymer matrix). PerioChip® has a size of approximately 5 mm × 4 mm × 0.35 mm. It has a mass of ~7 mg with 2.5 mg chlorhexidine digluconate and unspecified mass of glycerin and purified water in the biodegradable matrix. Although IVRT may not provide a direct correlation to *in vivo* performance of long-acting periodontal drug products because of the different conditions *in vitro* and *in vivo*, it can provide important information in the evaluation and comparison of periodontal drug products, development of these drug formulations, bioequivalence assessment of the generic forms of these products, and quality control in the manufacturing of the products.

The objective of the present study was to evaluate the key attributes that affect the drug release characteristics of gelatin matrix products (gelatin chips). Gelatin test products with Q1 and Q2 differences, different drug loading, and prepared with different manufacturing processes that could lead to different drug release behaviors were fabricated. Chlorhexidine (CHX) was the model drug used in these test products. Drug release was determined in IVRT. The physical and chemical properties were investigated by a texture analyzer and the destruction of the gelatin matrix using pepsin digestion, respectively. Drug release data were compared based on the attributes related to the fabrication of the gelatin products to investigate the key parameters affecting drug release characteristics. The knowledge obtained in the present study could serve as the database for the development and evaluation of gelatin-based drug products such as long-acting gelatin periodontal products and provide guidance to the performance assessment of these products.

Methods

Materials

Phosphate buffered saline (PBS), pH 7.4, consisting of 0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride, was prepared using PBS tablets (MP Biomedicals, LLC, Solon, OH) and deionized water (DI water). Simulated saliva, pH 8.0, consisting of 0.137 M sodium chloride, 0.0014 M potassium phosphate monobasic, 0.017 M sodium phosphate dibasic (Fisher Scientific, Fair Lawn, NJ) was prepared in DI water. PerioChip® (CHX gluconate, 2.5 mg) was manufactured by Drexel Pharma Technologies Ltd (Yokneam, Israel) and three different PerioChip® lots were used. CHX digluconate (20% solution in water), CHX powder, Type A porcine skin gelatin (300 Bloom), Type B bovine skin gelatin (225 Bloom), and glutaraldehyde (GTA) were purchased from Sigma-Aldrich (St. Louis, MO). Pepsin A (powder) was purchased from Acros Organics (Janssen Pharmaceutica, Geel, Belgium). Trimethylamine (HPLC grade), sodium phosphate monobasic, and o-phosphoric acid were purchased from Fisher Scientific (Fair Lawn, NJ).

Acetonitrile and methanol (HPLC grade) were from Pharmaco-AAPER (Shelbyville, KY). Release liner (Scotchpak 1022, 3.0 mil) was obtained from 3 M (St. Paul, MN). All materials were used as received.

Gelatin chip fabrication with drug loading by equilibration (Method A)

To evaluate the critical attributes that affect drug release from gelatin-based products, test products of GTA crosslinked gelatin were fabricated using a method modified from that described previously [16]. This method involved two steps after gelatin solution casting: crosslinking and then drug loading by equilibration in GTA and drug solutions, respectively. Briefly, 10 ml of 5% Type A or Type B gelatin with 0.25–4% glycerin was prepared by dissolving the reagents in deionized water at 50 °C with stirring. The solution was poured in a release liner mold (35 mm diameter) and allowed to evaporate at room temperature overnight. Gelatin chips were then punched out from the dried gelatin sheet from the mold with a cork borer and were placed in crosslinking solution of 0.5–8% GTA and 0.25–4% glycerin in PBS in a sealed container for 24 h at room temperature. After crosslinking, the gelatin chips were washed repeatedly (5X) in deionized water and dried. The gelatin chips were then loaded with CHX by equilibration in 2.5–20% CHX digluconate and 0.25–4% glycerin in water for 12–72 h. The same glycerin concentration was used in the gelatin, crosslinking, and drug loading solutions. The gelatin chips loaded with CHX were rinsed in 1 L of deionized water for 5–60 s, dried overnight, and then stored in sealed containers. The final products were dried gelatin chips that were translucent with different textures depending on the concentration of glycerin used (a plasticizer of the gelatin matrix). These test products had Q1 and Q2 differences including the types of gelatin (Types A and B), different ingredient (glycerin) concentrations, and different degrees of crosslinking of the gelatin matrices with GTA. In addition, the effect of varying drug (CHX) concentration in the fabrication process on product performance was also examined. These differences represent possible situations that can occur due to differences in the manufacturing of the drug products. Table 1 summarizes the conditions (attributes) examined in the present study.

Gelatin chip fabrication with direct drug loading (Method B)

A different manufacturing procedure with direct mixing of the gelatin, drug, and crosslinker instead of the two-step method of crosslinking and drug loading was also investigated. In the direct drug-loading method, the test products of GTA crosslinked gelatin were fabricated by the mixing of 0.5 g Type A gelatin, 1 ml 10% glycerin, and 0.39 ml to 3.0 ml 20% CHX digluconate (78 mg to 600 mg) in 8.21 ml to 5.6 ml water, respectively, at 50 °C. The mixture had a cloudy appearance believed to be due to the formation of CHX precipitate. Adjusting the pH of the mixture between pH 2.5 and 10 did not noticeably affect the cloudy appearance of the mixture. The mixture became clear when lower CHX concentration (final concentration less than ~2 mg/ml) was used in the mixture in the process. The mixture was then mixed with 0.4 ml 50% GTA. After brief stirring, the mixture (total volume ~10 ml) was immediately poured on a release liner mold (35 mm diameter) and allowed to evaporate at room temperature overnight. Gelatin chips were then punched out from the dried gelatin sheet from the mold with a cork borer and stored in sealed containers. The amounts of CHX added were selected to provide a similar range of CHX-to-gelatin ratios as those in the gelatin chips prepared in Method A for comparison.

IVRT screening for CHX release (Method I)

The gelatin chips prepared in the “Gelatin chip fabrication” section were placed in miniature plastic mesh baskets. Each basket was submersed in 100 ml simulated saliva in a 125-ml glass bottle under continuous stirring (700 RPM) at 37 °C with care being taken to keep the basket suspended in the solution above the stir bar. Samples were withdrawn from the bottles at 6, 12, 24, 96, and/or 120 h and were quantified by HPLC for the amounts of CHX released into the solution.

IVRT for CHX release (Method II)

The gelatin chips prepared in the “Gelatin chip fabrication” section were placed in miniature plastic mesh baskets with the baskets submersed in 10 ml simulated saliva in individual 20-ml glass scintillation vials. The baskets were suspended in the simulated saliva above the stir bars with continuous stirring (700 RPM) at 37 °C. At appropriate time intervals (e.g. 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48, 72, 96, 120, and 148 h), the baskets with the gelatin chips were removed from the scintillation vials and transferred to individual fresh vials containing simulated saliva. Samples were taken from the scintillation vials after the removal of the gelatin chips, and the concentrations of CHX in the samples were quantified by HPLC. For comparison, the release of CHX from PerioChip® was also determined. To investigate the effectiveness of stirring in the IVRT, the release of CHX from gelatin chips were examined at half the stirring rate (350 RPM) and compared with that at 700 RPM. The effect of stirring rates was evaluated using the gelatin chips of 5% gelatin, 2% GTA, 1% glycerin, and 15% CHX equilibration loading.

Physical properties of gelatin products

The gelatin products were subjected to testing using a texture analyzer (TA.XTPlus Texture Analyzer; Texture Technologies Corp, Hamilton, MA) with a cylinder probe *in vitro*. The gelatin products were tested in two configurations: compression and flexibility. Force vs. time curves were generated for each test condition. In the compression test, the gelatin chip was placed flat on a testing platform underneath an acrylic cylindrical probe of the instrument (12.7 mm × 35 mm, diameter × height). The force required to compress the gelatin product was then monitored as the probe traveled downwards for either 0.3 mm at a rate of 0.03 mm/s or continually downward until an instrument maximum force of 66 N was applied. The gradient (force/time of the linear profile near the end of the measurement) for each force vs. time curve was recorded. In the flexibility test, the gelatin product was fixed on its edge in a tensile gripe probe (TA-96B) with one-half of the product exiting the grip edge. The force required to flex the protruding gelatin product with the cylindrical probe was then monitored as the probe traveled downwards for 1 mm at a rate of 0.03 mm/s.

Gelatin chip digestion and chemical property

The digestion medium was 0.5% pepsin prepared by mixing pepsin powder in deionized water with pH adjusted with o-phosphoric acid, and the final medium had pH ~3.2. The gelatin chips were immersed in 2 ml pepsin medium with continuous stirring (700 RPM) in the vials at 37 °C for up to 11 days. Pepsin at acidic pH was used in the enzymatic digestion

study because trypsin at pH ~7 was found not as effective as pepsin to digest the gelatin products (data not shown).

HPLC assay

CHX concentrations in the samples were quantified using a Shimadzu HPLC system (Shimadzu Scientific Instruments, Inc., Addison, IL) at room temperature. The HPLC system consisted of two pumps (LC-20 AT), a variable wavelength UV absorbance detector (SPD-20A), an auto-injector (SIL-20A), and a Microsorb-MV100-5 C18 column (15 cm × 4.6 mm, 4.6 μ m, Varian, Lake Forest, CA). The mobile phase was 0.23 moles sodium phosphate mono-basic in 1.39 L water, mixed with 10 ml 0.5% trimethylamine, adjusted to pH 3.0 with o-phosphoric acid, and mixed with 0.6 L acetonitrile to form a water:acetonitrile (70:30) mixture, and the flow rate was 1.5 ml/min. The injection volume was 50 μ L, and the detection wavelength was 239 nm. Standard solutions of 1–50 μ g/ml CHX were prepared in simulated saliva to construct the calibration curve.

Data analysis

The data obtained in the present study generally are presented as means and standard deviations (SD). Statistical analyses were performed using Student's *t*-tests with Microsoft Excel (Redmond, WA) and one-way ANOVA with GraphPad and Bonferroni *post hoc* tests (La Jolla, CA), and a difference of $p < 0.05$ was considered statistically significant.

Results and discussion

Physical properties of gelatin products

Gelatin chips that were not crosslinked with GTA swelled and dissolved in aqueous medium such as water and simulated saliva. When the gelatin chips were crosslinked with GTA, considerably less swelling was observed. An increase in GTA concentration used in the crosslinking process gave rise to gelatin chips that swelled less in aqueous medium. However, the impact of GTA concentration diminished at GTA concentration higher than ~2%. Particularly, gelatin chips swelled and increased in weight by more than 6x when equilibrated in aqueous medium for 3 h without GTA crosslinking. In contrast, gelatin chips prepared using Method A and crosslinked with 2% GTA solution swelled and increased in weight by ~2x in 3 h. Similar gelatin swelling was observed (less than 20% difference) when the GTA concentration increased from 2% to 4% in Method A.

Gelatin without glycerin is brittle when it is dry. Glycerin is a plasticizer and affects the physical properties, including malleability, of gelatin. Gelatin products prepared by Method A (0.25%, 1%, and 4% glycerin, with and without crosslinking using 2% GTA) and Method B (1% glycerin with GTA crosslinking) were tested with compression-breakage texture analyses. No products examined were fragmented at maximum instrument forces up to 66 N. Method B products developed both minor and major fracturing in both compression and flexibility testing, suggesting that Method B products were more brittle than Method A products. Figure 1 shows the effects of varying glycerin concentrations on the force gradients (force/time) of gelatin products prepared by Methods A and B with GTA crosslinking. The force gradients for Method B products and 4% glycerin Method A

products were both significantly lower than 0.25% and 1% glycerin Method A products. Figure 2 shows the peak forces to flex gelatin products prepared by Method A of differing glycerin contents with and without GTA crosslinking. In Method A, 4% glycerin products prepared without GTA crosslinking were significantly more flexible than other products tested; the Method A products without GTA required approximately 10-fold less force to bend. The data of other products tested for flexibility in the figure show no significant differences, suggesting that GTA crosslinking reduces the plasticizing effects of glycerin.

Chemical properties of gelatin products

The results in the enzyme digestion study with pepsin indicate that gelatin chips prepared with Method A underwent slowest enzymatic degradation among the three manufacturing processes (Methods A, B, and PerioChip®), followed by gelatin chips prepared with Method B, which was slower than PerioChip®. Under the same GTA concentration condition, gelatin chips prepared with Method B began to disintegrate between 48 and 96 h, and gelatin chips prepared with Method A remained relatively intact after 11 days. PerioChip®, the reference, was the least resistant to digestion and completely disintegrated between 12 and 24 h in the pepsin solution. The varying degrees of resistance of the gelatin products to pepsin digestion could be attributed to the different degrees of gelatin crosslinking under the different manufacturing conditions.

Effect of gelatin type (fabricated by Method A) on CHX release

Two types of gelatin were investigated in the present study. Type A gelatin was obtained from acid-cured porcine tissue, and Type B gelatin was from lime-cured bovine tissue. Type A gelatin has an isoelectric point (pI) of 7.0–9.0 and Type B gelatin has a pI of 4.7–5.2 according to the supplier [17]. For example, Type A gelatin is close to its pI or is slightly positively charged and Type B gelatin is negatively charged at physiological pH. When the pH is close to the pI, the net charge of the gelatin protein is close to zero and the polymer collapses due to self-association, forming a more compact and stronger gel under low to normal ionic strength conditions [18,19]. In addition, the viscosity of the polymer is generally lower due to the decrease in polymer chain tangling (less fibrous) at the pI [19,20]. When the gelatin is charged, the gelatin swells due to charge repulsion of the ionizable functional groups and has increased bonding to water, causing the gel strength to decrease and viscosity to increase. The effects of Type A and Type B gelatins on CHX release from gelatin products were compared in IVRT Method I with the products fabricated using 5% gelatin and 1% glycerin with crosslinking using 0.5%, 2%, and 8% GTA and CHX loading using 12.5% CHX digluconate solution. Table 2 summarizes the CHX release data of the Type A and Type B gelatin products. The results show that CHX release from Type B gelatin products was consistently ~20% higher than that of Type A gelatin under the same conditions ($p < 0.05$, Student's *t*-test). In addition, CHX release from both Type A and Type B gelatin products decreased with increasing crosslinking from 0.5% to 8% GTA (see GTA effect in the next section).

IVRT Method II was also used to compare the release profiles of CHX from Type A and Type B gelatin products (Figure 3). The gelatin products were prepared with 5% gelatin, 1% glycerin, crosslinking using 2% GTA, and CHX loading using 12.5% CHX digluconate

solution. The results show ~20% higher cumulative amounts of CHX release from the products of Type B gelatin compared to Type A, consistent with the data from IVRT Method I. The cumulative amounts of CHX release from Type A and Type B gelatin products overlapped initially and then diverged; Type B gelatin provided a higher amount of CHX loading (total amount released) whereas Type A gelatin had a faster initial percent CHX release rate. Type A gelatin was the gelatin matrix to be examined for the effects of other attributes in the present study.

Effects of GTA and glycerin (fabricated by Method A) on CHX release

Table 3 presents the comparison of CHX release from the gelatin products fabricated under different GTA conditions using IVRT Method I. In this study, 1% glycerin was used and 15% CHX digluconate was the drug loading solution. The data from the gelatin type comparison study in the previous section, in which Type B gelatin was also used and 12.5% CHX digluconate was the loading solution, are also presented in the table. The results show that the GTA concentration (0.5% to 8% GTA) used in the crosslinking of 5% gelatin affected the cumulative amount of CHX release from the test products ($p < 0.05$, ANOVA). To further investigate the effect of GTA crosslinking on CHX release from the gelatin products, experiments were conducted using IVRT Method II (Figure 4). The results of IVRT Method II are consistent with those of Method I; increasing GTA concentration decreased the cumulative amount of CHX release from the test products. However, when the percent cumulative CHX release from the gelatin products were compared, GTA crosslinking did not significantly affect the percent CHX release vs. time profiles under the conditions studied. The IVRT data suggest that the GTA crosslinking conditions mainly affect CHX loading (total amount released) and not the rate of percent CHX release.

In addition to GTA, CHX release from gelatin products fabricated under different glycerin conditions was examined. In the glycerin study, 2% GTA was used for the crosslinking with 5% gelatin and the same 15% CHX-loading procedure was used to fabricate the products. The conditions of 0.25–4% glycerin did not significantly affect the cumulative amounts of CHX release from the gelatin products; no apparent relationship (or trend) was observed between cumulative amounts of CHX release and glycerin concentrations (CHX release varying between 114–146, 147–184, and 153–177 μg CHX per mg chip at 6, 24, and 120 h, respectively, for 0.25% to 4% glycerin; $p > 0.05$, ANOVA). Although glycerin was found to affect the physical properties of the gelatin products, its effect on CHX release from the products was minimal.

Effect of CHX loading concentration (fabricated by Method A) on CHX release

The effect of CHX digluconate concentration in the drug loading procedure on CHX release from the gelatin products was investigated using IVRT Method II (Figure 5). The results show that the total cumulative amounts of CHX release were somewhat proportional to the concentration of CHX digluconate in the drug loading solution (2.5% to 20% CHX) for the gelatin products fabricated with 5% gelatin, 1% glycerin, and 2% GTA crosslinking (Figure 5 insert). However, CHX loading concentration did not significantly affect the percent CHX release profiles of the products, except in the early part of the release profile for the product with 20% CHX loading condition. For comparison, the release profiles of CHX from

PerioChip[®] (marketed product or RLD) are also presented in the figure. The release of CHX from the RLD is comparable to that of the test product fabricated using 12.5–15% CHX digluconate loading solution.

Effects of CHX loading time and product rinsing time (fabricated by Method A) on CHX release

The effect of drug loading times on CHX release from the gelatin products was examined. The gelatin products were fabricated using 5% gelatin, 2% GTA crosslinking, 1% glycerin, and 10% CHX digluconate loading solution for 12, 15, 24, and 72 h of equilibration in the drug loading time study and 15% CHX digluconate loading solution in the rinsing time study. There was no significant difference between the cumulative CHX release profiles of different CHX loading times when compared in the same study (less than 10%, 2%, and 5% differences between the CHX release profiles when compared between 12 and 24 h, 15 and 24 h, and 24 and 72 h drug loading of the same gelatin batches, respectively; $p > 0.05$, ANOVA). This suggests no significant effect of CHX loading time on CHX release and that CHX loading was completed within 12 h under the conditions studied. The relatively fast CHX loading (compared to the duration of drug release) could be attributed to convective transport of CHX into the gelatin matrix when the dry gelatin chips swelled in the CHX digluconate solution at the beginning of the equilibration process.

The effect of water rinsing time at the end of product fabrication on CHX release from the gelatin products was also examined. The rinsing time (5, 10, 15, and 60 s) of gelatin chips with water at the end of the gelatin fabrication process did not significantly affect CHX release from the gelatin products (e.g. total cumulative amount released of 163 ± 10 , 166 ± 12 , 144 ± 21 , and 156 ± 3 mg CHX per mg chip, respectively; $p > 0.05$, ANOVA). This can be related to the short rinsing time (< 60 s) relative to the duration of the IVRT study (> 24 h).

Effect of direct mixing of gelatin product components (fabricated by Method B) on CHX release

The two methods of gelatin product fabrication (Methods A vs. B: equilibration drug loading vs. direct drug mixing, respectively) were compared in terms of total cumulative amounts of CHX release from the gelatin products. The direct mixing of 78, 106, 323, and 600 mg CHX digluconate with the gelatin corresponds to 11%, 15%, 35%, and 50% CHX digluconate in the gelatin chips (see table insert in Figure 6). Figure 6 presents the cumulative amounts of CHX released from the gelatin products fabricated using the direct drug mixing method (Method B) and determined in IVRT Method I. The data show a loss of CHX mass when Method B was used. Specifically, the cumulative amounts of CHX release from Method B gelatin products were consistently 2x lower than the amounts of CHX loaded in the products as determined from the mass balance of CHX used in direct drug mixing. A possible explanation is that Method B produced a CHX suspension in the gelatin matrix and the precipitated CHX could be slow to dissolve [21,22] and did not contribute to CHX release from the gelatin product within the duration of the IVRT study. However, in a recovery study, the extraction of CHX from gelatin products of Method B with the HPLC mobile phase indicated that the total amounts of CHX loaded in the gelatin products were not

significantly higher than the total cumulative amount release in IVRT (data not shown). Another possible explanation is the degradation of CHX under the Method B condition when all ingredients were added nearly simultaneously. The new peaks observed in the HPLC chromatograms of the IVRT samples that were not in those of Method A support the presence of degraded CHX products from the Method B experiments.

Comparison of IVRT methods: Methods I and II

A satisfactory IVRT method should be able to discriminate performance differences between drug products that are Q1 and Q2 different for manufacturing quality control and generic product evaluation. In addition to Q1 and Q2, other differences such as manufacturing process can also affect drug release. Therefore, it is important to examine the two IVRT methods (Methods I and II) in the present study, which are different in the following aspects. Method II had smaller dissolution volume and utilized frequent replacement of the dissolution medium at each sampling time point to maintain the sink condition. Hence, Method II had more sampling time points than Method I and was more sensitive to characterize drug release in the early time period. However, Method II was more labor-intensive. In addition, Method II with frequent sampling could lead to errors in the later part of the release study when the detection of small amounts of drug release was limited by the sensitivity of the assay method whereas Method I with the large dissolution volume was limited by assay sensitivity in the early part of the release study. As both IVRT methods were employed in the testing of gelatin products in the GTA study, CHX release data of Methods I and II from the GTA study were compared. Figure 7 presents the values of the amounts of CHX release in Method I divided by those in Method II (i.e. ratios of CHX amounts in Method I to Method II) of the same batch of gelatin products fabricated using 5% gelatin, 2% and 8% GTA, 1% glycerin, and 15% CHX digluconate loading solution. The data show that the amounts of CHX released in Methods I and II were not statistically different ($p > 0.05$, ANOVA) albeit that the release of CHX in Method I was slightly less than that in Method II (i.e. ratios < 1.0). Possible explanations of the difference between Methods I and II are (a) the assumption of sink condition in the dissolution chamber when the drug concentration in the dissolution medium is not significantly lower than drug solubility [23] and (b) variability due to HPLC assay with the low drug concentration in the dissolution medium in the early part of Method I and the later part of Method II. Nevertheless, the results suggest that the two IVRT methods in the present study were generally comparable for evaluating CHX release from the products.

The stirring speed of a dissolution apparatus can affect *in vitro* drug release from a test product. The CHX release profiles of the same batch of selected gelatin products (5% gelatin, 2% GTA crosslinking, 15% CHX digluconate loading solution, and 1% glycerin) at normal and half stirring speeds (700 and 350 RPM settings) were compared. In the analyses, the cumulative amounts of CHX release at normal stirring speed from the gelatin products were divided by those at half stirring speed of the same time points, and these ratios are presented in Figure 7. The results show that reducing the stirring speed to half of the normal rate did not significantly affect the release rate of CHX from the gelatin products (ratios ~ 1.0) with no significant differences ($p > 0.05$, ANOVA). This suggests that the stirring (stirring speed) in IVRT was sufficient in the present study for the gelatin products.

For comparison of batch-to-batch variability of the marketed product, Figure 7 also presents the results of CHX release from PerioChip[®] of three different lots. The data serve as a reference to check IVRT variability and to support the results comparing the IVRT methods (Methods I and II) and stirring speeds (700 and 350 RPM settings). The data in Figure 7 show less than 10% variability of CHX release from the marketed product (PerioChip[®]). Although no significant CHX release variability was observed for the marketed product, batch-to-batch variability was observed for the gelatin products fabricated in the present study (data not shown). Up to a maximum of 30% variability of CHX release could occur when the gelatin products were fabricated using the same method on different days (different batches).

Effects of manufacturing attributes on CHX release from gelatin products

The main attributes affecting the release of CHX from gelatin chip/implant products were gelatin type, GTA crosslinking, and CHX loading condition. The effects of other attributes such as glycerin, drug loading time, and water rinsing time on CHX release were found to be minimal under the IVRT conditions in the present study. The manufacturing procedure (gelatin crosslinking after gelatin solution casting and then drug loading vs. direct mixing of gelatin and drug and then crosslinker) was also found to influence (a) CHX release from the gelatin products, (b) product compressibility and flexibility, and (c) resistance to enzymatic digestion. The gelatin products fabricated by direct mixing provided significantly lower CHX release and less resistance to enzymatic degradation than the two-step gelatin crosslinking and drug loading method. The Q1 and Q2 differences (gelatin type and % GTA used in crosslinking) and different manufacturing processes (direct mixing vs. two-step gelatin crosslinking and drug loading) of the gelatin test products could lead to differences in drug release and other behaviors of the products.

Conclusion

Matrix drug products of Q1 and Q2 differences and different manufacturing processes could lead to different drug release performance. The knowledge of how quality attributes affect drug release from matrix dosage forms could assist the development of these drug products and their performance evaluations. The present study investigated the attributes related to the fabrication process of small gelatin matrix using CHX as the model drug. In the IVRT study, the attributes that affect the release profiles of CHX from the gelatin products were gelatin type, GTA crosslinking, and CHX digluconate concentration in drug loading. The effects of glycerin concentration, CHX loading time, and water rinsing time at the end of the product fabrication process on CHX release were not significant under the conditions studied. The findings of different key attributes that affect drug release in the present study demonstrate the value of a quality by design (QbD) approach for the gelatin products. The two gelatin fabrication methods investigated in this study (two different mixing procedures: two-step method of crosslinking and then drug loading vs. direct mixing of the components) created gelatin products of different physical properties, resistances to enzymatic digestion, and cumulative amounts of CHX release. These findings demonstrate the impact of gelatin fabrication process and the importance of controlling the mixing steps in this process because using the same ingredients (Q1 and Q2 equivalence) might not create matrix

products of the same chemical and drug release performance. Lastly, comparable cumulative amounts of CHX release were obtained in the two IVRT methods studied. This suggests that the IVRT methods were not an influencing factor for CHX release from the gelatin products and either method could be used to evaluate CHX release from the products.

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Figure 1.

Comparison of the force gradients (force/time) between gelatin products manufactured by Methods A and B with differing glycerin contents in the Texture Analyzer studies. Mean $\pm$ SD ($n = 3$). *Represents significant difference between the condition of Method A 4% glycerin and those of 0.25% glycerin and 1% glycerin and between the condition of Method A 4% glycerin and that of Method B 1% glycerin ($p < 0.05$, ANOVA); ** represents significant difference between the condition of Method B 1% glycerin and those of Method A 0.25% glycerin, 1% glycerin, and 4% glycerin ($p < 0.05$, ANOVA).

Figure 2.

Comparison of peak force to flex gelatin products manufactured by Method A with differing glycerin contents with and without GTA crosslinking (“with no GTA” = without GTA crosslinking) in the Texture Analyzer studies. Mean $\pm$ SD ($n = 3-4$). * represents significant difference between the forces used for the conditions of 0.25%, 1%, and 4% glycerin with GTA crosslinking and that of 4% glycerin without GTA crosslinking ($p < 0.05$, ANOVA).

Figure 3.

Effect of gelatin type in the fabrication process on cumulative amount of CHX release from the gelatin products. Data beyond 48 h are not shown because there are no significant changes of the data from 48 h to up to 6 days. Figure insert: percent cumulative CHX release vs. time profiles of the same data. Mean $\pm$ SD ($n = 3$).

Figure 4.

Effect of GTA concentration in the fabrication process on cumulative amount of CHX release from the gelatin products. Data beyond 48 h are not shown because there are no significant changes of the data from 48 h to up to 6 days. Figure insert: percent cumulative CHX release vs. time profiles of the same data. Mean $\pm$ SD ($n = 3$).

Figure 5.

Effect of CHX concentration of the drug loading solution in the fabrication process on (a) cumulative amount of CHX release and (b) percent cumulative CHX release from the gelatin products. Data beyond 48 h are not shown because there are no significant changes of the data from 48 h to up to 6 days. Figure insert: relationship between total cumulative amount CHX release from gelatin product and concentration of CHX digluconate (% CHX digluconate) in drug loading solution. Mean $\pm$ SD ($n = 3$).

Figure 6.

Cumulative amount of CHX release from the gelatin products fabricated using Method B. CHX added was calculated by: $\text{CHX per chip} = \text{CHX used} / (\text{CHX digluconate used} + 0.6 \text{ g})$. 0.6 g was the weight of the gelatin sheet after drying in the preparation when 10 ml gelatin solution without CHX digluconate was used. Mean $\pm$ SD ($n = 3$).

Figure 7.

Comparison of IVRT Methods I and II (closed squares, circles, diamonds, triangles) and of stirring rates in Method II (crosses) on the cumulative amount of CHX release from the same batch of the respective gelatin products. Comparison of CHX release from PerioChip® of different lots was the reference (open circles and triangles). The ratios of cumulative amounts of CHX release in Method I to those in Method II, the ratios of cumulative amounts of CHX release at normal stirring speed to those at half stirring speed, and the ratios of cumulative amounts of CHX release from lot #1 or lot #2 to those of lot #3 are plotted against time. The horizontal line indicates ratio = 1.0 and no difference between the two IVRT methods. Each symbol represents the data point from one set of experiments of the two IVRT methods compared. For the reference, each symbol represents the data point from two different lots of PerioChip®. Mean $\pm$ SD ($n = 3$).

Table 1.

Conditions and attributes examined for gelatin products fabricated for drug release experiments.

Attribute	Test parameters	Other conditions	Method used ^a
Method A			
Gelatin type	Type A and B	5% gelatin, 0.5%, 2%, 8% GTA, 12.5% CHX loading for 24 h, 1% glycerin	I
Gelatin type	Type A and B	5% gelatin, 2% GTA, 12.5% CHX loading for 24 h, 1% glycerin	II
Glutaraldehyde	0.5%, 1%, 2%, 4%, 8%	5% Type A gelatin, 15% CHX loading for 24 h, 1% glycerin	I
Glutaraldehyde	2%, 8%	5% Type A gelatin, 15% CHX loading for 24 h, 1% glycerin	II
Glycerin	0.25%, 0.5%, 1%, 2%, 4%	5% Type A gelatin, 2% GTA, 15% CHX loading for 24 h	I
CHX loading concentration	2.5%, 5%, 10%, 12.5%, 15%, 20%	5% Type A gelatin, 2% GTA, CHX loading for 24 h, 1% glycerin	II
CHX loading time	12 h, 15 h, 24 h, 72 h	5% Type A gelatin, 2% GTA, 10% CHX loading, 1% glycerin	II
Rinsing time	5s, 10s, 15s, 60s	5% Type A gelatin, 2% GTA, 15% CHX loading for 24 h, 1% glycerin	I
Method B			
CHX loading amount	78 mg, 106 mg, 323 mg, 600 mg CHX digluconate in 10 mL gelatin, glycerin, water mixture	Direct mixing of Type A gelatin, glycerin, CHX, and GTA to final concentrations: 5% gelatin, 1% glycerin, and 2% GTA in water	I
Reference, RLD			
PerioChip®	N/A	N/A	II

^aMethod I: 100 ml IVRT method; Method II: 10 ml IVRT method.

Table 2.

Effect of gelatin type on CHX release from the gelatin products at 6, 12, 24, and 96 h.

Type A vs. Type B	% difference ^a
GTA 0.5%	-22.5 (-18.9 to -24.7) ^b
GTA 2%	-20.4 (-16.0 to -23.9) ^b
GTA 8%	-19.9 (-12.9 to -24.7) ^c

The effect is expressed as percent difference between the cumulative amounts of CHX release of Type A gelatin products compared to those of Type B. The mean and range are presented for the 6, 12, 24, and 96 h time points ($n = 3$ each time point and condition).

^a% difference = (Type A value – Type B value)/Type B value $\times 100\%$. Negative values denote lower amounts of CHX release from Type A compared to Type B gelatin. Range shown in parentheses.

^bSignificant difference between Type A and Type B data ($p < 0.05$, Student's t -test) for all time points.

^cSignificant difference between Type A and Type B data ($p < 0.05$, Student's t -test) for all time points except at 96 h ($p = 0.07$).

Table 3.

Effect of GTA concentration in the fabrication process on CHX release from the gelatin products at 6, 24, 96, and 120 h.

	Type A gelatin, 15% CHX loading solution, % difference vs. 2% GTA ^a	Type A gelatin, 12.5% CHX loading solution, % difference vs. 2% GTA ^{a,b}	Type B gelatin, 12.5% CHX loading solution, % difference vs. 2% GTA ^{a,b}
0.5% GTA	12.9 (11.9 to 13.7) ^c	20.9 (19.9 to 22.3) ^d	24.1 (23.6 to 25.0) ^d
1% GTA	12.0 (10.6 to 13.5) ^c	— ^e	— ^e
4% GTA	-13.6 (-11.9 to -16.0) ^c	— ^e	— ^e
8% GTA	-27.9 (-25.5 to -32.3) ^d	-27.3 (-26.4 to -29.4) ^d	-27.7 (-25.8 to -29.1) ^d

The effect is expressed as percent difference between the cumulative amounts of CHX release for the 0.5%, 1%, 4%, and 8% GTA conditions compared to those of 2% GTA. The mean and range are presented for the 6, 24, 96, and 120 h time points ($n = 3$ each time point and condition).

^a% difference = (x % GTA value - 2% GTA value)/2% GTA value x 100%. Positive values denote higher amounts of CHX release compared to 2% GTA condition, and negative values denote lower amounts of CHX release compared to 2% GTA condition. Range of % difference shown in parentheses.

^bData from the Type A and Type B comparison experiments.

^cSignificant difference between the studied condition compared to that of 2% GTA ($p < 0.05$, ANOVA) for all time points, except 6 and 24 h.

^dSignificant difference between the studied condition compared to that of 2% GTA ($p < 0.05$, ANOVA) for all time points.

^eCondition was not studied.