Enzyme-triggered release of antimicrobials from gelatin microcapsules

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INTRODUCTION

The goal of microencapsulation is to create a barrier between a compound of interest and its direct environment. In this way it is possible to shield the encapsulated ingredient from the environment, but also to influence the release of the compound. Encapsulation and release systems, also called delivery systems, are used in a wide variety of applications, ranging from the slow release of fragrances in cosmetic formulations, or the protection of oxygen sensitive nutritional ingredients, to the controlled release of active pharmaceutical ingredients from pharmaceutical formulations. Enzyme triggered release of active ingredients is an interesting topic in food, pharmaceutical and medical applications. It would, for instance, enable the release of nutritional ingredients in specific parts of the gastrointestinal tract, or the release of antibiotics triggered by the presence of bacteria.

An interesting approach for enzyme-triggered release is the BioSwitch technology, in which active ingredients are contained within a crosslinked biopolymer matrix. Upon degradation of the matrix material by enzymes, the active ingredient is released to the environment (de Jong A.R. 2005 ; Slaghek T. 2008). This paper describes the development of gelatin microcapsules that allow enzyme-triggered release of antimicrobial compounds, which could be used in the prevention or treatment of decubitus wounds. Antiseptics, such as chlorhexidine or octenidine, are used to treat infected wounds, but their use may also have adverse effects. By making use of an enzyme-triggered delivery system, release of the antimicrobials can be tuned to occur only if the wound is infected. Important criteria for the optimization of such a release system are the amount of ingredient that can be loaded into the microcapsules, minimization of the spontaneous release in absence of enzymes and tuning of the triggered release in the presence of relevant enzymes.

MATERIALS AND METHODS

For the preparation of crosslinked gelatin microparticles, three different crosslinkers were used: glycerol diglycidyl ether (GDGE, Sigma-Aldrich), glutaraldehyde (GA, 25% in water, Sigma-Aldrich) or transglutaminase (TG, 1 µg/ml in water). The particles were prepared by the following procedure: 16 g of gelatin (Fluka, pH = 4.0-6.0, water content <15%, 170-190 Bloom) in 90 g of demineralized water was heated at 90°C for 15 minutes, after which the mixture was cooled to 40°C, 5.49 M NaOH was added (0.3 ml for GA or TG crosslinking, or 2.5 ml for GDGE crosslinking), and the mixture was stirred at 40°C for 60 minutes. Next, the crosslinking agent was added and the mixture was stirred at 40°C until gelation occurred or for a maximum of 60 minutes, after which the mixtures were kept at 40°C for 72 hours. The obtained gel was pressed through a sieve with meshes of approximately 1 mm^2 and washed at least three times in 5 l. of water until the pH was 7.0. The gel particles were then washed with ethanol and acetone and dried in air. The obtained powder was sieved and separated into 4 fractions with particle sizes varying between 75 µm and 1000 µm. For comparison, a different grade of gelatin, crosslinked for 24 hours using 2.5 ml of TG solution (1 µg/ml in water), was also used. This sample (TG-ref) was kindly donated by Aart de Jong (TNO Quality of Life).

Loading of the microcapsules with octenidine was performed for the fraction with a particle size range of 350 µm to 600 µm by stirring 200 mg of gelatin particles in a solution of 30 mg octenidine in 100 ml of water at 37°C for 4 hours. The degree of loading was evaluated at different time intervals by measuring the octenidine concentration in the solution using UV-VIS spectroscopy (absorption at 282 nm). After loading, the microcapsules were separated from the solution and washed with 2 ml of demineralized water. Spontaneous release of octenidine from the microcapsules was evaluated by placing them in 100 ml demineralized water under stirring and measuring the octenidine concentration in solution using UV-VIS spectroscopy (absorption at 282 nm) at regular time intervals. After the equilibrium of spontaneous release was reached, enzyme triggered release was evaluated by adding 1.0 ml of properase 1600L solution (Genencor, 1600 PU/L) and measuring the octenidine concentration in the solution at regular time intervals using UV-VIS spectroscopy (absorption at 282 nm, correcting for properase absorption).

RESULTS AND DISCUSSION

For the preparation of enzyme-triggered microcapsules, gelatin is selected as matrix material. As a result of production conditions, this material contains a large number of anionically charged groups to which positively charged ingredients may be complexed by polyionic interactions. Release of charged compounds from gelatin capsules has been previously described for both large molecules, e.g. proteins, and small molecules, such as clonidine hydrochloride (Vandelli M.A. 2001) or chlorhexidine digluconate (Einerson N.J. (2003). These systems were generally characterized by a relatively large initial release of the small molecules, followed by slow degradation of the matrix material. In our systems, we are aiming to minimize the initial release in absence of enzymes and creating increased release in the presence of enzymes.

Native gelatin slowly dissolves in water. In order to obtain stable microcapsules, the gelatin matrix should be crosslinked, either by chemical or enzymatic methods. For this study, we have selected three different crosslinking agents: two chemical crosslinkers, glycerol diglycidyl ether (GDGE) and glutaraldehyde (GA), and one enzymatic crosslinker, transglutaminase (TG). Preparation of gelatin microcapsules was performed by dissolving the gelatin in water, mixing with different amounts of the crosslinkers (see Table 1) and allowing gelation. After crosslinking, the gelatin was pressed through a sieve to obtain gel particles of the desired size, which were subsequently washed and dried.

Sample	Crosslinking agent	Amount
GDGE-1	GDGE	2 ml
GDGE-2	GDGE	3 ml
GA-1	GA	1 wt%*
TG-1	TG	1 ml
TG-2	TG	1.8 ml
TG-ref	TG	2.5 ml#

* based on dry mass of GA and gelatin
# different gelatin grade and reaction conditions

Table 1: Crosslinking conditions

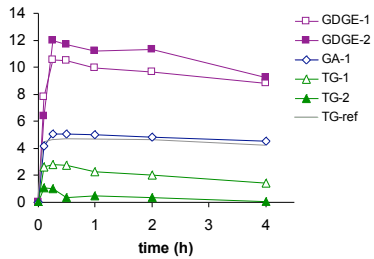

Figure 1: Octenidine loading after cross-linking at different conditions

The obtained gelatin particles were loaded with octenidine, an antimicrobial compound. Due to the opposite charges of octenidine and gelatin, octenidine binds to the gelatin matrix by polyionic interactions. As a result, the octenidine concentration in the surrounding solution is decreased, which is used as a measure for the loading capacity of the gelatin microcapsules. Using UV-VIS spectroscopy, loading of the gelatin particles with octenidine is evaluated in time (Figure 1). The GDGE crosslinked gelatin capsules were found to hold around 10−12 wt% of octenidine relative to their dry weight. The amount of crosslinking agent did not seem to have a large effect on the loading capacity. The loading capacity of the GA crosslinked microcapsules was around 5 wt%. After 4 hours, however, the gelatin capsules were found to start losing their integrity and the octenidine was released again. UV-VIS spectroscopy indicated that gelatin particles were dissolving. Some loading also seems to occur for the TG crosslinked microcapsules. However, after 1 hour the octenidine is released into the solution again, and UV-VIS spectroscopy showed the presence of gelatin in the solution. This indicated that the crosslinking by TG was probably incomplete, leaving the gelatin capsules still water soluble. For comparison, gelatin microparticles crosslinked using a higher concentration of TG for 24 hours were also loaded with octenidine (TG-ref in Figure 1), leading to similar loading capacities as the GA-1 capsules. Although these microcapsules consist of a different grade of gelatin than the other samples, and thus cannot be compared directly, these results demonstrate that TG crosslinking is a viable route for the preparation of stable, octenidine loaded gelatin microcapsules.

Ideal enzyme triggered delivery systems would show minimal release of the active component in the absence of relevant enzymes, while maximum release is obtained when the enzyme is present. Spontaneous release of octenidine from the produced gelatin microcapsules was evaluated by suspending them in demineralized water at 37°C and measuring the octenidine concentration in solution by UV spectroscopy. Results for these experiments are summarized in Figure 2. As was observed in the loading experiments, the GA crosslinked gelatin capsules slowly dissolved in water, thus releasing most of the octenidine within a few hours. Spontaneous release for the GDGE crosslinked microcapsules was around 16%; no significant effect of the amount of crosslinking agent was observed. The TG crosslinked capsules (TG-ref) only showed 7% of octenidine release in the absence of enzyme. However, since a different grade of gelatin was used for the preparation of these microcapsules, which might have a different charge density, these results cannot be compared directly to the other samples.

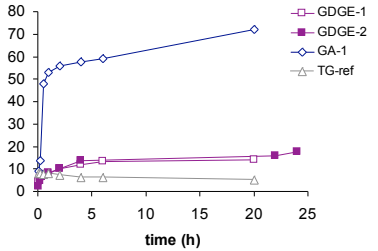

Figure 2: Release of octenide in the absence of enzyme

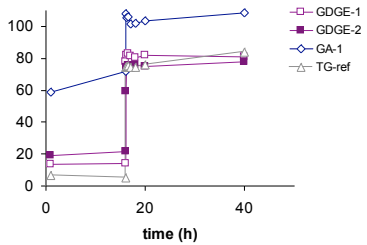

Figure 3: Release of octenide after addition of enzyme (at t=16h)

To test the efficacy of enzyme-triggered release, properase was used as a model enzyme, as it is known to degrade different grades of gelatin. In a later stage, enzymes more specific for infected wounds might be used. After equilibrium was reached for the spontaneous release experiments (after 16 hours), properase was added to the solution in which the gelatin capsules were placed and again octenidine release was monitored by UV spectroscopy (Figure 3). The GA crosslinked gelatin, already partly dissolved in water, rapidly broke down in the presence of properase, releasing the remaining octenidine into the solution. Also the GDGE crosslinked microcapsules were degraded by the enzyme and the maximum of 80% octenidine release was reached within 30 minutes. Again, the amount of GDGE used for crosslinking did not affect the rate or extent of octenidine release. The same amount of octenidine release was found for the gelatin capsules crosslinked using TG, which is a very promising result, taking into account the low spontaneous release observed for these microcapsules.

CONCLUSIONS

Enzyme-triggered release of octenidine from crosslinked gelatin microcapsules has been achieved. Stable gelatin capsules produced by GDGE crosslinking could be loaded with substantial amounts of octenidine antiseptic and spontaneous release of the compound in the absence of enzyme was below 15%. Addition of properase enzyme led to a rapid release of most of the octenidine, demonstrating effective break-down of the gelatin matrix. Gelatin microcapsules crosslinked using GA were found to slowly dissolve in water, indicating incomplete crosslinking of the gelatin matrix. Stability of the GA crosslinked particles might be improved by optimizing reaction conditions. TG crosslinking with 1.0 ml or 1.8 ml TG solution (1 µg/ml in water) also yielded soluble gelatin capsules unsuitable as matrix for octenidine release. Good results were obtained, however, with a different batch of gelatin crosslinked with a larger amount (2.5 ml) of TG solution. The obtained gelatin microcapsules exhibited limited release of octenidine in the absence of enzyme, whilst rapid release was produced upon addition of properase.

REFERENCES

De Jong, A.R. et al. (2005) *Active and intelligent packaging for food: Is it the future?* Food Additives and Contaminants 22 (10) 975-979

Einerson, N.J. et al. (2003) *Synthesis and physicochemical analysis of gelatin-based hydrogels for drug carrier matrices.* Biomaterials 24(3) 509-523

Slaghek T. (2008) *Switching on to biopolymers.* Chemistry and Industry 4 24–25

Vandelli M.A. et al. (2001) *Gelatin microspheres crosslinked with D,L-glyceraldehyde as a potential drug delivery system: preparation, characterisation, in vitro and in vivo studies.* International Journal of Pharmaceutics 215 175–184

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