Drug Development and Industrial Pharmacy, 2009; 35(11): 1384–1388

informa
healthcare

ORIGINAL ARTICLE

Enteric coating derived from marine sponge collagen

Martina Nicklas[1], Wolfgang Schatton[1], Sascha Heinemann[2], Thomas Hanke[2] and Jörg Kreuter[3]

[1]KliniPharm GmbH, Frankfurt, Germany, [2]Max Bergmann Center of Biomaterials and Institute of Materials Science, Dresden University of Technology, Dresden, Germany and [3]Institute of Pharmaceutical Technology, Biocenter Niederursel, Johann Wolfgang Goethe-University, Frankfurt, Germany

Abstract

Background: Enteric coating prevents oral dose forms from being digested in the stomach, which is required for drugs that are acid unstable, have an irritant effect on the stomach, or are designed to act in the small intestine. *Aim*: The objective of this study was to develop a novel gastroresistant delayed-release tablet coating based on the marine sponge *Chondrosia reniformis* Nardo and to investigate the technical feasibility of the coating process. *Method*: An aqueous gastroresistant coating dispersion on the base of freeze-dried sponge collagen 15% (w/w) as the film-forming agent was developed. The disintegration test for gastroresistant tablets (Ph. Eur.) was carried out at increasing coating levels to reveal the required collagen layer thickness. Reproducibility of the method, physical properties, and stability of the coated tablets were investigated. *Results*: Tablets coated with 13 mg/cm^2 of sponge collagen resisted more than 2 hours to 0.1 M hydrochloric acid, and disintegration of all tablets occurred within 10 minutes in phosphate buffer solution (pH 6.8). The method was reproducible, the mechanical properties of the coated tablets were satisfactory, and the obtained tablets could be stored for at least 6 months without loosing enteric properties. *Conclusions*: The novel coating based on the marine sponge collagen (using 12.9 mg/cm^2 coating material) complied with the requirements of Ph. Eur. for gastroresistant tablets. This coating material also meets the regulatory requirements for dietary supplements.

Key words: Chondrosia reniformis *Nardo; collagen; electron microscopy; enteric coating; marine sponge*

Introduction

Enteric coating of pellets, tablets, and capsules is frequently used since decades, prevents oral medications from being digested in the stomach, and leads to the controlled release of the active substance in the upper intestine. Such enteric properties, for example, are useful for substances that have an irritant effect on the stomach, for drugs that are acid unstable or are designed to act in the small intestine[1]. Materials commonly used for enteric coatings are anionic polymethacrylates [copolymer of methacrylic acid and either ethyl acrylate or methyl methacrylate (Eudragit®), polyvinyl derivatives (e.g., polyvinyl acetate phthalate), or cellulose-based polymers (e.g., cellulose acetate phthalate)]. Delayed release coating also is convenient for dietary supplements by reducing unpleasant flavors and odors and by enhancing intragastric acid stability of natural ingredients. Only natural polymers are acceptable as enteric coating materials for food additives and phytopharmaceuticals. There are a few enteric polymers of natural origin available, for example, natural resins such as shellac, zein, copal collophorium, and mixtures thereof. Shellac is the only material that is commonly used, but a major problem is its slow disintegration in higher pH media.

From solutions or dispersions of collagen continuous films can be obtained, which still possess the fibrous character of collagen[2]. The collagen from the marine sponge *Chondrosia reniformis* is stable against pepsin, trypsin, and collagenases[3,4] and insoluble in acid media[5–7] in contrast to bovine skin type I collagen. Because of its low immunogenicity and biodegradability[8], many cosmetic, medical, and pharmaceutical applications for collagen products exist[9–12]. Sponge collagen is safe in contrast to bovine collagen to transmissible spongiform encephalopathy and bovine spongiform encephalopathy[13]. The sponge *C. reniformis* Nardo is common throughout the Mediterranean Sea, can be easily kept in aquariums[14], and

Address for correspondence: Professor Jörg Kreuter, Institute of Pharmaceutical Technology, Biocenter Niederursel, Johann Wolfgang Goethe-University, Max-von-Laue-Straße 9, D-60438 Frankfurt am Main, Germany. E-mail: kreuter@em.uni-frankfurt.de

(Received 26 Jan 2009; accepted 1 Apr 2009)

ISSN 0363-9045 print/ISSN 1520-5762 online © Informa UK, Ltd.
DOI: 10.3109/03639040902939239

it is assumed that it is the only eatable sponge existing[15]. The objective of this study was to find out whether *Chondrosia* collagen can be used as a film-building polymer for enteric coatings as a new application.

Materials and methods

Materials

The marine sponge *C. reniformis* Nardo (Demospongiae: Hadromerida: Chondrosiidae) was harvested at the culture site of the Greek island of Kalymnos in the southeastern Aegean Sea and stored in 50% (v/v) ethanol until used. Sponge specimens were taken from two different cultures (see below).

Round biconvex placebo tablets comprising lactose, silicone dioxide, corn starch, microcrystalline cellulose, talc, and magnesium stearate were obtained from Rottendorf (Ennigerloh, Germany). Excipients used for the coating dispersion were triethyl citrate (Merck Schuchardt, Hohenbrunn, Germany) and talc (Caesar & Loretz, Hilden, Germany). All other reagents were of analytical grade and obtained as follows: potassium dihydrogen phosphate from Carl Roth (Karlsruhe, Germany), hydrochloric acid from Hedinger (Stuttgart, Germany), sodium hydroxide from Caesar & Loretz, Tris from Fluka (Buchs, Switzerland), and acetic acid from Sigma (St. Louis, MO, USA).

Composition and preparation of the coating dispersion

Chondrosia collagen was isolated by a previously described method[16] and freeze-dried using a Virtis Ultra 35 LE (Virtis Co., Gardiner, NY, USA). Two collagen batches, cultures from 2005 (batch 1) and from 2006 (batch 2), were used for the preparation of the coating dispersions: 200 g of freeze-dried collagen and 200 g purified water were mixed using a magnetic stirrer. The pH was adjusted to 7.4 using 0.1 M phosphate buffer. After 15 minutes, 20 g of triethyl citrate (for increasing flexibility and plasticity), 100 g of talc (anti sticking agent), and the required amount of purified water (total composition in Table 1) were gradually added under continuous stirring for 10 minutes to obtain a 15% (w/w) aqueous dispersion. A fine, homogenous suspension was obtained, which was passed through a 0.5-mm sieve. Stirring was sustained throughout the coating process.

Table 1. Composition of the coating dispersions batch 1 and batch 2 used to coat the placebo tablets.

Chondrosia reniformis collagen (freeze-dried)	200.4 g	200.3 g
Triethyl citrate	20.1 g	20.1 g
Talc	99.8 g	100.0 g
Water (including phosphate buffer)	1015.8 g	1013.1 g
Total mass	1336.1 g	1333.5 g
Total solids	320.3 g (23.97%)	320.4 g (24.03%)

Tablet coating

Five hundred grams of placebo tablets of a surface area of 113.1 mm^2 were preheated on trays for 30 minutes at 40°C. A spherical coating pan (diameter 20 cm at mouth) with adjustable motor speed (Erweka AR 401; Erweka Apparatebau GmbH, Heusenstamm, Germany) was used for the coating process. The operating parameters are given in Table 2. On the basis of theoretical estimation, 13 mg/cm^2 of freeze-dried sponge-derived polymer was applied. Samples of 12 tablets were taken corresponding to a theoretical coating thickness of 5, 7, 9, and 11 mg/cm^2 during the coating experiment and tested for enteric properties using the disintegration test according to Ph. Eur. to determine the required collagen layer thickness. Coating experiments were carried out for three batches of 500 g of tablets for both collagen batches.

Scanning electromicroscopy

Tablet hemispheres (horizontally cleaved) without coating and with a theoretical coating thickness of 13 mg/cm^2 were examined for their surface characteristics by scanning electron microscopy (SEM). The samples were mounted on stubs and coated with carbon in a Balzers SCD 050 coater (Balzers, Liechtenstein). A high tension of 10 kV was applied in a Philips ESEM XL 30 scanning electron microscope (Eindhoven, the Netherlands) working in high vacuum mode, and secondary electrons were detected for imaging.

Test for disintegration

The disintegration test according to Ph. Eur.[17] modified for gastroresistant tablets[18] was performed. After operating the disintegration tester (Erweka ZT 300; Erweka

Table 2. Operating parameters used to coat placebo tablets with *Chondrosia* collagen.

Parameter	Value
Tablet batch size	500 g
Spray gun	Ecospray®
Nozzle diameter	0.9 mm
Speed of rotation	35 rpm
Atomising pressure	4.5 kg/cm^2
Inlet air temperature	38°C
Outlet air temperature	28–33°C
Spray rate	2.3 g/min
Spraying time	151.5–153 minutes
Drying conditions	15 minutes at 20 rpm (speed of rotation) at 30°C

GmbH, Heusenstamm, Germany) for 2 hours at 37°C with 0.1 M hydrochloric acid as the liquid without the disks, the state of the six tablets was examined. The acid was replaced by phosphate buffer solution (pH 6.8), a disk was added to each tube, and the apparatus was operated until all tablets had disintegrated. The experiments were performed in duplicate for each batch.

Atomic force microscopy

The behavior of isolated *C. reniformis* sponge collagen in acidic and neutral buffered media was examined by atomic force microscopy (AFM).

The collagen (1 mg/mL) was suspended in 0.1 M acetic acid (pH 4) or 0.1 M Tris/HCl buffer (pH 7.4) under constant stirring at 4°C. Fifty microliters of the collagen suspensions were placed on the surface of titanium-sputtered glass disks. After adsorption for 30 minutes, the samples were rinsed with deionized water and air-dried. AFM imaging was performed in tapping mode® using a Nanoscope IIIa Bioscope (Digital Instruments/ Veeco, Santa Barbara, CA, USA). AFM images were taken at scan rates of 1.2 Hz, scanning 512 lines. Deflection and height images were taken simultaneously, and data visualization was assisted by WSxM software[19].

Physical properties of the enteric-coated tablets

The mechanical stability of the coated tablets (13 mg polymer/cm^2 based on theoretical estimation) was evaluated by the standard friability test (monograph 2.9.7)[20]. After 4 minutes in an Erweka friabilator (TA 200; Erweka GmbH, Heusenstamm, Germany), set at 25 rpm, 20 tablets taken at random were reweighed and the friability was calculated. Resistance to crushing in accordance with Ph. Eur. (monograph 2.9.8)[20], thickness and diameter of 10 tablets taken at random were examined by means of an Erweka TBH 30 (Erweka GmbH). Tablet hardness was determined on tablets oriented in the same way with respect to the direction of application of the force, and results are given in newtons (N). In addition to already mentioned parameters, weight uniformity was determined on 20 tablets according to Ph. Eur. method 2.9.5[20] using a Mettler Toledo AB204 analytical balance (Mettler-Toledo GmbH, Giessen, Germany). All experiments were carried out on tablets coated with 13 mg polymer/cm^2 and run in duplicates for each batch.

Reproducibility of coating

To test the reproducibility of the coating method, three batches of each collagen batch (total of six) were coated, and the enteric properties were evaluated by carrying out the modified test for disintegration.

Stability

It was investigated whether the enteric properties were affected by storage of the coated tablets (13 mg polymer/cm^2) at ambient conditions of 25 ± 5°C and 35 ± 10% relative humidity in commercial plastic sachets. After 6 months, the modified test for disintegration (see 'Test for disintegration') was performed to evaluate enteric properties.

Results and discussion

To develop an alternative gastroresistant coating derived from sponge collagen, the composition of the coating dispersion, the coating parameters, and the required coating thickness for enteric properties were explored using placebo tablets (disintegration within 15 seconds in water and in 0.1 M HCl). Preliminary tests at room temperature with an aqueous *Chondrosia* collagen dispersion (15%, w/w) in the absence of triethyl citrate and talc showed a continuous but rigid and brittle film. For this reason triethyl citrate (10% based on the weight of dry polymer) was added to improve the flexibility, and in addition talc (50% based on the weight of dry polymer) was added as an inert, antistatic substance to prevent the coating composition and the subsequent film from getting sticky. As a result, flexible and not friable films were formed. By using a neutral buffer solution for pH adjustment, such as 0.1 M phosphate buffer, dispersion of collagen in water was facilitated[5].

During coating the temperature of the tablets was below 38°C, being considerably below 60°C, the transition and shrinkage temperature of isolated collagen fibrils from *Chondrosia*[21]. Coated placebo tablets of a theoretical coating thickness of 5, 7, and 9 mg/cm^2 of both collagen batches disintegrated within 60 minutes in 0.1 M hydrochloric acid, whereas at least three tablets of each batch of a theoretical coating level of 11 mg/cm^2 resisted to the acid medium for 2 hours. Tablets coated with 13 mg/cm^2 of polymer complied with the specifications of delayed-release tablets of the Ph. Eur. by showing no signs of either disintegration or cracks after 2 hours. They were resistant for at least two further hours. After replacing the acid by phosphate buffer solution (pH 6.8), first signs of cracks or rupture were found after 3 minutes, and disintegration of all tablets occurred within 10 minutes. In contrast to soluble fibrillar collagen type I, which displays accelerated degradation into the monomer tropocollagen in acidic media (only 30% of initial amount still fibrillar after 2 days in 0.1 M HCl at 4°C; Heinemann et al.[5]), the percentage of fibrillar collagen of *C. reniformis*, measured by the Lowry protein assay, was about 100% after 7 days of acid treatment (0.1 M HCl, 4°C; Heinemann et al.[5]). The

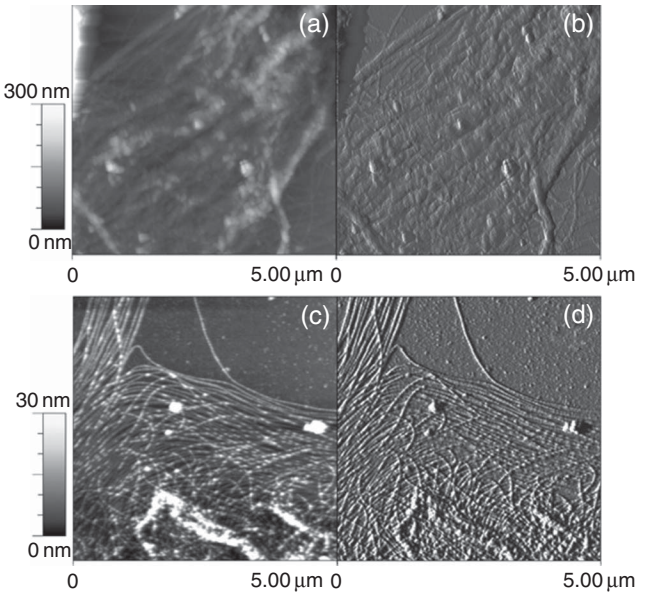

Figure 1. AFM picture of a bundle of *Chondrosia* collagen fibers after acid treatment: (a) Height and (b) deflection image. AFM picture of *Chondrosia* collagen fibers after separation into single fibrils after treatment in neutral buffered media: (c) Height and (d) deflection image.

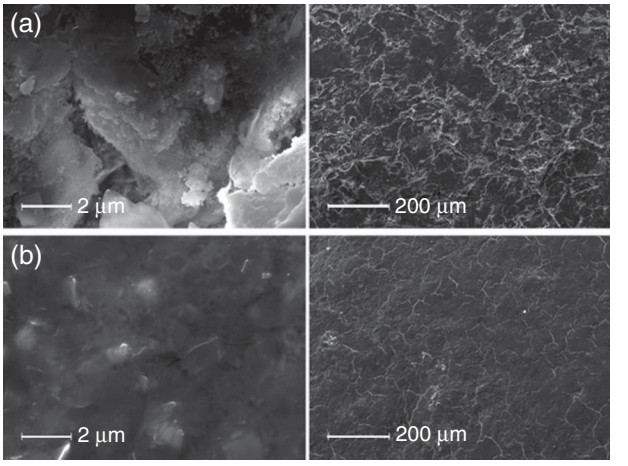

Figure 2. SEM photographs of uncoated tablet surface at different magnifications (a) and of the surface after coating with 13 mg/cm^2 of freeze-dried sponge collagen at different magnifications (b).

behavior of isolated *C. reniformis* sponge collagen in acidic and neutral buffered media was visualized using AFM (Figure 1). Collagen fibrils, right-handed superhelices, composed of lateral arranged triple-stranded tropocollagen and packed into tough bundles were not separated by acid treatment. AFM imaging displayed thick fibers of collagen fibrils arranged in firmly packed bundles (Figure 1a and b). Several fibrils are connected by minute filaments whose chemical composition is unidentified[6]. The rapid disintegration of coated tablets in buffer solution (pH 6.8) can also be explained by carrying out AFM. Heinemann et al.[5] demonstrated separation of the fibers into single collagen fibrils after treatment in neutral buffered media, and it was assumed that interfibrillar cross-links[21] were disconnected by the neutral solvent, and thus disaggregation took place as it is shown in Figure 1c and d. For all batches of tablets coated with 13 mg/cm^2 of polymer, time of resistance to the acid medium was at least 2 hours and disintegration at pH 6.8 always took place within 10 minutes, indicating reproducibility of the method. In contrast, shellac is not able to disintegrate within 60 minutes in phosphate buffer (pH 6.8)[22].

SEM photographs show the rough surface of the uncoated placebo tablets (Figure 2a) and the much smoother surface of the coated tablets (13 mg/cm^2 of freeze-dried sponge collagen based on theoretical estimation, Figure 2b) due to the film-building properties of the collagen. Although collagen fibers are not obvious in the micrographs (Figure 2b), disintegration test suggested that the collagen film still possessed the character and the physicochemical properties of the collagen fibers.

The parameters of the coated tablets (13 mg/cm^2 of polymer) were measured according to Ph. Eur. and showed satisfactory results. The friability of 20 tablets was below 0.15%, and tablets were of acceptable hardness (77.4 ± 4.5 N). In comparison to the uncoated tablets (65.6 ± 5.6 N), they were more resistant to crushing. Great uniformity regarding diameter (7.43 mm ± 0.32%), thickness (3.55 mm ±0.53%), and weight (163.4 mg ± 1.1%) indicated an evenly applied coating. During coating, no significant loss of coating dispersion was observed. The calculated value of actual coating thickness, based on determined weight gain, was 12.9 mg/cm^2 with both collagen batches. No variability was observed between collagen batches 1 and 2 regarding disintegration and physical properties of the coated tablets. The enteric properties were not affected by storage for 6 months at ambient conditions of 20–30°C and 25–45% relative humidity.

In conclusion, marine sponge collagen is well suited for delayed-release coating of tablets providing good mechanical properties and storage stability.

Acknowledgment

This project was supported by the European Commission (EU project '*Sponges*', No. 017800).

Declaration of interest: The authors report no conflicts of interest.

References

1. Wilding IR. (2000). Site-specific drug delivery in the gastrointestinal tract. Crit Rev Ther Drug Carrier Syst, 17:557–620.
2. Bräumer K. (1974). Das Faserprotein Kollagen. Appl Macromol Chem Phys, 40(1):485–92.
3. Junqua S, Robert L, Garrone R, Pavans de Ceccatty M, Vacelet J. (1974). Biochemical and morphological studies on the collagens of horny sponges. Ircinia filaments compared to spongines. Connect Tissue Res, 2:193–203.
4. Garrone R. (1978). Phylogenesis of connective tissue: Morphological aspects and biosynthesis of sponge intercellular matrix. In: Robert L, ed. Frontiers of matrix biology, vol. 5. Basel, München, Paris, London, New York, Sydney: S. Karger.
5. Heinemann S, Ehrlich H, Douglas T, Heinemann C, Worch H, Schatton W, et al. (2007). Ultrastructural studies on the collagen of the marine sponge *Chondrosia reniformis* Nardo. Biomacromolecules, 8(11):3452–7.
6. Garrone R. (1985). The collagen of the porifera. In: Bairati A, Garrone R, eds. Biolgogy of invertebrate and lower vertebrate collagens. Plenum Press: New York, 157–75.
7. Imhoff J-M, Garrone R. (1983). Solubilization and characterization of *Chondrosia reniformis* sponge collagen. Connect Tissue Res, 11:193–7.
8. Furthmayr H, Timpl R. (1976). Immunochemistry of collagens and procollagens. Int Rev Connect Tissue Res, 7:61–99.
9. Rössler B, Kreuter J, Ross G. (1994). Effect of collagen microparticles on the stability of retinol and its absorption into hairless mouse skin in vitro. Pharmazie, 49:175–9.
10. Friess W. (1998). Collagen – biomaterial for drug delivery. Eur J Pharm Biopharm, 45(2):113–6.
11. Kleinmann G, Larson S, Hunter B, Stevens S, Mamalis N, Olson RJ. (2007). Collagen shields as a drug delivery system for the fourth-generation fluoroquinolones. Ophthalmologica, 221(1):51–6.
12. Aishwarya S, Mahalakshmi S, Sehgal PK. (2008). Collagen-coated polycaprolactone microparticles as a controlled drug delivery system. J Microencapsul, 7:1–9.
13. Swatschek D, Schatton W, Müller WEG, Kreuter J. (2000). Maritime sponge collagen: Isolation, characterization and suitability for microparticle-preparation. Arch Pharm Pharm Med Chem, 333(1):31.
14. Nickel M, Brümmer F. (2003). In vitro sponge fragment culture of *Chondrosia reniformis* (Nardo, 1847). J Biotechnol, 100:147–59.
15. Grell KG, Gruner H-E, Kilian EF. (1993). Einführung Protozoa, Placozoa, Porifera. In: Gruner H-E, ed. Lehrbuch der Speziellen Zoologie, Band I: Wirbellose Tiere, 1. Teil. Gustav Fischer Verlag: Stuttgart, 278.
16. Schatton W, Schatton M, Swatschek D, Müller WEG, Kreuter J. (2007). Method for isolating collagen from marine sponges and producing nanoparticulate collagen, and the use thereof. European patent EP 1 259 120 B1.
17. European Pharmacopoeia. (2005). 5th ed. (Suppl. 3). Monograph 2.9.1. Stuttgart: Deutscher Apotheker Verlag, 4403–5.
18. European Pharmacopoeia. (2005). 5th ed. (Suppl. 8). Monograph 0478. Stuttgart: Deutscher Apotheker Verlag, 7049–52.
19. Horcas I, Fernandez R, Gomez-Rodriguez JM, Colchero J, Gomez-Herrero J, Baro AM. (2007). WSXM: A software for scanning probe microscopy and a tool for nanotechnology. Rev Sci Instrum, 78:013705.
20. European Pharmacopoeia. (2005). 5th ed. Monographs 2.9.5, 2.9.7 + 2.9.8. Stuttgart: Deutscher Apotheker Verlag, 293–4.
21. Garrone R, Huc A, Junqua S. (1975). Fine structure and physiocochemical studies on the collagen of the marine sponge *Chondrosia reniformis* Nardo. J Ultrastruct Res, 52(2):261–75.
22. Pearnchob N, Dashevsky A, Bodmeier R. (2004). Improvement in the disintegration of shellac-coated soft gelatine capsules in simulated intestinal fluid. J Control Release, 94(2–3):313–21.