RESEARCH ARTICLE

Preparation and characterization of shellac-coated anthocyanin pectin beads as dietary colonic delivery system*

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Scope: Anthocyanins are connected with various biological activities. A promising way to enhance the availability of anthocyanins for in situ effects in the lower intestine is colon-specific delivery.
Methods and results: Shellac and shellac/hydroxypropyl methylcellulose (HPMC) coated anthocyanin amidated pectin beads as dietary colonic delivery systems were successfully prepared by ionotropic gelation and fluid bed Wurster coating with aqueous shellac solution. Release characteristics, studied in vitro and ex vivo using simulated gastric fluid (SGF), ileostomy fluid and colostomy fluid (CF) revealed a retardation of anthocyanins during simulated passage of stomach and ileum as well as the desired release of pigments in the colon. Coating level was identified as an important parameter. By addition of 5 or 15% of the water-soluble polysaccharide HPMC to the shellac film, resistance in SGF was increased due to the plasticizer properties of the polymer. Incorporation of 15% HPMC (w/w based on shellac) into the shellac film additionally led to increased anthocyanin diffusivity and complete release as well as degradation of the formulation in CF.
Conclusion: In the used in vitro and ex vivo model system mimicking the human intestinal transit, the potential of shellac and shellac/HPMC coated anthocyanin amidated pectin beads as dietary colon targeting systems was demonstrated.

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1 Introduction

Anthocyanins are the most important group of water-soluble pigments and are responsible for the intensive red, purple and blue colors of different fruits and vegetables [1]. These flavonoids are connected with various biological activities, e.g. antioxidative, antiinflammatory and anticarcinogenic effects, improvement of risk factors for cardiovascular health as well as prevention of obesity [2, 3]. Furthermore, the results of several in vitro and animal studies suggest that anthocyanins may play a role in prevention and therapy of intestinal diseases such as acute diarrhea, inflammatory

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Abbreviations: APB, anthocyanin loaded pectin bead; **CF,** colostomy fluid; **cy3ara,** cyanidin-3-*O*-arabinoside; **cy3gal,** cyanidin-3-*O*-galactoside; **cy3glc,** cyanidin-3-*O*-glucoside; **dp3ara,** delphinidin-3-*O*-arabinoside; **dp3gal,** delphinidin-3-*O*-galactoside; **dp3glc,** delphinidin-3-*O*-glucoside; **GIT,** gastrointestinal tract; **HPMC,** hydroxypropyl methylcellulose; **IF,** ileostomy fluid; **mv3ara,** malvidin-3-*O*-arabinoside; **mv3glc,** malvidin-3-*O*-glucoside; **mv3gal,** malvidin-3-*O*-galactoside; **pn3ara,** peonidin-3-*O*-arabinoside; **pn3gal,** peonidin-3-*O*-galactoside; **pn3glc,** peonidin-3-*O*-glucoside; **pt3ara,** petunidin-3-*O*-arabinoside; **pt3gal,** petunidin-3-*O*-galactoside; **pt3glc,** petunidin-3-*O*-glucoside; **SEM,** scanning electron microscopy; **SGF,** simulated gastric fluid

*This work is dedicated to Dr. Dr. h.c. mult. Siegfried Hünig's 90th birthday.

bowel disease or colon cancer. An amelioration of experimental murine sodium sulfate colitis by supplementation of anthocyanin extracts was shown [4, 5]. The pigments were characterized as potent inhibitors of cyclooxygenase and lipoxygenase, key enzymes during inflammation processes [6–8]. An effective inhibition of carcinogenesis in the colon was induced by anthocyanins [9]. In addition, anthocyanins' antibacterial activity against pathogen bacteria as well as a prebiotic effect on gut microbiota was assessed [10–12]. Moreover, in ''traditional'' medicine anthocyanin-rich bilberries are well known for a long time as an effective drug for treatment of diarrhea [13].

Besides regular consumption of anthocyanin-rich foodstuffs, in vivo activities of the pigments are primarily determined by their availability. For instance, anthocyanins have to reach intestine and intestinal tissues to induce their above-mentioned effects in prevention and therapy of intestinal diseases. A variety of studies showed that anthocyanins are rapidly absorbed and excreted mostly as intact glycosides. Stomach and jejunum were characterized as predominant absorption sides [14]. Maximum pigment concentrations determined in human blood, e.g. after consumption of anthocyanin-rich berry products and/or extracts were only in the nM to low μM range. Furthermore, low anthocyanin urinary excretion was reported ranging from 0.003 to 1.9% of the intake [15]. With regard to this still unexplained, low bioavailability, systemic effects of anthocyanins seem to be limited. In contrast, an effective absorption of anthocyanins through the apical side of Caco-2 cells has been shown [16–18]. Local availability of the pigments in the intestinal tissue is increased and in situ effects appear to be more likely [17, 19]. However, during passage of the gastrointestinal tract (GIT) anthocyanins are not stable, as they are rapidly degraded to sugar, phenolic acids and aldehydes due to neutral pH conditions and activity of the gut microbiota [14].

A promising way to enhance the availability of intact anthocyanins for in situ effects in the lower intestine is colon-specific delivery. This technique serves to protect the active compound from degradation in the upper intestine, to transport it into the colon and release it there. Based on physiology and morphology of the human GIT, different approaches including pH-dependent, time- and pressure-controlled systems as well as biodegradable matrices have been developed to achieve a targeting of functional compounds. Microbiota-triggered drug release using biodegradable polymer matrices or coatings that resist digestion in the upper part of the GIT was found to be most effective with regard to target selectivity [20]. An approved biodegradable polymer for dietary colon targeting is cross-linked amidated pectin – a linear polysaccharide of partly amidated and methoxylated α-(1-4)-linked D-polygalacturonic acid residues [21]. Cross-linking of pectin is induced by divalent cations such as calcium. Due to electrostatic interactions between galacturonic acid residues and calcium ions as well as van der Waals contacts pectinate hydrogels are formed with ''shifted egg box'' configuration [22, 23]. In comparison to pectin they are characterized by increased hydrophobicity and decreased water solubility [24]. As amidated pectin is not metabolized by endogenous digestive enzymes, it is stable in saliva and gastric juice. In the terminal ileum, slight degradation is found due to microbial activity, but the main part of the polymer reaches the colon where it is completely degraded by the microbiota [25–27]. *Eubacterium eligens* and *Bacteroides thetaiotaomicron* being potential members of the microbiota able to degrade amidated pectin [28, 29]. The microbial degradation is not influenced by cross-linking [30]. In several studies, the potential of cross-linked amidated pectin as biodegradable colon drug carrier has been proven. Hence, Chambin et al. demonstrated colonic delivery of ketoprofen after encapsulation into amidated pectin beads [31] and Atyabi et al. have successfully prepared pectinate gel beads for delivery of macromolecules to the colon [32].

However, when amidated pectin is used for encapsulation of highly water-soluble active ingredients, e.g. anthocyanins, premature release due to diffusion of the active compound from the surface, pore formation and swelling is an obstacle [24, 33]. This problem can be solved by an additional protective coating that functions as a hydrophobic barrier in the upper GIT. An adequate hydrophobic coating material approved as a food additive is shellac. This natural, nontoxic, biodegradable, acidic polymer is obtained from the resinous secretion of the insect *Kerria lacca* [34]. Due to pK_a-values of 5.6–6.6 it is assumed to be stable in the stomach and to dissolve in the intestine [35]. Release properties of shellac coatings can be optimized by incorporation of pore formers such as, e.g. hydroxypropyl methylcellulose (HPMC). As a highly water soluble cellulose derivative HPMC is as well approved as a food additive [36].

The aim of the present work was to develop and evaluate the potential of shellac-coated anthocyanin-loaded amidated pectin beads as a dietary colonic delivery system. The effect of varying levels of the hydrophobic coating polymer was investigated. Furthermore, the incorporation of varying amounts of the pore former HPMC into the coating film was evaluated. In vitro and ex vivo release studies using simulated gastric fluid (SGF), ileostomy fluids (IFs) and colostomy fluids (CFs) were performed to mimic the human intestinal transit. The morphology of the prepared microspheres was characterized by scanning electron microscopy (SEM).

2 Materials and methods

2.1 Materials

Amidated pectin (AF 020; degree of esterification 29%, degree of amidation 20%) was purchased from Herbstreith & Fox KG (Neuenbürg, Germany). Bilberry extracts (Anthocyanin content 84%, 66%) were a gift from Kaden

Biochemicals (Hamburg, Germany). Calcium chloride was obtained from Acros Organics (Geel, Belgium) and glycerol from Riedel de Haën (Seelze, Germany).

Aqueous shellac solution (SSB® AQUAGOLD; 25% w/w shellac) and HPMC (Pharmacoat 606®) were provided by Synthapharm (Mülheim a. d. Ruhr, Germany). Talcum was purchased from Caesar & Loretz (Hilden, Germany).

Water for HPLC analysis was obtained from a Millipore™ water purification unit. Acetonitrile (HPLC gradient grade) was from Fisher Scientific (Dreieich, Germany), formic acid was purchased from Grüssing (Filsum, Germany). All chemicals for preparation of artificial gastric fluid and buffer solutions were of analytical grade and were purchased from Sigma-Aldrich (Schnelldorf, Germany). Methanol for extraction was obtained from Acros Organics and was redistilled before use. The standard 3,4,5-*trans*-trimethoxycinnamic acid was obtained from Sigma-Aldrich. HYDRANAL®-Composite 5 and HYDRANAL®-Methanol for Karl Fischer titration were purchased from Riedel de Haën.

2.2 Isolation of anthocyanin reference compounds

Anthocyanin reference compounds delphinidin-3-*O*-galactoside (dp3gal), delphinidin-3-*O*-glucoside (dp3glc), delphinidin-3-*O*-arabinoside (dp3ara), cyanidin-3-*O*-galactoside (cy3gae), cyanidin-3-*O*-glucoside (cy3glc), cyanidin-3-*O*-arabinoside (cy3ara), petunidin-3-*O*-galactoside (pt3gal), petunidin-3-*O*-glucoside (pt3glc), petunidin-3-*O*-arabinoside (pt3ara), peonidin-3-*O*-galactoside (pn3gal), peonidin-3-*O*-glucoside (pn3glc), peonidin-3-*O*-arabinoside (pn3ara), malvidin-3-*O*-galactoside (mv3gal), malvidin-3-*O*-glucoside (mv3glc) and malvidin-3-*O*-arabinoside (mv3ara) were isolated from aronia, bilberry and cranberry by preparative techniques. In brief, according to Kähkönen et al. bilberries were extracted twice with solvent consisting of water/methanol/formic acid 50:49:1 [37]. After centrifugation (1300 × *g*, 15 min) the supernatants were collected, solvent evaporated, lyophilized and the residue redissolved in 2% v/v formic acid in water. After lyophilization, commercial aronia concentrate and cranberry juice were directly solubilized in 2% v/v formic acid in water. These crude phenolic extracts were purified using Amberlite XAD-7 column chromatography [37]. The further fractionation was performed by preparative HPLC [7]. Identification of isolated anthocyanins was confirmed by retention times, UV-VIS spectra, MS and MS/MS data as well as information from literature [37–39].

2.3 Preparation of APB

Amidated pectin was dissolved in purified water at a concentration of 4% w/w. Glycerol as a plasticizer and bilberry extract were added at final concentrations of 10% w/w and 0.4% w/w under continuous stirring. The mixture was employed to prepare beads by laminar flow break-up technology using a laboratory scale microsphere production unit (Brace, Alzenau, Germany) consisting of pump, frequency generator, amplifier, vibration nozzle device, stroboscopic lamp and reaction tank. The experiments were performed at a flow rate of 6000 g/h. Vibration frequency was set at 125 Hz, amplitude was adjusted to 4.5, nozzle diameter was 1.5 mm. The droplets fell into the cross-linking solution (calcium chloride/isopropanol/water, 5:25:70 w/w/w) and instantly formed gel beads. These anthocyanin loaded pectin beads (APB) marked P4BG were recovered immediately, rinsed with water and gently dried in fluidized bed at 19°C.

2.4 Coating of APB

APB P4BG were hydrophobically coated with aqueous shellac solutions (coating solutions nos. 1–3; cf. bottom) using a fluidized bed coating apparatus (Wurster technique, bottom spray, GPCG 1, Glatt, Binzen, Germany). The coating solutions were prepared with commercially available aqueous shellac (SSB® AQUAGOLD; 25% w/w shellac). Coating solution no. 1 contained 10% glycerol as plasticizer and 10% talcum as anti-adhesive (w/w, based on shellac content). Using coating solution no. 1 three different coating levels were applied; the resulting materials were marked Sh8, Sh15 and Sh19 (Table 1). In addition to glycerol and talcum, coating solutions nos. 2 and 3 contained 5 and 15% of the pore former HPMC (w/w, based on shellac content), respec-

Table 1. Composition of shellac solutions applied for coating of APB

	Coating solution no. 1[a)]	Coating solution no. 2[b)]	Coating solution no. 3[c)]
Aqueous shellac (SSB® AQUAGOLD) (g)	450	450	450
Purified water (g)	450	450	450
HPMC (g)	–	5.6	16.9
Glycerol (g)	11.25	11.25	11.25
Talcum (g)	11.25	11.25	11.25

a) Preparation of Sh8, Sh15, Sh19 with varying levels of coating.
b) Preparation of HPMC5.
c) Preparation of HPMC15.

tively. Materials manufactured with coating solutions nos. 2 and 3 were assigned HPMC5 and HPMC15. The detailed composition of the coating solutions as well as the assignment of the coated materials is summarized in Table 1.

The coating solutions were homogenized by means of an ultra-turrax and stirred continuously during coating. The applied coating conditions were as follows: spray needle diameter 1.5 mm; air flow rate 96–99 m^3/h; inlet air temperature 50–52°C; product temperature 35–37°C; atomizing pressure 1.5 bar; spray rate 3–9 g/min. Batch sizes of 500 g were used for coating.

2.5 Characterization of APB

2.5.1 SEM

Beads were covered with platinum/palladium under vacuum by sputter technique (SCD 005, BAL-TEC, Schalksmühle, Germany). Examination of the bead surface was performed using a scanning electron microscope (Zeiss DSM 962, Carl Zeiss, Oberkochem, Germany) at 15 kV. The examinations were performed at two magnifications (50 × and 500 ×).

2.5.2 Determination of anthocyanin content

The anthocyanin content of the uncoated APB P4BG as well as the coated APB Sh8, Sh15, Sh19, HPMC5 and HPMC15 was determined after extraction. Briefly, APB were frozen in liquid nitrogen and ground in a mortar to a fine powder. An aliquot (42 mg) was transferred to a centrifuge tube and extracted with 2.5 mL 10% v/v formic acid in water under stirring and sonication (Transsonic T 460 Elma, Elma, Singen, Germany). The mixture was centrifuged at 2700 × *g* for 10 min (EBA-12, Hettich, Tuttlingen, Germany). The supernatant was collected. The extraction procedure was repeated four times with 2.5 mL of 5% v/v formic acid in methanol. All supernatants were combined, solvent removed by rotary evaporation and volume reconstituted to 5 mL with 10% v/v formic acid in water. After the sample was filtered through a disposable filter (polyvinyl polypyrrolidone, 0.45 µm, Roth, Karlsruhe, Germany) the anthocyanin content was assayed by RP-HPLC using an Alexys 100 LC-System (Antec Leyden, Zouterwoude, The Netherlands) consisting of two LC 100 pumps as well as an AS 100 autosampler. The system was equipped with a SPA-10A UV-VIS detector (Shimadzu Europe, Duisburg, Germany). Data acquisition and evaluation was performed with Alexys data system. A Hypersil ODS C18 column, 150 mm × 4.6 mm, with 5 µm particle size (Knauer, Berlin, Germany) was applied. The mobile phase consisted of (A) 10% v/v formic acid and (B) formic acid/water/acetonitrile 1:9:10. A gradient of 6% B at 0 min, 22% B at 40 min, 44% B at 60 min, 94% B from 63 to 68 min and 6% B from 71 to 80 min at a flow rate of 0.5 mL/min was applied. An injection volume of 100 µL was used. Anthocyanins were analyzed at 520 nm and quantified by means of calibration curves with anthocyanin reference compounds. Anthocyanin content refers to the sum of all single anthocyanin concentrations. All determinations were performed in duplicate.

2.5.3 Determination of water content

Water content in APB before and after coating was analyzed by means of volumetric Karl Fischer titration using a Karl Fischer apparatus (870 KF Titrino plus, Metrohm, Herisau, Switzerland). An exactly weighed sample of ground beads was suspended in HYDRANAL-Methanol® and titrated at room temperature using HYDRANAL-Composite5® as the Karl Fischer reagent. All determinations were performed in duplicate.

2.5.4 Determination of coating level

Coating level of the APB was calculated according to the following equation based on the differences in anthocyanin content (g/100 g) before (A_0) and after coating (A_C) as well as water content (g/100 g) before (W_0) and after coating (W_C):

$$\text{coating level } [\%] = [A_0(100 - W_C)/A_C] - 100 + W_0 \quad (1)$$

2.6 Drug release studies

Encapsulation properties of prepared APB were analyzed in an in vitro and ex vivo model system simulating the transit of human stomach, ileum and colon. Therefore, beads were incubated 3 h in SGF (pH 2.0), 4 h in IF (pH 6.3) and, finally, 15 h in CF (pH 6.2). IF and CF have been shown to represent a reliable model to mimic human intestinal metabolism [26, 40]. Incubation times were chosen according to transit times in the human GIT [41]. Applied pH conditions based on pH measurements of IFs and CFs as well as on literature data [42].

2.6.1 Subjects

IFs were provided by three healthy subjects (36–41 years old, all suffering from Crohn's disease without an involvement of the ileum) with a terminal ileostomy undergone colectomy 7–8 years prior to the study. CFs were provided by two healthy subjects (40 and 70 years old), both with left-sided colostomies after suffering from colon cancer or prestages undergone colectomy 15 and 32 years prior to the study. After removal of the affected part of the

colon no further symptoms for colon cancer were noticed. None of the patients was treated with antibiotics for the last 4 weeks.

2.6.2 Preparation of incubation media

2.6.2.1 SGF

The conditions in the stomach were simulated with SGF, pH 2.0, according to Gillatt et al. [43].

2.6.2.2. IF/CF

After removal, the ileostomy/colostomy bags were immediately placed in an anaerobic jar containing Anaerocult C® (Merck, Darmstadt, Germany) to create an anaerobic atmosphere. In the laboratory the jar was transferred into an anaerobic chamber (self-constructed) flushed with a N_2/CO_2 gas mixture (80:20 v/v). The IFs and CFs were pooled, respectively, diluted with the same volume of anaerobic carbonate-phosphate-buffer (pH 6.3 for IF, pH 6.2 for CF) according to Labib et al. [44], mixed with a glass rod and coarse particles were removed by filtration using glass wool. The filtrates were used as inoculi for drug release studies.

2.6.3 Incubation conditions

2.6.3.1 SGF and IF

An aliquot (252 mg) from each APB material (P4BG, Sh8, Sh15, Sh19, HPMC5 and HPMC15) was introduced in an incubation vessel containing 15 mL of SGF and incubated at 37°C under permanent stirring for 3 h. At the time points 0, 0.5, 1, 2 and 3 h samples (1 mL) of the supernatant medium were withdrawn. The samples were directly sonicated (Transsonic T 460 Elma, Elma), frozen in liquid nitrogen and lyophilized to stop the enzymatic reaction. After 3 h the SGF was replaced by 15 mL of IF. The incubation was continued for 4 h under the aforementioned conditions. At the time points 0, 0.5, 1, 2 and 4 h, samples (1 mL) of the supernatant medium were withdrawn and subsequently sonicated, frozen in liquid nitrogen and lyophilized. All experiments were performed in duplicate.

2.6.3.2 CF

For the materials Sh19, HPMC5 and HPMC15 incubation was continued in CF. Thus, after 4 h IF was removed. CF (15 mL) was introduced to the remaining APB. In order to create an anaerobic atmosphere, the incubation vessel was placed in an anaerobic jar containing Anaerocult C® (Merck). The incubation was continued for 15 h at 37°C under permanent stirring. Thereafter, the remaining APB were separated, immediately frozen in liquid nitrogen and lyophilized. All determinations were performed in duplicate.

2.6.4 Sample preparation

2.6.4.1 Samples after incubation in SGF/IF

The freeze-dried samples of the supernatant medium were extracted triply with 1 mL 5% v/v formic acid in methanol by vortexing and sonication for 5 min (Transsonic T 460 Elma, Elma). After centrifugation at 2700 × *g* for 10 min (EBA-12, Hettich), the supernatants were combined. The solvent was evaporated in nitrogen stream and the extract was dissolved in 1 mL of 10% v/v formic acid in water. The samples were filtered (polyvinyl polypyrrolidone, 0.45 µm, Roth) and standard 3,4,5-trimethoxycinnamic acid (1 mg/mL in methanol) was added to a final concentration of 50 µg/mL. Subsequently, the samples were subjected to HPLC analysis using an Agilent 1100 HPLC System (Waldbronn, Germany) consisting of HP 1100 gradient pump and HP 1100 photodiode array detector, equipped with a Wisp 710b autosampler (Waters, Eschborn, Germany). Data acquisition and evaluation were performed with HP ChemStation software. A Hypersil ODS C18 column, 150 mm × 4.6 mm, with 5 µm particle size (Knauer, Berlin, Germany) was applied. The mobile phase consisted of (A) 10% v/v formic acid in water and (B) acetonitrile. A gradient of 3% B at 0 min, 11% B at 40 min, 22% B at 60 min, 80% B from 65 to 70 min and 3% B from 75 to 80 min at a flow rate of 0.5 mL/min was applied. An injection volume of 25 µL was used. Anthocyanins and the standard 3,4,5-trimethoxycinnamic acid were analyzed at 520 and 280 nm, respectively.

2.6.4.2 Samples after incubation in CF

Lyophilized residues of APB were weighed, frozen in liquid nitrogen and ground in a mortar to a fine powder. Aliquots were transferred into a centrifugation tube and anthocyanin content was analyzed after extraction as described in Section 2.5.2. All experiments were performed in duplicate.

3 Results and discussion

3.1 Preparation and characterization of APB

APB were prepared by ionotropic gelation applying the laminar flow break-up technology based on the method of Hulst et al. [45]. A laminar jet of the anthocyanin-containing amidated pectin solution was pumped through a vibrating nozzle. Based on the overlay of the mechanical vibration the liquid broke up into uniform droplets. These droplets fell into the calcium chloride solution and instantaneously formed spherical hydrogel beads. The purple APB were dried gently in fluidized bed, whereas water content and bead size decreased sharply. To limit shrinkage and deformation of the pectin beads during the drying process, glycerol was included in the formulation as a plasticizer. SEM images (Fig. 1A and B) showed that dried APB P4BG had a near spherical shape with single indentations and a rough surface. Mean diameter of the dried beads was

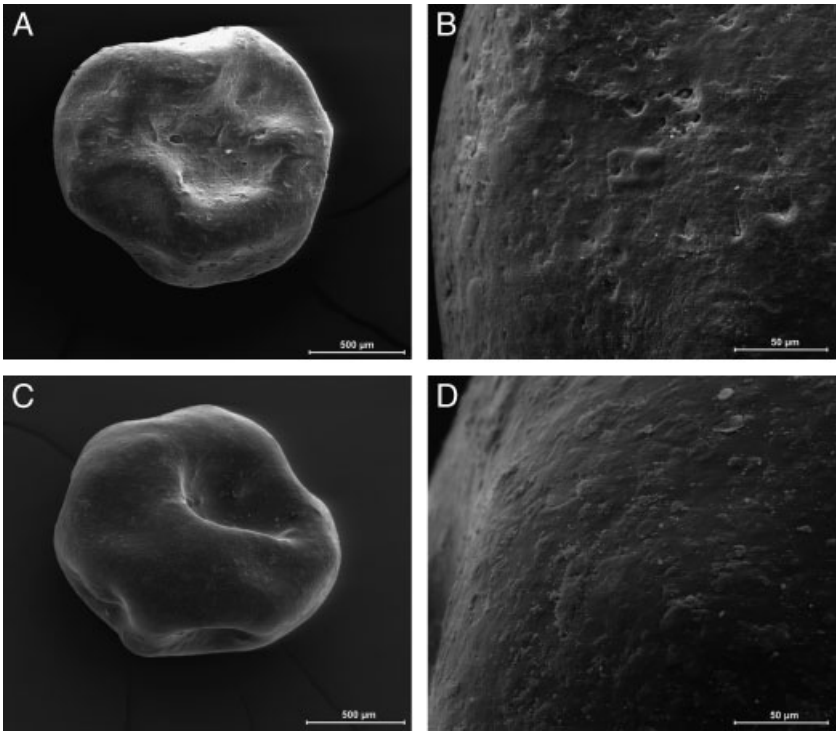

Figure 1. SEM picture of uncoated APB P4BG (A and B) and APB Sh19 coated with aqueous shellac (coating level 19%) (C and D).

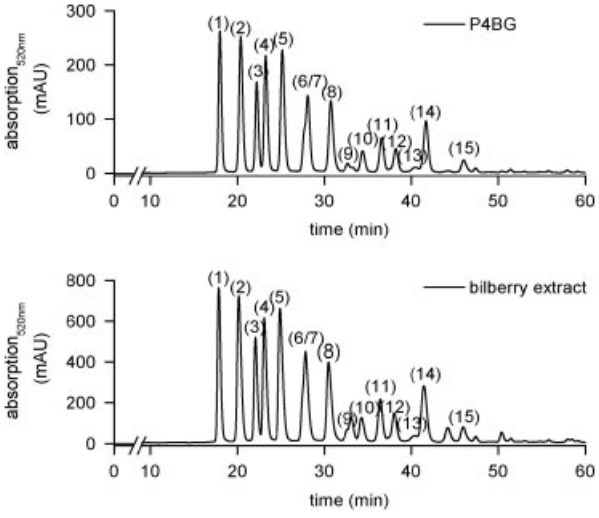

Figure 2. HPLC-chromatograms at 520 nm of the anthocyanin profiles of bilberry extract as well as uncoated APB P4BG after extraction. Peak identification: 1 dp3gal, 2 dp3glc, 3 cy3gal, 4 dp3ara, 5 cy3glc, 6 pt3gal, 7 cy3ara, 8 pt3glc, 9 pn3gal, 10 pt3ara, 11 pn3glc, 12 mv3gal, 13 pn3ara, 14 mv3glc, 15 mv3ara.

1.6 mm. Identification and quantification of anthocyanins incorporated was performed by HPLC-VIS using anthocyanin reference substances. In Fig. 2 the anthocyanin profiles of bilberry extract as well as APB P4BG after extraction are shown. As in the extract used for preparation, the 15 anthocyanins characteristic for bilberries are enclosed in the beads. Thus, encapsulation was successful and did not change the anthocyanin composition. Depending on the anthocyanin content of the used bilberry extracts, anthocyanin loading of the two batches of APB prepared was found 2.62 ± 0.05 g/100 g and 1.67 ± 0.01 g/100 g, respectively. This rather low anthocyanin concentration results from the high water solubility of the pigments that partly migrate into the cross-linking solution during preparation of APB. Low encapsulation efficiency around 50% has also been reported for immobilization of other soluble substances, e.g. theophylline and metronidazole, in pectin matrices [33, 46].

Until now research on the encapsulation of anthocyanins to enhance their stability and potentially provide controlled release of the bioactive pigments in the lower parts of the GIT is scarce. Cross-linked amidated pectin as a well studied encapsulation material for pharmaceutical drug delivery is, to the best of our knowledge, not applied for formulation of anthocyanins before. Instead, Frank and Schuchmann used submicron emulsion drops for encapsulation of blueberry extract [47]. In two other studies, anthocyanin extracts were incorporated in glucan as well as chitosan-sodium alginate gel [48, 49]. Anthocyanin release from chitosan-sodium alginate into SGF can be reduced by varying the ratio of the two polymers [49]. Further data on release characteristics of these targeting systems are not available.

3.2 Coating of APB

As unmodified beads of cross-linked amidated pectin P4BG lacked sufficient resistance against premature release

of highly water soluble anthocyanins, an additional hydrophobic coating was necessary. Thus, the APB were effectively coated by Wurster technique with aqueous shellac solution (coating solution no. 1) containing glycerol as plasticizer and talcum as anti-adhesive. Aqueous shellac solution, prepared by dissolution in the presence of alkali salts, was chosen for coating due to the storage stability of the films formed. In a study using paracetamol pellets, it was shown that after coating with ethanol shellac solution enteric resistance decreased sharply during 1 year of storage at 25°C and 60% rel. humidity. Pellets coated with aqueous alkaline shellac, however, did not show any change in release profile. Due to alkaline salt formation, reduction of free carboxylic residues as a result of inter- and intramolecular esterification is avoided [50, 51].

To test the influence on drug release, varying coating levels of 8, 15 and 19% were applied and calculated based on differences in water and anthocyanin content before and after coating. Prepared materials were marked Sh8 (coating level 8%), Sh15 (coating level 15%) and Sh19 (coating level 19%). The coating process of APB P4BG with aqueous shellac solution yielded glossy, colorless film coverings. As an example, Figs. 1C and D show SEM pictures of material Sh19. Film formation from single drops of coating suspension can be recognized.

Because of pK_a-values of 5.6–6.6 determined for commercially available shellac [35], the coatings should dissolve in the intestine due to the pH conditions in this part of the GIT. However, the data of some studies revealed that the polymer degrades too slowly in intestinal fluids resulting in a too low concentration of free drug. One possibility to modify the dissolution profile of shellac films is given by the incorporation of water-soluble pore formers such as HPMC [52–54]. To investigate the influence of pore former on the release characteristics, APB P4BG were coated by Wurster technique with aqueous shellac solutions (coating solutions nos. 2 and 3) containing 5 and 15% HPMC (w/w, based on shellac content), respectively. As for coating solution no. 1, glycerol and talcum were included in the coating dispersion as plasticizer and anti-adhesive, respectively. Prepared materials were marked HPMC5 as well as HPMC15. Coating levels of 16% (HPMC5) and 19% (HPMC15) were calculated. In contrast to pure shellac coatings, the shellac/HPMC films are characterized by the occurrence of round pores, resulting from HPMC embedded in the shellac matrix (Fig. 3). This typical structure of surface was also described for cellulose pellets coated with shellac/HPMC [54].

3.3 Drug release

Drug release studies are usually performed in buffer solutions at varying pH values to simulate the different parts of the GIT. Using simulated colonic fluid with 4% rat cecal content, Ravi et al. showed that in comparison to single buffer systems drug release is significantly increased when intestinal bacteria are available in the release model [55]. Especially for targeting systems where the drug release is

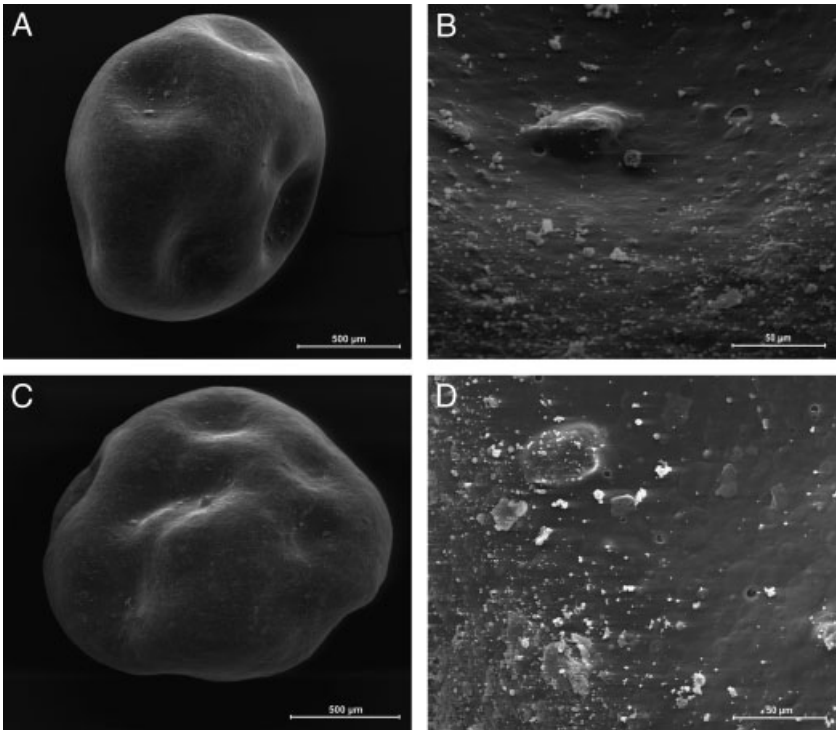

Figure 3. SEM picture of APB HPMC5 (A and B) and HPMC15 (C and D) coated with aqueous shellac solution containing 5 and 15% w/w HPMC (based on shellac), respectively.

triggered by the activity of colonic microbiota, sole buffer solutions are inappropriate. In the in vitro and ex vivo model system applied here for studying release properties, the effect of the enzymatic activity of human intestinal microbiota on the formulations' degradation is checked using IF and CF. These media have previously been shown to represent a reliable model to mimic human intestinal metabolism [26, 40]. Thus, to simulate the transit of human stomach, ileum and colon APB were incubated 3 h in SGF (pH 2.0), 4 h in IF (pH 6.3) and, finally, 15 h in CF (pH 6.2).

Due to neutral pH and microbial degradation, anthocyanins are not stable in IF and CF [14]. Degradation rates of the individual pigments differ and are influenced by hydroxylation and methylation pattern of the aglycon as well as the type of the conjugated sugar moiety. Mv3gal is known to be one of the most stable anthocyanins in IF. Kahle et al. determined 54.4% of the ingested mv3gal in IFs 0–8 h after patients consumed blueberries [56]. About 60% of mv3gal were recovered after 4 h of incubation in IF [57]. Thus, mv3gal was chosen as a marker of anthocyanin release from APB. Products from anthocyanin degradation, e.g. hydroxylated benzoic acids, were not determined in the incubation solutions.

3.3.1 Drug release from shellac-coated APB

In Fig. 4, the cumulative release of mv3gal from unmodified APB P4BG and APB coated with varying levels of shellac in SGF and IF as well as CF is shown as a function of time. From the uncoated APB P4BG 75% of mv3gal was already released in the simulated gastric environment. Due to dissolution of highly soluble anthocyanins from the surface of the formulation, water migrates into the matrix. This process creates pores and channels provoking swelling and enhancement of drug release [24]. Thus, the gastric resistance of the unmodified APB P4BG is insufficient. For colon drug delivery Maestrelli et al. prepared calcium cross-linked microspheres of amidated pectin loaded with theophylline. Similar to anthocyanins theophylline is highly acid soluble. In accordance with our results, incubation of theophylline-loaded pectin bead in SGF resulted in the fast release of the incorporated compound [33].

Coating of APB with 8% aqueous shellac as a hydrophobic barrier did not slow down the anthocyanin release. By increasing the coating level to 15 and 19%, the dissolution of anthocyanins in SGF was reduced to 35 and 18%, respectively, demonstrating that film coat thickness is inversely proportional to the release rate. In IF, anthocyanin release for uncoated APB P4BG and Sh8 reached 80 and 85%, respectively. For APB Sh15 and Sh19, decreased anthocyanin release in IF was determined resulting in a release rate in SGF and IF of mv3gal of 46 and 22%, respectively. Even after considering the anthocyanins' instability in IF, the results clearly show that the majority of the pigments remained in the formulations and are transferred into the simulated colon. As the formulation Sh19 showed the lowest anthocyanin release in SGF and IF beads were subsequently subjected to anaerobic incubation in CF. After 15 h of anaerobic incubation in CF, coated APB were widely degraded. Based on the anthocyanin content of the remaining beads, a cumulative release of $88 \pm 13\%$ of mv3gal was calculated (Fig. 4). This indicates that dissolution of the shellac coat as well as bacterial metabolization of the pectin matrix occurred and a targeting of the active compound to the simulated colon was achieved. The observed large range in pigment release is ascribed to differences in the shellac film surface of single beads causing differences in release characteristics [58].

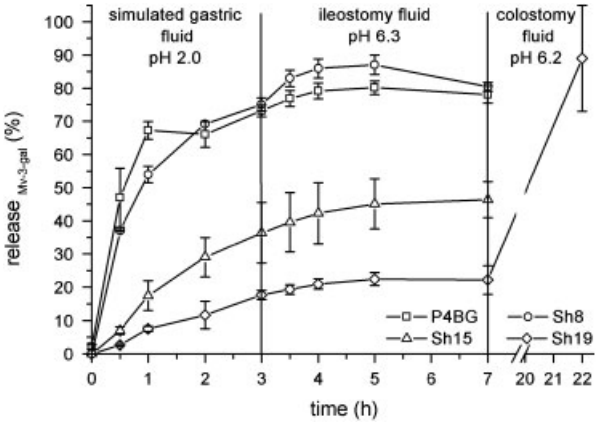

Figure 4. Representative release profile of mv3gal from uncoated APB P4BG and shellac coated APB Sh8 (coating level 8%), Sh15 (coating level 15%) and Sh19 (coating level 19%) in SGF, IF and CF. (Mean ± SD, $n = 2$).

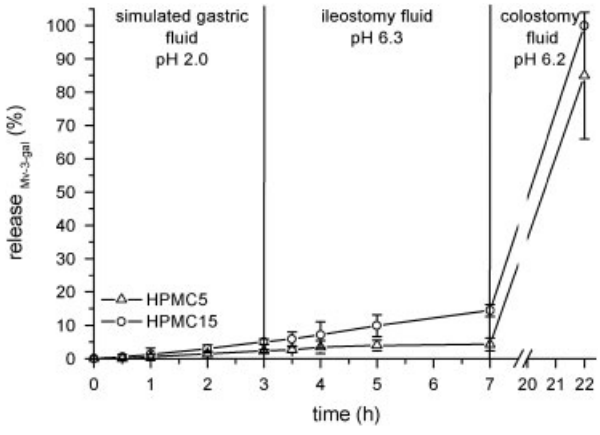

Figure 5. Representative release profile of mv3gal from APB HPMC5 and HPMC15 coated with aqueous shellac solution containing 5 and 15% w/w HPMC (based on shellac), respectively in SGF, IF and CF. (Mean ± SD, $n = 2$).

3.3.2 Drug release from APB coated with shellac containing 5% HPMC (HPMC5)

Figure 5 displays the cumulative release in SGF and IF as well as CF of mv3gal from material HPMC5 representing APB P4BG coated with shellac containing 5% (w/w, based on shellac) of the pore former HPMC. As indicated by a release rate of mv3gal below 5%, resistance in SGF is very high. In IF, anthocyanin release from formulation HPMC5 is very low as well. After adjacent incubation in CF material HPMC5 was widely degraded. From anthocyanin content of the remaining beads a cumulative release of mv3gal of $86 \pm 16\%$ was calculated. Thus, targeting of the active compound to the simulated colon was achieved as well with formulation HPMC5.

Comparison of the above-mentioned results with the incubation of sole shellac-coated APB Sh19 (Fig. 4) indicates that the ratio of pore former of 5% (w/w, based on shellac) is inadequate to achieve enlarged porosity and an increase in drug diffusivity from the shellac film. In contrast, an even higher resistance in SGF and IF and similar cumulative release was found. The improved gastric and ileal resistance does not result from the thickness of the hydrophobic film, as the formulation exhibits a similar coating level as APB Sh15. Instead, Qussi and Suess showed that addition of HPMC to shellac films increased their flexibility [59]. Thus, the plasticizer properties of HPMC leading to more stable coatings with lesser defects and intact surface may be causative.

3.3.3 Drug release from APB coated with shellac containing 15% HPMC (HPMC15)

Similar to HPMC5, the release profile in SGF, IF and CF of mv3gal from material HPMC15, APB P4BG coated with shellac containing 15% HPMC (w/w, based on shellac) is characterized by a high enteric resistance indicated by a release rate of mv3gal below 5% (Fig. 5). Compared to material HPMC5, an accelerated release in IF was found. Furthermore, after additional anaerobic incubation in CF material, HPMC15 was completely degraded and anthocyanins were fully released (cumulative release of 100%). Using HPMC15, targeting to the simulated colon, homogenous and complete release of the incorporated anthocyanins was achieved. Hence, by incorporation of 15% (w/w, based on shellac) of the pore former HPMC, the dissolution profile of the shellac film in SGF as well as in IF and CF was modified. Besides the increased gastric resistance stated above, diffusivity of the active compounds in IF as well as degradation of the whole formulation in the colon was accelerated. Dissolving the polysaccharide creates additional channels in the shellac film that accelerate water migration, release of active compound and degradation of the formulation [52–54]. Qussi and Suess. have found that porosity of a shellac coating increased from 4.7 to 8.7% by incorporation of 15% HPMC (w/w based on shellac) [54].

4 Concluding remarks

To summarize, shellac and shellac/HPMC-coated APB as dietary colon targeting systems were successfully prepared by ionotropic gelation and fluid bed Wurster coating with aqueous shellac solution. Release characteristics, studied in vitro and ex vivo using SGF, IF and CF revealed a retardation of anthocyanins during simulated passage of stomach and ileum as well as the desired release of pigments in the colon. Coating level was identified as an important parameter. By addition of 5 or 15% of the water-soluble polysaccharide HPMC to the shellac film, resistance in SGF was increased due to the plasticizer properties of the polymer. Incorporation of 15% HPMC (w/w based on shellac) into the shellac film additionally led to increased anthocyanin diffusivity and complete release as well as degradation of the formulation in CF. Further research is necessary to evaluate release characteristics of the prepared formulations in vivo as well as the potential of anthocyanin colon targeting systems in prevention and therapy of disorders of the colon.

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