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ORIGINAL ARTICLE

Preparation and Evaluation of a Bio-Erodible Bio-Adhesive Drug Delivery System Designed for Intraoral Extended Release of Flurbiprofen: In Vitro and In Vivo Assessments

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Abstract

Purpose Site-specific drug delivery for the local treatment of the diseases in the oral cavity is far superior to the systemic administration, for both safety and efficacy reasons. This study presents the design and development of an innovative bio-adhesive delivery system (BDS) based on a bio-erodible semi-interpenetrating polymeric network (semi-IPN) for intraoral extended release of flurbiprofen (FBP).

Method A unique combination of hydrolyzed gelatin (HG) cross-linked by tannic acid (TA) and Eudragit S, as the linear polymer, was used to compose the structure of the semi-IPN. The effect of structural factors, associated with the composition of the semi-IPN, on the release profile of FBP from the system was assessed using an in vitro dissolution method. The release rate of the drug in the oral cavity of healthy volunteers was also studied.

Results The degree of cross-linking of HG and the weight ratio between the cross-linked polymer and the linear polymer were found to be crucial factors affecting the release profile of FBP from the system. Likewise, the presence of a high concentration of plasticizer was essential for achieving a proper flexibility of the system as proved by a DSC method. The in vivo tests demonstrated a disparity between the volunteers in the FBP concentration found in the saliva, especially after 120 min post-administration of BDS. Both f1 and f2 tests indicated that the packaged BDS can be absolutely stable for at least 6 months at 30 °C.

Keywords Site-specific drug delivery · Semi-interpenetrating polymeric network (semi-IPN) · Topical treatment · Dissolution test · Hydrolyzed gelatin · Tannic acid · Eudragit S

Introduction

Intraoral delivery is generally considered as a preferred route for drug administration especially because of its convenience, accessibility, and the ease of implementation. Moreover, the intraoral drug delivery systems are self-administrable and can be removed to cease the drug delivery, if necessary, which increases the patient compliance even more [1]. The intraoral delivery for local treatment of diseases provides unique therapeutic advantages over the conventional systemic administration. Particularly, the lower dose, resulting in the fewer side effects, and high efficacy are the major benefits of such a route of administration for the local treatment of the diseases [2, 3]. The example of intraoral diseases, which can be treated locally, includes bacterial and fungal infections [4], aphthous [5], oral cancer [6], chemotherapy treatment-induced oral mucositis [7], ulcerative and vesiculobullous diseases of the oral mucosa [8], stomatitis [9], toothaches [10], periodontal and gingival diseases [11], xerostomia [12], hypersalivation [13], and sore throat [14].

The intraoral delivery systems are generally categorized based on the site in the oral cavity, where they are placed for the release action, including buccal (cheeks), sublingual, periodontal pocket and gingival (gums) [15].

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To date, a variety of intraoral delivery systems have been designed and developed including mucoadhesive tablets, films, patches, strips, lozenges, chewing gums, gels, paste, etc. [16–19].

Fast-dissolving delivery systems such as both fast-dissolving tablets (or orally disintegrating tablet—ODT) and films [20, 21], semisolid and liquid dosage forms present poor retention in the oral cavity, thus leading to a suboptimal therapeutic effect for the topical treatment [3].

The mucoadhesive drug delivery systems such as buccal or sublingual tablets, providing an extended retention time, are primarily intended for achieving the systemic effect [15]. Such mucoadhesive drug delivery systems are also generally hard and have a certain thickness creating a "foreign" feeling, so that patients are aware of their presence. As a result, these systems are often dislodged by the patient mostly due to the spontaneous movements of the tongue. Mucosal patches, which demonstrate good adhesion for extended periods of time, cause adverse reactions such as mucosal itching and irritation, or even necrosis in severe cases, at the patch sites [22, 23]. Thus, these preparations are also not completely satisfactory as a sustained- or controlled-release preparation for local treatment in the oral cavity.

These limitations, therefore, provide a challenge for designing new intraoral-specific drug delivery systems intended for prolonged drug administration.

In the present study, the attempt was made to develop a new concept of delivery system for intraoral extended release of active ingredients. Accordingly, a semi-interpenetrating polymer network (semi-IPN) constituting the underlying structure of this new delivery system was especially designed for this purpose.

Generally, an interpenetrating polymer network (IPN) is a heterogeneous polymer which is based on a combination of two or more networks which are interlocked but not covalently bonded to each other. In recent years, IPN systems have been used as potential carriers for drug delivery [24, 25]. A semi-IPN, on the other hand, is a combination of a cross-linked polymer and a linear (uncross-linked) polymer, which are also not covalently bonded to each other [26].

Tannic acid (TA) can react with gelatin to create a cross-linked network especially due to the formation of hydrogen bonds [27] as demonstrated in Fig. 1a. As a consequence, the gelatin solubility is considerably decelerated. In the present study, a combination of hydrolyzed gelatin (HG) cross-linked by TA, as the cross-linked polymer, and Eudragit® S 100 (Eud.S), which is a copolymer of methacrylic acid and methyl methacrylate (1:2) (Fig. 1b), as the linear polymer, was utilized to constitute the structure of the semi-IPN. Figure 1c schematically illustrates the structure of the semi-IPN in which the active material has been entrapped. The main function of the cross-linked polymer is the entrapment of the drug and the provision of a long-lasting release. Additionally, the cross-linked HG presents bio-erosion characteristics contributing to the effect of the controlled release mechanism. On the other hand, the linear polymer is responsible for preventing the burst effect of the release, especially in the first periods post-administration. This linear polymer, as an anionic polymer having a limited solubility at a pH below 7, may also inhibit the dissolution and erosion of the cross-linked polymer, thus providing additional means for the regulation of the release.

Since in such a system both processes of the cross-linking and the blending with linear polymer occur concurrently and two polymers are combined together in a molecular level while entrapping the active material, the drug is distributed within the system uniformly. As a consequence, the main advantage of this approach is expected to be an accurate drug content and a high uniformity of content between the dosage units.

Additionally, the system was designed to adhere onto a tooth to release the drug into the oral cavity, while undergoing a bio-erosion, to eliminate the possibility of any adverse effects which could arise upon the presence of the system.

The bio-adhesive delivery system (BDS) according to the present study, was primarily designed and developed for a controlled release of flurbiprofen (FBP), as the active ingredient, in the oral cavity. FBP is a non-steroidal anti-inflammatory drug (NSAID) with distinct analgesic and antipyretic activities [28]. FBP has been shown to be effective in reducing the severity and/or shortening of the duration of the chemotherapy-induced oral mucositis [29], the treatment of sore throat [30] and periodontitis [31].

This study aimed to explore the effect of different structural factors such as the cross-linking degree of the HG, the ratio between the cross-linked and the linear polymers in the semi-IP structure and the presence of plasticizer and solubilizer, on both the FBP release profile and the physical properties of the BDS.

Materials and Methods

Materials

Hydrolyzed gelatin (HG), tannic acid (TA), and Eudragit S 100 (Eud.S) (methacrylic acid–methyl methacrylate copolymer, 1:2), used for the preparation of the semi-IPN structure, were respectively purchased from Croda (Snaith, UK), Merck Millipore (MA, USA), and Evonik Industries (Essen, Germany). Triethyl citrate (TEC), used as the plasticizer, and Polysorbate 80 (Tween 80), used as the solubilizer, were respectively purchased from Kraemer & Martin GmbH (Sankt Augustin, Germany) and Sigma-

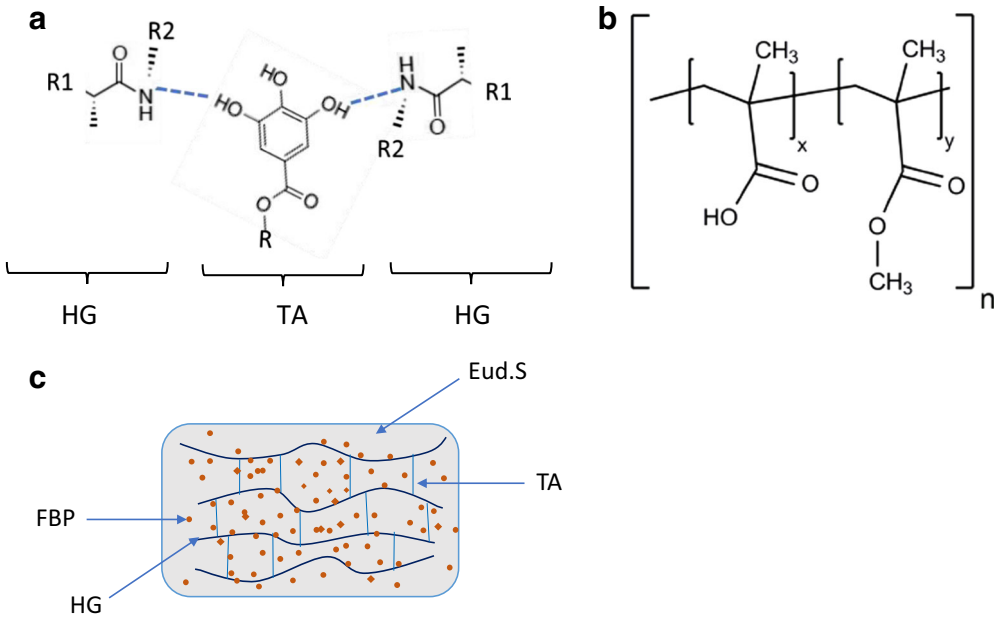

Fig. 1 **a** Hydrolyzed gelatin (HG) cross-linked by tannic acid (TA). **b** The chemical structure of Eudragit S 100 (Eud.S). **c** A schematic illustration of the bio-adhesive delivery system (BDS) based on a semi-IPN comprising a combination of a cross-linked polymer and a linear polymer, entrapping the active material (FBP)

Aldrich (MO, USA). Flurbiprofen (FBP) was supplied by Knoll AG/Abbott (IL, USA). Ethyl alcohol, a USP grade, was purchased from Merck Millipore (MA, USA). Purified water was USP grade.

Acetate buffer 50 mM (pH = 6) for HPLC analysis was prepared by dissolving 3.85 g ammonium acetate (analytical grade) (Merck Millipore, MA, USA) in 1000 ml purified water and adjusting the pH to 6.0 ± 0.1 using an analytical-grade glacial acetic acid (Merck Millipore, MA, USA). Phosphate buffer solution (PBS) for the in vitro dissolution test was prepared by first dissolving 110.2 g potassium dihydrogen phosphate (Sigma-Aldrich, Mo, USA) in 10 l purified water (USP) and then adding 150 g disodium hydrogen phosphate dihydrate ($Na_2HPO_42H_2O$) (analytical grade) (Sigma-Aldrich, Mo, USA) and lastly adjusting the pH to either 6.8 ± 0.1 or 7.5 ± 0.1 with sodium hydroxide solution 0.1 N (analytical grade) (Sigma-Aldrich, MO, USA). The diluting solution, for the preparation of either samples or standards for the HPLC analysis, was prepared by mixing acetonitrile (HPLC grade; Sigma-Aldrich, MO, USA) and acetate buffer (pH = 6) with the ratio of 70:30 *v*/*v*, respectively.

Preparation of the BDS

Different compositions of the BDS were formulated, as listed in Table 1, according to the weight ratio between HG and the cross-linker (TA), the weight ratio between the cross-linked HG (HG + TA) and the linear polymer (Eud.S), and the amount of the solubilizer used in the formulation. These ratios are respectively used to denote the formulation of the designed BDS prepared in this study. A denotation of 4-3-1.1, for instance, indicates a BDS formulation containing a weight ratio of 4 between the cross-linked HG and Eud.S (cross-linked HG/Eud.S), a weight ratio of 3 between HG and TA (HG/TA), and 1.1% (*w*/*w*) Polysorbate (solubilizer).

The BDS containing 15 mg FBP was prepared using two different steps as follows:

1. The preparation of semi-IPN structure; where the HG was in situ cross-linked by tannic acid while physically becoming intertwined and entangled with the linear polymer (Eud.S). At this step, FBP, plasticizer, and optionally solubilizer were also added to the composition of the semi-IPN to entrap the drug and adjust the physical and mechanical properties of the BDS. This step was performed in a Z-mixer (Model Sigma-15; Srugo, Israel) containing double Z arms. The Z-mixer was also equipped with a jacket heating/cooling unit (Model IC30W-H; Huber, Germany) and a condenser cooling unit (Model ICO12; Huber, Germany).

First, HG (2 kg) solution in purified water (1.8 kg) was prepared using a mechanical mixer (Romix, Israel) at 100–300 rpm which gradually increased to 600–800 rpm. After the total dissolution of HG and attainment of a clear solution, triethyl citrate (TEC) (1.690 kg) was added, while mixing, and mixed for about 15 min at the same mixing speed. Thereafter, FBP (1.040 kg) was added while mixing, and the mixing continued at the same speed for an additional 15 min. Where a solubilizer was needed, it was added after TEC at the same mixing speed and mixed also for 15 min prior to the addition of FBP.

The TA and Eud.S solutions, each one separately, were also prepared. TA (1 kg) was dissolved in purified water (1 kg) by mixing, using the mechanical mixer, at 100–300 rpm which gradually increased to 600–800 rpm. The mixing continued at the same speed for an additional 45 min. Eud.S (0.75 kg)

Table 1 Different BDS formulations, based on a semi-IPN comprising TA cross-linked HG and Eud.S, containing 15 mg FBP

Cross-linked HG/Eud.S–HG/TA–polysorbate										
Formulation	4–2–0	4–2.4–0	4–3–0	4–5–0	4–2–2.2	4–2.4–1.1	4–3–1.1	4.6–3–1	7.1–3–0.5	9.7–3–1.1
Ingredients						%				
HG	29.6	31.4	33.3	37.0	28.8	31.4	33.3	34.5	37.5	37.7
TA	14.8	12.9	11.0	7.3	14.4	12.9	11.1	11.5	12.5	12.6
FBP	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Eud.S	11.1	11.1	11.1	11.1	10.8	11.1	11.1	10.0	7.0	5.2
TEC	29.6	29.6	29.6	29.6	28.8	28.5	28.5	28.0	27.5	28.4
Polysorbate	0	0	0	0	2.2	1.1	1.1	1.0	0.5	1.1

HG hydrolyzed gelatin, *TA* tannic acid, *FBP* flurbiprofen, *Eud.S* Eudragit S 100, *TEC* triethyl citrate

solution was prepared in ethanol (2.250 kg) using the mechanical mixer, at 100–300 rpm, which gradually increased to 600–800 rpm. The mixing continued at the same speed for an additional 30 min. Thereafter, TEC (0.452 kg) was added into the Eud.S solution while mixing at 600–800 rpm which continued for about 15 min.

Next, both HG and the cross-linker (TA) solutions were transferred into the Z-mixer, which was set ahead at 25–30 °C, and mixed for 30 min. The temperature was then raised to 50 °C to evaporate the water under a reduced pressure (90–200 mbar) until a water content of less than 5%, which was measured by a loss-on-dry method, was achieved. At the end of this process, a dough-like composition was obtained. Thereafter, the solution of Eud.S was added and the dough was kneaded while drying under reduced pressure (90–160 mbar) until a residual solvent of less than 10% was obtained. The resulting dough was then discharged onto a polytetrafluoroethylene sheet and kept at room temperature for the dosing process (step 2).

2. The dosing process; where a continuous process including extrusion, cooling and cutting was consecutively used. First, the resulting dough was preheated at 60–65 °C for 2–3 h and subsequently extruded at 55–65 °C, according to the formulation type, using an extruder (Model CE 40/1; Proform, France). The resulting extrudant, which left the extruder as a long and thin strip with a width and a thickness of 5 and 3 mm, respectively, was directly placed on a conveyor belt that moved into a cooling tunnel (Model CB0807/2.5; Proform, France) to cool down to 5–10 °C. The cooled strip was then cut to the BDS units of 100 mg with the dimensions of 7/5/3 mm (length/width/thickness) using a pre-calibrated cutting machine (Model CCU2–80/250; Proform, France).

The resulting BDS units were then packaged in either polyvinyl chloride/polyvinyl dichloride/aluminum foil (PVC-PVDC) or in an aluminum/aluminum (Alu/Alu) blister using a packaging machine (Model EZ; Sepha, Ireland) and kept at either room temperature for further tests or other temperatures for the stability study as detailed below.

In Vitro Dissolution Study

The in vitro dissolution study of FBP-containing BDS was carried out using a Vankel dissolution tester (Vankel Technology Group Inc., NC, USA) which consisted of a dissolution bath (Vankel VK 7000/8) equipped with a monitoring system fitted with the paddles set, a heater/circulation system (Vankel VK 650A) set at 37 °C, a peristaltic pump (Vankel VK 810), an autosampler (Vankel VK 8000), and 1000-ml standard dissolution cells.

One unit of BDS was placed in a dissolution cell which contained 450 ml of PBS, pH = 6.8 or 7.5, which was preheated to 37 °C, and stirred by the paddle at 75 rpm. Three-milliliter samples were collected at predetermined time intervals for HPLC analysis and replaced with an equal volume of fresh dissolution medium each time.

The concentration of FBP in the dissolution samples was determined using a Waters LC Module 1 Integrated HPLC System (Waters, MA, USA), equipped with a UV detection and supported by a Millenium 2010 controller and integration software. The chromatographic separation was achieved by a Shendon BDS C18, 5 μm, 4.6 mm × 250 mm column (Phenomenex, California, USA) maintained at 27 °C. A mixture of acetonitrile (200 ml), methanol (250 ml), and acetate buffer (pH = 6) (550 ml) was used as the mobile phase which was filtered, through a 0.45-μm nylon membrane, and degassed prior to use.

The samples taken from the dissolution cells were first filtered through a 0.45-μm syringe filter prior to the injection into the HPLC system. The injection volume was fixed at 10 μl and the isocratic flow rate was 1.0 ml/min. The detection of FBP was carried out at λ_{max} = 247 nm. The run time was 18 min where FBP eluted, under these conditions, at 5.6 ± 0.8 min.

The actual amount of FBP in the sample was quantified using a calibration curve prepared by a serial dilution of a

primary FBP solution, which was previously prepared in the diluting solution, to yield a concentration range of 2–30 μg/ml (ppm).

The method was validated according to the International Conference on Harmonization (ICH) guidelines [32].

In Vivo Release Study

In vivo release studies were performed by applying the BDS to five healthy volunteers upon the volunteers' written consent. The clinical protocol was approved by an independent Ethics Committee in accordance with the ethical standards of the responsible committee on human experimentation (regional) and with the Helsinki ethical principles for medical research involving human subjects. Volunteers were instructed to stick the BDS onto the upper right canine (cuspid) tooth and hold lightly for 10 s without moistening it before the application. Volunteers were not restricted to any diet regimen before the study but they were requested to avoid consumption of water or food during the half-hour before the study. Only drinking was allowed ad libitum throughout the study but only 30 min after administration of the BDS, and also no drinking was allowed 10 min before the collection of salivary samples. Likewise, special care was taken to ensure that the tongue did not contact the BDS throughout the study to avoid abnormally mechanical erosion which could affect the release rate of FBP from the system. The adhesion retention time, disintegration and loss of fragments, and possible irritation were entirely monitored and recorded. Samples of saliva were collected at predetermined time intervals up to 240 min. The concentration of FBP in the saliva samples was determined by means of an HPLC method as set forth below.

First, the saliva proteins were precipitated by adding acetonitrile (300 μl) to 300 μl of sample. The sample was then mixed using a vortex for a few seconds and centrifugated at 5000 rpm for 10 min. The resulting supernatant was then injected into the HPLC system where a Hypersil BDS C18, 5 μm, 4.6 mm × 250 mm column (with a Hypersil C-18 guard column) was used for the chromatographic separation (Thermo Scientific, Massachusetts, USA). The concentration of FBP in the saliva was determined by a calibration curve prepared by spiking 50 μl of a series of FBP standard solutions (prepared in the diluting solution), ranging from 10 to 1050 μg/ml (ppm), in 450 μl human saliva and mixing using a vortex, for a few seconds, to yield standards in saliva in the range of about 1–105 ppm. Next, to each standard in saliva, acetonitrile (500 μl) was added and mixed using a vortex for a few seconds and lastly centrifugated at 5000 rpm for 10 min. The supernatant so obtained was finally injected into the HPLC system to obtain the calibration curve. The chromatography analysis was performed under conditions described earlier above.

FBP Residues in BDS

The residual FBP left in the in BDS after an application in the oral cavity of volunteers was determined—first, by dissolving the unite of BDS in the diluting solution (about 25 ml) in a 50-ml volumetric flask and diluting to the volume (50 ml) after the BDS completely dissolved. A sample was then taken and injected into the HPLC system after filtration through a 0.45-μm syringe filter.

Stability Study

Samples of the formulation of 4–2–0 (Table 1) were first packaged in either PVC-PVDC blister which was kept in individual sealed plastic vials including silica gel (PVC-PVDC/+SG) or in an Alu/Alu blister. The samples were then concomitantly kept at 25 ± 2 °C/60% RH ± 5% RH, 30 ± 2 °C/65% RH ± 5% RH, and 40 ± 2 °C/75% RH ± 5% RH for the stability study. Samples were then taken at time points of 1, 2, 3, and 6 months for a dissolution test as compared to the reference which was a freshly produced batch. In order to examine the dissolution profile comparison between the reference and the samples, two factors f_1 (the difference factor) and f_2 (the similarity factor) were used according to the following calculations [33, 34]:

$$f_1 = \left[\left(\sum_{t=1}^{n}|R_t - T_t|\right) \Big/ \left(\sum_{t=1}^{n} R_t\right)\right] \times 100$$

$$f_2 = 50 \times \log\left\{\left[1 + \left(\frac{1}{n}\right)\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} \times 100\right\}$$

where R_t and T_t are the cumulative percentage dissolved at each of the selected n time points of the reference and the test sample, respectively. The factor f_1 is proportional to the average difference between the two profiles, whereas factor f_2 is inversely proportional to the average squared difference between the two profiles, with emphasis on the larger difference among all the time points. When the two profiles are identical, $f_2 = 100$ and an average difference of 10% at all measured time points results in an f_2 value of 50. Any value of f_2 between 50 and 100 ensures similarity between two dissolution profiles. On the other hand, an f_1 value less than 15 (15–0) indicates insignificant difference.

Differential Scanning Calorimetry (DSC)

DSC analysis was carried out using a Mettler TA 4000 thermoanalyzer (Mettler Toledo, Greifensee, Switzerland) in a temperature range of − 120 to 250 °C. The samples for the analysis were previously dried in an oven at 40 °C for 20 h. A specimen of about 10 mg was then accurately weighed and placed in an aluminum pan and scanned in the temperatures

range with a heating rate of 10 °C/min under a continuous nitrogen gas flow. An empty perforated aluminum pan was used as the reference. The instrument was calibrated by indium as the standard.

Results and Discussion

Different formulations of BDS, as listed in Table 1, were prepared in order to explore the effect of the structural factors which could be decisive for controlling the release profile and the ultimate properties of the delivery system. These factors, which are associated with the semi-IPN structure and the composition of BDS, included the cross-linking degree of the HG, the ratio between two polymers in the semi-IPN structure, and the presence of the plasticizer and solubilizer in the BDS composition.

The Effect of HG/TA Weight Ratio

The physical cross-linking of HG is mainly intended to retard the solubility of the HG and to provide an extended release of the drug via the bio-erosion process of the system. The cross-linking also enhances the physical and mechanical properties of the polymer, particularly the rheological characteristics which play a major role during the kneading process involved in the preparation process of the semi-IPN.

In order to assess the effect of the degree of cross-linking on the release rate of FBP, various formulations differing in the ratio between HG and TA were prepared and tested using a dissolution method. To this end, the formulations 4–2–0, 4–2.4–0, 4–3–0 and 4–5–0 (Table 1) containing respectively an HG/TA weight ratio of 2.0, 2.4, 3.0 and 5.0 were prepared and tested in PBS pH = 7.5. Note that the weight ratio between cross-linked HG and Eud.S was 4 in all these formulations.

The cumulative percentage release of FBP, recorded up to 10 h, is graphically demonstrated in Fig. 2a. As one can realize, the decrease in the ratio between the HG and TA, indicating an increasing degree of cross-linking, decelerates the release rate of the drug from the system. While the higher degrees of cross-linking (e.g., the weight ratios of 2 and 2.4) provided a relatively extended release, a burst release was almost presented by the formulations with the weight ratios of 5 and 3. The ratio of 2.0 resulted in a typical controlled release where 81% of the drug was released in 10 h. Contrastively, the weight ratios of 5 and 3 yielded a significantly faster release where after only 2 h the drug release level reached about 84 and 95%, respectively, and after 4 h the FBP release reached 98–99%. This finding indicates that by adjusting the degree of cross-linking, one can control the release rate in accordance with the kind of the local disease. This can be elucidated by the fact that as the degree of cross-linking increases, more chains of the polymer are bound together and consequently the solubility of the polymer decreases [33]. Hence, the release profile of the drug from the system is concomitantly inhibited. On the other hand, a higher degree of cross-linking creates a network with a higher cross-linking density and thus the entrapment of the drug will be more effective. Furthermore, the degree of cross-linking not only affects the solubility rate of the cross-linked polymer but also that of the linear polymer. A higher density of cross-linking may lead to a higher entanglement of the linear polymer within the cross-linked polymer which in turn influences the dissolution of the linear polymer, thus hindering even more the release rate of the drug from the system.

The formulations 4–2–0, 4–3–0 and 4–5–0 were also tested in vivo where each formulation was applied in five volunteers by sticking the BDS onto the upper right Canine (Cuspid) tooth for 240 min. Thereafter, the BDS was removed and the residual FBP left in the system was quantified by an HPLC method as described in the Experimental section. The subtraction of the residual FBP found in the delivery system from the initial content of FBP can yield the drug's quantity released during 240 min of the application in the mouth cavity of the volunteer. The results of the average of the residual FBP values are presented in Fig. 2b, as compared to the comparable in vitro results. The same trend of the effect of cross-linker on the release profile was also evident in these series of experiments, despite the difference found in the release level between in vivo and in vitro dissolution tests in 240 min.

The Effect of the Solubilizer

Polysorbate 80 is a surface-active agent (surfactant) which may enhance the dissolution of FBP from the system, thus increasing the release rate. On the other hand, it may promote the wetting of the semi-IPN itself and thus facilitate its bio-erosion by a dissolution and/or disintegration process which in turn assists in the release of the drug. Additionally, since the solubility of FBP in water is fairly poor, the presence of Polysorbate 80 as a solubilizer may encourage a uniform dispersion of the active material into the semi-IPN system during the preparation process of the BDS. Based on these facts, therefore, different formulations containing Polysorbate 80 (Tween 80) were prepared to explore the effect of the solubilizer on the release rate of FBP from the system.

The formulations 4–2–2.2, 4–2.4–1.1 and 4–3–1.1 were tested by a dissolution method in PBS (pH 7.5) and compared to the corresponding formulations containing no solubilizer. The comparative release profiles, showing the effect of the solubilizer on the release profile of FBP from the system with different HG/TA weight ratios, are depicted in Fig. 3a–c**.** As expected, the release of FBP was significantly promoted by using the solubilizer in the formulation. For instance, according to Fig. 3b**,** while the cumulative release from the formulation 4–2.4–0 in 15 min was about 15%, the

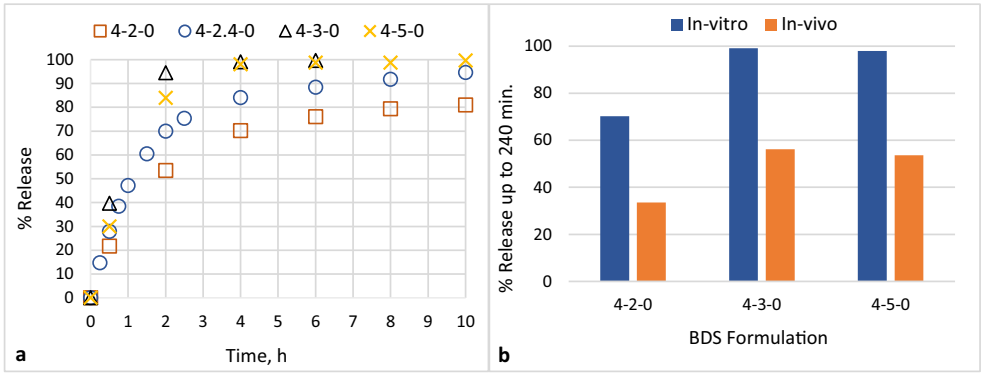

Fig. 2 The effect of HG/TA weight ratio (2.0, 2.4, 3.0 and 5) on the release profile of FBP **a** in vitro dissolution test carried out in PBS, pH = 7.5, **b** in vivo release after 4 h post-application compared to the in vitro release at the same time

formulation containing Polysorbate 80 (4–2.4–1.1) released 65% of the FBP content at the same time. The latter reached 100% release after approximately 4 h compared to 80% at the same time from the BDS formulation without Polysorbate (4–2.4–0). Figure 3a and c present the effect of the presence of the solubilizer on the FBP release rate for the formulations with HG/TA weight ratios of 2 and 3, respectively. The same trend of the solubilizer effect is demonstrated for these formulations as well. Based on these results, one can realize that the usage of the solubilizer in the formulation diminishes the effect of the cross-linking degree so that only the weight ratio of 2 (HG/TA) could provide a prolonged release lasting about 6 h (Fig. 3a). Note that at the same time only 76% of the initial FBP content could be released by the formulation containing no Polysorbate.

The Effect of Cross-Linked HG/Eud.S Weight Ratio

To explore the effect of the weight ratio between the cross-linked and the linear polymers in the semi-IPN structure on the release profile, different formulations (9.7–3–1.1, 7.1–3–0.5, 4.6–3–1, 4–3–1.1) varying in the weight fractions of the linear polymer (HG/Eud.S of 9.7, 7.1, 4.6, 4) were prepared and the release profile of these formulations was assessed using a dissolution method at pH 6.5. The results are demonstrated in Fig. 4a. In general, the increase in Eud.S content in the semi-IPN composition yields an enhancement in the release rate of FBP. It is noteworthy that this could happen despite the relatively acidic pH (6.5) of the dissolution medium where the solubility of Eud.S is the lowest or even null. This result indicates that the cross-linked HG is the release-controlling

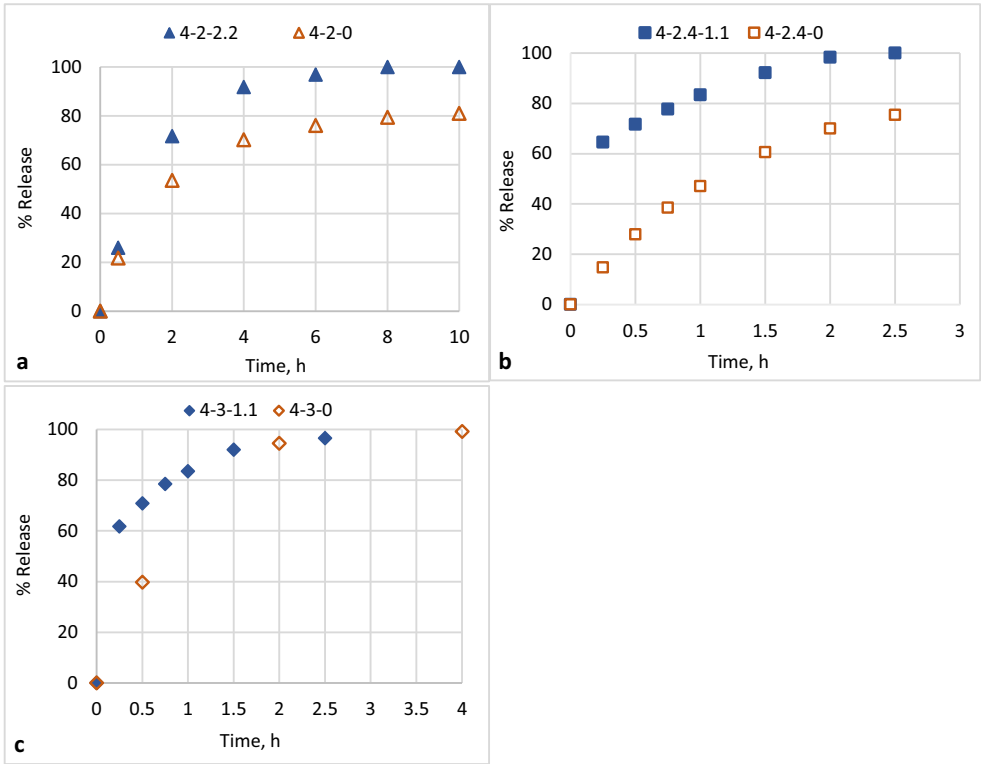

Fig. 3 The effect of the solubilizer on the dissolution release profile of FBP from BDS made with various HG/TA weight ratios. **a** HG/TA = 2, **b** HG/TA = 2.4, and **c** HG/TA = 3. Measured in PBS at pH = 7.5

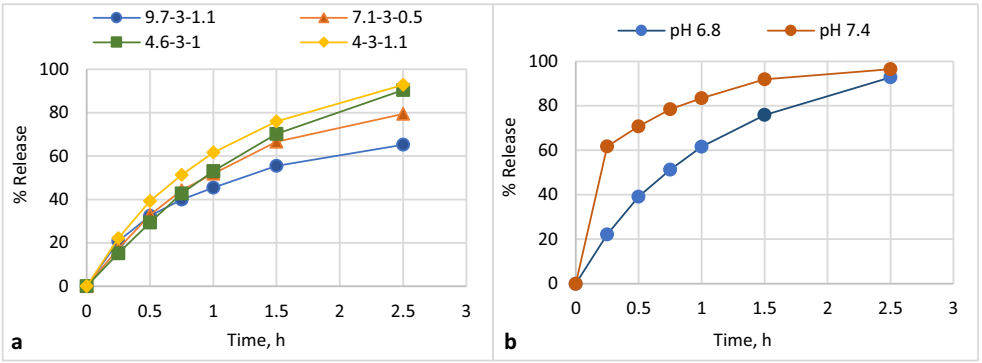

Fig. 4 **a** The effect of the weight ratio between the cross-linked HG to Eud.S (9.7, 7.1, 4.6, and 4) on the dissolution release profile of FBP measured in PBS, pH = 6.8. **b** The effect of the PBS pH on the release profile of FBP from the formulation of 4–3–1.1 (the weight ratio between the cross-linked HG to Eud.S was 4)

component in the designed BDS at this specific pH. The cross-linked HG as a network entraps the active material providing an extended release being attributed to the bio-erosion process, regardless the pH of the medium. Eud.S, on the other hand, is an enteric polymer whose solubility changes according to the pH of the dissolution [34, 35]. Therefore, the release profile of the system may change depending on the pH of the test medium. In order to show the effect of the pH on the release profile, formulation 4–3–1.1, having the highest content of Eud.S, was tested at two different pHs. The results are shown in Fig. 4b**.** As can obviously be seen, the release was faster at pH 7.4 than 6.8. This finding is associated with the fact that the dissolution of Eud.S is faster at a higher basic pH [35]. Moreover, whereas pH 7.4 resulted in a first order release, a semi-zero order release was presented by the test carried out at pH 6.8. These facts denote that at a certain pH the combination of the cross-linked HG and Eud.S as a semi-IPN can create a typical controlled release presenting a zero-order manner.

The Effect of the Plasticizer

A proper flexibility was strongly essential for conforming the designed BDS to the shape of the tooth and consequently increasing the patient's convenience and compliance. Likewise, the flexibility was necessary for ensuring the resistance of the system to the mechanical friction and shear forces expected during the application, thus lengthening the retention of the delivery in the oral cavity. Under these circumstances, therefore, the usage of TEC as a plasticizer in the formulation was definitely unavoidable.

A plasticized polymer can also generally present, due to its lower viscosity, a higher ability to spread onto a surface during the application, thus, the improvement in the adhesion properties. Likewise, a proper plasticizer diminishes the intermolecular attraction between the polymer's chains, which in turn results in an increase in the adhesion capabilities [36].

In this study, the effect of the plasticizer was studied using a DSC method. Figure 5 presents DSC thermograms of cross-linked HG (Fig. 5a) and two BDS formulations 4–3–0 (Fig. 5b) and 4–3–1.1 (Fig. 5c) containing TEC (~ 29% *w*/*w*). Their corresponding transition temperatures and heats are presented in Table 2.

Upon a heating scan carried out from − 120 to 200 °C, the thermograms of the cross-linked HG (Fig. 5a) showed a glass transition temperature (T_g) at 85.8 °C which ranges from 76.3 to 92.5 °C. The glass transition temperature is generally the temperature at which the polymer changes from the glassy state (hard) to the rubbery state (soft), as the result of the molecular segmental motion taking place in the disordered (amorphous) regions of the polymer's structure [37, 38]. This value of T_g is absolutely higher than the free gelatin as reported in different studies [39–41]. This can be due to the cross-linking bonds limiting the molecular segmental motion through the polymer chains.

The glass transition was followed by three consecutive endothermic peaks at 130.5, 160, and 165 °C. Since the decomposition temperature of gelatin is well above 200 °C [41], these endothermic peaks are, most likely, assigned to the dissociation and/or melting of crystalline regions with different degrees of ordering taking place as the result of the reaction between TA and HG.

The thermograms of two different BDS with the formulations of 4–3–0 and 4–3–1.1 are respectively demonstrated in Fig. 5b and c. In these thermograms, three different glass transitions, confined in different temperature ranges, can generally be recognized. The first glass transition occurring at − 59.5 °C (4–3–0) and − 50.0 °C (4–3–1.1) is associated with the excess of the plasticizer (TEC) in these formulations. The two other glass transitions, occurring at 48 and 75 °C, for the formulation 4–3–0, and at 45 and 76 °C, for the formulation 4–3–1.1, are respectively attributed to Eud.S and cross-linked HG. Accordingly, one can notice that the presence of plasticizer in the formulation could indeed significantly affect the T_g of both Eud.S as well as cross-linked HG. The T_g of Eud.S (unplasticized) has been reported to be above 120 °C [34, 42]. TEC is known as a proper plasticizer for Eud.S [42]; hence, it could properly lower the T_g to about 45 °C. Likewise, even though TEC is not known as a plasticizer for either HG or cross-linked HG, it could still somehow impact the T_g of the cross-linked HG and therefore it dropped to 75–76 °C (Fig. 5b, c). This could be due

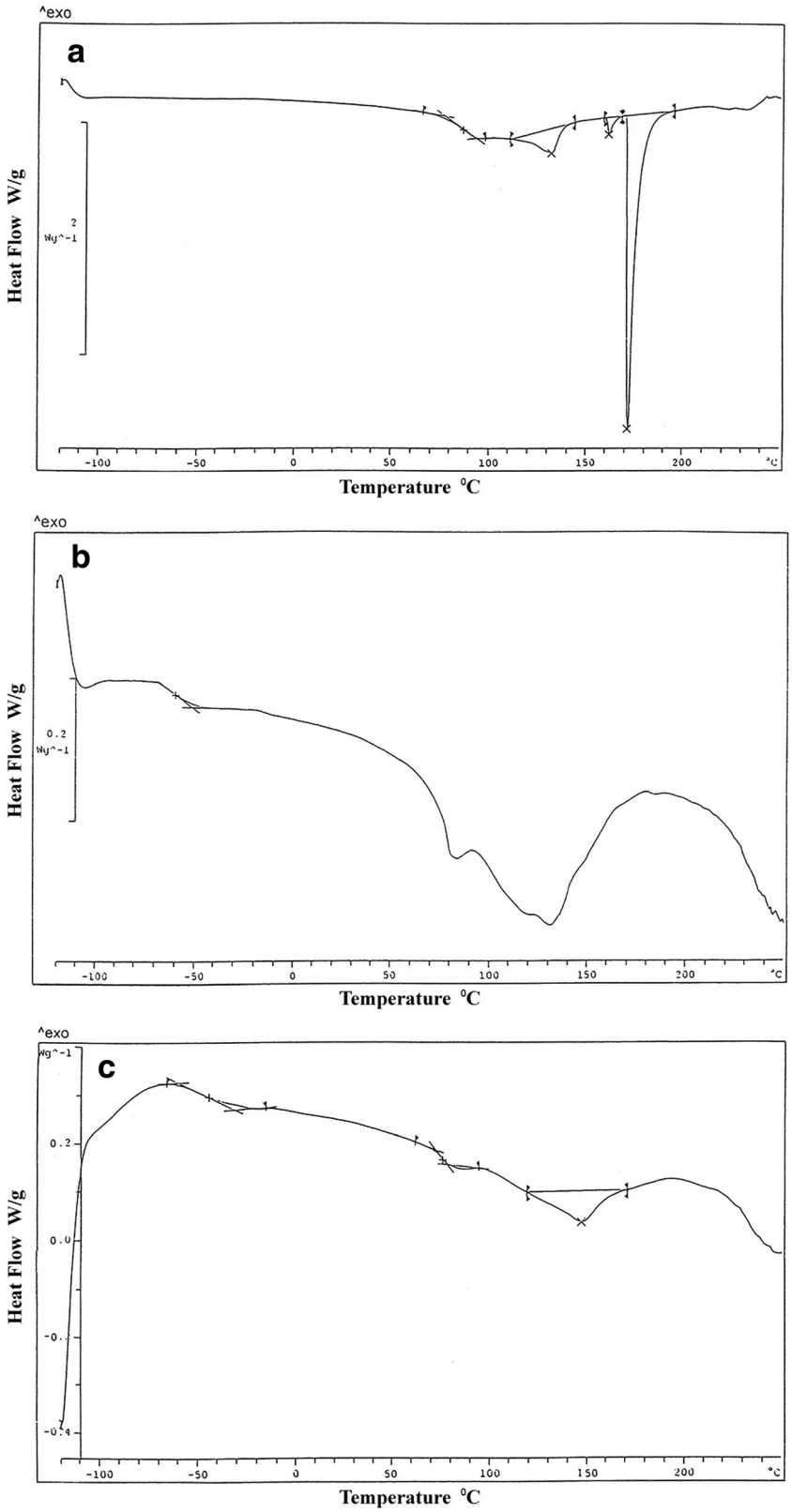

Fig. 5 DSC thermograms of hydrolyzed gelatin cross-linked by tannic acid (**a**) and two BDS formulations-4-3-0 (**b**) and 4-3-1.1 (**c**)

to a direct effect of TEC, as its concentration in the formulation is much higher than the most acceptable concentration ranges normally used in the conventional coating formulations. Alternatively, TEC could indirectly affect the cross-line HG T_g via softening the Eud.S penetrating into the cross-linked HG network which finally yields a higher free

Table 2 Thermal transitions occurring in TA cross-linked HG and two BDS formulations, detected by a DSC method

Sample	T_g (1) (°C)	T_g (2) (°C)	T_g (3) (°C)	Td1 (°C)	ΔHd1 (J/g)	Td2 (°C)	ΔHd2 (J/g)	T_m (°C)	ΔHm (J/g)
TA cross-linked HG	–	–	85.8	130	16.4	160	2.5	165	78.6
4–3–0	− 59.5	48.0	75.0	130	78.2	–	–	–	–
4–3–1.1	− 50.0	45.0	76.1			147.4	9.2	–	–

TA tannic acid, *HG* hydrolyzed gelatin, T_g glass transition temperature, *Td* disaggregation temperature, T_m melting temperature, *ΔHd* heat of disaggregation, *ΔH* heat of fusion

volume and thus a lower T_g. Note that the existence of two distinct glass transitions may verify a clear phase separation taking place between the linear and cross-linked polymers. Indeed, phase separation is a very well-known phenomenon occurring in different systems based on IPNs and semi-IPNs [43, 44]. During the preparation of the system, both phase separation and cross-linking reaction processes take place concurrently. In fact, the final state of the system is determined not by thermodynamic equilibrium but by the kinetic competition between the rate of phase separation and the rate of the cross-linking reaction. The size of the inhomogeneity depends on the outcome of this competition. If the cross-linking reaction is extremely fast, the system can be prepared homogeneously with no large regions of phase separation and vice versa.

It is noteworthy that as a result of the effect of the plasticizer on the Tg of both cross-linked HG and Eud.S, a lower processing temperature could be implemented during the preparation process of the BDS. Moreover, a significant decrease in the viscosity of the components could be observed which further facilitated the achievement of a uniform mixing of the components including the plasticizer itself.

An additional important point rising from Fig. 5b and c is the fact that the highly ordered microstructure of the cross-linked HG, which was mainly detected by the melting which occurred at 165 °C (Fig. 5a), does not appear anymore. However, the less ordered regions such as aggregates, showing a melting (disaggregation) at 130 °C and 147 °C for the formulations 4–3–0 (Fig. 5b) and 4–3–1.1 (Fig. 5c), respectively, still continue to exist. The effect of the solubilizer existing in the formulation 4–3–1.1 was mainly manifested by reducing the ability of the cross-linked HG to make the aggregation and consequently a reduced disaggregation heat could be detected in this formulation (Table 2) as compared to the formulation with the lack of a solubilizer (4–3–0).

The Stability of the BDS

Despite the affirmative and valuable contribution of the plasticizer, several adverse effects may arise, over time, during shelf life or stability study of the system. Leaching, for instance, can be a major problem encountered during shelf life as it can eventually result in the drastic alteration of all the characteristics of the polymeric drug delivery system, notably the incorporated drug release rate [45]. Likewise, at the temperature around the T_g of the plasticized polymers, the delivery system may lose its physical stability and thus significant changes in its shape, appearance, and dimensional constancy can result. A plasticized system may also present more sensitivity to humidity since the high free volumes being created between the polymer chains can constitute an appropriate space for absorbing water molecules. The excess of humidity in the formulation may further cause an additional softening effect and also initiate the degradation process of the susceptible components included in the formulation including the active ingredient.

Therefore, in light of these facts, it was in our interest to monitor the stability of the BDS over time especially at different temperatures. To this end, the formulation 4–2–0 packaged in either PVC-PVDC/+SG or in an Alu/Alu blister was tested for stability at 25, 30 and 40 °C. At time points of 1, 2, 3 and 6 months, samples were taken for dissolution tests as compared to the reference which was the batch tested at time 0. The dissolution tests were performed in PBS at pH = 7.5.

In order to explore the significance of the dissolution profile comparison between the reference and the samples, both f1 (the difference factor) and f2 (the similarity factor) test factors were used as described in the Experimental section. Note that an f2 value of 50 or greater (50–100) ensures sameness or equivalence of the two curves and, thus, the performance of the two delivery systems. Likewise, an f1 value less than 15 (15–0) indicates an insignificant difference.

Figure 6 plots the f1 and f2 values for the samples packaged in both packaging material types, PVC-PVDC/+SG (Fig. 6a) and in an Alu/Alu blister (Fig. 6c). The inspection of Fig. 6a indicates that, based on both f1 and f2 tests, the BDS was absolutely stable at least for 6 months at 30 °C. The samples kept at 40 °C for 6 months, on the other hand, failed because of the appearance reason since they became loose and lost their dimensional stability. Likewise, the samples kept at 40 °C for 3 months failed according to the f2 test. The release profile of these samples, as depicted in Fig. 6b, demonstrated a faster release rate than the samples kept at 25 °C and also than the reference samples. The main reason for this finding is associated with the T_g of the plasticized polymer which is very close to 40 °C. At the temperature close to the T_g of the

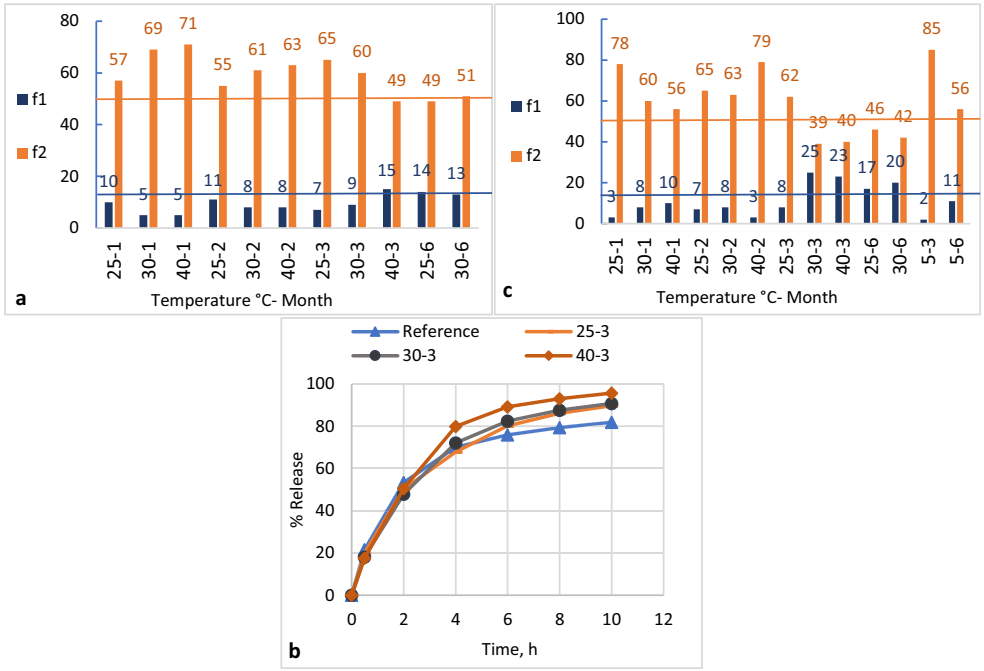

Fig. 6 Stability study of a BDS (4–2–0) stored at different temperatures (5, 25, 30 and 40 °C) for different time periods (1, 2, 3 and 6 months). **a** The results of f1 and f2 tests for the samples packaged in PVC-PVD/+SG. **b** The release profile of the samples packaged in PVC-PVD/+SG after 3-month storage at 40, 30 and 25 °C compared to the reference. **c** The results of f1 and f2 tests for the samples packaged in an Alu/Alu blister. The dissolution tests were carried out in PBS at pH = 7.5

plasticized polymer, due to the high mobility of the polymer chains, a migration of both the plasticizer and the drug to the surface of the system may take place which can subsequently enhance the release rate.

The aforementioned feature could be more prominent for the samples packaged in an Alu/Alu blister as can be seen in Fig. 6c. The samples kept at both 30 and 40 °C failed for the dissolution reason as they presented f1 and f2 values of above 15 and below 50, respectively. Such a finding can be elucidated by the fact that at these temperatures, the moisture trapped inside the Alu/Alu blister cavity is absorbed by the BDS, making it even softer and consequently the migration of the plasticizer and the drug to the surface could occur more easily, hence a faster dissolution rate of the drug could be obtained. It is interesting to notice that the samples packaged in the Alu/Alu blister and kept at 5 °C for up to 6 months (Fig. 6c) could absolutely present a high stability in terms of both dissolution rate and the appearance. This point supports the explanation given above regarding the instability of the system at higher temperatures.

In Vivo Release Kinetics Study

A BDS based on the formulation 4–2–0, containing 15 mg FBP, was used to study the in vivo release profile of the system. For this purpose, the formulation was applied in five individuals by sticking the system onto the upper right canine (cuspid) tooth. Samples of saliva were then taken after different predetermined time intervals up to 240 min post-administration for the HPLC analysis to quantify the concentration of FBP found in the saliva sample as described in the Methods section.

The results of this in vivo study are graphically illustrated in Fig. 7, where the average curve and standard deviation at each time point are also presented. Generally, two distinct stages, differing in the release rate, can be detected. The first is the stage in which the concentration of FBP in the saliva increases slowly over time up to about 90 min. The slow release rate nature of the system at this stage is the result of the fact that Eud.S still keeps its integrity and the system just gets wetted by the saliva. Therefore, the release is just a yield of the drug diffusion through the semi-IPN system.

The second stage, on the other hand, is characterized by a high concentration of FBP in the saliva, ranging from 120 to 240 min, with a mean C_{max} of approximately 17 ppm occurring at 180 min. This can happen since at this time point, Eud.S has already dissolved and the release occurs through both diffusion and erosion of, predominantly, the cross-linked HG processes. It is, however, noteworthy that the increase in the release rate at this stage, is accompanied by a prominent increase in disparity between the volunteers, as represented by standard deviations on the average curve in Fig. 7. For instance, whereas only about 6 ppm of FBP was found after 180 min with volunteer 1, volunteer 3 showed a concentration of about 40 ppm at the same time. Note that at this time point the standard deviation was found to be about 13. This high variability is most likely the result of the variation in pH of the saliva in the oral cavity of the volunteers. A normal healthy saliva in the oral cavity may have a pH ranging between 6.7 and 7.4 [46]. The release rate is enhanced only where the linear polymer (Eud.S) in the semi-IPN structure has already

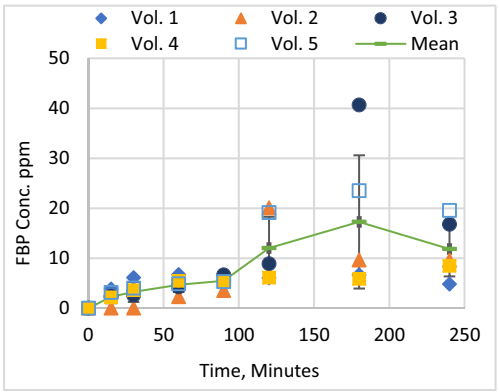

Fig. 7 The concentration of FBP found in the saliva of individuals and the average concentrations at different time points during the application of BDS (4–2–0) for 240 min

been dissolved, occurring only at an appropriate pH promoting the solubility of the polymer.

Conclusion

The present study showed for the first time that a semi-IPN structure can successfully be used for the preparation of an intraoral bio-adhesive system to provide an extended release of a drug in the oral cavity. The main advantage of the semi-IPN for this purpose is the controllability of both the retention time and the release profile according to a specific need by controlling the structural variables such as the type of both linear and cross-linked polymers, the ratio between them, and the degree of cross-linking of the cross-linked polymer. Accordingly, a specific semi-IPN based on a combination of TA cross-linked HG and Eud.S as a pH-sensitive linear polymer was explicitly found to be appropriate for the controlled release of FBP in the oral cavity. Although the study aimed to release FBP, the availability of the structural variables, associated with the semi-IPN structures, enables the implementation of such a delivery system for active materials rather than FBP. As such, for instance, a lower ratio between HG and TA, leading to a denser cross-linked polymer, may create the retardation needed for an extended release of a highly water-soluble active material and vice versa. Likewise, by selecting an appropriate type of the linear polymer having a specific pH solubility, the disparity in the concentration of the drug available in the saliva can be avoided. However, whatever the composition will be, no compromise in the flexibility and the adhesion properties of the system is acceptable. In other words, the presence of a plasticizer with specific concentration in the formulation will still remain necessary.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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