Research Article

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Therapeutic Delivery

Caffeic acid loading wound dressing: physicochemical and biological characterization

Aim: Caffeic acid has been described as active against bacteria commonly isolated from wound infections. However, its low stability and poor solubility reduce caffeic acid applicability as a pharmaceutical product. These parameters can be enhanced by complexation with cyclodextrin. The main goal of the present work was to incorporate caffeic acid on cyclodextrin-based hydrogels capable of controlled delivery, in order to be used as antibacterial wound dressing. **Materials & methods:** Cyclodextrin-based hydrogels were prepared by direct crosslinking of β-cyclodextrin or hydroxypropyl-β-cyclodextrin with 1,4-butanediol diglycidyl ether in the presence of hydroxypropyl methylcellulose. **Results:** The hydrogels obtained combine good physicochemical properties (viscoelasticity, superabsorbency and high ability to retain and deliver caffeic acid) with the preservation of caffeic acid' antibacterial activity and effect on fibroblasts, with gel–β-cyclodextrin the most suited. **Conclusion:** The hydrogels obtained could be useful as caffeic acid delivery-system devices for the treatment of wound infections.

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Hydrogels have been successfully applied as medical devices, especially for wound healing purposes [1]. Wound dressing hydrogels are polymeric networks with a 3D structure capable of absorbing high amounts of water. These materials, besides mechanical protection, may enhance the healing process by promoting gas exchange and reduce body fluid loss. The biological properties of hydrogels can be improved by the absorption of bioactive molecules on their network, such as growth factors to accelerate the healing process or antimicrobial agents to prevent infections [2]. However, hydrogels have poor properties regarding the controlled release of those bioactive molecules. Thus, cyclodextrin (CD), a truncated oligosaccharide with the ability to complex with a wide range of molecules, has been used to enhance the hydrogels' drug-delivery ability. CD-based hydrogels retain the suitable properties of polymeric networks (swelling, softness and mechanical properties) and, additionally, the capacity of CD to complex and sustainably release bioactive molecules [3].

Polyphenolics are extensively distributed among the plant kingdom, imposing plant odors, pigmentation and flavor and/or acting as plant defence mechanisms against tissue infections or injuries [4,5]. These plants' metabolites are present in human diet, and a wide range of biological effects have been attribute to them, such as antioxidant, anti-inflammatory, antimicrobial and antiviral effects [6–8]. Polyphenolics is the term applied to the set of molecules that share a common chemical skeleton (one or more aromatic rings with at least one hydroxyl group [OH] attached), but with different structures and functions [9].

Caffeic acid (3,4-dihydroxycinnamic acid) is a simple phenolic acid and has been reported as an antioxidant, antibacterial and fungicide [10–12]. This phenolic acid, as well as other polyphenolics, has been used as a food preservative and proposed as an antimicrobial agent for the pharmaceutical field. However, caffeic acid exhibits poor solubility and stability under environmental stress, reducing its applicability as an antibacterial

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Key terms

Hydrogel: Tri-dimensional networks of hydrophilic polymers with the ability to absorb from 10–20% (an arbitrary lower limit) to up to thousands of times their equivalent weight in water until the process reaches an equilibrium state.

Cyclodextrin: Produced by bacterial degradation of starch. They have a shape of a truncated cone due to the chair conformation of the glucopyranoside units which creates a micro-heterogeneous environment inside the cavity.

Caffeic acid: Also known as 3,4-dihydroxycinnamic acid, is a simple phenolic acid and has been reported as an antioxidant, antibacterial and fungicide.

Wound dressing: Cover the wound, providing physical protection against microorganism deposition, wound dehydration and external injuries.

Cellulose: Polysaccharide consisting of a linear chain of several hundred to many thousands of β(1→4) linked D-glucose units.

agent in the pharmaceutical field. Thus, encapsulation devices, such as CDs, have been suggested to ensure caffeic acid stability and improve its utilization as an antibacterial agent [13].

The main goal of the present work was to develop a hydrogel wound dressing based on cellulose and CDs, with the ability to retain and sustain the release of caffeic acid, for wound dressing proposes.

Material & methods

Materials

Caffeic acid (3,4-dihydroxycinnamic acid) was purchased from Sigma (St Louis, MO, USA), βCD (1135 g.mol^{-1}) and 2-hydroxypropyl-β-cyclodextrin (HPβCD, 1309 g.mol^{-1}) were acquired from AppliChem (St Louis, Missouri, USA). Hydroxypropyl methylcellulose (HPMC) Methocel® K4M (Mw 84,200, normal viscosity 400 mPas) was purchased from VWR Prolab (Carnaxide, Portugal) and 1,4-butanediol diglycidyl ether (BDGE), 50–60% in water, was purchased from Acros Organics (Geel, Belgium). All solutions were prepared with purified water, obtained by reverse osmosis (MiliQ®, Millipore, Madrid, Spain) with a resistivity above 18.2 MΩ.cm^{-1}.

Synthesis of gel-β and gel-HPβ

Hydrogels were synthesized through the method described by Lorenzo *et al.* [14]. Briefly, 2.5 g of each CD (βCD or HPβCD) was dissolved in 10 ml of NaOH (0.2 M). The solutions were maintained for 5 min, at 25°C and under mechanical agitation (200 rpm). After, 0.025 g of HPMC was added to each solution and the solubilization was improved by mechanical agitation (200 rpm) during 5 min, at 25°C. The crosslinking agent (BDGE, 2 ml) was added to 5 ml portions of each solution within petri dishes. The petri dishes were sealed with parafilm and kept for 2 min at 25°C and under mechanical agitation (200 rpm). To complete the crosslinking process, the plates were preserved at 50°C for 12 h. At this temperature, the CD and HPMC stability was assured. After cooling, hydrogels were immersed in ultrapure water for 12 h and 25°C to allow their swelling. Then, the hydrogels were transferred to HCl solution (10 mM) for a further 12 h and finally immersed in water for 7 days. The dry process was performed as followed: the hydrogels were kept at 25°C for 24 h and, after, were transferred to a desiccator until weight stabilization. At the end two hydrogels were obtained: βCD-co-HPMC (gel-β) and HPβCD-co-HPMC (gel-HPβ).

Preparation & characterization of caffeic acid load

For the hydrogel loading, a caffeic acid solution (2.3 ×10–1^{2} M, 2% ethanol) was prepared using H_3PO_4/NaOH buffer (pH 5 ± 0.5). To ensure that the caffeic acid was completely dissolved, the solution was maintained at 50°C, 200 rpm for 30 min. Dry hydrogel samples (90 ± 3 mg) were immersed in 5 ml of caffeic acid solution, 25°C and 60 rpm. The amount of phenolic acid in the solution was assessed by UV-Vis spectrophotometry, until the absorbance values stabilized. The caffeic loading was calculated based on the variation of the phenolic acid on the initial solution and on the equilibrium. The absorption spectra were measured at caffeic acid λ_{max} and recorded on a Jasco V560 spectrometer, using a 1 cm quartz cuvette.

The hydrogels with and without caffeic acid were characterized by differential scanning calorimetry (DSC), Fourier transform IR spectroscopy (FTIR) and contact angle.

DSC measurements were carried out in liquid nitrogen atmosphere using DSC-822e instrument (Mettler Toledo); (Greifensee, Switzerland). The calibration was made with indium as standard. Samples were weighed (2.5 ± 0.2 mg) and sealed in aluminium pans. Then, they were heated from 25 to 350°C at a scanning rate of 10°C.min^{-1}. Data were treated using LAB mettler star SW 8.1 software (Mettler-Toledo International Inc., Switzerland).

The FTIR-ATR analysis was performed as follows: samples (0.5 ± 0.05g) of each hydrogel were used. The IR spectra were recorded between 400 and 4000 cm^{-1}, in Avatar 360 FTIR spectrometer. The spectra of compounds alone (caffeic acid, βCD, HPβCD and HPMC) were, also, recorded, using the potassium bromide pellet technique.

Surface contact angles of hydrogels in contact with ultrapure water were measured using a contact angle

measurement apparatus (OCA15 Plus; Dataphysics, Burglengenfeld, Germany). A 3 µl water drop was placed over the clean hydrogel surface with an autopipette. All measurements were performed at room temperature.

Hydrogel characterization was made in triplicate for each hydrogel (gel-β, gel-β/caffeic acid, gel-HPβ, gel-HPβ/caffeic acid).

In vitro caffeic acid release

Dry load hydrogels (90 ± 3 mg) were immersed in 5 ml of synthetic sweat solution (0.5 g *l*-histidine monohydrochloride monohydrate, 5 g of sodium chloride, 2.2 g of sodium dihydrogen orthophosphate dihydrate, pH 5 ± 0.5), at 25 ºC. At predetermined timepoints, samples were taken until equilibrium was achieved. The amount of caffeic acid release was measured by the UV-Vis spectrophotometry, at caffeic acid λ_{max} and recorded on a Jasco V560 spectrometer, using a 1 cm quartz cuvette.

Antibacterial assessment

The hydrogels' antibacterial activity was tested against three bacteria: *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 11296). The bacteria were grown in tryptic soy agar (TSA, Merck, Germany) for 18 h at 37°C. Then, the cells were inoculated in tryptic soy broth (TSB, Merck, Germany) and incubated for 18 h at 37°C under agitation (120 rpm). Subsequently, the bacterial concentration of each strain was adjusted to 5×10^5 cells.ml^{-1}, via absorbance readings and corresponding calibration curve.

For the quantitative assess of hydrogel antibacterial capacity, samples with 90 ± 3 mg were immersed within 5 ml of 5×10^5 cells.ml^{-1} of each bacterium. Bacteria and medium controls were also included. The hydrogels and the bacteria were incubated for 18 h at 37°C. The number of viable cells was assessed by determination of the number of colony forming units, plating 10 µl of cell suspension from each replicate onto TSA, and incubated for 18 h at 37°C.

The assays were made in triplicate for each bacterium and hydrogel combination, at least in three independent assays.

Cytotoxicity assay

The hydrogels' *in vitro* cytotoxicity was carried out based on the method described on ISO 10993–10995:2009 – Biological evaluation of medical devices, part 5: Tests for *in vitro* cytotoxicity, by indirect contact [15]. The liquid extracts of the hydrogel were prepared as follows: hydrogels (90 ± 3 mg) were immersed within 5 ml of Dulbecco's modified Eagle's medium (DMEM) and then they were kept for 24 h at 25°C, on dark.

Fibroblast 3T3 (CCL 163), from American Type Culture Collection, were used in this study. Cells were cultured in DMEM supplemented with 10% of fetal bovine serum and 1% penicillin/streptomycin at 37°C, 5% CO_2. After achieving confluence, cells were passed at the density of 1×10^5 cells ml^{-1}, using trypsin. Then, cells were seeded at the density of 5×10^5 cells ml^{-1} (48-well plate) in 300 µl of DMEM complete medium. The 48-well plates were incubated for 24 h, 37°C and 5% CO_2 and after, the medium was replaced for 300 µl of each hydrogel extract and incubated for a further 24 h.

The cytotoxicity was tested by [3-(4,5-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] (MTS) (Promega, Madison, WI, USA) assay. This assay measured the cells viability by their capacity to uptake MTS and subsequent reduction by mitochondria, leading to the coloration of MTS. Thus, the liquid extract of hydrogels was removed, and a mixture of 6 µl of MTS and 294 µl of DMEM, without phenol, was added to each well. After 1 h, the absorbance value was measured, at 490 nm, and the results were expressed as a percentage of viable cells, using the number of cells grown on wells without hydrogel (control +) as 100%.

Statistical analysis

Statistical analysis was performed with OriginPro 8 SR0 (V8.70724, OriginLab Corporation, Northampton, MA, USA). The differences between groups were evaluated using the 2-way ANOVA variation test and the results were considered statistically significant for p-values < 0.0001.

Results

Caffeic acid-loaded gel-β & gel-HPβ

Figure 1 displays the caffeic acid-loading profiles of gel-β and gel-HPβ. The hydrogels obtained were capable of absorbing caffeic acid and achieving the equilibrium after 360 min. Interestingly, gel-β was capable of loading a higher amount of caffeic acid (0.6 g.ghydrogel^{-1}) when compared with the gel-HPβ (0.37 g.g$_{hydrogel}^{-1}$).

Physicochemical characterization of gel-β & gel-HPβ

The hydrogels with or without caffeic acid were characterized by DSC, FTIR and surface hydrophilicity.

The DSC thermograms of various products are shown in Figure 2. The DSC curve of caffeic acid displays a single sharp endothermic peak near 225°C, corresponding to its melting point. The CD (βCD and HPβCD), also, showed two defined peaks each, near 100°C and 275°C. In the case of HPMC, owing to its amorphous nature, a broad endothermic peak was

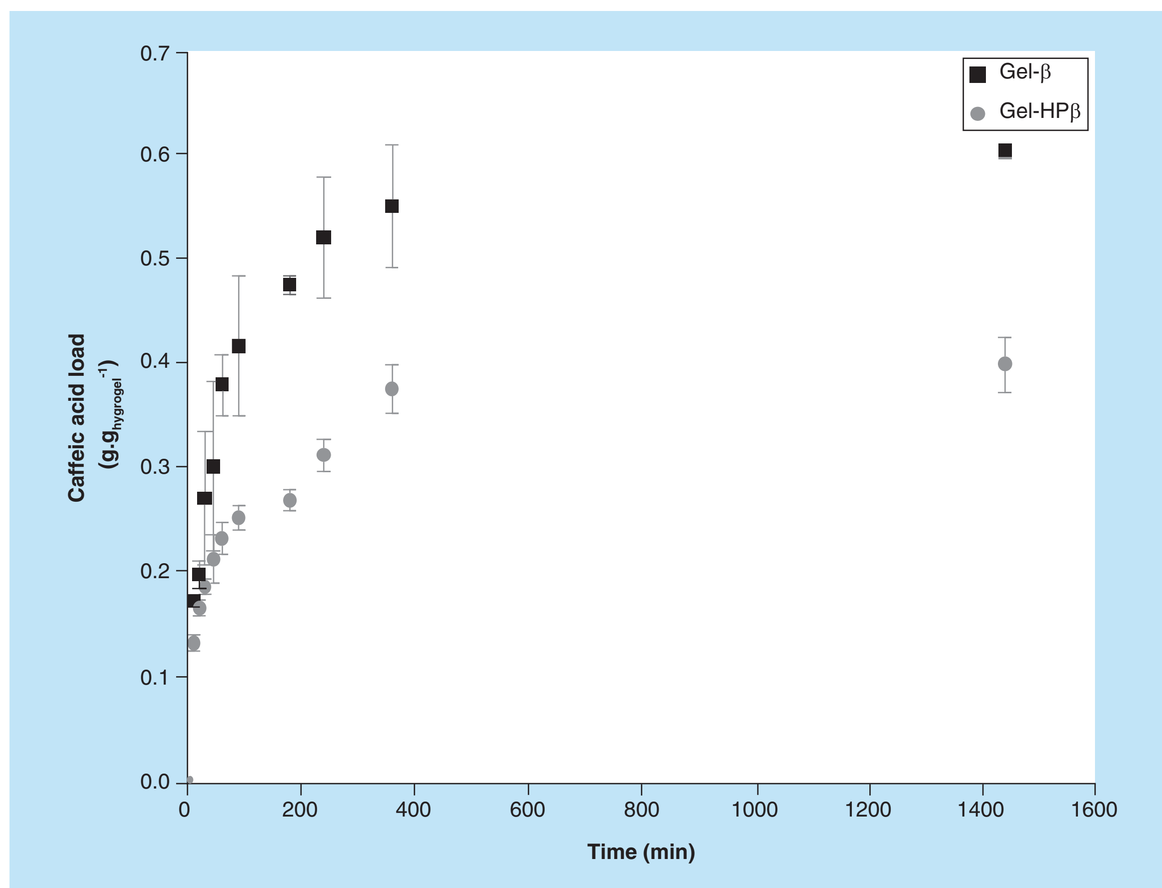

Figure 1. Caffeic acid loading in gel-β (black) and gel-HPβ (gray). The loading was performed during 24 h, 25 °C with caffeic acid dissolved on the buffer H_3PO_4/NaOH (pH 5 ± 0.1, 2% ethanol).
Gel-β: β-cyclodextrin-co-hydroxypropyl methylcellulose; Gel-HPβ: Hydroxypropyl-β-cyclodextrin-co-hydroxypropyl methylcellulose.

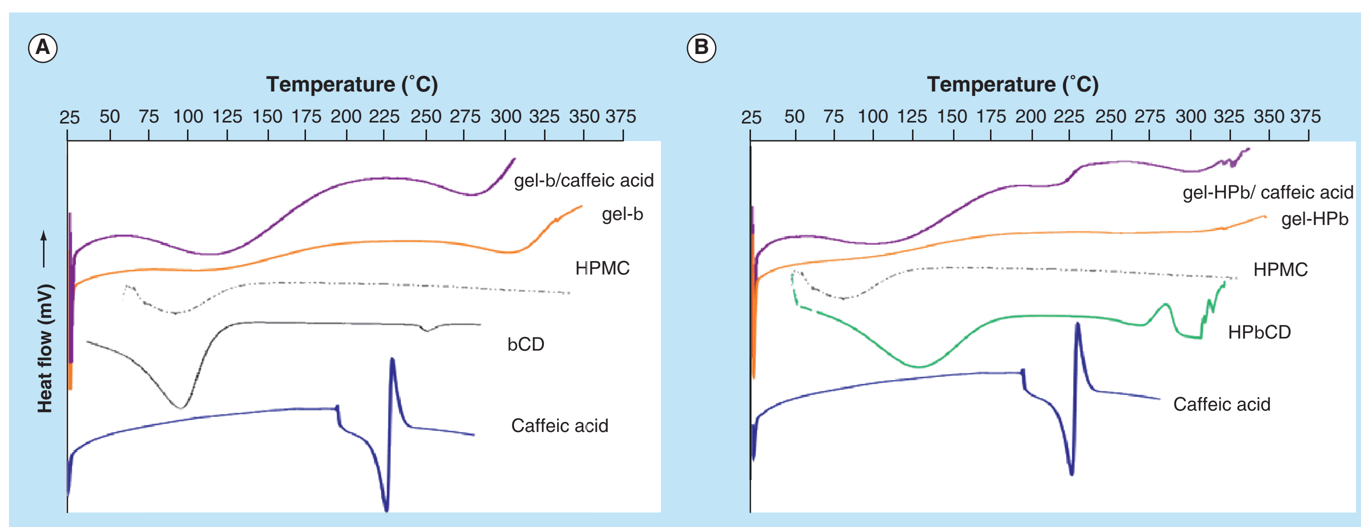

Figure 2. Differential scanning calorimetry thermograms of caffeic acid (blue), CD (black), HPMC (gray), gel (orange) and gel-loaded (purple). (A) with βCD and **(B)** with HPβCD.
CD: Cyclodextrin; gel-β: β-cyclodextrin-co-hydroxypropyl methylcellulose; gel-HPβ: Hydroxypropyl-β-cyclodextrin-co-hydroxypropyl methylcellulose; HPβCD: Hydroxypropyl-β-cyclodextrin; HPMC: Hydroxypropyl methylcellulose.
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observed at approximately 75°C. The hydrogels formation and crosslinking could be confirmed by the lack of obvious peak on their thermograms (orange line). Moreover, a nonobvious peak was obtained for loaded hydrogels.

Figure 3 present the FTIR spectra obtained from the gel-β and gel-HPβ with and without caffeic acid, and also, the network components alone (βCD, HPβCD, HPMC and caffeic acid). Both CD spectra show higher intensity on the following peaks (βCD/HPβCD): 3416/3447 cm^{-1} (O–H stretching), 2924 cm^{-1} (asymmetric vibrational stretching of C–H), 1645/1640 cm^{-1} (hydrogen interactions), 1157 cm^{-1} (C–O stretching) and 1029/1034 cm^{-1} (C–O–C) [3,16,17]. The HPMC spectrum showed a profile similar to the CD and the strong intensity peaks were identified: 3447 cm^{-1} (O–H stretching), 2924 cm^{-1} (asymmetric vibrational stretching of C–H), 1640 cm^{-1} (hydrogen interactions), 1115 and 1063 cm^{-1} (ether bond) [18,19].

The hydrogel spectra had similar profile regardless of the CD used for the synthesis of the polymeric network. However, they display some differences on the peaks intensity and shape when compared with the CD and HPMC spectra. The changes observed in the region of ether bond signals (between 1200 and 1000 cm^{-1}), and the decrease of peaks intensity at 3400 and 1649 cm^{-1} confirms that crosslinking occurred during the process.

The spectra obtained for the load gel-HPβ was similar to the gel without caffeic acid. However, the phenolic incorporation within the gel-β induced some changes on the spectra, more obvious between 2000 and 800 cm^{-1}. In this range, the caffeic acid peaks were present on the load gel-β spectra, making it more alike to the phenolic spectra than to the hydrogel.

The hydrophobicity of the hydrogel surface was assessed by the contact angle formed after a water drop was released on the material's surface. The gel-β had a contact angle of 85.68 ± 1.33° and the gel-HPβ 97.37± 1.63°. Thus, both surfaces can be considered hydrophilic, with the gel-β being more suitable for contact with water [20]. The caffeic acid loading slightly increased the hydrophilicity of the gel-β (78.73 ± 1.69°) and had no influence on the surface properties of the gel-HPβ (97.82 ± 1.68°).

In vitro caffeic acid release

Both polymeric networks were capable of sustaining the caffeic acid release for 48 h (Figure 4), with a burst at the first 60 min and a slower release until the equilibrium (360 min). After 2 days, the gel-HPβ released 1.2 $mg.g_{hydrogel}^{-1}$ of caffeic acid and the gel-β near half of that amount (0.77 $mg.g_{hydrogel}^{-1}$).

Antibacterial activity of gel-β & gel-HPβ

The hydrogel antibacterial activity evaluation revealed that both networks had comparable effects on growth of three bacteria (Figure 5). The gel-β and gel-HPβ without caffeic acid allowed the normal growth of the

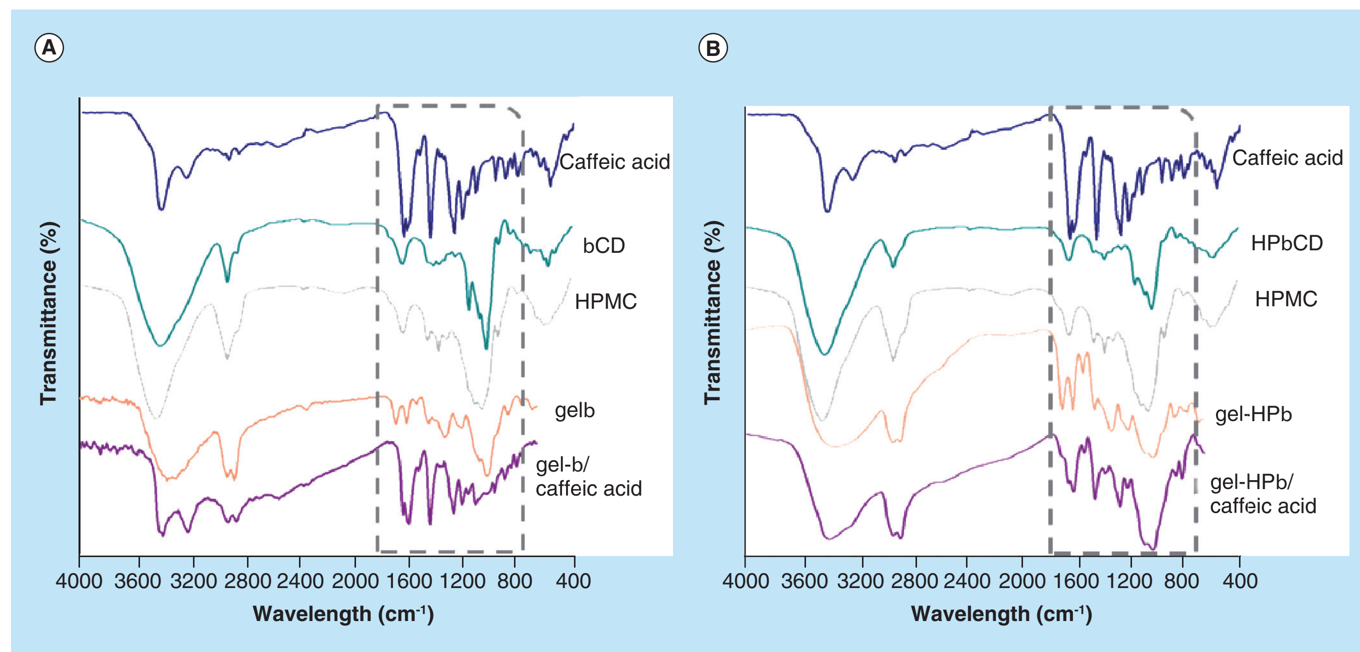

Figure 3. Fourier transform IR spectroscopy spectra of caffeic acid (blue), CD (green), HPMC (grey), gel (pink) and gel-loaded (purple).(A) with βCD and **(B)** with HPβCD.
CD: Cyclodextrin; gel-β: β-cyclodextrin-co-hydroxypropyl methylcellulose; gel-HPβ: Hydroxypropyl-β-cyclodextrin-co-hydroxypropyl methylcellulose; HPβCD: Hydroxypropyl-β-cyclodextrin; HPMC: Hydroxypropyl methylcellulose.
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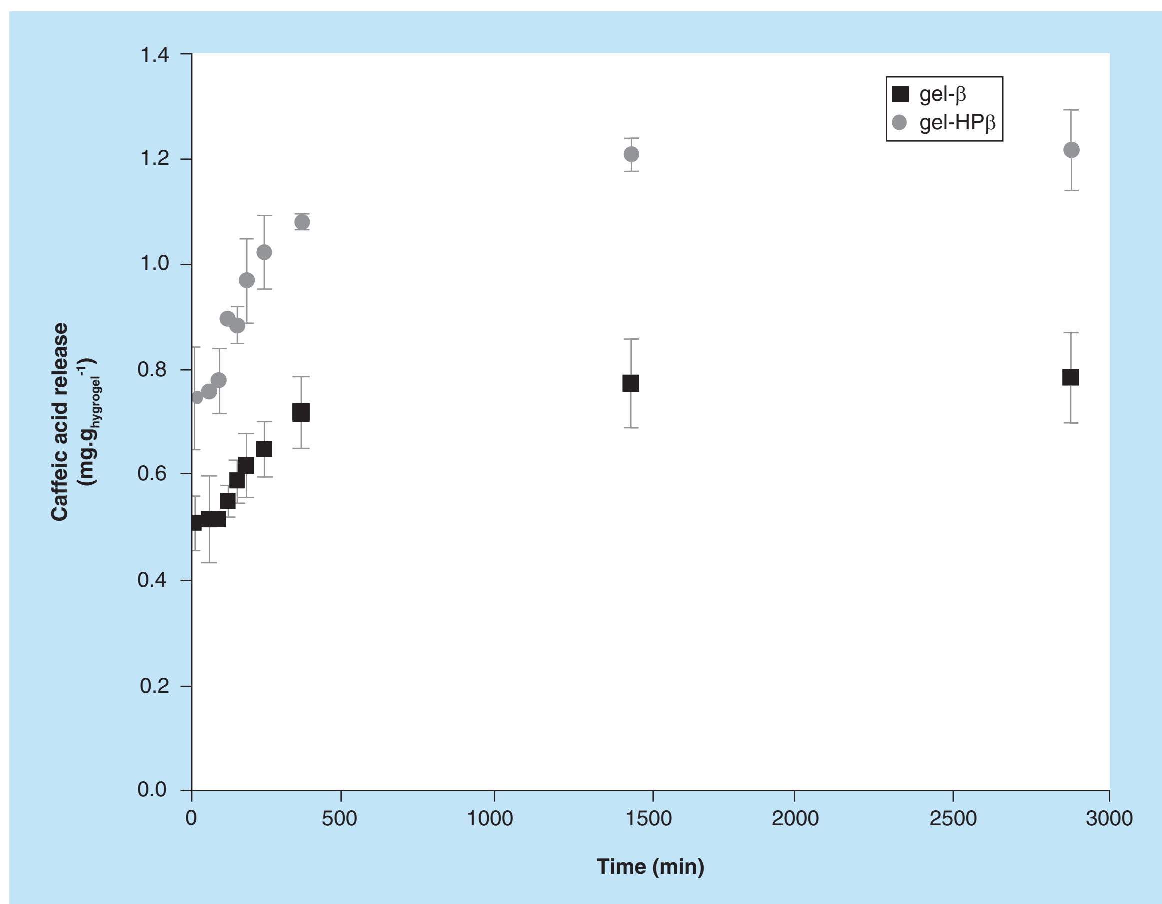

Figure 4. Caffeic acid released from gel-β (black) and gel-HPβ (gray) hydrogels; it was performed during 48 h, 25°C within synthetic sweat solution (pH 5 ± 0.1).
Gel-β: β-cyclodextrin-co-hydroxypropyl methylcellulose; Gel-HPβ: Hydroxypropyl-β-cyclodextrin-co-hydroxypropyl methylcellulose;

cells (near 6 log) and the hydrogels with caffeic acid caused a reduction of more than 3 log on the number of viable cells, when compared with the control. Apparently *S. epidermidis* was more susceptible to the antibacterial activity of both materials.

Effect of load hydrogels in fibroblasts proliferation

Figure 6 shows the hydrogels' effect on the proliferation of 3T3 fibroblasts. The gel-β and gel-HPβ allowed the perfect proliferation of the fibroblast. However, the caffeic acid loading induced a reduction on viable cells, gel-β/caffeic acid showed a decrease of 26% on viable cells and only 40% of viable cells were detected regarding the loaded gel-HPβ.

Discussion

Physicochemical characterization of gel-β & gel-HPβ

Previous work demonstrated that hydrogels could be obtained from the crosslinking of HPMC and βCD or HPβCD, using BDGE. The hydrogel formation occurred after 12 h when a ratio of 1 CD:1.25 BDGE:0.01 HPMC was used. The CD-based hydrogels obtained showed suitable characteristics for contact with skin injury. They were resistance but viscoelastic and with a smooth and continued surface. Moreover, both polymeric networks behaved as superabsorbent hydrogels and kept their shape after swelling [21]. Thus, the same conditions were used in the present work.

The hydrogels' loading properties result from the combination of several factors, such as network crosslinking degree, water uptake of the hydrogels (swelling) and interactions between guest and the network [22]. In addition, CD-based hydrogel ability to retain the guest molecule is also regulated by the formation of inclusion complexes (ICs) between the CDs and bioactive molecules. Thus, the guest molecule will be present trapped in the polymeric network and inside the CD's cavity [16,23].

The caffeic acid-loading capacity was dependent on the CD used for the hydrogel synthesis. Taking into account the swelling results (gel-HPβ > gel-β [21]) it was expected that gel-HPβ was capable of retaining

higher amount of caffeic acid in the aqueous phase. However, the gel-β absorbed more than twofold of caffeic acid, when compared with gel-HPβ. The interaction between caffeic acid and βCD or HPβCD was analyzed in a previous work. The native CD was reported to be the most suitable for the complexation with caffeic acid, at pH 5 [13]. Thus, the loading results and the ICs' stability (caffeic acid had higher affinity to βCD than HPβCD) suggests that CDs play a major role on the hydrogels' ability to load caffeic acid, and the βCD capacity to encapsulate caffeic acid was preserved after hydrogel synthesis (Figure 1).

The loading of larger molecules (diclofenac, ciprofloxacin, 3-methylbenzoic and estradiol) by similar hydrogels has been reported. All works stated that the IC, between the guest and the CDs, had a major contribution to the retention ability of CD-based hydrogels, which are in agreement with our results. Interactions between the guest molecule and HPMC were also referred, but with lower influence on the loading mechanism [3,17,23,24,25].

The crosslinking between CDs and HPMC, as well as the presence of caffeic acid on the hydrogels network, were confirmed by the DSC and FTIR analysis. The thermogram profiles of both hydrogels (gel-βCD and gel-HPβ) have a broad peak, as aresult of the crosslinking between the CD and HPMC. This suggested the presence of an amorphous structure, characteristics of hydrogel materials (Figure 2). Moreover, no significant differences were detected between gel-βCD and gel-HPβ thermograms, thus the crosslinking process was similar regardless of the CD. The thermograms of the load hydrogels (gel-β and gel-HPβ) lack the characteristic peak of caffeic acid (Figure 2). Therefore, the phenolic may be trapped in an amorphous or solid solution state in the polymeric network [26].

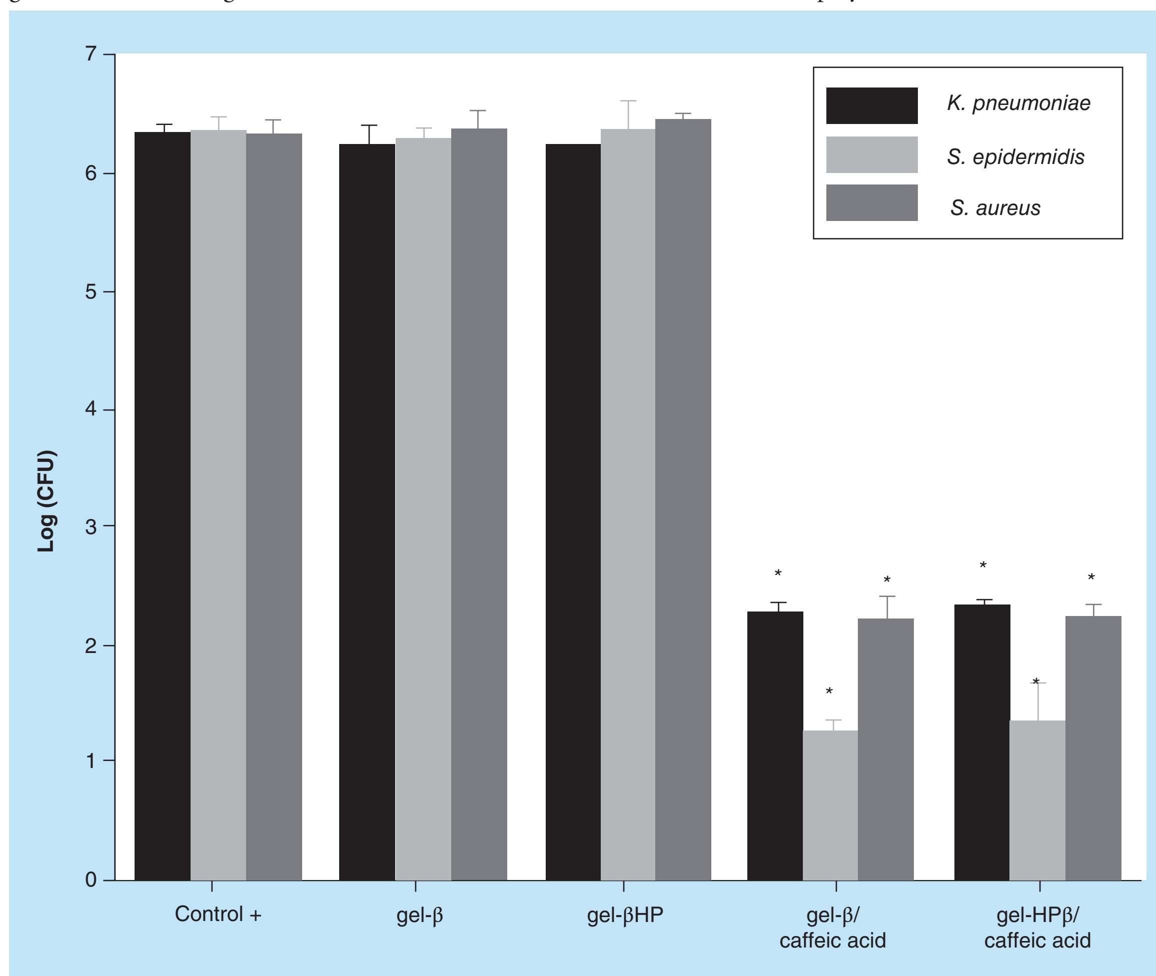

Figure 5. Antibacterial assessment of caffeic acid load hydrogels (90 ± 3 mg) by direct contact with the three bacteria (*K. pneumoniae, S. epidermidis and S. aureus,* 5 × 10^5 cel.ml^{-1}). The control + allowed the perfect growth of the cells. All data are expressed as mean + standard deviation (n = 9). *statistically different from the control +, $p < 0.0001$.
Gel-β: β-cyclodextrin-co-hydroxypropyl methylcellulose; Gel-HPβ: Hydroxypropyl-β-cyclodextrin-co-hydroxypropyl methylcellulose.

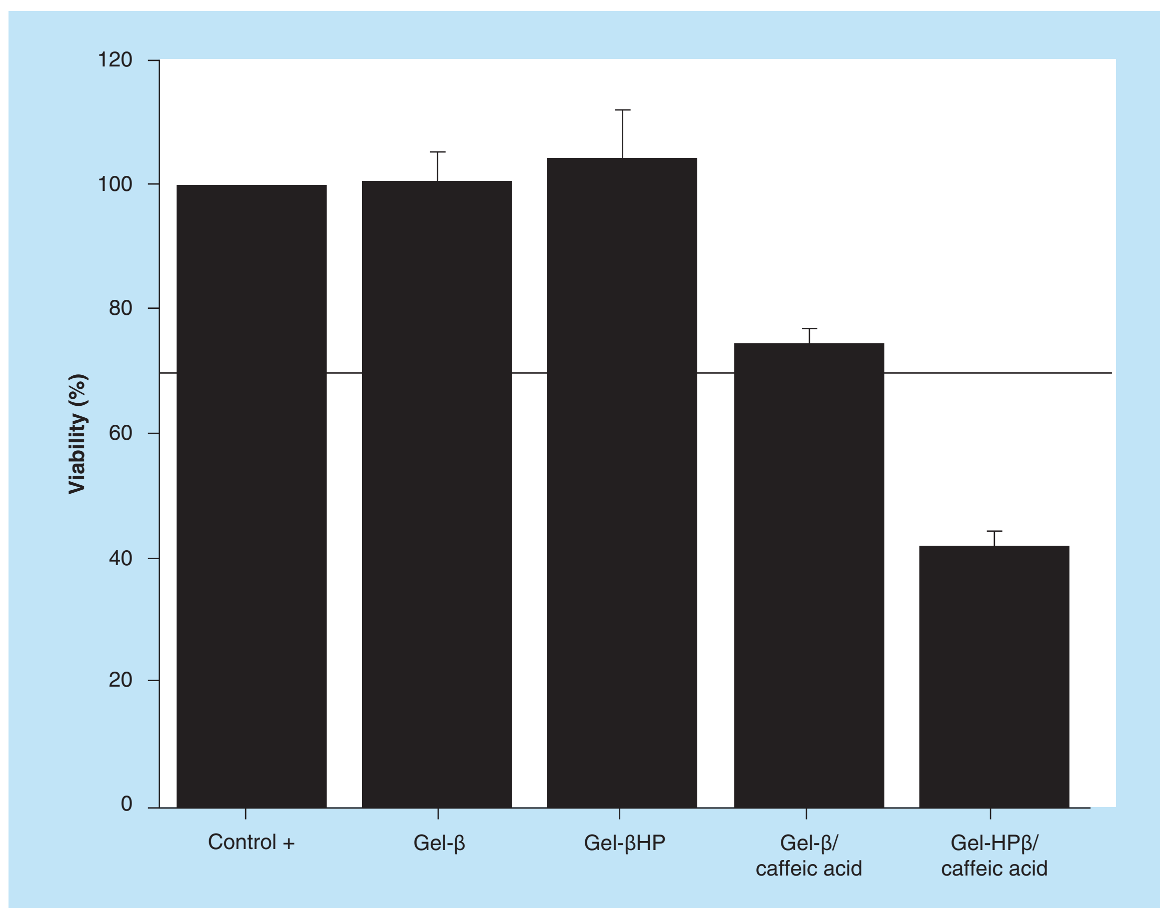

Figure 6. The viability of fibroblast 3T3 after 24 h of contact with liquid extracts from hydrogels (24 h within Dulbecco's modified Eagle's medium), measured with MTS assay. The control + allowed the perfect growth of the cells. All data is expressed as mean + standard deviation (n = 9). The line indicate 70% of cell viability.
Gel-β: β-cyclodextrin-co-hydroxypropyl methylcellulose; Gel-HPβ: Hydroxypropyl-β-cyclodextrin-co-hydroxypropyl methylcellulose.

Regarding the FTIR spectra (Figure 3), the alterations on the peaks' intensity and shape indicate that the polymeric network had less OH and hydrogen bonds, as a consequence of the crosslinking reaction [3,16]. Moreover, the differences between the loaded gel-β and gel-HPβ spectra may result from the presence of caffeic acid in different states on the polymeric networks. In the case of the hydrogel synthesized with HPβCD, the caffeic acid is probably adsorbed in the surface, since the spectra from gel-HPβ and gel-HPβ/caffeic acid had similar profiles. In opposition, the profile of gel-β and caffeic acid/gel-β had visible alterations indicating that the phenolic compound may be within the network, and interacting with the HPMC and/or βCD molecules.

Based on the results obtained, a reaction mechanism is proposed and illustrated in Figure 7. The BDGE has two epoxide groups able to react with the OH from CDs and HPMC, at alkaline pH (condition used), leading to the formation of stable ethers bonds. Thus, the decrease of the OH detected on the FTIR results were a consequence of their consumption for crosslinking reaction.

Furthermore, the physicochemical differences detected between the two hydrogels synthesized (gel-β and gel-HPβ) may arise from the CD' OH group involved in the reaction with BDGE. The CDs have a truncated cone shape (Figure 8) with 21 OH in the case of βCD, and HPβCD has 21 hydroxypropyl and/or OH groups. These groups have different reactivities, as demonstrated in Figure 8. Regarding βCD, the OH-2 and OH-3 (located on the wider side of the rim) are less reactive than the OH-6 (placed on the narrower side and directed away of the cavity), and the reactivity of the latter is improved by alkaline conditions. Moreover, the HPβCD has OH on their substituents with the same reactivity as the OH-6 [3].

Therefore, the gel-β was formed by the interaction of βCD' OH-6 and the epoxy group of BDGE, resulting in a network with lower mobility (Figure 7). In the case of gel-HPβ (Figure 7), CD may be linked to the polymeric structure by the OH-6 and/or by the OH

on the hydroxylpropyl groups, providing more flexibility to the polymeric network and, consequently, more viscoelastic and greater swelling.

Biological properties of gel-β & gel-HPβ

Wound dressing is expected to be biocompatible and, therefore, suitable for contact with injured skin without causing any harm to the user. The hydrogels' surface stated the interaction between the wound dressing and the skin. Those interactions can be predicted by the angle formed between the hydrogels' surface and water or other solvents [27].

The hydrogels' polymeric networks imply the ability of these materials to absorb large amounts of water, so it should be expected to obtain low contact angles, due to the hydrogels' hydrophilic nature [28]. According to the swelling results [21] gel-HPβ should have a lower contact angle than the gel-β, but the opposite was observed. The contact angle value reflects the degree of freedom of the molecules network to move, as a response to the environment change [28]. Therefore, the macromolecules of gel-HPβ have low mobility, decreasing their surface hydrophilicity [21]. Moreover, the enhanced gel-β hydrophilic surface after the caffeic acid loading can be a result of interactions between the caffeic acid and water. At pH 5, the caffeic acid is charged and able to interact with the water molecules [29]. Also, on the IC, the caffeic acid carboxylic group (charge group) is projected out of the βCD cavity, establishing interactions with the water molecules [13]. Thus, the gel-β surface hydrophilicity was increased by the interaction of caffeic acid with water.

Gel-β and gel-HPβ were able to sustain caffeic acid release for 2 days with similar profiles (Figure 4). An initial burst was detected, as a result of the transference of the caffeic acid present in the network aqueous phase to the solution. The remaining delivery was slower, since it depends on the dissociation rate between the CDs and the phenolic. At equilibrium, the gel-HPβ allowed higher release of caffeic acid, almost twice the amount observed for gel-β. At this

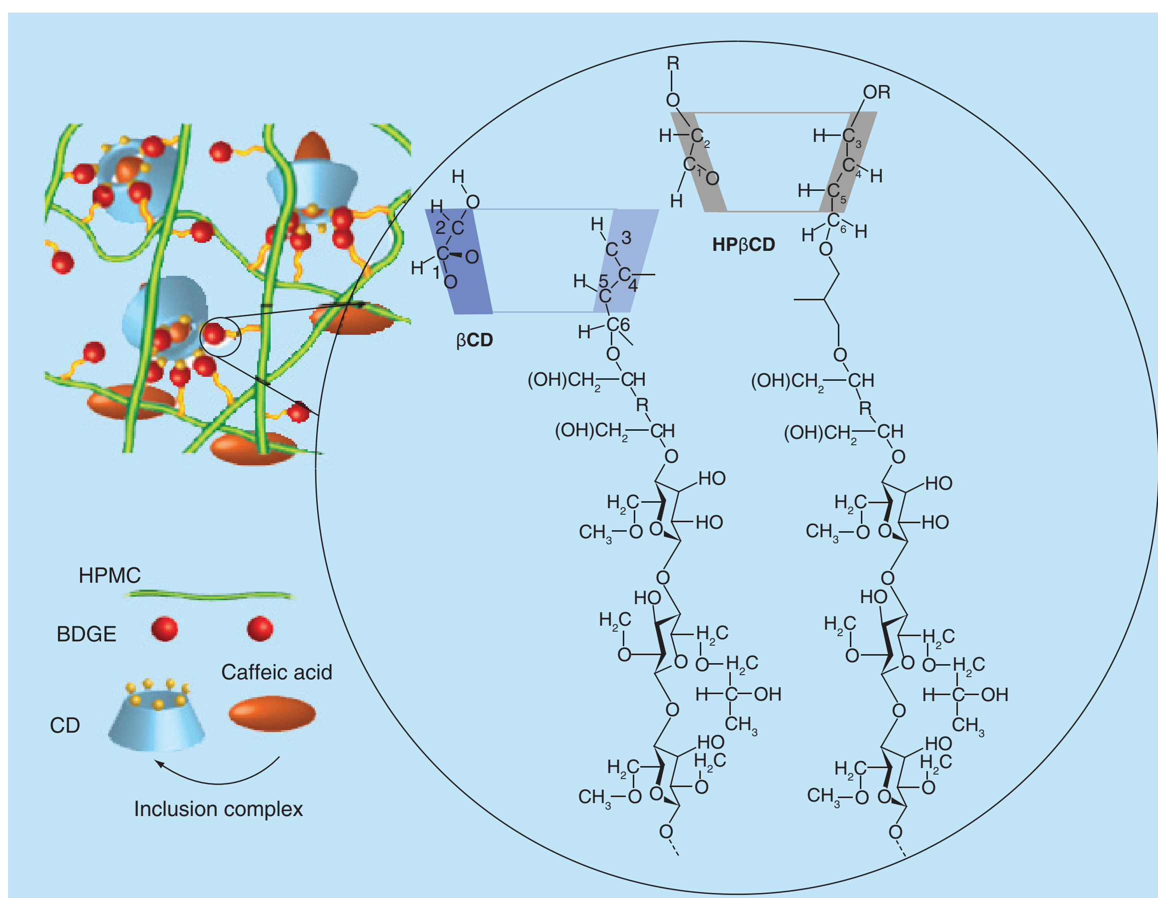

Figure 7. Reaction mechanism between the CYYC (βCD or HPβCD) and HPMC using BDGE, as crosslinking agent. The caffeic acid position within the CD cavities and adsorbed on the polymeric network are also represented.
BDGE: 1,4-butanediol diglycidyl ether; CD: Cyclodextrin; HPβCD: Hydroxypropyl-β-cyclodextrin; HPMC: Hydroxypropyl methylcellulose.

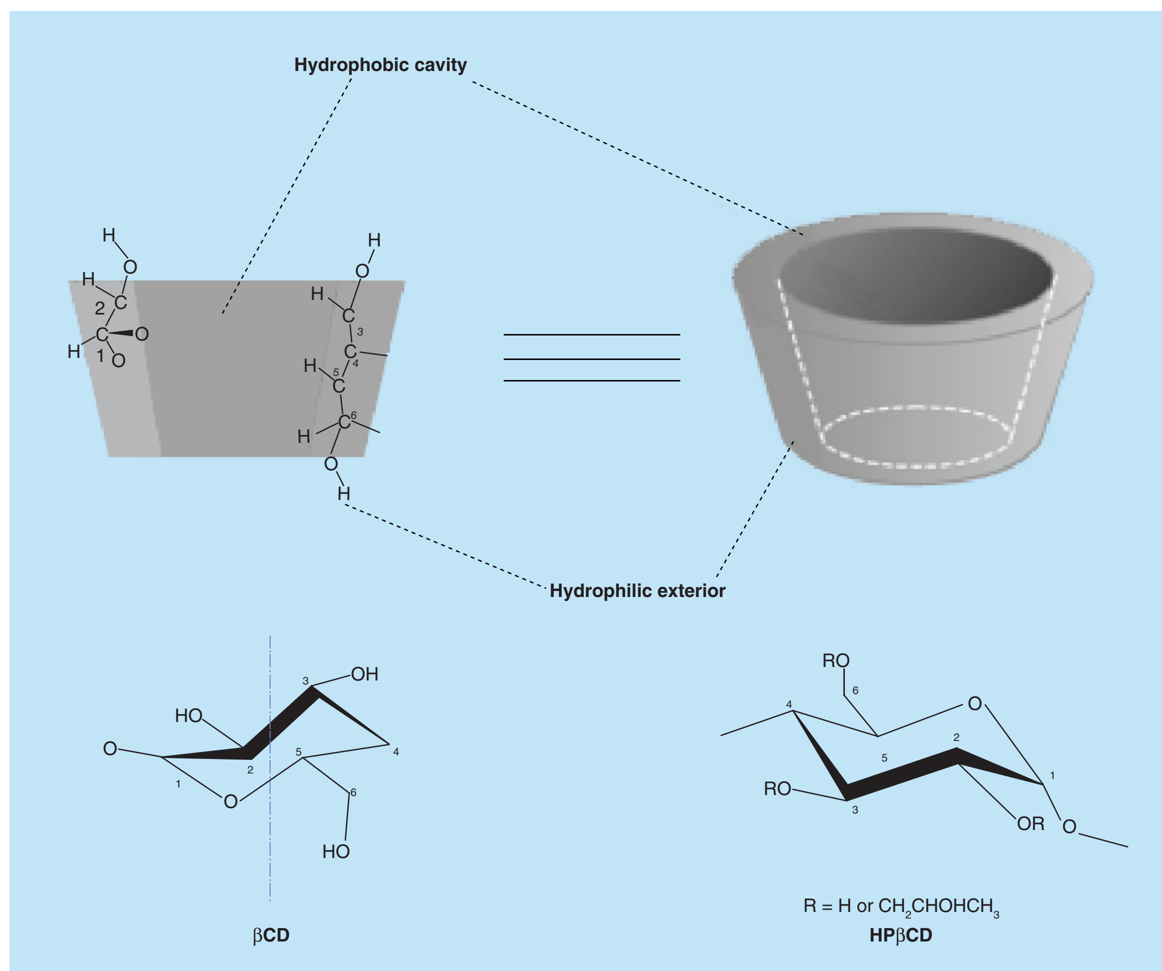

Figure 8. Position of the hydroxyl groups on the truncate shape of each cyclodextrin.
βCD: β-cyclodextrin; HPβCD: Hydroxypropyl-β-cyclodextrin.

point, two processes are involved in the release by CD-based hydrogels: the swelling degree and IC stability. The gel-HPβ had higher swelling capacity, which enhanced the caffeic acid mobility through the network. Additionally, the low gel-β release was a result of hampered caffeic acid dissociation from the CD cavity, since the complex formed with this CD was more stable than HPβCD [21]. This suggests that the sustained release was dependent on the CD ability to retain the caffeic acid in the network, as previously reported for CD-based hydrogels loaded with hydrophobic molecules [3,17,23,28].

The antibacterial properties of the produced hydrogels are directly linked to the material's ability to deliver the caffeic acid. Based on the caffeic acid release results (0.77 $mg.g_{hydrogel}^{-1}$ for gel-β and 1.2 $mg.g_{hydrogel}^{-1}$ for gel-HPβ), the concentration of caffeic acid in contact with bacteria was 77 or 120 mM. Gel-β and gel-HPβ were able to induce meaningful reduction on the bacteria growth regardless of the bacteria (Figure 5).

The antibacterial activity of caffeic acid appears to be deeply linked with the phenolic acid ability to reach the bacteria surface [30]. Thus, the caffeic acid loading into the polymeric networks allowed the caffeic acid interaction with electrons on the bacteria surface leading to a cascade of events. That interaction induced a change in bacterial electric potential and on the cytoplasmic pH. The proton donation causes destabilization and disruption of the cell membrane and, also, hyperacidification of cytoplasm [30]. Important enzymatic pathways can be disrupted leading to a decrease of bacteria growth or even total inhibition [12,31].

As stated above, hydrogels for wound dressing applications have to be friendly to the injury tissue, thus their cytotoxicity evaluation is crucial. Preliminary cytotoxicity assays of gel-β and gel-HPβ were performed based on the membrane integrity of the fibroblast cells, by MTS assay. This method has been often use for *in vitro* cytotoxicity evaluation of polymeric materials, and correlates the mitochondrial status with cell proliferation [2]. The caffeic acid effect on 3T3 fibroblasts was

described as dose-dependent, being safe for concentrations under 7mM [32]. Gel-HPβ allows only 40% of the cells to survive as a reflection of its higher ability to deliver caffeic acid (120 mM after 48 h). However, gel-β showed reasonable interactions with fibroblasts (viable cells above 70%), although the amount of caffeic acid release was 10-times the limit described for free phenolic compound. Accordingly, the caffeic acid loading into gel-β, apparently, reduced its cytotoxicity (Figure 6).

Conclusion

In conclusion, caffeic acid was successfully loaded into the CD-based hydrogels, and its release was kept for 2 days. Moreover, the loaded gel-β and gel-HPβ were capable of destroying most of the bacteria cells preserving the caffeic acid antibacterial activity. Nevertheless, gel-β appeared to be more suitable for healing wounds, since it was friendly to fibroblasts.

Future perspectives

Nowadays, wound dressings are complex materials that, behind the mechanical protection, are also capable of interacting with the injury tissue. These complex products improve the healing process by maintaining suitable conditions, allowing skin to establish integrity with appropriate cosmetic results. The incidence of chronic wounds related to diseases, population ageing and tissue infection caused by multiresistant strains of bacteria has been increasing. Thus, wound dressing research is now focused on the development of new materials with higher specificity in order to improve the quality of patients' life. One of the directions is the use of natural materials for wound dressing production for the development of a friendlier product. Therefore, the developed cellulosic CD-based hydrogels, especially the gel-β, have great potential as efficient carriers of caffeic acid, to be used as wound dressing.

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Executive summary

Objectives of the study

- The main goal of the present work was to incorporate caffeic acid on cyclodextrin-based hydrogels capable of controlling delivery in order to be used as antibacterial wound dressing.

Methods

- Cyclodextrin-based hydrogels were prepared by direct crosslinking of β-cyclodextrin or hydroxypropyl-β-cyclodextrin with 1,4-butanediol diglycidyl ether in the presence of hydroxypropyl methylcellulose. The hydrogels were characterized regarding their physicohemical and biological properties.

Conclusion

- The hydrogels obtained could be useful as caffeic acid delivery system devices for the treatment of wound infections.

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