Freeze-Dried Matrices for Buccal Administration of Propranolol in Children: Physico-Chemical and Functional Characterization

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Abstract

Buccal matrices represent a widely accepted dosage form permitting a convenient, easy, reliable drug administration and reducing administration errors. The aim of this study was the development of mucoadhesive buccal matrices for propranolol administration in children. Matrices were obtained by freeze-drying of drug loaded polymeric solutions based on gum tragacanth (GT), pectin (PEC), hydroxypropylmethylcellulose (HPMC), sodium hyaluronate (HA), gelatin (GEL), chitosan (CH) or a mixture of CH and HPMC (CH/HPMC). Matrices were characterized for drug solid state, morphology, water-uptake, mucoadhesion ability, *in vitro* drug release and permeation through porcine epithelium. The most promising formulations were tested for *in vitro* biocompatibility in human dental pulp fibroblasts. The preparative method and the polymeric composition influenced the drug solid state, as a complete amorphization as well as different polymorphic forms were observed. GEL and PEC guaranteed a fast and complete drug release due to their rapid dissolution, while for the other matrices the release was influenced by drug diffusion through the viscous gelled matrix. Moreover, matrices based on CH and CH/HPMC showed the best mucoadhesive properties, favoured the drug permeation, *in virtue* of CH ability to interfere with the lipid organization of biological membrane, and were characterized by a good biocompatibility profile.

Keywords: Pediatric; Polymeric drug delivery systems; Lyophilization; Solid-state; Mucoadhesive; Buccal delivery.

1. Introduction

Buccal mucosa represents an interesting site for drug systemic administration *in virtue* of several advantages with respect to the conventional oral route, such as by-passing the gastro-intenstinal environment and the first-pass metabolism in the liver. Moreover, buccal route could be an alternative way of drug administration for patients who cannot swallow the solid dosage forms. Buccal administration is also a needle-free procedure able to improve patient compliance.[1-4] All these advantages are of relevant interest especially for paediatric population, that would benefit particularly from an easily accessible and non-invasive administration route.[5, 6]

Development of adequate buccal formulations for children is challenging. In the recent years, several studies have been reported the development of different buccal formulations for drug administration in children, such as mini-tablets, films and matrices.[7-10]Among them, buccal matrices prepared by freeze-drying of different polymeric solutions or gels could represent an innovative and versatile dosage form since they are easy to handle, to apply and to remove, stopping the input of drug whenever desired.[10-12] The application of freeze-drying process in the development of solid matrices ensures to obtain stable pharmaceutical products with long shelf-life, as consequence of the low moisture content.[13] Moreover, due to their high porosity and specific surface area, freeze-dried polymeric matrices offer the advantage of rapid hydration associated with the development of a viscous network, thus ensuring drug release for extended period and improving bioavailability. The freeze-drying process offers also the possibility to enhance the dissolution properties of drug, producing solid dispersions and promoting drug conversion from crystalline to more soluble amorphous form. [13] However, these formulations can be accidentally swallowed or dissolved by saliva, thus determining a low residence time in buccal cavity. For this reason the use of mucoadhesive polymers, which can establish an intimate contact with the buccal mucosa for an extended period of time, could allow to prolong the formulation residence time at the application site (the maximum duration of buccal drug delivery is approximately 4-6 h)[3], thereby reducing the administration frequency.

Unfortunately, the effective barrier properties of the buccal mucosa mean that appropriate physico-chemical characteristics of drug are required. Propranolol is a β-blocker with suitable properties for transmucosal absorption: low molecular weight, medium apparent aqueous solubility, high first-pass metabolism and low systemic bioavailability.[14-16] Moreover, propranolol is commonly used as "off-label" antihypertensive drug, that not been well studied in paediatric clinical trials and dosing/safety/efficacy are largely extrapolated from trials in adults.[17] Despite the wide number of research articles reporting the development of buccal formulations containing propranolol,[18-25] only the previous work of our research group[26] proposed a buccal dosage form for paediatric use. In particular, bilayered buccal films, based on polyvinylpyrrolidone or polyvinylalcohol combined with different weight ratios of gelatin or chitosan, showed good mucoadhesion properties and were able to promote propranolol permeation through buccal mucosa.

In the present study we developed buccal matrices for the systemic administration of propranolol in paediatric patients able to adhere to the buccal mucosa, control drug release and facilitate its absorption. Shortly, the main steps concerned: 1) preparation by freeze-drying process of hydrophilic matrices based on non-toxic, non-irritant, widely available polysaccarides and proteins, such as gum tragacanth (GT), pectin (PEC), hydroxypropylmethylcellulose (HPMC), sodium hyaluronate (HA), gelatin (GEL), chitosan (CH) and a mixture of CH and HPMC (CH/HPMC); 2) drug solid state characterization; 3) evaluation of matrix mucoadhesive behaviour; 4) investigation of drug release and permeation ability; 5) assessment of biocompatibility profile.

2. Materials and methods

2.1 Materials

Type B gelatin from bovine skin (GEL; MW 50 kDa, isoelectric point in the range of pH 4.7-5.2), low-viscosity chitosan from shrimp shells (CH; MW 150 kDa, deacetylation degree 96-98 %, pKa = 6.3) and mucin from porcine stomach (type II, bound sialic acid ~1%) were obtained from Sigma-Aldrich (Milan, Italy). Pectin from citrus peel (PEC; MW 30-100 kDa, esterification degree 60 %,

pKa = 4.0) was purchased from Fluka (Milan, Italy). Hydroxypropylmethylcellulose (HPMC; Benecel™ K100M PHARM, MW 1000 kDa) was obtained from Ashland (Ashland, Switzerland). Sodium hyaluronate (HA; MW 1800-2300 kDa, D-glucuronic acid > 42 %, pKa = 2.9) and gum tragacanth (GT; MW 850 kDa, pKa = 3.0) were provided by ACEF (Piacenza, Italy). Propranolol hydrochloride (MW 295.807 Da, pKa = 9.5) was purchased from Polichimica (Bologna, Italy). Dulbecco's Modified Eagle Medium (DMEM), L-glutamine, fetal bovine serum (FBS), penicillin–streptomycin, thiazolyl blue tetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were from Merck KGaA (Darmstadt, Germany). Human dental pulp fibroblasts (HPFs) were kindly provided by Prof. M. Falconi and Prof. G. Teti (University of Bologna). All other chemicals were of analytical grade and were obtained from Carlo Erba (Milan, Italy).

Water-uptake and release studies were performed in buffer solution at pH 6.8 composed of 8.38 mM $Na_2HPO_4 \cdot 12H_2O$, 7.35 mM KH_2PO_4 and 94.11 mM NaCl. For permeation tests a buffer solution at pH 7.4 (PBS) based on 7.4 mM $Na_2HPO_4 \cdot 10H_2O$, 1.1 mM KH_2PO_4, 136 mM NaCl was used. For *in vitro* biocompatibility test an aqueous phosphate buffer at pH 7.2 composed of 171 mM NaCl, 3.3 mM KCl, 12.7 mM Na_2HPO_4, 1.4 mM K_2HPO_4 was used.

2.2 Preparation of freeze-dried matrices

Matrices were produced by freeze-drying of solutions with a polymer concentration of 1.5 % w/w. The polymeric solutions were prepared by dissolving GT, PEC, HPMC or HA in demineralized water at room temperature, while GEL was dissolved in demineralized water previously heated at 50 °C. CH on the other hand was dissolved in acetic acid solution (1 % w/w) at room temperature. On the basis of mucoadhesion and permeation results, a matrix based on CH and HPMC mixture (CH/HPMC) was also prepared by adding a CH solution to the HPMC one (polymer weight ratio 1:1) in order to obtain a final polymer concentration of 1.5 % w/w. The viscosity of polymeric solutions (0.5 % w/w, 25 °C) was measured thorugh a falling ball viscometer (HAAKETM Falling Ball Viscometer Type C, Thermo electron corporation, Karlsruhe, Germany). For the preparation of

loaded formualtions, an aqueous solution of propranolol hydrochloride was added to the different polymeric solutions (final drug concentration 1.66 % w/w), stirred for 24 h at 200 rpm and left overnight to eliminate air bubbles. Then, about 0.5 g of solution were placed into each cavity (diameter 13 mm) of a blister pack (Farmalabor, Canosa di Puglia, Italy), frozen overnight at -20 °C, freeze-dried at 0.01 atm and -45 °C (Christ Freeze Dryer ALPHA 1-2, Milan, Italy) and stored in a desiccator until use.

Matrices were measured for diameter and thickness through an electronic digital caliper (art. 1367 E 2900, Shanghai ShangErBo Import & Export Co., Shanghai, China). For the determination of drug content, each matrix was dissolved in 40 mL of PBS and the solution was analyzed by a previously reported HPLC method.[26]

2.3 Physico-chemical characterization

Differential scanning calorimetry (DSC) and Powder X-ray diffraction (PXRD) experiments were performed on loaded matrices to characterize the drug solid state. A Netzsch DSC200 PC differential scanning calorimeter (Netzsch, Germany) was used to perform calorimetric measurements (Temperature from 40 °C to 250 °C, heating rate of 5 °C/min), accordingly to the method described by Abruzzo and co-workers.[26]

The PXRD patterns of all samples were measured at 25 °C by using a Bruker D2 PHASER Diffraction System equipped with 1D high speed solid state LinxEye detector and Cu-Kα radiation (λ = 1.54056 Å) at 30 KeV and 10 mA. Analysis were conducted by placing the samples on a zero background sample holder, in the 2θ angle scan range of 5.0-50 degree. The step scan mode was performed with a step width of 0.02 and scanning speed of 8°/min. Data were evaluated with the Bruker program EVA.

Fourier trasform infrared (FTIR) spectra (4000-400 cm^{-1}, KBr pellets) of the samples were recorded on a model FTIR 4200 JASCO spectrophotometer, with a resolution of 4.0 cm^{-1}.

2.4 Moisture content

The moisture content was determined to evaluate the efficiency of freeze-drying to eliminate water from matrices.[27,28] The matrices were weighed after the freeze-drying (W_i) and then heated to 100 °C until a constant weight (W_f) was reached. The moisture content was estimated as percent weight loss following the equation below:

Moisture content % = $(W_i - W_f) \times 100/W_i$

2.5 Scanning electron microscopy (SEM)

SEM analysis were conducted to investigate the internal morphology of the matrices following the method reported by Abruzzo and co-workers.[26] Matrices were observed with LEO 420 (LEO Electron Microscopy Ltd., Cambridge, UK) using secondary electron imaging at 15 kV.

2.6 Water uptake ability

Water uptake studies were performed for 360 minutes by a gravimetric method following the procedure described by Bigucci and co-authors[29] in order to evaluate matrix hydration ability. Briefly, accurately weighted matrix was placed on a sponge previously soaked in phosphate buffer at pH 6.8, simulating human saliva pH.[30] Water uptake (WU) was determined as weight increase of the matrix for 360 minutes according to the following equation:

$WU\ (\%) = (W_{HM} - W_{DM}) \times 100/ W_{DM}$

where W_{HM} is the weight of hydrated matrix and W_{DM} is the weight of the dry matrix.

2.7 Mucoadhesion ability

Mucoadhesion ability is a crucial property to ensure a contact with the buccal mucosa and consequently to provide high drug concentration at the administration site. The force needed to pull out a freshly porcine esophageal mucosa from polymeric matrix was measured with an adapted tensiometer (Krüss 132869; Hamburg, Germany), accordingly to the method described in the work

reported by Abruzzo and co-workers[26] with slight modifications. Porcine esophageal mucosa was selected *in virtue* of its high similarity with the buccal one.[31] Briefly, mucosa was hydrated for 5 minutes with mucin suspension (0.05 % w/v) in phosphate buffer at pH 6.8. The mucosa was lowered until it just contacted the surface of the matrix. A $1x10^{-4}$ N force was applied to the matrix for 30 seconds. Then the mucosa was raised until it was separated from the layer with a withdraw speed of 0.5 mm/s. This point represents the adhesive bond strength between mucosa and matrix and is expressed as a positive force in Newton.

2.8 In vitro release studies

In vitro release studies were performed in order to evaluate the drug amount released from matrices over the time. The matrix was attached on a glass slide using cyanoacrylate adhesive and immersed in 40 mL of phosphate buffer at pH 6.8 under agitation (sink conditions were assured). The attachment on the glass slide is necessary to avoid matrix floating during the test.[11, 32] Aliquots of 1 mL were withdrawn at different time intervals until 360 minutes, replaced with fresh medium, and analyzed by HPLC.[26] Results are shown as percentage cumulative released drug plotted as a function of time.

2.9 In vitro permeation studies

To evaluate the cumulative amount of permeated drug from matrices through porcine esophageal epithelium,[33] a Franz-type static glass diffusion cell (15 mm jacketed cell with a flat ground joint and clear glass with a 12 mL receptor volume; diffusion surface area = 1.77 cm^2), equipped with a V6A Stirrer (PermeGearInc., Hellertown, PA, USA) was employed. Permeation studies were conducted accordingly with the method used by Abruzzo and co-workers.[26] Polymeric matrix was placed in the donor chamber on the esophageal epithelium, previously hydrated with 200 µL of phosphate buffer at pH 6.8, simulating human saliva pH. The receptor medium based on phosphate buffer at pH 7.4 was maintained at 37 ± 0.5 °C and continuously stirred. An aqueous solution (100

μL) of propranolol hydrochloride (6 mg/mL) was also tested for 360 minutes. The results of permeation studies are shown as percentage cumulative permeated drug plotted as a function of time.

To quantify the drug retained in the esophageal epithelium, permeation studies were conducted for 120 minutes in the presence of the most promising formulations selected on the basis of mucoadhesive and drug permeation properties (CH, PEC, GT and CH/HPMC). After this time, the excess of formulation was discharged with the use of a spatula, the epithelium was removed from the apparatus and was washed 3 times with 1 mL of phosphate buffer at pH 7.4. Then, it was cut into small pieces with scissors and placed under stirring at 200 rpm with 1 mL of 0.2% (v/v) H_3PO_4 overnight.[34] Each sample was vortexed for 3 min and centrifuged at 10,000 rpm for 15 min (GS-15R Centrifuge, Beckman Coulter, Milan, Italy). Finally, supernatants were filtered through filters with 0.45 μm pore diameter (MF-Millipore Membrane, Tullagreen, Carrigtwohill, Co. Cork, Ireland) and analyzed by HPLC.

2.10 Biocompatibility test in human dental pulp fibroblasts

The most promising formulations (CH, GT, PEC or CH/HPMC) were selected to investigate the *in vitro* biocompatibility in human pulp fibroblasts (HPFs) through MTT assay. Firstly, each formulation with (loaded formulations) or without propranolol hydrochloride (unloaded formulations) was immersed in 5 mL of DMEM- High glucose complete medium at 37 °C for 6 h. The semisolid formulations were then removed from medium and the obtained extracts were filtered through 0.45 μm Millipore filters. The extracts, as well as propranolol solution (obtained by dissolving the drug in the medium), were used for MTT assay.

A stock MTT solution (5 mg/mL in phosphate buffer at pH 7.2) was prepared and filtered through a 0.22 μm Millipore filter. HPFs were seeded into a 96-well culture plate in DMEM containing 10% FBS, 1% penicillin and streptomycin, according to the method reported by Zago and co-workers. [35] After 24 h, the medium was removed and cells were incubated for 6 h, three of which in the

presence of MTT solution at a final concentration of 0.5 mg/mL at 37 °C and 5% CO_2 in a humidified atmosphere. Subsequently, the medium was gentle removed and the blue violet formazan product was dissolved with DMSO. The absorbance of solutions was measured at 570 nm, using a multiwell plate reader (Wallac Victor 2, PerkinElmer, Waltham, Massachusetts, U.S.). Cell viability (% of control) is the ratio of the values of the cells treated with formulations and the values of the control.

2.11 Statistical analysis

Results are expressed as mean ± SD of three replicas, with except for permeation studies (five replicas) and MTT assay (four replicates). *t*-test was used to determine statistical significance of results ($p < 0.05$). One-way ANOVA ($p < 0.05$) followed by Bonferroni's test ($p < 0.05$) was used to assess statistical differences of biocompatibility results. The statistical analysis were performed with GraphPad Prism 5.0 software (San Diego, CA, USA).

3. Results and discussion

The objective of this work was to prepare freeze-dried matrices based on different polymers (CH, GT, PEC, HPMC, HA, GEL) for propranolol administration in children. Moreover, CH and HPMC, showing the best properties in terms of drug permeation and mucoadhesion, respectively, were mixed in order to explore the possibility to obtain a formulation with improved functionality.

All the prepared polymeric solutions allowed to obtain matrices by freeze-drying process. The matrices were easy to handle and to remove from blisters, with no or minimum damage. Moreover, they showed cylindrical shape with a diameter ranging between 12.68 and 13.21 mm and a thickness ranging between 2.76 and 3.11 mm. As reported in Table 1, the experimental drug content was similar to the theoretical one (8.3 mg) for each matrix, suggesting that the preparative method is adequate to prepare matrices with a fixed amount of propranolol hydrochloride.

3.1 Physico-chemical characterization

3.1.1 Differential scanning calorimetry (DSC)

The DSC profile of propranolol hydrochloride (Fig. 1h) showed its melting at 165.1 °C. The thermograms of freeze-dried matrices generally showed one endothermic peak at temperature lower than 100 °C, corresponding to the release of water molecules and an exothermic peak beyond 200 °C, corresponding to the polymer decomposition. Furthermore, the freeze-dried matrices, with except for GEL (Fig. 1d), showed a second endhotermic peak at a temperature lower than propranolol hydrochloride melting point accounting for a not-complete drug dissolution in the matrices. The absence of any endhotermic peak of the propranolol in the GEL profile suggested a complete drug amorphization. Finally, in the CH profile (Fig. 1c) the presence of a new peak at 113.5 °C was observed, probably due to a change of the drug physical state or a possible interaction between the drug and chitosan.

3.1.2 Powder X-ray diffraction (PXRD)

In order to better investigate the drug solid state, PXRD analysis were performed. It is known that the presence of the drug in different physical states can affect important functional properties, such as solubility, release, permeation, toxicity and subsequently therapeutic outcome. [36] The PXRD patterns of freeze-dried matrices confirmed a different degree of dispersion of the crystalline starting drug. Indeed, the way to achieve the maximum homogeneous drug dispersion in a polymeric matrix is to obtain a random drug distribution of particles rather than a simple dissolution of the crystalline drug into the polymeric matrix. In other words, amorphous matrices are expected when the maximum of drug dispersion is realized. As it is shown in Fig. 2a, the PXRD pattern of the crystalline polymorph II propranolol hydrochloride used in this work (characterized by the significant reflection positions at 8.5°, 9.2°, 12.6°, 12.9°, 16.8°, 17.3°, 18.7°, 19.4°, 19.8°, 21.3°, 25.2)[37, 38] turned into a PXRD pattern typical of amorphous state, when the drug is loaded into GEL. This result confirmed the drug amorphization observed in the DSC experiment. As reported in

literature, the presence of the drug in a higher energy solid state form, such as completely amorphous, has the potential of increasing the dissolution rate as less energy is required to break the drug crystalline structure during the dissolution process. [39] However, the amorphous state is usually less stable than the corresponding crystalline form and its tendency to undergo unwanted physical changes and also chemical degradation over the shelf life of the formulation is another crucial issue.[40]

A certain degree of drug crystallinity is kept when other polymers are used. In particular, for PEC, HPMC, CH/HPMC the same polymorphic form II of the propranolol hydrochloride was observed (with a degree of crystallinity increasing with the following order PEC< HPMC< CH/HPMC; Fig. 2b), meaning that no evident polymorphic modification was induced by the different polymeric composition.

On the contrary, the dispersion of the crystalline polymorph II into GT and HA not only produced a different degree of amorphization, but also an evident partial conversion of the drug into a different crystalline form. Indeed, the PXRD patterns recorded on both samples showed the characteristic peaks of the propranolol hydrochloride crystalline polymorph I, centred at 2θ 7.3°, 11.8°, 14.5°, 17.8° and 23.9° (Fig. 2c). However, a difference arised between the two polymeric matrices. In fact, as shown by the PXRD pattern in Fig. 2c, when the drug was dispersed into GT most of propranolol hydrochloride underwent the amorphization, while a minimum amount remained in its crystalline form. On the other hand, in the case of HA, the PXRD pattern proved that a discrete amount of drug in its crystalline form was only mixed into the matrix (as shown by the presence of the typical crystalline pattern of reflections of polymorph II).

As regard the presence of polymorph II (HPMC and CH/HPMC) and polymorph I (GT and HA), Bartolomei and co-workers defined that they are stable at room temperature even after grinding and compression. Moreover, the authors reported that polymorph I was characterized by higher solubility with respect to the polymorph II; this aspect could imply a faster drug release and permeation.[37]

When the matrix was prepared by using CH, the PXRD pattern showed the presence of crystalline drug into the matrix (Fig. 2d). This result was different than results observed in literature, where spray-dried chitosan microparticles loaded with propranolol hydrochloride have revealed the absence of drug crystals and the amorphous state of the polymer.[41] Moreover, the main reflection peaks observed in the PXRD pattern did not correspond to any of the drug crystalline polymorphs reported up to now. Indeed, the 2θ values of 6.4°, 11.2°, 12.2°, 14.2°, 15.3°, 16.3°, 20.5°, 21.7°, 22.9°, 24.7° and 26.6° are unique and indicative of the formation of a new propranolol hydrochloride polymorph. The presence of a new polymorph in CH could impact on functional properties of the matrix as well as toxicity on buccal mucosa. Since the new drug polymorph observed in CH matrix was not reported up to now, in the near future a deeper investigation will be necessary in order to determine its physico-chemical properties and stability during storage period.

3.1.3 Fourier trasform infrared (FTIR) measurements

The analysis of the frequencies of the fundamental bands attributed to propranolol hydrochloride in its polymorphic I and II forms has proved a partial conversion of the drug into a different crystalline form when dispersed into the polymeric matrices. Indeed, the FTIR spectrum (1700-700 cm^{-1} range) of the drug loaded into GEL showed both the fine structure and the specific bands, although in mixture, of the propranolol hydrochloride polymorphic form I. As shown in Fig. 3a, the two specific frequencies at 1142.6 and 928.5 cm^{-1} identificative of the propranolol hydrochloride form II, were changed and new bands appeared at 992.2 and 910.2 cm^{-1} clearly associated to the presence of form I.[37] This result has been not pointed out through the PXRD analysis, due to the highly amorphous nature of the polymeric dispersion. However, considering the set of matrices PEC, HPMC and CH/HPMC (Fig. 3b reports the FTIR spectrum of HPMC), where a certain degree of crystallinity of the drug is kept (as highlighted by PXRD analysis), the FTIR results confirmed that the propranolol hydrochloride crystalline form II was prevalent, with the evident typical frequency at 929.5 cm^{-1}.

As it has been revealed from the PXRD pattern of HA (Fig. 2c), the presence of both forms I and II of propranolol hydrochloride also resulted from the FTIR spectra, in which both specific peaks at 910.2 and 899.6 cm^{-1} were evident (Fig. 3c). Finally, the FTIR finger print of CH is shligthly different than that of polimorh II of propranolol hydrochloride, with the two specific frequencies at 1142.6 and 928.5 cm^{-1}, absent the first and moved to 931.4 cm^{-1} the second (Fig. 3d). Moreover, the distinct band at 960.3 cm^{-1} evident in the FTIR spectrum of polymorph II, splitted into the frequencies at 969.0 cm^{-1} and 961.3 cm^{-1}. The absence of any characteristic bands of the known propranolol hydrochloride polymorphs pointed towards the conclusion that a new propranolol hydrochloride polymorph is partially formed, as also evidenced by DSC and PXRD results. As discussed above, the presence of the drug in different physical states could affect the functional properties of the matrices.

3.2 Moisture content

Following freeze-drying process, all the formulations showed an acceptable moisture content ranging from 1.2 to 4.2 % (Table 1) which confirmed the efficiency of drying step and its ability to remove water from the formulations.[28]

3.3 Scanning electron microscopy (SEM)

The inner morphology has an important role in the hydration properties of matrices. Generally, a high porosity promotes the entry of water, which is expected to influence mucoadhesion ability, drug release and permeation. Fig. 4 showed the morphology of the matrices observed by SEM. The sublimation of the frozen water by freeze-drying process yielded to the formation of uniform network with different morphology. In particular, PEC, GT, HA, HPMC and CH/HPMC presented a porous network, CH showed a leaf-like morphology, while GEL was characterized by a more compact inner structure.

3.4 Water uptake ability

Suitable water uptake ability of a buccal formulation is an important property to control drug release and also to obtain effective mucoadhesion.[42] *In vitro* water uptake profiles of freeze-dried matrices are reported in Fig. 5. Matrices were characterized by different water uptake ability primarly depending on the polymeric composition. Specifically, matrices based on polymers containing charged carboxylic (PEC, GT, HA) or aminic (CH) groups showed higher water uptake values with respect to the non-ionizable HPMC ($p < 0.05$).[43,44] Moreover, all water uptake profiles were biphasic with a fast initial phase (within 15-30 minutes) followed by a phase with a reduced water uptake rate (HA, HPMC and CH/HPMC) or a plateau phase (CH, GT, PEC, GEL). Water uptake of HA was slow and after 15 minutes linearly increased over the time (878.12 ± 24.98 % at 180 minutes); at the end of the study its value was the highest among all the matrices. Indeed, despite its pKa (2.9), the initial hydration of HA led to the formation of a highly viscous layer that represented a diffusion barrier for the water influx, accordingly with results of Bertram and Bodmeier.[45] Similarly to HA, HPMC showed a slow and linear increase of hydration after 30 minutes. PEC and GT presented similar water uptake values over the time (895.17 ± 61.88 % for PEC and 870.00 ± 59.35 % for GT at 180 minutes), *in virtue* of their dissociation degree (pKa values are 4.0 and 3.0 for PEC and GT, respectively). CH showed a greater hydration ability (pKa= 6.3; 1116.20 ± 56.81 % at 180 minutes) with respect to PEC and GT, probably due to the high presence of positively charged amino groups generated by protonation in the acidic medium used for matrix preparation. The mixture CH/HPMC provided an intermediate water uptake behaviour with respect to CH and HPMC alone (808.80 ± 72.58 % at 180 minutes), accordingly to its polymeric composition. Differently from the polysaccharide matrices (PEC, GT, CH, HA and HPMC), the polypeptide nature of GEL (isoelectric point in the range of pH 4.7-5.2) provided a low water uptake (80.14 ± 38.82 % at 180 minutes) despite the presence of ionizable groups of aspartic acid, lysine, arginine and histidine. This result could be also attributed to the its more compact morphology (as shown in Fig. 4), that hinders the water entry inside the matrix.

3.5 Mucoadhesion ability

After buccal administration, matrices have to hydrate and polymeric chains have to interdiffuse into the mucus substrate and develop attractive forces.

Several polymeric properties influence the mucoadhesion ability, such as molecular weight, chain flexibility, charge, hydrogen bonding capacity, cross-linking density, and hydration ability.[46] Mucoadhesion results are reported in Table 1. HPMC showed a higher mucoadhesion ability than other polymers ($p < 0.05$), with the exception of CH and CH/HPMC ($p > 0.05$). HPMC is able to bind mucin *in virtue* of the chain entanglement and physical interlock with mucus and the presence of many hydrophilic groups that can establish hydrogen bonds.[47] Also the negatively charged polymers (GT, PEC, HA) could chemically and physically interact with mucin, even if the presence of negatively charged groups on polymeric chain led to repulsive electronic interactions with negatively charged sialic acid (pKa 2.6) and sulphate residues. As regard PEC, it was also reported that the electrostatic repulsion may cause uncoil of the polymeric chain, which facilitates entanglement and bond formation.[48] CH is characterized by the presence of OH and NH_2 groups able to establish hydrogen bonds and a linear chain with a sufficient flexibility to interact with mucin.[49] Moreover, the positively charged amino groups of CH are able to interact with negatively charged groups of mucus. GEL showed the lowest mucoadhesion ability probably due to its molecular weight (50 kDa) since it has been reported that a minimum polymer molecular weight of 100 kDa is required for mucoadhesion.[50] Moreover, GEL ability to adhere to the mucosa could be negatively influenced by its poor hydration (Fig. 5) that provided a limited interpenetration of peptide chains into the mucus layer.

3.6 In vitro release studies

The drug release from freeze-dried matrices can be affected by different factors such as their hydration, swelling and/or erosion/dissolution.[51] In addition, the solid state of the drug could impact

on drug release taking into account that generally, the amorphous form of a drug, characterized by a higher water solubility, is expected to have higher rate of dissolution.[39] As can be seen in Fig. 6, GEL and PEC allowed to obtain a fast and complete release of propranolol hydrochloride within 60 minutes. This behaviour could be mainly related to the fast hydration and dissolution of the matrices in the release medium.[52] Moreover, the complete or partial amorphization of propranolol hydrochloride into GEL and PEC could favour its solubilization. Matrices based on CH, GT, CH/HPMC, HPMC and HA showed a reduction of drug release with respect to GEL and PEC, probably as a function of a dual effect: the presence of propranolol in different physical state and its limited diffusion through the gelled matrix after hydration. However, despite the higher solubility of polymorph I (GT and HA) with respect to the polymorph II (CH/HPMC and HPMC), [37] results obtained in this study revealed that the rate limiting step of drug release was its diffusion through the gelled matrix, in agreement with previous findings. In fact, Portero and co-workers related insulin release from gelled chitosan sponges to the viscosity of polymeric solutions used for sponge preparation. They observed that an increase in the viscosity of starting chitosan solution determined a decrease of the drug release from sponges.[53] Similarly, in our work propranolol release from gelled matrices decreased with the increase of the viscosity of the starting polymeric solution (values reported in Table 1). More viscous solution produce network with higher viscosity after hydration in the release medium, that hinders the drug diffusion and release.[26] Specifically, in the case of CH, GT and CH/HPMC, showing a similar viscosity, no significant difference was observed in the released drug amount over the time, while HA provided the lowest drug release.

3.7 In vitro permeation studies

In vitro permeation studies were conducted to evaluate drug transport across the buccal epithelium to the systemic circulation. A sustained drug permeation over the time was obtained with all the formulations (Fig. 7). Matrices based on GEL, HA, HPMC, GT and PEC provided the permeation of a low drug amount within 360 minutes. The greater drug permeation observed in the case of CH

and CH/HPMC could be probably attributed to the well-known CH property to promote drug permeation across the buccal mucosa. In fact, as described in literature CH is able to interfere with the lipid organization of the buccal epithelium, thus enhancing drug transport.[54] The potential systemic capacity of the matrices can be predicted by the theoretical human plasmatic steady-state concentration (Css), using the following equation:

$$Css = (S \times J) / Cl$$

where J is the flux determined from the slope of the linear portion of the curve obtained by plotting the cumulative amounts of drug permeated per unit area against time, S is the application area on the buccal mucosa (or surface of matrices 1.33 cm^2) and Cl is the drug clearance (9.2 mL/min/kg).[55] Table 2 reports fluxes obtained with the different matrices and Css calculated considering a children of 6 years of age with body weigth around 25.7 kg. Accordingly to these theoretical data, it was possible to achieve propranolol concentrations within the therapeutic range with the use of matrices based on CH, GT, PEC or CH/HPMC.

3.8 Biocompatibility test in human dental pulp fibroblasts

The effect of unloaded and loaded matrices based on CH, GT, PEC or CH/HPMC on cell viability was determined by estimation of living cell competence to reduce thiazolyl blue tetrazolium bromide, also known as MTT assay.[56] Figure 8 shows the graphs relating to the viability of cells incubated with unloaded/loaded formulations and drug solution for 6 h. Unloaded matrices did not impair cell survival, thus demonstrating the safety of matrix composition. Regarding loaded formulations, the analysis of propranolol retained inside the epthelium showed that the maximum drug concentration was lower than 0.016 mg/ml. The extracts, as well as propranolol solution, were diluted in order to obtain a drug concentration equal to 0.016 mg/ml and used for MTT assay, accordingly to the method reported by Zanela da Silva and co-workers.[57] Cell viability incubated with the different loaded formulations and drug solution is not significantly different ($p > 0.05$) from viability of control cells. This result allow us to argue that the application of the selected

loaded matrices resulted safe for buccal use. Moreover, the new drug polymorph in CH, evidenced by DSC, PXRD and FTIR studies, did not influence cell viability, thus demonstrating its biocompatibility.

Conclusions

The developed matrices could represent innovative child-appropriate formulations, by combining the advantages of solid dosage forms and the improvement of children compliance. Freeze-dried matrices based on different polysaccarides and proteins were prepared and characterized for their physico-chemical and functional properties. Their hydrophilic nature guarantees the entry of water and the consequent formation of viscous networks with different drug release ability. Matrices based on CH and CH/HPMC showed good mucoadhesion properties, allowed to obtain the predicted plasma concentration at the steady state and possessed a good biocompatibility profile.

Considering that the organoleptic characteristics represent a crucial factor to guarantee the patient acceptability, especially for children, further studies will be performed in order to mask the propranolol bitter taste. In particular, the inclusion of some excipients suitable for pediatric use into the formulations, such as sweetening and flavoring agents, will be considered.

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Research Data: the raw research data used in preparation of the manuscript is not available publicly.

Declaration of interest: none.

Figure captions

Fig. 1. DSC thermograms of freeze-dried matrices and drug: GT (a), HA (b), CH (c), GEL (d), HPMC (e), PEC (f), CH/HPMC (g) and propranolol hydrochloride (h)

Fig. 2. X-ray diffraction patterns of propranolol hydrochloride together with (a) GEL (red), (b) CH/HPMC (green trace), PEC (light-blue trace) and HPMC (dark-red trace), (c) GT (blue trace) and HA (orange trace) and (d) CH (light violet trace)

Fig. 3. FTIR spectra in the fingerprint region (1700-700 cm^{-1}) of propranolol hydrochloride in its polymorphic II form (green trace) superimposed with (a) GEL, (b) HPMC, (c) HA and (d) CH (black trace). Specific peaks for the two polymorphic forms are pointed with arrows

Fig. 4. SEM images for the cross-section of the polymeric matrices

Fig. 5. *In vitro* water uptake profiles of the polymeric matrices

Fig. 6. *In vitro* release profiles of propranolol hydrochloride from polymeric matrices

Fig. 7. Permeation profiles of propranolol hydrochloride through esophageal porcine epithelium from drug solution and polymeric matrices

Fig. 8. Biocompatibility of propranolol hydrochloride, unloaded and loaded matrices assessed in human dental pulp fibroblasts by means of MTT assay

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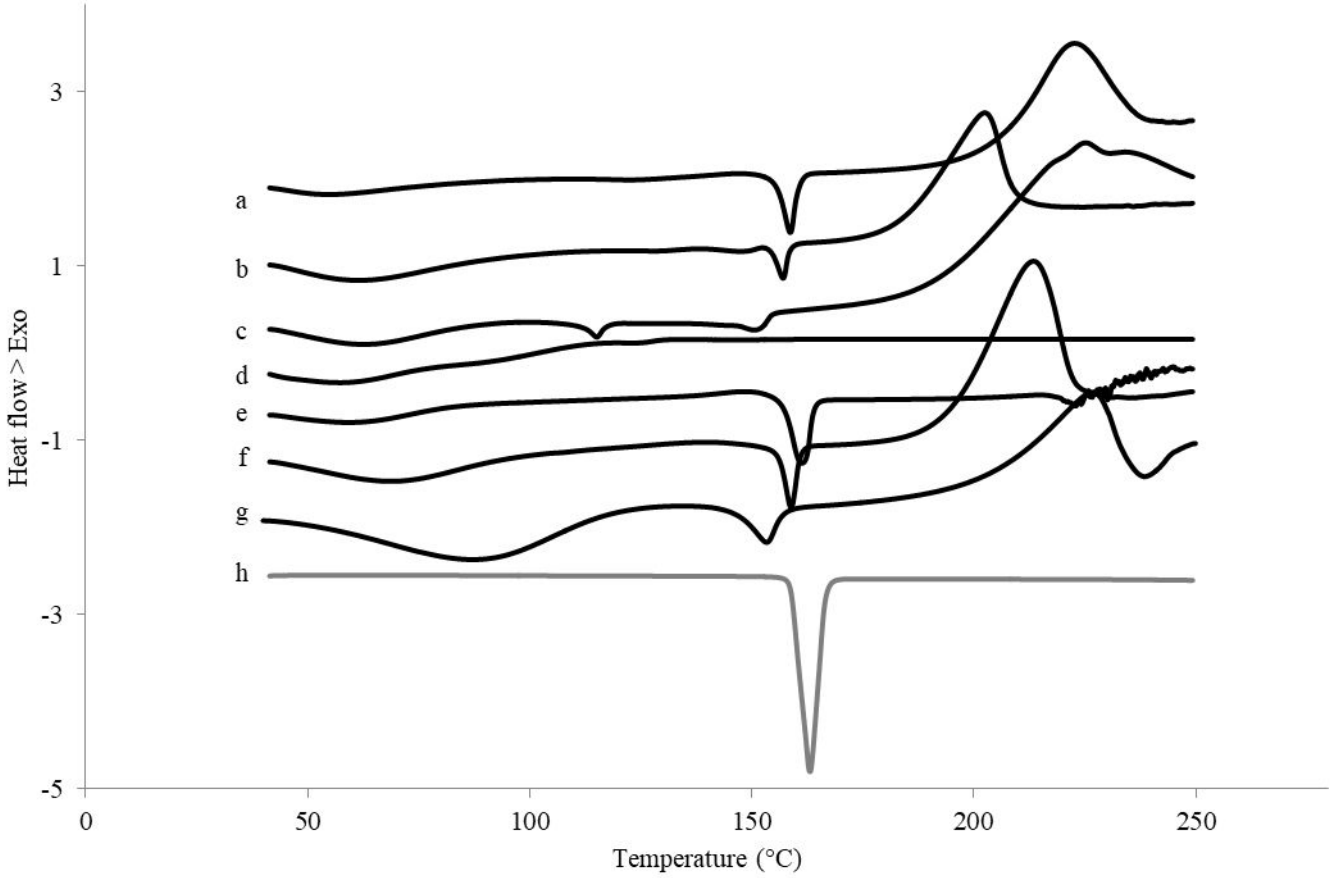
Table 1 Drug content, viscosity of polymeric solutions, moisture content % and mucoadhesive capacity expressed as detachment force

	Drug content (mg)	Viscosity (mPa x s)	Moisture content %	Detachment force (10^{-4} N)
GEL	8.19 ± 0.47	1.08 ± 0.01	2.7 ± 0.5	2.1 ± 0.3
GT	8.55 ± 0.29	19.38 ± 0.18	1.9 ± 0.3	3.6 ± 0.3
CH	8.08 ± 0.25	12.21 ± 0.06	1.7 ± 0.2	5.0 ± 0.4
PEC	7.98 ± 0.40	8.55 ± 0.25	1.3 ± 0.1	4.3 ± 0.3
HPMC	8.25 ± 0.48	77.69 ± 2.55	1.2 ± 0.3	5.7 ± 0.3
HA	8.16 ± 0.39	353.94 ± 3.67	4.1 ± 0.6	4.0 ± 0.4
CH/HPMC	7.97 ± 0.36	21.04 ± 0.5	1.3 ± 0.4	6.3 ± 1.1

Table 2 Values of flux (J) and predicted plasma concentration at the steady stated (Css) of the drug.

	J (μg/cm^2 h)	Css (μg/mL)
GEL	412.68 ± 19.26	0.038
GT	536.78 ± 26.84	0.050
CH	771.89 ± 40.88	0.070
PEC	543.48 ± 9.52	0.051
HPMC	371.12± 91.05	0.046
HA	469.84 ± 10.53	0.044
CH/HPMC	648.28 ± 27.16	0.060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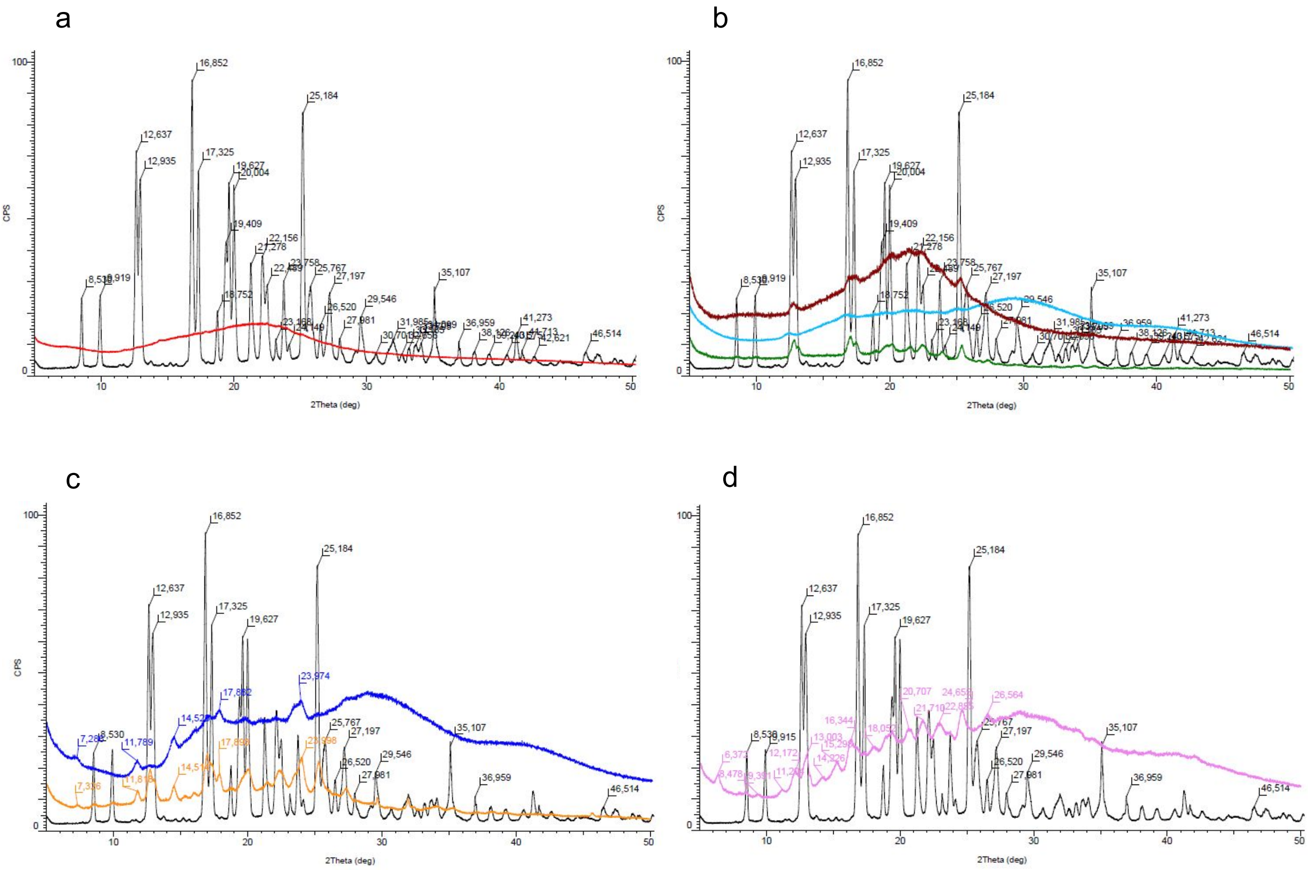
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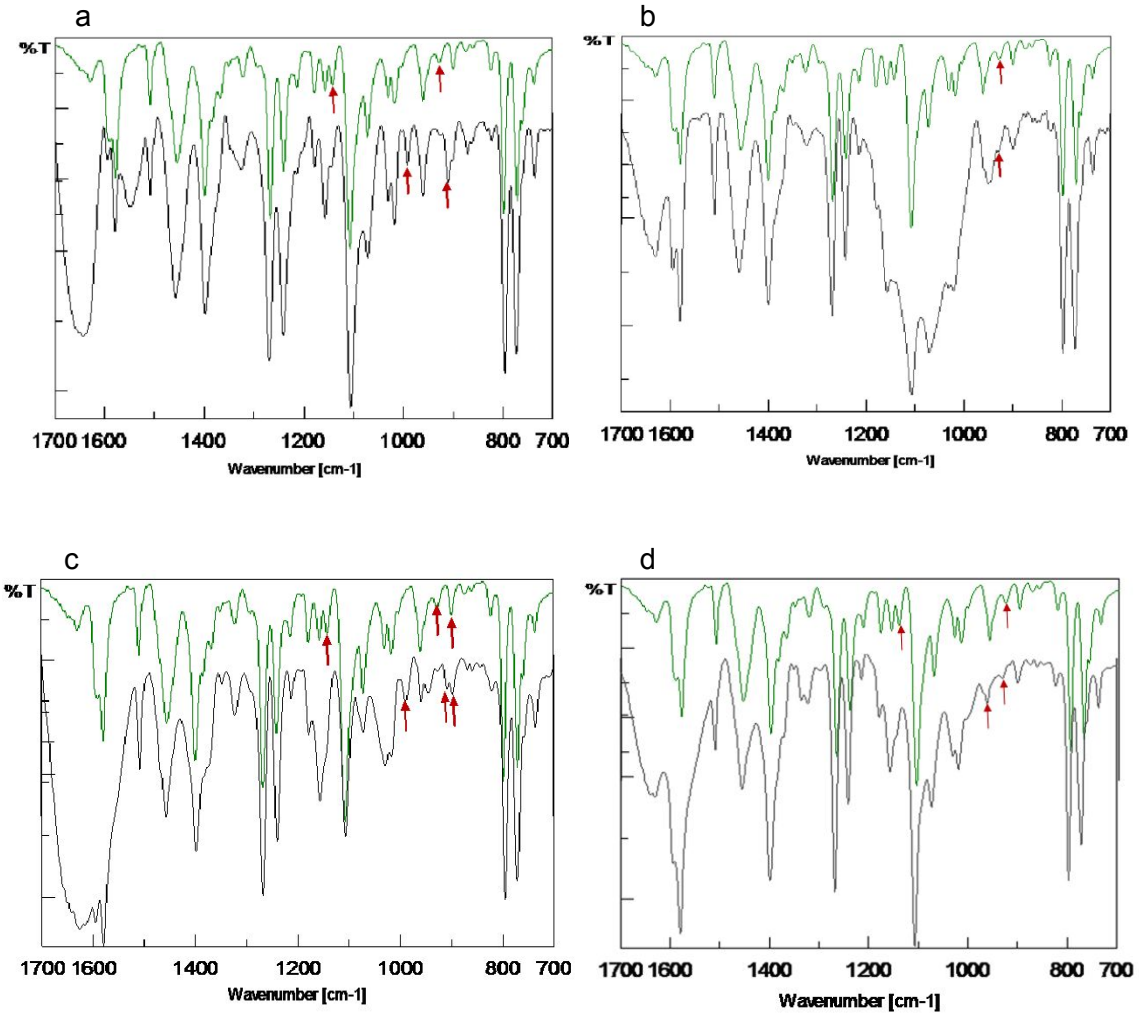
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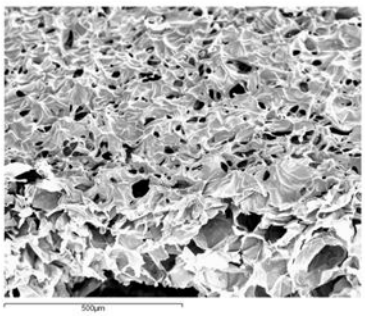

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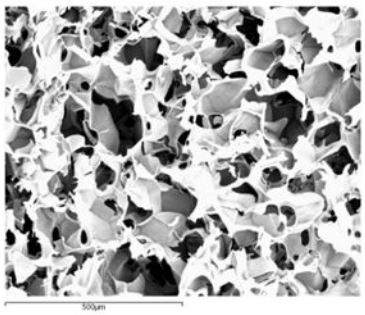

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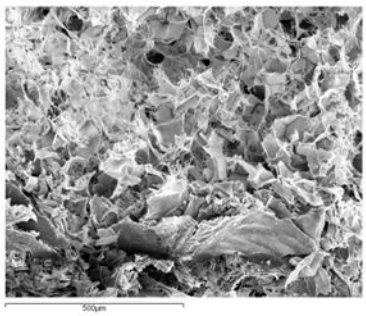

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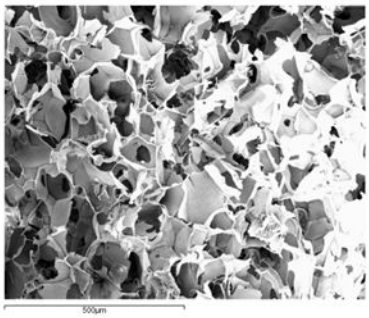

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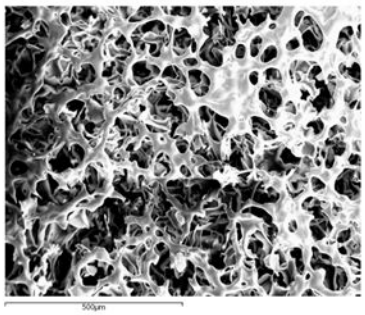

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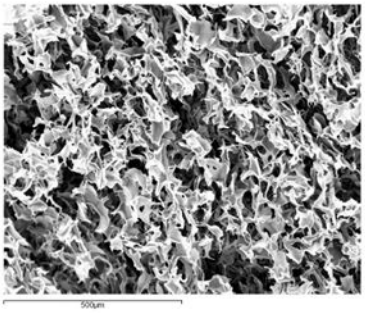

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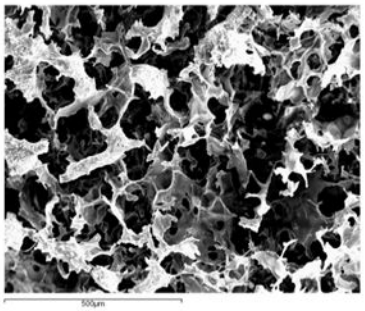

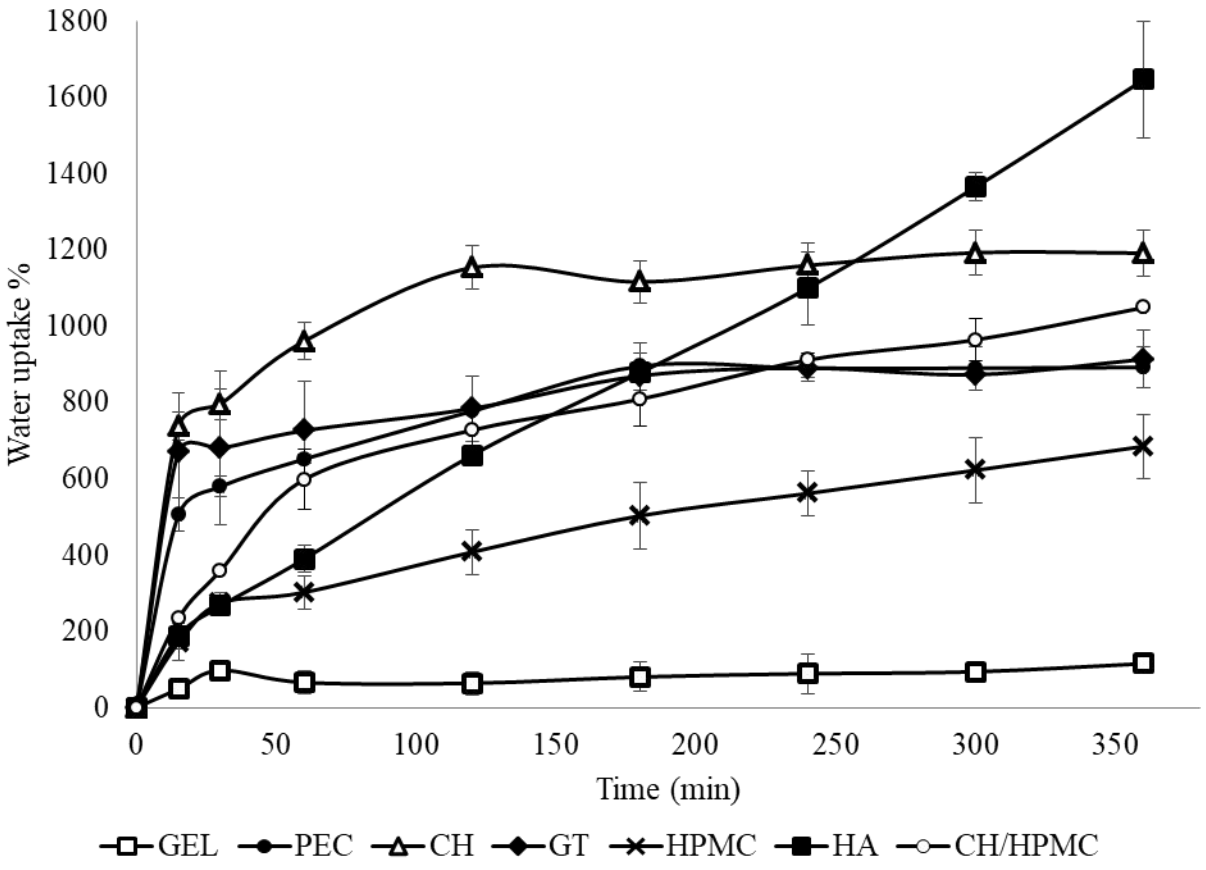
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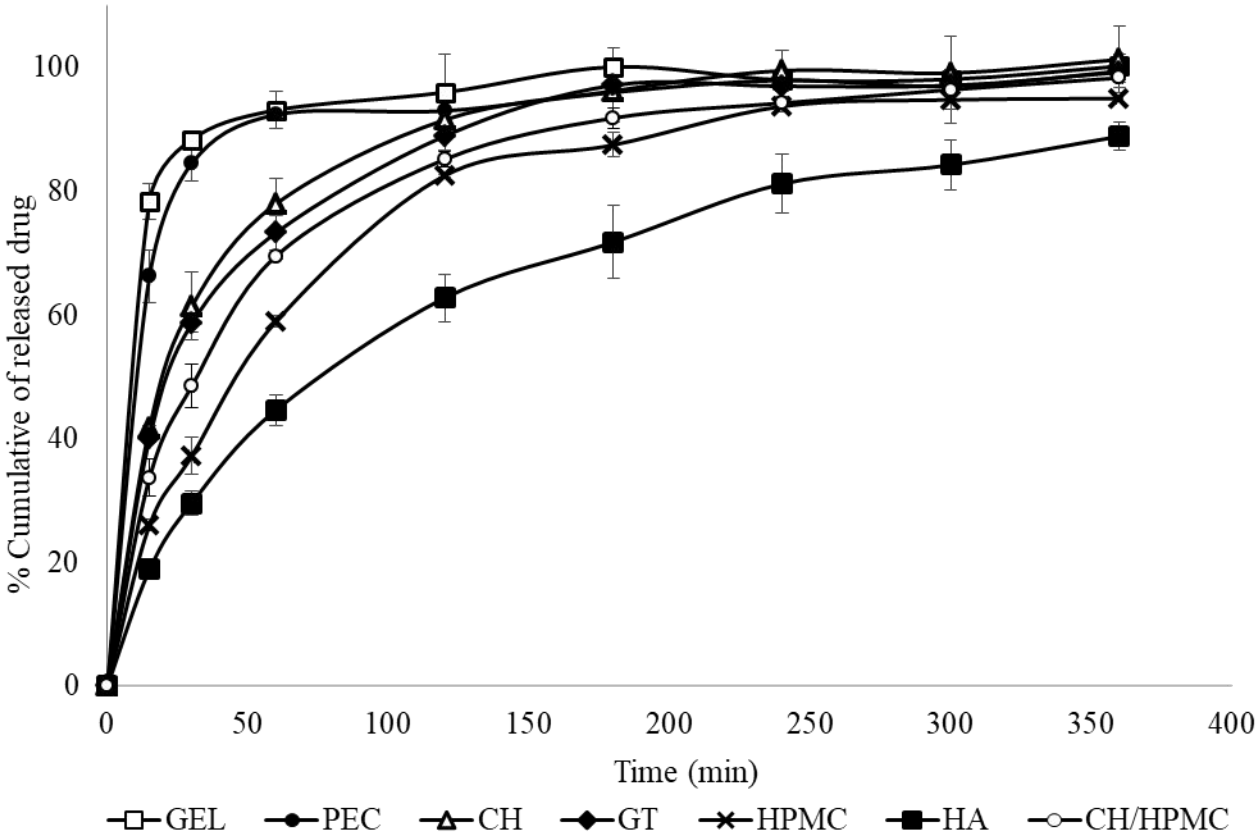
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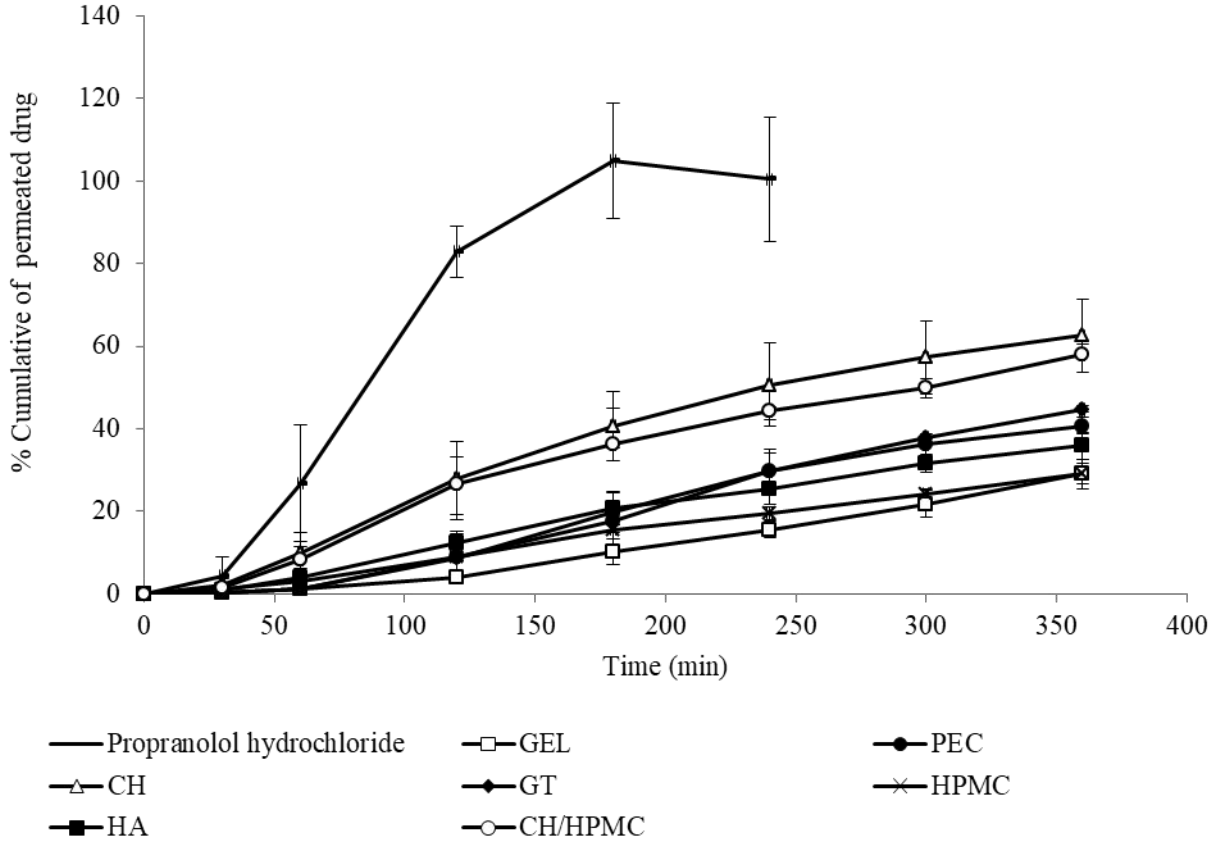
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Propranolol hydrochloride
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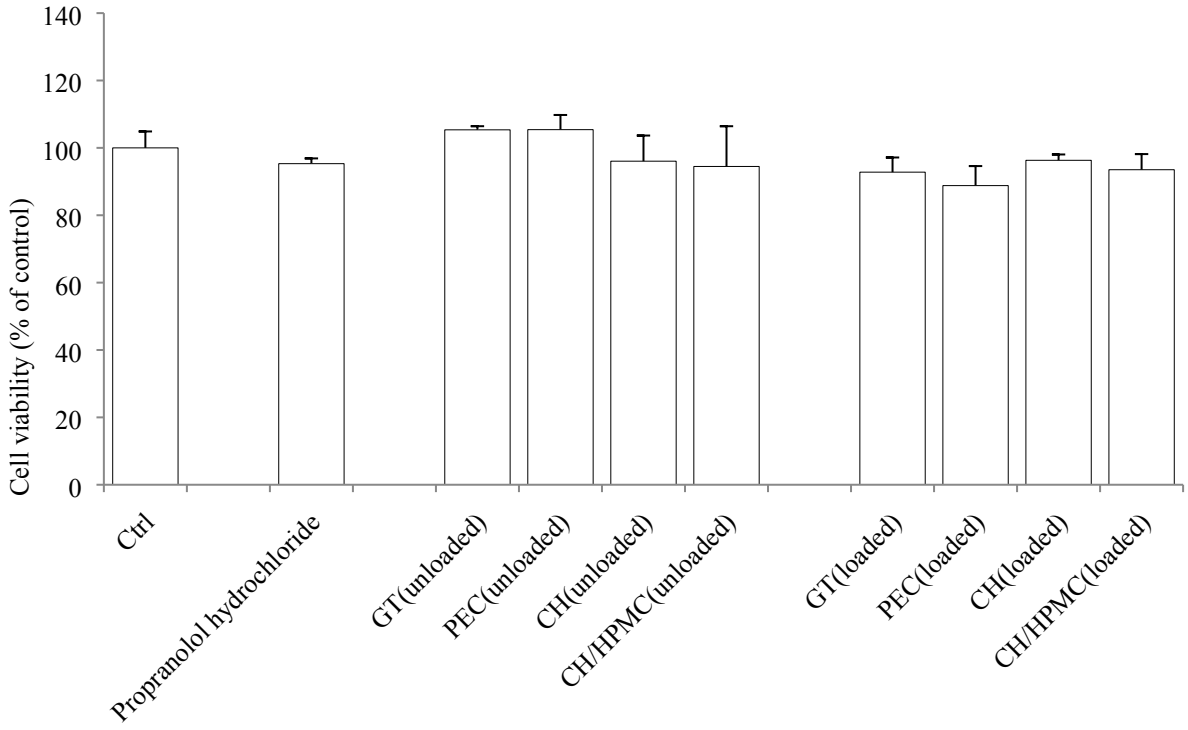
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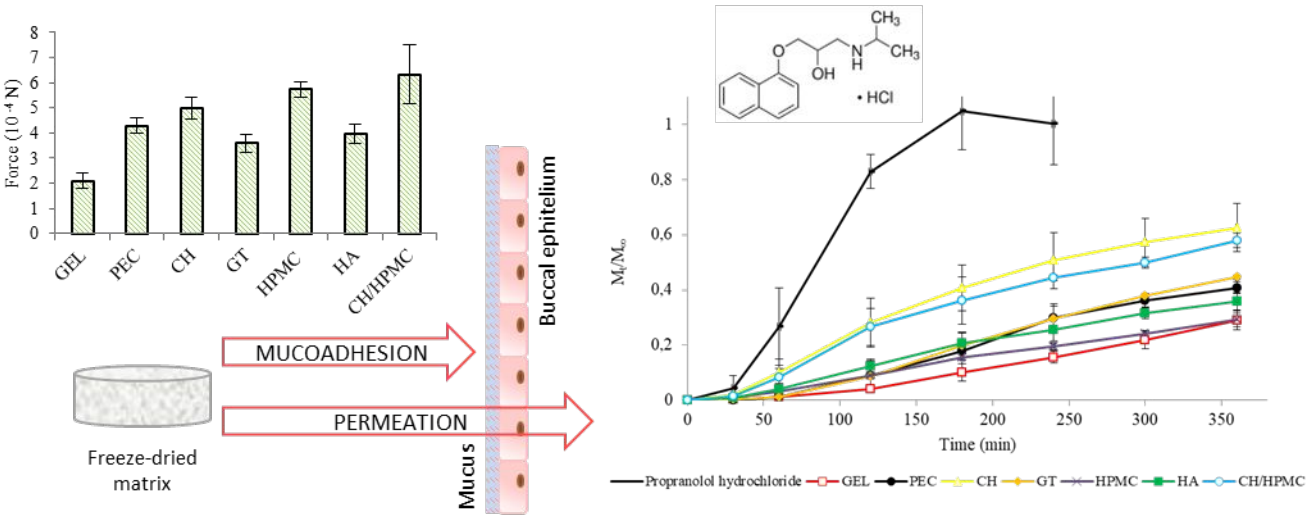
GT(loaded)
PEC(loaded)
CH(loaded)
CH/HPMC(loaded)


Force (10⁻⁴ N)
GEL
PEC
CH
GT
HPMC
HA
CH/HPMC
MUCOADHESION
PERMEATION
Freeze-dried matrix
Buccal ephitelium
Mucus
CH3
O
N
H
CH3
OH
• HCl
Mt/M∞
Time (min)
Propranolol hydrochloride
GEL
PEC
CH
GT
HPMC
HA
CH/HPMC