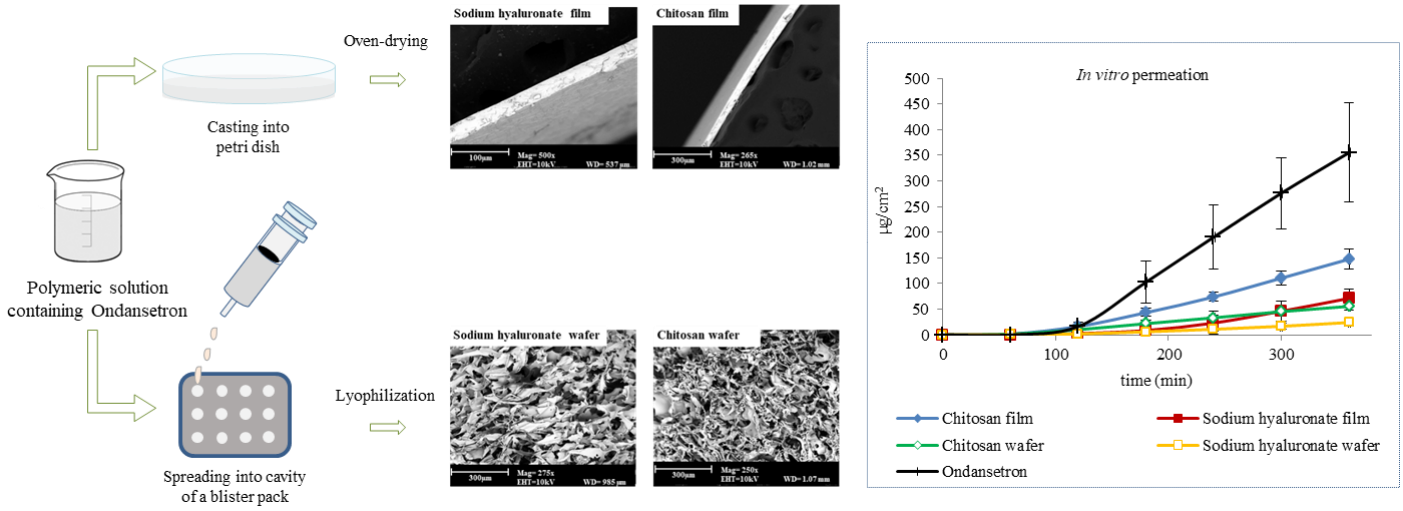
Ondansetron Buccal Administration for Paediatric Use: A Comparison Between Films and Wafers

***Graphical Abstract (for review)**



Ondansetron buccal administration for paediatric use: a comparison between films and wafers

Barbara Giordani[a1], Angela Abruzzo[a1]*, Cecilia Prata[b], Fiore Pasquale Nicoletta[c], Francesco Dalena[d], Teresa Cerchiara[a], Barbara Luppi[a], Federica Bigucci[a]

[a]*Department of Pharmacy and Biotechnology, University of Bologna, Via San Donato 19/2, 40127 Bologna, Italy*
barbara.giordani4@unibo.it; angela.abruzzo2@unibo.it; teresa.cerchiara2@unibo.it; barbara.luppi@unibo.it; federica.bigucci@unibo.it
[b]*Department of Pharmacy and Biotechnology, University of Bologna, Via Irnerio 48, 40126, Bologna, Italy; cecilia.prata@unibo.it*
[c]*Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Edificio Polifunzionale, 87036 Arcavacata di Rende, Cosenza, Italy*
fiore.nicoletta@unical.it
[d]*Department of Chemistry and Chemical Technologies, University of Calabria, 87036 Arcavacata di Rende, Cosenza, Italy*
dalena.ch@gmail.com

*Corresponding author: Angela Abruzzo, Department of Pharmacy and Biotechnology, University of Bologna, Via San Donato 19/2, 40127 Bologna, Italy.
Telephone number: +39 0512095615
angela.abruzzo2@unibo.it

[1]These authors contributed equally to the experimental work.

Abstract

The objective of this study was the development of different solid formulations, such as wafers and films, for buccal administration of ondansetron, a selective and potent antagonist of 5-hydroxytryptamine 3 receptors used in children for the treatment of nausea and vomiting. Wafers and films have been prepared drying an aqueous solution of pectin, hydroxypropyl methylcellulose, sodium hyaluronate, sodium carboxymethylcellulose, chitosan or gelatin, through lyophilization or oven. Formulations were characterized in terms of morphology, drug solid state and ability to hydrate, adhere to mucosa, release and favour the permeation of the drug through porcine esophageal epithelium, used as model of human buccal epithelium. The most promising formulations were tested for *in vitro* biocompatibility in human pulp fibroblasts. Films showed greater hydration and mucoadhesion abilities and allowed the release and the permeation of a greater amount of ondansetron with respect to wafers. Chitosan or hyaluronate provided films with the best mucoadhesion properties and good biocompatibility profile. Moreover, chitosan based film allowed to obtain the highest amount of permeated drug and could represent a novel child-appropriate dosage form able to combine the advantages of solid dosage form with the possibility to avoid the swallowing.

Keywords: films, freeze-dried wafers, ondansetron, buccal delivery, mucoadhesion, permeation

1. Introduction

Nausea and vomiting are distressing side effects of cancer chemotherapy, radiotherapy and surgical anaesthesia. Among the antiemetic agents currently available for children, ondansetron (ODS), a selective and potent antagonist of 5-hydroxytryptamine 3 (5-HT 3) receptors, has demonstrated superior efficacy, safety and pharmacoeconomic profile as compared to other antiemetics (Ye et al., 2001). This drug is nowadays administered either orally (oral tablets, orally disintegrating tablet, oral soluble film) or intravenously (injections) despite some drawbacks. In particular, although the drug is completely and rapidly absorbed after oral administration, its bioavailability is only 60% owing to hepatic first-pass metabolism (Wilde and Markham, 1996), while intravenous administration requires painful injections decreasing patients' compliance. Buccal route, through the mucosal membranes lining the cheeks, has received great attention as an alternative way for administration of ODS in children (Sudhakar et al., 2006). In fact, buccal mucosa allows drug to enter the systemic circulation through the external jugular vein, avoiding the first pass effect. Moreover, it is easily accessible, more resistant to damage than other mucosal membranes, well-vascularized and relatively permeable, and shows low enzymatic activity (Salamat-Miller et al., 2005). Although ODS has been classified as BCS Class III drug owing to its low permeability and high solubility, it shows some physicochemical and pharmacological properties suitable for buccal delivery (short half life, low dose and low molecular weight; Kumar et al., 2011) and its unionized form penetrates well through porcine buccal mucosa, as demonstrated by Mashru and co-workers (Mashru et al., 2005).

Currently, several polymeric formulations for the buccal drug administration, such as viscous solutions, gels, in situ gelling systems, tablets, wafers and films, were widely studied (Fonseca-Santos and Chorilli, 2018). Liquid viscous formulations and gels are rapidly removed from the buccal cavity, as a consequence of accidental swallowing, and they are difficult to administer as drops or sprays. Moreover, these formulations present some issues regarding the short term stability and consequently the use of preservatives and antioxidants is required in order to avoid the

microbial growth and spoilage, especially for products intended for multiple dosing. On the other hand, solid formulations can be retained inside the buccal cavity for a longer period and can ensure a more accurate drug dosing with respect to liquid formulations. Among the solid formulations, films are nowadays the preferred dosage form for transmucosal delivery of drugs in pediatric population (Krampe et al., 2016; Costa et al., 2019) and the mainly studied for delivery of ODS (Trastullo et al., 2016; Kumria et al., 2013). In fact, they are characterized by high thinness and flexibility, comfort of use, dose flexibility and in addition, by using mucoadhesive polymers, it is possible overcome the physiological removal mechanism of the oral cavity and improve the residence time at the application site (Borges et al., 2015).

An alternative approach for ODS delivery through buccal mucosa could be represented by mucoadhesive wafers obtained by freeze-drying, a technology traditionally used in the manifacture of product for parenteral administration and with great potential in the development of oral solid dosage forms (Siow et al., 2016). Wafers are more recent formulations compared to films and only few articles described their development for drug systemic administration through buccal mucosa. Wafers as well as films are able to guarantee easy administration, higher drug loading capacity and low residual moisture, that prevents microbial contamination or degradation of sensitive drugs (Costa et al., 2019).

Concerning excipients, several natural and synthetic mucoadhesive polymers have been studied for buccal films and wafers (Sandri et al., 2015; Russo et al., 2016; Costa et al., 2019). In order to obtain the best adhesion properties and guarantee an intimate and prolonged contact with the oral mucosa, they must show molecular weight above 100,000 Da, chain flexibily to promote the polymer chain diffusion through the mucus, hydrophilic properties to increase the contact with the mucosal surface and favour the mobility of the polymer chains, and functional groups capable of forming hydrogen bonds with the mucosal surface (Sosnik et al., 2014; Boddupalli et al., 2010; Salamat-Miller et al., 2005).

The design of children-appropriate medicine is a particular challenge considering that for many years scientific research, regulation, and formulation development have mainly focused on requires of adults (Preis and Breitkreutz, 2017). The novelty of this work is to fulfill the current demand for child-appropriate medicine by exploiting the advantages of solid dosage forms, such as the dose accuracy and the high physico-chemical stability, together with the possibility to avoid the swallowing and guarantee an easy administration. In particular, the aim of this study was to design mucoadhesive films and wafers for the systemic delivery of ODS through buccal mucosa and evaluate the influence of the polymeric composition as well as of the preparative method on functional properties. Wafers and films were based on a large number of polymers (pectin, hydroxypropyl methylcellulose, sodium hyaluronate, sodium carboxymethylcellulose, chitosan or gelatin) and prepared by dessication of an aqueous polymeric solution in two different ways: liophilization and oven drying (solvent casting method) for wafers and films, respectively. The formulations were then characterized in terms of morphology, drug solid state, hydration properties, mucoadhesion ability and biocompatibility. The formulations were also evaluated for drug release and permeation across porcine esophageal epithelium, used as model of human buccal epithelium.

2. Materials and methods

2.1 Materials

Pectin from citrus peel (PEC; MW 30-100 kDa, esterification degree 60 %, pKa = 4.0) and hydroxypropylmethylcellulose (HPMC; Benecel^{TM} K100M PHARM, MW 1000 kDa) were sourced from Fluka (Milan, Italy) and Ashland (Ashland, Switzerland), respectively. Sodium hyaluronate (HYA; MW 1800-2300 kDa, D-glucuronic acid > 42 %, pKa = 2.9) and sodium carboxymethylcellulose (CMC; MW 250 kDa, substitution degree 0.78, pKa = 4.3) were supplied from ACEF (Piacenza, Italy). Low-viscosity chitosan from shrimp shells (CH; MW 150 kDa, deacetylation degree 96-98 %, pKa = 6.3), type B gelatin from bovine skin (GEL; MW 50 kDa, 100– 115 mmol of free carboxyl groups per 100 g of protein, isoelectric point in the range of pH =

4.7-5.2), mucin from porcine stomach (type II, bound sialic acid ~1%), ondansetron hydrochloride dihydrate (ODS, MW 365.85 Da) and all other chemicals of analytical grade were commercially obtained from Sigma-Aldrich (Milan, 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 pulp fibroblasts (HPFs) were kindly provided by Prof. M. Falconi and Prof. G. Teti (University of Bologna). Hydration, mucoadhesion and release studies were conducted in aqueous buffer at pH 6.8, simulating human saliva pH, composed of 4.61 g/L KH_2PO_4 and 16.75 g/L $Na_2HPO_4x12H_2O$ (healthy saliva pH = 6.7-7.4; Marques et al., 2011), while *in vitro* permeation tests were conducted by using a saline solution (NaCl 9 g/L; Abruzzo et al., 2019; Trastullo et al., 2016). An aqueous phosphate buffer (PBS) at pH 7.2 with the following composition 10 g/L NaCl, 0.25 g/L KCl, 1.8 g/L Na_2HPO_4, 0.25 g/L K_2HPO_4 was used for *in vitro* biocompatibility test.

2.2 Preparation of films and wafers

Films and wafers were prepared by dessication of a polymeric solution by freeze drying and oven drying (solvent casting method), respectively. Polymeric solutions were prepared by mixing for 24 h at 200 rpm PEC, HPMC, HYA, CMC (at 25 °C) or GEL (at 50 °C) in water, while CH was solubilized in 1 % w/w acetic acid solution (at 25 °C). ODS was dissolved in water and added to the different polymeric solutions, thus obtaining final drug and polymer concentrations of 0.12 % w/w and 1.5 % w/w, respectively.

For film preparation, 13.3 g of each polymeric solution were placed on a Petri dish (diameter = 5 cm) and oven-dried at 70 °C for 3 h (heating oven FD series; Binder, Tuttlingen, Germany). Films were cut in discs of 13 mm in diameter, containing a theoretical amount of drug equal to 1.08 mg and stored in a desiccator until use.

For wafer preparation, 0.9 g of aqueous polymeric solution were spread into each cavity (diameter 13 mm) of a blister pack (Farmalabor, Canosa di Puglia, Italy), frozen overnight at -20 °C,

lyophilized at 0.01 atm and -45 °C (Christ Freeze Dryer ALPHA 1-2, Milan, Italy) and stored in a desiccator until use. The different formulations were named on the basis of polymeric composition as follows: PEC_F, $HPMC_F$, HYA_F, CMC_F, GEL_F, CH_F for films and PEC_W, $HPMC_W$, HYA_W, CMC_W, GEL_W, CH_W for wafers.

Formulations were weighted and diameter and thickness were measured through an electronic digital caliper (art. 1367 E 2900, Shanghai ShangErBo Import & Export Co., Shanghai, China). Drug content was measured by dissolving the formulation in 40 mL of saline solution (NaCl 9 g/L) and analyzed by the HPLC method previously reported (Trasullo et al., 2016).

2.3 Viscosity of polymeric solutions

Viscosity of aqueous polymeric solutions (0.5 % w/v) was measured at 25 °C through a falling ball viscometer (HAAKETM Falling Ball Viscometer Type C, Thermo electron corporation, Karlsruhe, Germany).

2.4 Differential scanning calorimetry (DSC)

DSC experiments were performed on polymeric films and wafers in order to investigate the solid state of ODS inside the formulations. Calorimetric measurements were conducted through a Netzsch DSC200 PC differential scanning calorimeter (Metzsch, Germany) using the following setting parameters: temperature from 25 °C to 250 °C, heating rate of 10 °C/min.

2.5 Scanning electron microscopy (SEM)

SEM analysis were carried out to investigate the morphology of the formulations by using a LEO 420 (LEO Electron Microscopy Ltd., Cambridge, UK) with a secondary electron imaging at 15 kV. Films and wafers were cut with a razor blade, fixed on supports and coated with gold-palladium under an argon atmosphere using a gold sputter module in a high-vacuum evaporator.

2.6 Hydration ability

Hydration process is a necessary step to allow the adhesion of the formulation with the biological membrane, the release of drug and consequently its permeation through the buccal mucosa. For this reason, the influence of the polymeric composition as well as the formulation type on the ability to uptake the water was investigated. The study was conducted through a gravimetric method following the procedure described by Bigucci and co-authors (Bigucci et al., 2015). Specifically, films or wafers were accurately weighted and placed on a sponge previously soaked in phosphate buffer at pH 6.8. Hydration ability was measured as weight increase of the formulation for 180 minutes, according to the following equation:

$$\text{Hydration ability } (\%) = (W_{HF} - W_{DF}) \times 100 / W_{DF}$$

where W_{HF} is the weight of hydrated formulation and W_{DF} is the weight of the dried formulation.

2.7 Mucoadhesion ability

The capability of a drug delivery system to adhere to the biological mucosa is important to assure an appropriate residence time at the application site, and consequently to guarantee the permeation of enough amount of drug. For this study, an adapted tensiometer (Krüss 132869; Hamburg, Germany) was used to measure the force needed to pull out a freshly porcine esophageal mucosa from the formulation. Porcine esophageal mucosa was used *in virtue* of its high similarity with the buccal one (Diaz del Consuelo et al., 2005a). Mucosa was hydrated for 5 minutes with mucin suspension (0.05 % w/v) in phosphate buffer at pH 6.8, fixed to a support (surface area 1 mm^2) with cyanoacrylate adhesive and then suspended from the tensiometer. Subsequently, the mucosa was lowered to make contact with the surface of the formulation placed on a glass slide. The adhesive bond strength was represented by the force (reported in Newton) required to separate the formulation from the mucosa.

2.8 *In vitro* release studies

The drug amount released over the time from the different formulations was assessed by *in vitro* release studies. Each film or wafer was attached on a glass slide using cyanoacrylate adhesive in order to avoid the formulation floating. The assembled system was placed in 40 mL of phosphate buffer at pH 6.8 under stirring (sink conditions were assured) and, at different time intervals until 360 minutes, aliquots of 1 mL were withdrawn, replaced with fresh medium, and analyzed by HPLC method. Results are shown as drug fractional amount released (ratio between the absolute cumulative amounts of drug released at time t (M_t) and infinite time (M_∞)) plotted as a function of time.

2.9 *In vitro* permeation studies

In vitro permeation tests were performed to determine the cumulative amount of drug able to diffuse from the formulation across the mucosa. 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. Porcine esophageal epithelium was used as a model of buccal membrane (Diaz del Consuelo et al., 2005b). It was isolated as described in our previous work (Abruzzo et al., 2017) and placed between the donor and the receiver chambers. The chambers were held together tightly with a cell clamp and sealed with parafilm to limit evaporation. The formulation was placed in the donor chamber on the esophageal epithelium, previously hydrated with 400 µL of phosphate buffer at pH 6.8 for 15 minutes. Saline solution was employed as receptor medium and maintained at 37 ± 0.5 °C under stirring. At predetermined time intervals until 360 minutes, samples (0.2 mL) were collected from the receiver chamber, replaced with the same amount of saline solution and analyzed using HPLC. An aqueous solution (400 µL) of ODS (2.7 mg/mL) was also tested. The results of permeation studies are shown as cumulative drug amount permeated (expressed as fractional amount) plotted as a function of time.

2.10 Biocompatibility test in human dental pulp fibroblasts

The most promising formulations, namely films and wafers based on CH and HYA, were selected to investigate the *in vitro* biocompatibility in human pulp fibroblasts (HPFs) through MTT assay. Firstly, each formulation in the presence or absence of ODS 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 resulting extracts were filtered through 0.45 μm Millipore filters. The extracts, as well as the solution of ODS in medium (0.216 mg/mL), were used for MTT assay.

A stock MTT solution (5 mg/mL in PBS) 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 (Zago et al., 2008). 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 design of a buccal drug delivery system involves the optimization of its composition and preparative procedure in order to obtain suitable properties, such as mucoadhesive characteristics, ability to release the drug and to promote its permeation. In this study, different polymers were selected on the basis of their peculiar features. In particular, PEC, HYA, CMC, CH and GEL show well-known properties of biocompatibility, biodegradability and mucoadhesivity (Laffleur, 2014; Cheung et al., 2015; Tedesco et al., 2016), while HPMC was selected also as controlled-release material (Kraisit et al., 2017; Do et al., 2014). Moreover, two simple and easily reproducible preparative techniques, solvent casting method and lyophilization, were adopted to obtain films and wafers, respectively. Both final formulations could represent a valid alternative to the conventional dosage forms for paediatric population, on account of their adequate handling, easy application inside the buccal cavity, ability to rapidly gelify and consequently decrease the discomfort (Lam et al., 2014; Berger er al., 2004).

3.1 Preparation of films and wafers

All the prepared polymeric solutions allowed to obtain films or wafers, easy to handle and to remove from the petri dish or blister without damage, with except to film based on GEL that was difficult to remove and susceptible to breakage. The macroscopical observation highlighted that all the films were thin, omogeneous and transparent, excluding CMC_F that showed an opalescent aspect. On the other hand all the developed wafers showed a cylindrical and regular shape.

Weight, thickness and drug content of the different formulations were determined and reported in Table 1. Our results demonstrated that no significant difference ($p > 0.05$) was present between the weight values of films and wafers, thus demonstrating the efficiency of the two employed preparative methods (Nair et al., 2013). The thickness was influenced by the preparative procedure

used for water removal from the polymeric solutions. In fact, the thickness of wafers (ranged from 3.69 mm to 5.92 mm) was higher ($p < 0.05$) than of films (ranged from 0.07 mm to 0.13 mm), as consequence of the freezing phase, which maintains the initial solution height, and the subsequent ice crystal sublimation under vacuum (Boateng et al., 2010). The thinness of films make them less obtrusive and more tolerable than wafers, thereby improving therapy compliance especially for younger patients (Montero-Padilla et al., 2017).

Finally, for each formulation the experimental drug content (Table 1) was close to the theoretical one (1.08 mg), suggesting that the preparative methods allowed to obtain an omogenous drug distribution inside the formulation.

3.2 Differential scanning calorimetry (DSC)

Fig. 1 shows the DSC profile of ODS overlapping with the profiles of films (Fig. 1A) and wafers (Fig. 1B). ODS showed a peak around 186 °C corresponding to the melting point of the drug and another peak at 109 °C due to the dehydration process, in agreement with the literature (Pattnaik et al., 2011). The thermograms of all the formulations showed one endothermic peak around 60-110 °C, related to the loss of water molecules and an exothermic peak beyond 200 °C, due to the polymer decomposition. For both films and wafers, the characteristic melting peak of ODS was absent, indicating that solvent casting method and lyophilization process induced drug transition from a cristalline to an amorphous state. Despite the disadvantages of the amorphous form, such as lower physical stability compared to crystals, the presence of ODS in this state could imply a better drug solubility and consequently an increase of its bioavailability (Rumondor et al., 2016).

3.3 Scanning electron microscopy (SEM)

Fig. 2 shows the internal structure and the surface morphology of the prepared formulations. SEM images highlighted differences between films (Fig. 2A) and wafers (Fig. 2B). In particular, films

showed a continuous and dense polymer sheet and a smooth surface. On the other hand, wafers were characterized by a sponge-like porous structure as a result of ice nucleation during lyophilization (Hou et al., 2003). Moreover, wafers displayed a different morphology as a consequence of the polymeric composition. In particular, PEC_W and $HPMC_W$ presented a network with spherically shaped pores; HYA_W, CMC_W and CH_W showed a leaf-like structure, while GEL_W was characterized by a more compact morphology.

3.4 Hydration ability

The hydration ability of buccal formulations has a crucial impact on drug release and mucoadhesive properties (Timur et al., 2019). Fig. 3 reports the hydration profiles of films (Fig. 3A) and wafers (Fig. 3B) in phosphate buffer at pH 6.8. As can be seen, the hydration of wafers was slower than films, probably due to higher thickness and lower contact between the porous surface and the sponge soaked with the buffer, that decreased the water diffusion inside the formulation.

Taking into account the polymeric composition, hydration ability could be influenced by the chemical structure of the polymer as well as by its ionization (Camponeschi et al., 2015). As can be seen in Fig. 3A, for all films the maximum hydration value was reached after 60 minutes. CH_F and HYA_F were characterized by highest hydration ability ($p < 0.05$) on the basis of their hydrophilic nature and of the presence of charged aminic and carboxyilic groups able to promote the water uptake (Kononova et al. 2019; Sandri et al., 2015). No significant difference was observed between CMC_F, PEC_F and $HPMC_F$ ($p > 0.05$).

Differently from films, hydration profiles of wafers were characterized by a fast initial phase within 20-30 minutes followed by a phase with a reduced water uptake rate, with except for GEL_W that reached a plateau after 60 minutes. The reduced water uptake rate was probably correlated to the internal porous structure of wafers in which the water slowly diffused. On the other hand, the more compact structure of GEL_W limited the water entry over the time. Moreover, wafers containing charged carboxylic (PEC, CMC, HYA) or aminic (CH) groups showed higher hydration ability with

respect to the neutral HPMC ($p < 0.05$, Timur et al., 2019; Bigucci et al., 2008; Berger et al., 2004). Finally, the polypeptidic nature of GEL (isoelectric point in the range of pH 4.7-5.2) provided a low hydration ability despite the presence of ionizable groups of aspartic acid, lysine, arginine and histidine.

3.5 Mucoadhesion ability

The formulation ability to adhere to a mucosal surface represents an important factor for the design of a buccal dosage form. A greater mucoadhesion and consequently, a longer residence time at the application site could result in high drug concentration in the absorption area and hence high flux, thus enhancing drug bioavailability, reducing daily dose frequency and improving patient compliance. Mucoadhesion process is generally based on an initial step of hydration followed by the interdiffusion and entanglement of polymeric chains into the mucus (Boddupalli et al., 2010).

Fig. 4 shows the mucoadhesion ability for films and wafers. As can be seen, the force necessary to detach films from the mucosa was higher with respect to wafers ($p < 0.05$), accordingly to previous findings about the hydration. Moreover, this result was in agreement with recent observation of Boateng (Boateng and Okeke, 2019), who reported that the low mucoadhesion property of wafers with respect to films can be attributed to the lesser contact with the mucosal surface of a network characterized by a sponge-like structure.

For both films and wafers, the presence of HYA and CH assured the best mucoadhesion to mucosa ($p < 0.05$), due to the greatest hydration ability and the presence of many groups that can establish hydrogen bonds with the mucus chains. Moreover, for CH based formulations the aminic groups were able to establish ionic interactions with the negatively charged sialic acid (pKa 2.6) and sulphate residues of mucin (Sosnik et al., 2014; Sandri et al., 2015; Russo et al., 2016). Furthermore, formulations based on PEC, CMC and HPMC showed a good mucoadhesive properties, *in virtue* of the presence of hydrophilic groups, the chain entanglement and physical interlock with mucus (Sosnik et al., 2014; Sudeendra et al., 2010; Sriamornsak et al., 2010). Finally,

the lowest mucoadhesion property of GEL_W ($p < 0.05$) was a consequence of its lowest hydration ability that limited the penetration of peptidic chains into the mucus layer.

3.6 *In vitro* release studies

In vitro drug release tests were performed in order to evaluate formulation ability to release the drug over the time. Fig. 5 shows the release profiles for films (Fig. 5A) and wafers (Fig. 5B) obtained in phosphate buffer at pH 6.8. Different factors, such as hydration ability, relaxation of the polymer chains, formation of viscous gel, drug dissolution and diffusion through the rehydrated formulation, were involved in the mechanism of drug release (Boateng et al., 2012). Viscosity of aqueous polymeric solutions was measured and viscosity values for PEC, HPMC, HYA, CMC, CH and GEL were 8.6 ± 0.3, 77.7 ± 2.6, 353.9 ± 3.7, 12.4 ± 0.2, 12.2 ± 0.1 and 1.1 ± 0.1 mPa x s, respectively. Generally, solutions with lower viscosity produce networks less viscous after hydration in the release medium, that could promote the drug diffusion and release.

As can be seen from the figures, films released ODS faster than wafers, due to their more rapid hydration that promoted drug diffusion (Viridénet al., 2009). The release profiles of all films, with except for HYA_F, were characterized by an initial fast phase followed by a plateau that was reached after only 180 minutes. On the other hand HYA_F provided a sustained release of ODS, in relation to the highest viscosity of the hydrated film, reaching the plateau after 180 minutes.

As regard release profiles of wafers, GEL_W and PEC_W allowed the release of the total amount of ODS after 90 and 120 minutes, respectively, as a consequence of the dissolution of the wafers. For the other wafers, a sustained release profiles were observed and the amount of the ODS released over the time increased with the decrease of the viscosity of the rehydrated formulation. In particular, CMC_W and CH_W, presenting a similar viscosity, showed overlapped release profiles ($p < 0.05$), while HYA_W, characterized by the highest viscosity, provided the lowest release over the time ($p < 0.05$).

3.7 *In vitro* permeation studies

In vitro permeation studies were performed to investigate ODS diffusion across the buccal epithelium. Fig. 6 shows the permeation profiles of ODS from an aqueous solution and from CH and HYA based films and wafers, selected on the basis of their best mucoadhesive properties among all the formulations. For both films and wafers, no significant difference was observed in the permeation profiles of PEC, HPMC, CMC, GEL with respect to HYA ($p > 0.05$; data not reported). The permeation profiles of the different formulations were lower than ODS solution profile ($p < 0.05$) and in all cases a sustained drug diffusion across the membrane was observed. Specifically, films provided the permeation of a greater amount of drug with respect to wafers ($p < 0.05$), as a consequence of their greater hydration and drug release ability. Moreover for both films and wafers, the presence of CH assured the permeation of a greater amount of drug ($p < 0.05$), in agreement with the well-documented ability of this polymer to interfere with the lipid organization in the buccal epithelium (Senel et al., 2000).

3.8 Biocompatibility test in human dental pulp fibroblasts

The effect of films and wafers, prepared with two different polymers (CH and HYA), on cell viability was determined by estimation of living cell competence to reduce thiazolyl blue tetrazolium bromide, also known as MTT assay (Mosmann et al., 1983). The viability of cells incubated with different formulations for 6 h is not significantly different ($p > 0.05$) from viability of control cells, as shown in Fig. 7. Considering that free ODS and formulations prepared without drug did not impair cell survival, we can argue that both ODS and selected formulations resulted safe for buccal use.

4. Conclusions

Polymeric films and wafers have been successfully prepared and proposed as buccal delivery systems for administration of ODS in children, on the basis of their ability to hydrate in contact with saliva thus reducing discomfort. Starting from the same polymeric solution, two formulations with different functional porperties were obtained by just changing the drying procedure. Specifically, films were able to greatly hydrate, adhere to mucosa and favour the drug release and permeation with respect to wafers. In addition, CH based film showed good biocompatibility profile, guaranted the highest amount of permeated drug and could represent a novel child-appropriate dosage form.

Acknowledgements

The authors would like to acknowledge Ilaria Russo and Jessica Montebelli for their valuable contribution and Prof. Gabriella Teti and Prof. Mirella Falconi for kindly providing the cell model.

Funding

This work was supported by the Italian Ministry for University and Scientific Research.

Declaration of interests

The authors declare that they have no conflict of interest.

References

Abruzzo, A., Cerchiara, T., Bigucci, F., Zuccheri, G., Cavallari, C., Saladini, B., Luppi, B., 2019. Cromolyn-crosslinked chitosan nanoparticles for the treatment of allergic rhinitis. Eur. J. Pharm. Sci. 131, 136-145.

Abruzzo, A., Nicoletta, F.P., Dalena, F., Cerchiara, T., Luppi, B., Bigucci, F. 2017., Bilayered buccal films as child-appropriate dosage form for systemic administration of propranolol. Int. J. Pharm. 531, 257-265.

Berger, J., Reist, M., Mayer, J. M., Feit, O., Peppas, N. A., Gurmy, R., 2004. Structural and interactions in chitosan hydrogels formed by complexation oraggregation for biomedical applications. Eur. J. Pharm. Biopharm. 57, 35-52.

Bigucci, F., Luppi, B., Cerchiara, T., Sorrenti, M., Bettinetti, G., Rodriguez, L., Zecchi, V., 2008. Chitosan/pectin polyelectrolyte complexes: selection of suitable preparative conditions for colon-specific delivery of vancomycin. Eur. J. Pharm. Sci. 35, 435-441.

Bigucci, F., Abruzzo, A., Cerchiara, T., Gallucci, M.C., Luppi, B., 2015. Formulation of cellulose film containing permeation enhancers for prolonged delivery of propranolol hydrocloride. Drug Dev. Ind. Pharm. 41,1017-1025.

Boateng, J., Okeke, O., 2019. Evaluation of Clay-Functionalized Wafers and Films for Nicotine Replacement Therapy via Buccal Mucosa. Pharmaceutics. 11(3).

Boateng, J.S., Matthews, K.H., Auffret, A.D, Humphrey, M.J., Eccleston, G.M., Stevens, H.N., 2012. Comparison of the in vitro release characteristics of mucosal freeze-dried wafers and solvent-cast films containing an insoluble drug. Drug Dev. Ind. Pharm. 38, 47-54.

Boateng, J.S., Auffret, A.D., Matthews, K.H., Humphrey, M.J., Stevens, H.N., Eccleston, G.M., 2010. Characterisation of freeze-dried wafers and solvent evaporated films as potential drug delivery systems to mucosal surfaces. Int. J. Pharm. 389, 24-31.

Boddupalli, B.M., Mohammed, Z.N.K., Nath, R.A., Banji, D., 2010. Mucoadhesive drug delivery system: An overview. J. Adv. Pharm. Tech. Res. 1, 381–387.

Borges, A.F., Silva, C., Coelho, J.F., Simões, S., 2015. Oral films: Current status and future perspectives: I - Galenical development and quality attributes. J. Control. Release. 2006, 1-19.

Camponeschi, F., Atrei, A., Rocchigiani, G., Mencuccini, L., Uva, M., Barbucci, R., 2015. New Formulations of Polysaccharide-Based Hydrogels for Drug Release and Tissue Engineering. Gels. 1, 3-23.

Cheung, R.C., Ng, T.B., Wong, J.H., Chan, W.Y., 2015. Chitosan: An Update on Potential Biomedical and Pharmaceutical Applications. Mar. Drugs. 13, 5156-86

Costa, J.S.R., de Oliveira Cruvine, K., Oliveira-Nascimento, L., 2019. A mini-review on drug delivery through wafer technology: Formulation and manufacturing of buccal and oral lyophilizates. J. Adv. Res. 20, 33-41.

Diaz del Consuelo, I., Pizzolato, G.P., Falson, F., Guy, R.H., Jacques, Y., 2005a. Evaluation of pig esophageal mucosa as a permeability barrier model for buccal tissue. J. Pharm. Sci. 94, 2777-2788.

Diaz del Consuelo, I., Jacques, Y., Pizzolato, G.P., Guy, R.H., Falson, F., 2005b. Comparison of the lipid composition of porcine buccal and esophageal permeability barriers. Arch. Oral Biol. 50, 981-987.

Do, M. P., Neut, C., Delcourt, E., Certo, T. S., Siepmann, J., & Siepmann, F., 2014. In-situ forming implants for periodontitis treatment with improved adhesiveproperties. Eur. J. Pharm. Biopharm. 88, 342-350.

Fonseca-Santos, B., Chorilli, M., 2018. An overview of polymeric dosage forms in buccal drug delivery: State of art, design of formulations and their in vivo performance evaluation. Mater. Sci. Eng. C Mater. Biol. Appl. 86, 129-143.

Hou, Q., Grijpma, D.W., Feijen, J., 2003. Preparation of interconnected highly porous polymeric structures by a replication and freeze-drying process. J. Biomed. Mater. Res. B. Appl. Biomater. 67, 732-40.

Kononova, S.V., Kruchinina, E.V., Petrova, V.A., Baklagina, Y.G., Klechkovskaya, V.V., Orekhov, A.S., Vlasova, E.N., Popova, E.N., Gubanova, G.N., Skorik, Y.A., 2019. Pervaporation membranes of a simplex type with polyelectrolyte layers of chitosan and sodium hyaluronate. Carbohydr. Polym. 209, 10-19.

Kraisit, P., Limmatvapirat, S., Nunthanid, J., Sriamornsak, P., Luangtana-Anan, M., 2017. Preparation and characterization of hydroxypropyl methylcellulose/polycarbophil mucoadhesive blend films using a mixture design approach. Chem. Pharm. Bull. 65, 284-294.

Krampe, R., Visser, J.C., Frijlink, H.W., Breitkreutz, J., Woerdenbag, H.J., Preis, M., 2016. Oromucosal film preparations: points to consider for patient centricity and manufacturing processes. Expert Opin. Drug Deliv. 13,493–506.

Kumar, M., Rajendra Prasad, M., Pramod, M., Prabhakar Reddy, V., 2011. Effect of permeation enhancer on ex-vivo permeation of ondansetron HCl buccal tablets. Int. J. Pharm. Sci. Res. 2(11), 2841-2845.

Kumria, R., Gupta, V., Bansal, S., Wadhwa, J., Nair, A.B., 2013. Oral buccoadhesive films of ondansetron: Development and evaluation. Int. J. Pharm. Investig. 3, 112-118.

Laffleur, F., 2014. Mucoadhesive polymers for buccal drug delivery. Drug Dev. Ind. Pharm. 40, 591-8.

Lam, J.K., Xu, Y., Worsley, A., Wong, I.C., 2014. Oral transmucosal drug delivery for pediatric use. Adv. Drug Deliv. Rev. 73, 50-62.

Marques, M.R.C., Loebenberg, R., Almukainzi, M., 2011. Simulated biological fluids with possible application in dissolution testing. Dissolut. Technol. 18,15-28.

Mashru, R.C., Sutariya, V.B., Sankalia, M.G., Sankalia, J.M., 2005. Effect of pH on in vitro permeation of ondansetron hydrochloride across porcine buccal mucosa. Pharm. Dev. Technol. 10, 241-247.

Montero-Padilla, S., Velaga, S., Morales, J.O., 2017. Buccal Dosage Forms: General Considerations for Pediatric Patients. AAPS PharmSciTech. 18, 273-282.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods. 65, 55-63.

Nair, A.B., Kumria, R., Harsha, S., Attimarad, M., Al-Dhubiab, B.E., Alhaider, I.A., 2013. In vitro techniques to evaluate buccal films. J. Control. Release. 166, 10-21.

Pattnaik, S., Swain, K., Mallick, S., Lin, Z., 2011. Effect of casting solvent on crystallinity of ondansetron in transdermal films. Int. J. Pharm. 406, 106- 110.

Preis, M., Breitkreutz, Jörg., 2017. Pediatric Drug Development and Dosage Form Design. AAPS PharmSciTech. 18, 239-240.

Rumondor, A.C.F., Dhareshwar, S.S., Kesisoglou, F., 2016. Amorphous Solid Dispersions or Prodrugs: Complementary Strategies to Increase Drug Absorption. J. Pharm. Sci. 105, 2498-2508.

Russo, E., Selmin, F., Baldassari, S., Gennari, C.G.M., Caviglioli, G., Cilurzo, F., Minghetti, P., Parodi, B., 2016. A focus on mucoadhesive polymers and their application in buccal dosage forms. J. Drug Deliv. Sci. Technol. 32, 113-125.

Salamat-Miller, N., Chittchang, M., Johnston, T.P., 2005. The use of mucoadhesive polymers in buccal drug delivery. Adv. Drug Deliv. Reviews. 57, 1666-1691.

Sandri, G., Rossi, S., Ferrari, F., Bonferoni, M.C., Caramella, C.M., 2015. Mucoadhesive polymers as enabling excipients for oral mucosal drug delivery. In: Rathbone, M.J., Senel, S., Pather, I. (Eds.), Oral Mucosal Drug Delivery and Therapy. Adv Del. Sci. Technol., 53–88.

Şenel, S., Kremer, M.J., Kaş, S., Wertz, P.W., Hıncal, A.A., Squier, C.A., 2000. Enhancing effect of chitosan on peptide drug delivery across buccal mucosa. Biomaterials. 21, 2067-2071.

Siow, C.R., Wan Sia Heng, P., Chan, L.W., 2016. Application of freeze-drying in the development of oral drug delivery systems. Expert Opin. Drug Deliv. 13, 1595-1608.

Sosnik, A., das Neves, J., Sarmento, B., 2014. Mucoadhesive polymers in the design of nano-drug delivery systems for administration by non-parenteral routes: A review. Prog. Polym. Sci. 39, 2030-2075.

Sriamornsak, P., Wattanakorn, N., Takeuchi, H., 2010. Study on the mucoadhesion mechanism of pectin by atomic force microscopy and mucin-particle method. Carbohydr. Polym. 79, 54-59.

Sudeendra, B.R., Hani, U., Gupta, R.K., Shivakumar, H.G., 2010. Development and characterization of bioadhesive vaginal films of clotrimazole for vaginal candidiasis. Acta Pharmaceutica Sciencia 52, 417–426.

Sudhakar, Y., Kuotsu, K., Bandyopadhyay, A.K., 2006. Buccal bioadhesive drug delivery-a promising option for orally less efficient drugs. J Control Release. 114, 15-40.

Tedesco, M.P., Monaco-Lourenço, C.A., Carvalho, R.A., 2016. Gelatin/hydroxypropyl methylcellulose matrices - Polymer interactions approach for oral disintegrating films. Mater. Sci. Eng. C Mater. Biol. Appl. 69, 668-674.

Timur, S.S., Yüksel, S., Akca, G., Şenel, S., 2019. Localized drug delivery with mono and bilayered mucoadhesive films and wafers for oral mucosal infections. Int. J. Pharm.559, 102-112.

Trastullo, R., Abruzzo, A., Saladini, B., Gallucci, M.C., Cerchiara, T., Luppi, B., Bigucci, F., 2016. Design and evaluation of buccal films as paediatric dosage form for transmucosal delivery of ondansetron. Eur. J. Pharm. Biopharm. 105, 115-21.

Viridén, A., Wittgren, B., Larsson, A., 2009. Investigation of critical polymer properties for polymer release and swelling of HPMC matrix tablets. Eur. J. Pharm. Sci. 36, 297-309.

Wilde, M.I., Markham, A., 1996. Ondansetron. A review of its pharmacology and preliminary clinical findings in novel applications. Drugs. 52, 773-794.

Ye, J.H., Ponnudurai, R., Schaefer, R., 2001. Ondansetron: a selective 5-HT(3) receptor antagonist and its applications in CNS-related disorders. CNS Drug Rev. 7, 199-213.

Zago, M., Teti, G., Mazzotti, G., Ruggeri, A., Breschi, L., Pelotti, S., Ortolani, M., Falconi, M., 2008. Expression of procollagen alpha1 type I and tenascin proteins induced by HEMA in human pulp fibroblasts. Toxicol. In Vitro. 22, 1153-1159.

Legend to Figures

Fig. 1 DSC thermogram of ODS overlapping with than of films (Fig. 1A) and wafers (Fig. 1B).

Fig. 2 SEM images of films (Fig. 2A) and wafers (Fig. 2B).

Fig. 3 *In vitro* hydration profiles for films (Fig. 3A) and wafers (Fig. 3B) in phosphate buffer at pH 6.8 until 180 minutes (mean ± SD, n= 3).

Fig. 4 Mucoadhesive capacity (expressed as detachment force) of films and wafers (mean ± SD, n= 3).

Fig. 5 *In vitro* release profiles of ODS from films (Fig. 4A) and wafers (Fig. 4B) in phosphate buffer at pH 6.8 until 360 minutes (mean ± SD, n= 3).

Fig. 6 Permeation profiles of ODS through esophageal porcine epithelium from drug solution and CH and HYA based films and wafers (mean ± SD, n= 5).

Fig. 7 Biocompatibility of ODS, CH and HYA based films and wafers assessed in human dental pulp fibroblasts by means of MTT assay (mean ± SD, n= 4).

***Conflict of Interest**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

***Credit Author Statement**

Barbara Giordani and **Angela Abruzzo:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing- Original draft preparation, Visualization. **Cecilia Prata**: Investigation, Writing- Original draft preparation. **Fiore Pasquale Nicoletta** and **Francesco Dalena**: Investigation. **Teresa Cerchiara** and **Barbara Luppi:** Writing- Reviewing and Editing. **Federica Bigucci**: Conceptualization, Methodology, Writing- Original draft preparation, Writing- Reviewing and Editing, Visualization, Supervision.

Table 1. Weight, thickness and drug content of films and wafers (mean ± SD, n = 4).

Formulation	Weight (mg)	Thickness (mm)	Drug content (mg)
PEC_F	12.0 ± 2.5	0.11 ± 0.09	1.26 ± 0.23
$HPMC_F$	12.1 ± 4.1	0.07 ± 0.04	0.97 ± 0.35
HYA_F	13.1 ± 3.1	0.13 ± 0.07	1.04 ± 0.21
CMC_F	13.8 ± 1.9	0.12 ± 0.07	1.01 ± 0.15
CH_F	14.0 ± 2.9	0.10 ± 0.02	1.00 ± 0.08
PEC_W	15.4 ± 1.4	5.86 ± 0.42	1.24 ± 0.18
$HPMC_W$	13.4 ± 1.9	3.69 ± 0.52	1.03 ± 0.08
HYA_W	14.6 ± 1.2	4.09 ± 0.48	1.06 ± 0.14
CMC_W	15.7 ± 1.3	5.57 ± 0.58	1.07 ± 0.12
GEL_W	15.8 ± 1.3	5.92 ± 0.30	1.18 ± 0.09
CH_W	15.6 ± 1.4	5.35 ± 0.19	1.13 ± 0.14

Figure 1

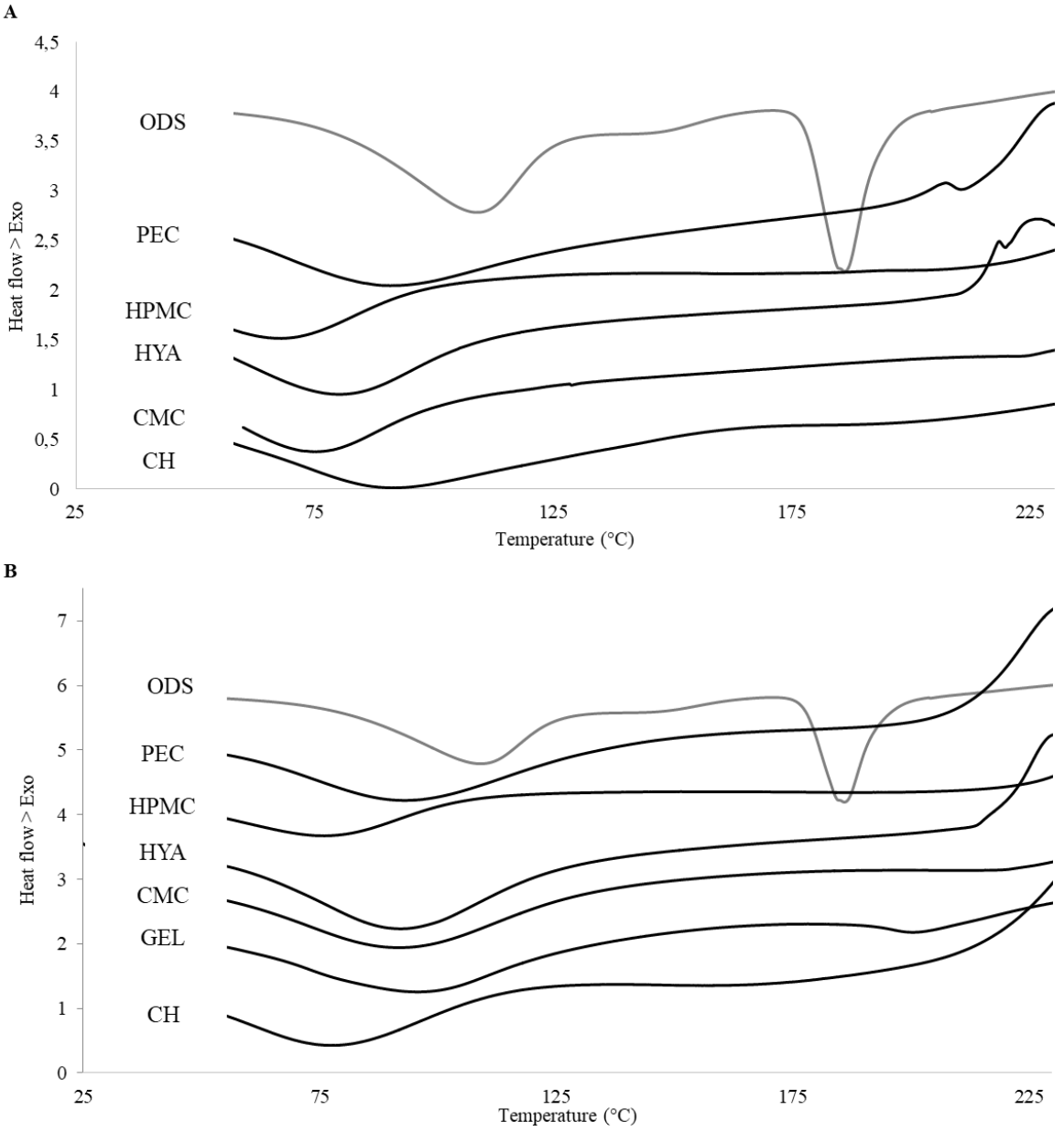

A
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
Heat flow > Exo
ODS
PEC
HPMC
HYA
CMC
CH
25
75
125
175
225
Temperature (°C)
B
7
6
5
4
3
2
1
0
Heat flow > Exo
ODS
PEC
HPMC
HYA
CMC
GEL
CH
25
75
125
175
225
Temperature (°C)

Figure 2

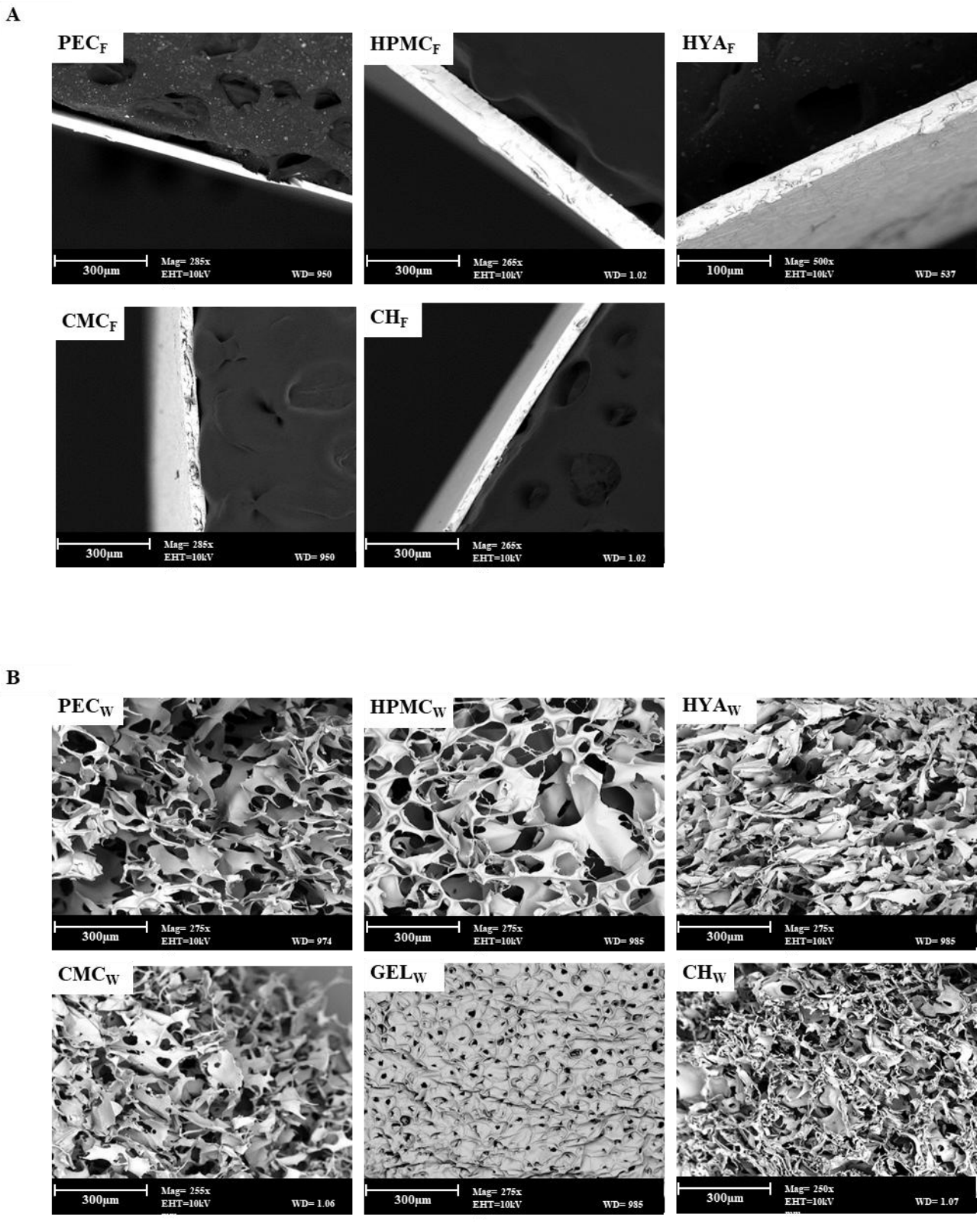
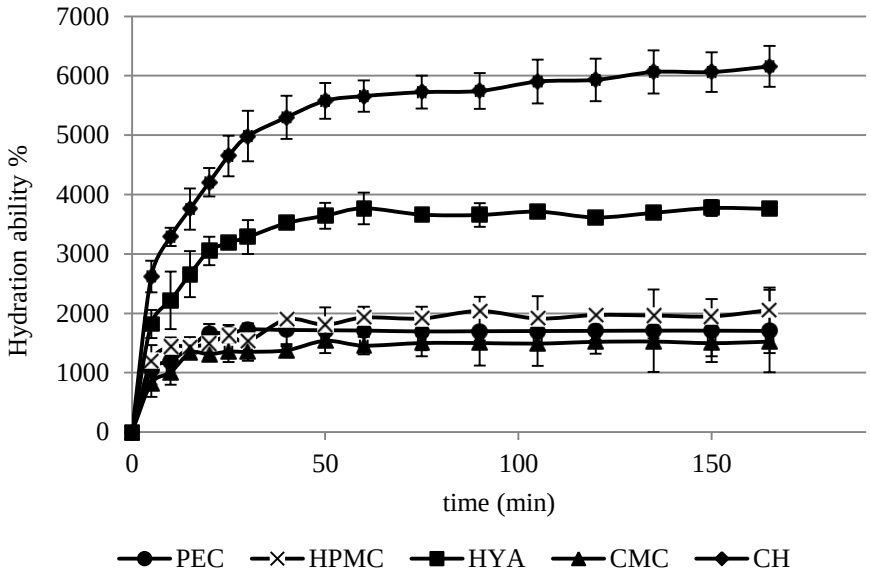

A
PEC$_F$
300µm
Mag= 285x
EHT=10kV
WD= 950
HPMC$_F$
300µm
Mag= 265x
EHT=10kV
WD= 1.02
HYA$_F$
100µm
Mag= 500x
EHT=10kV
WD= 537
CMC$_F$
300µm
Mag= 285x
EHT=10kV
WD= 950
CH$_F$
300µm
Mag= 265x
EHT=10kV
WD= 1.02
B
PEC$_W$
300µm
Mag= 275x
EHT=10kV
WD= 974
HPMC$_W$
300µm
Mag= 275x
EHT=10kV
WD= 985
HYA$_W$
300µm
Mag= 275x
EHT=10kV
WD= 985
CMC$_W$
300µm
Mag= 255x
EHT=10kV
WD= 1.06
GEL$_W$
300µm
Mag= 275x
EHT=10kV
WD= 985
CH$_W$
300µm
Mag= 250x
EHT=10kV
WD= 1.07

Figure 3

A


7000
6000
5000
4000
3000
2000
1000
0
Hydration ability %
0
50
100
150
time (min)
PEC
HPMC
HYA
CMC
CH

B

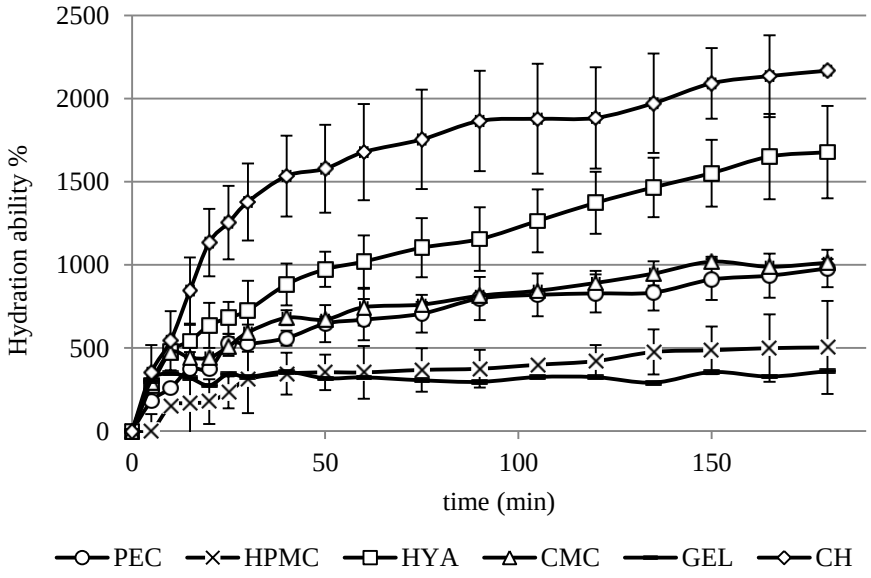

2500
2000
1500
1000
500
0
Hydration ability %
0
50
100
150
time (min)
PEC
HPMC
HYA
CMC
GEL
CH

Figure 4

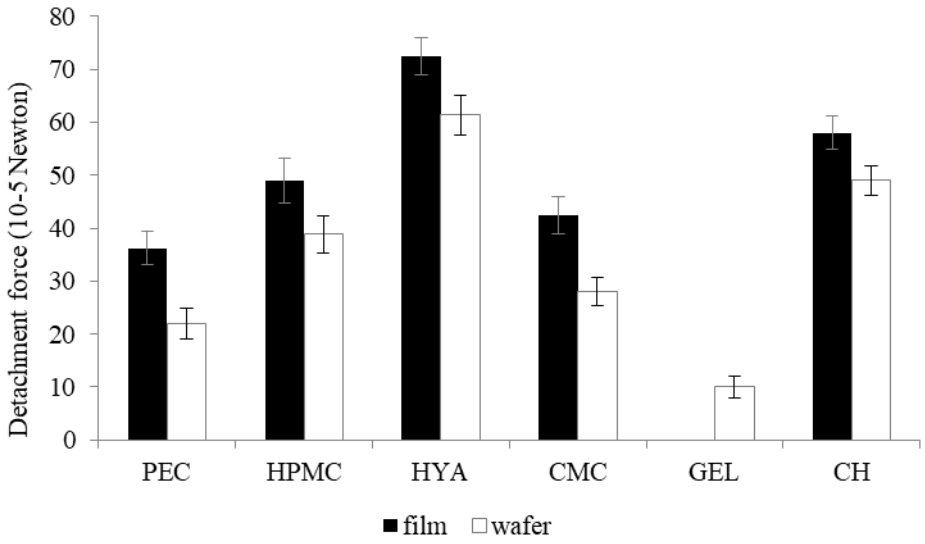
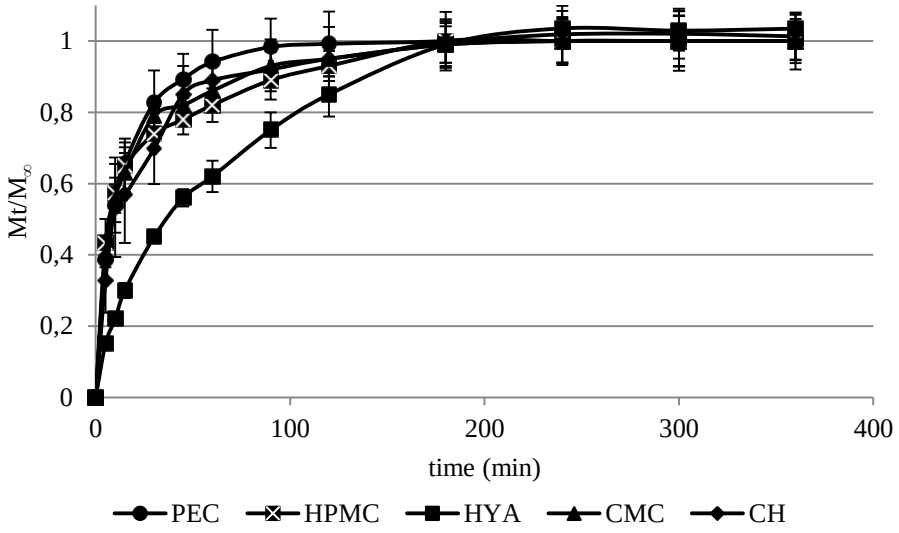

Detachment force (10-5 Newton)
80
70
60
50
40
30
20
10
0
PEC
HPMC
HYA
CMC
GEL
CH
film
wafer

Figure 5

A


Mt/M∞
1
0,8
0,6
0,4
0,2
0
0
100
200
300
400
time (min)
PEC
HPMC
HYA
CMC
CH

B

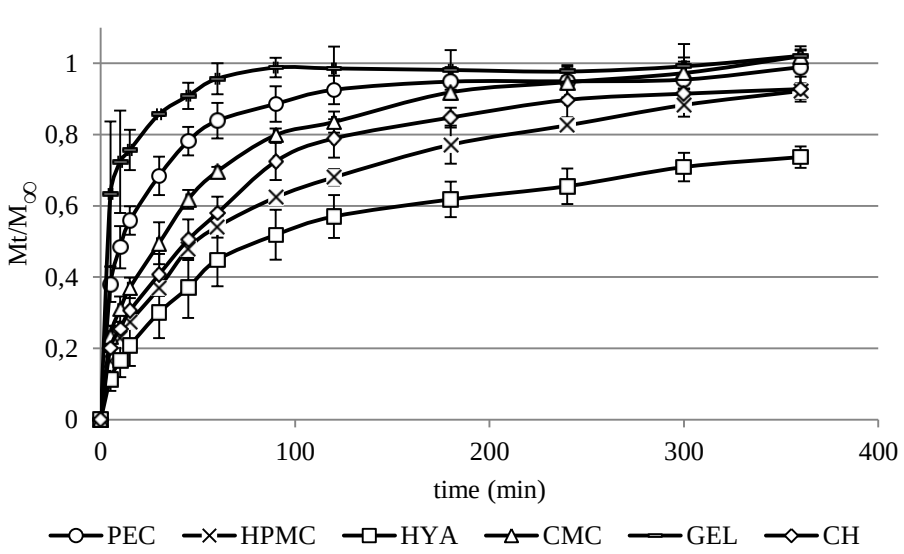

Mt/M∞
1
0,8
0,6
0,4
0,2
0
0
100
200
300
400
time (min)
PEC
HPMC
HYA
CMC
GEL
CH

Figure 6

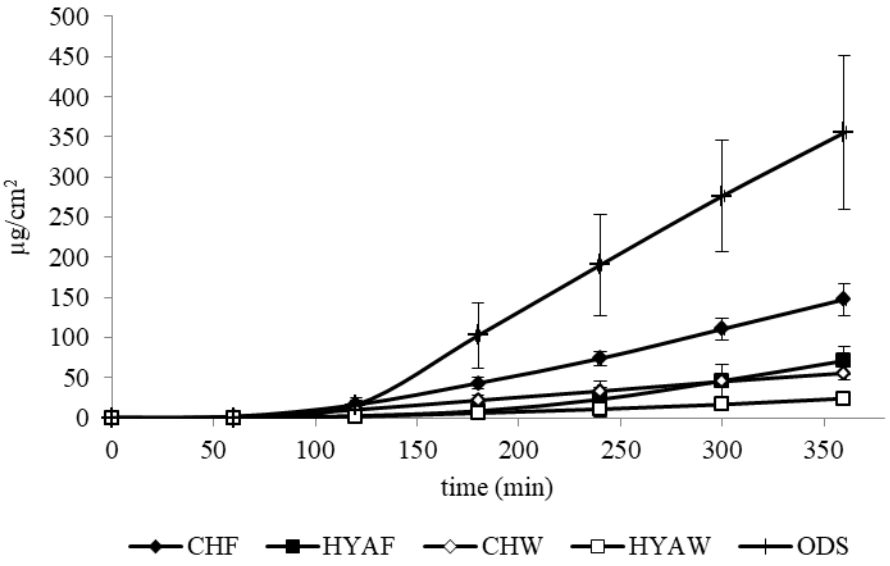
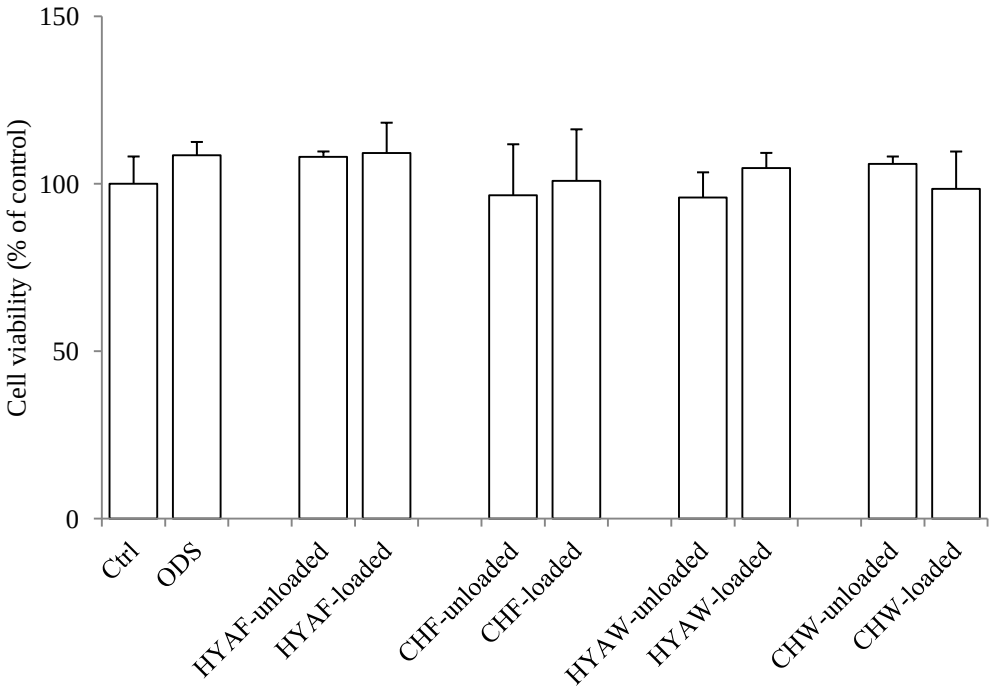

500
450
400
350
300
250
200
150
100
50
0
µg/cm²
0
50
100
150
200
250
300
350
time (min)
CHF
HYAF
CHW
HYAW
ODS

Figure 7


150
100
50
0
Cell viability (% of control)
Ctrl
ODS
HYAF-unloaded
HYAF-loaded
CHF-unloaded
CHF-loaded
HYAW-unloaded
HYAW-loaded
CHW-unloaded
CHW-loaded