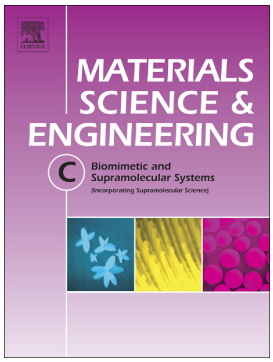


Interaction between mucoadhesive cellulose derivatives and Pluronic F127: investigation on the micelle structure and mucoadhesive performance

Jéssica Bassi da Silva, Rafaela Said dos Santos, Monique Bassi da Silva, Gustavo Braga, Michael Thomas Cook, Marcos Luciano Bruschi

PII: S0928-4931(20)33561-X

DOI: https://doi.org/10.1016/j.msec.2020.111643

Reference: MSC 111643

To appear in: *Materials Science & Engineering C*

Received date: 8 July 2020

Revised date: 15 September 2020

Accepted date: 15 October 2020

Please cite this article as: J.B. da Silva, R.S. dos Santos, M.B. da Silva, et al., Interaction between mucoadhesive cellulose derivatives and Pluronic F127: investigation on the micelle structure and mucoadhesive performance, *Materials Science & Engineering C* (2020), https://doi.org/10.1016/j.msec.2020.111643

Interaction between mucoadhesive cellulose derivatives and Pluronic F127: investigation on the micelle structure and mucoadhesive performance

Jéssica Bassi da Silva[a]; Rafaela Said dos Santos[a], Monique Bassi da Silva[b], Gustavo Braga[c], Michael Thomas Cook[d]; Marcos Luciano Bruschi[a,*]

[a]Postgraduate Program in Pharmaceutical Sciences, Laboratory of Research and Development of Drug Delivery Systems, Department of Pharmacy, State University of Maringa, Maringa, PR, Brazil.

[b]Department of Agronomy, State University of Maringa, Maringa, Brazil

[c]Department of Chemistry, State University of Maringa, Maringa, Brazil

[d]Research Centre in Topical Drug Delivery and Toxicology, School of Life and Medical Sciences, University of Hertfordshire, Hatfield, AL10 9AB, United Kingdom

*Corresponding author.

E-mail address: mlbruschi@uem.br (M.L. Bruschi).

ABSTRACT

Systems composed of bioadhesive and thermoresponsive polymers can combine *in situ* gelation with bio/mucoadhesion, enhancing retention of topically applied drugs. The effect of bioadhesive sodium carboxymethylcellulose (NaCMC) and hydroxypropyl methylcellulose cellulose (HPMC) on the properties of thermoresponsive Pluronic® F127 (F127) was explored, including micellization and the mucoadhesion. A computational analysis between these polymers and their molecular interactions were also studied, rationalising the design of improved binary polymeric systems for pharmaceutical and biomedical applications. The morphological characterization of polymeric systems was conducted by SEM. DSC analysis was used to investigate the crystallization and micellization enthalpy of F127 and the mixed systems. Micelle size measurements and TEM micrographs allowed for investigation into the interference of cellulose derivatives on F127 micellization. Both cellulose derivatives reduced the critical micellar concentration and enthalpy of micellization of F127, altering hydrodynamic diameters of the aggregates. Mucoadhesion performance was useful to select the best systems for mucosal application. The systems composed of 17.5% (w/w) F127 and 3% (w/w) HPMC or 1% (w/w) NaCMC are promising as topical drug delivery systems, mainly on mucosal surfaces. They were biocompatible when tested against *Artemia salina*, and also able to release a model of hydrophilic drug in a controlled manner.

Keywords: hydrogel; polymer blends; sodium carboxymethylcellulose; hydroxypropyl methylcellulose; poloxamer 407; drug delivery systems.

1. Introduction

Polymer blends are an advantageous strategy to develop new drug delivery systems, combining properties of the constituent biocompatible excipients to generate formulations with improved performance. Blends composed of bioadhesive and thermoresponsive polymers combine *in situ* gelation with mucoadhesion, enhancing local delivery of drugs through improved retention [1]. Pluronics® are triblock copolymers composed of poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) (PEO-PPO-PEO). They are non-ionic and amphiphilic materials able to interact with hydrophobic surfaces, such as biological membranes [2]. When in aqueous dispersion above a critical micellization temperature, Pluronics can self-assemble forming micelles, which have been used for the solubilisation of weakly-soluble drugs [3–5]. Their hydrophobic PPO core is able to incorporate water insoluble molecules and protect agents from external interactions [4]. Pluronic® F127 (F127) solutions also exhibit thermoreversible gelation, switching from liquid to gel when warmed due to the formation of the micelles. When the volume fraction of the micelles is high enough, the micelles pack into a face-centered cubic gel [6]. Therefore, formulations containing this polymer can present improved retention at the application site, which is important for local drug delivery systems, such as dermatological, oral or ophthalmic formulations. Despite its positive properties for pharmaceutical applications, F127 forms weak shear-thinning hydrogels [7], even considering its increased gel strength at elevated temperature [4]. There are significant drawbacks for thermogelling F127 dispersions in addition to its insufficient gel strength, including weak mucoadhesiveness and rapid dissolution, which limits its performance in drug delivery systems. Chemical modification or the incorporation of polymer additives have been demonstrated to enhance the properties of F127 hydrogels [6].

F127 preparations have been improved for many routes of administration by combination with other hydrophilic polymers (e.g. mucoadhesive polymers or other grades of Pluronic®) or particulates such as liposomes or nanoparticles [8–10]. Considering mucosal application (e.g. oral, ophthalmic, nasal, retal or vaginal routes), a common limitation is the humidity of the environment and presence of liquids, which reduces the adhesion of the preparation through overhydration, dissolution, and an increase in gelation temperature to above 37 °C following dilution in physiological fluids [11]. Following sublingual administration, for example, salivation, tongue movement and swallowing can quickly remove the pharmaceutical system from the application site [9,12,13]. To overcome poor retention and weak gel strength, synergistic interactions between F127 and other additives have been explored, including: poloxamer 188 [14,15], carbomers [1,16–18], polycarbophil [18], alginate [19], chitosan [20] and cellulose derivatives [21–24]. Moreover, mixing polymeric additives with F127 is attractive as the resultant materials would not require the regulatory burden that a chemical modification would impart [6].

Gels and ointments for topical application have been extensively formulated with F127, and most of them contain thickening agents such as carbomers or other poly(acrylic acid) derivatives [4,25]. Cellulose derivatives have also been suggested as good hydrophilic polymers to form polymeric blends with F127 since they do not present toxicity [16]. Cellulose is a plant polysaccharide in nature, and one of the most widely used excipients in pharmaceutical industry. It is composed of β(1 → 4) linear chains linked to D-glucose units, and as a pharmaceutical excipient is often covalently modified to form ether derivatives such as hydroxypropyl methylcellulose (HPMC) or sodium carboxymethylcellulose (NaCMC). These cellulose derivatives are known to be effective mucoadhesives and gel formers [25–27]. HPMC in solid dosage forms, such as

tablets, has demonstrated adhesive properties similar to that observed for poly(acrylic) acid derivatives and NaCMC. It is known that the mucoadhesive behaviour of HPMC may depend on the mucosal surface to which it is applied, with strong adhesion demonstrated onto the gastrointestinal tract [28]. It is believed that its mucoadhesive performance is related to entanglements between its macromolecular chains and the mucin glycoprotein network constituting secretory mucus [25]. NaCMC is often used as an excipient in solid, liquid or semi-solid pharmaceutical dosage forms, demonstrating intermediate mucoadhesive force among the most known mucoadhesive polymers [25,29].

Systems containing F127 combined with HPMC or NaCMC mucoadhesive agents have previously been evaluated for their rheological and mechanical characteristics for use as topical drug delivery systems [21–24,30]. The addition of cellulose derivatives to F127 hydrogels not only to contribute to the optimization of gel strength [24], but also to improve mucoadhesion [23]. For example, the addition of HPMC and NaCMC to F127 improved the retention of pilocarpine in the pre-corneal area, facilitating its retention and effect on the cornea [31–33]. However, a fundamental understanding of the processes leading to rheological synergism is missing. The effects of both cellulose derivatives on F127 micelle formation have not been studied and specific chemical interactions are not well understood.

The incorporation of drugs or additives to aqueous Pluronic® dispersions may influence its critical micelle concentration (CMC) and temperature (CMT), its aggregation number and the structure of its micelles [34–37]. At low temperature and copolymer concentration, both PPO and PEO are hydrophilic, presenting as unimers with a radius of about 2 nm. Above its CMC and CMT the block copolymers self-assemble into spherical micelles with a hydrodynamic radius of around 10 nm, which

the core, constituted of PPO portion, occupies 4-5 nm of this diameter and it is surrounded by a hydrophilic PEO portion [38]. The literature reports that increasing Pluronic® micelle size increases the drug loading ability of the micelles [39,40]. Moreover, there is some evidence indicating that the morphology of Pluronic micelles plays an important role in drug delivery and targeting application [34,37,41].

The objective of this study was to evaluate the effect of NaCMC and HPMC on F127 micellar properties and interactions within the system to gain insight into the mechanisms underpinning rheological synergism [24]. A computational analysis between the polymers and their interactions was studied, helping understand optimal conditions for forming *in situ* gelling systems. Mucoadhesion was also studied, and the release of a model hydrophilic drug was studied, to indicate suitability for future formulation development. Thus, this study investigates modification of the micelle structure of F127 by cellulose derivatives, looking at the mucoadhesive ability and the capacity of the system to release drugs for pharmaceutical and biomedical applications.

2. Materials and Methods

2.1. Materials

Pluronic® F127, erythrosine B, mucin (from porcine stomach, type II crude), and phosphate buffered tablets (pH 7.4) were purchased from Sigma-Aldrich (Sao Paulo, Brazil). HPMC K100 Methocel® (8.1% hydroxypropoxyl content and 22% methoxyl content) was donated from Colorcon Dow Chemical Company™ (Datford, United Kingdom). NaCMC (degree of substitution between 0.8 and 0.95) was purchased from Synth (Diadema, SP, Brazil). Ultra-purified water was obtained in-house using a Milli-Q water purification system (Millipore, Merck, Darmstadt, Germany). Unless specified, all reagents were used without further purification.

2.2. Preparation of systems

The thermogelling systems were prepared by dispersion of HPMC (3 or 4%, w/w) or NaCMC (1.0 or 1.5%, w/w) in purified water with mechanical stirring, at room temperature as described in Table 1. After completely dispersion of the cellulose derivative, an appropriate amount of F127 (17.5 or 20%, w/w) was added to the preparation and the mixture was stored at 5 °C for 48 h, to ensure complete polymer wetting. Afterwards, the polymeric system was stirred again to complete dispersion of polymers. The systems were kept at 5 °C for at least 24 h before analysis [1,42,43].

Table 1

Composition of the selected semi-solid formulations containing Pluronic® F127 (F127) and sodium carboxymethylcellulose (NaCMC) or hydroxypropryl methylcellulose (HPMC).

Concentrations (%, w/w)		
F127	NaCMC	HPMC
17.5	1.0	-
17.5	1.5	-
20.0	1.0	-
20.0	1.5	-
17.5	-	3
17.5	-	4
20.0	-	3
20.0	-	4

2.3. Scanning electron microscopy (SEM)

To perform the morphological analysis of the systems, the polymeric blends were subjected to instant freezing using liquid nitrogen at −80 °C for 20 min. Frozen samples were then lyophilized for 48 h using a Micro-196 modulo freeze dryer (Thermo Electron Corporation, Oregon, USA). Segments of the dried samples were carefully

deposited on stubs containing double-sided adhesive carbon tape. The samples were metallized by the deposition of a thin layer of gold using a metallizer Sputter Coater, model SCD 050 (Bal-Tec, California, USA), and evaluated using a SS550 Superscan scanning electron microscopy (Shimadzu, Tokyo, Japan).

2.4. Molecular modelling analysis

Molecular modelling studies of NaCMC, HPMC, NaCMC/F127, NaCMC/PEO, NaCMC/PPO, HPMC/F127, HPMC/PEO and HPMC/PPO were performed to evaluate the interaction of the cellulose derivative and F127 oligomers or fragments. PEO (-PEO_{12}-), PPO (-PPO_{12}-), F127 (-$(PEO)_5$-$(PPO)_3$-$(PEO)_5$-), NaCMC (-$(C_8H_{16}NaO_8)_2$-) and HPMC (-$C_{32}H_{60}O_{19}$-) were used. It was carried out using Orca 4.0 program [44] optimized in vacuum. Hartree-Fock (HF) method with implementations for long range interactions (HF-3c) was used to obtain the most stable geometric structure in macromolecular systems [45]. The advanced molecular editor Avogadro program version 1.1.1 [46], was applied for graphical visualization of the structures. The complexation energy (ΔE_{Comp}) between proportional fragments or oligomers of F127 copolymer and NaCMC or HPMC was determined according to Eq. (1) [47].

$$\Delta E_{Comp} = E_{F127/NaCMC\ or\ HPMC} - (E_{NaCMC\ or\ HPMC} + E_{F127}) \quad (1)$$

where, $E_{F127/NaCMC\ or\ HPMC}$, $E_{NaCMC\ or\ HPMC}$, and E_{F127} are the total electronic energy for the optimized structures of the complex between NaCMC or HPMC and the copolymers fragments or oligomers of F127; the amount of cellulose derivatives fragments, and F127 fragments or oligomers, respectively.

2.5. Differential scanning calorimetry (DSC)

Ca 35 mg of each formulation, 5 mg of lyophilized formulation, or 5 mg of the constituent polymers were placed in aluminium pans and hermetically sealed. DSC was performed using a DSC Q20 (TA Instruments®, Surrey, UK) at a heating rate of 5 °C/min between 5 and 40 °C for the hydrogels, and from 0 to 400 °C for the lyophilized systems, under a nitrogen atmosphere. The F127 crystallinity was calculated using DSC thermograms as the ratio of the measured polymer crystallization enthalpy to the product of the polymer weight fraction and the crystallization enthalpy of completely crystallized F127 [48]. The micelle formation temperature (CMT) was determined from the endothermic peak in the DSC thermograms of the hydrogels, which occurred between 10 and 20 °C.

2.6. Dynamic light scattering (DLS)

Micelle formation and critical micelle concentration (CMC) were studied using a Zetasizer Nano ZS (Malvern, UK) [49,50]. Samples were diluted in purified water. F127 dispersion was evaluated at concentrations ranging from 10 mg/mL to 0.01 mg/mL. The mixtures containing HPMC and NaCMC were kept at 1 mg/mL. The scattering intensity of the samples was evaluated using attenuator 10 at 37 °C and analysed using Malvern's Zetasizer® software over a range of concentrations.

2.7. Transmission electron microscopy (TEM)

The TEM analysis was performed using a JEM-1400 Transmission Electron Microscope (JEOL, Tokyo, Japan), with an accelerating voltage of 120 kV. Samples containing mixtures of 17.5% F127 and 3% HPMC or 1% (w/w) NaCMC were diluted

50-fold, and then negatively stained with 2% (w/v) uranyl acetate solution for observation. Samples were prepared at 37 °C to study the micelle formation.

2.8. In vitro mucoadhesion assessment

The mucoadhesive properties of the hydrogels were assessed using a TA.XT Plus texture analyser (Stable Micro Systems, UK) in tension mode. The polymeric blends were kept in a water bath for equilibration at 37 ± 1 ºC. Mucin disks were prepared by compression of 300–400 mg crude porcine mucin and then horizontally attached to a mobile cylindrical probe by a double-sided adhesive tape, and mucoadhesion determined using the tensile strength method. The probe was lowered at a speed of 1 mm/s until it reached the mucoadhesive hydrogel surface with a 0.03 N contact force, keeping the substrate just in contact with the hydrogel surface. The disk and formulation were kept in contact for 60 s, and the probe was withdrawn at a rate of 10.0 mm/s until complete detachment between mucoadhesive hydrogels and mucin disks [43]. The maximum force of detachment and the work of adhesion, which is the area under the force/distance curve, were determined using Texture Exponent 32 software (Stable Micro Systems, UK). All measurements were performed at least in three replicate samples, and the adhesion parameters calculated as mean values ± standard deviation.

2.9. Toxicity evaluation using Artemia salina

The toxicity study using *Artemia salina* was performed for the two formulations containing 17.5% F127 and 3% HPMC or 1% NaCMC, which were selected from mucoadhesion studies. Saline (3.5% w/v NaCl) was utilized to hatch *Artemia salina* cysts at 25 °C [51]. Cysts were deposited in a dark chamber, interconnected to an

illuminated chamber (using a lamp of 7 W), allowing migration of the nauplius (phototropism). After 48 h of incubation, the organisms were transferred to a Petri dish and used to evaluate the toxicity of the formulations containing 17.5% (w/w) F127 and 3% (w/w) HPMC or 1% (w/w) NaCMC. An aliquot of 1 mL (1:2 dilution), 2 mL (2:3 dilution) or 3 mL (3:4 dilution) of each stock dispersion were added to the well containing 20-25 nauplius, and the volume was made up 1.0 mL with saline water. After 30 min, the viability of microcrustaceans was calculated according to the Eq. (2):

$$Viability\ (\%) = N°\ \text{live}\ -\ N°\ \text{dead}/N°\ total\ x100 \quad (2)$$

Dead crustacea were quantified by counting immobile *Artemia salina.* Mobile crustacea were considered to be live [52].

2.10. In vitro evaluation of drug release profile

Erythrosine (ERI) was used as a hydrophilic drug model to investigate the ability of binary polymeric systems to control drug release. The analysis was performed using a modified Franz cell apparatus, with cellulose acetate membrane as support (molecular weight cut-off 12,400 kDa, Sigma-Aldrich, Sao Paulo, Brazil). The amount of 50 mL of phosphate buffered saline, pH 7.4, at 37 °C, stirred with a magnetic bar was used as receiver fluid. 2.0 mL was sampled and replaced with a new receiving fluid at 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h and 24 h [53]. The ERI concentration was evaluated by spectrophotometry (λ= 525 nm). The mechanism of ERI release from the ERI solution (1%, w/w) and from the two selected polymeric systems composed of 17.5% (w/w) F127 and 3% (w/w) HPMC or 1% (w/w) NaCMC and 1% (w/w) ERI was studied fitting the data to Higuchi, first-order, and Korsmeyer-Peppas equations.

ERI was quantified using a spectrophotometer (UV-1800, Shimadzu, Sao Paulo, Brazil) at λ_{max}= 525 nm. The Beer's law was obeyed over the concentration range of 0.06-8.0 μg/mL with a high degree of correlation (r = 0.9980). The proposed method is fairly sensitive with 0.096 and 0.291 μg/mL as detection and quantification limit, respectively. Fig. S1. displays the spectra recorded of ERI into a polymeric mixture at 6.0 μg/mL (A) and the spectrum of the polymeric matrix without the drug (B and C), demonstrating ERI peak at 525 nm with none being observed by polymeric system.

2.11. Statistical analysis

The effect of type and concentration of each cellulose derivative on the detachment force and work adhesion were statistically treated using two-way ANOVA. Individual differences between means were identified using Tukey's honestly significant difference *post-hoc* test. In all cases, $p < 0.05$ was taken to denote significance.

3. Results and discussion

The selection of the most suitable systems containing F127 and NaCMC or HPMC to be used for pharmaceutical and biomedical applications was made based on gelation temperature ($T_{sol/gel}$), and other rheological parameters previously described [24]. Eight formulations that have demonstrated suitable mechanical and rheological properties as topical drug delivery systems were selected to investigate F127-additive interactions. Four of them were composed of F127 (17.5 or 20%, w/w) and HPMC (3 or 4%, w/w) and the other four were composed of F127 (17.5 or 20%, w/w) and NaCMC (1.0 or 1.5%, w/w), which demonstrated a density of approximately 1 g/mL (Table S1).

3.1. Scanning electron microscopy (SEM)

Morphological analysis of the polymeric systems allows for a better comprehension of their structure and organisation. It may be a complementary tool following previous mechanical and rheological characterization of the hydrogels [24]. Fig. 1 and 2 display SEM images of the selected systems containing 17.5 or 20 % F127 and 3 or 4 % HPMC or 1 or 1.5% NaCMC.

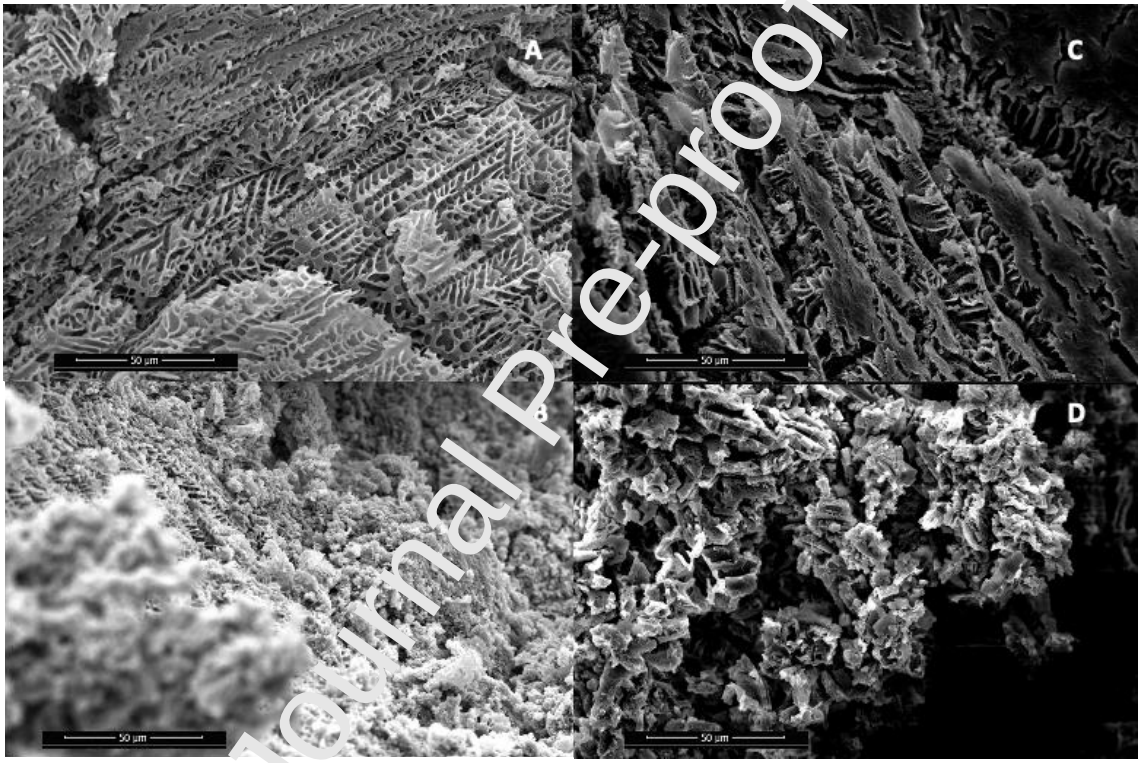


Fig. 1. Scanning electron microscopy (SEM) images of Pluronic® F127 (F127) and hydroxypropyl methylcellulose (HPMC) binary polymeric formulations at different concentrations and magnitude of 2000x: (A) 17.5% (w/w) F127 and 3% (w/w) HPMC; (B) 20% (w/w) F127 and 3% (w/w) HPMC; (C) 17.5% (w/w) F127 and 4% (w/w) HPMC; (D) 20% (w/w) F127 and 4% (w/w) HPMC.

The morphology of the formulation is often attributed to the interactions between polar groups of micellar copolymer and cellulose derivatives [54]. In general,

the polymeric platforms demonstrated a well organised morphology, which though heterogeneous, is well defined. Both polymeric combinations resulted in a sponge-like system, which is possible related to self-assembly of gel, as already described in the literature for other F127 blends [55,56]. Formulations containing F127 and HPMC (Fig. 1) showed a lamellar layout. A better-defined structure was observed at lower concentrations of F127, particularly in presence of low HPMC concentration. The system composed of 20% (w/w) F127 and 3% (w/w) HPMC appeared relatively dense, representative of a highly cohesive system, as also observed by texture profile analysis previously [24]. Moreover, systems composed of 20% (w/w) F127 and 4% (w/w) HPMC demonstrated amorphous morphology when compared to the other formulations, since hydrophobic associations and hydrogen bonds may contribute to this cohesion [55,57].

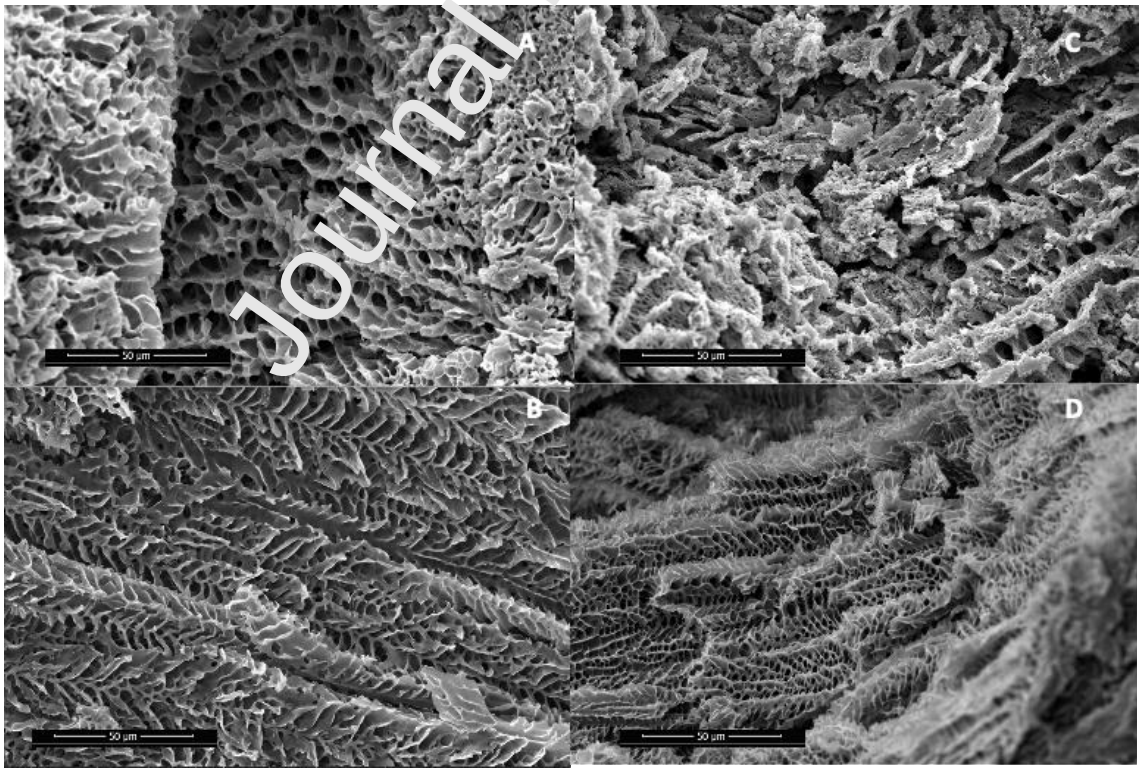


Fig. 2. Scanning electron microscopy (SEM) images of binary Pluronic® F127 (F127) and sodium carboxymethylcellulose (NaCMC) polymeric formulations at different concentrations and magnitude of 2000x: (A) 17.5% (w/w) F127 and 1.0% (w/w) NaCMC; (B) 20% (w/w) polox407 and 1.0% (w/w) NaCMC; (C) 17.5% (w/w) F127 and 1.5% (w/w) NaCMC; (D) 20% (w/w) F127 and 1.5% (w/w) NaCMC.

SEM images of the formulations of F127 and NaCMC (Fig. 2) demonstrated clear reduction in pore size when the concentration of F127 increased. This may be a consequence of the reduced volume fraction of trapped water, since the porous are result of the water sublimation during the lyophilization process. The establishment of hydrogen bonds between PEO moieties of F127 and carboxylic segments of NaCMC may also have contributed to the formation of channels, as already observed for mixtures of F127 and poly(acrylic acid) derivatives [55,58,59]. It may be suggested that NaCMC exhibited greater potential for interaction with water compared to HPMC formulations, showing better space to trap hydrophilic drugs [24]. Additionally, the SEM images evidenced possible change in the micelle dimensions for each cellulose derivative, which may also support interaction between thermoresponsive and mucoadhesive polymers.

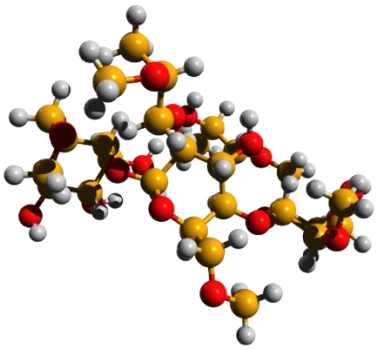
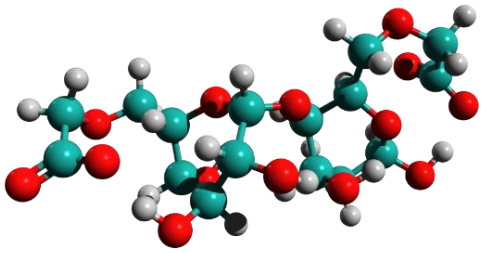
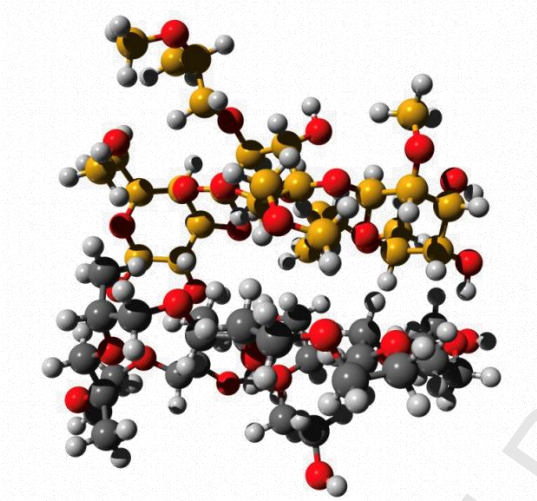
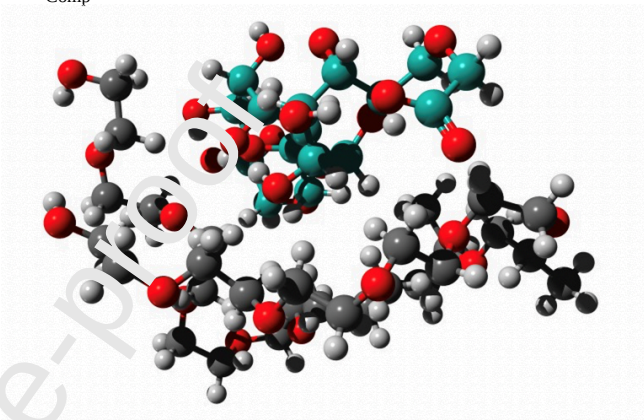
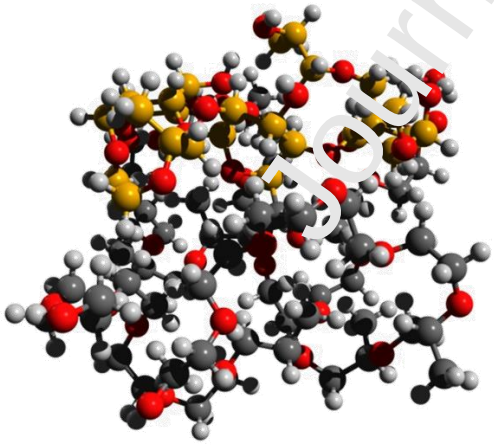
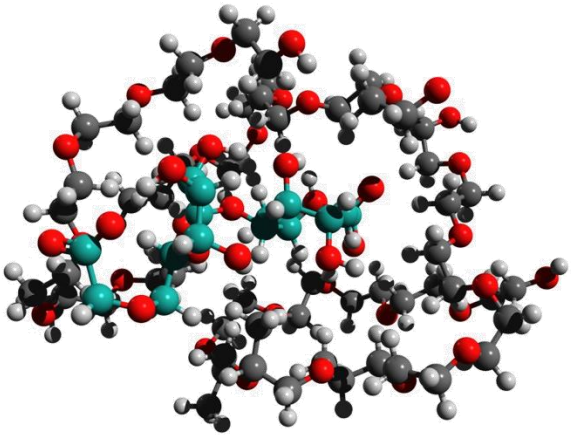
3.2. Molecular modeling analysis

Computational modelling allows for the comprehension of interactions involved between different molecules [60,61]. As interactions between F127 and NaCMC or HPMC are not completely understood [23,24,30]. This tool is useful to study, at a molecular level, how polymeric blends composed of cellulose derivatives and F127 behavior at simulated vacuum atmosphere. This contributes to the knowledge of how

these additives effect drug packing and micellization parameters of triblock F127, which are important to understand the formation of different nanostructures in aqueous solution [62]. Herein, chemical interactions were concerned in terms of ΔE_{Comp} between both mucoadhesive and thermoresponsive polymers, and the ΔE_{Comp} values for optimized structures are displayed in Table 2.

Table 2

Total electronic energy for the optimized fragment of sodium carboxymethylcellulose (NaCMC), hydroxypropyl methylcellulose (HPMC) and the complexes of 1:1 and 1:2 structure with Pluronic® F127 (F127) calculated at the HF-3c level of theory.

	
HPMC/1F127	**NaCMC/1F127**
HF-3c/hatrees= -4589.429974552590 [a]ΔE_{Comp} /kcal mol^{-1}= -30.13343577 	HF-3c/hatrees= -3898.496211700285 [a]ΔE_{Comp} /kcal mol^{-1}= -32.71768652 
HPMC/2F127	**NaCMC/2F127**
HF-3c/hatrees= -6756.52671808325 [a]ΔE_{Comp} /kcal mol^{-1}= -35.35806373 	HF-3c/hatrees= -6065.613438871278 [a]ΔE_{Comp} /kcal mol^{-1}= -50.79600363 

1 hartree = 627.5095 kcal/mol.

[a]For the calculations of ΔE_{Comp}/kcal mol^{-1} were used the values from HF-3c/hartrees.

Values of F127 fragments in *HF-3c/hartrees* were extracted from [61].

The complexation between F127 and both cellulose derivatives was favorable ($\Delta E_{Comp} < 0$). NaCMC presented ΔE_{Comp}= -32.718 kcal/mol, while HPMC showed values of -30.133 kcal/mol considering 1:1 proportion. The similarity of ΔE_{Comp} observed for them may indicate similar impact of both changing physical-chemical properties of F127. However, when the proportion between cellulose derivatives and F127 is increased to 1:2, it is possible to observe a more evident gap between NaCMC and HPMC mixtures. Considering the proportion 1:2, NaCMC presented ΔE_{Comp}= -50.796 kcal/mol, while HPMC showed values of -35.358 kcal/mol. In Table S2 there are the ΔE_{Comp} values for optimized cellulose derivatives with each segment of F127 (PEO and PPO). It indicated greater tendency of interaction between HPMC and PEO than HPMC and PPO, which increases the hydrophobicity of this segment. Meanwhile, NaCMC demonstrated higher interaction with PPO moiety. Since each cellulose derivative presents different hydrophobic/hydrophilic balance, the interactions stablished with F127 may occur in numerous different manners (e.g. hydrogen bonds, electrostatic or hydrophobic interactions) [63]. The findings also suggest a facilitated stabilization of the F127 fragment in presence of NaCMC in comparison to HPMC, which can facilitate interaction with the copolymer.

3.3. Differential scanning calorimetry (DSC)

DSC analysis is an important device to characterise polymers or their mixtures, as well as to study nanostructured systems [64]. It provides detailed information about enthalpy changes associated with thermally-induced process occurring within different substances, which may be used to understand alterations in F127 behaviour due to the

presence of additives [65]. The calorimetric profile of the lyophilised polymeric systems and the constituent polymers were evaluated in order to characterize them (Fig. 3).

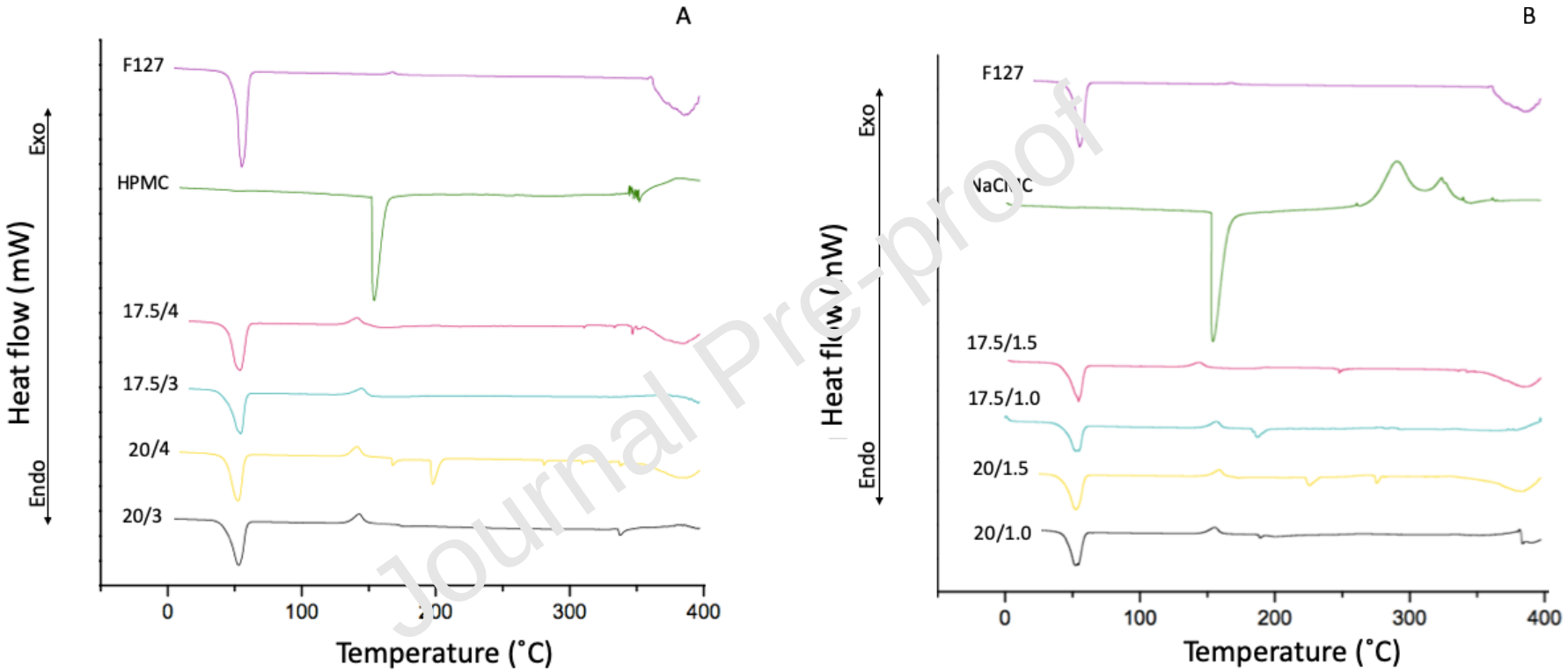


Fig. 3. DSC calorimetry thermogram of mono-polymeric and binary polymeric systems containing freeze dried (A) 17.5 or 20% (w/w) Pluronic® F127 (F127) and 3 or 4% (w/w) of hydroxypropyl methylcellulose (HPMC), or (B) Pluronic® F127 (F127) and 1 or 1.5% (w/w) sodium carboxymethylcellulose (NaCMC).

The DSC thermograms of the pure polymers showed fewer features compared to the mixtures. In DSC studies the cleavage of existing hydrogen bonds gives an endothermic signal and the formation of new bonds may give an exothermic one. F127 displayed an endothermic peak at 55.2 °C, associated with its melting point [66,67]. Moreover, a minor exothermic peak at about 150 °C was observed for it, suggesting a possible crystallization [68,69]. The cellulose derivatives also demonstrated a melting point of 154.01 °C for NaCMC and 154.16 °C for HPMC, in agreement with the literature [70]. Exothermic peaks were observed for both cellulose polymers above 300 °C, characterizing a degradative event.

Although small when compared to the pure copolymer, the endothermic peak of F127 at the same position (about 55 °C), was observed for all formulations (Fig. 3). Revealing the existence of F127 in crystalline state, in agreement with the literature [66]. Considering 100% F127 has a melting endotherm of 112.2 (J/g) magnitude, a reduction of F127 crystallinity was observed in NaCMC or HPMC formulations (Table S3). The addition of HPMC led to a non-concentration dependent reduction of F127 crystallinity in a range of 9.03-11.97%, while NaCMC demonstrated higher impact, decreasing its crystallinity by 7.66 to 28.64% depending on the formulation. The reduction in crystallinity may be linked to plasticisation of the F127 by HPMC or NaCMC as a result of intermolecular interaction, also predicted in this rank order *in silico*. As NaCMC had a greater impact on this parameter than HPMC, its reduced crystallinity may emphasize, therefore, its stronger interaction with F127, in agreement with computational modelling and with previously determined rheology [24]. The crystallinity refers to the degree of structural order of a solid, which influences its mechanical, thermal and chemical properties [68].

It is possible to observe that the exothermic peak at 150 °C viewed at isolated F127 (1.90 J/g) thermogram was intensified when the mixture was made with HPMC (13.46 J/g in average) and NaCMC (14.30 J/g in average). Depending on the heating rate of the method, crystalline organization and hydration degree of the polymers, DSC peak may be influenced, resulting in broad or sharp peak in comparison with the literature [71–73]. However, the endothermic characteristic signals of the cellulose derivatives (around 154 °C) were not observed on the dried polymeric blends. Lack of a melting peak indicates that the substance is present in an amorphous rather than a crystalline state [67,68,74,75], which give to the system increased flexibility [76]. The decrease or absence of crystallinity have been already revealed in some polymeric blends, as observed for PVA/ methylcellulose [77], PVA/ carboxymethyl cellulose [78] and PVP/ HPMC mixtures [76]. These observations may indicate that the ordered association of the cellulose derivatives molecules is strongly constrained by the presence of the F127 [77].

The thermograms are able to reflect the greater amount of F127 composing the binary system with predominance of its calorimetric profile when compared to HPMC and NaCMC. Additionally, both systems containing the greater amount of thermoresponsive and mucoadhesive polymer (yellow line in Fig. 3) exhibited extra small endothermic peaks at around 200 °C, which was not observed on the other studied mixtures. This may be related to crystallization and melting of possible stable phase or a solid-solid transition, since both polymers are used in the highest concentration [68,79].

Formation of micellar domains is always required for F127 gelation. DSC was also used to study the transition induced by temperature in the self-assembled aqueous polymeric dispersions [3,80], determining the CMT of the formulations. The presence of endothermic peaks comes from the desolvation of the hydrophobic portion of F127

with raising temperature [81]. Since this phenomenon leads to the micelle formation, the peak can be considered the CMT, while its integrating area may be determined as the enthalpy of micellization [10]. The literature reports a CMT for pure 20% (w/w) F127 dispersions at about 12 °C [15,82,83], and an enthalpy of micellization of 25.5 ± 2 J/g of F127 [84,85].

As showed in Fig. 4, all evaluated formulations demonstrated the micellization property imparted by F127, though no large difference was observed among their enthalpic behaviour (Table S4), in agreement with their previously determined $T_{sol/gel}$ [24,86]. The cellulose derivatives increased the CMT of F127, with values greater than 12 °C. HPMC-content formulations exhibited CMT (T_{peak}) around 14-16 °C, whereas NaCMC systems displayed CMT (T_{peak}) between 14 and 17 °C, with the values found inversely proportional to the polymer concentration. Each system demonstrated to begin the micellization at different temperatures (Table S4), with the lowest T_{onset} for the most concentrated systems. The mixture containing 20% (w/w) F127 and 4% (w/w) HPMC exhibited 10.2 °C as T_{onset}, while 20% (w/w) F127 and 1.5% (w/w) NaCMC showed 11.2 °C. Then, the 20/1 NaCMC formulation demonstrated 12.2 °C and 20/3 HPMC system presented 11.1 °C. Moreover, systems containing higher F127 concentration started the micellization process before those that were less concentrated.

The positive enthalpy indicates the transfer of unimers from solution to the micelle, which is an enthalpically unfavourable endothermic process [84]. Although a negative entropy contribution must be the driving force for the micellization of F127 block copolymer [87], direct interactions between cellulose derivatives and copolymer may also contribute to changes in its enthalpy. In comparison to raw F127 dispersions, both cellulose derivatives reduced the micelle formation endotherm of the copolymer around 0.8-fold, raising structural disorder [88]. Whilst no statistical significance was

observed, the total micellization enthalpy observed for HPMC was slightly less than that displayed for NaCMC formulations, further from the values described for pure F127. As the temperature increases, F127 micelles formation occur due to an entropically driven desolvation of PPO blocks [6,89], thereby, the reduction in total enthalpy reflects the decline in the energy consumed for PPO dehydration [81].

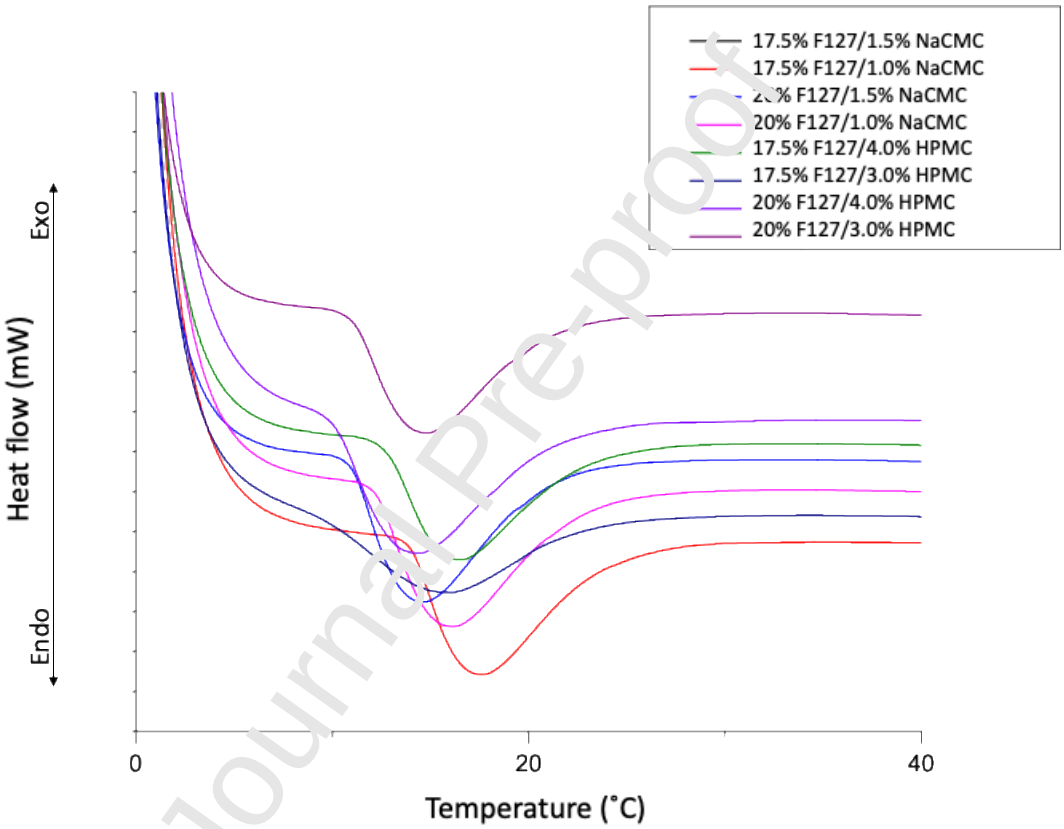


Fig. 4. DSC calorimetry thermogram of binary polymeric hydrogels composed of Pluronic® F127 (F127) and hydroxypropyl methylcellulose (HPMC) or sodium carboxymethylcellulose (NaCMC).

The importance of hydrophobic effect in the micellization enthalpy of F127 systems is already known [90,91]. Comparatively, the hydrodynamic volume occupied by NaCMC is frequently higher than that occupied by HPMC. As an ionic polymer,

NaCMC polymeric chains are able to be more extended. By Mark-Houwink-Sakurada relationship, an $\alpha = 0.87$ was found for NaCMC in 0.1 M NaCl [92], and $\alpha = 0.53$ for HPMC in aqueous solution [93]. Therefore, the micellization properties may be also affected by the greater interaction of NaCMC with water, providing a large steric bulk to interfere with micelle packing when compared to HPMC, which has relative hydrophobicity as a non-ionic species [24,38,94]. For instance, the addition of ethanol has been noted to reduce the enthalpy of micellization and increase the CMT of F127 in a similar way due to its structure breaking effect on water [95,96].

3.4. Dynamic light scattering (DLS)

DLS analysis of F127 dispersions was used to investigate the micellization process and aggregate formation. The values of light scattering intensity as a function of F127 concentration of F127 pure system, as well as, binary systems containing F127 and HPMC or NaCMC, at 37 °C, are displayed in Fig. 5. The light scattering intensity observed by F127 at concentrations below its CMC shows a nearly constant profile, indicative of background data. This intensity begins to increase linearly above its CMC, as the number of micelles in solution linearly increases with concentration, scattering light monotonically. Therefore, the intersection of the straight lines that best matched the data obtained corresponds to the CMC of the polymeric dispersion [49,50].

As exposed in Fig. 5., the intersection of best fit lines drawn through the data points corresponds to 0.227 mg/mL for monopolymeric F127 dispersion (Fig. 5. A), 0.122 mg/mL for the mixtures composed of F127 and HPMC (Fig. 5. B) and 0.169 mg/mL for the mixture containing F127 and NaCMC (Fig. 5. C). This intersection indicates the CMC of F127 copolymer in solution.

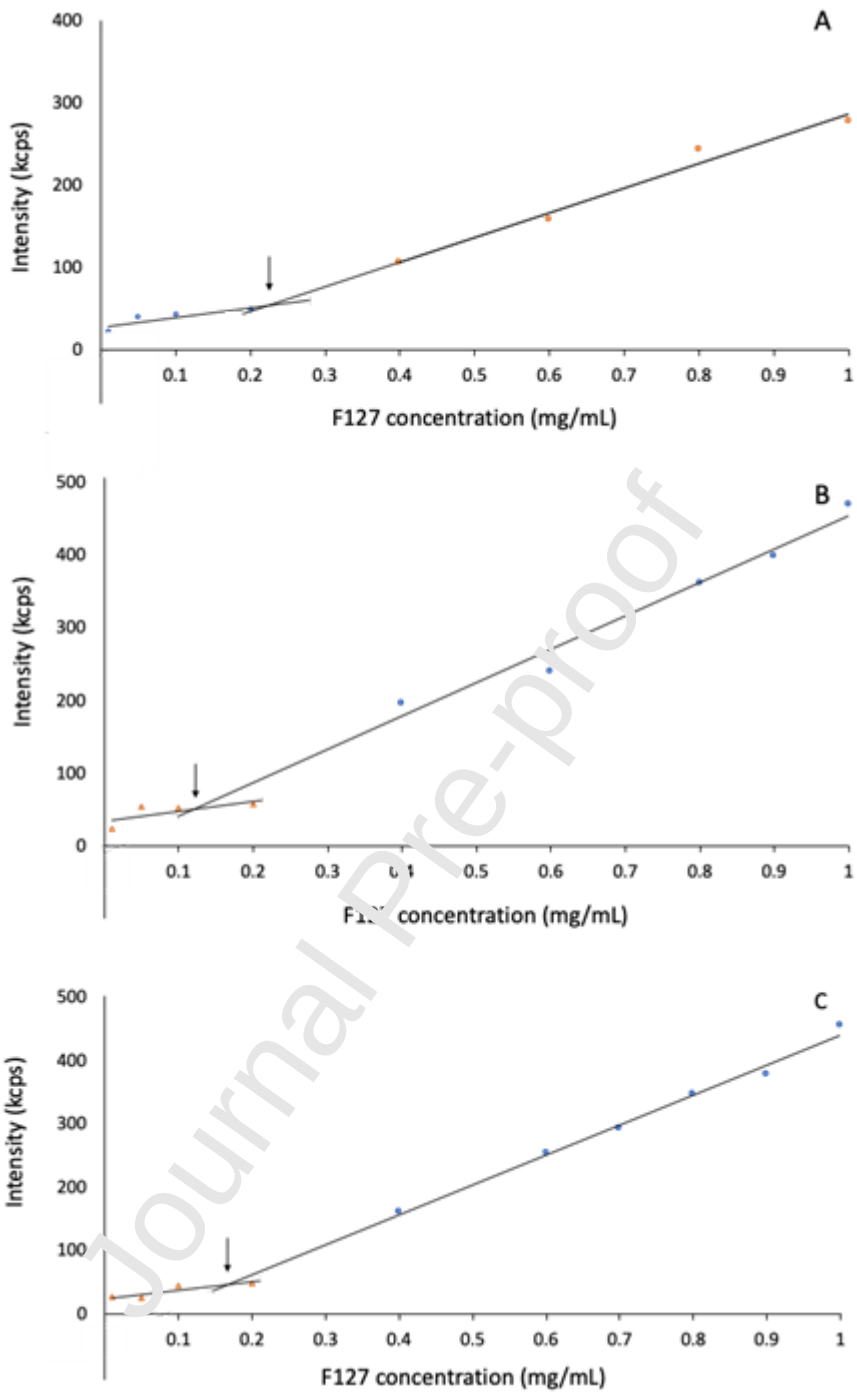


Fig. 5. Dynamic light scattering measurements of (A) Pluronic® F127 dispersion, and mixtures composed of Pluronic® F127 (F127) and (B) hydroxypropyl methylcellulose (HPMC) or (C) sodium carboxymethylcellulose (NaCMC) at 1 mg/mL and 37 °C.

Pluronic® F127 exists in solution as unimers, self-assembling into micelles at concentrations above the CMC, where the polymer self-aggregates [97]. Pure dispersions of F127 had a CMC of 0.227 mg/mL. As reported in previous studies, F127 CMC varies dependent on the temperature and method used. At 37 °C, by DPH fluorescence, a CMC of 0.16 mg/mL was observed for F127 [98,99], while by static light scattering CMC = 0.11 mg/mL was demonstrated [100]. Moreover, at 35 °C, a CMC = 0.25 mg/mL was also found [86]. Herein, the addition of both cellulose derivatives decreases the CMC of F127. The interaction between cellulose additives and F127 may replaces water solvating F127 chains, favoring the micelle formation, as well as competition for solvent effectively concentrating the polymer [69,95]. F127/NaCMC mixture demonstrated a CMC value of 0.169 mg/mL, while the F127 and HPMC mixture showed a CMC of 0.122 mg/mL, as displayed in Fig. 5 (B and C).

When increasing temperature, F127 aggregation becomes favoured, accompanied by a reduction in its CMC, which has been shown to be altered in the presence of additives [6,32]. Overall, this change mainly depends upon the lyophobic/lyophilic character of the solutes and the presence of cosolvents [3,35,40]. The addition of kosmotropic (order-making) sodium chloride in F127 dispersions reduces its CMC, while chaotropic (order-breaking) urea has the reverse effect. Sodium ions are able to induce hydrophobicity in the PPO moiety, reducing the hydrophilicity of PEO moieties favouring the micellization of the block copolymer at lower concentrations [32]. Moreover, the *in silico* study showed a good interaction of NaCMC with PPO and PEO (Table S2). The addition of the cellulose derivatives appears to act in this way. They may be able to reduce free water in the system, behaving as structure promoters, reducing the CMC of F127 copolymer [95,101].

In order to determine specific interactions between the polymers, FTIR-ATR analyses were performed for isolated polymers and the lyophilized binary systems in the lowest concentration evaluated (Fig. S2 and S3). Since F127 is the major polymer constituting the hydrogels, the FTIR of them were similar to the observed for raw F127. Bands around 3420 cm^{-1} shifts display the hydroxyl group, which had their intensity reduced for the hydrogels. It suggests that breakage of hydrogen bonds take place due to the presence of a lower amount of hydroxyl groups by their involvement in the interaction with the cellulose derivatives [102,103]. It was observed mainly for NaCMC preparations, confirming its high interaction with F127 by hydrogen bonds, while HPMC may use other ways to interact (e.g. hydrophobic interactions). Additionally, bands around 2880 cm^{-1} exhibiting C-H stretch vibration of F127 [104] were also more supressed for NaCMC systems, agreeing with computational modelling.

Interactions between the polymers, evidenced *in silico* and by spectroscopy, supports the cellulose derivatives behaviour on the F127 micellization process, since the interactions may increase the hydrophobicity of the copolymer. Moreover, hydrogen bonding between cellulose derivatives and PEO blocks of F127 may lead to decreased effective block length of PEO, thereby facilitating micellization [81].

With spherical architecture, the micelles of F127 can be characterized precisely by their radius [84]. By DLS, a diameter of 31.77 ± 1.43 nm (PDI of 0.166) was determined for F127 dispersion [105]. When mixed with NaCMC, a larger diameter for the micelles was observed (36.89 ± 1.56 nm), with a larger polydispersity index (PDI = 0.322). In the presence of HPMC, a diameter of 26.23 ± 0.20 nm was shown for the system, with a PDI of 0.136. In the literature, the favourable association of polyethylene glycol with PEO blocks in corona has been reported, leading to the formation of micelle clusters with increased diameter [91,106]. The corona specific dehydration induces inter

micellar attraction and cluster formation, whereas core specific dehydrations bringing sphere-to-rod micellar shape transition is also reported [34]. It is suggested that micellar clusters and heterogeneity of size may be related to changes in the corona [83], which may reflect the preference of NaCMC chains for this environment in comparison to HPMC, as already demonstrated for poly(acrylic acid) and F127 blends [81].

3.5. Transmission electron microscopy (TEM)

There are many techniques available to determine particle or micelle size, but only TEM currently allows the direct observation of the particles [107]. TEM images (Fig. 6) of F127 and HPMC or NaCMC mixtures were obtained at 37 °C. Although the particle sizes evaluated by TEM technique were slightly different in terms of absolute values from that observed by DLS, the distribution profile was the same. TEM samples were measured dry, whereas DLS was conducted in solution. Since the hydrodynamic diameter measured by DLS assumes a hydration layer surrounding the molecule, it is intuitive that the diameter measured by TEM is lower [83].

All the data evidenced a well-defined nanostructured system, with particles of 13.60 ± 2.97 nm for HPMC formulations and, 25.34 ± 4.69 nm micelles for NaCMC systems observed. The micelles exhibited a spherical shape and core-shell structure of F127 agreeing with what has been recently published [108]. The high polydispersity for NaCMC systems, as well as the low polydispersity for HPMC observed by DLS were confirmed by TEM (Fig. 6) The literature provides TEM images of F127 systems with highly homogeneous micelles of about 10 nm [56,83].

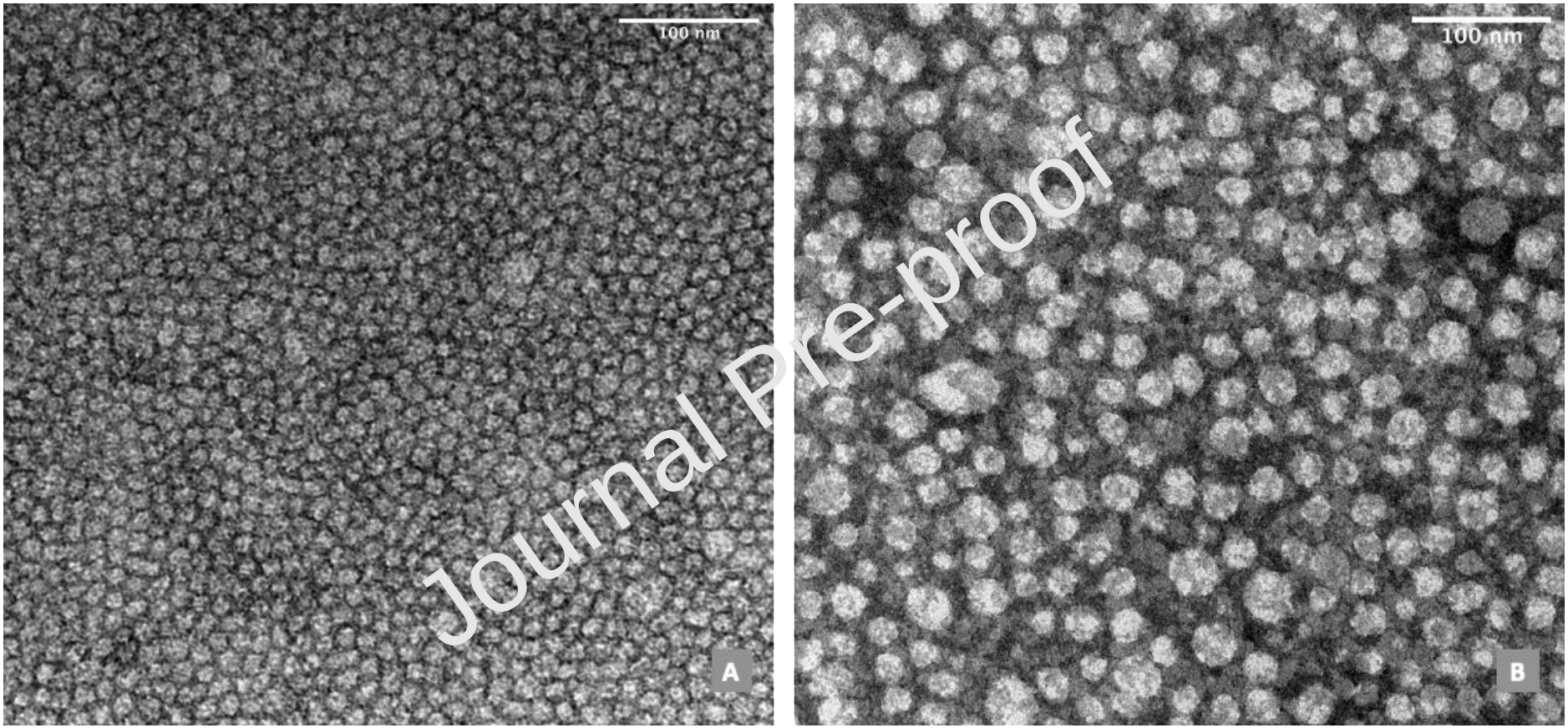


Fig. 6. TEM images of F127 and HPMC (A) or NaCMC (B) formulations made at 37 °C (A). Original magnification x150,000.

It is possible to observe that, at the same magnification, F127 system containing NaCMC are able to form smaller number of micelles with higher diameter, compared to HPMC-content system. Systems containing F127 and HPMC allowed the formation of a greater number of micelles with low diameter. Therefore, considering a same amount of F127, HPMC system may have a reduced aggregation number, which is defined as the average number of block copolymers chains comprising one micelle [69]. Furthermore, increased hydrophobicity and compression of corona have demonstrated apparent decrease of micelle size, building further dehydrated and dense micelles [84]. The finding may reflect the major presence of interactions stabilised between HPMC and PEO moiety of F127 (Table S2), which decrease the aggregation number [35], compressing the hydrophilicity of PEO segment.

The higher heterogeneity of size evidenced for NaCMC formulations by TEM micrographs, also supports the micellization disruption within those formulations, observed by DLS, since it may have a negative effect on the ordering micelles which occurs in a liquid crystalline fashion [83]. Besides changing the CMC of the F127 in distinct proportions, the two cellulose derivatives produced different sizes of nanoparticles, which may allow bespoke drug delivery and targeting applications. The solubilization of different drugs is governed by their partition coefficient; however, a greater size of micelles has been noted as a driving factor for increase in their drug loading capacity [37]. Meanwhile, Pluronic® micelles around 10-15 nm have demonstrated advantageous permeability in tissues in which it is increased, such as tumour or inflammatory sites, so that improving drug delivery [39].

3.6. Mucoadhesion

Alongside the development of topical dosage forms, defining mucoadhesive properties is an important step to characterize formulation-mucin interactions. Particularly, when prolonged residence time and decreased leakage of the formulation with mucosal secretion are required [12,17,109]. In pursuit of this, mucoadhesive excipients, such as the cellulose derivatives studied, enhance the retention of pharmaceutical dosage forms by numerous mechanisms [110]. The stronger the mucoadhesive force, the larger the amount of pharmaceutical systems remains in the targeted mucosal [109].

Among numerous methods described in the literature, tensile strength is the most frequently used to evaluate the mucoadhesive profile of different formulations, and therefore a useful comparator measurement [110]. Its determination is based on two parameters: detachment force and work of adhesion. One represents the measurements of maximum force to detach the dosage form, while the latter provides a wider evaluation of the sum of all established bonds, including cohesion in the formulation [12,43]. Fig. 7 displays the results obtained for the eight selected polymeric blends containing F127 and HPMC or NaCMC. After the first "contact" step, semi-solid systems are able to "consolidate" their mucoadhesion with further physical and chemical interactions. With respect to interactions, although electrostatic and ion-dipole interactions may play part of the process; due to the oligosaccharide sidechains of the mucin, hydrogen-bonding between cellulose derivatives and mucin is believed to play a higher role on the mucoadhesive performance of the preparations. Moreover, considering the backbone of the polymers, hydrophobic interactions may also occur due to the loss of entropy of water molecules as they solvate hydrophobic portions of the polymers in solution [111,112].

Within thermoresponsive hydrogels, either increase of formulation-mucosa interactions or *in situ* gelation approaches aim to improve mucoadhesion [111]. Previous studies suggest retention of F127 on mucosal membranes is due to its viscosity at 37 °C [113], which may be modified by the presence of additives [4]. F127 has shown to increase mucoadhesive force of polymeric systems, preventing their migration from mucosal tissue [109]. Hence, with respect to F127 concentration, systems composed of 20% (w/w) F127 exhibited mucoadhesive profile marginally higher than those containing 17.5%. By detachment force and work of adhesion, in most formulations there was not significative difference between both concentrations evaluated, with significance ($p<0.05$) only observed for systems containing 4% (w/w) HPMC.

Considering force of adhesion, formulations containing 1% (w/w) NaCMC or 3% (w/w) of HPMC demonstrated better mucoadhesive profiles when compared to the higher concentrations evaluated. The attributes that lead to improved retention in complex polymeric blends are multifaceted. However, it may be suggested the low viscosity of less concentrated systems allows for greater mobility of formulations to spread and wet a surface. Some studies have demonstrated HPMC is unable to bond to mucin, and its mucoadhesive profile related with chain entanglement and physical interlocking with mucus, which also become facilitated in less concentrated formulations [114]. For NaCMC, although it is able to develop strong hydrogen bonding with mucin, studies by surface plasmon resonance demonstrated that these interactions need to be followed by physical interlocking with mucus, being the combination of these two phenomena the most probable mechanism of its mucoadhesion [114,115]. Therefore, more diluted systems may allow for improved interpenetration of polymer and mucin chains, favouring the establishment of bonds

between mucoadhesive polymer and mucin when it comprises binary polymeric systems [30].

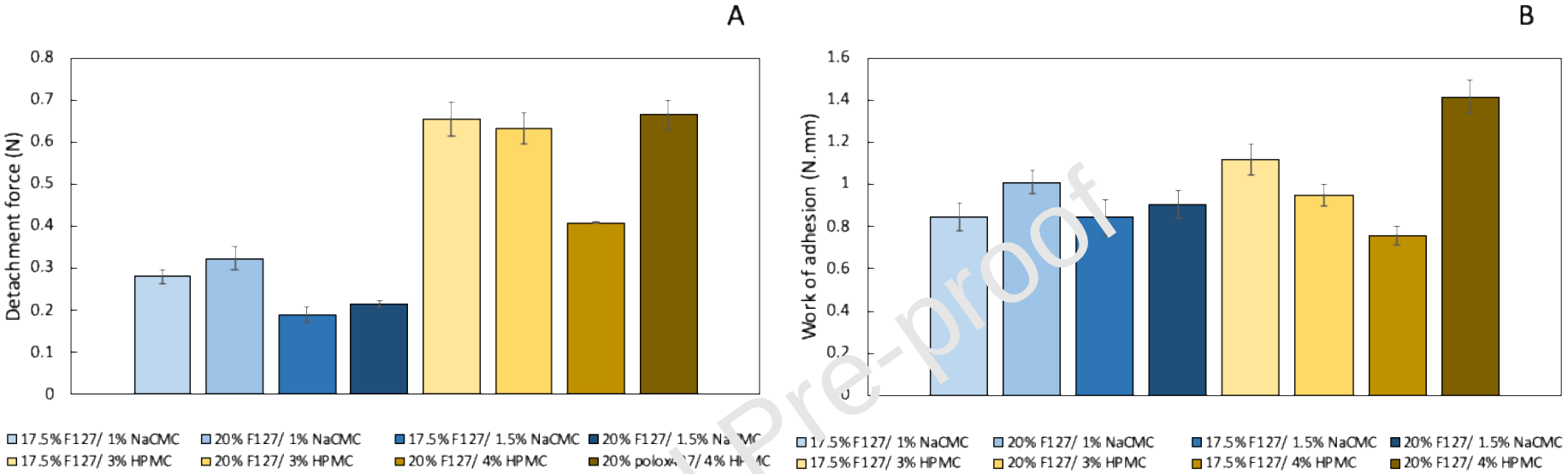


Fig. 7. Determination of mucoadhesion by the detachment force (A) and by the adhesion work (B) of the binary polymeric formulations of Pluronic® F127 (F127) and sodium carboxymethylcellulose (NaCMC) or hydroxypropyl methylcellulose (HPMC) at 37 °C, using a mucin disk as a substrate. Each value represents the mean (± standard deviation) of at least three replicates.

The outcomes from work of adhesion were not statistically different between most NaCMC and HPMC systems. However, a statistical difference was observed between 17.5% (w/w) F127/NaCMC systems and the 20/4 HPMC preparation, difference also being found between 17.5/1 NaCMC preparation and 17.5/3 HPMC systems ($p<0.05$). Considering this measurement, values marginally increased for NaCMC preparations comprising the highest amount of this polymer; however, it was not statistically significative ($p>0.05$). This may indicate a higher ability to hydrogen bond between NaCMC and mucin, when there is increased quantity of polymer. Meanwhile, for HPMC the same was not observed, reflecting the hampered physical interpenetration between mucoadhesive polymer and mucin chains in highly concentrated preparations. Still, 20/4 HPMC-content hydrogel reached raised values for this parameter, suggesting its larger viscosity may aid its retention [114].

As demonstrated in Fig. 7, NaCMC formulations presented half of the detachment force found for HPMC ones. In previous study of mucoadhesion of compressed polymer disks, HPMC has performed better mucoadhesiveness than NaCMC [116]. Furthermore, since HPMC presents higher hydrophobicity, it has lower ability to attract water to the system. The literature describes that water concentration influences the tensile strength method, and a super hydration state of some polymers can reduce their mucoadhesive performance [117–119].Therefore, this phenomenon may be related with the ability of cellulose derivatives to interact with water, and a higher swelling ability may not favour the adhesion process. It is known that NaCMC molecules with lower hydration degree demonstrate higher mucoadhesive properties, due to the water diffusion between gel and mucosa [120]. Another aspect influencing the lowest values observed for NaCMC may be its ionized state, where negative charges

lead to repulsive force when upon contact with also negatively-charged mucin chains [115].

The magnitude of the mucoadhesion is of great interest to the potential clinical utility of these formulations. Hence, evaluating this performance and based on all the background analyses, one formulation of each cellulose derivatives was selected. Considering their use as local drug delivery system, with good retention on the mucosal surface, the systems composed of 17.5% (w/w) F127 and 3% (w/w) HPMC or 1% (w/w) NaCMC were selected for toxicity and drug release study.

3.7. Artemia sanila toxicity study

The preliminary toxicity test using *Artemia salina* is an economic, fast, and reliable test to confirm the biocompatibility of the polymeric systems, which were already produced with biocompatible polymers. The reliability is related to sensitivity of the cysts to a toxic substance present in the medium solution [51,52].

The results obtained for 17.5% (w/w) F127 and 3% (w/w) HPMC or 1% (w/w) NaCMC are displayed in Fig. 8A. It was found both polymeric systems showed viability higher than 85% with 1, 2 or 3 mL of formulation. The systems were considered safe, because even at the higher evaluated concentration they kept nauplius viability, as expected. However, higher quantities of formulation promoted higher mortality for *Artemia salina*, which may occur by the entrapment of the nauplius into the polymeric systems, and not only by systems' toxicity.

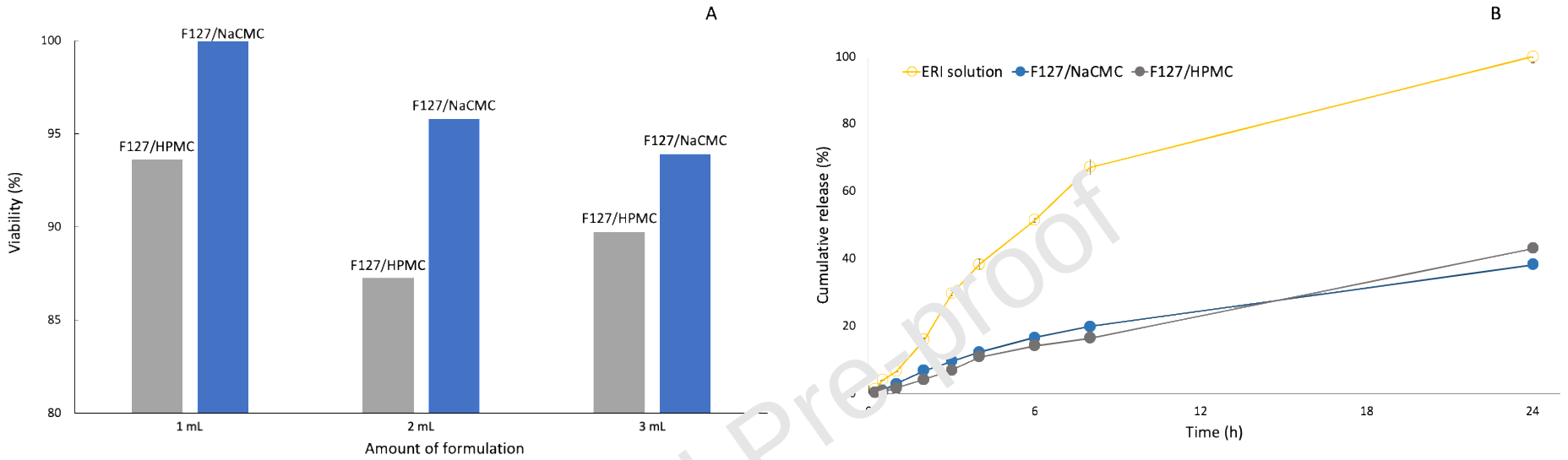


Fig. 8. (A) *Artemia salina* viability rate (%) from binary polymeric systems composed of 17.5% (w/w) Pluronic® F127 (F127) and 3% (w/w) hydroxypropyl methylcellulose (HPMC) or 1% (w/w) sodium carboxymethylcellulose (NaCMC). (B) *In vitro* release profile of ERI from 1% (w/w) aqueous solution, F127/HPMC and F127/NaCMC polymeric systems. Standard deviations are smaller than the symbols. In all cases, the relative standard deviation of replicate analyses was < 5%.

3.8. In vitro drug release profile

Drug release is an important ability in drug delivery systems since it controls magnitude and duration of therapeutic effect. It refers to the process in which drug solutes migrate from the polymeric system to the outer surface of the polymer and, then, to the receiving fluid. The *in vitro* release contributes to the rate and extent of drug bioavailability in the body, contributing to these factors without requiring alteration of drug chemistry [13,121].

Mathematical models of release are often zero- and first-order rate, but there are those that provide fast initial dose followed by zero- or first-order release of the sustained drug [121]. Their purpose is to simplify the complex release process, trying to predict the release mechanism from specific materials characterising the system [122]. The release of the drug can be theoretically predicted considering the amount and type of the drug, the polymer or other adjuvants, the size and shape of the pharmaceutical system [123]. Mixtures composed of F127 and other synergic polymers are frequently used in the development of drug delivery systems with controlled release [13,52,124], since different microphases of F127 can impart different microenvironments and, consequently, with drug release modulation [125]. ERI, a hydrophilic drug, was incorporated into the hydrogels, and the release was fitted by suitable equations for semi-solid systems, namely: first-order, Higuchi, and the Korsmeyer-Peppas model [121,126].

Table 3

Parameters of each mathematical model for release study of erythrosine (ERI) from a control solution, 17.5% (w/w) Pluronic F127 (F127) and 3% (w/w) hydroxypropyl methylcellulose (HPMC) or 1% (w/w) sodium carboxymethylcellulose (NaCMC) polymeric systems.

Formulations	Models						
	First order		Higuchi		Korsmeyer-Peppas		
	k	R^2	k	R^2	k	n	R^2
ERI solution	0.023 ± 0.002	0.842 ± 0.013	7.562 ± 0.536	0.929 ± 0.0127	3.207 ± 0.119	0.619 ± 0.019	0.530 ± 0.023
Polox407/HPMC	0.008 ± 0.001	0.983 ± 0.017	2.630 ± 0.179	0.842 ± 0.028	0.568 ± 0.053	1.073 ± 0.284	0.550 ± 0.075
Polox407/NaCMC	0.007 ± 0.000	0.899 ± 0.019	2.675 ± 0.057	0.930 ± 0.009	1.135 ± 0.047	0.571 ± 0.011	0.445 ± 0.014

The aqueous solution of ERI showed a total release (100%) after 24 h, with 50% of ERI released after 6 h. The hydrogels presented prolonged release at 37 °C, as none of them reached a complete release of ERI after 24 h of study (Fig. 8B). Both NaCMC and HPMC formulations demonstrated similar release profile, releasing less than 50% ERI after 24 h. Nonetheless, HPMC preparation shown ERI release slightly higher at 24 h, being about 43% compared to 38% of NaCMC.

For HPMC system, the best R^2 was found when fitting with the first order equation (0.983), while for NaCMC preparation and ERI solution, the best to fit the data was Higuchi model (R^2=0.930 and 0.929, respectively). The aqueous solution and NaCMC formulation, best fit by Higuchi kinetics, demonstrate that ERI release is mainly driven by the difference of concentration between formulation and bulk solution [30], meanwhile HPMC preparation shows the major influence of polymeric relaxation as fitted by first-order model.

Drug release from formulations can be controlled by different factors (e.g. dissolution, diffusion, osmosis, swelling, partitioning and erosion). Indeed, a hydrophilic drug is easily released through diffusion mechanism, while hydrophobic ones are normally related to swelling or erosion of the matrix [121]. The correlation coefficient for both formulations and control solution by Korsmeyer-Peppas equation were around 0.50 (Table 3). The low correlation coefficient obtained by this model may occur due to the amount of total ERI released, since its release did not attain at least 60%, the method was not accurately predicted.

For the aqueous solution, drug release is expected to be mainly controlled by the cellulose membrane, which is used as support for the formulation in the model. Herein, fitted by Higuchi kinetic, it confirmed the greater contribution of Fickian diffusion for ERI release through the membrane. NaCMC formulation, though mainly governed by

diffusion, presented the release of ERI also relying on the swelling state of the system. Therefore, it was obtained reduced k-value in comparison to the solution, which indicates the dissolution of gel sustaining this release [127]. The rearrangement of polymeric chains occurs slowly, and the diffusion process fosters a time-dependent effect. On the other hand, HPMC system displayed a first order kinetic for ERI release. The tension and untangling of the polymer chains occurs guiding the release of the drug [121], which could favour the higher release reached for HPMC system at the last time point comparing to the NaCMC matrix.

Therefore, in comparison to aqueous control, the hydrogels widely improved the controlled release of ERI, increasing its duration over an extended period. Moreover, the release kinetics also support the structuration of the systems, in agreement with the previous analysis. Within the hydrogels, the mesh size dictates the release of drugs through diffusion, that is altered though degradation and swelling of the matrix [128]. Being mainly governed by swelling and relaxation of the polymeric chains, with influence from Fickian diffusion (an intrinsic characteristic of the Franz cells model), HPMC seen to build up a less organized system than NaCMC.

NaCMC establishes well-structured bonds with F127, building an organized matrix. Thus, the well-formed channels, as shown by SEM imagens, allow for the permeating fluid into the system prioritizing the release of the drug due to the difference in concentration. Meanwhile, the interactions between HPMC and F127 impact in a release favoured by the relaxation of their chains. The amorphous matrix also observed by SEM micrographs swells over the time increasing the release of the payload, suggesting that polymer relaxation is the dominant mechanism for drug transport in this case [129].

4. Conclusions

The morphological characterization of the eight previously selected systems evidenced that 17.5% (w/w) F127 formulations had a well-defined structure, with clearer and higher porosity compared to the 20% (w/w) F127. A computational modelling demonstrated the interaction between F127 and cellulose derivatives are a favourable process, which is marginally favoured for NaCMC. Calorimetric characterization allows observation of a reduction of the F127 crystallinity when mixed with the cellulose derivatives, more evident for NaCMC. By DSC of the hydrogels, it was possible to observe the cellulose derivatives reduced the enthalpy of micellization of F127, increasing its CMT, with higher concentrations of F127 starting the micellization process first. NaCMC and HPMC reduced the CMC of F127, leading to a different size and quantity of micelles. When combined with F127, NaCMC reduced its CMC and produced lower number of micelles with a higher diameter. Although HPMC also decreased the CMC of F127, it was able to form micelles in higher quantity and reduced diameter, which was confirmed by DLS and TEM analyses. The findings present a rationale for understanding how cellulose derivatives affect the micelles formation, being key to understating how formulated micelles function as drug delivery systems. The mucoadhesion was efficient for all the evaluated systems. With greater chain mobility, less concentrated systems were better to attain higher mucoadhesive force on mucin disks. Formulations composed of 17.5% (w/w) F127 and 3% (w/w) HPMC or 1% (w/w) NaCMC were selected as the most appropriated systems to be used as local drug delivery systems, particularly considering their good retention on the mucosal surface. *Artemia salina* test confirmed the formulations are biocompatible, and the release study demonstrated they are able to release hydrophilic drugs, in a controlled way, by different mechanisms.

Acknowledgments

The authors would like to thank the financial supports of CAPES (*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior*/Coordination for the Improvement of Higher Education of Brazil; n° 88881.361580/2019-01-PDSE program), CNPq (*Conselho Nacional de Desenvolvimento Científico e Tecnológico*/National Counsel of Technological and Scientific Development of Brazil; n° 307442/2017-9) and FINEP (*Financiadora de Estudos e Projetos*/Financier of Studies and Projects of Brazil). Also, the authors thank ORCA program system version 4.0, principally Dr. Holger Kruse and Dr. Andreas Hansen for their help and knowledge with the implementation of HF-3C into the ORCA program suit.

Appendix A. Supplementary data

References

[1] S. Barbosa, D.S. Ferreira, J.B. da Silva, M. Volpato, F.B. Borghi-pangoni, R. Guttierres, M. Luciano, The importance of the relationship between mechanical analyses and rheometry of mucoadhesive thermoresponsive polymeric materials for biomedical applications, J. Mech. Behav. Biomed. Mater. (2017). doi:10.1016/j.jmbbm.2017.05.040.

[2] S. Nie, W.W. Hsiao, W. Pan, Z. Yang, Thermoreversible pluronic® F127-based hydrogel containing liposomes for the controlled delivery of paclitaxel: In vitro drug release, cell cytotoxicity, and uptake studies, Int. J. Nanomedicine. 6 (2011) 151–166. doi:10.2147/IJN.S15057.

[3] K. Al Khateb, E.K. Ozhmukhametova, M.N. Mussin, S.K. Seilkhanov, T.K. Rakhypbekov, W.M. Lau, V. V. Khutoryanskiy, In situ gelling systems based on Pluronic F127/Pluronic F68 formulations for ocular drug delivery, Int. J. Pharm. 502 (2016) 70–79. doi:10.1016/j.ijpharm.2016.02.027.

[4] G. Dumortier, J.L. Grossiord, F. Agnely, J.C. Chaumeil, A review of poloxamer 407 pharmaceutical and pharmacological characteristics, Pharm. Res. 23 (2006) 2709–2728. doi:10.1007/s11095-006-9104-4.

[5] M.M. Silva, D.R. Mota, C.B. Silva, H.P.M. de Oliveira, D.S. Pellosi, Synthesis of Pluronic-based silver nanoparticles/methylene blue nanohybrids: Influence of the metal shape on photophysical properties, Mater. Sci. Eng. C. 114 (2020) 110987. doi:10.1016/j.msec.2020.110987.

[6] M.A. Abou-Shamat, J. Calvo-Castro, J.L. Stair, M.T. Cook, Modifying the Properties of Thermogelling Poloxamer 407 Solutions through Covalent Modification and the Use of Polymer Additives, Macromol. Chem. Phys. 220 (2019) 1–19. doi:10.1002/macp.201900173.

[7] W. Wu, J. Liu, S. Cao, H. Tan, J. Li, F. Xu, X. Zhang, Drug release behaviors of a pH sensitive semi-interpenetrating polymer network hydrogel composed of poly(vinyl alcohol) and star poly[2-(dimethylamino) ethyl methacrylate], Int. J. Pharm. 416 (2011) 104–109. doi:10.1016/j.ijpharm.2011.06.015.

[8] W.K. Fong, R. Negrini, J.J. Vallooran, R. Mezzenga, B.J. Boyd, Responsive self-assembled nanostructured lipid systems for drug delivery and diagnostics, J. Colloid Interface Sci. 484 (2016) 320–339. doi:10.1016/j.jcis.2016.08.077.

[9] G. Dumortier, N. El Kateb, M. Sahli, S. Kedjar, A. Boulliat, J.C. Chaumeil, Development of a thermogelling ophthalmic formulation of cysteine, Drug Dev. Ind. Pharm. 32 (2006) 63–72. doi:10.1080/03639040500390934.

[10] B. Shriky, A. Kelly, M. Isreb, M. Babenko, N. Mahmoudi, S. Rogers, O. Shebanova, T. Snow, T. Gough, Pluronic F127 thermosensitive injectable smart hydrogels for controlled drug delivery system development, J. Colloid Interface Sci. 565 (2020) 119–130. doi:10.1016/j.jcis.2019.12.096.

[11] K. Edsman, J. Carlfors, R. Petersson, Rheological evaluation of poloxamer as an in situ gel for ophthalmic use, Eur. J. Pharm. Sci. 6 (1998) 105–112. doi:10.1016/S0928-0987(97)00075-4.

[12] M.T. Cook, V. V. Khutoryanskiy, Mucoadhesion and mucosa-mimetic materials—A mini-review, Int. J. Pharm. 495 (2015) 991–998. doi:10.1016/j.ijpharm.2015.09.064.

[13] M.L. Bruschi, D.S. Jones, H. Panzeri, M.P.D. Gremião, O. de Freitas, E.H.G. Lara, Semisolid Systems Containing Propolis fot the Treatment of Periodontal Disease: In Vitro Realease Kinetics, Syringeability, Rheological, Textural and Mucoadhesive Properties, J. Pharm. Sci. 99 (2007) 4215–4227. doi:10.1002/jps.

[14] L.M.B. de Francisco, H.C. Rosseto, L. de Alcântara Sica de Toledo, R.S. dos Santos, S.B. de Souza Ferreira, M.L. Bruschi, Organogel composed of poloxamer 188 and passion fruit oil: Sol-gel transition, rheology, and mechanical properties, J. Mol. Liq. 289 (2019). doi:10.1016/j.molliq.2019.111170.

[15] S.M. Querobino, N.C. de Faria, A.A. Vigato, B.G.M. da Silva, I.P. Machado, M.S. Costa, F.N. Costa, D.R. de Araujo, C. Alberto-Silva, Sodium alginate in oil-

poloxamer organogels for intravaginal drug delivery: Influence on structural parameters, drug release mechanisms, cytotoxicity and in vitro antifungal activity, Mater. Sci. Eng. C. 99 (2019) 1350–1361. doi:10.1016/j.msec.2019.02.036.

[16] S.C. Shin, J.Y. Kim, Enhanced permeation of triamcinolone acetonide through the buccal mucosa, Eur. J. Pharm. Biopharm. 50 (2000) 217–220. doi:10.1016/S0939-6411(00)00101-6.

[17] D.S. Jones, M.L. Bruschi, O. de Freitas, M.P.D. Gremião, E.H.G. Lara, G.P. Andrews, Rheological, mechanical and mucoadhesive properties of thermoresponsive, bioadhesive binary mixtures composed of poloxamer 407 and carbopol 974P designed as platforms for implantable drug delivery systems for use in the oral cavity, Int. J. Pharm. 372 (2009) 49–58. doi:10.1016/j.ijpharm.2009.01.006.

[18] S.B. De Souza Ferreira, J. Bassi da Silva, F.B. Borghi-Pangoni, M.V. Junqueira, M.L. Bruschi, Linear correlation between rheological, mucoadhesive and textural properties of thermoresponsive polymer blends for biomedical applications, J. Mech. Behav. Biomed. Mater. 55 (2015) 164–178. doi:10.1016/j.jmbbm.2015.10.026.

[19] A.A. Barba, G. Lamberti, L. Rabbia, M. Grassi, D. Larobina, G. Grassi, Modeling of the reticulation kinetics of alginate/pluronic blends for biomedical applications, Mater. Sci. Eng. C. 37 (2014) 327–331. doi:10.1016/j.msec.2014.01.034.

[20] T. Gratieri, G.M. Gelfuso, E.M. Rocha, V.H. Sarmento, O. de Freitas, R.F.V. Lopez, A poloxamer/chitosan in situ forming gel with prolonged retention time for ocular delivery, Eur. J. Pharm. Biopharm. 75 (2010) 186–193. doi:10.1016/j.ejpb.2010.02.011.

[21] D.S. Jones, A.D. Woolfson, J. Djokic, Texture profile analysis of bioadhesive polymeric semisolids: Mechanical characterization and investigation of interactions between formulation components, J. Appl. Polym. Sci. 61 (1996) 2229–2234. doi:10.1002/(SICI)1097-4628(19960919)61:12.

[22] H. Hägerström, K. Edsman, Interpretation of mucoadhesive properties of polymer, (2001) 1589–1599. doi:10.1211/0022357011778197.

[23] W. Wang, P.C.L. Hui, E. Wat, F.S.F. Ng, C.W. Kan, X. Wang, E.C.W. Wong, H. Hu, B. Chan, C.B.S. Lau, P.C. Leung, In vitro drug release and percutaneous behavior of poloxamer-based hydrogel formulation containing traditional Chinese medicine, Colloids Surfaces B Biointerfaces. 148 (2016) 526–532. doi:10.1016/j.colsurfb.2016.09.036.

[24] J.B. da Silva, M.T. Cook, M.L. Bruschi, Thermoresponsive systems composed of

poloxamer 407 and HPMC or NaCMC: mechanical, rheological and sol-gel transition analysis, Carbohydr. Polym. 240 (2020) 116268. doi:10.1016/j.carbpol.2020.116268.

[25] A. Sosnik, J. Das Neves, B. Sarmento, Mucoadhesive polymers in the design of nano-drug delivery systems for administration by non-parenteral routes: A review, Prog. Polym. Sci. 39 (2014) 2030–2075. doi:10.1016/j.progpolymsci.2014.07.010.

[26] A. Chowhan, T.K. Giri, Polysaccharide as renewable responsive biopolymer for in situ gel in the delivery of drug through ocular route, Int. J. Biol. Macromol. 150 (2020) 559–572. doi:10.1016/j.ijbiomac.2020.02.097.

[27] B. Fonseca-Santos, M. Chorilli, 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. 86 (2018) 129–143. doi:10.1016/j.msec.2017.12.022.

[28] D. Accili, G. Menghi, G. Bonacucina, P. Di Martino, G.F. Palmieri, Mucoadhesion dependence of pharmaceutical polymers on mucosa characteristics, Eur. J. Pharm. Sci. 22 (2004) 225–234. doi:10.1016/j.ejps.2003.12.011.

[29] P. Press, Handbook of Pharmaceutical Excipients, Sixth edit, 2009. doi:10.1017/CBO9781107415324.004.

[30] C. Pagano, S. Giovagnoli, L. Perioli, M.C. Tiralti, M. Ricci, Development and characterization of mucoadhesive-thermoresponsive gels for the treatment of oral mucosa diseases, Eur. J. Pharm. Sci. 142 (2020). doi:10.1016/j.ejps.2019.105125.

[31] H. Almeida, M.H. Amaral, P. Lobão, J.M.S. Lobo, In situ gelling systems: a strategy to improve the bioavailability of ophthalmic pharmaceutical formulations, Drug Discov. Today. 00 (2013). doi:10.1016/j.drudis.2013.10.001.

[32] P.R. Desai, N.J. Jain, R.K. Sharma, P. Bahadur, Effect of additives on the micellization of PEO/PPO/PEO block copolymer F127 in aqueous solution, Colloids Surfaces A Physicochem. Eng. Asp. 178 (2001) 57–69. doi:10.1016/S0927-7757(00)00493-3.

[33] A.H. El-Kamel, In vitro and in vivo evaluation of Pluronic F127-based ocular delivery system for timolol maleate, Int. J. Pharm. 241 (2002) 47–55. doi:10.1016/S0378-5173(02)00234-X.

[34] J. Dey, S. Kumar, S. Nath, R. Ganguly, V.K. Aswal, K. Ismail, Additive induced core and corona specific dehydration and ensuing growth and interaction of Pluronic F127 micelles, J. Colloid Interface Sci. 415 (2014) 95–102. doi:10.1016/j.jcis.2013.10.019.

[35] P.K. Sharma, S.R. Bhatia, Effect of anti-inflammatories on Pluronic® F127: Micellar assembly, gelation and partitioning, Int. J. Pharm. 278 (2004) 361–377. doi:10.1016/j.ijpharm.2004.03.029.

[36] K.T. Oh, T.K. Bronich, A. V. Kabanov, Micellar formulations for drug delivery based on mixtures of hydrophobic and hydrophilic Pluronic® block copolymers, J. Control. Release. 94 (2004) 411–422. doi:10.1016/j.jconrel.2003.10.018.

[37] V. Shah, B. Bharatiya, V. Patel, M.K. Mishra, A.D. Shukla, D.O. Shah, Interaction of salicylic acid analogues with Pluronic® micelles: Investigations on micellar growth and morphological transition, J. Mol. Liq. 277 (2019) 563–570. doi:10.1016/j.molliq.2018.12.142.

[38] C. Branca, G. D'Angelo, Aggregation behavior of pluronic F127 solutions in presence of chitosan/clay nanocomposites examined by dynamic light scattering, J. Colloid Interface Sci. 542 (2019) 289–295. doi:10.1016/j.jcis.2019.02.031.

[39] N.Y. Rapoport, J.N. Herron, W.G. Pitt, L. Pitina, Micellar delivery of doxorubicin and its paramagnetic analog, ruboxyl, to HL-60 cells: Effect of micelle structure and ultrasound on the intracellular drug uptake, J. Control. Release. 58 (1999) 153–162. doi:10.1016/S0168-3659(98)00149-7.

[40] Z. Ahmad, A. Shah, M. Siddiq, H.B. Kraatz, Polymeric micelles as drug delivery vehicles, RSC Adv. 4 (2014) 17028–17038. doi:10.1039/c3ra47370h.

[41] Y. Geng, P. Dalhaimer, S. Cai, R. Tsai, M. Tewari, T. Minko, D.E. Discher, Shape effects of filaments versus spherical particles in flow and drug delivery, Nat Nanotechnol. 2 (2007) 249–255. doi:10.1038/nnano.2007.70.

[42] I.R. Schmolka, Artificial skin I. Preparation and properties of Pluronic F127 Gels for treatment of burns, J. Biomed. MAter. Res. 6 (1972) 571–582. doi:10.1002/jbm.820060609.

[43] J. Bassi da Silva, V. V Khutoryanskiy, M.L. Bruschi, M.T. Cook, A mucosa-mimetic material for the mucoadhesion testing of thermogelling semi-solids, Int. J. Pharm. 528 (2017) 586–594. doi:10.1016/j.ijpharm.2017.06.025.

[44] F. Neese, Software update : the ORCA program system , version 4 . 0, (2017) 1–6. doi:10.1002/wcms.1327.

[45] R. Sure, S. Grimme, Corrected small basis set Hartree-Fock method for large systems, J. Comput. Chem. 34 (2013) 1672–1685. doi:10.1002/jcc.23317.

[46] M.D. Hanwell, D.E. Curtis, D.C. Lonie, T. Vandermeersch, E. Zurek, G.R. Hutchison, Avogadro : An advanced semantic chemical editor , visualization , and analysis platform, J. Cheminform. (2012) 33. doi:10.1186/1758-2946-4-17.

[47] R.S. Gonçalves, G. Braga, A.C.V. de Oliveira, G.B. César, T.T. Tominaga, E.H. Zampiere, I.R. Calori, F.A.P. de Morais, E.A. Basso, R.M. Pontes, N. Hioka, W.

Caetano, Hypericin Delivery System Based on P84 Copolymeric Micelles Linked with N‐(3-Aminopropyl)-2-pyrrolidone for Melanoma- Targeted Photodynamic Therapy´, ACS Appl. Polym. Mater. (2020). doi:10.1021/acsapm.0c00114.

[48] S.J. Lue, D.T. Lee, J.Y. Chen, C.H. Chiu, C.C. Hu, Y.C. Jean, J.Y. Lai, Diffusivity enhancement of water vapor in poly(vinyl alcohol)-fumed silica nano-composite membranes: Correlation with polymer crystallinity and free-volume properties, J. Memb. Sci. 325 (2008) 831–839. doi:10.1016/j.memsci.2008.09.015.

[49] A. Gupta, A.P. Costa, X. Xu, S.L. Lee, C.N. Cruz, Q. Bao, D.J. Burgess, Formulation and characterization of curcumin loaded polymeric micelles produced via continuous processing, Int. J. Pharm. 582 (2020) 119340. doi:10.1016/j.ijpharm.2020.119340.

[50] S. Horiuchi, G. Winter, CMC determination of nonionic surfactants in protein formulations using ultrasonic resonance technology, Eur. J. Pharm. Biopharm. 92 (2015) 8–14. doi:10.1016/j.ejpb.2015.02.005.

[51] S. Rajabi, A. Ramazani, M. Hamidi, T. Naji, Artemia salina as a model organism in toxicity assessment of nanoparticles, DARU, J. Pharm. Sci. 23 (2015) 1–6. doi:10.1186/s40199-015-0105-x.

[52] M. V. Junqueira, F.B. Borghi-Pangoni, S.B.S. Ferreira, B.R. Rabello, N. Hioka, M.L. Bruschi, Functional Polymeric Systems as Delivery Vehicles for Methylene Blue in Photodynamic Therapy, Langmuir. 32 (2016) 19–27. doi:10.1021/acs.langmuir.5b02039.

[53] M.V. Junqueira, F.B. Borghi-Pangoni, S.B. de S. Ferreira, M.L. Bruschi, Evaluation of the methylene blue addition in binary polymeric systems composed by poloxamer 407 and Carbopol 934P using quality by design: rheological, textural, and mucoadhesive analysis, Drug Dev. Ind. Pharm. 9045 (2016) 1–41. doi:10.1080/03639045.2016.1188111.

[54] G. Baek, C. Kim, Rheological properties of Carbopol containing nanoparticles, J. Rheol. (N. Y. N. Y). 55 (2011) 313–330. doi:10.1122/1.3538092.

[55] K. da S.S. Campanholi, G. Braga, J.B. da Silva, N.L. da Rocha, L.M.B. de Francisco, É.L. de Oliveira, M.L. Bruschi, L. V. de Castro-Hoshino, F. Sato, N. Hioka, W. Caetano, Biomedical Platform Development of a Chlorophyll-Based Extract for Topic Photodynamic Therapy: Mechanical and Spectroscopic Properties, Langmuir. 34 (2018) 8230–8244. doi:10.1021/acs.langmuir.8b00658.

[56] S.B. de S. Ferreira, G. Braga, É.L. Oliveira, J.B. da Silva, H.C. Rosseto, L.V. de C. Hoshino, M.L. Baesso, W. Caetano, C. Murdoch, H.E. Colley, M.L. Bruschi, Design of a nanostructured mucoadhesive system containing curcumin for buccal application: from physicochemical to biological aspects, Beilstein J.

Nanotechnol. 10 (2019) 2304–2328. doi:10.3762/bjnano.10.222.

[57] S.M. Carvalho, A.A.P. Mansur, N.S.V. Capanema, I.C. Carvalho, P. Chagas, L.C.A. de Oliveira, H.S. Mansur, Synthesis and in vitro assessment of anticancer hydrogels composed by carboxymethylcellulose-doxorubicin as potential transdermal delivery systems for treatment of skin cancer, J. Mol. Liq. 266 (2018) 425–440. doi:10.1016/j.molliq.2018.06.085.

[58] W. Li, B. Sun, P. Wu, Study on hydrogen bonds of carboxymethyl cellulose sodium film with two-dimensional correlation infrared spectroscopy, Carbohydr. Polym. 78 (2009) 454–461. doi:10.1016/j.carbpol.2009.05.002.

[59] Y.L. Lo, C.Y. Hsu, H.R. Lin, PH-and thermo-sensitive pluronic/poly(acrylic acid) in situ hydrogels for sustained release of an anticancer drug, J. Drug Target. 21 (2013) 54–66. doi:10.3109/1061186X.2012.725406.

[60] S. Mehta, H. Verstraelen, K. Peremans, G. Villeirs, S. Vermeire, F. De Vos, E. Mehuys, J.P. Remon, C. Vervaet, Vaginal distribution and retention of a multiparticulate drug delivery system, assessed by gamma scintigraphy and magnetic resonance imaging, Int. J. Pharm. 426 (2012) 44–53. doi:10.1016/j.ijpharm.2012.01.006.

[61] G. Braga, S. Souza, S. Barbosa, D.S. Ferreira, I.R. Calori, J.H. De Oliveira, D. Vanzin, M.L. Bruschi, R.M. Porto, P. Henrique, A.L. Tessaro, N. Hioka, Tautomeric and Aggregational Dynamics of Curcumin- Supersaturated Pluronic Nanocarriers, ACS App. Pol. Mat. (2020). doi:10.1021/acsapm.0c00589.

[62] C. Chen, R.A.L. Wylie, D. Klinger, L.A. Connal, Shape Control of Soft Nanoparticles and Their Assemblies, Chem. Mater. 29 (2017) 1918–1945. doi:10.1021/acs.chemmater.6b04700.

[63] I.R. Calori, G. Braga, C. Carvalho, D. Jesus, H. Bi, A. Claudio, Polymer scaffolds as drug delivery systems, Eur. Polym. J. 129 (2020).

[64] P. Gill, T.T. Moghadam, B. Ranjbar, Differential scanning calorimetry techniques: Applications in biology and nanoscience, J. Biomol. Tech. 21 (2010) 167–193.

[65] N.B. Naidu, K.P.R. Chowdary, K.V.R. Murthy, V. Satyanarayana, A.R. Hayman, G. Becket, Physicochemical characterization and dissolution properties of meloxicam-cyclodextrin binary systems, J. Pharm. Biomed. Anal. 35 (2004) 75–86. doi:10.1016/j.jpba.2004.01.003.

[66] M. El-Badry, M.A. Hassan, M.A. Ibrahim, H. Elsaghir, Performance of poloxamer 407 as hydrophilic carrier on the binary mixtures with nimesulide, Farmacia. 61 (2013) 1137–1150.

[67] L. Djekic, B. Čalija, Đ. Medarević, Gelation behavior, drug solubilization

capacity and release kinetics of poloxamer 407 aqueous solutions: The combined effect of copolymer, cosolvent and hydrophobic drug, J. Mol. Liq. 303 (2020). doi:10.1016/j.molliq.2020.112639.

[68] C. Leuner, J. Dressman, Improving drug solubility for oral delivery using solid dispersions, Eur. J. Pharm. Biopharm. 50 (2000) 47–60. doi:10.1016/S0939-6411(00)00076-X.

[69] A.M. Bodratti, P. Alexandridis, Formulation of poloxamers for drug delivery, J. Funct. Biomater. 9 (2018). doi:10.3390/jfb9010011.

[70] S.E. Bianchi, V.W. Angeli, K.C.B. De Souza, D. Dos Santos Miron, G. De Almeida Carvalho, V. Dos Santos, R.N. Brandalise, Evaluation of the solubility of the HPMC/PVA blends in biological fluids in vitro, Mater. Res. 14 (2011) 166–171. doi:10.1590/S1516-14392011005000033.

[71] P.J. Holdsworth, A. Turner-Jones, The melting behaviour of heat crystallized poly(ethylene terephthalate), Polymer (Guildf). 12 (1971) 195–208. doi:10.1016/0032-3861(71)90045-0.

[72] G. Wang, I.R. Harrison, Polymer melting: heating rate effects on DSC melting peaks, Thermochim. Acta. 231 (1994) 203–213. doi:10.1016/0040-6031(94)80023-5.

[73] P. Talik, J. Piotrowska, U. Hubicka, The Influence of Viscosity and Non-freezing Water Contents Bounded to Different Hydroxypropyl Celluloses (HPC) and Hydroxypropyl Methylcelluloses (HPMC) on Stability of Acetylsalicylic Acid, AAPS PharmSciTech. 20 (2019) 1–7. doi:10.1208/s12249-019-1406-z.

[74] Y. Xie, G. Li, X. Yuan, Z. Cai, R. Rong, Preparation and in vitro evaluation of solid dispersions of total flavones of Hippophae rhamnoides L, AAPS PharmSciTech. 10 (2009) 631–640. doi:10.1208/s12249-009-9246-x.

[75] D.P. Medarević, K. Kachrimanis, M. Mitrić, J. Djuriš, Z. Djurić, S. Ibrić, Dissolution rate enhancement and physicochemical characterization of carbamazepine-poloxamer solid dispersions, Pharm. Dev. Technol. 21 (2016) 268–276. doi:10.3109/10837450.2014.996899.

[76] H. Somashekarappa, Y. Prakash, K. Hemalatha, T. Demappa, R. Somashekar, Preparation and Characterization of HPMC/PVP Blend Films Plasticized with Sorbitol, Indian J. Mater. Sci. 2013 (2013) 1–7. doi:10.1155/2013/307514.

[77] J.S. Park, J.W. Park, E. Ruckenstein, Thermal and dynamic mechanical analysis of PVA/MC blend hydrogels, Polymer (Guildf). 42 (2001) 4271–4280. doi:10.1016/S0032-3861(00)00768-0.

[78] S. El-Sayed, K.H. Mahmoud, A.A. Fatah, A. Hassen, DSC, TGA and dielectric properties of carboxymethyl cellulose/polyvinyl alcohol blends, Phys. B

Condens. Matter. 406 (2011) 4068–4076. doi:10.1016/j.physb.2011.07.050.

[79] B. Perrenot, G. Widmann, Polymorphism by differential scanning calorimetry, Thermochim. Acta. 234 (1994) 31–39. doi:10.1016/0040-6031(94)85133-6.

[80] M.H.M. Nascimento, M.K.K.D. Franco, F. Yokaichyia, E. de Paula, C.B. Lombello, D.R. de Araujo, Hyaluronic acid in Pluronic F-127/F-108 hydrogels for postoperative pain in arthroplasties: Influence on physico-chemical properties and structural requirements for sustained drug-release, Int. J. Biol. Macromol. 111 (2018) 1245–1254. doi:10.1016/j.ijbiomac.2018.01.064.

[81] A.M. Pragatheeswaran, S.B. Chen, The influence of poly(acrylic acid) on micellization and gelation characteristics of aqueous Pluronic F127 copolymer system, Colloid Polym. Sci. 294 (2016) 107–117. doi:10.1007/s00396-015-3757-7.

[82] J. Jiang, C. Li, J. Lombardi, R.H. Colby, B. Rigas, M.H. Rafailovich, J.C. Sokolov, The effect of physiologically relevant additives on the rheological properties of concentrated Pluronic copolymer gels, Polymer (Guildf). 49 (2008) 3561–3567. doi:10.1016/j.polymer.2008.05.038.

[83] A.M. Pragatheeswaran, S.B. Chen, Effect of chain length of PEO on the gelation and micellization of the pluronic F127 copolymer aqueous system, Langmuir. 29 (2013) 9694–9701. doi:10.1021/la401639g.

[84] L.C. Pham Trong, M. Djabourov, A. Ponton, Mechanisms of micellization and rheology of PEO-PPO-PEO triblock copolymers with various architectures, J. Colloid Interface Sci. 328 (2008) 278–287. doi:10.1016/j.jcis.2008.09.029.

[85] M. Zhang, M. Djabourov, C. Bourgaux, K. Bouchemal, Nanostructured fluids from pluronic® mixtures, Int. J. Pharm. 454 (2013) 599–610. doi:10.1016/j.ijpharm.2013.01.043.

[86] P. Alexandridis, T. Alan Hatton, Poly(ethylene oxide)poly(propylene oxide)poly(ethylene oxide) block copolymer surfactants in aqueous solutions and at interfaces: thermodynamics, structure, dynamics, and modeling, Colloids Surfaces A Physicochem. Eng. Asp. 96 (1995) 1–46. doi:10.1016/0927-7757(94)03028-X.

[87] P. Alexandridis, J.F. Holzwarth, T.A. Hatton, Micellization of Poly(ethylene oxide)-Poly(propylene oxide)-Poly(ethylene oxide) Triblock Copolymers in Aqueous Solutions: Thermodynamics of Copolymer Association, Macromolecules. 27 (1994) 2414–2425. doi:10.1021/ma00087a009.

[88] A.L. Thompson, B.J. Love, Thermodynamic properties of aqueous PEO-PPO-PEO micelles with added methylparaben determined by differential scanning calorimetry, J. Colloid Interface Sci. 398 (2013) 270–272. doi:10.1016/j.jcis.2013.01.064.

[89] M. Vadnere, G. Amidon, S. Lindenbaum, J.L. Haslam, Thermodynamic studies on the gel-sol transition of some pluronic polyols, Int. J. Pharm. 22 (1984) 207–218. doi:10.1016/0378-5173(84)90022-X.

[90] S.K. Nixon, S. Hvidt, C. Booth, Micellization of block copolymer P94 in aqueous solution, J. Colloid Interface Sci. 280 (2004) 219–223. doi:10.1016/j.jcis.2004.07.031.

[91] N.M.P.S. Ricardo, N.M.P.S. Ricardo, F. de M.L.L. Costa, F.W.A. Bezerra, C. Chaibundit, D. Hermida-Merino, B.W. Greenland, S. Burattini, I.W. Hamley, S. Keith Nixon, S.G. Yeates, Effect of water-soluble polymers, polyethylene glycol and poly(vinylpyrrolidone), on the gelation of aqueous micellar solutions of Pluronic copolymer F127, J. Colloid Interface Sci. 363 (2012) 336–341. doi:10.1016/j.jcis.2011.10.062.

[92] W. Brown, D. Henley, Studies on Cellulose Derivatives, Die Makromol. Chemie. 79 (1964) 68–88. doi:10.1002/macp.1964.020790107.

[93] C. Clasen, W.M. Kulicke, Determination of viscoelastic and rheo-optical material functions of water-soluble cellulose derivatives, Prog. Polym. Sci. 26 (2001) 1839–1919. doi:10.1016/S0079-6700(01)00024-7.

[94] L.G. Weaver, R. Stockmann, A. Postma, S.H. Thang, Multi-responsive (diethylene glycol)methyl ether methacrylate (DEGMA)-based copolymer systems, RSC Adv. 6 (2016) 90923–90933. doi:10.1039/c6ra14425j.

[95] J. Armstrong, B. Chowdhry, A. Mitchell, A. Beezer, S. Leharne, Effect of cosolvents and cosolutes upon aggregation transitions in aqueous solutions of the poloxamer F87 (poloxamer P237): A high sensitivity differential scanning calorimetry study, J. Phys. Chem. 100 (1996) 1738–1745. doi:10.1021/jp951290c.

[96] C. Chaibundit, N.M.P.S. Ricardo, N.M.P.S. Ricardo, C.A. Muryn, M.B. Madec, S.G. Yeates, C. Booth, Effect of ethanol on the gelation of aqueous solutions of Pluronic F127, J. Colloid Interface Sci. 351 (2010) 190–196. doi:10.1016/j.jcis.2010.07.023.

[97] N. Pandit, T. Trygstad, S. Croy, M. Bohorquez, C. Koch, Effect of salts on the micellization, clouding, and solubilization behavior of pluronic F127 solutions, J. Colloid Interface Sci. 222 (2000) 213–220. doi:10.1006/jcis.1999.6628.

[98] L. Ci, Z. Huang, Y. Liu, Z. Liu, G. Wei, W. Lu, Amino-functionalized poloxamer 407 with both mucoadhesive and thermosensitive properties: preparation, characterization and application in a vaginal drug delivery system, Acta Pharm. Sin. B. 7 (2017) 593–602. doi:10.1016/j.apsb.2017.03.002.

[99] W. Zhang, Y. Shi, Y. Chen, J. Ye, X. Sha, X. Fang, Multifunctional Pluronic P123/F127 mixed polymeric micelles loaded with paclitaxel for the treatment of

multidrug resistant tumors, Biomaterials. 32 (2011) 2894–2906. doi:10.1016/j.biomaterials.2010.12.039.

[100] C.C. Perry, T.S. Sabir, W.J. Livingston, J.R. Milligan, Q. Chen, V. Maskiewicz, D.S. Boskovic, Fluorescence of commercial Pluronic F127 samples: Temperature-dependent micellization, J. Colloid Interface Sci. 354 (2011) 662–669. doi:10.1016/j.jcis.2010.10.028.

[101] J. Juhasz, V. Lenaerts, P.V.M. Tan, H. Ong, Effect of sodium chloride on physical characteristics of poloxamer 407 solutions, J. Colloid Interface Sci. 136 (1990) 168–174. doi:10.1016/0021-9797(90)90087-5.

[102] A. Hebeish, S. Sharaf, Novel nanocomposite hydrogel for wound dressing and other medical applications, RSC Adv. 5 (2015) 103030–103046. doi:10.1039/c5ra07076g.

[103] M. Dewan, G. Sarkar, M. Bhowmik, B. Das, A.K. Chattoapadhyay, D. Rana, D. Chattopadhyay, Effect of gellan gum on the thermogelation property and drug release profile of Poloxamer 407 based ophthalmic formulation, Int. J. Biol. Macromol. 102 (2017) 258–265. doi:10.1016/j.ijbiomac.2017.03.194.

[104] P. Shrimal, G. Jadeja, J. Naik, S. Patel, Continuous microchannel precipitation to enhance the solubility of telmisartan with poloxamer 407 using Box-Behnken design approach, J. Drug Deliv. Sci. Technol. 53 (2019) 101225. doi:10.1016/j.jddst.2019.101225.

[105] J.K. Valenzuela-Oses, M.C. García, V.A. Feitosa, J.A. Pachioni-Vasconcelos, S.M. Gomes-Filho, F.R. Lourenço, N.N.P. Cerize, D.S. Bassères, C.O. Rangel-Yagui, Development and characterization of miltefosine-loaded polymeric micelles for cancer treatment, Mater. Sci. Eng. C. 81 (2017) 327–333. doi:10.1016/j.msec.2017.07.040.

[106] C.P. Oliveira, M.F.N.P. Ribeiro, N.M.P.S. Ricardo, T.V.D.P. Souza, C.L. Moura, C. Chaibundit, S.G. Yeates, K. Nixon, D. Attwood, The effect of water-soluble polymers, PEG and PVP, on the solubilisation of griseofulvin in aqueous micellar solutions of Pluronic F127, Int. J. Pharm. 421 (2011) 252–257. doi:10.1016/j.ijpharm.2011.10.010.

[107] W.D. Pyrz, D.J. Buttrey, Particle size determination using TEM: A discussion of image acquisition and analysis for the novice microscopist, Langmuir. 24 (2008) 11350–11360. doi:10.1021/la801367j.

[108] R.S.C.M.Q. Antonino, T.L. Nascimento, E.R. de Oliveira Junior, L.G. Souza, A.C. Batista, E.M. Lima, Thermoreversible mucoadhesive polymer-drug dispersion for sustained local delivery of budesonide to treat inflammatory disorders of the GI tract, J. Control. Release. 303 (2019) 12–23. doi:10.1016/j.jconrel.2019.04.011.

[109] E. Baloglu, S.Y. Karavana, Z.A. Senyigit, T. Guneri, Rheological and mechanical properties of poloxamer mixtures as a mucoadhesive gel base, Pharm. Dev. Technol. 16 (2011) 627–636. doi:10.3109/10837450.2010.508074.

[110] J. Bassi da Silva, S.B. de S. Ferreira, O. de Freitas, M.L. Bruschi, A critical review about methodologies for the analysis of mucoadhesive properties of drug delivery systems, Drug Dev. Ind. Pharm. 9045 (2017) 1–67. doi:10.1080/03639045.2017.1294600.

[111] M.T. Cook, M.B. Brown, Polymeric gels for intravaginal drug delivery, J. Control. Release. 270 (2018) 145–157. doi:10.1016/j.jconrel.2017.12.004.

[112] G. Tejada, G.N. Piccirilli, M. Sortino, C.J. Salomón, M.C. Lamas, D. Leonardi, Formulation and in-vitro efficacy of antifungal mucoadhesive polymeric matrices for the delivery of miconazole nitrate, Mater. Sci. Eng. C. 79 (2017) 140–150. doi:10.1016/j.msec.2017.05.034.

[113] C. Charrueau, C. Tuleu, V. Astre, J.L. Grossiord, J.C. Chaumeil, Poloxamer 407 as a thermogelling and adhesive polymer for rectal administration of short-chain fatty acids, Drug Dev. Ind. Pharm. 27 (2001) 351–357. doi:10.1081/DDC-100103735.

[114] I. Bravo-Osuna, M. Noiray, E. Briand, A.M. Woodward, P. Argüeso, I.T.M. Martínez, R. Herrero-Vanrell, G. Ponchel, Interfacial interaction between transmembrane ocular mucins and adhesive polymers and dendrimers analyzed by surface plasmon resonance, Pharm. Res. 29 (2012) 2329–2340. doi:10.1007/s11095-012-0761-1.

[115] A. Sosnik, J. Das Neves, B. Sarmento, Mucoadhesive polymers in the design of nano-drug delivery systems for administration by non-parenteral routes: A review, Prog. Polym. Sci. 39 (2014) 2030–2075. doi:10.1016/j.progpolymsci.2014.07.010.

[116] S. Mortazavi, Investigation of various parameters influencing the duration of mucoadhesion of some polymer containing discs, J. Pharm. Sci. 10 (2002) 98–104.

[117] S.H.S. Gu, J.M.; Robinson, J.R.; Leung, Binding of acrylic polymers to mucin/epithelial surfaces: structure property relationships, Crit. Rev. Ther. Drug Carr. Syst. 5 (1988) 21–67.

[118] H. Blanco-Fuente, S. Anguiano-Igea, F.J. Otero-Espinar, J. Blanco-Méndez, In-vitro bioadhesion of carbopol hydrogels, Int. J. Pharm. 142 (1996) 169–174. doi:10.1016/0378-5173(96)04665-0.

[119] J. Bassi da Silva, S.B. de S. Ferreira, A.V. Reis, M.T. Cook, M.L. Bruschi, Assessing mucoadhesion in polymer gels: The effect of method type and instrument variables, Polymers (Basel). 10 (2018) 1–19.

doi:10.3390/polym10030254.

[120] D.S. Jones, A.D. Woolfson, A.F. Brown, Textural, viscoelastic and mucoadhesive properties of pharmaceutical gels composed of cellulose polymers, Int. J. Pharm. 151 (1997) 223–233. doi:10.1016/S0378-5173(97)04904-1.

[121] M.L. Bruschi, Strategies to Modify the Drug Release from Pharmaceutical Systems, 1st ed., Elsevier, 2015.

[122] Y. Fu, W.J. Kao, Semiconductor Packaging δ Connect to computer specimen Load cell, Expert Opin. Drug Deliv. 7 (2010) 429–444. doi:10.1517/17425241003602259.Drug.

[123] J. Siepmann, N.A. Peppas, Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC), Adv. Drug Deliv. Rev. 64 (2012) 163–174. doi:10.1016/j.addr.2012.09.028.

[124] F.B. Borghi-Pangoni, M.V. Junqueira, S.B. de Souza Ferreira, L.L. Silva, B.R. Rabello, W. Caetano, A. Diniz, M.L. Bruschi, Screening and In Vitro Evaluation of Mucoadhesive Thermoresponsive Systems Containing Methylene Blue for Local Photodynamic Therapy of Colorectal Cancer, Pharm. Res. (2015). doi:10.1007/s11095-015-1826-8.

[125] G.F. Picheth, T.C. Marini, P. Taladriz-Blanco, G.G. Shimamoto, G.J.V.P. dos Santos, F. Meneau, M.G. de Oliveira, Influence of Pluronic F127 microenvironments on the photochemical nitric oxide release from S-nitrosoglutathione, J. Colloid Interface Sci. 544 (2019) 217–229. doi:10.1016/j.jcis.2019.02.067.

[126] R.W. Korsmeyer, R. Gurny, E. Doelker, P. Buri, N.A. Peppas, Mechanisms ofSolute Release from Porous Hydrophilic Polymers, Int. J. Pharm. (1983) 25–35.

[127] R. Barse, C. Kokare, A. Tagalpallewar, Journal of Drug Delivery Science and Technology Influence of hydroxypropylmethylcellulose and poloxamer composite on developed ophthalmic in situ gel : Ex vivo and in vivo characterization, 33 (2016) 66–74. doi:10.1016/j.jddst.2016.03.011.

[128] C.A. Dreiss, Hydrogel design strategies for drug delivery, Curr. Opin. Colloid Interface Sci. 48 (2020) 1–17. doi:10.1016/j.cocis.2020.02.001.

[129] A.A. Kassem, R.M. Farid, D.A.E. Issa, D.S. Khalil, M.Y. Abd-El-Razzak, H.I. Saudi, H.M. Eltokhey, E.A. El-Zamarany, Development of mucoadhesive microbeads using thiolated sodium alginate for intrapocket delivery of resveratrol, Int. J. Pharm. 487 (2015) 305–313. doi:10.1016/j.ijpharm.2015.04.010.

Credit Author Statement

Jéssica Bassi da Silva: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft – review and editing. **Rafaela Said dos Santos:** Formal analysis. **Monique Bassi da Silva:** Formal analysis. **Gustavo Braga:** Data curation, Formal analysis, Investigation. **Michael Thomas Cook**: Funding acquisition; Investigation; Methodology; Supervision; Writing – original draft - review & editing. **Marcos Luciano Bruschi**: Conceptualization, Data curation, Methodology, Supervision, Funding acquisition, Project administration, Resources, Writing – original draft - review & editing.

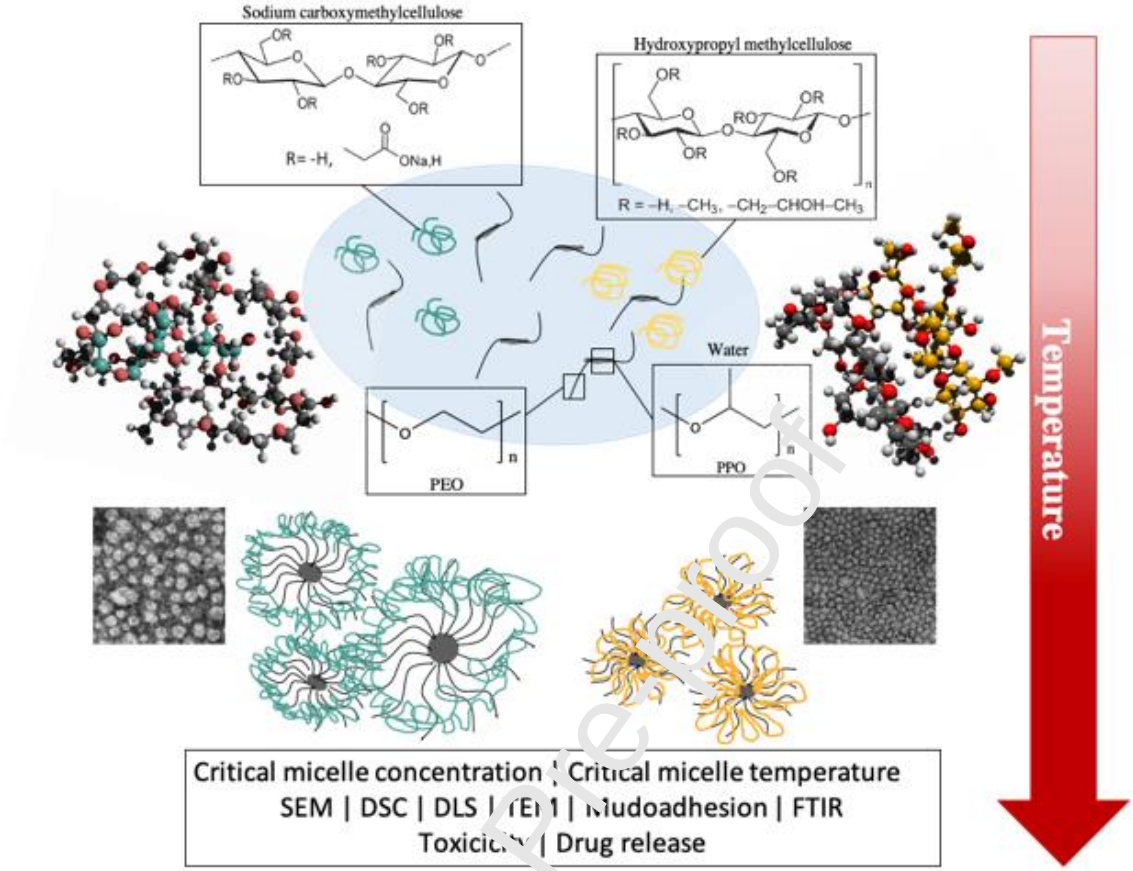
Declaration of interests

X 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:

-

Graphical abstract



Highlights

- Study of micellization and mucoadhesion of HPMC or NaCMC and Pluronic F127
- Cellulose derivatives altered enthalpy and critical micellar concentration of F127
- Formulations showed changing of hydrodynamic diameters of the aggregates
- Systems showed important mucoadhesion performance and dependent on polymer content
- 17.5% F127/3% HPMC or 1% NaCMC are promising as topical mucosal delivery systems

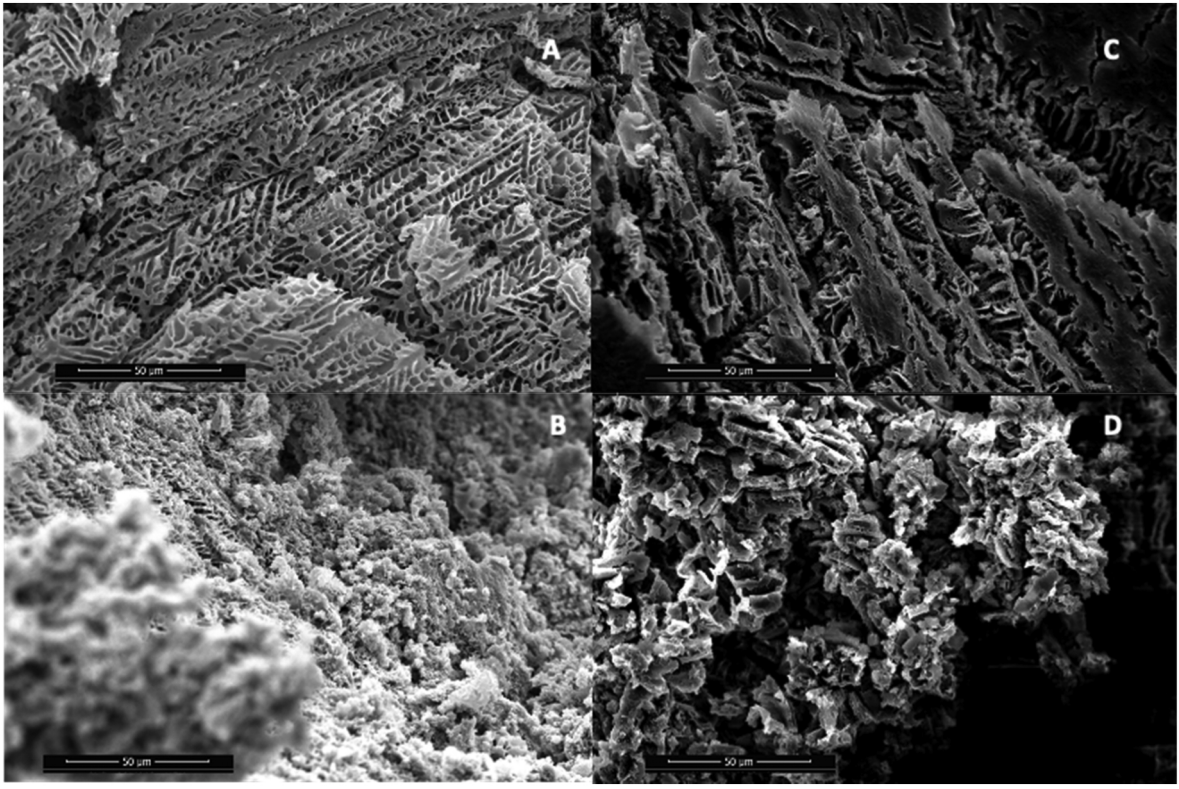


Figure 1

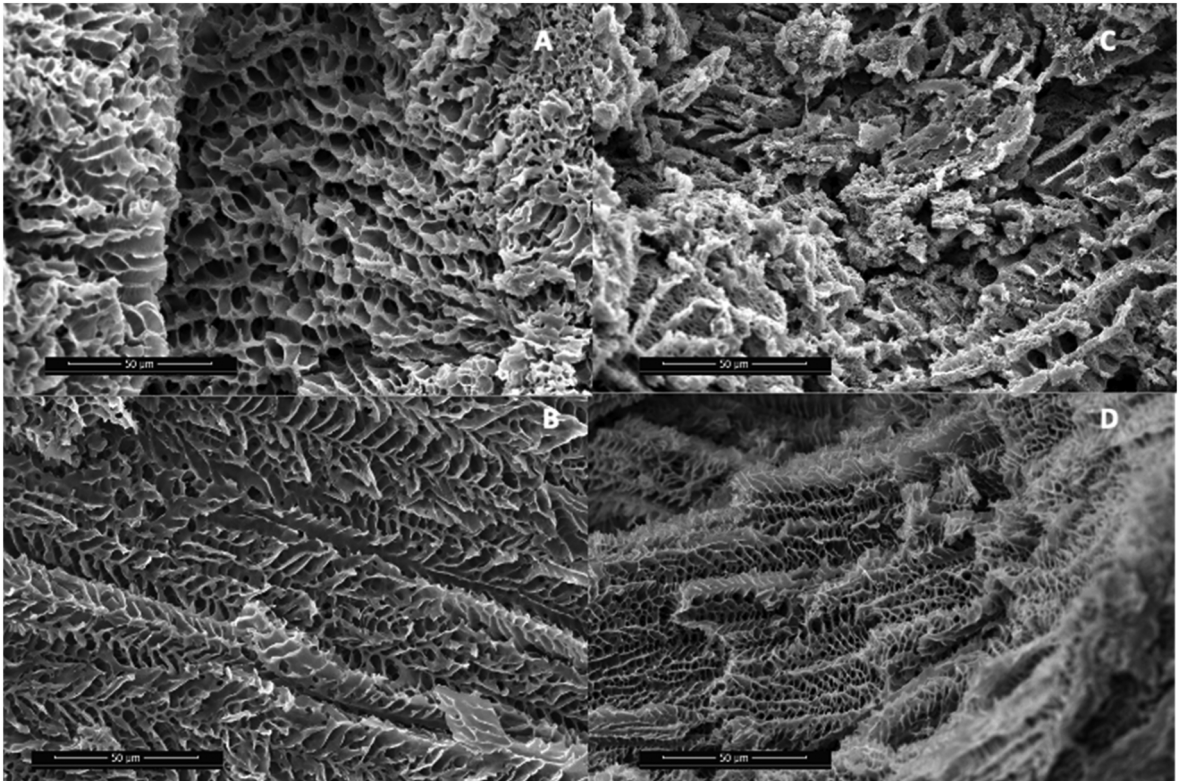


Figure 2

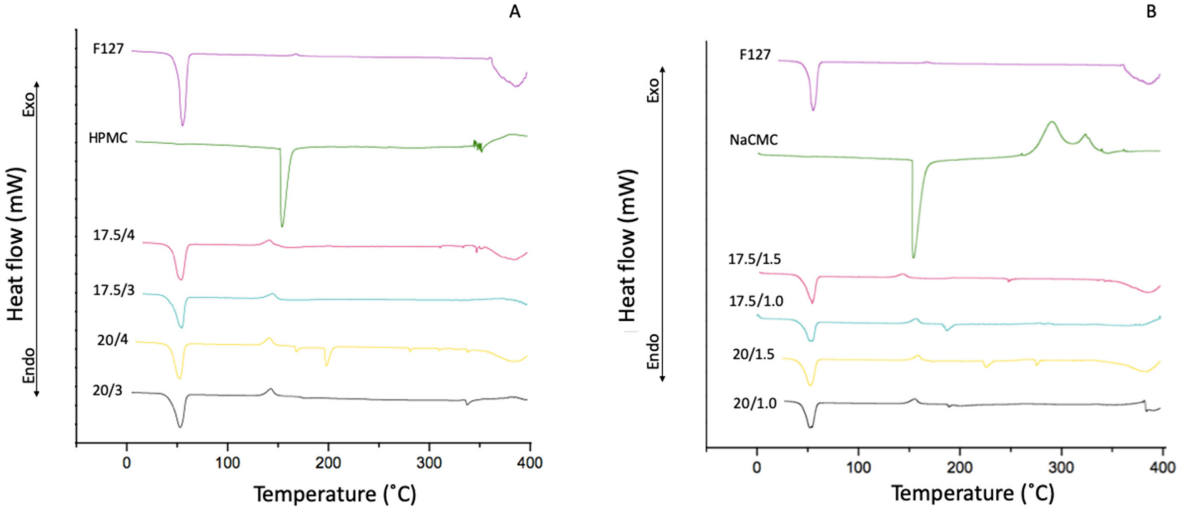

A
F127
HPMC
17.5/4
17.5/3
20/4
20/3
Exo
Endo
Heat flow (mW)
0
100
200
300
400
Temperature (˚C)
B
F127
NaCMC
17.5/1.5
17.5/1.0
20/1.5
20/1.0
Exo
Endo
Heat flow (mW)
0
100
200
300
400
Temperature (˚C)

Figure 3

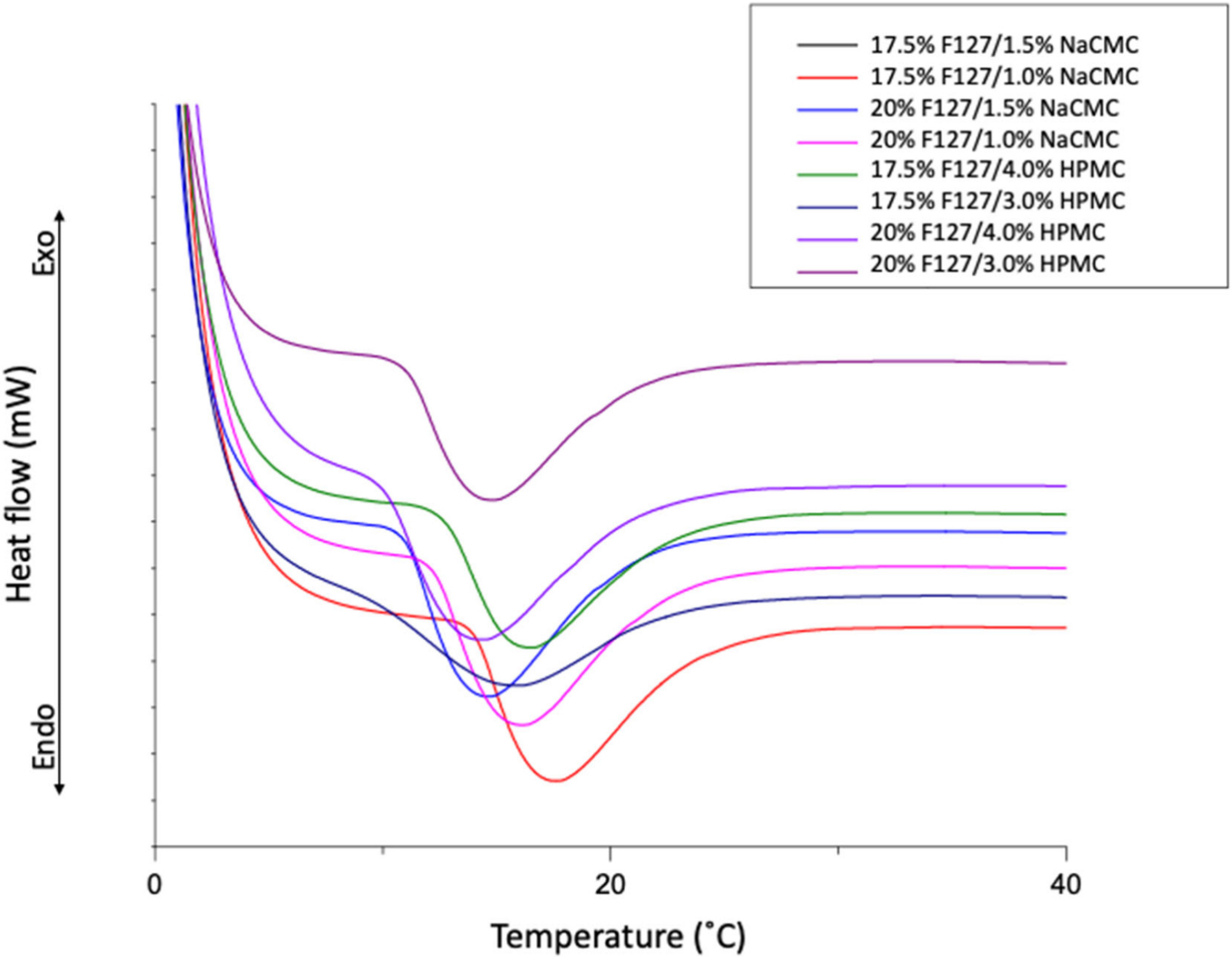
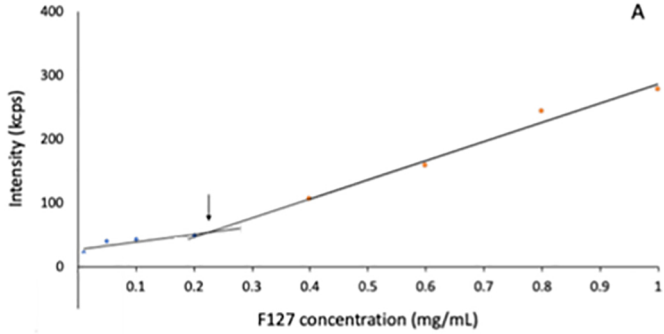
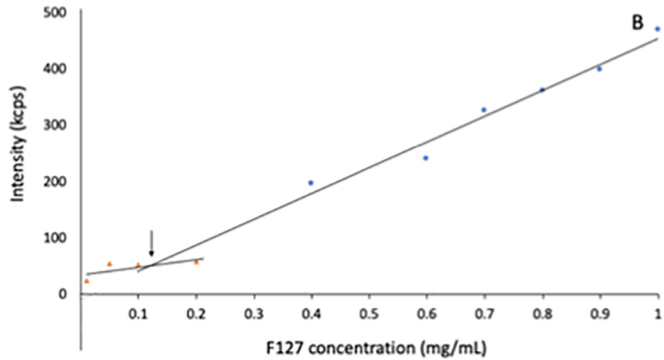

17.5% F127/1.5% NaCMC
17.5% F127/1.0% NaCMC
20% F127/1.5% NaCMC
20% F127/1.0% NaCMC
17.5% F127/4.0% HPMC
17.5% F127/3.0% HPMC
20% F127/4.0% HPMC
20% F127/3.0% HPMC
Exo
Heat flow (mW)
Endo
0
20
40
Temperature (˚C)

Figure 4


A
Intensity (kcps)
400
300
200
100
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
F127 concentration (mg/mL)


B
Intensity (kcps)
500
400
300
200
100
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
F127 concentration (mg/mL)

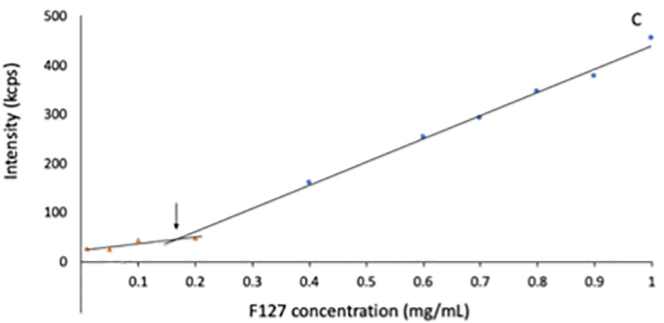

C
Intensity (kcps)
500
400
300
200
100
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
F127 concentration (mg/mL)

Figure 5

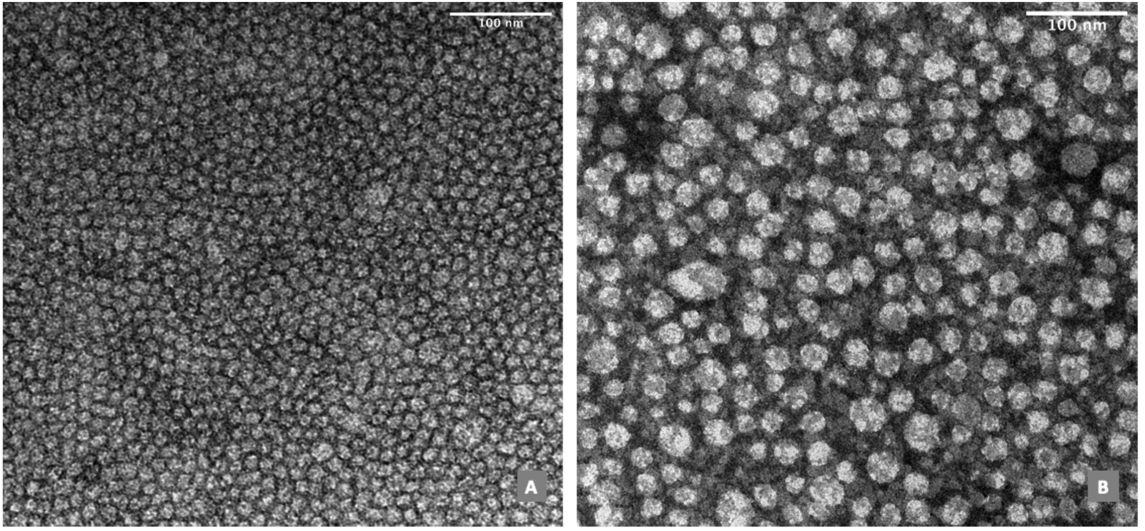


Figure 6

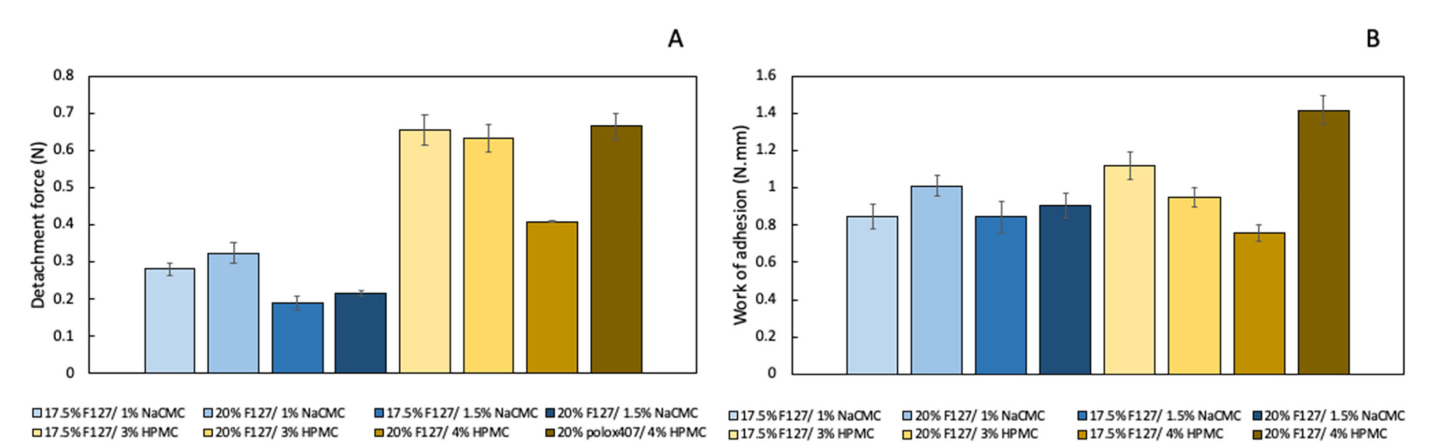
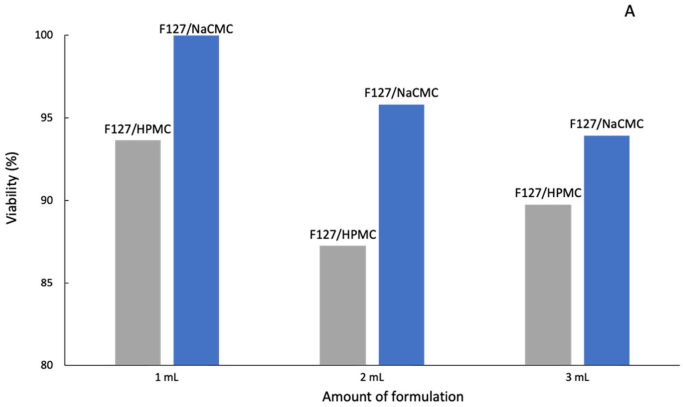

A
B
Detachment force (N)
Work of adhesion (N.mm)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
17.5% F127/ 1% NaCMC
20% F127/ 1% NaCMC
17.5% F127/ 1.5% NaCMC
20% F127/ 1.5% NaCMC
17.5% F127/ 3% HPMC
20% F127/ 3% HPMC
20% F127/ 4% HPMC
20% polox407/ 4% HPMC
17.5% F127/ 4% HPMC
20% F127/ 4% HPMC

Figure 7


A
Viability (%)
100
95
90
85
80
F127/NaCMC
F127/HPMC
F127/NaCMC
F127/HPMC
F127/NaCMC
F127/HPMC
1 mL
2 mL
3 mL
Amount of formulation

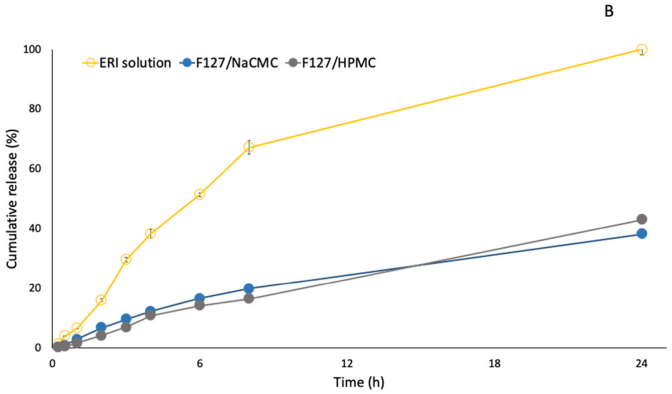

B
ERI solution
F127/NaCMC
F127/HPMC
Cumulative release (%)
100
80
60
40
20
0
0
6
12
18
24
Time (h)

Figure 8