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1 **Novel *in situ* gelling ophthalmic drug delivery system based on gellan gum**  
2 **and hydroxyethylcellulose: Innovative rheological characterization, *in vitro***  
3 **and *in vivo* evidence of a sustained precorneal retention time**

4 Pierre-Louis Destruel<sup>a,b,c</sup>, Ni Zeng<sup>d</sup>, Johanne Seguin<sup>c</sup>, Sophie Douat<sup>b</sup>, Frédéric Rosa<sup>b</sup>, Françoise  
5 Brignole-Baudouin<sup>e,f,g</sup>, Sophie Dufaÿ<sup>b</sup>, Amélie Dufaÿ-Wojcicki<sup>b</sup>, Marc Maury<sup>d</sup>, Nathalie Mignet<sup>c</sup>,  
6 Vincent Boudy<sup>b,c,\*</sup>

7  
8 <sup>a</sup> Unither Développement Bordeaux, ZA Tech Espace, av Toussaint Catros, Le Haillan, 33185, France

9 <sup>b</sup> Département recherche et développement pharmaceutique, Agence Générale des Equipements et Produits de  
10 Santé (AGEPS), AP-HP, 7 rue du fer à moulin, Paris, 75005, France

11 <sup>c</sup> Université de Paris, UTCBS, CNRS, INSERM, Faculté de Pharmacie, 4 av de l'observatoire, Paris, 75006,  
12 France, <http://www.cnrs.utcbs.fr/>

13 <sup>d</sup> Unither Pharmaceuticals, 3-5 rue St-Georges, Paris, 75009, France

14 <sup>e</sup> UMR CNRS 8638 - Chimie Toxicologie Analytique et Cellulaire, Université Paris Descartes, Sorbonne Paris  
15 Cité, Faculté de Pharmacie de Paris, 4 Avenue de l'Observatoire, Paris, 75006, France

16 <sup>f</sup> CNRS UMR 7210 - Inserm UMR\_S 968, Institut de la Vision, Paris, 75012, France

17 <sup>g</sup> Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts, INSERM-DHOS, CIC 503, Paris, 75012,  
18 France

19 \*Corresponding author: Boudy, V. (e-mail: [Vincent.boudy@aphp.fr](mailto:Vincent.boudy@aphp.fr); phone: 00 33 1 46 69 15 95)

20

21 **Abstract**

22 Achieving drug delivery at the ocular level encounters many challenges and obstacles. *In situ*  
23 gelling delivery systems are now widely used for topical ocular administration and recognized  
24 as a promising strategy to improve the treatment of a wide range of ocular diseases. The  
25 present work describes the formulation and evaluation of a mucoadhesive and ion-activated *in*  
26 *situ* gelling delivery system based on gellan gum and hydroxyethylcellulose for the delivery of  
27 phenylephrine and tropicamide. First, physico-chemical characteristics were assessed to  
28 ensure suitable properties regarding ocular administration. Then, rheological properties such  
29 as viscosity and gelation capacity were determined. Gelation capacity of the formulations and  
30 the effect of hydroxyethylcellulose on viscosity were demonstrated. A new rheological  
31 method was developed to assess the gel resistance under simulated eye blinking. Afterward,  
32 mucoadhesion was evaluated using tensile strength test and rheological synergism method in  
33 both rotational and oscillatory mode allowing mucoadhesive properties of  
34 hydroxyethylcellulose to be pointed out. Finally, residence time on the ocular surface was  
35 investigated *in vivo*, using cyanine 5.5 dye as a fluorescent marker entrapped in the *in situ*  
36 gelling delivery systems. Residence performance was studied by non-invasive optical imaging  
37 on vigilant rabbits, allowing eye blinking and nasolacrimal drainage to occur physiologically.  
38 Fluorescence intensity profiles pointed out a prolonged residence time on the ocular surface  
39 region for the developed formulations compared to conventional eye drops, suggesting *in*  
40 *vitro* / *in vivo* correlations between rheological properties and *in vivo* residence performances.

41

42 **Keywords**

43 Hydrogel, delivery system, ophthalmic, rheology, ocular residence time, optical imaging

## 1. Introduction

Ophthalmic drug delivery has raised a large interest over the last two decades. The pharmaceutical market for ophthalmic products is on the rise, with more and more drugs and medical devices in an increasing application number and yet, optimal drug delivery to the eye is still a knotty challenge in many cases [1,2].

The eye is a complex and sensitive organ. Achieving high ocular bioavailability through topical administration of active substances is a challenge as different mechanisms of protection against external aggressions (e.g. its annexes: eyelids and tear film) tend to eliminate the drug upon topical administration. Pre-corneal efflux factors such as nasolacrimal drainage and eye blinking significantly reduce absorption [3]. Indeed, under physiological conditions, the lacrimal fluid (7  $\mu\text{L}$ ) is completely renewed in five minutes and its secretion can reach a 400  $\mu\text{L}/\text{min}$  rate in case of irritation [4]. The active substances are then immediately diluted and subjected to rapid elimination of the ocular surface and conjunctival cul-de-sac. In addition, the ocular surface is periodically washed by the eyelids blinking. Furthermore, the blink rate exhibits a high interpersonal variability, averaging 20 blinks per minute [5,6]. These physiological factors often lead to limited contact time between the administered product and the cornea, conjunctiva and sclera. The ocular bioavailability following administration of liquid eye drop is rather low. Therefore, repeated administrations are frequently required to obtain the expected therapeutic effect.

Over time and following scientific and technological advances, many pharmaceutical dosage forms have been developed to overcome these physiological hindrances and enhance ocular residence time. Preformed gels [7], ointments [8,9] and inserts [10,11] are the most described of them, and have proven increased ocular bioavailability. However, semi-solid and solid

68 ophthalmic dosage forms often lead to patient discomfort due to high viscosities or irritations  
69 [12].

70 *In situ* gelling delivery systems are one of the most promising ophthalmic dosage forms [13].  
71 Instillation under liquid form allows an easy, safe and reproducible administration. Moreover,  
72 preparation and production at the industrial level are facilitated as compared to solid and  
73 traditional semi-solid forms. The major innovation of these smart hydrogels is related to a  
74 solution to gel phase transition, induced by different stimuli which can be found  
75 physiologically on the ocular surface such as temperature, pH or ionic strength [14].  
76 Immediately after administration, *in situ* activated hydrogels undergo a phase transition  
77 leading to gelation of the product. Gelation provides new viscoelastic properties, limiting pre-  
78 corneal elimination and leading to prolonged residence time on the ocular surface. Recently,  
79 the use of biopolymers exhibiting mucoadhesive properties increased the residence time of *in*  
80 *situ* gelling systems on the ocular surface [15]. Finally, prolonged residence time of the  
81 hydrogels on the ocular surface allowed an increase in the local absorption of the active  
82 ingredients, leading to an improved bioavailability and therefore to a better efficacy.  
83 Moreover, it has also been shown that the reduction of the pre-corneal elimination of *in situ*  
84 activated hydrogels leads to a decrease of the systemic absorption and therefore to a reduction  
85 of the side effects [16,17]. Hence, ophthalmic *in situ* gelling systems could potentially  
86 improve the treatment of pathologies affecting the anterior segment of the eye and can be  
87 considered as a new pharmaceutical strategy in the formulation of active ingredients with low  
88 absorption at the ocular level.

89 The *in situ* gelling delivery systems presented here are based on gellan gum and  
90 hydroxyethylcellulose (HEC). The gelling capacity of gellan gum in the ocular environment  
91 has already been demonstrated and the enhanced residence time on the ocular surface was  
92 proven *in vivo* for several gellan gum based formulations [18–21]. The combination with

93 HEC was conceptualized to enhance its viscosity, mucoadhesion and release properties, while  
94 reducing polymer concentration. HEC is also well tolerated as compared to other cellulosic  
95 polymers and exhibits suitable viscosity and lubricant properties [12]. All formulations are  
96 preservative free, as a large number of eye drop preservatives (e.g. benzalkonium chloride)  
97 exhibit an important toxicity [22–26]. The growing number of publications on this topic,  
98 market trends and expectations of patients and practitioners show that this strategy has a real  
99 potential.

100 The purpose of this study was to formulate and characterize a new *in situ* gelling system for  
101 the delivery of phenylephrine hydrochloride and tropicamide. Nowadays, phenylephrine and  
102 tropicamide are the two most used mydriatic active pharmaceutical ingredients. When  
103 mydriasis induction is required, prior eye examinations or ophthalmic surgeries, two  
104 predominant therapeutic strategies are available. Either the association of two liquid eye drops  
105 of phenylephrine and tropicamide (Neosynephrine<sup>®</sup> 10 % and Mydriaticum<sup>®</sup> 0.5 %  
106 respectively) or a solid ocular insert (Mydriaser<sup>®</sup>) combining phenylephrine and tropicamide.  
107 None of these two pharmaceutical dosage forms are optimal regarding topical ophthalmic  
108 administration. On one hand, due to the high pre-corneal elimination of conventional eye  
109 drops, the induction of mydriasis requires up to five drops in each eye. A five minutes interval  
110 between each drop is also required, leading to 30 to 45 minutes of patient care time prior eye  
111 surgery. On the other hand, the ocular insert must be administered by a trained medical staff  
112 and must be left in the conjunctival cul-de-sac for 30 to 45 minutes potentially leading to  
113 patient discomfort and local irritations. A possible alternative to reduce patients discomfort  
114 and increase the efficiency of treatments of the anterior segment of the eye could be *in situ*  
115 gelling systems.

116 Therefore, we developed an *in situ* gelling hydrogel made of gellan gum and  
117 hydroxyethylcellulose for the delivery of tropicamide and phenylephrine.

118 The first part of this work focuses on the formulation and physicochemical characterization  
119 [27]. Then, gelling behavior, rheological and mucoadhesive properties were evaluated. A new  
120 rheological method was developed to assess the gel resistance under simulated eye blinking.  
121 Finally, residence time on the ocular surface was assessed *in vivo* in rabbit to corroborate the  
122 hypothesis of prolonged drug delivery and validate the developed rheological methods.

123

## 124 **2. Materials and methods**

### 125 2.1. Materials

126 All the sol-gels were prepared using sterile water Versylene<sup>®</sup> purchased from Fresenius Kabi  
127 France (Sèvres, France). Phenylephrine hydrochloride of European Pharmacopoeia grade was  
128 a kind gift from Cheng Fong Chemical Co., Ltd. (Taipei, Taiwan). Tropicamide of European  
129 Pharmacopoeia grade was a free sample from Tokyo Chemical Industry Co., Ltd. (Tokyo,  
130 Japan). Deacylated gellan gum (Kelcogel<sup>®</sup> CG-LA) of pharmaceutical use grade was a free  
131 sample from CP Kelco (Atlanta, GA, USA). Hydroxyethylcellulose (Natrosol<sup>®</sup> 250 M) was  
132 generously provided by Ashland (Schaffhausen, Switzerland). Sodium citrate was purchased  
133 from Cooper (Ponthierry, France). The porcine gastric mucin type II was purchased from  
134 Sigma-Aldrich (St. Louis, MO, USA). The fluorescence probe (Cyanine 5) was synthesized in  
135 our laboratory as described previously [28].

136

### 137 2.2. Differential scanning calorimetry

138 Each component was placed in aluminum pans and hermetically sealed. Differential scanning  
139 calorimetry (DSC) and thermogravimetric analysis (TGA) of each individual component were  
140 performed using a Perkin Elmer Diamond DSC at a heating rate of 10 °C/min between 25 and  
141 200 °C.

142 Interaction analyses were done to assess the compatibility of phenylephrine hydrochloride  
143 with tropicamide and selected excipients. Binary mixes were analyzed with the following  
144 weight ratios: 1:3, 1:1 and 3:1.

145

### 146 2.3. Preparation of formulations

147 The hydrogels were prepared on a volume basis. Concentrations of all the components  
148 reported here are expressed as weight/volume percentages (% w/v). Gellan gum solutions  
149 were prepared by dispersing gellan gum (0.5 %) and sodium citrate (0.3 %) to a certain  
150 volume of sterile water while stirring. The solutions were then heated to 80 °C allowing the  
151 hydration of gellan gum chains. The clear solutions obtained were then cooled to room  
152 temperature. After volume adjustment of the water loss due to evaporation, the preparations  
153 were sterilized by filtering aseptically through 0.22 µm sterilizing filter under a laminar flow  
154 hood.

155 APIs stock solution containing HEC (0-0.71 %), phenylephrine (7.1%) and tropicamide  
156 (0.71%) in sterile water were prepared separately. After complete dissolution under magnetic  
157 stirring, the solution was filtered aseptically through 0.22 µm sterilizing filter under a laminar  
158 flow hood. A certain volume of the sterile gellan gum solution was then added dropwise  
159 under vigorous stirring to the resultant mixture to obtain homogeneous, sterile, *in situ* gelling  
160 ophthalmic formulations of phenylephrine and tropicamide. Final concentrations are reported  
161 in table 1.

162

### 163 2.4. Physicochemical characterization

164 Hereafter, the ophthalmic *in situ* gelling delivery systems previously prepared were  
165 characterized regarding the pH, osmolality and transparency to ensure a safe and non-irritant  
166 ocular administration and to maintain the patient's vision. Indeed, as an ophthalmic

167 formulation intended for topical use, the preparations should comply with preferable  
168 properties related to the physiology of the eye and ophthalmic delivery requirements [29,30].

169

#### 170 2.4.1. Transparency

171 The percent light transmittance in the visible light spectrum from 400 to 800 nm wavelength  
172 against water as a reference standard was measured using a Perkin-Elmer UV-Vis  
173 spectrometer Lambda 25. Measurements were done in triplicate and mean  $\pm$  SD value for  
174 each formulation was calculated.

175

#### 176 2.4.2. pH measurement

177 The pH of the formulations was measured using a Hanna HI 98240 pH meter. Prior  
178 measurement, the pH meter was calibrated using Mettler Toledo pH standards of 4.01 and  
179 7.00 at 25 °C. The calibration was then validated using a pH standard of 6.86. All  
180 formulations were tested for pH in triplicate and the mean  $\pm$  SD value was calculated.

181

#### 182 2.4.3. Osmolality

183 Osmolality of the formulations was tested using Roebbling Automatic Osmometer type 13.  
184 Prior to use, the apparatus was calibrated using distilled sterile water as blank (0 mOsm/kg)  
185 and standard solution of 300 mOsm/kg. Measurements were done in triplicate and mean  $\pm$  SD  
186 value for each formulation was calculated.

187

#### 188 2.5. Rheological studies

189 All the rheological analyses were performed on an Anton Parr MCR102 Rheometer and all  
190 the data were analyzed using the Anton Parr RheoCompass software. The geometry was a  
191 stainless steel cone/plate (diameter 50 mm, angle 1 ° and gap 100  $\mu$ m), which provided an



192 homogeneous shear of the sample. The cone was equipped with a solvent trap to avoid  
193 evaporation during measurement. Thanks to Peltier diodes which were placed in the lower  
194 plate, it was possible to control the temperature with a precision of 0.1 °C.

195

#### 196 2.5.1. Viscoelastic behavior and gelation assessment

197 Viscoelastic behaviors of the *in situ* gelling systems were evaluated, before and after addition  
198 of simulated tear fluid (STF), at 25 and 35 °C respectively. Oscillatory experiments were  
199 carried out and the storage modulus  $G'$  and loss modulus  $G''$  were measured. These  
200 experiments were carried out under frequency and amplitude values which belonged to the  
201 viscoelastic linear regime where  $G'$  remained invariant and where the sample did not undergo  
202 structural modifications. The frequency was 1 Hz and the amplitude 0.1 %. All rheological  
203 results are the mean  $\pm$  SD of n=6 experiments.

204 The STF was added at a 7:30 ratio corresponding to the ratio between the volume of the  
205 physiological tear fluid and the volume of the instilled formulation drops, the physiological  
206 volume of the tear fluid being 7  $\mu$ L and the average volume of an eye drop being 30  $\mu$ L [31].

207 The composition of STF is reported in table 2.

208

#### 209 2.5.2. Flow behavior and viscosity

210 Flow properties of the *in situ* gels mentioned previously were determined after addition of  
211 STF at 35 °C using a stress sweep. After 2 min of equilibration, the shear rate was increased  
212 gradually from 0.1 to 5000  $s^{-1}$  (upward curve). Then, the shear rate was maintained at 5000  $s^{-1}$   
213 for 30 seconds and subsequently decreased gradually from 5000 to 0.1  $s^{-1}$  (downward curve).

214 The results are the mean  $\pm$  SD of n=6 experiments.

215

216

217 The viscosity curves were fitted according to the Cross equation, Eq. (1):

$$218 \quad \eta = \eta_{\infty} + \frac{\eta_0 - \eta_{\infty}}{1 + (C\dot{\gamma})^m} \quad (1)$$

219 where  $\eta$  represents the apparent viscosity at a given shear rate (mPa.s),  $\dot{\gamma}$  is the shear rate ( $s^{-1}$ ),  
220  $\eta_0$  is the zero-shear viscosity (mPa.s) and  $\eta_{\infty}$  is the infinite-shear viscosity (mPa.s).  $C$  is the  
221 Cross time constant (s) and the reciprocal,  $1/C$ , gives a critical shear rate which is a useful  
222 indicator of the onset shear rate for shear thinning. The Cross constant  $m$  is dimensionless and  
223 corresponds to the measurement of the dependence degree of viscosity on shear rate in the  
224 shear-thinning region. A value of zero for  $m$  indicates a Newtonian behavior while values of  
225  $m$  tending to one show an increasing shear thinning behavior [33].

226

### 227 2.5.3. Gel resistance under simulated eye blinking

#### 228 2.5.3.1. Rotational measurements

229 This test was developed to predict the behavior of the preparation under eye blinking. To do  
230 so, the preparations were exposed alternatively to a high shear rate of  $5000 \text{ s}^{-1}$  for 1 second,  
231 immediately followed by measurements at a low-destructive shear rate of  $1 \text{ s}^{-1}$  for 1 min. The  
232 high shear rate was chosen to simulate the eye blinking as it was shown that the physiological  
233 eye blinking shear rate ranged between  $3000$  and  $40\,000 \text{ s}^{-1}$ . The low shear rate was selected  
234 to assess the viscosity behavior during the inter-blinking period, when the eye is open. This  
235 cycle was repeated ten times in a row to simulate ten blinking periods. The physiological  
236 inter-blinking period lasts 5 to 7 seconds, but a 1 min interval was required to allow sufficient  
237 precision of the measurement. Once again, this test was conducted after addition of STF. The  
238 results are the mean  $\pm$  SD of  $n=6$  experiments.

239

240

### 241 2.5.3.2. Oscillatory measurement

242 The same test was conducted replacing the low shear rate measurement phase by oscillatory  
243 measurements to predict the elastic behavior of the preparation under eye blinking. Thus, the  
244 preparations were exposed alternatively to a high shear rate of  $5000 \text{ s}^{-1}$  for 1 second,  
245 immediately followed by non-destructive oscillatory measurements at a frequency of 1 Hz  
246 and amplitude of 1 % for 1 min. The oscillatory measurements were selected to assess the  
247 elastic behavior during the inter-blinking period, when the eye is open. This cycle was  
248 repeated ten times in a row to simulate a ten blinking period. The physiological inter-blinking  
249 period lasts 5 to 7 seconds, but a 1 min interval was required to allow sufficient precision of  
250 the measurement. Once again, this test was conducted after addition of STF. The results are  
251 the mean  $\pm$  SD of  $n = 6$  experiments.

252

## 253 2.6. Bioadhesion: measurement of the mucoadhesive force

254 Evaluation of the mucoadhesive ability of the three formulations was performed using two  
255 complementary methods [34,35]. First, a tensile strength test was applied pointing out the  
256 surface interactions between the formulation and a mucin film acting as a biological substrate.  
257 Then, the rheological synergism method was used, showing the mucoadhesive effect due to  
258 internal interactions. These tests were run after addition of STF to assess the mucoadhesive  
259 capacity of the gelled formulations.

260

### 261 2.6.1. Tensile strength test

262 The mucoadhesive capacity of the formulations was evaluated by measuring the force  
263 required to detach the formulations from a mucin film in a tensile test [28,36]. The  
264 measurements were performed on an Anton Parr MCR102 Rheometer and all the data were  
265 analyzed using Anton Parr RheoCompass software. The geometry was a stainless steel

266 plate/plate (diameter 50 mm and gap 100  $\mu\text{m}$ ). A 5 % (weight/weight) porcine gastric mucin  
267 dispersion was previously prepared in sterile water. Mucin films were prepared directly on the  
268 lower plate: The mucin dispersion (300  $\mu\text{L}$ ) was placed and spread homogeneously on the  
269 plate. The plate was then heated for 3 min at 70  $^{\circ}\text{C}$  allowing the mucin dispersion to dry.  
270 Then, the hydrogel (400  $\mu\text{L}$ ) was carefully loaded on the lower plate, covering the mucin film.  
271 After a contact of 1 min with the hydrogel with a force of 0.3 N, the upper plate was moved  
272 upward at a constant speed of 10 mm/min until the complete break-up of the hydrogel. Six  
273 replicate analyses were performed for each formulation at 35  $^{\circ}\text{C}$ , using a fresh sample and a  
274 fresh mucin film in each case. The force of detachment as a function of displacement was  
275 recorded by the software. Then, the maximum detachment force ( $F_{\text{max}}$ ) was analyzed and  
276 compared with the data obtained from the gel strength study without mucin film. The  
277 difference between the two forces,  $\Delta F_{\text{max}}$ , indicates the mucoadhesion capacity of the  
278 hydrogel [28]. The results are the mean  $\pm$  SD of n=3 experiments.

279

## 280 2.6.2. Rheological synergism

281 This method was originally described by Hassan and Gallo [37] and adapted to ophthalmic  
282 formulations to take into account the dilution due to STF [38,39]. The geometry was a  
283 stainless steel cone/plate (diameter 50 mm, angle 1  $^{\circ}$  and gap 100  $\mu\text{m}$ ), which provided an  
284 homogeneous sample shear. The cone was equipped with a solvent trap to avoid evaporation  
285 during the measurement. Thanks to Peltier diodes which were placed in the lower plate, it was  
286 possible to control the temperature with a precision of 0.1  $^{\circ}\text{C}$ . All rheological results are the  
287 mean  $\pm$  SD of n=6 experiments.

288

### 289 2.6.2.1. Viscosity

290 Mucins (5 %, w/w) were dispersed in STF and then left to equilibrate overnight at 4 °C. The  
291 dispersion was then mixed with the formulations using the previous ratio of 30:7. The  
292 viscosities of the mucin dispersion, the three formulations and the formulations-mucin  
293 mixtures were then measured at 100 s<sup>-1</sup> and 35 °C with the rheometer. The viscosity due to  
294 mucoadhesion ( $\eta_b$ ) was calculated by Eq. (2):

$$295 \quad \eta_b = \eta_t - (\eta_m + \eta_p) \quad (2)$$

296 where  $\eta_t$  is the viscosity of the mixture,  $\eta_m$  is the viscosity of the mucin dispersion and  $\eta_p$  is  
297 the viscosity of the formulations. The mucoadhesive force was then calculated using the Eq.  
298 (3):

$$299 \quad F_b = \eta_b \times \gamma \quad (3)$$

300 where  $\gamma$  represents the shear rate at which the viscosity value was calculated.

301

#### 302 2.6.2.2. Oscillatory measurements

303 Here, the values of the elastic moduli ( $G'$ ) measured at 35 °C were used to evaluate the  
304 interactions between the formulations and the mucin dispersion [40]. The interaction was  
305 calculated as  $\Delta G'$  using Eq. (4):

$$306 \quad \Delta G' = G'_t - (G'_p + G'_m) \quad (4)$$

307 where  $G'_t$  is the elastic modulus of the mixture, and  $G'_p$  and  $G'_m$  represent respectively the  
308 elastic modulus of the formulation and the mucin dispersion. Herein,  $\Delta G'$  referred to as the  
309 mucoadhesive interaction term, is the elastic component interpreted as the interaction between  
310 the formulation and the mucins. In this study, the elastic modulus of the mucin dispersion was  
311 negligible, Eq. (4) was therefore simplified to Eq. (5):

312

$$313 \quad \Delta G' = G'_t - G'_p \quad (5)$$

314

315 The frequency was 1 Hz and the amplitude 0.1 %. These experiments were carried out under a  
316 stress value which belonged to the viscoelastic linear regime where  $G'$  remained invariant and  
317 where the sample did not undergo structural modifications.

318

### 319 2.7. *In vivo* evaluation of ocular residence time

320 This animal research project was authorized by Ministry of higher education and research, in  
321 conformity with the regulations of Committee on Ethics in the care and use of laboratory  
322 animals, with the reference Apafis #14792. Male New-Zealand albino rabbits were purchased  
323 from CEGAV (France). The *in vivo* experiments were performed in non-anesthetized rabbits  
324 kept in restraining boxes. Their heads were free of movements so that normal eye blinking,  
325 head and eye movements were allowed during the experiments.

326 *In situ* gels were prepared by adding 0.014 % of Cyanine dye 5.5 to obtain suitable  
327 fluorescence intensity [28]. One drop of preparation was carefully administered into animal's  
328 conjunctival cul-de-sac with a plastic transfer pipette. The contralateral eye was treated with a  
329 control solution containing the same amount of fluorescent probe. Prior administration, the  
330 fluorescence intensity of the *in situ* gels and the control solution were measured with a  
331 microplate fluorimeter Infinite F200 PRO (Tecan, France) and no significant difference was  
332 found (from  $39087 \pm 449$  to  $40358 \pm 997$ ). The weight of the drops from different  
333 formulations as well as control solution was measured, and no significant variations were  
334 found (from  $28.6 \pm 0.8 \mu\text{g}$  to  $30.1 \pm 1.2 \mu\text{g}$ ). A flexible scale was placed on the rabbit cheek,  
335 and pictures of both eyes were recorded using Fluobeam<sup>®</sup>, provided by Fluoptics (Grenoble,  
336 France). A laser was used to visualize the fluorescence signal. Acquisitions were performed  
337 immediately after administration (reference image) and until three hours after formulations  
338 administration.

339 Formulations A, B and C were compared with the control solution (phenylephrine 5 %,  
340 tropicamide 0.5 % and cyanine dye 0.014 % in sterile water). The kinetic data of fluorescence  
341 intensity was obtained by analyzing the images by open source processing program Image J  
342 [41]. Fluorescence intensity was measured selecting the ocular surface area only to avoid bias  
343 due to gel deposits on the edges of the eyelids and around the eyes. Results are mean  $\pm$  SD of  
344  $n = 3$  experiments, made on three different rabbits. Each rabbit had a minimum of 48 hours  
345 washout period between two experiments.

346 At the end of the protocol, rabbits were placed in foster care by an approved association, no  
347 sacrifice was needed.

348

## 349 2.8. Statistical analysis

350 The statistical significance of the obtained values was analyzed using one-way ANOVA and  
351 multiple T-tests ( $p < 0.05$ ) (Statgraphics centurion 18, Statgraphics Technologies, inc., The  
352 plains, VA, USA).

353

## 354 3. Results and discussion

355

### 356 3.1. Physicochemical characterizations

357 In addition to the unique rheological features of *in situ* gelling delivery systems, sol–gels  
358 intended for an ophthalmic use should meet the same requirements as conventional  
359 ophthalmic forms. The formulations must have optimal pH, clarity, and **osmolality**. The  
360 optimal pH for an ophthalmic formulation is near the physiological pH (7.0–7.4), although  
361 formulations with a pH from 4.0 to 8.0 shall be administered [29]. The formulation should  
362 also be isotonic to avoid irritation upon administration. Clarity is particularly important for  
363 sol-gels that are intended to remain in contact with the ocular surface. Indeed, the optical

364 absorption in the visible wavelength range [42] or transmittance [30,43] has to be explored to  
365 ensure that light is able to pass through the gel and that the vision of the patient is not altered.  
366 The pH and **osmolality** values for each formulation are shown in table 3.

367 The three formulations exhibited similar pH and **osmolalities**. With pH values between 6.45  
368 and 6.50 the pH were slightly acidic but close enough to neutrality and in the range of suitable  
369 pH for ophthalmic use. Regarding the **osmolality**, values from 430 to 440 **mOsm/kg** were  
370 found. At first glance, these results show a relatively large gap from isotonicity (300-320  
371 **mOsm/kg**). However, these high **osmolality** values are almost only due to the high proportion  
372 of phenylephrine hydrochloride in the formulations, which is required to reach a therapeutic  
373 mydriasis. It was shown that the osmolality of the commercially available mydriatic eye drop  
374 containing 10 % of phenylephrine hydrochloride was above 900 **mOsm/kg**. Therefore, while  
375 being relatively higher than physiological **osmolality**, the **osmolalities** of the formulations  
376 proposed here have been reduced by two fold compared with mydriatic eye drops used in  
377 routine.

378 The transmittance in the visible light spectrum was then measured. Results are shown in  
379 figure 1. The percent light transmittance of the three formulations was above 90 % over the  
380 entire visible domain making them appropriate for ophthalmic use. Besides, the increase of  
381 HEC concentration revealed a slight decrease in the transparency which may be attributed to  
382 the intrinsic properties of the polymer.

383

### 384 3.2. Differential scanning calorimetry

385 Heating DSC curve of phenylephrine hydrochloride showed a single endothermic peak at  
386 141.1 °C with no mass loss detected with TGA. This peak was identified as the fusion peak of  
387 phenylephrine hydrochloride (Fig. S1A). Regarding tropicamide, a single endothermic peak at



388 96.1 °C was detected on the heating DSC curve, with no mass loss on the TGA analysis. This  
389 peak was identified as the fusion peak of tropicamide (Fig. S1B).

390 The analysis of the binary mixes showed no significant differences in the peaks of PHE and  
391 TPC, as well as in the excipient's peaks. Therefore, no interactions between PHE, TPC and  
392 the excipients were evidenced (Tab. S1).

393

### 394 3.3. Rheological studies

#### 395 3.3.1. Viscoelastic behavior and gelation assessment

396 Oscillatory experiments were mainly used to determine whether the formulations were in a  
397 liquid or a gel state. The higher the  $G'$  value is, the more pronounced the elastic character is.  
398 Conversely the higher the  $G''$  value is, the more pronounced the viscous properties are. To  
399 extrapolate, one may consider that when the  $G''$  value is higher than the  $G'$  value, the  
400 preparation exhibits a viscous-dominant (i.e. liquid-like) behavior. Inversely, when the  $G'$   
401 value is higher than the  $G''$  value, the preparation exhibits an elastic-dominant (i.e. solid-like)  
402 behavior. Afterwards, the tangent of the phase angle, a useful quantifier of the presence and  
403 extent of elasticity in a fluid can be calculated as:  $\tan\delta = G''/G'$ . Thus, a viscous (liquid-like)  
404 state is observed when  $\tan\delta > 1$ . A gel (solid-like) state is observed when  $\tan\delta \leq 1$ . Beyond  
405 this critical gelation point, preparations do not necessarily appear as a cohesive and self-  
406 supporting gel but may appear as a fluid gel also referred as “weak gel” in the literature. This  
407 phenomenon occurs particularly with gellan gum based gels [21] and this behavior is highly  
408 sought regarding the ocular administration.

409 Storage modulus ( $G'$ ), loss modulus ( $G''$ ) and tangent of the phase angle ( $\tan\delta$ ) values in  
410 presence or absence of simulated tear fluid (STF) are reported in table 4.

411 Before addition of STF, the three formulations exhibited a liquid-like behavior with very low  
412 values of storage and loss moduli and tangents of the phase angle values greater than 1. The  
413 liquid-like behavior before administration is a prerequisite for *in situ* gelling delivery systems  
414 allowing a free flow and ability to form drops. Thus, formulations A, B and C are suitable for  
415 an easy, safe and reproducible administration to the eye.

416 After addition of STF, formulations A, B and C underwent a liquid / gel transition. Indeed, the  
417 storage moduli were increased by 30 folds and tangents of the phase angle shifted to values  
418 below 1. As reported in the literature [44], these measurements assessed the *in vitro* gelation  
419 due to ionic interactions between gellan gum chains and mono and divalent cations of the tear  
420 fluid. Therefore, formulations A, B and C should exhibit an *in situ* gelation following  
421 administration to the eye.

422

### 423 3.3.2. Flow behavior and viscosity

424 *In situ* gels presented a shear-thinning behavior (Fig. 2). **Viscograms** were fitted with the  
425 Cross model with good correlation coefficient values (A:  $R^2 > 0.986$ , B:  $R^2 > 0.998$ , C:  
426  $R^2 > 0.997$ ). A constant viscosity was obtained for very low shear rates, and then the viscosity  
427 decreased with the increase of the shear rate.  $\eta_0$ ,  $\eta_\infty$  and  $m$  values are reported in table 5, as  
428 well as the viscosity at  $5000 \text{ s}^{-1}$  which represent the viscosity under the shear of the eyelid.

429 From the Cross equation, no significant differences were shown between  $\eta_\infty$  values and  $\eta_0$   
430 values were in the same order of magnitude. Contrariwise, significant differences in the  
431 viscosity at  $5000 \text{ s}^{-1}$  were observed between formulation A, B and C. Thus, the three  
432 formulations exhibited similar values of  $\eta_\infty$  and  $\eta_0$  but different shear-thinning behaviors with  
433 different viscosity values over the stress sweep in the range of 0.1 to  $5000 \text{ s}^{-1}$ . These  
434 differences were highlighted with the Cross constant, formulation A being significantly more  
435 shear-thinning than formulations B and C with a greater  $m$  value.

436 Formulation A displayed a non-thixotropic behavior shown by the significant overlay of  
437 downward and upward curves (Fig. 2A). On the contrary, formulations B and C exhibited a  
438 thixotropic behavior shown by a significant gap between the downward and upward curves  
439 (Fig. 2B and 2C).

440 The three formulations exhibited flow behaviors and viscosities suitable for ocular  
441 administration. On one hand, formulation A exhibited a very low viscosity under high shear  
442 rates but recovered almost instantly when the deformation stopped. On the other hand,  
443 formulations B and C presented higher viscosities under high shear rates followed by a  
444 delayed and incomplete recovery at rest. The shear-thinning behavior presents real advantages  
445 for formulations intended to remain on the ocular surface. It allows a high viscosity at rest,  
446 when the eye is open, and a low viscosity under the shear of the eyelid, thus avoiding  
447 irritation and discomfort.

448

### 449 3.3.3. Gel resistance under simulated eye blinking

450 In order to complete the previous observations, two rheological experiments were developed.  
451 Formulations were submitted to a series of simulated eye blinking (i.e. high shear rate  
452 interspersed by rest periods) to predict the rheological behavior over time on the ocular  
453 surface.

454

#### 455 3.3.3.1. Rotational measurements

456 The first test allowed viscosity measurements during the inter-blinking intervals. At each  
457 simulated blinking, the viscosities reached a minimum due to the shear-thinning behavior and  
458 then recovered during the inter-blinking periods (Fig 3).

459 The results were in accordance with the flow behavior and thixotropy study. Also, the order of  
460 magnitude of the viscosity at  $1 \text{ s}^{-1}$  was the same than previously observed. As a non-

461 thixotropic hydrogel, viscosity of formulation A attained a plateau at its original value almost  
462 instantly during rest periods conducting to a steady viscosity all along the ten cycles of  
463 simulated blinking. Contrarily, as thixotropic hydrogels, viscosities of formulations B and C  
464 recovered without reaching the plateau during the inter-blinking intervals. This delay resulted  
465 in a slight loss of viscosity at each blinking. Thus, after two cycles, formulations B and C  
466 exhibited lower viscosities than formulation A which was the less viscous formulation  
467 originally.

468

#### 469 3.3.3.2. Oscillatory measurements

470 The second test allowed the measurement of storage ( $G'$ ) and loss ( $G''$ ) moduli during the  
471 inter-blinking intervals. The parameter  $\tan\delta$  was used to assess the gel state of the  
472 formulations. At each simulated blinking,  $\tan\delta$  reached a maximum, due to the partial  
473 deconstruct of the tridimensional network of the gel, and then recovered during the inter-  
474 blinking periods (Fig 4).

475 Formulation A exhibited a  $\tan\delta < 1$  all along the ten cycles. Thus, the gel network resisted to  
476 the high shear of the simulated blinking and formulation A remained at a gel state.

477 Formulation B also remained at a gel state all along the ten cycles except at the moment of  
478 high shear rate where  $\tan\delta \leq 1$ . Thus, it could be eliminated from ocular surface as a liquid at  
479 the moment of the blinking.

480 Formulation C exhibited a  $\tan\delta < 1$  for the rest period of the first two cycles. Then, the gel  
481 network was dismantled as  $\tan\delta > 1$  and the formulation was at a liquid state.

482 Thus, the elimination rate of the ocular surface of formulation C should be higher than  
483 formulation's B, itself higher than formulation's A. Therefore, formulation A exhibited the  
484 more suitable rheological properties to enhance the residence time on the ocular surface.

485

486 3.4. Bioadhesion: measurement of the mucoadhesive force

487 3.4.1. Tensile strength test

488 The maximum detachment forces of hydrogels ( $F_{\max}$ ) were measured and compared in the  
489 presence and in the absence of mucin film. The results are presented in figure 5. When  
490 comparing  $F_{\max}$  values with mucin to those without mucin, here expressed as  $\Delta F_{\max}$ , a  
491 significant increase was observed for all the formulations ( $p < 0.001$ ).

492 This indicated a positive interaction between hydrogels and mucin which was coherent with  
493 data found on the mucoadhesive properties of gellan gum and HEC in the literature [45].  
494 Therefore, hydrogels could be retained by interacting with the mucin associated to the ocular  
495 surface, leading to enhanced ocular retention time.

496

497 3.4.2. Rheological synergism

498 The mucoadhesive force of a formulation can be determined by a simple method which  
499 depends on the evaluation of the "rheological synergism" existing when mixing mucoadhesive  
500 polymers with mucin dispersions [46].

501 The mucoadhesive interaction term  $\Delta G'$  was found positive for all three formulations  
502 indicating a synergism on the elasticity of the gels and therefore, a positive interaction  
503 between hydrogels and mucin. The  $G'$  and  $\Delta G'$  values are summarized in table 6.  $\Delta G'$  values  
504 of formulations B and C containing HEC, were significantly higher than formulation's A,  
505 clearly exhibiting the strong effect of HEC on the mucoadhesion properties of hydrogels.  $\Delta G'$   
506 of formulations A, B and C are reported in figure 6A.

507 The mucoadhesive forces were positive for all three formulations. Positive mucoadhesive  
508 forces are due to viscosity synergisms in the mixtures indicating a mucoadhesive interaction

509 between hydrogels and mucin. The viscosity and mucoadhesive force values are summarized  
510 in table 7.

511 Viscosities of bioadhesion were significantly different between formulations A, B and C (Fig.  
512 6B). Formulation C was more mucoadhesive than formulation B, itself more mucoadhesive  
513 than formulation A. Again, the increase in the concentration of HEC was related to the  
514 increase of the mucoadhesive properties.

515 The three evaluation methods described above are complementary as they enlighten different  
516 aspect of mucoadhesion, which is a complex phenomenon [47,48]. More than ten different  
517 types of mucins are expressed on the ocular surface [49]. Two main categories are  
518 distinguished: the mucins associated to the membrane (i.e. cornea) as MUC1, MUC4 and  
519 MUC16 [50,51] and secreted mucins, dispersed in the tear fluid, like MUC2 and MUC5A. On  
520 one hand, tensile strength test highlighted the surface interaction between hydrogels and the  
521 mucin films. This method could be related to the first category of mucin as the interaction  
522 involved an interface, mucin film mimicking the cornea. On the other hand, rheological  
523 synergism involved a liquid phase interaction and could be related to the second category of  
524 mucin secreted in the tear fluid.

525

### 526 3.5. *In vivo* evaluation of ocular residence time

527 Fluorescence intensity of formulations A, B and C containing cyanine 5 dye were compared  
528 with the control solution of PHE and TPC containing the same concentration of cyanine after  
529 administration on non-anesthetized rabbits. **The impact of the addition of the dye on  
530 physicochemical properties of the formulations was assessed rheologically. No significant  
531 changes were shown on the viscosity and the gel resistance under simulated eye blinking.**

532 The evolution of the fluorescence intensity normalized by the initial fluorescence signal is  
533 plotted on figure 8. As we can see, there was a significant difference between the control

534 solution and the hydrogel formulations. As expected, the control solution was almost  
535 completely eliminated during the first 10 minutes following administration [1]. Formulations  
536 A, B and C showed a significantly higher retention of the fluorescent probe in the corneal  
537 region in comparison with the reference solution. During the first 10 minutes, a large  
538 variability was observed. It was probably due to the large volume of product instilled. Indeed,  
539 as said above, the volume of tear fluid on the ocular surface is about 7  $\mu\text{L}$ . Here, the drop  
540 volumes were about 30  $\mu\text{L}$ , resulting in a fast elimination of the surplus either by nasolacrimal  
541 drainage or by lacrimation. As we can see, a significant amount of *in situ* gel was found on  
542 the rabbit cheeks and eye contour (Fig. 7). Also, at the initial period, rabbits showed no sign  
543 of stress or discomfort due to administration. No increase in the blinking frequency was  
544 noted, confirming the limited volume capacity of the conjunctival cul-de-sac and ocular  
545 surface as the main factor for elimination [52]. The prolonged residence time on the ocular  
546 surface region is illustrated on figure 7.

547 After 20 minutes all the *in situ* gels tended to exhibit a slow decrease in signal intensity from  
548 the corneal region. After 3 hours, around 20 % remained on the rabbit's ocular surface, which  
549 suggested good resistance of the hydrogel formulations on ocular surface.

550 Area under curve (AUC) were calculated and showed a significant difference between *in situ*  
551 gelling formulations and the control solution ( $p < 0.05$ ) (Tab. 8). Formulation A exhibited the  
552 most prolonged residence time on the ocular surface which was coherent with *in vitro* gel  
553 resistance studies and previous studies on the importance on gelation versus viscosity  
554 enhancement [19]. Even though, no significant differences were shown between formulations  
555 due to large variability of *in vivo* experiments. Nevertheless, these results are promising as  
556 experiments were conducted on vigilant rabbits meaning that *in situ* gels were submitted to  
557 physiological eye blinking which is known to promote a fast elimination.

558 In the literature, a substantial amount of *in vivo* experiments are dedicated to preclinical  
559 studies on the residence performance of ophthalmic *in situ* gelling delivery systems  
560 [43,53,54]. Due to the low amount and transparency of the *in situ* gels, visual evaluation is  
561 delicate to carry out. In this context, various imaging methods are used [27]. The  
562 incorporation of a fluorescent marker provides accurate information about *in situ* gel's  
563 behavior *in vivo* [28]. Using a portable camera allowed relatively easy measurements and  
564 therefore, the animals could be kept vigilant. The imaging technique was non-invasive, and no  
565 sacrifice was needed to analyze the fluorescence intensity. As compared to gamma  
566 scintigraphy technique, which is often used to study the performance of ophthalmic  
567 hydrogels, the physical incorporation of the marker used in our study was easier and safer to  
568 realize.

569 Despite the significant increase in AUC and fluorescence intensity remaining after 3h (Tab.  
570 8), formulations A, B and C exhibited biphasic clearance patterns usually observed with liquid  
571 solutions [55], contrariwise with observations made by Rupenthal *et al.* [56] and Meseguer *et*  
572 *al.* [57] showing constant elimination rate for polymeric formulations. This could be due to  
573 the very low concentration of polymer used, which results in “liquid-like” gels also called  
574 weak gels [21]. Indeed, concentration of gellan gum was decreased by more than 3 fold  
575 compared with other studies. Still, *in situ* gelling delivery systems presented in this study  
576 exhibited a promising retention time on the ocular surface suggesting that elasticity (i.e.  
577 storage modulus ( $G'$ )) would play a leading role over other parameters such as viscosity or  
578 mucoadhesion in a certain extent.

579

#### 580 **4. Conclusion**

581



582 In this study, novel *in situ* gelling delivery systems containing a combination of  
583 phenylephrine hydrochloride and tropicamide were developed. All *in situ* gelling delivery  
584 systems exhibited suitable physico-chemical properties for ophthalmic use. Appropriate  
585 viscosities and *in situ* gelation capacities were demonstrated, along with shear-thinning  
586 behavior favorable to ocular administration. A new method to characterize rheological  
587 behavior under simulated eye blinking was developed and showed significant differences in  
588 the resistance of the gels. The addition of HEC enhanced the viscosity while decreasing the  
589 gels resistance to shear stress. Also, HEC reinforced the mucoadhesive properties of the  
590 formulations. Subsequently, ocular residence time was assessed *in vivo*, giving insights on the  
591 importance of each parameter. Indeed, the ocular residence time resulted in a combination of  
592 the effects individually assessed *in vitro*.

593 These new formulations are promising alternatives to conventional eye drops regarding their  
594 capacity to enhance residence time on the ocular surface for more than 3 hours, and therefore  
595 the drug bioavailability. Moreover, these innovative delivery systems could improve the  
596 treatment of several affections of the anterior segment of the eye. An enhanced bioavailability  
597 would lead to a better efficacy of the treatment while allowing a decrease in the frequency of  
598 administrations and therefore, in the quantity of drug administered. Also, several studies on *in*  
599 *situ* gelling delivery systems have shown reduced side effects with a decreased absorption at  
600 the systemic level. Finally, these delivery systems are an added value to drugs, making it  
601 possible to best meet patients and practitioners needs, to improve medical care and hence,  
602 patient compliance.

603

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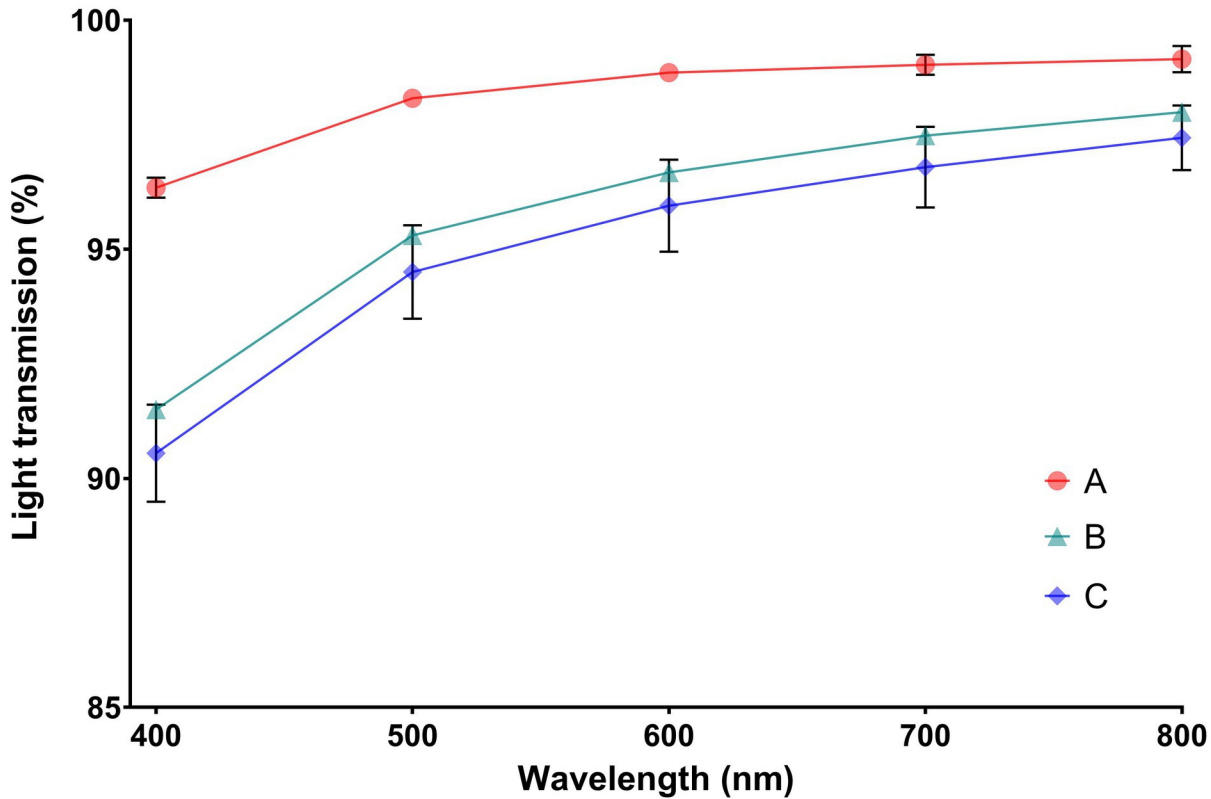
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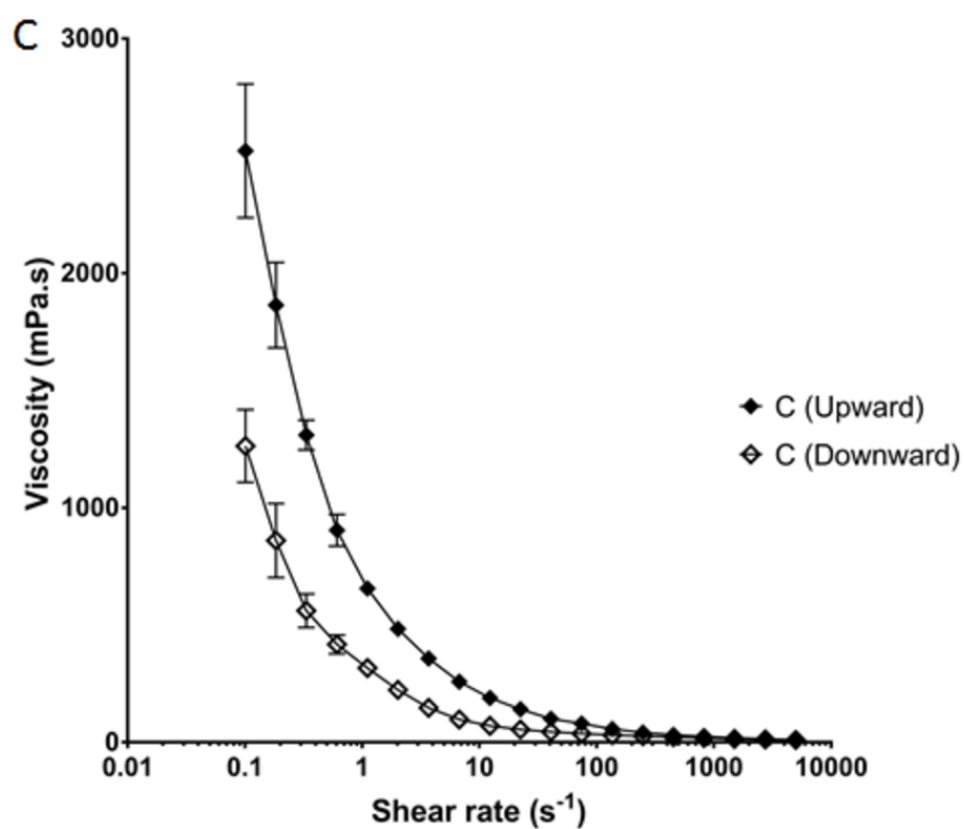
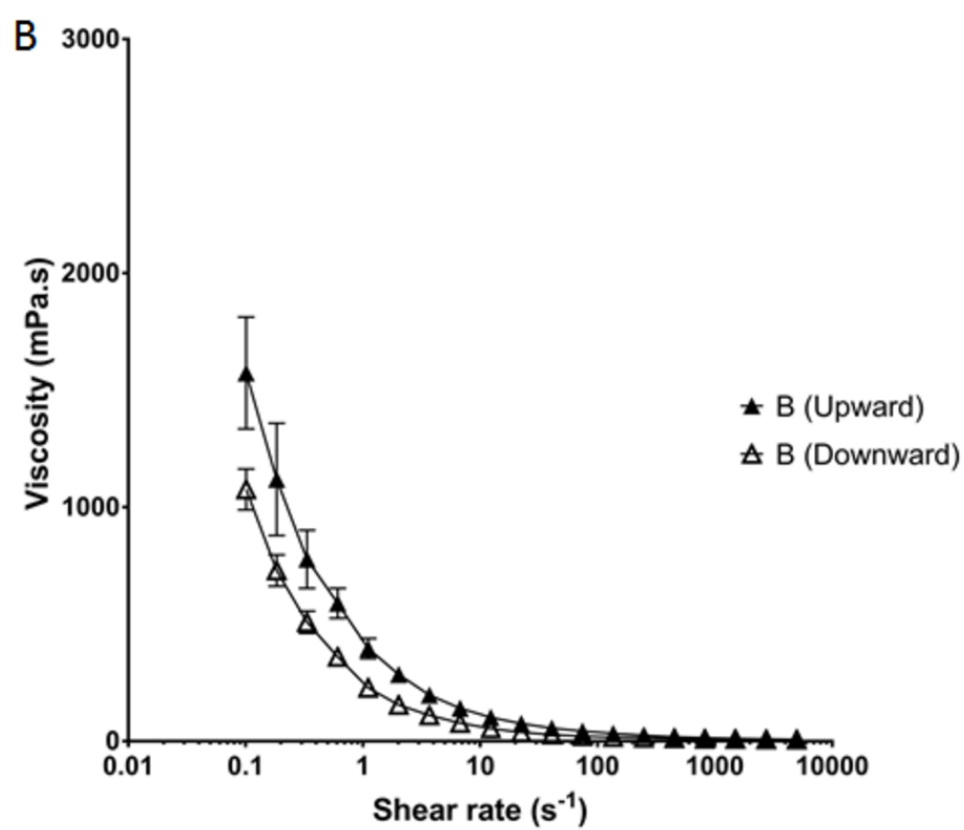
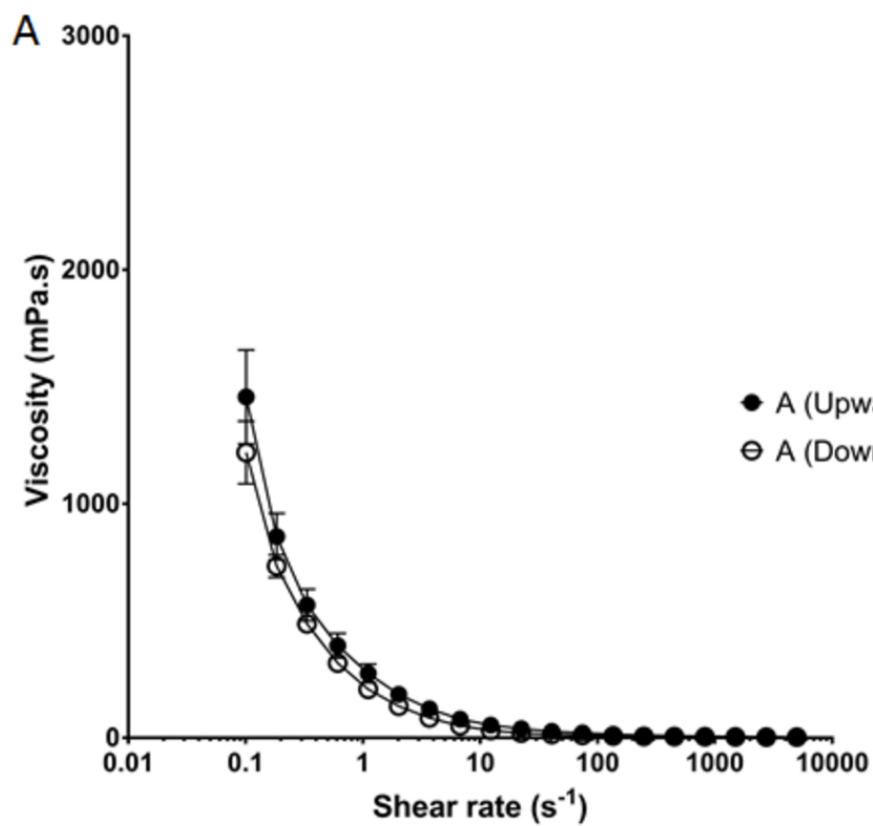
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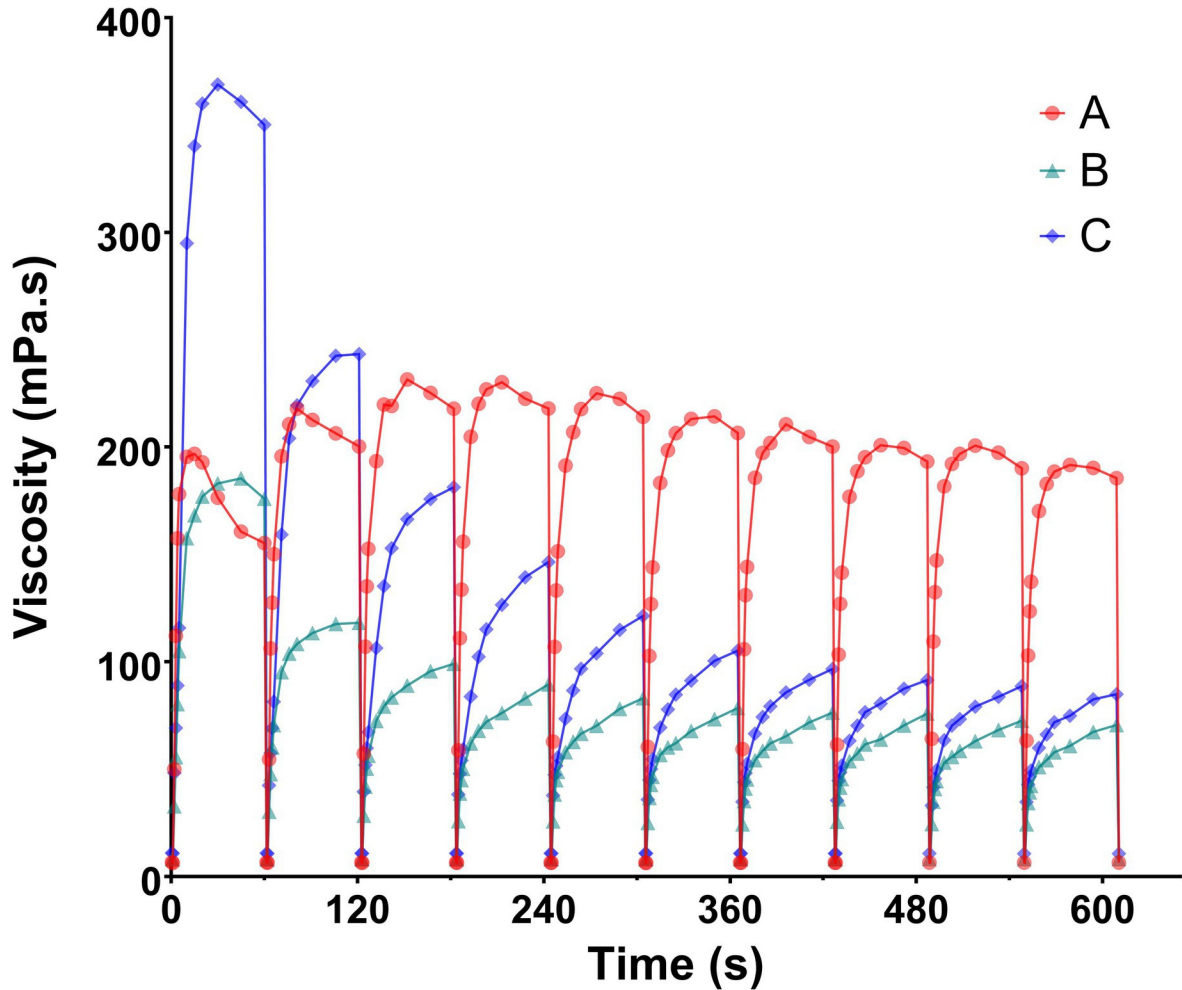
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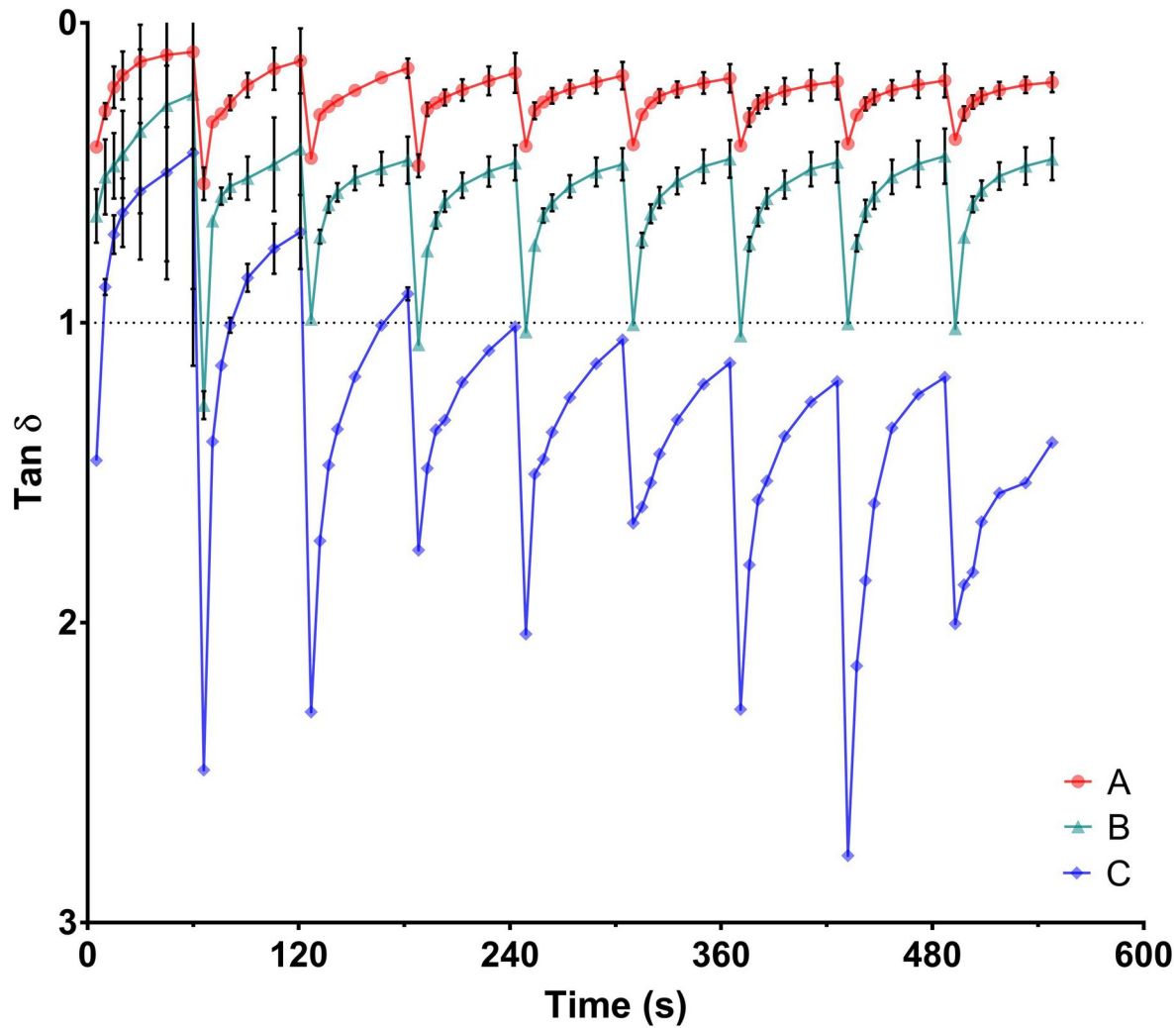
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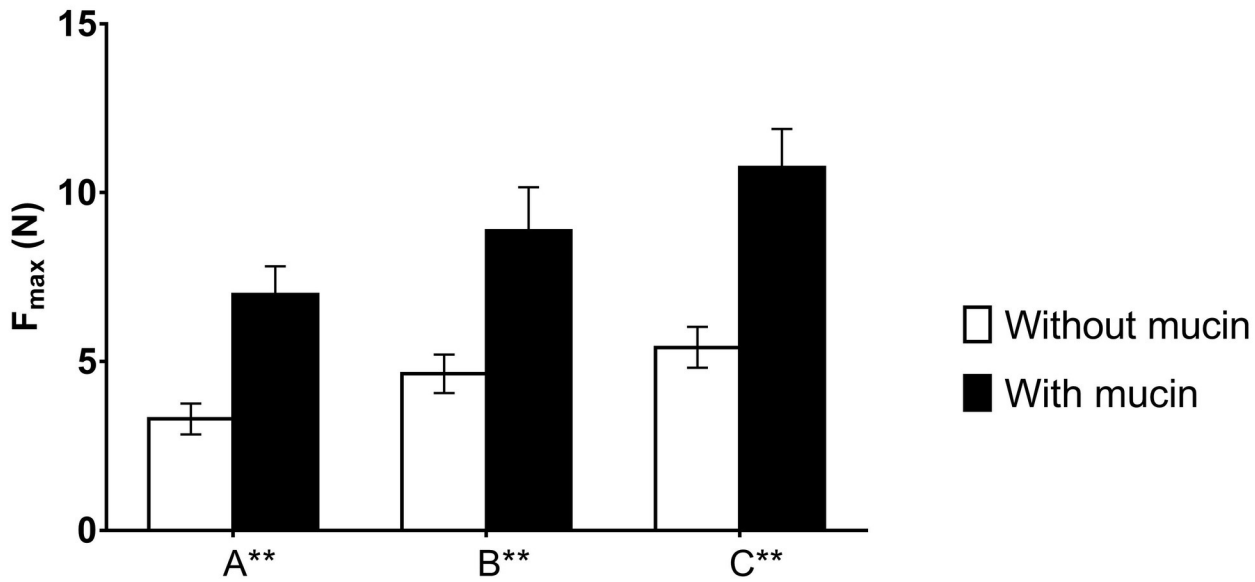


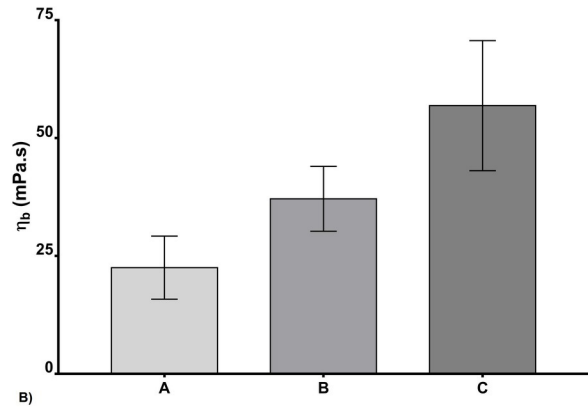
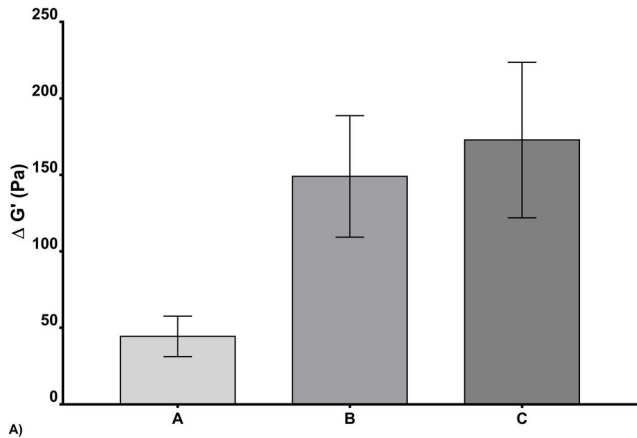








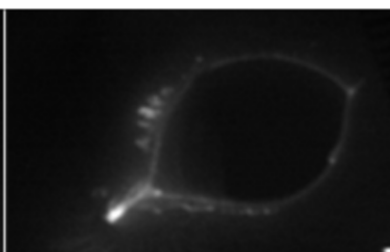
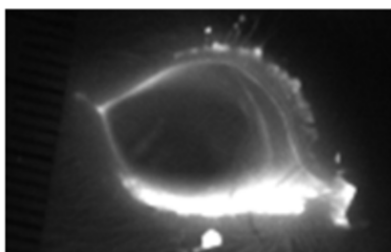




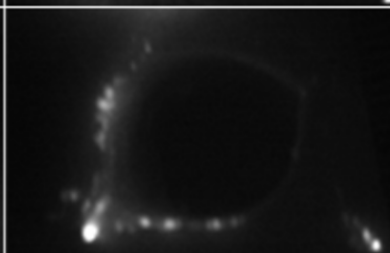
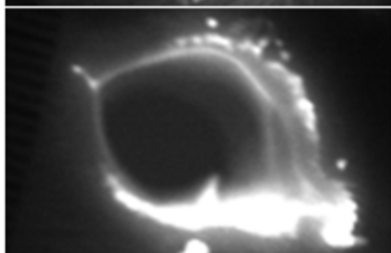
*In situ* gel

Control solution

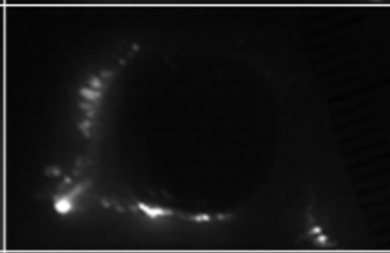
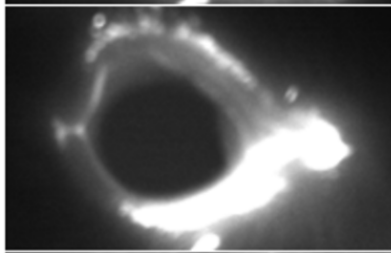
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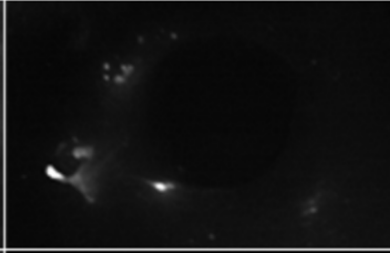
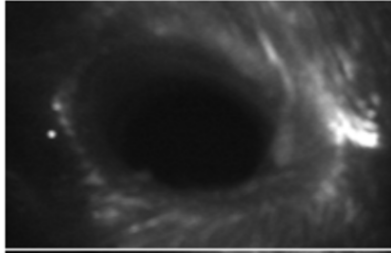
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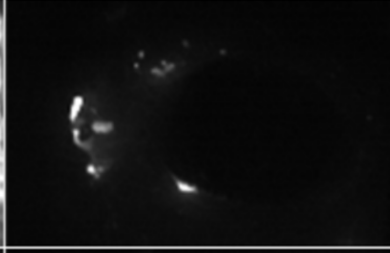
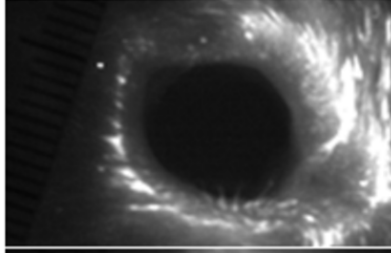
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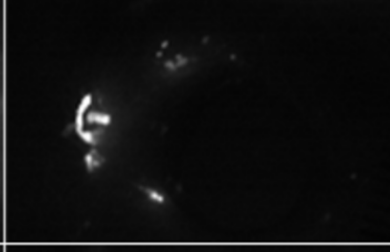
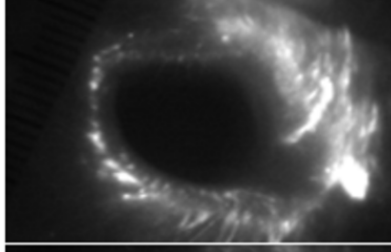
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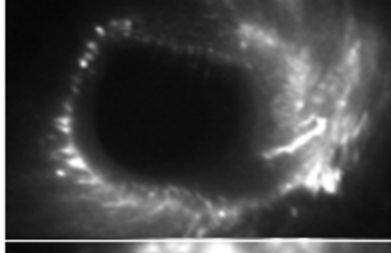
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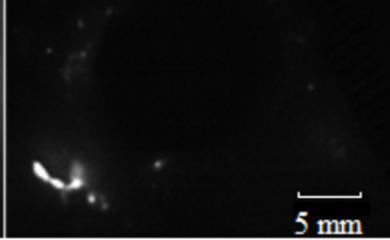
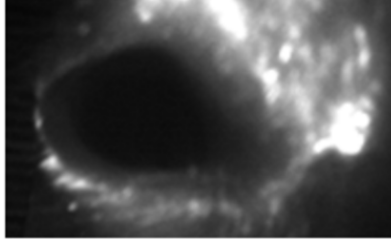
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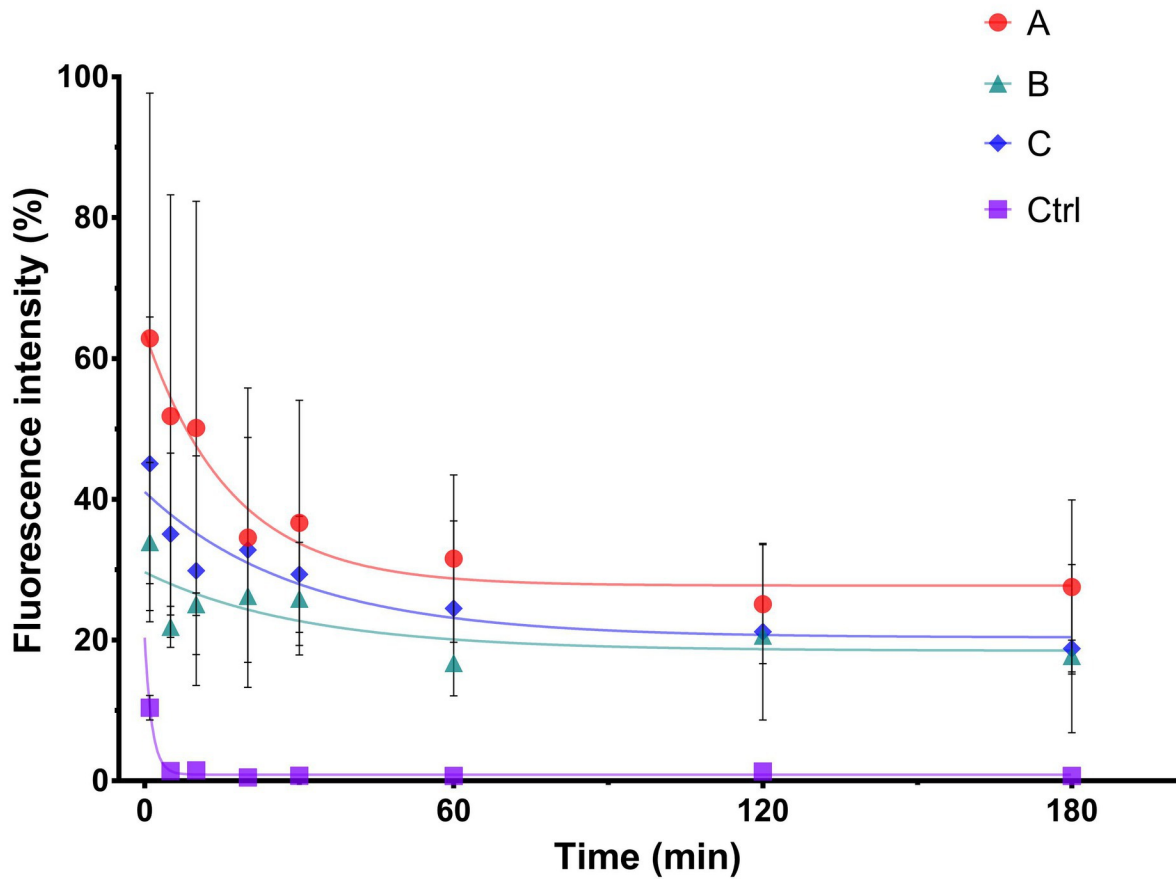
$t_{120\text{min}}$



$t_{180\text{min}}$



5 mm



**Table 1**Composition of the *in situ* gelling delivery systems

	Gellan gum (% w/v)	Hydroxyethylcellulose (% w/v)	Sodium citrate (% w/v)	Phenylephrine (% w/v)	Tropicamide (% w/v)
<b>A</b>	0.15	0	0.09	5.0	0.5
<b>B</b>	0.15	0.25	0.09	5.0	0.5
<b>C</b>	0.15	0.5	0.09	5.0	0.5

**Table 2**

Simulated Tear Fluid composition [30,32]

	Mass (g)
Sodium chloride	0.670
Sodium bicarbonate	0.200
Calcium chloride	0.008
Magnesium chloride	0.005
Potassium chloride	0.138
H <sub>2</sub> O	98.979

**Table 3**

pH and osmolality values

	pH	Osmolality (mOsm/kg)
<b>A</b>	6.46 ± 0.02	438.3 ± 2.1
<b>B</b>	6.49 ± 0.02	438.7 ± 5.0
<b>C</b>	6.50 ± 0.01	434.3 ± 5.0



**Table 4**

Values of  $G'$ ,  $G''$ ,  $\tan\delta$  and description of the viscoelastic behavior before and after gelation in presence or absence of simulated tear fluid (STF)

	Storage modulus, $G'$ (Pa)	Loss modulus, $G''$ (Pa)	Tangent of the phase angle, $\tan\delta$	Viscoelastic behavior
<b>A</b>	$0.117 \pm 0.023$	$0.217 \pm 0.011$	1.855	Liquid-like
<b>B</b>	$0.422 \pm 0.081$	$0.574 \pm 0.056$	1.360	Liquid-like
<b>C</b>	$0.622 \pm 0.091$	$1.122 \pm 0.071$	1.804	Liquid-like
<b>A + STF</b>	$17.545 \pm 1.061$	$2.262 \pm 0.366$	0.129	Weak gel
<b>B + STF</b>	$14.036 \pm 1.723$	$2.283 \pm 0.348$	0.163	Weak gel
<b>C + STF</b>	$18.154 \pm 3.403$	$3.468 \pm 0.631$	0.191	Weak gel

**Table 5**Values of  $\eta_0$ ,  $\eta$  at  $5000 \text{ s}^{-1}$ ,  $\eta_\infty$ , and  $m$  obtained from Cross model

	$\eta_0$ (mPa.s)	$\eta$ at $5000 \text{ s}^{-1}$ (mPa.s)	$\eta_\infty$ (mPa.s)	Cross constant $m$
<b>A</b>	$108613 \pm 14$	$3.9 \pm 0.2$	$3.2 \pm 0.5$	$0.66 \pm 0.04$
<b>B</b>	$81164 \pm 8$	$7.4 \pm 0.2$	$4.3 \pm 0.5$	$0.56 \pm 0.02$
<b>C</b>	$105085 \pm 20$	$10.9 \pm 0.6$	$4.8 \pm 0.4$	$0.52 \pm 0.03$

**Table 6**Values of mucoadhesive interaction term  $\Delta G'$  obtained from Eq. (5)

	$G'_t$ (Pa)	$G'_m$ (Pa)	$G'_p$ (Pa)	$\Delta G'$ (Pa)
<b>A</b>	$61.99 \pm 12.22$	$0.08 \pm 0.08$	$17.55 \pm 1.02$	<b><math>44.44 \pm 13.24</math></b>
<b>B</b>	$163.01 \pm 38.00$	$0.08 \pm 0.08$	$14.04 \pm 1.71$	<b><math>148.97 \pm 39.71</math></b>
<b>C</b>	$190.90 \pm 47.46$	$0.08 \pm 0.08$	$18.15 \pm 3.35$	<b><math>172.75 \pm 50.81</math></b>

**Table 7**

Viscosity of bioadhesion and force of bioadhesion values obtained from Eq. (2) and (3) respectively

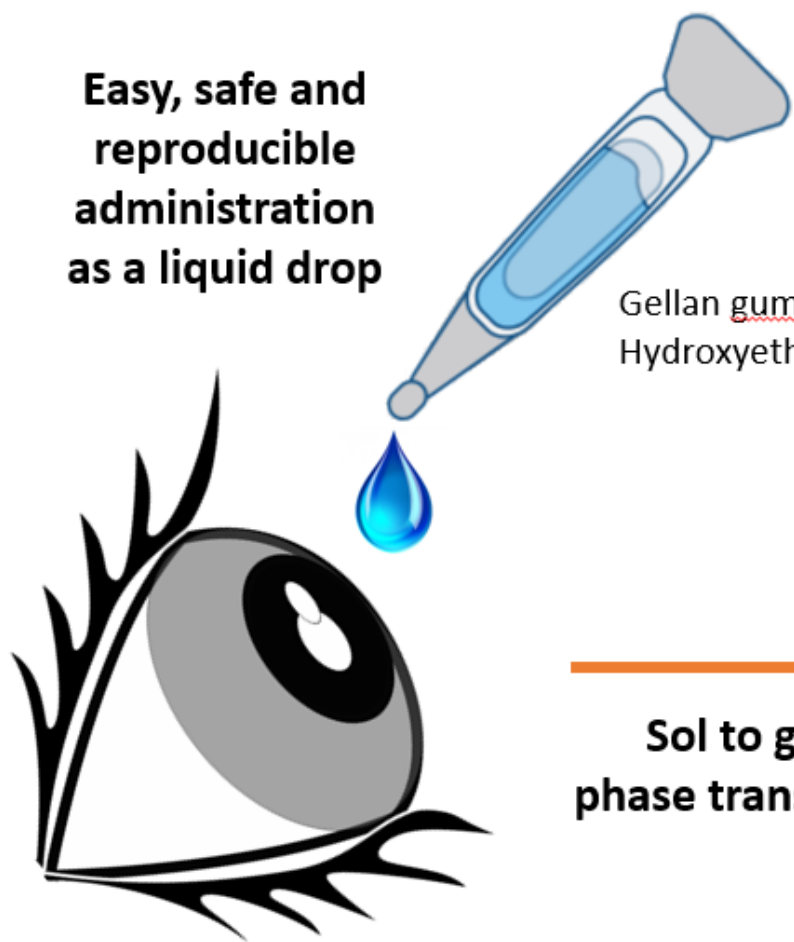
	$\eta_t$ (mPa.s)	$\eta_m$ (mPa.s)	$\eta_p$ (mPa.s)	$\eta_b$ (mPa.s)	$F_b$ (mPa)
<b>A</b>	43.89 ± 4.17	5.78 ± 1.31	15.60 ± 1.21	<b>22.51 ± 6.69</b>	<b>2251</b>
<b>B</b>	71.97 ± 4.10	5.78 ± 1.31	29.07 ± 1.47	<b>37.12 ± 6.88</b>	<b>3712</b>
<b>C</b>	122.34 ± 7.81	5.78 ± 1.31	59.68 ± 4.67	<b>56.88 ± 13.79</b>	<b>5688</b>

**Table 8**

AUC and fluorescence intensity remaining 3 hours after instillation

	AUC <sub>0-180min</sub> (% min)	Fluorescence intensity after 3h (%)
<b>A</b>	5567 ± 755	27.5 ± 12.4
<b>B</b>	3659 ± 151	17.7 ± 2.3
<b>C</b>	4325 ± 791	18.8 ± 11.9
<b>Control solution</b>	190 ± 51	0.7 ± 0.3

Easy, safe and reproducible administration as a liquid drop

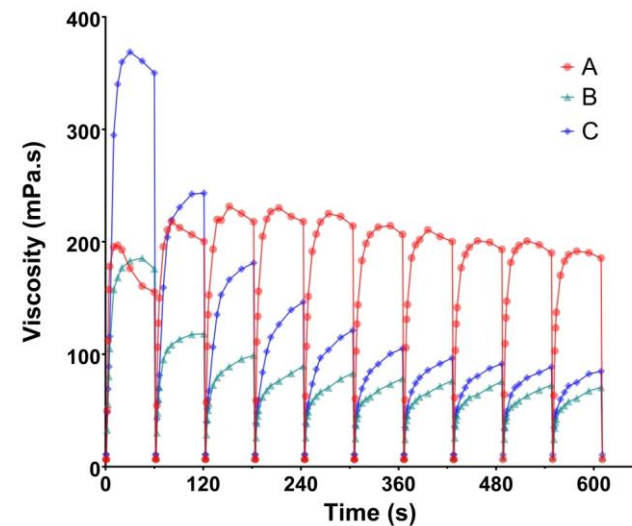


Gellan gum  
Hydroxyethylcellulose

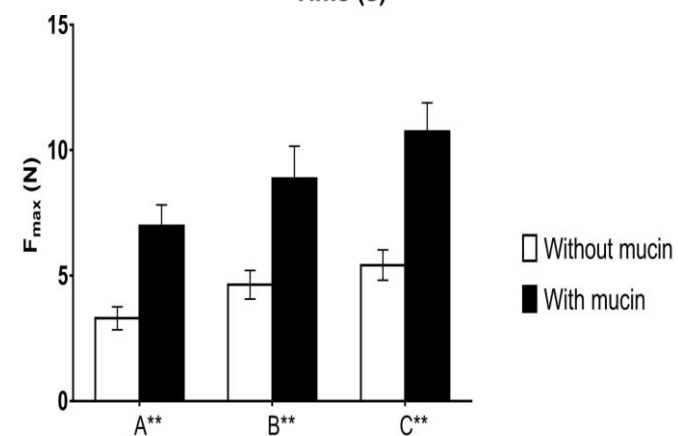
Sol to gel  
phase transition



Suitable rheological behavior



Mucoadhesion



Prolonged *in vivo* residence time

