

Acrylated Eudragit® E PO as a novel polymeric excipient with enhanced mucoadhesive properties for application in nasal drug delivery

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1 **Acrylated Eudragit[®] E PO as a novel polymeric excipient with enhanced**
2 **mucoadhesive properties for application in nasal drug delivery**

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11 **Abstract**

12 Eudragit[®] E PO (EPO) is a terpolymer based on *N,N*-dimethylaminoethyl methacrylate with
13 methylmethacrylate and butylmethacrylate, produced by Evonik Industries AG as a
14 pharmaceutical excipient. In this work, EPO was chemically modified through reaction with
15 acryloyl chloride. The successful modification of EPO was confirmed by FTIR, NMR-
16 spectroscopy, elemental and thermal analysis. The degree of acrylation was determined by
17 permanganatometric titration. The slug mucosal irritation test was used to demonstrate non-
18 irritant nature of EPO and its acrylated derivatives (AEPO). The mucoadhesive properties of
19 EPO and AEPO were evaluated using freshly excised sheep nasal mucosa and it was
20 demonstrated that acrylated polymers facilitated greater retention of sodium fluorescein on
21 mucosal surfaces compared to solution mixture of this dye solution with EPO as well as free
22 dye.

23 **Keywords:** Eudragit[®] E PO, mucoadhesion, acrylated polymers, slug mucosal irritation, nasal
24 drug delivery, nose-to-brain delivery

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28

29 1. Introduction

30 Drug delivery through mucosal routes of administration offers numerous advantages
31 such as improved bioavailability of active pharmaceutical ingredients, ease of therapy
32 application and in some cases the possibility of targeting particular organs (Andrews et al,
33 2009; Khutoryanskiy, 2011; Khutoryanskiy, 2014). In recent years, nasal administration has
34 gained a lot of interest due to the possibility for bypassing the blood-brain barrier and targeting
35 the brain directly through drug absorption via olfactory mucosa (Gänger et al, 2018; Pires et
36 al, 2018; Battaglia et al, 2018; Sonvico et al, 2018). This minimally invasive route to deliver
37 drugs directly to the brain could potentially offer new opportunities for treating various
38 neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's diseases
39 (Poovaiah et al, 2018).

40 Nasal cavity is an organ of human respiration, evolved to serve several functions,
41 including air conditioning and protection from various pathogenic microorganisms. The
42 protective function of the nasal cavity is achieved through mucociliary clearance, a
43 physiological mechanism that helps to trap dust and microorganisms present in the air within
44 the mucus blanket that is continuously produced and eventually moved into the digestive
45 system. This dynamic and sticky nature of the mucus layer ensures the prevention of potential
46 entry of microorganisms to the lungs (Washington et al, 2000; Hillery et al, 2001).

47 The mucus layer in the nasal cavity could act as a barrier that hampers the diffusion of
48 drugs to reach epithelial cells, which may reduce the efficiency of therapeutic agents
49 administered via intranasal route. One potential approach to improve the efficiency of drugs
50 administered via intranasal route is the use of mucoadhesive dosage forms, capable to ensure
51 longer residence in the nasal cavity (Ugwoke et al, 2005).

52 Cationic polymers are known to have excellent mucoadhesive properties due to their
53 ability to interact with negatively charged mucins via electrostatic attraction forces. Examples
54 of cationic polymers with proven mucoadhesive properties include chitosan (Sogias et al, 2008)
55 and some synthetic polymers of methacrylate nature with tertiary-amino- and quaternary
56 ammonium- functional groups (Keely et al, 2005; Fefelova et al, 2007). Some attempts were
57 reported to improve mucoadhesive properties of chitosan and other polymers through their
58 chemical functionalisation, for example, attachment of thiol- (Bernkop-Schnurch, 2004;
59 Bernkop-Schnurch, 2005), acrylate- (Davidovich-Pinhas et al, 2011; Shitrit et al, 2017),
60 methacrylate- (Kolawole et al, 2018), catechol- (Kim et al, 2015), maleimide- (Tonglairoom
61 et al, 2016; Shtenberg et al, 2017; Sahatsapan et al, 2018) and other groups (Ways et al, 2018).

62 Recently, we have reported the synthesis of mucoadhesive nanogels by polymerisation
63 of 2-dimethylamino)ethyl methacrylate in the presence of *N,N'*-methylene-bis-acrylamide as a
64 crosslinking agent (Brannigan et al, 2017). The resulting nanogels were subsequently modified
65 by the reaction with acryloyl chloride to introduce acrylated groups capable of forming
66 covalent linkages with thiols present in mucins under physiological conditions. These acrylated
67 nanogels exhibited superior mucoadhesive properties compared to the original poly((2-
68 dimethylamino)ethyl methacrylate) nanogels, when tested using bovine ocular mucosa.

69 Eudragit[®] E PO (EPO) is a linear cationic polymer manufactured and marketed by
70 Evonik Industries AG as a pharmaceutical excipient. EPO is a terpolymer that is composed of
71 *N,N*-dimethylaminoethyl methacrylate (DMAEMA), methylmethacrylate and
72 butylmethacrylate. The combination of these repeating units within this polymer ensures its
73 solubility in water only under acidic conditions (insoluble in the mouth), which is applicable
74 in the design of dosage forms with taste and odour masking. Once EPO coated dosage form
75 moves into the stomach the acidity of the gastric juice will ensure its quick dissolution and drug
76 release (Evonik technical notes, 2018). The ability of cationic EPO to form interpolyelectrolyte
77 complexes with various anionic polymers was also previously used in the design of solid
78 dosage forms for gastrointestinal delivery (Mustafin, 2011; Mustafin et al, 2011). Since EPO
79 is an approved pharmaceutical excipient and it does contain DMAEMA units in the terpolymer
80 structure, it opens up an interesting opportunity for its simple chemical modification using the
81 chemistry previously described by Brannigan and Khutoryanskiy (2017) with the aim to
82 prepare materials with enhanced mucoadhesive properties.

83 In the present study, we have modified EPO chemically through its reaction with
84 acryloyl chloride, which resulted in formation of acrylated polymers. The resulting products
85 were characterised using ¹H NMR and FTIR spectroscopy, thermal analysis,
86 permanganatometric titration and elemental analysis. The biocompatibility of parent EPO and
87 its acrylated derivatives were studied using slug mucosal irritation test. Liquid formulations
88 were prepared using EPO and its acrylated derivatives with sodium fluorescein as a model
89 compound and their retention on freshly excised sheep nasal mucosa was evaluated using
90 fluorescent microscopy.

91

92 **2. Experimental part**

93 **2.1. Materials**

94 Eudragit[®] E PO (EPO) with weight-average molecular weight 135,000 was received as a gift
95 from Evonik Röhm GmbH (Darmstadt, Germany). Acryloyl chloride was purchased from Alfa

96 Aesar (Lancashire, United Kingdom). Tetrahydrofuran anhydrous, deuterated chloroform
97 (CDCl_3), calcium chloride dehydrate, sodium chloride, potassium chloride, sodium fluorescein
98 were obtained from Sigma-Aldrich (Gillingham, United Kingdom). Sulfuric acid, potassium
99 permanganate and oxalic acid were received as a chemical standard from Uralhiminvest (UFA,
100 Russia). Dialysis membranes (Mw cut-off = 12-14 kDa) were purchased from Medicell
101 International Ltd (London, United Kingdom). Ultrapure water (Millipore, Bedford, MA,
102 U.S.A) was used for all aqueous solutions and all other chemicals were used as supplied
103 without modification.

104

105 **2.2. Methods**

106 **2.2.1. Synthesis of acrylated EPO**

107 Acrylated EPO was synthesized in a clean dry round-bottom flask with magnetic stirring.
108 Briefly, 2 g of EPO was dissolved in 100 mL tetrahydrofuran with permanent stirring at room
109 temperature. Acryloyl chloride was added dropwise to the resulting solutions with vigorous
110 stirring during 20 min at room temperature. In order to achieve 50 % and 25 % of acryloylation
111 2.88 mL and 1.44 mL of acryloyl chloride were used and the resulting samples are referred as
112 AEPO50 and AEPO25, respectively. The reaction mixtures were left for 72 hours at room
113 temperature with gentle stirring. The reaction mixtures were then transferred to a dialysis
114 membrane and dialyzed against deionised H_2O (5L deionised H_2O for 3 days changing the
115 dialysis media three times a day). The resulting products were freeze-dried using Heto Power
116 Dry LL 3000 freeze-drier (Thermo Electron Corporation).

117

118 **2.2.2. Preparation of artificial nasal fluid**

119 Artificial nasal fluid (ANF) was prepared according to the protocol described by Barbi et al.
120 (2014) with minor changes. Solution was prepared by dissolving 7.45 g NaCl, 1.29 g KCl and
121 0.32 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in 1000 mL deionised water. The solution was left stirring overnight at
122 room temperature. The artificial nasal fluid was kept at 37 °C in a water bath throughout the
123 experiments.

124 **2.2.3. Fourier transform infrared spectroscopy (ATR-FTIR)**

125 The ATR-FTIR spectra of EPO, AEPO25 and AEPO50 powders were recorded using a Nicolet
126 iS5 FTIR spectrometer (Thermo Scientific, U.S.A.) equipped with a DTGS detector. The
127 samples were directly mounted over the iD5 smart single bounce ZnSe ATR crystal and

128 scanned from 4000 to 400 cm^{-1} . OMNIC spectra software was used for the analysis of results.
129 Origin[®] software (Scientific Graphing & Analysis software, Version 7.5, OriginLab Corp.,
130 USA) was used for plotting graphs.

131

132 **2.2.4. ¹H nuclear magnetic resonance spectroscopy (¹H NMR)**

133 ¹H nuclear magnetic resonance spectra were recorded for EPO, AEPO25 and AEPO50 using a
134 DPX 400 MHz NMR spectrometer (Bruker, Germany). All samples were dissolved in
135 deuterated chloroform and transferred to 5 mm Norell tubes (Standard Series[™] 400 MHz
136 NMR). All chemical shifts were reported as δ in parts per million (ppm).

137 **2.2.5. Elemental analysis**

138 Elemental analysis was performed using Thermo Flash 2000 CHNS/O elemental analyzer
139 (Thermo Fisher Scientific, Paisley, UK). The vacuum dried samples (at 40 °C for 2 days) were
140 weighed into a crucible on a micro balance (Mettler Toledo XP6 Excellence Plus XP Micro
141 Balance, Switzerland). The crucibles with samples were packed and placed into the combustion
142 reactor via autosampler. Temperature in the oven was 900 °C, and a gas flow rate was 10
143 mL/min. Calibration of the instrument was performed with atropine standard (Thermo Fisher
144 Scientific, Paisley, UK). Eager Xperience Data Handling Software was used to analyze the
145 results.

146

147 **2.2.6. Thermal analysis**

148 Modulated differential scanning calorimetry (mDSC) experiments were carried out using a
149 Discovery DSC[™] (TA Instruments, New Castle, DE, U.S.A.), equipped with a refrigerated
150 cooling system (RCS90). These experiments were performed under dry nitrogen atmosphere
151 at 50 mL/min flow rate. Tzero[®] aluminum pans (TA Instruments, New Castle, DE, U.S.A.)
152 were used in mDSC experiments. Indium and n-octadecane were used as standards to calibrate
153 the DSC temperature scale. The modulation parameters used were: 2 °C/min heating rate, 40 s
154 period and 0.212 °C amplitude.

155 Thermogravimetric analysis (TGA) was performed using Discovery TGA[™] (TA Instruments,
156 New Castle, DE, U.S.A.). Samples (10-15 mg) heated in aluminum pans from 25 to 500 °C at
157 10 °C/min.

158 mDSC and TGA results were analysed using TRIOS™ software, version 3.1.5.3696 (TA
159 Instruments, New Castle, DE, U.S.A.).

160 **2.2.7. Back permanganometric titration**

161 Briefly, 30 mL of 0.2 N H₂SO₄ were placed in a conical flask with a Quickfit glass stopper.
162 Approximately, 50-100 mg of acrylated polymer were then added to H₂SO₄ and left stirring
163 until complete polymer dissolution. To this solution 10 mL of 0.1 N potassium permanganate
164 was added, followed with 4 mL of 0.1 N oxalic acid added from a microburette. These solutions
165 then were stirred and heated to 60 °C. This resulted in a change of solution colour from purple
166 to brown. The presence of small quantities of oxalic acid resulted in reduction of some MnO₄⁻
167 ions to Mn²⁺, which act as a catalyst and speed up the reaction of permanganate ions with oxalic
168 acid added subsequently. The reaction mixtures were then slowly titrated with 0.1 N oxalic
169 acid (4 drops per minute). Each titration was repeated in 5 times and the mean values were
170 calculated.

171 The degree of EPO acrylation was determined according to the formula:

$$172 \quad X = \frac{(V_1 - V_2) * K * T * 100 \%}{a},$$

173 where

174 V_1 —volume of oxalic acid, consumed in the control experiment, mL

175 V_2 —volume of oxalic acid, consumed in the experiment, mL

176 K —correction factor ($K=1.0000$),

177 T —a titre of oxalic acid to acrylated polymer ($T=1.2714$ mg/mL).

178 a —polymer sample weight, mg

179

180 **2.2.8. Slug mucosal irritation test**

181 *Limax flavus* slugs weighing 3-8 g were sourced locally in Harris Garden (Reading, UK). The
182 slug mucosal irritation test was conducted using slightly modified procedure reported by
183 Khutoryanskaya et al (2008). Solutions for slug mucosal irritation test were prepared by
184 dissolving 20 mg of EPO, AEPO25 and AEPO50 in 20 mL deionised water with pH adjusted
185 to 5.7 with 1 M NaOH or 1 M CH₃COOH solutions. Benzalkonium chloride (10 mg) was
186 dissolved in 100 mL deionized water and adjusted to pH=5.7 with 1 M NaOH to be used as a
187 positive control. Each slug was kept in 0.5-1 L glass beakers with a tissue paper moistened
188 with 20 mL ANF solution and left for two days at room temperature prior to experiments. Then

189 each slug was washed with 2 mL of ANF solution, excess of moisture on their body was
190 carefully removed with a tissue paper, and then they were put on Petri dishes with Whatman
191 filter paper moistened with 2 mL sample solutions. The samples included positive control (1
192 % benzalkonium chloride), negative control (ANF), as well as 1 mg/mL solutions of EPO,
193 AEPO25 and AEPO50. Slugs were kept in contact with the studied samples for 1 h, then they
194 were taken out, washed with 10 mL of ANF and carefully wiped with a tissue paper. All slugs
195 were then individually weighed before and after experiment using analytical balance. The
196 mucus production (MP) was determined as a slug body weight loss and calculated according
197 to the formula:

$$198 \quad \text{MP} = (m_b - m_a) / m_b \times 100 \%,$$

199 where m_a and m_b are the weights of a slug after and before each experiment, respectively.

200 All experiments were conducted using different slugs ($n=5$).

201

202 **2.2.9. Retention studies**

203 Experiments on retention of polymer formulations on nasal mucosal surfaces were conducted
204 using the fluorescent techniques developed and described by the Khutoryanskiy group earlier
205 (Irmukhametova et al, 2011; Štorha et al, 2013; Mun et al, 2016; Kaldybekov et al, 2018;
206 Ways et al, 2018). Sodium fluorescein solutions (0.001 mg/mL) were prepared in deionised
207 water and used as a medium for dissolving polymer samples. Then, 10 mg of EPO, AEPO25
208 or AEPO50 were dispersed in 10 mL of sodium fluorescein solutions and pH of these mixtures
209 was adjusted to pH=5.7. These dispersions were left for 24 h at room temperature with stirring
210 until complete dissolution and were protected from light by aluminium foil.

211 Sheep mucosal tissues are commonly used in the ex vivo studies on nasal drug delivery (Gavini
212 et al, 2008; Pund et al, 2013). Sheep heads were obtained from the local abattoir (Kazan,
213 Russia) and transported to the laboratory in a cold box (3-4 °C). The nasal septum tissue
214 containing mucosal lining (1.5×3 cm) was carefully dissected and extracted from each head
215 with scissors; it was washed with 1 mL of ANF and placed on a microscopy slide. All tissues
216 were used within 24 h after animal slaughter and each experiment was conducted in triplicate.

217 All experiments with retention of formulations on nasal mucosa were conducted at 37 °C in a
218 thermostat. Images of mucosal surfaces were taken using fluorescent microscope (Olympus
219 BX63), equipped with Alexa-488 filter. All images were of 4× magnification and were taken
220 at 512 ms exposure time and 1376-1038 pixels. Initially, fluorescence images of mucosal

221 tissues were recorded for each sample as a background fluorescence intensity. Then, 50 μ L
222 solutions of 1 mg/mL EPO, AEPO25, AEPO50 containing 0.001 mg/mL sodium fluorescein
223 were placed on mucosal surface and fluorescence images were recorded again. The mucosal
224 tissues were then transferred to a thermostat and irrigated with ANF using a syringe pump (0.43
225 mL/min). Fluorescence images of these mucosal tissues were taken at different time points.
226 ImageJ software was used for analysis of the resulting microscopy images by measuring the
227 pixel intensity after each wash. Results were presented as fluorescence intensity values versus
228 the volume of ANF. Background images were used to normalize the mean values by subtracting
229 the background fluorescence after each wash. The experiments were conducted in triplicate.
230 Solution of sodium fluorescein in deionised water (0.001 mg/mL) was used as a negative
231 control.

232

233 **2.2.10. Statistical analysis**

234 GraphPad Prism statistical analysis software (version 5.0) was used to analyze data acquired
235 during these experiments using one-way analysis of variance ANOVA and paired t-tests.
236 Results were presented as the mean \pm standard deviation and probability of $p < 0.05$ was
237 considered as significant. All measurements were reported in triplicate, unless otherwise
238 specified.

239

240 **Results and Discussion**

241 **Synthesis of acrylated EPO**

242 Previously, Brannigan and Khutoryanskiy (2017) have demonstrated that poly((2-
243 dimethylamino)ethyl methacrylate nanogels modified by reaction with acryloyl chloride
244 exhibited greater retention on ocular mucosa compared to unmodified polymers. Similar
245 modification is also possible for Eudragit[®] EPO, Eudragit[®] RL and Eudragit[®] S100 copolymers
246 containing 25 %, 10 % and 5 % of dimethylamino-groups, respectively (Mustafin, 2011;
247 Moustafine et al, 2011; Moustafine et al, 2013). To demonstrate this possibility Eudragit[®] EPO
248 was chosen for chemical modification using acryloylation according to the reaction scheme
249 shown in **Figure 1**. Two batches of acrylated EPO with 25 % and 50 % substitution of the
250 dimethylamino groups were synthesised (AEPO25 and AEPO50, respectively). (Figure 1 is
251 here).

252

253 **Characterisation of polymers using spectroscopic and thermal methods**

254 The successful modification of EPO was confirmed by FTIR-spectroscopy (**Figure 2**). The
255 FTIR-spectra of EPO, AEPO25 and AEPO50 show the characteristic bands for non-ionised
256 dimethylamino groups between 2770-2824 cm^{-1} (Moustafine et al, 2011), whose intensity
257 becomes weaker with acryloylation. However, the spectra of AEPO25 and AEPO50 also show
258 the presence of a new band at 1605 cm^{-1} indicating the attachment of additional carbonyl
259 groups to EPO. Moreover, the FTIR spectra of AEPO25 and AEPO50 demonstrate the bands
260 at 960-966 cm^{-1} and 989 cm^{-1} corresponding to quaternary ammonium groups (Moustafine et
261 al, 2012), which change depending on the degree of acryloylation.

262 (Figure 2 is here)

263 Additionally, we also used ^1H -NMR to confirm the chemical structure of modified polymers
264 (**Figure 3**). The spectra of AEPO25 and AEPO50 show the appearance of a new multiplet
265 between 5.98–6.44 ppm, which confirmed the presence of acryloyl groups. The intensity of
266 these peaks decreases due to the reduction in the degree of substitution of dimethylamino
267 groups. The appearance of a 5.98–6.44 ppm multiplet in the spectra of AEPO is generally
268 consistent with NMR characterisation of acrylated PDMAEMA previously reported by
269 Brannigan and Khutoryanskiy (2017), who used this method to determine the degree of
270 acryloylation. However, unfortunately, the complex mixture of signals resulting from different
271 repeating units of EPO leads to an overlap of many peaks; this made impossible to use ^1H -
272 NMR spectroscopy for quantitative determination of the degrees of acryloylation.

273 (Figure 3 is here)

274 Conjugation of acryloyl groups to EPO potentially should lead to some reduction in nitrogen
275 content in the samples, which could be studied using elemental analysis. According to **Table**
276 **1**, nitrogen content in EPO is 4.30 ± 0.12 wt %. AEPO25 and AEPO50 showed 3.60 ± 0.20 wt %
277 and 3.79 ± 0.24 wt % of nitrogen, respectively. This was a statistically significant reduction in
278 nitrogen content compared to unmodified EPO ($p < 0.05$); however, there was no significant
279 difference between AEPO25 and AEPO50 ($p > 0.05$). The lack of statistically significant
280 difference between AEPO25 and AEPO50 does not allow the calculation of the degree of
281 acryloylation based on elemental analysis data.

282 In the next step, the influence of the new acryloyl groups on the thermal behavior of EPO was
283 investigated. mDSC results demonstrate the presence of single glass transition events both in

284 EPO and AEPO samples (**Figure 4**). The parent EPO displayed the presence of a T_g at 49.5
285 °C, which is consistent with the previous reports (Moustafine et al, 2006; Menjoge and
286 Kulkarni, 2007; Claeys et al, 2013). A reduction of dimethyl amino groups content and their
287 partial replacement with quaternized nitrogen and acryloyl group resulted in copolymers with
288 substantial increase in glass transition temperatures: T_g of EPO increased from 49.5 °C to 94.5
289 °C and 81.9 °C for AEPO25 and AEPO50, respectively. The changes in T_g values of modified
290 polymers compared to parent material qualitatively indicate the successful derivatization of
291 EPO. Similar effects with increase in the T_g values upon reduction in the number of dimethyl
292 amino groups content in a terpolymer structure were previously reported by Claeys et al (2013).
293 A slightly unexpectedly lower T_g value of AEPO50 (81.9 °C) compared to AEPO25 (94.5 °C)
294 could potentially be related to the effects of quaternization, similarly to quaternized polymers
295 - Eudragit® RL and RS types, which are characterized by low T_g s (Eudragit® Application
296 Guidelines, 2012).

297 (Figure 4 is here).

298 TGA thermogram of parent EPO (**Figure 5**) showed the first weight loss event at 271.6–316.8
299 °C (29.6 %) possibly related to the removal of dimethylamino groups and formation of six-
300 membered cyclic anhydrides as proposed by Lin et al (1999). The second weight loss at 350.0-
301 475.0 °C (68.9 %) corresponds to a further complete decomposition of the terpolymer. The
302 acrylated derivatives of EPO show distinctly different thermal decomposition profiles
303 consisting of three degradation stages. In the case with AEPO, the first decomposition event
304 begins at around 40 °C and finishes at 200 °C resulting in a weight loss of 3.9 % and 4.0 % for
305 AEPO25 and AEPO50, respectively. This is likely related to the dehydration of a sample and
306 removal of some moisture. It is interesting to note that moisture content in the parent EPO was
307 practically not detectible, which may indicate that AEPO samples are more hydrophilic and
308 hygroscopic compared to EPO. The second decomposition stage in AEPO25 is observed at
309 200.0-337.5 °C (31.9 %), followed by the third weight loss at 337.5-475.0 °C (60.0 %).
310 AEPO50 displayed the second and third decomposition events at 200.0-337.5 °C (28.7 %) and
311 337.5-475.0 °C (62.6 %), respectively. Overall, the second degradation event of acrylated EPO
312 samples starts at 50-60 °C earlier compared to the first weight loss of parent EPO, but the final
313 decomposition stages of the synthesized samples occurred in the similar range (at 400-450 °C).
314 A decrease in the thermal stability of modified EPO is possibly related to the presence of

315 acryloyl groups, which are more chemically reactive and may undergo degradation at lower
316 temperatures.

317

318 (Figure 5 is here)

319

320 **Determination of the degrees of acryloylation**

321 Since it was not possible to determine the degrees of acryloylation of EPO using ^1H NMR (due
322 to the overlap of some characteristic signals in the spectrum) permanganatometric titration
323 technique was used. This was a back-titration method, where an excess of potassium
324 permanganate solution was used to oxidise unsaturated acryloyl groups in the polymer and
325 unreacted permanganate was titrated with oxalic acid. Oxalate reacts very slowly with
326 permanganate ions at room temperature, thus the solutions were titrated approximately at 60
327 °C to make this procedure more practical. In agreement with the manufacturer's specifications
328 (Eudragit® Application Guidelines, 2013) EPO contains 22.6 % of quaternary amino groups.
329 According to this data, the modified polymers (AEPO25 and AEPO50) should have 5.65 %
330 and 11.30 % of acryloyl groups, respectively, which was confirmed by permanganatometry
331 (**Table 1**).

332 (Table 1 is here).

333 **Toxicological Investigation**

334 In order to evaluate toxicological properties of modified polymers slug mucosal irritation test
335 was performed. This test was established and validated as a reliable method for preliminary
336 evaluation of irritation potential of chemicals to various mucosal membranes, including studies
337 of nasal irritation (Adriaens et al, 2001; Adriaens and Remon, 2002; Lenoir et al, 2011; Lenoir
338 et al, 2013). In this test, the first sign of good biocompatibility is colorless mucus, secreted by
339 slugs. Second, the amount of mucus production, which increased in stronger irritating
340 conditions (Khutoryanskaya et al, 2008; Adriaens et al, 1999; Adriaens and Remon, 2002). In
341 a positive control experiment (1% benzalkonium chloride) slugs suffered a severe irritation,
342 with 28.02 ± 2.70 % production of yellow mucus (**Figure 6**), which is consistent with the
343 previous reports (Khutoryanskaya et al, 2008). The slugs exposed to solutions with EPO
344 produce 4.55 ± 2.26 % colorless mucus, confirming non-irritating nature of this polymer. The
345 mucus production values recorded for AEPO25 and AEPO50 were 3.38 ± 1.37 and in 4.40 ± 2.29
346 %, respectively. No significant difference was observed between mucus production values

347 recorded for negative control, EPO, AEPO25 and AEPO50 ($p < 0.05$), indicating non-irritating
348 nature of modified EPO.

349

350 (Figure 6 is here).

351

352 **Mucoadhesion studies**

353 The retention studies with fluorescent detection of different mucoadhesive formulations on
354 different surfaces were described in previous publications (Irmukhametova et al, 2011; Storha
355 et al, 2013; Cook et al, 2015; Mun et al, 2016; Kaldybekov et al, 2018; Ways et al, 2018). This
356 flow-through test evaluating the retention of formulations on mucosal surfaces usually gives
357 good correlation with other methods (e.g. tensile studies) used to characterize mucoadhesive
358 properties (Kolawole et al, 2019). In the present work the retention properties of EPO,
359 AEPO25, AEPO50 solutions containing sodium fluorescein were studied on freshly excised
360 sheep nasal mucosa, irrigated with artificial nasal fluid (ANF). Fluorescent images of these
361 samples are presented in **Figure 7**.

362 (Figure 7 is here).

363 **Figure 8** shows the retention of EPO, AEPO25, AEPO50 solutions containing sodium
364 fluorescein on sheep nasal mucosa after analysis of the fluorescent images. It was established
365 that parent EPO exhibits mucoadhesive properties and retains the dye on mucosal surface better
366 compared to free sodium fluorescein. Approximately, 3.19 ± 1.40 % of fluorescence remained
367 on nasal mucosa after 60 min washing. This good retention of the dye mediated with EPO on
368 mucosal surfaces is likely to be related to its cationic nature that ensures electrostatic attraction
369 of this polymer to negatively charged mucosal surface. AEPO25 and AEPO50 facilitated even
370 greater retention of the dye on nasal mucosa compared to EPO: their retention after 60 mins of
371 washing is 6.34 ± 1.01 and 10.89 ± 3.48 %, respectively. This difference is statistically significant
372 ($p < 0.05$), demonstrating superior mucoadhesive performance of acrylated polymers.

373 (Figure 8 is here)

374 **Conclusions**

375 This study demonstrated successful chemical modification of Eudragit® E PO through reaction
376 with acryloyl chloride resulting in acrylated polymers. The structure and physicochemical
377 properties of these polymers were studied using FTIR and ^1H NMR spectroscopies, mDSC and

378 TGA thermal methods as well as by back permanganatometric titration. The slug mucosal
379 irritation test was used to demonstrate non-irritant nature of modified polymers. Acrylated
380 polymers exhibited superior mucoadhesive properties on nasal mucosa tissue compared to
381 parent Eudragit® E PO. Acrylated EPO can potentially be used as a mucoadhesive material for
382 formulation of dosage forms for transmucosal drug delivery. To the best of our knowledge, this
383 is the first study reporting the chemical modification of EPO with the aim to enhance its
384 mucoadhesive properties.

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393 **References**

394 Adriaens E., Dierckens K., Bauters T.G., Nelis H.J., van Goethem F., Vanparys P., Remon
395 J.P., 2001. The mucosal toxicity of different benzalkonium chloride analogues evaluated
396 with an alternative test using slugs. *Pharm. Res.* 18, 937– 942.

397 Adriaens E., Remon J. P., 2002. Evaluation of an Alternative Mucosal Irritation Test Using
398 Slugs. *Toxicol. Appl. Pharmacol.* 182, 169–175.

399 Andrews G., Laverty T.P., Jones D., 2009. Mucoadhesive Polymeric Platforms for
400 Controlled Drug Delivery. *Eur. J. Pharm. Biopharm.* 71, 505-518.

401 Barbi Mda S., Carvalho F.C., Kiill C.P., Barud Hda S., Santagneli S.H., Ribeiro S.J.
402 Gremião M.P., 2014. Preparation and Characterization of Chitosan Nanoparticles for
403 Zidovudine Nasal Delivery. *J. Nanosci. Nanotechnol.* 14, 1–10.

404 Battaglia L., Panciani P.P., Muntoni E., Capucchio M.T., Biasibetti E., De Bonis P.,
405 Mioletti S., Fontanella M., Swaminathan S., 2018. Lipid nanoparticles for intranasal
406 administration: application to nose-to-brain delivery. *Exp. Opin. Drug Deliv.* 15, 369-378.

407 Bernkop-Schnürch A., 2005. Thiomers: A new generation of mucoadhesive polymers.
408 *Adv. Drug Deliv. Rev.* 57, 1569-1582.

409 Bernkop-Schnürch A., Hornof M., Guggi D., 2004. Thiolated chitosans. *Eur. J. Pharm.*
410 *Biopharm.* 57, 9-17.

411 Brannigan R.P., Khutoryanskiy V.V., 2017. Synthesis and evaluation of mucoadhesive
412 acryloyl-acrylated PDMAEMA nanogels for ocular drug delivery. *Colloids and Surfaces*
413 *B: Biointerfaces.* 155, 538–543.

414 Claeys B., De Coen R., Geest B.G., de la Rosa V.R., Hoogenboom R., Carleer R.,
415 Adriaensens P., Remon J.P., Vervaeet C., 2013. Structural modifications of
416 polymethacrylates: Impact on thermal behavior and release characteristics of glassy solid
417 solutions. *Eur. J. Pharm. Biopharm.* 85, 1206-1214.

418 Cook M.T., Schmidt S.A., Lee E., Samprasit W., Opanasopit P., Khutoryanskiy V.V.,
419 2015. Synthesis of mucoadhesive thiol-bearing microgels from 2-(acetylthio)ethyl-
420 acrylate and 2-hydroxyethylmethacrylate: novel drug delivery systems for che-
421 motherapeutic agents to the bladder. *J. Mater. Chem. B* 3, 6599–6604.

422 Davidovich-Pinhas M., Bianco-Peled H., 2011. Alginate-PEGAc: a new mucoadhesive
423 polymer. *Acta Biomater.* 7, 625-633.

424 Dhondt M.M.M., Adriaens E., Van Roey J., Remon J.P., 2005. The evaluation of the local
425 tolerance of vaginal formulations containing dapivirine using the Slug Mucosal
426 Irritationtest and the rabbit vaginal irritationtest. *Eur J. Pharm. Biopharm.* 60, 419–425.

427 Dittgen M., Durrani M., Lehmann K., 1997. Acrylic polymers. A review of pharmaceutical
428 applications. *STP Pharm. Sci.* 7, 403–437.

429 Evonik Pharma Polymers. Eudragit® Application Guidelines. 12th Edition, Evonik Pharma
430 Polymers, Darmstadt. 2013, 44-111.

431 Evonik technical notes, EUDRAGIT® EPO ReadyMix - Evonik Industries,
432 <https://healthcare.evonik.com/sites/lists/NC/DocumentsHC/EUDRAGIT-E-PO>
433 ReadyMix-EN.pdf, accessed 07 Dec 2018

434 Fefelova N.A., Nurkeeva Z.S., Mun G.A., Khutoryanskiy V.V., 2007. Mucoadhesive
435 interactions of amphiphilic cationic copolymers based on [2-(methacryloyloxy) ethyl]
436 trimethylammonium chloride. *Int. J. Pharm.*, 339, 25-32.

437 Gavini E., Rassa G., Muzzarelli C., Cossu M., Giunchedi P., 2008. Spray-dried
438 microspheres based on methylpyrrolidinone chitosan as new carrier for nasal
439 administration of metoclopramide. *Eur. J. Pharm. Biopharm.*, 68, 245-252.

440 Gänger S., Schindowski K., 2018. Tailoring Formulations for Intranasal Nose-to-Brain
441 Delivery: A Review on Architecture, Physico-Chemical Characteristics and Mucociliary
442 Clearance of the Nasal Olfactory Mucosa. *Pharmaceutics*. 10(3), 116.

443 Hillery A.M., Lloyd A.W., Swarbrick J., 2001. *Drug Delivery and Targeting: For*
444 *Pharmacists and Pharmaceutical Scientists*. CRC Press, 496 p.

445 Irmukhametova G.S., Mun G.A., Khutoryanskiy V.V., 2011. Thiolated mucoadhesive and
446 PEGylated nonmucoadhesive organosilica nanoparticles from 3-
447 mercaptopropyltrimethoxysilane. *Langmuir* 27, 9551-9555.

448 Kaldybekov D.B., Tonglairoum P., Opanasopit P., Khutoryanskiy V.V., 2018.
449 Mucoadhesive maleimide-functionalised liposomes for drug delivery to urinary bladder.
450 *Eur. J. Pharm. Sci.* 111, 83-90.

451 Keely S., Rullay A., Wilson C., Carmichael A., Carrington S., Corfield A., Haddleton
452 D.M., Brayden D.J., 2005. In vitro and ex vivo intestinal tissue models to measure
453 mucoadhesion of poly (methacrylate) and N-trimethylated chitosan polymers. *Pharm. Res.*
454 22, 38-49.

455 Khutoryanskaya O.V., Mayeva Z.A., Mun G.A., Khutoryanskiy V.V., 2008. Designing
456 Temperature-Responsive Biocompatible Copolymers and Hydrogels Based on 2-
457 Hydroxyethyl(meth)acrylates. *Biomacromolecules* 9, 3353–3361.

458 Khutoryanskiy V.V., 2011. Advances in Mucoadhesion and mucoadhesive polymers.
459 *Macromol.Biosci.*11, 748-764.

460 Khutoryanskiy V.V., 2018. Beyond PEGylation: alternative surface-modification of na-
461 noparticles with mucus-inert biomaterials. *Adv. Drug Deliv. Rev.* 124, 140–149.

462 Khutoryanskiy V.V., 2014. Mucoadhesive materials and drug delivery systems. Wiley and
463 Sons.

464 Kim K., Ji K.K., Ryu J.H., Lee H., 2015. Chitosan-catechol: A polymer with long-lasting
465 mucoadhesive properties. *Biomaterials*, 52, 161-170.

466 Kolawole O.M., Lau W.M., Khutoryanskiy V.V., 2018. Methacrylated chitosan as a
467 polymer with enhanced mucoadhesive properties for transmucosal drug delivery. *Int. J.*
468 *Pharm.*, 550, 123-129.

469 Kolawole O.M., Lau W.-M., Khutoryanskiy V.V., 2019. Chitosan / β -glycerophosphate in
470 situ gelling mucoadhesive systems for intravesical delivery of mitomycin-C. *Int. J. Pharm.*
471 *X*, 1, 100007.

472 Lenoir J., Adriaens E., Remon J.P., 2011. New aspects of the Slug Mucosal Irritation
473 assay: predicting nasal stinging, itching and burning sensations. *J. Appl. Toxicol.*, 31, 640-
474 648.

475 Lenoir J., Bachert C., Remon J.P., Adriaens E., 2013. The Slug Mucosal Irritation (SMI)
476 assay: a tool for the evaluation of nasal discomfort. *Toxicol in Vitro*, 27, 1954-1961.

477 Lin S.Y., Yu H., Li M.J., 1999. Formation of six-membered cyclic anhydrides by thermally
478 induced intramolecular ester condensation in Eudragit[®] E film. *Polymer* 40, 3589-3593.

479 Menjoge A.R., Kulkarni M.G., 2007. Mechanistic investigation of phase behavior in
480 Eudragit[®] E blends. *Int. J. Pharm.* 343, 106-121.

481 Moustafine R.I., Zaharov I.M., Kemenova V.A., 2006. Physicochemical characterization
482 and drug release properties of Eudragit[®] EPO/Eudragit[®] L100-55 interpolyelectrolyte
483 complexes. *Eur. J. Pharm. Biopharm.* 63, 26–36.

484 Moustafine R.I., Bobyleva V.L., Bukhovets A.V., Garipova V.R., Kabanova T.V.,
485 Kemenova V.A., Van den Mooter G., 2011. Structural transformations during swelling of
486 polycomplex matrices based on countercharged (meth)acrylate copolymers (Eudragit[®]
487 EPO/Eudragit[®] L 100-55). *J. Pharm. Sci.* 100, 874-885.

488 Moustafine R.I., Bodrov A.V., Kemenova V.A., Rombaut P., Van den Mooter G., 2012.
489 Drug release modification by interpolymer interaction between countercharged types of
490 Eudragit[®] RL 30D and Eudragit[®] FS 30D in double-layer films. *Int. J. Pharm.* 439, 17-21

491 Moustafine R.I., Bukhovets A.V., Sitenkov A.Y., Kemenova V.A., Rombaut P., Van den
492 Mooter G., 2013. Eudragit® EPO as a complementary material for designing oral drug
493 delivery systems with controlled release properties: comparative evaluation of new
494 interpolyelectrolyte complexes with countercharged Eudragit® L100 copolymers. *Mol.*
495 *Pharm.*10, 2630–2641.

496 Mun E.A., Williams A.C., Khutoryanskiy V.V., 2016. Adhesion of thiolated silica
497 nanoparticles to urinary bladder mucosa: effects of PEGylation, thiol content and particle
498 size. *Int. J. Pharm.* 512, 32–38.

499 Mustafin R.I. 2011. Interpolymer combinations of chemically complementary grades of
500 Eudragit copolymers: A new direction in the design of peroral solid dosage forms of drug
501 delivery systems with controlled release (review). *Pharm. Chem. J.* 45, 285-295.

502 Mustafin R.I., Kabanova T. V., Semina I. I., Bukhovets A. V., Garipova V. R., Shilovskaya
503 E. V., Nasibullin Sh. F., Sitenkov A. Yu., Kazakova R. R., Kemenova V. A. 2011.
504 Biopharmaceutical assessment of a polycomplex matrix system based on Carbomer 940
505 and Eudragit EPO for colon-specific drug delivery. *Pharm. Chem. J.* 45, 491–494.

506 Pires P.C., Santos A.O., 2018. Nanosystems in nose-to-brain drug delivery: A review of
507 non-clinical brain targeting studies. *J. Control. Release*, 270, 89-100.

508 Poovaiah N., Davoudi Z., Peng H., Schlichtmann B., Mallapragada S., Narasimhan B.,
509 Wang Q., 2018. Treatment of neurodegenerative disorders through the blood-brain barrier
510 using nanocarriers. *Nanoscale*, 10, 16962-16983.

511 Pund S., Rasve G., Borade G., 2013. Ex vivo permeation characteristics of venlafaxine
512 through sheep nasal mucosa. *Eur. J. Pharm. Sci.*, 48, 195-201.

513 Sahatsapan N., Rojanarata T., Ngawhirunpat T., Opanasopit P., Tonglairoom P., 2018. 6-
514 Maleimidohexanoic acid-grafted chitosan: A new generation mucoadhesive polymer.
515 *Carb. Polym.*, 202, 258-264.

516 Shitrit Y., Bianco-Peled H., 2017. Acrylated chitosan for mucoadhesive drug delivery
517 systems. *Int. J. Pharm.*, 517, 247-255.

518 Sogias I.A., Williams A.C., Khutoryanskiy V.V., 2008. Why is chitosan mucoadhesive?
519 *Biomacromolecules*, 9, 1837-1842.

520 Sonvico F., Clementino A., Buttini F., Colombo G., Pescina S., Stanisguaski Guterres S.,
521 Raffin Pohlmann A., Nicoli S., 2018. Surface-Modified Nanocarriers for Nose-to-Brain
522 Delivery: From Bioadhesion to Targeting Pharmaceuticals. *Pharmaceutics*, 10(1), 34.

523 Shtenberg Y., Goldfeder M., Schroeder A., Bianco-Peled H., 2017. Alginate modified with
524 maleimide-terminated PEG as drug carriers with enhanced mucoadhesion. *Carbohydrate*
525 *Polym.*, 175, 337-346.

526 Štorha A., Mun E.A., Khutoryanskiy V.V., 2013. Synthesis of thiolated and acrylated
527 nanoparticles using thiol-ene click chemistry: towards novel mucoadhesive materials for
528 drug delivery. *RSC Adv.* 3, 12275–12279.

529 Tonglairoum P., Brannigan R.P., Opanasopit P., Khutoryanskiy V.V., 2016. Maleimide-
530 bearing nanogels as novel mucoadhesive materials for drug delivery, *J. Mater. Chem. B* 4
531 (40), 6581-6587.

532 Ugwoke M.I., Agu R.U., Verbeke N., Kinget R., 2005. Nasal mucoadhesive drug delivery:
533 Background, applications, trends and future perspectives. *Adv. Drug Deliv. Rev.* 57, 1640-
534 1665.

535 Washington N., Washington C., Wilson C., 2000. *Physiological Pharmaceutics: Barriers*
536 *to Drug Absorption.* CRC Press, 328 p.

537 Ways T.M., Lau W.M., Khutoryanskiy V.V., 2018. Chitosan and its derivatives for
538 application in mucoadhesive drug delivery systems, *Polymers* 10 (3), 267.

539 Ways T.M.M., Lau W.M., Ng K. W., Khutoryanskiy V.V., 2018. Synthesis of thiolated,
540 PEGylated and POZylated silica nanoparticles and evaluation of their retention on rat
541 intestinal mucosa in vitro, *Eur. J. Pharm. Sci.* 122, 230–238.

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544 Tables:

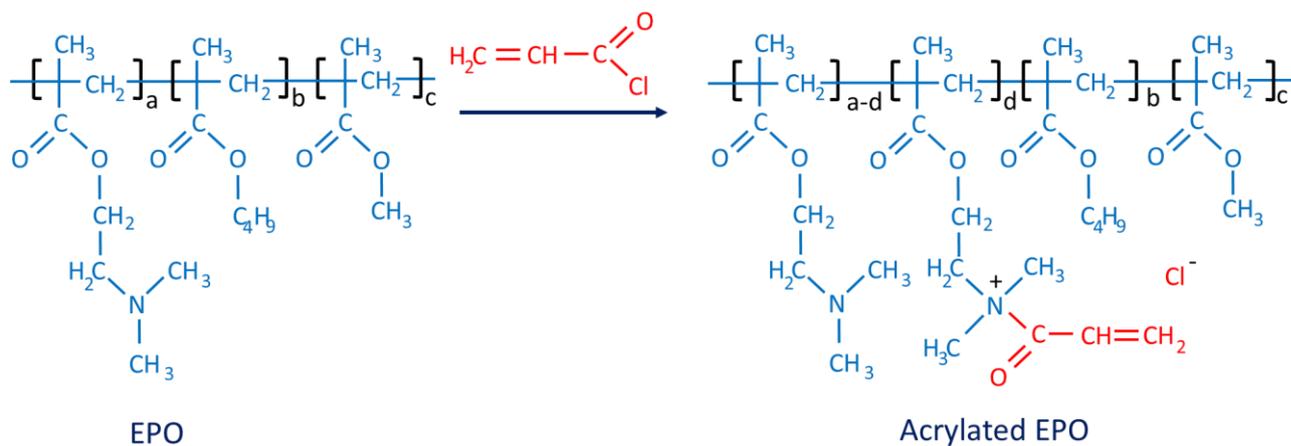
545

546 **Table 1. Quantitation and physicochemical properties of acrylated EPO**

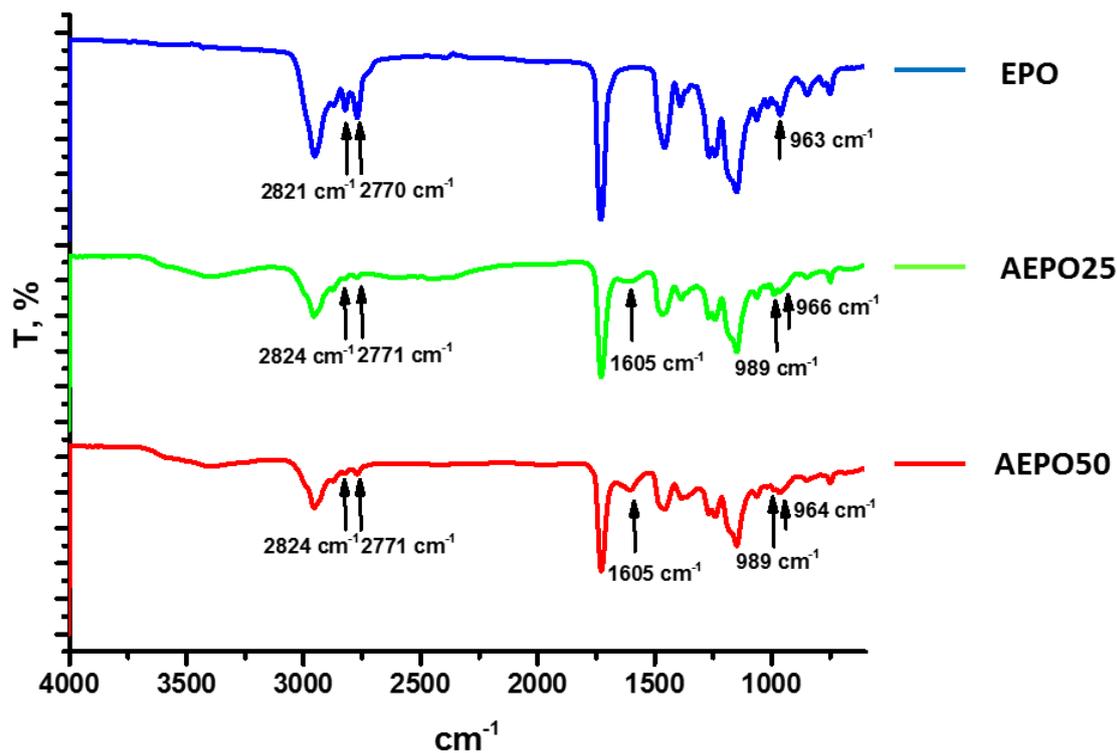
Sample	Acryloyl chloride (mL)	Content of acryloyl groups ^a (%)	Degree of acryloylation (%) ^b	Nitrogen content (%) ^c
EPO	0	0	0	4.30±0.12
AEPO25	1.44	5.7±0.4	25.1±1.6	3.60±0.20
AEPO50	2.88	11.3±0.2	50.0±0.8	3.79±0.24

547 ^{a,b}Determined by permanganatometric titration (n=5, $p<0.05$).^c Determined by elemental
548 analysis.

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552 **Figure 1.** Synthesis of acrylated EPO (25 °C, 72 h).553 **Figure 2.** FTIR spectra of EPO, AEPO25 and AEPO50.

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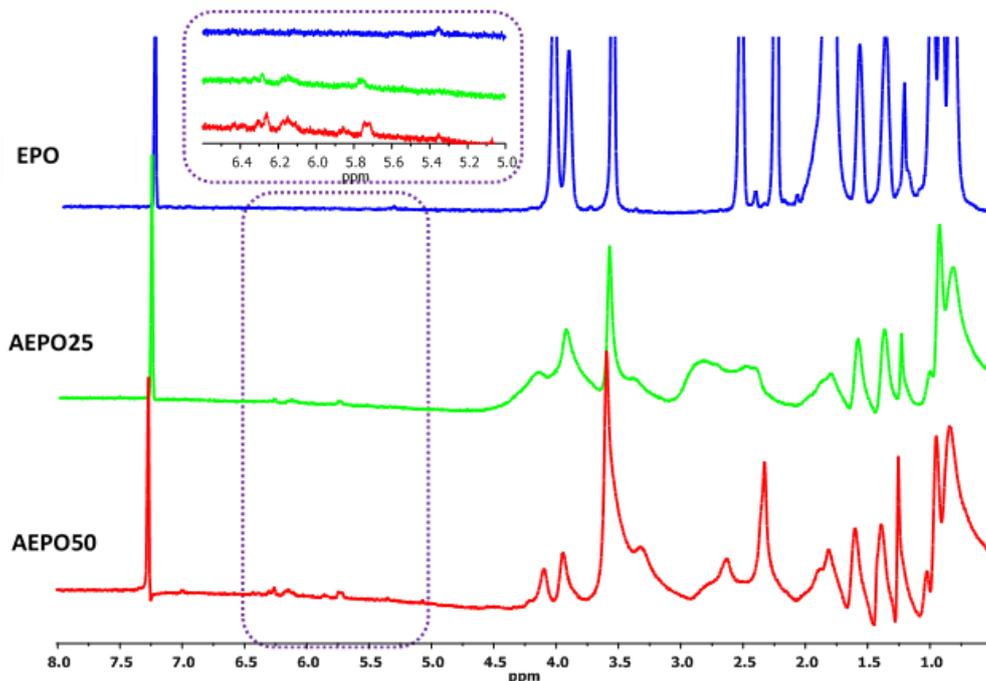
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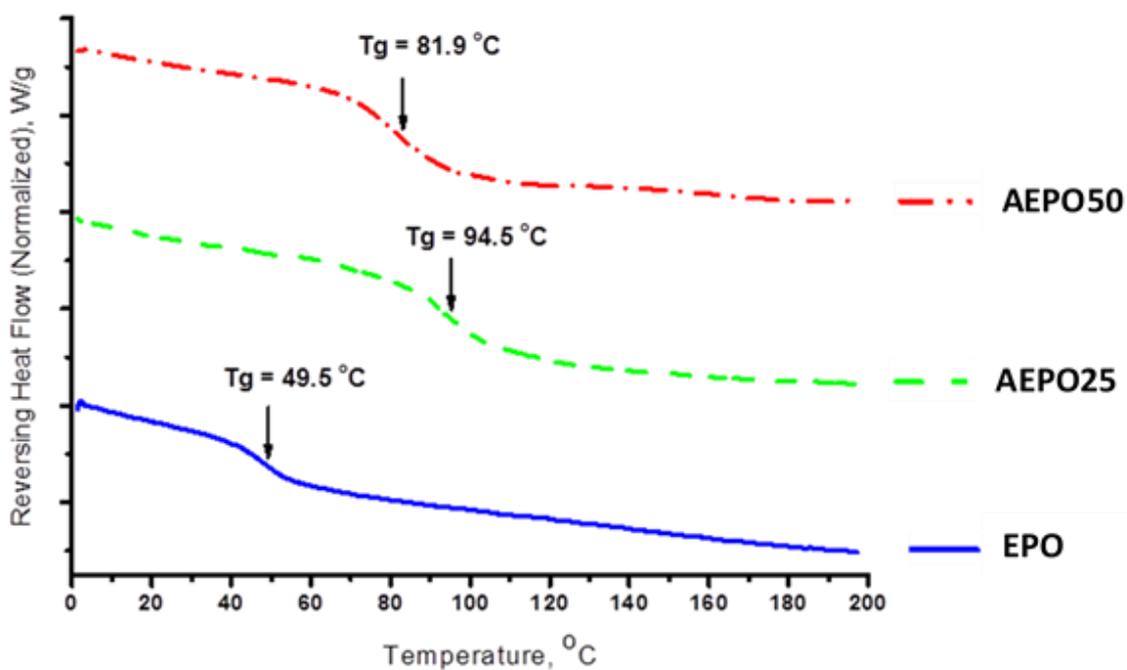
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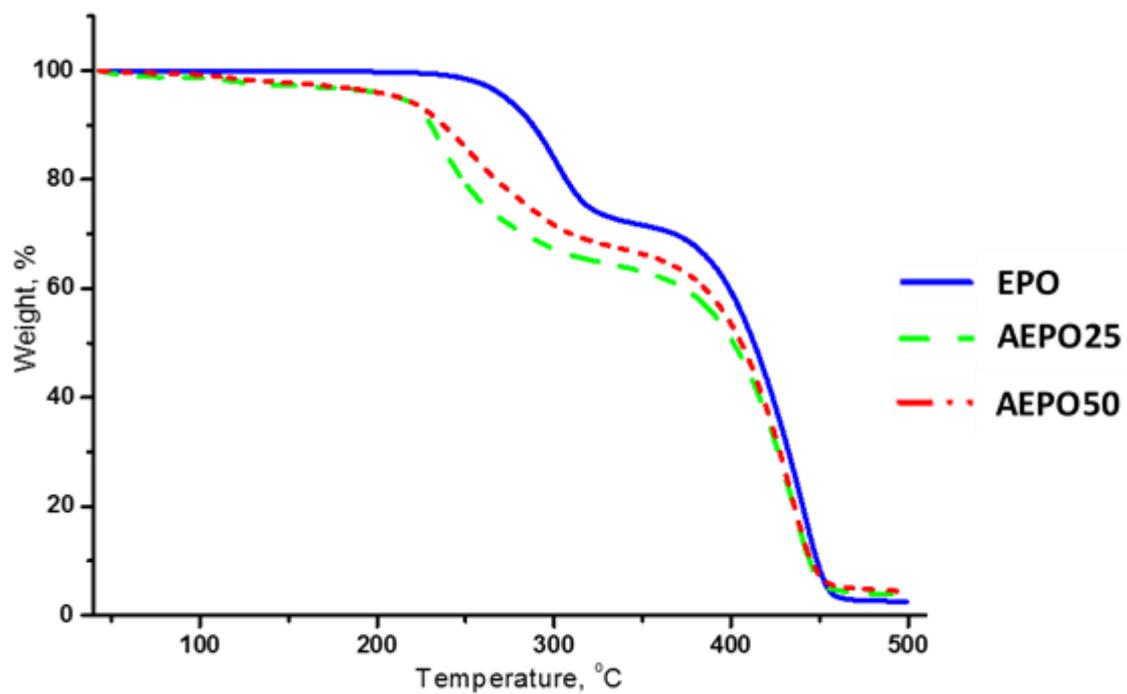
564 **Figure 3.** ^1H NMR spectra of EPO, AEPO25 and AEPO50 (CDCl_3 , 400 MHz).

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567 **Figure 4.** mDSC thermograms of EPO, AEPO25 and AEPO50.

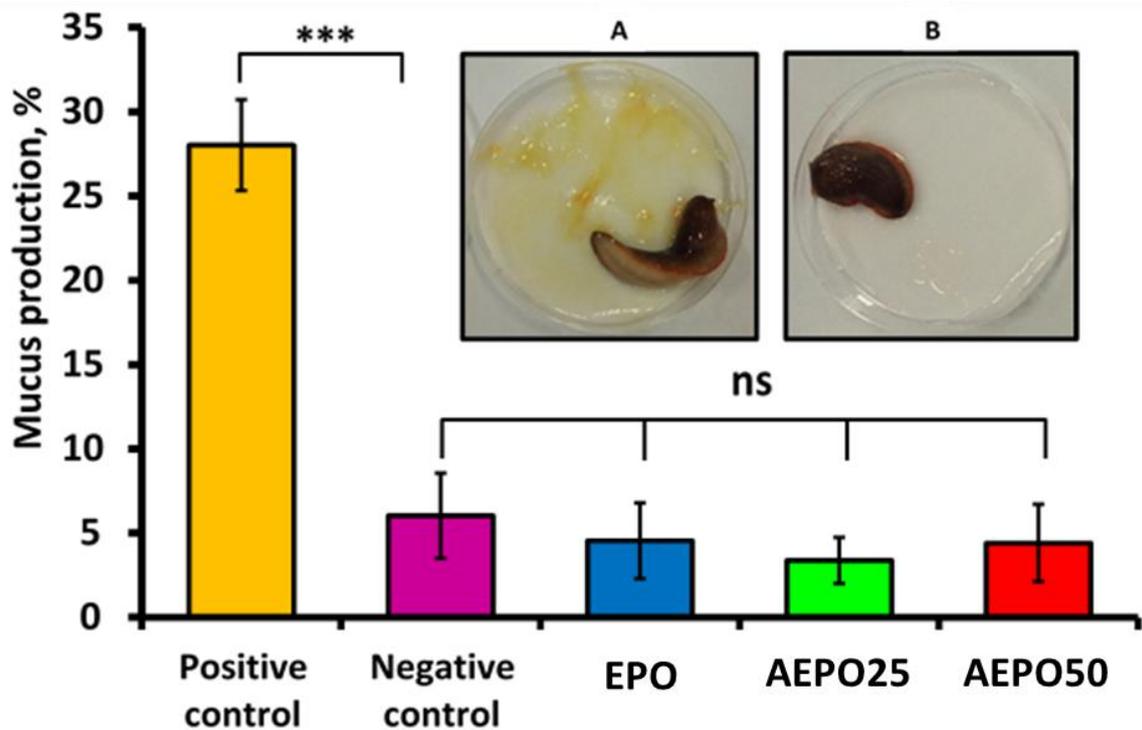


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569 **Figure 5.** TGA thermograms of EPO, AEPO25 and AEPO50.

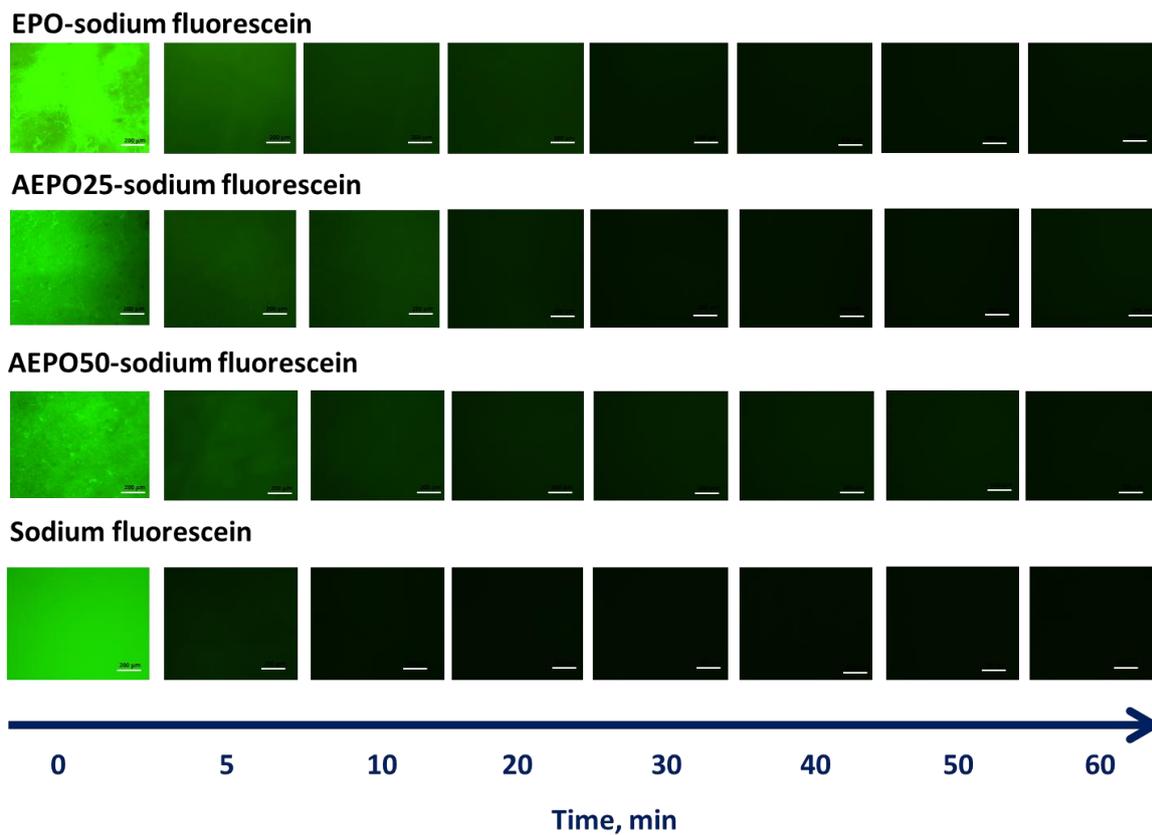
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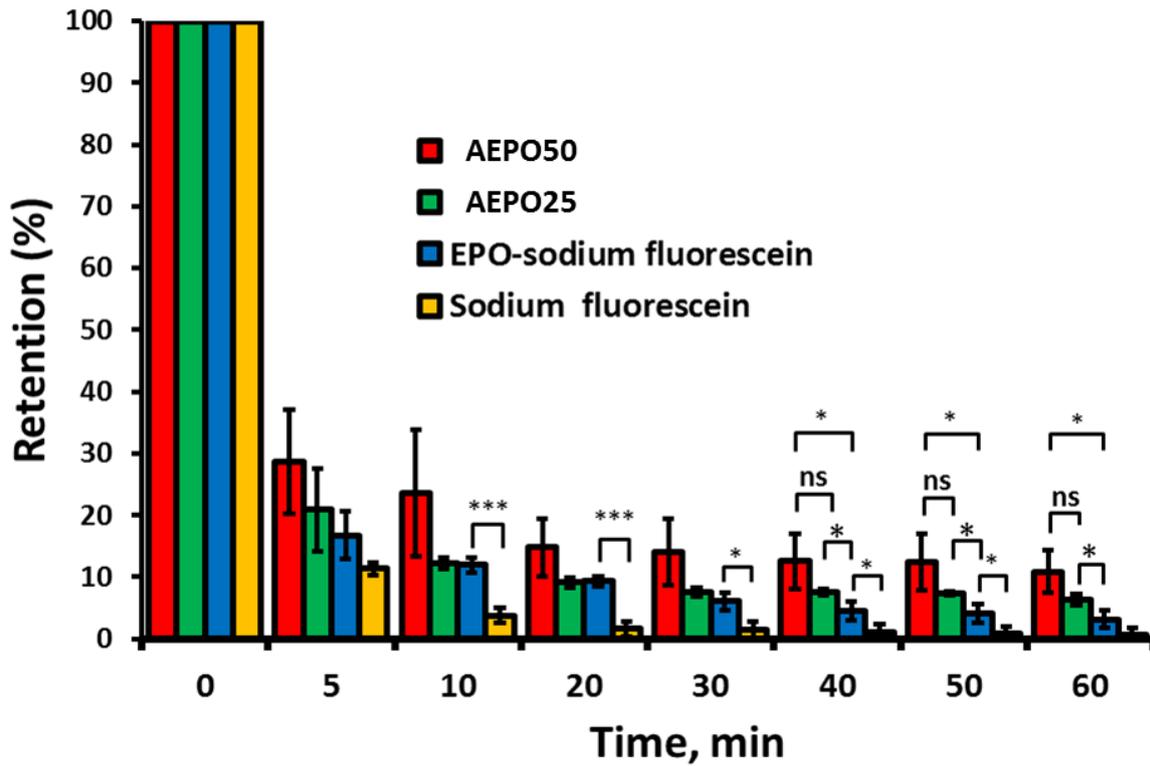
572

573 **Figure 6.** Mucus production by *Limax flavus* slugs in response to the contact with solutions of
 574 1 wt % benzalkonium chloride (positive control), ANF (negative control), 0.1 wt % EPO,
 575 AEPO25 and AEPO50 (pH=5.7). Data are expressed as mean \pm standard deviation (n=5). Inset:
 576 exemplar images of *Limax flavus* slugs in positive (A) and negative (B) controls experiment.



577

578 **Figure 7.** Fluorescent images showing retention of 1 mg/mL EPO, AEPO25, AEPO50
 579 solutions with 0.001 mg/mL sodium fluorescein, and pure 0.001 mg/mL sodium fluorescein
 580 solution on sheep nasal mucosa as washed with ANF. Scale bar is 200 μm .



582 **Figure 8.** Retention of 1 mg/mL EPO, AEPO25, AEPO50 solutions with 0.001 mg/mL sodium
 583 fluorescein and pure 0.001 mg/mL sodium fluorescein solution on sheep nasal mucosa as
 584 washed with different volumes of ANF (pH=5.7, n=3, mean \pm SD, “*” represents $p < 0.05$).

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