



# *A mucosa-mimetic material for the mucoadhesion testing of thermogelling semi-solids*

Article

Accepted Version

Creative Commons: Attribution-Noncommercial-No Derivative Works 4.0

da Silva, J. B., Khutoryanskiy, V. V., Bruschi, M. L. and Cook, M. T. (2017) A mucosa-mimetic material for the mucoadhesion testing of thermogelling semi-solids. *International Journal of Pharmaceutics*, 528 (1-2). pp. 586-594. ISSN 0378-5173 doi: <https://doi.org/10.1016/j.ijpharm.2017.06.025> Available at <http://centaur.reading.ac.uk/71723/>

It is advisable to refer to the publisher's version if you intend to cite from the work.

Published version at: <http://www.sciencedirect.com/science/article/pii/S0378517317305331>

To link to this article DOI: <http://dx.doi.org/10.1016/j.ijpharm.2017.06.025>

Publisher: Elsevier

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

[www.reading.ac.uk/centaur](http://www.reading.ac.uk/centaur)

## **CentAUR**

Central Archive at the University of Reading

Reading's research outputs online

1 **A mucosa-mimetic material for the mucoadhesion testing of thermogelling semi-**  
2 **solids**

3 Jessica Bassi da Silva;<sup>a</sup> Vitaliy V. Khutoryanskiy;<sup>b</sup> Marcos L. Bruschi;<sup>a</sup> Michael T.  
4 Cook<sup>c</sup>

5

6 <sup>a</sup>Postgraduate Program in Pharmaceutical Sciences, Laboratory of Research and  
7 Development of Drug Delivery Systems, Department of Pharmacy, State University of  
8 Maringá, Maringá, PR, Brazil.

9 <sup>b</sup>School of Pharmacy, University of Reading, Whiteknights, Reading, RG6 6AD, United  
10 Kingdom

11 <sup>c</sup>Research Centre in Topical Drug Delivery and Toxicology, Department of Pharmacy,  
12 Pharmacology, and Postgraduate Medicine, University of Hertfordshire, Hatfield, AL10  
13 9AB, United Kingdom

14

15 Abstract

16

17 Mucosa-mimetic materials are synthetic substrates which aim to replace animal tissue in  
18 mucoadhesion experiments. One potential mucosa-mimetic material is a hydrogel  
19 comprised of N-acryloyl-D-glucosamine and 2-hydroxyethylmethacrylate, which has  
20 been investigated as a surrogate for animal mucosae in the mucoadhesion testing of  
21 tablets and solution formulations. This study aims to investigate the efficacy of this  
22 mucosa-mimetic material in the testing of thermogelling semi-solid formulations, which  
23 transition from solution to gel upon warming. Two methods for assessing mucoadhesion  
24 have been used; tensile testing and a flow-through system, which allow for investigation  
25 under dramatically different conditions. It was found that the mucosa-mimetic material

1 was a good surrogate for buccal mucosa using both testing methods. This material may  
2 be used to replace animal tissue in these experiments, potentially reducing the number  
3 of laboratory animals used in studies of this type.

4

## 5 1. Introduction

6 Many factors can be considered in the development of drug delivery systems. For  
7 topical drug delivery, a key parameter for evaluating dosage forms is their bioadhesion  
8 and, in the case of mucosal membranes, mucoadhesion; the adhesive interaction  
9 between a material and a mucosal membrane (Khutoryanskiy, 2011; Smart, 2005).  
10 Mucoadhesion can improve the retention of the formulation on the mucosal surface,  
11 increasing the retention time at the site of action, improving drug absorption, and thus  
12 bioavailability (Khutoryanskiy, 2014). Novel mucoadhesive systems are of interest in  
13 oral transmucosal (Khan et al., 2016; Shojaei and Li, 1997), nasal (Nakamura et al.,  
14 1996; Ugwoke et al., 2005), ocular (Hornof et al., 2003; Ludwig, 2005), vaginal  
15 (Andrews et al., 2009; Friedl et al., 2013), and rectal drug delivery (Deđim et al., 2005),  
16 and these systems typically require evaluation on *ex vivo* animal mucosa (Cook and  
17 Khutoryanskiy, 2015; Ivarsson and Wahlgren, 2012).

18

19 One class of mucoadhesive formulations which is of interest is “thermogelling”  
20 materials, which transition from solution to gel upon warming from room to body  
21 temperature (de Araújo Pereira et al., 2013). Systems which gel *in situ* are easily  
22 administered and can improve the retention of dosage forms at desired place,  
23 potentially increasing patient compliance to treatment (Van Tomme et al., 2008).  
24 Poloxamer P407 (also known as Pluronic F127), an ABA triblock copolymer of  
25 poly(ethylene glycol) – *b* – poly(propylene glycol) – *b* – poly(ethylene glycol), is the

1 most commonly used thermogelling material (Nie, 2011), but has several drawbacks,  
2 such as weak gel strength and rapid dissolution (Wu et al., 2011). To attempt to enhance  
3 the mucoadhesion of poloxamer P407, bioadhesive polymers based on cross-linked  
4 poly(acrylic acid) (PAA), such as Carbopol<sup>®</sup> 971P, Carbopol<sup>®</sup> 974P and polycarbophil,  
5 have been incorporated into poloxamer dispersions (Chang et al., 2002; De Souza  
6 Ferreira et al., 2015; Jones et al., 2009; Junqueira et al., 2016). Poly(acrylic acid)  
7 derivatives were previously reported as mucoadhesive (Jabbari et al., 1993), and the  
8 cross-linked forms, carbopol and polycarbophil, impart high viscosity to formulations,  
9 enhancing mucoadhesion.

10

11 Over the years, the mucoadhesion process has been widely studied (Iqbal et al., 2012;  
12 Peppas and Huang, 2004; Sogias et al., 2008; Sosnik et al., 2014), and in order to assess  
13 it, many different *in vitro* and *ex vivo* techniques have been developed (Bassi da Silva et  
14 al., 2017; Cave et al., 2012; Withers et al., 2013). Nevertheless, the force required to  
15 detach a dosage form from mucosal tissue is still the most commonly used technique  
16 (Carvalho et al., 2010; Cook and Khutoryanskiy, 2015; Nair et al., 2013). This is  
17 typically determined with the use of a texture analyser. According to the dosage form,  
18 some techniques prove to be more suitable than others. Solid dosage forms require  
19 detachment force method and cannot be measured by rheological methods like liquid  
20 dosage forms, for example. On the other hand, there is flow-through method which can  
21 be used for solid dosage forms (Patel et al., 2012) and liquid dosage forms (Cook et al.,  
22 2015; Irmukhametova et al., 2011).

23

24 Generally, most methods use *ex vivo* tissues to assess mucoadhesion, a large amount of  
25 which is sourced from laboratory animals slaughtered for that tissue (Cook et al., 2015)

1 Moreover, when used animal sources, there is a lower reproducibility of the method,  
2 considering the greater variation between these tissues. Therefore, with the aim of to  
3 reduce the number of animals killed for this tissue, this work aims to demonstrate the  
4 efficacy of a synthetic alternative to animal tissue in mimicking mucosa for the testing  
5 of mucoadhesive semi-solids. This mucosa-mimetic material provides a testing  
6 substrate which is inexpensive and homogenous compared to animal tissue. A substrate  
7 containing 20 mol% N-acryloylglucosamine (AGA) and 80 mol% 2-  
8 hydroxyethylmethacrylate (HEMA) was identified as being an effective mimic for pig  
9 buccal mucosa when testing the mucoadhesion of tablets and liquid dosage forms (Cook  
10 et al., 2015; Hall et al., 2011). This study investigates the efficacy of this “mucosa-  
11 mimetic” material when testing for the mucoadhesion of semi-solid dosage forms using  
12 two different methodologies. This is the first reported use of a mucosa-mimetic material  
13 in studying the mucoadhesion of semi-solids, with previous research investigating solid  
14 (Eshel-Green et al., 2016; Hall et al., 2011; Khutoryanskaya et al., 2010) or liquid (  
15 Cook et al., 2015; Eshel-Green et al., 2016) dosage forms. The mucoadhesion of semi-  
16 solid dosage forms is driven by non-covalent interactions of macromolecules with  
17 mucins, as for liquid and solid dosage forms, but also by the rheology of the  
18 formulation and its ability to wet a surface (Smart, 2005). For validation of this mucosa-  
19 mimetic material it is imperative that this class of materials be investigated.

20

## 21 2. Materials and Methods

### 22 2.1 Materials

23 Carbopol<sup>®</sup> 971P, Carbopol<sup>®</sup> 974P and polycarbophil were purchased from Lubrizol  
24 (Brazil). Triethanolamine, 2-hydroxyethyl methacrylate (HEMA), N,N'-  
25 methylenebisacrylamide (MBA), ammonium persulfate (APS), N,N,N',N'-

1 tetramethylethylenediamine (TMEDA), acetonitrile (ACN), fluorescein isothiocyanate-  
2 dextran (FITC, 10 kDa), and phosphate buffered saline (PBS) tablets were all purchased  
3 from Sigma-Aldrich (U.K.). 2.5 mL polypropylene vials, fitted with screw-on septa,  
4 having 8 mm internal diameter, were also purchased from Sigma-Aldrich (U.K.).  
5 Acryloyl glucosamine (AGA) was synthesised using a previously published procedure  
6 (Cook et al., 2015). Unless specified, all reagents were used without further purification.  
7 Porcine buccal mucosa was sourced from Wetlab-MedMeat (U.K.) and kept frozen at -  
8 80 °C.

9

## 10 2.2 Hydrogel preparation

### 11 2.2.1 Mucoadhesive polymeric systems

12 Monopolymeric thermogelling systems were prepared by dispersing 20 % w/v  
13 poloxamer 407 in purified water at room temperature.

14 To produce the binary polymeric systems, Carbopol® 971P, Carbopol® 974P or  
15 polycarbophil (0.25, 0.20 and 0.25 %, w/w, respectively) were dispersed in purified  
16 water with stirring. The required amount of poloxamer 407 (20 %, 15 % and 15 %, w/w,  
17 respectively) was then added to this preparation and the mixture was stored at 4 °C for  
18 12 h, to ensure the complete polymer wetting. The polymeric systems were then stirred,  
19 to completely disperse the polymers. The preparations were then neutralized with q.s.  
20 triethanolamine, and kept at 4 °C for at least 24 h before analysis (Bruschi et al., 2007;  
21 de Araújo Pereira et al., 2013; Fabri et al., 2011; Jones et al., 2009; Schmolka, 1972).

22

### 23 2.2.2 Synthesis of mucosa-mimetic hydrogels

1 As previously described by Hall et al (2011), purified water, MBA, APS, TMEDA and  
 2 HEMA and AGA monomer(s) were added to glass vials (Table 1). The mixtures were  
 3 vortexed until complete dissolution of all ingredients. Ethanol was then added before  
 4 being mixed again and the mixtures were bubbled for 5 minutes with nitrogen. 2.0 mL  
 5 aliquots of reaction mixture were then transferred to 2.5 mL polypropylene vials fitted  
 6 with septa, which had been purged with nitrogen. The vials were then placed in a  
 7 preheated water bath at 60 °C, and reaction allowed to proceed for 3 h. The  
 8 polymerisation was terminated by cooling the vial with cold water. The hydrogels were  
 9 then purified by immersing samples in deionised water, which was changed daily, for  
 10 two weeks to remove any unreacted chemicals.

11

12 Table 1. Composition of feed mixtures for synthesis of mucosa-mimetic hydrogels

Sample	HEMA:AGA ratio (mol %)	HEMA (g)	AGA (g)	MBA (g)	APS (g)	TMEDA (g)
HEMA hydrogel 20 mol%	100:0	4.3337	-	0.0052	0.0380	0.0116
AGA hydrogel	80:20	3.4711	1.5393	0.0052	0.0380	0.0116

13

14

### 15 2.3 Equilibrium swelling degree measurements

16 Post-purification, a section of HEMA and AGA mucosa-mimetic hydrogels were  
 17 removed by scalpel and the swollen samples were weighed. These samples were placed  
 18 in small vials previously weighed and, allowed to dry in an oven at 50 °C for at least 48  
 19 h, and reweighed. The equilibrium swelling degree (ESD) was then calculated  
 20 according to equation 1:

$$21 \quad \text{ESD} = (W_s - W_d)/W_d \quad (1)$$



1 where  $W_s$  and  $W_d$  are the weights of the swollen and dry sample, respectively. Each  
2 experiment was repeated at least 3 times for each hydrogel.

3

#### 4 2.4 Density studies

5 The density of dry gels was calculated using the displaced volume of acetonitrile (ACN)  
6 in a 5 mL pycnometer (Sigma-Aldrich, U.K.). The empty pycnometer was weighed,  
7 filled with acetonitrile and reweighed, allowing for calculation of the pycnometer  
8 volume ( $V_{ACN+d}$ ). After that, a known amount of dried hydrogel ( $W_d$ ) was placed into a  
9 pycnometer, which was then filled with acetonitrile and weighed again ( $W_{ACN+d}$ ). The  
10 density of the sample ( $\rho_x$ ) may then be calculated using equations 2 and 3:

$$11 \quad V_{ACN+d} = (W_{ACN+d} - W_d) / \rho_{ACN} \quad (2)$$

$$12 \quad \rho_x = W_d / (V_{ACN+d} - V_{ACN}) \quad (3)$$

13

14 where  $\rho_{ACN}$  is the density of ACN (0.786 g/mL) and  $V_{ACN}$  is the volume of ACN.

15

#### 16 2.5 Equilibrium swelling volume measurements

17 First, the hydrogel swelling ratio ( $Q_m$ ) was calculated following the equation 4:

$$18 \quad Q_m = W_s / W_d \quad (4)$$

1 where  $W_S$  and  $W_D$  are the weights of the swollen and dry sample, respectively. Then,  
2 the equilibrium swelling volume (ESV) was calculated by equation 5:(Thomas et al.,  
3 2016)

$$4 \quad \text{ESV} = ((1 + \rho_x)/\rho_w) \cdot (Q_m - 1) \quad (5)$$

5 where  $\rho_w$  is the density of water.

## 6 2.6 Continuous shear (flow) rheometry

7 The continuous shear analysis of all thermogelling semisolid formulations without  
8 FITC-dex marker was performed at  $37 \pm 0.1$  °C. In flow mode, a controlled stress  
9 rheometer (MARS II, Haake Thermo Fisher Scientific Inc., Germany) with parallel steel  
10 cone-plate geometry (60 mm, separated by a fixed distance of 0.052 mm) was used.  
11 Samples were carefully placed to the inferior plate, and allowed to equilibrate for at  
12 least 1 min prior to start the analysis. Flow curves were evaluated over shear rates  
13 ranged from 0 to 2000  $s^{-1}$ . The flow properties of at least three replicates were  
14 measured, in each case.

15 The rheological properties of these formulations have been investigated previously  
16 using the following procedure, and are reported herein to discuss differences in  
17 formulations (Bruschi et al., 2007; de Araújo Pereira et al., 2013; Jones et al., 2009).  
18 The rheology of the formulations is a major factor in determining the retention, ease of  
19 application of the product and mucoadhesion of dosage forms, so it is important that the  
20 rheological properties of the materials be discussed. The ascending flow curves were  
21 fitted using the Power Law equation (equation 6).

$$22 \quad \sigma = k \cdot \gamma^n \quad (6)$$

1 where  $\sigma$  is the shear stress (Pa),  $k$  is the consistency index [(Pa.s)<sup>n</sup>],  $\gamma$  is the rate of shear  
2 ( $s^{-1}$ ), and  $n$  is the flow behavior index (dimensionless). The yield stress of the  
3 formulations was investigated by the following rheological models: Casson (equation 7)  
4 and Herschel–Buckley (equation 8) (Hemphill et al., 1993).

$$5 \quad \tau = \sqrt[n]{\left(\tau_0^n + (\gamma n_p)\right)^n} \quad (7)$$

6 where  $\tau$  is the shear stress (Pa),  $n$  is the flow behavior index (dimensionless),  $\tau_0$  is yield  
7 stress (Pa),  $\gamma$  is the rate of shear ( $s^{-1}$ ) and  $n_p$  is Casson plastic viscosity.

$$8 \quad \tau = \tau_0 + k.\gamma^n \quad (8)$$

9 where  $\tau$  is the shear stress (Pa),  $\tau_0$  is yield stress (Pa),  $k$  is the consistency index [(Pa  
10 s)<sup>n</sup>],  $\gamma$  is the rate of shear ( $s^{-1}$ ) and  $n$  is the flow behaviour index (dimensionless). Then,  
11 the hysteresis area of each binary polymeric system was calculated using RheoWin  
12 4.10.0000 (Haakes) software.

13 Moreover, the rheological analysis of formulations, with and without FITC-dex, was  
14 performed at 37 °C using an AR 1500 ex controlled stress/controlled rate rheometer  
15 (T.A. Instruments, UK), in flow mode, in conjunction with parallel steel plate geometry  
16 (40 mm, separated by a fixed distance of 600  $\mu$ m). The samples were carefully applied  
17 to the lower plate of the rheometer, ensuring that formulation shearing was minimized,  
18 and allowed to equilibrate for at least 3 min prior to analysis. In continuous shear mode,  
19 upward flow curve for each formulation were measured over shear rates ranged from 0  
20 to 500  $s^{-1}$ . In each case, the continuous shear properties of at least three replicates were  
21 determined.

## 22 2.7 Oscillatory rheometry

1 With the aim of determining the viscoelastic properties of the samples, firstly,  
2 oscillatory rheometry of all poloxamer-based thermogelling formulations was  
3 performed in oscillation mode, using the controlled stress rheometer described above  
4 (MARS II, Haake Thermo Fisher Scientific Inc., Germany) and, the same cone-plate  
5 (60 mm, separated by a fixed distance of 0.052 mm), at  $37 \pm 0.1$  °C. The samples were  
6 carefully applied to the plate, as already described. After linear viscoelastic region  
7 determination of each formulation, the frequency sweep analysis was evaluated from  
8 0.1 to 10.0 Hz. Thus, the storage modulus ( $G'$ ) was calculated using RheoWin  
9 4.10.0000 (Haakes) software. In each case, at least three replicates were evaluated (de  
10 Araújo Pereira et al., 2013; Jones et al., 2009).

11 Then, the possible interaction between poloxamer and Carbopol® 971P, Carbopol® 974P  
12 or polycarbophil was investigated by the difference between the dynamic modulus of  
13 the polymeric blends and the theoretical value of the modulus obtained by summation of  
14 the individual parts (Hemphill et al., 1993; Jones et al., 2009). Calculation of the  
15 interaction parameter for the binary mixtures was determined using the storage modulus  
16 values at 10.0 Hz of oscillatory frequency following the equation 9.

17

$$18 \quad \Delta G' = G'_{\text{mixture}} - (G'_{\text{poloxamer 407}} + G'_{\text{carbomer or polycarbophil}}) \quad (9)$$

19

## 20 2.8. Determination of gelation temperature ( $T_{\text{sol/gel}}$ )

21 Gelation temperatures of the thermogelling systems were determined as previously  
22 described (De Souza Ferreira et al., 2017). In oscillatory mode, with temperature ramp,  
23 using the same cone-plate previously described (60 mm). The determination of  $T_{\text{sol/gel}}$  of

1 each formulation were performed after determination of the linear viscoelastic region at  
2 5 °C and 60 °C. A temperature sweep analysis was performed over the temperature at  
3 5–60 °C range with defined frequency (1.0 Hz), and rate of heating 10 °C/min using a  
4 controlled stress (resident within the linear viscoelastic region).  $G'$ ,  $G''$ ,  $\eta'$  and  $\tan \delta$   
5 were calculated using RheoWin 4.10.0000 (Haakes) software. The temperature at which  
6 the elastic modulus was halfway between the values for the solution, and for the gel was  
7 called  $T_{\text{sol/gel}}$ .  $T_{\text{sol/gel}}$  was calculated for all binary system in which dynamic viscosity  
8 increased with increasing temperature and at least three replicate samples were  
9 evaluated in all cases (Andrews et al., 2009; Bruschi et al., 2007; de Araújo Pereira et  
10 al., 2013; Edsman et al., 1998).

11

## 12 2.8 Adhesion testing

### 13 2.8.1 Detachment test

14 The adhesive properties of the hydrogels were assessed using a TA.XT Plus texture  
15 analyser (Stable Micro Systems, UK). The hydrated 20 mol% AGA and HEMA  
16 hydrogels were kept immersed in deionised water (water bath) and equilibrated at  $37 \pm 1$   
17 °C for 0.5 h. Prior to measurements, the polypropylene vials containing hydrogels were  
18 cut away with a saw so that an 8 mm diameter cylinder of gel extended from the vial by  
19 approximately 2 mm. Poloxamer 407 20% (w/w) hydrogel and poloxamer 407/  
20 Carbopol® 971P, Carbopol® 974P or polycarbophil thermogelling systems were kept  
21 immersed in a water bath at  $37 \pm 1$  °C for 0.5 h and then, placed up to a hot plate which  
22 was equilibrated at  $37 \pm 1$  °C on the texture analyser.

23 The synthetic hydrogels or the animal mucosal tissue were attached to a mobile probe  
24 (cylindrical, P/6) using double sided adhesive tape. The probe was lowered at a speed of

1 1 mm/s until it reached the mucoadhesive hydrogel surface with a determinate contact  
2 force. The contact force of 0.03 N was applied to the poloxamer 407 20% (w/w) and  
3 poloxamer 407/Carbopol<sup>®</sup> 971P formulations, while to poloxamer 407/Carbopol<sup>®</sup> 974P  
4 and poloxamer 407/polycarbophil formulations a contact force of 0.002 N was applied  
5 with the aim of to keep the substrate just in contact with the hydrogel surface. A force  
6 larger than this drove the sample into the system so that contact was made on two faces.  
7 Substrate and formulation where kept in contact for 30 seconds, then the probe was  
8 withdrawn at a rate of 10.0 mm/s until complete detachment of the mucoadhesive  
9 hydrogels from the synthetic hydrogels or animal mucosal tissue was observed. The  
10 maximum force of detachment and the work of adhesion (the area under the  
11 force/distance curve) were determined using Texture Exponent 32 software (Stable  
12 Micro Systems, UK). All measurements were performed at least 6 times and the  
13 adhesion parameters calculated as mean values  $\pm$  standard deviation.

14 Adhesion of mucoadhesive hydrogels to animal mucosal tissues were studied using  
15 porcine buccal mucosa which were obtained from MedMeat (UK). These tissues were  
16 collected immediately after the slaughter of animals and were stored frozen at -20 °C.  
17 Before testing, the mucosal tissues were defrosted in water at 35–37 °C and the mucosa  
18 was excised from the cheek using a scalpel. In order to achieve the same surface area as  
19 the synthetic hydrogels during adhesion testing, mucosa was placed into a  
20 polypropylene sample vial and held in place with a screw-thread polypropylene cap  
21 with an 8mm diameter bore so that only an 8 mm diameter circle of mucosa came into  
22 contact with the thermogelling formulations. Detachment force and work adhesion was  
23 treated with two-way ANOVA (multiple comparisons), using Bonferroni post-hoc test.  
24  $P < 0.05$  was taken to be statistically significant.

25

## 1 2.8.2 Retention testing

2 Retention was studied using a flow-through system developed in-house (Cave et al,  
3 2012). The system consists of a channel containing a testing substrate (either ex vivo  
4 mucosa, 'mucosa-mimetic' or PTFE), over which a syringe-pump washes PBS. This  
5 system is then maintained at 37 °C within an incubator. FITC-dextran was added to  
6 thermogelling preparations at 1 mg/g to allow for fluorescence imaging. FITC-dextran-  
7 labelled formulations (20 µL) were then pipetted onto the testing substrate and allowed  
8 to warm over 2 minutes. This time was sufficient to allow for gelation to occur, as  
9 tested by inversion of substrate. The testing substrate was then imaged using a Leica  
10 MZ10F fluorescence stereomicroscope, equipped with a GFP filter set and monochrome  
11 camera, using an exposure time of either 11, 40 or 211 µs for HEMA mucosa-mimetic  
12 hydrogel, mucosa tissue and AGA mucosa-mimetic hydrogel, respectively. The PBS  
13 buffer eluent was then flowed over the testing substrate (4 mL/min), and images were  
14 taken at 1, 5, 10, and 15 mL elution volume. The quantity of polymer remaining on the  
15 surface of the testing substrate was then assessed using ImageJ. Briefly, the region on  
16 which the fluorescent polymers were pipetted was selected, and the brightness of the  
17 pixels measured. This brightness was then measured at the remaining time points, and  
18 the % fluorescence calculated with respect to the starting brightness value. Retention  
19 data was treated with two-way ANOVA (multiple comparisons), using Bonferroni post-  
20 hoc test.  $P < 0.05$  was taken to be statistically significant.

21

## 22 3. Results and Discussion

### 23 3.1 Synthesis and characterization of mucosa-mimetic hydrogels

24 Hydrogels of HEMA and 20 mol% AGA were produced in hydroalcoholic solution  
25 using free-radical polymerisation with a water-soluble cross-linker, MBA. ATR-FTIR

1 spectroscopy demonstrated that the HEMA hydrogel contained characteristic  
2 absorbances related to the HEMA monomer, such as the ester carbonyl stretch at 1700  
3  $\text{cm}^{-1}$  and broad alcohol vibration at  $\sim 3420 \text{ cm}^{-1}$ , with no residual monomer, as  
4 evidenced by the absence of a C=C absorbance at  $\sim 1640 \text{ cm}^{-1}$ . In addition to the HEMA  
5 absorbances, AGA had peaks at 1650 and  $1560 \text{ cm}^{-1}$ , related to the amine linking the  
6 sugar ring to the polymer backbone. These spectra are in accordance with those  
7 previously reported (Cook et al., 2015). In this study, 20 mol% AGA was produced as a  
8 reported “mucosa-mimetic” material, whilst HEMA will act as a control to indicate  
9 whether interactions with semi-solid dosage forms are identical for all hydrogels.

10

11

[FIGURE 1 HERE]

12

13 Figure 1. ATR-FTIR spectra of 100 % HEMA (green) and HEMA:AGA (80:20 mol%)  
14 (blue) hydrogels after drying.

15 In addition to spectroscopic analysis, the swelling properties of the two hydrogels were  
16 measured. ESD values are an indicator of the magnitude by which the hydrogel’s  
17 weight increases upon contact with water, ESV values indicate volume changes  
18 associated with swelling until equilibrium. In order to calculate ESV values, dried  
19 samples of hydrogels were analysed by pycnometry, which gives values of density.

20 ESD values presented in table 2 are comparable to previously published data (Hall et  
21 al., 2011), ESV values indicate that 20 mol% AGA hydrogel swelled to a greater extent  
22 than 100 mol % HEMA hydrogels. This is possibly a result of the large number of  
23 hydrogen-bonding groups on AGA, which may solvate to a greater extent than the  
24 HEMA pendant groups. The differences in equilibrium swelling volumes are likely to  
25 result in differences in entanglements and interaction between dosage form and gel due



1 to different mesh sizes and polymer volume fractions. Mesh size increase with ESV,  
 2 whilst polymer volume fractions decrease (Thomas et al., 2016).

3

4 Table 2. Swelling parameters and densities of 20 mol% AGA and HEMA hydrogels.

Sample	Parameters		
	Equilibrium swelling degree	Density, g/mL <sup>a</sup>	Equilibrium swelling volume
100 mol% HEMA	3.35 ± 0.59	1.79 ± 0.03	6.12 ± 0.38
20 mol% AGA <sup>b</sup>	3.80 ± 0.02	1.64 ± 0.16	7.25 ± 0.02

5 <sup>a</sup> of dried mass

6 <sup>b</sup> Difference in values for 20 mol% AGA and 100 mol% HEMA are statistically significant using two-tailed T-testing  
 7 (p < 0.01).

8

9

### 10 3.2 Production of thermogelling formulations

11

12 In order to determine whether 20 mol% AGA was capable of mimicking buccal mucosa  
 13 in the mucoadhesion testing of semi-solids, four thermogelling formulations were  
 14 prepared. These formulations were based on poloxamer P407, which undergoes a sol-  
 15 gel transition upon warming. Cross-linked poly(acrylic acid) derivatives were  
 16 incorporated into poloxamer P407 with the intention of producing a range of  
 17 formulations with different rheological and chemical properties. These formulations are  
 18 based on previously reported thermogelling systems (De Souza Ferreira et al., 2015;  
 19 Jones et al., 2009). The composition and rheological properties of the thermogelling  
 20 formulations are shown in table 3. The first formulation is 20 % poloxamer P407, which

1 undergoes a sol-gel transition at  $29.7 \pm 0.6$  °C, as determined by rheological method.  
2 Formulations F1, F2, and F3 include cross-linked poly(acrylic acids), giving a diverse  
3 range of rheological properties.  
4  
5 The rheological properties of these formulations have been investigated previously, and  
6 are reported herein to discuss differences in formulations (Bruschi et al., 2007; de  
7 Araújo Pereira et al., 2013; Jones et al., 2009). At all formulations, a shear-thinning  
8 behaviour (pseudoplastic flow), with yield value and hysteresis area was observed. In  
9 the most of cases the hysteresis area was characteristic of rheopectic material, which  
10 have the down-curve coming back above the up-curve and, this profile is quite common  
11 in binary polymeric systems at 37 °C (De Souza Ferreira et al., 2015; Jones et al., 2009).  
12 The addition of poly(acrylic acid) derivative decreased the consistency index of the  
13 systems when compared to the formulation containing just poloxamer (Table 3). The F2  
14 demonstrated higher consistency index, since this poly(acrylic acid) derived –  
15 Carbopol® 974P – has cross-link density larger than the others cross-linked poly(acrylic  
16 acid) type. On the other hand, the Carbopol® 971P has lower cross-linking density,  
17 therefore, it showed a low consistency index. Moreover, in flow rheology, greater yield  
18 values were detected for most of formulations, at 37 °C, as expected. Commonly, the  
19 yield value of the carbomers, has indicated an improvement of retention time of the  
20 blends containing bioadhesive and thermoresponsive polymers in the application site  
21 (De Souza Ferreira et al., 2015).  
22 According to the magnitude of the elastic moduli, in the oscillatory rheometry, the  
23 interaction parameter was derived. The rheological synergy, between poloxamer and  
24 poly(acrylic acid)s derived, provides evidence of adhesive interactions between them. In  
25 this sense, F1, F2 and F3 formulations, demonstrated strong interaction between the two

1 polymers. Thus, evidencing, beyond secondary bonds, the hydrogen bonds between  
2 carboxyl groups, which are widely distributed in acrylic acid chain, and hydroxyl  
3 groups of poloxamer (Jones et al., 2009). As already observed, in these concentrations  
4 (20/0.20; 15/0.25 and 15/0.25 %) the formulations containing respectively Carbopol<sup>®</sup>  
5 971P, Carbopol<sup>®</sup> 974P and polycarbophil, have demonstrated better interaction  
6 parameter (at 37 °C). This is consistent with the greater amount of poloxamer in  
7 micellar form, which can be available to form hydrogen bonds with carboxylic groups  
8 of poly(acrylic acid) (De Souza Ferreira et al., 2015; Khutoryanskiy and Staikos, 2009).  
9  
10 Moreover, using the increase of viscosity and elastic moduli ( $G'$ ), in oscillatory  
11 rheology analysis, the gelation transition temperature was determined. As known,  
12 poloxamer P407 is a thermoresponsive polymer and monopolymeric systems exhibit  
13 gelation temperature ( $T_{sol/gel}$ ) (Jones et al., 2009). Furthermore, when Carbopol<sup>®</sup> 974P  
14 (F2) and polycarbophil (F3) were used, the  $T_{sol/gel}$  increases, since the addition of other  
15 polymers can interfere in the micelles formation and change the gelation temperature.  
16 However, considering the suitable range of  $T_{sol/gel}$  from 25 °C to 37 °C, all formulations  
17 proved to be appropriate to mucosal application, becoming gel at body temperature  
18 (Bruschi et al., 2007; Gratieri et al., 2010; Yun Chang et al., 2002). These formulations  
19 also represent a diverse group of semi-solids with different viscosities, consistencies,  
20 flow behaviours and gelation temperatures. This diversity is important in validating the  
21 mucosa-mimetic material.

22

23 Table 3. Composition and rheological properties of thermogelling formulations.

24

Sample ID	Poloxamer P407 conc. (% w/w)	PAA type, conc. (% w/w)	Viscosity at 32 s <sup>-1</sup> d (Pa.s)	Consistency index (k) <sup>b,d</sup> (Pa.s)	Flow behavior index (n) <sup>b,d</sup>	Yield stress <sup>b,d</sup> (Pa)	Hysteresis area <sup>b,d</sup> (Pa.s)	Tsol/gel (°C)	Interaction parameter <sup>b,c</sup> (Pa)
Poloxamer <sup>a</sup>	20	N/A	12.530 ± 0.062	196.10 ± 12.27	0.14 ± 0.01	220.35 ± 4.05	-22991.67 ± 2464.22	28.67 ± 0.58	N/A
F1 <sup>a</sup>	20	Carbopol 971P, 0.20	26.277 ± 2.908	43.15 ± 0.50	0.85 ± 0.00	836.77 ± 5.25	857500.00 ± 7424.62	27.88 ± 0.06	1944.73 ± 381.93
F2	15	Carbopol 974P, 0.25	6.171 ± 0.331	139.40 ± 6.01	0.256 ± 0.005	120.61 ± 0.12	-17520.00 ± 8553.99	36.04 ± 0.06	2509.33 ± 215.85
F3 <sup>b</sup>	15	Polycarbophil, 0.25	6.935 ± 0.257	60.963 ± 3.307	0.359 ± 0.006	230.067 ± 4.484	-35990.00 ± 5507.62	36.42 ± 0.02	1927.03 ± 93.85

<sup>a</sup>(De Souza Ferreira et al., 2015)

<sup>b</sup>(De Souza Ferreira et al., 2017)

<sup>c</sup>Interaction parameter between poloxamer and cross-linked poly(acrylic acid) by storage modulus (G') at 10 Hz

<sup>d</sup> at 37°C

1

2 Flow rheograms for each formulation are shown in Figure 2. All formulations show

3 responses to shear which are typical for pseudoplastic semi-solids. This is also reflected

4 in their reported flow-behaviour indices (Table 3), which all had values lower than one,

5 calculated to fitting to a power-law model. Marked differences in viscosities are

6 apparent between the formulations (Figure S1, ESI).

7 Formulations were also prepared with 1 mg/g FITC-dextran (10 kDa) incorporated, in

8 order to conduct flow-through experiments (Figure 2, orange). With the aim of

9 observing the structural changes or marker interactions with the binary systems, the

10 flow rheology was studied. There were no apparent changes in flow rheology profile

11 when FITC-dextran was incorporated into pluronic and F1 formulations, but there were

12 observable increases in viscosity for formulations F2 and F3. This is consistent with

13 increased physical cross-linking in these systems, which are more influenced by the

14 presence of the marker. Despite an increase in the viscosity may modify the retention

1 time of the formulations during the flow-through experiments, the viscosity has not  
2 been greatly changed between formulations with and without FITC-dextran and,  
3 interferences were not observed in the results.

4

5 [FIGURE 2 HERE]

6 Figure 2. Flow rheograms for a) pluronic, b) F1, c) F2, and d) F3 formulations with  
7 (orange circles) and without (black circles) 1 mg/g FITC-dextran (10 kDa). Data  
8 presented as mean  $\pm$  standard deviation (N = 3).

9

### 10 3.3 Mucoadhesion testing

11 The adhesion of thermogelling formulations was determined using two methods, texture  
12 analysis and a flow-through system. Texture analysis measures the force required, or  
13 work needed, to remove a dosage form from a substrate (e.g. mucosal membrane), and  
14 is the standard method of testing the mucoadhesion of solid dosage forms. In order to  
15 measure adhesion of semi-solid dosage forms by this method, modification had to be  
16 made to standard procedures. 100 mol% HEMA and 20 mol% AGA hydrogels were  
17 formed in polypropylene vials, which were then cut back so that the hydrogel extended  
18 by approximately 2 mm from the end of the vial. This gave a flat surface of hydrogel on  
19 which to measure adhesion. The vial was then attached to the probe of a texture  
20 analyser (Figure 3a). This allowed the thermogelling systems to be maintained on a hot-  
21 plate at the base of the texture analyser whilst the adhesion of hydrogels to their surface  
22 was determined (Figure 3b). This gave a force-distance relationship from which either  
23 the maximum force required to remove the dosage form or the area under the force-time  
24 curve could be determined, giving the force and work of adhesion, respectively (figure  
25 2c).

1 [FIGURE 3 HERE]

2 Figure 3. Modifications made to a texture analyser allow for testing substrates  
3 (hydrogels, mucosa, and polypropylene) to be pressed against thermogelling semisolid  
4 formulations (a). Removal of the testing substrate from the formulations (b) gives a  
5 force-time curve from which values of force and work of adhesion can be measured (c).

6  
7 The adhesion of thermogelling formulations to hydrogels, mucosa, and a control of  
8 polypropylene is shown in Figure 4. The mucosa-mimetic 20 mol% AGA hydrogel and  
9 buccal mucosa gave mean values of adhesion which were closest to buccal mucosa,  
10 giving values of adhesion which were not statistically significant in two out of four  
11 formulations, for work and force of adhesion, as determined by two-way ANOVA.  
12 Kruskal–Wallis testing gave no significant differences between 20 mol% AGA and  
13 mucosa, but experimental replicates were not sufficient to determine conclusively  
14 whether non-parametric statistics were required. Generally, values of adhesion were  
15 higher in the control hydrogel, 100 mol% HEMA, than the mucosa-mimetic 20 mol%  
16 AGA hydrogel and the buccal mucosa. Average % deviations from mean mucosa values  
17 across all formulations for buccal mucosa, 20 mol% AGA, 100 mol% HEMA, and  
18 polypropylene, were 5 %, 23 %, 79 %, and 52 %, respectively.

19  
20 [FIGURE 4 HERE]

21 Figure 4. Force (a) and work (b) of adhesion of thermogelling formulations to  
22 hydrogels, buccal mucosa, and polypropylene, as determined by texture analysis. Data  
23 represented as mean  $\pm$  standard deviation (N = 6). “ns” designates no statistical  
24 significance, \* indicates  $p < 0.05$ , and \*\*\* indicates  $p < 0.001$ , using two-way ANOVA  
25 with Bonferroni post-hoc.

1  
2 The metric by which adhesion is measured from force-displacement curves during  
3 texture analysis impacted on results. Whilst poloxamer samples gave comparable rank-  
4 orders of adhesion using either work or force, F1-3 gave different rank orders  
5 depending on which value was used. The force of adhesion reflects the force required to  
6 overcome the adhesive forces between testing substrate and the thermogelling  
7 formulations, as fracture always occurred at the interface between the two materials.  
8 The work of adhesion is often the preferred metric for measuring mucoadhesion, as it is  
9 seen as being more relevant to the “bedside” application. When determining the work of  
10 adhesion, adhesive interactions are confounded by cohesive forces within testing  
11 substrate and formulation, and thus the elasticity and plasticity of the materials will be  
12 reflected in this value.

13  
14 Focusing on the values of force of adhesion allows for discussion of the adhesive forces  
15 alone. The adhesion of 100 mol% HEMA hydrogel to all formulations is higher than, or  
16 equal to, the adhesion of those formulations to the mucosa-mimetic 20 mol% AGA  
17 hydrogel. This can be rationalized by consideration of the physicochemical properties of  
18 the hydrogels. 100 mol% HEMA contains fewer functional groups capable of hydrogen  
19 bonding than the 20 mol% AGA hydrogel per monomer unit, and has lower degrees of  
20 swelling (Table 2). The mucoadhesion of poloxamer and PAA is often attributed to the  
21 formation of hydrogen bonds between polymer and secretory mucins on the surface of  
22 tissue. As adhesion to 100 mol% HEMA is higher than 20 mol% AGA it is not likely  
23 that adhesion can be simply attributed to hydrogen bonding; there may be additionally  
24 complementary chemical interactions, such as van der Waals forces or the so-called  
25 “hydrophobic effect”, wherein the poor solvation of hydrophobic moieties promotes

1 their interaction (Smart, 2005). An additional factor affecting adhesion to the hydrogels  
2 is the water content, reflected in equilibrium swelling values. Differences in swelling  
3 values likely reflect differences in hydrogel mesh sizes, which in turn will affect the  
4 interpenetration and entanglement of polymer across the formulation-hydrogel interface  
5 (Sahlin and Peppas, 1997). Additionally, the high water content of the hydrogels may  
6 have a lubricative effect, leading to a reduction in adhesion to the more swollen 20  
7 mol% AGA hydrogel. This would also effectively reduce the concentration of polymer  
8 in the hydrogel, lowering number of monomer units with which interaction can occur  
9 per unit area. It is believed that 20 mol% AGA is able to mimic buccal mucosa due to  
10 the chemical similarity of the AGA monomer with oligosaccharide side-chains adorning  
11 the mucin glycoproteins secreted on the mucosal tissue. It is likely that physical  
12 properties, such as swelling degrees, also play a role in this mimicry (Hall et al., 2011).

13

14 Adhesion of formulations to polypropylene was generally equal to or greater than  
15 adhesion to 20 mol% AGA and buccal mucosa. Formulations cannot adhere to  
16 polypropylene via entanglement or by polar interactions, so it is believed that this  
17 adhesion is a result of the absence of water from the polypropylene. The formulations  
18 are able to wet the surface of polypropylene sufficiently to allow adhesion without the  
19 lubricative effect of water. Polypropylene has been used to evaluate the mucoadhesion  
20 of wetted polymer films, but was not found to be a good mimic of mucosa in this study  
21 (Choi et al., 1999).

22

23 The retention of thermogelling semi-solids on HEMA, 20 mol% AGA, buccal mucosa,  
24 and PTFE, a “non-stick” control, was also determined using a flow-through method  
25 previously developed (Figure 5) (Cave et al., 2012; Cook et al., 2015; Irmukhametova et



1 al., 2011; Withers et al., 2013). There was no statistically significant difference in  
2 retention values between 20 mol% AGA and buccal mucosa for the poloxamer, F2, and  
3 F3 formulations at any washing volume (Figure 4a, 4c, and 4d, respectively). However,  
4 there were significant differences at 1 and 5 mL volumes, using formulation F1. All  
5 formulations were retained most poorly on PTFE, indicating that the retention is not  
6 simply the result of the rheology of the semi-solids, dissolution of the gel, or release of  
7 FITC-dextran from the formulation.

8 [FIGURE 5 HERE]

9 Figure 5. The retention of FITC-dextran labelled pluronic (a), F1 (b), F2 (c), and F3 (d)  
10 formulations on 100 mol% HEMA, 20 mol% AGA, buccal mucosa, and PTFE, using a  
11 flow-through method. Data presented as mean  $\pm$  standard deviation (N = 3). “ns”  
12 designates no statistical significance, \* indicates  $p < 0.05$ , and \*\* indicates  $p < 0.01$ ,  
13 using two-way ANOVA with Bonferroni post-hoc.

14

15 The adhesion of semi-solids to surfaces is a complex phenomenon, arising from several  
16 different interactions (Figure 6) (Smart, 2005). Polymer-solvent interactions dictate, in  
17 part, dissolution of the dosage form, as well as the favourability of interaction with the  
18 surface. Cohesive interactions within the dosage form affect its rheology, which in turn  
19 modulates retention. Polymer-substrate interactions allow for adhesive bonding between  
20 gel and surface, and stabilize polymer-polymer entanglements where polymer mobility  
21 is possible. In the flow-through model (figure 5), formulations have greater adhesion to  
22 hydrogel and mucosa than to PTFE. This is likely a result of polymer entanglement with  
23 the surfaces, and concomitant formation of non-covalent bonds. Both 20 mol% AGA  
24 and 100 mol% HEMA are good mimics of buccal tissue using this method, with 20  
25 mol% AGA performing marginally better. Adhesion to 20 mol% AGA is consistently

1 lower than to 100 mol% HEMA, which may be attributed to greater swelling degrees, or  
2 an increased hydrophilicity modulating formulation-hydrogel interactions. It is  
3 conceivable that the lower hydrophilicity of 100 mol% HEMA indicates that  
4 hydrophobic interactions improve mucoadhesion, but this is confounded by factors such  
5 as competition for hydrogen-bonding groups with water.

6

7

[FIGURE 6 HERE]

8

Figure 6. An overview of the factors dictating the mucoadhesion of semi-solids.

9

#### 10 4. Concluding remarks

11 Reducing the use of animals in research is a key goal of many researchers worldwide.

12 The development of mucoadhesive formulations typically requires the use of *ex vivo*

13 animal tissue, which could be reduced were there a validated synthetic substrate capable

14 of mimicking mucosa. The adhesion of thermogelling semi-solid formulations to 20

15 mol% AGA, a potential mucosa-mimetic material, 100 mol% HEMA, buccal mucosa

16 and controls has been studied with the aim of supporting the use of 20 mol% AGA

17 hydrogels as mucosa-mimetic materials. A 20 mol% AGA hydrogel was shown to be a

18 good surrogate for buccal mucosa using two methods of studying mucoadhesion.

19 Controls also allow for study of mucoadhesive interactions, indicating that

20 mucoadhesion occurs in these dosage forms largely as a result of polymer entanglement

21 and polymer-surface interactions, and is not simply governed by the rheology of the

22 dosage forms.

23

#### 24 **References**

25 Andrews, G.P., Donnelly, L., Jones, D.S., Curran, R.M., Morrow, R.J., Woolfson, A.D.,

1 Malcolm, R.K., 2009. Characterization of the rheological, mucoadhesive, and drug  
2 release properties of highly structured gel platforms for intravaginal drug delivery.  
3 *Biomacromolecules* 10, 2427–2435.

4 Bassi da Silva, J., Ferreira, S.B. de S., de Freitas, O., Bruschi, M.L., 2017. A critical  
5 review about methodologies for the analysis of mucoadhesive properties of drug  
6 delivery systems. *Drug Dev. Ind. Pharm.* in press.

7 Bruschi, M.L., Jones, D.S., Panzeri, H., Gremião, M.P.D., de Freitas, O., Lara, E.H.G.,  
8 2007. Semisolid Systems Containing Propolis for the Treatment of Periodontal  
9 Disease: In Vitro Release Kinetics, Syringeability, Rheological, Textural, and  
10 Mucoadhesive Properties. *J. Pharm. Sci.* 96, 2074–2089.

11 Carvalho, F.C., Bruschi, M.L., Evangelista, R.C., Palmira, M., Gremião, D., 2010.  
12 Mucoadhesive drug delivery systems. *Braz. J. Pharm. Sci.* 46, 1–18.

13 Cave, R.A., Cook, J.P., Connon, C.J., Khutoryanskiy, V. V., 2012. A flow system for the  
14 on-line quantitative measurement of the retention of dosage forms on biological  
15 surfaces using spectroscopy and image analysis. *Int. J. Pharm.* 428, 96–102.

16 Chang, J.Y., Oh, Y.K., Choi, H. gon, Kim, Y.B., Kim, C.K., 2002. Rheological  
17 evaluation of thermosensitive and mucoadhesive vaginal gels in physiological  
18 conditions. *Int. J. Pharm.* 241, 155–163.

19 Choi, H.K., Kim, O.J., Chung, C.K., Cho, C.S., 1999. A mucoadhesive polymer  
20 prepared by template polymerization of acrylic acid in the presence of  
21 poly(ethylene glycol) macromer. *J. Appl. Polym. Sci.* 73, 2749–2754.

22 Cook, M.T., Khutoryanskiy, V. V., 2015. Mucoadhesion and mucosa-mimetic  
23 materials—A mini-review. *Int. J. Pharm.* 495, 991–998.

24 Cook, M.T., Schmidt, S. a., Lee, E., Samprasit, W., Opanasopit, P., Khutoryanskiy, V.  
25 V., 2015. Synthesis of mucoadhesive thiol-bearing microgels from 2-

1 (acetylthio)ethylacrylate and 2-hydroxyethylmethacrylate: novel drug delivery  
2 systems for chemotherapeutic agents to the bladder. *J. Mater. Chem. B.* 3 , 6599-  
3 6604

4 Cook, M.T., Smith, S.L., Khutoryanskiy, V., 2015. Novel glycopolymer hydrogels as  
5 mucosa-mimetic materials to reduce animal testing. *Chem. Commun.* 51, 14447–  
6 14450.

7 de Araújo Pereira, R.R., Ribeiro Godoy, J.S., Stivalet Svidzinski, T.I., Bruschi, M.L.,  
8 2013. Preparation and characterization of mucoadhesive thermoresponsive systems  
9 containing propolis for the treatment of vulvovaginal candidiasis. *J. Pharm. Sci.*  
10 102, 1222–1234.

11 De Souza Ferreira, S.B., Da Silva, J.B., Borghi-Pangoni, F.B., Junqueira, M.V.,  
12 Bruschi, M.L., 2017. Linear correlation between rheological, mechanical and  
13 mucoadhesive properties of polycarbophil polymer blends for biomedical  
14 applications. *J. Mech. Behav. Biomed. Mater.* 68, 265–275.

15 De Souza Ferreira, S.B., Moco, T.D., Borghi-Pangoni, F.B., Junqueira, M.V., Bruschi,  
16 M.L., 2015. Rheological, mucoadhesive and textural properties of  
17 thermoresponsive polymer blends for biomedical applications. *J. Mech. Behav.*  
18 *Biomed. Mater.* 55, 164–178.

19 Değim, Z., Değim, T., Acartürk, F., Erdoğan, D., Ozoğul, C., Köksal, M., 2005. Rectal  
20 and vaginal administration of insulin-chitosan formulations: an experimental study  
21 in rabbits. *J. Drug Target.* 13, 563–72.

22 Edsman, K., Carlfors, J., Petersson, R., 1998. Rheological evaluation of poloxamer as  
23 an in situ gel for ophthalmic use. *Eur. J. Pharm. Sci.* 6, 105–112.

24 Eshel-Green, T., Eliyahu, S., Avidan-Shlomovich, S., Bianco-Peled, H., 2016. PEGDA  
25 hydrogels as a replacement for animal tissues in mucoadhesion testing. *Int. J.*

1 Pharm. 506, 25–34.

2 Fabri, F.V., Cupertino, R.R., Hidalgo, M.M., Monteiro Weffort de Oliveira, R.M.,  
3 Bruschi, M.L., 2011. Preparation and characterization of bioadhesive systems  
4 containing propolis or sildenafil for dental pulp protection. *Drug Dev. Ind. Pharm.*  
5 1446–1454.

6 Friedl, H.E., Dünnhaupt, S., Waldner, C., Bernkop-Schnürch, A., 2013. Preactivated  
7 thiomers for vaginal drug delivery vehicles. *Biomaterials* 34, 7811–7818.

8 Gratieri, T., Gelfuso, G.M., Rocha, E.M., Sarmiento, V.H., de Freitas, O., Lopez,  
9 R.F.V., 2010. A poloxamer/chitosan in situ forming gel with prolonged retention  
10 time for ocular delivery. *Eur. J. Pharm. Biopharm.* 75, 186–193.

11 Hall, D.J., Khutoryanskaya, O. V., Khutoryanskiy, V. V., 2011. Developing synthetic  
12 mucosa-mimetic hydrogels to replace animal experimentation in characterisation  
13 of mucoadhesive drug delivery systems. *Soft Matter.* 7, 9620.

14 Hemphill, T., Campos, W., Pilehvari, A., 1993. Yield-power law model more accurately  
15 predicts mud rheology. *Oil Gas Journal.* 91, 1–9.

16 Hornof, M., Weyenberg, W., Ludwig, A., Bernkop-Schnürch, A., 2003. Mucoadhesive  
17 ocular insert based on thiolated poly(acrylic acid): Development and in vivo  
18 evaluation in humans. *J. Control. Release* 89, 419–428. Iqbal, J., Shahnaz, G.,  
19 Dünnhaupt, S., Müller, C., Hintzen, F., Bernkop-Schnürch, A., 2012. Preactivated  
20 thiomers as mucoadhesive polymers for drug delivery. *Biomaterials* 33, 1528–35.

21 Irmukhametova, G.S., Mun, G.A., Khutoryanskiy, V. V., 2011. Thiolated mucoadhesive  
22 and PEGylated nonmucoadhesive organosilica nanoparticles from 3-  
23 mercaptopropyltrimethoxysilane. *Langmuir* 27, 9551–9556.

24 Ivarsson, D., Wahlgren, M., 2012. *Colloids and Surfaces B : Biointerfaces Comparison*  
25 of in vitro methods of measuring mucoadhesion : Ellipsometry , tensile strength

1 and rheological measurements. *Colloids Surfaces B Biointerfaces* 92, 353–359.

2 Jabbari, E., Wisniewski, N., Peppas, N.A., 1993. Evidence of mucoadhesion by chain  
3 interpenetration at a poly (acrylic acid)/mucin interface using ATR-FTIR  
4 spectroscopy. *J. Control. Release.* 26, 99-108.

5 Jones, D.S., Luciano, M., Freitas, O. De, Palmira, M., Gremião, D., Helena, E., Lara,  
6 G., Andrews, G.P., 2009. Rheological , mechanical and mucoadhesive properties  
7 of thermoresponsive , bioadhesive binary mixtures composed of poloxamer 407  
8 and carbopol 974P designed as platforms for implantable drug delivery systems for  
9 use in the oral cavity. *Int. J. Pharm.* 372, 49–58.

10 Junqueira, M.V., Borghi-Pangoni, F.B., Ferreira, S.B. de S., Bruschi, M.L., 2016.  
11 Evaluation of the methylene blue addition in binary polymeric systems composed  
12 by poloxamer 407 and Carbopol 934P using quality by design: rheological,  
13 textural, and mucoadhesive analysis. *Drug Dev. Ind. Pharm.* 9045, 1–41.

14 Khan, S., Trivedi, V., Boateng, J., 2016. Functional physico-chemical , ex vivo  
15 permeation and cell viability characterization of omeprazole loaded buccal fi lms  
16 for paediatric drug delivery. *Int. J. Pharm.* 500, 217–226.

17 Khutoryanskaya, O., Potgieter, M., Khutoryanskiy, V. V., 2010. Multilayered hydrogel  
18 coatings covalently-linked to glass surfaces showing a potential to mimic mucosal  
19 tissues. *Soft Matter* 551–557.

20 Khutoryanskiy, V. V. (Ed.), 2014. *Mucoadhesive Materials and Drug Delivery Systems.*  
21 John Wiley and Sons, Ltd.

22 Khutoryanskiy, V. V, 2011. *Advances in mucoadhesion and mucoadhesive polymers.*  
23 *Macromol. Biosci.* 11, 748–64.

24 Khutoryanskiy V.V. and Staikos G. (Eds) *Hydrogen-bonded Interpolymer complexes:*  
25 *Formation, Structure and Applications.* World Scientific, 2009

1 Ludwig, A., 2005. The use of mucoadhesive polymers in ocular drug delivery. *Adv.*  
2 *Drug Deliv. Rev.* 57, 1595–639.

3 Nair, A.B., Kumria, R., Harsha, S., Attimarad, M., Al-dhubiab, B.E., Alhaider, I.A.,  
4 2013. In vitro techniques to evaluate buccal films. *J. Control. Release.* 166, 10–21.

5 Nakamura, F., Ohta, R., Machida, Y., Nagai, T., 1996. In vitro and in vivo nasal  
6 mucoadhesion of some water-soluble polymers. *Int. J. Pharm.* 134, 173–181.

7 Nie, S., 2011. Thermoreversible Pluronic® F127-based hydrogel containing liposomes  
8 for the controlled delivery of paclitaxel : in vitro drug release , cell cytotoxicity ,  
9 and uptake studies. *Int. J. Nanomedicine.* 151–166.

10 Patel, V.F., Liu, F., Brown, M.B., 2012. Modeling the oral cavity : In vitro and in vivo  
11 evaluations of buccal drug delivery systems. *J. Control. Release* 161, 746–756.

12 Peppas, N.A., Huang, Y., 2004. Nanoscale technology of mucoadhesive interactions.  
13 *Adv. Drug Deliv. Rev.* 56, 1675–87.

14 Sahlin, J.J., Peppas, N.A., 1997. Enhanced hydrogel adhesion by polymer  
15 interdiffusion: use of linear poly(ethylene glycol) as an adhesion promoter. *J.*  
16 *Biomater. Sci. Polym. Ed.* 8, 421–436.

17 Schmolka, I.R., 1972. Artificial skin I. Preparation and properties of pluronic F127 gels  
18 for treatment of burns. *J. Biomed. Mater. Res.* 6, 571–582.

19 Shojaei, A.H., Li, X., 1997. Mechanisms of buccal mucoadhesion of novel copolymers  
20 of acrylic acid and polyethylene glycol monomethylether monomethacrylate. *J.*  
21 *Control. Release.* 47, 151–161.

22 Smart, J.D., 2005. The basics and underlying mechanisms of mucoadhesion. *Adv. Drug*  
23 *Deliv. Rev.* 57, 1556–1568.

24 Sogias, I.A., Williams, A.C., Khutoryanskiy, V. V, 2008. Why is chitosan  
25 mucoadhesive? *Biomacromolecules* 9, 1837–1842.

- 1 Sosnik, A., das Neves, J., Sarmiento, B., 2014. Mucoadhesive polymers in the design of  
2 nano-drug delivery systems for administration by non-parenteral routes: A review.  
3 Prog. Polym. Sci. 39, 2030–2075.
- 4 Thomas, R.C., Chung, P.E., Modi, S.P., Hardy, J.G., Schmidt, C.E., 2016. Sacrificial  
5 crystal templating of hyaluronic acid-based hydrogels. Eur. Polym. J. 87, 487–496.
- 6 Ugwoke, M.I., Agu, R.U., Verbeke, N., Kinget, R., 2005. Nasal mucoadhesive drug  
7 delivery : Background , applications , trends and future perspectives Adv. Drug  
8 Deliv. Rev. 57, 1640–1665.
- 9 Van Tomme, S.R., Storm, G., Hennink, W.E., 2008. In situ gelling hydrogels for  
10 pharmaceutical and biomedical applications. Int. J. Pharm. 355, 1–18.
- 11 Withers, C.A., Cook, M.T., Methven, L., Gosney, M. a, Khutoryanskiy, V. V, 2013.  
12 Investigation of milk proteins binding to the oral mucosa. Food Funct. 4, 1668–74.
- 13 Wu, C., Gaharwar, A.K., Chan, B.K., Schmidt, G., 2011. Mechanically Tough Pluronic  
14 F127 / Laponite Nanocomposite Hydrogels from Covalently and Physically Cross-  
15 Linked Networks. Macromolecules. 44, 8215–8224.
- 16 Yun Chang, J., Oh, Y.K., Soo Kong, H., Jung Kim, E., Deuk Jang, D., Taek Nam, K.,  
17 Kim, C.K., 2002. Prolonged antifungal effects of clotrimazole-containing  
18 mucoadhesive thermosensitive gels on vaginitis. J. Control. Release 82, 39–50.  
19  
20

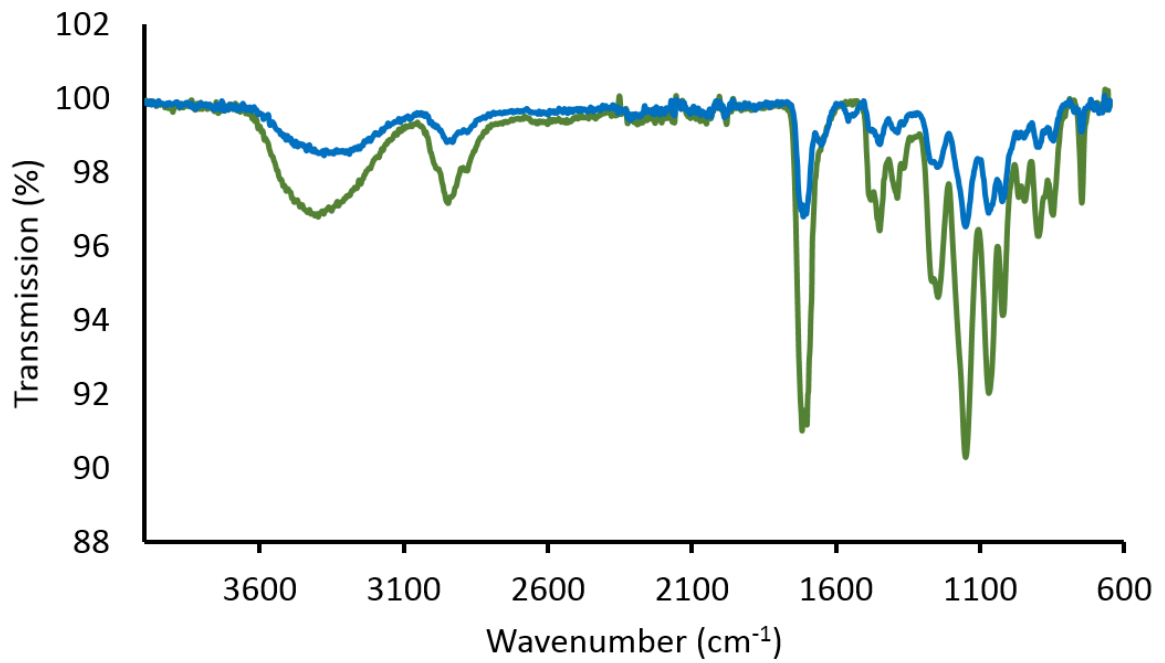


1

2

3 Figure 1. ATR-FTIR spectra of 100 % HEMA (green) and HEMA:AGA (80:20 mol%)

4 (blue) hydrogels after drying.

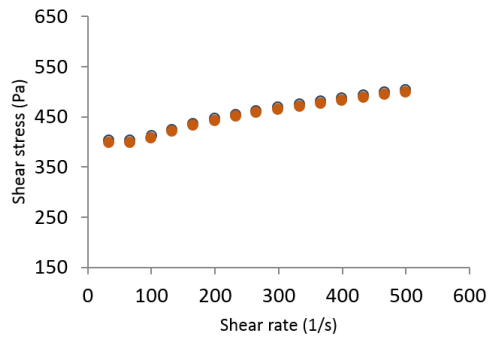


5

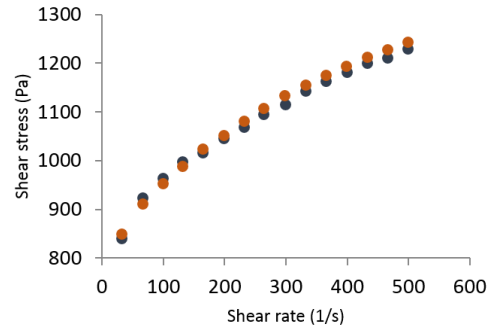
6

1  
2 Figure 2. Flow rheograms for a) pluronic, b) F1, c) F2, and d) F3 formulations with  
3 (orange circles) and without (black circles) 1 mg/g FITC-dextran (10 kDa). Data  
4 presented as mean  $\pm$  standard deviation (N = 3).

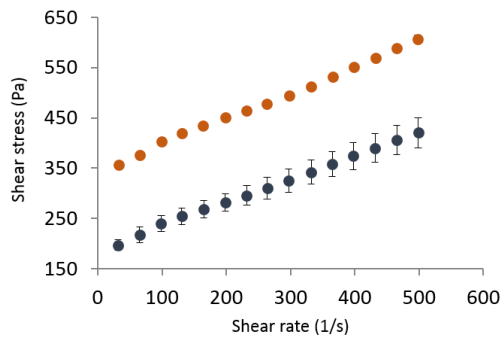
a)



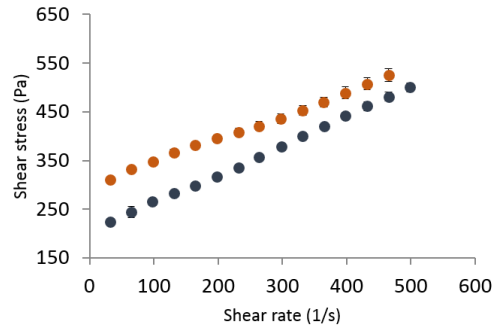
b)



c)



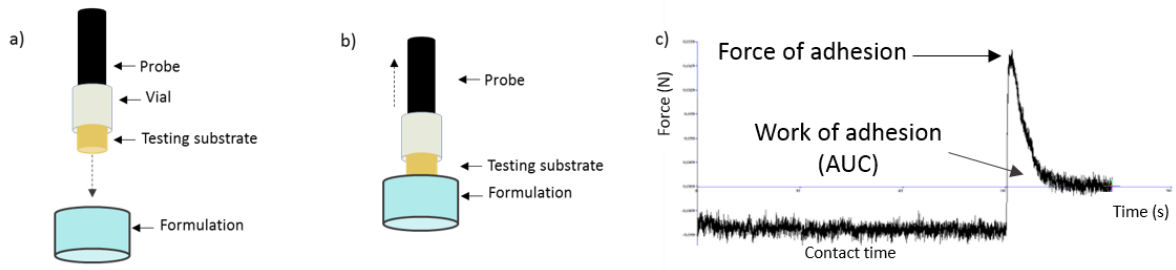
d)



5

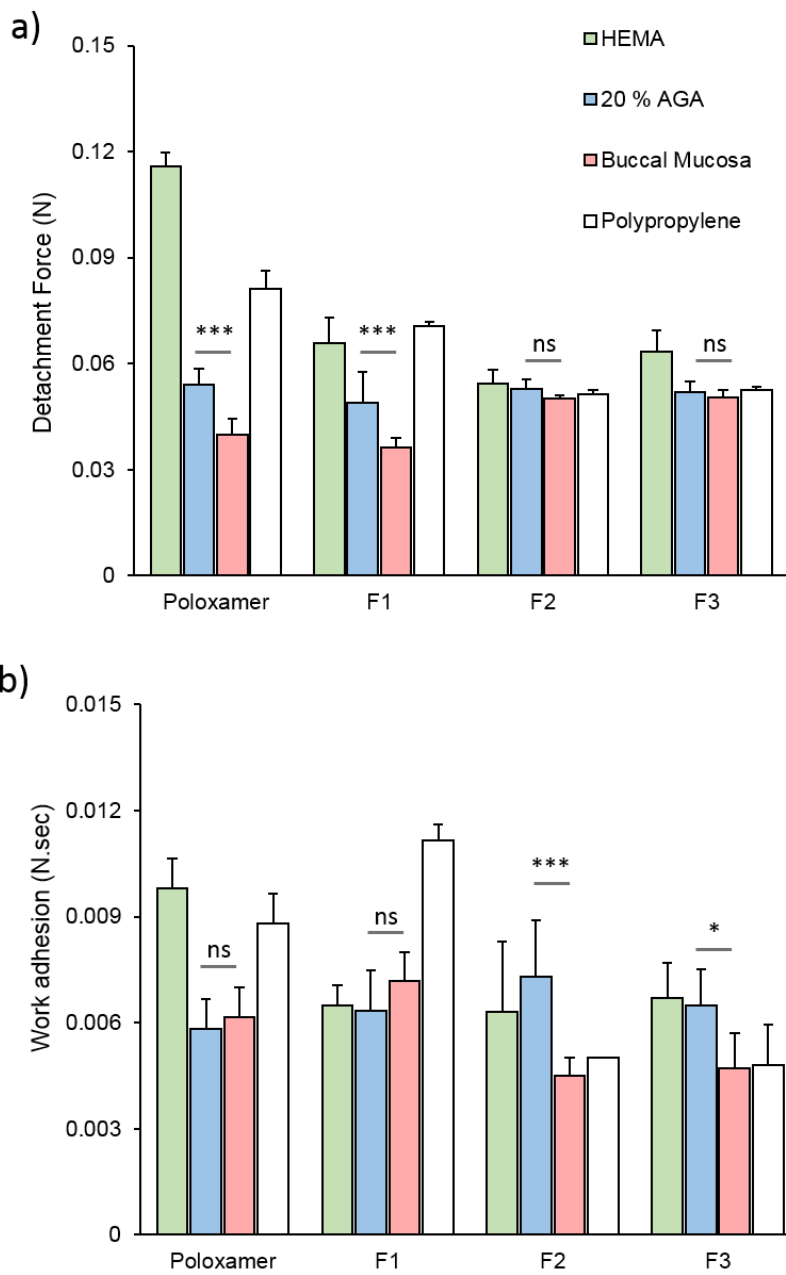
6

1  
2 Figure 3. Modifications made to a texture analyser allow for testing substrates  
3 (hydrogels, mucosa, and polypropylene) to be pressed against thermogelling semisolid  
4 formulations (a). Removal of the testing substrate from the formulations (b) gives a  
5 force-time curve from which values of force and work of adhesion can be measured (c).



6  
7

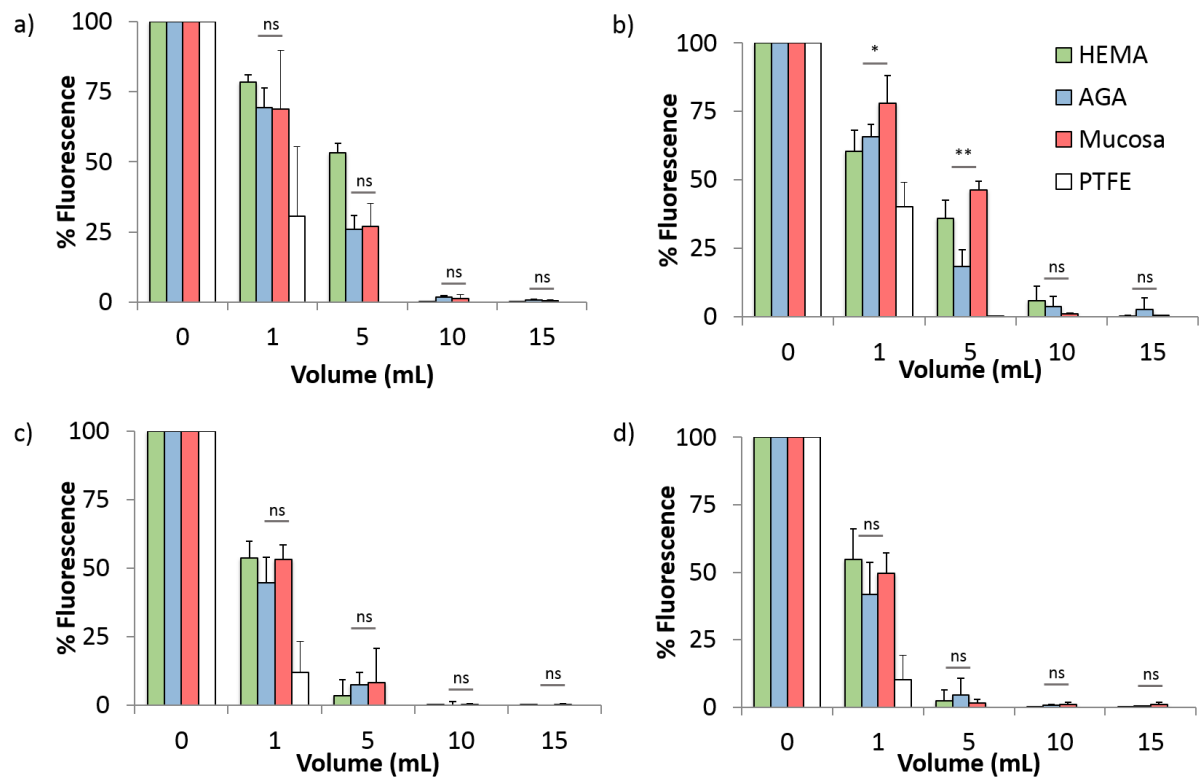
1  
 2 Figure 4. Force (a) and work (b) of adhesion of thermogelling formulations to  
 3 hydrogels, buccal mucosa, and polypropylene, as determined by texture analysis. Data  
 4 represented as mean  $\pm$  standard deviation (N = 6). “ns” designates no statistical  
 5 significance, \* indicates  $p < 0.05$ , and \*\*\* indicates  $p < 0.001$ , using two-way ANOVA  
 6 with Bonferroni post-hoc.



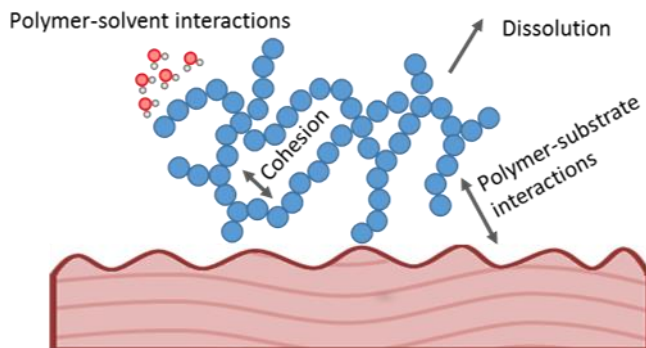
7

8

1  
 2 Figure 5. The retention of FITC-dextran labelled pluronic (a), F1 (b), F2 (c), and F3 (d)  
 3 formulations on 100 mol% HEMA, 20 mol% AGA, buccal mucosa, and PTFE, using a  
 4 flow-through method. Data presented as mean  $\pm$  standard deviation (N = 3). “ns”  
 5 designates no statistical significance, \* indicates  $p < 0.05$ , and \*\* indicates  $p < 0.01$ ,  
 6 using two-way ANOVA with Bonferroni post-hoc.



7  
 8 Figure 6. An overview of the factors dictating the mucoadhesion of semi-solids.



9  
 10  
 11