In vitro evaluation of some parameters involved in mucoadhesion of aqueous polymeric dispersions

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ABSTRACT

Context. The mucoadhesive formulations are constantly developing due to their relevance in the drug delivery to various districts of the organism.

Objective. The purpose of this study was to find a direct link between physicochemical properties of the polymers and their adhesive ability in order to offer guidelines for the development of mucoadhesive semisolid formulations.

Materials and methods. Twelve polymers were dispersed in water and characterized with regard to their mucoadhesiveness; apparent viscosity; contact angle on solid surface; hydrodynamic diameter of their molecules. The adhesive properties were related to the other measured parameters.

Results and discussion. The data seem to indicate the existence of an optimal value of viscosity, around 5-6 Pa s, to obtain the highest mucoadhesiveness of the polymeric dispersions. Regarding the molecular sizes, the best mucoadhesive performances seem to be given from polymers with a hydrodynamic diameter lower than 350-400 nm. In any case, the ability to wet the surface by the polymeric dispersion seems to play an essential role in bioadhesion process, capable of strongly limiting the phenomenon.

Conclusions. Performing simple in vitro measurements it seems possible to identify the best polymeric concentration to obtain a semisolid formulation with good mucoadhesive properties.

INTRODUCTION

In recent years, the mucoadhesive polymeric formulations have received constant attention for their relevance as delivery systems. Bioadhesive dosage forms have been developed for buccal, nasal, ocular, vaginal, rectal and oral application (1).

All bioadhesive carriers require polymers, either synthetic or natural, which have the ability to adhere to biological surfaces (2). In particular, in semisolid/liquid formulations the concentration of the polymer is a peculiar factor in the development of a strong adhesive bond (3). Thus, the mucoadhesive properties are related to the capacity of polymers to undergo hydration, and each polymer requires optimum water content for maximal mucoadhesion (4). However, other factors also play an important role in the adhesive bond strength as the molecular weight of polymer and a good wetting of the substratum by formulation (5).

Aim of the present study was to find a direct link between physicochemical properties of the polymer and its adhesive ability. For this purpose a large number of polymers was investigated and chosen among those most frequently used in the pharmaceutical field, making sure to include a wide representation even if limited to the traditional first-generation: hydrophilic macromolecules containing numerous hydrogen bond forming groups. They are also called 'wet' adhesives in that they are activated by moistening and have the characteristic of adhering non-specifically, that is the adhesion may occur at sites other than those intended (6,7). First-generation mucoadhesive polymers are usually divided in three main subsets according to their origin, natural, semi-synthetic or synthetic, including non-ionic and ionic structures. Typical examples are carbomers, alginates and the cellulose derivatives. These have been largely used as they are available 'off-the-shelf' with regulatory approval, but in the last few years, new materials have been

investigated that allow specificity, or prolong and strengthen the mucoadhesion process. Novel second-generation mucoadhesive polymers are sometimes represented by existing modified polymers, while in other cases they are new materials. Some types, like lectins, are able to bind directly to mucosal surface structures circumventing the major disadvantage of the traditional adhesives and being less susceptible to mucus turnover rate. Positively charged and second-generation polymers were not included in this study because they are still hardly present in commercial formulations.

The study was aimed at finding correlations between the mucoadhesive properties of aqueous dispersions of polymers and some their easily determinable parameter, such as apparent viscosity, spreading properties, and molecular size of the polymers themselves, in order to guide the pharmaceutical technologist in the development of bioadhesive semisolid dosage forms.

MATERIALS AND METHODS

Materials

Xanthan gum, XG (Xantural® 75; CP Kelco U.S. Inc., Atlanta, GA); tamarind gum, TG (Glyloid®; Dainippon Sumimoto Pharma Co. Ltd, Osaka, J); polyvinyl alcohol, PVA (MW = 108,000 Da; 99.7 mol% hydrolyzed; Polysciences Europe GmbH, Eppelheim, D); sodium alginate, ALG (Protanal® LF10/60FT; FMC BioPolymer, Wallingstown, IRL); hydroxypropylmethylcellulose, HPMC1 and HPMC2 (Methocel® E15LV and K4M; Colorcon Ltd., Dartford, UK); carboxymethylcellulose, CMC1 and CMC2 (Blanose® 7LF and 7HF; Hercules, Wilmington, DE); hydroxyethylcellulose, HEC (Natrosol® 250HX; Ashland Inc, Covington, KY); polyacrylic acid, PAA1 and PAA2 (Carbopol® 940 and 971P

NF; Lubrizol Inc., Cleveland, OH); polyethylene oxide, PEO (Sentry® Polyox Resin WSR-301 NF; Dow Chemical Co., Union Carbide Benelux, Antwerpen, B); hog gastric mucin, HGM (Carl Roth GmbH + Co. KG, Karlsruhe, D).

Polymer dispersion

The polymeric dispersions used throughout the study were generally prepared by stirring appropriate amounts of polymer at room temperature in ultra-pure water (MilliQ, Millipore). PVA dispersions were obtained by stirring at 80 °C or by autoclaving at 121 °C. The concentrations of the polymeric dispersions investigated for mucoadhesion, viscosity and contact angle are listed in Table I. The range of concentrations was chosen in order to highlight the fluctuations in the adhesiveness of the product.

Mucoadhesion

Turbidimetric titration

Appropriate amounts of HGM were dispersed in ultra-pure water to form a colloidal dispersion (1 mg/ml), which was sonicated for 15 min and subsequently centrifuged at 500 rpm for 10 min. The supernatant was retained and used for the experiments. Such concentration of mucin allowed obtaining dispersions with low initial values of absorbance at 550 nm. Freshly prepared mucin dispersions were always used in the experiments.

Mixtures containing fixed amount of mucin dispersion and polymer dispersion (0.5 mg/ml) were prepared by gently stirring for 1 h and underwent turbidimetric analysis by an UV/vis spectrometer (UV-2101 PC, Shimadzu, Japan) at 550 nm. Polymer/mucin weight ratios ranged from 0.002 to 0.225. Three titrations were made for each polymer.

Tensile (detachment) method

The mucoadhesive properties of the aqueous polymeric dispersions were evaluated by measuring their work of adhesion (W) on a mucous surface by tensile test. The apparatus consisted of a testing cell (two cylindrical sections, upper and lower) connected to a tensile apparatus fitted with force and elongation transducers, whose output was fed to a computer equipped with data acquisition software (Handyscope2, TiePie Engineering, The Netherlands) (8). The mucous layer consisted of 0.125 ml of a 28.0 % w/w aqueous dispersion of HGM uniformly spread on wet filter-paper disks of 12 mm diameter tightly secured to both cell sections. Following application, the mucin layers were superficially dried for 5 min by cold air blown and then 0.050 ml of the semisolid sample under study were thinly layered onto upper mucous surface. The lower cell section was slowly raised and put into contact with the sample; after 2 min of contact the cell sections were moved away at constant speed (1.25 mm/min) up to complete separation. Analysis of the resulting force *vs*. distance curves (work of adhesion, W) was performed using Prism® software (GraphPad Software Inc., La Jolla, CA). All W values were normalized with respect to the adhesion area; eight repetitions were made for each polymer.

Rheological measurements

The rheological behavior of the polymeric dispersions was determined at 25 °C by a Rheostress RS 150 apparatus (Haake, Germany) equipped with coaxial cylinders (Z40 and Z41) and cone-plate (C60/4-P61), at shear rates ranging from 0 to 500 s^{-1}. All polymeric dispersions exhibited a pseudoplastic flow, described by the Ostwald-de Waele power law: $\tau = KD^N$ and their apparent viscosity, η', was calculated for $D = 1\ s^{-1}$, i.e. the value of

the viscosity plateau when shear rate approaches zero. This calculation takes in account the weaker interactions of the macromolecules that are significant when the fluid is at rest, such as during the onset of adhesive bonds.

Dynamic light scattering

Dynamic light scattering (DLS) measurements were conducted at 25 °C by Submicron Particle Size (N4 Plus, Coulter, FL) equipped with a He-Ne laser (output power 10 mW at wavelength 632.8 nm). The experiments were carried out at 90° and the CONTIN fit was performed to obtain the hydrodynamic diameter distributions.

Samples were dispersed in ultra-pure water (0.5 mg/ml) at room temperature under stirring for 48 h. In order to eliminate the effect of dust particles, a 0.45 μm membrane was used to filter the samples and the diluent water into a dust-free cell before the measurements. A few drops of the dispersion were added to diluent water contained in an appropriate cuvette. The intensity measured was below 10000 counts/sec for diluent water and between 5 10^4 and 1 10^6 counts/sec for the samples. Two runs of triplicate measurements were made.

Contact angle

The spreading coefficient of all the polymeric dispersions was analyzed by contact angle measurements.

An optical contact angle-measuring instrument (OCA 15, DataPhysics Instrument GmbH, Germany) was used to determine static contact angles of the polymeric dispersions on a microscope slide. The system consisted of a high-resolution CCD video camera and a

six-fold power zoom lens with integrated fine focusing; the images were recorded and analyzed by SCA 20 software.

The sessile drop method was used: briefly, it consists of placing a known volume of dispersion on the glass surface. When the spreading of the droplet attains an equilibrium state the contact angle is determined. Ten measurements were made for each polymeric dispersion.

Data analysis

The correlation degree between two variables was evaluated as Pearson Product Moment Correlation (Pearson's r). Data analysis (area under the curve; linear regression; 95% confidence band of the best-fit line) was performed by Prism software (GraphPad Software Inc., S. Diego, CA).

RESULTS AND DISCUSSION

Mucoadhesion

It is known that turbidimetric titration is useful to study polymer-mucin interactions (9-11) so this technique has been used as a simple method to monitor the mucoadhesive interactions.

The addition of polymers to mucin dispersion was accompanied by an increase in solution turbidity until a maximum value, corresponding to different polymer/mucin weight ratios, after which the turbidity decreases gradually for further polymer addition. Increased turbidity as a result of mixing of mucin and polymer is related to aggregation

phenomena between polymer and mucin due to strong interactions between the two components. The peak of the titration curve indicates that all the possible sites of interaction between the polymer and mucin have been saturated, the precipitation of the aggregates is complete and then the stoichiometric ratio has been reached; a further addition of polymer is accompanied by a decrease in turbidity for dilution of the dispersion. An example of titration curve is illustrated in Figure 1 and the polymer/mucin stoichiometric ratios are listed in Table II.

Table I summarizes the results of the mucoadhesion tensile tests, as mean values of the work (W, J/cm^2; n = 8) required for detachment of two hydrated mucin surfaces between which the polymeric dispersions were placed.

The majority of the methods used to assess the adhesive interactions between a mucous substrate and a semisolid formulation employs a tensile approach (e.g. 12-16). In these cases, however, it is important to investigate very well whether the separation of the system adhesive/mucus takes place inside one of the two components (cohesive failure) or at the interface between the two (adhesive interaction). For this purpose the surface of separation was carefully inspected during and after each measurement to verify where the fracture occurred when the detachment force was applied. Visual inspection pointed to the occurrence of separation at lower mucin/polymer interface and in a clear-cut manner. The upper interface is probably stronger for the pressure exerted on the polymer during the distribution on the mucous surface, as a kind of preload and friction during contact (17-19). Although several authors have demonstrated the possibility of breakage within the polymeric dispersion (cohesive failure) (20-22) the tensile test is still considered useful for practical purposes from the researchers for determining a value of bioadhesion and to compare it with that obtained by other methods (15,23-26). In our experiments, during the tensile test we always observed a clear-cut break of the mucin/polymer system without

formation of threads, which would mean a lengthening of the polymeric material and then a cohesive failure: the detachment appeared to start from the edges of the interface and to advance towards the center, often leaving a typical fingering on the mucous surface. The fingering may produce a force-distance curve with a slightly different shape, but the area included approximates the calculated work of adhesion very well (22). The resulting force/distance curve showed a final segment not too elongated, with a rapid drop of force to the point of complete detachment, confirming a clear-cut break of the mucin/polymer system. The analysis of the shape of the force/distance curve is of primary importance to assess the reliability of the results and many researchers have studied this topic (e.g. 18,22). In some instances the final segment of the curve is highly elongated and often characterized by several peaks and valleys; it is reasonably considered that this behavior reflects the tendency of the polymer to be elastic and to detach in small portions, overcoming the cohesive forces (18).

Finally, taking into account the cohesiveness of the mucous substrate, if the failure had taken place within the mucin network, the measured W values would have to be the same in all the experiments. Therefore, we consider the values obtained from the tensile test an acceptable approximation of the mucoadhesive properties of the polymeric dispersions.

Observing our data, we note that the W values increase until a maximum followed by a decrease with increasing polymer concentration. For each polymer, the maximum work of adhesion (W_{max}) measured is in close correlation (Pearson's r = 0.675) with the polymer/mucin weight ratio at the stoichiometric point observed by turbidimetric titration (Figure 2), confirming that all the adhesive sites were saturated and no further interactions were possible between the molecules. The good agreement between the two series of measurements confirms, once again, their reliability.

Only data related to XG dispersion deviate from the general trend; the work of adhesion measured by tensile method seems too low with respect to interactions observed by turbidimetry.

The correlation between W_{max} and MW, as illustrated in Figure 3, demonstrates that the mucoadhesiveness of the dispersions tends to decrease by increasing the polymer MW (Pearson's r = 0.700) although the polymers studied have an average MW in the ideal range to obtain the highest mucoadhesion (10^4 to 10^6 Da) (27,28) and all W_{max} values are quite similar, in a close range.

Also in this case XG dispersion deviates from the general trend because of its low value of W_{max}.

Rheological measurements

Apparent viscosity values of each polymeric dispersion are listed in Table I. It is noteworthy that, while the concentrations at which W_{max} develops are highly variable from polymer to polymer, the η' value corresponding to W_{max} is instead in a very narrow range. This close dependence of mucoadhesion by the viscosity of polymeric dispersion is well illustrated in Figure 4: W_{max} values are given by dispersions with an apparent viscosity ranging from 4.9 to 6.5 Pa s in concentrations between 0.08 and 14.00 % w/w.

Many researchers have investigated the relationship between the bioadhesive strength of polymeric dispersions and their rheological characteristics. While direct correlations between the rheological parameters and the adhesion work were not found by some authors (12,29), others pointed out a certain relationship between the bioadhesive performance and the flow properties (15,13), both taking in account the Loss Factor (tan δ) and the viscosity (30,31). In particular, Shin and co-workers found that the increase up to

2.0 % of the polyacrylic acid concentration in gels caused increased viscosity and bioadhesiveness, but above this limit the bioadhesive property did not increase further. Unfortunately, the authors tested only one concentration higher than 2.0 % and therefore were not able to check the progress of bioadhesion on varying this parameter. It is particularly interesting to note that the highest bioadhesive strength of the gels was measured for polyacrylic acid dispersion with viscosity ranging from 4 to 6 Pa s. The dependency of the adhesive properties on viscosity of a semisolid formulation is therefore not clearly fixed up and still debated by researchers: the results of this work seem at least set an optimal value of viscosity of polymeric dispersion to which the highest mucoadhesiveness can be obtained. This statement may also be reflected in the rheological analysis of some commercial preparations known for their mucoadhesive properties, such as Gelclair®, Miphil® or Lacrinorm®. These products show apparent viscosity ranging from 5.2 to 5.9 Pa s, when measured under the conditions applied in this study (data not published).

Dynamic light scattering

The results obtained from the analysis by dynamic light scattering (DLS) as hydrodynamic diameter for the different polymers studied are reported in Table III. Some polymers showed bimodal size distribution, so in the table are listed the average sizes of both populations. In the Figure 5 the hydrodynamic diameter measures of the polymers according to their average molecular weight cited in literature or in manufacturers' technical data sheet are illustrated (32-37). The two series of values are rather well correlated, with Pearson's r = 0.874, if the PEO smallest population and ALG and PAA2 largest population (gray circles) are excluded from the calculation; when all data are

considered the correlation drops to a Pearson's r value of 0.324. PEO seems to fit better with the other measures when its largest population is selected while ALG and PAA2 when the smallest sizes are taken in account. This could be due to several factors: PEO resins have a very long linear chain structure, so as to present a high average MW but a size smaller than macromolecules with branched chains (32). Anyway, such a structure allows PEO chains to easily form an interpenetrating network with mucus to reach remarkable level of adhesion in line with its MW (4). For ALG and PAA2 the phenomenon is not explained by the authors, and may be the subject of further interesting investigation: their structures are similar to those of other polymers under study and then it does not seem to be a plausible cause.

Observing the data collected, it can be noted that the mucoadhesive properties of the polymers under study tend to decrease with increasing the hydrodynamic diameter (Figure 6; Pearson's r = 0.345), also if the correlation is not so good. It is evident how PAA2 (large population) and PEO (small population) are the cause of this poor correlation, probably still linked to their not defined molecular sizes, as previously discussed. This trend seems indicate that there is a threshold value of hydrodynamic diameter beyond which a reduction of mucoadhesive properties happens; this value might be identified about 350-400 nm. Among tested materials PAA1 and PEO showed the largest molecular sizes, 469.8 and 1022.9 nm respectively, and consequently a lower mucoadhesiveness, with W_{max} values below $5 \cdot 10^{-5}$ J/cm^2, as expected on the basis of our experimental data. Besides, it is known that the size as well as the spatial conformation of the molecule considerably influences the formation of bioadhesive bonds (27,28). Moreover, the mobility of macromolecular chains appears to be an important parameter to take into account for predicting the adhesive ability of polymers: molecules with a large hindrance or low mobility lose their ability to diffuse

and interpenetrate through the glycoprotein network of the mucous layer leading to a poor coupling of the binding sites.

Again, XG dispersion is the one that is farthest from this pattern, showing a too low work of adhesion in relation to its molecular size.

Contact angle

The values of contact angle obtained from the polymeric dispersions show, for all substances tested, a linear correlation with the concentration of the polymer indicating an apparent pattern to lower wettability by dispersions at higher concentration. Such behavior is certainly related to the increase in the viscosity of the dispersions linked to the increase of the concentration of polymer. The dependence of the final spreading on the viscosity of dispersion is an aspect first of all related to the non-Newtonian behavior of polymer flow and can be mainly attributed to viscous dissipation effects (38,39). Also, according to the physics of the adhesion of a liquid to a plane, nonporous, solid surface, the value of work of adhesion for these dispersions varies as a function of contact angle value producing a parabolic curve with a maximum at the value of W_{max} (40). This trend is also maintained correlating W_{max} values with the relevant measurements of contact angle determined for the same polymer concentration (Figure 7). The graph highlights that high contact angle values are witnessing a decrease in adhesiveness, with an optimal value in the range of 40-70 deg, and indicates that the wettability by the polymeric dispersion has a primary importance to obtain high adhesion values (41). After all, the wetting theory is perhaps the oldest established theory of adhesion and it is considered by someone the one with the greatest weight: for adhesion to occur the adhesive must wet the substratum and the better ability of polymers to spread on the surface is usually associated with excellent

mucoadhesive performance (5).

XG dispersion shows the highest contact angle value (78.48±1.38 deg), this time in line with other data. This result can explain the low adhesive performance of XG, which seemed not related with the other measured parameters, confirming the importance of the wettability in bioadhesion process. The spreading and retaining of polymeric dispersions on mucosa surfaces depend on the equilibrium of surface energies, along with the rheology of the liquid (7). In the data showed the rheological factor was cancelled because similar for all polymer dispersions at W_{max} and then the dependence on the wettability is pointed out becoming predominant.

CONCLUSION

A considerable amount of studies on mucoadhesion has been carried out over the years, but despite this, there is still a need for further investigations. Even though a broad understanding of the properties that favor mucoadhesion has been reached, it is still difficult to systematize them, since different study methods has been used and different aspects of the mucoadhesive process investigated; besides the mechanism responsible for the mucoadhesion can also be dependent on the formulation type. On the other hand, there is no doubt that mucoadhesion is an important factor to consider when choosing a formulation.

To date it is known that certain properties of the polymers such as charge, hydrophilicity, molecular weight and even the peculiarities that they provide to the formulations can affect the formation and strength of adhesive bond.

The concentration of active polymer in semisolid formulations has always been considered a factor influencing the strength of mucoadhesion essentially related to the

number of polymeric chains available for interactions (3,42,43) or without the authors gave detailed interpretations of the phenomenon (20,23). The data produced in this paper seem to indicate that the concentration of polymeric dispersion is significant only because related to viscosity that this imparts to the formulation. In our opinion, the relationship found between the viscosity of polymeric dispersion and the mucoadhesiveness might facilitate the optimization of semisolid formulations, from the point of view of the mucoadhesive performance, leading to the development of more effective mucoadhesive semisolid dosage forms.

An element worthy of consideration is also the molecular size of the polymer, although from the data of this investigation its role in mucoadhesion process does not seem clear: this parameter comes out from MW and conformation of chains of the polymer and must be a limit to consider. The investigation of this aspect will be the subject of future experiments.

In any case, the ability of the polymeric dispersion to wet the surface plays an essential role in bioadhesion and it can strongly limit the phenomenon.

Of course, like all in vitro tests, these approaches can be useful as simple experiments carried out to select the final formulations to submit to in vivo tests. In fact, it is commonly accepted that the in vivo behavior of a mucoadhesive system is strongly influenced by the boundary conditions of the mucosa with which the system is in contact, such as pH, roughness, washing away, which are not easily reproducible in vitro, even using native mucus or entire mucosal tissue.

Declaration of interest

The authors report no declarations of interest.

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Table I - Physico-chemical parametrs of polymeric dispersions under investigation

Polymer	**Concentration** (% w/w)	**Work of adhesion** (W, J/cm^2 10^5±S.E.)	**Apparent viscosity** (η', Pa s)	**Molecular weight** (Da)
XG	0.50	1.832±0.103	0.429	2 10^6
	1.00	2.811±0.169	1.280	
	1.50	3.087±0.238	3.771	
	2.00	*3.972±0.308*	*6.506*	
	2.50	3.303±0.271	12.562	
	3.00	3.120±0.280	16.523	
	5.60	2.988±0.327	35.270	
	7.00	2.690±0.251	51.501	
TG	2.50	2.726±0.161	2.974	6 10^5
	2.60	*6.322±0.427*	*5.613*	
	2.70	5.028±0.326	8.157	
	2.80	3.540±0.195	8.740	
PVA	2.00	2.667±0.478	0.003	1.08 10^5
	4.00	3.283±0.085	0.030	
	7.00	3.598±0.320	0.219	
	10.00	4.656±0.311	0.424	
	13.00	*5.288±0.434*	*5.208*	
ALG	3.00	4.920±0.132	2.307	8.5 10^5
	3.20	4.261±0.396	4.380	
	3.50	*5.208±0.246*	*6.023*	
	3.70	3.850±0.259	7.960	
	4.00	4.976±0.119	9.491	
HPMC1	1.30	4.578±0.169	0.004	6.03 10^4
	3.20	5.304±0.318	0.030	
	6.50	5.424±0.342	0.281	
	7.50	5.676±0.412	0.438	
	12.00	5.983±0.285	4.120	
	14.00	*6.424±0.161*	*6.069*	
HPMC2	1.50	2.075±0.103	1.971	3.9 10^5
	1.70	3.682±0.215	2.830	
	1.90	4.123±0.185	5.370	
	2.00	*5.582±0.234*	*5.753*	
	2.10	1.616±0.038	6.309	
	2.70	1.313±0.119	15.031	
	3.00	1.297±0.033	31.622	
CMC1	7.00	5.073±0.350	3.177	9 10^4
	8.00	5.159±0.201	5.309	
	8.05	*5.728±0.289*	*6.516*	
	8.10	5.196±0.241	7.129	
	8.50	4.865±0.390	9.354	
	9.00	4.734±0.259	12.794	
CMC2	1.20	3.957±0.145	5.383	1 10^6
	1.25	*5.036±0.223*	*6.310*	
	1.30	4.411±0.124	6.966	
	1.50	3.786±0.096	8.790	
	2.00	3.539±0.284	13.900	
HEC	1.90	3.533±0.333	4.550	1 10^6
	2.00	4.166±0.258	5.728	
	2.05	*5.620±0.391*	*6.339*	
	2.10	4.109±0.155	6.745	
	2.30	4.256±0.289	8.872	
	2.50	3.747±0.158	12.560	

PAA1	0.02 0.035 0.05 0.055 *0.08* 0.10 0.20 0.25	3.539±0.302 4.107±0.295 4.157±0.136 4.227±0.426 *4.742±0.561* 4.028±0.225 3.839±0.314 3.014±0.420	0.005 0.037 0.254 0.429 *5.976* 13.021 35.975 46.251	$4\ 10^6$
PAA2	0.30 *0.40* 0.50 0.55 0.80	4.060±0.372 *5.227±0.435* 4.708±0.210 3.289±0.258 3.613±0.244	3.589 *4.977* 5.623 5.943 10.544	$1.25\ 10^6$
PEO	1.70 *1.80* 1.90 2.00 2.10 2.20	4.653±0.193 *4.750±0.355* 4.681±0.219 4.278±0.121 4.382±0.226 4.340±0.207	4.214 *4.943* 5.333 6.166 7.211 7.464	$4\ 10^6$

Table II - Polymer/mucin stoichiometric ratios calculated by turbidimetric titration.

Polymer	Polymer/mucin weight ratios
XG	0.0706
TG	0.1010
PVA	0.0814
ALG	0.0102
HPMC1	0.1020
HPMC2	0.0050
CMC1	0.0880
CMC2	0.0316
HEC	0.0550
PAA1	0.0376
PAA2	0.0102
PEO	0.0376

Table III - Molecular sizes obtained by dynamic light scattering (DLS). For the polymers showing bimodal size distribution average sizes of the two populations are reported.

Polymer	**Hydrodynamic diameter** (nm±S.E.)
XG	203.11 ±30.41
TG	77.82 ±4.14
PVA	12.72 ±1.41 132.90 ±25.43
ALG	100.92 ±5.59 531.62 ±58.77
HPMC1	58.03 ±7.34
HPMC2	69.98 ±5.94
CMC1	18.86 ±3.09
CMC2	110.00 ±23.04
HEC	39.28 ±6.49 193.46 ±42.75
PAA1	469.80 ±78.17
PAA2	304.81 ±30.19 1771.70 ±260.87
PEO	32.92 ±8.94 1022.90 ±226.19

Figure captions

Fig. 1 - Turbidimetric titration of 1 mg/ml mucin by polymer dispersions. The arrows indicate the maximal value of turbidity corresponding to polymer/mucin stoichiometric ratio.

Fig. 2 - Correlation between the two mucoadhesion measurement series: W_{max} obtained by tensile method and polymer-mucin ratio by turbidimetry. Dashed lines = 95% confidence bands of regression line; Pearson's r = 0.675. Gray circle = excluded from the calculation of r

Fig. 3 - Correlation between maximal work of adhesion and molecular weight of polymers under study. Dashed lines = 95% confidence bands of regression line; Pearson's r = 0.700. Gray circle = excluded from the calculation of r

Fig. 4 - Apparent viscosity (circles) and concentration (triangles) values of polymeric dispersions that produce W_{max}

Fig 5 - Relation between size and weight of polymeric molecules under investigation. Dashed lines = 95% confidence bands of regression line; Pearson's r = 0.874. Gray circles = excluded from the calculation of r

Fig 6 - Correlation between maximal work of adhesion and molecular size of polymers under investigation. Dashed lines = 95% confidence bands of regression line; Pearson's r = 0.345. Open circles = largest population for polymers with bimodal size distribution. Gray circle = excluded from the calculation of r. For clarity the label only for the more interesting points was reported.

Fig 7 - Correlation between maximal work of adhesion and contact angle of polymeric dispersions studied

Figure 1

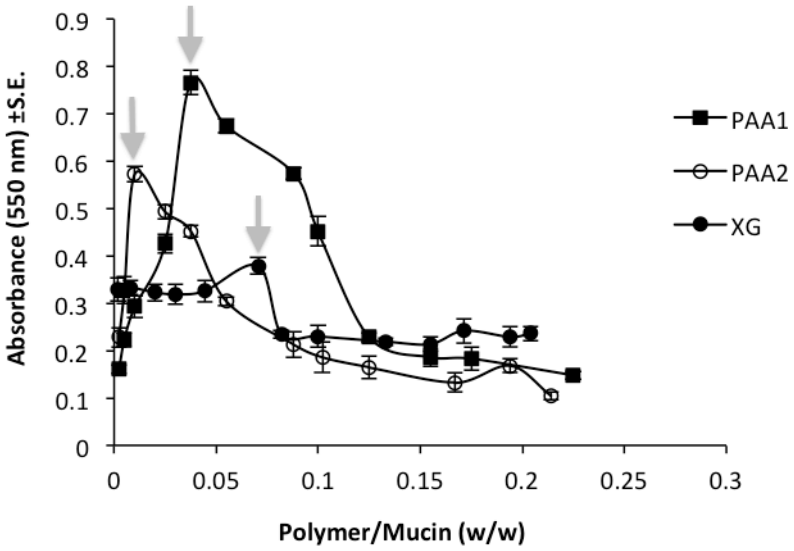


Figure 2

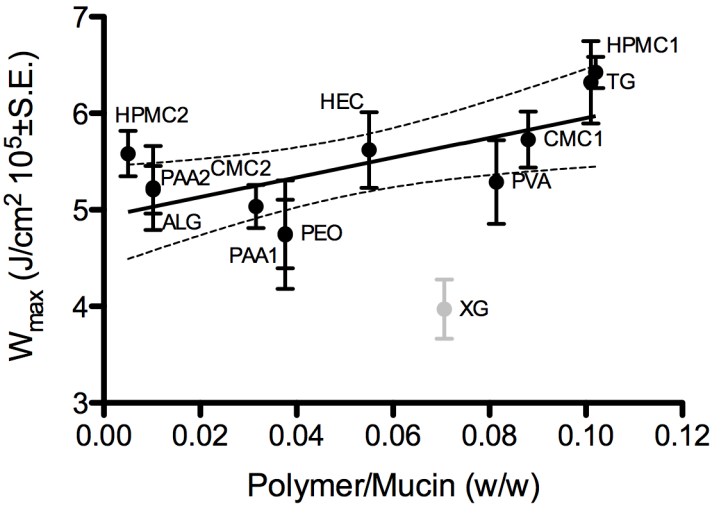


Figure 3

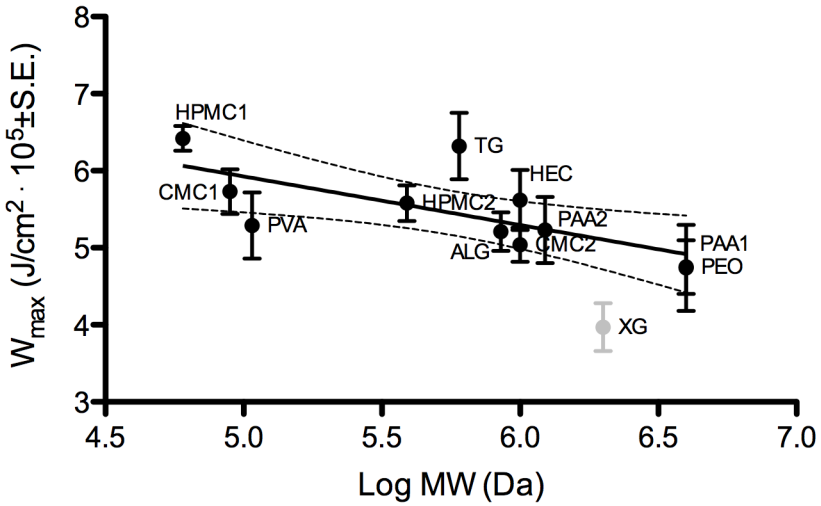


Figure 4

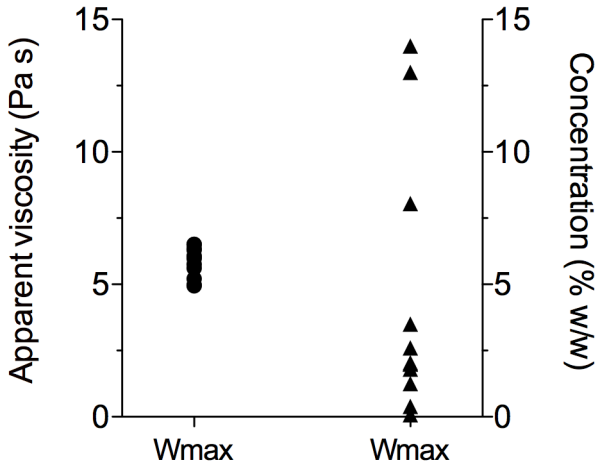


Figure 5

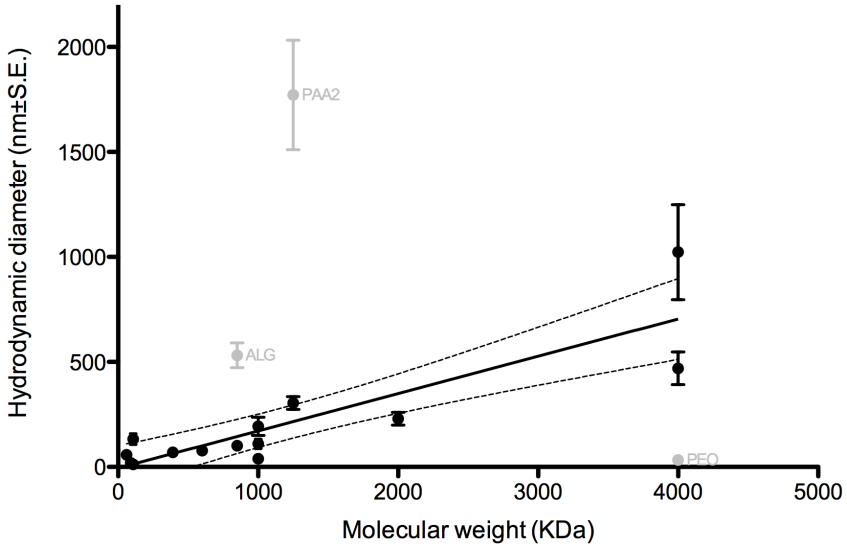


Figure 6

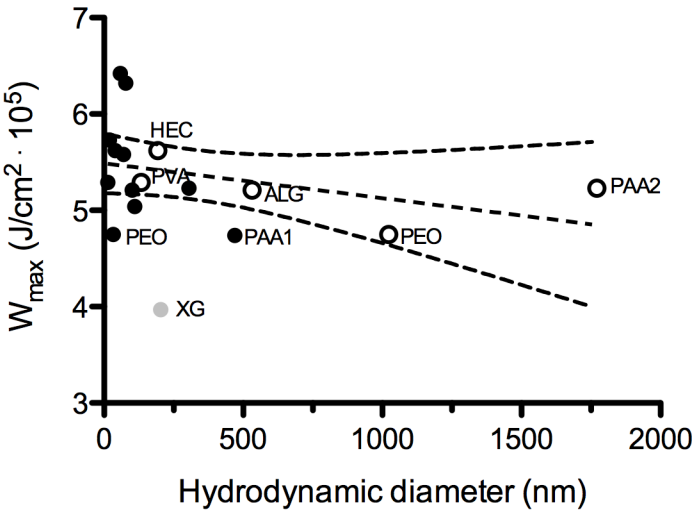
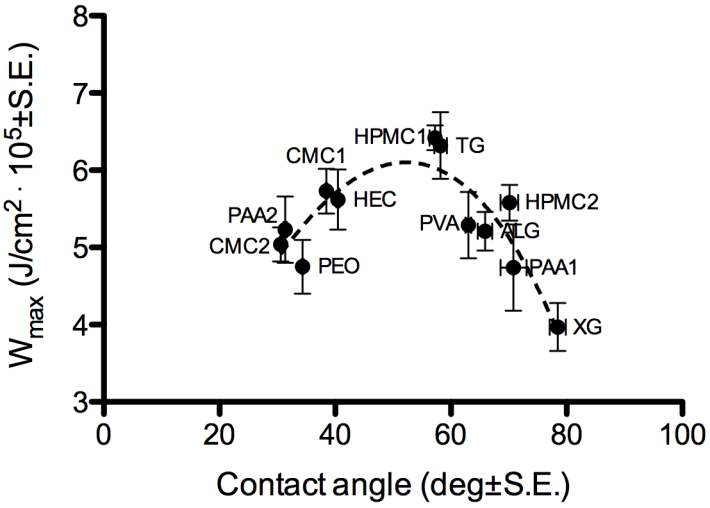


Figure 7


8
7
6
5
4
3
0
20
40
60
80
100
W_{max} (J/cm^2 · 10^5±S.E.)
Contact angle (deg±S.E.)
HPMC1
TG
CMC1
HEC
PAA2
CMC2
PEO
PVA
HPMC2
XG
PAA1