Can thiolation render a low molecular weight polymer of just 20-kDa mucoadhesive?

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Abstract
The objective was to investigate whether even low-molecular weight polymers (LMWPs) can be rendered mucoadhesive due to thiolation. Interceded by the double catalytic system carbodiimide/N-hydroxysuccinimide, cysteamine was covalently attached to a copolymer, poly(4-styrenesulfonic acid-co-maleic acid) (PSSA-MA) exhibiting a molecular weight of just 20 kDa. Depending on the amount of added N-hydroxysuccinimide and cysteamine, the resulting PSSA-MA–cysteamine (PC) conjugates exhibited increasing degree of thiolation, highest being "PC 2300" exhibiting 2300.16 ± 149.86 μmol thiol groups per gram of polymer (mean ± SD; n = 3). This newly developed thiolated polymer was evaluated regarding mucoadhesive, rheological and drug release properties as well from the toxicological point of view. Swelling behavior in 100 mM phosphate buffer pH 6.8 was improved up to 180-fold. Furthermore, due to thiolation, the mucoadhesive properties of the polymer were 240-fold improved. Rheological measurements of polymer/mucous mixtures confirmed results obtained by mucoadhesion studies. In comparison to unmodified polymer, PC 2300 showed 2.3-, 2.3- and 2.4-fold increase in dynamic viscosity, elastic modulus and viscous modulus, respectively. Sustained release of the model drug codeine HCl out of the thiomers was provided for 2.5 h (p < 0.05), whereas the drug was immediately released from the unmodified polymer. Moreover, the thiomers was found non-toxic over Caco-2 cells for a period of 6- and 24-h exposure. Findings of the present study provide evidence that due to thiolation LMWPs can be rendered highly mucoadhesive as well as cohesive and that a controlled drug release out of such polymers can be provided.

Introduction
Mucoadhesive controlled release dosage forms are known for extending residence time at absorption site, expanding legitimate contact with absorptive membranes and exerting control over the rate and amount of drug release¹,². Mucoadhesive capability of a dosage form depends upon the nature of the mucosa and physicochemical attributes of the polymer. Strong hydrogen bonding groups, preferably anionic charges, adequate flexibility for penetration into mucus network and optimum molecular weight are generally attributes of polymers exhibiting comparably high mucoadhesive properties³. On one hand, it is generally believed that the threshold obligatory to successful mucoadhesion is at least 100 kDa and mucoadhesive forces tend to increase with increasing molecular weight of the polymer⁴. Although polymer mucus interpenetration represents an important parameter for high mucoadhesive properties, on the other hand, it decreases with increasing molecular weight. In contrast, low-molecular weight polymers (LMWPs) show high penetration into the mucus⁵,⁶. Nevertheless, they are not considered a choice as mucoadhesive polymers as they dissolve very fast, exhibit insufficient cohesive properties and lead consequently to a too fast drug release⁷.

As thiolated polymers can strongly increase their molecular weight and consequently cohesive properties by the formation of disulfide bonds even after interpenetration with the mucus has taken place⁸, they might allow combining the benefits of both high-molecular weight polymer and LMWPs in order to achieve high mucoadhesive properties.

In order to verify this working hypothesis, it was the aim of this study to synthesize novel thiolated LMWP and to evaluate their resulting mucoadhesive properties. Accordingly, the sulfhydryl compound cysteamine was immobilized as ligand to
poly(4-styrenesulfonic acid-co-maleic acid) (PSSA-MA) via amide bond formation, a low-molecular weight \( M_w \sim 20 \text{kDa} \) anionic copolymer exhibiting numerous carboxylic acid groups for conjugation\(^{10,11} \). The resulting PSSA-MA–cysteamine (PC) conjugate was evaluated \textit{in vitro} with regard to its mucoadhesive properties. In addition, the influence of thiolation on further essential features such as swelling behavior, rheological properties and drug release were analyzed. The results of this study should provide evidence that comparatively LMWPs can exhibit sufficiently high mucoadhesive properties due to thiolation.

### Materials and methods

#### Materials

Poly(4-styrenesulfonic acid-co-maleic acid) [1:1 styrene/maleic acid mole ratio], average molecular weight 20 kDa, \( N \)-hydroxysuccinimide (NHS), cysteamine, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC), 2,4,6-trinitrobenzensulphonic acid, 5,5'-dithiobis(2-nitrobenzoic acid) Triton\(^{x} \) X-100 and sinapinic acid (SA) were purchased from the Sigma–Aldrich (Vienna, Austria). Trifluoroacetic acid (TFA) was obtained from Acros Organics (Geel, Belgium). All the other components were of analytical grade.

#### Synthesis of polymer–cysteamine conjugates

The covalent attachment of cysteamine to PSSA-MA was accomplished by the formation of amide bonds between the primary amino groups of the sulphydryl compound and carboxylic acid groups of the copolymer. One gram of PSSA-MA was hydrated with 100 ml of demineralized water. The carboxylic acid moieties of the polymers were activated for 10 min by the addition of EDAC in a final concentration of 100 mM and NHS in concentrations as shown in Table 1. pH was adjusted within the range of 6–7. Cysteamine was added in amounts as listed in Table 1 and pH re-adjusted to the range of 6–7. Reaction mixtures were incubated overnight under continuous stirring at room temperature. The resulting polymer–cysteamine conjugates were isolated in the dark by dialyzing at 10\(^{\circ}\)C 2 times against 0.2 mM HCl, 2 times against the same medium but containing 1% NaCl and then exhaustively twice against 0.2 mM HCl. Control samples were prepared and isolated in exactly the same way as polymer–cysteamine conjugates but EDAC and NHS were omitted during the coupling reaction. All the samples were lyophilized by drying frozen aqueous polymer solutions at 10\(^{\circ}\)C and 0.01 mbar (Christ Beta 1-8K; Osterode am Harz, Germany). Thiol group contents and disulfide bonds were determined, and intramolecular disulfide bonds formed during synthesis were broken with the reducing agent NaBH\(_4 \) (1 mg/10 \( \mu \)mol of disulfide bonds). For this purpose, polymer was dissolved in \( \sim 100 \) ml of demineralized water and the pH was adjusted to 7–8. Then NaBH\(_4 \) was added to polymer solution and the stirring was continued for 60 min. In the following, polymer conjugate was dialyzed 2 times against 0.2 mM HCl in dark at 10\(^{\circ}\)C and lyophilized under same conditions as above. Afterward, PC conjugates and controls were stored at 4\(^{\circ}\)C until further use.

#### Determination of the thiol group content

Ellman’s reagent was used to quantify free thiol groups immobilized on PC as described previously by our research group\(^{12} \).

#### Disulfide bond test

This test was performed to quantify disulfide bonds that may have been formed due to oxidation by air/atmosphere during the thiolation process. Disulfide content was determined after reduction with NaBH\(_4 \) and addition of Ellman’s reagent, as elaborated by our research group previously\(^{12} \).

#### Determination of unbound cysteamine

Primary amino groups were determined by using a colorimetric assay technique with 2,4,6-trinitrobenzenesulphonic acid (TNBS) for thimer conjugates and control as described by our research group previously\(^{13} \).

#### Fourier transport infrared spectroscopy (FTIR) studies

Perkin Elmer Spectrum 100 ATR-IR spectrometer (Perkin Elmer, Waltham, MA) in an arrangement with a Spectrum software version 6.3.1.0134 (Perkin Elmer) was set up to obtain the spectrum. Both the unmodified PSSA-MA copolymer and PC were recorded with 10 scans in a wave number range from 4000 to 650 cm\(^{-1}\) and a resolution of 1 cm\(^{-1}\). Measurements were performed at 22\(^{\circ}\)C\(^{14} \).

#### MALDI-TOF-MS analysis

The mass spectra of polymers were collected with Bruker Daltonics Ultraflex I MALDI-TOF/TOF instrument (Bremen, Germany) using a stainless steel target (MTP 384 target, Bruker Daltonics). For the mass determination of unmodified PSSA-MA copolymer and PC conjugates, SA was used as a matrix. First 1% (m/v) solution of all the polymers was prepared in water and then 1\( \mu \)l of each sample was spotted on a stainless steel target, followed by 1\( \mu \)l of saturated SA solution [SA in water/acetonitrile (50/50, v/v) containing 0.1% TFA]. All the measurements were recorded in positive linear mode. Mass spectra were documented by summing up 500 laser shots. Laser power was set at 70–80% of its maximal intensity, using a 337-nm nitrogen laser at 50 Hz. The Flex Analysis version 2.4 software (Bruker Daltonics GmbH, Bremen, Germany) provided by the manufacturer was used for data processing\(^{15} \).

#### Cytotoxicity test – resazurin assay

To estimate potential cytotoxic effects of these newly developed thiolated copolymers, resazurin assay was performed on a Caco-2 monolayer\(^{16} \). Cells were cultured on 24-well plates at a density of 1 \( \times 10^7 \) cells/ml in 500\( \mu \)l minimum essential medium (MEM) for

| Polymer (g) | EDAC [final concentration (mM)] | NHS [final concentration (mM)] | Added cysteamine (g) | Thiol groups (\( \mu \)mol/g of polymer) | Total amount of thiol groups (\( \mu \)mol/g of polymer) |
|-------------|----------------------------------|-------------------------------|----------------------|----------------------------------------|------------------------------------------------|
| PC 315      | 1                                | 100                           | --                   | 0.5                                    | 314.92 ± 64.09                                    |
| PC 1608     | 1                                | 100                           | 20                   | 0.5                                    | 1608.86 ± 92.70                                   |
| PC 2300     | 1                                | 100                           | 40                   | 1                                      | 2300.16 ± 149.86                                  |
| Control     | 1                                | --                            | 0                    | 1                                      | 32.43 ± 1.15                                      |

Table 1. Concentrations of various components used for coupling reaction in order to form polymer–cysteamine conjugates and degree of thiolation in PSSA-MA–cysteamine conjugate.
14 days at 37 °C and 5% CO₂. Thereafter, cells were incubated with 0.5% (m/v) of thiomers, unmodified PSSA-MA and controls. The pH of all the samples was kept constant at 7.4. Untreated cells served as positive control and a 4% solution of Triton X-100 was used as negative control. After 6 and 24 h of incubation at 37 °C, cells were washed twice with phosphate buffer saline (10 mM, pH 7.4) and medium was replaced by 500 μl of resazurin solution. Cells were incubated for 3 h along with resazurin solution at 37 °C. Thereafter, 100 μl were taken from the supernatant of each well and transferred to a 96-well plate, and the resulting fluorescence (λex = 540 nm and λem = 590 nm) was measured by a micro plate reader. Cell viability was calculated as percentages of the positive control as follows:

\[ \text{Cell viability} \% = \frac{\text{Average absorbance value of each triplicate}}{\text{Positive control}} \times 100 \]

**Tablets manufacture**

Unmodified PSSA-MA and PC were compressed into a flat-faced tablets weighing 30 mg each, with a diameter of 5.0 mm (single punch eccentric press-Paul Weber, Germany). The compaction pressure of 10 kN was maintained constant during the preparation of all the tablets.

**Swelling behavior studies**

The swelling behavior studies were carried out to determine the water-absorbing capacity of the tablets using a gravimetric method. Test tablets were fixed to a needle and immersed in a beaker containing 100 mM phosphate buffer pH 6.8 at 37 °C. At predetermined time points, the swollen tablets were taken out of the incubation medium, excess water was removed and the amount of water uptake was determined gravimetrically. The swelling ratio was then calculated according to the following equation:

\[ \text{Water uptake} \% = \frac{W_\text{at}}{W_0} \times 100 \]

where \( W_\text{at} \) is the weight of taken up water at time \( t \), found by subtracting weight of dry tablet from total weight of tablet along with water at time \( t \) and \( W_0 \) is the initial weight of the dry tablet.

**In vitro assessment of mucoadhesive characteristics**

**Rotating cylinder method**

Tablets weighing 30 mg each of unmodified PSSA-MA and PC conjugates were attached to a freshly excised intestinal porcine mucosa, that was fixed on a stainless steel cylinder (diameter: 4.4 cm, height 5.1 cm, apparatus 4-cylinder, USP). Thereafter, the cylinder was placed in the dissolution apparatus, according to the USP, entirely immersed with 900 ml of 100 mM phosphate buffer pH 6.8 at 37 °C and agitated with 100 rpm. The detachment of the test tablets was determined visually during an observation time of 3 h.

**Rheological evaluation of polymers with mucus**

Rheological studies were carried out by evaluating the adhesion properties of thiomers, unmodified PSSA-MA and control. 500 μl of polymer solutions (1% m/v thiomers, unmodified PSSA-MA in 100 mM phosphate buffer pH 6.8) were mixed with 500 mg of native small intestinal porcine mucus and incubated for 2 h at room temperature. Control consisted of 500 μl of 100 mM phosphate buffer pH 6.8 alone, without any of the polymer. Afterward, 500 μl of the mixtures of thiomers, unmodified PSSA-MA and control with mucus were transferred to a cone-plate rheometer (Haake Mars Rheometer, 379-0200, Thermo Electron GmbH, Karlsruhe, Germany) to measure the dynamic viscosity and other parameters. The oscillating measurements were performed with a shear stress in the range of 0.2–500 Pa and the temperature was maintained at 37 ± 0.1 °C.

Dynamic oscillatory test within the linear visco-elasticity region were performed at 1 Hz frequency. The elastic modulus (\( G' \)), the viscous modulus (\( G'' \)) and the dynamic viscosity (\( \eta^* \)) were calculated, for both unmodified PSSA-MA and thiomers, and analyzed to determine the effect of thiolation on these parameters.

**In vitro evaluation of release studies**

Release studies were performed with minor modifications to a method already established in our research group. The model compound used was codeine HCl (cod-HCl) in a concentration of 10% (w/w). 108 mg of each of unmodified PSSA-MA and thiomers was dissolved in 40 ml of demineralized water and homogenized with 12 mg of cod-HCl. The mixture was stirred for 1 h and then frozen at −80 °C and lyophilized. Tablets were compressed out of the lyophilized polymer/drug mixture as described earlier. The in vitro release rate from these drug delivery systems was analyzed by dissolution study. The dosage form was placed in a beaker (Schott Duran 25 ml, Germany) containing 15 ml of release medium (100 mM phosphate buffer pH 6.8). The beakers were covered, placed on oscillating surface and incubated in an oven maintained at 37 ± 0.5 °C. Aliquots of 200 μl were withdrawn every 30 min and continued for 6 h and replaced with an equal volume of release medium equilibrated at 37 °C. Afterward, samples were centrifuged to separate any undissolved portion. The amount of cod-HCl released was determined via HPLC analysis (LaCHROM Elite) using a prontoSIL C18-column (250 mm × 4.6 mm, 5 μm; Bischof chromatography, Leonberg, Germany). Acetonitrile/0.05% TFA (10/90) was used as eluent at a flow rate of 1.0 ml/min (UV detector at 210 nm, injection volume 20 μl). The amount of released cod-HCl was calculated by interpolation from a standard curve. Cumulative corrections were made for previously removed samples.

**Statistical data analysis**

All the results are displayed as mean of at least three experiments ± SD. Statistical data analysis was performed using the Student’s t-test, two tails with 95% confident interval (p value <0.05) as the minimal level of significance.

**Results**

**Characterization of PC conjugates**

The covalent attachment of cysteamine to PSSA-MA was achieved by the formation of amide bonds between the primary amino group of the amino acid and a carboxylic acid group of the polymer as depicted in Figure 1. In order to obtain different amounts of cysteamine immobilized on the PSSA-MA backbone, NHS and cysteamine were added in increasing concentrations while keeping the carbodiimide concentration constant as listed in Table 1. Maximum thiol content was found by using 40 mM NHS. As depicted in Table 1, “thiol groups” represents amount of free thiol groups without considering disulfide bonds. New conjugates showed minimum 314.92 ± 64.09 μmol thiol groups per gram polymer (PC 315), another conjugate with intermediate 1608.86 ± 92.70 μmol thiol groups per gram polymer (PC 1608), and maximum 2300.16 ± 149.86 μmol thiol groups per gram polymer (PC 2300). Outcomes of disulfide bond test are shown as

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*References omitted for brevity.*
conjugates; polymer – poly(4-styrenesulfonic acid-co-maleic acid) (PSSA-MA); 1-ethyl-3-(3 dimethylaminopropyl) carbodiimide hydrochloride (EDAC) and N-hydroxy succinimide (NHS).

Figure 1. Schematic diagram for synthesis of polymer–cysteamine conjugates; polymer – poly(4-styrenesulfonic acid-co-maleic acid) (PSSA-MA); 1-ethyl-3-(3 dimethylaminopropyl) carbodiimide hydrochloride (EDAC) and N-hydroxy succinimide (NHS).

“total amount of thiol groups” in Table 1 that includes free thiol groups as well as thiol groups obtained after breaking disulfide bonds with NaBH₄. The greater the difference between “thiol groups” and “total amount of thiol groups” is, the more disulfide bonds are present in thiomer. Efficiency of dialysis process was perceived by quantifying unbound cysteamine with TNBS assay. The analysis revealed <2% (w/w) of unbound cysteamine of the total mass of the thiolated polymers. The lyophilized PC conjugates appeared as white to pale yellow, odorless and amorphous powder. This was soluble in aqueous medium with mild stirring or freely soluble in alkaline medium of pH ≥ 10 and forms a very low viscosity solution.

FT-IR studies
The FT-IR spectra of unmodified PSSA-MA and PC are shown in Figure 2. The main bands that were assigned as characteristic of unmodified PSSA-MA are: 3415 cm⁻¹ (O–H stretching), 1571 and 1407 cm⁻¹ (C=O stretching unconjugated), while thiolated PSSA-MA is characterized by 3278 cm⁻¹ (N–H stretching), 2549 cm⁻¹ (S–H stretch) and 1624 cm⁻¹ (C=O stretching, conjugated, amide II). Band assignment was done according to Maqelin et al. ¹¹, Barth ²² and Bhatia and Ahuja ²³.

MALDI-TOF-MS analysis
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was used for an in-detail analysis of PSSA-MA and PC conjugates. MALDI-TOF-MS is appropriate for polymer analysis because of its accuracy and simplicity of the mass spectra. Average molecular weights of the conjugates were determined in order to evaluate that they were still in range of 20 kDa. The masses determined by MALDI-TOF-MS are shown in Figure 3.

Cytotoxicity studies
In principle, resazurin assay determines the effects of the compounds/polymers on metabolic activity of mitochondria of Caco-2 cells. All the tested polymers did not tempt severe cytotoxicity within 6 and 24 h. Based on these results, >80% cells were found viable after incubation with the polymers, as depicted in Figure 4. Statistically, cell viability of thiomer conjugates was found significantly different (p<0.05) as compared to control after an incubation period of 24 h.

Swelling behavior and cohesive properties
Swelling behavior of mucoadhesive polymers has pronounced influence on their stability, release of entrapped drug, adhesive and cohesiveness properties. The water uptake of unmodified PSSA-MA and thiolated conjugates are presented in Figure 5. Water uptake studies showed immediate disintegration of unmodified PSSA-MA tablets within 45 s, in 100 mM phosphate buffer pH 6.8 at 37 °C. Tablets comprising the PSSA-MA thiomers started to swell straightaway after being immersed and revealed an abrupt water uptake for 5–10 min. It was followed by slow erosion in case of more thiolated derivatives (PC 2300 and PC 1608) leading to complete disintegration in 45–60 min. In disparity tablets made from PC 315 were more resistant to erosion, after the initial water uptake they appeared like jelly beads that disintegrated very slowly and later fell from the needle at 90 min. Tablets made from all the thiomers were resistant to erosion. Among them, PC 315 tablets were comparatively more stable likely due to the highest content of disulfide bonds after synthesis.

In vitro evaluation of mucoadhesive properties

Rotating cylinder method
On one hand, unmodified PSSA-MA tablets instantly separated and disintegrated in 30 s, thiomers tablets on the other hand remained attached to the intestinal mucosa for minimum of 10 min (PC 315) and maximum of 180 min (PC 2300). Time until tablets were detached from the mucosa is demonstrated in Figure 6.

Rheological investigations of polymer/mucus mixtures
Mucus layer is a blend of some electrolytes, lysozymes, cells, glycoproteins and water but the main component of interest in mucus are mucin glycoproteins being linked to each other through disulfide bonds²⁴,²⁵. In case of thiolated polymers, covalent bonds between the macromolecules and mucus affect the rheological properties.

Figure 7 displays the results of rheological investigations of polymer solutions with native porcine mucus. Only a negligible change was observed among the blank and unmodified PSSA-MA. On the other hand, difference of observed parameters rose consistently with increasing thiolation. The highest values were noted for PC 2300, showing 2.3-fold increase in each dynamic viscosity (η*) and elastic modulus (G*) and a 2.4-fold in viscous modulus (G″) as compared to unmodified PSSA-MA.

In vitro evaluation of release studies
The percentage of cod-HCl released from tablets of thiomers and control (PSSA-MA) was plotted as a function of time as
illustrated in Figure 8. Unmodified PSSA-MA tablets disintegrated in 45 s and drug release appeared following immediate-release pattern. However, thiomers tend to slowdown the release of the model drug and maximum effect was observed for PC 2300, 50% of the drug released in almost 1 h. For all the polymers, 100% level of cod-HCl release reached within 6 h. Apparently, water uptake behavior of the thiomers is in good correlation with the release of the drug. Statistically, the sustaining effect of PC 2300 was found significant ($p<0.05$).

**Discussion**

Thiolated PSSA-MA is a biodegradable polymer representing ion exchange resin that has been chemically modified by the covalent
attachment of thiol groups. It had been discussed previously in a study by Shahnaz et al., and the more EDAC is added, the more thiol groups are immobilized. In this study, it is further revealed that increasing the concentration of NHS, while maintaining EDAC constant, increases the degree of thiolation. These results are in line with a study in which the rate of L-cysteine immobilization to hyaluronic acid was also shown to be strongly dependent on the amount of NHS used for the coupling reaction.

The FT-IR spectra of PC appeared different to unmodified PSSA-MA. These changes in spectrum of unmodified polymer, particularly N–H stretch and shift in C=O stretch because of secondary amide, confirm the conjugation of cysteamine with PSSA-MA. Very weak signal for S–H stretch may be attributed to its very low amount in the thiolated polymer. MALDI-TOF-MS spectra of PSSA-MA and its thiolated conjugate confirmed that there was no significant difference between their average molecular weights. The formation of inter-macromolecular disulfide bonds as a consequence of thiol group oxidation could therefore be excluded. Furthermore, the chemical structure of conjugates was confirmed by 1H NMR studies showing additional signals in the range of 3.3–3.0 ppm for the introduction of methylene groups (data shown in supplementary figure S1).

Water uptake studies demonstrated that the covalent attachment of thiol groups has significant influence on the swelling behavior of the polymer. Exact water uptake is not possible as a matter of definition. The eroded portions of thiomer tablets were not included into calculations, and therefore actual water uptake is likely higher than the observed values. The observations of study are in good accordance with results obtained for mucoadhesion studies performed with tablets containing the unmodified PSSA-MA and corresponding unmodified polymer. It could be proven that thiolation of PSSA-MA enhanced its adhesion time by forming covalent disulfide bonds between the polymer and mucus. In general, we could show that an improved adhesive property of PSSA-MA is result of increased stability and decreased solubilization. However, cohesiveness of polymer tablets influenced the residence time at the mucosal surface, since tablets were mainly not separated (apart from PC 315, in this case tablets detached) but gradually swollen and eroded. Previously, effect of thiolation on anionic polymers of different molecular weights has been investigated. These studies compared the mucoadhesive and cohesive properties of various poly(acrylic acid)–cysteine conjugates with a molecular weight ranging from

**Figure 4.** Results of resazurin assay on Caco-2 cells monolayers after incubation time of 6 h (black bars) and 24 h (white bars) with PSSA-MA and its conjugates with cysteamine (PC 315, PC 1608 and PC 2300). Triton X-100 acts as negative control while white MEM as positive control. Results are expressed as means ± SD of three experiments.

**Figure 5.** Swelling performance of thiomer tablets (30 mg) in 100 mM phosphate buffer solution pH 6.8 at 37 °C. PC 315 ($\pi$), PC 1608 ($\equiv$) and PC 2300 (*). Indicated values are the means ± SD of three experiments. ($) Pointing the time when erosion of matrix tablets start.

**Figure 6.** Mucoadhesion behavior of PSSA-MA and thiomers (PC 315, PC 1608 and PC 2300); studies were performed on rotating cylinder using 100 mM phosphate buffer pH 6.8 at 37 °C. Displayed results are the means ± SD of three experiments.

**Figure 7.** Rheology studies of PSSA-MA and its conjugates with cysteamine (PC 315, PC 1608 and PC 2300) and blank; performed at 37 °C. Elastic modulus (black bars), viscous modulus (grey bars) and dynamic viscosity (white bars) were calculated and designated values are the means ± SD of three experiments.

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2 to 450 kDa. As compared to previous studies on mucoadhesion of anionic polymers, we could show pronounced mucoadhesive properties for an at least 2-fold smaller polymer than the so far tested smallest polymer. The mucoadhesive properties of thiolated chitosan 20 kDa have been shown earlier both as coating for nanoparticles and in tablet form, although there was no focus on the mucoadhesive properties of LMWPs.

Higher increase in viscosity of thiomers proved the concept of disulfide bond formation between thiolated polymers and mucus. Furthermore, an increase in the elastic modulus (\(G''\)) and viscous modulus (\(G'\)) reveals the transformation from a liquid-like action to a solid (gel)-like response for thiomers.

Outcomes of release studies, combined with mucoadhesive and swelling properties, make PC conjugate a proficient excipient for a number of drug delivery systems. They can serve the purpose for preparation of sponge-like nasal inserts as studied by Bertram and Bodmeier. They can also be a suitable candidate for preparation of sponge-like nasal inserts as studied by Bertram and Bodmeier. Furthermore, an increase in the elastic modulus (\(G''\)) for their support and funding.

**Conclusion**

With this study, we could demonstrate that due to thiolation LMWP can exhibit high mucoadhesive properties as well as high cohesive properties and can provide a sustained drug release. These results might change the paradigm that just polymers with a molecular weight >100 kDa can be regarded as mucoadhesive. Evidence provided within this study that LMWP can exhibit high mucoadhesive properties due to thiolation might open the door to numerous novel polymeric excipients of comparatively low molecular weight.

**Declaration of interest**

The authors report no conflicts of interest. The authors would like to acknowledge Higher Education Commission of Pakistan (HEC) and the Austrian Agency for International Cooperation in Education and Research (OAD) for their support and funding.

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Supplementary material available online
Supplementary Figure S1.