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# Synthesis and evaluation of mucoadhesive acryloyl-quaternized PDMAEMA nanogels for ocular drug delivery

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KEYWORDS: Mucoadhesion, nanogels, quaternization, PDMAEMA, ocular drug delivery

ABSTRACT: Poly((2-dimethylamino)ethyl methacrylate) (PDMAEMA) nanogels were synthesized via surfactant-free free-radical polymerization technique in aqueous conditions utilizing *N,N'*-methylene-bis-acrylamide (MBA) as a crosslinking agent. The PDMAEMA nanogels were subsequently quaternized with acryloyl chloride in order to yield mucoadhesive materials which incorporate two mucoadhesive concepts; electrostatic interactions and covalent bond forming acrylate groups. The native PDMAEMA nanogels were found to exhibit good mucoadhesive properties on *ex vivo* bovine conjunctival tissues, which was found to increase proportionally with the degree of quaternization. With a view to determine the ocular drug delivery capabilities of the materials, both quaternized and native nanogels were loaded with pilocarpine hydrochloride *via* an absorption method, and their *in vitro* release profiles were analysed. The nanogels were found to exhibit a high loading capacity (>20% of total weight) and a sustained release over 6 hours.

Total number of words: 3825

Tables: 2

Figures: 4

Scheme: 1

## INTRODUCTION:

Mucoadhesive materials are defined as materials which exhibit an attractive interaction, or adherence, at the interface between the material and the mucosal gel layer of a mucosal membrane.<sup>1</sup> Mucosal membranes are moist tissue linings which envelop all cavities and canals which communicate with the exterior *i.e.* genitourinary tract, respiratory passages, aural and ocular passages, *etc.*<sup>2</sup> Mucoadhesive materials have received a substantial attention from pharmaceutical sectors in recent decades owing to their unprecedented ability to act as drug delivery systems to mucosa, increasing the residence times of therapeutic agents at the site of delivery.<sup>3-5</sup>

Since the inception of mucoadhesion as a drug delivery mechanism, a plethora of polymeric materials have been shown to exhibit mucoadhesive capabilities, however these materials avail of a limited number of transient attractive interactions *e.g.* hydrophobic interactions, Van der Waals forces, hydrogen bonding and ionic interactions.<sup>6,7</sup> More recently, efforts have been made to improve the mucoadhesive properties of polymeric materials through the addition of covalent bond forming groups *i.e.* thiols, maleimides, catechols, *etc.*<sup>8-11</sup> In the past decade, there has been some interest in the utilization of acrylated polymers as covalent bond forming mucoadhesive functionalities.<sup>12</sup> First reported in 2010, acrylated polymers exhibit mucoadhesive properties as a consequence of their ability to undergo a Michael-type reaction with thiol/sulfide groups present in the cysteine-rich termini of the mucin glycoproteins at the mucosal surface.<sup>13</sup>

An area which has gained a substantial attention in the field of mucosal drug delivery recently, is the utilization of nanogel systems. Nanogels are defined as nanosized particles composed of a physically or chemically crosslinked polymer network which exhibits the ability to swell when in a ‘good’ solvent, typically water.<sup>14</sup> Nanogels offer a unique platform for mucosal drug delivery

as they can facilitate high drug loading capacities in addition to exhibiting excellent biocompatibility, enhanced colloidal stability and large surface areas with tunable chemical and physical properties.<sup>15-17</sup>

Herein, we describe the synthesis of crosslinked poly((2-dimethylamino)ethyl methacrylate) (PDMAEMA) nanogels and the subsequent quaternization with acryloyl chloride yielding mucoadhesive nanogels which incorporate two mucoadhesive concepts; electrostatic interactions with negatively-charged mucins and covalent bond formation between acrylated moieties and thiol-groups present in cysteine-rich fragments of mucins. These dual-functional materials were analyzed to determine their mucoadhesive properties, drug loading capacities and in vitro release profiles. To our knowledge, the utilization of acryloyl chloride in the quaternization of tertiary amines to yield ‘dual-functional’ mucoadhesive nanogels has never been reported before.

## EXPERIMENTAL

### *Materials and methods*

Poly(vinyl pyrrolidone) (PVP) ( $M_w = 10,000 \text{ g}\cdot\text{mol}^{-1}$ ), azobisisobutyronitrile (AIBN), methanol and *N,N'*-methylene-bis-acrylamide were purchased from Sigma Aldrich. Acryloyl chloride and *N,N*-dimethylaminoethyl methacrylate (DMAEMA) were purchased from Alfa Aesar (Lancashire, United Kingdom). Dialysis membranes ( $M_w$  cut-off = 12–14 kDa) were purchased from Medicell International Ltd (London, United Kingdom). All reagents were used as received unless otherwise stated. <sup>1</sup>H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Ultrashield 400 plus NMR spectrometer at 298 K. All chemical shifts were reported as  $\delta$ , in parts per million (ppm) and referenced against the chemical shift of residual solvent resonances (DMSO:  $\delta = 2.50$  ppm). Fourier transform infrared (FT-IR) spectra were

recorded using a Perkin Elmer spectrum 100 infrared spectrophotometer at a wave number range of 500–4000  $\text{cm}^{-1}$ . Thermogravimetric analysis (TGA) was conducted on TGA Q50 and the data was subsequently analysed using TA universal analysis software. Samples were placed in aluminium crucibles and were subject to a heating rate of 10  $^{\circ}\text{C}\cdot\text{min}^{-1}$  from 25  $^{\circ}\text{C}$  to 500  $^{\circ}\text{C}$ , under a nitrogen atmosphere. The z-average hydrodynamic diameter, polydispersity (PDI) and zeta-potential were determined using a Nano Zetasizer at 25  $^{\circ}\text{C}$ . Samples for z-average and PDI determination were made to a concentration of 0.1  $\text{mg}\cdot\text{mL}^{-1}$  in deionized  $\text{H}_2\text{O}$ . Samples for zeta-potential determination were made according to literature (0.25  $\text{mg}\cdot\text{mL}^{-1}$  of 0.005M NaCl aqueous solution) and conducted in a disposable folded capillary cell.<sup>24</sup> Transmission electron microscopy (TEM) was performed on a 200 kV Philips CM 20 TEM microscope. Samples were transferred onto a carbon-coated copper grid and subsequently stained with uranyl acetate. Bovine eyeballs were obtained from a local abattoir on the day of slaughter, and transported in phosphate buffer (pH 7.4) maintained at 4-8  $^{\circ}\text{C}$ . The conjunctival tissues were removed and washed using phosphate buffer and used within 24h of dissection. Fluorescence images were taken using a Leica MZ10F fluorescence stereomicroscope fitted with a GFP filter and subsequently analysed using ImageJ software. Simulated tear fluid was composed of NaCl (6.7 g),  $\text{NaHCO}_3$  (2.0 g),  $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$  (0.08 g) dissolved in 1000 mL of deionized water.<sup>18</sup> Unless stated otherwise, all experiments were repeated 3 times and the data presented as mean  $\pm$  standard deviation. The statistical significance of the differences in retention experiments was examined using a two-way ANOVA statistical analysis, performed on GraphPad Prism v5.0 using Tukey's post hoc test, where  $p < 0.05$  was considered to be statistically significant.

### *Synthesis of quaternized PDMAEMA nanogels*

Poly(*N,N*-dimethylaminoethyl methacrylate) (PDMAEMA) nanogels were synthesized *via* a method modified from a previous study.<sup>19</sup> In a clean dry round-bottom flask (RBF) fitted with a stirrer bar, septum and nitrogen flush, DMAEMA (1.6 g, 10.18 mmol), MBA (8 mg,  $5.19 \times 10^{-2}$  mmol) and AIBN (4 mg,  $2.43 \times 10^{-2}$  mmol) were dissolved in 2 mL of ethanol with stirring (100 rpm) before the addition of 18 mL of deionized H<sub>2</sub>O and PVP as an emulsifying agent (160 mg,  $1.60 \times 10^{-2}$  mmol). The polymerization solution was allowed to equilibrate for 10 min under a nitrogen purge before being heated to 70 °C for 2 h. After the polymerization was completed, the reaction solution was transferred to a dialysis membrane and dialyzed against deionized H<sub>2</sub>O (4L deionized H<sub>2</sub>O for 5 days changing the dialysis media twice daily), yielding a cloudy nanogel suspension (16 mg·mL<sup>-1</sup> in deionized H<sub>2</sub>O). PDMAEMA nanogels were stored at 4-8 °C and were lyophilized prior to <sup>1</sup>H NMR and FT-IR spectroscopic analysis. <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O)  $\delta$  4.45–3.95 (m, 2H, -CH<sub>2</sub>-N(CH<sub>3</sub>)<sub>2</sub>), 3.34 (m, 2H, -C(O)O-CH<sub>2</sub>-), 2.89 (m, 6H, CH<sub>2</sub>-N(CH<sub>3</sub>)<sub>2</sub>), 1.87–1.11 (m, 5H, -CH<sub>2</sub>-C(CH<sub>3</sub>)<sub>2</sub>-).

In a clean dry glass vial fitted with a stirrer bar and nitrogen flow, 10 mL of the PDMAEMA nanogel solution was cooled using a salt water ice-bath before the dropwise addition of acryloyl chloride (40% quaternization = 2 mL,  $24.62 \times 10^{-2}$  mmol, 18% quaternization = 1 mL,  $12.31 \times 10^{-2}$  mmol, 8% quaternization = 0.5 mL,  $6.16 \times 10^{-2}$  mmol) with vigorous stirring. The reaction was allowed to proceed for 20 min before being warmed to room temperature with gentle stirring for a further 12 h. Quaternized PDMAEMA nanogels (qPDMAEMA) were subsequently purified by dialysis against deionized H<sub>2</sub>O to yield a slightly opaque nanogel solution (12 mg·mL<sup>-1</sup> in deionized H<sub>2</sub>O). Quaternized PDMAEMA nanogels were stored in dark conditions at 4-8 °C under nitrogen and were lyophilized prior to <sup>1</sup>H NMR

and FT-IR spectroscopic analysis.  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  5.99 (m, 3H,  $-\text{C}(\text{O})\text{O}-\text{CH}=\text{CH}_2$ ), 4.27–3.70 (m, 2H,  $-\text{CH}_2-\text{N}(\text{CH}_3)_2$ ), 3.29 (m, 2H,  $-\text{C}(\text{O})\text{O}-\text{CH}_2-$ ), 2.98 (m, 6H,  $\text{CH}_2-\text{N}(\text{CH}_3)_2$ ), 1.68–0.91 (m, 5H,  $-\text{CH}_2-\text{C}(\text{CH}_3)-$ ).

#### *Retention of PDMAEMA and qPDMAEMA nanogels on ex vivo bovine conjunctival tissue*

In order to determine the mucoadhesive properties of PDMAEMA and qPDMAEMA nanogels on *ex vivo* bovine conjunctival tissue, a modified in-house method previously developed by the group was employed (Scheme S1 ESI†).<sup>20</sup> To an aqueous solution of fluorescein sodium ( $1 \text{ mg}\cdot\text{mL}^{-1}$  in deionized  $\text{H}_2\text{O}$ ), 10 mg of freshly lyophilized nanogels were added. The fluorescently loaded nanogels were retrieved by centrifugation (13000 rpm) before being reconstituted in 1 mL of deionized  $\text{H}_2\text{O}$ . To freshly dissected bovine conjunctival tissue ( $5 \times 5 \text{ cm}^2$ ), 20  $\mu\text{L}$  of the fluorescent nanogels were deposited before being transferred to a sloped channel, incubated at 35 °C, and subsequently washed with simulated tear fluid ( $500 \mu\text{L}\cdot\text{min}^{-1}$ ). At pre-determined intervals, fluorescence images were taken of the conjunctival tissue and analyzed for pixel intensity using ImageJ software. The data obtained was presented as a percentage of nanogel remaining on the conjunctival tissue based on the original pixel intensity.

#### *Loading and in vitro release of pilocarpine hydrochloride from PDMAEMA and qPDMAEMA nanogels*

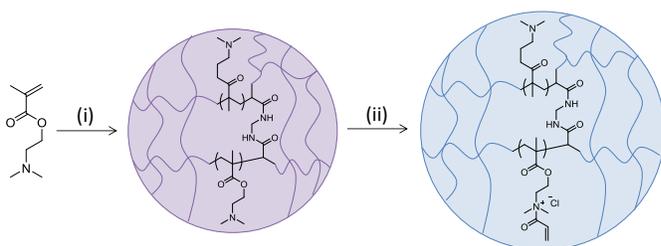
Using sacrificial samples for each time-point, freshly lyophilized nanogels (12 mg) were rehydrated in 1 mL of a 0.5% aqueous solution of pilocarpine-HCl with gentle stirring. At predetermined time-points, nanogels were removed by centrifugation and washed with 1 mL aliquots of deionized water ( $\times 2$ ), by repeated resuspension and centrifugation. Aqueous washings

were combined with the supernatant liquid, lyophilized and reconstituted to 1 mL before being filtered (0.45  $\mu\text{m}$  syringe filter) and analyzed by UV-vis spectrophotometry ( $\lambda = 220 \text{ nm}$ ) in order to determine the active pharmaceutical ingredient (API) not adsorbed into the nanogel matrix. The *in vitro* release of pilocarpine-HCl was conducted using a modified method outlined by Vong *et al.*<sup>21</sup> Using sacrificial samples; 25 mg of loaded nanogels were suspended in 10 mL of simulated tear fluid and incubated at 35 °C with gentle agitation (30 rpm). At predetermined time-points, nanogels were removed by centrifugation and the supernatant fluid was collected and passed through a 0.45  $\mu\text{m}$  syringe filter before being analyzed by UV-vis spectrophotometry. For both loading and release studies, results were reported as a percentage of pilocarpine-HCl adsorbed and released based on the original quantity of drug in solution.

## RESULTS AND DISCUSSION

### *Synthesis of quaternized PDMAEMA nanogels*

#### **Scheme 1. Synthesis of quaternized PDMAEMA nanogels<sup>a</sup>**



<sup>a</sup>Reagents and conditions: (i)  $\text{H}_2\text{O}:\text{EtOH}$ , DMAEMA, MBA, PVP, AIBN, 70 °C, 2h (ii) Acryloyl chloride, 0-25 °C, 12h.

The successful synthesis of the nanogels, and post-polymerisation modification, was confirmed by  $^1\text{H}$  NMR and FT-IR spectroscopy. In agreement with the literature, the  $^1\text{H}$  NMR spectrum

exhibited characteristic peaks at 2.89, 4.32, 3.42 and 2.10-0.65 ppm, attributed to the  $-CH_3-$  protons of the dimethylamino- functionality, the ethyl side-chain, and the hydrocarbon backbone, respectively (Fig. 1). The FT-IR spectrum further confirms the synthesis of the crosslinked PDMAEMA nanogels *via* the presence of absorbance bands which are attributed to the carbonyl functionality of the PDMAEMA ( $1735\text{ cm}^{-1}$ ) and MBA crosslinking agent ( $1645\text{ cm}^{-1}$ ). Additionally, an absorbance band ascribed to the tertiary dimethylamino- functionality is present at  $1140\text{ cm}^{-1}$  (Fig. S1). The tertiary amino- functional groups of PDMAEMA were modified post polymerization by the direct addition of acryloyl chloride to the nanogel suspension at reduced temperatures. The successful quaternization of the PDMAEMA nanogels (qPDMAEMA) was confirmed *via*  $^1\text{H}$  NMR spectroscopy through a characteristic shift of the methyl protons from 2.89 ppm to 2.98 ppm, in combination with the appearance of a multiplet between 5.98-6.44 ppm attributed to the alkene functional group of the acryloyl chloride.

Table 1. Synthesis of quaternized PDMAEMA nanogels.

Nanogel	Acryloyl chloride (mL)	Degree of quaternization <sup>a</sup> (%)	Yield (%)
PDMAEMA	0	0	69
qP8	0.5	8	57
qP18	1.0	18	60
qP40	2.0	40	54

<sup>a</sup>Determined by  $^1\text{H}$  NMR spectroscopy ( $\text{D}_2\text{O}$ , 400 MHz, 298 K).

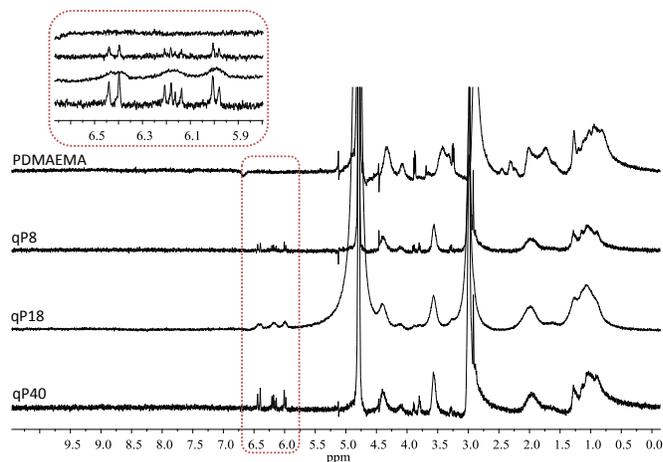


Figure 1.  $^1\text{H}$  NMR spectra of native and quaternized PDMAEMA nanogels ( $\text{D}_2\text{O}$ , 400 MHz, 298 K).

Additionally, the FT-IR spectra exhibit the evolution of an absorbance band at  $2630\text{ cm}^{-1}$  associated with the presence of a quaternary amine with a chloride counter-ion whilst retaining the PDMAEMA functionalities. Interestingly it was found that by varying the quantity of acryloyl chloride used during post-polymerization modification, the degree of quaternization could be modulated. The degree of quaternization was determined by  $^1\text{H}$  NMR spectroscopy, using the integration ratio of dimethylamine protons to acryloyl protons (Fig. 1, Table 1). Furthermore, the thermogravimetric analysis (TGA) of the native PDMAEMA revealed the presence of a single inflection at  $370\text{ }^\circ\text{C}$  relating to the degradation of the polymer matrix, however, qP40 quaternized nanogel exhibited an initial loss of mass between  $125\text{--}370\text{ }^\circ\text{C}$  before complete degradation of the polymer matrix, attributed to the degradation and loss of the quaternized acryloyl group (Fig. S2).

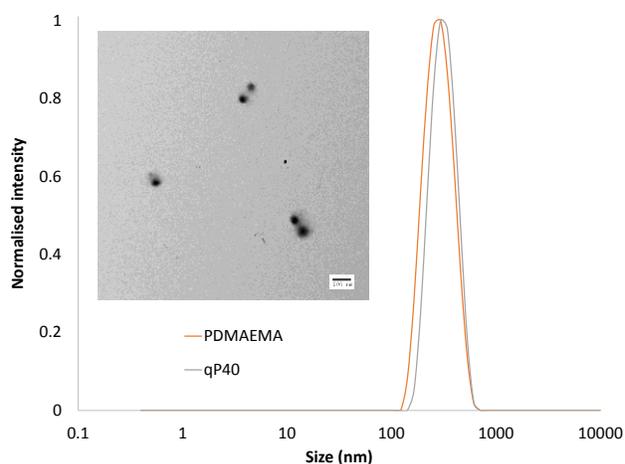


Figure 2. Hydrodynamic diameter of native PDMAEMA and 40% quaternized nanogels, as determined by dynamic light scattering. Inserted image: TEM image of the qP40 nanogel (Scale bar 100 nm).

Table 2. Physicochemical features of native PDMAEMA and quaternized nanogels.

Nanogel	Degree of quaternization <sup>a</sup> (%)	Z-average <sup>b*</sup> (nm)	PDI <sup>b*</sup>	Zeta potential <sup>b**</sup> (mV)
PDMAEMA	0	278 ± 2	0.143	25.6 ± 0.1
qP8	8	296 ± 3	0.149	28.9 ± 0.2
qP18	18	311 ± 2	0.144	32.3 ± 0.3
qP40	40	331 ± 3	0.143	33.8 ± 0.4

<sup>a</sup>Determined by <sup>1</sup>H NMR spectroscopy (D<sub>2</sub>O, 400 MHz, 298 K). <sup>b</sup>Determined by dynamic light scattering (DLS) (Nano Zetasizer, 25 °C). \*0.1 mg·mL<sup>-1</sup> in deionized H<sub>2</sub>O. \*\*0.25 mg·mL<sup>-1</sup> of 0.005M NaCl aqueous solution.

The size and shape of the PDMAEMA nanogels, and their post-polymerization modified analogues, were confirmed using dynamic light scattering (DLS) analysis and transmission electron microscopy (TEM). It was found that the unmodified PDMAEMA nanogels exhibited a unimodal size distribution with reasonable polydispersities ( $PDI < 0.2$ , Table 2), with a z-average of  $278 \pm 2$  nm. Furthermore, in agreement with literature, the PDMAEMA nanogels exhibited moderate colloidal stability with a zeta-potential ( $\zeta$ ) measurement  $>20$  mV (Table 1). Interestingly, after modification with acryloyl chloride, it was found that both the z-average and  $\zeta$ -potential increased proportionally to quaternization, whereas PDI was found to decrease after an initial increase (Table 2). This is a consequence of an increase in hydrophilicity with quaternization, leading to the nanogels swelling and increasing in colloidal stability. It was noted that of both the quaternized and native PDMAEMA nanogels exhibited a spherical nature by TEM in addition to a decrease in size as a consequence of dehydration.

#### *Retention of PDMAEMA and qPDMAEMA nanogels on ex vivo bovine conjunctival tissue*

In a study by Keely *et al.*, linear PDMAEMA-based materials were found to exhibit excellent mucoadhesive properties on both isolated rat intestinal sacs and human mucus-producing E12 monolayers. Furthermore, the PDMAEMA-based materials were found to exhibit a minimal cytotoxic effect.<sup>22</sup> More recently, Long *et al.* showed that quaternized polymeric amines showed no significant cytotoxicity up to 25 mol%.<sup>23</sup> These studies offered a reputable precedent for the synthesis and application of acryloyl chloride quaternized PDMAEMA nanogels for mucoadhesion to ocular conjunctive tissues.

Utilizing an in-house method previously developed by the group, the mucoadhesive properties of PDMAEMA and quaternized PDMAEMA nanogels on ex vivo bovine conjunctival tissue

were determined (Scheme S1, Fig S3). In order to allow for viable experimental times, the rate of conjunctival tissue washing with simulated tear fluid was two orders of magnitude greater than natural tear production ( $2 \mu\text{L}\cdot\text{min}^{-1}$ ). Complementary to previous studies of PDMAEMA-based materials, the native PDMAEMA nanogels offered a good degree mucoadhesion with  $>60\%$  retention after 60 min of continuous washing (Fig. 3). This is a consequence of the inherent cationic nature of PDMAEMA interacting with sialic/sulfuric acid residues of the mucin glycoproteins.

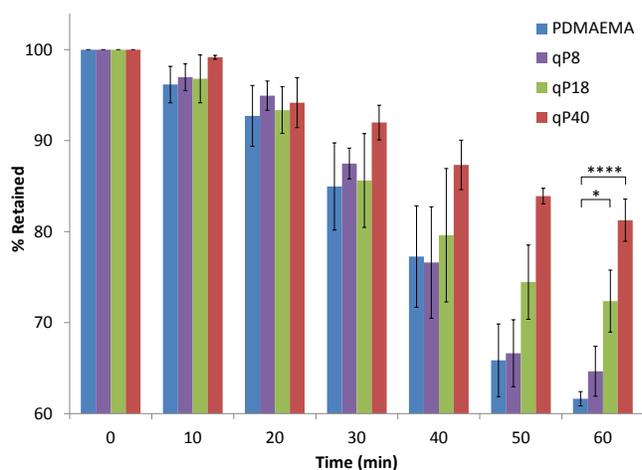


Figure 3. Percentage retention of PDMAEMA and quaternized PDMAEMA nanogels on *ex vivo* bovine conjunctival tissue. Data expressed as mean  $\pm$  standard deviation ( $n = 3$ ). \*Statistically significant difference ( $P < 0.05$ ). \*\*\*\*Statistically significant ( $P < 0.0001$ ).

It was found that once quaternized; the qPDMAEMA nanogels exhibited an increase in retention on the mucosal surface. Furthermore, it was noted that the percentage retained increased proportionally with the degree of quaternization, with 8% quaternization exhibiting  $\approx 65\%$  retention, 18% quaternization exhibiting  $\approx 70\%$  retention and 40% quaternization exhibiting

≈ 80% retention after 60 min washing. A statistical two-way analysis of variance (ANOVA) was conducted on the retention data and it was found that the enhanced final retention of both 18% and 40% quaternized nanogels were statistically significant when compared to the native PDMAEMA materials. This enhancement of the mucoadhesive properties after quaternization is attributed to both an increase in the zeta-potential (Table 1), which relates to the positive surface charge of the nanogels, and the formation of covalent bonds between the acryloyl functionality and thiol-bearing cysteine residues present on the surface termini of the mucin glycoproteins.

#### *Loading and in vitro release of pilocarpine hydrochloride from PDMAEMA and qPDMAEMA nanogels*

In order to demonstrate the capabilities of the PDMAEMA and qPDMAEMA nanogels as potential drug delivery systems for ocular drug delivery, pilocarpine hydrochloride was loaded into the native PDMAEMA and 40% quaternized PDMAEMA nanogels, as they exhibited the greatest statistical difference in their mucoadhesive properties. This was achieved using a simple adsorption method *via* rehydration of freeze-dried nanogels in a 0.5% w/v aqueous solution of pilocarpine·HCl. It was found that both the PDMAEMA and quaternized PDMAEMA nanogels exhibited a loading efficiency 89% and 81%, respectively. Furthermore, PDMAEMA nanogels exhibited maximum loading capacity of 22.3% of the total weight (297  $\mu\text{g}\cdot\text{mg}^{-1}$  nanogel) while qP40 nanogels exhibited a maximum loading capacity of 20.3% of the total weight (270  $\mu\text{g}\cdot\text{mg}^{-1}$  nanogel) (Fig S4). This process was employed to yield loaded nanogels which were subsequently used to determine the in vitro release profile of the nanogels.

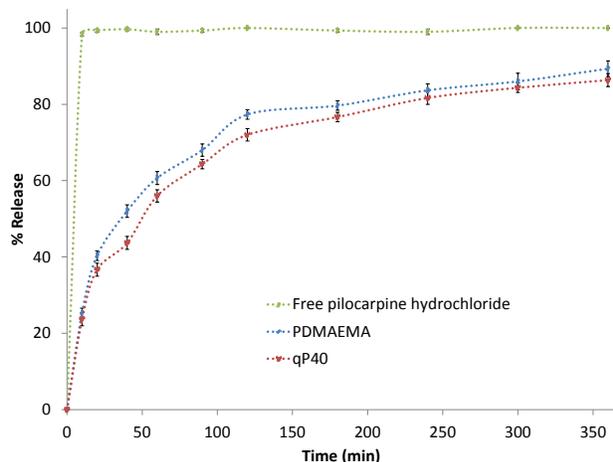


Figure 4. Release profiles of pilocarpine hydrochloride from PDMAEMA and qP40 nanogels. Data expressed as mean standard deviation (n = 3).

As suspected, the release profiles of both nanogels exhibited a large burst release (>50%) of the API within the first hour. This burst release was followed by a sustained release over the subsequent 5 hours, with a maximum API release between 85-90% (Fig. 4). This initial burst release is attributed to a combination of surface absorbed pilocarpine·HCl and the large diffusion gradient between the nanogels and the release medium. As is evident from the release profiles, no statistical difference was observed between the native and quaternized nanogels, which demonstrates that although having a significant effect on the mucoadhesive properties of the materials, quaternization does not negatively affect the release profile of the nanogels.

## CONCLUSION

In summary we have reported the facile synthesis of ‘dual-functional’ mucoadhesive nanogels utilizing a native PDMAEMA nanogel modified through quaternization with acryloyl chloride. From this we have concluded that the combination of static interactions and covalent-bond

forming mucoadhesive functionalities allows for the development of materials which exhibit superior mucoadhesive properties than the native nanogels. This therefore offers a novel route to materials with superior mucoadhesive properties in a facile and cost effective manner.

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## ASSOCIATED CONTENT

### **Supporting Information.**

FT-IR spectra and TGA thermograms of nanogels, equations for drug loading calculations, release kinetics and statistical analysis.

## AUTHOR INFORMATION

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### **Author Contributions**

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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### **ABBREVIATIONS**

DMAEMA, (2-dimethylamino)ethyl methacrylate; PDMAEMA, poly((2-dimethylamino)ethyl methacrylate); PVP, poly(vinyl pyrrolidone); AIBN, azobisisobutyronitrile, MBA, *N,N'*-methylene-*bis*-acrylamide;

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