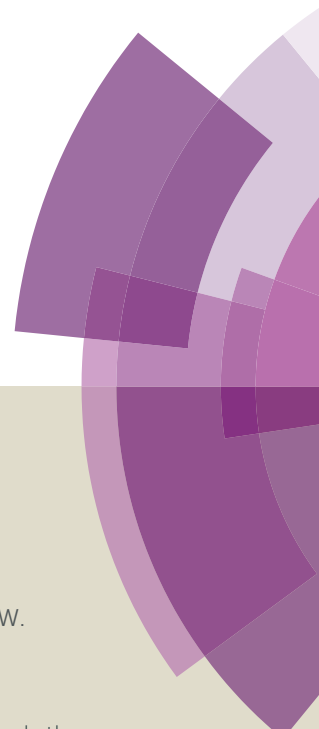


Journal of Materials Chemistry A

Accepted Manuscript



This article can be cited before page numbers have been issued, to do this please use: J. Yang, I. Bos, W. Pranger, A. Stuiver, A. Velders, M. A. Cohen Stuart and M. Kamperman, *J. Mater. Chem. A*, 2016, DOI: 10.1039/C5TA09437B.



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

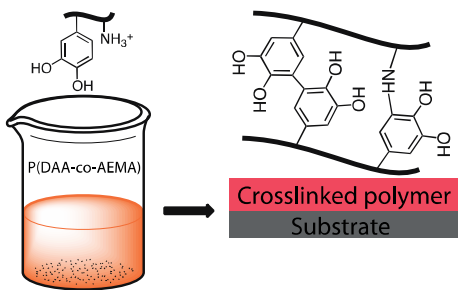
You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

A clear coat from a water soluble precursor: a bioinspired paint concept

Juan Yang, Inge Bos, Wim Pranger, Anthonie Stuiver, Aldrik Velders, Martien A. Cohen

Stuart, Marleen Kamperman



Mussel-inspired copolymer P(DAA-co-AEMA) forms water-resistant coating upon pH increase by self-crosslinking reaction of catechols and amines.



Journal Name

ARTICLE

A clear coat from a water soluble precursor: a bioinspired paint concept

Juan Yang,^a Inge Bos^a, Wim Pranger^b, Anthonie Stuver^b, Aldrik H. Velders^c, Martien A. Cohen Stuart^a, Marleen Kamperman^{a†}

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Traditional paints consist of hydrophobic polymers dissolved in hydrocarbons; they are appreciated for their rheological properties and the smooth and glossy films they form upon drying and crosslinking, but are now largely banned because of the hazards associated with the solvents. In terms of health, water borne paints based on colloidal resin dispersions are an improvement but these systems lack the rheological and film forming properties of traditional paints. We present here a bio-inspired alternative that combines the best of both worlds: a water soluble polymer that can crosslink by mild oxidation to a fully water resistant and adhesive coating. Using free radical polymerization, we copolymerized two water soluble monomers, namely borax-protected dopamine acrylamide (DAA-*p*) and 2-aminoethylmethacrylamide (AEMA) in various proportions. We determined the reactivity ratio of these monomers using an in situ ¹H NMR monitoring method and found values of 0.0 and 0.46 for DAA-*p* and AEMA, respectively. From this we conclude that in the polymers DAA-*p* pairs do not occur, while DAA-AEMA diads are relatively frequent. After removing the protective borax by hydrolysis, we obtain polymers which are soluble in water at low pH (pH 2) but which undergo rapid oxidative crosslinking when the catechol groups of DAA react with amines from AEMA. This leads to a water resistant, mildly hydrophobic film with a water/air contact angle of about 40 degrees which adheres well to glass substrates.

Introduction

Natural organisms often use coatings to protect themselves against corrosive factors in the environment, e.g. mechanical impact, moisture, and temperature. Examples include mussel byssal threads¹, squid beak^{2, 3}, and protective cuticles on plants⁴. The production of the protective coating, generally, involves two steps: i) secretion of fluidic and pliable proteins, and ii) fast curing to form hard and tough solids¹. For instance, mussels produce viscous liquid mussel foot proteins (mfps) at pH < 5 inside granules. Upon being secreted into the seawater, at pH 8, the mfps solidify in a few minutes to form very tough and hard cuticle⁵. The formed cuticle (~ 5 μm in thickness)⁶ coats the exposed byssus surfaces, and protects the byssus against microbial attack and rough sea waves⁷⁻⁹. A similar hydrophobic coating has been observed in squid beak, i.e. *Dosidicus* beak. The beak is composed of chitin fibers, proteins and polyphenolic compounds. The polyphenolic compounds form a dense crosslinked network, providing an efficient hydrophobic coating around the soft and hydrophilic chitin nanofibers, preventing the chitin from softening by water adsorption.²

For mussels, the fast cuticle curing can occur due to the efficient, versatile and complicated crosslinking chemistry of mfps¹⁰. As reported by Waite, et al., significant amounts of catecholic amino acid 3,4-dihydroxyphenylalanine (DOPA) are present in mfps¹¹. The catechol moiety in DOPA forms crosslinks in a variety of pathways. Briefly, catechols are easily oxidized to form reactive *o*-quinones. The *o*-quinones can then either react with catechols via dismutation reaction, or with amines via Michael type addition or Schiff base reaction, or with thiols via Michael addition to form crosslinks¹⁰. In addition, catechols can interact with transition metals by forming strong coordination bonds to give catechol-metal complexes¹². The crosslinking of mfps happens in response to the change in environment, i.e., the higher pH in seawater, and the exposure to oxygen.

Inspired by the fascinating properties of natural coatings, and the versatile chemistry of catechols, much effort has been devoted to fabricating thin catechol-functionalized films for various applied fields such as biomedical engineering and antimicrobial coatings¹³⁻¹⁵. A common fabrication method is layer-by-layer (LBL) assembly, i.e. alternating deposition of catechol-containing polymer and amine-containing polymer on substrates. The reaction between catechol and amine is employed as a tool to ensure the stability of the multilayer films. Detrembleur and co-workers have published several interesting articles on LBL.¹⁶⁻¹⁸ For instance, they prepared films by alternatively depositing, from aqueous solution, oxidized catechol-containing polymer pox(DOPA) and amine-containing polymer poly(allylamine) (PAH). The reaction between the quinones in pox(DOPA) and primary amines in PAH yielded films with long-term durability. The films can further be modified to target different applications. By doping with silver nanoparticles, the films were

^a Laboratory of Physical Chemistry and Soft Matter, Wageningen University, Dreijenplein 6, 6703 HB Wageningen, the Netherlands.

^b Akzo Nobel Decorative Coatings B.V., Rijkssstraatweg 31, 2171 AJ Sassenheim, the Netherlands

^c Laboratory of BioNanoTechnology, Wageningen University, Dreijenplein 6, 6703 HB Wageningen, The Netherlands

† Corresponding author. Email: marleen.kamperman@wur.nl.

Electronic Supplementary Information (ESI) available: [Additional NMR and UV spectra and viscosity data]. See DOI: 10.1039/x0xx00000x

used as antibacterial coatings. The films can also be grafted with enzymes (e.g. DispersinB) or poly(ethylene glycol) to have antibiofilm and anti-adhesion properties, respectively.¹⁶ Xu et al. have also reported similar LBL work. They prepared multilayered films by alternatively immersing a substrate in an aqueous solution of poly(acrylic acid-dopamine) (PAA-dopamine) and PAH. By immersing the final film into an aqueous solution of 1 mM NaIO₄, crosslinking was triggered. The crosslinked films showed high stability under extreme conditions, e.g. extreme pH and high ionic strength.¹⁹

Besides LBL assembly that involves multiple-step deposition, a one-pot deposition strategy of aqueous solutions containing catecholamine-containing polymers on various substrates has also been developed. For instance, Messersmith et al. prepared films by immersing various substrates (e.g. Au, Pt, PS, PMMA) into an aqueous solution of copolymer p(N-(3,4-dihydroxyphenethyl) methacrylamide-co-aminoethylmethacrylamide) (catechol content 10.6 wt%) at pH 8.3 for 24 h. The films were further used for DNA immobilization.²⁰

In this study, we aimed at reproducing the working mechanism of mussels by developing a catecholamine polymer for water-borne paint applications. The current solvent-based alkyd-paints are still considered superior in terms of rheology, but harmful for the environment since organic solvents are used. Our catecholamine polymer, in contrast, is water soluble, and should be a promising alternative having similar rheological characteristics as classical solvent-based paints. Therefore, the requirements the polymer should fulfil were 1) being able to self-crosslink in response to an external trigger such as pH; 2) being water-soluble at acidic pH; 3) being water-insoluble at basic pH. We synthesized a co-polymer by free radical polymerization of borax-protected dopamine acrylamide (DAA-*p*) and 2-aminoethylmethacrylamide hydrochloride (AEMA). The monomer reactivity of DAA-*p* and AEMA was investigated by in-situ ¹H NMR measurements. At low pH, the polymer is soluble in water and forms a stable solution. Polymer films were prepared by increasing the pH and casting the polymer solutions on substrates. The increase in pH triggers crosslinking reactions, which resulted in crosslinked, water-insoluble films. The chemistry and morphologies of the films were investigated by UV-VIS, and AFM measurements.

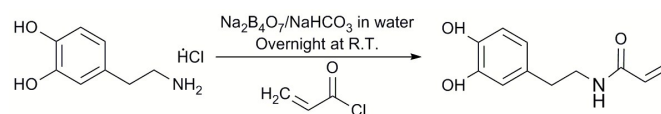
Materials and Experiments

Materials: All reagents were used as received unless mentioned otherwise. Dopamine hydrochloride, methacrylic anhydride (94%), 2-methoxyethyl acrylate (98%), basic alumina, deuteriochloroform (99.96%), tetrahydrofuran (98.5%), ethyl acetate (99%), dimethylformamide (99.8%), 2,2'-Azobis(2-methylpropionitrile) (AIBN) (98%), 2,2'-Azobis(2-methylpropionamidine)dihydrochloride (95%), dichloromethane (98.5%), methanol (98.5%), 3,4-dimethoxyphenethyl amine (97%), triethylamine (>99.5%) and acryloyl chloride (97%) were purchased from Sigma-Aldrich. Sodium hydroxide solution (1 M) and anhydrous magnesium sulfate (MgSO₄, 97%) were purchased from Merck. Hydrochloride solution (1 M) was purchased from Fluka. Hexane (p.a.) and diethyl ether (p.a.) were purchased from Biosolve. Sodium tetraborate decahydrate (p.a.) and sodium bicarbonate were purchased from J.T.Baker. 2-Aminoethyl methacrylamide hydrochloride was purchased from Polysciences.

Experiments

Dopamine acrylamide (DAA) monomer synthesis

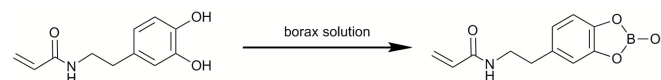
Dopamine acrylamide (DAA) was synthesized by reaction of dopamine hydrochloride and acryloyl chloride in a mixture of tetrahydrofuran (THF) and Milli-Q water, see Scheme 1. 20 g of sodium tetraborate decahydrate and 8 g of sodium bicarbonate were added to 200 ml Milli-Q water. The aqueous solution was bubbled with N₂ for 20 min, followed by addition of 10 g of dopamine hydrochloride. Next, 8.6 ml of acryloyl chloride in 50 ml of THF was added dropwise. The pH of the solution was adjusted to above 8 by adding drops of 1 M NaOH solution. The reaction mixture was stirred overnight (around 14 h) under continuous N₂ bubbling at room temperature. After the reaction, a slightly pinkish slurry was formed with a white solid present at the bottom of the flask. The reaction mixture was filtered by vacuum filtration and the clear, slightly pinkish solution was washed twice with 100 ml ethyl acetate. The obtained aqueous solution was acidified by 1 M HCl to a pH of around 2, followed by extraction with 100 ml ethyl acetate repeated three times. The brownish organic solution obtained from extraction was collected and dried over MgSO₄. Afterwards, the volume of the solution was reduced to around 50 ml by rotary evaporation and precipitated into 500 ml hexane. The formed suspension was stored at 4 °C overnight to aid crystallization. After 14 h, the resulting brownish solids were collected, dissolved in 100 ml ethyl acetate and precipitated in 1000 ml hexane. The final solid obtained by filtration of the suspension was dried in a vacuum oven overnight at room temperature and the yield was 54%.



Scheme 1. The synthesis pathway of DAA

DAA Protection (DAA-*p*)

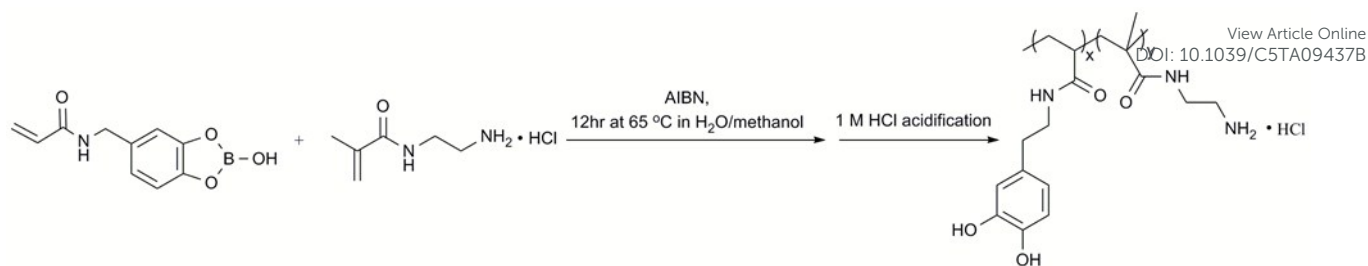
The protection of DAA, see Scheme 2, was carried out by adding 3 g DAA dissolved in a mixture of ethanol/H₂O (10 ml ethanol and 20 ml Milli-Q water) to 289.5 ml of degassed aqueous solution of sodium tetraborate decahydrate (11.0420 g, 2 molar equivalents compared to catechol moieties). The reaction mixture was kept under nitrogen for one hour under continuous stirring. After that, the product was lyophilized and used without any further purification.



Scheme 2. The synthesis of protected DAA (DAA-*p*) from DAA and sodium tetraborate decahydrate solution

Polymer synthesis

Co-polymerization of DAA-*p* and 2-aminoethyl methacrylamide hydrochloride (AEMA) was carried out by free radical polymerization at 65 °C for 10 hours, see Scheme 3. DAA-*p* (0.74 g), and AEMA (2.3 g, DAA-*p*/AEMA=8:92 mol/mol) were added to 14.06 ml Milli-Q water in a 50 ml three-neck round bottom flask. The reaction mixture was heated up to 65 °C, and after that, AIBN

Scheme 3. Polymer synthesis by free radical polymerization of DAA-*p* and AEMA

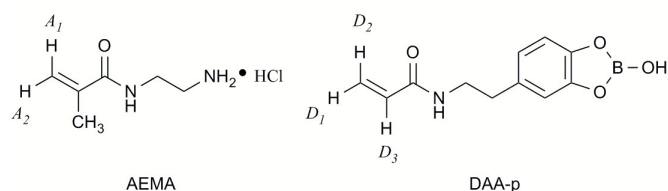
(0.02493 g, 0.152 mmol) in 1.7 ml DMF was added. It is important to note that at room temperature, DAA-*p* was not fully dissolved. The solubility of DAA-*p* increased significantly when the temperature reached 55 °C. After the reaction had been kept under nitrogen overnight under continuous stirring, the reaction mixture was allowed to cool down. Then 1M HCl was added to acidify the mixture to pH 1. The mixture was transferred to a dialysis membrane (MWCO = 1 kDa) and dialysed against acidic Milli-Q (0.001 M HCl) for two days. The product was lyophilized and stored at 4 °C until further use. The yield was 58%.

Static light scattering (SLS)

SLS was used to determine the molar mass of the copolymer. The measurements were carried out at 20 °C with an ALV light scattering setup using an ALV5000/60X0 External Correlator with a Cobolt Samba-300 DPSS Laser operating at 100mW at a wavelength of 660 nm and an ALV / HIGH QE APD Single Photon Detector. Copolymer solutions in 1 mM HCl aqueous solution with a concentration of 0.26 g/l were measured at different angles. The angle was varied using an ALV-125 goniometer with increasing steps of 5° between 20° and 120°. The molecular weight of the copolymers was deduced from a partial Zimm analysis of the collected data.

Polymerization kinetics studied by ¹H NMR

The reactivity ratio of DAA-*p* and AEMA was determined by in-situ ¹H NMR, as described by Aguilar et al.²¹ The experiments were carried out in a 500 Avance III NMR spectrometer, operating at 500.137 MHz, equipped with a 5 mm TXI probe. To perform quantitative experiments, the following measurement conditions were used: A pulse sequence of 9 μs equivalent to a 90° tip angle and a 53 s delay time were applied to allow the total relaxation of the protons and to process the individual data. The spinning rate of the sample was set to 9 Hz during shimming, and for each datum only one acquisition (FID) was used to ensure that the measurement corresponds to instantaneous composition and not to an average. The measurement was carried out at 56 °C. A solution of DAA-*p* and AEMA (total monomer concentration is 5 wt%) in 10 mM Na₂CO₃ in D₂O (0.75 ml) was added in a screw-capped thin NMR tube under argon. Three sets of experiments were performed, and the feed molar fractions of DAA-*p* were 6, 10 and 15 mol%, respectively. Dioxane (10 μl) was added to be used as an internal reference for integration purposes. V50 (1.19 mg, 4.39 μmol) was used as the initiator.



Scheme 4. Chemical structure of the comonomers used in the copolymerization including the nomenclature of the vinyl protons

The NMR spectra were processed and the signals were integrated using the software Mestrenova 10.0.2. The concentrations of unreacted DAA-*p* and AEMA were determined as follows.

$$[DAA - p] = \frac{D_1 + D_2 + D_3}{3R} \quad (1)$$

$$[AEMA] = \frac{A_1 + A_2}{2R} \quad (2)$$

where D_1 , D_2 and D_3 correspond to the protons assigned to the vinyl group of DAA-*p*, and A_1 and A_2 are the protons of the vinyl group of AEMA. R is the reference peak of dioxane, since dioxane is not expected to react, and therefore the peak should stay constant. These values were normalized to the initial feed monomer concentration $[DAA-p]_0$ and $[AEMA]_0$.

¹H NMR spectroscopy

¹H NMR of the polymers was performed in 0.1 M DCl/D₂O on a Bruker AMX-400 spectrometer (400 MHz) at 298 K.

Polymer film preparation

For AFM, UV-vis measurements, optical microscopy, contact angle measurements and the cross-cut tape test, polymer films were prepared using a casting method. For AFM measurements, glass cover slips, 18 mm long x 18 mm wide, were used as substrates for the polymer films. A 10 mg/ml polymer solution was prepared using 0.001 M HCl (aq) as the solvent. Four polymer films were prepared under the following conditions: a) low pH; b) high pH, by adding 1M NaOH; c) high pH, in the presence of a moderate amount of NaIO₄ (NaIO₄/ catechol = 1/ 100); d) high pH, in the presence of a large amount of NaIO₄ (NaIO₄/ catechol = 1/ 10). A volume of 0.6 ml of the respective polymer solution was spread onto a pre-cleaned coverslip, and left to dry under well-ventilated conditions for more than 24 h. For UV-vis measurements and the cross-cut tape test, 4.5 cm long x 1.25 cm wide quartz slides and 7.5 cm long x 2.6 cm glass slides were used as substrates, respectively. Polymer solutions were prepared in the same way as for the AFM measurements; 1.04 ml of polymer solution was spread onto the pre-cleaned quartz slide for UV-vis measurements; and 3.61 ml of polymer solution was spread onto the pre-cleaned glass slide for the cross-cut tape test. The

polymer films were left to dry under well-ventilated conditions for more than 24 h.

Polymer films were also deposited on stainless steel (2.4 cm x 1.4 cm) and on polystyrene (2.6 cm x 1.8 cm) following the same procedure under condition b) as described above. 0.62 ml and 0.87 ml of polymer solution were spread onto pre-cleaned stainless steel and polystyrene substrates, respectively. The films were left to dry under well-ventilated conditions for more than 24 h.

UV-VIS spectroscopy

UV-visible characterization was performed on a Shimadzu UV-2600 spectrophotometer at wavelengths from 200 to 600 nm. For the calibration of the catechols, five solutions of dopamine hydrochloride in water with different concentrations (0.18 – 0.25 μM) were prepared. The spectra of the samples were measured (wavelength scan) in a quartz cuvet. The absorbance intensity at $\lambda = 280$ nm for the samples was used to calculate the extinction coefficient ε .

Atomic force microscopy

The roughness of the polymer films was analyzed using a Digital Instruments NanoScope V equipped with a silicon nitride probe (Veeco, NY, U.S.A.) with a spring constant of 0.4 N/m in ScanAsystTM imaging mode. Images were recorded between 1.5 Hz and 1024 samples/line. Images were further processed with Nanoscope Analysis 1.20 software (Veeco Instruments Inc. 2010, U.S.A.). A first order flattening was used for all the images.

Contact angle measurements

The wettability of the polymer films was measured by automated static water contact angle measurements with a Krüss DSA 100 goniometer. The volume of the drop of deionized water was 3.0 μL . The reported values are the average of at least two droplets, and the relative error is less than $\pm 3^\circ$.

Optical microscopy

Cross-sections of the polymer films on the glass substrates were imaged using an Olympus BX 60. Images were obtained using a 40x magnification.

FTIR

A 10 mg/ml polymer solution was prepared using 0.001 M HCl (aq) as the solvent. Subsequently, four polymer samples were prepared under the following conditions: a) low pH; b) high pH, by adding 1M NaOH; c) high pH, in the presence of a moderate amount of NaIO_4 ($\text{NaIO}_4/\text{catechol} = 1/100$); d) high pH, in the presence of a large amount of NaIO_4 ($\text{NaIO}_4/\text{catechol} = 1/10$). The four polymer solutions were allowed to react for 24 h and lyophilized thereafter. The ATR-FTIR measurements were carried out using a Bruker Tensor 27 FTIR spectrometer.

Rheology measurements

250 mg PAEMA was dissolved in 1 ml 1mM HCl solution. The pH was measured using a Schott pH meter. The initial pH was around 2. After measuring the viscosity of this solution, the pH was increased to around 9 by adding 1.0 M NaOH. Subsequently, the viscosity was

measured at pH 9 followed by an additional viscosity measurement at pH 12. The viscosities were measured using an Anton Paar MCR 301 rheometer with a CC10/TI probe. The temperature of the Peltier plate was set to 25 $^\circ\text{C}$. Between loading the sample and the start of the measurements there was a waiting time of minimal 5 minutes. The apparent viscosity was measured at shear rates ranging from 0.01 to 1000 1/s, with four data points per decade and a measuring time of 10 seconds per data point. For each pH, the measurement was done twice; in between the measurement there was a waiting time of minimal 5 minutes. Data points with viscosities below the low-torque limit of the rheometer were removed from the graphs.

Cross-cut tape test

The cross-cut tape test ASTM D 3359 was used to measure the adhesion between the dried polymer film and the glass substrate. Briefly, a crosshatch pattern (eleven cuts for each direction with cuts 1 mm apart) was made using a sharp razor blade that cut completely through the film to the substrate. The detached flakes of coating were removed using a soft brush. A pressure-sensitive tape was applied over the crosshatch cut and the tape was pressed into place using a pencil eraser to ensure good contact between the tape and the coating. After 60 s of application, the tape was quickly removed by pulling it off as close to an angle of 180° as possible. Adhesion was assessed based on the percentage of coating area removed from the substrate by the tape and rated on a 0B to 5B scale. To be specific, 5B, 4B, 3B, 2B, 1B and 0B indicate that 0%, less than 5%, 5-15%, 15-35%, 35-65% and more than 65% of the coating area has been removed, respectively. The test was repeated three times.

Results and discussion

Polymer synthesis

In this work, we developed a catecholamine co-polymer by free radical copolymerization in aqueous conditions of two vinyl comonomers, one containing borax protected catechol (DAA-*p*) and the other containing amines (AEMA). An essential step is the protection of the catechols before polymerization. As reported in our previous work, using vinyl monomers containing unprotected catechols would result in the formation of a crosslinked polymer²². The reason is the radical scavenging effect of catechols. More specifically, during radical polymerization, the catechols in one polymer chain can react with propagating radicals in another polymer chain. Therefore, to synthesize a linear catechol-containing polymer by radical polymerization, the catechols should be protected. Different protection methods are possible, dependent on the specific requirement of the system. In our case, we have tried two methods, namely methoxy-protection and borax-protection. For the methoxy-protection, i.e., protecting the catechols with methyl groups, we have successfully synthesized the protected polymers (Fig. S1). Deprotection requires using BBr_3 and strict control of temperature at -65°C . This deprotection strategy brings difficulty due to the following reasons. Firstly, the catechol deprotection by BBr_3 needs to be performed in the absence of water, since BBr_3 reacts violently with water. However, our copolymer is only soluble in a mixture of organic solvent and water, thereby making the deprotection hard. Secondly, the removal of methoxy groups requires strict control of reaction conditions, i.e. proper amount of BBr_3 , only organic solvent, and very low

temperature. An addition of inappropriate amount of BBr_3 may result in partial deprotection of catechols²³. In view of these problems, we considered borax protection as an alternative. In this work, we start from the synthesis of vinyl monomers containing borax-protected catechols DAA-*p*. The stability of the complex of borax with catechol is dependent on pH.²⁴ Generally, the pH has to be high (> 9) to ensure sufficient stability of the borax-catechol complex against dissociation or oxidation. In our synthesis, we keep the pH above 9 by using 10 mM sodium carbonate aqueous solution as the solvent. After 10 h of reaction, we deprotected the borax-catechol complex by lowering the pH to around 2 using a 1 M HCl aqueous solution. After dialysis, the polymeric product was obtained. To check the effectiveness of the deprotection procedure, the polymer and DAA-*p* aqueous solutions were investigated by UV-VIS (Fig. S2). DAA-*p* showed a peak at 286 nm, which was ascribed to the borax-catechol complex (Fig. S2). For the polymer, in contrast, an absorbance peak at 280 nm was detected, which is typically ascribed to free catechols¹⁹. Therefore, the catechol groups in our polymer have been completely deprotected.

Polymer characterization

^1H NMR was used to characterize the copolymer, and the spectrum is shown in Fig. 1. By comparing the integration area of peaks at $\delta = 7.2\text{--}6.6$ ppm that can be assigned to the three protons of the aromatic ring in DAA (positions a), and peaks at $\delta = 3.75\text{--}2.8$ ppm of the alkyl groups in both monomers (positions e, f, b, c), we calculated the DAA composition in the final polymer to be around 7.2 mol%. This result agrees well with that from UV-VIS, obtained as follows. To calculate the DAA composition in the final polymer, we made a calibration curve based on dopamine hydrochloride. By preparing dopamine hydrochloride solutions with different concentrations in 1 M HCl aqueous solution, we obtained the extinction coefficient of catechol as $2500\text{ m}^2\cdot\text{mol}^{-1}$. By using the Lambert-beer law, the catechol concentration was obtained, and the DAA composition in the final polymer was determined to be 8.2 mol%.

The determination of the molecular weight was carried out by static light scattering. The copolymer has a weight-averaged molecular weight M_w of 4912 kg/mol, corresponding to a weight-averaged degree of polymerization of about 29000.

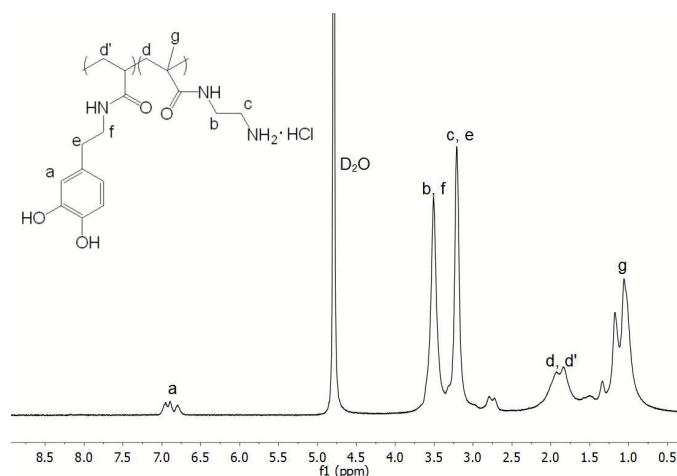


Figure 1. ^1H NMR spectra of poly(DAA-AEMA) in 0.1 M DCl/D₂O

Monomer reactivity

View Article Online

DOI: 10.1039/C5TA09437B

Generally, free radical polymerization is expected to generate random copolymers. This polymerization technique is favorable in our system, since a random copolymer would lead to an even distribution of crosslinks throughout the material. To check the randomness of the copolymer, we studied the reactivity ratios of the comonomers. The reactivity ratio r is a kinetic parameter that characterizes the monomer sequence distribution in a copolymer. r_1 is defined as the ratio of the rate constant for a reactive propagating radical M_1 adding its own type of monomer M_1 to the rate constant for addition of the other monomer M_2 . In our study, it was determined by in-situ ^1H NMR. The comonomer concentrations were monitored as a function of time. Fig. 2, 3 and 4 show representative spectra of the DAA-*p*/ AEMA solutions during polymerization at different reaction times. As shown in Fig. 3, the peaks ascribed to the vinyl groups of DAA-*p* ($\delta = 6.52\text{--}6.36$ ppm, and $\delta = 6.02\text{--}5.96$ ppm) and AEMA ($\delta = 5.8\text{--}5.75$ ppm, and $\delta = 6.06\text{--}6.02$ ppm) decreased in time (marked in green). The aromatic peaks at $\delta = 7.0\text{--}6.75$ ppm (marked in blue) became broader with increasing time. Meanwhile, broad peaks that can be ascribed to the $-\text{CH}_2-$ groups in the polymer main chain, e.g., $\delta = 1.4\text{--}1.1$ ppm (marked in blue), increased. These changes in the spectra in time clearly indicate that the polymerization takes place rapidly at elevated temperatures. We studied three reactions having feed molar ratios of DAA-*p* of 6, 10 and 15 mol%, respectively. By integrating the peaks ascribed to the vinyl groups of DAA-*p* and AEMA, as shown in Fig. 3, the monomer concentrations can be obtained as a function of time. From the variation of the monomer concentrations, the reactivity ratios of the comonomers can be obtained by fitting the experimental data to an integral form of the copolymer composition equation²⁵:

$$\frac{M_2}{M_{20}} = \left(\frac{M_{20}M_1}{M_{10}M_2} \right)^{r_2/(1-r_2)} \times \left(\frac{(r_1-1)(M_1/M_2)-r_2+1}{(r_1-1)(M_{10}/M_{20})-r_2+1} \right)^{(r_1r_2-1)/((1-r_1)(1-r_2))} \quad (3)$$

where M_i is the instantaneous molar concentration of monomer i (which can be calculated by comparing the integration of the protons peaks of the vinyl groups in monomer i to that of dioxane), r_i is the reactivity ratio of monomer i , and M_{i0} is the initial molar concentration of monomer i .

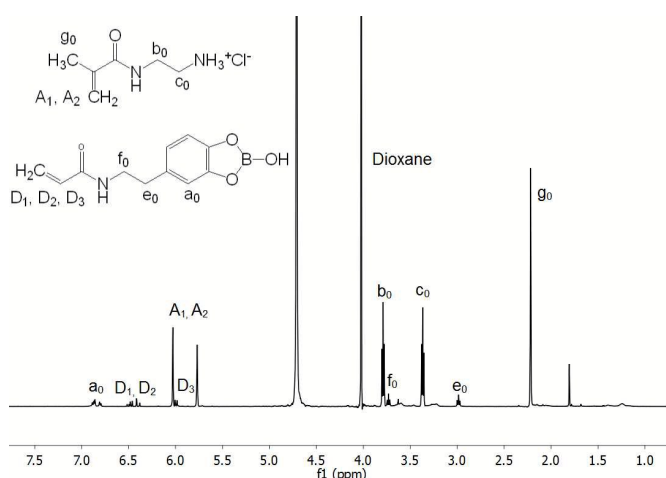


Figure 2. ^1H NMR spectra of the copolymerization reaction DAA-*p* and AEMA with an initial feed molar ratio of DAA-*p*: AEMA = 15: 85

after reaction at 56 °C for 12 min. The characteristic peak of D₂O is at 4.79 ppm.

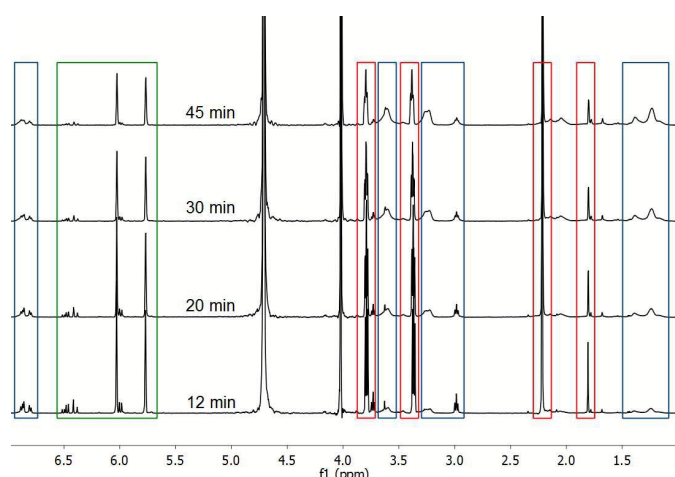


Figure 3. Representative ¹H NMR spectra of the copolymerization reaction DAA-p and AEMA with an initial feed molar ratio of DAA-p: AEMA = 15: 85. The peaks marked with green boxes indicate the vinyl protons of the monomers; red boxes show that the protons in the alkyl groups decrease with time, and blue boxes indicate peaks that broaden.

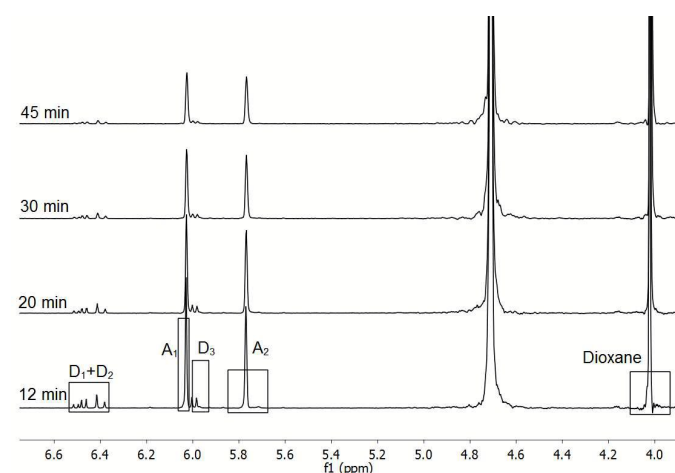


Figure 4. Details of the vinyl region of representative spectra for the copolymerization reaction DAA-p and AEMA with an initial feed molar ratio of DAA-p: AEMA = 15: 85.

The method takes any experimental point M_2 , M_1/M_2 as starting point for the rest of the reaction. More specifically, for each reaction, the first 5-10 points were used as the initial M_{20} , M_{10}/M_{20} for the rest of the reaction. By using the least-squares optimization to fit each set of experimental data to eq (3), we obtained 5-10 reactivity ratios of r_{DAA-p} and r_{AEMA} . The obtained reactivity ratios that fit each set of experimental data are highly correlated²¹, and r_{DAA-p} vs r_{AEMA} followed a straight line (Fig. 5). And for each reaction, as shown in Fig. 5, we obtained one straight line. The point where the lines intersect gives the final values of reactivity ratios, i.e., 0 for r_{DAA-p} and 0.46 for r_{AEMA} . As all three lines intersect in one point, this indicates that a reliable reactivity ratio has been obtained. A value of 0 for the reactivity ratio of DAA-p indicates that the DAA-p propagating radicals cannot add a DAA-p monomer and therefore always add an AEMA monomer, while $r_{AEMA} = 0.46$ indicates that, AEMA propagating radicals add to DAA-p in about half of the cases.

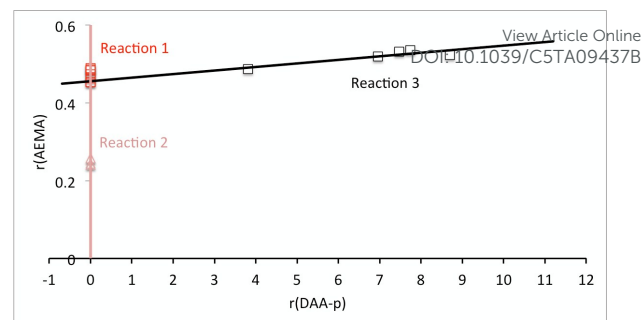


Figure 5. Reactivity ratios of DAA-p (r_{DAA-p}) vs AEMA (r_{AEMA}) of three reactions with the feed ratio of DAA-p as 6 mol%, 10 mol% and 15 mol%, respectively.

Based on the obtained reactivity ratios, we calculated the copolymer composition as a function of comonomer composition according to the following equation²⁵:

$$F_1 = \frac{r_1 f_1^2 + f_1 f_2}{r_1 f_1^2 + 2 f_1 f_2 + r_2 f_2^2} \quad (4)$$

where F_i is the mole fraction of M_i in the copolymer, and f_i is the feed molar fraction of monomer i . By applying the r_i we obtained above, we obtain the relation between F_{DAA-p} and f_{DAA-p} (Fig. 6). For our copolymer, the reactivity ratios of both monomers are less than unity, and $r_{DAA-p} r_{AEMA} = 0$, giving rise to an azeotropic copolymerization. The F_{DAA-p} / f_{DAA-p} plots cross the line representing $F_{DAA-p} = f_{DAA-p}$. At the crossover point or azeotropic point, i.e. $f_{DAA-p} = 0.35$, the copolymerization occurs without change in the feed composition. For feed compositions near the azeotropic point, the copolymer composition distributions are narrow at low conversions. At high conversions, the copolymer composition drifts to either comonomer, depending on the initial feed, whether it contains more DAA-p or more AEMA. The copolymer composition distribution becomes wider when the initial feed composition deviates further from the azeotropic point. In our copolymer system, with an initial feed molar ratio of DAA-p: AEMA = 8: 92 as shown in Fig. 7, at low conversion, it is expected to present a moderate alternating structure. At high conversion, when DAA-p has been largely consumed, more AEMA is incorporated into the copolymer chains.

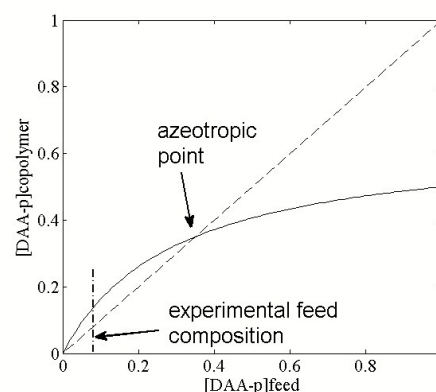


Figure 6. Copolymer composition as a function of monomer feed fraction for poly(DAA-p-AEMA) system. The dashed diagonal line represents $F_{DAA-p} = f_{DAA-p}$. The black arrow points to the azeotropic

point. The dash dotted line indicates the feed composition for our copolymer with an initial DAA-*p* percentage of 8 mol%.

- At low conversion



- At high conversion



Figure 7. Hypothesized monomer distribution scheme in the copolymer for P(DAA-*co*-AEMA). Solid circle, DAA-*p*; empty triangle, AEMA.

Polymer films

We aimed at developing a linear, water-soluble polymer containing two types of functional groups, i.e., catechols and amines. By triggering the catechol crosslinking chemistry, this polymer should be able to change from water-soluble to water-insoluble. To verify the hypothesis, two polymer films were prepared on a pre-cleaned glass substrate, one at low pH (~ 2) and one at high pH (~ 11.5). The crosslinking of the polymer can be enhanced by adding the oxidizing agent sodium periodate (NaIO_4). To study the effect of the degree of crosslinking on the solubility behavior, two extra polymer films were prepared by adding different amounts of NaIO_4 , i.e. 1 mol% and 10 mol% of catechol, respectively. The thickness of the four polymer films were measured by optical microscopy, and shown in Table 1. A representative sample is shown in Fig. 8. The polymer can also be deposited on other substrates, such as polystyrene and stainless steel (Fig. S3).

Table 1. Thickness of polymer films as determined by optical microscopy. The standard deviation of the thickness of each sample is based on three measurements at different locations in the same film.

Sample	Thickness (μm)
P(DAA- <i>co</i> -AEMA) low pH	9.1 ± 0.4
P(DAA- <i>co</i> -AEMA), high pH	11.9 ± 0.7
P(DAA- <i>co</i> -AEMA), high pH, 1/100 NaIO_4	11.1 ± 0.2
P(DAA- <i>co</i> -AEMA), high pH, 1/10 NaIO_4	12.6 ± 0.5

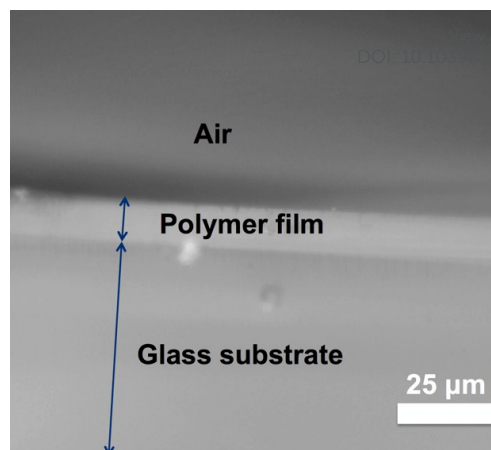


Figure 8. Optical microscopy image of polymer film P(DAA-*co*-AEMA) prepared at high pH) on a pre-treated glass substrate. The thickness of the polymer layer was measured with optical microscopy ($\times 40$ magnification).

The wettability and swelling behavior of the four polymer films on the glass substrate were studied by contact angle measurements, as shown in Fig. 9 and Table 2. Upon depositing one water droplet, the uncrosslinked film, prepared at low pH, started to absorb water, and swelled in a few seconds as shown in Fig 9 (a). After some time, the water droplet was fully absorbed and the polymer film assumed an irregular shape. The quick swelling behavior clearly indicates that the polymer is still soluble in water. This behavior is corroborated by UV-vis measurements. As shown in Fig. 10, P(DAA-*co*-AEMA) prepared at low pH exhibited a distinct peak at 280 nm, which is ascribed to unreacted catechol groups. A very broad and tiny peak at around 350 nm also appeared, which might be due to slow oxidation during the evaporation of water during film formation.

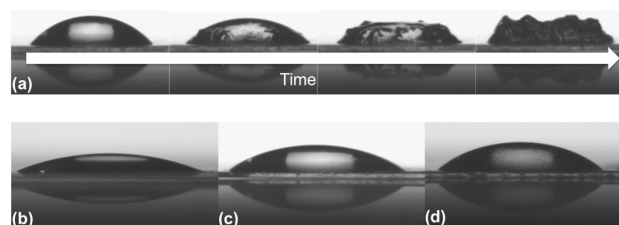


Figure 9. Static water contact angles of the four polymer films: a) uncrosslinked polymer film, P(DAA-*co*-AEMA) prepared at low pH (~ 2), b) slightly crosslinked polymer film, P(DAA-*co*-AEMA) prepared at high pH (~ 11.5), c) moderately crosslinked polymer film, P(DAA-*co*-AEMA), pH 11.5, NaIO_4 /catechol=1/100 (mol/mol), d) highly crosslinked polymer film, P(DAA-*co*-AEMA), pH=11.5, NaIO_4 /catechol=1/10

Table 2. Static water contact angle of four polymer films

Sample	Contact angle ($^{\circ}$)
P(DAA-co-AEMA) low pH	-
P(DAA-co-AEMA), high pH	30 ± 2.4
P(DAA-co-AEMA), high pH, 1/100 NaIO ₄	41 ± 0.2
P(DAA-co-AEMA), high pH, 1/10 NaIO ₄	46 ± 0.2

Increasing the pH before film casting, i.e., P(DAA-co-AEMA) prepared at pH 11.5, resulted in a polymer film that did not swell upon loading the film with a water droplet, as shown in Fig. 9(b). The polymer film was stable in the presence of water during the measurement, and exhibited a stable contact angle of around 30° . The different behavior between the low and high pH treatment can be interpreted as follows. Firstly, at high pH 11.5, when the pH is higher than the pK_a of the amino group in AEMA (~ 9.6)²⁶, AEMA is deprotonated, and free amines are formed. The *o*-quinones formed by oxidation from the catechols, can then react with the free amines by either a Schiff-base reaction or a Michael-type addition, or with catechols by dismutation reactions.¹⁰ We have shown in a separate study that the reaction between *o*-quinones and amine is predominant over the dismutation reaction with catechols. In the present study, we used UV-vis and FTIR measurements to corroborate this finding. As shown in Fig. 10, the peak at $\lambda = 280$ nm shifted to a slightly higher wavelength, $\lambda = 288$ nm. Meanwhile, a strong peak at $\lambda = 340$ nm appeared. The peak at $\lambda = 340$ nm has been ascribed to the formation of the reaction intermediate of catechol crosslinking.²⁷ The absorbance spectra of the film did not change over the course of 71 hours (Fig. S4), indicating that the polymer film is chemically stable for at least 3 days. Furthermore, in the FTIR spectrum of the polymer as shown in Fig. 11, the polymer prepared at low pH showed peaks at 1163 cm^{-1} ascribed to C-N stretch for aliphatic amines, and a broad peak above 3000 cm^{-1} for phenolic O-H stretch. These two peaks both decreased significantly for the other three crosslinked samples, indicating that the OH (on the aromatic ring of catechols) and amines had reacted. Secondly, in our catecholamine polymer, at low pH, the amines are protonated and charged, which favors the solubility in water. By increasing the pH, the deprotonation of the amines leads to a loss of charge, which reduces the solubility. This decrease in solubility upon pH increase is reflected by a decrease in viscosity. As shown in Fig. S5, the viscosity of PAEMA decreases fourfold when the pH increases from 2 to 12. However in general, to switch from water-soluble to water-insoluble a larger change in viscosity is expected²⁸, which indicates that the formation of the water-insoluble network is mainly due to the crosslinking reactions. The effects of catechol crosslinking-chemistry on the polymer solubility have also been observed in catechol-modified chitosan. The uncrosslinked polymer was soluble in water. By exposing the polymer to NaIO₄ solution, the crosslinking chemistry between catechol and the amines in chitosan was triggered, resulting in a water-insoluble polymer.²⁹

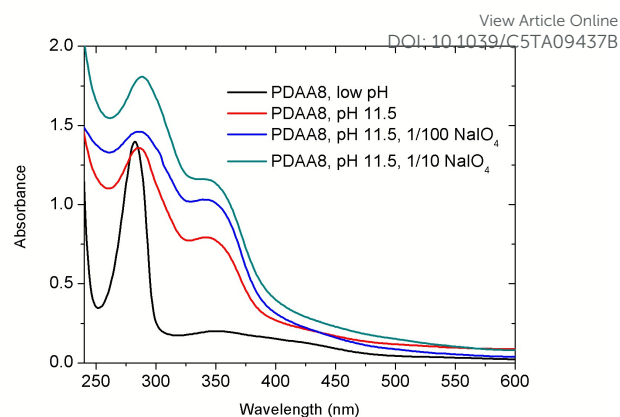
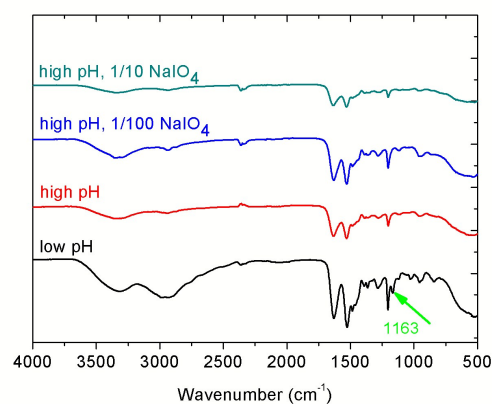


Figure 10. UV-vis spectra of polymer films deposited on quartz slides with different degree of crosslinking.

Figure 11. FTIR spectra of four polymers prepared at different conditions: a) at low pH (black line); b) high pH (red line); c) high pH, 1/100 NaIO₄ (blue line); d) high pH, 1/10 NaIO₄ (dark cyan line). The green arrow indicates the small peak at 1163 cm^{-1} .

The crosslinking rate of the catecholamine polymer film can be enhanced by the addition of NaIO₄. As shown in Fig. 9(c), upon adding a small amount of NaIO₄ (NaIO₄/catechol = 1/100), the polymer film showed a higher static water contact angle of 41° . This increase indicated a more crosslinked polymer film. The higher degree of crosslinking is also reflected in the UV-vis spectra: the peak is shifted to the right, i.e., $\lambda = 290$ nm, and the intensity of the peak at $\lambda = 340$ nm is increased. A further addition of more NaIO₄ (NaIO₄/catechol = 1/10) yielded a polymer film with even higher intensity of absorbance at $\lambda = 340$ nm (Fig. 10), and a higher static contact angle of 46° (Fig. 9(d)).

The effect of crosslinking on the morphology of the polymer film was studied by AFM measurements, as shown in Fig. 11. As shown in Fig. 12(a), the uncrosslinked polymer film showed a very smooth surface with negligible roughness. For the crosslinked films, however, as shown in Fig. 12 (b), (c) and (d), the roughness of the films increased. The roughnesses for the various crosslinked films did not differ much, indicating that a more crosslinked film does not necessarily lead to a rougher surface. It is known that the roughness of the surface has an amplifying effect on the contact angle. According to the Wenzel equation, for an intrinsic water/air contact angle (referring to a perfectly flat surface) smaller than 90° ,

roughness decreases the apparent contact angle, so that the surface seems to be more hydrophilic.³⁰ In our case, we observed that the contact angles for crosslinked films are lower than 90°. By taking into account the amplifying effect of roughness, we can conclude that the intrinsic contact angle for the crosslinked films is most likely higher than the data we currently obtained. The effect of crosslinking on the intrinsic contact angle should still follow the same trend as the one we observed.

In addition, our polymer films attach well to the glass substrate. By performing a cross-cut tape test, we measured the adhesion between the polymer film prepared at pH 11.5 and the glass substrate. Three tests at different locations of the film were performed. We found that, according to ASTM D 3359, the adhesion is rated as 4B. This means that only less than 5% of the coating area has been removed by the tape, indicating that our coating attaches well to the glass substrate. The good performance of the polymer film in the cross-cut adhesion test also implies a good mechanical stability of the film.

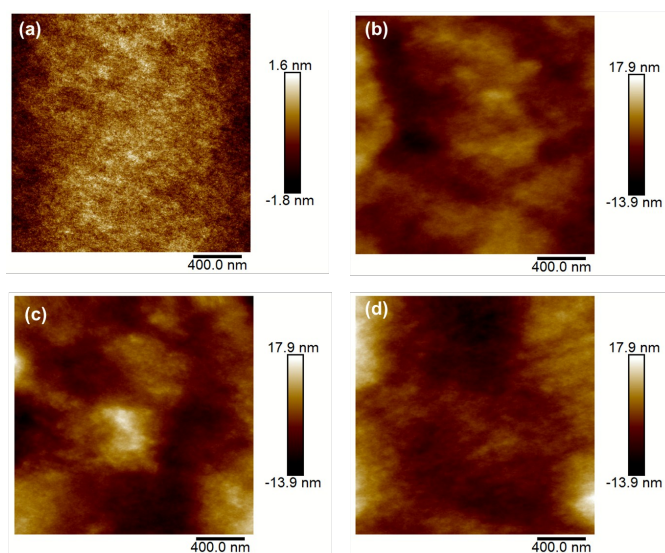


Figure 12. AFM images of P(DAA-co-AEMA) films, a) uncrosslinked film, P(DAA-co-AEMA) at low pH; b) slightly crosslinked film, P(DAA-co-AEMA), at pH 11.5; c) moderately crosslinked film, P(DAA-co-AEMA), pH 11.5, NaIO₄/ catechol=1/100; d) strongly crosslinked film, P(DAA-co-AEMA), pH 11.5, NaIO₄/ catechol=1/10.

Conclusions

In conclusion, we developed a one-pot coating system using the pH-responsive catecholamine polymer P(DAA-co-AEMA). We synthesized the copolymer by free radical polymerization of two monomers, DAA-*p* and AEMA, in aqueous conditions. The polymer works similarly as mussel proteins, i.e., being water-soluble at low pH (pH 2), and becoming water-insoluble at high pH (pH 11.5). The water-solubility switch was ascribed to the self-crosslinking ability of the polymer at high pH. Moreover, the crosslinking of the polymer is affected by the polymer sequence distribution. Using an in-situ ¹H NMR monitoring method, we studied the sequence distribution by looking at the reactivity ratios of the monomers DAA-*p* and AEMA. Both the reactivity ratios of the monomers are lower than unity, i.e., $r_{\text{DAA-}p} = 0$, $r_{\text{AEMA}} = 0.46$, giving rise to a moderately alternating polymer structure.

Acknowledgements

View Article Online

DOI: 10.1039/C5TA09437B

We would like to thank Remco Fokkink for help with dynamic and static light scattering measurements. We gratefully acknowledge the financial support of AkzoNobel.

References

1. J. H. Waite, *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 1990, 97, 19–29.
2. A. Miserez, D. Rubin and J. H. Waite, *Journal of Biological Chemistry*, 2010, 285, 38115–38124.
3. A. Miserez, T. Schneberk, C. Sun, F. W. Zok and J. H. Waite, *Science*, 2008, 319, 1816.
4. H. Bargel, K. Koch, Z. Cerman and C. Neinhuis, *Funct. Plant Biol.*, 2006, 33, 893–910.
5. N. Holten-Andersen, M. J. Harrington, H. Birkedal, B. P. Lee, P. B. Messersmith, K. Y. C. Lee and J. H. Waite, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, 108, 2651.
6. L. Vitellaro Zuccarello, *Tissue Cell*, 1981, 13, 701–713.
7. J. H. Dreisbach and J. R. Merkel, *J. Bacteriol.*, 1978, 135, 521–527.
8. G. I. Zardi, K. R. Nicastro, C. D. McQuaid and J. Erlandsson, *Mar. Biol.*, 2008, 153, 853–858.
9. J. R. E. Harger, *Veliger*, 1970, 12, 401–414.
10. J. Yang, M. A. Cohen Stuart and M. Kamperman, *Chem. Soc. Rev.*, 2014, 43, 8271–8298.
11. J. H. Waite and M. L. Tanzer, *Science*, 1981, 212, 1038.
12. M. J. Harrington, A. Masic, N. Holten-Andersen, J. H. Waite and P. Fratzl, *Science*, 2010, 328, 216–220.
13. J. Ji, J. H. Fu and J. C. Shen, *Adv. Mater.*, 2006, 18, 1441–1444.
14. Y. D. Wang, P. P. Joshi, K. L. Hobbs, M. B. Johnson and D. W. Schmidtke, *Langmuir*, 2006, 22, 9776–9783.
15. T. Boudou, T. Crouzier, K. F. Ren, G. Blin and C. Picart, *Adv. Mater.*, 2009, 22, 441–467.
16. E. Faure, C. Falentin-Daudré, T. S. Lanero, C. Vreuls, G. Zocchi, C. Van De Weerd, J. Martial, C. Jérôme, A.-S. Duwez and C. Detrembleur, *Advanced Functional Materials*, 2012, 22, 5271–5282.
17. E. Faure, P. Lecomte, S. Lenoir, C. Vreuls, C. Van De Weerd, C. Archambeau, J. Martial, C. Jérôme, A.-S. Duwez and C. Detrembleur, *Journal of Materials Chemistry*, 2011, 21, 7901–7904.
18. A. Charlot, V. Sciannamea, S. Lenoir, E. Faure, R. Jérôme, C. Jérôme, C. Van De Weerd, J. Martial, C. Archambeau, N. Willet, A.-S. Duwez, C.-A. Fustin and C. Detrembleur, *Journal of Materials Chemistry*, 2009, 19, 4117–4125.
19. J. Wu, L. Zhang, Y. Wang, Y. Long, H. Gao, X. Zhang, N. Zhao, Y. Cai and J. Xu, *Langmuir*, 2011, 27, 13684–13691.
20. H. O. Ham, Z. Liu, K. H. A. Lau, H. Lee and P. B. Messersmith, *Angewandte Chemie International Edition*, 2011, 50, 732–736.
21. M. R. Aguilar, A. Gallardo, M. d. M. Fernández and J. S. Román, *Macromolecules*, 2002, 35, 2036–2041.
22. J. Yang, J. Keijsers, M. Heek, A. Stuijver, M. A. Cohen Stuart and M. Kamperman, *Polym. Chem.*, 2015, 6, 3121–3130.
23. E. D. Vickery, L. F. Pahler and E. J. Eisenbraun, *J. Org. Chem.*, 1979, 44, 4444.
24. M. Rafiee and D. Nematollahi, *Electrochim. Acta*, 2008, 53, 2751–2756.

ARTICLE

Journal Name

25. G. Odian, *Principles of Polymerization*, Wiley-Interscience, 4th edn., 2004.
26. Z. C. Deng, H. Boucekif, K. Babooram, A. Housni, N. Choytun and R. Narain, *J. Polym. Sci. A Polym. Chem.*, 2008, 46, 4984-4996.
27. B. P. Lee, J. L. Dalsin and P. B. Messersmith, *Biomacromolecules*, 2002, 3, 1038-1047.
28. Y. Li and J. C. T. Kwak, *Langmuir*, 2002, 18, 10049-10051.
29. J. H. Ryu, S. Jo, M.-Y. Koh and H. Lee, *Advanced Functional Materials*, 2014, 24, 7709-7716.
30. D. Quere, *Phys. A*, 2002, 313, 32-46.

View Article Online
DOI: 10.1039/C5TA09437B