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# Supporting information

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# Origin of surface charge of double network hydrogels prepared by sequential polymerization

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# Supporting Information

## 1.1 Experimental section

### 1.1.1 Materials

The monomers 2-acrylamido-2-methylpropanesulfonic acid (AMPS, 1st network) was obtained by Toagosei Co., Ltd., Japan. The acrylamide (AAm, 2nd network), the crosslinker N,N'-methylenebis(acrylamide) (MBAA), and initiator 2-oxoglutaric acid were obtained from Wako Pure Chemical Industries, Ltd., Japan. All reagents were used as received, and all solutions were prepared in double-distilled water.

### 1.1.2 Single network, double network, and co-polymer hydrogel synthesis

All hydrogels were synthesized based on the formulations in **Table 1** and **Table S1** via free-radical polymerization by 365 nm-UV light for 8 hours in an argon atmosphere. The double network (DN) hydrogels were prepared as previously described using glass slides as a molding material, and no compression in the polymerization steps was applied to create a 2nd network surface layer[1].

Briefly, the 1st network was synthesized from an aqueous solution 1 M AMPS containing 3 mol% crosslinker MBAA and 1 mol% 2-oxoglutaric acid as initiator, poured between two glass plates spaced by 1 mm. The poly 2-acrylamido-2-methylpropanesulfonic acid (PAMPS) hydrogel rectangles of different sizes were cut out to reach volume ratios $V_r$ of 0,02 to 0,001 ($V_r$=$V_{PAMPS}$/$V_{AAm}$) of PAMPS hydrogel ($V_{PAMPS}$) in AAm 2nd network precursor solution ($V_{AAm}$). For the sample PAMPS-w, an as-prepared PAMPS hydrogel with 2 cm³ (geometry: *l*×*b*×*h*, 4×5×0,1 cm; 2 mL) washed in 100 mL water ($V_r$ 0,02) for 15 days. Here, the 100 mL washing water was exchanged daily, and ionic conductivity was measured. PAMPS and PAMPS-w hydrogels were equilibrated for 3 days in 50mL of 2nd network precursor solution containing 2,0 M AAm monomer, 0,1 mol% MBAA crosslinker, and 0,1 mol% 2-oxoglutaric acid. The PAMPS hydrogels soaked in AAm solution were sandwiched between two glass plates and polymerized to DN hydrogels (PAMPS/PAAm). After equilibration and removal of the PAMPS

hydrogel from the 2nd network precursor solution, this waste solution was used to synthesize polyacrylamide (PAAm) single network (SN) hydrogels (PAAm-ps).

Further, a control group out of poly(AAm-co-AMPS) hydrogel was synthesized to demonstrate how the PAMPS concentration affects the electric potential. Here, the PAMPS concentration was regulated by the concentration of AMPS in the mixture in the range of 20 – 0,02 mM. The mixture solution contains 2,0 M AAm, 0,1 mol% MBAA crosslinker, and 0,1 mol% 2-oxoglutaris acid. Since this control group tries to mimic the residual AMPS content in the 2nd network precursor solution, it is also referred to as $C_{AMPS,R}$ for the reason of simplification. To synthesize the poly(AAm-co-AMPS) hydrogels, the aqueous AAm and AMPS precursor solutions were mixed by the ratios in **Table S1**. The mixtures were polymerized between two glass plates spaced by 1 mm. Before experimental use, all hydrogels were equilibrated in water and in $1x10^{-4}$ M NaClaq for MET measurement.

**Table S1: Poly(AAm-co-AMPS) hydrogel formulation with varied $C_{AMPS,R}$ concentration.**

| Total volume (mL) | Volume of PAAm precursor sol.* (mL) | Volume of 1 M AMPS precursor sol. (µL) | $C_{AMPS,R}$ (mM) | $C_{AMPS,R}/C_{AAm}$ (M/M) |
|---|---|---|---|---|
| 50 | 49 | 1000 | 20 | 1/98 |
| 50 | 49,5 | 500 | 10 | 1/198 |
| 50 | 49,9 | 100 | 2 | 1/998 |
| 50 | 50 | 10 | 0,2 | 1/10000 |
| 50 | 50 | 1 | 0,02 | 1/100000 |

* 2,0 M AAm, 0,1 mo% crosslinker, and 0,1 mol% initiator.

### 1.1.3 Determining unreacted $C_{AMPS,R}$ of the hydrogel via the ionic conductivity of bath solution

The unreacted AMPS monomer concentration ($C_{AMPS,R}$) in the hydrogel bathing solution was monitored on a conductivity meter (FE30 KIT, Mettler Toledo). The evaluation included the correlation between ionic conductivity and the concentration of the dissolved AMPS monomers via a dilution series ranging from $10^{-1}$ to $10^{-6}$ M, using water or 2nd network precursor solution containing AAm monomer (formulation in **Table 1**) as diluting agent (**Figure 1A**). Hereby, we assume that other stable and highly charged molecules in the hydrogel precursor solution, such as crosslinker and initiator, are totally consumed during the 8 hours of polymerization because they are used at a much smaller concentration

than the monomer. In the AAm solution, the lower detection limit of $C_{AMPS}$ lies at a concentration of 0,1 mM, as the conductivity approaches a constant value of 340 µS/cm, which is characteristic for the pure 2nd network precursor solution of 2 M AAm containing 0,1 mol% crosslinker and 0,1 mol% photoinitiator (**Table S2**). The calibration curves for AMPS in water and AMPS in 2nd network precursor solution were fitted by Origin (OriginLab Corp.) software. With these calibration curves, we calculated $C_{AMPS,R}$ in the washing water of the PAMPS-w hydrogel immersed in 100 mL water and the equilibration of PAMPS and PAMPS-w hydrogel in 2nd network precursor solution of AAm at multiple time points. Hydrogels were stored at 25 °C in the dark on a horizontal shaker plate at 60 rpm. It needs to be noted that the calculated $C_{AMPS_R}$ was corrected by the daily decrease in conductivity of the control AAm bath solution containing no PAMPS hydrogel since the UV/VIS reactive 2-oxoglutaric acid is consumed slightly over time.

### 1.1.4 Determine surface charge density $C_G$ via the microelectrode technique (MET)

To investigate the effect of $C_{AMPS,R}$ on the hydrogel surface charge density $C_G$ the electric potential difference $\Delta\varphi$ between the inside and outside of the hydrogel was measured via MET[2]. Before the sampling, hydrogels were washed in water until the washing water reached a conductivity of less than 2,0 µS/cm. Then the hydrogel was immersed in the bath aqueous solution of NaCl 1 x $10^{-4}$ M ($C_S$) (Debye length is 30,4 nm) until it reached equilibrium at a conductivity of ~15 µS/cm at 25 °C. The hydrogels were probed with a fiber-filament glass capillary microelectrode filled with 3 M KCl of 180 nm outer diameter and 128 nm inner diameter (P-1000 capillary puller - Sutter Instrument Co., U.S.). The microelectrode was inserted at a speed range of 0,5 – 2 µm/s. The surface charge density $C_G$ at the hydrogel surface was calculated via **Eq. 1** by using the averaged electric potential differences ($\Delta\varphi$ between blue arrows in **Figure 3**, n = 3) between the bathing solution (**Figure 3**, white area) and the hydrogel surface region (**Figure 3**, light grey area). The activity coefficients are considered as 1 ($\gamma_S$, $\gamma_G$ = 1) since the mobile ion concentration in salt solution and the surface layer of DN hydrogels are very low.

### 1.1.5 Determination of the volumetric swelling ratio of hydrogels with varied $C_{AMPS,R}$ in the precursor solution

The volumetric swelling ratio of hydrogels $Q = (t_1/t_0)^3$, was determined from the thickness of hydrogels in the as-prepared state after polymerization of PAAm ($t_0$) and in the equilibrated state in 1x10$^{-4}$ M NaCl solution ($t_1$) for PAAm-ps and Poly(AAm-co-AMPS) hydrogels. For PAMPS/PAAm DN hydrogels, the surface layer's swelling ratio should be considered. The bulk DN hydrogel hardly swells in the 1x10$^{-4}$ M NaCl solution, as the volumetric swelling ratio $Q = (t_1/t_0)^3$ of the as-prepared PAMPS/PAAm hydrogel ($t_0$) (which is the same as the thickness of PAMPS hydrogel equilibrated in the AAm 2$^{nd}$ network precursor solution) and the thickness of PAMPS/PAAm hydrogel swollen in 1x10$^{-4}$ M NaCl solution ($t_1$) is almost 1 (**Figure S3**). A recent study has shown that the soft surface layer of the second network swells uniaxially normal to the surface since the lateral swelling is constrained by the rigid bulk DN hydrogel [3]. A recent study has also shown that the volume of PAAm hydrogel with uniaxial swelling is the same with the isotropic swelling[4]. Thereby, we assume that $Q$ of the surface second network layer in PAMPS/PAAm DN gel is the same as that of PAAm-ps.

### 1.1.6 Mechanical testing via micro-indentation

The hydrogel elastic modulus ($E$) was measured on a tensile-compressive mechanical tester (Autograph AG-X, Shimadzu Co., Japan) in air at 25°C. A sphere-shaped micro-indenter (r = 250 μm, material: stainless steel) probed the hydrogel at a deformation velocity of 250 μm/min for 1 min. The Hertzian model[5] for rigid sphere indenters, with an assumed Poisson's ratio for the hydrogels of 0,5, was used to determine $E$ from the micro-indentation loading curves. We made sure to limit the effect of hydrogel drying on the mechanical testing by saturating the air with water via a humidifier placed next to the tensile tester and reducing the measurement time down to 1 minute.

## 1.2 Supporting Data

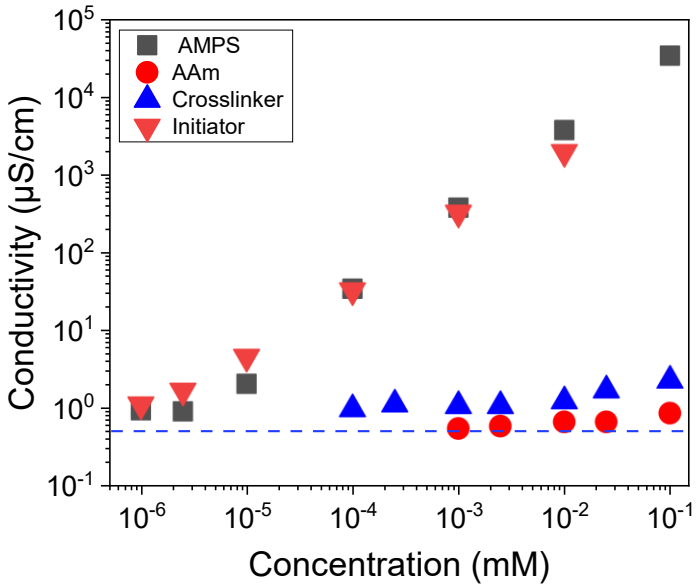


**Figure S1: The relation of conductivity to the concentration of agents used in the 1st and 2nd network precursor solution.** The Conductivity of AMPS, AAm, the crosslinker N,N'-methylenebis(acrylamide), and the initiator 2-oxoglutaric acid in deionized water measured at 25°C (the blue dotted line represents the conductivity of deionized water). The conductivity measurements cannot account for the hydrolysis of acrylamide and hydrolysis. However, even if there are impurities and hydrolysis in AAm, the conductivity of AAm solutions is not higher than 1 µS/cm. Therefore, we can conclude that for the conductivity measurements, the effects of hydrolysis and impurities in AAm are negligible because the conductivity in the precursor solution comes from the initiator.

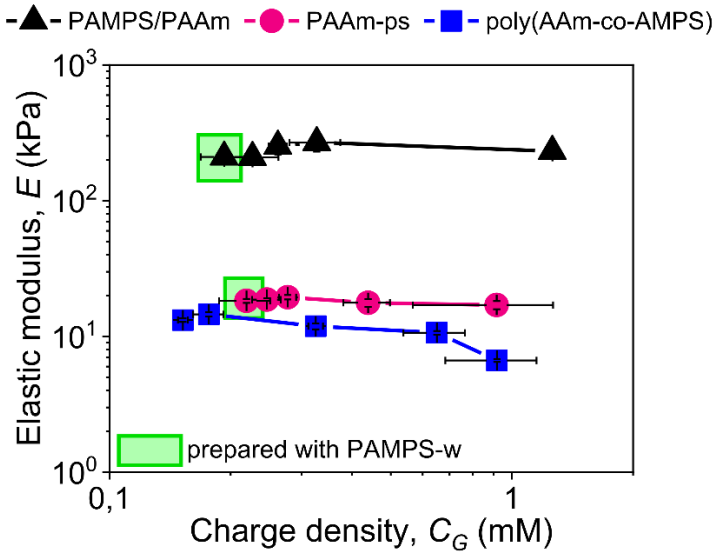


**Figure S2: Relation between surface layer charge density $C_G$ calculated from $\Delta\varphi$ and the elastic modulus $E$ on the surface layer of hydrogels measured by micro-indentation**. We found that $E$ measured in the bulk of the PAMPS/PAAm DN hydrogels is almost constant in the range of 200 – 300 kPa, regardless of charge density increase from 0,19 and 1,26 mM. The PAAm-ps hydrogel group behaves similarly but shows a lower elastic modulus range at 15 - 20 kPa, which is characteristic of SN PAAm hydrogels of this formulation. Only the poly(AAm-co-AMPS) hydrogel series showed a slight decrease of $E$ with the increase of $C_G$, which is attributed to the swelling, as shown in the main article (**Figure 4B**) and the supporting information (**Figure S3**).

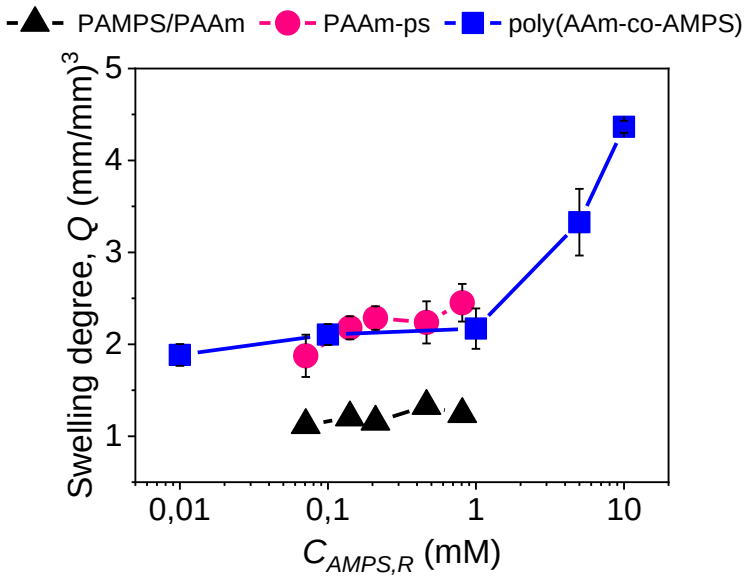


**Figure S3: The swelling ratio *Q* of the bulk hydrogels in the state of MET measurement against $C_{AMPS,R}$ in the precursor solution of the PAAm network.** The decreased swelling of the DN hydrogels after 2nd network polymerization results from the reduced swelling of the 2nd network confined by the 1st network in the bulk PAMP/PAAm DN hydrogel. However, the swelling ratio of the surface second network layer in PAMPS/PAAm DN hydrogels should be similar to that of PAAm-ps hydrogels. Therefore, $C_GQ$ of the DN hydrogels was calculated, using *Q* of the corresponding PAAm-ps hydrogels.

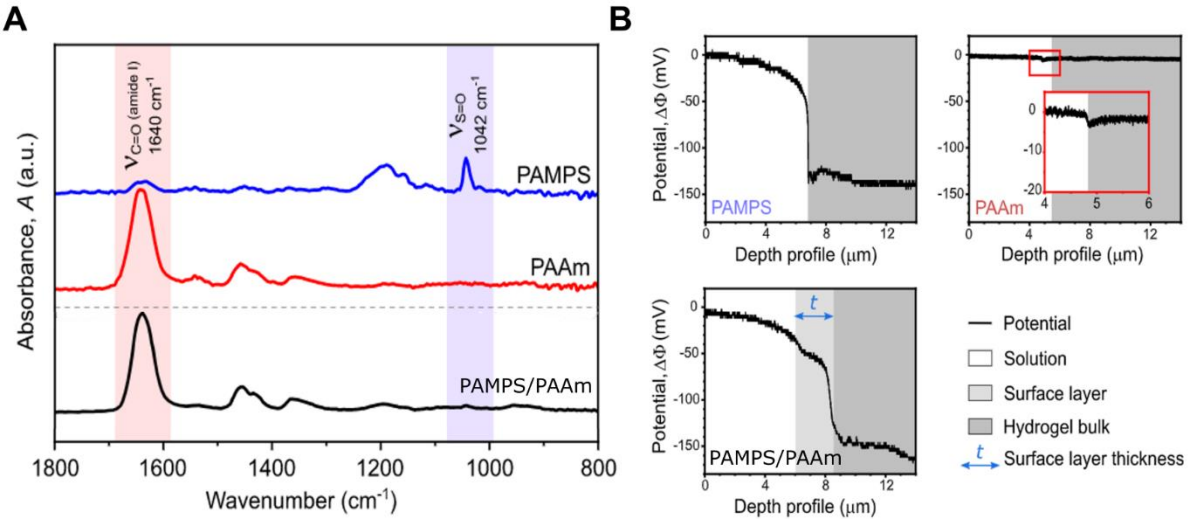


**Figure S4: Comparison between ATR-FTIR and Microelectrode technique measurements (MET).** (A) ATR-FTIR profiles and (B) MET measurements of single network PAMPS, PAAm, and the corresponding double network hydrogel (PAMPS/PAAm) demonstrate that the concentration of AMPS groups in the double network hydrogel surface layer was too low to be detected by ATR-FTIR, while the MET measurement was able to clearly detect a drop in surface layer potential of -50mV. The figure is reproduced with permission from "Modulation and Characterization of the Double Network Hydrogel Surface-Bulk Transition" (DOI:10.1021/acs.macromol.9b01399). Copyright 2019 ACS.

**Table S2: Summary of results**

| $V_r$ (mL/mL) | Conductivity (µS/cm) | $C_{AMPS,R}$ (mM) | $C_{AMPS,R}/C_{AAM}$ (M/M) | $Q$ (mm/mm)$^3$ | $\Delta\varphi$ (mV) | $C_G$ (mM) | $C_GQ$ (mM) | $E$ (kPa) |
|---|---|---|---|---|---|---|---|---|
| PAMPS/PAAm | | | | | | | | |
| 0,02 | 510,2 | 0,81 | 1/2419 | 1,24 ± 0,02 | -65,0 ± 0,9 | 1,26 ± 0,04 | 3,09 ± 0,28$^{\$}$ | 231,5 ± 10,4 |
| 0,01 | 437,5 | 0,46 | 1/4304 | 1,33 ± 0,05 | -30,2 ± 3,7 | 0,33 ± 0,05 | 0,73 ± 0,13$^{\$}$ | 269,0 ± 39,3 |
| 0,003 | 384,1 | 0,21 | 1/9495 | 1,16 ± 0,03 | -24,7 ± 1,3 | 0,26 ± 0,01 | 0,60 ± 0,05$^{\$}$ | 252,7 ± 35,5 |
| 0,001 | 369,6 | 0,14 | 1/14271 | 1,20 ± 0,01 | -20,8 ± 4,1 | 0,23 ± 0,04 | 0,49 ± 0,08$^{\$}$ | 209,0 ± 7,8 |
| 0,02* | 356,2 | 0,07 | 1/28000 | 1,12 ± 0,05 | -16,7 ± 3,2 | 0,19 ± 0,02 | 0,36 ± 0,06$^{\$}$ | 209,9 ± 8,1 |
| PAAm-ps | | | | | | | | |
| 0,02 | 510,2 | 0,81 | 1/2419 | 2,45 ± 0,20 | -55,6 ± 9,0 | 0,92 ± 0,35 | 2,24 ± 0,86 | 17,1 ± 1,2 |
| 0,01 | 437,5 | 0,46 | 1/4304 | 2,24 ± 0,23 | -37,8 ± 3,2 | 0,44 ± 0,06 | 0,98 ± 0,16 | 17,7 ± 1,1 |
| 0,003 | 384,1 | 0,21 | 1/9495 | 2,28 ± 0,13 | -26,1 ± 1,3 | 0,28 ± 0,01 | 0,63 ± 0,05 | 19,5 ± 0,7 |
| 0,001 | 369,6 | 0,14 | 1/14271 | 2,18 ± 0,12 | -23,0 ± 2,2 | 0,25 ± 0,02 | 0,54 ± 0,05 | 18,7 ± 0,4 |
| 0,02* | 356,2 | 0,07 | 1/28000 | 1,87 ± 0,23 | -19,9 ± 3,5 | 0,22 ± 0,03 | 0,41 ±0,07 | 18,3 ± 0,6 |
| PAAm | | | | | | | | |
| -- | 340 | -- | -- | 1,98 ± 0,12 | -9,2 ± 1,6 | 0,14 ± 0,01 | 0,28 ± 0,02 | 15,1 ± 1,0 |
| poly(AAm-co-AMPS) | | | | | | | | |
| -- | 4600 | 20 | 1/98 | 4,37 ± 0,07 | -56,3 ± 5,9 | 0,92 ± 0,23 | 4,00 ± 1,02 | 6,7 ± 0,2 |
| -- | 2350 | 10 | 1/198 | 3,33 ± 0,36 | -47,8 ± 4,7 | 0,65 ± 0,11 | 2,16 ± 0,44 | 10,7 ± 0,3 |
| -- | 738 | 2 | 1/998 | 2,17 ± 0,22 | -30,3 ± 1,1 | 0,33 ± 0,01 | 0,71 ± 0,08 | 11,9 ± 0,6 |
| -- | 395 | 0,2 | 1/10000 | 2,11 ± 0,11 | -14,5 ± 2,2 | 0,18 ± 0,02 | 0,37 ± 0,04 | 14,6 ± 0,5 |
| -- | 360 | 0,02 | 1/100000 | 1,88 ± 0,12 | -10,8 ± 0,7 | 0,15 ± 0,01 | 0,29 ± 0,02 | 13,2 ± 0,4 |

*Sample prepared associated with PAMPS-w. $^{\$}C_G$ multiplied by $Q$ from the corresponding PAAm-ps gel.

## 1.3 References


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