

HOKKAIDO UNIVERSITY

Title	Origin of Surface Charge of Double Network Hydrogels Prepared by Sequential Polymerization			
Author(s)	Frauenlob, Martin; Guo, Honglei; Kurokawa, Takayuki; Gong, Jian Ping			
Citation	ACS Macro Letters, 12(7), 860-865 https://doi.org/10.1021/acsmacrolett.3c00160			
Issue Date	2023-07-18			
Doc URL	http://hdl.handle.net/2115/92799			
Rights	This document is the Accepted Manuscript version of a Published Work that appeared in final form in ACS Macro Letters, copyright c American Chemical Society after peer review and technical editing by the publisher. To access the final edited and published work see https://pubs.acs.org/articlesonrequest/AOR-TMEDFJGWUG5ATJBWGSRV.			
Туре	article (author version)			
Additional Information	There are other files related to this item in HUSCAP. Check the above URL.			
File Information	ACS_macro_origin_of_DN_charge_SI.pdf			



1	Supporting information
2	to
3 4	Origin of surface charge of double network hydrogels prepared by sequential polymerization
5	Martin Frauenlob ^{1,2} , Honglei Guo ³ , Takayuki Kurokawa ^{3,*} and Jian Ping Gong ^{3,4} *
6 7	¹ Graduate School of Life Science, Hokkaido University, N21W11, Kita-ku, Sapporo, Hokkaido 001- 0021, Japan
8 9	² Group of Biomaterials and Microfluidics Core Facility, Institut Pasteur, 25-28 Rue du Docteur Roux, 75015 Paris, France
10 11	³ Faculty of Advanced Life Science, Hokkaido University, N21W11, Kita-ku, Sapporo 001-10 0021, Japan
12 13	⁴ Institute for Chemical Reaction Design and Discovery (WPI-ICReDD), Hokkaido University, 8 N21W10, Kita-ku, Sapporo 001-0021, Japan
14	
15	*Corresponding author (E-mail: <u>kurokawa@sci.hokudai.ac.jp; gong@sci.hokudai.ac.jp</u>)
16	

Supporting Information 17 18 Experimental section 19 1.1 Materials 20 1.1.1 The monomers 2-acrylamido-2-methylpropanesulfonic acid (AMPS, 1st network) was obtained by 21 Toagosei Co., Ltd., Japan. The acrylamide (AAm, 2nd network), the crosslinker N,N'-22 23 methylenebis(acrylamide) (MBAA), and initiator 2-oxoglutaric acid were obtained from Wako Pure 24 Chemical Industries, Ltd., Japan. All reagents were used as received, and all solutions were prepared 25 in double-distilled water. Single network, double network, and co-polymer hydrogel synthesis 26 1.1.2 27 All hydrogels were synthesized based on the formulations in Table 1 and Table S1 via free-radical 28 polymerization by 365 nm-UV light for 8 hours in an argon atmosphere. The double network (DN) 29 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 layer¹. 30 31 Briefly, the 1st network was synthesized from an aqueous solution 1 M AMPS containing 3 mol% 32 crosslinker MBAA and 1 mol% 2-oxoglutaric acid as initiator, poured between two glass plates spaced 33 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 34 hydrogel (V_{PAMPS}) in AAm 2nd network precursor solution (V_{AAm}). For the sample PAMPS-w, an as-35 prepared PAMPS hydrogel with 2 cm³ (geometry: *l×b×h*, 4×5×0,1 cm; 2 mL) washed in 100 mL water 36 (Vr 0,02) for 15 days. Here, the 100 mL washing water was exchanged daily, and ionic conductivity was 37 measured. PAMPS and PAMPS-w hydrogels were equilibrated for 3 days in 50mL of 2nd network 38 39 precursor solution containing 2,0 M AAm monomer, 0,1 mol% MBAA crosslinker, and 0,1 mol% 2-40 oxoglutaric acid. The PAMPS hydrogels soaked in AAm solution were sandwiched between two glass 41 plates and polymerized to DN hydrogels (PAMPS/PAAm). After equilibration and removal of the PAMPS 42 hydrogel from the 2nd network precursor solution, this waste solution was used to synthesize
43 polyacrylamide (PAAm) single network (SN) hydrogels (PAAm-ps).

44 Further, a control group out of poly(AAm-co-AMPS) hydrogel was synthesized to demonstrate how the 45 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 46 47 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 48 C_{AMPS,R} for the reason of simplification. To synthesize the poly(AAm-co-AMPS) hydrogels, the aqueous 49 50 AAm and AMPS precursor solutions were mixed by the ratios in Table S1. The mixtures were 51 polymerized between two glass plates spaced by 1 mm. Before experimental use, all hydrogels were 52 equilibrated in water and in 1×10^{-4} M NaClaq for MET measurement.

_	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

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

54 * 2,0 M AAm, 0,1 mo% crosslinker, and 0,1 mol% initiator.

55 1.1.3 Determining unreacted $C_{AMPS,R}$ of the hydrogel via the ionic conductivity of bath 56 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 CAMPS lies at a concentration of 64 0,1 mM, as the conductivity approaches a constant value of 340 µS/cm, which is characteristic for the 65 pure 2nd network precursor solution of 2 M AAm containing 0,1 mol% crosslinker and 0,1 mol% 66 photoinitiator (Table S2). The calibration curves for AMPS in water and AMPS in 2nd network precursor 67 68 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 69 of PAMPS and PAMPS-w hydrogel in 2nd network precursor solution of AAm at multiple time points. 70 71 Hydrogels were stored at 25 °C in the dark on a horizontal shaker plate at 60 rpm. It needs to be noted 72 that the calculated C_{AMPS R} was corrected by the daily decrease in conductivity of the control AAm bath 73 solution containing no PAMPS hydrogel since the UV/VIS reactive 2-oxoglutaric acid is consumed 74 slightly over time.

75 1.1.4 Determine surface charge density C_G via the microelectrode technique (MET)

76 To investigate the effect of $C_{AMPS,R}$ on the hydrogel surface charge density C_G the electric potential 77 difference $\Delta \varphi$ between the inside and outside of the hydrogel was measured via MET². Before the 78 sampling, hydrogels were washed in water until the washing water reached a conductivity of less than 79 2,0 μ S/cm. Then the hydrogel was immersed in the bath aqueous solution of NaCl 1 x 10⁻⁴ M (C_s) (Debye 80 length is 30,4 nm) until it reached equilibrium at a conductivity of ~15 µS/cm at 25 °C. The hydrogels 81 were probed with a fiber-filament glass capillary microelectrode filled with 3 M KCl of 180 nm outer 82 diameter and 128 nm inner diameter (P-1000 capillary puller - Sutter Instrument Co., U.S.). The microelectrode was inserted at a speed range of $0.5 - 2 \mu m/s$. The surface charge density C_G at the 83 84 hydrogel surface was calculated via Eq. 1 by using the averaged electric potential differences ($\Delta \phi$ between blue arrows in Figure 3, n = 3) between the bathing solution (Figure 3, white area) and 85 86 the hydrogel surface region (**Figure 3**, light grey area). The activity coefficients are considered as 1 (γ_s , γ_G = 1) since the mobile ion concentration in salt solution and the surface layer of DN hydrogels are 87 88 very low.

89 1.1.5 Determination of the volumetric swelling ratio of hydrogels with varied $C_{AMPS,R}$ in the 90 precursor solution

The volumetric swelling ratio of hydrogels $Q = (t_1/t_0)^3$, was determined from the thickness of hydrogels 91 92 in the as-prepared state after polymerization of PAAm (t_0) and in the equilibrated state in 1x10⁻⁴ M 93 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⁻ 94 ⁴ M NaCl solution, as the volumetric swelling ratio $Q = (t_1/t_0)^3$ of the as-prepared PAMPS/PAAm 95 hydrogel (t₀) (which is the same as the thickness of PAMPS hydrogel equilibrated in the AAm 2nd 96 network precursor solution) and the thickness of PAMPS/PAAm hydrogel swollen in 1x10⁻⁴ M NaCl 97 98 solution (t_1) is almost 1 (Figure S3). A recent study has shown that the soft surface layer of the second 99 network swells uniaxially normal to the surface since the lateral swelling is constrained by the rigid 100 bulk DN hydrogel ³. A recent study has also shown that the volume of PAAm hydrogel with uniaxial 101 swelling is the same with the isotropic swelling⁴. Thereby, we assume that Q of the surface second 102 network layer in PAMPS/PAAm DN gel is the same as that of PAAm-ps.

103 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 ($r = 250 \mu m$, material: stainless steel) probed the hydrogel at a deformation velocity of 250 $\mu m/min$ for 1 min. The Hertzian model⁵ 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.

111

112 1.2 Supporting Data



113

114 Figure S1: The relation of conductivity to the concentration of agents used in the 1st and 2nd network 115 precursor solution. The Conductivity of AMPS, AAm, the crosslinker N,N'-methylenebis(acrylamide), 116 and the initiator 2-oxoglutaric acid in deionized water measured at 25°C (the blue dotted line 117 represents the conductivity of deionized water). The conductivity measurements cannot account for 118 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 119 120 for the conductivity measurements, the effects of hydrolysis and impurities in AAm are negligible 121 because the conductivity in the precursor solution comes from the initiator.



122

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

130 (Figure 4B) and the supporting information (Figure S3).



131

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.







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.

147

139

148 Table S2: Summary of results

Vr	Conductivity	$C_{AMPS,R}$	$C_{AMPS,R}/C_{AAM}$	0	Δφ	C_{G}	$C_G Q$	Ε
(mL/mL)	(uS/cm)	(mM)	(M/M)	\sim (mm/mm) ³	(mV)	(mM)	(mM)	(kPa)
PAMPS/PAAm						()		
0.02	510.2	0.81	1/2/10	1.24 ± 0.02	-65.0 ± 0.9	1.26 ± 0.04	$3.09 \pm 0.28^{\circ}$	2315 ± 104
0.01	437.5	0.46	1/2419	$1,21 \pm 0,02$ $1,33 \pm 0.05$	-30.2 ± 3.7	0.33 ± 0.05	$0.73 \pm 0.13^{\circ}$	269.0 ± 39.3
0.003	384.1	0.21	1/0405	$1,55 \pm 0,03$	-247 ± 13	0.26 ± 0.01	$0.60 \pm 0.05^{\circ}$	$252,0 \pm 35,5$ $252,7 \pm 35,5$
0,005	369.6	0,21	1/9493	$1,10 \pm 0,03$ $1,20 \pm 0.01$	$24,7 \pm 1,5$ 20.8 ± 4.1	0.23 ± 0.01	$0.49 \pm 0.08^{\circ}$	$202,7 \pm 33,3$ 200.0 ± 7.8
0,001	256.2	0,14	1/142/1	$1,20 \pm 0,01$	$-20,8 \pm 4,1$	$0,23 \pm 0,04$	$0.36 \pm 0.06^{\circ}$	$209,0 \pm 7,8$
0,02*	550,2	0,07	1/28000	$1,12 \pm 0,03$	$-10,7 \pm 3,2$	0,19 ± 0,02	0,50 ± 0,00	209,9 ± 8,1
PAAIII-ps								
0,02	510,2	0,81	1/2419	$2,45 \pm 0,20$	$-55,6 \pm 9,0$	$0,\!92\pm0,\!35$	$2,24 \pm 0,86$	$17,1 \pm 1,2$
0,01	437,5	0,46	1/4304	$2{,}24\pm0{,}23$	$\textbf{-37,8} \pm \textbf{3,2}$	$0{,}44 \pm 0{,}06$	$0,\!98\pm0,\!16$	$17,7 \pm 1,1$
0,003	384,1	0,21	1/9495	$2,\!28\pm0,\!13$	$-26,1 \pm 1,3$	$0{,}28 \pm 0{,}01$	$0,\!63\pm0,\!05$	$19,5\pm0,7$
0,001	369,6	0,14	1/14271	$2,18 \pm 0,12$	$-23,0 \pm 2,2$	$0,\!25\pm0,\!02$	$0{,}54 \pm 0{,}05$	$18,7 \pm 0,4$
0,02*	356,2	0,07	1/28000	$1,\!87\pm0,\!23$	$-19,9 \pm 3,5$	$0{,}22\pm0{,}03$	$0,41 \pm 0,07$	$18,3\pm0,\!6$
PAAm								
	340			$1,98 \pm 0,12$	$-9,2 \pm 1,6$	$0,\!14\pm0,\!01$	$0{,}28\pm0{,}02$	$15,1 \pm 1,0$
poly(AAm-co-AMPS)								
	4600	20	1/98	$4,37 \pm 0,07$	$-56,3 \pm 5,9$	$0,92 \pm 0,23$	$4{,}00\pm1{,}02$	$6,7 \pm 0,2$
	2350	10	1/198	$3,33 \pm 0,36$	$-47,8 \pm 4,7$	$0,65 \pm 0,11$	$2,\!16\pm0,\!44$	$10,7 \pm 0,3$
	738	2	1/998	$2,17 \pm 0,22$	$-30,3 \pm 1,1$	$0,33 \pm 0,01$	$0{,}71\pm0{,}08$	$11,9 \pm 0,6$
	395	0,2	1/10000	$2,11 \pm 0,11$	$-14,5 \pm 2.2$	$0,18 \pm 0,02$	$0,\!37\pm0,\!04$	$14,6 \pm 0,5$
	360	0,02	1/100000	$1,\!88\pm0,\!12$	$-10,8 \pm 0,7$	$0,15 \pm 0,01$	$0{,}29\pm0{,}02$	$13,2 \pm 0,4$

149 *Sample prepared associated with PAMPS-w. ${}^{S}C_{G}$ multiplied by Q from the corresponding PAAm-ps gel.

151 1.3 References

Frauenlob, M.; King, D. R.; Guo, H.; Ishihara, S.; Tsuda, M.; Kurokawa, T.; Haga, H.; Tanaka, S.;
 Gong, J. P. Modulation and Characterization of the Double Network Hydrogel Surface-Bulk
 Transition. *Macromolecules* 2019, *52* (17), 6704–6713.
 https://doi.org/10.1021/acs.macromol.9b01399.

(2) Guo, H.; Kurokawa, T.; Takahata, M.; Hong, W.; Katsuyama, Y.; Luo, F.; Ahmed, J.; Nakajima,
T.; Nonoyama, T.; Gong, J. P. Quantitative Observation of Electric Potential Distribution of
Brittle Polyelectrolyte Hydrogels Using Microelectrode Technique. *Macromolecules* 2016, 49
(8), 3100–3108. https://doi.org/10.1021/acs.macromol.6b00037.

- 160 (3) Kiyama, R.; Yoshida, M.; Nonoyama, T.; Sedlačík, T.; Jinnai, H.; Kurokawa, T.; Nakajima, T.;
 161 Gong, J. P. Nanoscale TEM Imaging of Hydrogel Network Architecture. *Advanced Materials*162 **2023**, *35* (1), 2208902. https://doi.org/10.1002/adma.202208902.
- 163 (4) Haque, M. A.; Kurokawa, T.; Nakajima, T.; Kamita, G.; Fatema, Z.; Gong, J. P. Surfactant
 164 Induced Bilayer-Micelle Transition for Emergence of Functions in Anisotropic Hydrogel.
 165 Journal of Materials Chemistry B 2022, 10 (41), 8386–8397.
 166 https://doi.org/10.1039/D2TB00172A.
- 167 (5) Hertz, H. On the Contact of Elastic Solids. J. Reine Angew. Math. 1881, 92, 156–171.

168

¹⁵⁰