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

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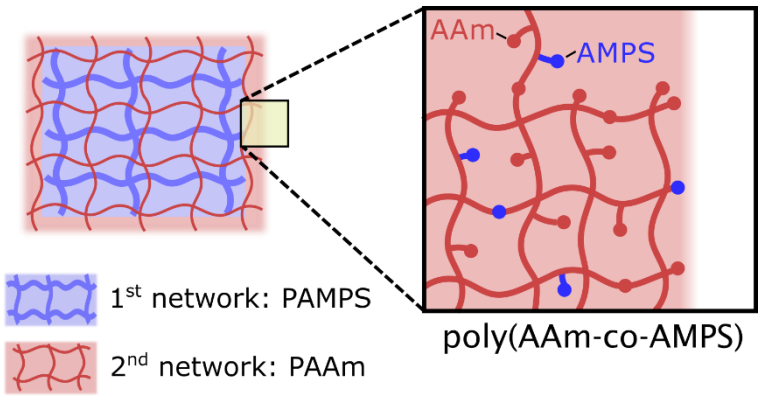
Abstract

Understanding the physicochemical properties of hydrogel surfaces and their molecular origins is important for their applications. In this paper, we elucidate the molecular origin of surface charges in double-network hydrogels synthesized by two-step sequential polymerization. Synthesis of hydrogels by free-radical polymerization does not fully complete the reaction, leaving a small amount of unreacted monomers. When this approach is used to synthesize double network (DN) hydrogels by two-step sequential polymerization from charged monomers for the first network and neutral monomers for the second network, the unreacted 1st network monomers are incorporated into the 2nd network. Since the surface of such DN hydrogels is covered with a µm-thick layer of the neutral second network, the incorporation of a small amount of charged monomers into the 2nd network increases the surface charge and, thereby, their repulsive/adhesive properties. Therefore, we propose a method to remove unreacted monomers and modulate the surface charge density of DN hydrogels.

Keywords: double-network hydrogel, co-polymer, free-radical polymerization, surface charge density, Donnan potential



Double network (DN) hydrogels are macromolecules with high water storage and load-bearing capacity[1–3], applied in industrial[4,5] and biomedical applications[6,7] because of their excellent mechanical performance and their similarity to biological tissue. The DN hydrogel has an interpenetrating structure of two separate hydrophilic polymer networks of contrasting architecture and mechanical properties; typically, a 1st network that is a highly crosslinked polyelectrolyte and at a low molar concentration functions as a rigid and brittle network, and a 2nd network consisting of a loosely crosslinked electrically neutral polymer that is soft and ductile[8]. The DN hydrogel is synthesized via a two-step sequential free-radical polymerization where in the first step, the rigid 1st network is synthesized from an electrolyte monomer such as 2-acrylamido-2-methylpropanesulfonic acid (AMPS) of high-concentration in the presence of crosslinker and photo-initiator. In the second step, the as-prepared poly 2-acrylamido-2-methylpropanesulfonic acid (PAMPS) hydrogel is immersed in an aqueous precursor solution containing the 2nd network monomers such as acrylamide (AAm), low amount of crosslinker and a photo-initiator. After the 1st network hydrogel reaches swelling equilibrium in the precursor solution of the second network, the 2nd polymerization is performed in the presence of the 1st polyelectrolyte network[9].

Having a charged polyelectrolyte as the 1st network allows the synthesis of tough hydrogels but limits the regulation of the surface charge concentration[10]. This is an obstacle to overcome because these charges of hydrogels play a crucial role in surface applications such as lubrication and adhesion[11]. E.g. when hydrogels are used as implants, charged surfaces lead to the accumulation of proteins, which in consequence leads to cell adhesion and an inflammatory response[12,13]. Therefore, the regulation of the surface properties of DN hydrogels is crucial for their applicability.

Previous approaches to control the DN hydrogel surface properties deploy the hydrophobicity/hydrophilicity of polymerization molding substrate[14], the interfacial modification, and subsequent grafting of polymer brushes[15] or micro-patterning[16,17]. In our previous study, we described the formation of a surface layer of the 2nd network polymer on top of the DN hydrogel bulk based on the electrostatic repulsion between the glass molding substrate and 1st network polyelectrolyte

hydrogel[14]. The surface of such DN hydrogels, however, exhibits a charge density higher than expected from the neutral polymer network for the DN hydrogels.

An explanation is that the increased charge density is related to the incorporation of unreacted monomers from the 1st network polymerization step in the neutral 2nd network. In most cases of free-radical polymerization, like the 1st network synthesis, the reaction does not run to completion[18,19]. Since high monomer concentration (~ M) was used for the first network formation, the unreacted residual monomer as high as sub-mM order could be present even for a reaction of 99.99% completion. For single network (SN) hydrogels, these residual monomers are removed to an undetectable level by washing the hydrogels in water after polymerization in multiple cycles, and the amount of residual monomers is at a minimum at use. However, in the DN hydrogel synthesis, the 1st network hydrogel is immediately after synthesis immersed in the 2nd network precursor solution (**Scheme 1A**). This is performed to reach the high molar ratio of 20/1 – 30/1 between the 2nd and the 1st network required for excellent mechanical performance[9]. Because of this two-step sequential polymerization, the unreacted 1st network monomers could freely equilibrate in the 2nd network precursor solution. With the subsequent synthesis of the 2nd network, the unreacted 1st network monomers could be incorporated in the 2nd network so that the 2nd network is a co-polymer with a small fraction of the 1st charged monomers.

To verify this assumption, we deployed electric conductivity measurements on monomer solution and electric potential measurements on DN hydrogels and corresponding SN hydrogels. We deployed the microelectrode technique (MET) to precisely quantify the charge concentration at the surface layer of DN hydrogels. MET measures the Donnan potential of a hydrogel equilibrated in a bath solution through the insertion of an extremely thin capillary electrode into the hydrogel[20,21].

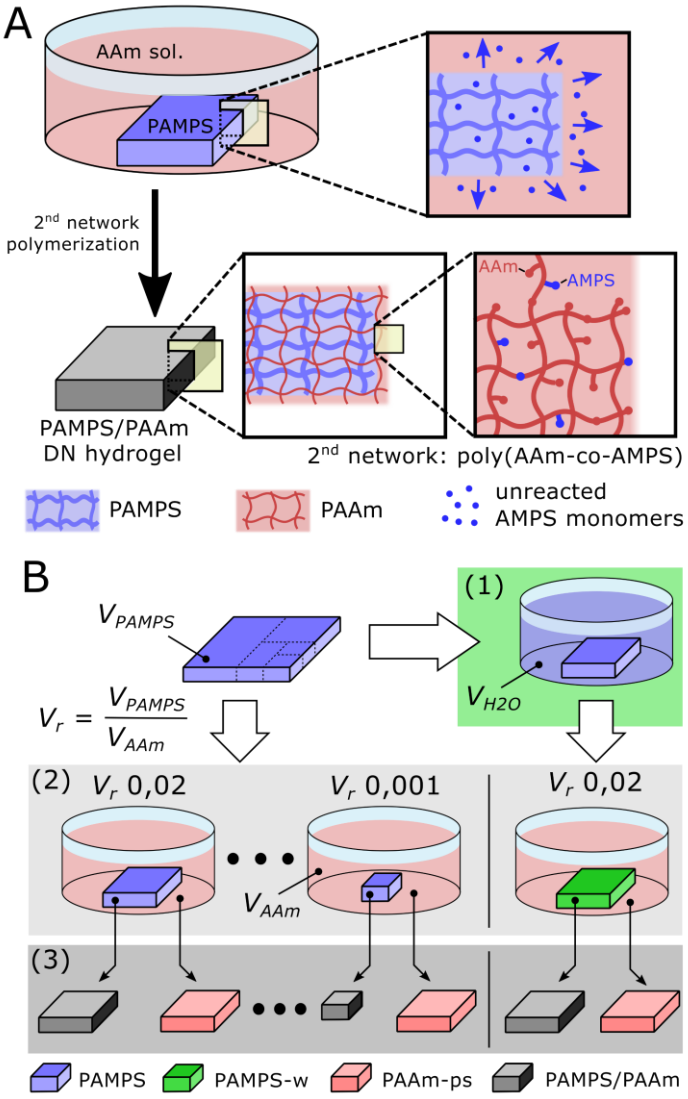
We synthesized DN hydrogels using PAMPS as the 1st network and polyacrylamide (PAAm) as the 2nd network. Here we varied the volume ratio V_r of the as-prepared PAMPS 1st network hydrogel (V_{PAMPS}) and the 2nd network precursor solution (V_{AAm}) that the PAMPS hydrogel is immersed in (**Scheme 1B**). By doing so, we expect to change the concentration of unreacted AMPS monomers in the 2nd network

precursor solution. First, we measure the conductivity of the 2nd network precursor solution with different volume ratios V_r and estimate the unreacted AMPS monomers concentration ($C_{AMPS,R}$) in the 2nd network precursor solution. Second, we measure the electric potentials ($\Delta\varphi$) and estimate the charge concentration (C_G) in the surface layer of the DN hydrogels. These results (C_G) were compared to that of the SN hydrogels synthesized from the 2nd network precursor solution (PAAm-ps). A DN hydrogel where the residual 1st network AMPS monomers were removed from the PAMPS hydrogel by a washing step with water (PAMPS-w) prior to immersion in the 2nd network precursor solution was also measured for comparison. Third, the charge concentration (C_G) of DN hydrogels and SN hydrogels from the 2nd network precursor solution (PAAm-ps) was compared to that of co-polymer hydrogels (poly(AAm-co-AMPS)) synthesized from a solution containing a similar concentration of the unreacted AMPS monomers concentration ($C_{AMPS,R}$). These comparisons allow us to understand the magnitude of impact the incorporation of unreacted 1st network monomers in the 2nd network has on the DN hydrogel surface charge density. The hydrogel formulations are found in **Table 1** and **Table S1,** and details on the experimental methods are found in the **supporting information**. For the reason of simplification, the AMPS concentrations in the precursor solution of the co-polymers (poly(AAm-co-AMPS)) are also referred to as $C_{AMPS,R}$.

Table 1: Hydrogel sample codes and formulations

Sample code	PAMPS - 1st network			PAAm - 2nd network		
	Monomer (M)	Crosslinker* (mol%)	Initiator* (mol%)	Monomer (M)	Crosslinker (mol%)	Initiator* (mol%)
PAMPS (-w)	1	3	1	-	-	-
PAAm (-ps)	-	-	-	2	0,1	0,1
PAMPS/PAAm	1	3	1	2	0,1	0,1

*The mol% of crosslinker N,N′-methylenebis(acrylamide), and initiator 2-oxoglutaric acid are relative to the concentration of monomer.



Scheme 1: (A) The scheme and (B) the methodology to prove the incorporation of unreacted residual 1st network monomers (AMPS) in the 2nd polymer network (PAAm). Performed by (1) monitoring the removal of unreacted monomers of as-prepared PAMPS hydrogel, (2) the detection of unreacted AMPS monomers in the 2nd network precursor solution, and (3) the quantification of surface charge density of the synthesized PAMPS/PAAm DN and corresponding SN hydrogels.

Monomers of polyelectrolytes such as AMPS are highly soluble in water and unreacted monomers could be extracted from the hydrogel by washing with water. The concentration of the unreacted monomer ($C_{AMPS,R}$) can be determined by measuring the ionic conductivity of the hydrogel washing water[22]. We can use this method to monitor the transport of AMPS from the hydrogel into the bathing solution. Initially, we established the calibration curves in **Figure 1A** for the correlation between the ionic conductivity and the AMPS concentration (C_{AMPS}) in water and in 2nd network precursor solution. These calibration curves show that the detection down to 2 x 10^{-3} mM of C_{AMPS} in water and down to 0,1 mM of C_{AMPS} in AAm is possible.

PAMPS hydrogels were synthesized as described in the **supporting information,** and unreacted monomers were removed via immersing PAMPS hydrogel (V_{PAMPS}) in water (V_{H2O}) at a volume ratio V_r of 0,02. For samples equilibrated in water without daily water exchange, conductivity constantly stayed high at $C_{AMPS,R}$ of 0,6 mM. On the sample with daily water exchange (PAMPS-w), where the ionic conductivity measurements of the washing water were performed after 24 hours of incubation, **Figure 1B** shows that $C_{AMPS,R}$ in H_2O decreased fast and after 5 -7 days stayed constant below 0,05 mM, corresponding to the ionic conductivity of fewer than 2 µS/cm. The results indicate the necessity to wash hydrogels for at least 5 - 7 days to reduce the concentration of unreacted residual AMPS molecules from the 1st network polymerization. It needs to be mentioned that the conductivity measurements give the overall results of electric conduction from both cationic and anionic species.

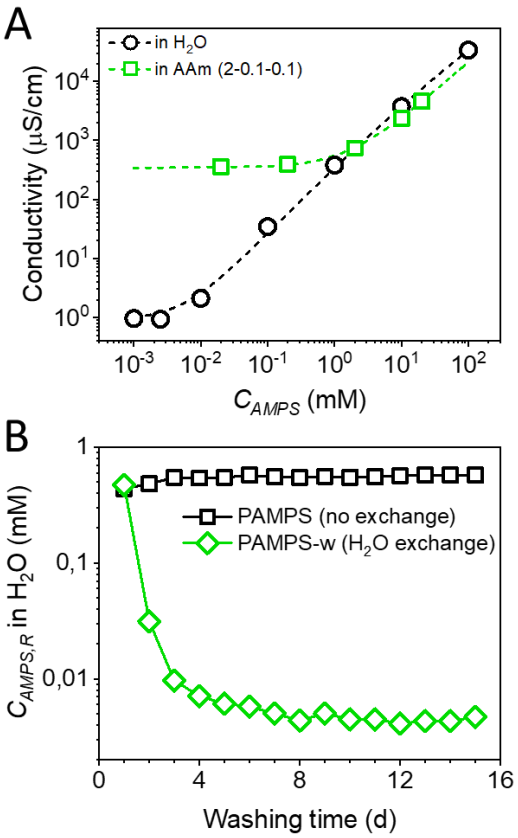


Figure 1: Determine $C_{AMPS,R}$ in the hydrogel washing procedure for PAMPS-w. (A) Calibration curves for the ionic conductivity of AMPS in water and in the 2nd network precursor solution with various concentration C_{AMPS}. (B) The reduction of $C_{AMPS,R}$ in the washing water of the PAMPS-w hydrogel (V_r 0,02) by daily water exchange, indicating the removal of unreacted monomers.

To investigate if the residual AMPS amount reaches a detectable concentration in 2nd network precursor solution, the washed PAMPS-w and the as-prepared PAMPS hydrogels were immersed in 2 M AAm containing 0,1 mol% crosslinker and 0,1 mol% photoinitiator and ionic conductivity was measured for 3 days. **Figure 2A** visualizes the increase of conductivity in the AAm solution with immersion time. By immersing bigger volumes of PAMPS hydrogels (V_{PAMPS}) in AAm solution (V_{AAm}) at volume ratios V_r (=V_{PAMPS}/V_{AAm}), we achieved higher conductivities at the equilibrium time. This demonstrates that residual AMPS monomers diffuse into the AAm solution and that the equilibrium $C_{AMPS,R}$ depends on the volume ratio V_r between the immersed 1st network hydrogel and 2nd network precursor solution. By using the calibration curve shown in **Figure 1A**, we obtain the equilibrium $C_{AMPS,R}$ for various V_r (**Figure 2B**). The tested volume ratios V_r of 0,01 and 0,02 are widely applied ratios in the laboratory format because these ratios generate less 2nd network precursor waste solution. We see that $C_{AMPS,R}$ reaches 0,46 – 0,81 mM, which could affect the charge density of the DN hydrogel. This amount of $C_{AMPS,R}$ relates to a conversion rate from monomer to polymer of approximately 95 %. In contrast, when the washed PAMPS-w hydrogel is immersed in AAm solution, the conductivity stays stable and shows comparable results to the conductivity of pure AAm solution (**Table S2**). This shows that the PAMPS-w washing procedure effectively reduces $C_{AMPS,R}$ in the 2nd network precursor solution to a concentration below the conductivity measurement detection limit of 0,1 mM shown in **Figure 1A**. It should be noted that due to the small volume ratio V_r (< 0,02), the concentration of AAm monomers in the precursor solution of the second network hardly changes by the immersion of the PAMPS network. The effect of impurities in the 2nd network precursor solution and hydrolysis of the polymer on the conductivity can be neglected because its conductivity is mainly defined through the initiator and residual AMPS (**Figure S1**).

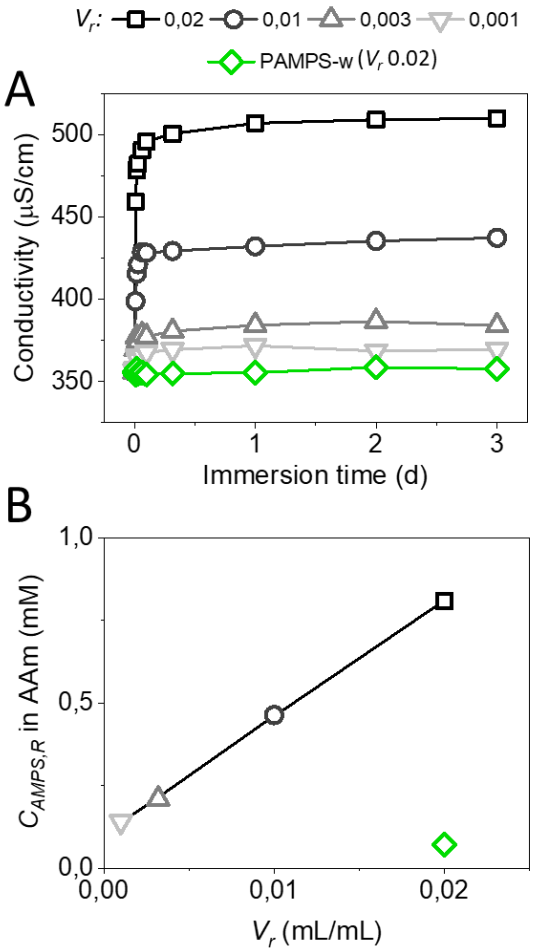


Figure 2: Determining the concentration of the 1st network monomer in the 2nd network precursor solution. (A) The time profiles of ionic conductivity after the immersion of the as-prepared PAMPS hydrogels in AAm solutions with volume ratios (V_r) ranging from 0,02 to 0,001. (B) The equilibrium residual AMPS monomer concentration in AAm solutions $C_{AMPS,R}$ as a function of the volume ratios (V_r). $C_{AMPS,R}$ was calculated from conductivity. For comparison, the results after the immersion of the pre-washed PAMPS-w at V_r 0,02 was also shown.

To determine if higher concentrations of $C_{AMPS,R}$ in the 2nd network precursor solution increase the DN hydrogels surface charge density C_G after synthesis, we deployed electric potential measurements by using MET. When a polyelectrolyte hydrogel is equilibrated in a salt solution, a characteristic distribution of small mobile ions inside and outside of the gels is created, which generates an electrostatic potential difference (Donnan potential) at the gel-solution interface $\Delta\varphi$[20,21]:

$$\Delta\varphi = \frac{RT}{zF} ln\left(\frac{\gamma_S \times C_S}{\gamma_G \times C_G}\right) \quad \text{(Eq. 1)}$$

Here, R is the gas constant, T is the absolute temperature, z is the valence of the small mobile ions, F is the Faraday constant, γ and C are the activity coefficient and the concentration of the small mobile ion of interest, respectively, in bath solution (γ_S, C_S) and in the gel (γ_G, C_G). By measuring the Donnan

potential of the hydrogel in a low salt solution, we can estimate the counter ion concentration C_G in the surface layer of DN hydrogels. This C_G equals the charge concentration incorporated in the surface layer according to the charge neutrality condition.

We found (**Figure 3**, 1-3) that the electric potential difference to the bath solution at the DN hydrogel surfaces $\Delta\varphi$ increased with the $C_{AMPS,R}$ (low to high) while the bulk electric potential (dark grey area) was constantly low at values around -150 to -160 mV. The DN hydrogel synthesized with the washed PAMPS-w hydrogel (green box) had the lowest $\Delta\varphi$ of -16,7 ± 3,2 mV among the DN hydrogels, which indicates that the residual AMPS monomers are incorporated in the 2nd network of PAAm. The difference in electric potential between the surface layer and the bulk of DN hydrogel indicates the presence of a surface layer of several μm-thick, which is consistent with the previous study[14].

The surface electric potential could also be influenced by the limited swelling of the 2nd network confined in the 1st network structure and a change of distribution of ions in the gel. To investigate the influence of confinement, SN AAm hydrogels were synthesized from the residual 2nd network precursor solution 3 days after the immersion of the PAMPS hydrogel (PAAm-ps). The electric surface potentials of the PAAm-ps hydrogels (**Figure 3**, 4-6) are similar to the potentials found in the surface layer of the corresponding PAMPS/PAAm DN hydrogels, showing that the surface layer of the DN hydrogel is swelling freely and thus not affect the electric potential at the surface. It needs to be mentioned that based on this measurement, we cannot account for non-uniform swelling that does not affect the electric potential.

To strengthen the claim that $C_{AMPS,R}$ regulates $\Delta\varphi$ at the DN hydrogel surface, a control group of poly(AAm-co-AMPS) hydrogels were tested (formulation in **Table S1**). The co-polymer hydrogels, having similar concentrations of AMPS in feed with that of residual AMPS in the second network precursor solution of DN hydrogels, show similar electric surface potentials (**Figure 3**, 7-9) to the ones of the PAMPS/PAAm DN hydrogels.

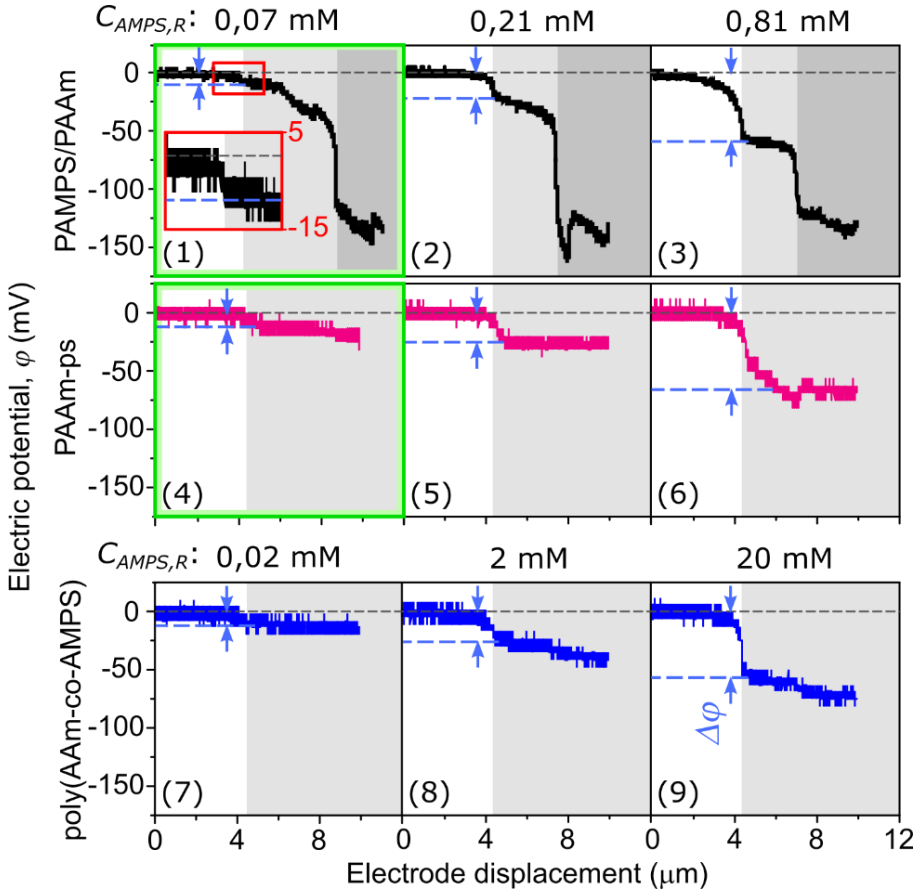


Figure 3: Electric potential profiles of hydrogels $\Delta\varphi$ measured by MET. DN hydrogels (PAMPS/PAAm, 1-3), the corresponding PAAm hydrogel synthesized from the AAm precursor solution (PAAm-ps, 4-6), and the co-polymer hydrogel (poly(AAm-co-AMPS), 7-9). The profile backgrounds in white, grey, and dark grey indicate the potential in the bathing solution, in the hydrogel surface layer, and in the hydrogel bulk, respectively .The potential differences between blue arrows indicate the electric surface potential $\Delta\varphi$ of hydrogels relative to bath solution (C_s = 10^{-4} M NaCl aq. solution). (1 and 4) The green frames indicate the samples prepared with pre-washed PAMPS-w as the 1st network.

When we compare the charge density C_G, calculated from the electric potential $\Delta\varphi$ (Eq. 1) between the sample groups, it is observable that at the same $C_{AMPS,R}$, the co-polymers show a lower charge density than that of PAMPS/PAAm DN hydrogels and PAAm-ps hydrogels (**Figure 4A**). To investigate where the discrepancy in the electric potential measurements originates from, the hydrogel swelling after 2nd network polymerization in the MET bath solution Q was correlated with the $C_{AMPS,R}$ since hydrogel swelling is linked to the fixed ions of the hydrogel. Here it was found that in the range of 0,07-0,81 mM $C_{AMPS,R}$, PAAm-ps, and Poly(AAm-co-AMPS) hydrogels have almost the same Q of about 2, independent of $C_{AMPS,R}$ (**Figure 4B**). This result indicates that a small amount of charges incorporated into the PAAm hydrogels hardly influence their swelling ability. This conclusion is also confirmed by

the results of macroscopic mechanical properties, which were constant in the observation range of C_G (**Figure S2**).

Next, we investigate the correlation between the true surface charge density incorporated in the PAAm network and $C_{AMPS,R}$ in the precursor solution. Since the hydrogels swelled in the bath solution for performing the MET, the values C_G measured by MET should be corrected, taking into consideration the swelling. It is assumed that the surface layer of DN hydrogel should have the same swelling ratio as that of PAAm-ps, although the bulk of DN hydrogel hardly swells (**Figure S3**). So, for DN hydrogel, the Q of PAAm-ps was used in the calculation of C_GQ of its surface layer. The plot of C_GQ against $C_{AMPS,R}$ is shown in **Figure 4C**, where C_GQ stands for the true charge density incorporated in the PAAm hydrogel at the as-prepared state. With this plot, we can elucidate if all the charges in the PAAm gels are from residual monomer of $C_{AMPS,R}$ or not. For the co-polymer, when $C_{AMPS,R} \leq 0{,}2$ mM, C_GQ is bigger than $C_{AMPS,R}$, because C_GQ in this region is dominated by the weak charges of the PAAm network. When $C_{AMPS,R} > 0{,}2$ mM, C_GQ is smaller than $C_{AMPS,R}$ which can be accounted for by a low polymerization efficiency of the AMPS monomer at the tested low $C_{AMPS,R}$ and polymer ratios of $C_{AMPS,R}/C_{AAm} \leq 1/98$ (**Table S2**). Surprisingly, we found that the C_GQ of PAMPS/PAAm and PAAm-ps is always higher than $C_{AMPS,R}$. This deviation with the co-polymer indicates an underestimation of $C_{AMPS,R}$ in the second network monomer solution.

This could be caused by two connected effects. First, the underestimation of $C_{AMPS,R}$. The incomplete reaction of the 1st network gives not only unreacted AMPS monomers but also oligomers and sols that can dissolve in the 2nd network precursor solution. These oligomers also contribute to electric conductivity but less per monomeric unit in comparison with monomers. Therefore, the $C_{AMPS,R}$ from conductivity using AMPS monomer solution as calibration curve could be underestimated[23]. Second, the oligomers and sols could also be incorporated into the second network. It is known that by free radical polymerization, some vinyl groups of the 1st network crosslinker have not reacted[8]. Through these unreacted crosslinkers, the oligomers or sols could also be incorporated into the PAAm-ps and PAMPS/PAAm hydrogels.

In summary, the results in **Figure 3 and 4** clearly show that the surface charge density of the PAMPS/PAAm DN gels could sensitively change with the polymerization procedures and should be carefully quantified by MET. The plot of C_GQ against $C_{AMPS,R}$ could be used as a calibration curve to get the required surface charge density of the hydrogels for a well-controlled experiment. Here we emphasize that the amount of unreacted first network monomer copolymerized in the second network is in mM concentration, and it is too low to be detected by ATR-FTIR (**Figure S4**)[14]. This work also demonstrates that MET is a powerful method to characterize the electric properties of hydrogels.

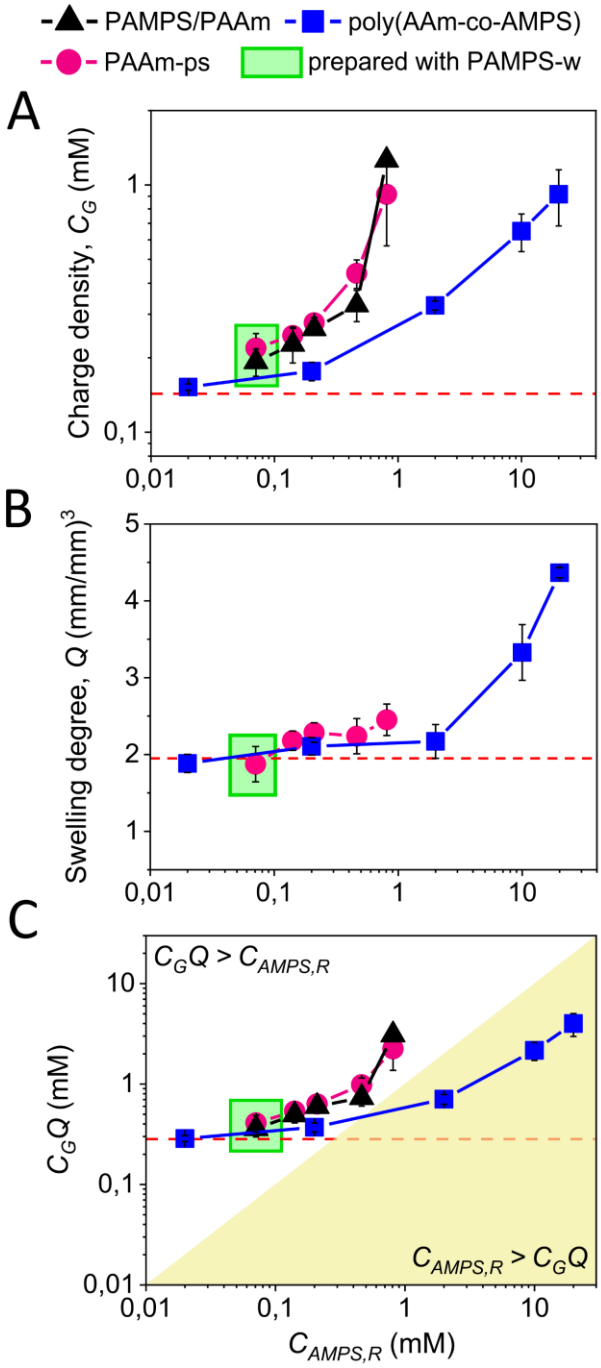


Figure 4: True Charge density C_GQ in the surface layer of hydrogels. (A) C_G in the surface layer as a function of the residual AMPS concentration $C_{AMP,R}$ in the precursor solution of gels. (B) The swelling ratio Q of the bulk hydrogels in the state of MET measurement against $C_{AMPS,R}$ in the precursor solution of the PAAm network. For PAMPS/PAAm, only the Q of the surface layer is relevant, so its Q of the bulk is not shown to avoid confusion. (C) True surface charge C_GQ in the surface layer of hydrogels as a function of $C_{AMP,R}$ in the precursor solution of gels. For PAMPS/PAAm, the Q of PAAm-ps was used in the calculation C_GQ for its surface layer. The orange-yellow background in (C) indicates the area $C_{AMP,R} > C_GQ$. The red dashed lines in (A, B, C) indicate the results of pure PAAm (**Table S2**). The Error ranges in the figure indicate STD for n = 3 - 5 measurements per hydrogel. The activity coefficients in the bath solution (γ_S) and in the gel (γ_G) are set to 1 in calculating C_G from $\Delta\varphi$ using equation 1.

We documented here, to our knowledge for the first time, that unreacted monomers from the 1st network polymerization diffuse into the 2nd network precursor solution, which in consequence, leads to the incorporation of a small amount of monomers of the first network into the second network by the two-step sequential polymerization, resulting in the formation of poly(AAm-co-AMPS) in the 2nd network. Although the concentration of AMPS monomer (< 1 mM) and the molar ratio of AMPS to AAm (< 1/1000 M/M) are very low, this incorporation of unreacted 1st network monomers in the second network leads to an increase of true charge density C_GQ from 0,28 of pure PAAm to 3,09 mM at the surface of the DN hydrogel. Further, the increase of C_GQ was even higher than expected, which could be related to the incorporation of unreacted oligomers or sols in the 2nd network. These concentrations at first seem negligible. However, it was shown that such low charge densities lead to the adhesion of molecules[24]. We demonstrated that it is possible to remove the residual AMPS monomer and synthesis DN hydrogels with a PAAm hydrogel layer by first, an additional washing step of the PAMPS hydrogel after the 1st network polymerization as performed with the PAMPS-w hydrogel. Second, the immersion of the as-prepared PAMPS hydrogel in the 2nd network precursor solution at a volume ratio (V_r) of less than 0,001 has a similar effect to the washing but would waste a lot of reagent solution. Further, it is possible to regulate the charge density at the surface of the DN hydrogel while maintaining the swelling ratio and bulk elastic modulus constant by adding a small amount of AMPS ($C_{AMPS,\,R}$ < 1 mM or $C_{AMPS,\,R}/C_{AAm}$ < 1/1000) in the 2nd network. Such separable modulations of surface charge density and elastic modulus should greatly merit the understanding of soft interface interaction between hydrogels and cells.

Supporting Information Available: Contains experimental details, figures on additional measurements, and a summary data table.

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