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Cryo-SEM: Direct Evidence of Water and Counterion Release upon Complexation

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Polyelectrolyte hydrogels are polymeric networks of charged groups, and they retain a high water content. These gels been applied extensively in drug delivery due to their ability to complex with oppositely charged molecules [1-2]. Polyelectrolyte complexation within microgels is traditionally studied by confocal microscopy using fluorescently labeled molecules [3]. However, the labeling changes molecular physicochemical properties and consequently can alter complexation behavior, especially for small molecules. Here we study the distribution of unlabeled cationic antimicrobials complexed within anionic microgels using EDS in the cryo-SEM. We observe direct evidence for water and counterion release during complexation.

Poly(acrylic acid) (PAA) microgels, both as synthesized as well as after complexation loading with the L5 cationic antimicrobial (+6 charge, 2274 g/mol), were soaked in 0.01 M sodium phosphate buffer at pH 7.4 and cryo-fixed by high-pressure freezing (Leica HPM-100 HPF) [4-5]. After freeze fracturing, the specimens were imaged at -135 °C using a Zeiss Auriga Cross-Beam FIB-SEM equipped with a Leica VCT-100 cryo-transfer system. In order to generate topographic contrast for imaging, specimens were sublimated at -95 °C for 10 min, 40 min, or 12 h.

Figs. 1A and 1B show typical cryo-SEM images of as-synthesized and L5-complexed microgels after 10 min sublimation. The process removes ~1 μm of ice, leaving fractured microgels protruding from the surrounding ice. As-synthesized microgels are homogeneous throughout the cross-section surfaces while L5-complexed microgels exhibit a shell layer with a few microns thickness. To further analyze the difference in composition and antimicrobial distribution within the L5-complexed microgels, carbon, oxygen and nitrogen X-ray line scans were collected across the fracture surface using EDS (Oxford Max-80 SDD detector) with 1 keV incident electrons. Profiles of the C/O intensity ratio reflect hydration levels in these gels [6]. The C/O ratios of 0.1 and 7.1 indicate highly hydrated and highly dehydrated microgel states, respectively. As shown by Fig. 1C, the as-synthesized microgels and the surrounding ice exhibit similar O intensities, indicating a very high water content within the microgel. The slightly higher C intensity reflects the fact that the hydrated microgel contains a small fraction of polymer. In contrast, L5-complexed microgels shows very different C, O and N intensities in their shell (Fig. 1D). The N intensity is unique to amine groups, and the spike in N intensity indicates that the L5 mainly complexes in the shell layer. The C/O ratio in the core is close to that of as-synthesized microgels, indicating the core is still highly hydrated. In contrast, the C/O ratio in the shell is very high and comparable to that of dried microgels. Thus, a significant of water is lost in the complexed shell. Further examination of the N and Na X-ray intensities (Fig. 2) using 2 keV electrons across a fully sublimed sample shows that the Na concentration is reduced in the shell, consistent with the theory that entropic gain from counterion release drives the complexation process [7].

To conclude, the L5 cationic antimicrobial peptide complexes primarily, if not exclusively, with the surface layer of PAA anionic microgels. This process is accompanied by the release of water and counterions [8].

References:

[1] Yong Wu *et al,* Journal of Polymer Science Part B: Polymer Physics **54** (2016), p. 64.
[2] Qichen Wang *et al,* ACS Applied Materials and Interfaces **4** (2012), p. 2498.
[3] Helena Bysell *et al,* Langmuir **12** (2006), p. 5476.
[4] Jing Liang *et al,* Microscopy and Microanalysis **22** (2016), p. 1898.
[5] Yong Wu *et al,* Microscopy and Microanalysis **20** (2014), p. 1348.
[6] Jing Liang *et al,* Polymer **116** (2017), p. 1.
[7] Kathrin Wagner *et al,* Langmuir **16** (2000), p. 303.
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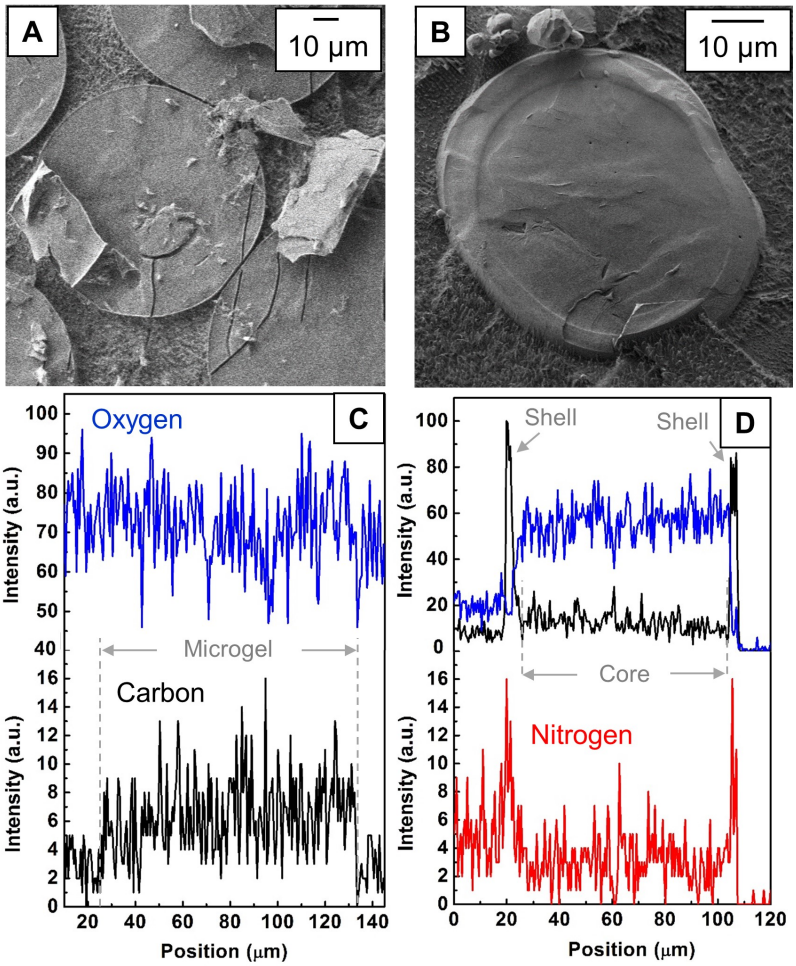

Figure 1. Cryo-SEM images of (A) as-synthesized and (B) L5-complexed microgels under 10 min sublimation; X-ray intensity line profiles of carbon (black), oxygen (blue) and nitrogen (red) across a fractured surface of (C) as-synthesized and (D) L5-complexed microgels after 10 min sublimation.

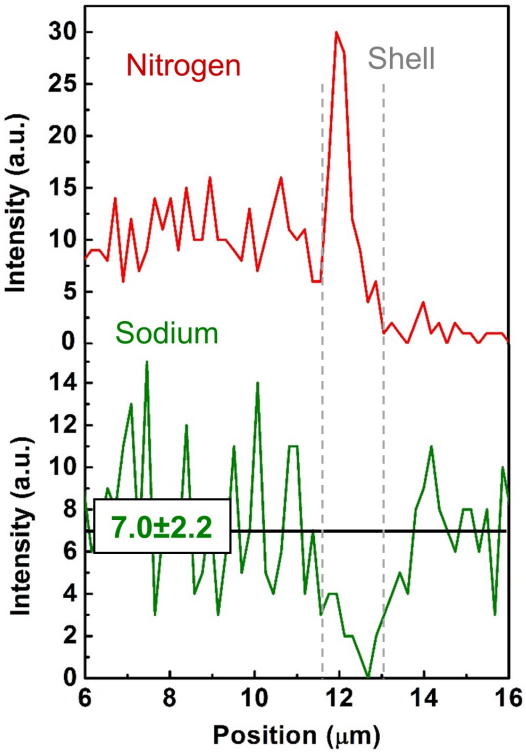

Figure 2. X-ray intensity line profiles of nitrogen (red) and sodium (green) across the shell layer of fully sublimed L5-complexed microgels.

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