Measuring Microgel Swell Ratio by cryo-SEM

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Abstract:

The swell ratio is a key parameter characterizing the structure and properties of a hydrogel. In macroscopic gels, the swell ratio can be determined by straightforward measurements of the gel weight in the dry and hydrated states. However, measuring the swell ratio characteristic of microgels (gel particles with dimensions of 0.1-100 μm) is substantially more challenging because of their small size and polydispersity. We use cryo-scanning electron microscopy (cryo-SEM) to measure microgel diameter both in the frozen-hydrated and fully dry states using pH-responsive poly(ethylene glycol)-co-acrylic acid microgels. The volume swell ratios characteristic of the various microgels are essentially the same as those measured from otherwise-identical macroscopic gels. Hence we can conclude that, at least in this case where the macroscopic gel and microgel synthesis methods are similar, the simple measurement of a macroscopic swell ratio provides a reasonable approximation to the microgel swell ratio.

Perhaps the most significant quantity characterizing a hydrogel is its swell ratio. The swell ratio is a macroscopic manifestation of the gel mesh size and crosslink density. These molecular-level parameters in turn control mechanical and transport properties critical to applications such as tissue engineering and drug delivery. The relationship between swell ratio and mesh size was initially developed by Flory and Rehner [1, 2]. The volume swell ratio, Q , is given as:

$$Q = \frac{V_s}{V_d} \quad \dots (1)$$

where V_s is the volume of swollen network at equilibrium and V_d is the volume of unswollen (dry) network. For a macroscopic hydrogel, determining the volume swell ratio is straightforward from measurements of the hydrogel weight in the dry, m_d , and water-swollen, m_s , states:

$$Q = 1 + \frac{\rho_p}{\rho_s} \left(\frac{m_s}{m_d} - 1 \right) \quad \dots (2)$$

where the polymer and solvent densities are ρ_p and ρ_s , respectively. The gel mesh size can then be calculated from the swell ratio using the Flory-Rehner theory, and this approach has been applied to a number of neutral polymer gel systems such as PEG and poly(vinyl alcohol) [3, 4]. Peppas et al. [5] further modified the Flory-Rehner theory to address polyelectrolyte gel systems where issues of charge, pH, and ionic strength can contribute substantially to the gel-swelling behavior.

Microgels are gel particles with sizes in the range of 0.1-100 μm [6]. They are being used increasingly in a variety of colloid, drug-delivery, and surface-modification applications [7-10], which, just as in their macroscopic gel counterparts, require control of the mesh size. However, in contrast to a macroscopic gel whose dimensions enable reasonably easy and accurate measurements of the dry and swollen weights, the swelling behavior of microgels is much more difficult to characterize. Dynamic light scattering, for example, only provides the intensity-averaged hydrodynamic diameter and does so just for relatively small (sub-micron sized) microgels. Weight measurements can be made using samples containing a large number of microgels to mimic a macroscopic sample, but fully removing interstitial water between microgels is difficult and consequently can lead to errors in measuring the swollen weight.

Here we ask the simple question of how well a measurement of swell ratio from a macroscopic gel compares to that of a microgel synthesized under identical conditions - composition, solvent, UV exposure, etc. - except for the finely divided nature of the microgel. We use cryo-SEM to make direct measurements of microgel size in the frozen-hydrated (swollen) and dehydrated (dry) states, and, at least in the particular case involving poly(ethylene glycol)-co-acrylic acid (PEG-co-AA) gels and microgels, we find excellent agreement between the swell ratio characteristic of the macroscopic gels and the microgels.

PEG-co-AA gels and microgels were synthesized by free radical polymerization. The microgels were formed by membrane emulsification using a precursor solution of 2 mL PEG diacrylate (PEGDA; $M_n = 575$ g/mol; Sigma), 200 μL acrylic acid (Sigma), and 100 μL photo-

initiator (Darocur 1173; Sigma) all dissolved in 10 mL dichloromethane. The precursor solution was dispersed as microscopic droplets in 100 mL deionized water (DI) by forcing the precursor solution through a Shirasu Porous Glass (SPG) membrane [11]. The resulting emulsion was exposed to 450 W UV light for 15 minutes. After solvent removal, the microgels were washed by repeated centrifugation and re-suspension in DI water. Macroscopic hydrogels with dimensions on the order of 1 cm were prepared by exposing 1 mL of precursor solution to 450 W UV light for 15 minutes. To better mimic the microgel synthesis conditions as well as prevent dichloromethane evaporation, the precursor solution was covered by 10 mL of DI water during photo-polymerization. The resulting gels were soaked in DI water and heated to 50 °C while dichloromethane was removed by vacuum. The gels were then soaked for 3 days in a large bath of DI water. The swollen weight was obtained after soaking the sample in either pH 3 or pH 9 in 0.01 M phosphate buffer for 48 hours. The dry weight of the macroscopic gels was obtained by dehydrating the gels at 50 °C for 48 hours. Prior to the hydrated weight measurement, excess surface buffer was removed by gently patting the gel surfaces with filter paper.

Cryo-SEM used frozen-hydrated samples were prepared by high-pressure freezing (HPF). 5 μ L of microgels suspended in pH 3 or pH 9 buffer (0.01 M phosphate buffer) were frozen using a Leica EM HPM100 HPF system. High-pressure freezing promotes water amorphization while freezing by plunging in liquid nitrogen (LN2) at atmosphere pressure tends to create crystalline ice, which can induce artifactual specimen structure [12, 13]. HPF microgel samples were cryo-fractured and cryo-transferred to a Zeiss Auriga Cross-Beam FIB-SEM equipped with a Leica VCT-100 cryo-transfer system. Frozen-hydrated samples were imaged using 1 keV electrons at a temperature of -135 °C and a vacuum of 2×10^{-6} mbar. Dried samples were imaged

at room temperature. To remove water from the frozen-hydrated microgel suspensions, the sample temperature was raised to $-95\text{ }^{\circ}\text{C}$ to sublime water into the microscope vacuum. We used sublimation times of 10 min and 40 min to remove relatively small amounts (microns) of water from the sample surface. Samples were re-cooled to $-135\text{ }^{\circ}\text{C}$ for cryo-SEM observation. Alternatively, fully dehydrated specimens were produced by warming the specimen to $-95\text{ }^{\circ}\text{C}$ for several hours until the cryo-stage's LN₂ supply was exhausted, whereupon the specimen slowly warmed to room temperature over a period of approximately 12 h. The water content of the microgels after these three sublimation processes was followed by monitoring the intensity ratio of carbon and oxygen X-rays collected by energy dispersive X-ray spectroscopy (Oxford Max-80 SDD EDS system).

Figure 1 presents typical SEM images showing the microgels after each of the three different sublimation treatments. Importantly, the microgels appear smooth and spherical.

Figure 1 – Cryo-SEM (A, B) and room-temperature SEM (C) images of frozen-hydrated microgel suspensions (pH 3) after sublimation treatments of: (A) 10 min; (B) 40 min; and (C) 12 h. The insets indicate the ratios of carbon and oxygen X-ray intensities.

10 min of sublimation removes $\sim 1\ \mu\text{m}$ of water, leaving the microgels significantly embedded in ice (Fig. 1A). The microgel C/O X-ray intensity ratio (Fig. 1A inset) is 0.44. Note that this ratio does not directly correspond to the composition ratio, and we did not pursue the various corrections and calibrations required to make this conversion. Furthermore, 1 keV electrons travel only about 100 nm into the specimen. X-ray generation is consequently localized primarily to the near-surface region of the individual microgels analyzed. After the 40 min sublimation treatment (Fig. 1B) the topmost layer of microgels in the frozen-hydrated is almost entirely free from surrounding ice. Nevertheless, the microgel C/O X-ray intensity ratio (Fig. 1B inset) under the 40 min sublimation is essentially the same (0.43) as that after 10 min. This observation indicates that water is preferentially sublimated from the ice surrounding the individual microgels, while bound water remains within the exposed microgels. However, even this bound water is removed when the microgels are subjected to the extended sublimation process followed by warming to room temperature under the high vacuum of the microscope (Fig. 1C). Under this condition, the C/O X-ray intensity ratio increases to almost 36. We measured a similar ratio from thick microgel specimens, which were dried for 3 days at 50 °C and then analyzed under identical SEM imaging and EDS X-ray collection conditions. We can thus be confident that the 12 h sublimation treatment produces fully dried microgels. Importantly, the dried microgels maintain their spherical structure and smooth surface, suggesting that the de-swelling process is isotropic.

The microgel size distribution was measured directly from many different images of specimens subjected to the partial and full sublimation treatments such as those in Figures 1B and 1C, respectively. Since the freeze-fracture process produces a specimen surface presenting

fractured microgels, some of which have an exposed face diameter less than that of an exact hemisphere, care was taken to measure the diameter of a fractured microgel only when its maximum diameter was visible. Figure 2 presents size-distribution histograms, together with a Gaussian fit to each data set, for dry and frozen-hydrated microgels that had been swollen either in pH 3 or pH 9 buffer. Each dataset on these histograms represents measurements made from over 100 different microgels. The frozen-hydrated microgels swollen in pH 9 buffer have swell ratios larger than those swollen in pH 3 buffer because of the increased swelling at pH values higher than the pKa of AA carboxyl group (~4.5).

Data from Figure 2 were used to

calculate the volume swell ratio using equation (3), where d_s and d_d are the average hydrated and dried microgel diameter, respectively:

$$Q = \frac{V_s}{V_d} = \left(\frac{d_s}{d_d}\right)^3$$

...(3)

Figure 2 - (A) Room-temperature SEM image of dried microgels. (B) Cryo-SEM image of frozen-hydrated microgels after a 40 min sublimation treatment. The scale bar represents 50 μm for both A and B. Size-distribution histograms of dried (12 h sublimation) and frozen-hydrated (40 min sublimation) at: (C) pH 3; and (D) pH 9. The solid and dashed lines represent Gaussian fits to each data set (>100 measurements per data set).

Measurements of macroscopic gel weights in the dry (m_s) and hydrated (m_d) conditions were used to calculate the volume swell ratio using equation (2), assuming the PEG-co-AA copolymer density (ρ_p) is 1.12 g/cm^3 and water density (ρ_s) is 1 g/cm^3 . The results are summarized by Table 1. The swell ratios

Table 1: Volume swell ratios

	pH 3	pH 9
Microgel ^a	3.3 ± 1.3	4.0 ± 2.1
Macroscopic gel ^b	3.4 ± 0.1	3.9 ± 0.1

^a The microgel average diameter $\pm$ standard deviation are derived from Gaussian fits in Figure. 2.

^b The average $\pm$ standard deviation are each derived from weight measurements of 5 gels.

characterizing the bulk gels are almost identical to those of the microgels.

Following reports in the literature [14-16], we also measured the hydrated microgel swell ratio by analyzing the weight of microgel pellets after centrifugation. As we would do with a hydrated bulk gel, we removed excess water by gently patting the pellets with filter paper. We used 5 different pellets for each pH condition, and, after measuring the hydrated weight, we then dried the pellets at 50°C for 2 days. Using this method together with equation (2), we measured microgel volume swell ratios of 6.2 ± 0.5 at pH 3 and 16.3 ± 0.7 at pH 9. These values are substantially higher than those reported in Table 1. We attribute those differences to excess interstitial water in the hydrated microgel pellet that is difficult to remove because of the substantially higher surface area associated with the microgels relative to a macroscopic gel.

To further confirm the accuracy of the cryo-SEM measurements, we followed a set of the same microgels from the frozen-hydrated state to the fully dehydrated state. The set of microgels is shown in Fig. 3. These were hydrated in pH 9 buffer. Unlike the statistical approach reported

in Figure 2, image data such as that in Figure 3 enable direct measurements of microgel size and thus determine the swell ratio characteristic of individual microgels. The microgel sizes after the 10 min and 40 min sublimation treatments are again identical. They undergo isotropic deswelling with the 12 h sublimation/warming process, each ending with a substantially smaller dry size. We again used equation (3) to determine the microgel-specific pH 9 swell ratio. By analyzing the cross-sectional diameters of ten different microgels in the hydrated and dry states, the volume swell ratio obtained was 4.1 ± 0.5 . This value is essentially the same as that reported in Table 1.

In conclusion, we have used cryo-SEM to make very specific and accurate

measurements of microgel swell ratio in the PEG-co-AA system. These ratios compare extremely well with those measured from macroscopic hydrogels synthesized from the same precursor solution but without the membrane-emulsification process. In this particular case, we can thus conclude that making the far simpler measurement of a swell ratio from a macroscopic

Figure 3 – Cryo-SEM (A, B) and room-temperature SEM (C) images of the same set of microgels after sublimation for: (A) 10 min; (B) 40 min; and (C) 12 h. The same two microgels, labeled 1 and 2, are marked in each image.

gel can provide an accurate characterization of the network properties of both the macroscopic gel and microgel. Extrapolating beyond this specific case, the relationship between macroscopic gels and microgels is likely to be maintained for similar compositions and similar microgel synthesis methods. Hence we can conclude that, at least in this case where the macroscopic gel and microgel synthesis methods are similar, the simple measurement of a macroscopic swell ratio provides a reasonable approximation to the microgel swell ratio.

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