

Structural Changes and Aggregation of Human Influenza Virus Upon Acidification as Measured by Light Scattering

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Abstract

The pH-induced change in the structure and aggregation state of the PR-8 and X-31 strains of intact human influenza virus have been studied *in vitro* by static and dynamic light scattering. Reducing the pH from 7.4 to 5 produces a large increase in the intensity of light scattered to low angles. A modest increase in the polydispersity parameter from dynamic light scattering accompanies that increase, as does a change in how the polydispersity parameter varies with scattering angle. The scattering profiles never match those of a single, solid spherical particle, but the deviations are modest at pH 7.4. At pH 5, scattering profiles calculated for multiple spherical particles come much closer to matching the experimental data than those computed for single particles. While these changes, indicating aggregation, develop over a period of minutes to hours after acidification, a nearly instantaneous change in virus conformation is the first response of the virus particles to lower pH.

Theory

Intensity is proportional to the concentration and mass, reduced by the particle form factor. As shown in the equation

$$I(q) = I_0 K_{opt} c M P(q)$$

In these experiments concentrations are constant so an increase in the intensity of scattered light is likely due to a change in mass caused by aggregation. These changes in aggregation can also be seen by comparing scattering to the particle form factor. For example the form factor for a sphere is

$$P_1(q) = \frac{3}{x^3} (\sin x - x \cos x)^2$$

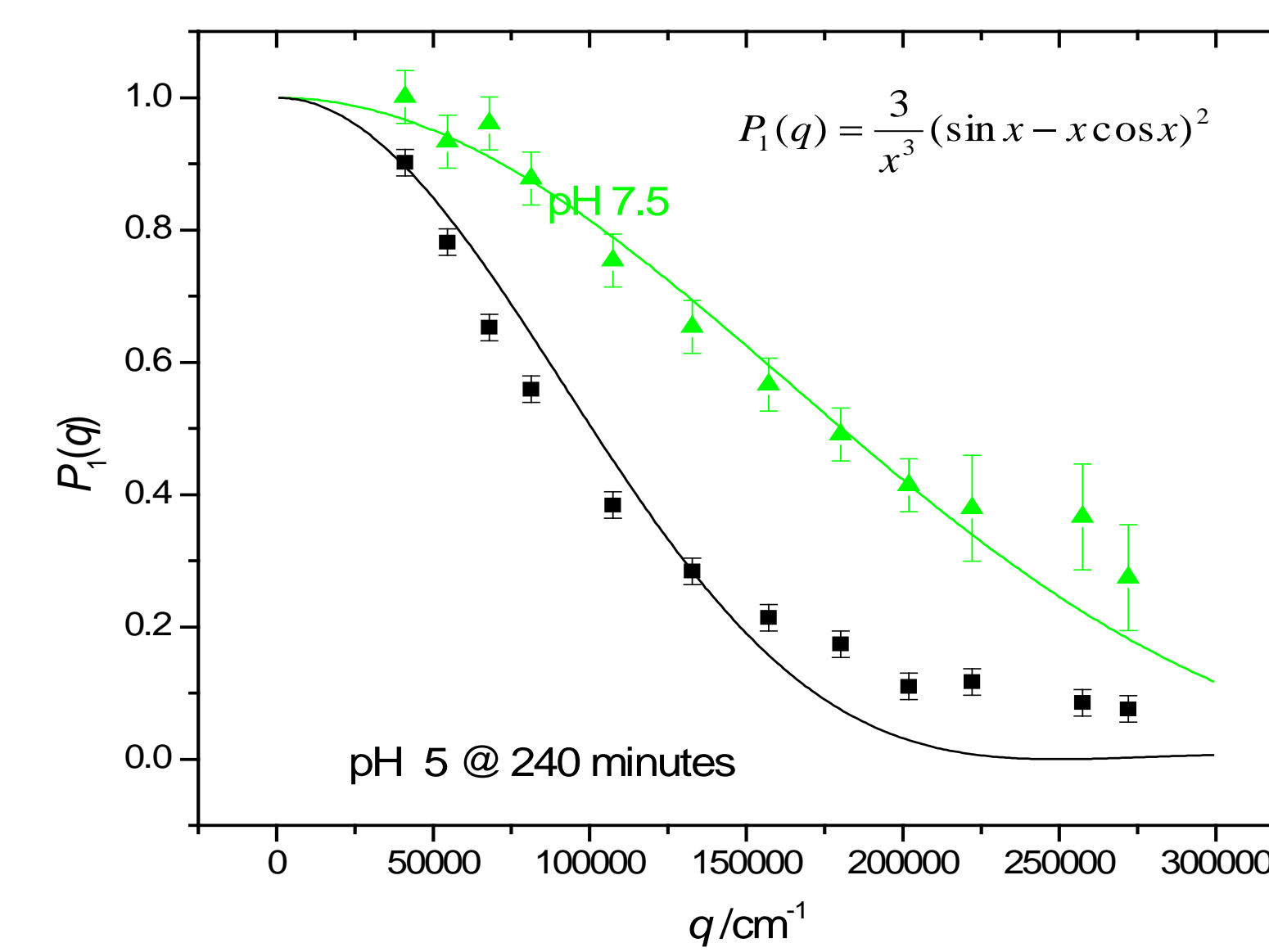
where $x = Rq$. The scattering from an N -particle, freely-rotating aggregate, normalized to that at $q=0$ is the product of the form factor for the individual particle and a structure factor, $S(q)$, that reflects the interparticle distances, r_{ij} :

$$P_N = P_1 S(q)$$

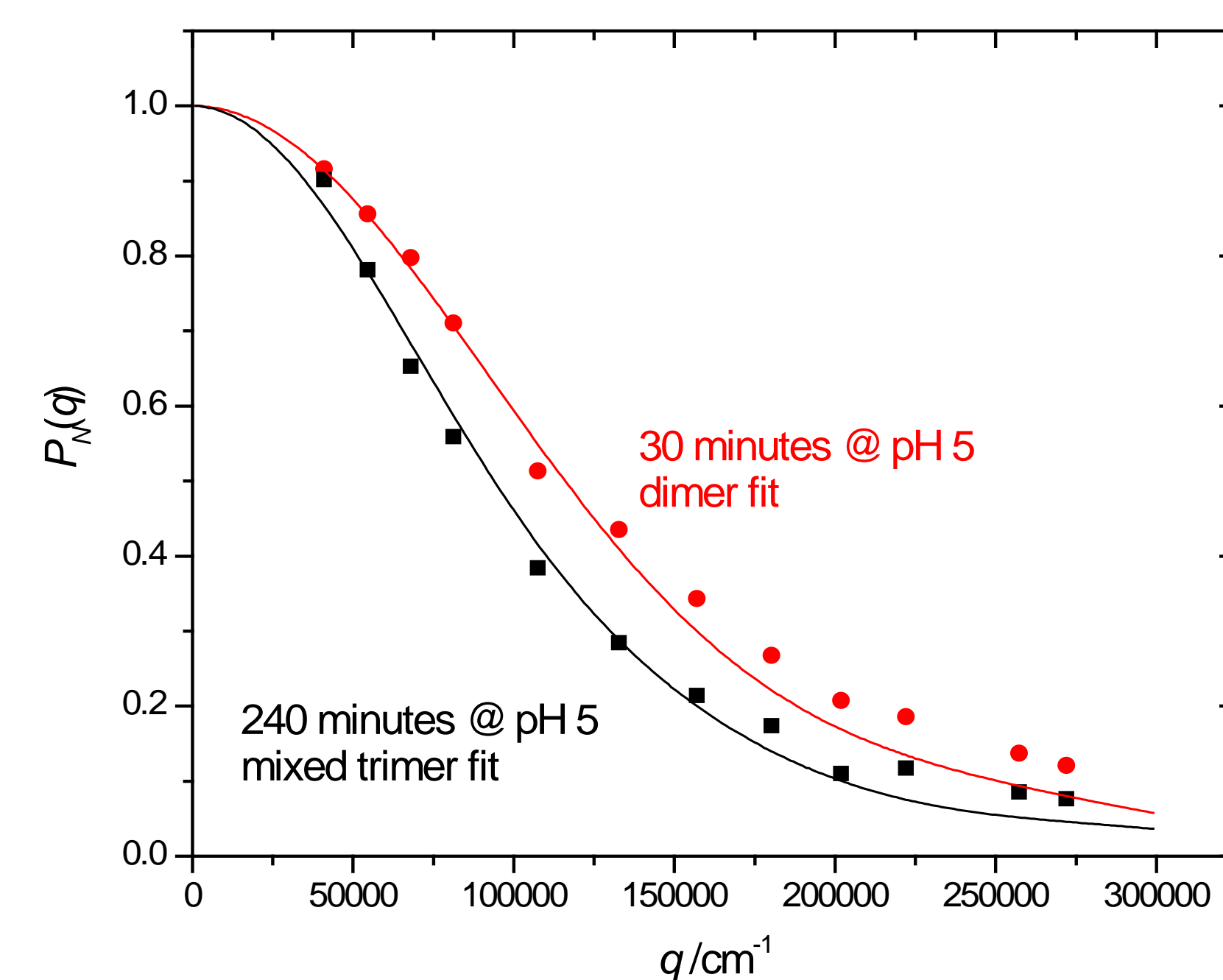
$$S(q) = \frac{1}{N^2} \sum_{i,j} \frac{\sin qr_{ij}}{qr_{ij}}$$

For example, the normalized scattering intensity for a linear trimer made of touching spheres is:

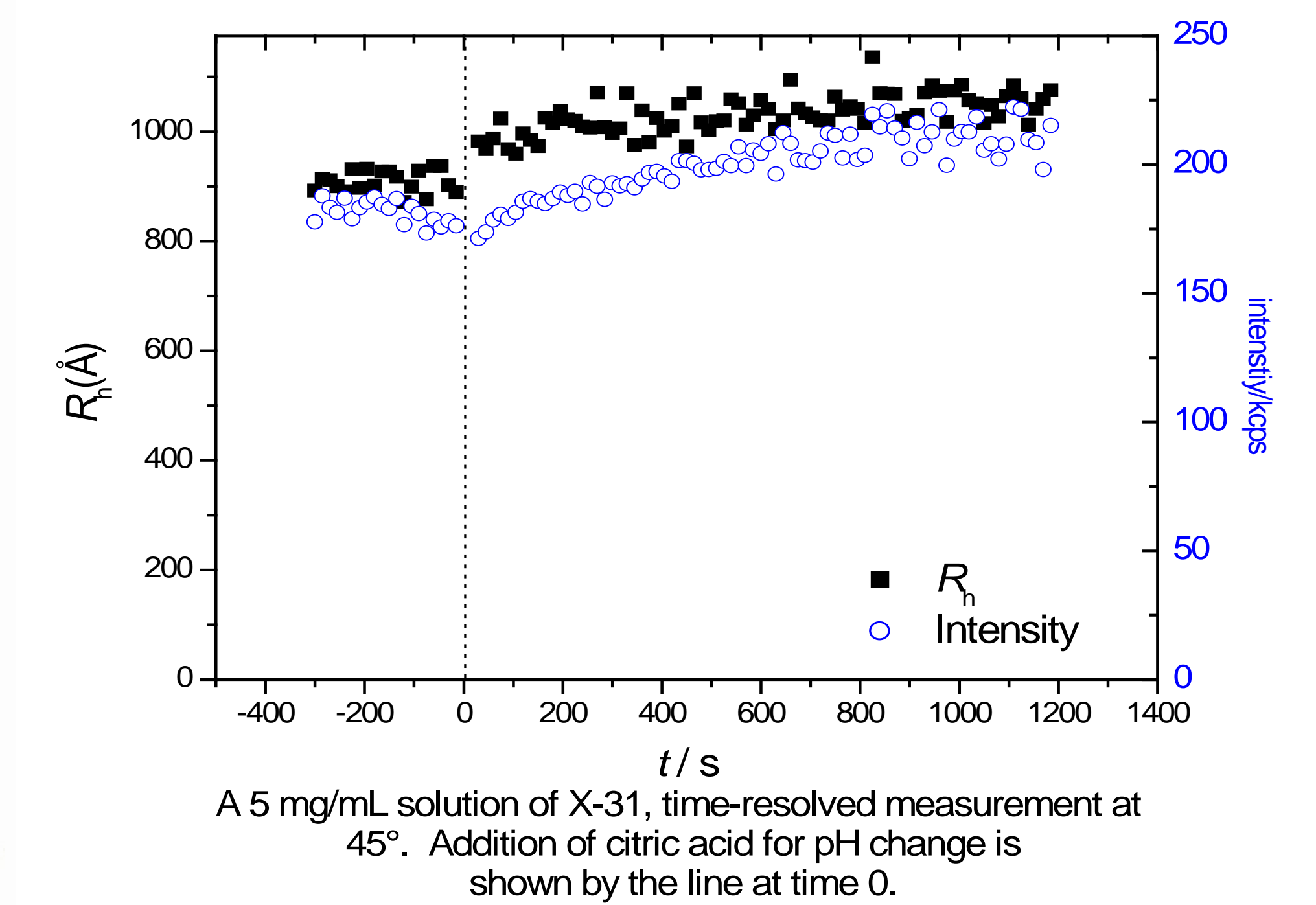
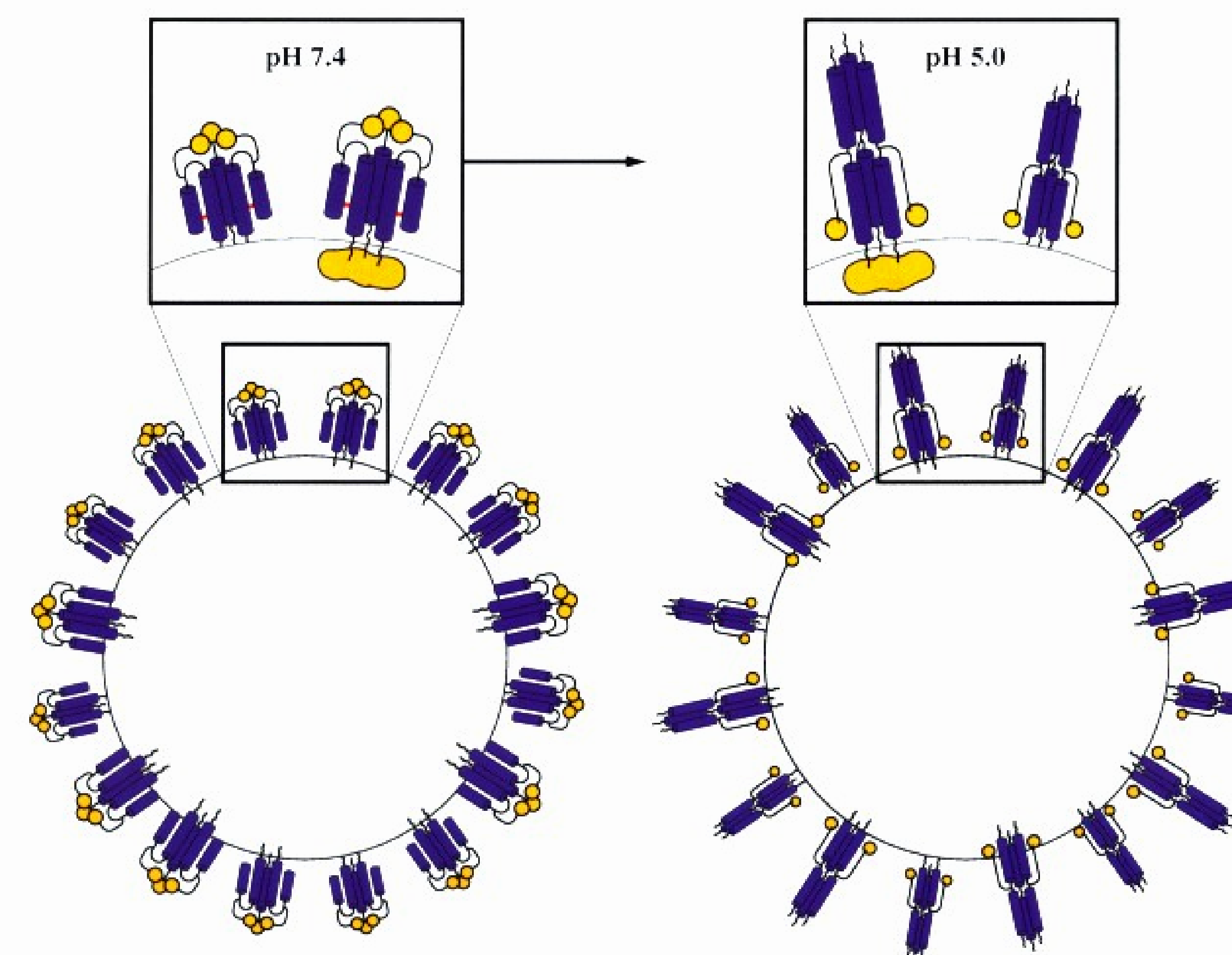
$$P_3(q) = \frac{P_1(q)}{9} \left[3 + 4 \cdot \frac{\sin(2qR)}{2qR} + 2 \cdot \frac{\sin(4qR)}{4qR} \right]$$



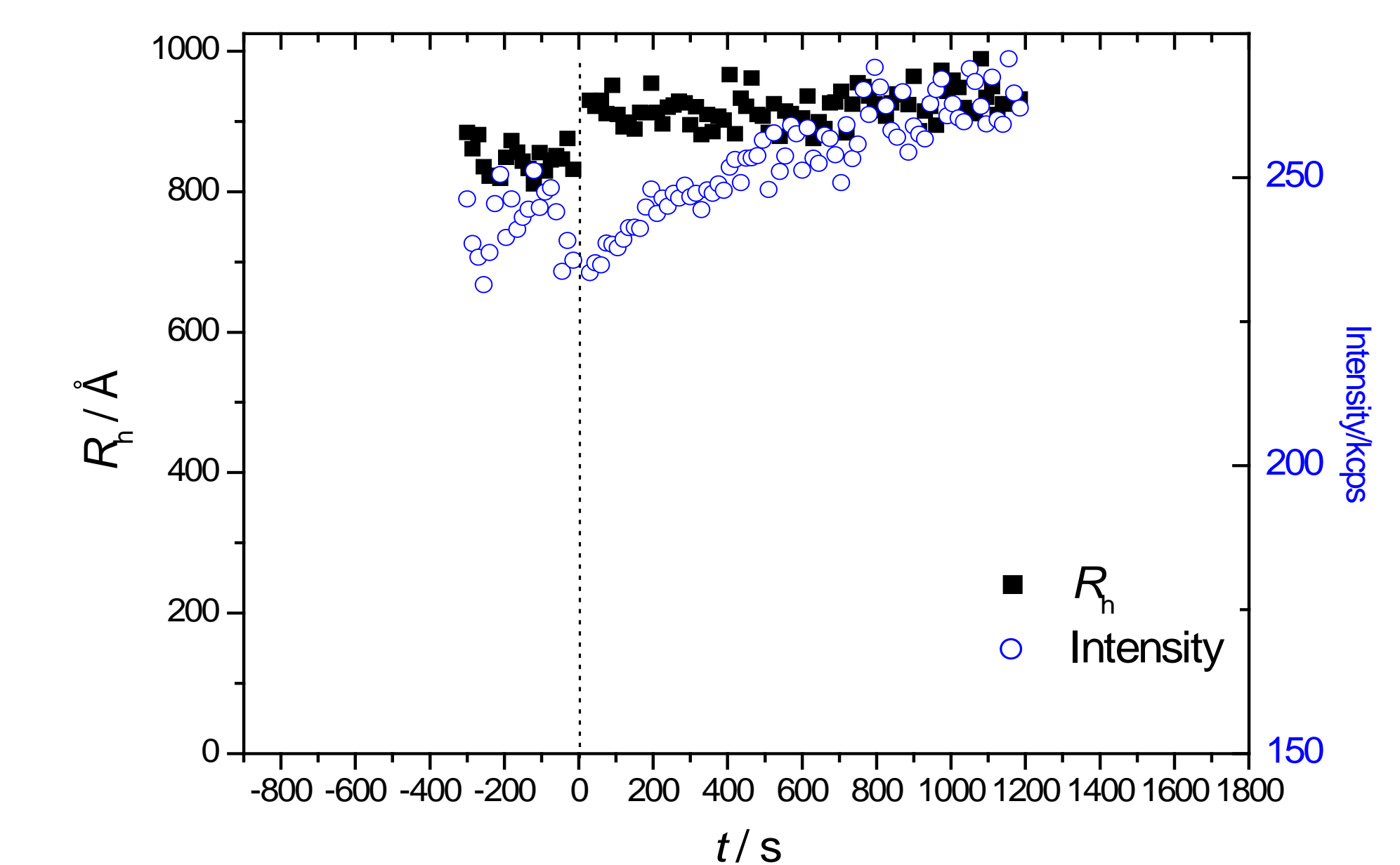
Single-sphere fits are not completely satisfying for virus particles at any measured condition, but come much closer at pH 7.5 than at lower pH.



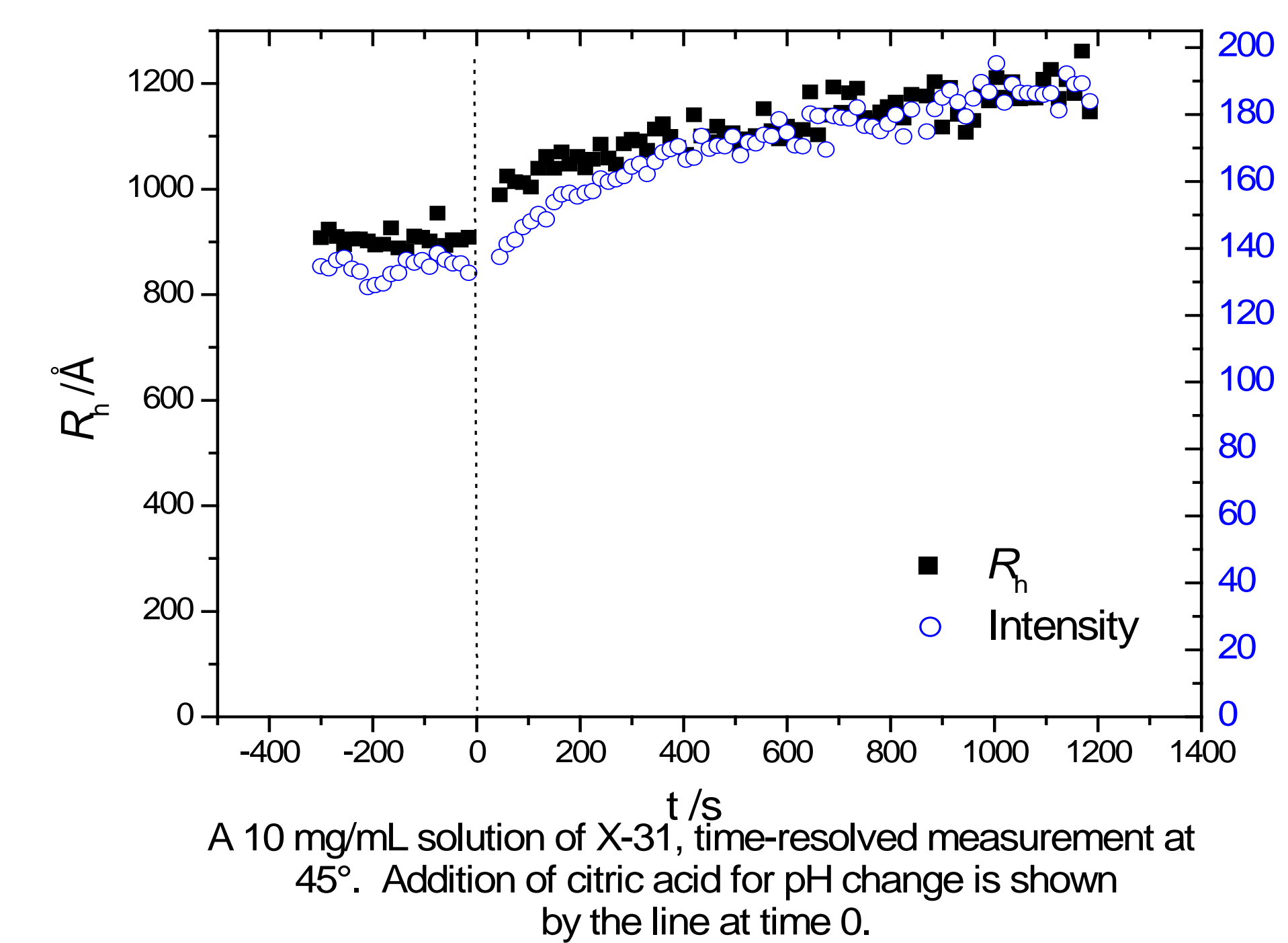
A fit for a dimer of solid spheres, $R=1000\text{\AA}$, is overlaid on data for PR-8 after 30 minutes at pH 5. The fit for a 50:50 mixture of linear and trigonal trimers is overlaid on the data for PR-8 after 240 minutes at pH 5.



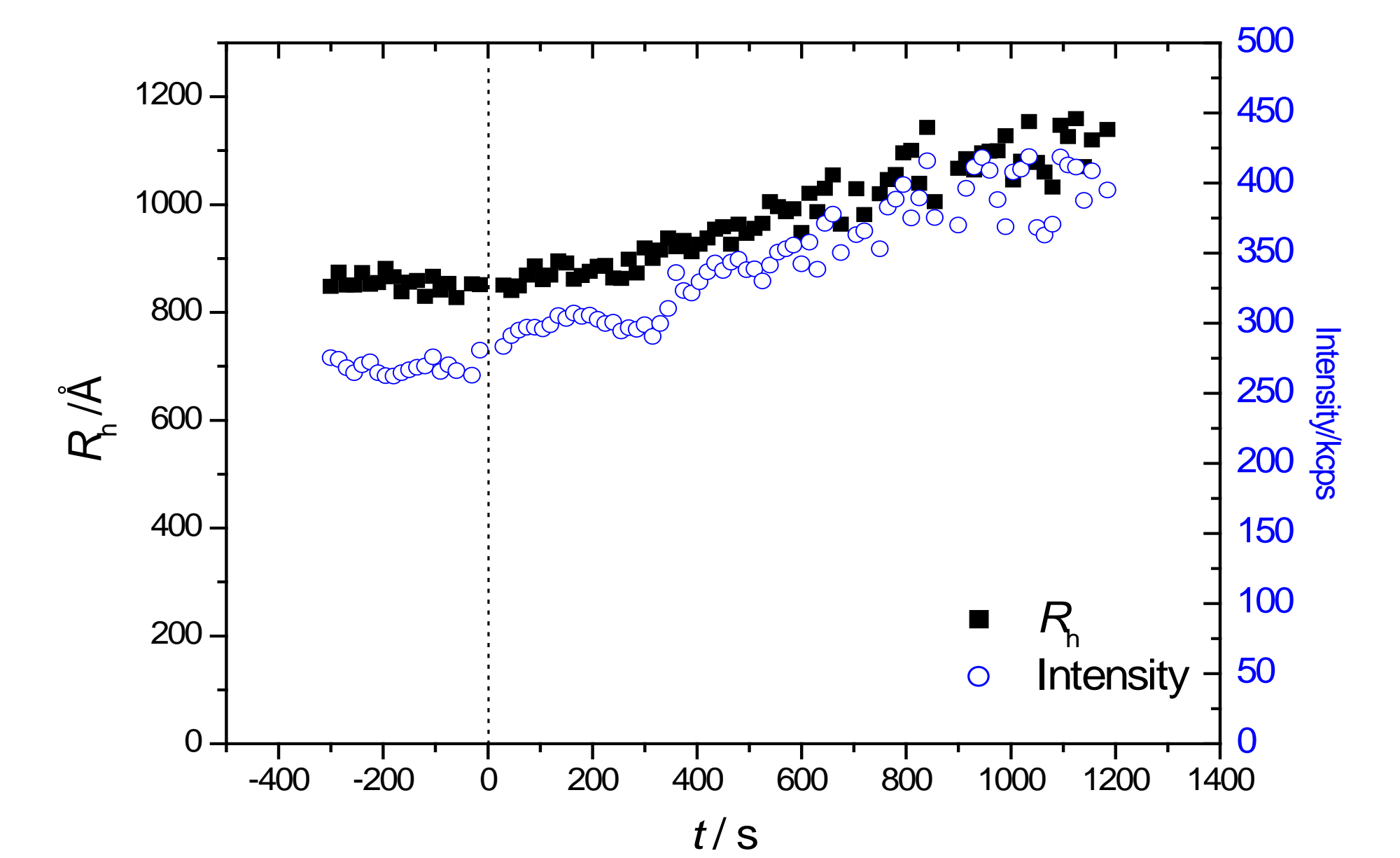
A 5 mg/mL solution of X-31, time-resolved measurement at 45°. Addition of citric acid for pH change is shown by the line at time 0.



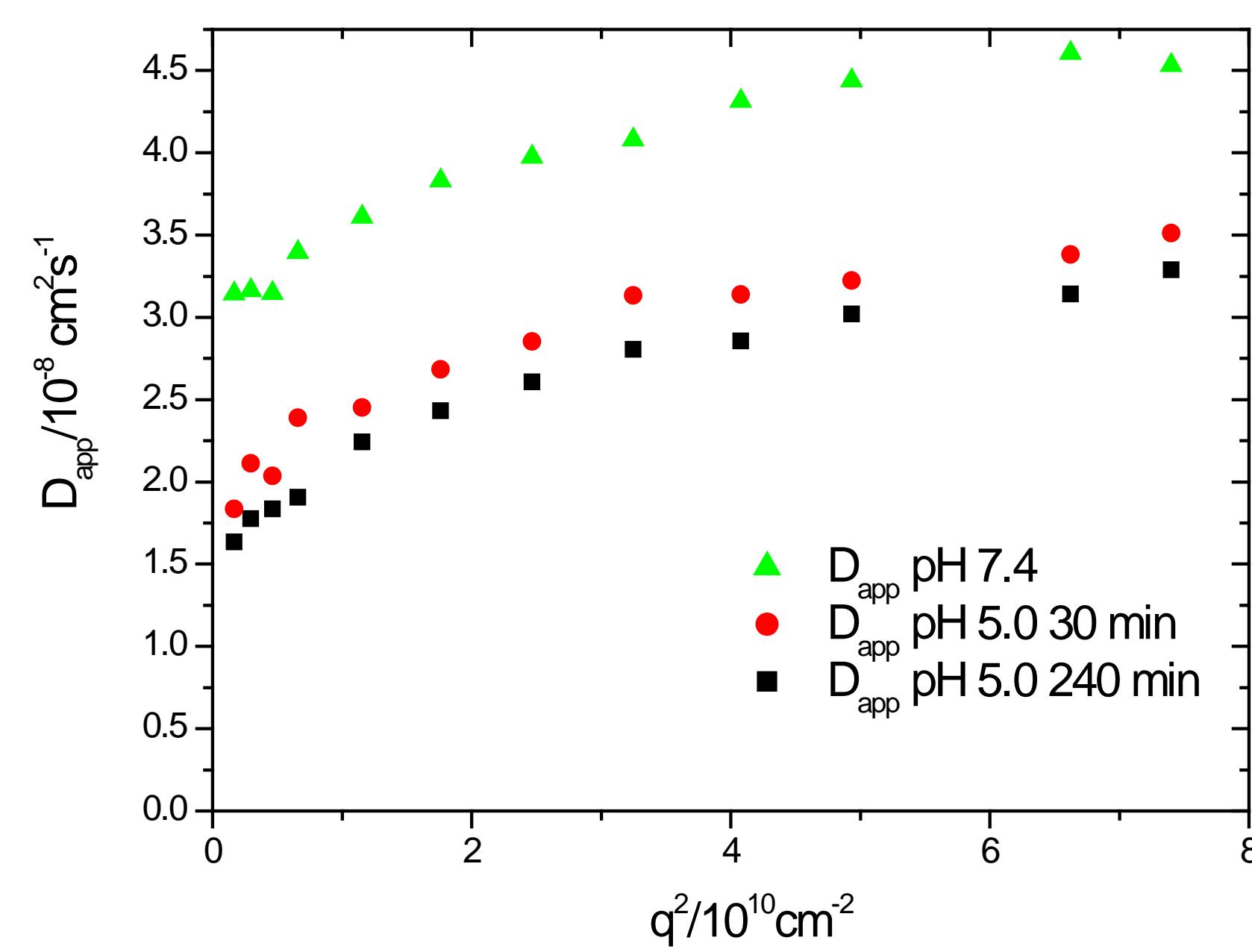
A 2 mg/mL solution of X-31, time-resolved measurement at 45°. Addition of citric acid for pH change is shown by the line at time 0.



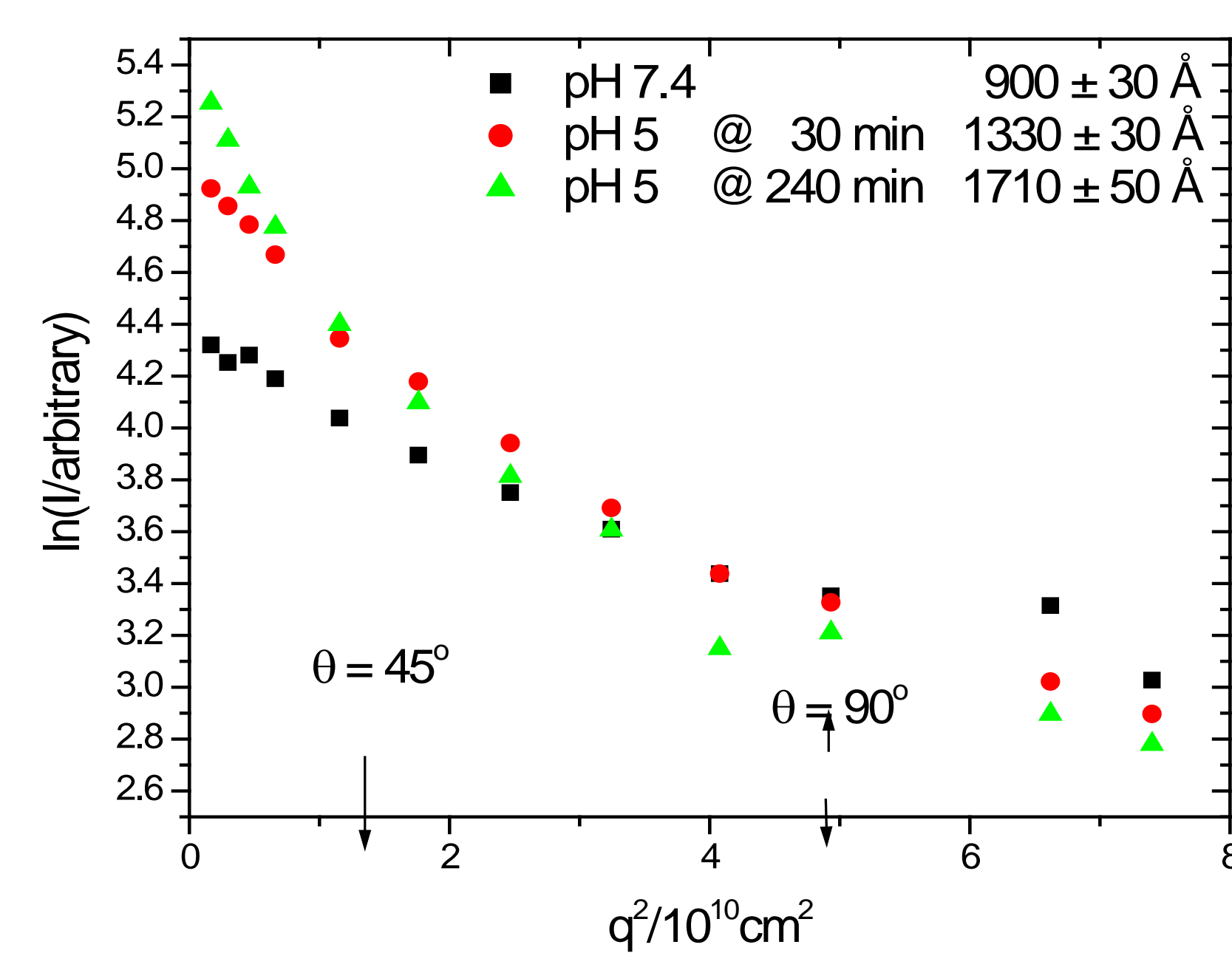
A 10 mg/mL solution of X-31, time-resolved measurement at 45°. Addition of citric acid for pH change is shown by the line at time 0.



Latex particles in water and the addition of citric acid at time 0.



Apparent diffusion by conventional scattering of PR-8 at 10 mg/mL concentration and pH 7.5(p) and at 30(l) and 240(n) minutes after acidification to pH 5.0.



Guinier plots for PR-8 at 10 mg/mL done on a conventional instrument

Conclusion

Suspended at physiological pH, influenza virus particles are polydisperse with respect to size and possibly aggregated slightly. This confirms, under very gentle conditions, the observations from electron microscopy. Upon acidification to pH 5, the particles aggregate over an extended period time. The data support the formation of dimers and trimers of the particles that existed at pH 7.4, although these were probably not entirely unaggregated and certainly not uniform. Time-resolved measurements show that aggregation is preceded by a rapid conformational change involving an expansion of about 100 Å in hydrodynamic radius. One may look to small angle X-ray or small angle neutron scattering measurements for short range structural details (17), but these methods are not well suited to following rapid changes. It may prove fruitful to follow structural alterations by simultaneous, time-resolved, multi-angle SLS and DLS measurements. For example, the quotient R_g/R_h can reveal information about shape change, as can the depolarized scattering signal. The present study is silent on these issues, as it is on the reversibility of pH-induced changes and behavior upon acidification to other pH values. Reliable methods to suspend the virus particles without any aggregation would be very valuable to such studies.

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