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A Novel Quantitative Fluorescence Spectroscopy Method to Estimate the Surfactant-Induced Split Ratio of Influenza Virus

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Most of the seasonal flu vaccines are produced after chemical inactivation and non-ionic surfactant treatment of the viruses with an aim of producing a vaccine product that has a low level of reactogenicity with high potency. Surfactants cause viruses to 'split' due to the membrane solubilisation and they further stabilize unbound membrane proteins. Consequently, this 'splitting' process affects the formulation stability and potency of flu vaccines greatly. Hence, finding the ideal splitting conditions and being able to estimate the split ratio quantitatively is of utmost importance for rapid preparation of flu vaccines. Here, we present a quantitative method, employing both steady-state and time-resolved fluorescence spectroscopy, to estimate the split ratio of flu virus following surfactant treatment. A lipophilic fluorescent dye was used to probe the molecular interactions and track changes in micro-environments. The fluorescence spectra of the dye shift towards the red side of the spectrum after the surfactant is added, suggesting disappearance of hydrophobic environments due to membrane solubilization. Results from both methods correlated well and showed that there are three distinct molecular environments with emission maximums at ~589, 630 and 670 nm and with fluorescence lifetimes of 4.45, 2.21 and 0.650 ns, respectively. Subsequently, we calculated the split-ratio of the virus using the percentage of dye in different micro-environments from both data sets. This study forms the basis of an in situ method to quantify split viruses during vaccine manufacturing and will facilitate the rapid development of the flu vaccine in a more controlled manner.

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