Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary Information

(“Fluorescence lifetime imaging of optically levitated aerosol: a technique to quantitatively map the viscosity of suspended aerosol particles”, Fitzgerald et al.)

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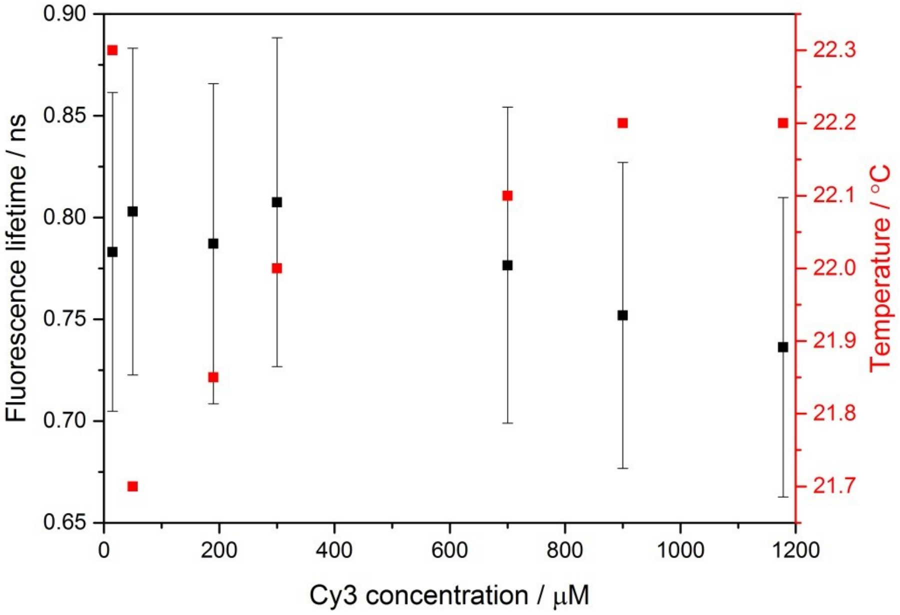
A. Effect of Cy3 dye concentration on fluorescence lifetime

Fig. S1



Control test for effect of dye concentration on fluorescence lifetime: Black squares: Change in fluorescence lifetime of sucrose droplets containing different concentrations of Cy3 (15, 50, 190, 300, 700, 900 and 1178 µM). Red squares: change in temperature surrounding the droplets.

To check that there was negligible effect of the dye concentration on the fluorescence lifetime as a result of self-quenching, sucrose/Cy3 solutions were made up that covered the range of the Cy3 concentrations that would be present in the trapped aerosol when equilibrated over the range from high to low RH, i.e. the starting Cy3 concentration in the bulk sucrose solution will have increased once the trapped aerosol equilibrated to a lower RH and lost water. The time resolved fluorescence traces of Cy3 in these solutions were measured to ensure that as the Cy3 concentration increased, the fitted lifetime did not significantly decrease; the latter would suggest that the fluorescence is being quenched.

The range of concentrations to be tested was estimated by considering the sucrose volume fraction and the difference between a bulk 0.75 M solution and a completely dry solution at 0% RH (provides an upper limit for the dye concentration).

The sucrose volume fraction when completely dry will equal 1.

The sucrose volume fraction (at 0.75 M) will equal:

$$C_{\mathrm{suc}} \times \left(\frac{Mr_{\mathrm{suc}}}{\rho} \right) = 0.16 \tag{1}$$

Where 'C_{suc}' is the concentration of sucrose in the bulk solution (0.75 mol L^{-1}), 'Mr_{suc}' is the molar mass of sucrose (342 g mol^{-1}, 'ρ' is the density of the sucrose solution (1.59x10^{3} g L^{-1}).

Therefore the greatest concentration increase for the dye is:

$$1 \div 0.16 = 6.2 \tag{2}$$

The highest dye concentration in the aerosol at the driest RH is therefore:

$$6.2 \times C_{\max} \tag{3}$$

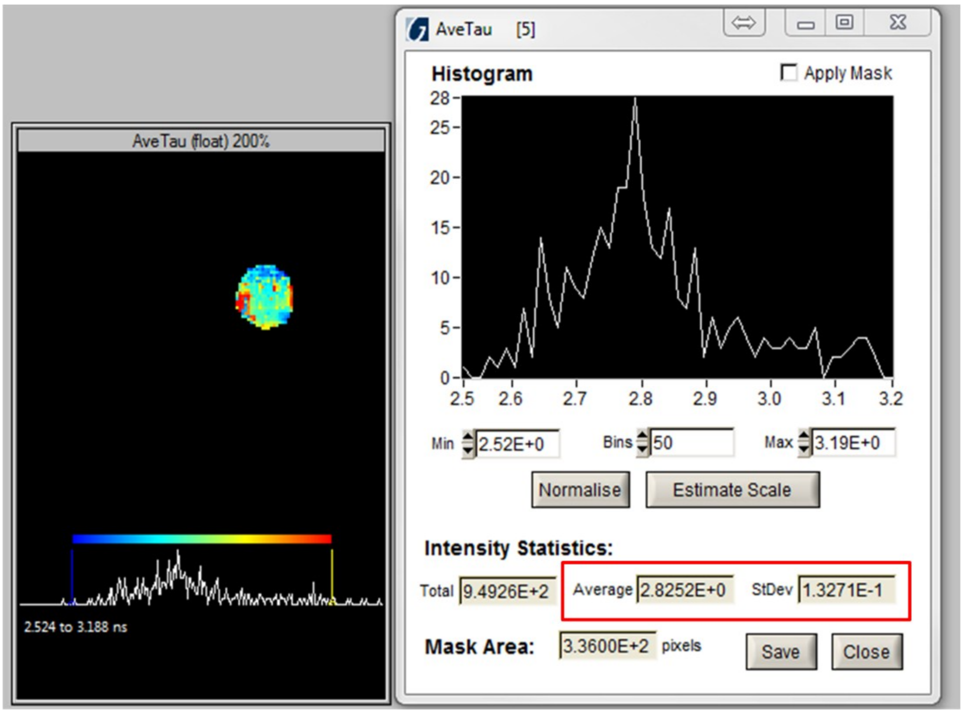
where '$C_{\max}$' is the highest dye concentration used in the bulk sucrose solution (190 µM). Therefore sucrose solutions were made up with Cy3 concentrations ranging from 50 µM to 1178 µM. Millilitre droplets of the solution were placed on a coverslip and sealed in a stainless steel chamber in which the temperature was monitored and the lifetime measured. Fig. S1 shows the fluorescence lifetimes for sucrose/Cy3 solutions with Cy3 concentrations of 15, 50, 190, 300, 700, 900, 1200 µM.

The temperature variation within the chamber is also shown as significant changes in temperature may also affect the lifetime. These measurements show that there was no significant decrease in the fluorescence lifetime as a result of increasing Cy3 concentration, demonstrating that at these concentrations the lifetime of Cy3 is not affected by aggregation or quenching.

B. Estimation of variability in measured fluorescence lifetimes and therefore viscosities

The FLIM image showing the distribution of the average fluorescence lifetimes is calculated and produced by the TRI2 software using equation (2). Alongside the calculated FLIM image is a histogram representing the frequency of the lifetimes in the image, plus the standard deviation of the spread in lifetimes across the image. An example image and histogram is shown in Fig. S2. These were the parameters used for quoting average lifetimes taking into account variation, i.e. τ_{av} ±

Fig. S2



Min 2.52E+0 Bins 50 Max 3.19E+0

Intensity Statistics:

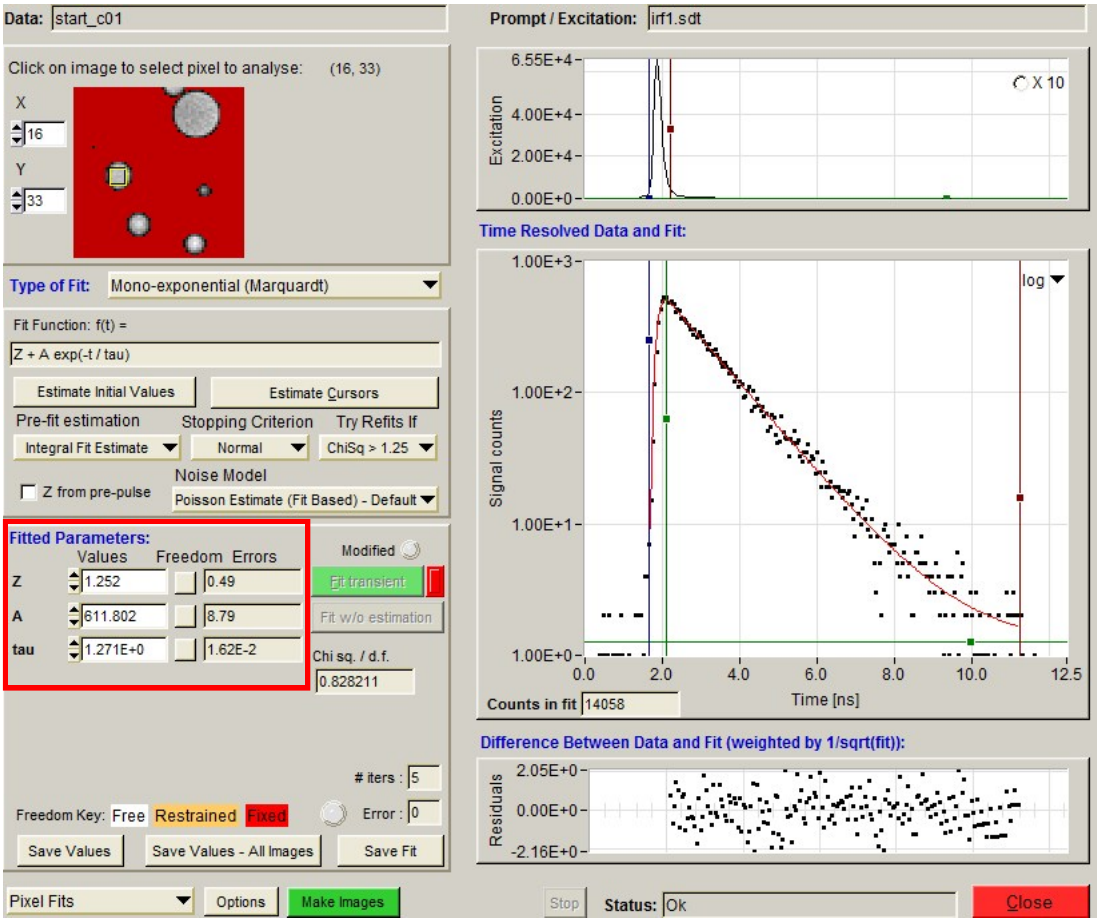
Total 9.4926E+2 Average 2.8252E+0 StDev 1.3271E-1

Mask Area: 3.3600E+2 pixels

Example screenshot from TRI2 software:
Average fluorescence lifetime image (left) of the trapped inhaler particle plus Cy3 (Fig. 7C, main text) and histogram (right) displaying the average lifetime and standard deviation of the lifetimes across the FLIM image (red box), used to quote Average lifetime ± StDev.

In the case of single decays that could be fitted using a monoexponential model, (i.e. where all pixels in an image were binned together to produce a decay that was fitting with a single lifetime component), the error bars for the measurement were provided by the goodness of fit as determined by TRI2 software (Fig. S3) to represent variability. For decays that required a biexponential model, the error associated with the average lifetime was derived from errors for the fitting parameters (A_1, A_2, τ_1, τ_2) obtained in TRI2, using standard propagation of error rules.

Fig. S3



Data: start_c01

Prompt / Excitation: irf1.sdt

Click on image to select pixel to analyse: (16, 33)

X: 16

Y: 33

Type of Fit: Mono-exponential (Marquardt)

Fit Function: f(t) = Z + A exp(-t / tau)

Pre-fit estimation: Integral Fit Estimate

Stopping Criterion: Normal

Try Refits If: ChiSq > 1.25

Noise Model: Poisson Estimate (Fit Based) - Default

☐ Z from pre-pulse

Fitted Parameters:

	Values	Freedom	Errors
Z	1.252	☐	0.49
A	611.802	☐	8.79
tau	1.271E+0	☐	1.62E-2

Chi sq. / d.f.: 0.828211

Counts in fit: 14058

iters: 5

Error: 0

Status: Ok

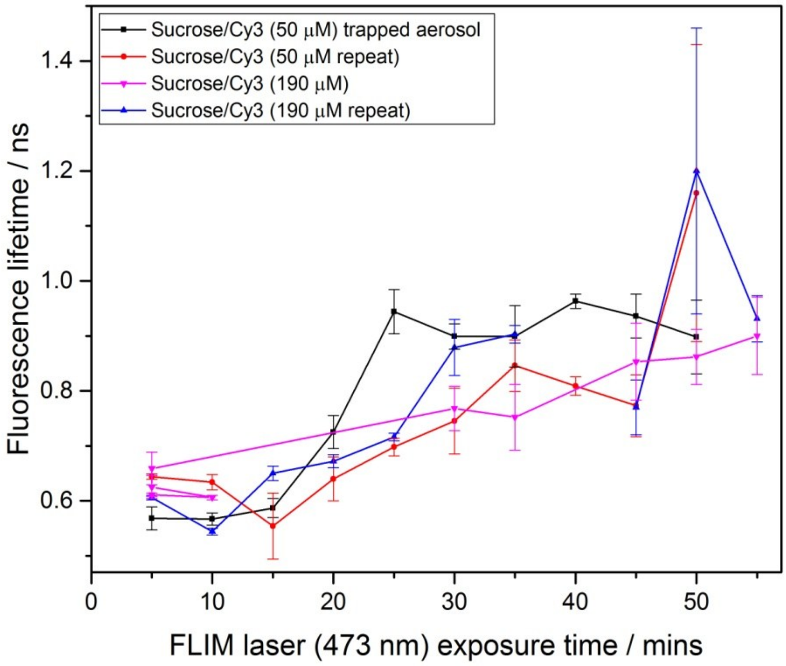
An example screenshot of the TRI2 user-interface for processing time-resolved image data. Outlined in red are the fitting parameters for the single fluorescence decay and the associated errors.

Once the lifetime is converted into a viscosity using the calibration in Fig. 1B in the main text, the variability in the viscosity then must be accounted for. The way this was represented in this manuscript was to firstly add and subtract the standard deviation (extracted from the FLIM image histogram) to and from the average lifetime. These two values were then converted into viscosities to provide an upper and lower limit of the spread in viscosity. These limits could then be converted into viscosity ± values by subtracting the lower limit from the calculated viscosity to obtain the 'minus' value and by subtracting the calculated viscosity from the upper limit to obtain the 'plus' value.

C. Effect of the FLIM laser on trapped aqueous aerosol

It was important to investigate whether the FLIM laser was having an influence on the trapped aerosol. It was found that prolonged exposure from the SP excitation lasers resulted in a lengthening of the fluorescence lifetime in all chemical systems investigated. Fig. S4 shows an example of this effect on aqueous sucrose samples. Trapped sucrose/Cy3 aerosol were exposed to the 473 nm FLIM laser (0.5 mW) while acquiring a series of images at a fixed RH (~83% RH). The increase was ~0.2 ns after 30 minutes of imaging. The change was independent of the dye concentration. On a case-by-case basis, a compromise was found between laser power (<0.5 mW), acquisition time (<30 minutes) and fluorescence intensity output, so that the experiments could be carried out within conditions which did not lead to changes > ~0.2 ns.

Fig. S4



Change in average fluorescence lifetime ± standard deviation, with exposure to 473 nm SP fluorescence excitation laser (RH ~ 83%), for suspended sucrose/Cy3 aerosol with 50 and 190 µM Cy3 concentration.

D. Literature estimates of sucrose viscosity

Two example literature estimates for sucrose viscosity were used to compare to the deposited and trapped measurements of sucrose/Cy3. Laliberté (2007) published a model for calculating the viscosity of 74 different aqueous solutions over a range of temperatures and concentrations. This study developed parameters using over 9000 data points from previous studies that looked into the viscosity of one solute in water. The model was validated by comparing the calculated values for more than one solute in a system to published experimental values. Equations 4-6 (outlined below) show Laliberté's parameterisation for the viscosity of a sucrose solution using the mass fraction of the solute (MFS). Secondly, the simple empirical equation using the mole fraction of the sugar (7) by Génotelle (1978) as outlined by Longinotti (2008), was used.

To link the literature estimates for the water content dependent viscosity of sucrose with RH, the MFS at different RHs was calculated by first using the equation (8) taken from the study by Zobrist et al. (2011) to calculate the water activity at a range of MFS values and then multiplying by 100 to find the RH at these MFS values. A plot of MFS against RH provides a trendline equation from which values of RH for given MFS values could be estimated. The output from the two literature parameterisations could then be plotted alongside the corresponding RH.

- Equation to calculate viscosity of pure water as function of temperature (Knox and Reid 2008). This correlation was established by Laliberté using viscosities from *Recommended Reference Materials for the Realization of Physicochemical Properties* (Marsh 1987):

  $$\eta_w = \frac{T+246}{0.05594T + 5.2842T + 137.37} \tag{4}$$

  Where the 'η_w' is viscosity of pure water (mPa.s), and the 'T' is temperature (°C)

- Equation to calculate viscosity of solute. This was found to adequately represent the viscosities of the solutes included in Laliberté's data set:

  $$\eta_i = e^{\left(\frac{v_1 w_i^{v2} + v_3}{(v_4 T + 1)(v_5 w_i^{v6} + 1)}\right)} \tag{5}$$

  Where the 'η_i' is viscosity of the solute (mPa.s), 'T' is temperature (°C), 'v_1' to 'v_6' are solute specific dimensionless constants and 'w_i' is mass fraction of solute.

- To calculate the viscosity of the final solution (rearranged from Laliberté, 2007). This equation was found by Laliberté to provide a sufficient estimation of viscosity of an aqueous solution containing one solute:

  $$\eta_m = \eta_i^{w_i} . \eta_w^{w_w} \tag{6}$$

  Where 'w_w' is mass fraction of water

- Génotelle's empirical equation for sucrose viscosity.

  $$log_{10} \frac{\eta}{\eta^*} = a_1 + a_2 x + \phi(b_1 + b_2 x^n) \tag{7}$$

$$\phi = \frac{30 - T}{91 + t}$$

Where the *'η'* is viscosity of the solute (mPa.s), *'a_1'*, *'a_2'*, *'b_1'*, *'b_2'* and *'n'* are coefficients of the Génotelle equation, *'Φ'* is reduced temperature, *'x'* is mole fraction of the sugar and 1mPa.s was used for η^*.

- This equation is a revised version of a previous effort by Zobrist et al. (2008) to outline a parameterisation for the water activity of aqueous sucrose solutions as a function of temperature and concentration.

$$a_w(T, w) = \frac{(1 + aw)}{(1 + bw + cw^2)} + (T - T^\theta)(dw + ew^2 + fw^3 + gw^4) \quad (8)$$

Where *'a'* to *'g'* are the revised fit parameters, and $T^\ominus$ = 298.15 K.

E. Calculating heating effect of optical trap on suspended aerosol

To estimate the particle heating through absorption of the laser radiation incident on the particle, we used an approach similar to that employed by Tong et al. (Knox and Reid 2008, Tong et al. 2014). Characteristics of the laser beam that had to be considered were wavelength, power and beam profile, as well as absorption characteristics of the particle and surrounding medium. The equation used was:

$$\Delta T = \frac{\left(1 - exp\left(-2r\varepsilon c\right)\right)P_p}{4\pi r K} \tag{9}$$

Where 'ΔT' is the steady state rise in particle temperature (K), 'r' is the particle radius (2.5×10^{-4} cm), 'ε' is the upper limit molar extinction coefficient, 'c' is the concentration of the particle, 'P_p' is the average laser power available for absorption in the particle (2.5×10^{-2} W), 'K' is the thermal absorption of air (2.58×10^{-4} W m^{-1} K^{-1}).

The first calculation was for the heating effect on pure water. An absorption coefficient value for water of 0.12 cm^{-1} was taken from Hale et al. (1973). This was used in place of the product of the extinction coefficient and the concentration. The particle radius = 1×10^{-4} cm. This calculation estimated a steady state rise in particle temperature of 1.85 K for pure water. This provides an estimate for the upper limit of the heating effect on the aqueous salbutamol sulphate/NaCl aerosol.

The next calculation was for pure sucrose. The particle radius = 2.5×10^{-4} cm. 4.65 M was the concentration of the particle estimated from dividing the density of sucrose (1.59 g cm^{-3}) by its molar mass (342 g mol^{-1}). This is an upper limit sucrose concentration. The molar extinction coefficient (2.13×10^{-2} M^{-1} cm^{-1}) was estimated using the Beer-Lambert law and the absorbance at 1064 nm in a UV-Vis absorption spectrum of an aqueous sucrose solution (0.75 M)/Cy3 (70 µM). This calculation estimated a steady state rise in particle temperature of 1.5 K. This has been accounted for in Fig. 4 by calculating the sucrose viscosity at different RHs using the Génotelle and Laliberté parameterisations using a temperature of 23.5 °C, which is the average laboratory temperature elevated by 1.5 °C.

F. Estimating the equilibration time for sucrose aerosol

The e-folding time (τ), as outlined by Shiraiwa et al. (2011), was used to estimate the equilibration time for aerosol of known size and viscosity (equ. 10). This defines the time after which the concentration in the particle core deviates by less than a factor of 1/e from the equilibrium value. This formula has been combined with the Stokes-Einstein equation (equ. 11) to provide a direct relationship between the e-folding time, the dynamic viscosity of the particle, radius of the diffusing species and the particle diameter (equ. 12).

For our purpose, τ has been estimated using an effective molecular radius of the diffusion species water (~1 Ångström radius).

$$\tau = \frac{d_p^2}{4\pi^2 D} \tag{10}$$

Where

$$D_{org} = \frac{kT}{6\pi a v} \tag{11}$$

Therefore

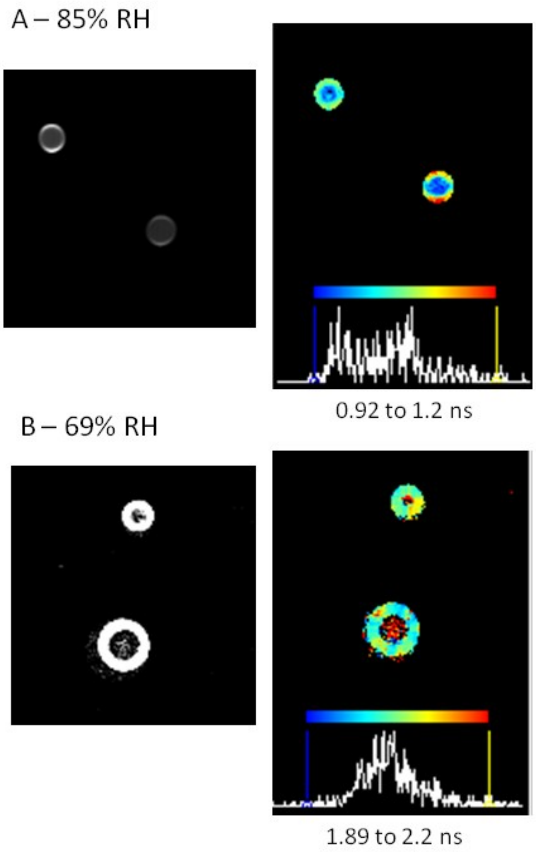
$$\tau = \frac{d_p^2 3 a v}{2\pi k T} \tag{12}$$

Where 'τ' is the e-folding time of equilibration, 'd_p' is the particle diameter, 'D' is the diffusion coefficient, 'k' is the Boltzmann coefficient (1.38×10^{-3} J K^{-1}), 'T' is the temperature (K), 'a' is the effective molecular radius (m), 'v' is the dynamic viscosity (Pa.s).

F. Example FLIM images of potential phase separation of salbutamol sulphate/NaCl/Cy3 droplets deposited on a coverslip

To compare our trapping results to equivalent coverslip experiments, we measured the fluorescence lifetime of salbutamol sulphate/NaCl/Cy3 droplets on a coverslip at RHs higher than the crystallisation point previously measured for NaCl on a coverslip (51% RH) (Hosny et al. 2013). It was observed that the droplets, although starting spherical, changed their morphology and phase separated in some cases on the slide. Examples of which are shown in Fig. S5. This resulted in skewed lifetimes and therefore is a justification for a trapping technique away from a surface.

Fig. S5

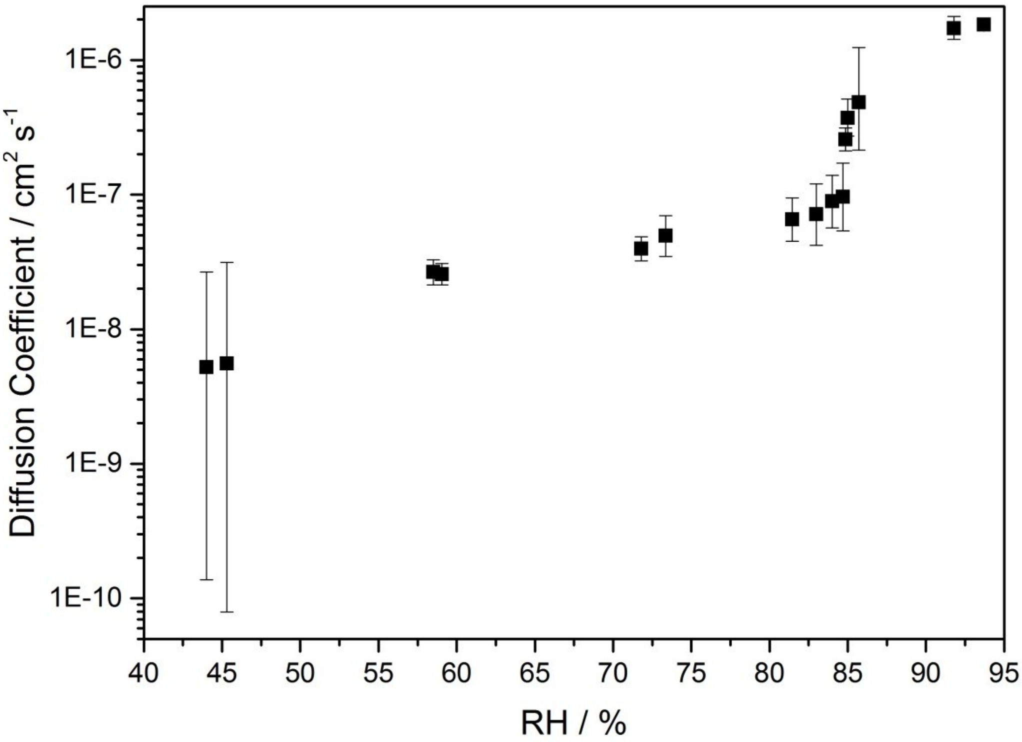


Fluorescence intensity (left) and FLIM images (right) of salbutamol sulphate/NaCl/Cy3 droplets deposited on a coverslip, equilibrated at different RHs. A: 85% RH; B: 69% RH. Image scale is 250 µm^2.

H. Conversion of measured salbutamol sulphate/NaCl viscosities into diffusion coefficients for water

The measured viscosities were converted into diffusion coefficients using the Stokes-Einstein equation (equ. 11). The effective molecular radius of water (*a*) was set to 1 Å.

Fig. S6



Diffusion coefficients for water through salbutamol sulphate/NaCl aerosol as a function of RH: Calculated using the Stokes-Einstein relation and measured viscosities (Fig. 5B).

I. References:

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