application note

Exploring the Concentration Limits of DLS: Characterisation of Vitamin B1

Introduction

Vitamin B1 (thiamine) is a water-soluble compound with a molecular weight of 300.84 g/mol (Da) and a structure consisting of a methylene bridge linking a pyrimidine ring and a thiazole ring (figure 1) [1]. It is found in a variety of foods including wholegrain cereal, nuts, eggs and liver. It is an essential vitamin in maintaining the health of the brain, nerves and cardiovascular system. Signs of vitamin B1 deficiency include poor concentration, loss of appetite and exhaustion [2].

Dynamic light scattering (DLS) is a technique suitable for the size characterization of nanoparticles and molecules such as proteins and polymers [3-5]. The technique measures the time-dependent fluctuations in the intensity of scattered light that occur due to the random movement of the particles or molecules undergoing Brownian motion. The velocity of this Brownian motion is measured and is called the translational diffusion coefficient (D) which can be converted into a hydrodynamic diameter (D_H) using the Stokes-Einstein equation [3-5].

Vitamin B1 is an ideal candidate in exploring the concentration limits of DLS measurements due to its small size and low molecular weight. This application note summarizes a series of DLS measurements performed on various concentrations of vitamin B1 solutions.

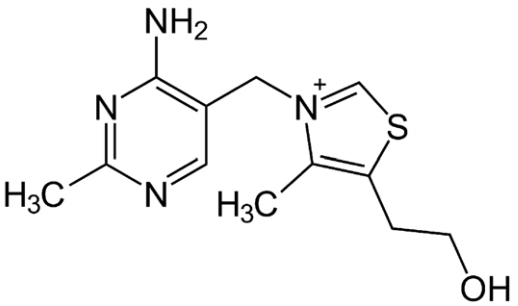

Figure 1: The structure of vitamin B1 (thiamine) consists of a methylene bridge linking a pyrimidine ring and a thiazole ring.

Experimental

Vitamin B1 solutions (400mg/ml to 10mg/ml) were prepared in deionised water and then filtered through 0.1μm pore size filters (Whatman Anotop) prior to measurement. Measurements of all the vitamin B1 concentrations were made on a Zetasizer Nano S using a detection angle of 173°. All measurements in this study were taken at a temperature of 25°C with at least 3 repeat measurements on each sample taken to check for result repeatability. The Nano S uses a 4mW He-Ne laser operating at a wavelength of 633nm.

The viscosity of each sample concentration was determined on an A and D SV-10 vibroviscometer at 25°C [6].

The intensity size distributions were obtained from analysis of the correlation functions using the Protein Analysis algorithm in the instrument software. This algorithm is based upon a non-negative least squares fit [7,8]. These intensity particle size distributions were converted into volume using Mie theory [9]. The optical properties of the vitamin B1 molecules were not considered important because their size is significantly lower than the incident laser wavelength used.

Results and Discussion

The lower concentration limit of the DLS technique depends on the amount of excess scattered light produced. This excess is the difference in scattering between the molecule or particle being studied and the dispersant it is prepared in. This in turn depends on a number of factors such as the refractive indices of the molecule and dispersant, the size of the molecule or particle being measured, the power and wavelength of the laser used, the sensitivity of the detector and the optical configuration of the instrument. The backscatter detection (173°) used in the Zetasizer Nano S in combination with fiber optics ensures very high signal to noise ratios even at very low concentrations.

Table 1 summarizes the results obtained from various concentrations of vitamin B1. The data displayed

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includes the sample concentration, the derived count rates (the normalized count rates) and the peak mean diameters from the intensity and volume size distributions. The results shown are the means and standard deviations obtained from 3 repeat measurements. The results obtained show very good repeatability for each of the concentrations. There is a gradual increase in the size obtained with decreasing sample concentration from 400mg/ml to 20mg/ml. This increase in size may be due to the structure factor which becomes significant at higher concentrations [10].

The extrapolation of these intensity and volume peak mean values to zero concentration enable infinite dilution values to be calculated (figure 4). This approach is recommended in the ISO13321 [4] for samples which show a systematic concentration dependence in the particle size results obtained. The sizes obtained for the intensity and volume mean data extrapolated to zero concentrations are 0.66nm and 0.61 respectively.

The lowest concentration of sample on which successful measurements can be made is 20mg/ml. Deionised water would be expected to give a count rate of between 20 and 30kcps on a Zetasizer Nano S. The count rate of 42.5kcps obtained for the 20mg/ml vitamin B1 sample is sufficient for repeatable results to be obtained. Figure 2 shows the intensity size distributions of the 20mg/ml sample obtained from 3 repeat measurements with the corresponding volume size distributions shown in figure 3, demonstrating the repeatability of the results obtained even at such low concentrations.

The 10mg/ml concentration has a count rate of 31.3kcps which is similar to that expected for DI water. The results obtained from this concentration were multimodal and not repeatable.

Table 1: Summary of the results obtained from a series of vitamin B1 concentrations. The table shows the derived count rates in kilo counts per second (kcps) and the peak mean diameters (in nm) from the intensity and volume size distributions. The results shown are the means and standard deviations obtained from 3 repeat measurements.

Vitamin B1 Concentration (mg/ml)	Derived Count Rate (kcps)	Intensity Peak Mean (nm)	Volume Peak Mean (nm)
400	279.2 ± 0.8	0.37 ± 0.005	0.36 ± 0.006
300	238.6 ± 1.3	0.44 ± 0.009	0.42 ± 0.012
200	189.8 ± 0.4	0.54 ± 0.001	0.52 ± 0.007
100	115.6 ± 2.2	0.59 ± 0.009	0.53 ± 0.023
80	121 ± 1.0	0.62 ± 0.020	0.57 ± 0.025
40	68.5 ± 1.2	0.65 ± 0.035	0.61 ± 0.029
20	42.5 ± 1.3	0.63 ± 0.039	0.58 ± 0.026
10	31.3 ± 2.9	-	-

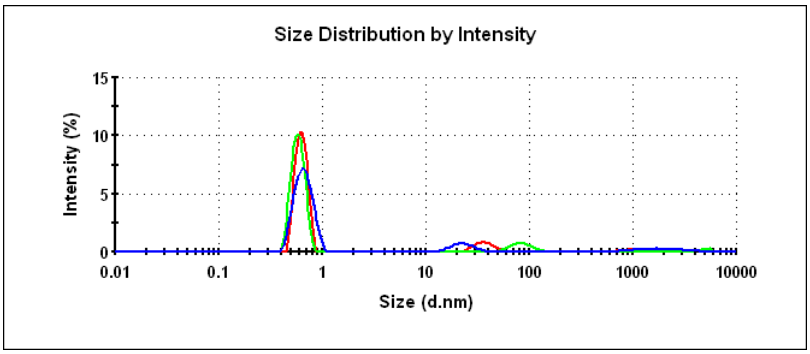

Figure 2: Intensity size distributions obtained for 20mg/ml vitamin B1.

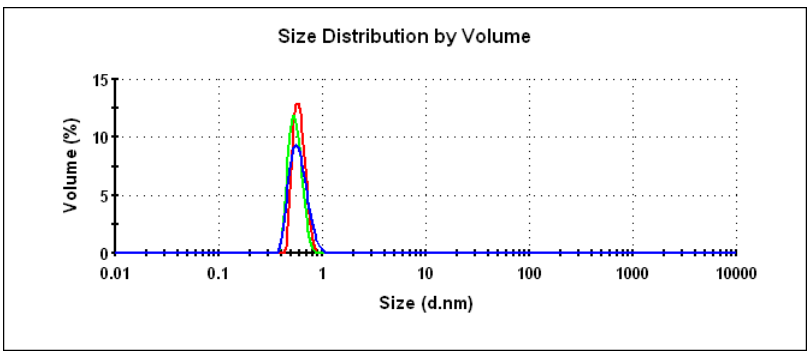

Figure 3: Volume size distributions obtained for 20mg/ml vitamin B1.

Figure 5 shows the correlation functions obtained from the 100, 80, 40, 20 and 10mg/ml vitamin B1 concentrations respectively. The plots show the gradual decrease in the intercept (signal to noise) with decreasing concentration. This is to be expected as the excess scattering decreases with concentration. Correlation functions obtained from the 200, 300 and 400mg/ml (not shown) concentrations had even higher intercepts.

Conclusions

The lower concentration limit of the DLS technique depends on the amount of excess scattered light produced by the particles or molecules studied. Probing the concentration limits of the technique can be done by measuring a suitable sample of small size and low molecular weight such as vitamin B1 used in this study. This also indicates that measurements at a high concentration to produce more scattering can give results that ignore concentration dependent effects, and that for a full and correct characterization of the material, a wide range of concentrations should be studied.

The sensitivity of the instrument is also a key factor in determining the lowest concentration and size limits measureable. The optical configuration used in the Zetasizer Nano S ensures very high signal to noise ratios even at very low concentrations as demonstrated from the correlation functions obtained in this study.

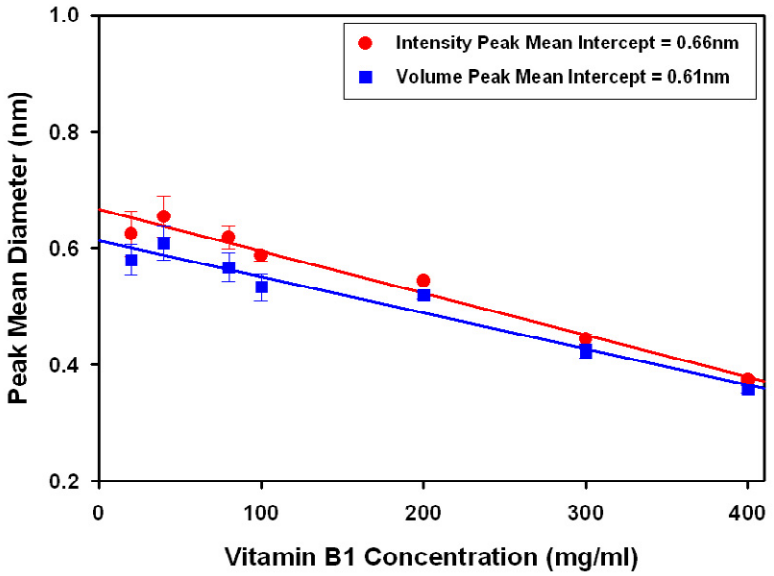

Figure 4: A plot of the intensity and volume peak means obtained as a function of sucrose concentrations. The data is extrapolated to zero concentration and the intercepts for each plot are recorded.

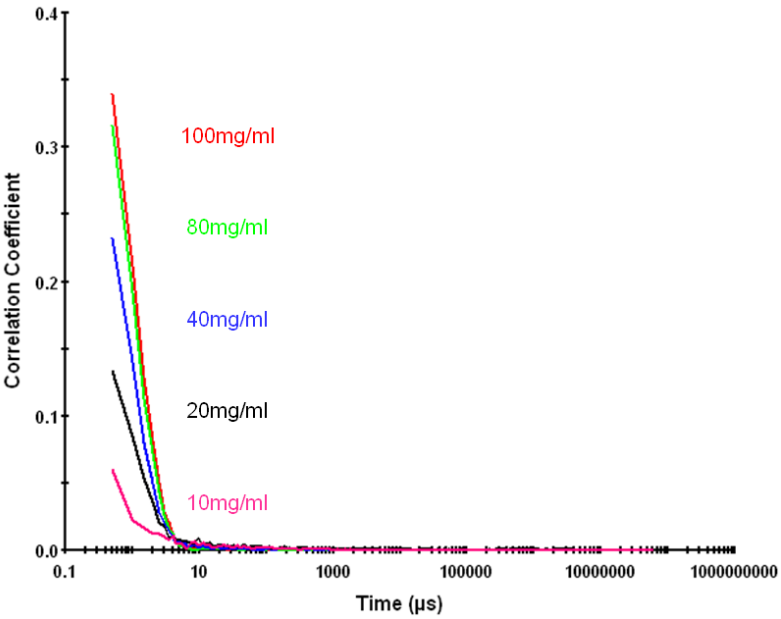

Figure 5: Correlation functions obtained from measurements of various concentrations of vitamin B1; 100, 80, 40, 20 and 10mg/ml respectively.

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