Fluidic Analytics

Application note

Interleukin-2 stability in changing buffer and temperature conditions

The stability of human interleukin-2 under changing conditions of buffer and storage temperature was evaluated using the Fluidity One. By observing size and concentration over a 24-hour period, we find that samples prepared in PBS have a tendency to aggregate, while those in acetic acid remain monomeric over the observation period.

Authors
Maren Butz[1], Sean Devenish[1]

www.fluidic.com

1. Fluidic Analytics Ltd. Cambridge, UK

Introduction

The ability to conduct reliable and repeatable experiments on protein molecules relies on beginning with consistent and stable samples. A wide range of factors can affect protein stability, including temperature, pH, salt concentration, preservatives and cosolutes (1). Finding the optimum storage conditions for a protein of interest is the essential first step to conducting robust studies and analyses.

Here we evaluate human interleukin-2 (IL-2), a cytokine with a range of roles in the immune system and homeostasis (2), for stability under changing environmental conditions. A Fluidity One instrument was used to monitor changes in the size (hydrodynamic radius, R_h) and concentration to evaluate overall stability, and particularly propensity to aggregate.

Methods

50 µg of lyophilized human IL-2 recombinantly expressed in E. coli (Sigma-Aldrich code SRP3085) was dissolved in 10 mM acetic acid to produce a 1 mg/mL stock solution.

Aliquots of this stock solution were diluted to 100 µg/mL with either PBS or 100 mM acetic acid, then stored either at room temperature (RT) or at 4 °C as described in Figure 1.

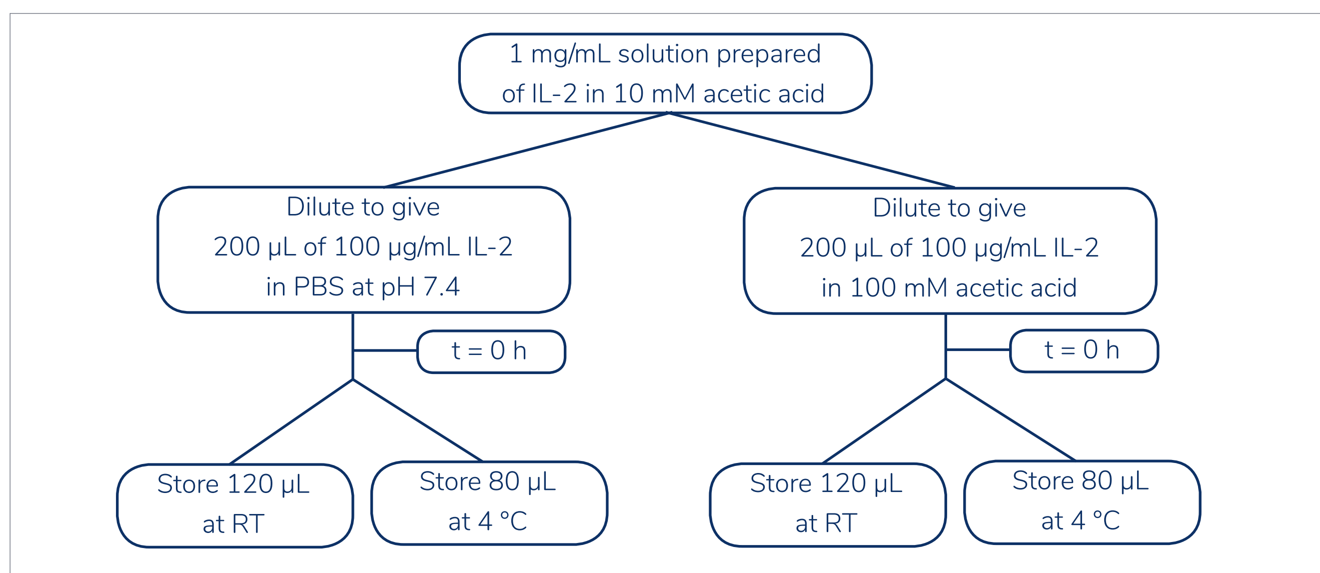

Figure 1: Figure 1 Sample preparation sequence prior to testing

An aliquot from each sample type was removed and tested using a Fluidity One prototype over the course of 24 hours; initial samples (t = 0 h) were run within ten minutes of sample preparation. The R_h and concentration were measured in each test.

After incubation for 24 hrs, each sample was mixed by flicking the tube several times before loading to obtain a "mixed" reading. Each sample was then centrifuged for 20 minutes at their respective storage temperatures at a maximum speed of 13.3 krpm to obtain a "centrifuged" reading.

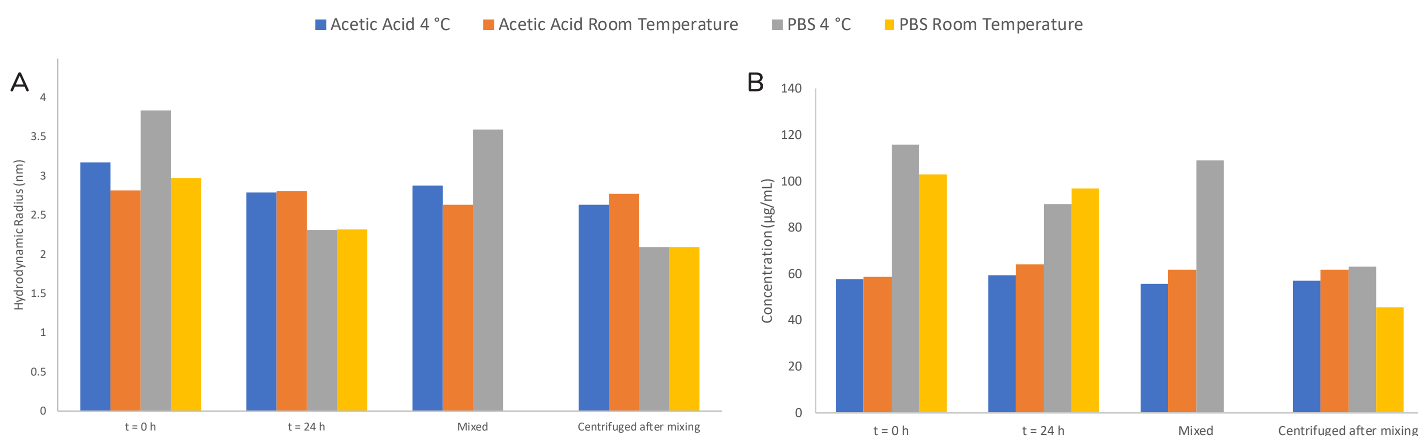

Figure 2: Results of size (A) and concentration (B) for the different storage conditions of IL-2 over time

For the PBS sample stored at room temperature mixing resulted in a size outside of the instrument range for the used flow rate, so manual data analysis was used to estimate the average R_h of the sample at 10 nm; the concentration was also manually derived and found to be 107 µg/mL.

Results

The samples prepared in acetic acid show very little variation in size or concentration across the test, at both chilled and room temperature.

The lower concentration measured for acetic acid compared to PBS samples is an artefact of the low pH of the sample buffer reducing labelling efficiency by 20% (confirmed independently with BSA in acetic acid; data not shown) and incomplete sample mixing.

For the samples prepared in PBS we observe a marked decrease in both size and concentration over the first 24 hours. This is consistent with aggregation; once an aggregate reaches a large enough size to drop out of solution it can no longer be detected, so the measured concentration decreases. The observed size decrease indicates the presence of small aggregates early in the time course, which are removed by further aggregation to leave only soluble monomers at the end.

This theory was tested via mixing and centrifuging, to bring the aggregates back into solution and then remove them again. In both PBS samples we observe that size and concentration increase after mixing, then decrease after centrifuging – consistent with the prediction.

Conclusion

The size and concentration of IL-2 prepared in different buffers and stored under different temperature conditions was evaluated using the Fluidity One.

From the results it appears that IL-2 forms aggregates within 24 hrs when prepared in PBS, but not when prepared in acetic acid. Temperature was found to have little effect on the stability of samples incubated in acetic acid.
For samples stored in PBS the amount of un-aggregated IL-2 was not affected by temperature, but the size of aggregate species was significantly higher for the sample stored at room temperature.

The Fluidity One provides a simple means to evaluate stability over varying buffer and temperature conditions, which could be employed when working with new proteins and peptides, or could be employed immediately prior to use of critical samples to ensure their integrity.

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References

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+44 (0)1223 560 432
welcome@fluidic.com
www.fluidic.com
@fluidicanalytic

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