Supplementary Materials

S1. Preparation and Validation of Reference Standards

Hydrazine sulfate (1.0 g, ≥99.0%, Sigma-Aldrich) was dissolved in ultra-pure water (PURELAB Ultra, ELGA LabWater, pH 7, 18.2 MΩ.cm), made up to 100 mL and left to stand for 4-6 hours. In a 100 mL volumetric flask, hexamethylenetetramine (2.5 g, ≥99.0%, Sigma-Aldrich) was dissolved in ultra-pure water (25 mL). Hydrazine sulfate solution prepared earlier (25 mL) was added to the flask, mixed and left to stand for 24 hours. This primary opalescent suspension was mixed thoroughly before use. Primary opalescent suspension (15 mL) was made up to 1000 mL with ultra-pure water to give the standard of opalescence. The reference suspensions I-IV were prepared according to Table S1.

Table S1. Preparation of reference suspensions according to British Pharmacopoeia[23].

Reference Suspension	Opalescent values (NTU)	Component (mL)		Degree of Opalescence
		Standard of opalescence	Ultra-pure water	
I	3	5	95	Clear (≤ Ref I)
II	6	10	90	Slightly opalescent (≤ Ref II)
III	18	30	70	Opalescent (≤ Ref III)
IV	30	50	50	Very opalescent (≤ Ref IV)

A calibration curve (Figure S1b) was prepared to provide a robust assignment of the category of opalescence for subsequent visual experiments. The calibration curve was fit-for-purpose with an R^2>0.99. The absorbance readings for four reference suspensions of different opalescent values were repeatable, producing similar plots. The coefficient of variance (%CV) for mean absorbance of Ref I-IV across three batches was 3.3%, 2.3%, 2.2% and 2.5% respectively. The range of %CV was narrower than that typically obtained, for example Mahler et al. achieved a %CV range between 1.2 and 11.8%[25]. Four degrees of opalescence (clear, slightly opalescent, opalescent and very opalescent) as shown in Figure S1a gave a more reproducible categorization of insulin samples compared to the sole visual description of appearance[25,29]. According to the BP requirements for parenteral, soluble insulin injection must be free from turbidity (clear)[30]. Hence, any insulin sample that was unclear or had absorbance reading greater than average absorbance of Ref I failed the test, which was determined to be an absorbance of 0.0174.

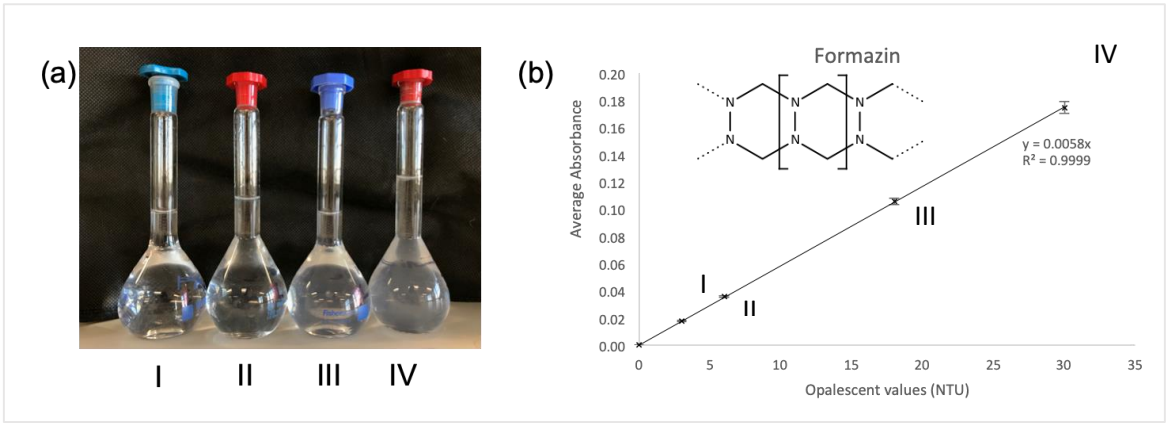


Figure S1. (a). Four reference suspensions prepared according to the BP. Figure S1(b). Average absorbance of three batches of reference suspensions (formazin) plotted against opalescent values (NTU). UV calibration linear regression: Average absorbance at 350nm = 0.0058×opalescent values (R^2 = 0.9999). Data points shown are the mean of n = 3 measurements, with error bars indicating the standard deviations on these values.

S2. Comparison of Insulin Solutions Stored in Pure Water and HCl

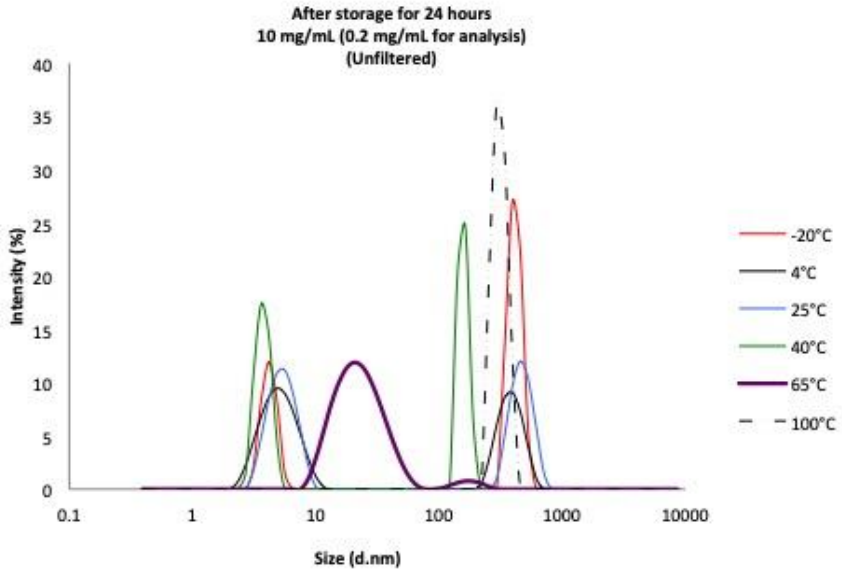
The effect of dilution by acid (0.01M HCl) on representative stability samples was investigated to allow comparison with the insulin solutions prepared using ultra-pure water (Table 8.2).

Table S2. Comparison of insulin solutions,10 mg/mL, stored for 24 hours at different temperatures, diluted to 0.2 mg/mL for analysis with either ultra-pure water or 0.01M HCl.

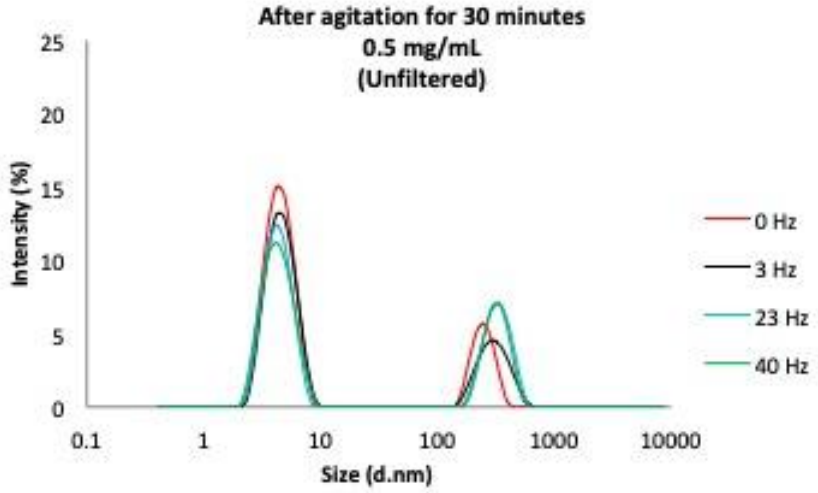
Temperature (°C)	**Absorbance @350mn diluted with ultra-pure water, mean ×10^{-3}, n=3, ± ×10^{-3} s.d.**	**Absorbance @350mn diluted with 0.01M HCl, mean ×10^{-3}, n=3, ± ×10^{-3} s.d.**	**Degree of opalescence by absorbance**
-20	1.3 ± 0.1	1.8 ± 0.1	Clear (≤ Ref I)
4	1.3 ± 0.1	1.9 ± 0.1	Clear (≤ Ref I)
25	2.5 ± 0.2	1.9 ± 0.1	Clear (≤ Ref I)
40	2.9 ± 0.3	2.6 ± 0.2	Clear (≤ Ref I)
65	9.7 ± 1.2	3.6 ± 0.8	Clear (≤ Ref I)

This shows that using either ultra-pure water or 0.01M HCl to dilute the 10 mg/mL insulin samples did not have an effect on the degree of opalescence, and all solutions passed the BP quality tests when stored at temperatures between -20 and 40°C. The absorbances measured at 350nm were all below the value of 17.4 ×10^{-3}, which represents the cut off for opalescence reference one. Thus, the stability of insulin was maintained independently of whether ultra-pure water or 0.01M HCl was used for dilution.

S3. DLS Size Distribution Curve for Insulin



(a)



(b)

Figure S3. (a) Size distribution diameter in nm for diluted insulin samples (0.2 mg/mL, unfiltered before analysis) after 10mg/mL insulin was being stored at respective temperatures for 24 hours. (b)Size distribution diameter in nm for 0.5 mg/mL insulin samples (unfiltered before analysis) after being agitated at respective agitation frequencies for 30 minutes.

S4. Vibration Measurement Tests

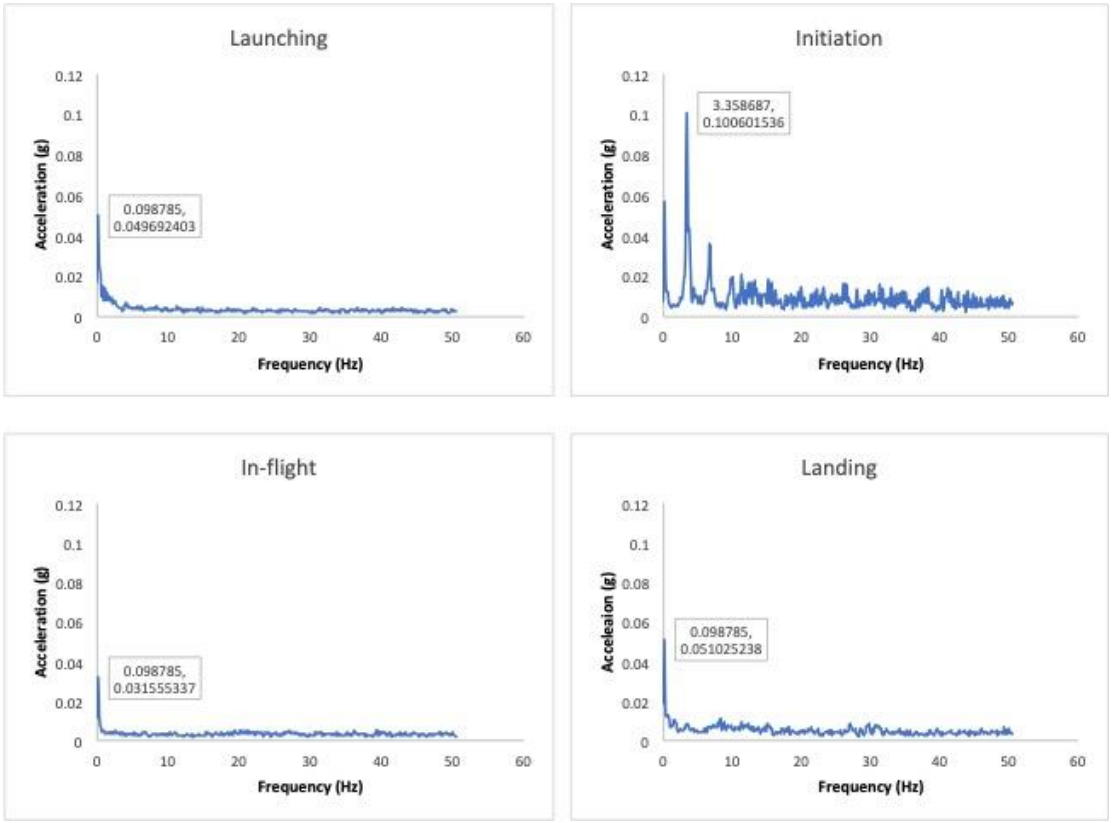


Figure S4. The most frequently observed vibration frequency with respective acceleration magnitudes (g) during launching, initiation, in-flight and landing.