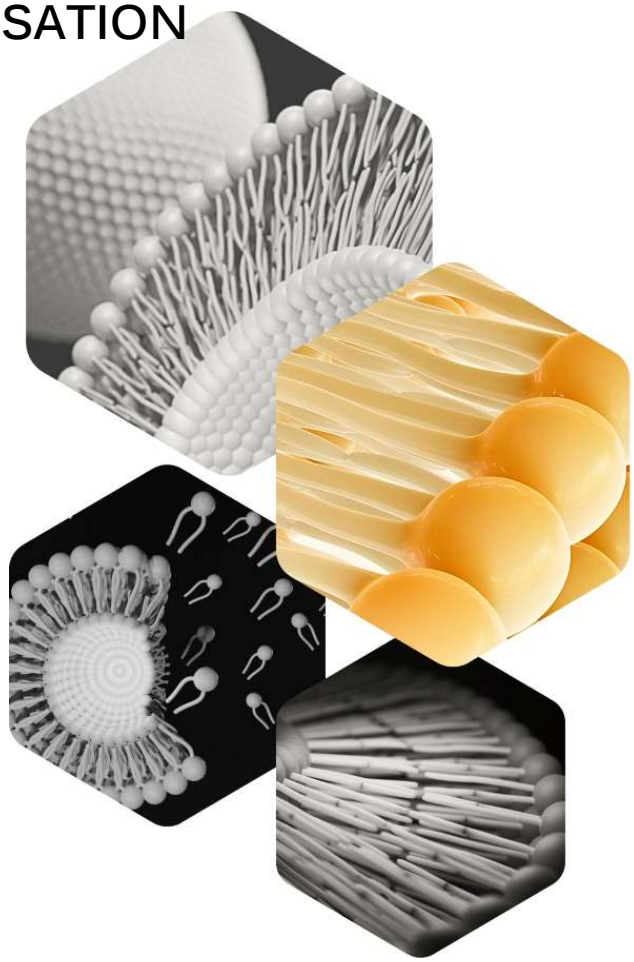
LIPOSOME CHARACTERISATION



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LIPOSOME CHARACTERISATION ANALYSIS

Certificate code:	GMP-QC-1106-01	**Internal Sample:**	GMP-110601A
Page No.:	Page 1 of 7	**External Sample:**	N/A
Sampled on:	10/06/24	**Reported on:**	11/06/24
PO reference:	N/A	**Number of samples:**	1

S. NO.	PRODUCT	BATCH NO.	UNITS	DETAILS
1.	Vitamin C liposomal [redacted]	29032024-00000-00025	1	External Request

PURPOSE

Quality testing of commercially marketed liposomal product.

DEPARTMENT

One bottle from the referenced batch was forwarded to the Quality Control Department.

METHODS

The following characterisation methods were employed as part of QC testing to verify the quality and authenticity of the product.

1. **Physical Appearance**:

   Description: Visual assessment of the powder to observe colour, smell and taste. Consistency in these attributes indicates uniform quality.

   Method: Powders were inspected visually, using organoleptic evaluation.

2. **Particle Size Analysis (Dynamic Light Scattering - DLS)**:

   Description: DLS measures the size distribution of particles in suspension. It is critical for determining the uniformity and quality of liposomal formulations.

   Method: Samples were diluted in a suitable solvent and subjected to DLS to measure the hydrodynamic diameter and polydispersity index (PDI), indicating size uniformity.

3. **Moisture Content**:

   Description: Moisture content affects the stability and shelf life of the product. Low moisture content is generally preferred for dry formulations.

   Method: An Infrared Moisture Analyser was used to determine the percentage of water content in the samples.

4. **Powder Re-Dispersibility**:

   Description: Re-dispersibility assesses how well the powder dissolves in water, an indicator of emulsifying property of liposomal products.

   Method: The dissolution time of the powder in water was measured manually to ensure it dissolves within a specified timeframe.

5. **Lipid Content (Sudan Test)**:

   Description: The Sudan test is a qualitative test to confirm the presence of lipids, which are essential components of liposomal structures.

   Method: Samples were stained with Sudan dye, which binds to lipids/ fatty acids, confirming their presence.

6. **Lipid Extraction and Quantification**:

   Description: This test measures the actual lipid content, providing a quantitative assessment of the lipids present in the product.

   Method: Lipids were extracted from the samples using an organic solvent, dried, and weighed to determine the lipid content per gram of sample.

7. **Electron Microscopy (TEM) Imaging**:

Description: TEM provides high-resolution images of liposomal vesicles, allowing for the visualisation of their size and structure.

Method: Samples were prepared and imaged at high magnifications (20,000x) to observe the liposomal vesicles' morphology.

RESULTS

a) Physicochemical properties

The [redacted] product consisted of creamy white powder with no smell, and exhibited acidic taste, which is characteristic taste of Vitamin C. Powder exhibited excellent flow and compressibility properties. The product was readily soluble in water and the size was within nanometre range (295nm, 0.551) as determined via Zeta Sizer. Assay was performed for nutrient content analysis, which also met the labelled claim (as mentioned on COA, i.e. not less than 65%). Moisture content was also in acceptable range i.e. <2%.

Results are displayed in table 1, as given below;

Table 1: Physicochemical properties of Bart Vitamin C powder

SR. NO.	PARAMETER	METHOD	RESULT
1	Appearance, taste and smell	Organoleptic	Creamy white powder, no smell, acidic taste
2	Flowability	Angle of repose and Car's index	Excellent
3	Size and PDI	DLS Zeta sizer	295nm, 0.551
4	Assay/ Drug content	HPLC	748.85mg/g
5	Moisture content (%)	IR moisture analyser	1.75%
6	Powder re-dispersibility	Manual	Dissolves within 5min

Figure 1: Dispersibility profile in water. [redacted] was readily soluble in water and dissolved within 5 minutes.

b) Sudan Test

Sudan III reacts with the lipids or triglycerides to stain red in colour. Lysochromes such as Sudan III bind to lipids but do not stick to any other substrate, subsequently confirming the presence or absence of lipids. Water was used as a negative control and 10% almond oil as a positive control with [redacted] as the test product. All products were treated with Sudan reagent using the standard protocol.

Under negative control, Sudan reagent dispersed in water instantaneously.

Under positive control, a very distinct and sharp red ring was formed on the top layer, as shown in figure 2 below.

Under the [redacted] product, a loosely dispersed red ring was formed on the top layer, reflecting a low amount of lipids. Lipids, less dense than water and insoluble in water, form a prominent and dense red layer or globules above the water, as seen with the positive control.

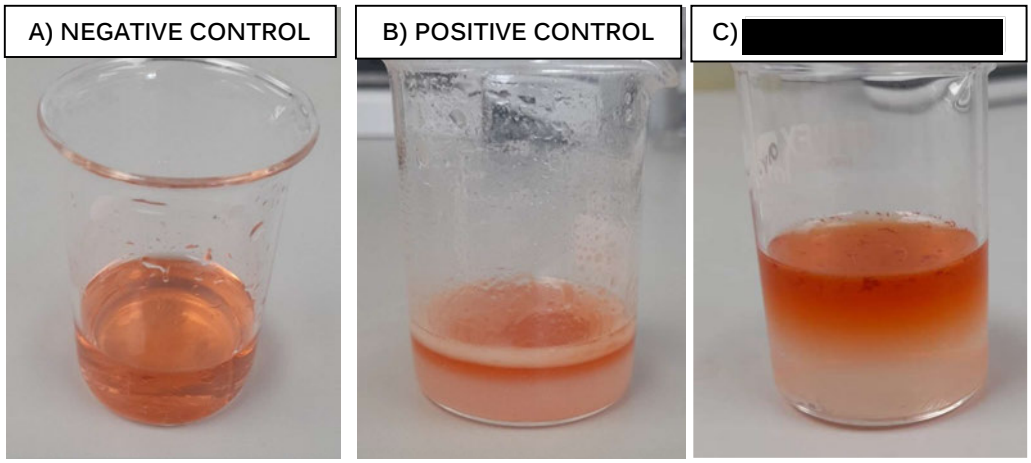

Figure 2: Sudan test for lipid content:

a) Water - Negative control - no red ring formation
b) Oil - Positive control - sharp red ring formation
c) [redacted] - Test product - loosely dispersed red band

c) Lipid Extraction

The Sudan test provided an initial indication of low lipid content. The next step was to extract lipid from the product and quantify it.

A clear solution was obtained, showing only traces of lipid. Following air-drying of the extracted solution, the amount of lipid quantified was very low, 0.32mg/g of the sample. This is much less than the amount of lipid found in quality liposomal products.

Figure 3: Lipid Extraction test. Sample is representative of [redacted] where clear, transparent liquid reflects absence or minimal concentration of lipid.

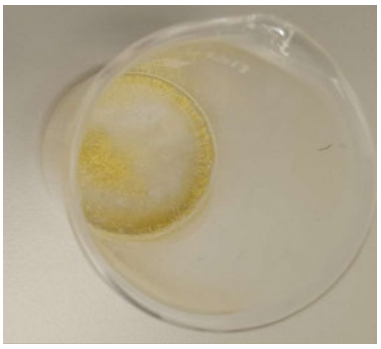

Figure 4: Air dried samples: [redacted] found to contain Vitamin C crystals with traces of lipid.

Table 2: Weight of Extracted Lipid

SAMPLE	WEIGHT OF LIPID EXTRACTED
[redacted]	0.00032g or 0.32mg/g sample

d) TEM imaging

The test product, [redacted] was sent for the third-party, TEM imaging analysis, to provide a visual representation of morphology, size and distribution of liposomes. The TEM images displayed a low presence of nanoparticles of arbitrary shape. The sample was also found to be full of nanocrystals <10-20 nm.

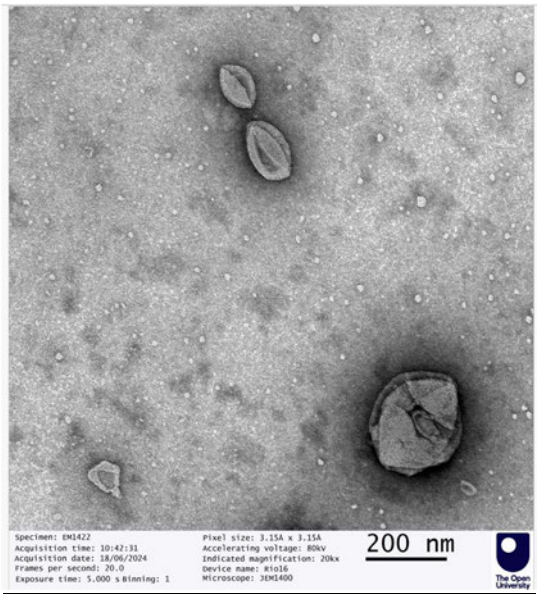

Figure 5: Transmission Electron Microscopic images at 20kx magnification: [redacted] powder TEM image showing minimal, irregular shaped structures.

FINDING AND CONCLUSION

The test sample, [redacted], displayed ideal physicochemical properties, as required for nanomaterials / nanosized particles.

The active content was within the claimed range.

The absence of a defined quantity of lipid, as required for liposomal formulation, questioned the validity of the claim that this is a liposomal product. Both Sudan and lipid extraction tests showed only trace presence of lipids, confirming that the product is actually nanosized dispersion and does not contain true liposomes. TEM images displayed irregular structures rather than well-formed liposomes.

Based on these results, the [redacted] product is regarded as non-liposomal.

In conclusion, based on the results obtained from all tests, it can be confirmed that the product under investigation only partially meets the required quality attributes for a liposomal product, and is therefore regarded as non-liposomal.

	NAME	POSITION	DIGITAL SIGNATURE	DATE
Performed by	Elnaz Salehian	R &D Scientist	Elnaz Salehian	11/06/2024
Reviewed by	Dr. Hanan Abdalmaula	Lead Scientific Researcher	Dr. Hanan Abdalmaula	11/06/2024
Approved by	Dr. Chloe Bradbury	Head of Medical Affairs	Dr. Chloe Bradbury	18/06/2024