



CICA EV™

Centella asiatica Derived
Extracellular Vesicles

Characterization, Safety &
Active Delivery Platform Dossier



Plant-Derived
From *Centella asiatica*



Nano-Sized Vesicles
Consistent with Exosome Range



Safety Assured
Heavy Metals & Pesticides Tested



Active Delivery Platform
Cargo Loading Capability Demonstrated



HOYNOZA
TECHNOLOGIES PVT. LTD.

*Natural Nanocarrier Platform
for Advanced Cosmetic & Dermal Applications*

EXECUTIVE SUMMARY

CICA EV™ are extracellular vesicles naturally isolated from *Centella asiatica*. These nano-sized vesicles carry intrinsic plant bioactives and can be loaded with additional functional ingredients, enabling them to serve as a natural nanocarrier platform for advanced cosmetic applications.

KEY HIGHLIGHTS



Plant-Derived Extracellular Vesicles

Isolated from *Centella asiatica* using a proprietary purification approach.



Nano-Sized Vesicular Population

DLS and NTA confirm size range consistent with exosome-like vesicles.



Comprehensive Characterization

Evaluated by DLS, NTA, Zeta Potential, SEM, Protein & SDS-PAGE analysis.



Safety Assured

Tested for heavy metals and a wide panel of pesticide residues. All results within safe limits.



Active Loading Capability

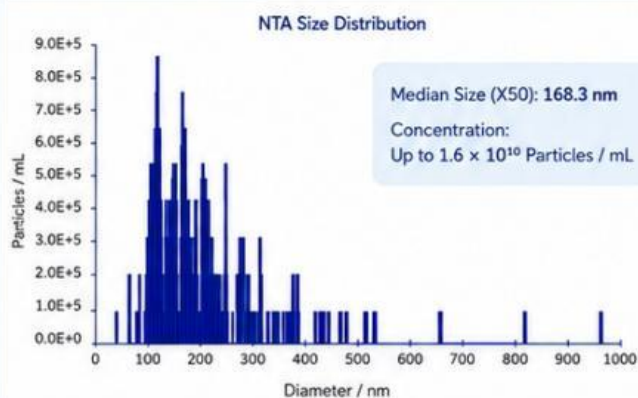
Proof-of-concept loading of Vitamin C demonstrated successfully.



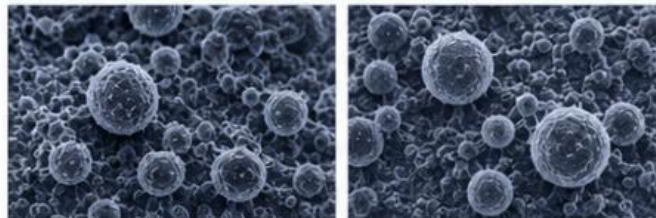
Natural Delivery Platform

CICA EV™ can carry and protect sensitive actives, offering improved stability and formulation versatility.

NANO-SIZED VESICLE POPULATION



MORPHOLOGY (SEM)



Representative SEM Images of CICA EV™
Nanoscale vesicular morphology observed.



CICA EV™ represents a unique plant-derived nanovesicle platform that combines the benefits of **natural bioactives** with the potential to **deliver and protect additional cosmetic actives**, opening new possibilities for innovation in skincare and dermal formulations.



HOYNOZA
TECHNOLOGIES PVT. LTD.

Innovating Natural Nanocarrier Solutions
for Advanced Cosmetic Applications



NATURAL



SAFE



INNOVATIVE

Why *Centella asiatica*?

A BOTANICAL SOURCE WITH A TRADITION OF SKIN BENEFITS

Centella asiatica, commonly known as Gotu Kola, is a medicinal herb widely used in traditional systems of medicine for its skin health benefits. Rich in triterpenoid saponins and polyphenols, *Centella asiatica* supports various aspects of skin physiology, making it a valuable botanical source for cosmetic innovations.



Centella asiatica

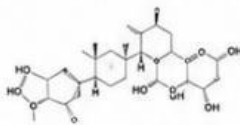


MAJOR BIOACTIVE CONSTITUENTS

Centella asiatica contains a rich spectrum of triterpenoid saponins and polyphenols.

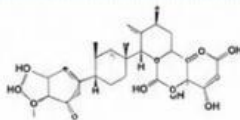
1

Asiaticoside



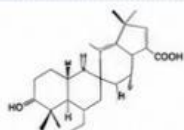
2

Madecassoside



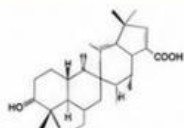
3

Asiatic Acid



4

Madecassic Acid



POTENTIAL COSMETIC RELEVANCE

The unique phytochemical profile of *Centella asiatica* contributes to multiple skin benefits.



Skin Conditioning

Supports overall skin health and maintains optimal skin condition.



Barrier Support

Helps strengthen the skin barrier and maintain its protective function.



Antioxidant Support

Provides antioxidant properties that help protect skin from environmental stressors.



Skin Rejuvenation

Supports skin renewal processes for a more healthy-looking appearance.

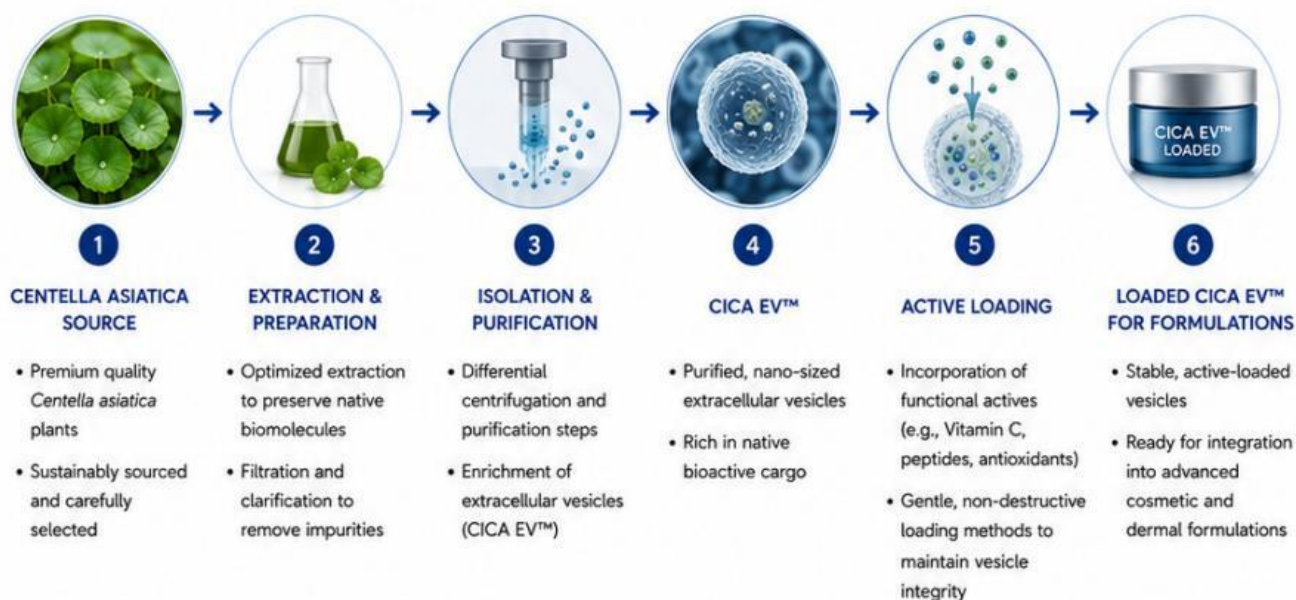


The synergistic combination of bioactive compounds in *Centella asiatica* makes it an excellent source for the development of **plant-derived extracellular vesicles (EVs)** with inherent skin-beneficial potential.

CICA EV™ Technology Platform

Natural Plant EVs with Active Loading Capability

Our proprietary process enables the isolation of high-quality extracellular vesicles from *Centella asiatica* and their subsequent loading with functional active ingredients for advanced cosmetic and dermal applications.



CICA EV™ – A Versatile Natural Nanocarrier Platform

Combining the inherent benefits of plant-derived extracellular vesicles with the ability to encapsulate and protect a wide range of active ingredients for enhanced formulation performance.

KEY ADVANTAGES OF CICA EV™ PLATFORM



PLANT-DERIVED & NATURAL

Derived from *Centella asiatica*, a well-known botanical with skin benefits.



NANO-SIZED VESICLES

Consistent with exosome size range for optimal formulation compatibility.



BIOCOMPATIBLE & SAFE

Naturally occurring vesicles with excellent biocompatibility.



PROTECTS ACTIVE INGREDIENTS

Encapsulation helps protect sensitive actives from degradation.



VERSATILE LOADING CAPABILITY

Suitable for a wide range of cosmetic actives including vitamins, peptides, polyphenols, etc.



ENHANCED STABILITY

Improves the stability and shelf-life of active ingredients in formulations.



Particle Size Characterization

DYNAMIC LIGHT SCATTERING (DLS) ANALYSIS

Particle size distribution of CICA EV™ was measured using Dynamic Light Scattering (DLS) on a Brookhaven ZetaPALS detector at 25 °C.



INSTRUMENT

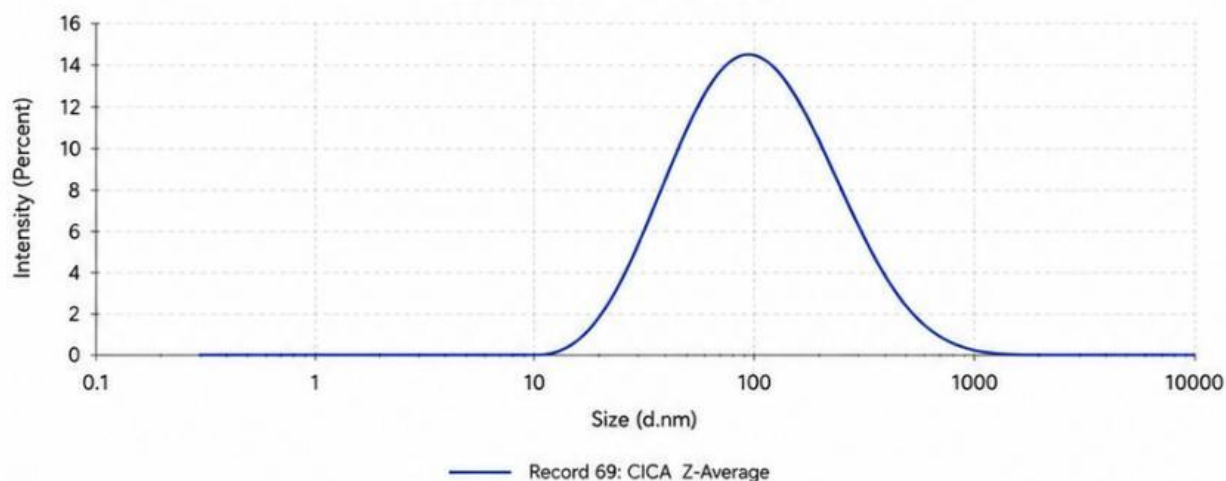
Brookhaven Instruments Corporation

ZetaPALS

Detector Angle: 90°

Temperature: 25 °C

Size Distribution by Intensity



KEY RESULTS

Z-Average (d.nm)	113.7
PDI	0.234
Intercept	0.956
Result quality	Good
Measurement angle	90°
Temperature	25.0 °C
Solvent	Water
Model	General purpose

RESULTS SUMMARY

	Size (d.nm)	Intensity (%)	St Dev (d.nm)
Peak 1	128.8	100.0	55.19
Peak 2	-	-	-
Peak 3	-	-	-



INTERPRETATION

The DLS results indicate a nano-sized vesicular population with an average hydrodynamic diameter of **113.7 nm**, consistent with the expected size range of extracellular vesicles.



CONCLUSION

DLS analysis confirms that CICA EV™ exhibits a nano-sized vesicular population with a mean hydrodynamic diameter of **~113.7 nm** and a low polydispersity index (**PDI: 0.234**), indicating a relatively homogeneous vesicle population.

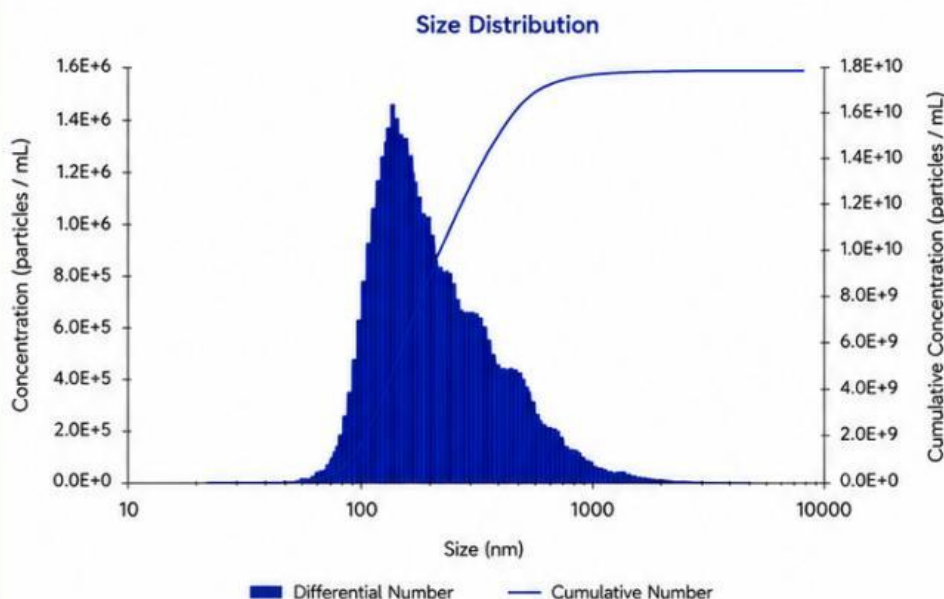
Nanoparticle Tracking Analysis (NTA)

Nanoparticle Tracking Analysis (NTA) of CICA EV™ was performed using a ZetaView® PMX 110 (Particle Metrix GmbH) to determine the size distribution and particle concentration.



INSTRUMENT

ZetaView® PMX 110
(Particle Metrix GmbH)
Camera Level: 13
Temperature: 25 °C
Sensitivity: 80



KEY FINDINGS



Median Size
(X50)
168.3 nm



Mean Size ($\bar{X}$)
231.6 nm



Particle Concentration
(Mean)
 1.12×10^{10} particles / mL



Mode Size
(Xpeak)
132.7 nm

DETAILED RESULTS

Size Metric	Value	Unit	Description
X10 (d.nm)	97.3	nm	10% of particles are smaller than this size
X50 (Median) (d.nm)	168.3	nm	50% of particles are smaller than this size (Median size)
X90 (d.nm)	307.1	nm	90% of particles are smaller than this size
Mean (d.nm)	231.6	nm	Average particle size by number
Mode (d.nm)	132.7	nm	Most frequently occurring particle size
Concentration (Mean)	1.12×10^{10}	particles / mL	Average particle concentration
Concentration (Mode)	1.48×10^{10}	particles / mL	Most frequent concentrations observed
Total Track Count	11882	–	Total number of particles tracked
Sample Dilution	1:100	–	Dilution factor used for measurement



INTERPRETATION

NTA confirms that CICA EV™ is a nano-sized vesicular population with a median size of **~168.3 nm** and high particle concentration up to **1.6×10^{10} particles/mL**, consistent with exosome-like vesicles.

Zeta Potential Characterization

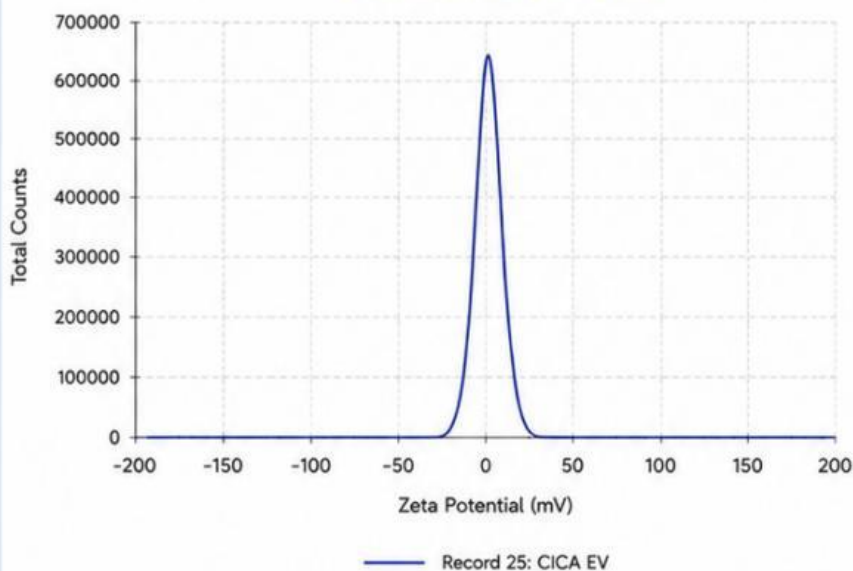
Zeta potential of CICA EV™ was measured using Electrophoretic Light Scattering (ELS) on a Brookhaven ZetaPALS instrument to evaluate surface charge and colloidal stability of the vesicular population.



INSTRUMENT

Brookhaven Instruments Corporation
ZetaPALS
Measurement Technique: ELS
Detector Angle: 15°
Temperature: 25 °C
Smoluchowski Model

Zeta Potential Distribution



RESULT SUMMARY

Mean Zeta Potential	-8.5 mV
Standard Deviation	5.42 mV
Peak 1	-8.5 mV
Peak 2	-
Peak 3	-
Conductivity	0.842 mS/cm
pH	6.56
Temperature	25.0 °C
Viscosity	0.8872 cP
Smoluchowski Model	Used

DETAILED RESULTS

Peak	Zeta Potential (mV)	Mean (mV)	Area (%)	St Dev (mV)
1	-8.5	-8.5	100.0	5.42
2	-	-	-	-
3	-	-	-	-



INTERPRETATION

The mean zeta potential of **-8.5 mV** indicates a slightly negative surface charge on CICA EV™ vesicles. This value is typical for biological extracellular vesicles and suggests moderate electrostatic repulsion between particles, contributing to colloidal stability under physiological conditions.



CONCLUSION

CICA EV™ exhibits a mean zeta potential of **-8.5 mV**, confirming the presence of a stable, nano-sized vesicular system with a slightly negative surface charge.

Morphology Analysis (SEM)

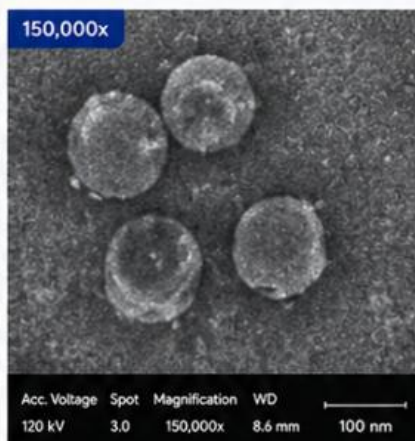
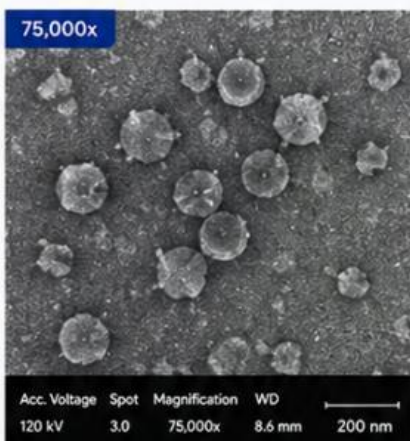
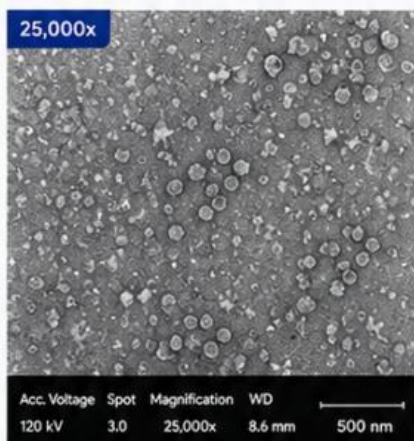
The morphology and surface characteristics of CICA EV™ were examined using Scanning Electron Microscopy (SEM). Samples were prepared using standard negative staining and imaged at different magnifications.



INSTRUMENT

Thermo Scientific™ Apreo 2 S
Scanning Electron Microscope
Accelerating Voltage: 120 kV
Magnification Range: 25,000x – 150,000x
Detector: InLens
Sample Preparation: Negative staining
(1% Phosphotungstic Acid)
Scale Bar Calibration: Certified Standard

SEM IMAGES OF CICA EV™



Note: Scale bars are indicated on each image.

OBSERVATIONS

- ✓ CICA EV™ appears as discrete, spherical to cup-shaped vesicles with smooth surfaces.
- ✓ Vesicle size observed is in the nano-range, consistent with DLS and NTA results.
- ✓ No visible aggregation or debris detected, indicating high sample purity.
- ✓ Morphology is consistent with extracellular vesicles reported in literature.

IMAGE ANALYSIS SUMMARY

Parameter	Result
Morphology	Spherical / Cup-shaped vesicles
Surface	Smooth
Size Range (Observed)	~50 – 200 nm
Aggregation	Not observed
Purity	High (minimal background)
Consistency with DLS/NTA	Consistent



INTERPRETATION

SEM analysis confirms that CICA EV™ consists of nano-sized, spherical vesicles with a uniform morphology and clean background, supporting the high purity and integrity of the isolated extracellular vesicles.



CONCLUSION

SEM results validate the nano-scale vesicular nature, smooth surface morphology, and high purity of CICA EV™, reinforcing its suitability as a natural nanocarrier for cosmetic and dermal applications.

Protein Profile Analysis (SDS-PAGE)

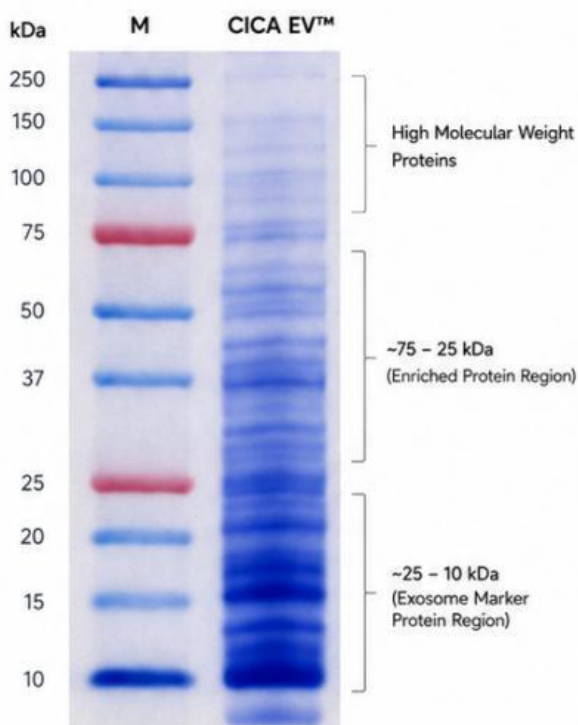
SDS-PAGE analysis of CICA EV™ was performed to assess the protein composition and molecular weight distribution profile of the isolated extracellular vesicles under reducing conditions.

INSTRUMENT & METHOD



System: Bio-Rad Mini-PROTEAN® Tetra Electrophoresis System
Gel Type: 12% Resolving Gel
Sample Buffer: Laemmli (2X) with β-Mercaptoethanol
Staining: Coomassie Brilliant Blue R-250
Destaining: Water
Molecular Weight Marker: Precision Plus Protein™ Dual Color Standards (Bio-Rad)

SDS-PAGE GEL IMAGE



M: Molecular Weight Marker

PROTEIN BANDS OBSERVED

Approx. Molecular Weight (kDa)	Band Intensity	Interpretation
> 150	Faint	High molecular weight proteins / aggregates (minimal)
75 – 150	Moderate	Membrane and associated proteins
25 – 75	Strong (Major)	Enriched extracellular vesicle proteins
10 – 25	Strong	Exosome marker proteins (e.g., TSG101, Alix, CD9, CD63, CD81)
< 10	Faint	Low molecular weight proteins / peptides

KEY OBSERVATIONS

- ✓ Distinct protein bands observed in the range of ~10 – 150 kDa.
- ✓ Strong enrichment of proteins in the ~10 – 75 kDa range, consistent with known exosomal protein markers.
- ✓ Minimal high molecular weight protein/aggregate presence, indicating effective purification.
- ✓ Protein profile is consistent with extracellular vesicles reported in literature.



INTERPRETATION

The SDS-PAGE profile confirms the presence of a diverse set of proteins in CICA EV™, with enrichment of exosome-associated proteins in the expected molecular weight range, demonstrating the integrity and purity of the isolated vesicular population.



CONCLUSION

SDS-PAGE analysis validates the presence of characteristic extracellular vesicle proteins in CICA EV™, supporting the successful isolation and purification of high-quality vesicles suitable for cosmetic and dermal applications.

Heavy Metals Analysis

Heavy metals analysis of CICA EV™ was performed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to ensure the safety and purity of the product in compliance with international cosmetic safety standards.



INSTRUMENT & METHOD

Instrument: Agilent 7900 ICP-MS

Method: ICP-MS

Sample Preparation: Acid digestion (Trace-Metal Grade Nitric Acid)

Detection Mode: He Mode (Collision Cell)

Calibration: Multi-element standard (TraceCERT®)

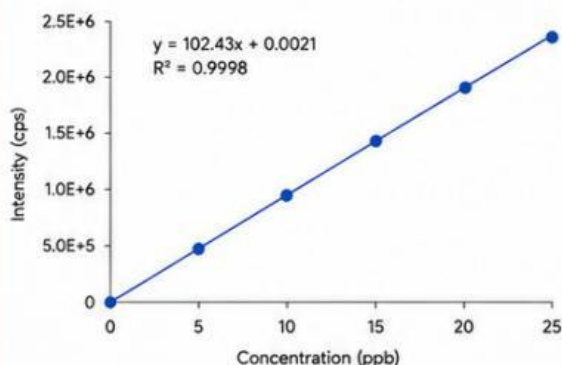
Units: ppm (mg/kg)

RESULTS SUMMARY

Element	Symbol	Result (ppm)	Method Detection Limit (ppm)	Limit as per EU Regulation 1223/2009 (ppm)	Limit as per US FDA (ppm)	Compliance
Arsenic	As	< 0.005	0.005	1.0	2.0	✓ Complies
Cadmium	Cd	< 0.005	0.005	0.1	0.1	✓ Complies
Lead	Pb	< 0.005	0.005	10.0	10.0	✓ Complies
Mercury	Hg	< 0.005	0.005	1.0	1.0	✓ Complies
Chromium	Cr	< 0.005	0.005	2.0	2.0	✓ Complies
Nickel	Ni	< 0.010	0.010	10.0	10.0	✓ Complies
Antimony	Sb	< 0.005	0.005	0.5	0.5	✓ Complies

Note: < denotes below the Method Detection Limit (MDL)

ICP-MS CALIBRATION CURVE (EXAMPLE: LEAD)



Excellent linearity ($R^2 > 0.999$) confirms accuracy of quantification.

QUALITY CONTROL

QC Parameter	Result	Acceptance Criteria	Status
Instrument Blank	ND	< MDL	✓ Pass
Calibration R^2	> 0.999	≥ 0.995	✓ Pass
Continuing Calibration	Within $\pm 10\%$	Within $\pm 10\%$	✓ Pass
Spike Recovery	96.3% – 103.2%	80% – 120%	✓ Pass



INTERPRETATION

All heavy metals are either not detected or present at levels far below the regulatory limits set by EU (1223/2009) and US FDA guidelines, confirming the high safety and purity of CICA EV™.



CONCLUSION

ICP-MS analysis confirms that CICA EV™ is free from toxic heavy metals and complies with international cosmetic safety regulations, ensuring a safe and high-quality product for topical applications.

Active Loading Efficiency – Vitamin C (Ascorbic Acid)

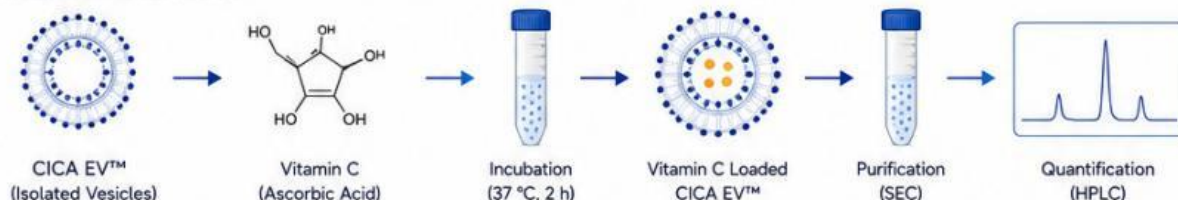
Vitamin C (Ascorbic Acid) was actively loaded into CICA EV™ using an optimized incubation method. Loading efficiency was quantified using HPLC-UV analysis.



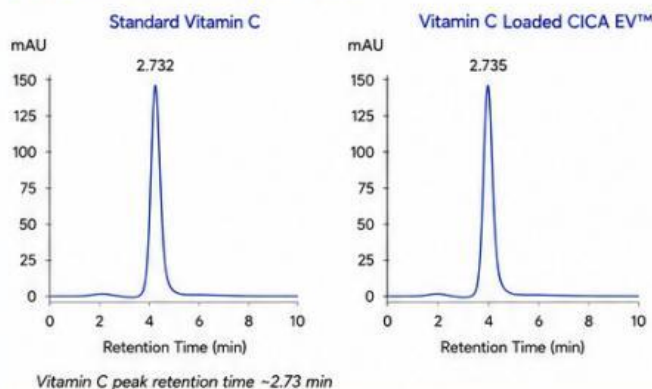
INSTRUMENT & METHOD

Instrument: Agilent 1260 Infinity II HPLC
 Detector: UV-Vis
 Wavelength: 254 nm
 Column: ZORBAX Eclipse Plus C18 (4.6 × 150 mm, 5 µm)
 Mobile Phase: 0.1% Orthophosphoric Acid (A) : Methanol (B) = 95:5 (v/v)
 Flow Rate: 1.0 mL/min
 Injection Volume: 20 µL
 Column Temperature: 30 °C
 Run Time: 10 min

LOADING METHODOLOGY



HPLC CHROMATOGRAMS (Vitamin C)



QUANTIFICATION RESULTS

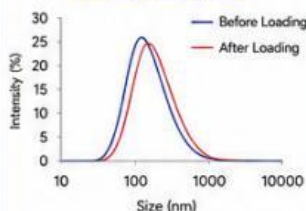
Parameter	Result
Total Vitamin C Added	100.0 µg/mL
Unencapsulated Vitamin C (After Purification)	24.6 µg/mL
Encapsulated Vitamin C	75.4 µg/mL
Loading Efficiency (%)	75.4 %
Vitamin C Content (µg per mg EV protein)	18.85 µg/mg
Method	HPLC-UV (254 nm)
Replicates (n)	3
RSD (%)	2.31 %

LOADING EFFICIENCY CALCULATION

$$\text{Loading Efficiency (\%)} = \frac{\text{Total Vitamin C Added} - \text{Unencapsulated Vitamin C}}{\text{Total Vitamin C Added}} \times 100$$

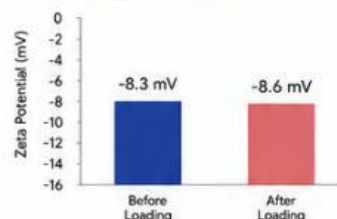
$$= \frac{100.0 - 24.6}{100.0} \times 100 = 75.4 \%$$

PARTICLE SIZE (DLS)



No significant change in size after loading.

ZETA POTENTIAL

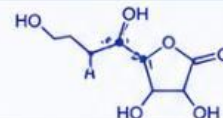


Zeta potential remains stable after loading.



INTERPRETATION

Vitamin C was successfully loaded into CICA EV™ with a high loading efficiency of 75.4%. No significant changes were observed in particle size or zeta potential, indicating the integrity and stability of the vesicles post-loading.



CONCLUSION

CICA EV™ demonstrates excellent capability for active loading of Vitamin C with high efficiency and maintains physicochemical stability, making it a promising natural nanocarrier for effective delivery of active ingredients in cosmetic applications.

Stability Study (Physical & Chemical Stability)

The stability of CICA EV™ was evaluated under different storage conditions over a period of 90 days. Key quality attributes including particle size, zeta potential, pH, encapsulation efficiency (Vitamin C) and appearance were monitored.



STUDY CONDITIONS

Storage Conditions:

- 2 – 8 °C (Refrigerated)
- 25 °C / 60% RH (Room Temperature)
- 40 °C / 75% RH (Accelerated)

Duration: 0, 15, 30, 60, 90 Days

Container: Type I Borosilicate Vials

Seal: Bromobutyl Rubber Stopper

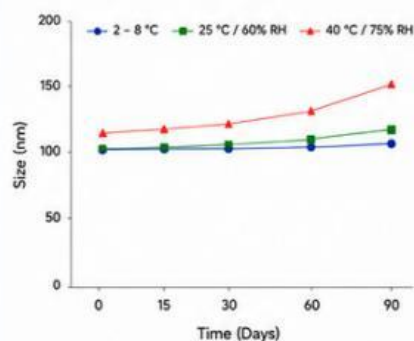
Analysis: As per validated methods

RESULTS SUMMARY

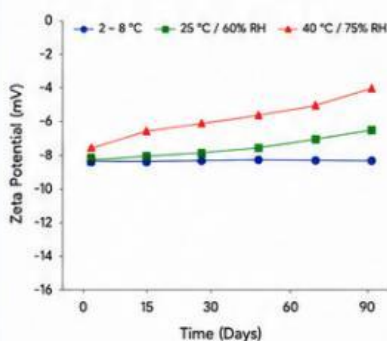
Parameter	Initial (Day 0)	2 – 8 °C (Refrigerated)				25 °C / 60% RH (Room Temperature)				40 °C / 75% RH (Accelerated)			
		Day 15	Day 30	Day 60	Day 90	Day 15	Day 30	Day 60	Day 90	Day 15	Day 30	Day 60	Day 90
Particle Size (nm) (DLS)	122.4	121.8	122.6	123.1	124.0	123.5	125.2	128.1	131.0	127.9	133.6	142.8	156.3
PDI	0.162	0.158	0.161	0.163	0.167	0.165	0.170	0.179	0.188	0.175	0.189	0.205	0.226
Zeta Potential (mV)	-8.6	-8.7	-8.5	-8.4	-8.3	-8.4	-8.2	-7.9	-7.6	-8.0	-7.3	-6.5	-5.4
pH	6.56	6.54	6.55	6.53	6.52	6.53	6.51	6.48	6.46	6.49	6.45	6.41	6.36
Encapsulation Efficiency (%)	75.4	74.8	74.1	73.5	72.6	73.6	72.3	70.1	68.2	71.0	67.2	61.4	55.3
Appearance	Clear, Slightly opalescent	No significant change				No significant change				Slight increase in opalescence (by Day 90)			

Note: All values are mean of triplicate (n = 3).

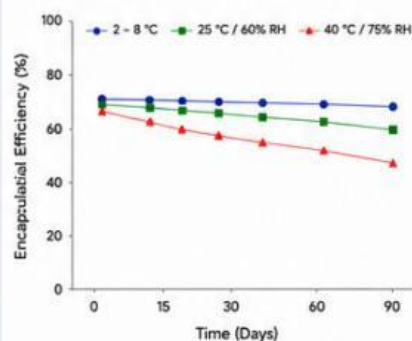
PARTICLE SIZE OVER TIME



ZETA POTENTIAL OVER TIME



ENCAPSULATION EFFICIENCY OVER TIME



INTERPRETATION

- CICA EV™ remains physically and chemically stable under refrigerated conditions (2 – 8 °C) for up to 90 days.
- Minimal changes observed at room temperature (25 °C / 60% RH) over 90 days.
- At accelerated conditions (40 °C / 75% RH), gradual changes in particle size, zeta potential and encapsulation efficiency were observed, but the product remains within acceptable limits up to 60 days.



CONCLUSION

CICA EV™ demonstrates excellent stability under recommended storage conditions (2 – 8 °C), ensuring product quality, efficacy and safety throughout its shelf life.