Rapid Reconstitution of Electrostatic Spray-Dried ErythroMer to Enable Cold-Chain-Free Resuscitation at the Point of Injury

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Aims

Aim 1. Optimize the electrostatic spray-drying process for ErythroMer nanoparticles.

Aim 2. Evaluate the impact of ESD on ErythroMer physicochemical properties and functional performance.

Aim 3. Assess the reconstitution characteristics, storage stability, and scalability of ESD-produced ErythroMer powders.

Background

A Shelf-stable RBC Substitute is Needed for when **Stored Blood is Unavailable** (prehospital, RDCR, austere settings, mass casualties, developing world)

MINUTES MATTER: Every minute of delay in replacing lost blood increases mortality by 5%, yet most EMS / medics do not carry blood due to perishability and cold chain requirements

Prior HBOC Attempts Failed

- **Efficacy: Poor O_2 Delivery** (capture O_2 in lung, but do not release O_2 to tissue)
- **Safety: Vasospasm** (sequester endogenous vasodilator nitric oxide (NO)

ErythroMer™ RBC Analogue

Bio-synthetic "Artificial Cell"

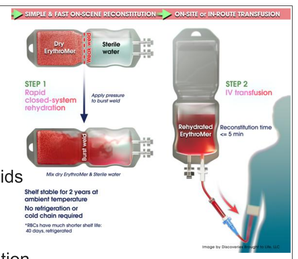
- ✓ **Nanoparticle-encapsulated** human **hemoglobin (Hb)**
- ✓ **Universal Option** for all blood types

Bioengineered to Mimic RBCs

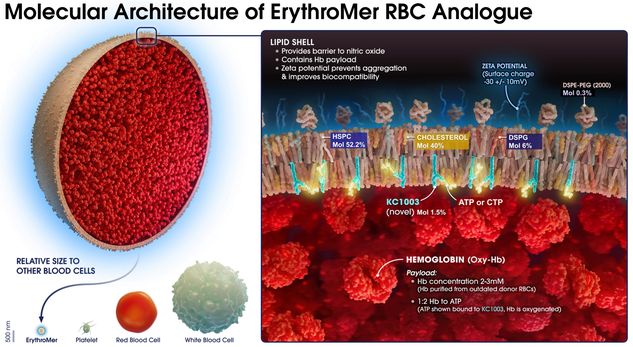
- ✓ **Oxygen Delivery** lasts ~8-10 hours
- ✓ **Biodigestible** to 'natural' peptides and lipids

Electrostatic spray-dried, Sterile Powder

- ✓ **Long-term storage**, no need for refrigeration
- ✓ **Rapid reconstitution** with water for injection



Molecular Architecture of ErythroMer RBC Analogue

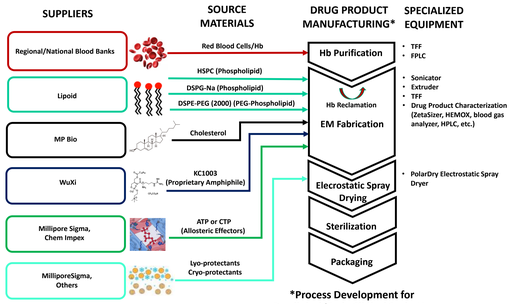


Elecrostatic spray drying for extended shelf-life

- ✓ Rapid reconstitution
- ✓ Effective oxygen transport
- ✓ No disruption of normal vascular tone
- ✓ Retention of Hb payload
- ✓ Preservation of Hb oxidative state with minimal metHb formation

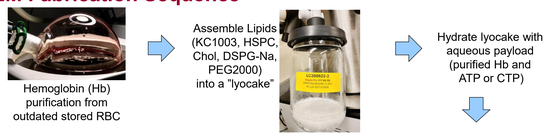
Methods

1 Source Materials, Processes & Equipment Overview

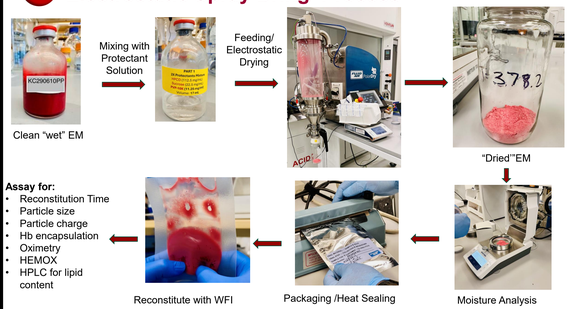


*Process Development for Preclinical/Early Clinical Scale-Up

2 EM Fabrication Sequence



3 Electrostatic Spray Dring Process



Assay for:

- Reconstitution Time
- Particle size
- Particle charge
- Hb encapsulation
- Oximetry
- HEMOX
- HPLC for lipid content

4 ESD Process Conditions/ Optimization

Table 1. Primary Electrostatic Spray Drying (ESD) Process Conditions

Parameter	Run 010826-1	Run 010826-2
Drying Gas Flow (Nm³/hr.)	5	5
Inlet Temperature (°C)	70	90
Atomizing Pressure (kPa)	300	300
Applied Voltage (kV)	1.5	1.5
Magna Flo Settings	100 psi / 10 s / 2%	100 psi / 10 s / 2%
Nozzle Purge Pressure (kPa)	3.3	3.3
Pump Rate (RPM)	12 → 7	8

Table 2. Secondary Process Conditions for ESD Runs

Parameter	Run 010826-1	Run 010826-2
Average Feed Rate (g/min)	1.31	1.27
Average Outlet Temperature (°C)	28.1	32.1
Process Time (hr:min)	0:56	0:52

Table 3. Feedstock compositions for ESD Runs

Feed Volume (mL)	Run 010826-1	Run 010826-2
EM Volume (mL)	50 mL	50 mL
Protectant Volume (mL)	25 mL	25 mL

Post-ESD Characterization

Morphology of EM (Cryo-TEM)

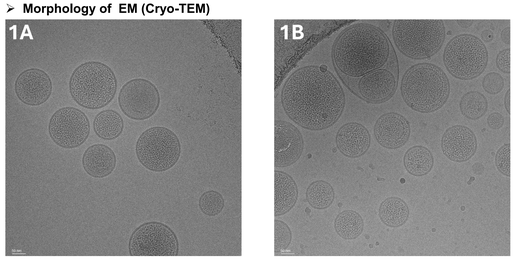


Figure 1A. Cryo-TEM image of ESD-dried EM

Figure 1B. Cryo-TEM image of lyophilized EM

- ✓ ESD-dried EM→ unilamellar particles with a narrower size distribution
- Lyophilized EM→ greater multilamellarity including particle-within-particle structure

Morphology of EM (SEM)

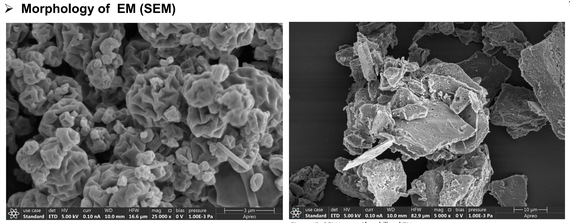


Figure 1C. SEM images of ESD-dried EM

Figure 1D. SEM image of lyophilized EM

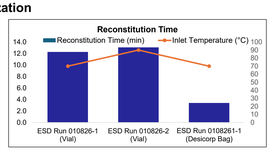
- ✓ ESD-dried EM→ spherical morphology with uniform size distribution
- Lyophilized EM→ irregular, porous, flake-like clusters

Results

EM Formulation Process and Post-ESD Reconstitution Characterization

EM ID	Drying Method	HPCD (mg/mL)	Sucrose (mg/mL)	Inlet Temperature (°C)	Reconstitution Time (mm:ss)	Particle Size (nm)	PDI	Zeta Potential (mV)
KC251210-PP	Wet EM	N/A	N/A	N/A	N/A	177.1	0.06	-45.11
Lyo260116	Lyophilization	140	24	N/A	10:12	173.3	0.05	-49.45
KC251210-PP	ESD 010826-1	140	24	70	12:16	187.7	0.04	-47.61
KC251210-PP	ESD 010826-2	140	24	90	13:05	186.8	0.23	-48.65
KC251210-PP	ESD 010826-1-(Desicorp Bag)	140	24	70	3:23	184.6	0.07	-44.41

HPCD: Hydroxypropyl-β-cyclodextrin

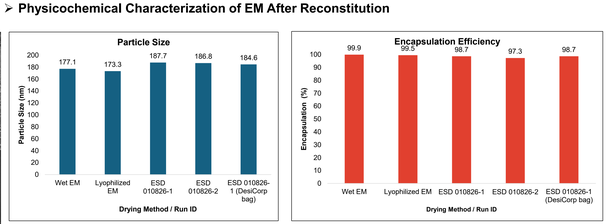


- ✓ Reconstitution in a DesiCorp® bag enabled faster preparation compared with vial-based reconstitution of ESD-dried material or conventional lyophilized at **70 °C** inlet temperature.

Moisture Analysis (LoD%)

Parameter	Run 010826-1	Run 010826-2
Moisture Content (LoD%)	4.09	4.05

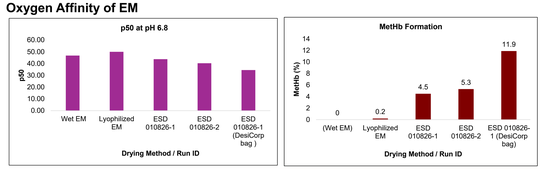
Physicochemical Characterization of EM After Reconstitution



- ✓ Small particle size (<200 nm) with narrow PDI (<0.1) and >98% hemoglobin retention after ESD at **70 °C** inlet temperature.

Results (cont.)

Oxygen Affinity of EM



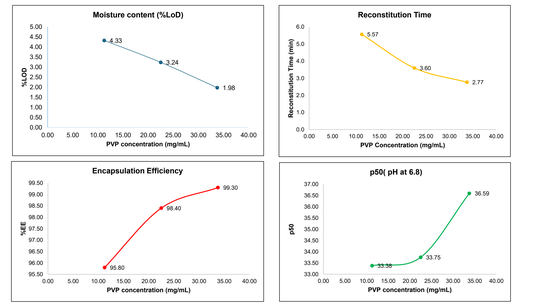
- ✓ High p50 values of (41-43 mmHg) of ESD –dried EM
- ✓ Preservation hemoglobin oxygen-binding properties
- ✓ Effective oxygen release capacity.
- ✓ Need optimization/improvement to decrease metHb <1%

Improvement Through Optimization of Process Parameters and Protective Excipients

Pre-ESD Characterization	%EE	Size (nm)	p50 (pH 6.8)	%MetHb
EM: KC260610PP	99.9	170	45.76	0.0

ESD Run ID	HPCD (mg/mL)	Sucrose (mg/mL)	PVP (mg/mL)	Inlet Temperature (°C)	Product Temperature (°C)	Size(nm)	%MetHb
Run 063026-1	112.5	22.5	11.25	65	30.9	189	8.4
Run 063026-2	112.5	22.5	22.5	65	30	186	8.5
Run 070126-1	112.5	22.5	33.75	65	29.7	196	5.7

HPCD: Hydroxypropyl-β-cyclodextrin, **PVP(10 kDa)** :Polyvinylpyrrolidone



- ✓ Lower moisture content, rapid reconstitution (<3 min), >99% encapsulation efficiency (%EE), and high retention of p50 values with concentration-dependent PVP addition at 65°C inlet temperature.

Conclusions

- ❖ Electrostatic Spray Drying (ESD) successfully produced dry EM powder while preserving the critical attributes of encapsulated hemoglobin (Hb).
- ❖ ESD-dried EM demonstrated rapid reconstitution (<3 min) compared with conventionally lyophilized EM reconstituted in a glass vial, highlighting its potential for time-critical resuscitation.
- ❖ ESD-dried EM exhibited favorable physicochemical characteristics and potential for prolonged circulation.
- ❖ Hemoglobin retention exceeded 99% with addition of PVP, while moisture level remained below 2%. Oxygen-binding functionality was preserved, with p50 values maintained between 33-36 mmHg at pH of 6.8 despite high level of metHb (>5%).
- ❖ Strategies under evaluation include incorporation of different concentration of protective excipient, and adjustment of spray-drying process parameters.
- ❖ Continuous ESD drying reduces processing time from days to hours compared with batch lyophilization, supporting commercial-scale production
- ❖ Future studies will focus on improving room-temperature stability, evaluating post-reconstitution stability, and optimizing scalability for larger-batch production.

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