German Rare Donor Program

A Novel Technology for Preservation of Human Erythrocytes

Abbreviated version 10 May 2005

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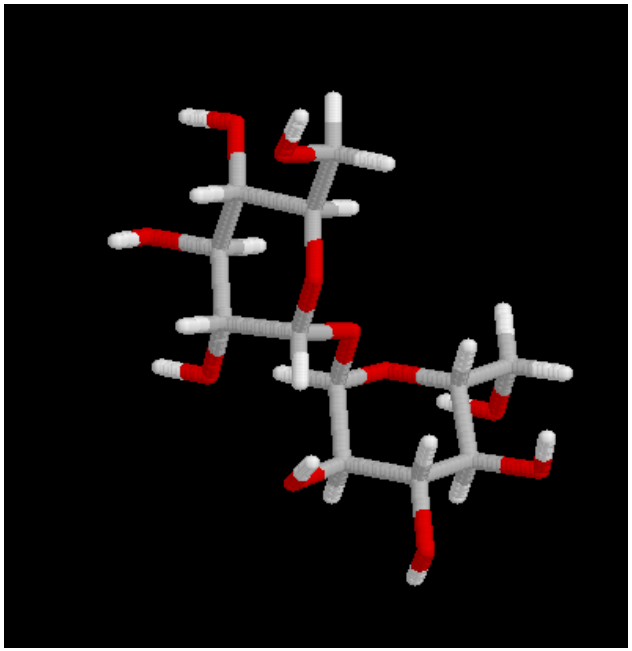
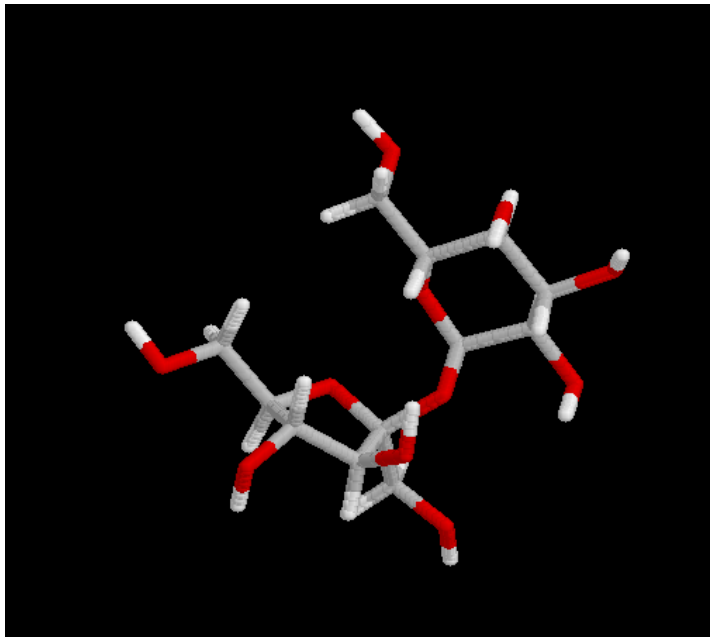
A common feature of the anhydrobiotic organisms is that they can synthesize large quantities of disaccharides, often sucrose or trehalose.



Trehalose



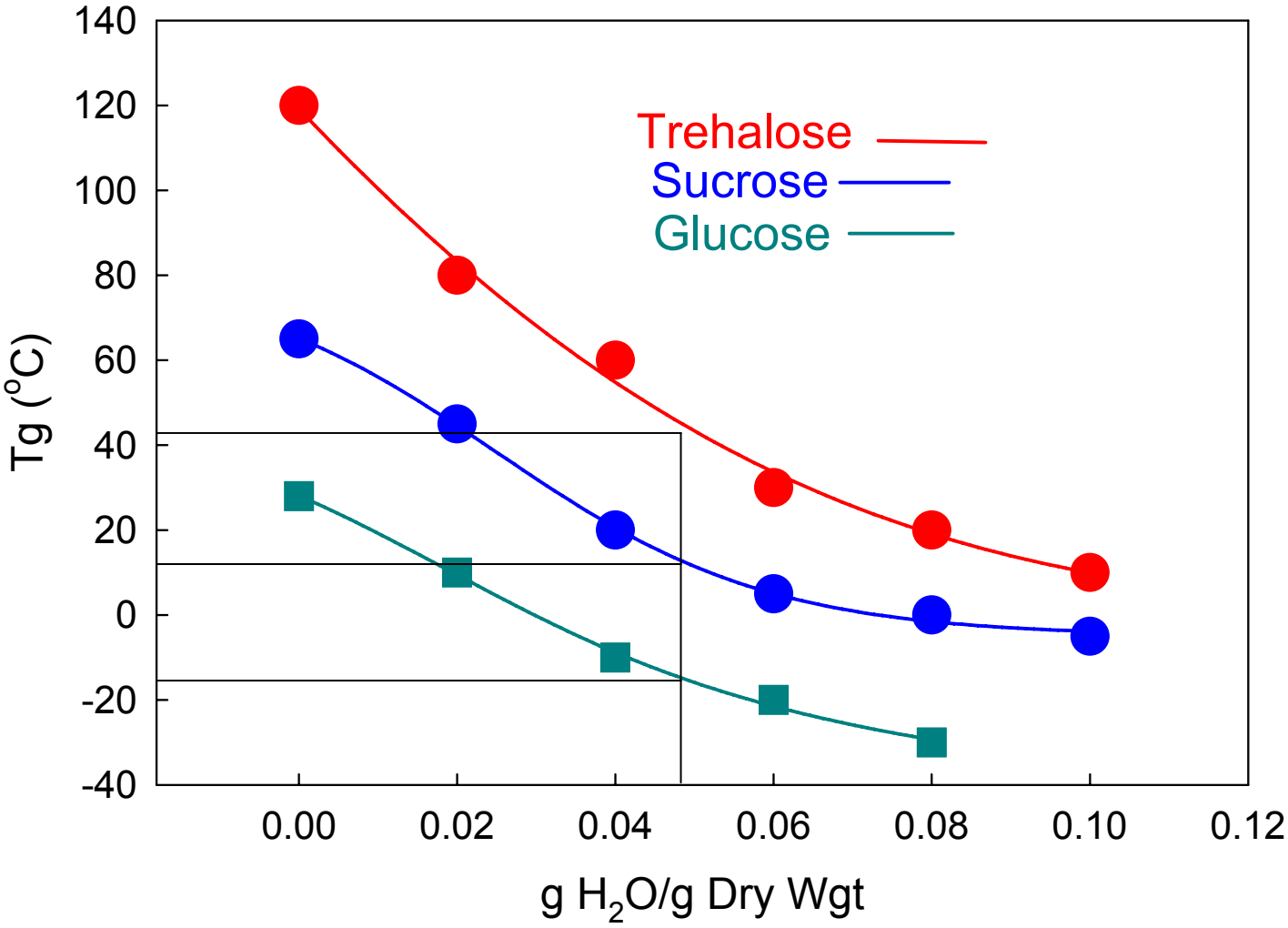
Sucrose

- High glass transition temperature
- High chemical, acid and thermal stability
- Low hygroscopicity

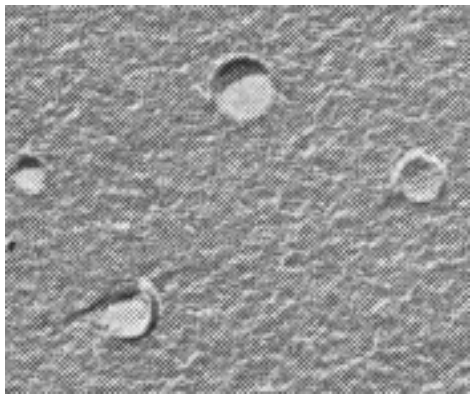
Trehalose Preserves Dry Biomolecular Assemblages

Assemblage	*Dried without trehalose*	*Dried with trehalose*
Membranes	Fusion, phase transitions, leakage	No fusion or leakage
Proteins	Denatured	Structure maintained
DNA	Fragmentation into short segments	No fragmentation

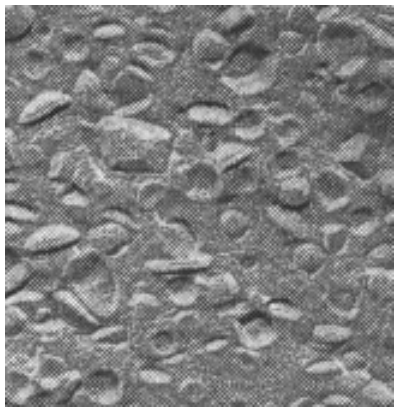
Crowe et al. (1984. Science) began establishing the mechanism.


140
120
100
80
60
40
20
0
-20
-40
Tg (°C)
Trehalose
Sucrose
Glucose
0.00
0.02
0.04
0.06
0.08
0.10
0.12
g H2O/g Dry Wgt

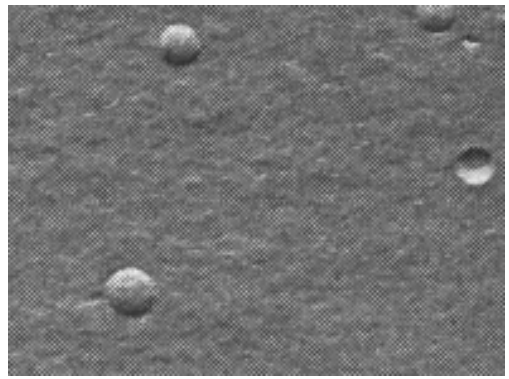
Some real world applications from studies on anhydrobiosis: liposomes for drug delivery.



Hydrated liposomes



Liposomes dried with trehalose



Liposomes dried with trehalose and rehydrated

The dry liposomes are shipped in serum bottles.

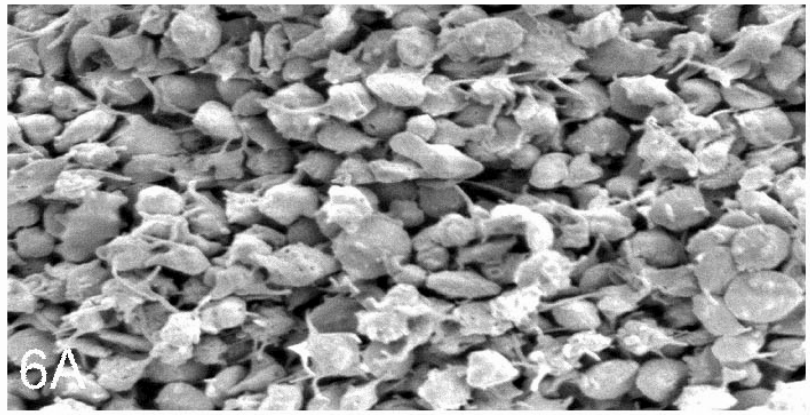
Crowe, J.H. and L.M. Crowe. 1992. Preservation of liposomes by freeze drying. *In*: Gregoriadis, G. (ed). *Liposome Technology*. 2nd Edition. CRC Press.

Crowe, J.H. and L.M. Crowe. 1989. Method for preserving liposomes. U.S. Patent Number 4,857,319.

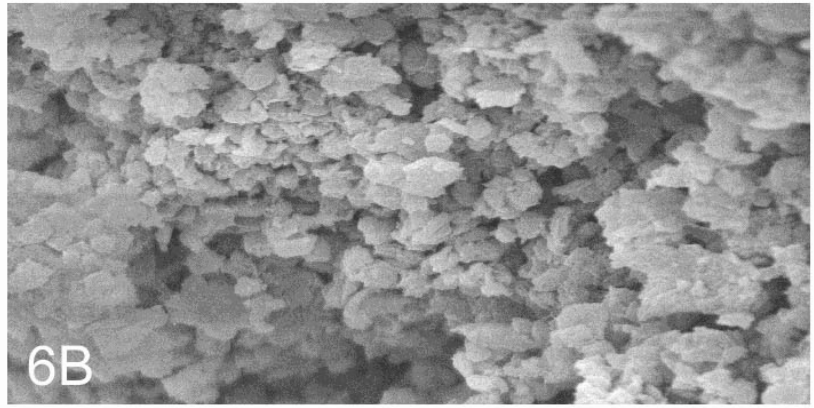
Ongoing work at the Center for Biostabilization:

1. Human Blood Platelets

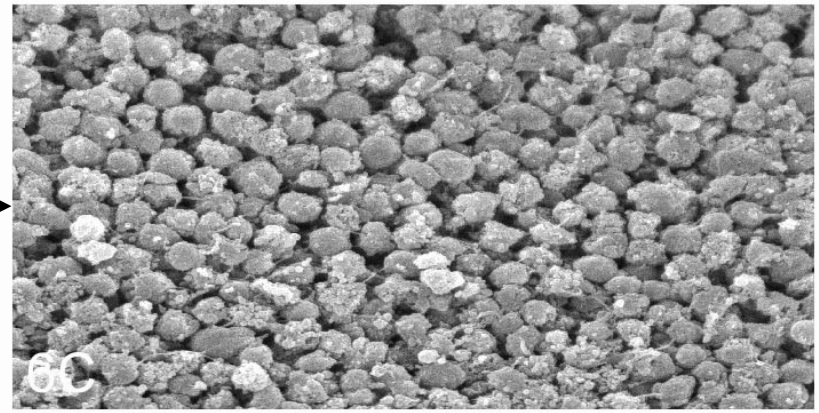
Fresh platelets, obtained from Sacramento Blood Center →



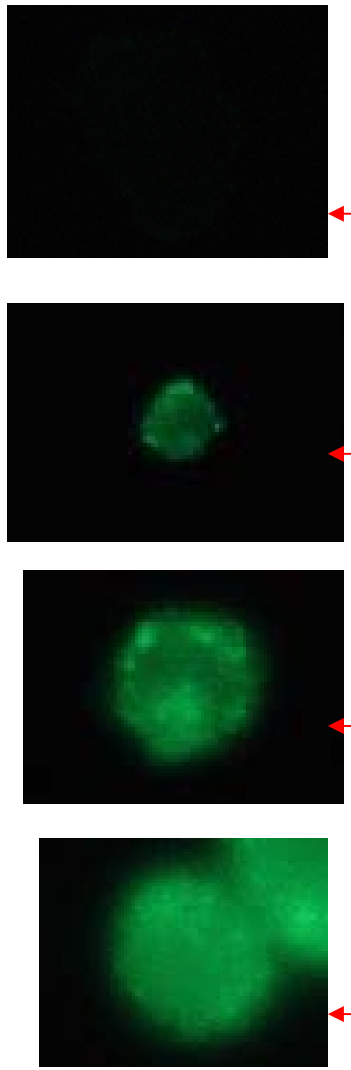
Freeze-dried without trehalose, rehydrated →



Freeze-dried with trehalose, rehydrated. They are intact in the rehydrated state. →



W.Wolkers et al. Cell Pres.Technol.3, 2004



Fluorescence images showing distribution of lucifer yellow placed outside after:

15 min

1 hr

3.5 hr

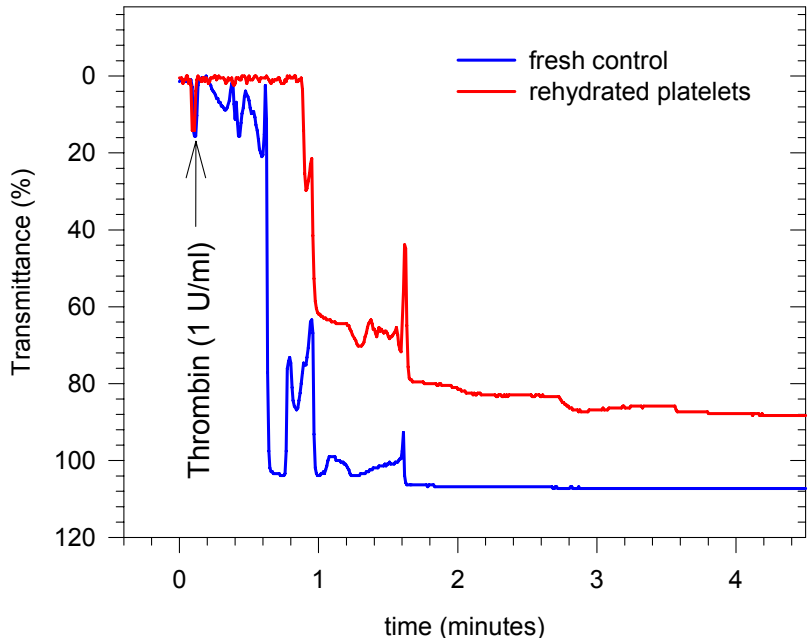
5 hr

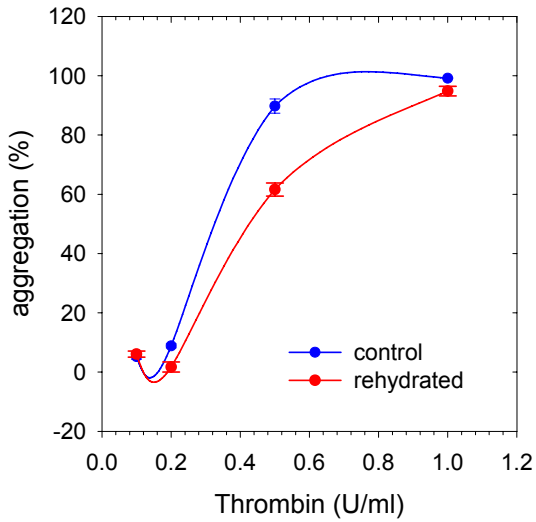
Trehalose enters platelets by an endocytotic pathway regulated by temperature.

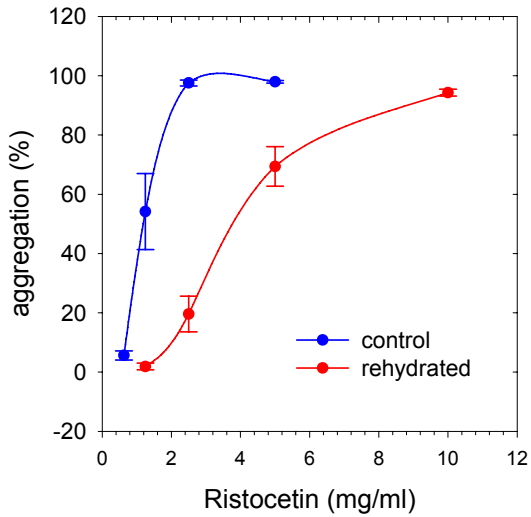
W.Wolkers et al. BBA 1612, 2003

Rehydrated platelets respond normally to agonists

- Stimulation with agonists results in proper clot formation
- Dose response curves of rehydrated platelets fall within normal physiological range
- Freeze-dried platelets are stable at room temperature for at least 700 days

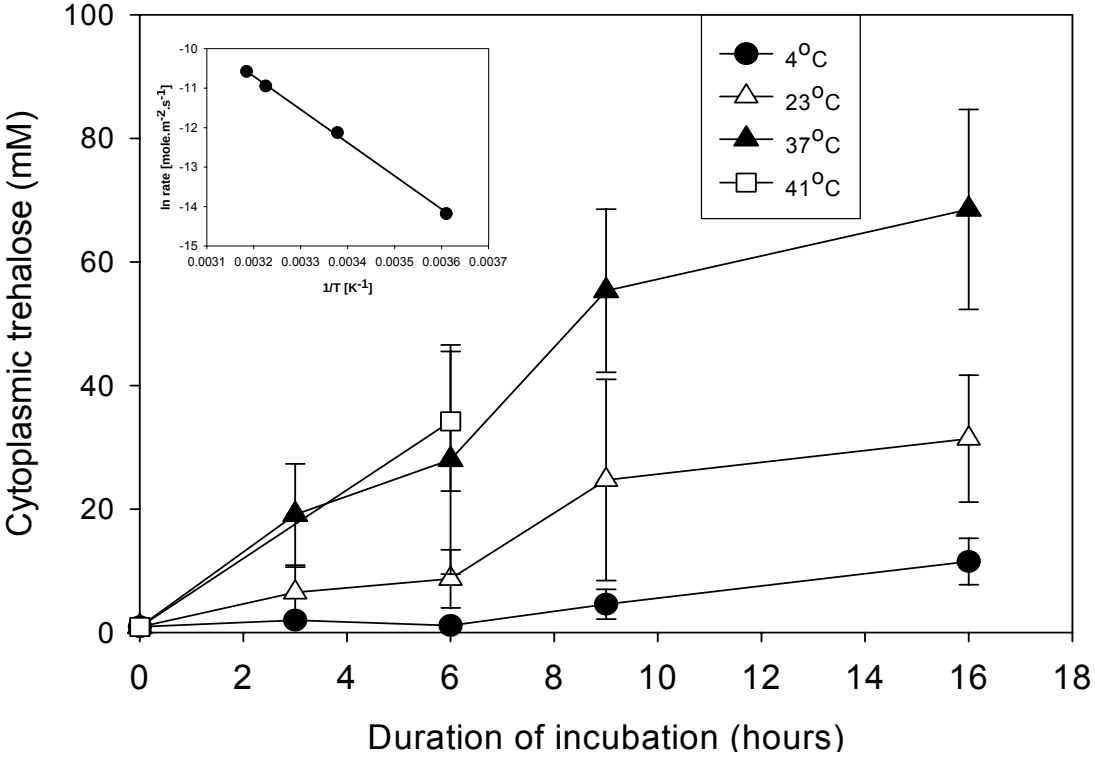


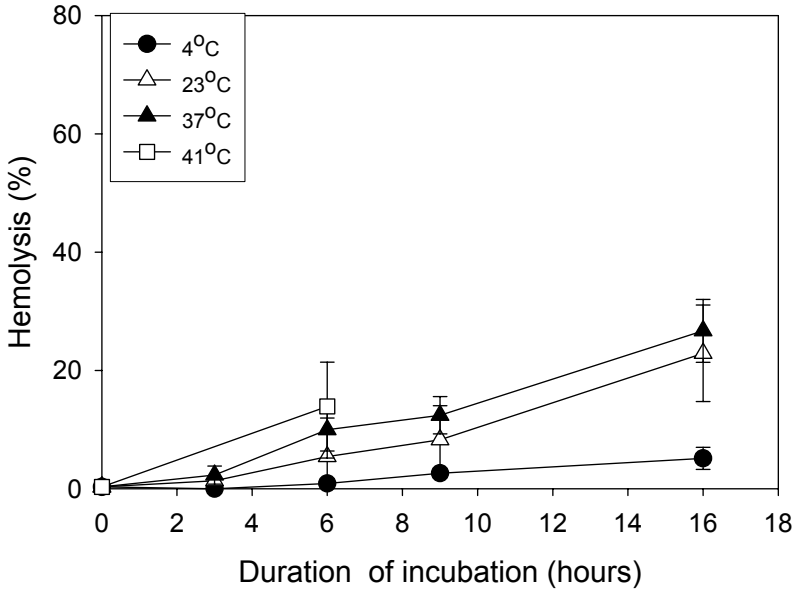




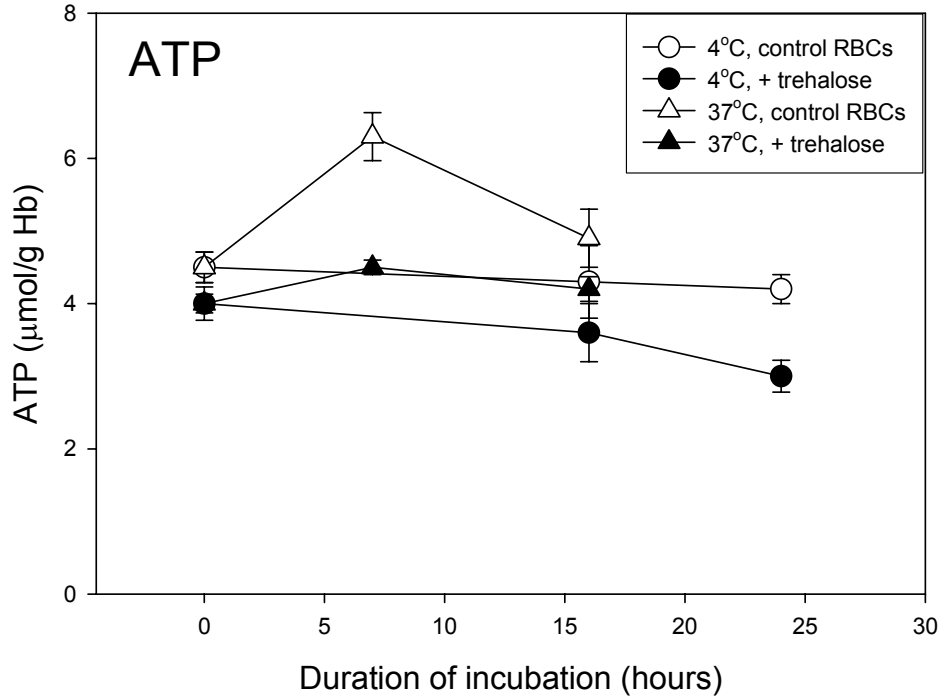
W.Wolkers et al. BBA 1612, 2003

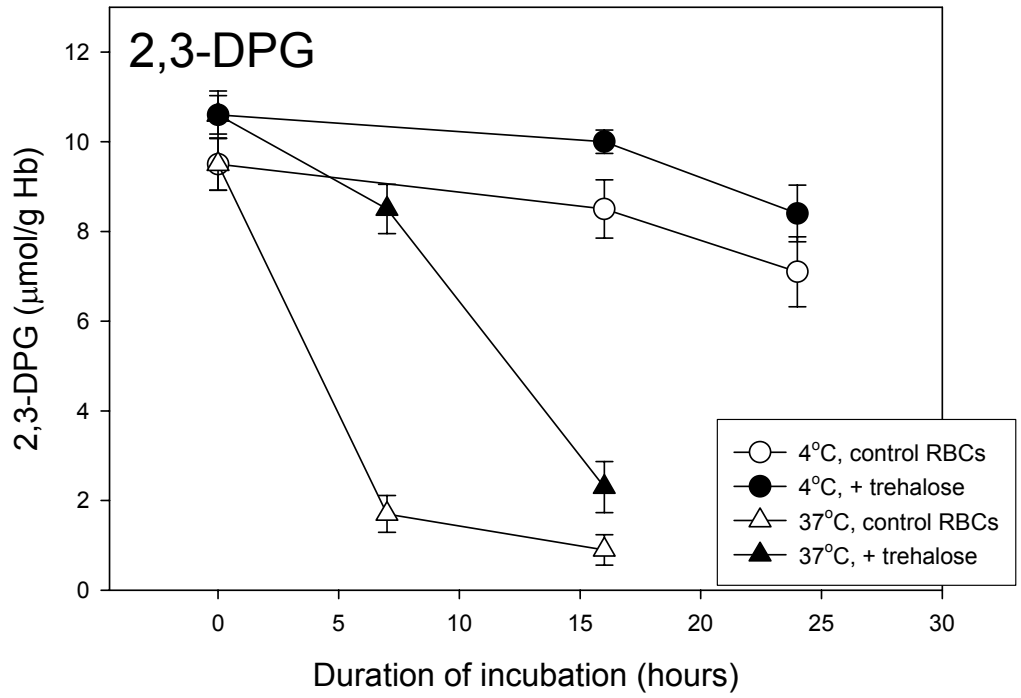
Trehalose uptake as a function of time and temperature





Satpathy et al. Cryobiology 2004

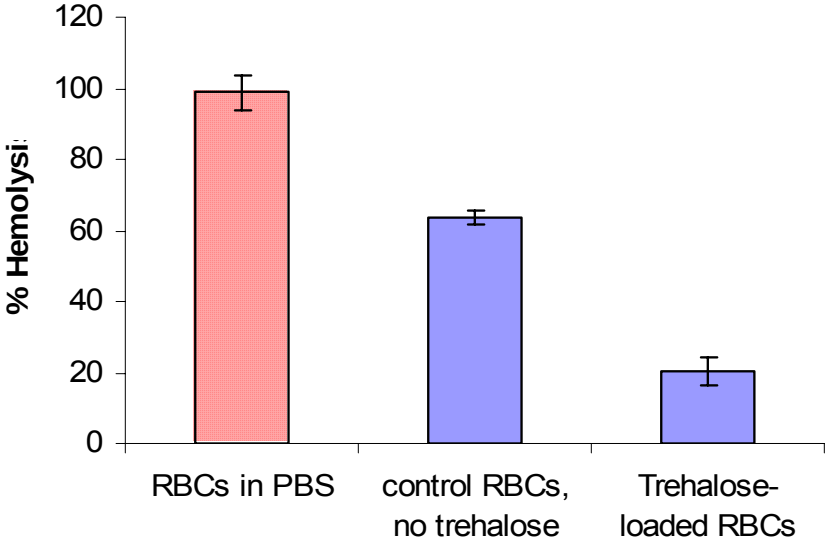


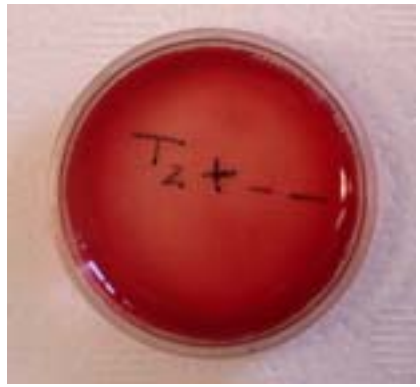


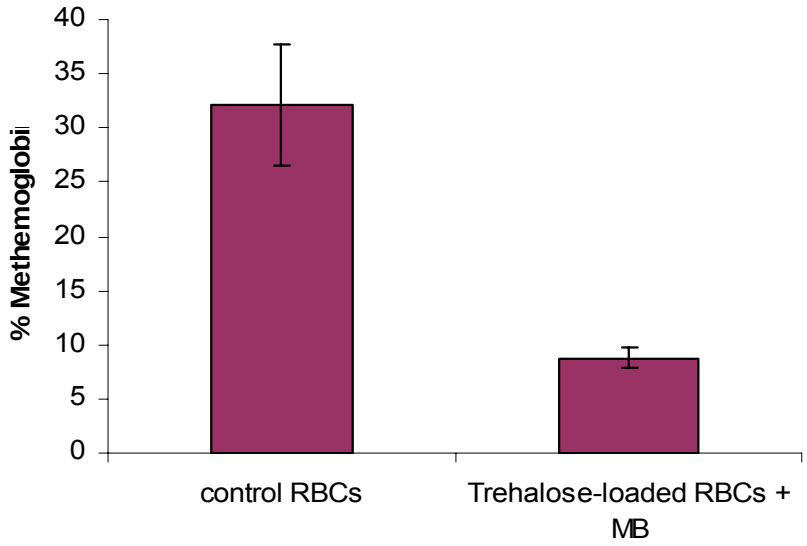
Level of ATP and 2,3-DPG in RBCs during loading in 800 mM trehalose/100 mOsm ADSOL/K-phosphate (pH 7.2)

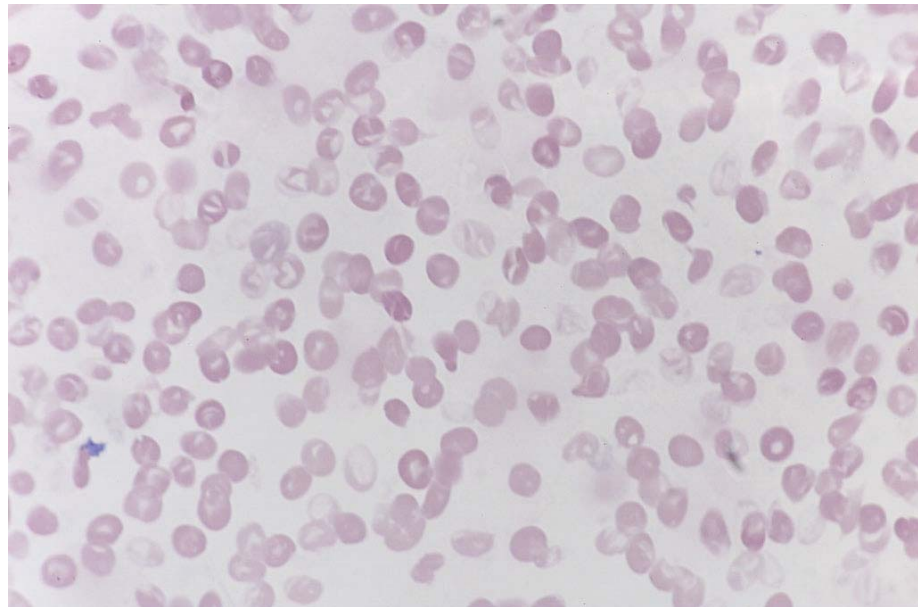
physiological concentrations:
ATP = 3.65-4.45 µmol/g Hb
DPG = 10.5-16.2 µmol/g Hb

Post-rehydration hemolysis and percent methemoglobin in freeze-dried RBCs

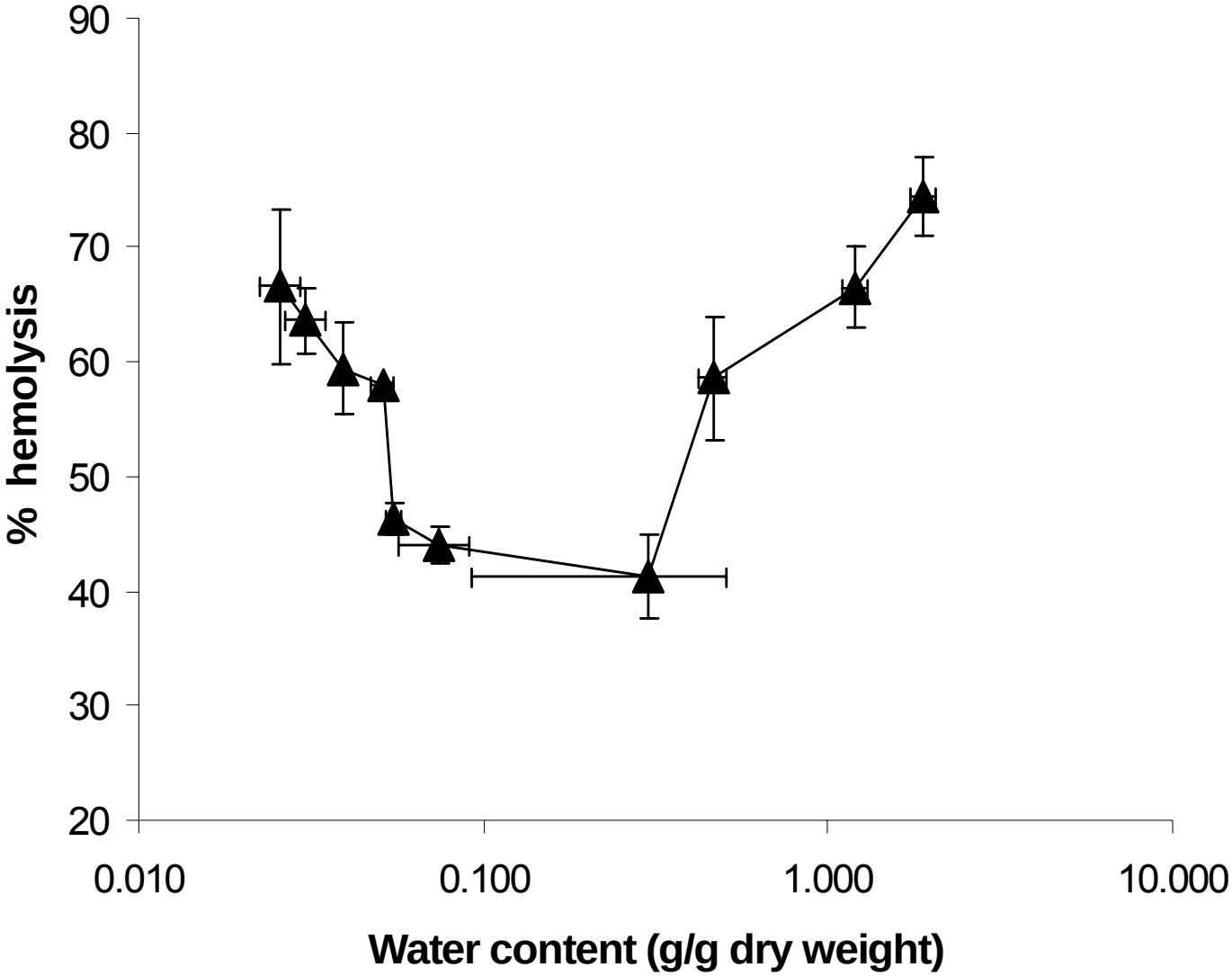




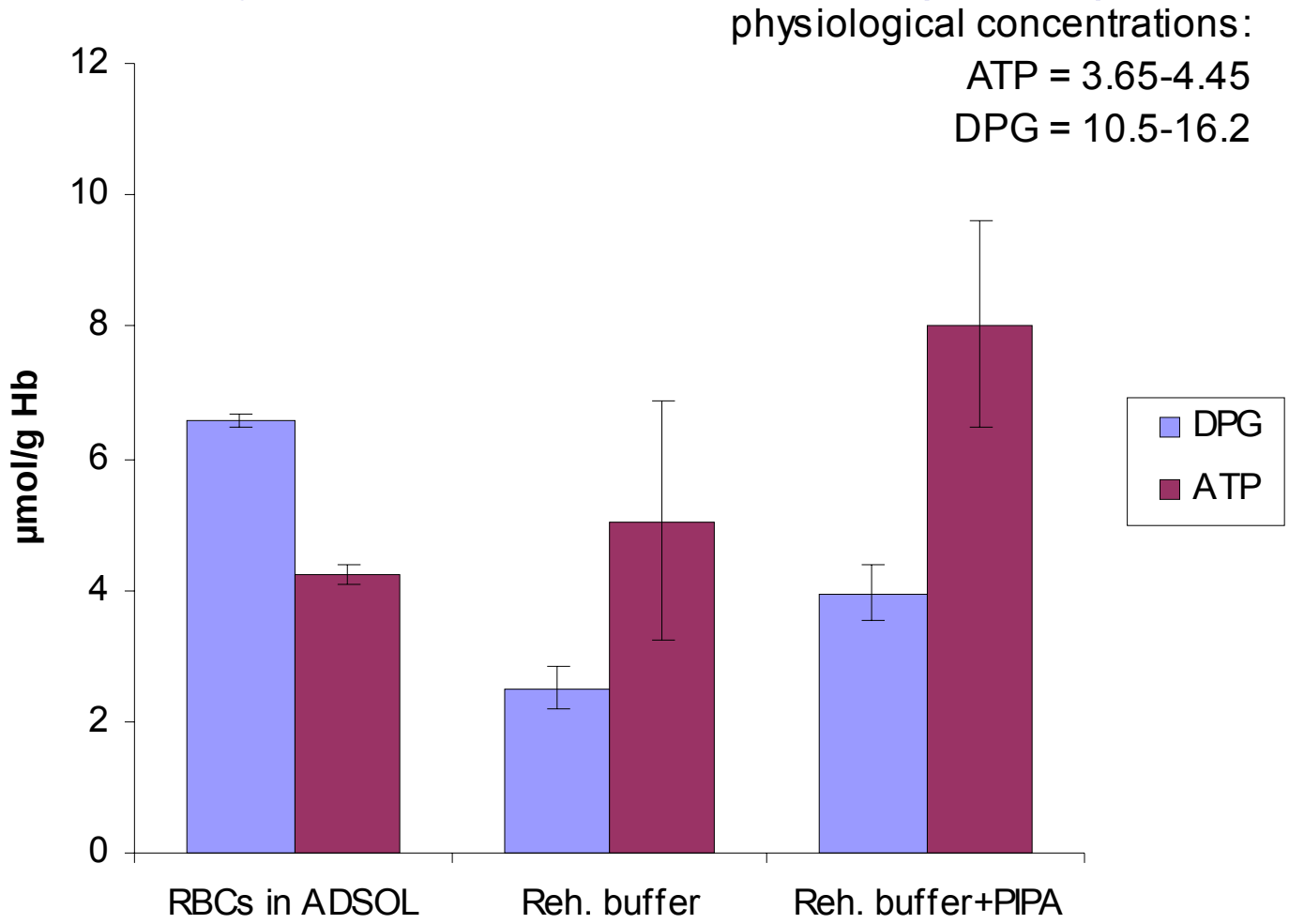




Percent hemolysis of freeze-dried RBCs as a function of the residual water content

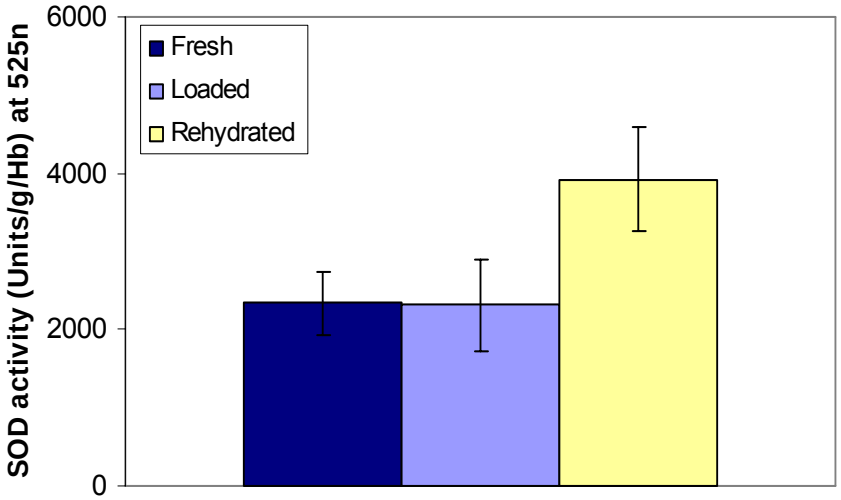


Level of ATP and 2,3-DPG in freeze-dried and rehydrated RBCs. The rehydration buffer is supplemented with phosphate, inosine, pyruvate, and adenine (PIPA).

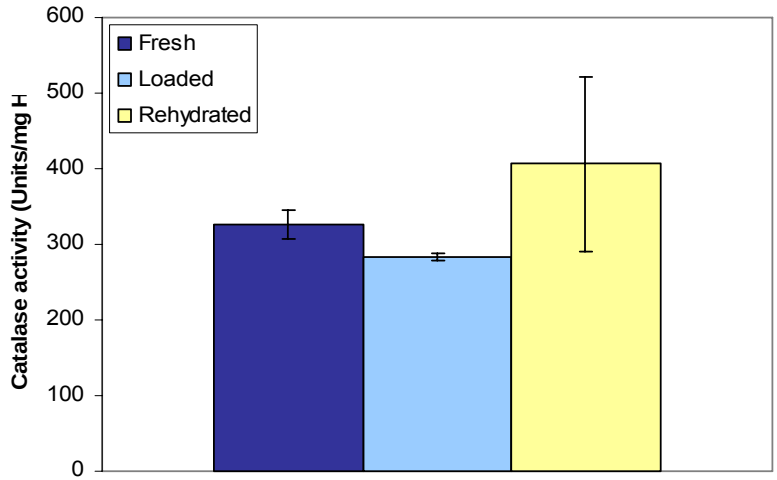


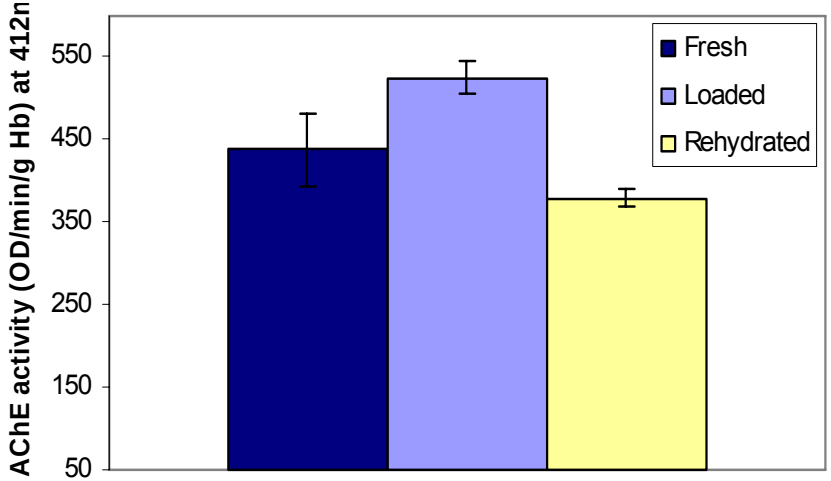
Superoxide dismutase (SOD), catalase and acetylcholine esterase (AchE) activity in freshly isolated, trehalose loaded, and freeze-dried and rehydrated RBCs

SOD

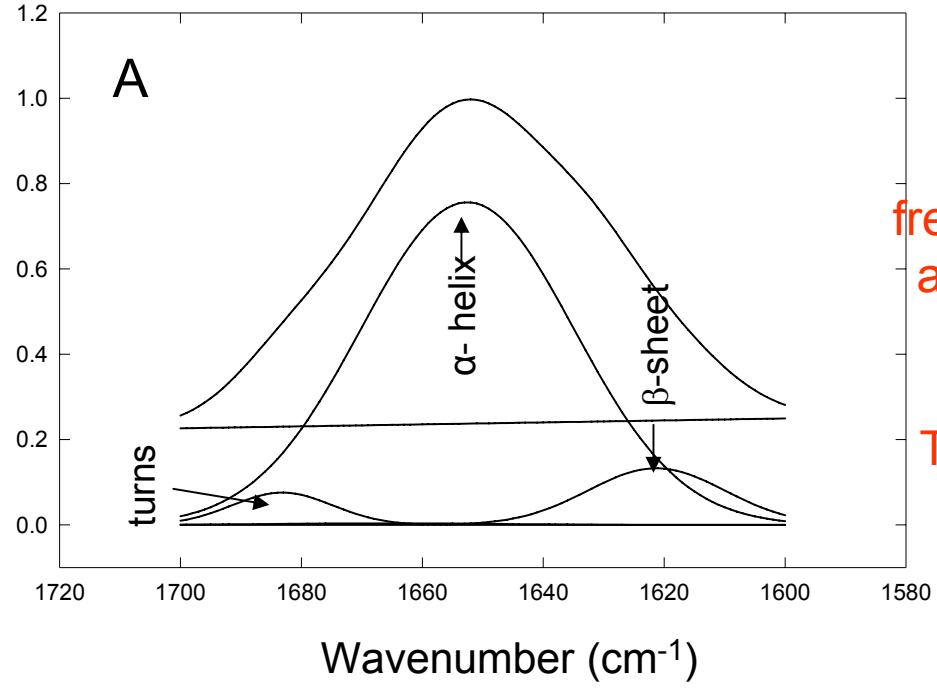


Catalase



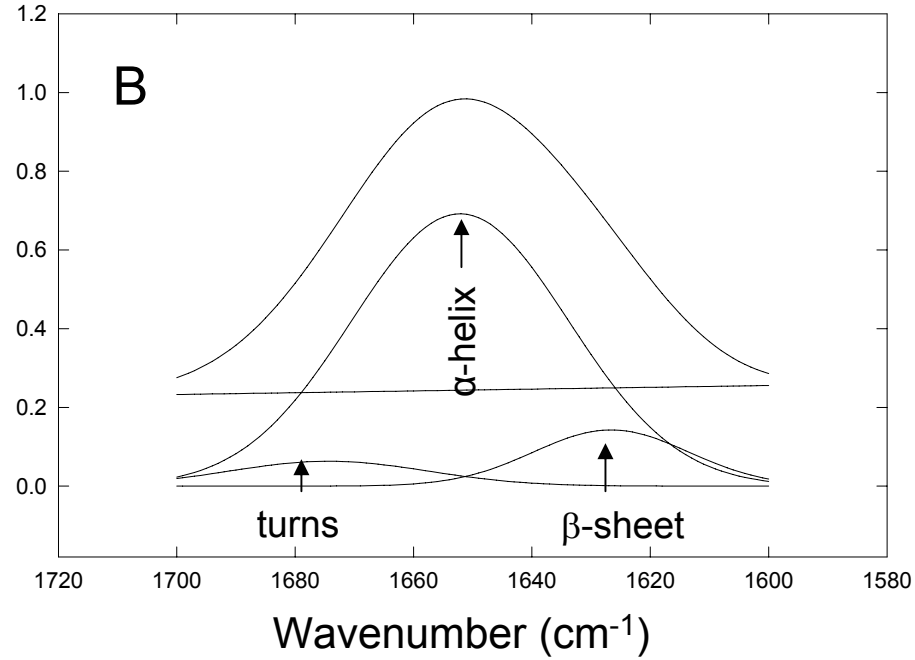


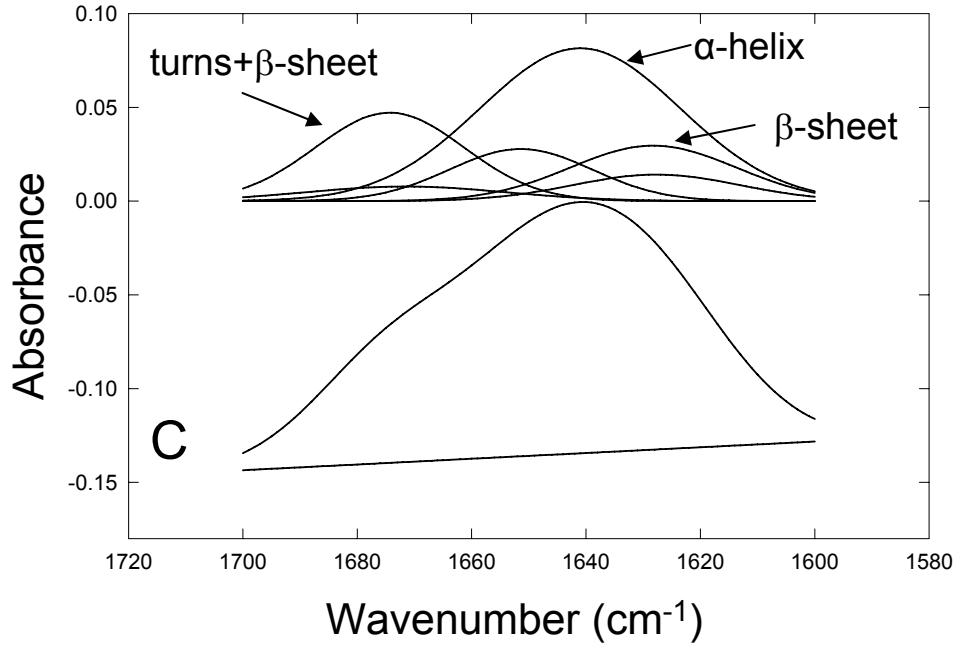
AchE



Secondary structure of hemoglobin from fresh RBCs (A), freeze-dried with trehalose (B), and freeze-dried without trehalose (C) RBCs.

Trehalose preserves the secondary structure of hemoglobin during freeze-drying





Lipid profiles of fresh (—) and rehydrated (—)RBCs

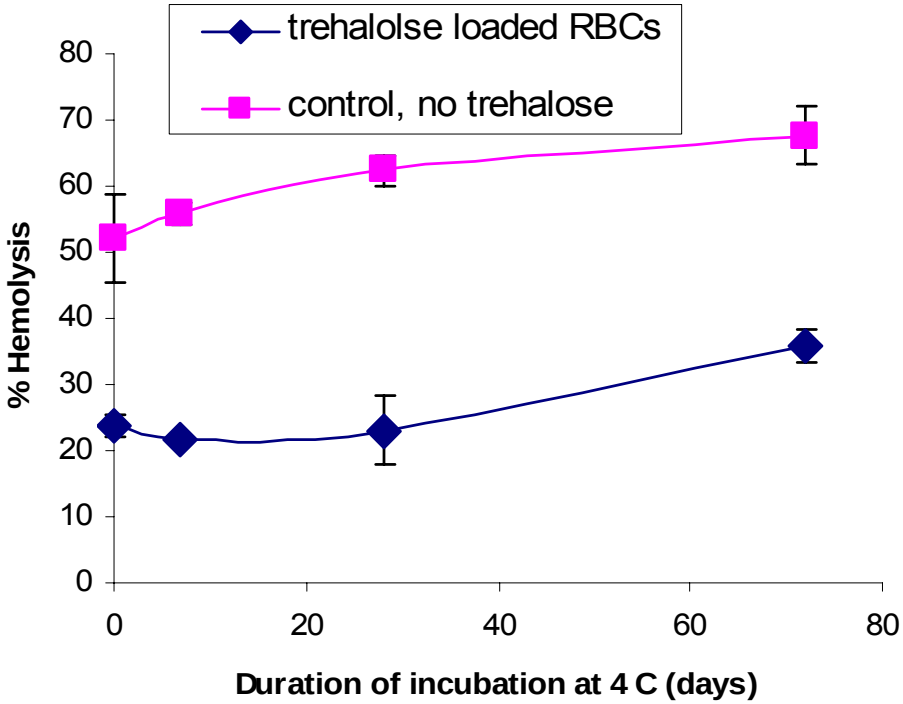
Fresh RBCs, chol:phospholipids, 55:45
Freeze-dried RBCs, chol:phospholipids, 65:35

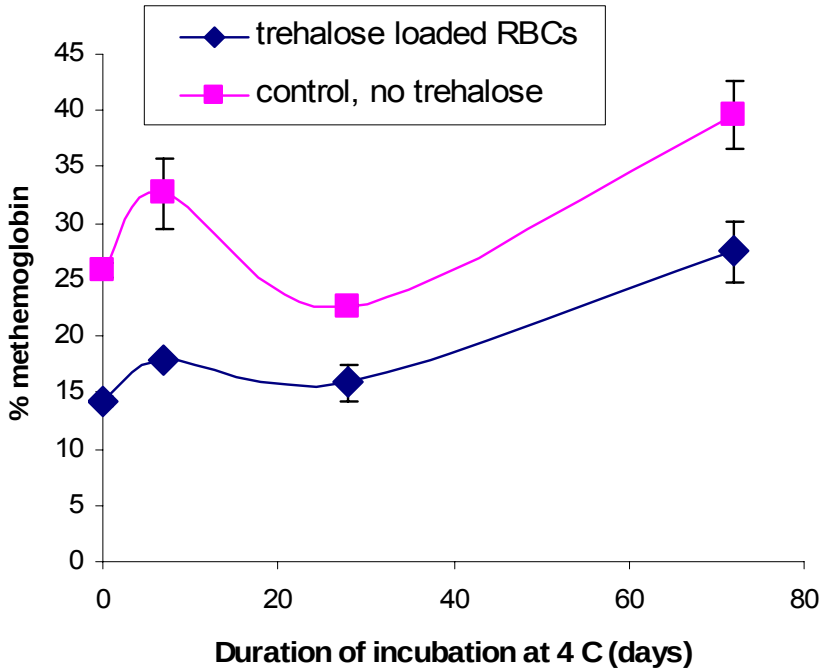
Chol

PC, SM

PE, PS

Long-term stability of freeze-dried RBCs





Acknowledgement

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