Australian Government

Australian Nuclear Science and Technology Organisation

Silk powders – metal binding properties and their potential for controlled drug release

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Applications and Properties of Silk Powders

Applications [1-3]

- Cosmetic products
- Nutritional foods
- Fibre treatment, Fillers in films
- Wound care, Enzyme immobilisation
- Composite scaffold for cell growth
- Drug delivery

Properties [4-8]

- Biocompatible, biodegradable
- Ease of processing
- Good mechanical properties
- Faster kinetics of metal binding

[1] X. Wang, E. Wenk, A. Matsumoto, L. Meinel, C. Li, D.L. Kaplan, *Journal of Controlled Release*, **2007**, 117 (3), 360. [2] R. Rajkhowa, L. Wang, X. Wang, *Powder Technology*, **2008,** 185 (1): 87. [3] E. Wenk, A. Wandrey, H. Merkel, L. Meinel, *Journal of Controlled Release*, **2008** (accepted for publication) [4] Hakimi, O., D.P. Knight, F. Vollrath, P. Vadgama, Composites Part B: Engineering Bio-engineered Composites 2007, 38, (3), 324-337. [5] Minoura, N., S.-I. Aiba, M. Higuchi, Y. Gotoh, M. Tsukada, Y. Imai, Biochemical and Biophysical Research Communication 1995, 208, (2), 511-516. [6] Tsubouchi, K., T. Ninagawa, Patent 11104228, 1999. [7] Tsubouchi, K. Patent 123683, 2004. [8] Wang, Y., H.-J. Kim, G. Vunjak-Novakovic, D.L. Kaplan, Biomaterials 2006, 27, (33), 6064-6082.

Processing of Silk powders

Silk powder is prepared from silk fibroin:

- Mechanical attrition
- Regeneration from solution

Regeneration from solution	Mechanical attrition
Non-uniform	Uniform
Partially water-soluble powder	Water insoluble
β crystalline structure changes	Retain β crystalline structure
Long processing time	Short processing time
Higher cost	Lower cost

Varieties of Silk powder

- Eri silk (AES1, AES3)


- Mulberry silk (AMS1)


Different morphology

And

amino acid composition

Aim

How does the processing and variety of silk effect:

- Metal binding (Co^{2+}, Cu^{2+}, Cd^{2+})
  - Effect of pH
  - Rate of uptake
  - Loading capacity

  } Radiotracer

- Porosity
  - PALS (Positron annihilation lifetime spectroscopy)

    0.1-10nm (micropore)
  - BET gas absorption

    10-50nm (mesopore)
  - SEM (Scanning elelctron microprobe)

    >50nm (macropore)

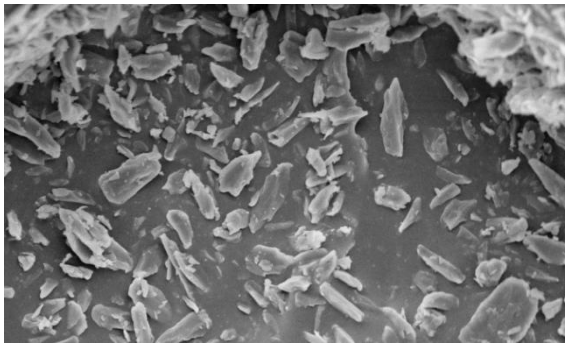
SEM (Scanning Electron Microscope) imaging

Eri: normal degumming, wet-ball mill, spray dry

9µm

10µm

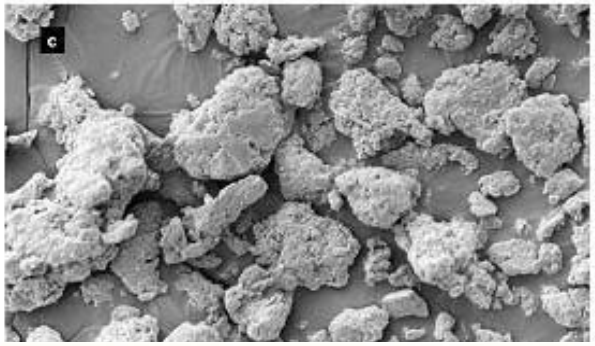
Eri: intensive degumming, wet-ball & air-jet mill, spray dry



4.5µm

10µm

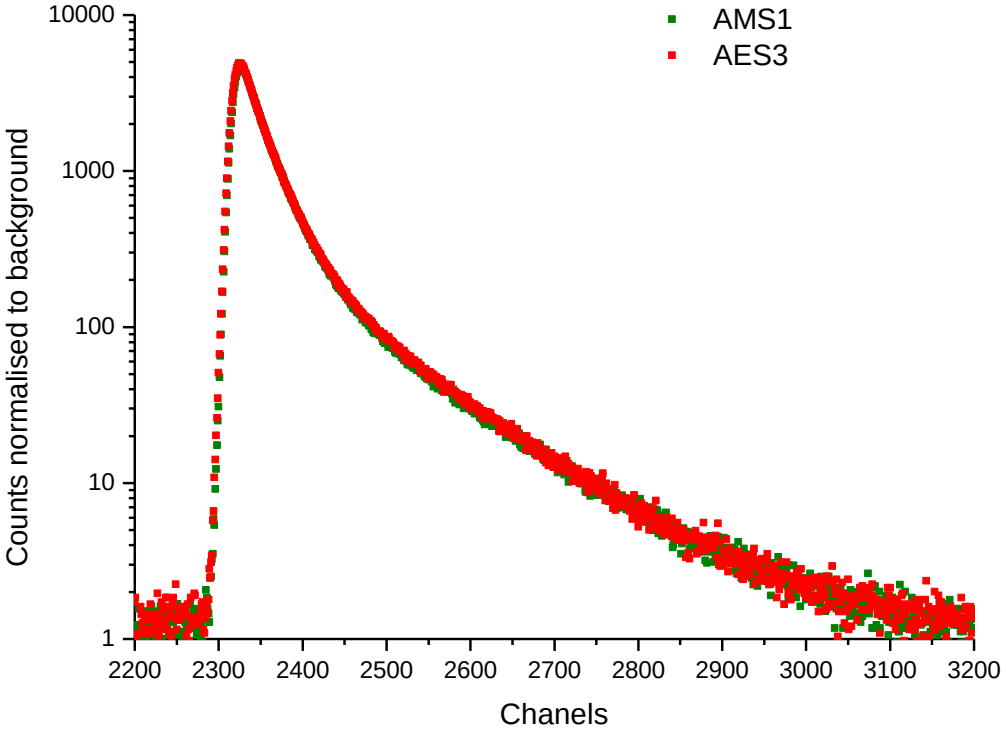
Mulberry: commercial, without further processing



5µm

10µm

Porosity



Silk sample analysed	Pore size (nm)
Eri: normal degumming (AES1)	0.48
Eri: intensive degumming (AES3)	0.48
Mulberry: commercial (AMS1)	0.47

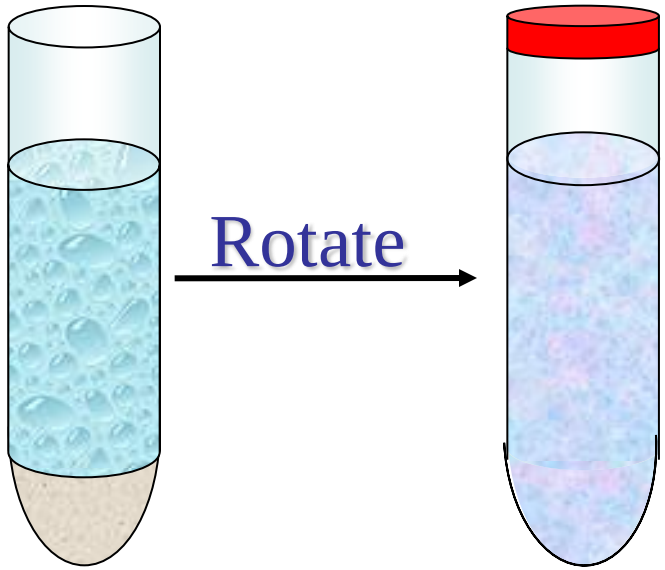
- PALS shows no change in microporosity on processing
- BET shows no significant change in surface area on processing

Basic protein structure appears to be conserved

Experimental conditions

- $^{64}Cu/Cu^{2+}$, $^{57}Co/Co^{2+}$, $^{109}Cd/Cd^{2+}$
  - $^{64}Cu/Cu^{2+}$: fast kinetics; soft & hard donors
  - $^{57}Co/Co^{2+}$: slow kinetics; hard donors
  - $^{109}Cd/Cd^{2+}$: slow kinetics; soft donors
- pH: 3 to 9
- Temperature: 23°C
- $[M^{2+}] = 10^{-3}$ to 10^{-6}M

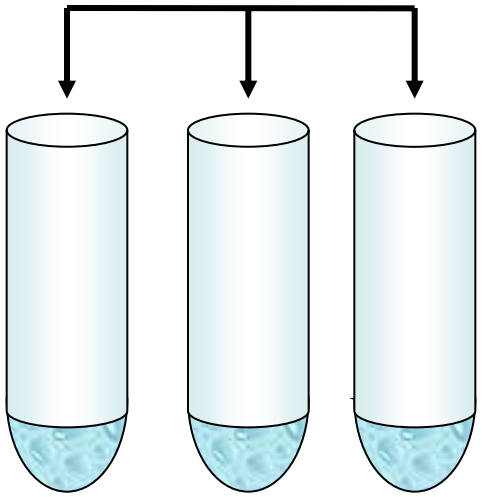
Experimental procedure



Rotate

Centrifuge

3 x 20µL

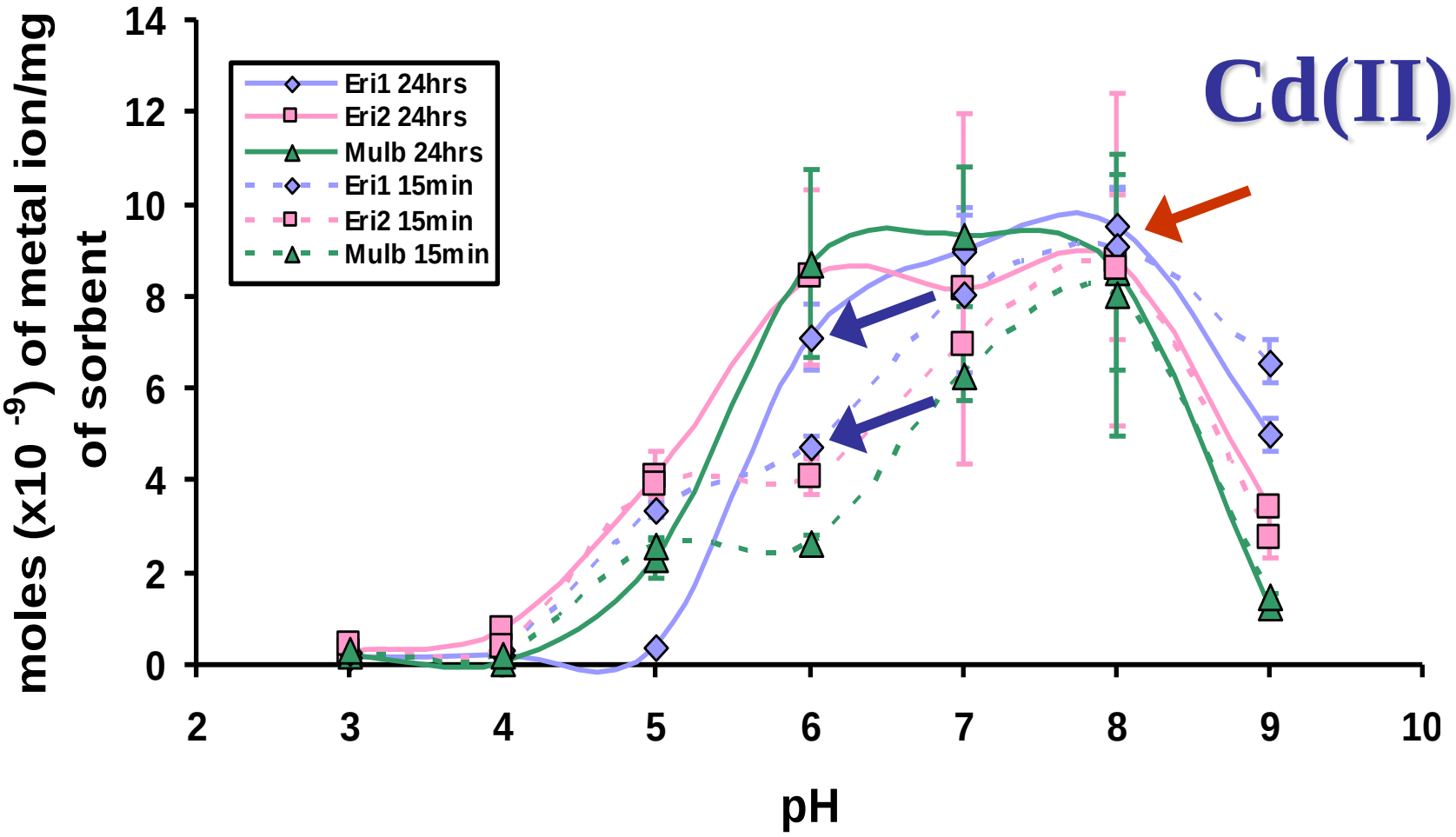


Activity counted
In γ counter

~10mg silk powder
(x4)

+ 1mL
(metal ion +
radiometal ion +
buffer)

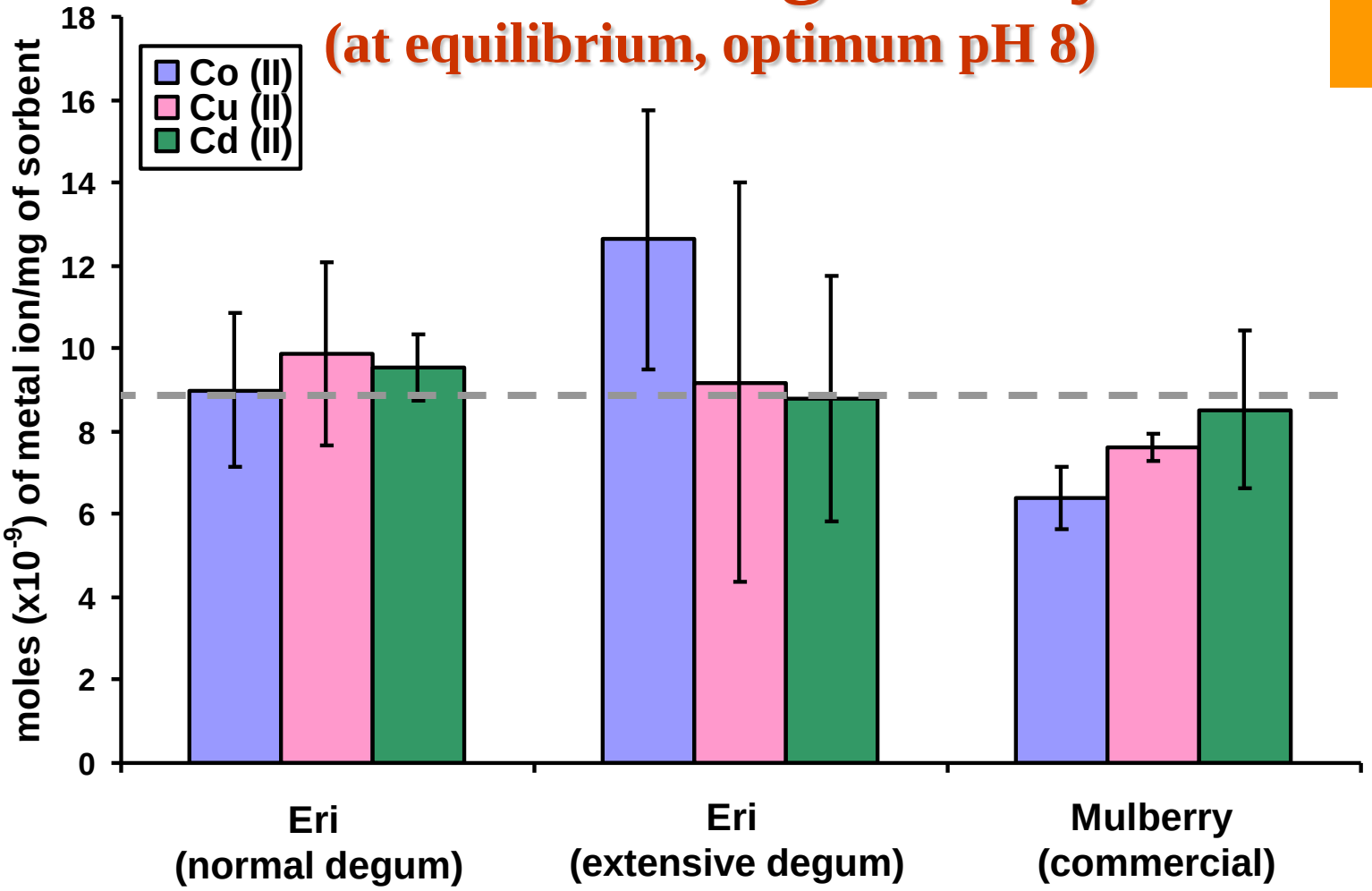
Effect of pH on Metal Binding



[M^{2+}] = 10^{-4}M; powder 10 mg; Temp.23°C; Total Vol: 1.0 mL; centrifuge; 5000 rpm

Metal binding affinity

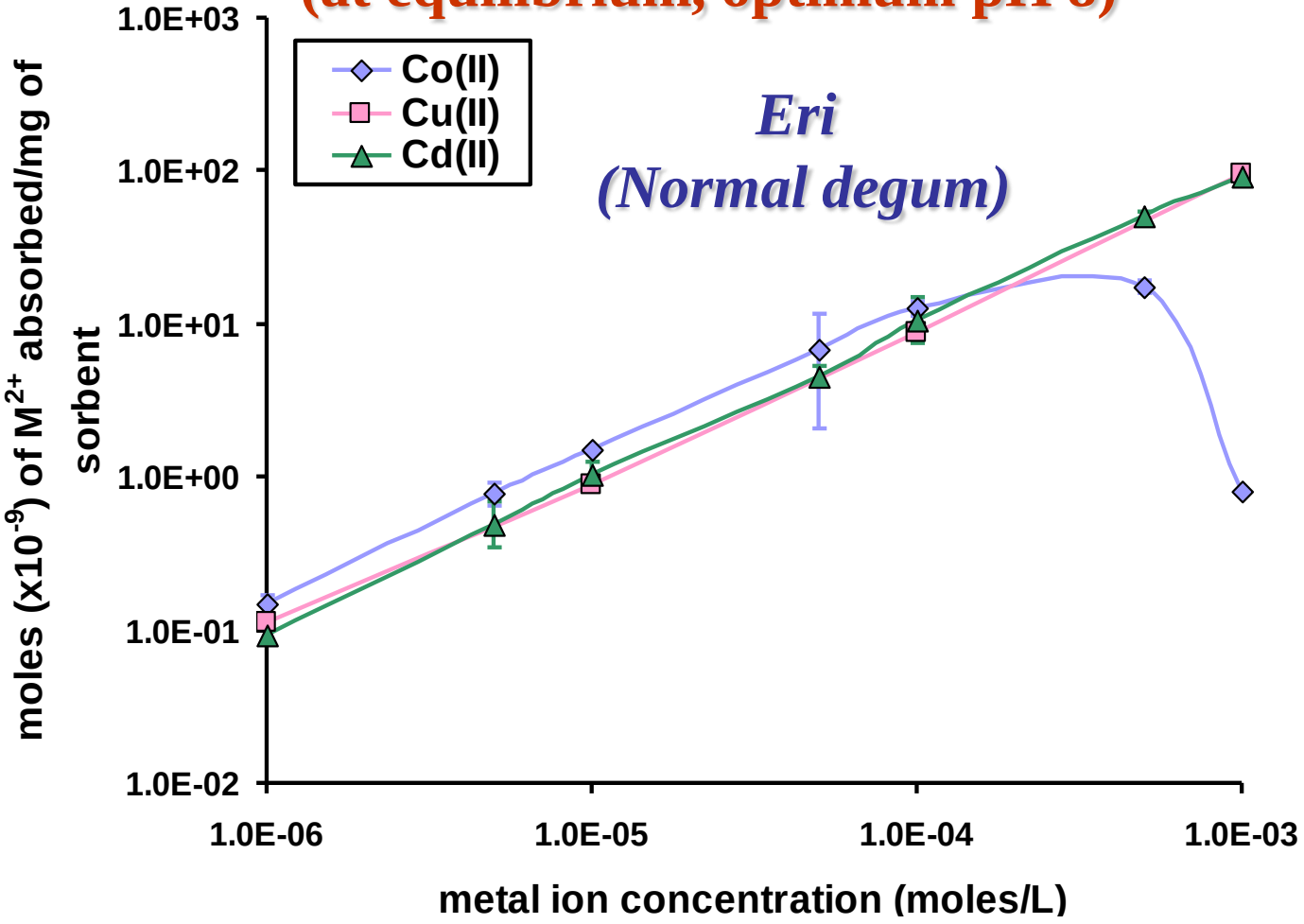
(at equilibrium, optimum pH 8)



$[M^{2+}] = 10^{-4}M$; powder 10 mg; Temp.23°C; Total Vol: 1.0 mL; centrifuge; 5000 rpm

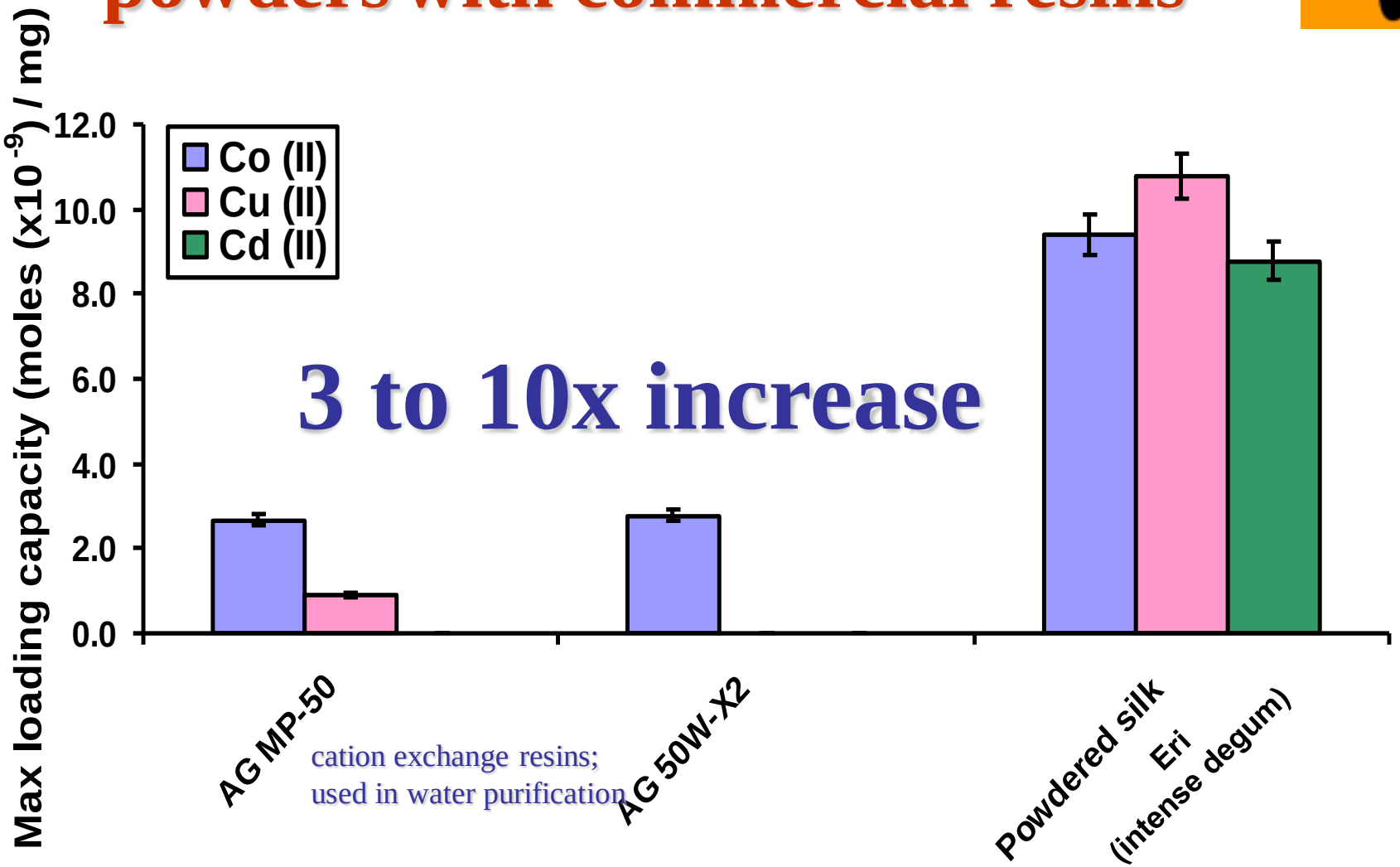
Loading capacity

(at equilibrium, optimum pH 8)



$[M^{2+}] = 10^{-3}$ to 10^{-6}M; powder 10 mg; Temp.23°C; Total Vol: 1.0 mL; centrifuge; 5000 rpm

Comparison of metal loading on silk powders with commercial resins



cation exchange resins;
used in water purification

$[M^{2+}] = 10^{-4}M$; powder 10 mg; Temp.23°C; Total Vol: 1.0 mL; centrifuge; 5000 rpm

Conclusion

- Processing does not affect the basic protein structure or porosity.
- Metal binding varies with pH; independent of silk variety and processing method.
- Higher (3 to 10x) loading capacity than commercial resins.
- Rapid, high-throughput radiotracer assay was developed (~ 800 readings; 10 sec each).

Future directions

- **Binding with larger nuclear probes (drug binding and controlled release)**

- **Incorporate into composite material; evaluate performance (selectivity, loading capacity)**

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Thank you