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Title: CE-MXRF: THE POWER OF SEPERATION WITH BENCH TOP ELEMENT SENSITIVE DETECTION

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CE-MXRF: The Power of Separation with Bench Top Element Sensitive Detection

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Capillary electrophoresis (CE) is a proven separation technique that offers highly efficient separation, rapid analysis, and minute sample consumption. When combined with a element specific detection scheme, it can be used for chemical speciation of biologically and environmentally relevant species such as metal containing proteins.

In this study, a new tool was developed for separation and elemental detection. Specifically, a simple CE apparatus was constructed using a thin-walled fused Si capillary and interfaced with a bench top micro x-ray fluorescence (MXRF) system. X-ray excitation and detection of the separated sample volumes was performed using an EDAX Eagle II micro x-ray fluorescence system equipped with a Rh target excitation source and **a** SiLi detector. It was demonstrated that the system could be used for the separation and detection of two metals from one another, specifically Cu^{2+} and Co^{2+}. Free Co^{2+} could also be isolated from Co^{2+} bound to cyanocobalamin (Vitamin B-12). Other systems that were explored were the separation of two organics, ferritin from cyanocobalamin as well as the separation of the different Cu and Zn isoforms of metallothinein. CE-MXRF was also used to separate the important serum isoforms of transferrin.

Direct comparisons were made between CE-MXRF system and other elemental separation techniques such as CE-PIXE, CE-synchrotron-XRF, and CE_ICPMS.

CE-MXRF: The Power of Separation with Bench Top Element Sensitive Detection

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Capillary Electrophoresis with Element Specific Detection

CE

1) High separation efficiencies

2) Small sample volume requirements (nL), of both sample and reagents

3) Rapid sample throughput

CE + Elemental Specific Detection

5) Chemical speciation-quantitative evaluation of different forms of a specific element in a complex matrix

6) Separation and detection possible in environmentally and biologically relevant environments which contain important metal complexes

Common CE Elemental Detection Methods

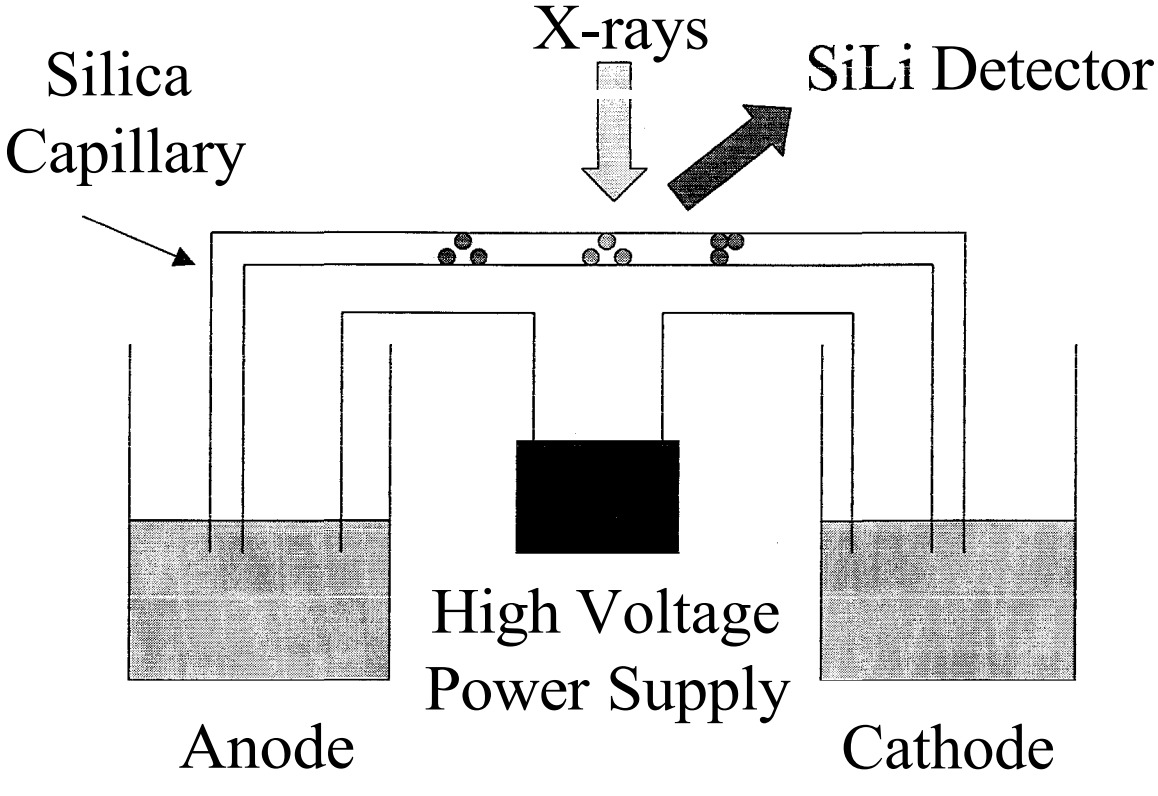
Method	Typical Detection Limits	Advantages	Disadvantages
PIXE (e.g. C. Vogt et al. J. Chromatogr. A 727 (1996) 301-310.)	**10^{-7}-10^{-5}M**	• Simultaneous multi-elemental analysis, **Z>13**	• Limited Access • Radiolysis inside capillary requires **decoupling** of separation and detection • Requires **an** etched I 0 μm Si window
Synchrotron-XRF (e.g. S.E. Mann et al. Anal. Chem. 2000, 72, 1754-1758.)	**~10^{-4} M**	• **Simultaneous, on-line, multi-** elemental detection, **Z>17** • **Nondestructive**	• Limited Access • Requires a polyethylene window
ICPMS (e.g. V. Majidi et al. Analyst, May 1998, Vol 123 (803-808).)	**10^{-11}-10^{-9} M**	• **Simultaneous, on-line, multi-** elemental detection • Isotope specific detection	• Destructive, • Complicated interface • Affected by buffer/ matrix **effects** • Strong Fe interferrences

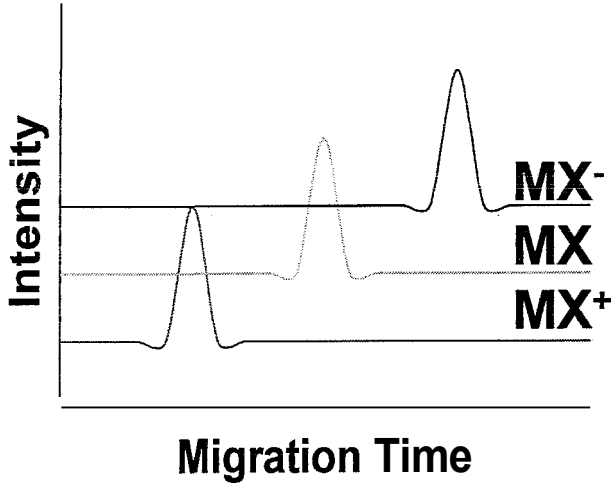
CEMXRF: A New Tool for Separation and Elemental Detection

Goal: To develop a new too! for separation and elemental detection

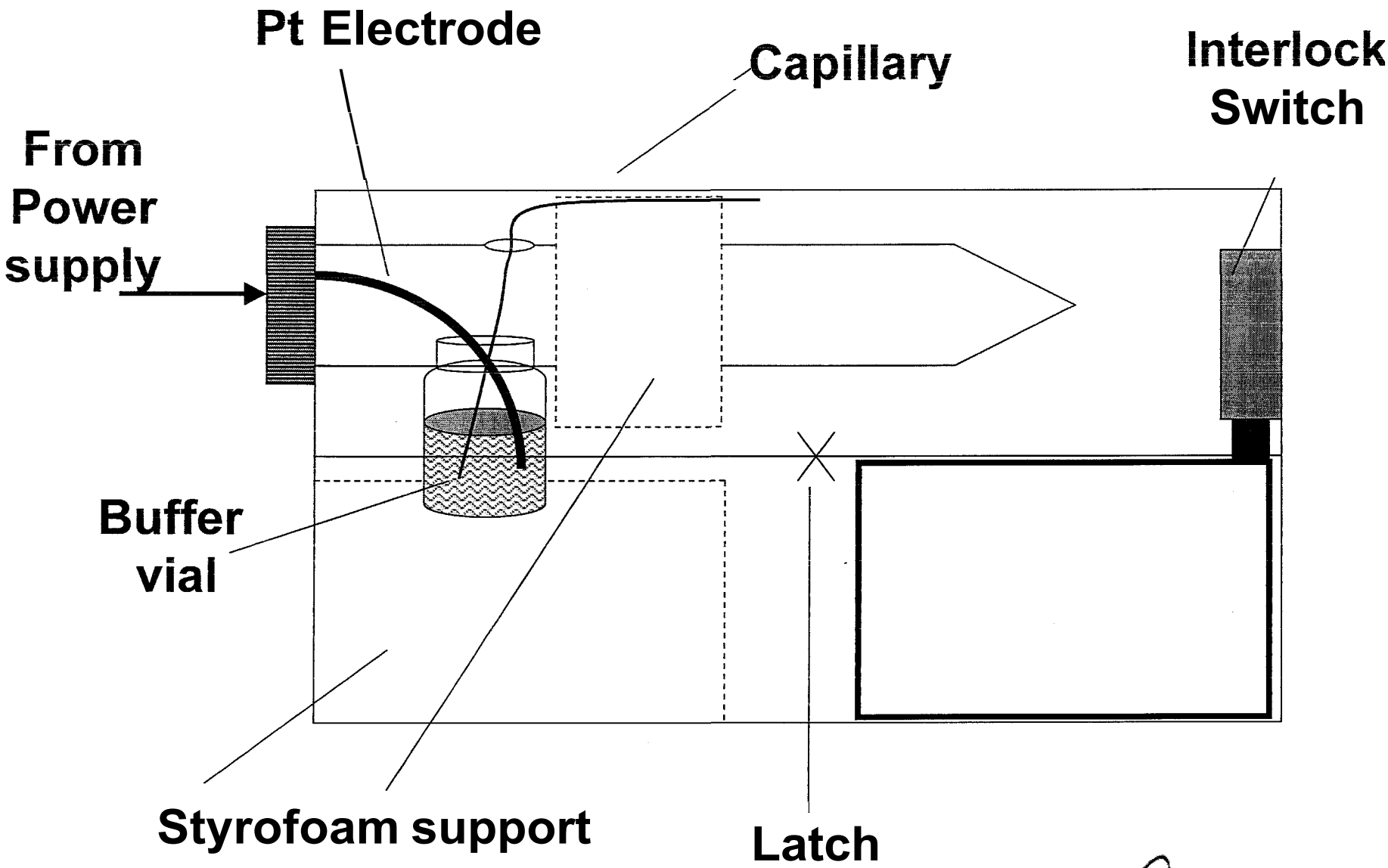
- **Biologically relevant species such as metal containing proteins**
- **Combinatorial library analysis; detection and quantification of binding events between unlabeled proteins and other small molecules**
- **Nondestructive**
- **Bench top Instrumental Interface**

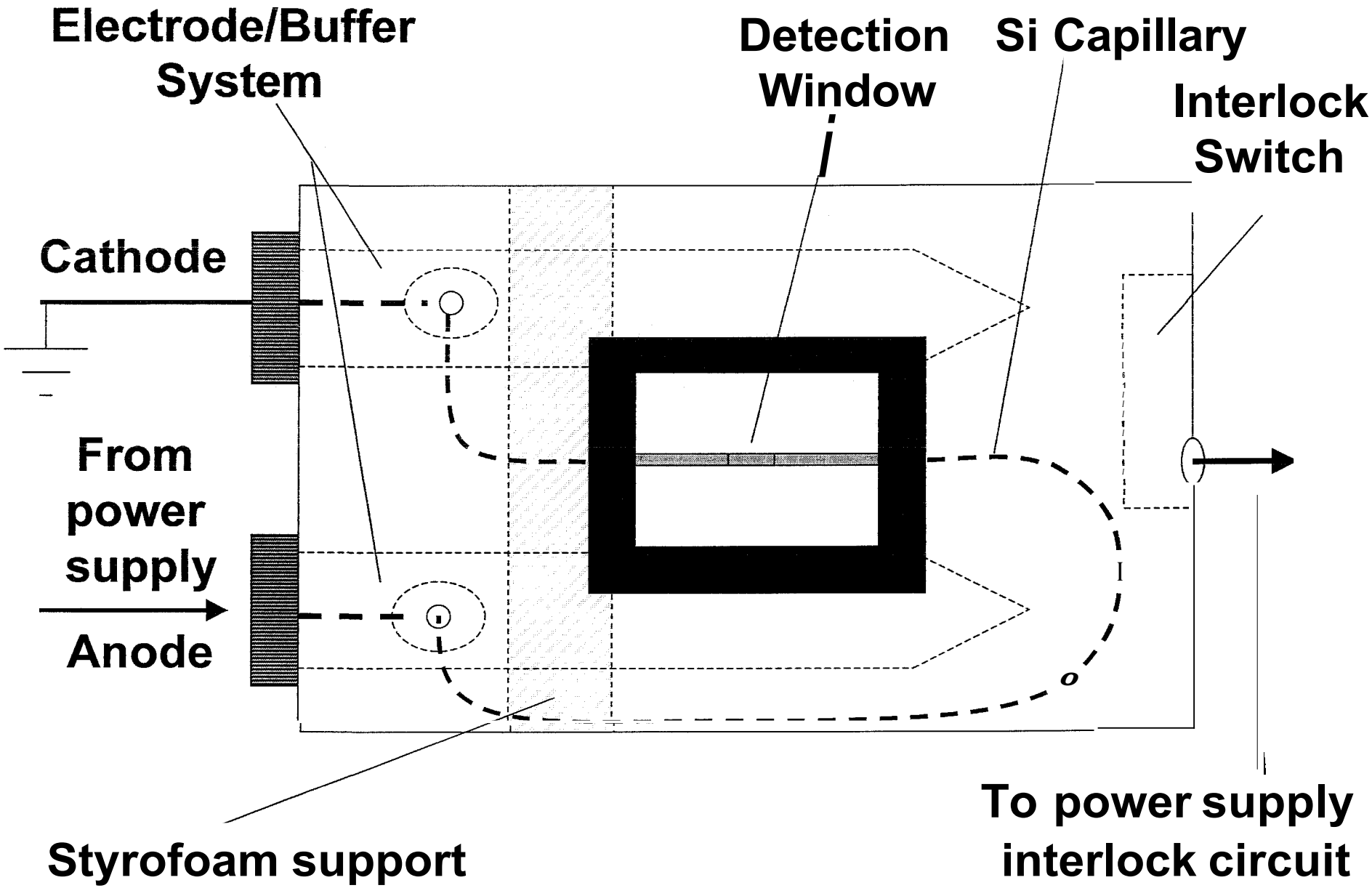
CE-MXRF

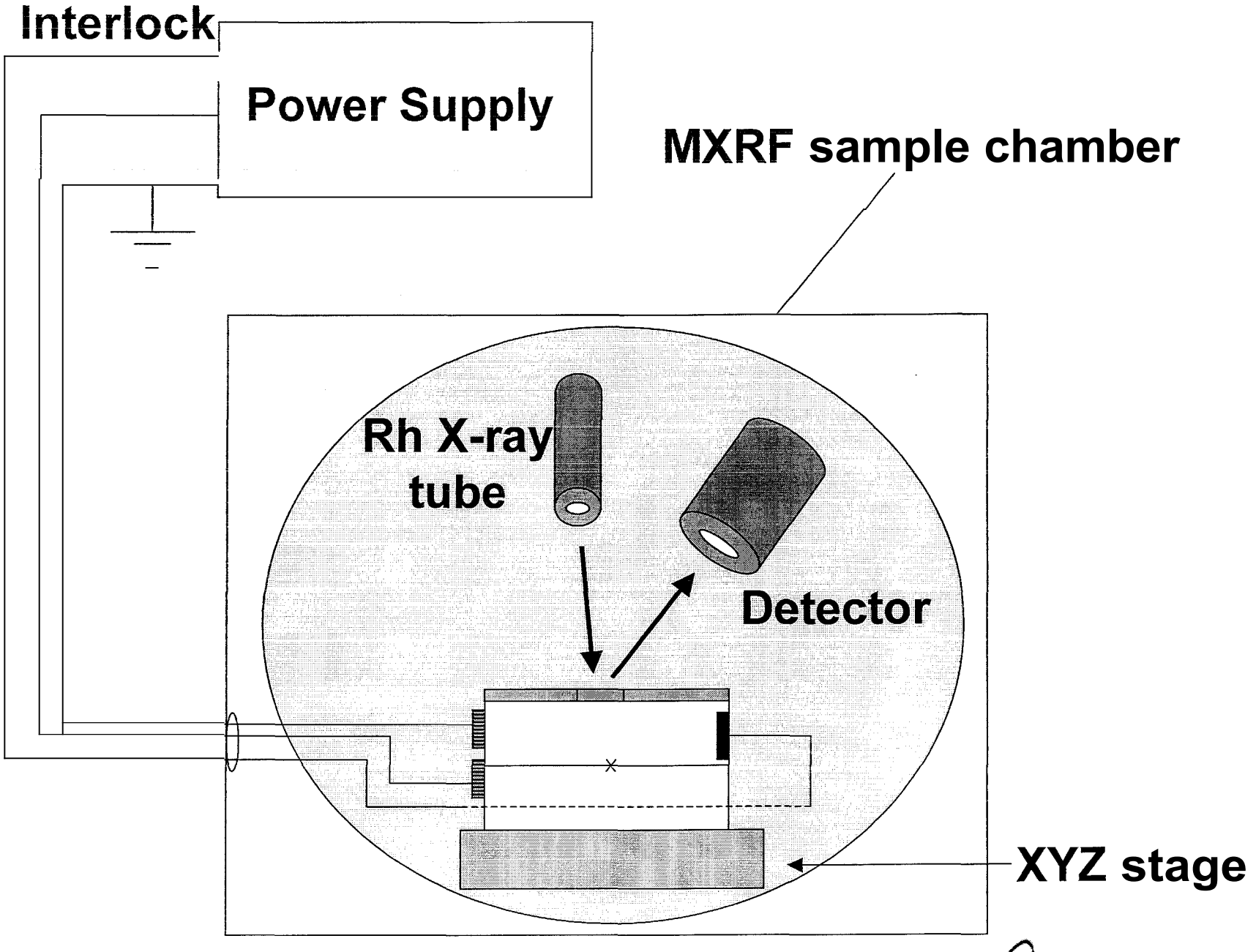




Simultaneous, on-line, multi-elemental detection, $Z \geq 11$


Pt Electrode
Capillary
Interlock Switch
From Power supply
Buffer vial
Styrofoam support
Latch


Electrode/Buffer System
Detection Window
Si Capillary
Interlock Switch
Cathode
From power supply
Anode
Styrofoam support
To power supply interlock circuit


Interlock
Power Supply
MXRF sample chamber
Rh X-ray tube
Detector
XYZ stage

Selection of Capillary Material

Si Tubing	Dimensions			Detection Limit (mg/mL)*			
	O.D. (mm)	I.D. (mm)	Wall Thickness (mm)	Cu	Zn	Fe	Co
TSP167100	0.164	0.097	0.067	0.084	0.154	0.088	0.112
TSP250350	0.362	0.256	0.106	0.046	0.072	0.009	0.031
TSP530660	0.666	0.534	0.132	10.008	0.021	0.010	10.025
TSP075375	0.363	0.075	0.288	1.983	0.488	0.935	1.248

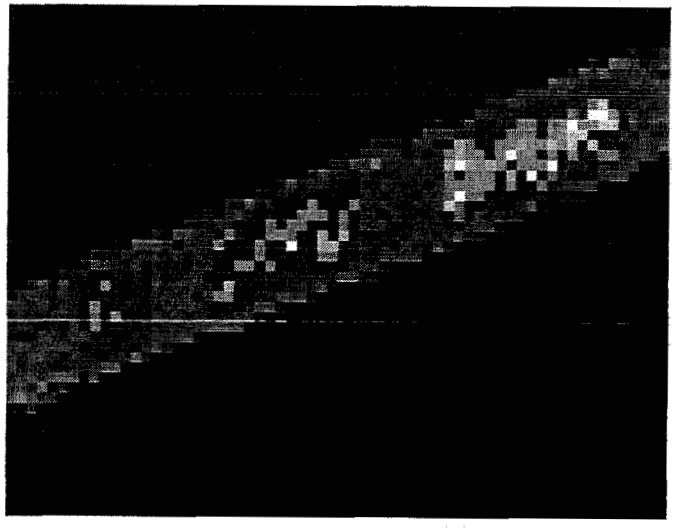
*** Based on sample volume assuming a 0.050 mm MXRF spot size**

Higher energy X-rays allow detection of species through thin-walled fused silica capillary

The Polyimide Coating Dilemma

Issue:

- **–15 μm polyimide coating gives stability to fragile Si capillary**
- **Many means for detection cannot penetrate coating**

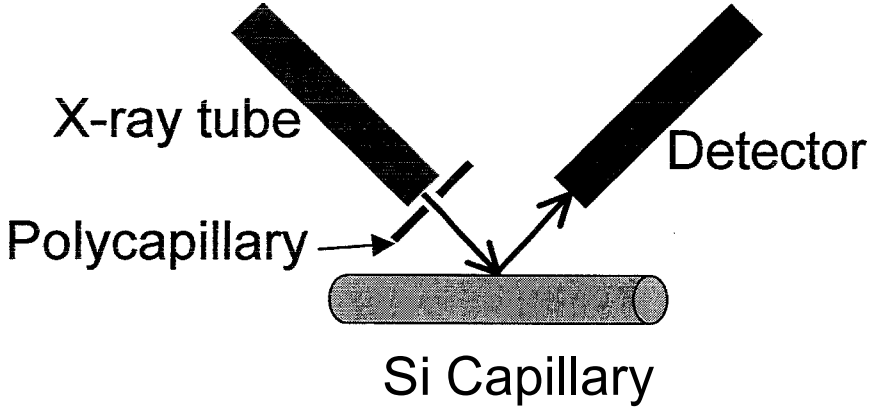


1 ppm 10 ppm 100 ppm

Polyimide Coating Present?	Detection Limit (mg/mL)			
	Cu	Zn	Fe	Co
Yes	0.076	10.064	0.01	10.073
No	0.084	0.154	0.088	0.112

Polyimide coating does not interfere with MXRF detection.

Instrumental Parameters



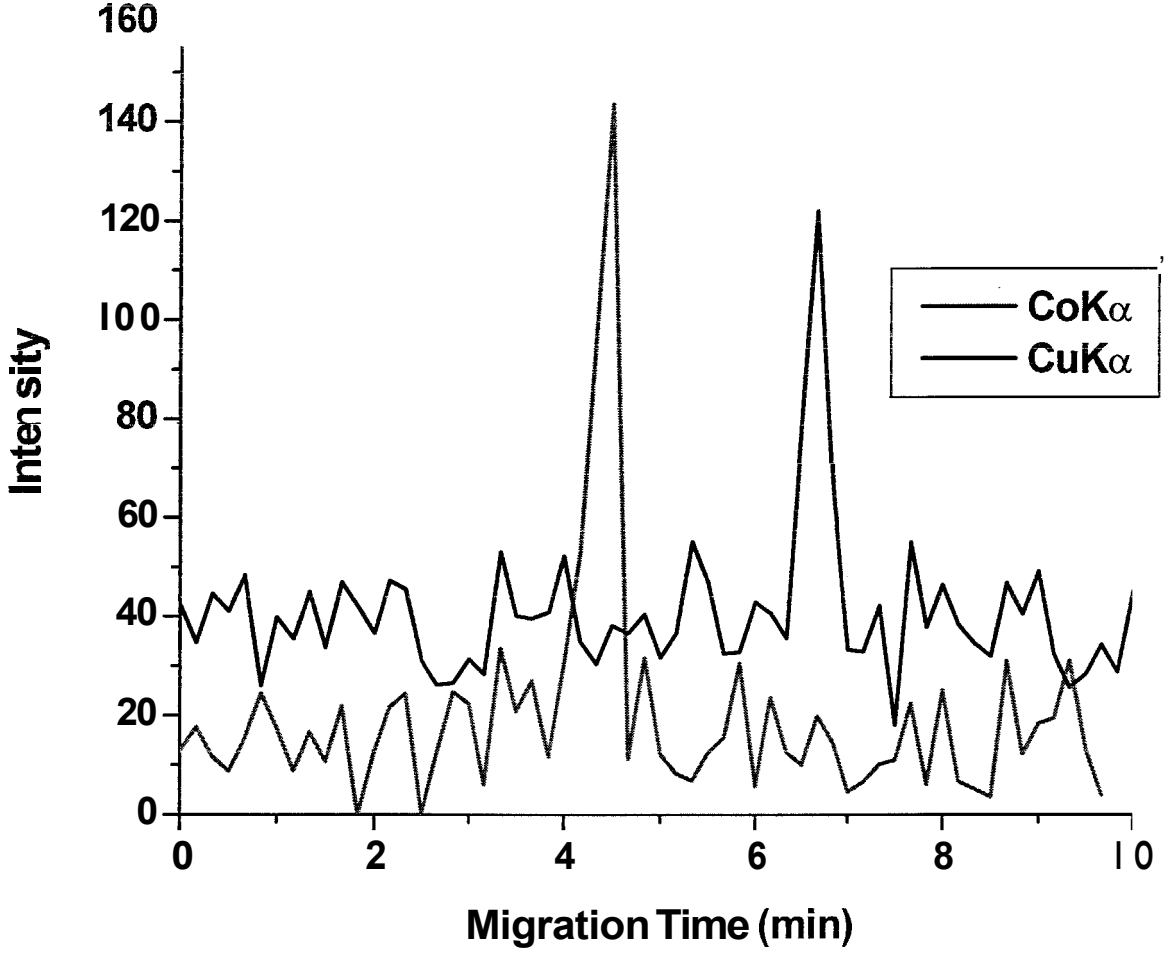
MXRF

- EDAX Eagle II
- Rh X-ray tube, 40 kV, 1000 μA
- Polycapillary focusing optic: 30 μm minimum spot size
- No Vacuum
- SiLi detector
- Spectral Acquisition Rate: $0.1s^{-1}$

CE

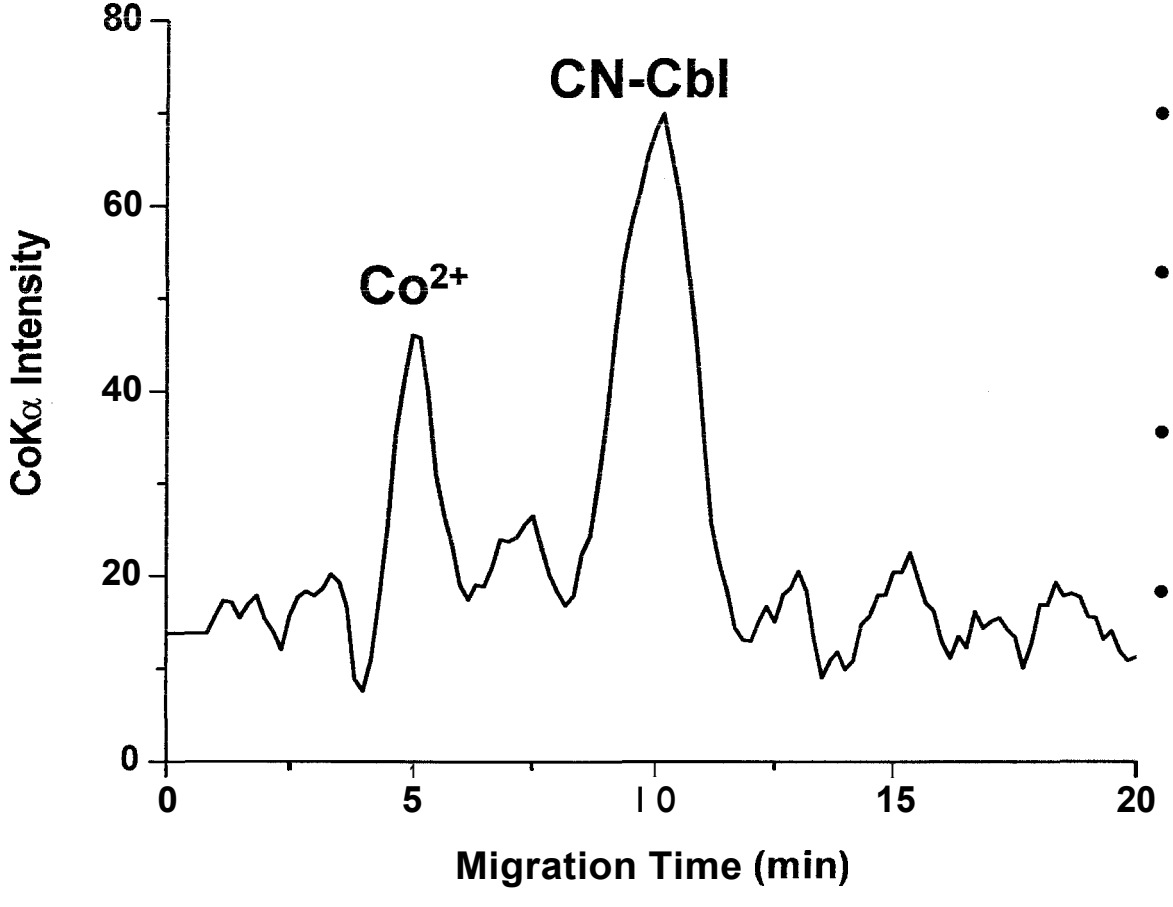
- Fused Silica Capillary; 100 μm o.d., 170 μm i.d.
- Capillary length 70 cm (61 cm to detection window)

0.1 mg/mL Cu^{2+} and Co^{2+} in de-ionized H_2O



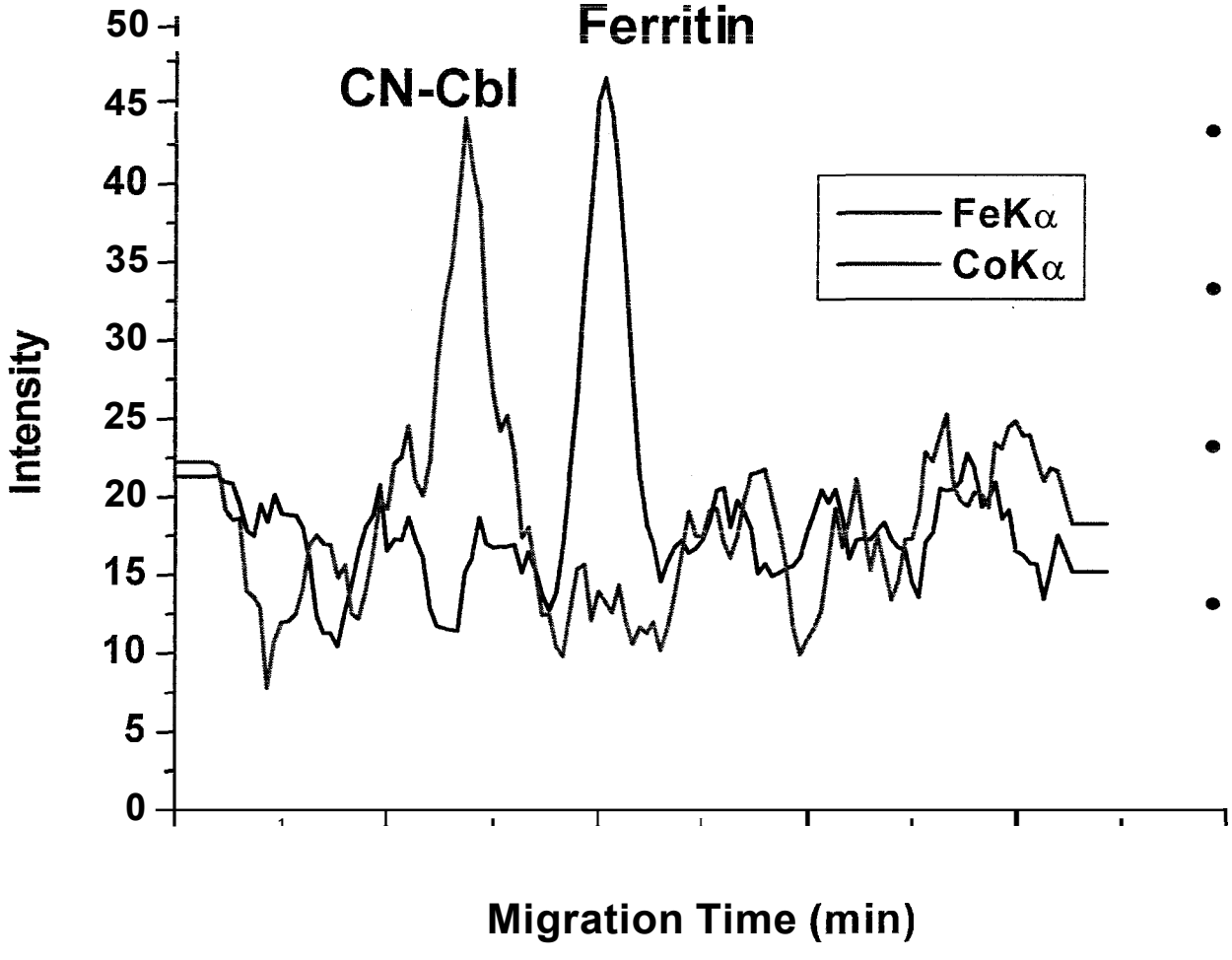
- 70 cm capillary (100 μm id, 170 μm od)
- 50 mM NH_4Ac run buffer, pH 4.5
- 4 s pressure injection at 380 mbar
- 10 KV running potential

0.2 mg/mL Co^{2+} and 13.8 mg/mL Cyanocobalamin (CN-Cbl)



- 70 cm capillary (100 μm id, 170 μm od)
- 75 mM Trizma run buffer, pH 8.0
- 4 s pressure injection at 380 mbar
- 10 KV running potential

1.16 mg/mL Ferritin and 13.8 mg/mL Cyanocobalamin (CN-Cbl)



- 70 cm capillary (100 μm id, 170 μm od)
- 100 mM Trizma run buffer, pH 8.5
- 4 s pressure injection at 253 mbar
- 9.5 KV running potential

Summary

- A simple CE apparatus was constructed using thin-walled fused Si capillary and interfaced with a bench top MXRF system
- CEMXRF was successfully used for metal/metal, free metal/metal complex, multiple metal complex, and biological isoform separations.
- CEMXRF overcomes many shortcomings of other CE-elemental analysis techniques
  - Nondestructive
  - Bench top analysis with simple interface
  - Fe detection
  - Minimal matrix interferences

The Next Step…

- Optimize System to decrease operating detection limits
  - Monochromatic excitation
  - Capillary modification
- Explore possibilities of simultaneous, dual detection methods
- Further explore other biologically relevant systems

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