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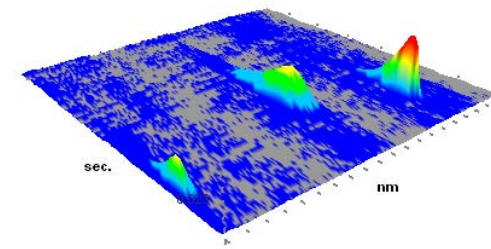
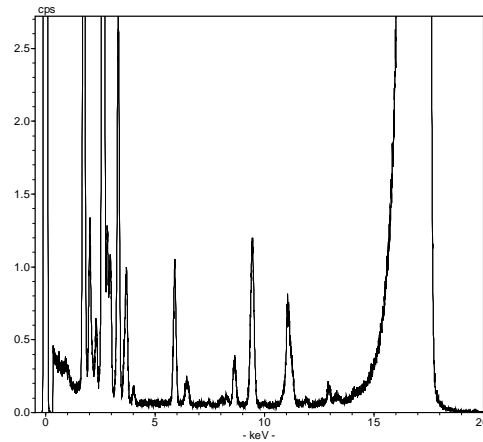
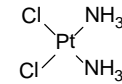


Challenges and Innovations in the (Bio)analysis of Metal Complexes

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Challenges and Innovations in the (Bio)analysis of Metal Complexes

- Applications, samples and techniques
- GF-AAS / HR-CS AAS
- TXRF



Heavy Metal Impurities

Metals as catalysts and reagents in API production

Metals of Interest / Concentration Limits

examples	<u>oral</u> exposure		<u>parenteral</u> exposure	
	µg/day	/ ppm	µg/day	/ ppm
Pt, Pd	100	10	10	1
Ru, Ir	100	10	10	1
Ni, Cr	250	25	25	2.5
Cu, Mn	2500	250	250	25
Fe, Zn	13000	1300	1300	130

Detection Limits

Atomic Absorption Spectroscopy (AAS)

F-AAS: ppm range

GF-AAS: ppb – low ppm

Inductively Coupled Plasma (ICP)

ICP-OES: ppb – low ppm

ICP-MS: ppt – low ppb

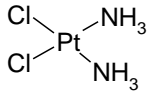
X-ray Fluorescence Spectroscopy XRFS

ppb – low ppm

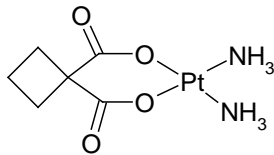
Metal Based Drugs: Current Status

➤ 2000 drugs registered in Germany

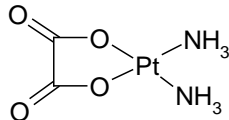
Tumor Therapy: Cisplatin, Carboplatin, Oxaliplatin, Arsenic trioxide



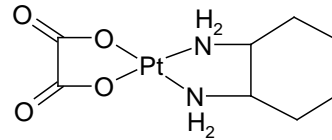
Cisplatin



Carboplatin



Nedaplatin

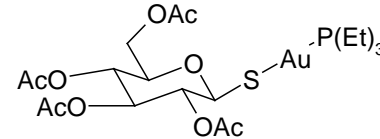


Oxaliplatin



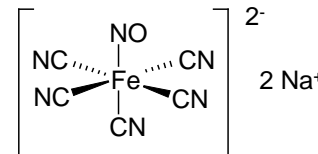
source: U. Warnke, Pharm. Unserer Zeit, 2006, 35, 110.

Rheumatoid Arthritis: Gold Complexes



Hypertensive Emergency: Sodium Nitroprusside

Leishmaniosis : Antimony Complexes



Psychiatric Disorders: Lithium Salts

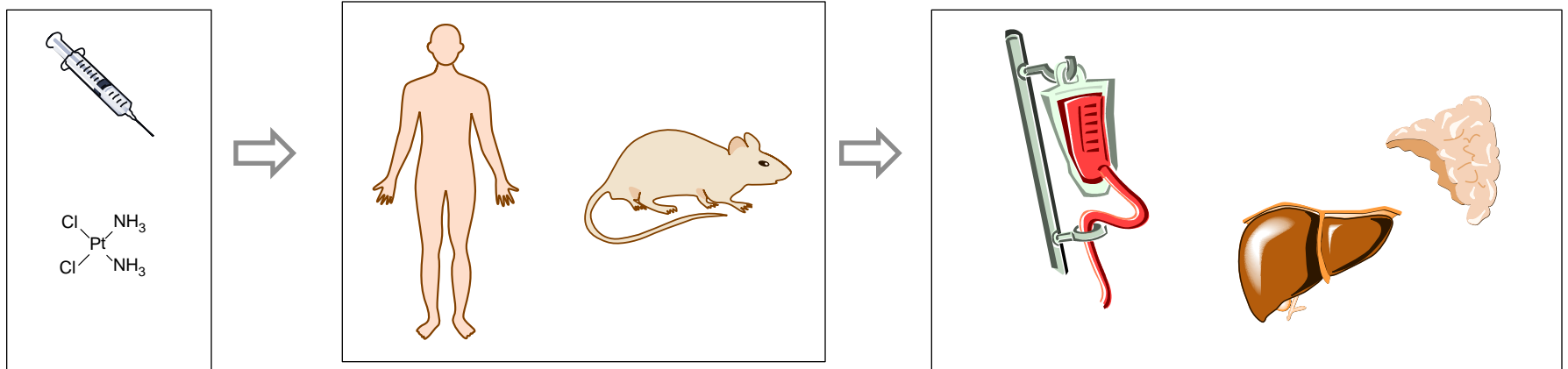
Diagnostics and Radiopharmaceuticals: Technetium- and Gadolinium Complexes



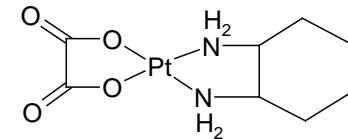
source: www.cephalon.com.

Bioanalysis of Metals and Metal Based Drugs

Clinical Pharmacokinetics / Animal Studies



Examples for applications

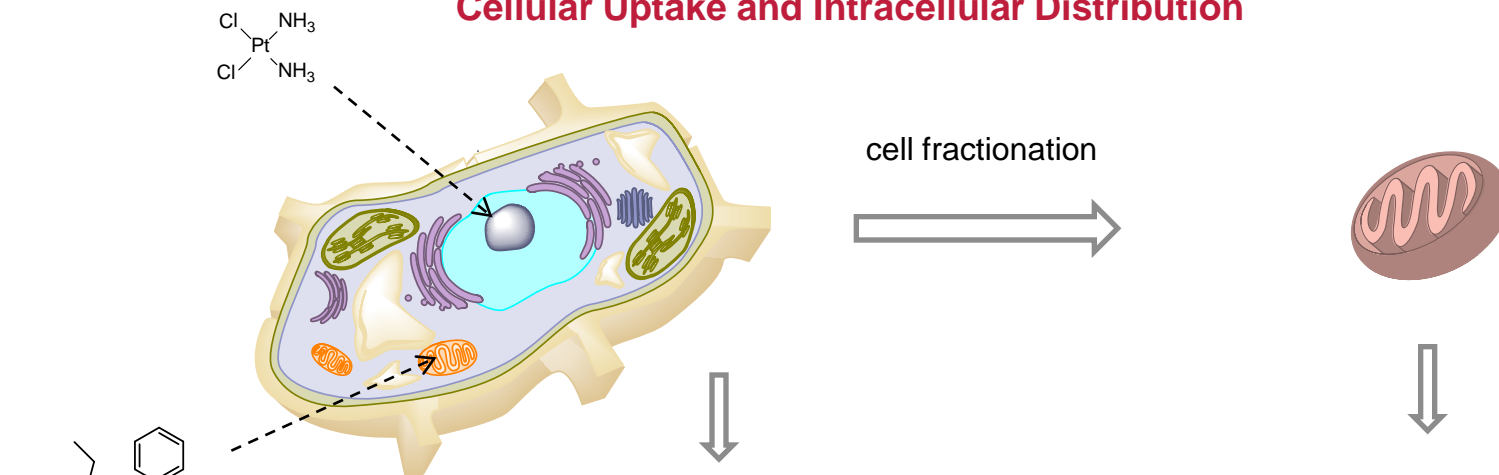


Platinum in patients receiving **Oxaliplatin**: blood, serum urine analysed by GF-AAS

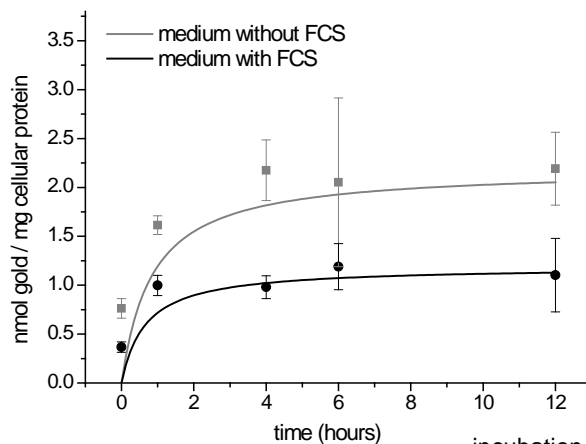
Platinum in patients in a phase I and pharmacokinetic study with **Oxaliplatin**: serum and urine analysed by ICP-MS

Bioanalysis of Metals and Metal Based Drugs

Cellular Uptake and Intracellular Distribution

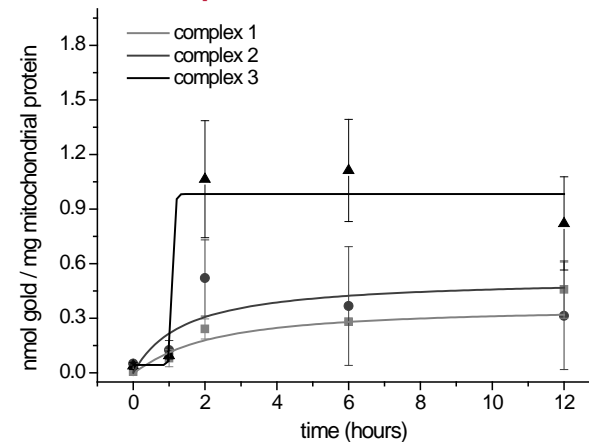


Cellular uptake

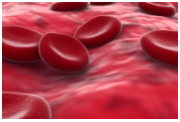


incubation with 3.0 μM of gold(I) complexes

Uptake into mitochondria



Bioanalysis of Metals and Metal Based Drugs



to be considered in AAS and ICP bioanalysis:

Samples

biological fluids

blood, serum, urine...

tissues

cell suspensions

Common Problems

proteins and salts

recovery rates

spectral interferences

Sample Preparation

no pretreatment

chemical pretreatment

(e.g. acid digestion,
microwave or ultrasound treatment)

dilution

Mode of Calibration

external or internal standard

standard addition method

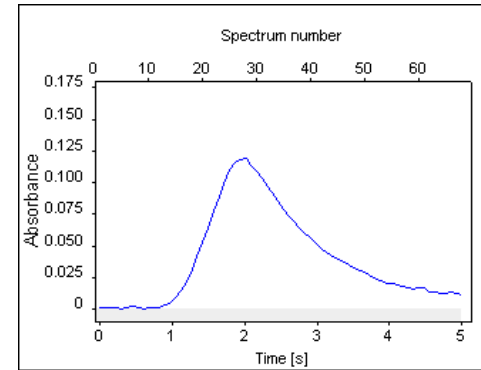
matrix matched calibration

Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS)

Principle

Specific wavelength absorption of ground state atoms

Measurement of integrated absorbances or signal heights



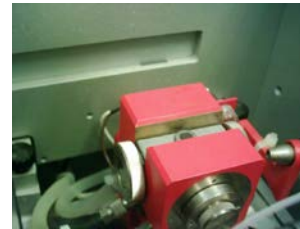
Key features

Samples are atomized (vaporised) in an atomizator

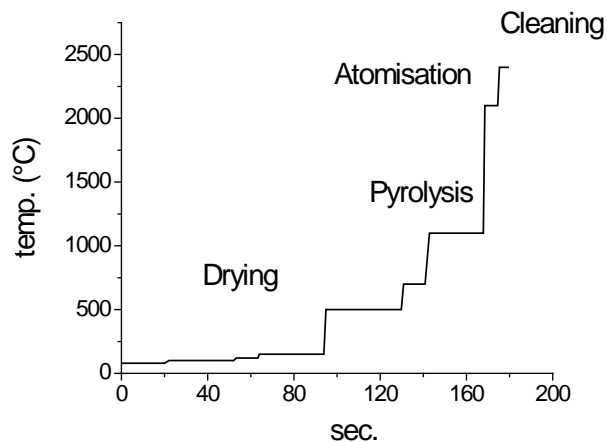
Line emitter light source (e.g. hollow cathode lamp)

Background correction (e.g. D₂ lamp)

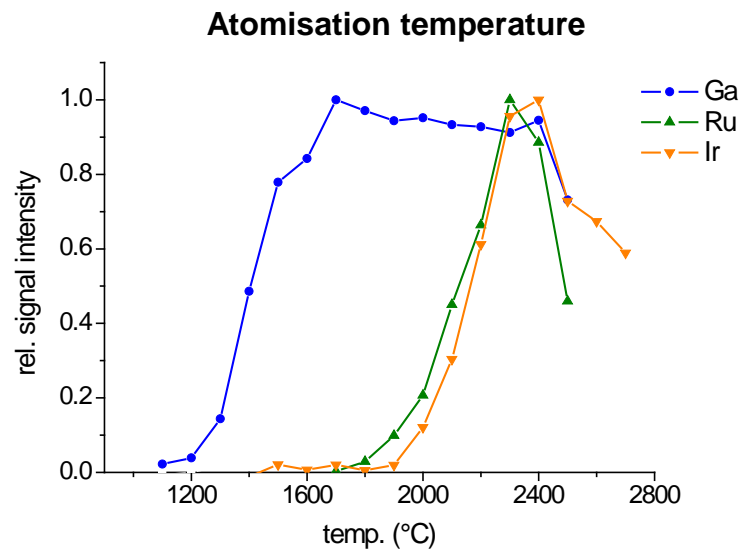
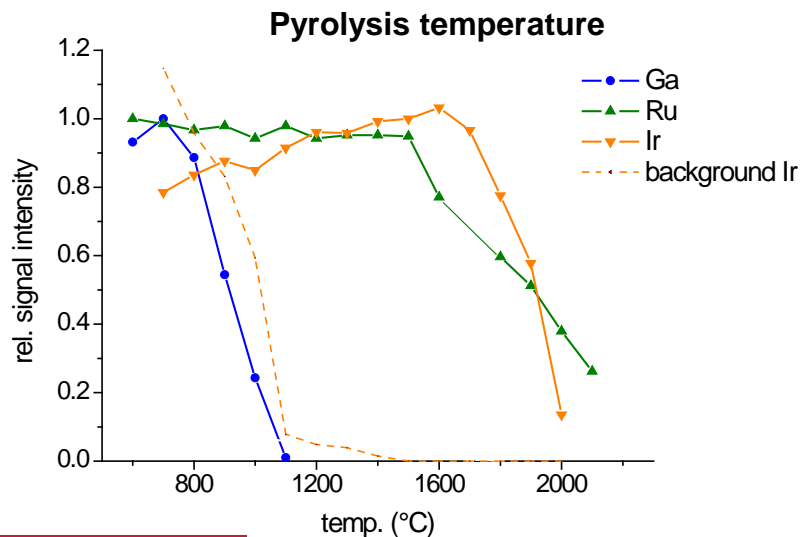
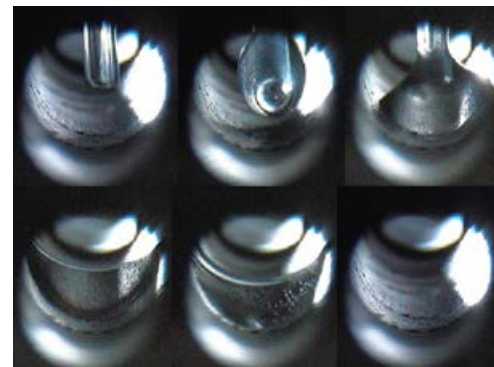
Modifiers (e.g. Mg/Pd)



GF-AAS: Optimization of the Furnace Program



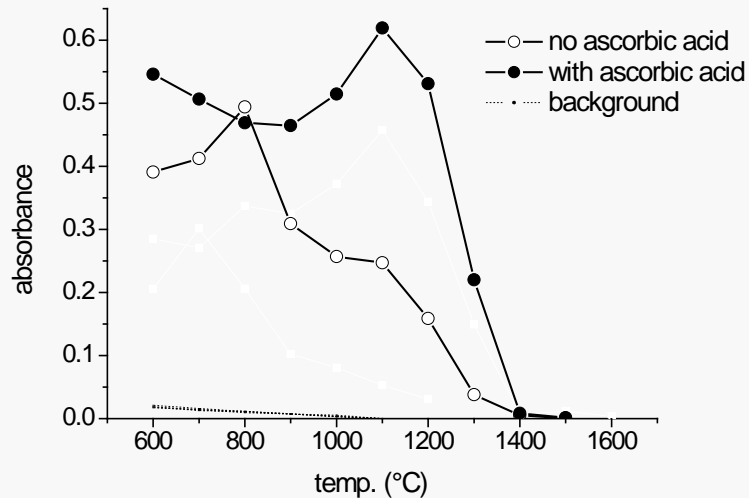
Injection and Drying



AAS Method Development: Modifier / Matrix Effects

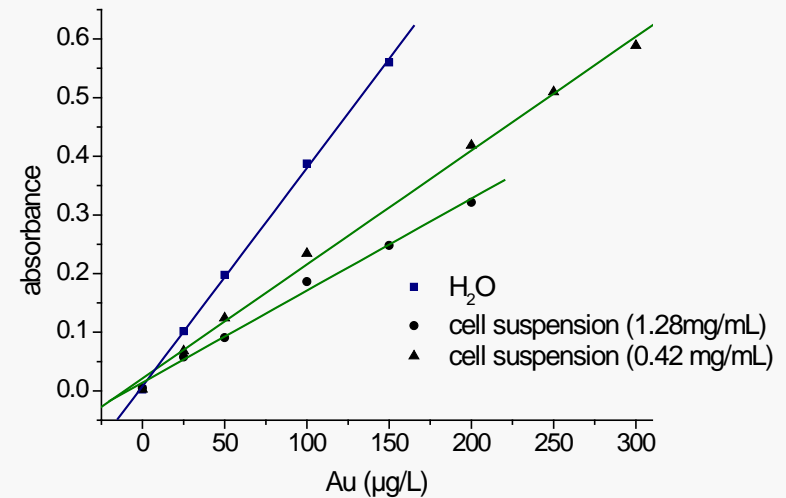
Using Modifiers

Pyrolysis temperature for Au



Matrix Effects

Au in water or cell suspensions

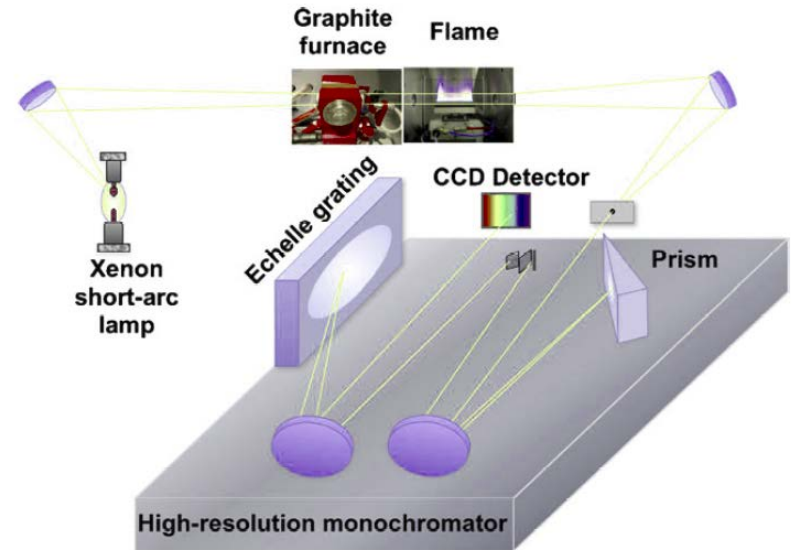


High-Resolution Continuum Source AAS (HR-CS AAS)

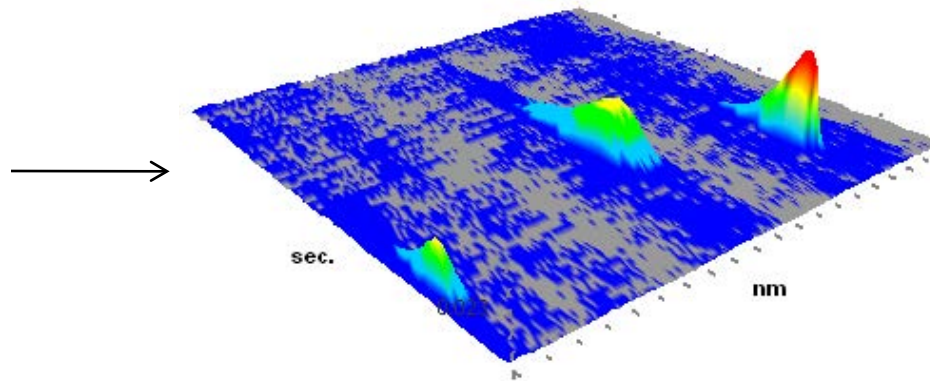
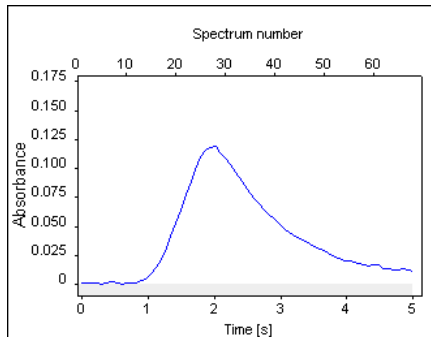
Characteristics of HRCS-AAS

- Continuum light source
- Improved monochromator and detector
- Simultaneous background correction
- Broader spectral range (< 1 nm)
- Determination of non metals and diatomic species

(e.g. F, Cl, Br, I, P, S, PO, NO)



source: Resano et al., *Anal. Bioanal. Chem.* 2011, 399, 323

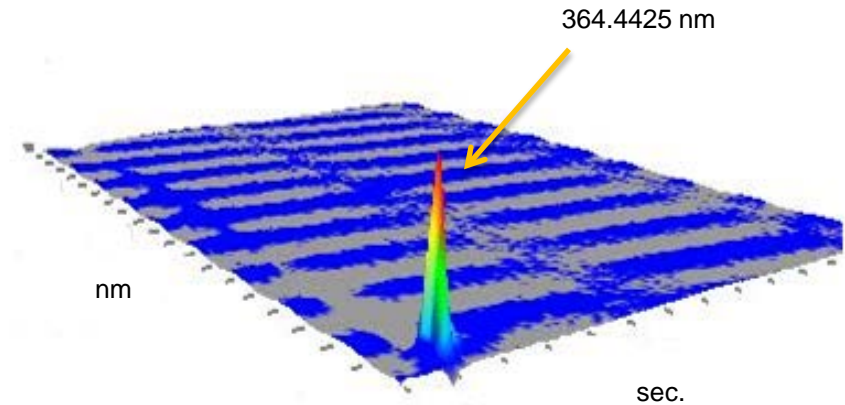
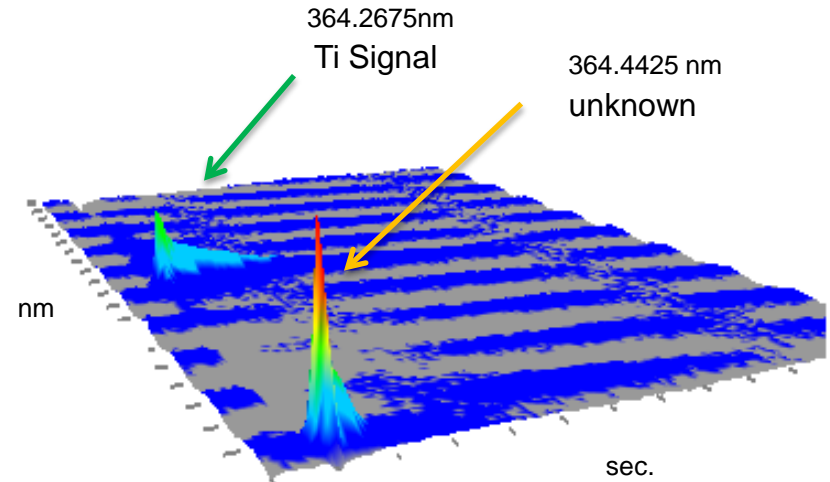
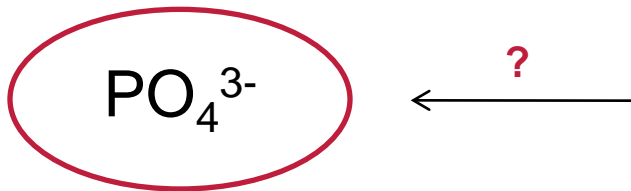
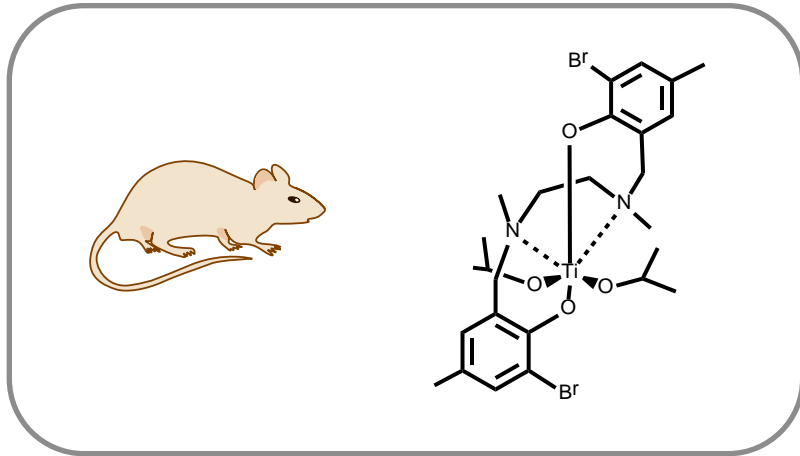


345.846 nm (Ni)
346.044 nm (Re)
346.165 nm (Ni)

a Re complex with Ni impurities

High-Resolution Continuum Source AAS (HR-CS AAS)

Mesurement of Ti in urine (mice)



DNA stock solution

Total Reflection X-Ray Fluorescence Spectrometry

Principle

Fluorescence emission after X-ray excitation of inner shell electrons

Key Features

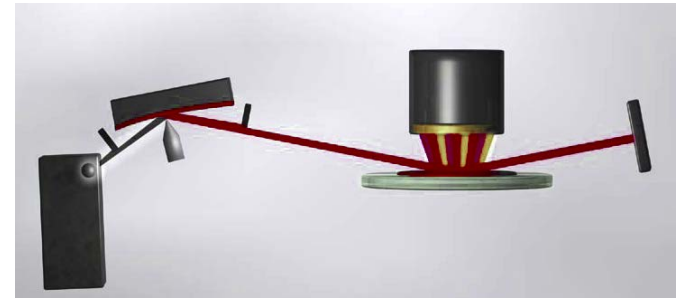
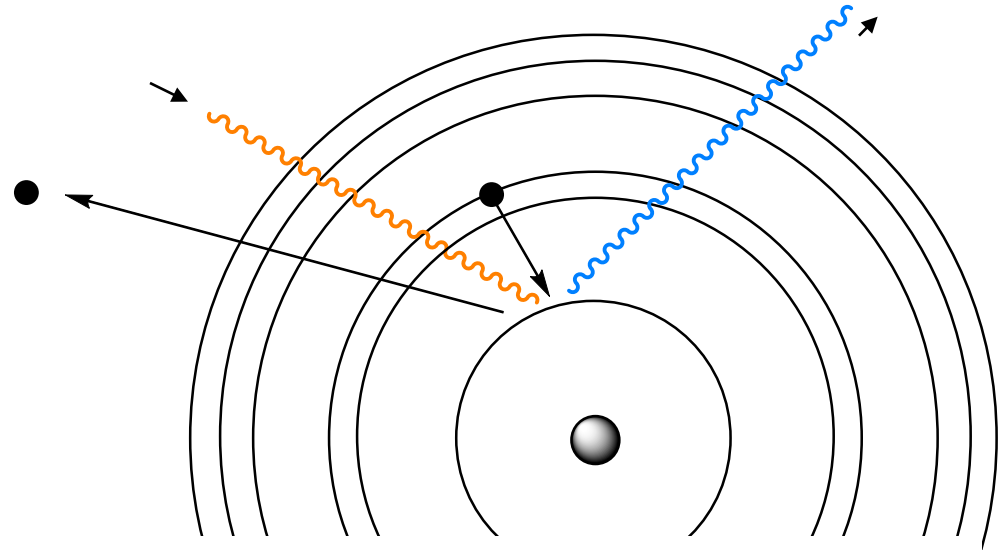
Samples are applied as thin-layers

Beam angle almost 0

Qualitative and quantitative Analysis

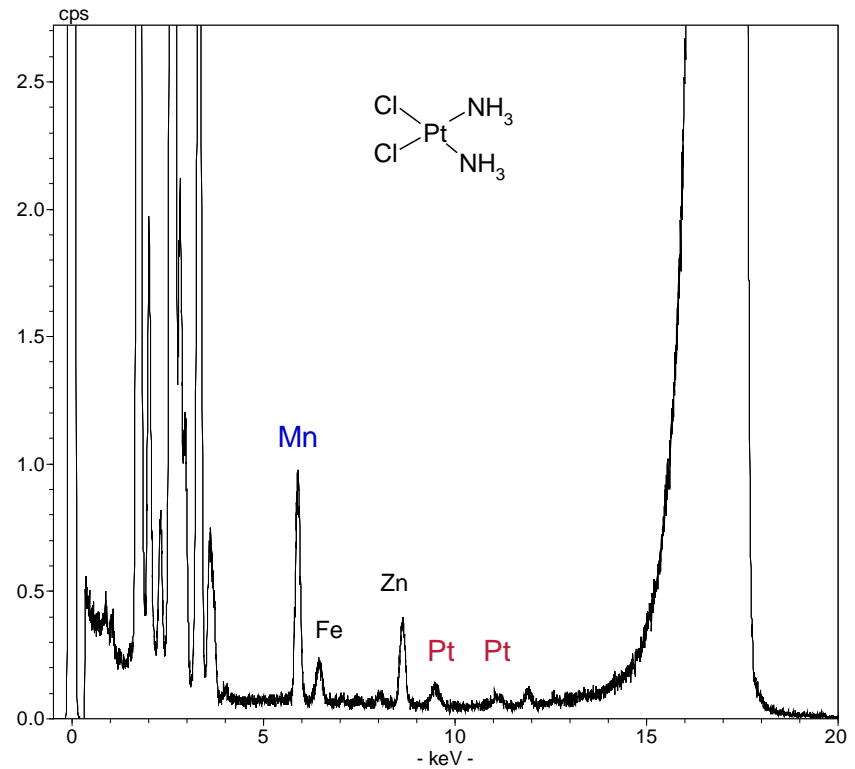
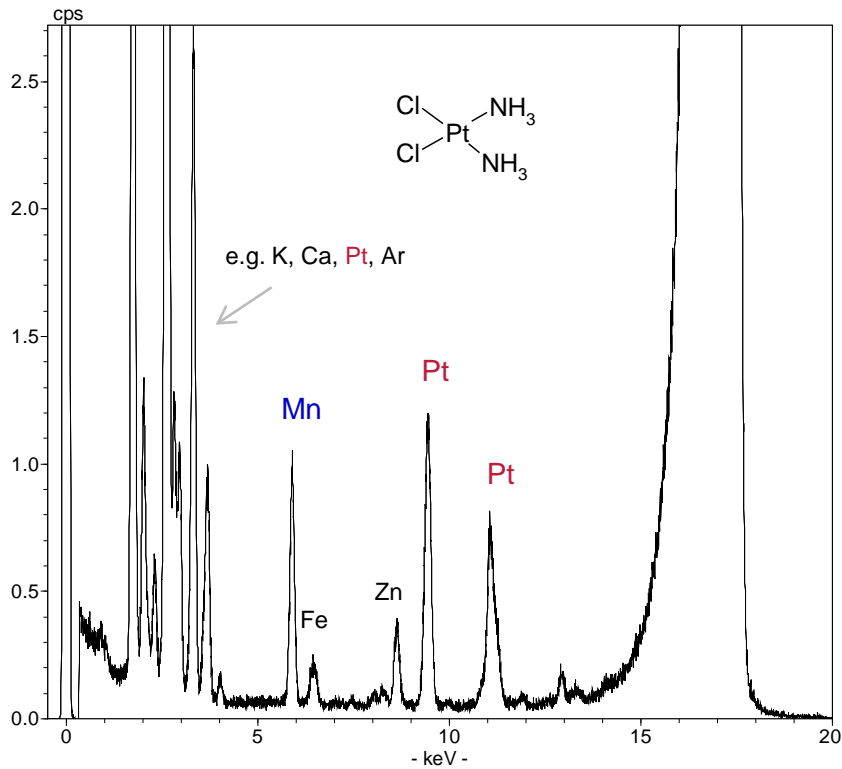
Multielement analysis

LODs: ppb range



Bildquelle: www.bruker.com

Total Reflection X-Ray Fluorescence Spectrometry



Cisplatin

Picofox S2, measuring time: 500 s

samples: **Pt 1 ppm** (5.1 μM) and **62.5 ppb** (0.3 μM) in HT-29 cell suspension (0.5mg/mL)

additives: internal standard **Mn**, 1% PVA

Conclusions

Challenges

Detection Limits

Sample Matrix

Methods



Vielen Dank für die Aufmerksamkeit !

g
croscopy
o-XANES, SXRF)

Raman Microspectroscopy

