CHEM 309 Instrumental Analysis Summary

Overview

Goals: understand strengths and weaknesses; similarities and differences. Make the best choice with respect to cost, accuracy, and time.

Qualitative Analysis	Infrared Spectroscopy (IR), Nuclear Magnetic Resonance Spectroscopy (NMR), Mass Spectrometry (MS), Atomic Emission Spectroscopy (AES)
Quantitative Analysis	Beer's law, ultraviolet/visible spectroscopy (UV/Vis), Atomic Absorption Spectroscopy (AAS), Chromatography (GC, LC, etc.), Mass Spectrometry (MS, ICP-MS the best for inorganic), Atomic Emission Spectroscopy (AES), Florescence/Phosphoresce (F/P), Electrochemistry (Potentiometry, Coulometry)

1. Quant. Techniques

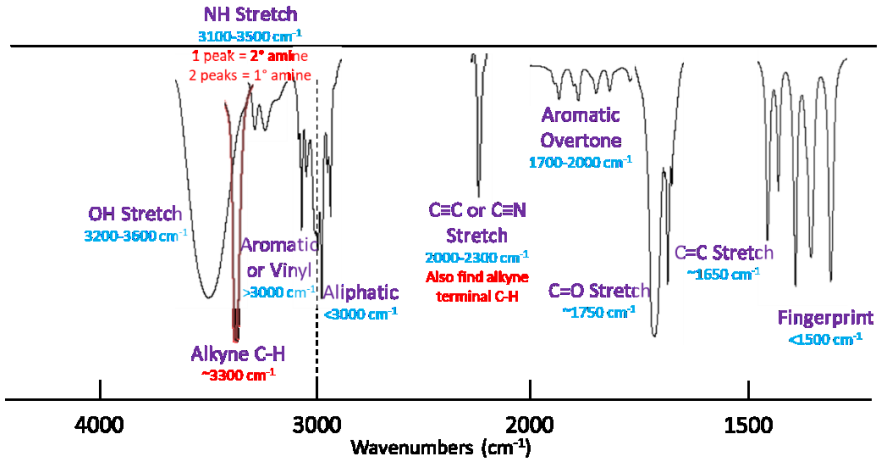
Method	Interpretation
Peak heights/areas	
Calibration curve	Create standards of known solution, run the instrument under known conditions, use peak height or peak area to create curve. Run unknown under same conditions and interpolate.
Beer's law	$A = \varepsilon_\lambda bc = -\log\left(\frac{I}{I_0}\right) = -\log(T)$ Absorption is additive! Linearity concerns: high concentration→dilute; error in %T→keep 15%<T<65%, 0.18<A<0.82; bandwidth in monochromator→small bandwidth, measure λ_{max}; stray light causes low abs.→cut down on optics, black interior; photometric S/N→signal averaging; source flicker→best source or dual beam
Internal standards	Fix the variation in injection volume; compound that has similar structure to analyte can be added in known concentration to each standard and unknown; ratio of peak areas or peak heights eliminates sample to sample variation in injection volume
Standard addition	Know what the analyte is but don't know how much. Adding different (known) amount of analyte into the unknown, plot absorbance vs amount of standard added, x-intercept = negative of initial concentration. One addition: $c_0 = \frac{A_o C_1}{(A_1 - A_0)}$ where C_1 is the amount of standard added.

Concentration Units: ppm (part/whole × 10^{-6}), ppb (10^{-9}).

Analytical Techniques	Method
Chromatography	Peak heights/areas, internal standards, calibration curve
Mass Spec	Peak heights/areas, must account for ionization and detection efficiencies.
Chromatography + MS	Peak area. Selected-ion monitoring (MS) and selected reaction monitoring (MS/MS) used to increase S/N.
Spectroscopy	Beer's Law, Standard Addition, calibration curve

2. Qual. Techniques

Analysis	Interpretation

Chromatography	Compare retention times to those of known standards under the same conditions. Not the best for true qual., but the use of MS improves this dramatically.
Mass Spec	1. Find isotopes from peak ratios (2m/z apart) (1) ^{79}Br:^{81}Br = 1:1, two Br = 1:2:1 for $M^{+\cdot}$ (2) ^{35}Cl:^{37}Cl = 3:1, two Cl = 9:6:1 for $M^{+\cdot}$ (3) ^{32}S:^{34}S = 25:1 for $M^{+\cdot}$ (4) ^{12}C:^{13}C=1:N%, N = number of Carbon (1m/z apart, M+1 peak) 2. Evidence of N: odd MW, odd number of N 3. Look at fragments (EI-MS) through (1) α-cleavage, i-cleavage, (O, N, S, X, C=O)→odd fragments (2) heteroatom rearrangement (O, N, S, X), McLafferty rearrangement (C=O)→even fragments (3) functional groups: aromatics, aliphatic, carbonyl-containing, alcohols, amines 4. IHD = 0.5 * [2C + 2 – H – X + N]. Oxygen does not contribute to IHD – try 1 oxygen if not formula works.
IR	 Evidence of N: NH stretch Evidence of O: OH stretch, C=O stretch
NMR	^{13}C: count the number of unique carbons. Peak (m) at 75-80 is solvent. C=O peak (aldehyde, ketone) >~200 ppm ^{1}H: count the number of unique types of hydrogens; use integral to figure out number of hydrogens in each peak; use splitting and chemical shift (ppm) to figure out positions. Aldehyde (s) shifts 9-10 ppm.

3. Chromatography

3.1 Theory

Separation of compounds. Works under shopping mall analogy where people come in and out of shops on a people mover.

Stationary Phase	Solid	Liquid on solid support	Resin	Polymer
Mobile Phase	Liquid or gas	Gas or liquid	Liquid	Liquid
Chromatography	Adsorption	Partition	Ion-exchange	Size exclusion
Example	TLC, Column	GC, LC		

Plate Theory

(1) Theoretical plates (N): $N = 16\left(\frac{t_R}{w_b}\right)^2$ where t is the retention time and w is the peak width. Larger N, more separation. N depends on the length of the column.
(2) Height equivalent to a theoretical plate (HETP, H): H = L/N. Smaller H means a more efficient separation. Dependent on: technique – GC/LC/CE etc., choice of mobile and stationary phases, temperature, and flow rate

Rate Theory

The cause of peak broadening explained by the Van Deemter equation $H = A + \frac{B}{\bar{u}} + C\bar{u}$ where $\bar{u}$ is the average linear velocity (cm/s). A = eddy diffusion, B = longitudinal diffusion, C = mass-transfer broadening.

Retention factors and resolution

Retention factors gives the separability of compounds; resolution R = 1.5 baseline resolved.

3.2 GC

Instrumentation: Gas tank – flow control – column on the oven – detector - readout
Operation modes (temperature): isothermal or T gradient (more volatile things elute first)
Sample vaporized upon injection.

Detectors	TCD	FID	ECD	FPD	MS
Universality	Most	Almost			Mostly
Destructive	No	Yes			
Sensitivity	Poor	More than TCD			
Comments	Requires reference flow; Least useful	Detect ion current; Most widely used	Selective for X, NO_2, P analytes	Selective for S and P analytes	Ionized by electron impact
Qual./Quant.	Quant.				Qual.

3.3 LC

HPLC Instrumentation: solvent reservoirs – pump – sample loop – column - detector
Mobile phase (solvent composition): isocratic, linear ramp, gradient
Operation modes: normal phase (non-polar elutes first), reverse phase (polar elutes first)

Sample introduction	Notes
Pumps	Syringe pump, reciprocating pump
Injectors	Sample loop, manual or automatic sampling
Columns	Small material, low flow, high sensitivity

Packed column; sample is push at high pressure.

Detectors	RID	UV/Vis	Fluorescence	MS
Universality	Most			Yes
Destructive	No	No	No	Yes
Comments	Compare refractive index; need reference flow; no gradient	Beer's law	Molecules absorb light and emit at a different λ; very selective;	Electrospray compatible

			very sensitive; tagging pre-column and post column	
Qual./Quant.	Quant.			Qual.

3.4 Ion exchange
Stationary phase is polymer with charged side groups, substituted polystyrenes often used
Separation based on charge density

3.5 Size exclusion
Stationary phase is cross-linked polymer with very large, uniform pores
Large MW elute first

4. Electrophoresis

4.1 Gel
Cross-linked polymer, voltage applied across gel; anions migrate towards cathode (down). Only works for charged species. Useful for DNA and protein.
Separation based on charge density (size).

4.2 Capillary Electrophoresis (CE)
Instrumentation: high voltage supply, detector, capillary, buffer
Capillary is fused silica, small sample volumes, and higher voltages leads to very efficient separation (highest theoretical plates). Doesn't work for neutrals. No stationary phase.
Detectors: UV/Vis, fluorescence, MS
Two different flows: electrophoretic flow (ep), and electro-osmotic flow (eo). eo flow > ep flow

5. Mass Spectrometry

Instrumentation: sample introduction – sample ionization – mass analyzer – ion detection – vacuum system – computer
Mass spec measures mass/charge (m/z). It operates at low pressure.
Resolution: $R = M/\Delta M$ to resolve two peaks with same nominal mass but different exact mass. High res. > 10,000. Atomic weights uses average mass in periodic table, no use in MS.

Phase	Method	Comments
Sample introduction	Batch inlet	Only works for volatile, no ionization
	Direct probe	Less volatile
	GC inlet	Jet separator separate analyte from mobile phase, no ionization
Ion sources	Electron impact (EI)	Use with above introduction methods. Creates molecular ion $M^{+\cdot}$ at 70 eV, hard ionization, many fragments
	Chemical ionization (CI)	Soft ionization, pseudo-molecular ions
Combined introduction and ionization sources	Electrospray ionization (ESI)	Soft ionization, pseudo-molecular ions, singly and multiply charged ions. Sample introduced as pre-formed ions in liquid. Compatible with HPLC, CE. Excellent for bio molecules.

	Matrix-assisted laser desorption ionization (MALDI)	Matrix assisted LD, soft ionization, pseudo-molecular ions, singly charged ions

Mass Analyzer	Basis of separation	Mass range	Resolution	Comments
TOF	Velocity	Unlimited	Low (10^3-10^4)	Distribution of KE from ions leaving the source – fixed with reflectron
Reflectron TOF	Velocity	Unlimited	Very high (50,000)	Often combined with MALDI-RTOF
Quadrupole filter	m/z	Up to ~1000	Unit mass	Increase 2U/V (slope in a,q space), increase resolution; cheapest. Often GC-jet separator-EI-Quad
Paul ion trap	m/z	50 – 2000 (4000)	Low (10^3-10^4)	Similar to 2D quad in 3D (Mathieu stability diagram); end cap and ring electrodes
Linear ion trap	m/z	50 – 2000 (4000)	Low (10^3-10^4)	2D trap
Magnetic sector (B)	mv/z	Up to ~2000 amu (higher with higher B)	Medium (1000)	
Double sector (EB)	KE then mv/z	Same as B	Much higher 10^4	First high res instrument, outdated and used in isotopic dating
ICR	m/z	100 - 200,000	v.v. high > 10^6	Ions undergo cyclotron motion and trapped. Ion frequencies Fourier Transformed. Very expensive (>$1M)
Orbitrap	m/z	50 – 2000 (4000)	v.v. high > 10^5 – 10^6	Ions in precession; newest, motion undergo FT; very expensive (>$700,000)
Tandem Mass Spec	MS/MS in space: triple quad (select precursor in Q1, knock apart in Q2, scan for products in Q3). Q1, Q3 mass resolving, Q2 r.f. only. MS/MS in time: ion traps, ICR. Modes: product mode, parent mode, natural loss mode, selected reaction monitoring (SRM)			

Detectors needed for quads, sectors, ion traps, TOF.

Detectors	Comments
Electron multiplier	Most common, cascade of electrons, very sensitive (ion counting)
Daly detector	Ions→electrons→photons→PMT

6. Spectroscopy

Electromagnetic radiation	Spectroscopy
Radiowaves	NMR
Microwave	Microwave
Infrared	IR

Visible/Ultraviolet	Electronic

Selection rules: (1) $\Delta s=0$, (2) $E_{photon} = h\nu = \Delta E_{transition}$, (3) symmetry:
Instrumentation: radiation source – wavelength selection (scanning/broadband) – sample containment – radiation detector – signal processing and readout (Fourier Transform and S/N)

Sources		
IR	Visible	UV
Globar (SiC) Nichrome wire	W filament (320-3500 nm) Hollow Cathode Lamp (line source, metal dependent) Laser (line source, metal dependent, three- and four-levels)	Xe arc lamp (250-600 nm) H_2 (D_2) lamp (160-375 nm)
Wavelength Separators (Monochromators)		
Filter (broad band, low-pass, high-pass, notch available) Prism (non-linear dispersion) Grating (linear dispersion, need to filter higher order harmonics, stray light, costly, compact)		
Detectors		
IR	UV-Visible	
Thermocouple (measure voltage) Golay cell (measure pressure)	Photovoltaic (photon ejects electrons from semiconductor, not powered) Phototube (photon ejects electrons from metal cathode, powered) Photomultiplier tube (PMT) (photon ejects electrons from metal cathode, powered) Photodiode (can be used in arrays to measure multiple wavelengths simultaneously)	

Spectroscopy	Qualitative	Quantitative	Selectivity	Sensitivity	Linearity of Beer's law
Flame AAS	No (lamp)	Excellent (1-2% rel. precision)	Very, provided interferences are accounted for	LOD = 0.3 ppb – 20 ppm	Rel. poor.
GFAAS	No (lamp)	v. good (1-5% rel. precision)	Very, provided interferences are accounted for	LOD = better than flame	Ok
AES	Excellent	Excellent (1-2% rel. precision)	Very, provided interferences are accounted for	LOD = sub ppb	Excellent
UV-Vis absorption	Poor	v. good (1-3% rel. precision)	Not great due to broad bands	10^{-4} – 10^{-7} M depending on e	Ok
F/P	Poor	Excellent	Very	LOD = sub ppb	Best

6.1 Atomic Absorption Spectroscopy (AAS)
Instrumentation: source (HCL) – chopper – sample introduction (nebulizer and flame atomizer or graphite furnace atomizer) – monochromator (grating) – detector (PMT)

Transition between electronic states (uv/vis or higher); line spectra in gas phase; atomic transitions are sharp (natural linewidth ~10^{-5}nm + Doppler broadening + pressure broadening)

We want flame atomization, no excitation, ionization, nor oxidation; atomization efficiency depends on flame (fuel, oxidant, part of flame, temperature), metal, concentrations, etc.

GFAAS over FAAS

Strength	Low LOD, higher sensitivity, small sample volumes, no noise from flame
Weakness	Cost, time, less precise

Interferences
Chemical (→use modifiers): (1) anions make poorly volatile salts→add releasing agent; (2) avoid oxidation→add a different metal to get reduced preferentially/releasing agent; (3) avoid ionization→add a different alkali metal
Spectral overlap (molecular impurities)→have second D_2 lamp

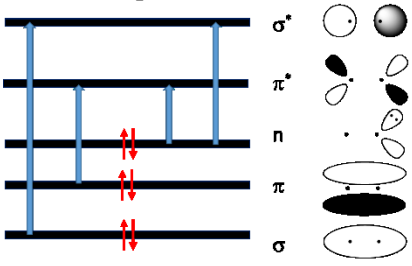
6.2 Atomic Emission Spectroscopy (AES)
Instrumentation (single wavelength scanning): source – grating – detector
Instrumentation (multiple wavelength): sample – ICP – [grating – prism – CCD detector] (Echelle Monochromator)

AAS vs. ICP-AES

Criteria	AAS	ICP-AES
Qualitative analysis	Poor (change lamps)	Excellent
Quantitative analysis	1 at a time	Multiple elements simultaneously
Limit of detection (LOD)	FAA: ppm-ppb GFAA: ppb-ppt	ppm-ppt
Linearity	Relatively poor (1-3 orders of magnitude)	Excellent, ppb-%
Precision	FAAS: 1-2%; GFAAS: ~5%	1-2%
Technical requirements	low	high
Cost	~$30-70K	~$100-200K

6.3 Molecular Absorption Spectroscopy (UV/Vis)
Instrumentation (photometer, one λ): lamp – lens – bandpass filter – cell – photodetector – readout
Instrumentation (spectrophotometer, λ selection, single beam): light – diffraction grating – aperture – sample – detector
Instrumentation (spectrophotometer, double beam): D_2 and Tungsten lamp – filter – monochromator – beam splitter – sample + reference – photodiode – readout
Instrumentation (all λ): Tungsten and D_2 lamp – lens – shutter – sample – lens – slit – grating – diode array

Transition between electronic, vibrational, rotational states. Gas phase electronic spectra resolved, solution spectra broadened.

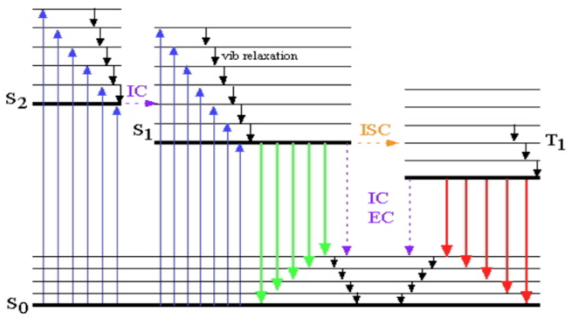


σ → σ*: high energy, not useful analytically
n → σ*: λ_{max} ~ 150 – 250 nm, solvent cutoff
π → π*: λ_{max} ~ 200 – 400 nm, high energy
n → π*: λ_{max} ~ 400 – 700 nm

Chromophore, auxochrome, bathochromic shift (red shift due to conjugation and resonance, more analytically useful), hypsochromic shift (in polar solvent)

6.4 Molecular Emission (F/P)
Instrumentation (fluorometer, no scanning): source – filters – detector
Instrumentation (spectrofluorometer, scanning, single or double beam): source – excitation grating monochromator – sample – emission grating monochromator – detector

IC = Internal conversion (between states of same multiplicity)
ISC = Intersystem crossing (between states of different multiplicity)
IVR = Intramolecular vibrational relaxation
F = radiative transition between two states of same multiplicity
P = radiative transition between two states of different multiplicity; heavy atom effect →spin-orbit coupling→ISC→P



At high concentration, self-quenching, self-absorption will decrease F power.

6.5 Infrared Spectroscopy (IR)
Instrumentation (scanning): IR source – sample + reference – chopper – monochromator – detector
Instrumentation (Fourier Transform): IR source – Michelson interferometer – sample – detector
Vibration mode: bend, symmetric stretch, asymmetric stretch.

$$\tilde{\nu}_{obs} = \frac{1}{2\pi c}\left(\frac{k}{\mu}\right)^{\frac{1}{2}}$$

Selection rules: Δv = 1, change in overall diploe moment.

6.6 Nuclear Magnetic Resonance Spectroscopy (NMR)
Instrumentation: shim coils, excite coils, detect coils, de-coupling coils (in ^{13}C NMR).
Nuclei have quantized spins (I), add a magnetic field, energy becomes $E = -\gamma\hbar m_I B_0$. Radio frequency EM radiation is used to promote ½ of the excess ground state spins to the excited state, and then spins relaxed through spin-lattice (T_1) or spin-spin (T_2).

Chemical shift (shield, de-shield, effective magnetic field, downfield, upfield)
$\delta = \frac{\nu_{sample} - \nu_{TMS}}{\nu_{reference}} \times 10^6$ where ν_{TMS} is the reference frequency of B_0.

Magnetic anisotropy, spin-spin coupling and splitting.

7. Electrochemistry

Balancing redox reaction from Gen Chem.: half reactions → mass balance (expect O and H) → balance deficient O side with H_2O → balance deficient H side with H^+ → balance charge by adding electrons → add all up → if in base, add OH^- to both sides cancel out H^+ in balanced reaction

Concentration effects: $E = E^0 - (0.0592/n) \log Q$, Q put in gas phase (in pressure) and ion phase (in concentration).

7.1 Potentiometry

Instrumentation: Galvanic (Voltaic) cells with reference electrode, indicator electrode, voltage measuring device (high Ω)

$\Delta E_{cell} = E_{ind} - E_{ref}$

Reference electrodes	Standard hydrogen electrode (SHE) $2\ H^+ + 2\ e^- \rightarrow H_2$ (E^0=0V) Dangerous, overpotential problem
	Saturated calomel electrode (SCE) $Hg_2^{2+} + 2\ e^- \rightarrow 2\ Hg\ (l)$ ($E^0 = 0.788V$)
	Ag/AgCl $Ag^+ + e^- \rightarrow Ag(s)$ ($E^0 = 0.80$ V) $AgCl + e^- \rightarrow Ag + Cl^-$ ($E^0 = 0.228$ V)
Indicator electrodes	Direct type (1st order): a metal sensing its own ion in solution
	Indirect type (2nd order): metal electrode responding to its ligand product (anion)
	3rd order: response of metal indicator electrode to another cation through a common anion.
	Inert electrodes: used in ½ reactions involving only ions
	Membrane indicator electrodes: combination of reference electrode and membrane electrode immersed in known concentration of analyte

7.2 Coulometry

Instrumentation: Electrolytic cells

Potential Problems	Explanation	Fixation
iR Drop	Flow of electrons through electrolytic circuit encounters a resistance	Keep Ω low – use lots of electrolyte Keep i low – don't force current
Concentration polarization overpotential (η_c)	[ ] of electroactive species is depleted near the electrode (concentration effect)	Large surface area electrodes and stir solution
Activation overpotential (η_k)	Voltage develops due to charging of electrode because of slow e^- transfer across solution electrode boundary (kinetic effect)	Large surface area electrodes
$\Delta E = E_{cathode} - E_{anode} + (\eta_{cc} + \eta_{ck}) + (\eta_{ac} + \eta_{ak}) - iR$		

Modes of operation	Explanation
Constant potential	Hold ΔE constant, current changes over time, measure $\int qdt$.
Constant current	Hold current fixed, (ΔE changes), measure $i \times t$; add mediator, used in titration
Electrogravimetry	Constant i, measure mass (product deposited at cathode)