Analytical Cumulative Examination

Reversed phase chromatography (RPC) is ubiquitous in analytical chemistry.

1. What is the mechanism by which substances are separated in RPC?

Most organic compounds have some degree of hydrophobicity. The ease with which hydrophobic species are dissolved in polar, hydrogen bonding solvents is a function of the solvophobic effect. Hydrogen bonding in polar solvents causes surface tension and makes it more difficult to create a cavity in the solution in which the hydrophobic species can reside. This solvophobic effect causes hydrophobic molecules to associate intra- and interspecifically and in the course of doing so reduce their hydrophobic contact area with polar solvents. The greater the hydrophobic contact area with the solution and the more hydrophobic a molecule is per unit of contact area, the greater the solvophobic effect

In RPC, columns generally have an octadecyl stationary phase. Surface tension in the polar mobile phase provides the driving force (solvophobic effect) that causes hydrophobic analytes to associate differentially and reversibly with the octadecyl stationary phase. The strength of this interaction is diminished by adding a miscible, but less polar organic solvent to the polar initial mobile phase. Running a gradient of mobile phase ranging from pure, polar stationary phase (which is usually water) to a high percentage of more non-polar organic solvent (such as acetonitrile or methanol) allows a broad spectrum of organic compounds to be separated by RPC.

2. It would be common with a series of hexapeptides of identical amino acid composition that RPC could resolve the mixture into multiple groups. How would this occur in light of your answer above?

Amino acids vary substantially in hydrophobicity based on the chemical structure of their side chain. Those that have alcohols, sulfhydryl groups, amines, and carboxyl groups in their side chains are more polar. The presence of aromatic groups and large numbers of methylene groups in side chains increases hydrophobicity. Tryptophan and phenylalanine are among the more hydrophobic amino acids.

Most peptides, especially a peptide with six amino acids will have sufficient hydrophobicity to interact with the octadecyl stationary phase on an RPC column when the mobile phase is water. However, the hydrophobic contact area with the RPC column will vary by the position of amino acids in the peptide sequence, even when the amino acid composition is identical. Placing two hydrophobic amino acids beside each other in the peptide sequence produces greater hydrophobic contact area than when they are widely

separated by polar amino acids. This near neighbor effect allows peptides of the same amino acid composition to be separated in most, but not all cases.

3. There is currently great interest in capillary RPC columns. Why is this? What are the advantages of capillary chromatography columns?

It is frequently the case that one has a limited, fixed amount of sample for analysis. The concern then is how to get the maximum signal from the limited amount of analyte in the sample.

When substances are eluted from a chromatography column they are diluted by the mobile phase. The degree to which they are diluted is directly proportional to the square of column radius. This means that if we place the same amount of sample on columns of different column radius, detection sensitivity will be inversely proportional to the square of their column radius.

The diameter of a common RPC column is 4.6 mm while that of a miniature RPC column is 1 mm, but analyte concentration from the 1 mm column will be 21 times higher. Going to a 100 um diameter capillary column, analyte concentration will be 2100 times higher than from a 4.6 mm column. Clearly, there is over a thousand fold gain in sensitivity by doing nothing more than using a capillary column in the analysis.

4. How is mass spectrometry being used to identify peptides eluting from RPC columns?

The sequence of the human genome and that of many other organisms is known. Based on our knowledge of the genetic code it is possible to predict the amino acid sequence of proteins derived from genes of known sequence. Moreover, the molecular weight and sequence of all tryptic peptides derived from these proteins can be predicted.

Mass spectrometry can determine the molecular weight of peptides and in many cases it is possible through collision induced dissociation and tandem mass spectrometry to partially deduce the amino acid sequence of a peptide based on fragment ions generated through cleavage at peptide bonds.

Peptides and proteins can be identified from this experimental data by going back to genomic data and seeing if one can find a match in terms of both peptide molecular weight and peptide sequence. In view of the fact that most proteins contain more than 30 tryptic peptides, if it is possible to find 3-15 peptides from the same protein it is probably present in a sample.

No Biochemistry crib available

December 8, 2007

Written by Professor Skrynnikov

INORGANIC CHEME EXAM — CRIB

December 8, 2007

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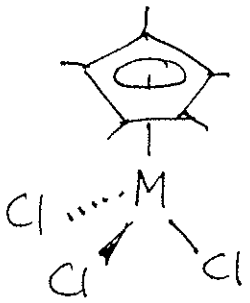
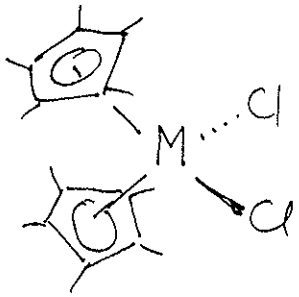
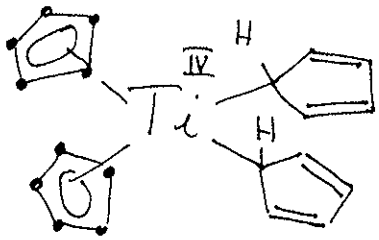


electron count 12



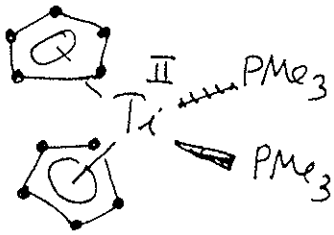
electron count 16

(i)



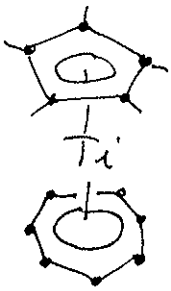
electron count 16

(ii)



electron count 18

(iii)



electron count 16

While most bis-Cp complexes of the 1st transition series have the ferrocene type of sandwich structure ($(C_5H_5)_2Ti$ and $(C_5H_5)_2Mn$ are exceptions), many of the 2nd and 3rd transition series do not. The <u>early transition elements</u> Zr, Hf, Nb, Ta, Mo and W have more complicated structures (like Nb), which most likely reflects the comparative ease of oxidizing the M(II) oxidation state, as would be present in a $(\eta^5\text{-}C_5H_5)_2M$ structure. In the case of niobocene, there are two structural forms, both of which contain contain diniobium units and Nb in a higher oxidation state. Osmocene, like its ferrocene congener, has a stable 18-electron count and, therefore, has a sandwich structure. If rhenocene were $(\eta^5\text{-}C_5H_5)_2Re$, it would have a 17-electron count; it dimerizes by forming a Re-Re bond to give a more stable 18-electron molecule.

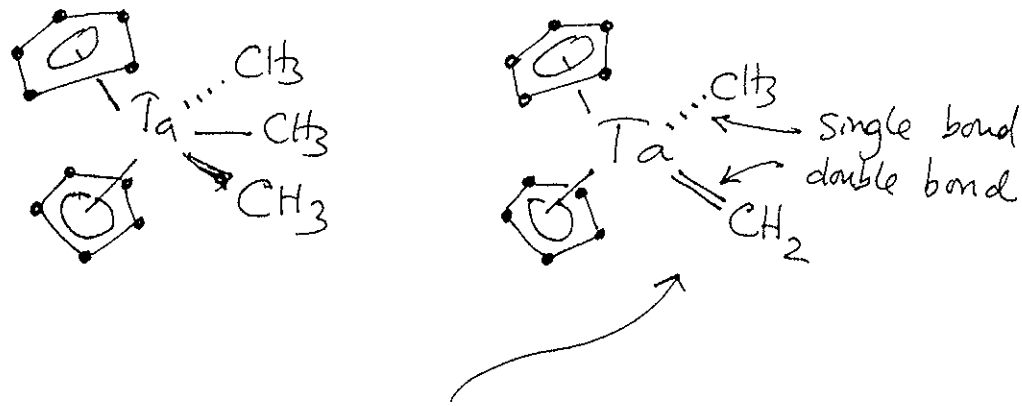
3) (i) $2\,TaCl_5 + 3\,Me_2Zn \longrightarrow 2(CH_3)_3TaCl_2 + 3ZnCl_2$

(ii) $(CH_3)_3TaCl_2 + 2(C_5H_5)Tl \longrightarrow (\eta^5\text{-}C_5H_5)_2Ta(CH_3)_3 + 2TlCl\downarrow$

(iii) $(\eta^5\text{-}C_5H_5)_2Ta(CH_3)_3 + \underset{\text{(electrophile)}}{Ph_3C^+BF_4^-} \longrightarrow [(\eta^5\text{-}C_5H_5)_2Ta(CH_3)_2]^+BF_4^- + Ph_3CCH_3$

(iv) $[(\eta^5\text{-}C_5H_5)_2Ta(CH_3)_2]^+BF_4^- + \underset{\text{(strong base)}}{Li[N(SiMe_3)_2]}^{*} \longrightarrow (\eta^5\text{-}C_5H_5)_2Ta(CH_3)(CH_2) + (Me_3Si)_2NH + Li^+BF_4^-$

* Other strong bases such as $Me_3P{=}CH_2$ or NaOMe can be used

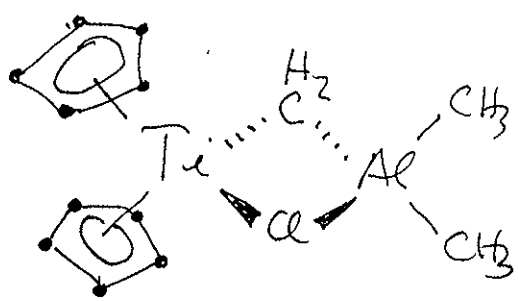


<u>Special significance</u>: example of an alkylidene (carbene) complex formed by an <u>intermolecular</u> α-H abstraction reaction (of the cationic dimethyl intermediate).

4). Tebbe's reagent is $Cp_2Ti(CH_2)(Cl)Al(CH_3)_2$

$$(\eta^5\text{-}C_5H_5)_2TiCl_2 + 2\,Al(CH_3)_3 \longrightarrow$$
$$(\eta^5\text{-}C_5H_5)_2Ti(CH_2)(Cl)Al(CH_3)_2 + CH_4 + AlCl(CH_3)_2$$

or $$(\eta^5\text{-}C_5H_5)_2Ti(CH_3)_2 + AlCl(CH_3)_2 \longrightarrow$$
$$(\eta^5\text{-}C_5H_5)_2Ti(CH_2)(Cl)Al(CH_3)_2 + CH_4$$

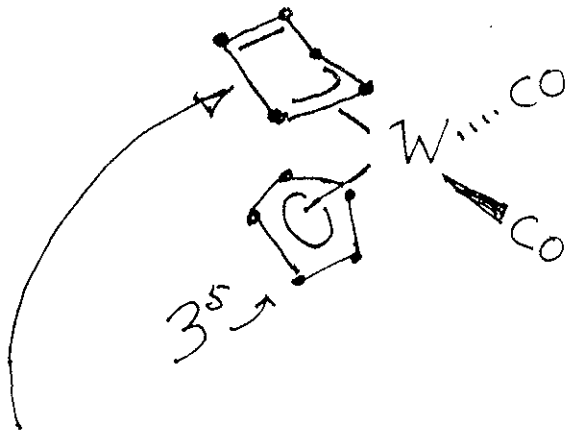


Tebbe's reagent is used for methylene tranfer as in the homologation of olefins and the conversion of ketones to terminal olefins.

5) A good example of a complex that contains a η^3-C_5H_5 ligand is

$$(C_5H_5)_2W(CO)_2$$

If both C_5H_5 ligands were η^5-bound this compound would have a 20-electron count for the metal. In order not to exceed the stable 18-electron count one of the Cp rings binds as an η^3-ligand

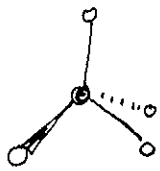


Electron count	
W	6
2×CO	4
η^5-Cp	5
η^3-Cp	3
	18-e

η^3- non-planar (folded) ring which behaves like an allyl-ene

B (i) $TiCl_4$ (VCl_4 is another example; d^1)

$$Ti \xrightarrow[\text{heated tube}]{Cl_2} TiCl_4 \text{ (can be distilled)}$$

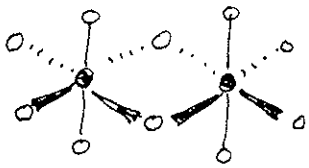


Tetrahedral

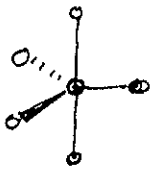
Ti(IV) is d^0; no $d \leftrightarrow d$ transitions but $Cl_\pi \rightarrow Ti_d$ electron-transfer transitions occur to give orange-yellow color.

(ii) MCl_5 M can be Nb, Ta, Mo, W or Re

Nb, Ta, Mo & Re prep. by chlorination of the metal powder in a heated tube furnace *i.e.* $M + Cl_2$.
WCl_5 is formed by thermolysis of WCl_6 or reduction of this same halide.
Structures in the solid-state are



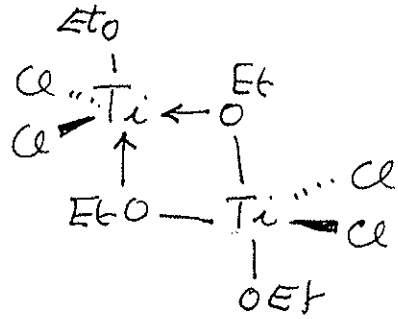
In gas phase



(trigonal bipyramidal)

Nb, Ta are d^0.
Mo, W are d^1 – pairwise interactions form M–M bond.
Re is d^2 – pairwise interactions form Re=Re bond.

(iii) e.g $TiCl_4 + 2EtOH \longrightarrow TiCl_2(OEt)_2$



Ethoxide bridged dimer Ti has approx. trigonal bipyramidal geometry.

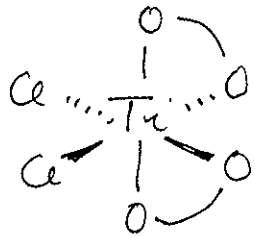
Ti(IV) is d^0; no d↔d transitions and compound is colorless.

(iv) e.g $TiCl_2(acac)_2$

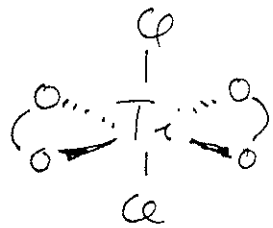
$$TiCl_4 + 2\,Hacac \xrightarrow{\text{toluene}} TiCl_2(acac)_2 + 2\,HCl$$

$TiCl_2(acac)_2$ can have a <u>cis</u> or <u>trans</u> octahedral structure.

<u>cis</u>



<u>trans</u>



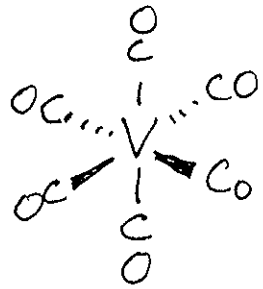
Ti(IV) is d^0; no d↔d transitions and compound is a pale yellow color.

(v) $V(CO)_6$ is the only example.

$$VCl_3 + CO + xs\ Na \xrightarrow[120^\circ;\ \text{high } p]{\text{diglyme}} [Na(\text{diglyme})_2]^+[V(CO)_6]^-$$

$$V(CO)_6 \xleftarrow[\text{2) sublime at } 50^\circ]{\text{1) } H_3PO_4}$$

$[V(CO)_6]^-$ can also be formed by the $Na^+C_{14}H_{10}^-$ (sodium anthracenide) reductive carbonylation of $VCl_3(THF)_3$.

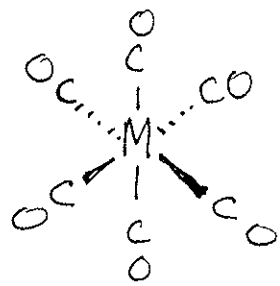


V(0) is d^5 and paramagnetic. $V(CO)_6$ is a 17-electron paramagnetic molecule. Easily reduced to $[V(CO)_6]^-$ which is diamagnetic (18-e).

(vi) $[M(CO)_6]^{2-}$ for M = Ti, Zr or Hf

$$MCl_4 \xrightarrow[\text{or cryptand, CO, } -70^\circ C \text{ in THF}]{K^+C_{10}H_8^-,\ \text{crown ether}} [M(CO)_6]^{2-}$$

$K^+C_{10}H_8^-$ is a strong reducing agent; crown ether or cryptand complexes the K^+ ions.



18-e species; diamagnetic.

No Organic crib available

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Written by Professor Thompson

No Physical crib available

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Written by Professor Towns