No Analytical crib available
January 15, 2011
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Ph# 40498

No Organic crib available
January 15, 2011
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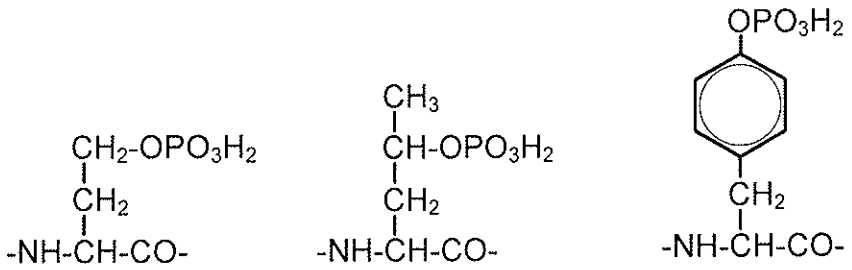
Biochemistry Cume On Post-Translational Modification
January 15, 2011

There are more than 100 types of post-translational modifications (PTMs) found in proteins. One of these is phosphorylation. It has been reported that proteins can be phosophorylated at up to 50 or more sites.

1. What types of phosphorylation are seen in proteins, e.g. which amino acids are modified, how does phosphorylation occur, and what are the chemical structures of the products?

Answer.



phosphoserine phosphothreonine phosphotyrosine

Phosphorylation generally results from a kinase phosphorylating a specific serine, threonine, or tyrosine residue through addition of a phosphate group from ATP.

2. What are some of the functions of these various types of phosphorylation, e.g. what biological role do they play.

Answer. Phosphorylation can act as a molecular switch, causing a protein to go from one state to another, in structural organization as needed in assembling molecular complexes, signal transduction, regulation, triggers apoptosis and necrosis, enzyme activation, molecular recognition, as an allosteric effector, and in energy metabolism.

3. There can be many different isoforms of a phosphoprotein. How would you separate them from each other for identification?

Answer. Isoforms in this case can vary by charge, by mass, and also by position of a phosphate ester group. By far the best way to separate phosphoproteins would be by isoelectric focusing using an immobiline slab gel at 4-10 °C for 24 hr. Under these conditions separation of isoforms that vary in total number of phosphate groups is easy. Isoforms that vary in position can also be separated because the environment in which the phosphate group is located can

cause small differences in pI. SDS gel electrophoresis gives very poor resolution because relative differences in molecular weight between isoforms are so small.

Anion exchange chromatography (AEC) where elution is achieved at pH 7-8 in either the ascending salt gradient mode of elution or a pH gradient can be used. Ionic strength gradient elution is the highest resolution. AEC is less favorable because separation is dominated by charge at a specific location on the surface of a protein and not all the phosphate residues will be in that area.

Mass spectrometry is the least favorable because most instruments lack the requisite resolution and many large proteins difficult to ionize although credit was given for MS as an answer. The are very few reports in the literature of using MS to separate phosphorylated proteins.

4. How would you identify the sites of phosphorylation in each isoform you have resolved, e.g. where has phosphorylation occured in the primary structure and what amino acid(s) are involved.

Answer. The approaches most widely described in the literature are based on mass spectrometry (MS). A sample in which the phosphoprotein of interest has either been enriched or purified is the best. Samples are tryptic digested and the phosphopeptides are often selected from the digest through either IMAC, titanium oxide affinity chromatography, or with an antibody in the case of tyrosine phosphate. Phosphopeptides thus resolved are then subjected to gradient elution reversed phase chromatography, electrospray ionization, and some version of MS/MS. The first dimension of MS may be achieved is generally achieved with either an ion trap or quadrapole selector. Time-of-flight (TOF) is also used occasionally. The molecular ion is then fragmented by collision induced dissocation (CID) and the fragment ions analyzed in an dimension of MS, often with a TOF but quadrapole selectors are used as well. Post-CID fragments ions generally indicate the presence of phosphate and at least particial or total sequence of the peptide. The position of the phosphate ester in the sequence is revealed as well. The greatest problem in indentifying phosphate esters in a protein is when multiple phosphate groups are contained within a single tryptic peptide. Such peptides are much more difficult to ionize and fragmentation is very complex.

Location of phosphate esters in a protein by analysis of the intact proteins is also used, but much less frequently, and generally only with proteins that have a low degree of phosphorylation. The problem in this "top-down" approach is that fragmentation does not always occur at the location of phosphorylation, the fragment obtained may be difficult to sequence, and you many have to go to higher dimensions of MS to obtain phosphorylate site information.

Many of you used antibodies as an approach to locate phosphorylation. There are very few cases where this has been reported. First, you have to already know where phosphorylation occured and have prepared an Ab that recognizes that site alone. Second, it would only be with tyrosine phosphate. I know of no case where it is possible to see threonine or serine phosphorylation sites in a protein. And third, very few antibodies recognize sequence around a

site of phosphorylation. It is for this reason one can obtain an Ab that recognizes tyrosine phosphate in general, but there are very few where people claim to be recognizing a site of phosphorylation.

5. How would you quantify the total amount of a particular protein and the fractional amount of the protein in each of the phosphorylated isoforms.

Answer. The total amount of protein might be obtained by an immunological assay that does not discriminate between isoforms, by SDS-PAGE with Sypro Ruby staining or with a Western blot. Isoforms could be quantified in several ways. One would be through gel staining after IEF using any of the stains noted above. Western blotting would be best because it would not report unresolved protein that had comigrated with the protein. Some of you suggested use of 2-D electrophoresis using IEF in the first dimension and SDS-PAGE in the second dimension. This is even better, especially with Western blotting.

Mass spectral analysis of all the tryptic peptides described above can also be used but is far less desirable. The problem with MS is that the great difference in ionization efficiency between peptides. Because two peptides give the same ion current in the MS does not mean they are of the same concentration. The solution to this is through the use of isotopically labeled internal standards in the socalled multiple reaction monitoring approach. No one described this approach.

Theorectially multiple, separate immunological assays could be used, one for the total protein and others for the isoforms; but I don't know that anyone has ever reported such a case.

Inorganic Cumulative Exam – Hint / Partial Soln

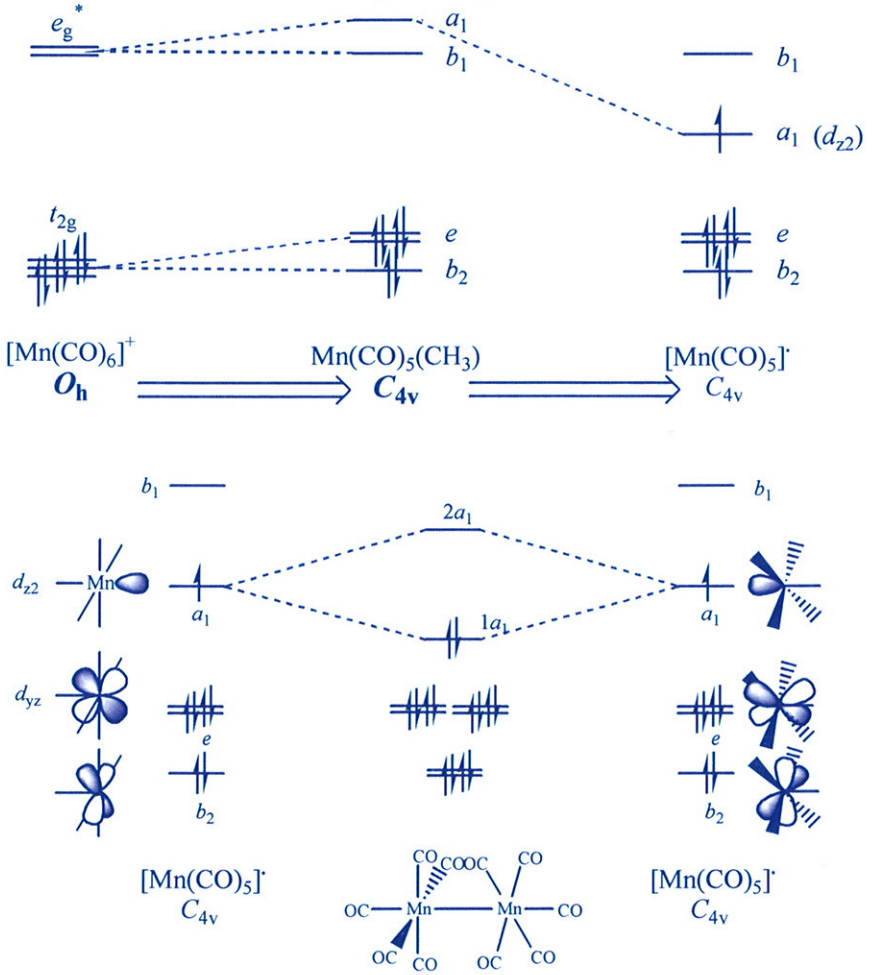
January 15, 2011

1 (20 pt) One of the grand challenges in combating global warming is to reduce CO_2 to CH_4 and other fuels. (A, 5pt) How many electrons are required to reduce CO_2 to CH_4? (B, 15 pt) During the stepwise reduction of CO_2, the first step ($CO_2 \rightarrow CO_2^{-1}$) is known to be the most energetically demanding. Provide a detailed rationale based on electronic structures.

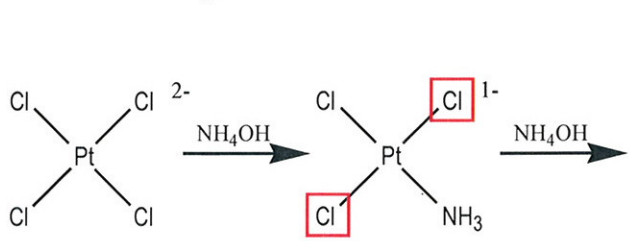
A: CO_2: +4; CH_4, -4; 8 e^-

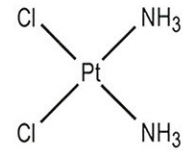
B: CO_2 is linear (Lewis dot structure??); CO_2^{-1} is bent (Lewis dot structure??); very large reorganization energy

2 (30 pt) Photolysis of $MeMn(CO)_5$ results in a transient radical $Mn(CO)_5$, which dimerizes to yield $Mn_2(CO)_{10}$. Construct the MO diagrams (*d*-orbitals only) for each of three species using the *symmetry descent* technique and discuss the nature of Mn-Mn bonding. (Hint: think about the *d*-orbital splitting in $M(CO)_6$; Useful character tables on the following page; NO CREDIT without the use of symmetry)

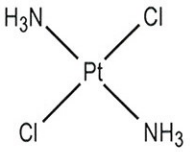


3 (20 pt) *Cisplatin* (*cis*-$PtCl_2(NH_3)_2$) is the most celebrated inorganic antitumor drug. (A, 15 pt) Please devise the synthetic routes with concise rationale for both *cis*- and *trans*-$PtCl_2(NH_3)_2$ assuming the availability of K_2PtCl_4, $[Pt(NH_3)_4]Cl_2$, and common laboratory reagents such as HCl(*aq*), NH_4OH, NaOH(*aq*) and HNO_3(*aq*). (B, 5 pt) Predict the color of $PtCl_2(NH_3)_2$ with a brief justification.





dominant (the trans-effect of Cl^- activates ligand shown in red square)



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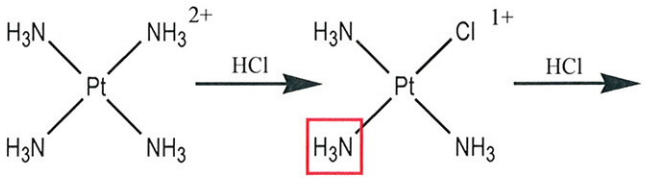

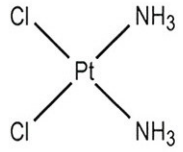

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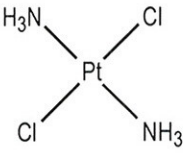


dominant (the trans-effect of Cl^- activates ligand shown in red square)

Both $PtCl_2(NH_3)_2$ are off-white (light yellow) since possible transitions (*d-d*, and LMCT) are all very high energy.

4 (30 pt) Chemistry Nobel Laureates from the last four decades include four inorganic chemists: Ernst Otto Fischer and Geoffrey Wilkinson (1973), Henry Taube (1983) and Richard R. Schrock (2005). Their prize-winning contributions have been featured prominently in modern inorganic textbooks. Please describe the prize-winning contributions of any two Laureates of your choice in details (one page per Laureate).

The Nobel Prize in Chemistry 1973 was awarded jointly to Ernst Otto Fischer and Geoffrey Wilkinson *"for their pioneering work, performed independently, on the chemistry of the organometallic, so called sandwich compounds"* For the rest of details see Huheey's 4th Ed. Pp.669-686

The Nobel Prize in Chemistry 1983 was awarded to Henry Taube *"for his work on the mechanisms of electron transfer reactions, especially in metal complexes"*. For the rest of details see Huheey's 4th Ed. Pp. 557-570

The Nobel Prize in Chemistry 2005 was awarded jointly to Yves Chauvin, Robert H. Grubbs and Richard R. Schrock *"for the development of the metathesis method in organic synthesis"*. For the rest of details see Huheey's 4th Ed. p.658;

CUMULATIVE EXAM FOR PHYSICAL CHEMISTRY

NAME_____

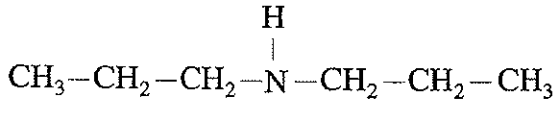
Solved Exam

This exam consists of two (2) questions plus a unit conversion Table as the last page. Be sure your exam booklet is complete.

Assume, **unless otherwise stated**, that throughout this exam, the harmonic oscillator-rigid rotor approximation provides a valid description of the rotational and vibrational motions of all molecules.

Assume, **all atomic masses are equal to the mass number**. Thus assume, for example, that the atomic mass of $^{16}O = 16.0$ amu, the atomic mass of $^{2}H = 2.0$ amu, and so on.

1. Consider the nonlinear DPA molecule. It has the following chemical structure:



(1.1)

a. Answer questions (i)-(iii) by writing in the correct numbers

(i) T **translational degrees of freedom** where

$T =$ 3

(ii) R **rotational degrees of freedom** where

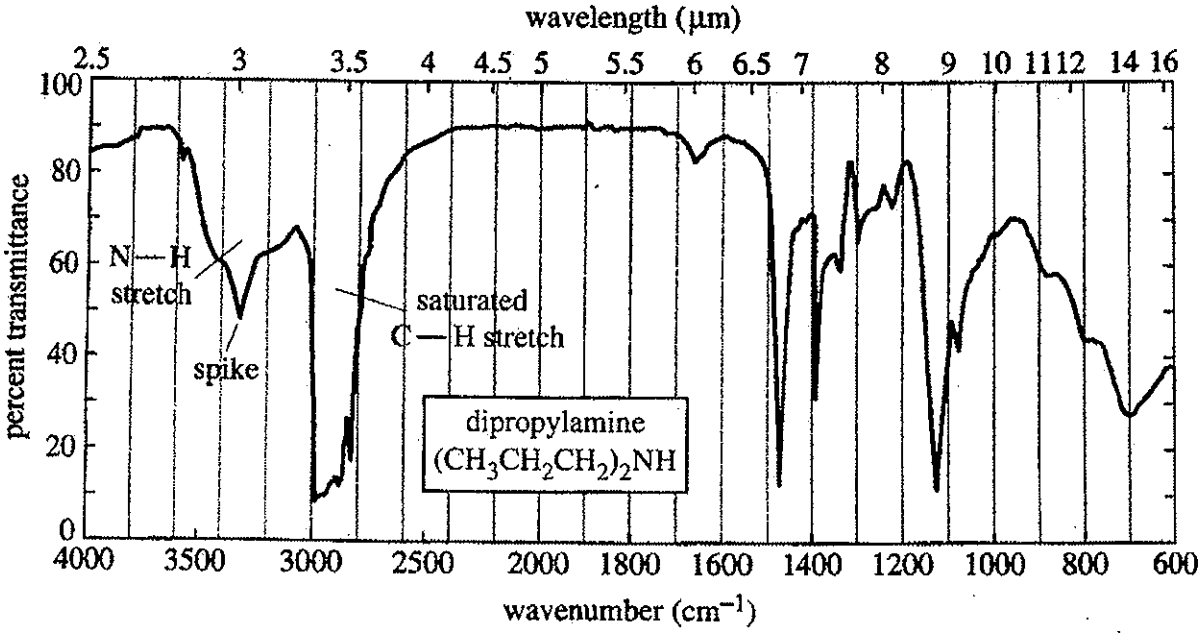
$R =$ 3

(iii) V **vibrational degrees of freedom** where

$V =$ 60

$3N-6 = 3(22)-6 = 60$ normal modes

The rest of Problem One will deal with two ideal gases. Each gas is composed of N molecules in a volume V at temperature T. For **gas 1**, all of the molecules are dipropylamine (DPA). For **gas 2**, one-half of the molecules are DPA and one-half are a different substance X. Below is the IR spectrum of DPA.



b. From statistical thermodynamics, one may show for **either gas** that **at high T** the constant volume heat capacity $C_v(T)$ may be written as

$$\lim_{T\to\infty} C_V(T) = ynR \tag{1.2}$$

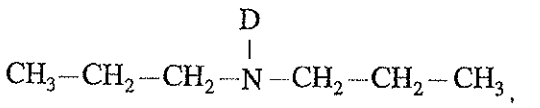
where $n = N/N_A$ is the total number of moles, where $R = N_A k$ is the gas constant, and where y is a numerical parameter.

(i) What is the numerical value of y for gas 1?

$y = 6$ since

$C_v \approx \left(\frac{3}{2} + \frac{3}{2} + 60\right) nR$

(ii) If the substance X is the **deuterated isotope** of DPA



(1.3)

what is the numerical value of y for gas 2?

$y = 63$

Since $C_V = \left[\frac{3}{2}(63) + \frac{3}{2}63\right]nR$

(iii) If the substance X is the **fluorine molecule** F_2, what is the numerical value of y for gas 2?

$y = 3325$

since

$C_V = \left[\left(\frac{1}{2}63 + \right.\right.$ $1/2\left(\frac{3}{2} + \frac{2}{2} + 1\right)$ $\left.\left.\right]\right.$ $nR = \quad R$

c. Will the IR spectrum of gas 2 be the same as or different from the IR spectrum of gas 1, shown in Figure 1.1, if

(i) substance X is the deuterated isotope of equations 1

$y = 63.5$

Due to isotope effect, N-H peak will be halved in intensity and a new N-D peak will form around 24,09 cm^{-1}.

$\mu(N-H) = 0.933$ $\mu(N-D) = 1.75$

$\tilde{\nu}_e(N-H) \sim 33.00\ cm^{-1}$

$\frac{\omega_e(N-H)}{\omega_e(N-D)} = \sqrt{\frac{\mu(N-D)}{\mu(N-H)}}$ $\therefore \tilde{\omega}_e(N-D) \sim 24.09\ cm^{-1}$

Spectrum will be same because F_2 is IR inactive. (~~Wrong~~)

However, the peak intensities will be halved because there is only 50% of dipropylamine in the given volume.

2.

For a generic ideal gas reaction $aA + bB \leftrightarrow cC + dD$, statistical thermodynamics gives the following result for the equilibrium constant $K_{eq}(T)$

$$K_{eq}(T) = \frac{\left[q_C^{\oplus}(T)/N_A\right]^c \left[q_D^{\oplus}(T)/N_A\right]^d}{\left[q_A^{\oplus}(T)/N_A\right]^a \left[q_B^{\oplus}(T)/N_A\right]^b} \exp\left[-\frac{\Delta E_0}{RT}\right]. \qquad 2.1$$

In equation (5.1), all of the $q^{\oplus}(T)$'s are of the form

$$q^{\oplus}(T) = Q^{tr}(T, V^{\oplus}) Q^{rot}(T) Q^{vib}(T) g_0^{el}, \qquad 2.2$$

where the superscript $\oplus$ denotes one mole of gas at pressure p = 1 bar; while

$$\Delta E_0 = -N_A\left[cD_{C,0} + dD_{D,0} - aD_{A,0} - bD_{B,0}\right], \qquad 2.3$$

where $D_{c,0}$, etc., are the **negatives** of the **ground state energies** for atoms and the **ground state quantum dissociation energies** for molecules. Note that in all these equations, N_A is Avogadro's number.

In this problem we will consider $K_{eq}(T)$ for the following isotopic substitution reaction

$$D + HCl \rightleftarrows H + DCl \qquad 2.4$$

where $H \equiv {}^1H$, $D \equiv {}^2H$, and $Cl \equiv {}^{35}Cl$. Note that infrared spectroscopy gives for the HCl molecule

$$\tilde{\omega}_e(HCl) = 2889.7\ cm^{-1} \text{ and } \tilde{B}_e(HCl) = 10.59\ cm^{-1}. \qquad 2.5$$

a. Show that for the reaction of Eq. (2.4), Eqs. (2.1) and 2.3) specialize to

$$K_{eq}(T) = \left[\frac{q_H^{\oplus}(T) q_{DCl}^{\oplus}(T)}{q_D^{\oplus}(T) q_{HCl}^{\oplus}(T)}\right] \exp\left[-\frac{\Delta E_0}{RT}\right] \qquad 2.6$$

where

$$\Delta E_0 = -N_A\left[E_{H,0} + D_{DCl,0} - E_{D,0} - D_{HCl,0}\right] \qquad 2.7$$

where $E_{H(D),0}$ is the ground state energy of the H(D) atom.

$a = b = c = d = 1$

By Eqn. 4.1, we get

$$K_{eq}(T) = \frac{\left[q_H^{\oplus}(T)/N_A\right]^1 \left[q_{DCl}^{\oplus}(T)/N_A\right]^1}{\left[q_D^{\oplus}(T)/N_A\right]^1 \left[q_{HCl}^{\oplus}(T)/N_A\right]^1} \exp\left[-\frac{\Delta E_0}{RT}\right]$$

$$= \frac{q_H^{\oplus}(T)\, q_{DCl}^{\oplus}(T)}{q_D^{\oplus}(T)\, q_{HCl}^{\oplus}(T)} \exp\left[-\frac{\Delta E_0}{RT}\right].$$

b.

(i) (12 pts) Show that Eq. (2.6) may be reexpressed as

$$K_{eq}(T) = \left(\frac{M_H M_{DCl}}{M_D M_{HCl}}\right)^{3/2} \left(\frac{\mu_{DCl}}{\mu_{HCl}}\right) \left[\frac{1-\exp(-\Theta^{vib}_{HCl}/T)}{1-\exp(-\Theta^{vib}_{DCl}/T)}\right] \exp\left[\frac{-\Delta E_0}{RT}\right] \qquad 2.8$$

where M_H is the mass of a hydrogen atom, M_{DCl} is the total mass of a DCl molecule, and so on and where μ_{DCl} and μ_{HCl} are, respectively, the reduced masses of HCl and DCl.

Other Side

(ii) Show that at T = 300K to an excellent approximation Eq. (2.8) reduces to

$$K_{eq}(T) = \left(\frac{M_H M_{DCl}}{M_D M_{HCl}}\right)^{3/2} \left(\frac{\mu_{DCl}}{\mu_{HCl}}\right) \exp\left[\frac{-\Delta E_0}{RT}\right] \qquad (2.9)$$

To an excellent approx. at T = 300K for HCl and DCl $\exp[-\Theta^{vib}/T] = 0$ yielding Eq. (2.9) from (2.8).

(b)

$$K_{eq}(T) = \left[\frac{Q_H^{trans}(T)\, Q_{DCl}^{trans}\, Q_{DCl}^{vib}\, Q_{DCl}^{rot}}{Q_D^{trans}(T)\, Q_{HCl}^{trans}\, Q_{HCl}^{vib}\, Q_{HCl}^{rot}}\right] \exp\left[-\frac{\Delta E_0}{RT}\right]$$

$$g_{0,H}^{el} = g_{0,D}^{el}$$
$$g_{0,HCl}^{el} = g_{0,DCl}^{el}$$

$$= \frac{\left[\frac{2\pi M_H kT}{h^2}\right]^{3/2} V \left[\frac{2\pi M_{DCl} kT}{h^2}\right]^{3/2} V \left[1-\exp(-\theta_{DCl}^{vib}/T)\right]^{-1} \left(\frac{k}{hc}\right) \frac{8\pi^2 \mu_{DCl} r_e^2 c}{hT}}{\left[\frac{2\pi M_D kT}{h^2}\right]^{3/2} V \left[\frac{2\pi M_{HCl} kT}{h^2}\right]^{3/2} V \left[1-\exp(-\theta_{HCl}^{vib}/T)\right]^{-1} \left(\frac{k}{hc}\right) \frac{8\pi^2 \mu_{HCl} r_e^2 c}{hT}} \exp\left[-\frac{\Delta E_0}{RT}\right]$$

$$= \left[\frac{M_H M_{DCl}}{M_D M_{HCl}}\right]^{3/2} \left[\frac{1-\exp(-\theta_{HCl}^{vib}/T)}{1-\exp(-\theta_{DCl}^{vib}/T)}\right] \left[\frac{\mu_{DCl}}{\mu_{HCl}}\right] \exp\left[-\frac{\Delta E_0}{RT}\right]$$

$$= \left[\frac{M_H M_{DCl}}{M_D M_{HCl}}\right]^{3/2} \left[\frac{\mu_{DCl}}{\mu_{HCl}}\right] \left[\frac{1-\exp(-\theta_{HCl}^{vib}/T)}{1-\exp(-\theta_{DCl}^{vib}/T)}\right] \exp\left[-\frac{\Delta E_0}{RT}\right]$$

(iii) Show that Eq. (2.7) may be reexpressed as

$$\Delta E_0 = \frac{1}{2} N_A hc[\tilde{\omega}_e(DCl) - \tilde{\omega}_e(HCl)]. \tag{2.10}$$

Show all of your reasoning. (For example, it is not sufficient to merely state $E_{H,0} = E_{D,0}$. If you assume this relation it must be justified.)

Because the mass of nuclear is much larger than the mass of electron, so the nuclear is almost fixed while the electrons are moving around the nuclear at high speed, therefore the ground state energy of atoms or molecules depends on the electric distribution only.

That means the ground state energy of isotope is the same.

So. $E_{H,0} = E_{D,0}$, $D_{HCl,e} = D_{DCl,e}$.

By Equ. 4.7 we get

$$\Delta E_0 = -N_A[E_{H,0} + D_{DCl,0} - E_{D,0} - D_{HCl,0}]$$

$$= -N_A[D_{DCl,0} - D_{HCl,0}]$$

Since $D_0 = D_e - \frac{1}{2}hc\tilde{\omega}_e$

So $\Delta E_0 = -N_A\left[\left(D_e^{DCl} - \frac{1}{2}hc\tilde{\omega}_e(DCl)\right) - \left(D_e^{HCl} - \frac{1}{2}hc\tilde{\omega}_e(HCl)\right)\right]$

$= -N_A\left[\frac{1}{2}hc\tilde{\omega}_e(HCl) - \frac{1}{2}hc\tilde{\omega}_e(DCl)\right]$ because $D_e^{DCl} = D_e^{HCl}$

$= \frac{1}{2}N_A hc[\tilde{\omega}_e(DCl) - \tilde{\omega}_e(HCl)]$

c. Compute the numerical value of $K_{eq}(T = 300K)$ from Eqs. (2.9) and (2.10) and given information. Recall $\Theta^{VIB} = \frac{hc}{k}\tilde{\omega}_e \Rightarrow \Theta^{VIB}(K) = 1.44\tilde{\omega}_e(cm^{-1})$.

Since $\tilde{\omega}_e \propto \frac{1}{\sqrt{\mu}}$

So $\frac{\tilde{\omega}_e(DCl)}{\tilde{\omega}_e(HCl)} = \left[\frac{\mu_{HCl}}{\mu_{DCl}}\right]^{1/2} = \left[\frac{\frac{1\times35}{1+35}}{\frac{2\times35}{2+35}}\right]^{1/2} = 0.7169$.

$\therefore \tilde{\omega}_e(DCl) = 0.7169 \times \tilde{\omega}_e(HCl) = 0.7169 \times 2889.7\,cm^{-1} = 2071.5\,cm^{-1}$

By Eqs. (4.10) we get.

$\Delta E_o = \frac{1}{2}N_A hc[\tilde{\omega}_e(DCl) - \tilde{\omega}_e(HCl)]$

$= \frac{1}{2} \times 6.02214\times10^{23}\,mol^{-1} \times 6.626\times10^{-34}\,J\cdot s \times 3.00\times10^{8}\,m\cdot s^{-1} \times (2071.5 - 2889.7)\,cm^{-1}$

$= -4897.26\,J/mol$.

$RT = 8.3145\,J\,mol^{-1}K^{-1} \times 300K = 2494.35\,J/mol$.

By Eqs. 4.9,

$K_{eq}(T=300) = \left[\frac{1}{2}\cdot\frac{37}{36}\right]^{3/2}\left[\frac{36\times2}{37}\right]\exp\left[-\frac{-4897.26\,J/mol}{2494.35\,J/mol}\right]$

$= 5.11$