CUMULATIVE EXAMINATION IN ANALYTICAL CHEMISTRY Crib

Oct 24, 2009

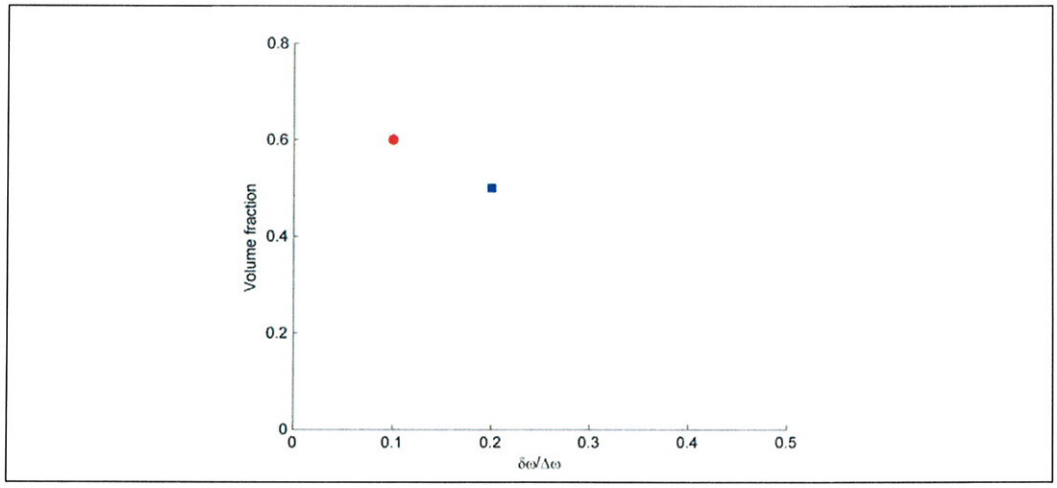
After each individual exam is graded, 4 questions with the lowest score will be dropped. In other words, you only need to answer 8 questions out of 12 to earn a perfect total score.

The exam is based on the article by Zabow *et al.* (e-mailed to all of you one week prior to the exam; hardcopy of this article is also attached with this exam booklet).

1. The review of Zabow's paper, written by Richard Bowtell (Nature 453:993), says: "Optical imaging routinely uses multicolored contrast agents ranging from traditional chemical dyes and fluorophores to specially engineered quantum dots. ... Now Zabow and colleagues are bringing color to MRI". Why is it advantageous to have multiple "colors" in MRI?

The amount of information obtained from the single MRI procedure can be increased – e.g. one could simultaneously run a 'battery of diagnostic tests'. Especially important is a potential ability to co-localize, for example, certain types of cells. It is also possible to co-localize the areas with elevated concentration of different enzymes, etc. (Zabow describes how the disks can be activated by specific enzymes)

2. The figure below is plotted in the same axes and has the same meaning as Fig. 1c in the Zabow's paper.



Two points in the plot (red circle and blue square) represent two different implementations of the "Zabow's disk assembly". Which one is preferable? Please, justify.

Red circle is preferable – it utilizes 60% of the volume, producing a narrow line with a

large shift (linewidth to shift ratio 0.1)

3. The diffusion is a mixed blessing in the context of Zabow's experiment. Please, give the reasons as to why fast diffusion is good and, at the same time, bad.

It is good since it effectively boosts the sensitivity – the diffusion 'pumps' water molecules through the disk assembly, providing a chance to saturate a greater number of spins. At the same time, it is bad, since it degrades spatial resolution. Furthermore, it leads to line-broadening (if the water molecule hops too rapidly between the inside and outside of the cell).

4. The experimental curve in Fig. 4a of the Zabow's paper contains what appears to be a strong signal rising toward 0 kHz. This signal does not correspond to the theoretical prediction (red line in Fig. 4a). What is this signal?

Bulk water (D_2O) – as opposed to the cavity water.

5. At p.1059, Zabow writes: "...elementary magnetostatics gives $\Delta\omega = (\gamma J_S/2)[(S-h/2)/((S-h/2)^2+R^2)^{1/2} - (S+h/2)/((S+h/2)^2+R^2)^{1/2}]$. For thin disks with $h \ll 2S \approx R$, this reduces to:

$$\Delta\omega = -\gamma J_S \left(\frac{hR^2}{2(R^2+S^2)^{3/2}} \right).$$"

Please, show mathematical calculations, demonstrating how the first result can be reduced to the second.

Start with denominators, e.g. $\sqrt{(S-h/2)^2+R^2} \approx \sqrt{S^2+R^2-Sh}$ (here we neglected small quadratic term, h^2). Now $\sqrt{S^2+R^2-Sh} = \sqrt{S^2+R^2}\sqrt{1-[Sh/(S^2+R^2)]}$ and then, using Taylor series, $\sqrt{1-[Sh/(S^2+R^2)]} \approx \{1-(1/2)[Sh/(S^2+R^2)]\}$. The rest is simple algebra.

Generally, the formula $\sqrt{1+x} \approx 1+(x/2)$ which holds for small x is very useful.

6. At p. 1059, Zabow writes: " (magnetic torque on the disks) equates to pressures of order 10^{-8} to 10^{-6} N μm^{-2}. By comparison, even within cellular cytoplasm, yield stresses are only in the range 10^{-13} to 10^{-9} N μm^{-2}". Please, comment on this passage. Why Zabow brings up the cellular cytoplasm? What is the yield stress?

Zabow makes this argument to indicate that magnetic torque is sufficient to orient the disk even in the most viscous (biological) environment. Cellular cytoplasm is such super-viscous environment. In fact, it can be thought of as a gel. You have to apply certain minimal pressure to make things move in the gel (i.e. make the gel yield), hence "yield stress".

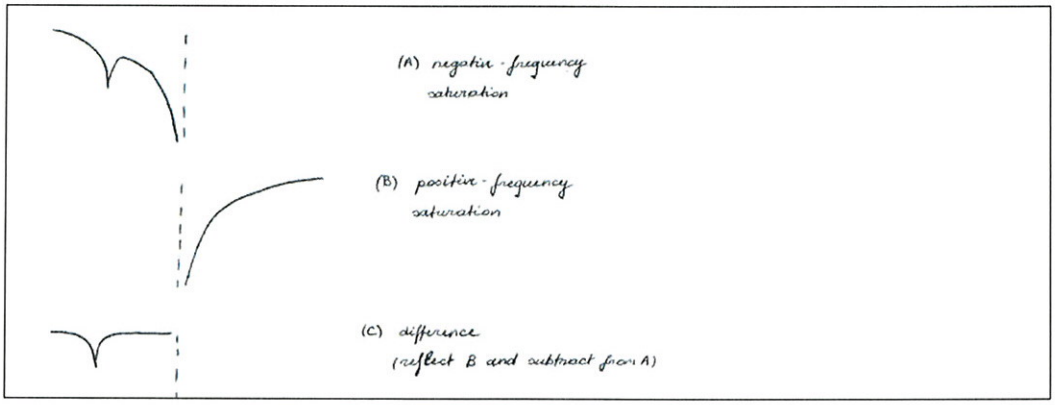
7. Fig. 5 in Zabow *et al.* shows two SEM images – one with low magnification, another with high magnification. How does one control the magnification of the SEM image (please, explain the concept)?

To obtain higher magnification, the electron beam should be focused on a smaller area of the sample (achieved by means of electrostatic lenses, etc.).

8. In the visible spectrum, red light has the lowest frequency, blue has the highest, and green is in between. In Zabow's experiment, however, blue has the lowest frequency, green the highest, and red is in between (see Fig. 3d). Why the discrepancy?

Of course, coloring of signals according to the resonance shifts is a matter of convention – one could choose any coloring scheme (in practice, one has to make sure that the coloring scheme is easy on the eye and emphasizes important features of the MRI image).

9. In the caption of Fig. 4, Zabow writes: "because the surrounding water broadening is approximately symmetric (... the broadened water) background can be eliminated by considering differences between corresponding positive- and negative-frequency saturation". Please, discuss this experimental scheme and draw the sketches of the z-spectra: with positive-frequency saturation, negative-frequency saturation, and the difference spectrum.



10. The disks used in Zabow's work are made from nickel, which is ferromagnetic; this leads to negative frequency shifts (see Figs. 1 and 4). Assume that the disks are made from antiferromagnetic material. What can be said of the frequency shifts?

Frequency shifts will be small – and still negative. Antiferromagnetic materials have a pattern of alternating spins. When exposed to the external fields they become magnetized in the same way as ferromagnetic materials – only weakly.

11. At p. 1059, Zabow writes: "A final gold sputter-coating further enhances ... access to thiol-based chemistry for specific surface functionalization if desired". Please, elaborate on this statement.

Please, see Chem.Rev. 104, p. 293 for discussion of thiol coating of gold nanoparticles.

12. At p. 1058, Zabow writes: "The potential of spectral shifting is indicated by recent interest in PARACEST (PARAmagnetic Chemical Exchange Saturation Transfer, ref. 7) molecular complexes, whose chemical shifts can generate off-resonance MRI contrast through proton exchange". While you probably have not read the cited paper, try to intuit the concept of the PARACEST experiment based on this brief description.

In Zabow's experiment, bulk water protons exchange in and out of the disk cavity. In the PARACEST experiment, bulk water protons exchange in and out of OH and NH exchangeable sites of a paramagnetic complex. The presence of paramagnetic center in the complex ensures that the resonances of said OH and NH protons are strongly shifted (and/or relaxed). Hence, as you can see, the details are different, but the fundamental concept behind PARACEST and the Zabow's experiment is similar.

Cumulative Exam Questions for Biochemistry (October 2009) Crib

1. Explain why each of the following reagents can denature most globular proteins. (5 points each):
   a. Urea (8M)- urea competes for H-bonds that stabilize protein secondary and tertiary structures. It also has a slight hydrophobic bond destabilizing capacity.
   b. pH 2- low pH will titrate all ionizable groups to their protonated state. By doing so, it will both break all salt bridges (ion pair interactions) and charge up the protein to a strongly positively charged state, leading to charge repulsion between many ionizable groups.
   c. sodium dodecyl sulfate- SDS will compete for hydrophobic interactions that stabilize the hydrophobic cores of most proteins. It will also bind along the polypeptide backbone at ~1.4g SDS per gram protein and unfold the protein due to the high negative charge density imposed on the backbone.
2. Calculate the diameter of a perfectly spherical globular protein of molecular weight 30,000. Show your work and state your assumptions. (20 points)

   Assume the density of an average globular protein is 1.3 g/cc.

$$30{,}000\text{g/mole} \times \frac{1cc}{1.3g} \times \frac{1mole}{6.02x10^{23}\,molecules} \times \frac{(10^7\,nm)^3}{1cc} = \text{Volume}$$

   $V = 38.33\ nm^3 = 4/3\pi\ r^3$

   $r = 2.09$ nm

   diameter = $2r = 4.2$ nm

3. <u>α-helical structure</u>

   C=0 of each amino acid is H-bonded to the N-H of the amino acid four residues downstream in the sequence.

   There is a 1.5Å rise along the helical axis for each amino acid

   All side chains extend radially outward

   There is an amino acid every 100° around the helical axis or 3.6 residues per complete turn

   The screw sense is right-handed or clockwise

   <u>β-sheet structure</u>

The axial distance between contiguous amino acids along the same strand is ~ 3.3Å

The sheet is stabilized by H bonds connecting peptide carbonyl and amide NH residues between adjacent strands

Side chains alternate pointing vertically above and below the sheet

Adjacent strands in a sheet can run either parallel or anti-parallel to each other

4. See text book

5. a) List all ionizable amino acids starting from most acidic to most basic terminal COOH

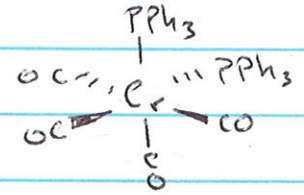
Terminal COOH-1
Asp - 2
Glu - 4
His – 5
Terminal - NH_3^+-1
Cys - 2
Tyr - 2
Lys - 6
Arg - 6

Start with low pH form of all amino acids and titrate mentally with NaOH until you achieve an equal number of positive and negative charges. In the above case, the protein at low pH will begin with 18 positive charges and neutrality will be reached only after one of the six lysines has been titrated to its neutral form. Using the Henderson-Hasselbalch equation we can calculate when 1 of 6 lysines is titrated to its high pH form:

$pH = pKa + \log[Lys^0]/[Lys^+] = 10.7 + \log 1/5 = 10.7 - 0.7 = 10$

Inorganic Cumulative Exam Crib
Purdue University
October 24, 2009

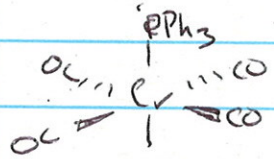
1. Two possible isomers:



cis Point group C_{2v}

C_{2v}	$\hat{E}$	C_2	σ_v	σ_v'
Γ_{4CO}	4	0	2	2

reduces to $2A_1 + B_1 + B_2$ all IR active



trans Point group D_{4h}

D_{4h}	E	$2C_4$	C_2	$2C_2'$	$2C_2''$	i	$2S_4$	σ_h	$2\sigma_v$	$2\sigma_d$
Γ_{4CO}	4	0	0	2	0	0	0	4	2	0

reduces to $A_{1g} + B_{1g} + E_u$

↑ IR active

Thus, trans- $Cr(PPh_3)_2(CO)_4$.

2. Dissociative $\Delta S^{\ddagger} > 0$

$$Pt(Ph)_2(SMe_2)_2 \rightleftharpoons [Pt(Ph)_2(SMe_2)] \xrightarrow{Phen} Pt(Ph)_2(Phen)$$

---

3. FeF_6^{3-} Fe^{III} d^5 high spin

e_g ↑ ↑

t_{2g} ↑ ↑ ↑

d-d transitions are spin-forbidden.

CoF_6^{3-} Co^{III} d^6 high spin

↑↓ ↑ ↑ e_g

↑↓ ↑ ↑ t_{2g}

spin-allowed.

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4. a. $[Co(NH_3)_5F]^{2+} + F^- + CO_2 + H_2O$

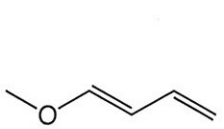
b. $RC(=O)-O^-$ MgX^+

c. $W(CO)_6$ reductive disproportionation of CO_2

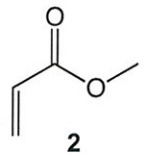
d. R_3P, R_3P, Ni, $C{=}O$

Organic Cumulative Exam Crib
10/24/09
Cycloaddition

A [4+2] cycloaddition between (*E*)-1-methoxybuta-1,3-diene (**1**) and methyl acrylate (**2**) provides a racemic mixture of (1*R*,2*S*)- and (1*S*,2*R*)-methyl 2-methoxycyclohex-3-enecarboxylates as the major product in 54% isolated yield (*J. Am. Chem. Soc.*, **1996**, *118*, pp 12555–12561). Here minimization of the dipole moment in the transition state (or favorable mixing of highly polarized HOMO of the diene and LUMO of the dienophile) is responsible for the exclusive regioselectivity, and a secondary orbital overlap could be used to explain the observed diastereoselectivity (64:36) preference.



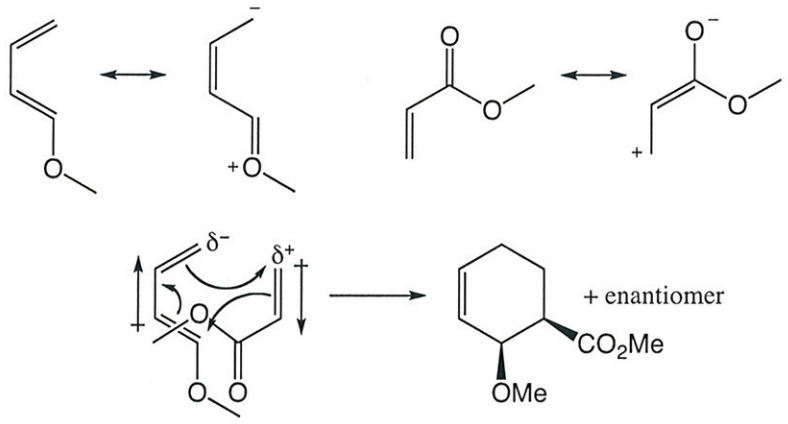
1



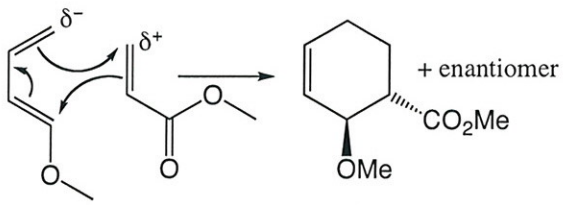
2

Answer the following questions about this or related reactions:

1) (**15 pts**) Using appropriate structural models, account for both the regio- and stereochemical outcome of this process.



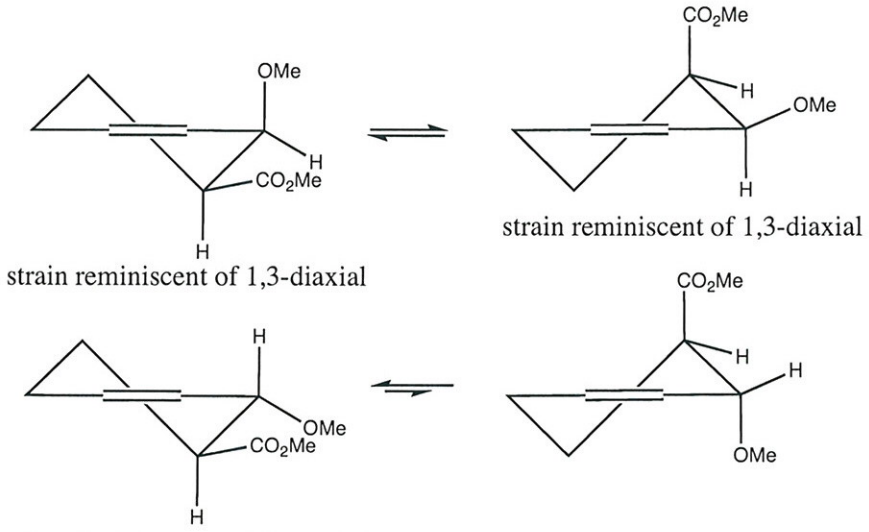
endo major



exo minor

While an FMO analysis was expected, dipole moment qualitative evaluation and *endo*/*exo* demonstration would have been sufficient.

2) (**10 pts**) On the basis of conformational preferences, compare relative stabilities of the major and minor products in this cycloaddition.



Any reasonable attempt to analyze disubstituted cyclohexenes was credited.

3) (**10 pts**) Although many [4+2] cycloadditions proceed via essentially concerted mechanisms, a short-lived zwitterionic intermediate is expected to form in this reaction under most conditions. Propose control experiments that would confirm:

a. relatively short ($\leq 10^{-11}$ s) lifetime of the intermediate;

Use a substrate that can report on stereospecificity of this cycloadition. For example, if (*E*)- or (*Z*)-methyl but-2-enoate as a dienophile results in the maintenance of the substituent geometry (*trans* or *cis*, respectively) in the cycloadduct,the short lifetime of the intermediates can be inferred.

b. its zwitterionic (as opposed to diradical) nature.

- **Observe an extent of a solvent polarity effect on the rate of cycloaddition: transition state leading to the zwitterionic intermediate will be stabilized by polar solvents and the rate of cycloaddition will be enhanced (No rate enhancement is expected for a radical pathway)**

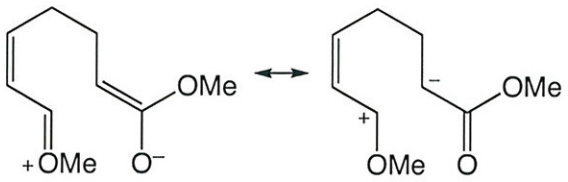
- **Trap the intermediate with a protic solvent, for example.**

4) (**15 pts**) As with other reactions, there is expected to be a "retro" cycloaddition process following the reverse course.

   a. Provide conditions that would favor the formation of the diene **1** and dienophile **2** from the corresponding cycloadduct.

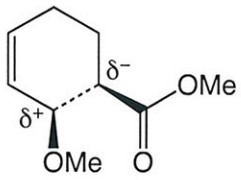
   Reverse process is favored entropically, hence higher temperature should shift the equilibrium toward the diene and dienophile

   b. Sketch the intermediate expected in the "retro" process.



same as in the forward process

   c. Sketch the transition state for the rate-limiting step in the reverse reaction.



5) (**30 pts**) In the following list identify a reaction variable from each pair that would feature a faster cycloadduct formation rate under otherwise identical conditions. Provide a brief explanation that supports your choice:

   a. methoxyethene (methyl vinyl ether) vs methyl acrylate (**2**) as dienophiles

   cycloaddition is faster with methyl acrylate: lower HOMO–LUMO energy gap

   b. 1-methoxycyclopenta-1,3-diene vs (*E*)-1-methoxybuta-1,3-diene as dienes

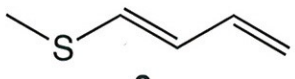
   cycloaddition is faster with 1-methoxycyclopenta-1,3-diene:

diene is fixed into the reactive *s*-cis conformation

c. (*Z*)-1-methoxybuta-1,3-diene vs (*E*)-1-methoxybuta-1,3-diene as dienes

cycloaddition is faster with (*E*)-1-methoxybuta-1,3-diene: (*E*)-diene is expected to have a higher population of *s*-cis conformers

d. compound **3** vs (*E*)-1-methoxybuta-1,3-diene as dienes



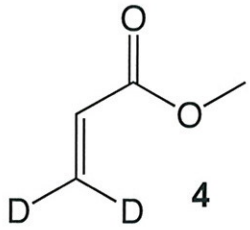
3

cycloaddition is faster with (*E*)-1-methoxybuta-1,3-diene: more orbital mixing between a methoxy substituent and diene HOMO resulting in a higher HOMO and a higher dipole moment.

e. toluene vs acetonitrile as solvents

cycloaddition is faster in a more polar acetonitrile: higher stabilization for the transition state (zwitterionic-like) in the rate-limiting step.

f. methyl acrylate **2** vs dideutero derivative **4** as dienophiles

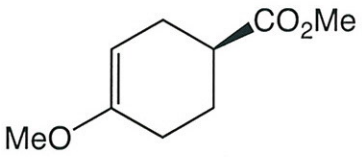


4

cycloaddition is faster with methyl acrylate: sp^2 to sp^3 hybridization change results in a more crowded rate-limiting transition state with 4 as dienophile

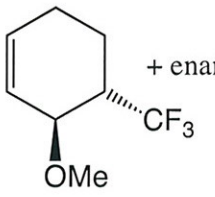
6) (**10 pts**) Draw major products that are expected to be formed in the related cycloadditions involving the reactants listed below. Please be specific about regiochemical and stereochemical outcome in each case.

a. 2-methoxybuta-1,3-diene and dienophile **2** (*Tetrahedron* **1987**, *43*, 3765–3786)



+ enantiomer

b. diene **1** and 3,3,3-trifluoropropene (trifluoromethylethene) as dienophile (*JOC* **1982**, *47*, 2051–2055);



+ enantiomer

no possibility for secondary orbital overlap

7) (**10 pts**) Recent developments in Chemical Biology and Bioorganic Chemistry have solved the problem of obtaining the products, which would normally be disfavored by orbital interactions (i.e., (1*R*,2*R*)- or (1*S*,2*S*)-methyl 2-methoxycyclohex-3-enecarboxylate in this reaction) via a variety of somewhat related approaches. *Briefly* describe a solution for this problem from literature (e.g., *Science* **1993**, 262, pp. 204 - 208) or devise your own plan to accomplish this formidable feat.

Raising a catalytic antibody or evolving a Diels-Alderase against a transition-state analogue may allow the cycloaddition to yield the *exo* product as the major one.

Solutions
PChem Exam 10/24/09
Crib

1. (15 pts)

$$\psi_1 = \frac{1}{\sqrt{3}}\psi_{2s} + \sqrt{\frac{2}{3}}\psi_{2p_z}$$

$$\psi_2 = \frac{1}{\sqrt{3}}\psi_{2s} + \frac{1}{\sqrt{2}}\psi_{2p_x} - \frac{1}{\sqrt{6}}\psi_{2p_z}$$

$$\psi_3 = \frac{1}{\sqrt{3}}\psi_{2s} - \frac{1}{\sqrt{2}}\psi_{2p_x} - \frac{1}{\sqrt{6}}\psi_{2p_z}$$

2. (25 pts) The probability is found from the integral:

$$\int_0^{a_0} R_{1s}^2(r)\, r^2\, dr$$

Since $\Psi_{100} = \frac{1}{\sqrt{4\pi}} R_{1s}(r) = \frac{1}{\sqrt{\pi}}\left(\frac{1}{a_0}\right)^{3/2} e^{-r/a_0}$

then $R_{1s}(r) = 2\left(\frac{1}{a_0}\right)^{3/2} e^{-r/a_0}$

The integral then becomes:

$$\frac{4}{a_0^3}\int_0^{a_0} r^2 e^{-2r/a_0}\, dr$$

It was given that $\int_0^1 x^2 e^{-2x}\, dx = \frac{1}{4} - \frac{5}{4}e^{-2}$

Therefore:

$$\frac{4}{a_0^3}\int_0^{a_0} r^2 e^{-2r/a_0}\, dr = 4\int_0^1 x^2 e^{-2x}\, dx$$

$$= 1 - 5e^{-2}$$

$$= 0.323$$

or (32.3%)

Solutions
PChem exam 10/24/09

3a. $[\hat{L}_z, \hat{L}_+] = \hat{L}_z\hat{L}_+ - \hat{L}_+\hat{L}_z$

(10 pts)

$= \hat{L}_z(\hat{L}_x + i\hat{L}_y) - (\hat{L}_x + i\hat{L}_y)\hat{L}_z$

$= \hat{L}_z\hat{L}_x + i\hat{L}_z\hat{L}_y - \hat{L}_x\hat{L}_z - i\hat{L}_y\hat{L}_z$

$= (\hat{L}_z\hat{L}_x - \hat{L}_x\hat{L}_z) - i(\hat{L}_y\hat{L}_z - \hat{L}_z\hat{L}_y)$

$= [\hat{L}_z, \hat{L}_x] - i[\hat{L}_y, \hat{L}_z]$

$= i\hbar\hat{L}_y - i(i\hbar\hat{L}_x)$

$= \hbar(\hat{L}_x + i\hat{L}_y)$

$[\hat{L}_z, \hat{L}_+] = \hbar\hat{L}_+$

3b. $[\hat{L}^2, \hat{L}_+] = \hat{L}^2\hat{L}_+ - \hat{L}_+\hat{L}^2$

(10 pts)

$= \hat{L}^2(\hat{L}_x + i\hat{L}_y) - (\hat{L}_x + i\hat{L}_y)\hat{L}^2$

$= \hat{L}^2\hat{L}_x + i\hat{L}^2\hat{L}_y - \hat{L}_x\hat{L}^2 - i\hat{L}_y\hat{L}^2$

$= (\hat{L}^2\hat{L}_x - \hat{L}_x\hat{L}^2) - i(\hat{L}_y\hat{L}^2 - i\hat{L}_y\hat{L}^2)$

$= \underbrace{[\hat{L}^2, \hat{L}_x]}_{=0} - i\underbrace{[\hat{L}_y, L^2]}_{=0} = 0$

Solutions
PChem exam 10/24/09

4. (25 pts) Assume a surface of area A, perpendicular to the direction of motion, x. A molecule with $v_x > 0$ will strike the surface in a time interval Δt if it is within a distance $d \leq v_x \Delta t$. Therefore, we need to know the number of molecules in the volume $A v_x \Delta t$ with $v_x > 0$. This number is is the volume multiplied by the number density of molecules or $\Delta v_x \Delta t N$, where $N = \frac{n N_A}{V} = P/kT$. We need to sum over all possible velocities and the probability distribution of velocities:

$$\text{number striking surface} = NA\Delta t \int_0^\infty v_x f(v_x) dx$$

collision frequency is number per unit time per area:

$$Z = N \int_0^\infty v_x f(v_x) dx$$

$$= P/kT \int_0^\infty \left(\frac{m}{2\pi kT}\right)^{1/2} v_x e^{-mv_x^2/2kT} dv_x$$

using the integral given in the question this becomes:

$$Z = \frac{P}{kT}\left(\frac{m}{2\pi kT}\right)^{1/2} \frac{kT}{m}$$

$$Z = \frac{P}{(2\pi m kT)^{1/2}}$$

Solutions
PChem comp 10/24/09

5. a. an increase in pressure will [decrease] the diffusion constant because the mean free path is inversely proportional to pressure: $\lambda = \frac{kT}{\sqrt{2}\sigma P}$

(15 pts)

b. an increase in temperature will [increase] the diffusion constant because the mean speed is directly proportional to temperature: $\bar{c} = \left(\frac{8kT}{\pi m}\right)^{1/2}$

c. increasing the size of the gas molecules will increase the collision cross section, σ, which will decrease the mean free path. This will [decrease] the diffusion coefficient.