Department of Chemistry
Cumulative Examinations

December 9, 2017

You may choose to answer any exam from any area covered in the examination booklet. Each exam may contain multiple parts. You may answer more than one exam but each exam is scored separately and is treated as an individual examination result. Thus, answering parts of two exams with a score of 50% would not yield a 100% grade for this cumulative exam. Instead you would receive 50% on each examination attempted.

This booklet contains five examinations.

1) Analytical Cumulative Examination, Pages 1-5
2) Biochemistry Cumulative Examination, Page 6
3) Inorganic Cumulative Examination, Pages 7-9
4) Organic Cumulative Examination, Page 10
5) Physical Cumulative Examination, Page 11

On your examination booklet:

1) Print your student ID number.
2) Print the Exam Booklet number.
3) Print the question number you are answering.
4) Print the Exam Date.

Do not write your name anywhere on the examination booklet. Each exam will be scored anonymously. If you attempt more than one exam, you must use a separate examination booklet for each examination.

When you complete the examination, return the examination and your answer booklet to the proctor. Exam results will be posted on bulletin board #2B on the north side of the hall near BRWN 2124.
Announced topic:
The upcoming Analytical Chemistry cume will be on the following publication.

“Separation of small interfering RNA stereoisomers using reversed-phase ion-pairing chromatography” by Li Li, Tony Leone, Joe P. Foley, and Christopher J. Welch, J. Chromat. A 2017, 1500, 84-88.

Questions:

1. (10) Summarize in your own words the abstract of the manuscript. Please note that clarity, organization, and logical flow will be considered in addition to scientific content in the evaluation of the scoring.

2. (15) In your own words, prepare an introduction, including the big-picture motivation for the work, the previous related efforts that have paved the foundation, the primary remaining gap to be addressed in the present study, and the methods described in the manuscript to address the gap.

3. (10) Provide a caption and discussion for Figure 2.

4. (10) Provide a caption and discussion for Figure 3.

5. (15) Provide a caption and discussion for Figure 8.

6. (15) Provide a physical description of IP-RP-UHPLC, including a molecular-scale sketch of the key molecular interactions responsible for chiral separation.

7. (15) Describe the general mechanism of action for siRNAs as potential therapeutic candidates. What is the biochemical role of siRNA, and how might addition of a targeted siRNA be used to target a disease such as cancer?

8. (10) What is the key difference between an enantiomer of a molecule versus a diastereomer?
Full length article

Separation of small interfering RNA stereoisomers using reversed-phase ion-pairing chromatography

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ABSTRACT

1. Introduction

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E-mail addresses: li_li@merck.com (L. Li).
2 diastereomeric sense single RNA strands

\[
\begin{align*}
\text{GGAUCCU\textsuperscript{5}UAP\textsuperscript{3}A} & \quad \text{GGAUCCU\textsuperscript{5}UAP\textsuperscript{3}A} \\
\text{GGAUCCU\textsuperscript{5}UAP\textsuperscript{3}A} & \quad \text{GGAUCCU\textsuperscript{5}UAP\textsuperscript{3}A}
\end{align*}
\]

4 diastereomeric antisense single RNA strands

\[
\begin{align*}
\text{GGAUCCU\textsuperscript{5}UAP\textsuperscript{3}A} & \quad \text{GGAUCCU\textsuperscript{5}UAP\textsuperscript{3}A} \\
\text{GGAUCCU\textsuperscript{5}UAP\textsuperscript{3}A} & \quad \text{GGAUCCU\textsuperscript{5}UAP\textsuperscript{3}A}
\end{align*}
\]

Fig. 1. (a) Structure of the Apoll gene-targeting siRNA duplex studied in this investigation. Nucleotides marked with asterisks contain chemically modified ribose substituents in which the 2'-OH is replaced with 2'-methoxy. In the nucleotides highlighted in red, naturally occurring phosphodiester linkages have been replaced with phosphorothioate linkages. (b) Six single-stranded siRNA species at high temperatures. (c) Eight diastereomeric siRNA duplexes at low temperatures. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. The impact of ion pairing reagents on the separation of siRNA diastereomers

vided a much poorer separation, with broad and overlapping peaks. Comparing the results in Fig. 2a and b, the cyan column appears to
3.3. The impact of organic modifier on the separation of siRNA stereoisomers

The siRNA duplex was analyzed using gradient elution, where ACN, MeOH, THF, and IPA were evaluated as the organic modifier in the mobile phase. The organic solvent imparts hydrophobicity to the mobile phase, which impacts the separation of the oligonucleotide. As shown in Fig. 4, the use of ACN resulted in a marked improvement in the separation of the sense and antisense diastereomers relative to MeOH. Poor separations were obtained using mobile phases with IPA or THF (data not shown). The overall retention time with ACN as organic modifier is significantly shorter compared to MeOH, reflecting the fact that ACN is a stronger solvent than MeOH for reversed-phase separations.

Double-stranded siRNA forms a duplex in an aqueous environment. The binding force between the two strands is largely attributed to hydrogen bonding, hydrophobic and ionic interactions. The separation conditions reported here calls for high column temperature (e.g., 80°C) with inclusion of organic modifier in the mobile phase. Under these conditions the siRNA duplex is denatured during the separation, i.e., the sense and antisense strands are dissociated from one another and are migrating down the column independently and with a reduced secondary structure. In addition, it is possible that ACN is more effective in disrupting the siRNA duplex, hence improving the separation of the sense and antisense stereoisomers. Solution calorimetry experiments showed that the siRNA duplex has an annealing temperature of 57°C in 0.1 M pH 7 TEAA buffer solution (Fig. 5). The annealing temperature dropped to 48 and 53°C, respectively, when 10% ACN or MeOH was added to the TEAA buffer solution, supporting the hypothesis that ACN is more effective in disrupting the interstrand interactions of the siRNA duplex.

3.4. Method optimization for the separation of siRNA stereoisomers

The separation conditions were further optimized by evaluating the impact of column temperature (50–80°C), the concentration of the TEAA ion pairing reagent (0.02–0.5 M), the flow rate (0.16–0.22 mL/min), and the gradient steepness. Fig. 6 shows the overlaid chromatograms with column temperature at 60, 70 and 80°C, suggesting that a high temperature above the annealing temperature of the siRNA favors stereoisomer separation. Clearly, chromatographic resolution of diastereomeric phosphorthioates within single strands, as opposed to duplex siRNA structures, seems to be preferred. The separation was also evaluated at different concentrations of the ion-pairing agent. 0.1 M TEAA was selected as the optimal concentration. The flow rate and gradient steepness were then fine-tuned to further optimize the resolution of all species. The optimized conditions enabled a baseline resolution of all components as shown in Fig. 7. The limit of detection (LOD) for the final method was determined by injecting a series of low level siRNA sample solutions. The LOD was estimated to be 4.2 ng with detection by UV at 260 nm.

3.5. Separation of desulfurization products of siRNA stressed with iodine

Fig. 3. Effect of the organic modifier on the separation of siRNA stereoisomers by reversed-phase ion-pairing chromatography. Conditions: Column and oven temperature as in Fig. 3, other conditions as in Fig. 2.

Fig. 4. Effect of the organic modifier on the separation of siRNA stereoisomers by reversed-phase ion-pairing chromatography. Conditions: Column and oven temperature as in Fig. 3, other conditions as in Fig. 2.

Fig. 5. Differential Scanning Calorimetry (DSC) showing siRNA duplex annealing temperature in 0.1 M TEAA (pH 7) buffer.

Fig. 6. Effect of column temperature on the separation of siRNA stereoisomers. Conditions: UHPLC column: BEH C18; Column oven temperature as indicated in the legend, other conditions as in Fig. 2.
Fig. 7. Optimized separation of the stereoisomers of the sense and antisense strand of siRNA using ion-pairing HPLC. Final conditions: Columns and oven temperature as in Fig. 2; Mobile phase A is 0.1 M TEAA: The gradient elution program was from 6% to 12% B in 10.5 min, followed by steeper gradients of 12% to 14% B and 14–30% B in 5.5 min and 7 min, respectively. Other conditions as in Fig. 2.

Fig. 8.

4. Conclusions

A reversed-phase ion-pairing chromatography method was developed for the baseline separation of multiple stereoisomers of a double-stranded siRNA containing three phosphorothioate modification sites. Key chromatographic parameters relevant to diastereomer separation included the structure of the ion pairing reagent, the organic modifier, and the chemistry of the stationary phase. Together with acetoniitrite as the organic modifier, TEAA provided a superior separation efficiency and selectivity than its structural analogs such as EAA, DEA As or TMAA. ACN is a key component which facilitates diastereomer separation while other organic modifiers including MeOH, THF or IPA failed in this aspect. Solution state DSC analysis of siRNA suggested that ACN can effectively disrupt the self-association of siRNA double strands, which appeared to be important to stereoregion separation. The optimized separation method was applied to an siRNA sample deliberately stressed with ionic solution to induce desulfurization, where up to six degradation products were resolved from the parent siRNA stereoisomers.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2017.04.008.

References

The 2017 Nobel Prize in Chemistry has been awarded to three scientists who pioneered the cryo-EM method. The three essential components of cryo-EM are “cryo”, “EM” and “3D”. Please answer the following questions about cryo-EM.

1) (20 points) “cryo”
   a) What does “cryo” mean in the context “cryo-EM”? (5 points)
   b) What problems does “cryo” solve? (5 points)
   c) How to achieve “cryo” state? (5 points)
   d) What limitations do you see for current “cryo” method? (5 points)

2) (20 points) “EM”
   a) Does “EM” in “cryo-EM” mean “SEM” or “TEM”? Explain your choice (5 points)
   b) Concisely describe the optics of an EM instrument. Include the major components and relate them to the counterparts of a light microscope. (10 points)
   c) Why EM can achieve much higher resolution than light microscopes? (5 points)

3) (25 points) “3D”
   a) Explain why an EM image only contains 2D information of a 3D structure. (5 points)
   b) How to recover 3D information from 2D images? Divide your answers into four parts
      i) General principle (5 points)
      ii) How is this principle realized for 2-D crystal samples (5 points)
      iii) How is this principle realized for 1-D helical samples (5 points)
      iv) How is this principle realized for single particles (5 points)

4) (20 points) The recent “resolution revolution” in cryo-EM and its impacts to structural biology is what led to the 2017 Nobel Prize in Chemistry awarded to cryo-EM. Describe four critical advances in recent years that have contributed to this “revolution”.

5) (15 points) No method can be the ideal method for all problems. Describe how you are going to decide the best structural method (cryo-EM, X-ray crystallography, or NMR) if you want to solve the atomic structure of a new protein sample.
Directions. Read each question carefully and answer to the best of your knowledge. Do not ramble on trying to get points as this will only make things worse.

Spectrochemical Series:

$I^- < Br^- < SCN^- < Cl^- < NO_2^- < N_3^- < F^- < OH^- < O_2^- < H_2O < NCS^- < CH_3CN < py < NH_3 < en < bpy < NO_2^- < PPh_3 < CN^- < CO \sim PF_3$

1. (10 points) Give the total electron count and the type of carbene ligand for Grubbs' catalyst (below).

2. (20 points) Classify each the following molecules into its respective point group.

(A) POCl_3 (O along z-axis)

(B) PtF_4^{2-}

(C)

(D) PF_5
3. (10 points) An **aqueous** solution of iron(II) sulfate is paramagnetic. When an excess of cyanide ion is added to the solution it becomes diamagnetic. What is the **identity of each species and explain (or show)** the reason for the observed magnetism?

4. (15 points) **Match** the element with its medical or biological application:
   (a) Ca ________ (1) Urease
   (b) Mo ________ (2) Anti-Arthritic
   (c) Mn ________ (3) Oxygen Evolving Complex
   (d) Tc ________ (4) Vitamin B12
   (e) Fe ________ (5) Insulin Mimetic
   (f) Co ________ (6) Hemocyanin
   (g) Ni ________ (7) MRI
   (h) Pt ________ (8) Xanthine Oxidase
   (i) Cu ________ (9) Cisplatin
   (j) Au ________ (10) Muscle contraction
   (k) Zn ________ (11) Myoglobin
   (l) V ________ (12) Cell membranes
   (m) P ________ (13) Hydrolases
   (n) Li ________ (14) Cardiolite
   (o) Gd ________ (15) Depression

For each multiple choice questions below, give the correct letter, as well as an explanation rationalizing your choice. **No credit will be given without a CORRECT supporting explanation.**

5. (5 points) Which of the following is the **most acidic**?
   (A) [Ni(H₂O)₆]²⁺ (B) [Co(H₂O)₆]²⁺ (C) [Fe(H₂O)₆]²⁺ (D) [Mn(H₂O)₆]²⁺

6. (5 points) Which of the following complexes is **most likely** to have the highest electron affinity, i.e. **most likely to accept an electron**?
   (A) Fe(CO)₅ (B) Mn(CO)₆ (C) Cr(CO)₆ (D) V(CO)₆

7. (5 points) Which of the following complexes is **most likely** to have the lowest ionization energy, i.e. **most likely to remove an electron**?
   (A) Fe(CO)₅ (B) Mn(CO)₆ (C) Cr(CO)₆ (D) V(CO)₆

8. (5 points) Which of the following has the **most** unpaired electrons?
   (A) ClAuPPh₃ (B) [Co(H₂O)₆]²⁺ (C) Ni(CO)₄ (D) Mn(CO)₆

9. (5 points) Which of the following complexes is **most likely** paramagnetic?
   (A) Cr(CO)₆ (B) PtCl₄(PMe₃)₂ (C) AuCl₄⁻ (D) V(CN)₄
10. (5 points) Which of the following is most likely an 18-electron complex?
   (A) Zn(H₂O)₆²⁺  (B) Pt(bpy)₂²⁺  (C) OTc(NCS)₂(PF₅)₂  (D) Os(en)Cl₃(N)

11. (5 points) Which of the following would most likely have a full t₂g molecular orbital?
   (A) Nb(PMe₃)₆  (B) W(CN)₆  (C) Tc(CO)₃(bpy)Br  (D) Fe(H₂O)₆²⁺

12. (10 points) Give a brief explanation for the following observations.
   (A) The Cu(I) complex, Cu₄(SR)₄, R = alkyl, but it doesn’t matter, is dark red.
   (B) (η⁵-C₅H₅)₂Co is a strong reducing agent.
TOPIC: Papers by Tim Jamison in ACS journals in 2017

In a recent paper (J. Am. Chem. Soc. 2017, 139, 13969) Jamison and co-workers described a β-selective hydrocarboxylation of styrenes with CO₂

\[ \text{Ar} + \text{CO}_2 \rightarrow \text{ArCO}_2\text{H} \]

1) The authors note that the advantage of their method is that it gives β-addition, instead of α-addition (Markovnikov). The Markovnikov carboxylation can be carried out with transition metal catalysis, as in this reaction reported by Greenhalgh and Thomas (J. Am. Chem. Soc. 2012, 134, 11900)

\[ \text{Ph} + \text{CO}_2 \rightarrow \text{PhCO}_2\text{H} \]

Provide a mechanism for this α-carboxylation reaction.

2) The Jamison approach to achieve β-carboxylation is to use CO₂⁻ (the anion radical). Describe the electronic structure of CO₂⁻

3) The full reaction conditions for the reaction are shown below

\[ \text{Ph} + \text{CO}_2 \rightarrow \text{PhCO}_2\text{H} \]

a) The preferred amine reductant is pentamethylenepiperidine, but other amines work. Given this information, propose a mechanism for the generation of CO₂⁻

b) Given the formation of CO₂⁻, propose a mechanism for the formation, being sure to account for why p-terphenyl is catalytic.

c) Dicarboxylation also occurs in the reaction. Indicate the product that is formed and propose a mechanism.

4) This reaction is not something that is easily done. What does Jamison’s group do in their experiments that makes this reaction possible for THEM but not for most everyone else?
Physical Cume Exam

December 9, 2017

For a particle of mass \( M \) in a one-dimensional box of length, \( L \), the energy levels and wave functions are given by:

\[
E_n = \frac{\hbar^2 n^2}{8ML^2} \quad n = 1, 2, 3, \ldots
\]

\[
h = \text{Planck's constant}
\]

\[
\psi_n(x) = \sqrt{\frac{2}{L}} \sin\left(\frac{n\pi x}{L}\right) \quad 0 \leq x \leq L
\]

This simple model has been applied to the \( \pi \)-electrons in linear conjugated hydrocarbons. Consider 1,3,5-Hexatriene \( \text{C}_6\text{H}_8 \) (as a linear molecule with 6 \( \pi \)-electrons and assume the length 10 \( a_0 \), where \( a_0 \) is Bohr radius. In atomic units \( \hbar = M_{\text{electron}} = a_0 = 1 \))

1. Estimate the ionization energy and electron affinity of \( \text{C}_6\text{H}_8 \) (with the approximation of non-interacting electrons), explain your results. What happens if the electrons are interacting?

2. Sketch the molecular orbitals for this molecule? Explain.

3. Explain why the quantum number \( n \) does not start from zero \( (n=0) \).

4. Calculate the probability of each \( \pi \)-electron to be in the left 1/3 of the molecule.

5. What are the missing terms in this simple model? Explain how you would improve this model to get better results?

6. Why the wave function is \( \sqrt{\frac{2}{L}} \sin\left(\frac{n\pi x}{L}\right) \) and not \( \sqrt{\frac{2}{L}} \cos\left(\frac{n\pi x}{L}\right) \). Explain how you choose a good variational wave function for a given problem, give an example.

7. Estimate the average momentum of the electrons in this molecule. Explain how you calculate the kinetic and potential energy of the electrons.

8. Explain the degeneracy of the energy levels if we extend the system to a particle in a two dimensional box.

9. Can this model be used to describe the rotational motion of the carbon atoms? Explain your results.

10. In your previous calculation of ionization, electron affinity, and the average velocity, of the \( \pi \)-electrons which one will not change if you raise the temperature from \( T = 0^\circ \text{k} \) to \( 300^\circ \text{k} \)? Explain why.
### Periodic Classification of the Elements

![Periodic Table Image]

(Numbers in parentheses are the mass numbers of the most stable isotopes.)