Reversible Changes

Prelab Assignment

Read the entire experiment. Submit your completed prelab questions on Labflow before you begin the lab, according to the deadline set in the syllabus.

Experimental Overview

This is a 2- part experiment to explore the effects of acid, base and temperature on a reaction that goes in both directions simultaneously. You will use the color of the solution to help determine which chemical species is in greater abundance.

Introduction

Chemical reactions commonly take place in both directions, both forward and reverse. When a reaction commonly goes in both directions, the reaction arrows are written as in Reaction 1.

$$A + 2\,B \rightleftharpoons C + D \qquad \text{Reaction 1}$$

Chemists plan their reactions and reaction conditions (such as temperature, pressure and solvent) to maximize the forward reaction and produce the greatest amount of product. The favored reaction direction depends on the concentration of the reactants for that direction. If one of the reactants of the intended reaction is used by a side reaction, less of the intended product will be formed. In fact, if enough of a side reactant is present, it can cause the intended reaction to go backwards, Reaction 2. In this reaction A can react with either B or E. If the wanted products are C and D, then the reaction with E is a side reaction.

$$\begin{array}{l} A + 2\,B \rightleftharpoons C + D \\ + \\ E \\ \downarrow \\ M \end{array} \qquad \text{Reaction 2}$$

Understanding the energetics and kinetics of the reaction helps chemists to plan their reactions. An exothermic reaction will proceed in the forward direction best when run at lower temperatures. An endothermic reaction uses heat as a reactant; therefore, higher temperatures will encourage the reaction to proceed. Heat can be considered a reactant in an endothermic process, Reaction 3.

$$\text{Reactants} + \text{heat} \rightarrow \text{Products} \qquad \text{Reaction 3}$$

Application

DNA exhibits a hierarchical structure from the molecular level up to larger assemblies. The core molecular helix of DNA is usually wrapped around histones, which then form wound structures known as chromatin, before assembling into the large formations called chromosomes.

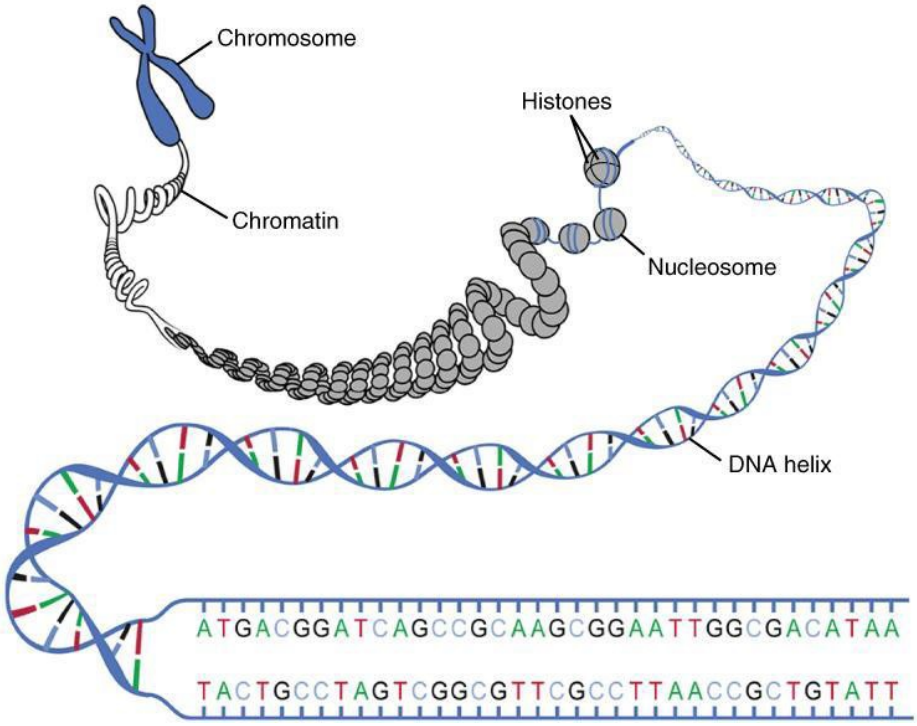


Figure 1 - Hierarchical structure of DNA[1]

When DNA is functioning properly, the larger formations partially unravel to allow genes to be accessed. This change in structure is reversible and the DNA can be wound to form the chromosomal structure again.

Small molecules undergo reversible changes as well. Certain reactions or change in conditions can change a small molecule into a different chemical form. Adjusting the conditions or using a different reaction can regenerate the initial chemical species. Alcohols for example undergo reversible changes when dissolved in water. The proton (hydrogen ion) on the oxygen can fluctuate between bonding to the alcohol oxygen atom or associating with a water molecule in an ionized form, Reaction 4. This process happens rapidly and occurs continually at ambient temperatures.[2]

Reaction 4

Water alcohol water + hydrogen ion alcohol - hydrogen ion

These reversible changes are usually possible when there is a small difference in energy for the process. If the energy difference is larger the change can be irreversible.

Chemical changes to DNA, such as reactions with cisplatin molecules, also result in an irreversible change.[3] As described in an earlier experiment, the chemical bonds formed between cisplatin and DNA are strong, resulting in DNA that can no longer function properly.

Irreversible changes also occur with small molecules. Consider the reaction between calcium chloride and sodium phosphate. Both reagents dissolve in water, but when combined, the strong calcium phosphate bonds that form cause the product to precipitate out of solution. This process does not easily proceed in the opposite direction, where the calcium phosphate would dissolve again, so it can be considered irreversible.

Chemical changes to a cobalt-containing molecule will be studied in this experiment to determine if the processes are reversible, and how they can be described with reaction equations.

References

1. OpenStax, Rice University. 3.3 The Nucleus and DNA Replication. https://cnx.org/contents/FPtK1zmh@8.25:9TxHOD3O@4/The-Nucleus-and-DNA-Replicatio (accessed Jul 16, 2018).
2. Chemistry LibreTexts by California State University. 7.2 The Acidity Constant. https://chem.libretexts.org/Textbook_Maps/Organic_Chemistry/Book%3A_Organic_Chemistry_with_a_Biological_Emphasis_(Soderberg)/Chapter_07%3A_Organic_compounds_as_acids_and_bases/7.2%3A_The_acidity_constant (accessed Jul 18, 2018).
3. Jamieson, E. R.; Lippard, S. J. Structure, Recognition, and Processing of Cisplatin-DNA Adducts. *Chem. Rev.* **1999**, 99, 2467-2498.

Procedure

Safety Note

- Wear safety goggles at all times in the laboratory.
- If any strong acids or bases come in contact with your skin, rinse immediately with lots of water. Always wash your hands before leaving the laboratory.

<u>Part I. Chromate/ Dichromate Ion Equilibrium</u>

In the presence of the hydrogen ion (H^+), chromate ions (CrO_4^{2-}) react to produce dichromate ions ($Cr_2O_7^{2-}$) according to the following reaction.

$$CrO_4^{2-}\,(aq) + H^+\,(aq) \rightleftharpoons Cr_2O_7^{2-}\,(aq) + H_2O\,(l)$$

1. In a clean small test tube, place 1 to 2 mL of 1 M K_2CrO_4. Add several drops of 3 M H_2SO_4. Shake the test tube to mix the solution. Record your observations.

2. Add several drops of 6 M NaOH, until you observe a change (mixing after each drop). Observe and record changes.

3. Add several drops of 3 M H_2SO_4 until you observe a change.

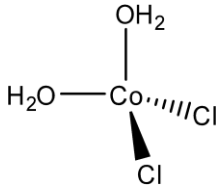
4. Add several drops of 6 M NaOH until you observe a change.

5. Explain the results; include equations for reactions. Why do you observe each of the changes?

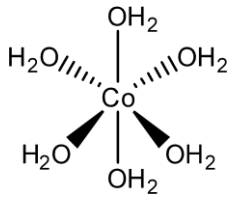
6. How does the addition of H_2SO_4 affect the chromate/dichromate equilibrium? How does the NaOH affect the equilibrium?

<u>Part II. Temperature Dependence in a Complex Ion Equilibrium</u>

Metal ions such as Co^{2+} will form complex ions, where ligands surround the metal ion. A ligand is either a molecule or ion that donates electron density to the metal ion to form a bond. Examples of ligands include H_2O, NH_3, Cl^-, and OH^-. $CoCl_2(H_2O)_2$ is a complex metal ion with two Cl^- (chloro) and two H_2O (aqua) ligands surrounding the Co^{2+} in a tetrahedral geometry. $Co(H_2O)_6^{2+}$ is also a complex ion with six water molecules (aqua ligands) surrounding the central metal ion, Co^{2+}, in an octahedral geometry. Ligands are not attached permanently to the central metal ion, but can dissociate and return, depending on interactions with other substances in the solution and the energy of the system.



$CoCl_2(H_2O)_2$



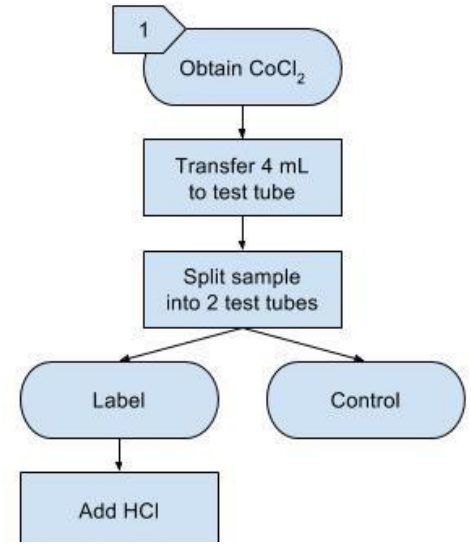
$Co(H_2O)_6^{2+}$

In aqueous solution, we can observe the conversion between the tetrahedral complex ion, $CoCl_2(H_2O)_2$, which is blue, and the hydrated octahedral complex ion, $Co(H_2O)_6^{2+}$, which is pink, according to the following equilibrium.

$$\underset{\text{blue}}{CoCl_2(H_2O)_2} + 4\,H_2O \rightleftharpoons \underset{\text{pink}}{Co(H_2O)_6^{2+}} + 2\,Cl^-$$

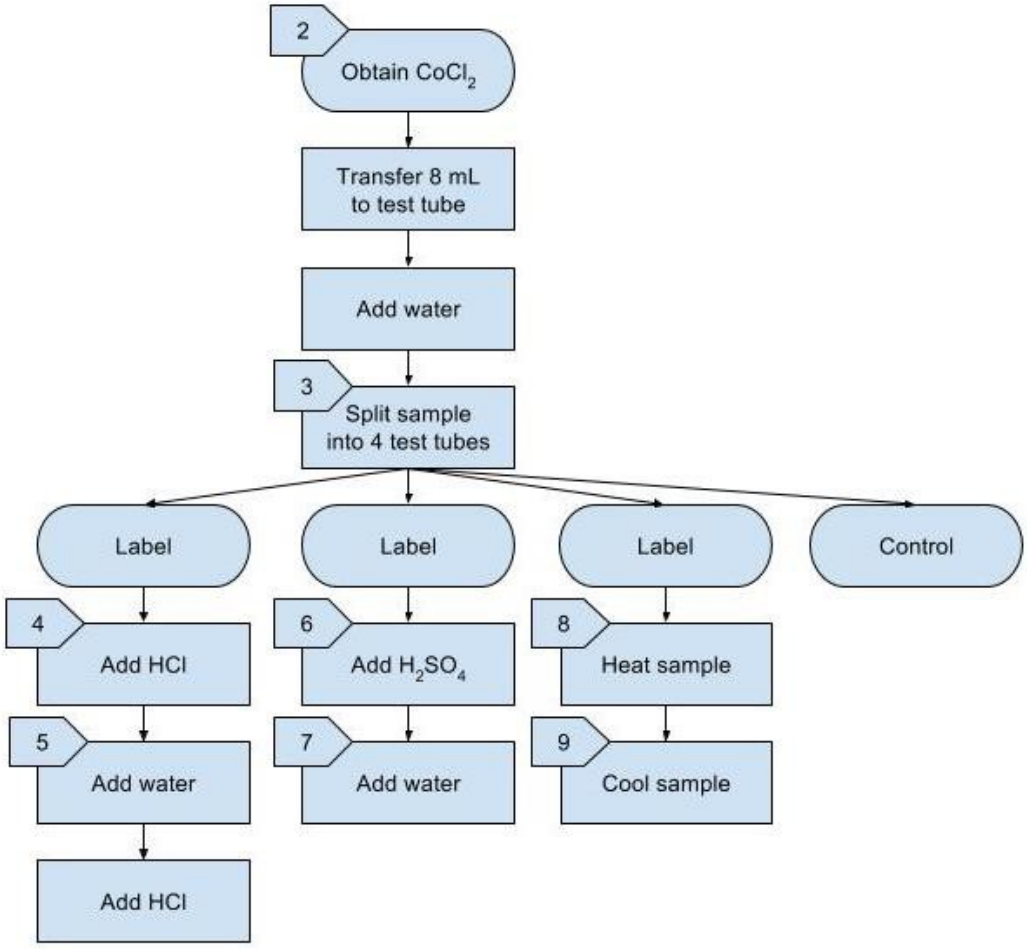
By dissolving $CoCl_2 \cdot (H_2O)_6$ in methanol, the solution obtained is between blue and pink.

Part II Procedure: (Flowcharts are provided for clarity)



Flowchart: Part 2, Step 1

1. Place 4 mL of 0.15 M $CoCl_2 \cdot (H_2O)_6$ (dissolved in methanol) into a small test tube. Split this sample into two test tubes so one can be a control. Add 6 M HCl dropwise to one of the test tubes and record any observations.
2. Place 8 mL of 0.15 M $CoCl_2 \cdot (H_2O)_6$ (dissolved in methanol) into a small test tube. Using a dropper, add just enough water to turn the solution pink. If you do not observe a color change in later steps, you may have added too much water and you will need to start here again.
   How much water did you add?
3. Split this sample into 4 test tubes. One sample from this step, and one from step 1 will be controls for visual comparison to the other samples to monitor changes.
4. To one of the test tubes, add 6 M HCl, dropwise, until you observe a color change. Record your observations and explain your results.
5. Add water to the same sample from step 4 and record any observations. Then add HCl again dropwise and record any observations.
6. With a different sample from step 3, add H_2SO_4 dropwise and record any observations.
7. With the same sample from step 6, add water and record any observations.
8. Heat a different test tube from step 3 in a beaker of hot water. You can heat the beaker of water using a hot plate. Observe and record the color change.
9. If heating the solution caused the equilibrium to shift in one direction, cooling the solution is expected to shift the equilibrium in the reverse reaction. Cool the solution in an ice water bath. Observe and record the color change. Explain your results; include equations showing reactions.



Flowchart: Part 2, Steps 2 through 9

10. Clean all glassware and equipment. Dispose of all waste properly. Refer to the *Chemicals Utilized* table to determine the correct quantities of disposed materials and record these values on the waste sheets. Be sure to clean up any materials spilled during the experiment. Please leave the laboratory in better condition than at the start of the experiment.

Chemicals Utilized Table

Chemical Name	Amount	Waste Type

Data Analysis Questions

- Draw out all ions in solution for each step.
- In Part I, why do we use different concentrations for sulfuric acid and sodium hydroxide, 3 M H_2SO_4 versus 6 M NaOH?
- In Part II, does the reaction produce or consume heat? Write the equation for the equilibrium reaction including the heat (is heat on the reactant or product side)?

Section Development: Discussion/ Conclusions

You are going to develop the discussion section of a technical report. First, read the full discussion section below to see an example of what you will be producing. Then, answer the following questions to help you develop the discussion. You will type a formal discussion section in paragraph form (question #9).

Before you type your discussion section, read the following discussion section from a published article. Consider the following questions as you read the included discussion section:

- Are the results listed?
- How do the authors discuss their work in the context of other researchers' works?
- What comparisons are made?
- What statements are made with certainty and what statements are made that suggest uncertainty?

DISCUSSION

Until relatively recently,[2-4, 27] DNA was considered to be a static component of the genetic apparatus, but recent *in vitro* [2-4] and *in vivo* [1, 28] discoveries support the concept that DNA is a conformationally dynamic molecule that plays an active role in its expression.

We have demonstrated that certain segments of DNA exist *in vivo* in a balance between B- and Z-helices that presumably is influenced by environmental factors (such as ionic conditions, protein binding, supercoiling, drugs, or carcinogens). Transcription, replication, toroid formation, and DNA-protein interactions are processes that can influence the level of supercoiling *in vivo*. We believe that the amount of B form observed includes a component that is provided by the reversibility of the B-Z equilibrium, but it also reflects the substantial portion of the plasmid pool that is involved in these processes.

Our two *in vivo* assays are based on very different methods. The M·*Eco*RI assay evaluates the helical nature of all GAATTC sites in any plasmid form (linearized, fragmented, supercoiled, etc.), whereas the linking number assay focuses only on the supercoiled plasmids. However, their results agree surprisingly well. This implies that the amounts of nicked, linear, or fully relaxed plasmid DNA *in vivo* are small.

Our results show that the existence of a DNA secondary structure *in vivo* depends both on the thermodynamic stability of this structure and on prevailing biological processes and interactions that provide transient relaxation of supercoils. Also, each conformational state of a sequence of DNA may have different biological properties, thus at least doubling the repertoire of DNA capabilities and strengthening the presumed role of DNA structure in gene regulatory processes.

Zacharias, W., Jaworski, A., Larson J.E., and Wells, R.D., The B- to Z-DNA Equilibrium in vivo is Perturbed by Biological Processes, PNAS, 85, 19 (1988), 7069-7073.

(The full paper is posted to Labflow if you are interested in reading further. Superscripts refer to references in the original work.)

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1. In 2-3 paragraphs, summarize your Results. Make sure to include your data, including concentrations and volumes. This data might be presented in tabular format, if appropriate.

2. In a full report, the Results section will have already explained what you saw, in great detail. At this point you are analyzing your results. (Do not repeat what you would state in the Results section.) Tell whether your results should be trusted. Were there any measurements that did not make sense? Did the data seem to agree? Include your notes here:

3. Restate your Hypothesis. In detail, tell which parts of your data support or reject your hypothesis.

4. The main point of your discussion is to explain the effect of the independent variable on your dependent variable. Identify the independent and dependent variables and how they relate to each other.

5. In general, the discussion section of a technical report interprets your results in relation to the background information. Since your background information focuses on the underlying causes and molecular level understandings of what is happening in the reaction, your discussion should link your macroscopic observations with the correct molecular-level behaviors.

Possible questions you may try to answer in your discussion include:

Why is acid more effective than temperature at changing the color?
What is the underlying mechanism?

Make a few notes that help you link what you saw with what this means.

6. Discuss your findings in light of the findings of others who have conducted similar experiments, and remember to cite those primary references correctly.

7. Explain what could have gone wrong or where your research could go in the future.

8. In a complete paper, the conclusions section will state whether your data support your hypothesis (This will not go into your discussion, but it is good to organize your thoughts and make sure your discussion is building to this point.) In 1-2 sentences, succinctly state your conclusion.

9. After you have drafted your thoughts, use the answers to the questions above (in numbers 1-5) to write 2-3 paragraphs interpreting your results. Keep in mind the context of the Results section within the paper.

Submit your responses to the **Prelab** and **Section Development** questions 1-9 via Labflow.