Slide 1

Nitrogen in All Its Forms

A very versatile little atom

Slide 2

Nitrogen

Nitrogen is in the center of the periodic table. Unlike the left side which favors cations or the right side which favors anions, Nitrogen is right in the center.

Nitrogen has 7 different possible "oxidation states" (pseudo-charges)

Slide 3

Common Nitrogen Compounds

NH_3 (-3)
N_2 (0)
N_2O (1)
NO (2)
N_2O_3 (3)
NO_2 (4)
N_2O_5 (5)

Slide 4

What does nitrogen do?

Nitrogen is an essential building block of biological molecules:

DNA is a blueprint for protein production.

DNA is expressed via the proteins it codes.

Proteins are made up of amino acids.

Amino acids have a carboxylic **acid** on one end and an **amine** (nitrogen) group on the other.

Slide 5

An amino acid

An amine is just substituted ammonia and can be recognized by an $-NH_2$ group somewhere in the molecule.

CH_3NH_2 "methylamine"

An amino acid is another "amphoteric" molecule which means…

Slide 6

It is both an acid and a base!

The acid end is a carboxylic acid which can be recognized by a –COOH group

An amino acid has an $-NH_2$ group (a base) and a -COOH group (an acid) in the same molecule.

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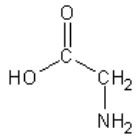
Biologically important amino acids

Proteins are made up of a small subset of biologically important amino acids called "α-amino acids" The "alpha" means that the amine group and the carboxylic acid are attached to the same carbon atom.

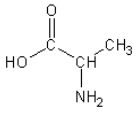
Slide 8

glycine



Slide 9

Alanine



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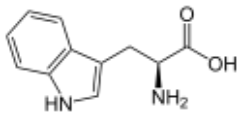
Tryptophan – Happy Thanksgiving!



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Biology rules

The biological importance of amino acids results in both opportunities and problems for nitrogen.

1. To make amino acids, plants need nitrogen. (Animals mostly absorb the amino acids from what they eat – plants!)
2. When organisms die, their decay releases the nitrogen back into the environment.

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Aquatic & soil systems

Plants need to acquire nitrogen from the environment and incorporate it into its amino acid production.

Not all forms of nitrogen can easily be incorporated.

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"Good Nitrogen" – The Plant Perspective

For a plant, nitrogen must be in a form the plant can absorb and use.

What properties would a molecule need to have?

1. Water soluble
2. Membrane permeable

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"Good Nitrogen"

NH_4^+ ($NH_3 + H_2O$)

NO_3^-

Other forms of nitrogen aren't directly usable, but "nitrogen-fixing bacteria" can make them usable.

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Bad Nitrogen

NO and NO_2 (sometimes called NO_x)

They are responsible for acid rain, ozone depletion and are greenhouse gases.

They are the products of combustion of nitrogen containing compounds.

Slide 16

Even The Good can be The Bad

What's the difference between a poison and a drug?

The Dose!

Even beneficial forms of nitrogen can be harmful if present in too large a concentration.

Slide 17

Bad in Excess

NH_3 (NH_4^+)

Why?

It's a base – changes the pH of water/soil.

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Bad in Excess

NO_3^- - EPA limit of 10 mg/L

NO_2^- - EPA limit of 1 mg/L

Binds to hemoglobin causing methemoglobinemia in infants (a dangerous type of anemia)

Slide 19

Biodegradation of waste

In polluted waters, as organic contamination is biodegraded, ammonia concentration peaks early and then decreases during biodegradation. Nitrite (bad nitrogen) peaks later and then tails off even quicker. Nitrate rises later in the process.

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Nitrogen profiles

Looking at the RELATIVE concentrations of different nitrogen compounds can give you some indication of where in its natural evolution a water system is.

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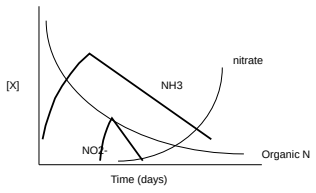
Nitrogen cycle



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Testing for Nitrogen compounds

There is a test for "total Nitrogen" which includes everything except N_2.

The other nitrogen species are oxidized (HEY! It's redox chemistry!!!) to nitrate, then reduced to nitrite (along with any native nitrate).

Addition of a dye allows for very sensitive colorimetric analysis.

Slide 23

Colorimetry – What is it?

Analysis of the quantity of a chemical compound based on the color intensity of the sample.

Color intensity ↔ [colored species]

If you measure the intensity of a specific color, e.g. 450 nm, using a photodetector and white light, then the chromophore's concentration is directly proportional to the intensity of the color. All you need is a calibration curve.

Slide 24

Why is my shirt this color?

My shirt is salmon today. Why is it salmon colored?

It is salmon colored because my shirt hates red and maybe a little yellow light but LOVES blue light and likes yellow light! It absorbs all the blues and leaves the red and a little yellow.

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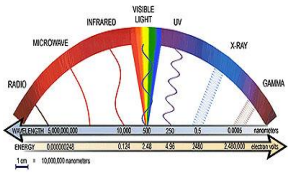
UV-Visible Spectroscopy

Solving Crimes and Saving Lives

Slide 26

Slide 27

The Electromagnetic Spectrum



Slide 28

If I shine radiation on a sample – what happens?

- It could pass right through
- It could bounce off
- It could be absorbed by the sample

Slide 29

If I shine radiation on a sample – what happens?

- It could pass right through
  - Matter is mostly empty space, photons are electromagnetic. If it doesn't interact it goes right through. Think of a window!
- It could bounce off
- It could be absorbed by the sample

Slide 30

If I shine radiation on a sample – what happens?

- It could pass right through
  - Matter is mostly empty space, photons are electromagnetic. If it doesn't interact it goes right through.
- It could bounce off
  - If a photon collides with the matter and gets redirected (think of a mirror)
- It could be absorbed by the sample

Slide 31

If I shine radiation on a sample – what happens?

- It could pass right through
  - Matter is mostly empty space, photons are electromagnetic. If it doesn't interact it goes right through.
- It could bounce off
  - If a photon collides with the matter and gets redirected (think of a mirror)
- It could be absorbed by the sample
  - The photon interacts with the matter - the photon energy must be absorbed

Slide 32

Why does a substance absorb light?

- Conservation of energy
  - Photons have energy, the sample must accept this energy
  - On a molecular level, it is the particles of the sample that must receive this energy
  - Different types of radiation are absorbed by different particles

Slide 33

What is actually absorbing the light?

- UV-Vis radiation is tuned to the electrons in the sample
- Absorption of a UV-Vis photon results in an electron in the sample gaining energy

Slide 34

Atomic Orbitals

- If you think of a single atom, it consists of a nucleus (protons and neutrons) surrounded by electrons.
- The electrons can occupy different orbitals that have different energies

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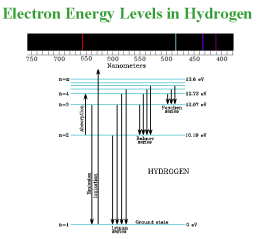

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Why is the world colored?

- If all light were absorbed by all matter, the world would be black.
- If all light were reflected by all matter, the world would be white.
- Because some light is absorbed by some matter, things look colored
  - We see the light that is NOT absorbed by matter

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Chromophores

- The "colored parts" of molecules
- In a molecule that has many atoms, the electron orbitals are created by a combination of electrons from many different atoms mixing together ("molecular orbitals")
- Some combinations of atoms are more likely to absorb UV-Vis light than others
- These "colored parts" of molecules are somewhat independent of the rest of the molecule

Slide 38

Conjugated pi systems – alternating double and single bonds

- Strongly absorbing of UV and visible light
- The number of bonds is related to the wavelength of light absorbed
- The wavelengths of light absorbed and the amount of light absorbed is a signature of the molecule.

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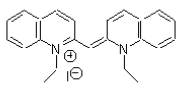
1,1'-diethyl-2,2'-cyanine iodide (PIC – Pseudo IsoCyanine Iodide)



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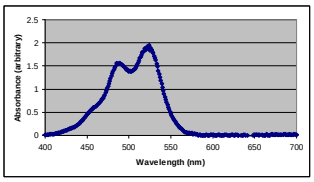
PIC UV-Vis spectrum



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Light gets absorbed as it passes through because the molecules absorb it.

Increasing "b" means more molecules have to be passed, so more light gets absorbed.

Decreasing "b" means fewer molecules have to be passed, so less light gets absorbed.

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Beer's Law

The thicker the glass, the darker the brew, the harder it is for light to get through…

Actually, Beer was a guy and his law applies to the absorption of light by materials.

Slide 43

Everything absorbs something

Even colorless solutions absorb some light, just not visible wavelengths (ROY G. BIV). They might absorb infrared light or ultraviolet, or radio waves or X-rays.

Colored solutions, of course, absorb colored light.

Slide 44

Not a big surprise

So, colored solutions absorb light.

How much? What do you think the absorption depends on?

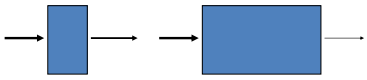
The thicker the glass, the darker the brew… ☺

Slide 45

Not a big surprise

The thicker the glass…

A "thicker glass" has more solution that the light must pass through.



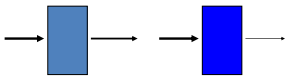
More solution means more light absorbing molecules means less light gets through!

Slide 46

Not a big surprise

The darker the brew…

A "darker brew" has a higher concentration of absorbers.



More colored molecules means more light absorbing molecules means less light gets through!

Slide 47

In a phrase

$A=\varepsilon bC$

Where A = "absorbance" of light
ε = "molar absorptivity"
b = the thickness of the solution
C = the concentration of the solution

"molar absorptivity" is a property of the material. Some molecules are just natural better light absorbers.

A = log scale of light absorption

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In practice

$A=\varepsilon bC$

If I know ε for a molecule and I know how thick my "beaker" is, then if I measure A I know C.
ε = "molar absorptivity"
b = the thickness of the solution
C = the concentration of the solution

That's the algebra…there's also the graphing theory.

Slide 49

Graphing…a lost art.

A=εbC

What does that equation "look like"?

How about now: A=[εb]C = mC

It's a straight line! I graph A vs. C and the slope is "m"

Slide 50

If I don't know ε

If I know ε and b, I could just use the algebra.

But I don't need to know ε or b if I make a graph…called a "calibration curve".

I make a series of solutions where I know the concentration and then I measure the absorption of light using a "spectrophotometer". Make a graph and I have a calibration curve.

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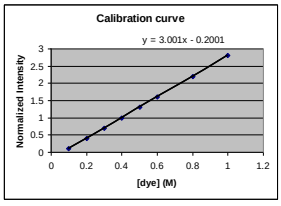
Calibration curve



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What's a spectrophotometer?

Just a light measuring device…we'll use one in lab. It has a lamp and a wavelength selecting device. It compares the light intensity with and without the sample to see what % of the light gets absorbed by the sample.

Slide 53

Total Nitrogen Ain't All That

Total Nitrogen content isn't as important as the concentration of individual species: the "good nitrogen" and the "bad nitrogen".

There are individual tests for individual types of nitrogen.

Slide 54

Testing for ammonia

3 main tests:

1. Phenate Addition – Direct and Distilled
2. Volumetric Analysis
3. Ammonia Selective Electrode

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Phenate addition

Adding an alkaline phenol (C_6H_5OH) solution along with hypochlorite (ClO^-) and a manganous salt (Mn^{2+}).

The manganese is a catalyst for the reaction:

$2\ C_6H_5OH + ClO^- + NH_3 \rightarrow C_{12}H_9NO_2 + \ldots$

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$C_{12}H_9NO_2$

Indophenol (short for indigo phenol) is a very blue dye. It can be quantified via colorimetry.

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Interference

Colorimetric analysis is subject to possible errors from:

1. Native color – other "blue" species
2. Turbidity – scattered light

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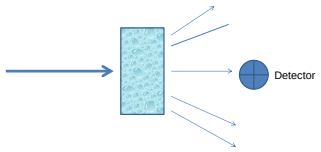
Scattered Light



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Phenate addition with distillation

To prevent interference, the waste water sample can first be distilled. The ammonia is volatile and will be distilled off early with some water as long as the pH stays in the 9.5 range.

How would you keep the pH steady?

Add a buffer (standard borate buffer has pH=9.5)

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Phenate Addition after Distillation

So, you would take the original sample (say 1 L), add buffer, and then distill off approximately 20-50% of the sample. Since the ammonia comes off early, 99% of it has been distilled off in the first 20% of the distillate.

Then you can do the phenate addition and the colorimetric analysis.

You need to correct the value for “concentration”!!!

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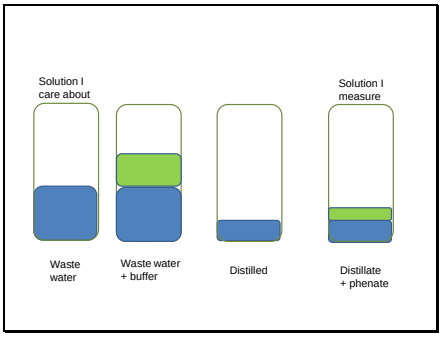

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Sample Problem

1000 mL of waste water is mixed with 1000 mL of a standard borate buffer (pH =9.5). The resulting sample is distilled until 500 mL of distillate is collected. Phenate addition and colorimetric analysis of the distillate determines the indophenol concentration to be 1.2×10^{-4} M. What is the ammonia concentration of the waste water?

Slide 63

Solution

$$\frac{1.2 \times 10^{-4} \text{ mol indo}}{1 \text{ L}} = \frac{1.2 \times 10^{-4} \text{ mol } NH_3}{1 \text{ L}}$$

$$\frac{1.2 \times 10^{-4} \text{ mol } NH_3}{1 \text{ L}} * \frac{17.1 \text{ g } NH_3}{1 \text{ mol } NH_3} = \frac{0.00205 \text{ g}}{1 \text{ L}}$$

2.05 mg/L

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Dilution is the solution

2.05 mg/L is NOT the answer

UNITS! UNITS! UNITS!

$\frac{2.05\ mg\ NH_3}{L\ ANALYZED}$ NOT $\frac{2.05\ mg\ NH_3}{L\ waste\ water}$

We did stuff before we analyzed it.

Slide 65

NOT like a titration

When we did titrations, we never worried about the stuff we were adding and the resulting solution. Why not?

Ping Pong Balls!

A+B = C the water doesn't do anything, only the As and Bs matter.

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Colorimetry cares

We aren't titrating.

What do we observe when we do the colorimetry experiment?

Color – specifically intensity of color.

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Kool-Aid

If you have half a glass of strawberry Kool-Aid, it is red. What if you add half of a glass of water to it?

Less red!

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It's all in what you look for:

In a titration, we are looking for the NUMBER of atoms. That doesn't change when you dilute it.

In colorimetry, we are looking for the COLOR of the solution. That does change when you dilute it.

Slide 69

Recall the analysis

1000 mL of waste water is mixed with 1000 mL of a standard borate buffer (pH =9.5). The resulting sample is distilled until 500 mL of distillate is collected. Phenate addition and colorimetric analysis of the distillate determines the indophenol concentration to be 1.2×10^{-4} M. What is the ammonia concentration of the waste water?

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Dilution is the solution

$\frac{2.05 \text{ mg } NH_3}{\text{L analyzed}}$ * 0.500 L analyzed = 1.025 mg NH_3

There must have been a total of 1.025 mg NH_3 in the sample analyzed. That ammonia was originally in 1 L of the waste water.

$\frac{1.025 \text{ mg } NH_3}{1 \text{ L waste water}}$ = 1.025 mg NH_3/L

THAT'S the answer!

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Volumetric Analysis

Rather than do a colorimetric analysis, you could do a titration of the distillate.

It has to be distilled first. Why?

To get the volatile ammonia away from any other acid/base species that would mess up the titration.

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Ion selective electrode

There are ammonium selective ions. (They have a membrane permeable only to the ammonium ions). Works like a pH meter – an electrode that is selective of H^+.

You just stick in the probe and read a voltage. The voltage is directly proportional to the concentration. (like a pH meter!)

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So, we have choices!

1. Colorimetry
2. Titration
3. Ion-selective electrodes

We're focusing mostly on the colorimetry because that is what is new!

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Other Nitrogen species

You can test for:

1. Organic nitrogen – bound in proteins
2. Nitrite – WE DID THIS IN LAB!
3. Nitrate
4. Total Nitrogen

I am not going to bother you with the chemistry. The tests are all similar to the phenate test (create a colored compound from the nitrogen of choice) or the ion-selective electrode method.

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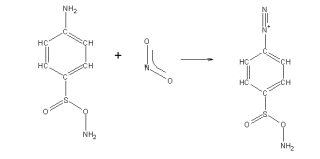
Nitrite test – or what you really did in lab!

Unbalanced (there's some water and protons)



Sulfanilamide nitrite a diazonium salt

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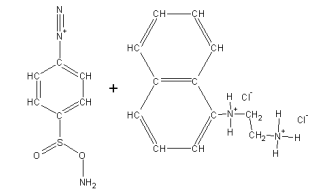
Then we add color…



Diazonium salt

N-(1-naphthyl)-ethylenediamine dihydrochloride

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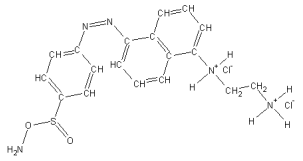
The actual colored compound.

This is the actual “pink” you saw in lab!



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Once you have color, you can do colorimetry!

Make a calibration curve.
Compare your solution to the calibration curve.

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Why Test for Nitrogen?

1. Some forms are good for plants. Important for agricultural regions.
2. Some forms are harmful (NO_x, NO_2^-, NO_3^-) to humans and other animals.
3. Biology is reversible.

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Biology is Reversible

Living beings take up nitrogen and use it in the formation of amino acids that create proteins that allow for expression of our genetic make-up.

If it is a resource for building, it is also a presence in wastes.

Living beings excrete waste nitrogen predominantly as either ammonia or urea $CO(NH_2)_2$.

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Biology is Reversible

Nitrogen is an indirect indicator of animal waste (feces or urine) as well as decay of deceased organisms.