TABLE OF CONTENTS

- Coming up With an Idea - pg. 6-7
- Background Research and journey of experimental design pg. 8-108
- The time period of ~~so~~ turbidimeter building pg. 109 - 128
- The experimental procedure pg. 130-144
~~- Other analysis~~
- Inverse debye length formula pg. ~~14~~ 145-147
- Discussion ~~(Brief)~~ pg. & Analysis pg. 148-152

Sep 27, 2025

- Today I came across a cool study!
- I came accross a link of Isef (International Science and engineering fair) abstracts. it was ~~on~~ "synergistic effects of Polysaccharides on Protein adsorbtion and tribological behaviours on Orthokeratology lenses."
- In this article I learned that proteins from our tears can deposit on contact lenses which can initate the eyes, or even ~~cause~~ lead to corneal scratches and injections.
- This is something that I ~~might~~ want to research more about.
- This also is ~~somewhat~~ related to my project last year where I researched on near monks' affect on myopia developement, while still being new and unfamilian.

- Today I shadowed Dr. Andrea Lasky, who is ~~a~~ practicing optometrist at Mission Eye Care from noon ~~to 3~~ 4pm
- I am so grateful to ~~that~~ I was able to have this wonderful opportunity, ~~this~~
- ~~After~~ Ever since my science fair project from last year, I have been super interested in optometry and vision science.
- Dr. Lasky is a specialist in speciality contact lenses, so I saw a lot of patients with contacts, which made me realize that there are so many things with contacts! Which I never realized as ~~a~~ ~~one~~ someone who wears glasses.
- This developed my interest in contact lenses and deepend my existing passion for vision!
- I cannot thank Dr. Lasky enough for so kind to let me shadow her!

- I am thinking of doing a project (experiment) on protein deposition on contact lenses. ~~This is something that I~~ ~~D~~

- I want do an experiment as it will help me learn experimental design, give me hands-on ~~scient~~ scientific experience.

- This will combine biochem and vision science - ~~two~~ of my favourite subjects.

- My mission is to find a specific problem, controversy, or gap in the knowledge and investigate it.

Nov 12, 2025

- Today I am going to some basic surface level background research

The topics I will study are:

- Soft contact lens materials

- Tear film chemistry

- Contact Lens care regimens

Contact Lens Materials -

"Types of soft contact lens materials"

-By Good Optometry

~~site~~

Notes:
- silicone hydrogel are oxygen permeable
- hydrogel are softer
- daily disposables are more hygiene safe
- ~~bi~~ bi-weekly and monthly need cleaning

Nov 12 ~~2025~~ 2025

"Best Contact solutions"

- Doctor Eye Health

Notes
- there are ~~multi~~ multi-purpose solutions that are used for cleaning, disinfecting, rinsing, and storing
- Enzymatic cleaners are harsher protein removal cleaners
- Hydrogen peroxide based cleaners are preservative free but require neutralization

Nov 16, 2025

Today I will learn about contact lens material more in-depth.

Article 1: Contact lens 101 Part 1 - Contact Lens Materials (full citations in my online project platform

Notes:

- History of contact Lenses
  - In 1887, the first contact lens made with glass that covered the whole eye was made
  - 1948 -> PMMA plastic contacts made for first time
  - ~~Polymethyl methaacrylate -> GPs (Rigid Gas permeable)~~
  - Polymethyl methacrylate (PMMA) similar to GPs today but did not allow oxygen to pass through
  - Hydrogel -> Hydrophilic polymer
  - In 1985 -> hybrid lenses -> rigid centre with a soft surrounding
  - ~~1987~~ 1995 -> Daily disposables
  - 2002 -> silicone hydrogel -> oxygen permeable

- Hydrogel lenses are soft
  - poly HEMA -> main material hydrogels ~~polyhydroxythyle~~
  - poly-hydroxyethyl methacrylate.
  - higher water content = more oxygen permeability in hydrogels
  - silicone hydrogels -> more silicone = more oxygen
  - ~~Silicone hydrogel~~ Silicone can be added in Gas permeable lenses to improve oxygen permeability.
  - Plasma treatment or other wetting agents can be used to improve the wettability in silicone hydrogel
  - silicone hydrogels reduce the chance of corneal hypoxia -> increases risk eye infection ~~and~~, abnormal blood vessel growth on the cornea and swelling.

Nov 16, 2025

Today I will learn about contact lens material more in-depth.

Article 1: Contact lens 101 Part 1 - Contact Lens Materials (full citations in my online project platform

Notes: #

- History of contact Lenses
  - In 1887, the first contact lens made with glass that covered the whole eye was made
  - 1948 -> PMMA plastic contacts made for first time
  - ~~Polymethyl methacrylate -> GPs (Rigid Gas permeable)~~
  - Polymethyl methacrylate (PMMA) similar to GPs today but did not allow oxygen to pass through
  - Hydrogel -> Hydrophilic polymer
  - In 1985 -> hybrid lenses -> rigid centre with a soft surrounding
  - ~~1987~~ 1995 -> Daily disposables
  - 2002 -> silicone hydrogel -> oxygen permeable

- Hydrogel lenses are soft
  - poly HEMA -> main material hydrogels ~~polyhydroxyethyle~~
  - poly-hydroxyethyl methacrylate.
  - higher water content = more oxygen permeability in hydrogels
  - silicone hydrogels -> more silicone = more oxygen
  - ~~Silicone hydrogel~~ Silicone can be added in Gas permeable lenses to improve oxygen permeability.
  - Plasma treatment on other wetting agents can be used to improve the wettability in silicone hydrogel
  - silicone hydrogels reduce the chance of corneal hypoxia -> increases risk eye infection ~~and~~, abnormal blood vessel growth on the cornea and swelling.

Nov 18, 2025

- Today I was thinking that for my science fair experiment, I can test either different contact lens materials or cleaners and see how much protein they clean or attract.

- For this I could use the bradford assay that changes color to ~~blue~~ blue (the intensity depends on protein concentration in the solution and build a spectrophotometer to measure the absorbance of the solution from Arduino!

Nov 19, 2025

- Today I will look into how to build a spectrophotometer from arduino.

Youtube video: "Arduino Based Spectrophotometer Demonstation Trial"

By: Isabelle Jane Romo.

Notes:

~~Ardu~~

Materials -

- Arduino uno - have
- female to male jumper wires - have
- male to male jumper wires - no.
- motor - yes
- Led - IDK
- Breadboard - yes
- photodiode - no.

Building a low-cost spectrophotometer of visible light using Arduino and MATLAB

BY @FilipeAlmeidaTech

Notes:

- ± need cuvettes?

~~Soure~~

~~Then I looked at~~

Nov 25, 2025

-Today I will research about the tear film.

"The antatomy of the tear film"

The tear film consists of the mucin layer, aqueous layer, and the lipid layer

Dec 15, 2025

Today I will do more specific ~~problem~~ research to get a specific problem.

First Website →
Title: Types of solutions for cleaning contact lenses
Published Jun 26, 2024
Platform: Eye Jones

Dec 20, 2025
Notes:
Multipurpose cleaners
- versitile
- clean, disinfect + store
- simple (combines steps)
- rinse, clean, & disinfect with one
- ability to remove deposition and microorganism
- contain (typically) disinfectants like [polyhexamethylene, biguanide, or] [polyquaternium → Food.
Pros → Convinience, disinfecting, cost-effective
Cons → Some users may feel sensetive or get allergic to the preservatives
~~Not as effe~~ Mostly work on common pathogens; not as good as hydrogen peroxide based cleaners.

→ soft contact lenses

- ~~Boss~~ Bausch + Lomb, renu advanced multipurpose cleaners are popular for hygine and comfort. ↓
- RGP lenses ~~do require~~ require more specific
  - Even though some cleaners say that the solution is no rub, rubbing helps remove more debris.
  -

Hydrogen Peroxide (H_2O_2)
- uses anti-microbial and bubbling properties of H_2O_2
- break down organic materials like proteins and lipids
- the bubbling action help loosen up deposits - providing a deeper clean.
- After the lenses are soaked in the solution, a neutralizing solution to convert H_2O_2 to into saline. ~~to~~
- Effective against a great range of microorganisms
- Good for sensetive eyes as preservative free
- More effective at removing proteins

Cons → If the neutralization doesn't happen properly (~~not~~ instructions not ~~follo~~ followed correctly) will be painful
→ longer soak time for 6 hours = low convinience.

⇒

~~Brands in Australia~~

Enzymatic cleaners
- especially for protein removal
- break down protein
- used on a weekly basis (typically)

Analysis:
some questions I have after reading this article:

What factors ~~influence~~ influence ~~the~~ protein deposition on hydrogel lenses?

~~At~~ @ On searching this question on google I came across this article

Title: How and Why of contact lens deposits.
By: Heidi Wagner

Publishing date: May 15 2020

Notes
- Because daily contact lenses, deposits on reusables are overlooked
- In 2019, daily disposables accounted for 35% of lens prescribing in the U.S.A
- Planned replacement of contact lens make deposition less prevalent and ~~severe~~ severe.
-

~~Undert~~
- deposits can cause ocular pathology like papillary conjuctivitis, punctate keratitis, corneal inflamatory events, and microbial keratitis.

Underlying mechanisms
- ~~even~~ deposits are influenced by patient compliance, individual tear chemistry, and environment.
- Understanding these factors help minimize discomfort

Identifying Desposits

Dec 22, 2025

Today I will continue my notes on the article from Saturday.

Indentifying Deposits
- deposits begin ~~in~~ within mins
- deposits are best indentifyied by seeing biomicroscopy of the lens on the eye with various ranges of ~~luminousi~~ illumination and magnifications
- Another way is through a lens loupe
- Deposits can be distinguished by color shape, and location.

~~Coo~~ Common types of deposits
- proteins & lipids → ~~lysosome~~ lysozyme binds to the lens surface, and change its structure which impairs its function.
- This is called denaturation
- Factors include lens substrate, temperature, and PH.
- Proteins create an opaque film
- Lipids create a shiny and slimy layer
- These happen in SCLs and GPs

Lens Calculi

↓

-(gelly bumps)
- SCLs → happen
- these are distinct, localized elevations on the anterior surface for SCLs
- composed of Ca, Lipids, and mucoproteins

Aquestion I had → What are mucoproteins?
Answer → contains carbohydrates and is part of mucous and are ~~mucous~~ viscuous proteins
↓
Link: https://www.britannica.com/science/protein/Plant-protein#ref593835

- lens calculi causes the ~~aqueos~~ aqueous tear layer to deplete which results in a hydrophobic zone, causing more deposition.
- Uncommon in monthly replacement or more freaquently.

~~These bumps~~
-These are seen in high water ionic (group IV) hydrogel lens materials.

-

I skipped a few subheadings as they were not directly related to my project idea.

Environmental Debris
- makeup particles are a common source of deposits
- Lotions and hairspray can also bind to the lens.
- Can be prevented with hygine
- Other debris like leaf litter can also contaminate the lens.
- GPs lenses have less wettability.
- This problem can be solved by plasma cleaning.
- Some medication can lead to discolonation in soft contact lens.
- some glaucoma drops can also cause discolonation

Lysozyme
- some benefits as it has antibacterial and antiinflamatory
- Lactoferrin in the tears work with lysozyme to inhibit gram positive and gram negative bacteria.
- Further studies are needed to better comprehend this
- sometimes increases comfort in HEMA lenses

Lens Materials
↓

- HEMA → Hydroxyethyle methacrylate
- Lysozyme works more actively on hydrogels than silicone hydrogel
- There should be lens materials that bind good deposits and repel bad ones
- FDA classifies hydrogels as ionic (group 3 & 4) and non-ionic (group 1 & 2)
- Group 2 and 4 have a higher water content (>50%) than 1 & 3
- Silicone hydrogels have a higher oxygen permeability
- polymethyl methacrylate (PMMA) sh deposit less lysozyme than hydrogel
- lysozyme is more prevalent in ionic group 4 materials
- Hydrogels contain ~~methacrylic~~ methacrylic acid to increase water content and O_2 permeability.
- HEMA based lenses are "magnets" for protein deposition as (MA) is negatively charged, ~~and~~ which binds to positively charged protein.

~~I will continue my notes for this website on another day.~~

~~I will find another website to learn more and cross-verify.~~

Dec 23, 2025

Today I will continue my notes for the article on 'Lens Materials'

Notes on Lens materials

- Hydrogels are HEMA based
- when silicone was added to the material to create silicone acrylate lens material.
- This resulted in more oxygen permeability but more protein deposition
- Then flourine was added to improve wettability of flourosilicone acrylate.
-

Question I had while reading the article what are Hyper-Dk and ~~Hyper-k~~ Moderate-Dk lens material.

Hyper-Dk → More oxygen permeability

Dk value ≥100

Dk measures how much oxygen is able to pass through the lens

- usually used in specialty

↓

~~Continued~~ Notes on Lens Wear & Care

- lens wear ~~care~~ care should be tailored to the patient.
- multipurpose ↘ cleaners
  - Cleaning removes loosely binded deposits
  - Rinsing removes debris
  - Disinfection inhibits microbes.
  - FDA discourages no-rub lens systems

A queseption I had while reading this article:

What does digital cleaning mean?

~~for website name~~:

Digital cleaning means rubbing the lens to further remove debris in the lens cleaning process

- Surfactant or enzymatic cleaners are rarely ~~needed~~ indicated for bi-weekly/monthly contacts but may needed for heavy depositers.
- surfactant cleaners remove loosely binded lens debris, and ~~unbinded~~ protein.

~~= In contemporary~~ unbound

- In contemporary systems use surfactants and wetting agent.
- some anecdotal reports suggest that lens ↗ residue ↘ proprietary wetting agent.
- This can be solved ↘ related by switching to a hydrogen peroxide product without the wetting agent.
-

A question I had while reading the article:

What does "topping off" mean in the context of lens care?

Answer: Means adding new solution into the old (used one).

↓

Website: Healio.com (full link in citations project platform)

~~- GPs = SCLs in 1 cleaning d -~~

- Boston original -> 2 bottle system has a ~~seper~~ separate abrasive cleaner.
- GPs
-

I will continue my notes for this website tommorow.

~~Right now~~

Dec 24, 2025

- While reading the article: "How and Why of contact lens deposition", A dilemma or problem I ~~found~~ learned ~~out~~ about that I found interesting was that Lysozyme is a good thing when it is native, but when it becomes denatured it becomes harmful.

- I was thinking I could do a project on this so I searched on google: "How to measure denatured lysozyme vs. native lysozyme and came accross this chapter from a book

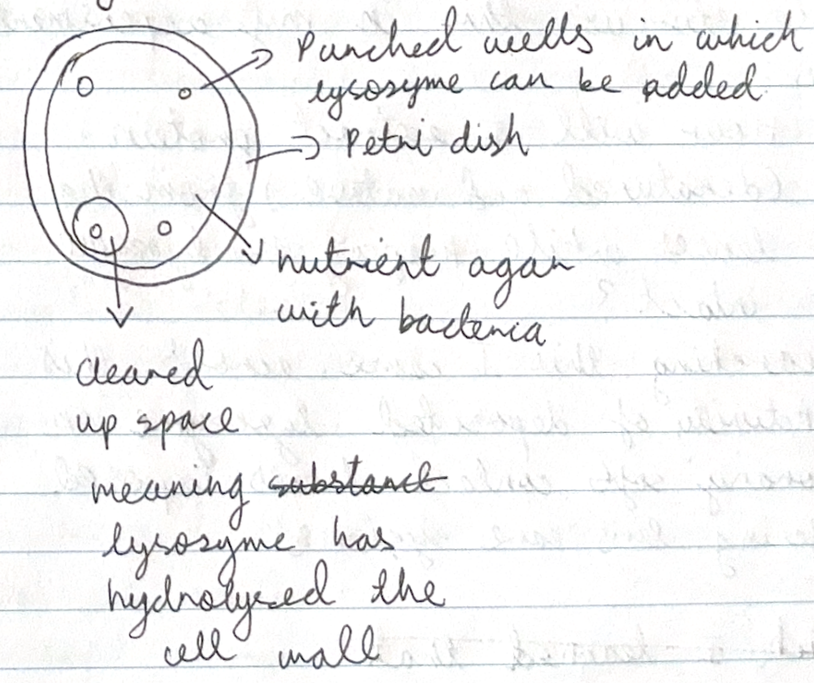
Link: Ableweb.org/biology labs/wp/content/uploads/volumes/vol-3/11-Snyder.pdf

Name -> Chapter 11 -> Two reliable + inexpensive lysozyme assays for teaching enzymology and ~~microbio~~ microbiology.

By: John A. Snyder, Donald Fritsch

Notes:

- Lysozyme can hydrolyze the cell wall of some bacteria -> break down chemically with H_2O
- Most assays for lysozyme are turbidimetric (quantifying cloudiness)
- 1st Radial diffusion assay (similar to most antibiotic assays)
- Diagram



- the diameter the cleared ring is proportional to the concentration of lysozyme in the solution that diffused out of the well.
- This is labelled as the "intra-agar lysis assay" (Nace et al 1965) or the lysoplate assay (Ossenman and Lawlor 1966).
- 2nd way includes spectrophotometry measures a suspension of bacteria buffer
- 1st method involves dead cells,
- 2nd needs live bacteria
-

One question I had about how I will be able to use this in my experimental design is:

> How will I extract protein (denatured and native) from the lenses while keeping the lysozyme intact?

After searching this I came across this study: "Activity of deposited lysozyme on contemporary soft contact lenses exposed to differing lens care systems"

~~In which I learned that~~

Notes:

- ~~pa~~ participants were told to use either peroxide 3% system, or polyquaternium-1 and myristamidopropyl dimythylamine containing systems.
- participants were using ~~silicone hydrogel~~ senofilcon A silicone hydrogel contacts.
- protein was extracted with 50 is to 50 0.02 triflouroacetic acid I

After searching bout this chemical I learned that it is ~~dangerous~~ risky for ~~jo~~ junior high students to use. and not as accesible

To add, most protein ~~extr~~ removing cleaners is a denaturant, meaning that while removing the protein, it denatures the active protein as well. However enzymatic cleaners break the proteins into ~~amio~~ amino acids which cannot be detected in a protein assay like the bradford or BCA as it is not protein anymore.

I learned this after searching: Which contact lens solution remove lysozyme effectively without altering the state of lysozyme.

-

Dec 29, 2025

Today I found this ~~as~~ article study which peeked my interest and had information which could be relevant to my project.

Article name: ~~A~~Effect of contact lens solutions in ~~stabilizing~~ stabilizing the activity of tear lysozyme. (full citation in my online project platform)

Notes:

- lysozyme helps maintain ocular surface homeostasis
- CLs manufacturers include ingredients in the blisten packaging solution which help ~~stabe~~ stabilizing the tear film.
- the experiment included exposure to test solutions ~~and~~ measurement of lysozyme activity ~~using~~ followed ~~micrococ~~ micrococcus luteus turbidity ~~te~~ test.
- the study did not provide additional detail as it was a free version only including the abstract.

Now I will continue my notes from the article - "The how and why of contact lens deposition.

Notes:
- surface treatments can be used to make the experience better for patients (Hydra-PEG is a waterbased treatment used for scleral and GPs for more comfort)
- The coating ~~provides~~ is helps inhibit deposits and fogging
- 'Tangible clean' is a MPS is used for lenses with Hydra-PEG and other SCLs
- ~~abras~~ Abrasive cleaners should not be used with plasma ~~treatment~~ treated lenses, Hyper-DK lenses, and Hydra-PEG
-

Dec 30, 2025

Today I will be watching this youtube video: "Maintaining tear film Homeostasis in soft contact Lens wear."

By the youtube channel: @ Eyes On Eyecare Optometry.

Notes:
- There are a ton of contact lens drop-outs (meaning people who discontinued wearing CLs.
  43% discontinuation rate in Canada.
- A study that I can look at after this lecture: CLEAR - Effect of contact lens materials and designs on the anatomy and physiology of the eye.
- Pros of CLs → freedom, confidence, sports, peripheral ~~version~~ vision.
- Contact lenses can be designed for specific activities
- One single bottle of MPS average weight is equivalent to 2.5 years of daily disposable CLs
- Daily disposables might not be as wasteful as they are often recycled

- Contact lenses divide the tear film into pre and post lens tear film
- Faster thinning of tear film and ~~two~~ evaporation of the tear film = soft contact lenses.
- All CLs thin the film, but SCLs evaporate the tear film
- Some patients experience dry eye only with CLs
- Pre-Lens Tear Film

↓

Outer lipid coating
- aqueous - mucin components

Pre CL → Post

Post-Lens Tear Film

↓

Thin aqueous - mucin layer

↓

oxygen transmitted to the cornea

- No. 1 → reason drop out is discomfort 50%
- Lipid layer - prevents tears from evaporating
- Aqueous layer - the bulk of the film
- Mucin layer - helps spread the tear ~~across~~ across the eye.

Ø Another youtube video → "Tear Film & Contact Lens Interaction"

By: Dr. Saud Javed

Notes:
- there is an ~~adhes~~ adhesive force between the water molecules of the film and the molecules of the CLs
  - Tears have a refractive index!
  - CLs affect cornea
  - CLs damage corneal epithelial cells
  - can
  - lysozyme, polysaccharides, chlorides, phosphates, and ~~ot~~ calcium
  - Low PH of tears = more protein deposition. High PH = high minerals
  -
- After searching on google, I found a really surprising fact that I didn't find earlier, is that the bradford assay is ~~not th~~ sensitive to the detergents and surfactants present in CL cleaners. This will hamper my results. Plus DC (detergent compatible) bradford assay is not particularly safe for students and is above my budget.

- I didn't find anything about this earlier so I am planning on testing an environmental factor (the salinity of saline)'s affect on lysozyme denaturation

Dec 31, 2025

- Today I will continue planning and researching for my experimental project on lysozyme denaturation

First I have a few questions I will research for my background research

- What is lysozyme? It's ~~structural~~ and chemical composition, properties, and how it denaturations?

- What environmental factors affect lysozyme denaturation in lens care ~~so~~?

- ~~Why~~ What problem does the factor ~~pose~~ pose for CL wearers?

- How do these factors denature lysozyme?

"What Is the Role Of Lysozyme In Tear Film?
- Optometry knowledge base"

Notes:
- ~~Vital component of the~~
- ~~Primary~~ Primarily located in the aqueous layer
- It breaks down the cell walls of gram positive bacteria, reducing infection risk.
- Lysozyme has antifungal properties
- It degrades chitin that is found in the cell walls of fungi.
- Lysozyme may have antiviral properties
- ~~Stabilizes~~ Stabelizes the tear film which interact with lipids & proteins like lactoferrin and lipocalin
- These interactions help maintain the surface tension of the tear film which is crucial for preventing the tears from evaporating.
- Stable tear film is ~~crucial~~ crucial for keeping eyes lubricated and have clear vision
- Low lyrozyme can lead to dry eye and hepetic infection

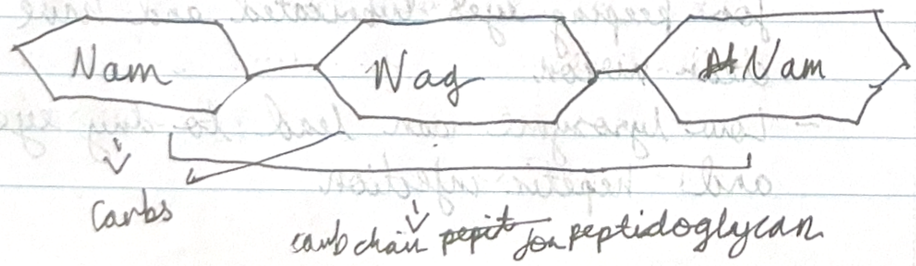
"Lysozyme Mechanism"

By: @ Catalyst University

Notes:

- In the cell walls of bacteria there is a polymer called peptidoglycan.
- Peptidoglycan is half protein and so half carbs
- ~~Carbohydrates are made from~~ WAG (N-~~ace~~ ~~acetyglu~~ acelyglucosamine) ~~and~~ ~~which~~ which is a monosaccharide that provides structure to the polymer (derivative of glucose)
- NAM (N-acetylmuramic acid) more complex sugar derived from WAG. It is unique to bacterial cell walls and contains a peptide chain

Diagram



- Lysozyme splits all these repeating units of nag & nam
- This damages the cell wall, making it easier for our body to kill the bacteria
- This does not act on the protein part only the carbohydrate part
- Lysozyme only breaks the Nam-Nag bond NOT the Nag-Nam bond
- Glutamic acid and aspartate?
- Glu sits at the top of the nam and asp at the bottom

Another Video to along with this one:
"What Is Lysozyme And How does it Work"

By: @ chemistry for everyone

~~#~~ -Notes

- lysozyme binds to the ~~sug~~ sugar chains and catalyses a hydrolysis reaction
- Hydrolysis is ~~use~~ uses water to break ~~mol~~ bonds
- The internal pressure between the cell wall causes it to burst leading it to die

- Lysozyme is a model in chem for enzyme catalysis
-

Another video to support my understanding

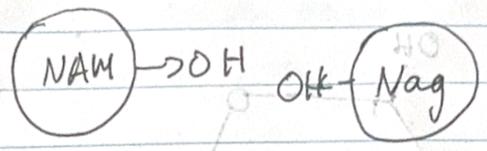
"Lecture 25: Mechanism of Lysozyme, Chemistry of Lysosyme"

By: @ Chemistry by guptasir

Notes:

- Lysozyme is an antibacterial enzyme
- Lysozyme in chemistry is called N-acetylmuramide glycanhydrolase
- Peptidoglycan consists of sugars and amino acids.
- Lysozyme is a single chain globular protein containing 129 amino acids
- 1922 Alexander Flemming ↑ discovered
- The ~~bond~~ linkage between two sugars is called glycosidic linkage ~~(is a specific covalent bond)~~
- Lysozyme ~~rep~~ ruptures this bond
- This is a two-step displacement reaction
- First Nag comes then Nam

- Then:



- the two main amino acids that are involved in this reaction:
  - Aspartic acid 52
  - Glumatic acid 35

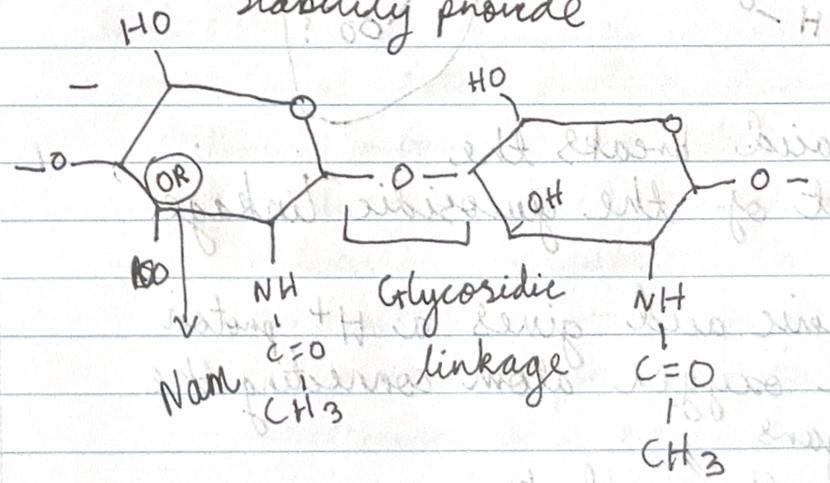
↓

Do the real thing → initiate

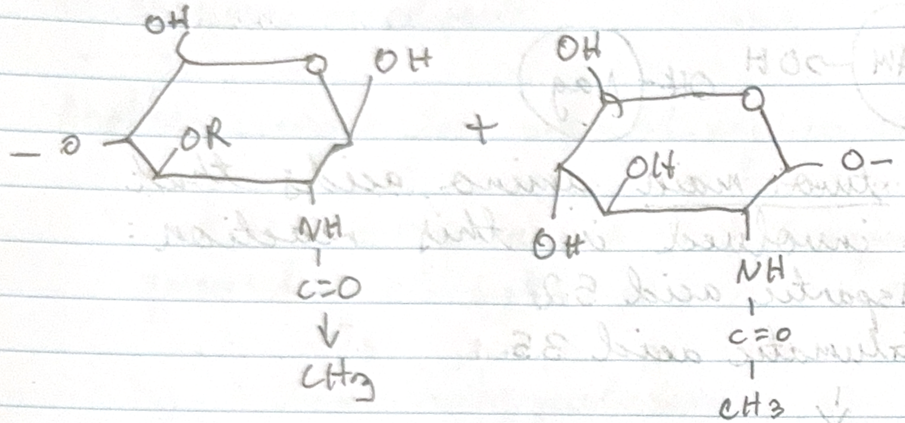
- Tryptophan 61
- Tryptophan 62
- Aspartic acid 101
- Asp 102

↓

Stability provide



After lysozyme

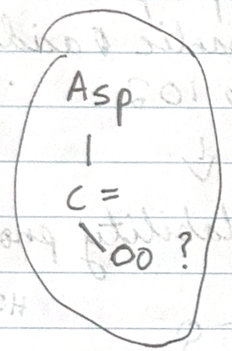


First lysozyme then water will be involved

Glumatic acid

Glutamic

- ~~Glumatic~~ acid breaks the Nam part of the glucosidic linkage



- The glutamic acid gives a H^+ proton to the oxygen atom connecting the two sugars
- This causes the bond to snap
- Half of the sugar chain is stuck to the enzyme

- Then Asp 52 has a negative charge which helps it hold onto the nag sugar ~~preventing it from~~
- Next, ~~A water molecule~~ Glu 35 grabs ~~a water molecule~~ a H^+ from a water molecule
- The remaining OH^- breaks the link between Asp52 and Nag.
- Helping the Glu 35 & Asp 52 to break the next bond.

~~Affect of~~

"The truth about Lysozyme"

Article ?

Notes:

- 90% of total protein deposits
- Biotrue = Bauss + Lomb, Opti-free replenish by Alcon, Complete - Easy rub
- Molecules: Sulfobetaine in Biotrue is a zwitterion which protects lysozyme to prevent it from denaturing.
- zwitterion is a molecule with both + and - changes but a net change of 0
- $ Block copolymer tetronic 1107 are

attracted to denatured protein helping to remove them.

- Opti-free replenish uses negatively charged citrate to pull protein from the lens.

- citrate has a negative charge 10 times greater than that of the lens

"~~Hof...~~" - Hofmeisten series

- from wikipedia

~~Notes:~~

- salts interact ~~can~~ with protein and can denature it

~~- lysozyme → low static~~

-

- "Correlation of lysozyme activity and stability in the presence of Hofmeisten series anions."

Notes:

- How salts affect lysozyme activity depends on the the concentration of anions and their position in the Hofmeisten series.
- ~~Kosmotropic~~ Kosmotropic anions (sulfates and acetate) increase stability and activate the enzyme
- Chaotropic anions (bromide, thiocyanate, and perchlorate, including chloride) inhibits enzyme activity.
- strong correlation between stability and activity

-

Jan 1, 2025

- Today I will find more specific information on how conicity affects lysozyme denaturation.

- In order to find specific and relevant info for my project I will use google scholar.

"Lysozyme & Lipid deposition on silicone hydrogel materials."

Notes

- lower levels of lysozyme on silicone hydrogel than hydrogel ~~as it~~ but a ~~d~~ greater degree of them were denatured

~~"An overview of anti~~

"An overview of ~~antibacterial~~ antimicrobial activity of lysozyme and its functionality in cheese"

Notes: ~~p~~ of

- pH is dependant ~~sal~~ salt concentration
- High salt = bad
-

"~~No salting-in of lysozyme chloride observed at low ionic strength over a large range of pH.~~"

~~Notes:~~ (this study did not make any sense)

"~~The dependance of lysozyme activity on pH and ionic strength.~~

Notes: ~~The~~ (Again same problem)

Jan 2, 2025

- Today I emailed Dr. Lasby (an optometrist who is a specialist in CLs whom I shadowed for an interview request
- She replied saying that my topic idea wasn't something she doesn't encounter in her clinical practice, so she said that she can refer me to a research scientist who can help answer my questions.

- I reached out to a professional for help with my background research as ~~understand~~ I couldn't find many sources that actually made sense (as I am a Grade 9 student and most of the ~~male~~ studies and concepts are university-level.
- For that reason I am planning on using

- Chat Gpt and google AI tommorow to help me translate ~~rese~~ university level science language to grade 9 language

- This will help me gain a deeper understanding.

- On top of that today I asked google AI if there is anything that I should be aware of after giving it a description of my project and the protocol for ~~Intra agar plate~~ radial diffusion assay and it said that having a lab or someone to contact and ask for guidance with proper protocol would be good as radial diffusion assay requires precision. So, I emailed Ufc and MRC.

" Biological and Clinical Implications of Lysozyme Deposition, activity, and Denaturation"

- Surface vs. Bulk activity of lysozyme " deposited on hydrogel contact lens materials "

" Specific Ion Effects: Why the properties of lysozyme in Salt solutions follow a Hofmeisten series."

Notes:
- Lysozyme deposites and denatures more on ionic and high water content (hydrophilic)
- surface lysozyme is the lysozyme on the CL surface
- Bulk lysozyme absorbed into the CL
- Lysozyme is + charged, ions → lot can disrupt the ionics among ~~the protein~~ part of the protein particles causing protein to denature
- Ø Lysozyme
- Lysozyme's structure is stabilised by
- Salting in → low salt → better solubility
- Salting out → Caggregation & denaturation

- surface lysozyme -> attatched
- bulk lysozyme -> free lysozyme in solution
- Lysozyme's ~~activity~~ structure is stabilized by
  1 ionic (electrostatic interactions between charged amino acids
  2. Hydrogen bonds
  3. Hydrophobic interactions
  4. Van der ~~Walls~~ Waals forces

- Ionic strength is how many ~~cha~~ ions are dissolved in a substance and how ~~mut~~ much they interfere with electrostatic forces
- Na^+ and Cl^- ions surround charged amino acids screens meaning shields the charges disrupting the forces keeping proteins shape.
- Lysozyme's structure is what gives it its antimicrobial.
- ~~surface~~ bulk lysozyme ~~is~~ -> particles have it easy ~~to~~ rotate
- Ionic strength can also decreases debye length (which is how far charges can ~~not~~ attract each other) before salt blocks them

- chloride can bound to ~~to~~ positive lysozyme

- 0mM -> inactive

- 5 - 50mM At 7.0 pH about 50mM best

- Greater than 250mM activity decreases
  -> NaCl promote strong attractions between lysozyme molecules -> causing clumping

~~Extra~~ Other sources being used
"HEW lysozyme salting by high-concentration NaCl solutions followed by titration calorimetry.

~~"No salting in of lysozyme~~

- I had a doubt that free lysozyme and surface lysozyme would probably react differently ~~so~~, so I looked deeper into it and I found that surface lysozyme denatures faster.

- I will think about how I can rephrase my thinking and approach to solving the lysozyme denaturation problem using my experimental design.

Jan 3, 2025

- Today I looked deeper into my experimental design and asked google AI questions I had about ~~how the buffer contains~~ which PH-7 buffer I should use which contains the least ~~amount of~~ ionic strength. It recommended potassium phosphate buffer.

~~Jan 5, 2025~~

Jan 5, 2025

- Today, I talked to Ms. Easton (science fair coordinator) about getting materials - she recommended me to find my materials from bioshop, but I had difficulties today in making an account.

I also read Dr. Losby's response that she sent me with AI responses to my questions

Jan 6, 2025

- Today, I read through a "two reliable and inexpensive ways to ~~measu~~ observe lysozyme enzyme activity" pdf. The main protocol I am following and visualized steps and noted down questions. -> I also made a list of ~~mater~~ ALL the stuff very detailed I need.

- Today, I talked to Mr. Webster (science fair coordinator) about my experiment and he advised me to contact Mr. William Huddleston, Mr. Robert Barclay, Ms. Heather Addy, Ms. Zenobia Ali (professors at ufc) who get help me with a lab, guidance, and

materials that I would need.

Jan 8, 2025

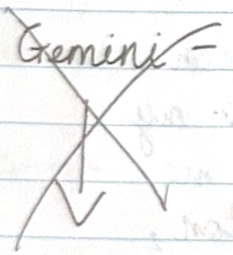
- Today I will ~~find what~~ see where I can find all the materials needed at bioshop and amazon ~~as~~ just in case I don't get a response from professors.

- I will also look into using gemini for finding sources.

- I ~~will~~ have learned some prompt engineering for specific science research questions.

- I want deep and statistical insights into the mechanism.

- ~~Gemini~~ - Actually I should look into the materials first.



-I need bacto powdered agar, lysozyme (lyphillized) and micrococcus luteus (dead and dried) from bioshop.

-I need other equipment from amazon.

-I found lysozyme on bioshop but I don't know if it is in ~~lypho~~ lyphillized ~~fo~~ form

Micrococcus luteus did not show up in bioshop so I will check other places so I can talk to Mr. Webster tomamorow about getting materials.

I found one at ~~thermosci~~ ~~thermo~~ fisher scientific, but it is not dead.

- I also found one in probabs.

- The lysozyme at thermofisher is quite ~~exp~~ expensive but available at bioshop

- The M. luteus is also expensive, ~~so~~

~~- I will go to U of C and MRU after school tommorow to talk in person to inquire.~~

- Hopefully, I get a reply soon!

- I emailed 3 more professors (sceduled for tommorow morning) and followed up.

Jan 9, 2025

- Today I got a reply from Dr. William Huddleston and Ms. Carla Slavik.

- Dr. William Huddleston said that he is unable to ~~ooo~~ accommodate for a ~~to~~ lab. He told me to email some of his colleagues ~~that~~ who.

- Ms. Carla Slavik said that the professors she has contacted don't have the experience of ability to help me. She said that she will pass my message down to other professors in the biology department to see if they are interested.

- I replied saying that I understand that a lab and/or supervisor might not be available, The main issue is obtaining the M. luteus. I asked them if they can at least provide me some materials and equipment, that would be great!

- I also emailed the professors that Dr. William Huddleston gave me.

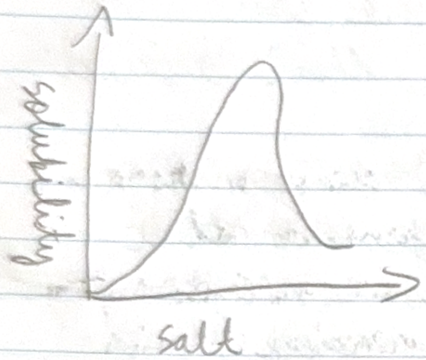
Jan 10, 2025

- Today I will continue digging deeper into my background research and finalizing my experimental design.

Youtube video: "Salting in and salting out | How does salting out happen? | What is salting in of proteins?

By channel: Animated biology with arpan

Notes:
- mild concentration → nothing else
  ↓
  solubility



- Aspantate and Glutamate are negatively charged

- salting-in is when when adding salt improves protein solubility

- ~~at~~ in lower ionic strength the Na^+ ions interact ~~wit~~ with the charges in the protein resulting in more water molecules being attracted to the protein.

- In higher ionic strength the Na^+ ions attract water molecules to themselves, leaving the protein

Salting-out protein gets precipitated

Salting-in → more interactions in solute - solvent and vice versa

Jan 11, 2025

- Today I will continue my deep background research, refining my experimental design, sending emails to Dr. Gordan Chan (Dr. Robert Barclay recommended) and Dr. Lasky - ~~asking~~ asking if she is available for a general interview on CLS as this help me ~~at~~ enhance my contextual understanding.

Youtube vid - Salting in and salting out of proteins.

By: Shomu biology

Notes:
- used ~~in~~ to determine precipitate of proteins.
- The proteins will settle down with high concentration

- Proteins stick together rather than the solvent → high
- The hydrophillic amino acids ~~interact with water in protein aqueous solution~~ in the surrounding and vice versa
↓
- Hydrophobic core
- salt interact with protein → stabelize the protein → increasing solubility
* proteins come close in together (dump) (precipitate)
- The hydrophillic surrounding causes the salt-water molecules create a hydrophilic shell.

Video: Protein structure and folding

By: ~~Anobba~~ Amoeba sisters

Notes:
- 1st level → primary → amiono acids are formed by peptide chains.
- 2nd structure →

Alpha helix 

Beta pleated structure → 

- depends on hydrogen bonds
- 3rd tertiary
- disulphite bonds, van der waals forces
4rd → more than one chain
-

Youtube video: What is protein denaturation

By: Kiki ~~st~~ sanford

Notes:
- Amino acids form chains and forces among the chain makes the protein to fold.
- The Kinetic energy vibrates ~~cau~~ causing bonds within the folding to break

~~Hydrolysing and denaturing protein~~

~~BY: chenProfJH~~

Protein structure

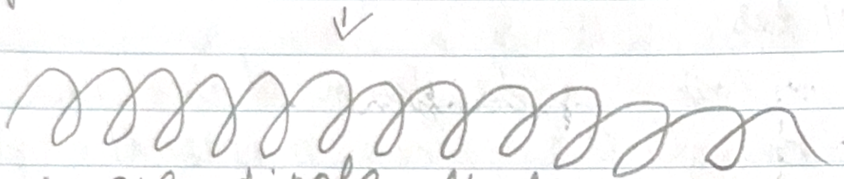
By: Professor Dave Explains

- Proteins → polypeptides

- Proteins are enzymes

- Proteins are formed when amino acids form peptide bond through a dehydration reactions

- Alpha helix



? What are dipole-dipole bonds ?

Lysozyme is globular protein, which means that it is folded a lot.

Factors that denature proteins

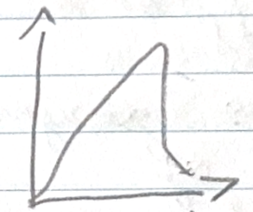
By: Walter Jahn.

Notes:

- ~~Add~~ Adding heavy metals can denature protein

Video: GCSE Biology - Enzymes - How temperature and pH Affect Rate of reaction:

Notes:



~~Video: Salting out~~

~~By: Andrew K~~

~~Notes:~~

Denaturation of proteins - biochemistry

By:

Now I am going to use gemini to find specific information ~~&~~ g and give me sources.

I am going to use techniques for getting better scientific results from AI like providing context define its role etc.

Question one: Information on (from sources) ~~(links from~~ that patients experience discomfort due to lysozyme denaturation ~~fro~~ BECAUSE of uncontrolled levels of Salinity.

Notes:

- The sources it gave me:

Source: "Medical new today (2024) and the cleaveland clinic."

Notes:

This article wasn't that relevant and had info I already knew.

Jan 13, 2025

- Today I talked to Mr. Webster ~~and~~ about how I am going to do my experiment as the professors I contacted haven't replied or are unable to help with me getting a lab or even M. Luteus ~~I~~.
- ~~M. Lut~~

- M. luteus is expensive, and my school ~~has it~~ is an ~~organization can't get~~ junior high school cannot buy bacteria so that is not working.

- I found some M. luteus that is cheap as it is not advanced and I believe delivers to private residences

- For a back-up plan we (Mr. Webster) talked about using tripsin (modeling lysozyme) and instead of using bacteria, I can use milk protein as tripsin breaks down protein, however, tripsin and lysozyme react in slight different ways to sodium chloride. Using tripsin to conclude about lysozyme is not scientifically just.

- I found one from pro labs that is only $20.

- But that one only delivers to institutions.

- I found out that the M. luteus in fisher scientific is dead.

- I will ask Mr. Webster if the school can purchase it if it is non viable.

Jan 14, 2025

- Today I talked to Mr. Webster after school, and he said that even if M. luteus is non-viable, the school still might not be able to get is it biological.

- We both discussed an alternative.

- Getting lysozyme also could be difficult.

- I knew that lysozyme is abundant in egg white so I can extract lysozyme from egg white if lysozyme is not available

Mr. Webster and I came up with the idea that we can observe denaturation as increase turbidity.

- I ~~re~~ learned in my background research about ~~lyso~~ protein denaturation that when eggs are cooked the protein denatures giving it a opaque color.

- With this version of the experiment I can be resourceful with my materials (I will make a spectrophotometer from arduino - I have arduino uno already) and focus on denaturation and aggregation than enzyme activity.

Jan 15, 2025

- Today I will look into seeing where I could go to request for a lab in-person ~~today~~.

- It would be great if I am able to execute my lysoplate assay.

- The biological science centre in in the science a building. I will go tommorow after school to ~~enquire~~ inquire.

Jan 16, 2025

- I went to the biological science centre to inquire.

- I talked to Dr. Marie Fraser whom I emailed previously.

- She said that labs don't allow below 15.

- She said that the bacteria also needs to be used safely, and they

use an auto-clave for it.

- She can provide lysozyme ~~or~~, I need to talk to M. Webster about it.

- So, the lysoplate assay might not work.

- So, I am planning on executing the turbidity test.

- For that I need to carefully plan my experimental design.

-

- Now I will research into contact lens solutions to be able to form a clear problem statement for your experiment and use my experiment to make real-world impact.

Book: "Clinical Manual of Contact Lenses"

Chapter - "Lens problem solving" and "CL care"

Notes:

-

Actually I could not find any relevant sections in the free google books preview

Article - "Lens care update - protein removal without enzymes"

Notes:

- 70% of SCLs are made from ionic material
- Solutions contain positively charged elements which complete with protein to be on the lens surface
- Similar to water softener calcium

from H_2O with potassium.

- Some elements with form ionic bonds with protein in such a way that protein can get out easily

- Plasma polymerisation coating reduces protein build-up

- salt tablets?

Article - Nancy parker - What is contact lens solutions and what is in it

Notes:

- MPSs solutions contain surfactant disinfectants, wetting agents and preservatives.

Article: "Contact lens spectrum"

Notes:

- At some point every CL wearer displays at least 1 hygine risk
- >80% of CL wearers practice non-compliance

Jan 17, 2025

- Today I will look into contact lens compositions to be able to form an experiment that is scientifically significant

Study: "Physical properties of contact lens solutions"

Notes:
- 275 - 300 osmolarity
- 7.0 - 7.35 pH
- Not all solutions have same formulation

Osmolarity means - ~~number~~ amount of dissolved particles in the substance

Study: "Affect of osmolarity change in MPS induced by an incorrect lens cleaning procedure."

Notes:
- Increase in osmolarity can affect lens comfort

* Study: "Role of hypo-osmotic saline drops in ocular comfort during CL wear."

Notes:
- 60% ~~prefer~~ preferred -> hypo-osmotic
- 33% - preferred -> hyper-osmotic
-

~~Study~~:

Study: "Osmolality and pH of commercially available contact lens solutions and eye drops"

Notes:
- These are (osmolality and pH) are not indicated on package
- 192 - 364
- 6.35 - 6.26, 7.99 - 8.00
- significant.

Osmolality and osmolarity are ~~meas~~ the amount of dissolved particles NOT ionic strength directly. Ionic strength is the ions dissolved.

I will use gemini to find sources and simplify them.

Google search question:

Ionic strengths of different Cl solutions and NaCl concentrations and how osmoprotectants protect lysozyme denaturation.

- 0.9% NaCl physiological

Ionic strength formula: $I = \frac{1}{2} \sum_{i=1}^{n} c_i z_i^2$

- Osmoprotectants like glycerin

c_i = molar concentration

z_i = its charge

~~- Theory~~

Sources
- Wikipedia - Ionic strength
-

Jan 18, 2025

- Today I will continue my research on contact lens solution composition, osmoprotectants, and finalize aspects of my experiment so I can
  - Submit my ethics and due care and get started

~~source~~

Youtube video: Multi-purpose solution for contact lenses? | Optometrist tutorial

By: Antino The Optometrist

Notes:
- Don't use saline to store lenses, only for rinsing
- Hydrogen peroxide is a strong oxidant
- 6 hours of neutralizing

Youtube video: "Denaturation of Protein - Biomolecules | Class 12 Chemistry"

By: Magnet Brains Education

Notes:
- Hydrogen bonds are disturbed when protein denatures
- Denatured protein cannot be brought back easily
- secondary and tertiary structure is destroyed making it loose its function

- I will use gemini to find sources as I was having trouble finding sources that have exactly what I need: analysis of contact lens compositions (especially the salt and osmoprotectants)

~~Source~~

- I also used google AI mode to find specific sources

~~Additional~~ Additionally, I searched at which concentration of NaCl lysozyme start loses it ~~[illegible]~~ structure, retains it, and completely looses its structure.

Source: "Enhance drug loading efficiency of contact lenses via salt induced modulation."

Notes: (I only read conclusion and results)
- and then asked gemini to simplify into something I could fully comprehend)

- drug loading is putting some medicine into contact lens

(Wasn't relevant)

Source:

Notes:
- CL solutions have ~~st~~ erithritol and glucerin which protect protein from osmotic stress - too much salt.

Jan, 19, 2025

- Today, I will complete my ethics and Due Care Form and Finalize a few aspects of my experiment.

I asked gemini ~~for~~ some questions ~~I~~ for my experimental design.

"Do I need an incubator."

- As temperature can influence lysozyme aggregation.

Answer: Incubator is essential for controlling temp

~~From~~ Source:"

Another question - how long will the aggregation take?

- Even months if ~~aggregates~~ room temp incubator = fake

~~From~~ Source: "Influence of incubation temp and time."

Q-What is the concentration of lysozyme in tears

Source: "Lysozyme content of tears in normal subjects and in patients with external eye injections"

↓

Average was 1.7 mg/ml

For the source - Influence of incubation temperature on protein aggregation =

it was hard to understand so asked ~~gpt~~ gemini to explain.

I am thinking of peeping incubator at 37° to mimic eye temperature.

Also, I will keep lysozyme concentration at 1.7 mg/ml to mimic physiological conditions.

I will measure the turbidity ~~every~~ at certain time intervals

Another source: "Thermally induced fibrillar aggregation of hen egg white lysozyme"

says that @ room temperature took a long time for aggregation.

Higher temperatures were fast

Fibrillar means aggregation that becomes thread like.

The time internals I will measure turbidity is something I will ask H.-. Webster tomorrow.

I will fill out my form now!

Jan 20, 2025

- Today I went to U of C biochem centre, to talk to Dr. Marie Fraser for lysozyme, pH buffer, and an incubator, but she wasn't here, so I will go tommorow

- Today I also submitted ~~it~~ ~~to~~ the Ethics + Dare care after reviewing it with Mr. Webster.

- Now I will work on background and experimental precedure.

I put the same prompt/search into chat gpt, gemini, and a simple google search to cross-verify

"Give me sources that explicitly state the levels of lysozyme aggregation at different NaCl concentrations at pH-7, and 37°C vs. room temp"

~~to~~ Source: "Solubility lysozyme ~~chloride~~ in the presence of aqueous ~~so~~ chloride salts: common ion and its role on solubility and crystal thermodynamics."

Notes:
- Ternary - one solvent - two solutes
  - protein, salt, water
- preferential - interaction coefficient - how much protein wants to be with the salt.
- Salt partioning - ions ~~sh~~ split (some are in the water and some stick to the crystal protein
- the formula:
-

Source:

Notes:
- Cloud point temperature ↗ when a clear solution becomes clear when it cools.
- ~~Formula no. 1~~ - The interaction potential ~~(U(r))~~
- ~~$\frac{E(I)}{K_B} = a\sqrt{I} + b$~~ (E = ~~protein attraction~~

- for ~~monovale~~ ~~monola~~ monovalent cations → as concentrations increase the cloud point temp increases
- at low I for a salt, difference in CPT can be explained in terms of repulsive electrostatic interactions but at higher I, difference are with hydration forces
- NaCl was ~~used~~ chosen as a reference salt
- NaCl affect on Lysoz. or related to ~~hydro~~ hydration forces
-

~~Source: "solubility of Lysozyme in the pro~~
- lysozyme aggregates 1-4M
- lysozyme concentration was kept

Source: Protein Phase Behaviour in Aqueous solutions: Crystallization, Liquid - Liquid phase separation, Gels and aggregates.

~50mg/ml, pH 7 at room temp (20-25°C)

I had a few questions regarding my science fain project that I wanted ~~to~~ ask google AI and find soumes on.

1. ~~Do I need to clean~~ What is the best cleaning practice for the test tubes and cuvette?

Answer: (AI + my thinking)

- I myself ~~for~~ got is fresh test tubes at amazon, so I don't have to worry about cross - contamination.

However the quartz cuvettes are expensive so can't buy ~~as~~ new ones for each sample.

For cuvettes I need to rinse them ~~with~~ with distilled water and maybe ethanol. I can use lens wipes which I already have to wipe cuvettes.

For the test tubes I will clean them with ~~salt~~ soap and water.

I am also going to learn more about data analysis for my experiment

~~So~~ Video: Hypothesis Testing and The Null Hypothesis, Clearly Explained.

Notes:
-helped me understand context

Khan academy - Idea behind hypothesis testing.

Notes:
~~- The null hypothesis is~~

Video: Hypothesis Testing Explained

Notes:
- Null hypothesis → we assume if everything is right
- Alternative hypothesis - what we test about population

-

"What is P value in statistics In Simple Language."

↓
Ytube video

P value are the liklihood from 0-1 that data was ~~pure luck~~ chance

- "How to calculate the standard deviation" -> The organic chem tutor

Wots
find the mean of the mean

$$s = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}} \qquad \bar{x} = \frac{\text{sum}}{n}$$

~~90, 91, 93~~

~~$s = \sqrt{(90 -}$~~

Standard deviation is how much the readings differ from the mean

Jan 23, 2025

- Today my ethics and due care was approved, actually it was approved a few days earlier but they declined it as I didn't have a title yet.

- I chose my title as "No More salty Sight."

- Gemini recommended me 'salty sight' from which I made my title as "No more salty sight"

- I asked gemini to ~~make~~ suggest ~~titles~~ titles that would be catchy.

- Also, on ~~Friday~~ Wednesday, I got materials like L.E.D CI need a UV led as it will detect smaller changes in help turbidity and male-to-male wires from school.

- I also ~~told~~ asked Ms. Easton (my science teacher) to email Dr. Marie Fraser to ask how they can deliver the materials to our school

- They materials I needed that weren't available at school, I ordered from amazon like quartz cuvettes, photodiodes, and test tubes WITH stoppers and ~~received~~ received today.

- Today, I ~~built~~ got the uv led to light up.

- I calculated how many ohms resisten I need and I got 65 ohms.

Formula:

$$\frac{\text{Volts from USB port (5)} - \text{Forward voltage of led (UV=3.7)}}{0.02A}$$

$$= \frac{5 - 3.1}{0.02}$$

$$= \frac{1.3}{0.02}$$

$$= 65$$

However, I didn't have 65 ohms resister, so I thought how about ~~how I can combine~~ how can I combine 10 ohms resisters to get 10 ohms? (slightly higer resistence is okay.

I searched this up and learned that if I arrange the resistens in a series, then the resistence will add ~~up~~ up.

Then it worked!

- I wore sunglasses when looking at the UV led as it is uv rays.

- Photos of this stage and sources I used are in my project platform.

Jan 25, 2025

- Today I looked into wiring and coding the photodiode (BPW34) for the ~~sesso~~ light sensing.

- In a website (links are in my project platform) for the wiring I read that the photodiode alone only has a small range.

- It would need a transmittance amplifier on op amp to amplify the voltage signal.

- ~~The~~

- I found a sensitive, high precision sensor that has a photodiode, amplifier, and a feedback resistor making it beginner friendly.

- So I am planning to use that.

I also found a cardboard box lying around at home that I painted black that will be my light-tight box. I also planned out that I will stick the breadboard on the sides of the box and the arduino ~~boro~~ board will remain outside and will be connected to the breadboards with female to male and male to male jumper wires.

Jan 26, 2025

"How does OPT101 light sensor work → give me sources and how to wire and code opt101 with arduino uno r3, → sources, and give me sources that assess the precision of OPT101. To help measure, transmittance of a substance light"

→ ~~copilot~~ copilot, google AI, chatgpt, and gemini

(this way I can cross-verify)

and get ~~p~~ multiple percepectives and more sources

NOTE: 4

Gemini - ~~I used~~

Sources -

"Texas Instruments - Opt101 datasheet revision b"

Notes:

Because, this was quite technical, I asked chatgpt to help me explain the concepts.

↓

Current → Voltage

Key features → high precision

- Red light sensitivity
- Reduces noise
- Has a feeback resiston
- single power supply

Analog pin works well with arduino UNO

~~Connect~~

The chip can sense from infared to uv light

~~Decoding~~ -

GND = negative supply

5V = positive supply

4→ resiston (placed between input + output)
↳ connect to pin 5

Pin 2 → already connected

I will look into tutorials on youtube on arduino pins and the chip as well

VCC - V5

GND - GND

OUT - A0

Additional sources I used:

"Beer-lambert law" (I remember this formula from another video + matched)
→ Youtube video →

"Spectrophotometry and Beer's law"
© ProfessorDave Explains

Notes:
- Kinetics (~~the rate of a~~ → the rate of a chem reaction
- ~~a~~

~~Actually this video was more aimed for spec~~

~~"Transmittance and Absorbance"~~



$A = \varepsilon \cdot L \cdot C$

$\log(I_0/I) = A : \varepsilon \cdot c \cdot L$



I_0 = intensity of incident light
I = intensity of emitted light
A = absorbance
ε = Molar coefficient

- I will choose a red led for my experiment as the OP+101 has the maximum sensitivity to 650nm wavelength which is red so it will give me more accurate results.

- The beer lambert law doesn't fully apply for my project because I am not testing any specific "chemical light behaviour" as such.

The way I want to measure transmittance is by taking the 100% light value by running the turbidimeter with just ~~water and lysozyme~~ water, and with buffer and salt to see the light transmittance with the salt concentrations and see the change in transmittance with lysozyme aggregation.

Now I will watch a tutorial on opt101 with arduino.

" CJMCU 101 OPT101 Analog light sensor with arduino [Source code and source ~~sound~~ circuit diagram]

- 180 ohm resistor x5

- 2 ohm resistor

- What is the melding?

1M and OUT → A2 with 1M ohms resistor

(I checked with google ai and it says not to do the)

Jan 27, 2025

- Today I got bad news that Dr. Marie Fraser cannot provide the materials in the time frame that I need.

- So I will exctract lysozyme from egg white (as from my background research I learned that egg whites contain lysozyme)

- Mr. Webster told me that I can use ~~bicarbo~~ carbonates that we have in school for buffer.

- From my research I found out that I can add some vinegar for neutralizing the base.

Jan 28, 2026

- Today I will look into how I can purify lysozyme from egg white

source "Isolation and purification of lysozyme from egg whites."

Because the ~~concepts~~ terms were hard to understand, I asked Gemini to help me simplify the article

Notes:
- The study was not something that I could do at a middle school level.

Youtube video "Lysozyme preparation:

- They used lab equipment that I don't have

"Rapid separation of lysozyme from chicken egg whites by reductants and thermal treatment."

Notes:
- Lysozyme in egg whites have the most thermal stability than other proteins in eggs especially at lower pH
- Egg whites mixed with water then heated up and then the precipitate was separated.
- Egg whites were diluted.
  70°C → Maximum 78% retaintion
-

I can skip cystine and other chems as I don't have them.

~~Do~~ Feb 3, 2025

- ~~Today~~ I will decide which table salt I will use

- The kitchen table salt usually contains iodine and stuff so I want a salt that is as pure sodium chloride as possible.

- From the windsor MSDS it says that it is 99% NACl which is pure enough for my experiment

~~- Now I will research~~

~~- Now I w~~

- Because I got my opt101 light sensor today, I will ~~learn how~~ to wire it today and code it!

But ~~before that~~ in order to do that I will learn arduino pins and coding!

Feb 6, 2025,

- In the last couple of days, ~~I~~ I ~~got~~ received the opt101 from amazon, and picked up some materials from school like wires, an extra breadboard and a red led.

- I also cut two pieces of cardboard that fit perfectly in the box and made cuts in them that would be aligned with the led and sensor

- However the opt101 sensor was being really illogical and inconsistent in its readings, I thought that using a capicitor would make the result more stable but it didn't.

So I tried using a photodiode that I had and it gave me a max of 50 which is a good enough range for my project.

I switched to a Red led so I calculated the resistance which gave me 140 Ohms

the wiring I used for the led was anode connected to series circuit (100 ohms + 10^{x4} ohms) and that connected to pin 12 of arduino and cathode connected to GND of arduino.

- The code was pinMode(12, OUTPUT); and digitalWrite(12, HIGH) in void setup.

- Then, I alligned the LED and the sensor (OPT101 at that time) using a toothpick for observing trajectory.

- I tried using a capicitor but that didn't work

- I used cardboard piecies that exactly fit into the box to focus the light onto the cuvette and a cuvette holder.

- I switched to a photodiode that worked perfectly and gave me 0-5 o range

"The kinetic study on thermal denaturation of hen egg white lysozyme precipitation"

Notes:
← ~~This~~ Was not relevant.

"Heat stability of egg white proteins"

Notes:
- Heat stable at pH-7
- 60°C pH-7 (lysozyme 0% inactivated
- cystine and ovalbumin increase lysozyme's heat sensitivity

- Heating egg white for 3.5 mins at 62°C → damaged 6% of dysozyme

"Egg protein composition"

Notes:
- Hen eggs are 3.4% lysozyme.
- Rapidly decreases after 0.5M at pH 7.
- Lysozyme is more heat stable ~~than~~ in diluted solutions.
- disulphide exchange reactions usually causes gel formation.

- Today I picked up the materials I need from school today.

- Today I will continue research and visualize my experiment
  ↓
- Oh! I also need a funnel

Questions I want to answer:

~~What~~ How does concentration, pH, time, and temperature affect lysozyme response to various salt concentrations

Continued notes
- they used ascorbic acid and salt but ≠ need a neutral pH and 0% initial salt.

- ovomucoid → is a giant protein (covered in sugar chains)
  osmoprotectant - small

S-Ovalalbumin - the heat stable ovalbumin
↓
~~Ovo glyco~~

Ovoglycoprotein — minnor protein
↓
glycoprotein

Flavoprotein - (ovoflavoprotein)

Also contain -> glucose 0.5% and free carbs

some minerals and vitamens.

Feb 10, 2025

- Today I will research more about the science behind egg white purification. and what to expect in my data.

- "The thermodynamic and gelation properties of lysozyme and ovalbumin"

Notes
- lysozyme is more heat-stable than ovalbumin at lower pH.
- Ova's isoelectric point is 4.5 pH

" PH and heat induced structural changes of chicken ovalbumin in relation with antigenic properties

Notes:
- ~~Ovalbumin~~ ~~Ovalbumin~~ Ovalbumin =

Major egg white protein

* Advances on the impact of thermal processing on structure and antigenicity of chicken ovomucoid."

~~Notes~~:

-

"Functional properties and extraction techniques egg white protein."

#Notes

- Ovomucoid contains
- glycoprotein and sialic acid
- 9 disulfide bonds
- stable when heated pH 7 or below

"Ovomucoid"

Notes:

Feb 12, 2025

"Gelation Properties of Albumen proteins, singly and in combination"

Notes:

- Ovalbumin, globulin, and have temp of denaturation at 72°C
- Conalbumin - 57.3°C
- Lys - 81.5°C
- Ovomucin and Ovomucoid - no coagulation ability
- Ovomucoid increased coagulation temp of lysozyme

"HEW Lysozyme salting by high-concentration NACl solution followed by titration calorimetery.

Notes:

- 0-1.5mM lysozyme in 0.1 sodium acetate buffer (pH -4.25) up to 0.9M

~~Feb~~ Feb 12, 2026 (conti

From my research I concluded that 60°C for 10 minutes with a 1:1 dilution with distilled water ~~would be~~ pH around 4.5, and 0.5M NaCl would be the best because at that parameters I will get around my desired concentration and lysozyme is most stable at pH and temp while other proteins except ovomucoid (Ovomucoid is slightly negatively charged and acts as a protein stabilizer like mucins in our eyes.

Steps of protein extraction ~~pro~~ precedure I did today.

1. Callibarate pH meter with buffer solutions in the case
2. Calibarate digital weighing scale as per package instructions
3. ~~Put~~ Pour ten egg white of 17 eggs after seperating the yolk. Measure the weight of the bowl first

4. Measure the volume of the egg white on a kitchen scale
5. Subtract the volume measured in ~~step no. 4~~ step no. 3 from step no. 4
6. ~~Mix Mix 0.5M NACl of the double the amount of egg white~~
6. Measure 0.5M salt for the double volume of the egg white (30.86 g)
7. Measure distilled water of the egg ~~white~~ whites' volume
8. Mix the salt with the water with a stirring rod until fully dissolved
9. Mix the water with salt with egg white
10. Measure ~~[0.6]~~ a few ~~g~~ of ascorbic acid
11. Measure the pH of the egg whites [8.9 pH] -8.7 (fluctuated)

12. Add ascorbic acid little by little in a few drops of distilled water until pH is 2-3 and mix with a stirring rod until fully dissolved

~~13. Then add~~

13. Then add the ascorbic acid solution gradually into the egg white mixture until pH is 4.4-4.6

14. Mix gently and thoroughly

~~15. Set up a double boiler~~

15. Fill a pot with tap water ~~and set up a double boiler~~

16. ~~Place~~ Put the pot on the stove and turn the heat on high flame

~~17. Measure temperature continously~~

17. Measure temperature continously with a thermometer

18. Turn the flame off when temperature reaches 60°C

19. Pour the egg white mixture into another pot and set-up a double boiler: see picture in the procedure's section in the project platform.

~~20.~~ 21. Measure the temperature continously of the egg white ~~for~~ mixture

~~21.~~ 20. Turn the flame on low heat and turn off the stove

22. Set a timer for 10 mins once the liquid reaches 60°C

~~23. If~~

If the temp starts dipping down turn the flame on low ~~or~~

23. Once the time is over and ~~it~~ parts of the liquid has precipitate and coagulate, pour the hot liquid through a strainer lined with coffee filters into a seperate bowl

24. Wait until all that is ~~hot~~ left in the strainer looks thick and white

25. ~~She~~ Filter the extract a few times again in the same process.

The extract looks slighty cloudy and has a ~~she~~ yellowish tint from vitamin b.

26. Store the extract in the refrigirator

THE EXTACT IS READY!!!!

Feb 13, 2025

4 - Today I started my escperiment and this was my procedure:
- Callibarate pH meter and mean gloves
5 - Warm the extract ~~the 5~~ with the same double boiler system to 37°C

7 - ~~D~~ Once the ~~to~~ ~~each~~ lysozyme extract is at the temperature
- use a pippette to measure 4 ml of extract in a graduated cylinder.
- Pour the liquid from the graduated cylinder

into a test tube labeled "O.SM 2" with a sharpie.

- Put the ~~cuvette~~ test tube with a test tube stopper in a pot filled with tap water heated to 37°C.

- set a timer for 3 mins

~~- Use~~

- Once the timer is over, take the test tube out of the hot water bath

- Then, use a pippette to transfer the solution to a cuvette.
~~pipette~~ draw the solution from the side of the test tube and pippette the solution ~~in~~ into the cuvette from the side to avoid bubbles but some bubbles were formed

- Carefully place the cuvette in the cuvette holder of the PIX turbidimeter and close the box

- Save and label multiple readings

- Repeat the steps for the 5 mins, 10 mins, 25 mins, 40 mins, and 60 mins time intervals

- ~~Wash~~ Rinse the cuvette and pippette with warm distilled water and dry them to maintain thermal equillibrium and avoid contamination between samples and timings.

- When I had a break between my measurements I placed the cuvette and pippette in a ziplock bag

- When trials ~~has~~ with salt were tested, salt was measured and added to the ~~test~~ test tube with a funnel.

- Then, the test tubes were inverted 15-20 times.

- Then same steps apply

- Today I also calculated the electrostatic sheilding of the electrolytic solution using the inverse debye length formula as part of the DLVO theory.

$$K^{-1} = \sqrt{\frac{\epsilon_0 \epsilon_r K_B T}{2 N_A e^2 I}} \qquad K^{-1} = \sqrt{\frac{K_B T \epsilon_0 E_r}{2 q^2 N_A I}}$$

K^{-1} = the inverse debye length (~~to~~ the extention of lysozyme's field

E_r = ~~relative~~ dielectric constant of water at given temp (37°C = 74)

ϵ_0 = permetivity of vacuum

I = Ionic strength

$$K^{-1} = \sqrt{\frac{K_B T \epsilon_0 E}{2 q^2 N_A I}}$$

T- absolute temperature in Kelvin

N_A - Avocado's number

q = elementary charge K_B - Boltzmann constant

K_B - ~~converts~~ (1.380649 × 10^{-23}) the proportional ratio between temp in kelvin and thermal energy in joules

N_A - converts moles into individual ions ($6.02214076 \times 10^{23}$)

$\epsilon_0 = 8.85418 \times 10^{-12}$ F/m $q = 1.602176634 \times 10^{-19}$

→ Compute

$$K^{-1} = \left(\frac{(1.380649 \times 10^{-23}) \times (310\,K) \times (8.85418 \times 10^{-12}) \times 74}{2[1.602176634 \times 10^{-19}]^2 \times (6.02214076 \times 10^{23}) \times 0.5} \right)^{\frac{1}{2}}$$

$$K^{-1} = \sqrt{\frac{2.804303688659 \times 10^{-30}}{3.091730893032 \times 10^{-60} \times 0.5}}$$

$$K^{-1}_{(0.5)} = \left(\frac{2.804303688659 \times 10^{-30}}{1.545865446516 \times 10^{-60}} \right)^{\frac{1}{2}}$$

$$K^{-1}_{(1)} = \left(\frac{\text{same no.}}{\text{same no as without I}} \right)^{\frac{1}{2}}$$

$$K^{-1}_{(3)} = \sqrt{\frac{\text{same no.}}{9.275192677909 \times 10^{-60}}}$$

$$K^{-1}_{(5)} = \sqrt{\frac{\text{same no.}}{1.545865446515 \times 10^{-59}}}$$

$K^{-1}_{(0.5)} = 1.34687308 \times 10^{15}$

$K^{-1}(1) = 9.523830894 \times 10^{14}$

$K^{-1}(3) = 5.498586314 \times 10^{14}$

$K^{-1}(5) = 4.259186674 \times 10^{14}$

I made a mistake!

I made a mistake in the calculation so I did this again

$K^{-1}_{(0.5M)} = 0.4259$ nm $K^{-1}_{(3)} = 0.1739$ nm

$K^{-1}(1) = 0.3012$ nm $K^{-1}(5) = 0.1347$ nm

Feb 20, 2026

- Today I did the single Factor ANOVA on Excel. I got a very significant p-value!

- For my distilled water control group I originaly only did one trial but since performing experiments in triplicate ~~so I~~ did two more trials today.

Feb 21, 2026

- Today, I worked on my written report and did t-Tests ~~for my~~ to compare the protein samples and distilled water samples

Feb 23, 2026

- Today I worked on my writing for bgd research, procedure, and analysis.

- I also did a post-hoc tuckey test using formulas to see which salt groups have the most significant difference

Oh and for my hypothesis, I forgot to mention this, but my hypothesis is that the relative change (%) between inverse debye lengths would be lower than the actual change as other factors such as ~~no~~ high zeta potential of lysozyme, low concentration, ovomucoid, and lysozyme's hydration sheild around lysozyme will prevent aggregation. aggregation turbidity will increase as salt increases.

Feb 24, 2026

- Today I worked on my writing for my report

Feb 26, 2026

- Today, I worked on my report

Feb 27, 2026

- Today I worked on collecting images for my writing in report from my phone

Feb 28, 2026

- Today worked on report

Mar 1 2026
- Today I ~~took~~ selected images for my participant image, headon image, and participant image.

- Mar 2, 2026
- Today I did a one sample t-test to compare percentage change between salt concen to theoretical debye length one.

- Mar 3, 2026
- Edited my report

- Mar 4, 2026
- Final adjustment before submission.

For a brief description of my analysis and conclusion, application, and observations

The biggest difference was between 1M and 3M. At 1M and 0.5M no significant aggregation occured.

Between 0.5M to 1M the Non-Inverse debye length factors played

a significant role in preventing aggregation.

For 3M and 5M after about 10 mins some crystalization started to occur. After 25 mins all particles settled at the bottom but dissolved after pipeting.

~~The My alter~~

- The rate of aggregation ~~was~~ stayed ~~at~~ nearly flat. Maybe the main aggregation occured before 3 min.

My ~~ate~~ alternative hypothesis in general was true for final readings

but not for the rate of aggregation
The change in (%) between 3M-5M and 1M-3M was not statistically ~~significant~~ different than inverse debye length change

For future work, I would use cheesecloth instead and OPTIO1.

I will follow my original plan for lysoplate for future esclension.

~~My application is for better ionic strength contact~~

My application is about contact lenses are ocular so health that my bgd research talkes about