

EXPERIMENT - 1 : CELL DISRUPTION BY MECHANICAL LYSIS

AIM: In this experiment, we aim to study the various methods of mechanical lysis including glass beads & ultrasonication.

MATERIALS:

1. Cell suspension for cell lysis
2. Lysis buffer
3. Glass beads
4. Pipettes
5. Vortex
6. Centrifuge
7. Falcon Tubes
8. Cuvette & Spectrophotometer

THEORY:

Mechanical lysis of cells can be performed by different methods such as Glass beads, French press, ultra-sonication etc.

The method of glass beads involves vortexing the cells with glass beads. The mechanical forces exerted by the beads burst the cells open thereby separating the intracellular contents.

Ultra-sonication is the use of ultrasonic sound waves to lyse the cells. When cells in suspension are exposed to ultra-sounds, their cell membranes get destabilized and proteins get de-aggregated, thereby lysing the cell.

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PROCEDURE:

(i) Glass Beads

1. A 10ml suspension of cells was taken in a 15ml falcon and glass beads were added to it.
2. This solution was then vortexed for 10mins and ~~the~~ 1ml sample transferred to an MCT.
3. The remaining suspension was centrifuged for another 10mins and the sample collected.
4. This was repeated a total of 3 times.
5. The 3 1ml samples were centrifuged @ 4°C, 8000 RPM for 15 mins and supernatants transferred to fresh MCTs.
6. The lysis buffer was set as the blank and samples were measured for absorbance @ 280 nm.
7. Since the initial absorbance was much higher than 1, the samples were diluted 10x and re-measured.

(ii) Ultra Sonication

1. 35ml of cell suspension was taken in a 50ml falcon.
2. The falcon was kept in ice in the sonicator such that the probe is at the center of the tube and reaches upto half the volume in the tube.
3. The sonicator was run for 20 mins and a 2ml sample was taken out after every 10 mins.
4. The samples were centrifuged @ 4°C, 8000 RPM for 15 mins and the supernatants transferred to fresh MCT.
5. Absorbance was measured @ 280 nm.

RESULT : from the experiment, we get the max release absorbance as 9.31 & Time constant = 1.188 mins

DISCUSSION:

From this experiment, we observe that increasing the vortex time increases the cell lysis & thus protein content in the solution. This however starts saturating after a point upto the point where all the cells have lysed & hence there is no more increase in the protein content of the cells.

The ultrasonication observations weren't available for more than 1 time point - However, the cell lysis from ultrasonication appears to be much lesser than the one with glass beads ($A_{600} = 5.82$ vs 8.64 after 30 mins)

The k_m for the above was calculated based off the best fit curve

R^2 for the linear fit in the $\ln \left(\frac{1-R}{R} \right)$ vs $t^{1/2}$ curve

OBSERVATIONS

(i) Sample (A)

(a) NaOH

$$\frac{2N}{IN} \quad \frac{IN}{0.5N}$$

$$0.365 \quad 0.257$$

$$0.273 \quad 0.189$$

$$0.321 \quad 0.155$$

$$0.326 \quad 0.113$$

$$0.281 \quad 0.321$$

$$0.264 \quad 0.259$$

$$0.271 \quad 0.342$$

$$0.271 \quad 0.271$$

$$0.289 \quad 0.359$$

$$0.275 \quad 0.275$$

$$0.282 \quad 0.180$$

$$0.276 \quad 0.180$$

$$0.273 \quad 0.180$$

(b) SDS

$$4\% \quad 2\%$$

$$0.107 \quad 1\%$$

$$0.321 \quad 0.113$$

$$0.286 \quad 0.113$$

$$0.264 \quad 0.113$$

$$0.271 \quad 0.113$$

(c) Lysisyme

$$0.1mg/ml \quad 0.5mg/ml$$

$$0.264 \quad 0.1mg/ml$$

$$0.271 \quad 0.1mg/ml$$

(ii) Sample (B)

(iii) Sample (C)

(iv) Sample (D)

(v) Sample (E)

(vi) Sample (F)

(vii) Sample (G)

(viii) Sample (H)

(ix) Sample (I)

(x) Sample (J)

(xi) Sample (K)

(xii) Sample (L)

(xiii) Sample (M)

(xiv) Sample (N)

(xv) Sample (O)

(xvi) Sample (P)

(xvii) Sample (Q)

(xviii) Sample (R)

(xix) Sample (S)

(xx) Sample (T)

(xxi) Sample (U)

(xxii) Sample (V)

(xxiii) Sample (W)

(xxiv) Sample (X)

(xxv) Sample (Y)

(xxvi) Sample (Z)

EXPERIMENT-2 : Cell Disruption By Non-Mech.METHODS

AIM : To study methods of non-mechanical lysis on C. trachomatis

& Give bacteria.

MATERIALS :

1. Tris HCl

2. Cells required for disruption

3. SDS

4. Lysisyme

5. NaOH

6. MilliQ Water

7. Spectrophotometer

PROCEDURE

1. Cells suspended in 2000µl Tris-HCl were taken for lysis.

2. NaOH lysis was performed using 2N, IN & 0.5N NaOH in each tube.

3. SDS lysis performed using 4%, 2% & 1% SDS solutions.

4. Finally, lysisyme lysis was performed using solutions of conc. Imidyl, Oligoflow, O.1mg/ml respectively.

5. The stock solutions of each were input, and the blank and absorbance readings were taken after 20 mins of cold centrifuge of the sample.

CALCULATIONS :

From the observations, Abs (B) > Abs (A) in most cases (except some).

Also Abs 2N > IN > 0.5N > 1% > 0.1mg/ml.

This indicates that Sample B is most probably Gram-ve since it lysed much faster than A in most cases.

RESULT: We observed that Absorbance due to cell lysis increased upon increasing concentration and also that Absorbance of $B > A$
 $\therefore B$: Gram Negative
 A : Gram Positive

DISCUSSION:

The results for the experiment were close to what was expected \rightarrow Abs \uparrow as conc. of lysate \uparrow
Also, since Gram-ve bacteria have a thinner cell wall, they lyse faster thereby increasing the protein content \uparrow absorbance.

A few readings for sample B are found to be lesser than A. This could be due to:

- Inadequate incubation or lysate concentration
- Error while measuring absorbance.

These can be resolved by providing an adequate incubation period & cleaning the cuvette properly post each measurement.

EXPERIMENT-3 : FILTRATION OF ANINCOMPRESSIBLE SOLID

Aim: To filter an incompressible solid

INTRODUCTION:

Darcy's law defines the ability of a fluid to pass through a porous material. It is analogous to Ohm's law. The cake resistance has 2 components $\rightarrow R_m \& R_c$, which refer to membrane & cake resistances respectively.

$$V = \frac{K \Delta P}{\mu L}$$

V: flow velocity ; ΔP : Pressure drop ; L = Thickness
 K: Proportionality const. ; μ : Viscosity of fluid.

MATERIALS :

- 1. Mortar & Pestle
- 2. Chalk
- 3. Measuring cylinders
- 4. Conical flask
- 5. Whatman filters
- 6. Funnel

PROCEDURE:

1. 2 chalk pieces were ground & 5g powder added to 25ml H₂O
2. Solution poured into a burette & placed over a funnel w/ filter paper
3. 5 readings were taken over 90 mins for the volume of water eluted.

OBSERVATIONS

<u>Time (mins)</u>	<u>Volume (ml)</u>	<u>Volume eluted (ml)</u>
0	100	100
10	100	60
20	100	25
25	100	50
30	100	60
40	100	15
50	100	100
60	100	113
70	100	130
80	100	145
90	100	145
100	100	145

CALCULATIONS:

$$\text{Area of the filter : } \pi(4)^2/4 = 12.48 \text{ cm}^2$$

$$\frac{t/v}{\sqrt{mL}}$$

0.4
50

$$t_0 = 0.0013 N + 0.3714$$

卷之三

0.538 13

b) 135:0

$$\therefore K_1 = 0.0013 = \mu \nu g / 2 \rho R^2 \Rightarrow \alpha = \frac{\rho^2 R^2 K_1}{8 \mu}$$

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$$\nu = 0.01 \text{ Poise} (\text{Pa}\cdot\text{s}) = 10^3 \text{ Pa}\cdot\text{s}$$

$$g = 8.66 \text{ g/cm}^3$$

$$\therefore x = \frac{2 \times 1000 \times 0.0013}{6.66 \times 10^{-3}} = \boxed{\underline{\underline{8000.395555555555}}}$$

$$R_m = \frac{1000 \times 12.48 \times 0.3414}{10^{-3}} = 4.68 \times 10^6 \text{ cm}^{-1}$$

$$F_{\text{down}} \propto \frac{N}{V} \propto \frac{V_A}{R}$$

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RESULT: The resultant $\alpha = 4872.06 \text{ cm}^2/\text{g}$ &
 $R_m = 4.6 \times 10^6 \text{ cm}^{-1}$

DISCUSSION:

In this experiment we calculated the specific cake resistance & filter membrane resistances. The filter membrane resistance obtained was much higher than expected ($\approx 10^6 \text{ cm}^{-1}$). This could be due to various experimental errors such as improper time interval of measurement or Non-constant Pressure due to water level not being maintained.

Even the R^2 obtained is relatively low ($R^2 \approx 0.82$) indicating few faulty readings.

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OBSERVATIONS

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Time (min)

Volume

Volume eluted

Time (min)	Volume	Volume eluted
0	300 ml	-
5	195 ml	105 ml
10	150 ml	150 ml
15	125 ml	175 ml w/ 10 ml
20	100 ml	200 ml remaining
25	80 ml	220 ml total
30	65 ml	235 ml total
35	50 ml	255 ml total
40	40 ml	275 ml total
45	30 ml	285 ml total
50	140 ml	295 ml total
55	120 ml	315 ml total
60	105 ml	330 ml total
65	100 ml	335 ml total
70	95 ml	340 ml total
75	90 ml	345 ml total

Cake Diameter \approx 3.5 cm
Cake Area \approx 9.62 cm^2 
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EXPERIMENT : Vacuum Filtration of Yeast Solution

AIM: To filter a yeast suspension under constant vacuum.

INTRODUCTION: Define the ability of a fluid to pass through a porous material. It is analogous to Darcy's law where discharge is proportional to pressure difference.

$$Q = \frac{\pi r^2 V}{\mu L} \Delta P$$

Where,

 V = fluid flow velocity L = thickness K = Proportionality const. μ : Viscosity of liquid ΔP = Pressure drop across bedMATERIALS:

1. Cell suspension
2. Measuring cylinder
3. Conical flask
4. Vacuum filter set-up
5. Filters
6. Water

PROCEDURE:

1. Set up the filtration unit & ensured no leakage
2. Filled the filter chamber upto 300 ml w/ yeast-cake
3. The pump was turned on & readings were taken after every 5 mins (till 75mins)
4. Re-filled to 200 ml after 30 mins to ensure adequate discharge.

CALCULATIONS

Diameter of lake = 3.5 cm

Area of lake $\approx 9.62 \text{ cm}^2 (\pi D^2/4)$

<u>A t/V</u>	<u>V/A</u>
0.058	10.91
0.641	15.59
0.824	18.18
0.962	20.78
1.093	22.86
1.28	24.42
1.320	26.50
1.394	28.58
1.519	29.62
1.63	30.66
1.679	32.74
1.75	34.30
1.866	34.81
1.98	35.33
2.09	35.85

$$\frac{At}{V} = 0.0628 \frac{V}{A} - 0.3162$$

$$\therefore K = 0.0628 = \frac{\mu \alpha g}{2P} \Rightarrow \alpha = 2PK/\mu g$$

$$B = (-) 0.3162 = \frac{\mu R_m}{P} \Rightarrow R_m = PB/\mu$$

Given: $\mu = 0.012 \text{ Poise (Pa.s)}$

$$\Delta P = 600 \text{ mmHg} = 8 \times 10^4 \text{ Pa}$$

$$\rho = 0.00195 \text{ g/ml}$$

$$\alpha = 5.7 \times 10^9 \text{ cm/g}$$

$$R_m = (-) 2.108 \times 10^7 \text{ cm}^4$$

RESULTS: $\alpha = 5.7 \times 10^9 \text{ cm g}^{-1}$, $R_m = (-) 2.1 \times 10^7 \text{ cm}^{-1}$

DISCUSSION :

In this experiment we observed that the value of R_m came out to be -ve \Rightarrow The filter supposedly facilitated the outflow rather than preventing it. This can be rectified by assuming a negligible R_m . If the R_m is ~~is~~ $\ll 1$, the experimental errors lead to a -ve intercept in the curve.

Taking an $R_m \approx 0$, we get the equation as :

$$\frac{A t}{V} = 0.0518 \frac{V}{A}, R^2 = 0.994$$

This is a much better fit since the R^2 in the previous case was found to be 0.984

Thus, in this experiment, we assume the membrane resistance to be much lesser than the specific cake resistant i.e. $\alpha \gg R_m$

EXPERIMENT 5 : DENSITY GRADIENT CENTRIFUGATION

AIM: To estimate terminal velocity & separate beads using density gradient centrifugation.

INTRODUCTION:

Centrifugation uses the properties of the particles & machine in order to separate particles in suspension based on their density, shape & size. DGC is used widely to fractionate animal, plant & bacterial cells.

MATERIALS REQUIRED:

- | | |
|--------------------------|------------------------------|
| 1. Sodium Alginate (A&B) | 4. Centrifuge & Pipette |
| 2. CaCl_2 | 5. Millipore |
| 3. Sucrose Solution | 6. Crystal violet & Safranin |

PROCEDURE:

(a) Bead Preparation:

- 2 Beakers of 10ml CaCl_2 taken & A&B added drop by drop
- Beads placed @ 4°C for 20min
- Kept on tissue for drying

(b) Gradient Preparation:

3 Gradients prepared :	(1)	(2)	(3)
	0%	0%	0%
	15%	20%	25%
	30%	40%	30%
	45%	60%	35%

- Kept undisturbed for 15min

(c) Centrifugation:

- 2 beads each of A & B added to all 3 gradients,
- Centrifuged @ 10000 RPM, 20mins

OBSERVATIONS:

(i) Gradient 1 : All beads at the bottom of 45% layer
 (Intermixed) ∵ Beads $> 45\%$

(ii) Gradient 2 : All beads between 40-60% layers at the intersection
 \therefore Beads $\in (40\% - 60\%)$

(iii) Gradient 3 : All beads at the bottom of 35% layer
 \therefore Beads $> 35\%$

CALCULATIONS:

1. Based on observations, we can assume the density of the beads to be \sim Density of 50% sucrose solution
 $S_{50\% \text{ sucrose}} = 1.23 \text{ g/ml}$

$$2. Vg = \frac{\pi d^2}{18 \mu} (S_s - S) g$$

Suspension : 40% sucrose : $\mu = 6.15 \times 10^{-3} \text{ Pa}\cdot\text{s}$
 $S = 1.176 \text{ g/ml}$

Solid : $\sim 50\%$ sucrose : $S_s = 1.23 \text{ g/ml}$

$$d = 4 \text{ mm} = 4 \times 10^{-3} \text{ cm}$$

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$$\therefore V_g = \frac{4^2 \times 10^{-2} \times (1.23 - 1.176)}{18 \times 6 \cdot 15 \times 10^{-3}}$$

$$V_g = 7.804 \times 10^{-3} \text{ m/s} = 0.78 \text{ cm/s}$$

Since both beads lay in the same regime, the V_g for both is found to be constant & same.

RESULTS:

(i) Density of Bead A = 1.23 g/cc

Density of Bead B = 1.23 g/cc

(ii) V_g for Bead A = 0.78 cm/s

V_g for Bead B = 0.78 cm/s

DISCUSSION:

- We observed that the density & hence V_g for both beads was similar. This could be due to improper measurements or non-uniform bead sizes.
- Improper gradient preparation would lead to gradient mixing & hence not give a correct result. Gradient was found to be mixed post centrifugation, leading to a erroneous result.
- The bead sizes were not uniform and an average bead diameter was taken for V_g calculations.

EXPERIMENT 6 : LIQUID-LIQUID EXTRACTION

AIM: To calculate the partition coefficient of 2 dyes using liquid-liquid extraction

INTRODUCTION: Liquid-liquid extraction is a separation process used extensively in the extraction of biochemicals especially antibiotic purification. In LLE, a desired solute can be extracted from a solution by contacting it with another solvent which the solute has higher solubility in. The distribution of the solute in the 2 solvents is governed by the solvents' partition coefficient (K).

$$K = (x/y)$$

Where x = conc. in light phase

y = conc. in heavy phase.

MATERIALS REQUIRED:

1. Dye Solutions
2. Test Tubes
3. Separating funnels
4. Measuring cylinders
5. Chloroform
6. Spectrophotometer

PROCEDURE :

(a) Preparation of standard curve.

1. Dilutions of Dye A & B were prepared
2. Absorbance was measured at 590 nm for dye A and 595 nm for dye B
3. Curve for OD vs conc. was plotted to obtain the standard curve for both dyes

(b) Liquid-Liquid Extraction:

1. 10ml dye was taken in a separating funnel
2. 2ml Chloroform was added to the separating funnel
3. ~~The~~ The funnel was shaken for 2 min
4. The stand was placed undisturbed for 5 mins & the colour observed.
5. The organic phase was collected while the aqueous phase was collected in a separate test tube & OD was measured @ 590nm using 1ml.
6. Absorbance was measured for all 4 standard experiments and for both dyes

CALCULATIONS

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EXPERIMENT F : ABSORPTION

Aim: To plot the breakthrough curves for adsorption. (a)

MATERIALS:

1. Spectrophotometer
2. Activated Charcoal
3. Rhodamine Dye
4. Adsorption Column
5. Peristaltic Pump

INTRODUCTION

Adsorption involves transfer of substances from one phase to another using conc. difference as the driving force which can be diffusion, convection or conduction. Once a solution is passed through an adsorption col, the output conc. is modified as a function of interaction of the particles in the solution & adsorbent.

A plot of Conc. vs Time gives us adsorption capacity. Interaction btw the Adsorbent & particles in solution ↓ and particles are expelled in the solution. The curve plotted is S-shaped and is called the BREAKTHROUGH CURVE

PROCEDURE(a) Standard Curve Preparation

Prepare standard curve @ OD 510nm

OBSERVATIONS : Group B

(a) Bed length : 2 cm

(b) Sample concentration = 20 mg/L

(c) STANDARD CURVE

Conc. (mg/L)	OD ₁	OD ₂	Avg.
20	1.562	1.563	1.5625
15	1.163	1.164	1.1635
10	0.784	0.783	0.7835
5	0.369	0.371	0.370
2.5	0.121	0.121	0.121
1.25	0.061	0.061	0.061
0.625	0.031	0.031	0.031
0.312	0.016	0.016	0.016
0.156	0.008	0.008	0.008
0.078	0.004	0.004	0.004
0.039	0.002	0.002	0.002
0.019	0.001	0.001	0.001
0.009	0.0005	0.0005	0.0005

(d) BREAKTHROUGH CURVE

Time (min)	OD
10	0.011
20	0.017
30	0.006
40	0.001
50	0.003
60	0.16
70	0.174

$$\text{Sample conc. } (C^*) = 20 \text{ mg/L}$$

(e) ADSORPTION

- The adsorption column was filled with crushed charcoal upto 2 cm
- Water was passed for 10 min & effluent discarded.
- The solution was known & stoppers closed after 10 mins.
- The effluent is then collected till clear & discarded.
- Effluent collected every 10 min & stored in 2 ml N.T.S.

5. OD measured for all samples at 510 nm

CALCULATIONS :

$$\text{Equation of Std. Curve : } OD = 0.0799^* \text{Conc} - 0.03 = 0.0799^* \text{Conc} + 0.03$$

$$\therefore \text{Conc.} = \frac{OD - 0.03}{0.0799}$$

OD	Concentration (out) (mg/L)
0.011	0.141
0.017	0.218
0.006	0.077
0.001	0.0128
0.003	0.0385
0.16	2.0565
0.174	2.2365

DATA FOR OTHER GROUPS

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Time (min)	C_{out}/C^*
10	0.007
20	0.0109
30	0.0038
40	0.00064
50	<u>0.0019</u>
60	0.1028 ← Breakthrough
70	0.1118
80	Column Scale - Up
90	Fataly Number : 2019.BB.100.b4 XX = 64 Wh
100	
110	
120	
130	
140	
150	
160	
170	
180	

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DISCUSSION

The breakthrough curves obtained (L-HS) suggest considerable variance in profiles obtained.

(a) GROUP-A : We observe an initial decrease in concentration of adsorbate. This is followed by a minima which then increases.

This could be due to improper washing of the column since the adsorbent might be saturated.

Once time proceeds, the adsorbent gets free and starts adsorbing which then proceeds to a normal breakthrough curve with $t_b \approx 50$ mins

(b) GROUP-B : We observe an initially almost constantly low affluent concentration which suddenly shoots up at ($t_b = 50$ mins) around 50 mins. This represents a fairly accurate breakthrough curve. Initially low conc represents a good amount of desorption which then shoots up and saturates as the adsorbent starts saturating.

(c) GROUP-C : We observe a breakthrough around $t_b = 40$ mins following which the concentration increases steadily. This curve doesn't achieve saturation, implying that the adsorbent isn't completely packed after 40 mins.

(d) GROUP-D : We do not observe a standard breakthrough. The curve increases, saturates b/w 20-40 mins then increases linearly again. This could be due to

Improper column washing or faulty experimental reading

(e) GROUP-E: We observe a breakthrough at $t_B = 20$ mins which starts saturating around $t = 80$ mins. It appears to be a standard breakthrough curve.

RESULTS:

We observe the breakthrough points for the different groups to be:

- (a) Group A: $t_B = 50$ mins
- (b) Group B: $t_B > 50$ mins
- (c) Group C: $t_B = 40$ mins
- (d) Group D: $t_B = 40$ mins or $t_B = 10$ mins
- (e) Group E: $t_B = 20$ mins

OBSERVATIONS(a) Standard curve

Conc (g/l)	Area
1	6830665
2	3169516
3	10295004
4	13657603
5	16629500

3169516

10295004

13657603

16629500

6830665

1

2

3

4

5

6830665

10295004

13657603

16629500

3169516

1

2

3

4

5

6830665

10295004

13657603

16629500

3169516

1

2

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6830665

10295004

13657603

16629500

3169516

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6830665

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16629500

3169516

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CALCULATIONS

$$\text{Area} = \frac{3 \times 10^6 \times \text{Conc.} - 5614.2}{(\text{Area} + 5614.2) / (3 \times 10^6)}$$

As per std curve :
Conc =

1. Start injection @ low flowrate. Wash the column for 1 hr.
2. Adjust the baseline
3. Place the vials in samples & make sequence for the samples making sure to set the vials correctly.
4. Start the sequencing run and analyse the results

CALCULATIONS

1. Standard curve given on the LHS

2. $t_R = \text{Retention time}$

$t_R = 1^{\text{st}} \text{ solvent peak } (\sim 8.55)$
Adjusted retention time $t_R' = t_R - t_s$

$$\therefore t_R - t_s \propto K$$

t_s

$$\therefore K^*(\text{const}) = \frac{t_R - 8.5}{8.5}$$

$$\therefore \boxed{\frac{t_R}{8.5} - 1 = K^* C}, \quad \Rightarrow \text{constant}$$

3. Entry numbers : 2019B810064

$\times \times : 64$ times dilution

$$C_1 = 4.877 \rightarrow 0.0762 \text{ g/L}$$

$$C_2 = 2.2861 \text{ g/L}$$

$$C_3 = 1.9152 \text{ g/L}$$

- As per std curve : Peak Area $3 \times 10^6 \times \text{conc.}$
- $A_1 = 3 \times 10^6 \times 0.0762 \approx 228600$ (exact = 228561.29)
 - $A_2 = 3 \times 10^6 \times 0.0357 \approx 107100$ (exact = 107073.57)
 - $A_3 = 3 \times 10^6 \times 0.311 \approx 93300$ (exact = 99689)

Hence, the peak area decreases by fold.

4. Since retention times for $B < A$, we can conclude the B was run at a higher flow rate. This also led to much smaller peak areas for B since the column doesn't get separated well due to little time for interaction with the stationary phase

5. Analyte 1: $\text{Conc}_c = 4.877 \text{ g/L}$

$$\text{Conc}_d = 1.08 \text{ g/L}$$

$$\text{Fold change} = \frac{4.877}{1.08} = \underline{4.51} \times \text{decrease}$$

Analyte 2: $\text{Conc}_c = 2.28 \text{ g/L}$

$$\text{Conc}_d = 0.513 \text{ g/L}$$

$$\text{Fold change} = \frac{2.28}{0.513} = \underline{4.44} \times \text{decrease}$$

Analyte 3: $\text{Conc}_c = 1.915 \text{ g/L}$

$$\text{Conc}_d = 0.35 \text{ g/L}$$

$$\text{Fold change} = \frac{1.915}{0.35} = \underline{5.47} \times \text{decrease}$$

DISCUSSION:

Samples A & B consisted of 2 peaks below the % threshold which have been neglected. Sample C & D contained 1 peak below % which has been similarly neglected. From the results, B appears to have run at a higher flow rate than A while C & D ran at the

same flow rate. Analytes in Sample C were at a 4.5-5.5 X concentration as opposed to Sample D as evidenced by the larger peak areas and heights.

RESULTS: In this experiment, we successfully analyzed the results of 4 HPLC runs.

Teacher's Signature: _____

EXPERIMENT 9PROTEIN PRECIPITATION BYAMMONIUM SULPHATE

AIM: To study protein precipitation under various Amm. Sulphate concentrations

THEORY: Precipitation is the conversion of soluble proteins into insoluble forms. It leads to formation of amorphous solids & can be forced by changing salt conc., temp & pH.

For protein precipitation, one of the most widely used methods is salt addition such as $(\text{NH}_4)_2\text{SO}_4$

MATERIALS REQUIRED:

- | | |
|----------------------|---------------------------------|
| 1. Cell suspension | 5. Vortex |
| 2. Ammonium Sulphate | 6. Centrifuge |
| 3. Ice | 7. Falcon Tubes |
| 4. Pipetter | 8. Spectrophotometer & cuvettes |

PROCEDURE:

1. Lysis bacterial cells by sonication & centrifuge @ 10,000 RPM for 20 mins
2. Collect 1ml of supernatant and add $(\text{NH}_4)_2\text{SO}_4$ to make 35%, 40%, 45%, 50% & 55% salt conc. solutions.
3. The solutions were vortexed & incubated for 20 mins
4. The solutions were centrifuged @ 4°C, 8000 RPM for 15 mins & Abs recorded @ 280 nm

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OBSERVATIONS:

After adding 10 ml of dilute HCl to 10 ml of NaOH
the solution turned yellowish green.

After

1 min

After

2 min

After

3 min

After

4 min

After

5 min

After

6 min

After

7 min

After

8 min

After

9 min

After

10 min

After adding 10 ml of dilute HCl to 10 ml of NaOH
the solution turned yellowish green.
After 1 min the color turned orange-yellow.
After 2 min the color turned orange.
After 3 min the color turned orange-yellow.
After 4 min the color turned orange.
After 5 min the color turned orange-yellow.
After 6 min the color turned orange.
After 7 min the color turned orange-yellow.
After 8 min the color turned orange.
After 9 min the color turned orange-yellow.
After 10 min the color turned orange.

DISCUSSION

The color change observed in the above reaction
is due to the formation of a complex between
the metal ion and the ligand.

The reaction is a reversible equilibrium reaction
between the metal ion and the ligand.
The equilibrium constant for this reaction is
given by the equation:
$$K = \frac{[M\text{-L}]}{[M][\text{L}]}$$

Teacher's Signature: _____