Answer Key

CHEM1501 Midterm Practice Problems

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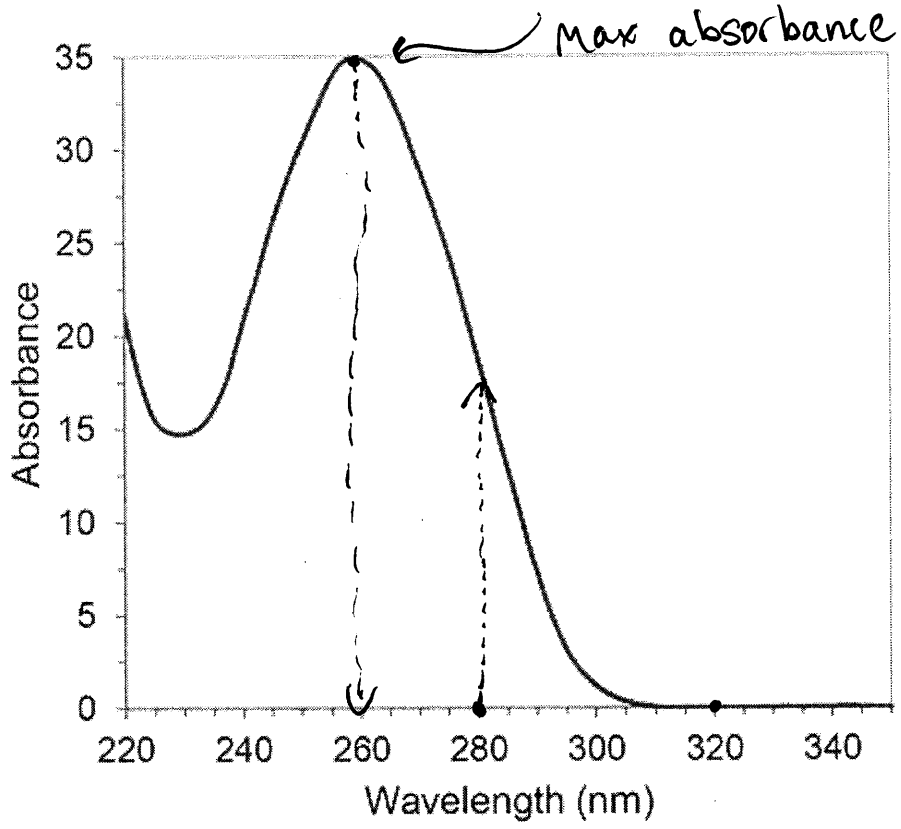
Question 1: The following is a spectrum graph for purified nucleic acids, e.g. DNA and RNA.



a.) Please identify the λ_{max} for DNA/RNA:

$\lambda_{max} = 260\,nM$

b.) For these kinds of purified nucleic acids the absorbance at 280 nm often represents the amount of protein content, while the absorbance at 320 nm can represent general background noise in the solution. Calculate the approximate ratio between protein content and nucleic acid content:

320 nM = 0 Abs.
280 nM ≈ 17.5 Abs.

$$\frac{260}{280} = \frac{35}{17.5} \simeq 2.0 \text{ to } 2.2$$

You may also calculate the 280/260 ratio for this question.

Assume for the remaining questions that the calculated ratio indicates an adequate nucleic acid purification

Question 2: There is a mysterious tube in the lab. You measure the absorbance on a spectrophotometer, with the machine providing the "raw" values in the table below.

sample	260 Raw	280 Raw	320 Raw	260	280	260/280	ng/µl
blank	.072	.069	.060	n/a	n/a	n/a	n/a
tube	.084	.075	.061	?	?	?	?

Recall that the 320 nm signal is general background noise - which allows you to ensure that samples have the same amount of background signal. The amount of nucleic acid/protein material should be calculated in relation to the amount absorbed by a tube with the liquid which the nucleic acid is dissolved in (blank).

a.) Calculate the amount of the 260 and 280 nm signal attributed to the nucleic acids in the tube?

$260 = 260_{tube} - 260_{blank} = .084 - .072 = .012$

$280 = 280_{tube} - 280_{blank} = .075 - .069 = .006$

b.) Is this an "adequate nucleic acid purification," when comparing the 260/280 ratio to the optimal ratio as calculated in **1-b**?

$\frac{260}{280} = \frac{.012}{.006} \approx 2.0$ Yes, this is an adequate nucleic acid purification.

Question 3: The Beer-Lambert Law is a relationship between absorbance and concentration: $A = \varepsilon lc$. If A = absorbance of nucleic acids, $\varepsilon_{DNA} = 0.020\ (\mu g/mL)^{-1}cm^{-1}$ and l = path length. You have been provided a diagram of the equipment you utilized to make these measurements.

a.) Calculate the concentration of nucleic acids in this tube:

260 nm Abs = .012

$\varepsilon_{DNA} = .020 \frac{mL}{\mu g\, cm}$

$l = (.5mm)\left(\frac{1cm}{10mm}\right) = .05cm$

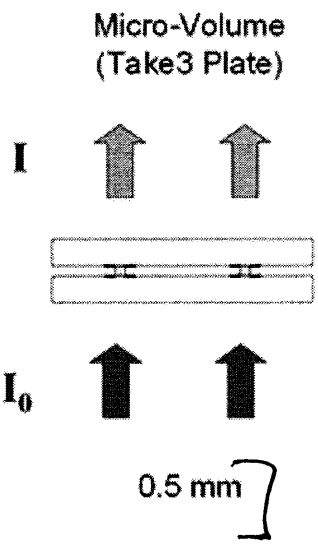
$.012 = (.020)(.05)C$

$C = 12 \frac{ng}{\mu l}$ ← why $\frac{ng}{\mu l}$?

unitless ↓

$.012 = \left(.020 \frac{mL}{\mu g\, cm}\right)(.05cm)\, C$

$C = 12 \frac{mg}{mL} \cdot \left(\frac{10^3 ng}{1mg}\right)\left(\frac{1mL}{10^3 \mu l}\right) = 12 \frac{ng}{\mu l}$



Question 4: After completing your experiment on the spectrophotometer, you have 25 µl left in the mystery tube. The label states: "LINC00173 // DNA // 3093 bases." You assume that this means the tube contains identical particles of DNA 3092 bases long called LINC00173.

Each base is on average 650 g/mol. You wish to phosphorylate the 5' ends of the DNA strands; each DNA strand has one 5' end. You will design a reaction totaling 50 µl to accomplish this task. You have been provided the following instructions:

- 10 units (or 1 µl) of T4 PNK-enzyme can phosphorylate 300 pmol of 5′ termini
- You should have a final concentration of 1X T4 PNK Buffer
- You should have a final concentration of 1 mM ATP
- Nuclease-free water is not involved in the reaction; it is simply to ensure that the final reaction volume is 50 µl.

You have access to the reagents described in the following table:

T4 PNK	? 0-1 µl
10X T4 PNK Buffer	? 5 µl
10 mM ATP	? 5 µl
DNA ~~(20 mcl)~~	? 25 µl
Nuclease-free Water	? 14-15 µl

a.) Calculate all the necessary volumes to perform a successful phosphorylation reaction:

$10X(X\ \mu l) = 1X(50\ \mu l)$ } Amount of 10X T4 PNK Buffer required

$X = 5\ \mu l$

$10mM(X\ \mu l) = 1mM(50\ \mu l)$ } Amount of 10 mM ATP required

$X = 5\ \mu l$

DNA = 25 µl ⇒ given in the mystery tube

How much enzyme? $25\ \mu l\ DNA \cdot \left(\frac{12\ ng}{1\ ne}\right) = 300\ ng\ DNA$

$3092\ bases\ DNA \cdot \left(\frac{650\ g}{1\ mol\ base}\right) = 2009800\ \frac{g}{mol\ LINC00173} \cdot \left(\frac{10^9\ ng}{1\ g}\right)$

$= 2.0098 \times 10^{15}\ \frac{ng}{mol\ LINC00173}$

$$300\ ng\ DNA \cdot \left(\frac{1\ mol\ LINC00173}{2.0098\times10^{15}\ ng}\right)\left(\frac{6.022\times10^{23}\ molecules\ LINC00173}{1\ mol\ LINC00173}\right)$$

$$= 8.989\times10^{10}\ molecules\ LINC00173$$

$$(8.989\times10^{10}\ molecules\ LINC00173)\left(\frac{1\ 5'\ termini}{1\ molecule}\right)$$

$$= 8.989\times10^{10}\ 5'\ termini$$

$$8.989\times10^{10}\ 5'\ termini \cdot \left(\frac{1\ mol\ 5'\ termini}{6.022\times10^{23}\ termini}\right) = 1.49\times10^{-13}\ mol\ 5'\ termi$$

$$1.49\times10^{-13}\ mol\ 5'\ termini\left(\frac{10^{12}\ pmol}{1\ mole}\right) = .149\ pmol\ 5'\ termi$$

$$(.149\ pmol\ 5'\ termi)\left(\frac{1\ \mu l\ T4\ PNK}{300\ pmol\ 5'}\right) = .000498\ \mu l\ T4\ PNK$$

Note: you need very little enzyme to perform this reaction. All amnes up to 1 µl accepted if you address the feasibility of pipetting out .000498 µl.

Nuclease-free Water:

$$50\ \mu l - 25\ \mu l - 5\ \mu l - 5\ \mu l - (0-1\ \mu l) =$$

$$14-15\ \mu l$$