BCH 372 Names: _____
Modern Concepts in Biochemistry Laboratory _____ _____
(30 points) _____
_____

Datum Sheet for Laboratories 11 and 12
Ion Exchange Chromatography - Part A and Part B

1. Give the LDH activity of dialyzed resuspended 65% ammonium sulfate pellet fraction (**D65P**). Show the volume that was assayed, the initial velocities of the three replicate runs, the mean, and the activity (2 points).

   volume assayed: _____

   V_o values (ΔA_{340}/min) _____ _____ _____

   average V_o (ΔA_{340}/min) _____

   average V_o (μmole/min) _____

   activity (μmole/min ml) _____

2. Give the LDH activities in **μmoles/min ml** based on the assays with 50 μl volumes of the wash fractions from both the DEAE Cellulose and CM Cellulose columns (4 points).

fraction #	DEAE Cellulose	CM Cellulose
1	_____	_____
2	_____	_____
3	_____	_____
4	_____	_____
5	_____	_____
6	_____	_____

3. Give the following information about the pooled fractions that were obtained from the two ion-exchange columns during the washing step. Fill out a separate section for each pool. Some groups may have only two pools, but others may have three or four (6 points)

Wash Pool 1

column resin _____

fractions pooled _____

volume of pool (ml) _____

V_o values (ΔA_{340}/min) _____ _____ _____

average V_o (ΔA_{340}/min) _____

average V_o (μmole/min) _____

activity (μmoles/min ml) _____

total units recovered _____

Wash Pool 2

column resin _____

fractions pooled _____

volume of pool (ml) _____

V_o values (ΔA_{340}/min) _____ _____ _____

average V_o (ΔA_{340}/min) _____

average V_o (μmole/min) _____

activity (μmoles/min ml) _____

total units recovered _____

Wash Pool 3

column resin _____

fractions pooled _____

volume of pool (ml) _____

V_o values (ΔA_{340}/min) _____ _____ _____

average V_o (ΔA_{340}/min) _____

average V_o (μmole/min) _____

activity (μmoles/min ml) _____

total units recovered _____

Wash Pool 4

column resin _____

fractions pooled _____

volume of pool (ml) _____

V_o values (ΔA_{340}/min) _____ _____ _____

average V_o (ΔA_{340}/min) _____

average V_o (μmole/min) _____

activity (μmoles/min ml) _____

total units recovered _____

4. Give the LDH activities in **µmoless/min ml** based on the assays with 50 µl volumes of the fractions that were obtained during elution with 0.03 M bicine buffers containing different concentrations of NaCl. Use as many lines as necessary to show elution of most of the LDH activity (6 points).

fraction #	DEAE Cellulose	CM Cellulose
7	_____	_____
8	_____	_____
9	_____	_____
10	_____	_____
11	_____	_____
12	_____	_____
13	_____	_____
14	_____	_____
15	_____	_____
16	_____	_____

5. Give the following information about the pooled fractions that were obtained from the ion-exchange columns during the elution of proteins with buffers containing increasing concentrations of NaCl washing step. Fill out a separate section for each pool. Some groups may have only two elution pools, but others may have three or four (6 points)

Salt Elution Pool 1

column resin _____

fractions pooled _____

volume of pool (ml) _____

V_o values (ΔA_{340}/min) _____ _____ _____

average V_o (ΔA_{340}/min) _____

average V_o (μmole/min) _____

activity (μmoles/min ml) _____

total units recovered _____

Salt Elution Pool 2

column resin _____

fractions pooled _____

volume of pool (ml) _____

V_o values (ΔA_{340}/min) _____ _____ _____

average V_o (ΔA_{340}/min) _____

average V_o (μmole/min) _____

activity (μmoles/min ml) _____

total units recovered _____

Salt Elution Pool 3

column resin _____

fractions pooled _____

volume of pool (ml) _____

V_o values (ΔA_{340}/min) _____ _____ _____

average V_o (ΔA_{340}/min) _____

average V_o (μmole/min) _____

activity (μmoles/min ml) _____

total units recovered _____

Salt Elution Pool 4

column resin _____

fractions pooled _____

volume of pool (ml) _____

V_o values (ΔA_{340}/min) _____ _____ _____

average V_o (ΔA_{340}/min) _____

average V_o (μmole/min) _____

activity (μmoles/min ml) _____

total units recovered _____

6. Attach to this datum sheet the two complete elution profiles for the ion-exchange columns. Each elution profile should have LDH activity (in **μmoles/min ml**) plotted as a function of fraction number (3 points).

7. Summarize the results of the ion-exchange chromatography by filling out the following table. **Insert the number** of each of your pools. Some groups may have only one wash pool or one elution pool for a particular column (3 points).

Column	Total Units Loaded	Percentage of Units Recovered Wash Pool	Wash Pool	Elution Pool	Elution Pool
DEAE-	_____	_____	_____	_____	_____
CM-	_____	_____	_____	_____	_____