

By putting your name on this paper, you agree to abide by the university's code of academic integrity in completing the quiz. Use no books, cell phones, communication with others etc. Use your calculator for calculations only.

Student's name Key

**Problem** (20 points). Suppose you have a mixture of five proteins listed in the table below.

#	Protein	pI	Mol. Weight, kDa
A	Ubiquitin	6.4	8.5
B	Lysozyme	11.0	13
C	Myoglobin	7.0	16.7
D	Ovalbumin	4.6	45
E	Serum albumin	4.9	68.5

(a) Indicate the order in which these proteins will elute from a gel-filtration column (starting with the one that elutes first). You can use letters A-E (see table) for simplicity.

E, D, C, B, A

(b) You load this mixture on a cation exchange column (i.e. column that bears negatively charged groups). The buffer you use for this column is acetate buffer, pH 4.9.

-- List proteins that will appear in the flow-through (i.e. will not bind to the column).

D, E

-- In order to elute those proteins that are immobilized on the column, you then apply a linear salt gradient, with NaCl concentration gradually increasing from 0 to 1 M. Indicate the order in which the proteins bound to the column will elute as the salt concentration increases.

A, C, B

(c) You repeat the same procedure as in (b) but now you use anion exchange column (that bears positively charged groups), and the buffer is TRIS (pH 8.0). Answer the same questions as in (b).

-- Flow through: B

-- Elute: C, A, E, D

(d) You load this protein mixture and run it on the SDS PAGE. Predict the order they will migrate on the SDS gel, starting with the fastest.

A, B, C, D, E

(e) You are interested in further studies of lysozyme. Based on the results of your experiments above, devise a reliable procedure for its purification from this mixture of proteins.

There are several possibilities, probably the simplest one is to use anion-exchange column (as in Problem C) with buffer at pH > 7 (but lower than 11). Lysozyme will be the only protein in the flow-through.