2.2 **Bioassays** Discuss the relative importance of the bioassay during the different phases of research and development of a new bioactive molecule, including discovery research, laboratory process development, and large-scale process development.

2.3 **Enzymatic Activity Assay for L-Alanine Dehydrogenase** L-Alanine dehydrogenase can be assayed by using the following reaction:

$$\text{L-Alanine} + \text{NAD}^+ + \text{H}_2\text{O} \xrightleftharpoons{\text{L-alanine dehydrogenase}} \text{pyruvate} + \text{NADH} + \text{NH}_4^+$$

For this assay, a sample containing this enzyme was diluted 1:1000 and added to a solution containing L-alanine, NAD, and carbonate buffer at pH 10.0 and 25°C. Absorbance readings versus time were taken using a path length of 10 mm and wavelength of 340 nm as shown in Table P2.3.

TABLE P2.3

Time (min)	A_{340}
2.0	0.27
2.5	0.33
3.0	0.40
3.5	0.47
4.0	0.53
4.5	0.60
5.0	0.67

From these data and the molar absorptivity for NADH ($\varepsilon = 6.2 \times 10^2$ liter mol^{-1} mm^{-1} at 25°C and 340 nm), calculate the enzyme concentration in units per milliliter. (*Note:* NADH absorbs at 340 nm, but NAD^+ does not.) If the purified enzyme has a specific activity of 30 U/mg and the enzyme solution that was assayed has a protein concentration of 1.1 mg/ml, what is the purity of the enzyme in the sample and what is its specific activity? (Data from H. U. Bergmeyer and M. Grassl, "Handling of reagents," in *Methods of Enzymatic Analysis*, vol. II, H. U. Bergmeyer, ed., p. 102, Verlag Chemie, Weinheim, 1983.)

2.4 **Troubleshooting purity methods** A recombinant protein produced in a production scale process typically has a level of host cell protein (HCP) by ELISA of 30 ppm. The most recent batch showed a shift in HCP to 80 ppm. What steps can you take to confirm this result?

2.5 **Operating Problems During the Electrophoresis of Proteins** For the following problems that can occur in the analysis of proteins by gel electrophoresis, what do you think is the cause of the problem, and how do you think the problem can be solved?

(a) "Smile effect"—band pattern curves upward at both sides of the gel
(b) Lateral band spreading
(c) Skewed or distorted bands
(d) Vertical streaking of protein bands

2.6 **Protein Charge During Gel Electrophoresis** A solution of 0.1% sodium dodecyl sulfate (SDS) binds so strongly to polypeptide chains that one detergent molecule is sufficient to saturate two amino acid residues (R. Scopes, *Protein Purification*, 3rd ed., p. 297, Springer-Verlag, New York, 1994). For a protein with a molecular weight of 60,000 in 0.1% SDS, estimate the effect on the electrical charge per protein molecule.

2.7 **Problem in the Determination of Molecular Weight by Gel Electrophoresis** A purified recombinant protein is analyzed for molecular weight by SDS-PAGE at pH 8.5. From the protein sequence deduced from the gene that was expressed in bacteria, the protein is expected to have a molecular weight of 44,000. However, the molecular weight of the protein is found by SDS-PAGE to be 52,000. Explain the reason or reasons for this difference in molecular weight. What calculation could you make to help explain this discrepancy?

2.8 **Temperature in a Vertical Electrophoresis Gel** You are designing a mini–vertical gel electrophoresis system to separate proteins. The gel is 7 cm high, 8 cm wide, and 0.075 cm thick and runs at 200 V and 60 mA. The glass support plates on either side of the gel are each 0.200 cm thick. To finalize the design, you need to decide whether the system should be placed in an air bath or a water bath. Estimate the maximum temperature in the gel for both cases using thermal conductivities and heat transfer coefficients found in the literature. Which bath do you recommend be used?