

PROBLEMS

8.1 Coagulation

- a. Section 8.6.3 described how the addition of acid can trigger the coagulation (clumping) of proteins in milk or egg. The suggested mechanism was a reduction of the effective charge on the proteins and a corresponding reduction in their mutual repulsion. The addition of salt also promotes coagulation, whereas sugar does not. Suggest an explanation for these facts.
- b. Cheese-making dates from at least 2300 BCE. More recently (since ancient Roman times), cheese-makers have used a milk-curdling method that does not involve acid or salt. Instead, a proteolytic (protein-splitting) enzyme (chymosin, or rennin) is used to cut off a highly charged segment of the κ -casein molecule (residues 106–169). Suggest how this change could induce curdling and relate it to the discussion in Section 8.6.2.

8.2 Isomerization

Our example of buffalo as a two-state system (Figure 6.8 on page 220) may seem a bit fanciful. An example of a more realistic example from biochemistry is the isomerization of a phosphorylated glucose molecule from its 1-P to its 6-P form (see Figure 8.12), with $\Delta G^0 = -1.74$ kcal/mole. Find the equilibrium concentration ratio of glucose-P in the two isomeric states shown.

8.3 pH versus temperature

The pH of pure water is not a universal constant; rather, it depends on the temperature: At 0°C, it's 7.5, whereas at 40°C, it's 6.8. Explain this phenomenon and comment on why your explanation is numerically reasonable.

8.4 Difference between F and G

- a. Consider a chemical reaction in which a molecule moves from gas to a water solution. At atmospheric pressure, each gas molecule occupies a volume of about 24 L/mole, whereas in solution, the volume is closer to the volume occupied by a water molecule, or 1/(55 mole/L). Estimate $(\Delta V)_p$, expressing your answer in units of $k_B T_r$.
- b. Consider a reaction in which two molecules in aqueous solution combine to form one. Compare an estimate of $(\Delta V)_p$ with what you found in (a) and comment on why we usually don't need to distinguish between F and G for such reactions.

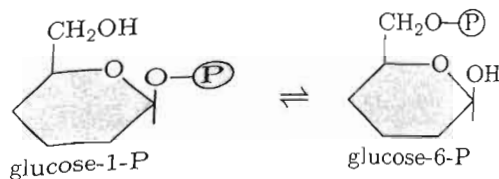


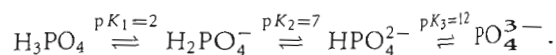
Figure 8.12: (Molecular structure diagrams.) Isomerization of glucose-P. [Adapted from Alberts et al., 2004.]

8.5 *Simple dissociation*

Section 8.3.2 gave the dissociation pK for acetic acid as 4.76. Suppose that we dissolve a mole of this weak acid in 10 L of water. Find the pH of the resulting solution. What fraction of acetic acid molecules is dissociated?

8.6 *Ionization state of inorganic phosphate*

Chapter 2 oversimplified somewhat in stating that phosphoric acid (H_3PO_4) ionizes in water to form the ion HPO_4^{2-} . In reality, all four possible protonation states, from three H's to none, exist in equilibrium. The three successive proton-removing reactions have the following approximate pK values:



Find the relative populations of all four protonation states at the pH of human blood, around 7.4.

8.7 *Electrophoresis*

In this problem, you will make a crude estimate of a typical value for the electrophoretic mobility of a protein.

- Model the protein as a sphere of radius 3 nm, carrying a net electric charge $q = 10e$, in pure water. If we apply an electric field of $\mathcal{E} = 2 \text{ volt cm}^{-1}$, the protein will feel a force $q\mathcal{E}$. Write a formula for the resulting drift velocity and evaluate it numerically.⁷
- In the experiment discussed in Section 8.3.4 on page 312, Pauling and coauthors used an electric field of 4.7 volt cm^{-1} , applied for up to 20 hours. For a mixture of normal and defective hemoglobin to separate into two distinguishable bands, they must travel different distances under these conditions. Estimate the separation of these bands for two species whose charges differ by just one unit and comment on the feasibility of the experiment.

8.8 T_2 *Grand partition function*

Review Section 8.1.2 on page 298.

- Show that the distribution you found in Your Turn 8B is the one that minimizes the grand potential of system a at T, μ , defined by analogy with the usual free energy (Equation 6.32 on page 224) as

$$\Psi_a = \langle E_a - \mu N_a \rangle - TS_a. \quad (8.38)$$

- Show that the minimal value of Ψ thus obtained equals $k_B T \ln Z$.
- Optional:* For the real gluttons, generalize your result in (a) and (b) to systems exchanging particles and energy, and changing volume as well (see Section 6.5.1).

⁷ T_2 Actually, one uses a salt solution (buffer) instead of pure water. A more careful treatment would account for the screening of the particle's charge (Section 7.4.3' on page 284); the result contains an extra factor of $(3/2)(\lambda_D/a)$ relative to your answer.

Notes for problem 8.7

8.3.4 Electrophoresis can give a sensitive measure of protein composition

Even though the analysis in Section 8.3.3 was rough, it did explain one key qualitative fact about the experimental data (Figure 8.1): At some critical ambient pH, a protein will be effectively neutral. The value of pH at this point and, indeed, the entire titration curve are fingerprints characteristic of each specific protein.

Section 4.6.4 on page 142 explained how putting an electric field across a salt solution causes the ions in that solution to migrate. Similar remarks apply to a solution of *macroions*, for example, proteins. It is true that the viscous friction coefficient ζ on a large globular protein will be much larger than that on a tiny ion (by the Stokes formula, Equation 4.14 on page 119). But the net driving force on the protein will be

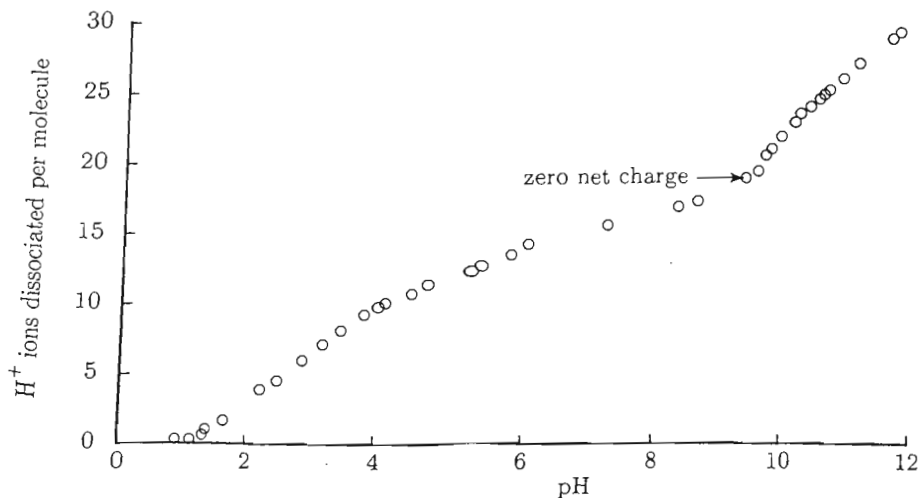


Figure 8.1: (Experimental data.) The protonation state of ribonuclease depends on the pH of the surrounding solution. The arrow shows the point of zero net charge. The vertical axis gives the number of H^+ ions dissociated per molecule at $25^\circ C$, so the curves show the protein becoming deprotonated as the pH is raised from acidic to basic. [Data from Tanford, 1961.]

huge, too: It's the sum of the forces on each ionized group. The resulting migration of macroions in a field is called **electrophoresis**.

The rule governing the speed of electrophoretic migration is more complicated than the simple qE/ζ used in our study of saltwater conductivity. Nevertheless, we can expect that an object with zero net charge has zero electrophoretic speed. Section 8.3.3 argued that any protein has a value of ambient pH at which its net charge is zero (called the protein's isoelectric point). As we titrate through this point, a protein should slow down, stop, and then reverse its direction of electrophoretic drift. We can use this property to separate mixtures of proteins.

Not only does every protein have its characteristic isoelectric point; each *variant* of a given protein will, too. A famous example is the defective protein responsible for sickle-cell anemia. In a historic discovery, Linus Pauling and coauthors showed in 1949 that the red blood cells of sickle-cell patients contained a defective form of hemoglobin. Today we know that the defect lies in parts of hemoglobin called the β -globin chains, which differ from normal β -globin by the substitution of a *single amino acid*, from glutamic acid to valine in position six. This tiny change (β -globin has 146 amino acids in all) is enough to create a sticky (hydrophobic) patch on the molecular surface. The mutant molecules clump together to form a solid fiber of fourteen interwound helical strands inside the red cell and give it the sickle shape for which the disease is named. The deformed red cells in turn get stuck in capillaries and then damaged; finally they are destroyed by the body, with the effect of creating anemia.

and sickle-cell hemoglobin could be distinguished by their electrophoretic mobility. In this trial, the hemoglobin was bound to carbon monoxide, and its mobility μ (in $\text{cm s}^{-1}/(\text{volt cm}^{-1})$) was measured at various values of pH. *Circles*: Normal hemoglobin. *Squares*: Sickle-cell hemoglobin. (Solid black symbols represent trials with dithionite ion present; open symbols are trials without it.) [From Pauling et al., 1949.]

In 1949, the sequence of β -globin was unknown. Nevertheless, Pauling and coauthors pinpointed the source of the disease in a single molecule. They reasoned that a slight chemical modification in hemoglobin could produce different dissociation constants. Isolating normal and sickle-cell hemoglobin, they indeed found that even though the corresponding titration curves look similar, the two proteins' isoelectric points differ by about a fifth of a pH unit (Figure 8.2). The sign of this difference is just what would be expected for a substitution of valine for glutamic acid: The normal protein is consistently more negatively charged in the range of pH shown than the defective one because

- It has one more acidic (negative) residue, and
- That residue (glutamic acid) has $pK = 4.25$, so it is dissociated throughout the range of pH shown in the graph.

In other physical respects, the two molecules are alike; for example, Pauling and coauthors found that both had the same sedimentation and diffusion constants. Nevertheless, the difference in isoelectric point was enough to distinguish the two versions of the molecule. Most strikingly, Figure 8.2 shows that at pH 6.9, the charges of the normal and defective proteins have opposite signs, and so the two proteins migrate in opposite directions under an electric field. (You'll show in Problem 8.7 that this difference is indeed big enough to separate proteins.)

T₂ Section 8.3.4' on page 336 mentions some more advanced treatments of electrophoresis.