Partitioning and Diffusion of Macromolecules in Charged Gels

by

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Submitted to the Department of Chemical Engineering
in July 7, 1995 in partial fulfillment of
the requirements for the degree of
Doctor of Philosophy in Chemical Engineering

Abstract

The macromolecular partition and diffusion coefficients are equally important in describing diffusion through gel membranes. The goals of this thesis were to determine what effects electrostatic, hydrodynamic and steric interactions have on the partitioning and diffusion of macromolecules in agarose gels. To accomplish this measurements of the partitioning and diffusivity of proteins and Ficolls were made in charged and uncharged agarose gels and theoretical predictions were developed for the effects of electrostatic interactions on the partitioning of charged spherical macromolecules in random fiber arrays.

The effects of electrostatic interactions on the diffusion and equilibrium partitioning of fluorescein-labeled proteins in charged gels were examined using fluorescence recovery after photobleaching (FRAP) and gel chromatography, respectively. Measurements were made with bovine serum albumin (BSA), ovalbumin, and lactalbumin in SP-Sepharose (6% sulfated agarose), in phosphate buffers at pH 7 and ionic strengths ranging from 0.01 to 1.0 M. Diffusivities in individual gel beads (D) and in the adjacent bulk solution (D_∞) were determined from the spatial Fourier transform of the digitized two-dimensional fluorescence recovery images. Equilibrium partition coefficients (Φ) were measured by recirculating protein solutions through a gel chromatography column until equilibrium was reached, and using a mass balance. Diffusion in the gel beads was hindered noticeably, with $D/D_\infty = 0.4 - 0.5$ in each case. There were no effects of ionic strength on BSA diffusivities, but with the smaller proteins (ovalbumin and lactalbumin) D_∞ increased slightly and D decreased at the lowest ionic strength. In contrast to the modest changes in diffusivity, there were marked effects of ionic strength on the partition coefficients of these proteins. We conclude that for diffusion of globular proteins through gel membranes of like charge, electrostatic effects on the effective diffusivity ($D_{eff} = \Phi D$) are likely to result primarily from variations in Φ, with only small contributions from the intramembrane diffusivity.

A theory has been developed to predict the effects of electrostatic interactions on the equilibrium partition coefficient (Φ) of spherical macromolecules in gels, the gels being modulated as random arrays of fibers. The partitioning theory derived by Ogston (Trans. Faraday Soc. 54:1754-1757, 1958) for neutral macromolecules and fibers was extended by using a Boltzmann factor, containing an electrostatic free energy, to modify the probability of fitting a sphere in a space between fibers. This approach, which is limited to dilute solutions of macromolecules, is approximate in that the only electrostatic

interactions considered are those between the sphere and the nearest fiber. The electrostatic free energy was calculated from finite-element solutions to the linearized Poisson-Boltzmann equation for a sphere interacting with a long cylinder, both with specified surface charge densities. Free energies calculated for many combinations of sphere raqdius, fiber radius, separation distance, Debye length, and the surface charge densities of the sphere and fiber are presented as a correlation involving the various dimensionless parameters. When the sphere and fiber have like charges, Φ decreases with increases in the sphere size, the volume fraction of fibers, the Debye length, and either surface charge density; results are presented to illustrate each of these effects. Predictions from the theory are in good agreement with recent measurements of Φ for proteins in moderately charged gels.

To characterize the microstructure of the agarose gel a new technique was developed to measure the hydraulic permeability of reinforced gel membranes, allowing calculation of the Darcy permeability (κ) of the gel. The method was applied to agarose with concentrations ranging from 2.0 –7.3%. To create membranes which would be thin enough to yield easily measured filtration rates at modest applied pressures, yet be able to withstand handling, gels were cast on woven polyester meshes. The resulting membranes had thicknesses of 70-100 μm and a fractional open area of 0.32. To correct for the presence of the mesh, finite-element solutions were obtained for the pressure field in the three-dimensional region occupied by the gel. For the particular meshes employed here, the hydraulic permeability of the reinforced membrane was calculated to be 0.47 – 0.55 times that for a layer of pure gel, the exact value depending on the thickness of the composite membrane. The principal determinant of κ was the agarose concentration, but there was a secondary effect of applied pressure. The Darcy permeability extrapolated to zero applied pressure (κ_o) varied from 616 nm^2 for 2.0% agarose to 22 nm^2 for 7.3% agarose. At a given gel concentration, the value for κ_o was as much as twice the value for κ measured at the maximum pressure difference of 20 kPa. The method used should be adaptable to a variety of other gel materials.

The diffusivities of uncharged macromolecules in gels (D) are typically lower than in free solution (D_∞), due to a combination of hydrodynamic and steric factors. To examine these factors, we measured D and D_∞ for dilute solutions of several fluorescein-labeled macromolecules, using an image-based fluorescence recovery after photobleaching (FRAP) technique. Test macromolecules with Stokes-Einstein radii (r_s) of 2.1 – 6.2 nm, including three globular proteins (bovine serum albumin, ovalbumin, latalbumin) and four narrow fractions of Ficoll, were studied in agarose gels with agarose volume fractions (ϕ) of 0.038 – 0.073. The gels were characterized by measuring the hydraulic permeability of supported agarose membranes, allowing calculation of the Darcy permeability (κ) for each gel sample. The diffusivity ratio D/D_∞ , which varied from 0.20 to 0.63, decreased with increases in r_s or ϕ. Thus, as expected, diffusional hindrances were most severe for large macromolecules and/or relatively concentrated gels. According to a recently proposed theory for hindered diffusion through fibrous media, the diffusivity ratio is given by the product of a hydrodynamic factor (F) and a steric factor (S). The functional form is $D/D_\infty = F(r_s/\kappa^{1/2}\, S(f)$, where $f = [(r_s + r_f)/r_f]^2\phi$ and r_f is the fiber radius. Values of D/D_∞ calculated from this effective medium theory,

without use of adjustable parameters, were in much better agreement with the measured values than were predictions based on other approaches.

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