

1. (21 pts) CoCrMo alloys form a passivating oxide on their surface that renders them useful for orthopaedic implants. Such implants remain susceptible, however, to fretting corrosion, which occurs as a consequence of repetitive rubbing of the implant against a second hard surface (e.g., another implant component or bone). To study fretting corrosion, Contu et al. (*Corrosion Sci.* **2005**, *47*, 1863) employed a tribo-electrochemical cell, illustrated below, employing a CoCrMo alloy (66:28:6 wt ratio Co:Cr:Mo) as the working electrode, Pt wire as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode (SCE is +0.231V on the standard hydrogen electrode (SHE) scale.) A ceramic tube in contact with the sample was rotated periodically to create a rubbing action on the surface. Dissolved oxygen was removed from electrolyte solutions by bubbling with argon gas for 24 h prior to the experiments. The figure below shows data for the open circuit potential (OCP) measured before, during and after 3 rotations of the ceramic tube in buffered solutions at pH 4 (solid) and pH 7 (dashed).

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See Fig. 1 and Fig. 2(a) in Contu, F., B. Elsener, and H. Böhni. "Corrosion Behaviour of CoCrMo Implant Alloy During Fretting in Bovine Serum." *Corrosion Science* 47 (2005): 1863-1875.

a) (4 pts) Why does the OCP first drop rapidly then rise rapidly and plateau following each abrasion of the alloy surface by the ceramic tube?

Stresses on the oxide layer at the alloy surface induced by rubbing cause breaks in the oxide film that expose pure metal to the electrolyte solution, increasing the magnitude of the measured OCP. Immediately upon exposure, the bare metal begins to react with water to reform an oxide layer at the surface. With increasing time, the oxide growth becomes limited by ion diffusion through the oxide and the potential plateaus.

b) (6 pts) Write the expected anodic half-reaction(s) for this alloy at pH 4. Based on this reaction and the Nernst equation, provide an expression for the measured electrical potential across the rubbed alloy surface.

Expected anodic reactions include:



The Nernst equation for metal oxidation is

$$E = E^0 + \frac{RT}{nF} \ln \frac{[M^{n+}]}{[M]}$$

Noting that the SCE electrode is +0.231V from the standard hydrogen electrode (SHE) scale, the standard electrochemical potentials ΔE^0 for Co and Cr can be obtained from Table 5 on pg. 432. The atomic fraction of Co and Cr are 65 and 31%, calculated from the weight fractions provided.

The resulting expressions for the measured potentials from these reactions would be:

$$E(V) = -0.511 + \frac{0.0591}{2} \log \frac{[\text{Co}^{2+}]}{[\text{Co}]} = -0.506 + 0.0295 \log [\text{Co}^{2+}]$$

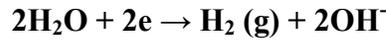
$$E(V) = -0.961 + \frac{0.0591}{2} \log \frac{[\text{Cr}^{2+}]}{[\text{Cr}]} = -0.946 + 0.0295 \log [\text{Cr}^{2+}]$$

c) (4 pts) What is the expected cathodic half reaction at pH 4 and pH 7 for this experiment?

Since dissolved oxygen has been removed from the electrolyte solution, the cathodic half-reactions will involve other species:

For pH 4: $2\text{H}^+ + 2\text{e} \rightarrow \text{H}_2 (\text{g})$

For pH 7 and higher one might alternately observe the reaction:



d) (2 pts) Why is the OCP measured at pH 4 higher than the value measured at pH 7?

The anodic half-reaction can only take place when there is a corresponding cathodic reaction that consumes the electrons created. The associated potential for the cathodic reaction will depend upon pH. For example, the Nernst equation for the hydrogen evolution reaction is:

$$\Delta E = \Delta E_{\text{H}_2}^0 + 0.0591 \log a_{\text{H}^+}$$

where $\Delta E_{\text{H}_2}^0$ would be 0 V for the SHE. Since pH is defined as:

$\text{pH} = -\log a_{\text{H}^+}$, the measured potential will drop as pH increases, because the concentration (activity) of H^+ ions is lower.

e) (2 pts) Why does the open-circuit potential after rubbing plateau to a different value than the original measured OCP for this alloy?

The difference in equilibrium potential reflects a change in the structure or chemistry of the oxide layer formed after rubbing.

f) (3 pts) The figure below provides data from cathodic polarization (increase in the cathodic reaction relative to anodic reaction) experiments at pH 4 for the CoCrMo alloy in electrolyte solution (phosphate buffer) vs. serum. Shown is the measured current density as a function of the potential. Provide an explanation for the reduced current measured in serum compared to buffer. Would this be a favorable or unfavorable effect *in vivo*?

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See Fig. 4(a) in Contu, F., B. Elsener, and H. Böhni. "Corrosion Behaviour of CoCrMo Implant Alloy During Fretting in Bovine Serum." *Corrosion Science* 47 (2005): 1863-1875.

Protein molecules in serum would be expected to adsorb on the metal surface, impeding the kinetics of the cathodic reaction. This would be a favorable effect in vivo as the reduced current means a reduced corrosion rate of the alloy.

2. (19 pts) Catalase is an enzyme (mol. wt.: 247kDa, diameter: 10.5 nm) found in liver cells that catalyses the conversion of hydrogen peroxide to water and oxygen. Its catalytic activity is of interest for biosensor applications. As an approach for fractionating catalase from biological solutions, Jia and coworkers (*Int. J. of Biol. Macromol.* **2005**, 37, 42) prepared chitosan microspheres coated with a covalently attached dye, Cibacron Blue F3GA, which exhibits strong binding to catalase. The figure below shows an SEM image of the chitosan particles. The wet density of the spheres was found to be 1.328 g/mL. Adsorption studies were performed to determine the amount of catalase adsorbed to the particles as a function of solution concentration. An adsorption isotherm for pH 7 is shown below.

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See Fig. 1 and 4 in Shentu, Jingling, Jianmin Wu, Weihua Song, and Zhishen Jia. "Chitosan Microspheres as Immobilized Dye Affinity Support for Catalase Adsorption." *International Journal of Biological Macromolecules* 37 (2005): 42-46.

a) (5pts) Assuming a Langmuir model, determine the affinity constant K_a and the maximum surface coverage Γ_{\max} for CAT adsorption on the dye-linked chitosan beads.

[CAT] (mg/mL)	1/[CAT] (mL/mg)	Γ (mg/g)	1/ Γ (g/mg)
0.03	33.	2.5	0.4
0.13	7.7	7	0.14
0.53	1.9	17.5	0.057
1.13	0.89	23.5	0.043
1.80	0.56	24	0.042
2.50	0.40	24.5	0.041

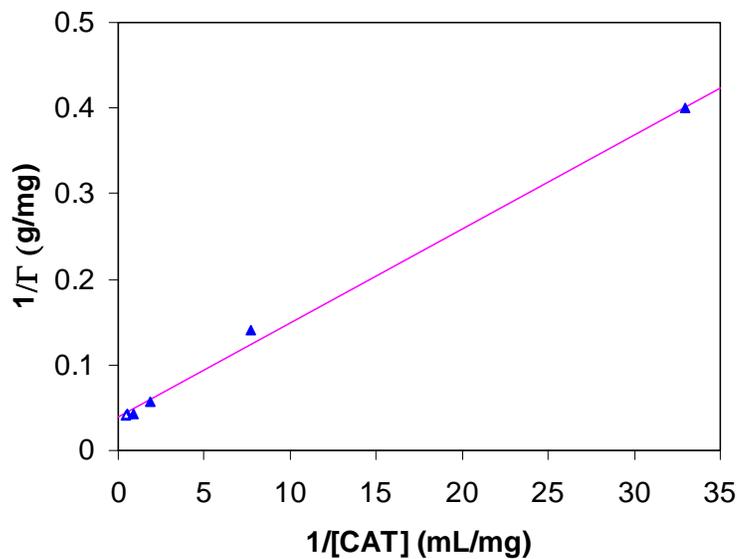
Noting that $v = \Gamma/\Gamma_{\max}$, the Langmuir equation can be expressed as:

$$\frac{1}{\Gamma} = \frac{1}{[P]K_a\Gamma_{\max}} + \frac{1}{\Gamma_{\max}}$$

Performing linear regression on the data gives a best-fit line of:

$$1/\Gamma = 0.011/[CAT] + 0.039$$

from which we obtain $\Gamma_{\max} = 25.6 \text{ mg/g}$ and $K_a = 3.55 \text{ mL/mg}$



b) (6 pts) From the obtained value for Γ_{\max} , calculate the effective area per protein, stating any assumptions you make.

The effective area per protein can be computed from:

$$A_{\text{eff}} = \frac{M_{\text{protein}}}{N_{\text{Av}}\Gamma_{\max}}$$

where Γ_{\max} is given in units of mass/area. Currently we have this value in mg/g, however. We must therefore make an estimate of the surface area per gram of chitosan beads and we know the wet density of the beads is 1.328 g/cm^3 . We will make the assumption that the area available from protein adsorption is just the surface area of the spherical beads.

$$\frac{A_{\text{bead}}}{V_{\text{bead}} \rho_{\text{bead}}} = \frac{4\pi r^2}{\frac{4}{3}\pi r^3 (1.328 \text{ g/cm}^3)} = 7.5 \times 10^{16} \text{ nm}^2 / \text{g}$$

$$\Gamma_{\max} = \frac{25.6 \text{ mg/g}}{7.5 \times 10^{16} \text{ nm}^2 / \text{g}} = 3.41 \times 10^{-19} \text{ g/nm}^2$$

$$A_{\text{eff}} = \frac{M_{\text{protein}}}{N_{\text{Av}} \Gamma_{\max}} = \frac{2.47 \times 10^5 \text{ g/mol}}{6.02 \times 10^{23} \text{ molec/mol} (3.41 \times 10^{-19} \text{ g/nm}^2)}$$

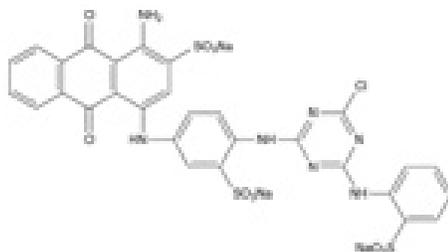
$$A_{\text{eff}} = 1.2 \text{ nm}^2 / \text{molec}$$

c) (4 pts) How does your value compare to the expected molecular area for catalase, given the protein dimensions? Explain the possible origin of discrepancies.

Catalase is relatively large with a diameter of ~10.5 nm, giving an expected surface area of $\pi r^2 = 87 \text{ nm}^2$. This is much larger than the computed area per molecule from the extracted value for Γ_{\max} . The discrepancy between these values indicates that there is much more surface area per particle than just the outer surface of the chitosan sphere. This could occur if the particles were porous, as the higher magnification SEM image suggests.

d) (2 pts) At pH 7, both CAT and the dye Cibacron Blue F3GA have a net negative charge. Explain why CAT adsorption onto the chitosan particles still occurs.

Though the net charge on CAT is negative, one would still expect positive charged groups on the protein outer surface that exhibit attractive electrostatic interactions with the dye. The structure of Cibacron Blue F3GA (below) also contains amine groups that could be positively charged, interacting with negatively charged moieties on the protein surface.



e) (2 pts) The data below shows the amount of adsorbed CAT as a function of ionic strength of the solution (increasing NaCl content). Explain why a decrease in adsorption is observed with increasing salt content in solution.

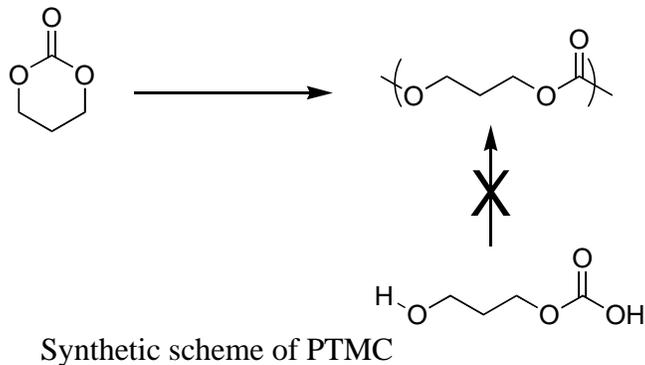
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See Fig. 3 in Shentu, Jingling, Jianmin Wu, Weihua Song, and Zhishen Jia. "Chitosan Microspheres as Immobilized Dye Affinity Support for Catalase Adsorption." *International Journal of Biological Macromolecules* 37 (2005): 42-46.

This data suggests that the adsorption of CAT to Cibacron Blue F3GA is predominantly electrostatic in nature. As the ionic strength increases, charge shielding leads to decreased adsorption.

3) (10 pts) Aliphatic polycarbonates have been gaining much attention as degradable polymers, due to biocompatibility or bioresorbability, while aromatic polycarbonates are hardly hydrolysable. Feijin and coworkers have reported *in vitro* and *in vivo* degradation of poly(trimethylene carbonate) (PTMC), an aliphatic polycarbonate with a glass transition temperature of $T_g = -15^\circ\text{C}$, and found interesting aspects of its degradation (*Biomaterials*, 2006, 27, 1741).

a) (3 pts) PTMC is synthesized by ring-opening polymerization of 1,3-dioxan-2-one rather than by polycondensation of carbonic acid mono-(3-hydroxy-propyl)ester as shown in the scheme below. Why?



With polycondensation of the linear monomer, a by-product H_2O has to be removed at high temperature and under vacuum conditions, and during the polymerization, the entropy of the system decreases. By comparison, ring-opening of the cyclic monomer does not produce by-products and the reaction proceeds under mild conditions. Due to ring-strain, ring-opening is enthalpically favorable and increases available conformations (and therefore entropy). The ΔG value of most ring-opening polymerizations is negative.

b) (2 pts) Various PTMCs were implanted in the femur and tibia of rabbits. Mass losses and molecular weight (MW) changes were monitored as a function of time. Here, x of PTMC_x stands for MW (kDa) of TMC. As can be seen in Figs 1 and 2, in the initial degradation stage, the mass of the polymeric materials decreased with time; however, MW values did not change significantly. Explain what happened.

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See Fig. 1 and 2 in Zhang, Zheng, Roel Kuijer, Sjoerd K. Bulstra, Dirk W. Grijpma, and Jan Feijen. "The In vivo and In vitro Degradation Behavior of Poly(trimethylene carbonate)." *Biomaterials* 27 (2006): 1741-1748.

The *in vivo* degradation of PTMC proceeded by enzymatic hydrolysis. In the initial degradation stage, surface erosion took place, and the polymer was hydrolyzed from the surface by enzyme(s), while the bulk polymer stayed intact. Thus, molecular weights did not change much during that period.

c) (2 pts) Table 2 shows water contact angles of PTMC surfaces. The results indicate that low MW PTMC became more hydrophilic than high MW PMTC after conditioning in water for 1 week at 37°C, while both polymer surfaces presented similar wetting properties before conditioning. What is a likely explanation for these results?

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See Table 2 in Zhang, Zheng, Roel Kuijer, Sjoerd K. Bulstra, Dirk W. Grijpma, and Jan Feijen. "The In vivo and In vitro Degradation Behavior of Poly(trimethylene carbonate)." *Biomaterials* 27 (2006): 1741-1748.

During conditioning, rearrangement of polymer chains occurred readily, and more hydrophilic segments could face toward the water phase. Especially, hydrophilic chain ends preferred to come out from the bulk. As a result, polymer surfaces became more hydrophilic. In comparison to high MW polymer surface, low MW surface had more polymer chain ends, resulting in a more water wettable surface.

d) (3 pts) Mass loss of PTMCs in lipase solution is shown in Figure 3. Mass loss rate of high MW PMTC was larger than that of low MW PMTC. These *in vitro* results were compared with the *in vivo* results (Fig. 1 above). In general, hydrolysis of high MW polymers is slower than for low MW polymers because high MW polymer surfaces are more hydrophobic, making H⁺ and/or OH⁻ ion less accessible to the surface. In this study, high MW PTMC presented a more hydrophobic surface after conditioning, as shown in Table 2. What is a plausible reason for the increased degradation of high MW PMTC in the presence of lipase?

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See Fig. 3 in Zhang, Zheng, Roel Kuijer, Sjoerd K. Bulstra, Dirk W. Grijpma, and Jan Feijen. "The In vivo and In vitro Degradation Behavior of Poly(trimethylene carbonate)." *Biomaterials* 27 (2006): 1741-1748.

In the *in vitro* and *in vivo* experiments, enzyme(s) played an essential role in PTMC degradation. Lipases have hydrophobic domain(s) and will adsorb to a hydrophobic surface. In the case of low MW PTMC, the more hydrophilic surface suppressed the enzyme attack, which resulted in a slower degradation.