Lecture 10: Bioengineering applications of hydrogels: Molecular Imprinting and Drug Delivery

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Last Day: polyelectrolyte gels
Polyelectrolyte complexes and multilayers
Applications in bioengineering
Theory of ionic gel swelling

Today: Molecular imprinting
Hydrogels in drug delivery

Supplementary Reading: S.R. Lustig and N.A. Peppas, 'Solute diffusion in swollen membranes. IX. Scaling laws for solute diffusion in gels,' *J. Appl. Polym. Sci*. **36**, 735-747 (1988)
T. Canal and N.A. Peppas, 'Correlation between mesh size and equilibrium degree of swelling of polymeric networks,' *J. Biomed. Mater. Res*. **23**, 1183-1193 (1989)

Molecular Imprinting[1,2]

Concepts of molecular imprinting

- Molecular imprinting is the design of polymer networks that can recognize a given target molecule and bind it preferentially in the presence of an excess of irrelevant molecules, some of which may have very similar molecular structures
    - Seeks to mimic specificity in biological recognition obtained through protein-protein interactions
- Steps to the preparation of molecularly-imprinted networks:
    1. mixing of binding monomers and target molecule
        - target can be mixed directly with liquid monomers in bulk or co-dissolved in a non-interfering solvent
        - monomers bind target
            - covalent interactions
            - non-covalent bonding
            - metal coordination
        - mixture usually at high concentration (e.g. 50% w/vol solutions): enforces close interactions of target with binding monomers and leads to a tight network that holds the position of functional groups in position of template binding
    2. polymerization of monomers in place
        - usually photopolymerization (rapidly 'trap' structure)
    3. washing for removal of target molecule from network pockets

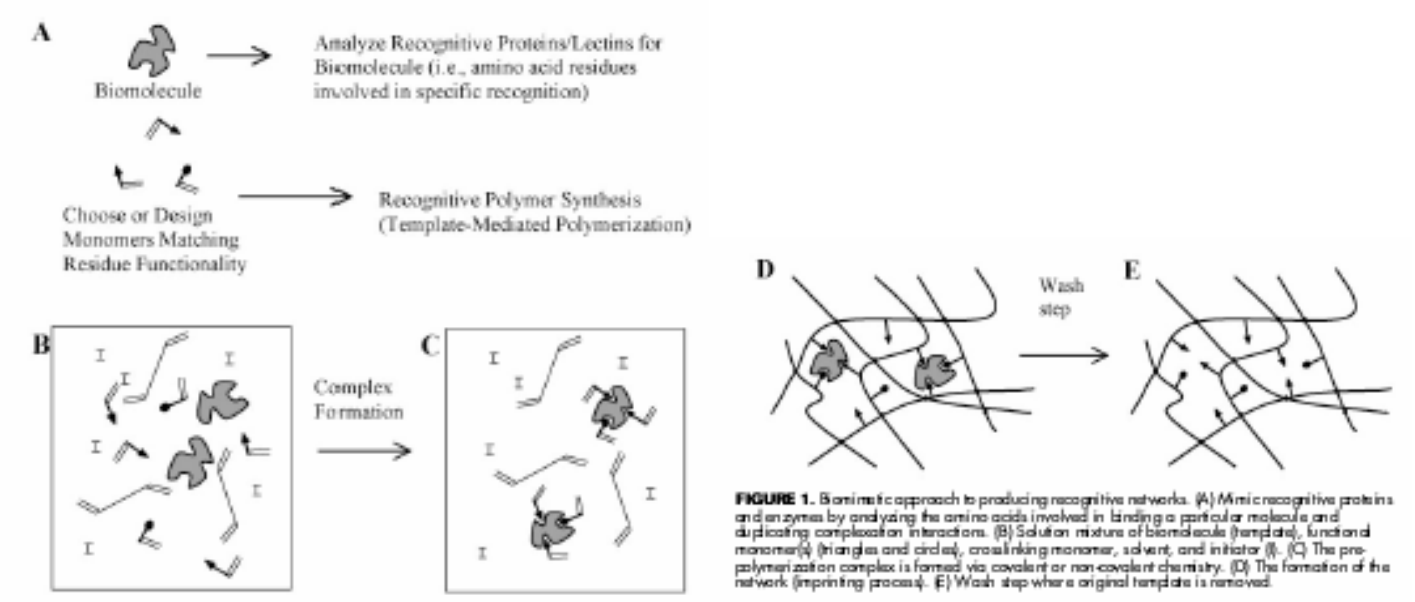


FIGURE 1. Biomimetic approach to producing recognitive networks. (A) Mimic recognitive proteins and enzymes by analyzing the amino acids involved in binding a particular molecule and duplicating complexation interactions. (B) Solution mixture of biomolecule (template), functional monomer(s) (triangles and circles), crosslinking monomer, solvent, and initiator (I). (C) The pre-polymerization complex is formed via covalent or non-covalent chemistry. (D) The formation of the network (imprinting process). (E) Wash step where original template is removed.

- types of target molecules:[1]
  - small-molecule drugs
  - steroids
  - nucleic acids
  - amino acids
  - metal ions
  - proteins

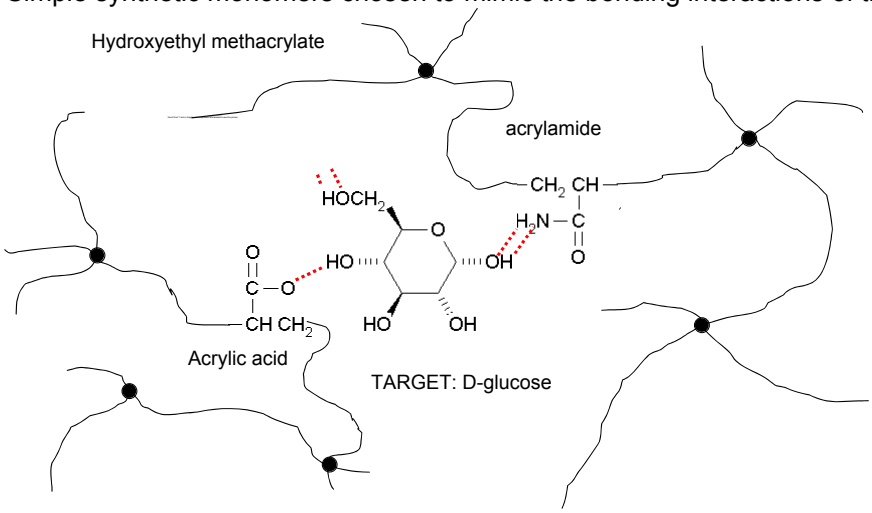
Structure of Molecularly-Imprinted Networks

- structure of molecularly-imprinted networks
  - imprinted networks can be confined to a thin surface layer or prepared in bulk
    - surface networks usually perform better for capture of large molecules like proteins

- simple synthetic components for recognition networks
  - monomers:
    - methacrylic acid
    - itaconic acid
    - acrylamides
    - 4-vinyl pyrrolidone
    - β-cyclodextrin
    - other designed monomers
  - cross-linkers
    - ethylene glycol dimethacrylate
    - PEG dimethacrylate
  - 'chain effect'[3]
    - binding of monomers to macromolecular templates causes a reduction in chain termination and thus an overall increase in reaction rate

- Example of molecular recognition: molecular imprinting of D-glucose (Peppas)
  - Monomers chosen as analogs of the amino acid residues that bind to glucose in vivo:
    - WHAT RECEPTORS BIND GLUCOSE?
      - Aspartate

- Glutamate
- Asparagines
- Serine
  - Draw structures on board
- Simple synthetic monomers chosen to mimic the bonding interactions of these amino acids:



Specificity of binding:

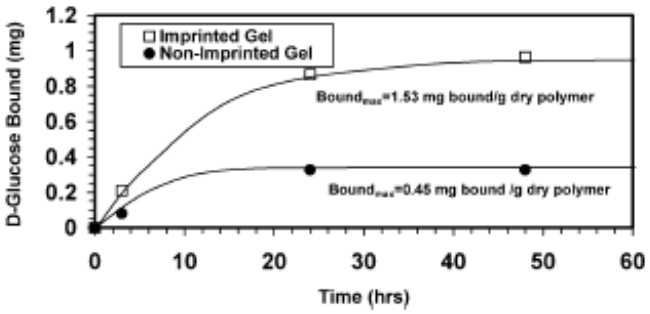


FIGURE 6. Kinetic D-glucose binding study in water. Acrylamide-PEG200DMA copolymers of 67% crosslinking ratio prepared in DMSO (*T* = 24°C).

Issues:

Tightly cross-linked networks hold functional group positions for better recognition but restrict entry of target into network

Limited complexity in recognition units

TABLE 1. Competitive D-Glucose Substrate Binding in Water: Acrylamide-PEG200DMA Copolymers with 67% Crosslinking Ratio (Mole/Mole Monomers). Polymer Prepared in DMSO

Competitive substrate	*Imprinted intensity*	*Non-imprinted intensity*
Fluorescent analogue only (concentration = F_g)	223 ± 11.81	50.97 ± 0.77
Fluorescent analogue and glucose (100 × F_g)	99.44 ± 4.36	80.77 ± 3.95
Fluorescent analogue and glucose (3000 × F_g)	49.81 ± 1.09	48.85 ± 0.88
Fluorescent analogue and galactose (100 × F_g)	220 ± 10.10	n.a.
Fluorescent analogue and galactose (3000 × F_g)	209.06 ± 10.46	n.a.
Fluorescent analogue only (PEG only network)	n.a.	56.57 ± 0.90

- Improving recognition by surface templating (Ratner[4,5])
  - Protein adsorbed to mica surface, coated with disaccharide, then coated with $\mathbf{C_3F_6}$ film by radiofrequency glow-discharge plasma treatment
  - Sugar coating protects protein from denaturation on dehydration

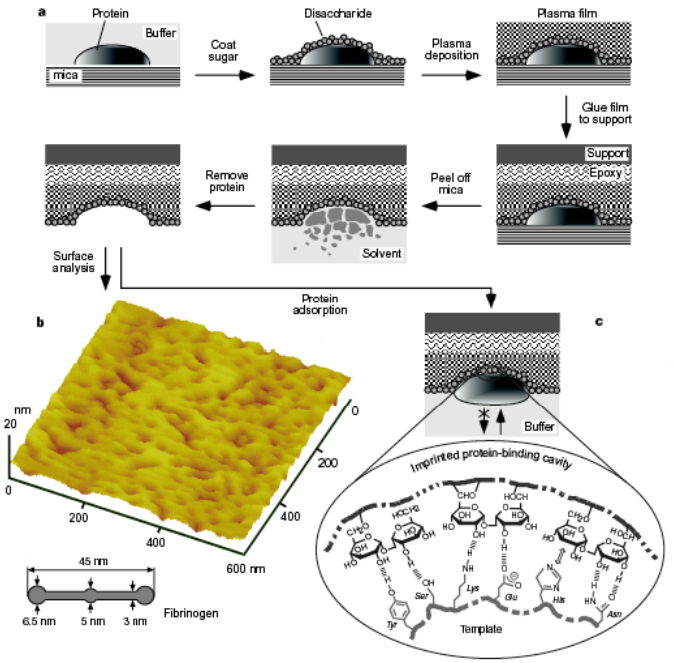
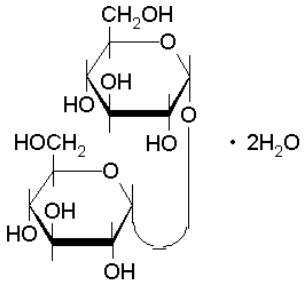
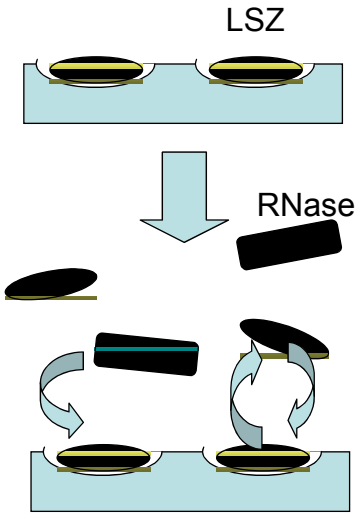


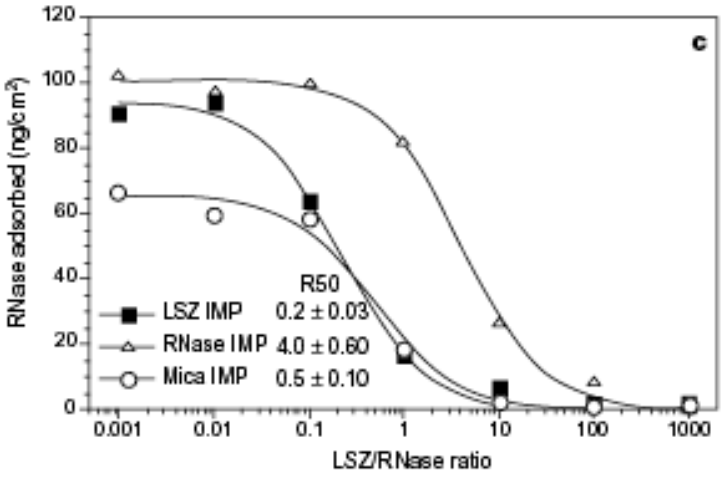
Figure 1 Protocol for template imprinting of proteins. (See refs 16 and 29 for details.) **a**, Template protein was adsorbed onto a freshly cleaved mica in citrated phosphate-buffered saline (CPBS), pH 7.4. A 1–10 mM solution of disaccharide was spin-cast to form a 10–50 Å sugar overlayer. The sample was put into the in-glow region of a 13.56 MHz RFGD reactor. Plasma deposition of C_6F_{11} was conducted at 150 mtorr and 20 W for 3–6 min, forming a 10–30 nm fluoropolymer thin film. The resulting plasma film was fixed to a glass coverslip using epoxy resin and oven-cured. Mica was peeled off and the sample was soaked in a NaOH/NaClO (0.5/1.0%) solution for 0.5–2 h for dissolution and extraction of protein. A nanopit with a shape complementary to the protein was created on the imprint surface. **b**, A tapping mode AFM image of the surface of a fibrinogen imprint, together with a drawing of fibrinogen. **c**, Mechanisms for the specific protein recognition of template-imprinted surfaces. A nanocavity-bound template protein is prevented from exchange with other protein molecules in the solution because of steric hindrance and an overall strong interaction; the latter is due to many cooperative weak interactions, involving hydrogen bonds, van der Waals forces and hydrophobic interactions for example.



Trehalose (disaccharide)

- Resulting recognition:





LSZ in solution can exchange with imprinted LSZ, but Rnase cannot displace LSZ on surface

LSZ = lysozyme

- Utilizing in-situ formability of photopolymerized hydrogels for lab-on-a-chip applications
  - Photopolymerized Bulk templates (Peppas):

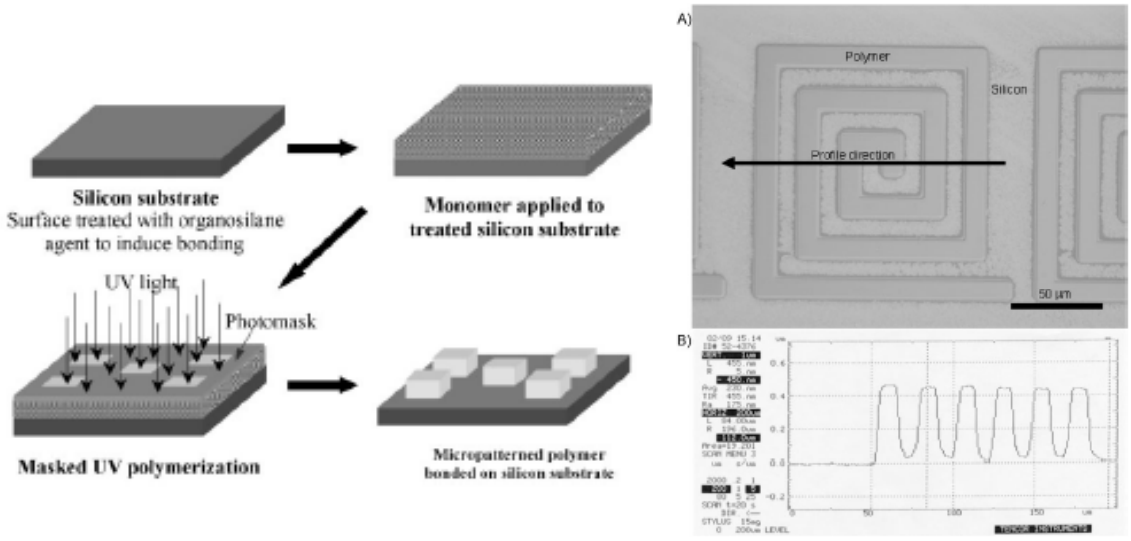


FIGURE 12. Micropatterning on silicon. (A) Environmentally sensitive hydrogel patterned in a spiral shape onto a silicon substrate. Image captured with an optical microscope in Nomarski mode. (B) Profilometry data for the path shown in (A). Height of pattern is shown to be 450 nm.

- Plasma-deposited surface templates patterned by microcontact printing (Ratner):

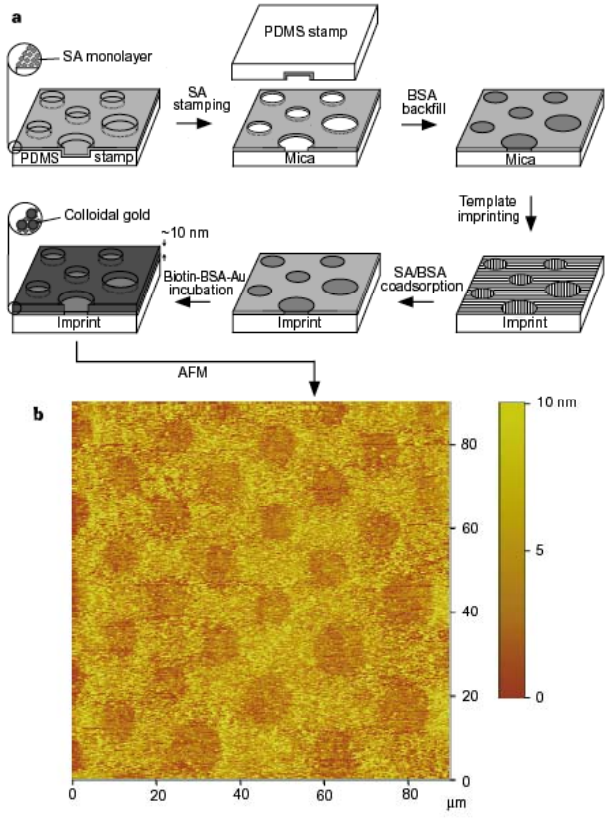


Figure 4 Visual demonstration of template recognition by imprints of protein mixtures patterned with microcontact printing[29,30]. **a**, Streptavidin (SA) in PBS buffer (pH 7.4) was adsorbed onto a poly(dimethylsiloxane) (PDMS) stamp that has micrometre-scale circular indentations, followed by a buffer and water rinse and drying under a stream of nitrogen. The stamp was put into contact with a mica surface for ~5 s, transferring a monolayer of streptavidin to mica only in the contacted regions. The mica surface was then exposed to albumin (BSA) in PBS.

This adsorption blocks the uncovered surface area. A template imprint of this SA/BSA patterned surface was prepared as shown in Fig. 1. Binary protein adsorption onto the imprint was performed from a SA/BSA (1/1) solution in PBS for 4 h. The imprint was incubated 1 h with a solution of biotin–BSA labelled with 10-nm colloidal gold to reveal the surface distribution of adsorbed streptavidin with AFM (**b**).

Hydrogels in drug delivery

- What

Control of drug release kinetics by hydrogel structure[6,7]

- Release from stable hydrogels is controlled by diffusion of solute through the network
- Diffusion is described by Fick's second law:

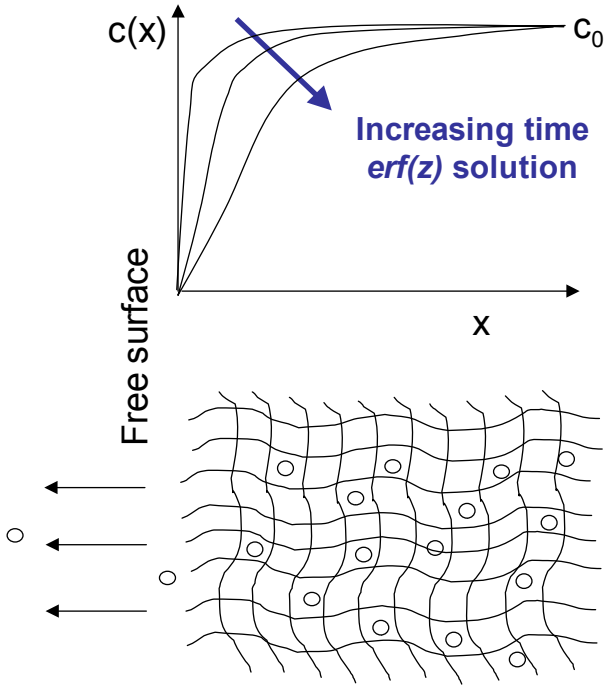
Eqn 1

$$\frac{\partial C}{\partial t} = D_{gel}\frac{\partial^2 C}{\partial x^2}$$

- Recall the solution to Fick's second law for a semi-infinite slab contacting a perfect sink:

Eqn 2

$$\frac{c_0 - c(x)}{c_0} = 1 - erf\left(\frac{x}{2\sqrt{tD}}\right)$$



- Diffusion of drugs through a network is controlled by the mesh size (ζ)

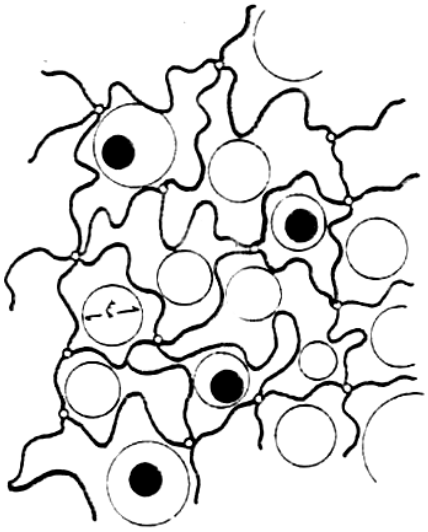
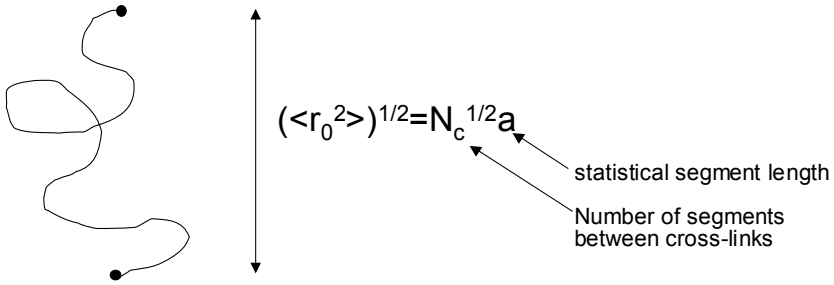


Fig. 3. Crosslinked structure of a polymer gel, showing effective chains of the structure defined by crosslinks. The effective area for diffusion for the solute is characterized by an average mesh size ξ. The smaller solutes, illustrated as dark circles, must pass between the macromolecules.

- The mesh size is related to the network swelling Q and the end-to-end distance between cross-links:



Eqn 3 $$\left(\bar{r}_0^2\right)^{1/2} = \left(\frac{2M_c}{M_0}\right)^{1/2} C_n^{1/2} l$$

- …assuming a polymer chain that has 2 carbon-carbon bonds per repeat unit
- derived from random walk chain statistics
  - Where *l* is the bond length in the polymer backbone
  - M_c is the molecular weight between cross-links
  - M_0 is the molecular weight per repeat unit
  - Where C_n is the characteristic ratio for the polymer chain

Eqn 4 $$\xi = \frac{\left(\bar{r}_0^2\right)^{1/2}}{\phi_{2,s}^{1/3}} = Q^{1/3}\left(\bar{r}_0^2\right)^{1/2} = C_n^{1/2} Q^{1/3} N^{1/2} l$$

- *Q* is the degree of swelling = $V_{\text{dry polymer}}/V_{\text{swollen polymer}}$
- *N* is the degree of polymerization between cross-links

- The mesh size is related to the diffusion constant of a solute in the network
- Eyring theory of diffusion:

Eqn 5 $$D = T\nu e^{-\frac{\Delta G^*}{kT}} = T\nu e^{-\frac{\Delta H^*}{kT}} e^{\frac{\Delta S^*}{k}}$$

- Where ΔG^* is the activation energy, ΔH^* is activation enthalpy, and ΔS^* is activation entropy
- N = translational oscillating frequency of solute molecule (jump rate!)
- T = temperature
- k = Boltzman constant

- The ratio of diffusion constant in the gel to that in solution is:

Eqn 6
$$\hat{D} = \frac{D_{gel}}{D_0} = \frac{e^{\frac{\Delta S^*_{gel}}{k}}}{e^{\frac{\Delta S^*_0}{k}}}$$

- Where ΔS^*_{gel} is the activation entropy for diffusion in the gel and ΔS^*_0 is the activation entropy in for diffusion in the solvent
- This assumes the activation enthalpy and oscillation frequencies for diffusion are approximately the same in the gel and pure solvent (reasonable for dilute and chemically inert systems)

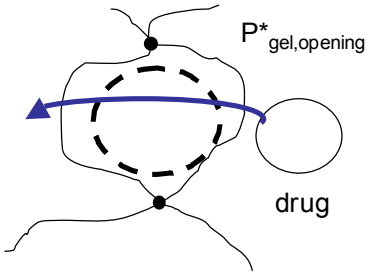
- The activation entropies are:

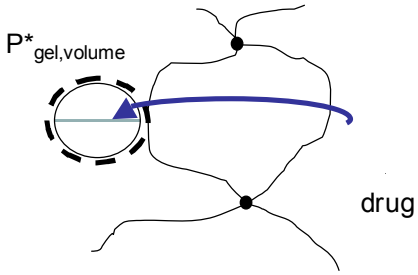
Eqn 7 $\Delta S^*_{gel} = k \ln P^* - k \ln P_0$

Eqn 8 $\Delta S^*_0 = k \ln P^*_0 - k \ln P_0$

Eqn 9
$$\hat{D} = \frac{P^*_{gel}}{P^*_0} = \frac{P^*_{gel,opening} P^*_{gel,volume}}{P^*_{0,volume}}$$

- Where P*volume is the probability that a solute-sized volume of free space exists to jump into
- P*opening is the probability that the network has a solute-sized gap to jump through





Eqn 10
$$P^*_{gel,opening} = \frac{\xi - r}{\xi} = 1 - \frac{r}{\xi}$$

- Where r is the radius of the solute (drug) and ξ is the network mesh size

- The probability of a volume to jump into is an exponential of the ratio of the solute size to the available free volume per mole:

Eqn 11
$$P^*_{gel,volume} \sim e^{-\frac{v^*}{v_{free,gel}}}$$

Eqn 12
$$P^*_{0,volume} \sim e^{-\frac{v^*}{v_{free,1}}}$$

- Where vfree is the specific free volume and v* is the volume of the solute (drug)
- Refs for free volume theory applied here:

- Yasuda et al. Makromol. Chem. 26, 177 (1969)
- Peppas and Reinhart, J. Membrane Sci. 15, 275 (1983)

- Now:

Eqn 13
$$\frac{P^*_{gel,volume}}{P^*_{0,volume}} = e^{-\left(\frac{v^*}{v_{free,gel}} - \frac{v^*}{v_{free,1}}\right)}$$

- The free volume in a swollen gel is approximately vfree,1 since the free volume contribution from polymer is extremely low (2.5% even in solid polymers at 25°C)

Eqn 14 $v_{free,gel} = \phi_1 v_{free,1} + \phi_2 v_{free,2}$

- Therefore:

Eqn 15 $v_{free,gel} \sim \phi_1 v_{free,1} = (1-\phi_2) v_{free,1} = (1-1/Q) v_{free,1}$

  - Where Q is the swelling degree = $V_{swollen\ gel}/V_{dry\ gel} = 1/\phi_2$

- Therefore:

Eqn 16
$$\frac{P^*_{gel,volume}}{P^*_{0,volume}} = e^{-\left(\frac{v^*}{(1-\frac{1}{Q})v_{free,1}} - \frac{v^*}{v_{free,1}}\right)} = e^{-\frac{v^*}{v_{free,1}}\left(\frac{1}{Q-1}\right)} \approx e^{-\left(\frac{1}{Q-1}\right)}$$

  - $v^*/v_{free,1} \sim 1$ for most polymers, experimentally

- Therefore:

Eqn 17
$$\hat{D} \cong \left(1 - \frac{r}{\xi}\right) e^{\left[\frac{-1}{(Q-1)}\right]}$$

- And thus finally:

Eqn 18
$$D_{gel} \cong D_0 \left(1 - \frac{r}{\xi}\right) e^{\left[\frac{-1}{(Q-1)}\right]}$$

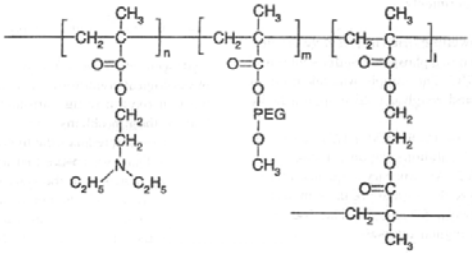
  - Insulin: MW – 5900 g/mole; hydrodynamic radius = 16 Å

Design of glucose-responsive drug delivery microgels for treatment of diabetes[8-10]

- Work by Podual and Peppas
- Immobilized glucose oxidase enzyme within pH-responsive polyelectrolyte gel network along with encapsulated insulin

  - Network composed of DEAEM, PEGMA, and TEGDMA
  - GOD covalently tethered to network
  - Insulin entrapped in network
  - Polymerized gels as microspheres

- Synthesis of microgels?



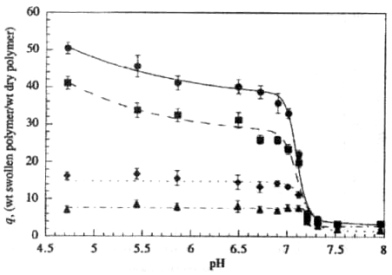
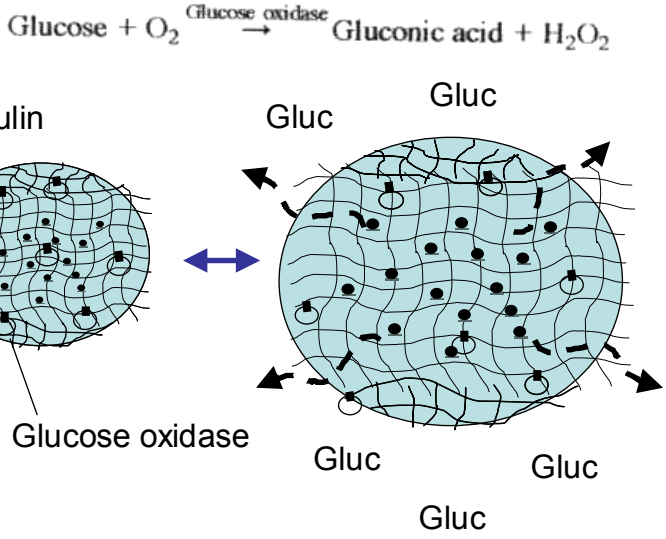


Fig. 3. Effect of crosslinking ratio on equilibrium weight swelling ratio of P(DEAEM-g-EG) hydrogels immobilized with GOD and catalase at 37°C. Hydrogels were prepared with 50:50 molar ratio of PEG molecular weight 200), 3.3×10^{-4} g GOD/g of polymer. The graphs shown are for $X = 0.005$ (●), 0.01 (■), 0.02 (◆) and 0.04 (▲).



- Fast variation in swelling due to microgel dimensions
- Mesh size responds in a similar manner, using theory described above:

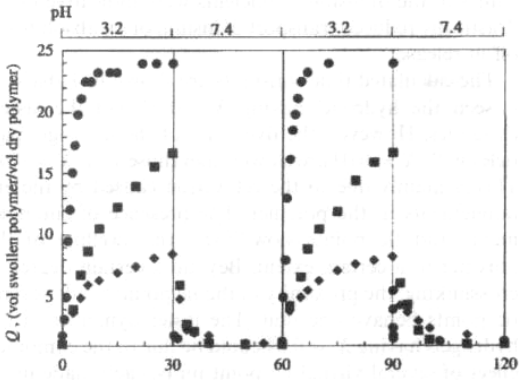


Fig. 8. Pulsatile swelling response of P(DEAEM-g-EG) microspheres with particle size = 160 μm, $E = 3.3 \times 10^{-4}$ g/g of polymer and $M_{PEG} = 200$ to alternate pH changes between pH 7.4 and 3.2 shown at the top of the plot. The dynamic response was the fastest for the microparticles with $X = 0.02$ (●), followed by $X = 0.03$ (■). The particles of crosslink density $X = 0.04$ (◆) were the slowest in responding to the changes in pH.

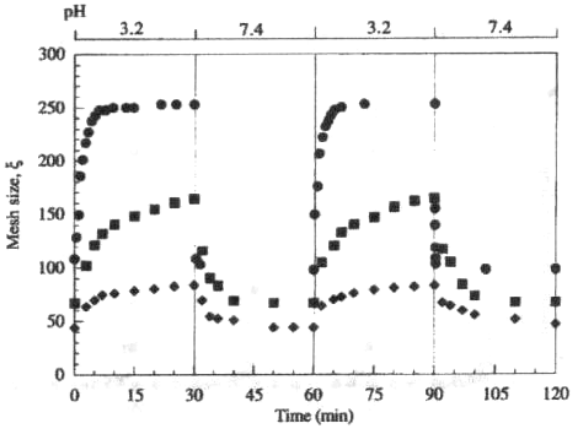


Fig. 9. Change in the mesh size of P(DEAEM-g-EG) microspheres of particle size = 160 μm, $E = 3.3 \times 10^{-4}$ g/g polymer, and $M_{PEG} = 200$ to alternate pH changes between pH 7.4 and 3.2. The mesh sizes are shown for hydrogels with $X = 0.02$ (●), $X = 0.03$ (■) and $X = 0.04$ (◆).

- The gels thus designed respond to concentrations of glucose in the surrounding medium, dynamically:

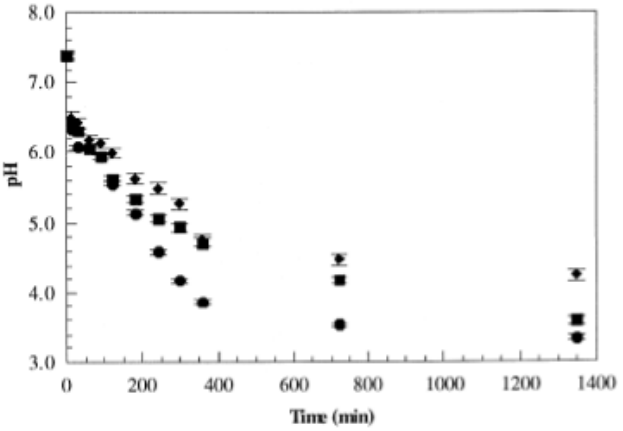


Fig. 3. Change in the pH of glucose solutions due to the oxidation of glucose to gluconic acid by GOD immobilized in P(DEAEM-g-EG) gels. The reduction in pH is shown for 200 (●), 100 (■) and 50 mg/dl (♦) of glucose.

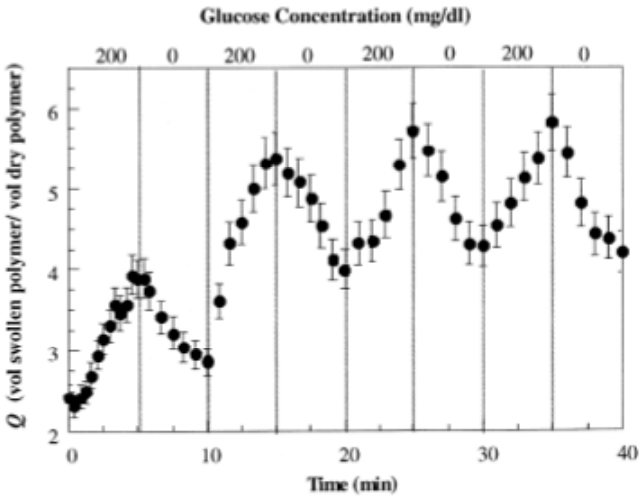


Fig. 6. Variation of the volume swelling ratio of 160-μm particles with time due to a pulsatile concentration of glucose in the swelling medium. These particles have a nominal crosslinking ratio, $X = 0.02$, and enzyme loading, $E = 3.3 \times 10^{-4}$ g/g of polymer. The concentrations of glucose used in this study are shown on top of the plot.

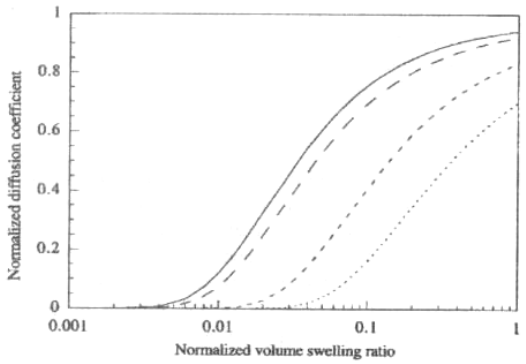


Fig. 5. Normalized insulin diffusion coefficient through P(DEAEM-*g*-EG) at 37°C as a function of the volume swelling ratio normalized with respect to the maximum volume swelling ratio. The diffusional behavior is for gels with initial crosslinking ratio of $X = 0.005$ (—), $X = 0.01$ (– –), $X = 0.02$ (– – –) and $X = 0.04$ (· · ·).

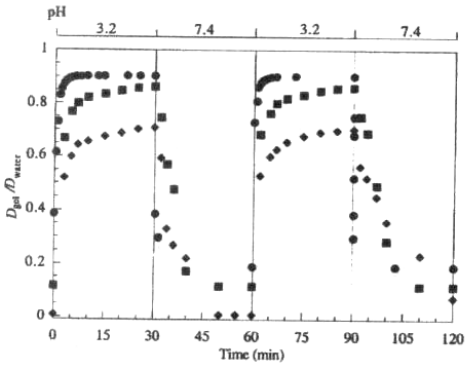


Fig. 11. Variation of the diffusion coefficient of insulin in P(DEAEM-g-EG) microspheres of particle size = 160 μm, $E = 3.3 \times 10^{-4}$ g/g polymer, and $M_{PEG} = 200$ to alternate pH changes between pH 7.4 and 3.2. The diffusion coefficient is normalized against the diffusion coefficient of insulin in water which was calculated to be 1.41×10^{6} cm^2/s. The data are shown for hydrogels with $X = 0.02$ (●), $X = 0.03$ (■) and $X = 0.04$ (♦).

References

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