

# Stabilization and Release Effects of Pluronic F127 in Protein Drug Delivery

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This paper explores the biochemistry of the surfactant hydrogel Pluronic F127 in its capacity as a protein drug delivery mechanism. Computer modeling techniques were used to simulate the behavior of the surfactant in a hydrous environment and to predict its interaction with the test enzyme, urease. Folding behavior was observed in the surfactant model. The theoretical calculations were tested using fluorescent probing and derivitization techniques on the surfactant and test protein. Results indicated aggregate disruption capabilities for the surfactant, and also confirmed hydrophobic residues as the likely interaction site for surfactant-protein binding. The hydrogel's ability to time-release the protein from its matrix was examined, and the gel was found to be an effective release mechanism for a polypeptide solute. Thus, Pluronic F127 was affirmed as a powerful mechanism for protein drug delivery, and more insight was gleaned into the mechanisms of its operation.

In modern times, protein drugs have had an ever-increasing ability to efficiently combat disease in the human body,<sup>1</sup> and delivery of polypeptide agents, whether insulin or interferons, to biological targets has thus become a task of great importance to doctors and patients alike. For this reason, it is the goal of many medical researchers to preserve the bioactivity of specific protein drugs and find methods for their delivery. Non-ionic surfactants are an attractive vehicle for protein drugs. The polypeptide stabilizing abilities of surfactants have been verified by past experimentation; the addition of surfactants (such as Tweens and Pluronics) has been shown to increase the ability of polypeptides to remain bioactive.<sup>2,3</sup>

Keeping polypeptides in their active or native state is often a difficult

task. The thermodynamic fragility of proteins makes it possible for them to physically denature due to changes in temperature or pH, or simply as a result of agitation of their medium.<sup>3,4,5</sup> Lyophilization, or freeze drying, is another process which can severely damage a protein's ability to function.<sup>6</sup> Even proteins in ideal conditions are subject to forces of degradation. In the process of folding from primary to tertiary structure, a polypeptide passes through many intermediate states which have hydrophobic residues exposed to the aqueous environment. If intermediate structures encounter each other, they can form protein aggregates, whereby the polymers exclude their hydrophobic residues from the surroundings by conglomerating together and thus minimizing the potential energy of their system more effectively than the native state.<sup>2,7</sup> Aggregates are mainly non-bioactive states which remain in equilibrium concentration in any protein solution, and are one of the large-

est obstacles to maintenance of protein drug bioactivity.<sup>3</sup>

There are many theories as to how non-ionic surfactants are able to help maintain a protein's native state. One hypothesis is that the surfactant, as an amphipathic (simultaneously hydrophilic and hydrophobic) compound, can compete with the enzyme for a denaturing surface, for surfactants have been shown to specifically halt surface-induced denaturation when added to a protein solution.<sup>4,6</sup> Other investigations suggest that the surfactant can help decrease the potential energy of a surfactant-adsorbed protein through weak hydrophobic bonding, thus lowering the Gibbs free energy of the system and increasing the native conformation's stability.<sup>2</sup>

Pluronic F127 is one such surfactant with an additional property in aqueous solution: at or above room temperature, a solution of F127 will convert from its liquid state to that of a non-fluid hydrogel. Such a characteristic immediately suits it to protein drug

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delivery: a semisolid solution of protein drug and surfactant could allow the polypeptide to remain concentrated *in vivo* while also protected by the surfactant matrix. PF127 is a polymer of polyoxyethylene (PEO) and polyoxypropylene (PPO) with two 96-unit hydrophilic PEO chains surrounding one 69-unit hydrophobic PPO chain.<sup>8</sup>

Pluronic F127 has already demonstrated significant ability to preserve polypeptide bioactivity *in vitro* and *in vivo*. At various temperatures (including that of a physiological medium), a 14% w/w PF127 solution has been shown to greatly diminish the ability of denaturant influences to affect the stability of a test enzyme.<sup>5</sup> *In vivo* assays have also been made of PF127's effectiveness. Wout *et al.* observed the capability of an internally injected PF127 solution in rats to nearly quadruple the sustained catalytic ability of the dissolved urease enzyme over the course of several days.<sup>5</sup>

To ascertain the biochemical nature of PF127 as a protein drug delivery device, assays were made to explore its interaction with a test protein. Computer models were used to examine the hydrophobicity of the surfactant and the protein and thus better understand the interaction between the two compounds. Fluorescent polarization and probing procedures elucidated *in vitro* how PF127 binds to a polypeptide and what the result of this binding is with respect to the folding state of the protein. Trials were also run which examined the most important physical characteristic of the hydrogel: its ability to release the enzyme from its matrix over time. For a drug delivery device

to be effective, it must be capable of allowing biologically appreciable amounts of polypeptide to diffuse into the body.

The test protein chosen was urease, an enzyme which catalyzes the con-

version of urea to ammonia and carbon dioxide. The bioactivity of urease had already demonstrated to be well protected from denaturant influences by PF127,<sup>5</sup> and it thus was an ideal tool for further exploring the surfactant.

Through an examination of the biochemistry behind the surfactant's stabilizing abilities and a quantification of PF127's ability to polypeptide, this

study demonstrates Pluronic F127 to be a strong candidate for the basis of a protein drug delivery method.

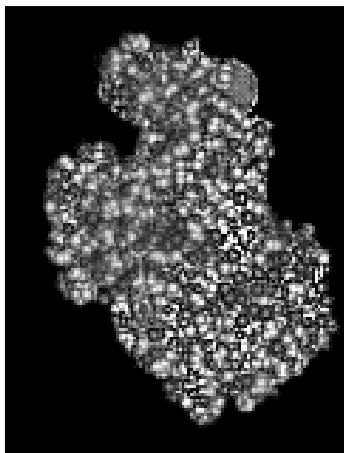
## Materials and Methods

Pluronic F127 was obtained as a generous donation from the BASF Group (Mount Olive, NJ). Urease was procured in powder and 230  $\mu$ M-unit tablet forms from Sigma Chemical Co. (St. Louis, MO). All aqueous solutions were created using deionized distilled water. Fluorescent spectrophotometry was performed in a Perkin-Elmer (Norfolk, CT) LS 503 Fluorescent Spectrophotometer. UV spectrophotometry was performed in a Shimadzu UV spectrophotometer. Fluorescein isothiocyanate (FITC), 1,8-anilinonaphthalene sulfonate (ANS), and dansyl chloride (DaCl) were obtained from Molecular Probes, Inc. Phosphate buffer solutions (PBS, pH 7.4) were prepared according to standard procedure.

## Computer Modeling

Using Quanta97 v. 97.0711 (Molecular Simulations Inc. 1986-1998 All Rights Reserved), and obtaining a urease profile from the Brookhaven Protein Data Bank at <http://www.pdb.bnl.gov>, it was possible to simulate the interactions of the polymers in a computer environment.<sup>9</sup> The program's molecule builder was first used to create the Pluronic F127 polymer, which was then simulated in a hydrous environment. A Powell integration method was used to predict aspects of its folding structure in water. This method is a type of conjugate gradients method which takes into account previous values of the energy gradient in the calculation of its displacement vector, and also imposes restraints on gradient values to avoid numerical overflow.<sup>10</sup> An 8 Å thick surface hydration cloud was simulated surrounding the polymer, and the model was allowed to progress towards energetic equilibrium (with the root mean square, RMS, force on the molecule diminishing as the molecule's conformation neared the base of the potential well and the RMS force readings began to fluctuate with no further trend of increase or decrease). A second, similar energy minimization was also performed on the same model surfactant, with the molecule beginning the minimization process artificially bent once over on itself. Its initial condition was thus one of being folded in the water-solute cloud. The system was then again allowed to progress to the minimal energy, equilibrated state.

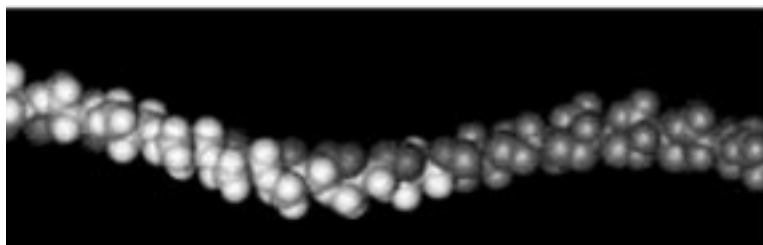
To verify the hydrophobicity assessment made by Quanta, an alternate method of hydrophobicity testing was employed. Water and ethane were both used as a probe molecules to test the potential energy of the molecular system when they interacted with the reportedly hydrophobic urease residues to observe the program's energy predictions.



**Figure 1.** Energy contour map for the interaction of water and the B-100 proline residue on urease.



**Figure 2a.** A polymer pluronic in a regular helix conformation without a solvent.



**Figure 2b.** The polymer chain in water is allowed to twist down a potential gradient. The shaded region is hydrophilic.

### 1,8-Anilino-naphthalene Sulfonate and Urease Fluorescent Spectrophotometry

1,8-anilino-naphthalene sulfonate (ANS) is a hydrophobic fluorescent probe which itself only fluoresces when in a hydrophobic environment. It emits with abnormally high intensity when in contact with a polypeptide.

PBS solutions of urease at 0, 1, 2, 3, and 4 unit/mL concentrations were prepared. A 4 mL sample of each solution was placed in a corresponding test tube and each tube received 20  $\mu$ L of stock ANS solution. Fluorescent spectrophotometry was performed on samples from each tube on a range of 400 nm to 560 nm, with all samples excited at the same intensity.

A second experiment was performed which included ANS, urease, and surfactant. 20 mL PBS PF127 solutions of 5 mM, 0.5 mM, 0.05 mM, and 5  $\mu$ M were prepared, and each one received 20  $\mu$ L of standard ANS solution and 5 mg of urease powder in 10 mL of PBS. The resulting samples were scanned on range of wavelengths corresponding to these ANS trials. A standard curve for fluorescence of an ANS-PF127 mixture was created using surfactant concentrations of 1 mM, 0.1

mM, 0.01 mM, and 1  $\mu$ M.

### FITC-Urease Tagging and Polarization Fluorescent Spectrophotometry

A sodium bicarbonate/sodium carbonate buffer (9.8 mL) at pH 8.5 was mixed with 460  $\mu$ M units of urease and 0.2 mL of FITC at room temperature for 60 minutes. By this process, a fluorophore was bonded to the protein, giving it fluorescent properties. The solution was placed in a dialysis tubing with molecular weight cutoff of 6-8 kDa. The bag was stirred in a PBS bath for 36 hours to remove unbound FITC from the bag. The resulting solution was then taken from the bath and lyophilized.

Solutions of PF127 in PBS were prepared at concentrations of 0.9 mM, 0.09 mM, 9  $\mu$ M, and 0.9  $\mu$ M. Five mg of FITC-urease was dissolved in each sample of PF127 solution. The samples were then each scanned from 490 nm to 620 nm, and the peak emission frequency was noted. A blank sample without any surfactant was also prepared and tested similarly. Subsequently, readings were taken using polarization crystals at the noted peak frequency for the blank, such that polarization values were measured,

where the value is:

$$\frac{(I_V - I_H)G}{(I_V + I_H)G}$$

where  $I_H$  and  $I_V$  are the intensities of vertical and horizontal polarization states and  $G$  is a grating factor specific to the incident wavelength which depends on the parameters of the measuring system.<sup>13</sup>

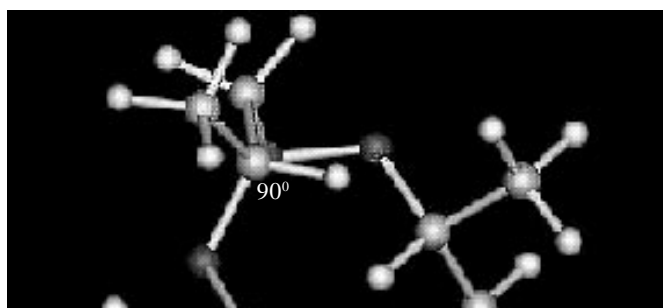
### DaCl Tagging and Polarization Fluorescent Spectrophotometry

A similar polarization study was conducted where the fluorophore, instead of being present in FITC on the enzyme was instead used to derivatize hydroxyl groups on the surfactant. Thus, the surfactant gained fluorescent properties. The DaCl was bonded to the surfactant chains in an anhydrous methanol solution and then precipitated in an ethyl ether medium, which was evaporated once the polymer had come out of solution.

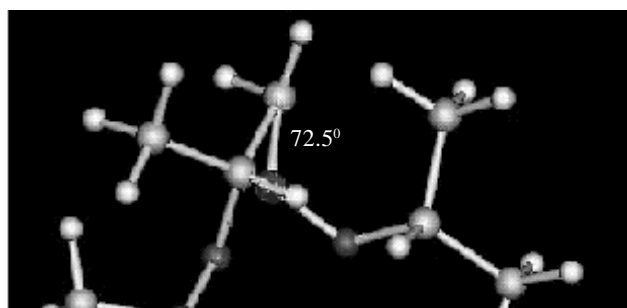
A polarization study was performed using the DaCl fluorescent surfactant. Ten mL solutions of 1 unit/mL urease and DaCl-F127 concentrations ranging from 1 mM to 1  $\mu$ M were placed in a fluorescent spectrophotometer and the polarization values were recorded.

### Diffusion of Urease from PF127 Hydrogel

An aqueous solution of PF127 (20% surfactant by mass) was prepared. The surfactant required approximately 36 hours to dissolve at 4°C. One 230  $\mu$ M unit tablet of urease was added in 20 mL surfactant solution and mixed until full dissolution. Of eight 50 mL beakers, four were filled with 5 mL of the surfactant-urease solution and four received 5 mL pure surfactant solution as controls. Each beaker was placed in a 37°C bath, to allow for hydrogel formation. Fifty mL PBS was poured over the gels, and UV spectrophotometric measurements were taken at a wavelength of 280 nm every hour for 8 hours, using a PBS blank as a background for the readings. Each reading



**Figure 3a.** Test polymer with structure similar to PF127 bent along its hydrophobic center block.



**Figure 3b.** Test polymer after allowed to slide down the potential gradient to equilibrium.

was in fact the mean of 5 readings taken over the course of one minute.

After 8 hours, samples of the buffer solutions in the beakers were assayed for the bioactivity of the enzyme present in the solutions. Five hundred  $\mu\text{L}$  of urease solution was taken from each of the four beakers containing urease in the release study and distributed among four test tubes. The standard test for urea nitrogen was performed on the samples using phenol nitroprusside and sodium hypochlorite solutions.

## Results and Data

### Potential Energy Interaction Map on Enzyme Surface

The first task accomplished with Quanta was an examination of the theory of mutual potential energy minimization as the mechanism of binding between urease and PF127. Figure 1 shows the potential energy for the system at each interaction coordinate when a hydrophobic surface residue of the protein was allowed to interact with a water molecule in a computer environment. A similar interaction was plotted for a hydrophobe and the residue, and the potential energy of the system was lowered in comparison to the water-urease map. These results confirm the notion that two hydrophobic compounds can decrease the total potential energy of a molecular system by excluding each other from the hydrous environment.

The surfactant's hydrophobic segment might perform an interaction of that type with the surface of a polypeptide (Figure 1).

### PF127 Folding Structure

The original expectation in the enlisting of Quanta as a resource for this experiment was to predict the exact binding stoichiometry of PF127 to urease. Although Quanta had too many limitations to create a complex folding structure for an PF127 polymer, it was feasible to view the initial stages of the hydrophobic interactions which are theorized to cause bundling of the polypropylene region of PF127. Figure 2a shows a model of PF127 in a normal, helical structure, without any solvate structure surrounding it. In contrast, Figure 2b demonstrates the effect that energy minimization of the same molecule dissolved in water has on its structure. This result, while not as drastic as was initially hoped, does lend credence to the position that the surfactant can fold in on itself, thus minimizing the surface area of its hydrophilic block and therefore making it more likely that the polymer could present a hydrophobic unit to the surface of a polypeptide for bonding.

It was found, however, that further evidence for a folding behavior could be observed in the model when a test polymer of the composition 3PEO-3PPO-3PEO was helped along toward a theorized, folded equilibrium by artificially bending the molecule along

the center of the hydrophobic region and then minimizing the system's potential energy. Figure 3 shows the bending angle of the molecule for unminimized and minimized versions of the bent molecule. As one can distinguish from Figures 3a and 3b, when following a potential gradient from a root mean square force of approximately 3000  $\mu\text{dynes}$  to one of 0.237  $\mu\text{dynes}$ , the minimized molecule actually bent further, from a starting angle of  $90.0^\circ$  to the relative stability of  $72.5^\circ$ . This result is even more conclusive corroboration of the hypothesis that the surfactant folds in aqueous solution, compacting its hydrophobic unit such that it would be more amenable to docking with a hydrophobic residue on the surface of a protein.

### 1,8-Anilinonaphthalene Sulfonate Fluorescent Probing of Urease and PF127

Standard backgrounds of the interactions of ANS with PF127 and urease were acquired and the data are displayed in Table 1. Since ANS fluoresces only when it is in a hydrophobic region, it is very useful for discerning the nature of the surfaces of the protein and surfactant. ANS is also useful for this trial because of its tendency to fluoresce especially intensely when in contact with a polypeptide.<sup>11</sup> Table 1a clearly indicates widespread presence of hydrophobicity on the surface of Pluronic F127, and 1b similarly indicates such a contingent on the sur-

face of urease. It is the interaction of these two surfaces which the third trial run with ANS explored.

Figure 4 graphs the intensity of the fluorescent emission of ANS in a solution of both urease and PF127. This trial provides the most definitive confirmation that the binding between urease and PF127 is one dependent on the interaction of their hydrophobic surface regions. At high surfactant concentration, the fluorescent intensity is very high because PF127 has a large hydrophobic surface to offer the probe. Figure 4, however, demonstrates an increase in ANS fluorescent intensity over one interval of PF127 concentration decrease. This result can be explained by a process where, as surfactant concentration dipped low enough, all of the surface residues on the polypeptide previously occupied by the surfactant were suddenly available to the waiting ANS. Since ANS fluoresces intensely when in contact

<b>-log [F127]</b>	<b>Int. of ANS-PF127 solution</b>
0	95.82
1	72.00
2	48.02
3	32.66

**Table 1a.** Background of ANS fluorescent emission due to Pluronic 127 in solution.

<b>[urease] (mM units/mL)</b>	<b>Int. of ANS-urease solution</b>
0	40.76
10	242.3
20	252.3
30	289.2
40	317.0

**Table 1b.** Similar background for the interaction of urease and 1,8 ANS.

with a protein, this displacement of surfactant by ANS showed up as the increase in intensity seen in Figure 4.

### FITC-Urease Emission Polarization in PF127 Solution

FITC-urease was examined using a fluorescent polarization measuring technique. The FITC-urease was excited with the absorption wavelength of FITC, and the polarization of the emitted light was measured. For a higher spin rate, the protein would go through more radians of rotation during an excited state, and the light would have a lower polarization value. The trial thus gives a quantitative description of the molecule's tumbling rate in solution. Figure 5 shows the polarization values at different F127 concentrations.

The results displayed in Figure 5 invite an intriguing conclusion. If the polarization of light emitted by the fluorophore decreases as the surfactant concentration increases, that indicates that tumbling rate rises with PF127 concentration. Such a result may seem baffling at first, since increased binding of PF127 should surely slow the tumbling rate. If, however, the protein in absence of the surfactant were clumping together to form slowly tumbling aggregates, then the breaking up of these aggregates and the resulting increase in tumbling rate, could be seen as a direct consequence of the addition of surfactant. Thus, Figure 5 provides evidence that PF127 can disrupt protein aggregates.

### Dansyl Chloride-PF127 Emission Polarization in Urease Solution

These trials were conducted in a similar fashion to those of the FITC-tagged urease. When using dansyl chloride (DaCl), however, one is derivatizing the surfactant and not the enzyme. The polarization of the fluorophore emission is thus dependent on the tumbling of the PF127,

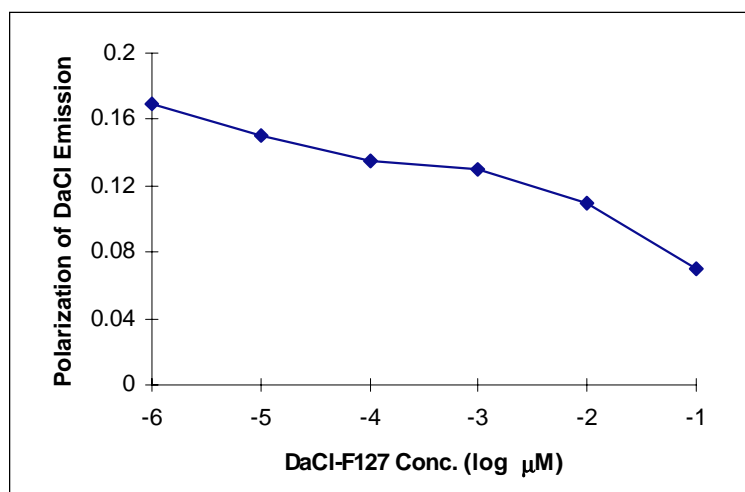
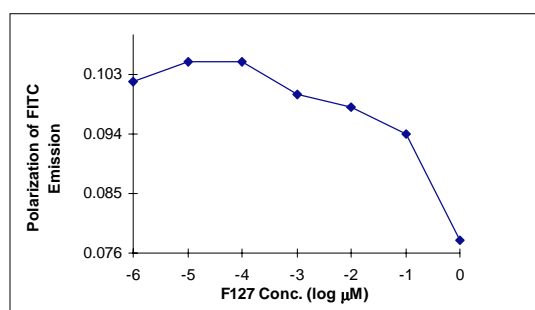
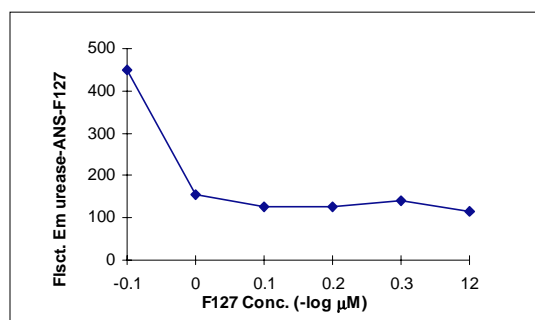
not on that of the enzyme. Nevertheless, Figure 6 confirms the results shown in Figure 5. Again, as surfactant concentration increases, the tumbling of the fluorophore (weakly bound to protein aggregates in solution) increases as more aggregates are disrupted by the higher surfactant concentration. Figure 6 demonstrates a strong confirmation of the theory brought on by the results of the FITC trial of Figure 5.

### Diffusion of Urease from the PF127 Matrix

Figure 7 shows the absorbance at 280 nm (protein absorbance wavelength) of buffer solution above a urease-PF127 hydrogel over an eight hour period. The trial was performed to test the surfactant hydrogel's ability to release appreciable concentrations of enzyme from its matrix into the surrounding fluid. As can be seen in Figure 7, the urease concentration in the solution increased almost linearly over time. Further verification that the hydrogel was indeed releasing significant amounts of the enzyme came from a bioactivity assay of samples taken from the trial's end products. Upon the addition of urea and the appropriate indicators, the solution turned a visible blue, indicating significant urea catalysis. Thus, not only does PF127 help to stabilize the native state of polypeptides, but it is also a useful way of time releasing enzymes in medically useful concentrations.

### Formation of PF127 Hydrogel

As an additional assessment of the hydrogel's properties, an assay of the viscosity of an aqueous F127 solution was performed to observe the process by which it shifts from liquid to semisolid matrix, i.e. the process of gelation.<sup>9,12</sup> Figure 8 displays the results, which were obtained by an experimenter at NEU Pharmacy Labs. Figure 8 assures the solidity of the



**Figure 4.** (Top Left) Fluorescence Emission intensity of a urease-ANS-F127 solution at 480 nm.

**Figure 5.** (Bottom Left) Polarization of fluorescent emission by FITC-derivatized urease indicates a decrease in tumbling rate.

**Figure 6.** (Above) Polarization of fluorescent emission by DaCl-derivatized PF127 increases with decrease in surfactant concentration.

PF127 hydrogel at the physiological temperature of 37°C, and thus that it could remain in a semisolid state, keeping dissolved enzymes concentrated and protected in the human body.

## Discussion

The trials have produced data which suggest that Pluronic F127 would indeed be an effective drug delivery device. The computer modeling performed on Quanta gave a useful assessment of what kind of behavior was expected from urease and F127 in their interplay. The surfactant molecule, when submerged in water, tended to minimize its energy by folding, compacting the polyoxypropylene units exposed to water and coiling them together into a smaller hydrophobic unit with less surface area exposed to the hydrous environment.

Given the computing power available and the nature of the modeling program, intervention in the parameters of the system was necessary to obtain the best folding result. The differences between the undissolved, linear structure of PF127 and the

minimized one are apparent (there is a several angstrom loss of length from the unminimized to folded state), but the artificially bent models are more telling. In this case, the system had been deliberately initiated in a doubled-over conformation, and one which was certainly not initially in any kind of equilibrium, but rather experienced a strong twisting root mean square force 10,000 times the magnitude of that of the equilibrated state. Yet, as the system moved down its potential gradient, bonds stretching and converting into more energetically attractive states, the net effect was a decrease in the bending angle of the surfactant model. Thus, while an initial hill had to be surmounted to push the system in the folding direction, the tendency of the molecule in water does seem to be for the hydrophobic block to contract and twist in on itself while presenting hydrophilic tails to the hydrous environment.

Having verified the contractile nature of the hydrophobic region, it is feasible to associate the region with the surfactant's stabilizing ability. A more compact hydrophobic unit might make a mutual exclusion of water by

surfactant and hydrophobic residue a much more favorable and practicable arrangement. It is clear from the evidence provided from the ANS probing that there is definitely a significant presence of hydrophobic residues on the surface of urease (Table 1b). Because the probe only fluoresces in a hydrophobic environment, we can infer that the observed emission comes from the weak binding of ANS with hydrophobic residues on the protein's surface.<sup>11</sup> True, F127's hydrophobic region is larger than an ANS probe, but the general character of the protein's surface nevertheless now seems to be one receptive to weakly bonding hydrophobic molecules.

ANS probing of the urease-F127 interaction gave some insight into the interplay between the two molecules. When the experiment was originally designed, the hope was that less surfactant would begin to allow more ANS to bind to hydrophobic regions on urease, thus increasing the fluorescent intensity of the sample and indicating less binding of F127 to urease as the concentration of F127 went down.

The results obtained, while not as simple as the original expectation, nev-

ertheless suggest that the surfactant's binding to urease does indeed depend on the interaction of the two polymers' hydrophobic regions. The ANS fluorescent intensity decreased for a certain range of surfactant concentrations, then increased again once the F127 concentration was low enough, and finally decreased in the blank sample with no F127. It would seem that, for most of the F127 concentration range, the background from the binding of ANS to F127 hydrophobic regions was too high. Indeed, it may be possible that F127 has a higher affinity for the ANS, and thus the first part of the curve is not particularly helpful. Nevertheless, as F127 concentration reached 5  $\mu\text{M}$ , the fluorescent intensity increased noticeably.

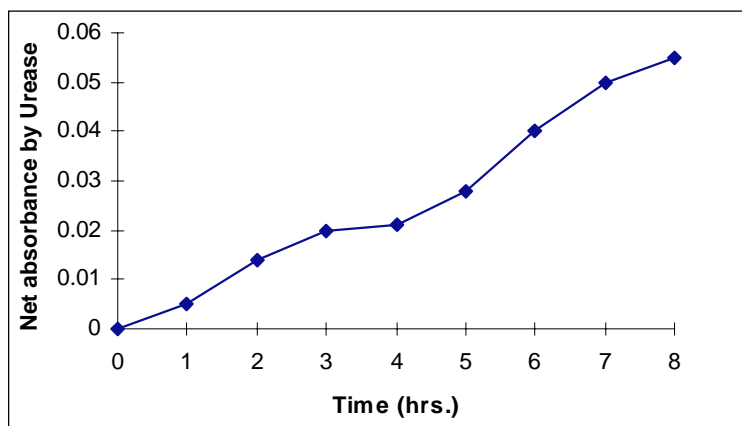
To see how this result verifies dependence of urease-F127 binding on

hydrophobicity, one must remember that ANS is abnormally fluorescent when bound to proteins.<sup>11</sup> Thus, with high concentration of surfactant, the PF127 binds to ANS and causes it to emit, but also binds to most of the available hydrophobic residues on urease. Once the surfactant concentration falls such that ANS can compete with it, however, the fluorescent probe then has new opportunity to bind the urease surface, displacing the surfactant. The combined ANS fluorescence due to urease and F127 adds up to increased fluorescence because ANS fluoresces brightly from the surface of the protein urease. The ANS probing therefore verifies what the computer modeling had lead us to postulate: that surfactant and protein bind via an interaction between their respective hydrophobic surfaces regions.

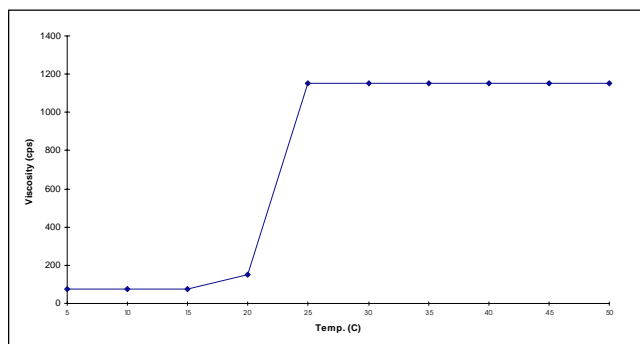
This evidence from the ANS probing might at first be confusing in light of the results of the FITC-urease polarization measurements. The polarization of light emitted by a fluorescing molecule is a measure of that molecule's rate of tumbling. The faster the polymer spins, the more it turns while in the excited state, and the less polarized the light emitted. Thus, if one claims that the surfactant binds to the protein, such binding should increase its rotational inertia. This in turn would cause a decrease in tumbling rate which translates directly into an increase in polarization of emitted light.<sup>13</sup> Thus, the expectation was that polarization of FITC-urease would rise with PF127 concentration.

The result of the trial was exactly opposite to what was expected. The polarization values decreased as the surfactant concentration is increased, almost in a mirror image of the expected behavior (see Figure 6). We explain these results as consequences of protein aggregation. It is clear that it is faulty to assume that most of the urease in solution is in a solitary state until interacting with F127. Rather, there must have been a high concentration of aggregate polypeptide in solutions of protein used. A solution with a high concentration of aggregated FITC-urease would have had a very low average tumbling rate; aggregates are high volume complexes which rotate slowly in solution.<sup>13</sup> One would thus expect highly polarized light for low F127 concentration. The surfactant, meanwhile, if helping to minimize the potential of the native state, would assist in decreasing the equilibrium concentration of the aggregate state, thus raising the polarization of the emission. The findings for this trial, therefore, are in exact agreement with the theory that surfactants assist in stabilizing the native state and disrupting non-bioactive protein aggregates.

The dansyl chloride-tagged F127 investigations corroborated the findings of the FITC fluorometry. While the



**Figure 7.** Net absorbance at 280 nm of the buffer solution above a urease P-127 hydrogel.



**Figure 8.** Measuring the viscosity of 70% w/w aqueous solution of F127 as a function of temperature.

fluorophore was now on the surfactant and not on the enzyme, almost identical results were obtained for a trial of varying surfactant concentration and constant urease concentration. Again, it would seem that the surfactant tumbles along with the protein, but with increased concentration disrupts greater and greater numbers of unwieldy protein aggregates and provides more stability for the native state. Further investigations of this aggregate disruption using non-denaturing electrophoresis would be the logical next step in demonstrating the aggregate disruption.

Examination of the hydrogel's ability to release the protein gave promising results with respect to the possibility of F127 becoming a drug delivery device. While proteins are large macromolecules, the properties of the hydrogel permitted the diffusion of urease into the buffer solution above the layer of gel at a linear rate. Furthermore, the buffer solution was quite capable of ample catalysis of urea to ammonia and carbon dioxide after 8 hours, indicating a strong concentration of bioactive urease. With this initial linear rate of release we might expect some kind of sigmoidal long-term behavior as the protein concentration in the hydrogel approached that of the urease presence in the buffer solution. What is important, regardless of the exact diffusion rate, however, is that the surfactant clearly can release the protein in a time-scale which lends itself to use in the human body. This hydrogel is a material which retains proteins within its matrix but allows for gradual, quantifiable diffusion of the drug into the surrounding medium in significant concentrations.

Finally, the viscosity data regarding the aqueous solution of surfactant are a useful way of viewing the delivery power of the hydrogel. Because its viscosity is so high at body temperature, a protein-drug containing gel could conceivably be injected into a

patient and remain concentrated for hours without flowing throughout the body. Though F127 is water soluble and would eventually dissolve, its hydrogel would nevertheless be able to dramatically increase the amount of bioactive polypeptide made available to a body in which it released a protein drug. The gelation is one of many positive characteristics for Pluronic F127 as a drug delivery device.

## Conclusion

Pluronic F127 is a surfactant molecule with highly beneficial characteristics that make it a strong candidate for a protein drug delivery device. Its interaction with polypeptides is most likely one of minimization of potential energy by mutual exclusion of hydrophobic residues from the aqueous medium, as was predicted by computer modeling and verified by fluorescent probing. It has helpful abilities with regard to aggregate disruption and native state stabilization, and also harbors a hydrogel character which could allow it to give a time-released drug delivery. These data allow for a better understanding of the mechanisms of protein stabilization and release, and will hopefully assist in the design and implementation of increasingly powerful protein-based therapies.

## Acknowledgments

A deep debt of gratitude is owed to Dr. Mansoor Amiji for his generous devotion of time, resources, and wisdom to the construction of this project. The Center for Excellence in Education also deserves tremendous thanks for its confidence in young scientists that made this paper possible. And, finally, a warm thank you is extended to Michael Broxton, without whom the computer modeling would have run aground very quickly.

## References

- <sup>1</sup> Bam, Narendra B., T. W. Randolph, and J. L. Cleland. "Stability of Protein Formulations: Investigation of Surfactant Effects by a Novel EPR Spectroscopic Technique." *Pharmaceutical Research*. **12**, 2-11 (1994).
- <sup>2</sup> Bam, Narendra B., T. W. Randolph, and J. L. Cleland. "Molten Globule Intermediate of Recombinant Human Growth Hormone: Stabilization with Surfactants." *Biotechnological Progress*. **12**, 801-809 (1996).
- <sup>3</sup> Katakam, M., L. N. Bell, and A. K. Banga. "Effect of Surfactants on the Physical Stability of Recombinant Human Growth Hormone." *Journal of Pharmaceutical Sciences*. **84**, 713-716 (1995).
- <sup>4</sup> Manning, M. C., K. Patel, and R. T. Borchardt. "Stability of Protein Pharmaceuticals." *Pharmaceutical Research*. **6**, 903-918 (1989).
- <sup>5</sup> Wout, Z. G., E. A. Pec, and T. P. Johnston. "Biological Activity of Urease Formulated in Poloxamer 407 After Intraperitoneal Injection into the Rat." *Journal of Pharmaceutical Sciences*. **81**, 626-630 (1992).
- <sup>6</sup> Chang, B. S., B. S. Kendrick, and J. F. Carpenter. "Surface-induced Denaturation of Proteins during Freezing and Its Inhibition by Surfactants." *Journal of Pharmaceutical Sciences*. **85**, 1325-1330 (1996).
- <sup>7</sup> Pearlman, Rodney and Y. John Wang. *Formulation, Characterization, and Stability of Protein Drugs*. New York: Plenum Press (1996).
- <sup>8</sup> Schmolka, I. R. and I. G. Lundsted. "The Synthesis and Properties of Block Copolymer Polyol Surfactants." *Block and Graft Copolymerization*. New York: John Wiley and Sons (1986).
- <sup>9</sup> Vadnere, Madhu, G. Amidon, S. Lindenbaum, and J. L. Haslam. "Thermodynamic studies on the gel-sol transition of some pluronic polyols." *International Journal of Pharmaceutics*. **22**, 207-218 (1984).
- <sup>10</sup> Sternberg, Michael J. E. *Protein Structure Prediction: a Practical Approach*. New York: IRL Press (1996).
- <sup>11</sup> Haughland, Richard P. *Molecular Probes*, Inc (1992).
- <sup>12</sup> Peppas, Nikolaos A. *Hydrogels in Medicine and Pharmacy: vol. 1*. Boca Raton, FL: CRC Press (1996).
- <sup>13</sup> Qaqish, Roula B., and M. Amiji. "Synthesis of Fluorescent Chitosan Derivative and its Application for the Study of Chitosan-Mucin Interactions" (1997).