Surfactant Effect on Intercellular Transport of DNA, Proteins, and Electrolytes

D. Assan*, X. Gao**, J. Lai*** and S. Leung****

*Civil & Environmental Engineering Department, Idaho State University, Pocatello, ID, USA, assadavi@isu.edu
**Civil & Environmental Engineering Department, Idaho State University, Pocatello, ID, USA, gaoxian@isu.edu
***Biomedical & Pharmaceutical Sciences Department and Biomedical Research Institute, Idaho State University, Pocatello, ID, USA, lai@pharmacy.isu.edu
**** Civil & Environmental Engineering Department and Biomedical Research Institute, Idaho State University, Pocatello, ID, USA, leunsolo@isu.edu

ABSTRACT

Surfactants play a key role on bodily functions at molecular level, such as cell signaling and signal transmission. Their importance in cell survival is well recognized, but the surfactant effect on intermolecular/interfacial mass transport is not well understood and the available information of the subject is very limited quantitatively. In this report, we systemically studied the effect of surfactants on intercellular transport of DNA, proteins, and some key electrolytes with an artificial cellular system constructed with a semipermeable membrane. The permeabilities of these biomolecules and electrolytes across the semipermeable membranes in the artificial cell system were measured. DNA, three metabolically important enzyme proteins (namely, glutamate dehydrogenase (GDH), lactate dehydrogenase (LDH), and malate dehydrogenase (MDH)) of various molecular masses, and key electrolytes (Ca^{++}, Cl^{-}, K^{+}, and Na^{+}) were employed to study the transport behaviors through a semipermeable membrane in an artificial cell system at a pH range (6.5-7.4) and concentrations relevant to body functions. The effect of surfactant charges and membrane pore sizes were also observed. Results of this study provide quantitative understanding of intermolecular interfacial mass transport that is important in the design of many fore-coming applications of biotechnology.

Keywords: surfactant, DNA, protein, electrolyte, membrane

1 INTRODUCTION

In current advances of biotechnology, various models of introduction of DNA, proteins and electrolytes into cells to alter their gene expression have important biomedical and bioengineering applications, for examples, in cancer/disease therapies and special drug delivery. However, the molecular mechanisms underlying how these molecules cross cell and nuclear membranes are poorly understood.

Our research group has systematically investigated the individual transport properties of DNAs, proteins, and electrolytes through a semipermeable membrane under various conditions that are important to biological functions; in this study, we investigated the effects of anionic, cationic, and non-ionic surfactants, as well as CNT (carbon nanotubes) on the interfacial transport across a semipermeable membrane of these biomolecules and electrolytes at pH 6.5 to 7.4. The effect of different membrane pore sizes was also investigated.

It is the goal of our research group to integrate, in a stepwise manner, studies of many of the environmental factors that are influential to cellular DNA, proteins, and electrolyte interfacial transport that can be used for proper control of such molecules in artificial organ development, tissue engineering, and drug delivery.

2 MATERIALS AND METHODS

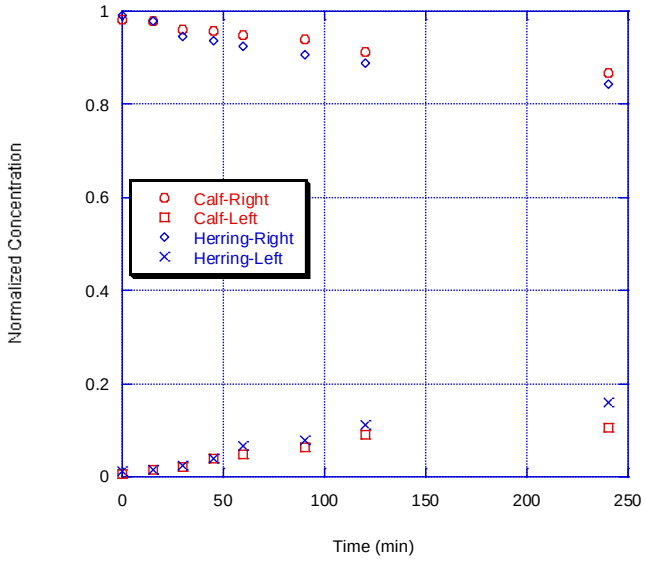
The DNA for this study came from Herring sperm and calf thymus (Sigma), the DNA contained 6.1 % of sodium. The cationic surfactants used were C-573 (low molecular weight) and C-581 (high molecular weight) (Cytec Industries, Inc.). The anionic surfactants were IB-45 (hydrophilic) and TR-70 (hydrophobic) (Cytec Industries, Inc.). Non-ionic surfactant was Triton-X 100 (Sigma). Enzyme proteins of MDH (malate dehydrogenase), LDH (lactate dehydrogenase), and LDH (glutamate dehydrogenase) were ordered from Sigma. Carbon nanotube (CNT) solution was prepared by multiple-wall nanotube (MWNTs, < 8 nm) at 50 mg/l (CheapTubes.com, Brattleboro, VT). Four electrolytes were used to observe the permeability of the electrolytes permeated across a polycarbonate membrane (1-5 μ), they are K^{+}(0.01 M), Na^{+}(0.1 M), Cl^{-}(0.1 M), and Ca^{++}(0.00025 M). The concentrations of electrolytes were similar to what would be found inside human body fluids.

DNA and enzyme proteins were measured by UV-Vis spectroscopy, electrolytes were measured by electrodes. Experimental setup of the artificial separation cell and surfactant concentrations were similar to studies that we previously reported [1, 2]. Membrane used in the

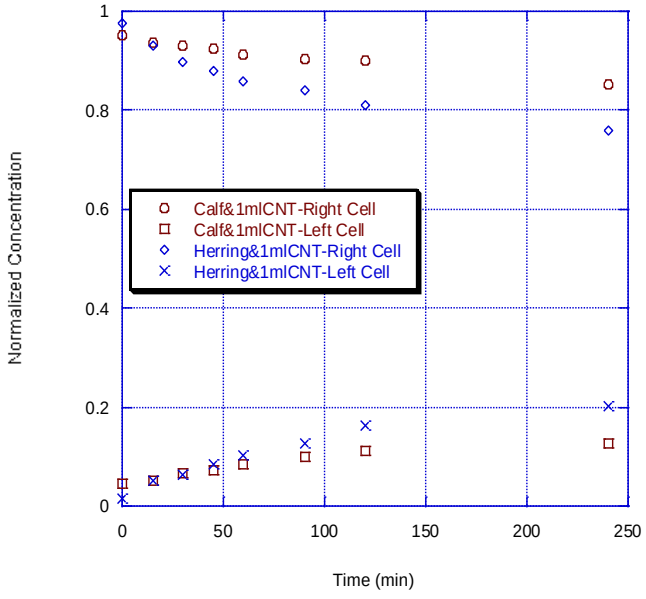
separation cell was polycarbonate with pore sizes of 0.1, 1, and 5 microns.

3 RESULTS AND DISCUSSION

3.1. Molecular Size Differential of DNAs



(a)



(b)

Figure 1: (a). Transporting rates of calf and herring DNA with 5 micron membrane at pH 6.95 without the effect of surfactants. Herring DNA had higher permeability in the absence of surfactants ;(b). same conditions as in (a) except with an addition of 1ml of CNT solution (per 100 ml of DNA solution). CNT has increased the permeability of DNA, in particular with herring DNA.

Figure 1(a) shows that herring DNA was lighter than calf thymus DNA, according to diffusional theory and CNT has drastically increased the permeability of herring DNA. For some unknown reasons at this time, the permeability of calf thymus was not affected by the addition of CNT which somewhat served as a lubricant in the permeation process across the cell membrane (Figure (b)).

3.2. Effect of Surfactants on DNA Permeability

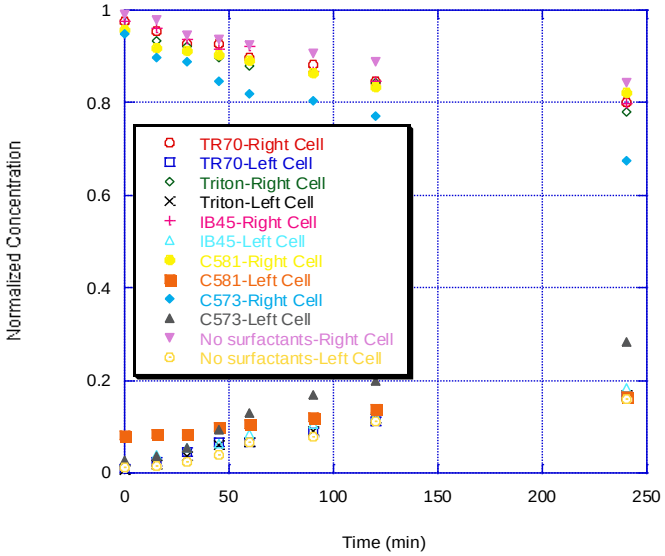


Figure 2:. Transporting rates of herring DNA with 5 micron membrane at pH 6.95 with the effect of 5 different (cationic, anionic, and non-ionic) surfactants.

As shown in Figure 2, the light molecule of cationic surfactant (C573) was by far the most effective on the mobility of the herring DNA (thus permeability), which also coincided with literature observation that the presence of cations could affect mobility of DNA up to 40% [3].

3.3. Effect of Hydrophilic Surfactant on Permeability of Different Proteins

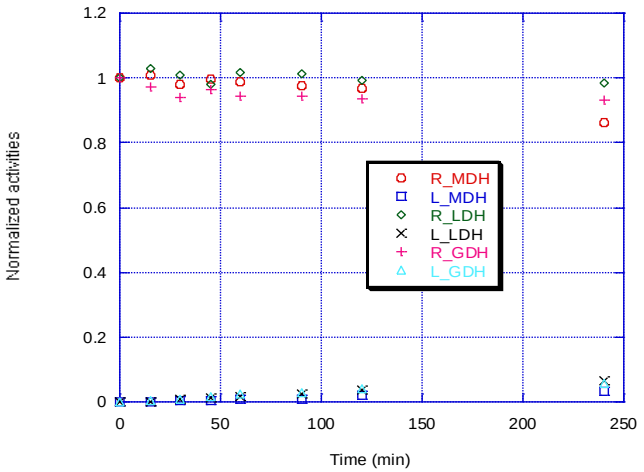
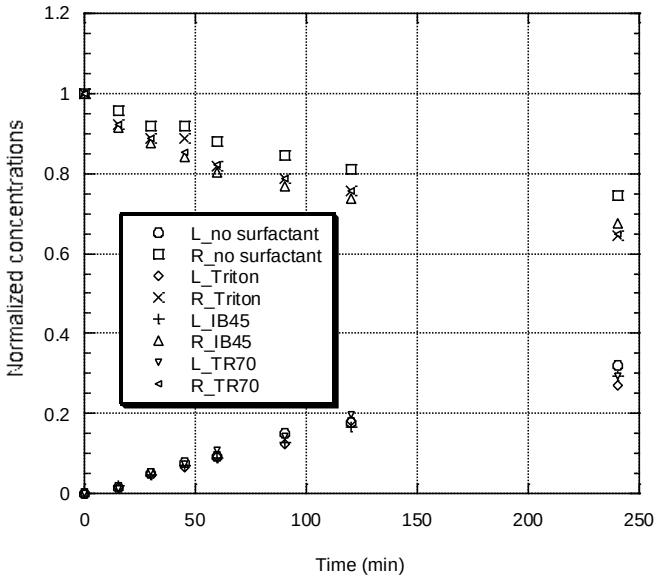


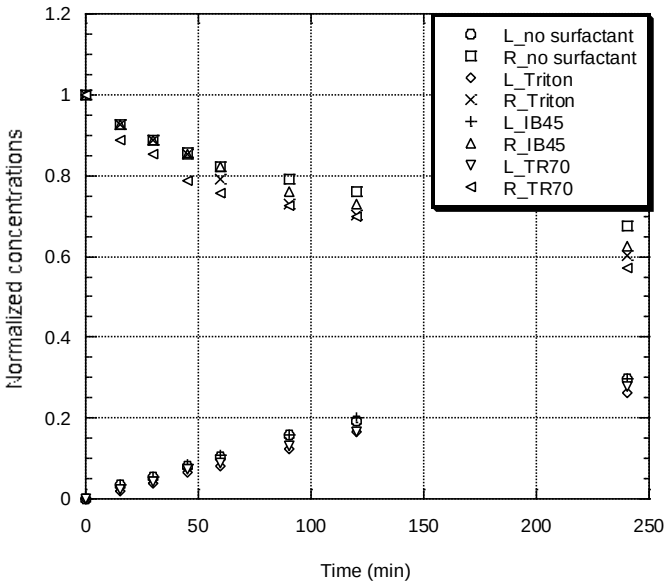
Figure 3: Transporting rate of 3 different enzyme proteins at pH 6.95 with 0.1 ppm IB45 surfactant in solution. Membrane pore size was 1 micron.

When only considering the mass of the proteins that passed through the membrane (left-side of separation cell), the order of mobility of the proteins then was LDH>GDH>MDH which is deviated from the size of the molecules (GDH>LDH>MDH). Was this deviation the consequence of the added anionic surfactant (IB45) remains to be resolved.

3.4. Effect of Different Surfactants on Permeability of Electrolytes



(a)



(b)

Figure 4: Effect of surfactant on the permeation of (a). 0.1 M of Na^+ at pH 6.95; (b). 0.1 M of Cl^- at pH 6.95.

When concentrations of an electrolyte were within the same magnitude, we found that the permeability of a single-valance electrolyte (Cl^-) was about the same within the separation cell regardless of the difference in concentrations. All single-valence electrolytes accumulated about 30% of its initial mass in the dilute side of the separation cell after 4 hours, the divalent electrolyte (Ca^{++}) accumulated about 20% of the total mass after 4 hours. For all the electrolytes, permeation of mass was faster from the concentrated side to the diluted side without surfactant; at this time, it is difficult to differentiate which surfactant has more retardation effect to the permeation of the electrolytes, it appeared that surfactant would have more effect to multivalent electrolyte than that to the monovalent counterpart.

4 CONCLUSIONS

The permeability of herring DNA was faster than the calf thymus DNA with the polycarbonate membrane in the absence of surfactant, which coincides with the molecular diffusional theory. The CNT drastically increased the permeability of herring DNA, but hardy had any influence on calf thymus DNA which was somewhat surprising. Low molecular cationic surfactant was more effective in increasing the permeability of herring DNA. Membrane pore size might be the limiting factor in the DNA migration, the CNT and surfactant addition appeared to be secondary controlling factors in our observations. As for enzyme proteins, the migration order was LDH > GDH >MDH. Although the difference among the three studied proteins were not vastly significant, the ranking order is

totally defying the prediction of molecular theory, therefore, there were other factors in the permeation of enzyme proteins that are yet to be considered. On the other hand, surfactants appeared to be hindering the migration of electrolytes from the concentration side to the dilute side in the separation cell, and none of the electrolytes in the study have larger molecular size than the herring DNA. The effect of surfactants to the biomolecules was totally different from the electrolytes.

5 ACKNOWLEDGMENTS

This study was supported by a DOD USAMRMC Project Grant (Contract #W81XWH-07-2-0078).

REFERENCES

[1] S. Leung and J. Lai, "Differential Effects of Anionic Surfactants on Activities of GDH, LDH, and GDH", Biochemical Engineering Journal, 25(1): 79-88, 2005

[2] J.C.K. Lai and S. W. Leung, “Protein and Cell Signaling with Biomaterials: Interfacial Transport”. In Encyclopedia of Biometerials and Biomedical Engineering (Wnek GE & Bowlin GL, eds.), pp.1-11, Marcel Dekker. New York, NY, Mar.27, 2006

[3] E. Stellwagen, Q Dong, and N. Stellwagen, “Monovalent Cations Effect the Free Solution Mobility of DNA by Perturbing the Hydrogen-Bonded Structure of Water”, Biopolymers, 78(2): 62-68, 2005