UNDERGRADUATE LABORATORY MODULE ON SKIN DIFFUSION

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Biological sciences play an increasingly important role in chemical engineering education.[1-8] At Georgia Tech, we officially added biological education to the curriculum in 2003, when the department was renamed the School of Chemical and Biomolecular Engineering and a biotechnology track was added to the undergraduate curriculum for interested students. Currently, 25-30% of our undergraduates receive B.S. degrees via the biotechnology track. Within biology-related fields, chemical engineers play a role in developing biopharmaceuticals, biomaterials, biofuels, green chemistry, and, as discussed in this article, novel drug delivery systems. Chemical engineers also model biological processes from the molecular level to the systems level.

Hands-on laboratory education related to the biological sciences can help prepare students for engineering careers in biotechnology or medicine. We have developed a skin diffusion laboratory module for the unit operations laboratory class that aims to teach students about biological tissue handling, molecular diffusion, fluorescence-based assays, and the importance of the skin barrier to health. Until now, five other bio-related unit operation laboratory modules have been available to students: fermentation, protein separation, protein growth, glucose isomerization, and enzyme kinetics.

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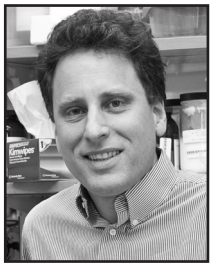

In addition to drug delivery, diffusion across skin is an important topic in toxicology and occupational safety.

Although these labs exposed students to a number of critical topics in biotechnology, none of them are directly related to medicine or involve the handling of biological tissue. For students interested in medical applications of chemical engineering, there was a need to expand the scope of bio-unit operations lab options.

To address this need, we developed and implemented a new laboratory module focused on diffusion of molecules across skin. This lab was designed to introduce students to transdermal diffusion by having them assess the permeability and lag time of fluorescent compounds crossing mouse skin. Our objectives for this lab were to (i) expand students' knowledge of diffusion, permeability coefficients, and lag times; (ii) give students hands-on, applicable experience handling biological tissue; (iii) teach students about the physics and applications of fluorescence; and (iv) introduce students to the importance of the skin barrier to health and safety.

Diffusion of compounds across skin is an important topic in health science and chemical engineering. Most notably, pharmaceuticals can be delivered to the skin for a local dermatological effect using topical creams and ointments (*e.g.*, local anesthetics, anti-fungal creams) or for a systemic response using transdermal patches (*e.g.*, nicotine for smoking cessation, hormones for birth control). It is estimated that more than one billion transdermal patches are manufactured each year.[9] In addition to drug delivery, diffusion across skin is an important topic in toxicology and occupational safety. The CDC estimates that 13 million workers in the United States are exposed to chemicals that can be absorbed through the skin. Contact dermatitis is the most common skin-related occupational illness, with costs over US$1 billion per year.[10] Transdermal hazards in other occupations can lead to cancer, hepatotoxicity, neurotoxicity, reproductive disorders, and death.

The skin is not only an important organ in health-related contexts, but it also has interesting properties as a diffusion barrier.[11] The outermost layer of skin is called stratum corneum. Although it is only 10 – 15 μm thick in humans, the stratum corneum is the rate-limiting barrier to entry of most compounds into the body. This tissue is organized in a brick-and-mortar structure, in which the bricks are cell remnants composed largely of cross-linked keratin and the extracellular mortar consists of lipids organized in multilamellar bilayers. Below stratum corneum is the viable epidermis, which measures 50 – 100 μm thick and contains densely packed keratinocytes and other cells in a conventional aqueous extracellular matrix, but lacks blood vessels. Deeper still is the 1 – 2 mm thick dermis, which contains blood vessels for systemic drug absorption, as well as hair follicles and sweat glands. Despite the complex organization of skin, skin permeability is often modeled by assuming that the stratum corneum is the only significant barrier and that transdermal delivery can be treated as one-dimensional diffusion through a uniform slab.[12]

LABORATORY DESCRIPTION

Design of the Laboratory Module

This lab enables students to study the permeability and lag time of two fluorescent compounds diffusing across mouse skin: sulforhodamine B (molecular weight: 559 Da, excitation: 585 nm, emission: 607 nm) and fluorescein isothiocyanate-conjugated to dextran (FITC-dextran, molecular weight: 4000 Da, excitation: 495 nm, emission: 521 nm). Both model permeants were purchased from Sigma-Aldrich (St Louis, MO). The mouse skin was purchased from Pel-Freez (Rogers, AR). Two skin conditions were tested: full-thickness mouse skin or mouse skin that had been tape stripped to remove the stratum corneum. There was also a negative control that had full-thickness skin but no model permeants in order to determine background noise due to endogenous fluorescent compounds extracted from the skin.

These two model compounds were chosen because of their differing permeabilities in skin. A model-based estimate of the permeability of sulforhodamine B in human epidermis is $2.6 \times$ 10-5 cm/h[12] (based on a molecular weight of 559 Da and log octanol-water partition coefficient of 2.2[13]). The expected permeability of FITC-dextran in full-thickness human or mouse skin is vanishingly small because skin permeability decreases as a very strong, nonlinear function of increasing molecular weight.[12]

After removing the stratum corneum barrier, both compounds are expected to permeate through tape-stripped skin at measurable levels. Although absolute skin permeability will depend on experimental conditions, the ratio of skin permeability values for sulforhodamine and FITC-dextran collected at the same experimental conditions should be less variable. Based on Stokes-Einstein theory, the ratio of the permeability coefficients in tape-stripped skin should approximately equal the inverse ratio of the hydrodynamic radii of the molecules in water, assuming no steric hindrance to diffusion in the viable epidermis. For sulforhodamine B (see footnote 2 in Reference 14, applying the Joback method[15] instead of the Lydersen method to determine hydrodynamic radius to account for sulfur-based functional groups) and dextran (see Appendix in Reference 16 to determine hydrodynamic radius), this ratio is estimated as 2.6 : 1 for sulforhodamine B : FITC-dextran (see Appendix).

The skin samples were placed in a diffusion cell in contact with a donor solution containing both model compounds on

the outer surface of the skin and a receiving solution containing phosphate-buffered saline (PBS, Sigma Aldrich) on the underside, as in Reference 12. Both compounds were studied simultaneously in each skin chamber to reduce the number of skin samples needed for the lab. Control samples had PBS donor solutions. Students, working in groups of two or three, set up the diffusion chambers, and returned at 4, 5, 6, and 7 hours after to collect samples of the receiving solution. These samples were used to assay the flux of each molecule using a fluorometer.

The fixed equipment we purchased includes a -80 °C chest freezer ($7,000, Model 5708, Thermo Fisher Scientific, Waltham, MA), a digital balance with milligram resolution ($2,200 AL204, Mettler Toledo, Greifensee, Switzerland), a fluorometer ($5,500, Glo-Max Multi Jr., Promega, Sunnyvale, CA), five 7 inch × 7 inch stirplates ($1,250, Thermo Fisher Scientific, Waltham, MA), nine diffusion cells with clamps ($2,000, Permegear, Hellertown, PA) and a hair clipper ($20, GMT189CGB, Conair, Stamford, CT). The total cost of this equipment is approximately US$18,000, but if a laboratory has access to a pre-existing -80 °C freezer, fluorometer, and balance, the fixed equipment cost drops to approximately $2,800. It is also possible to run this experiment using synthetic membranes, detecting permeants by UV-vis spectroscopy, or other means. For biological experiments where molecules extracted from skin increase noise, however, alternative detection methods have poor detection limits or larger cost compared to fluorometry. The operating costs for the lab are dominated by the mouse skin costs. Obtaining nine skin samples costs approximately US$30 per lab.

Laboratory Procedure

The first task of the lab was to prepare fluorescent compound solutions to be used as the donor solutions and create calibration curves for the two model permeants. The students also created a calibration curve for sulforhodamine B at FITC-dextran's excitation wavelength and used it to correct the FITC-dextran calibration curve, because sulforhodamine B is weakly excited at FITC-dextran's excitation wavelength.

The students also prepared mouse skin samples for the experiment. The skin samples were thawed, cut and made planar, and shaved using a hair clipper. To obtain tape-stripped skin samples after shaving, students repeatedly applied Scotch tape (3M, St. Paul, MN) to the skin and peeled the tape off rapidly using forceps until the skin appeared shiny, which indicated complete stratum corneum removal.[17] One-square-inch sections of full-thickness or tape-stripped skin were then cut out.

Each skin sample was clamped between two identical glass chambers, as shown in Figure 1. The diffusion cells have donor and receiving chambers with volumes of 3.4 mL each for introducing or drawing out fluid. The chambers also have stirbar wells that contain custom stirbars (Permegear, Hellertown, PA). The chamber in contact with the outer surface of the skin was considered the donor chamber, and the opposite chamber was the receiving chamber.

The study was designed for the students to set up three cells containing control skin, three cells containing full-thickness skin samples, and three cells containing tape-stripped skin samples. The negative control samples used full-thickness skin and had PBS in both the donor and receiving chamber. The other two groups had PBS in the receiving chamber and a solution containing 1×10^{-4} M of each of the fluorescent model compounds in the donor chamber. Diffusion cells were covered with aluminum foil to protect them from light.

Four hours after the diffusion cells were set up, liquid samples of the full chamber volume were drawn from each receiving chamber and transferred to plastic cuvettes, and the receiving chambers were quickly replenished with saline. The fluorescence of each sample was measured at two excitation wavelengths, first at 495 nm for FITC-dextran and then at 585 nm for sulforhodamine B. The sampling was repeated at 5, 6, and 7 hours after set up.

DATA ANALYSIS

Although skin is anisotropic, diffusion across skin is sometimes modeled as diffusion across an isotropic membrane as a simplification.[18] The governing equation is a modified version of Fick's first law of diffusion[11]:

$$J = k_p \cdot \Delta C_d \approx k_p \cdot C_d \qquad (1)$$

where J is the steady state flux of the permeant (mol cm^{-2} h^{-1}), k_p is the permeability coefficient (cm h^{-1}), ΔC_d is the permeant

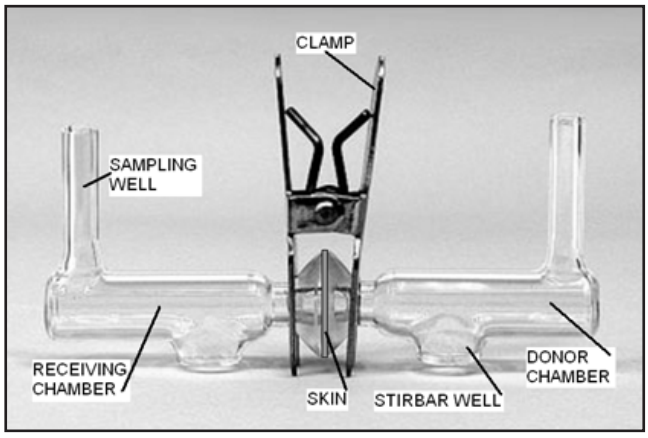

Figure 1. *Diffusion cell set-up. A skin sample is placed between two identical glass chambers. A pinch clamp holds the chambers together and keeps the skin in place. The donor chamber contains the model permeant solution. The receiving chamber contains phosphate-buffered saline as well as any permeant molecules that cross the skin. The receiving chamber is emptied for analysis and replaced with fresh solution periodically.*

concentration gradient across the skin (mol cm^{-3}), and C_d is the permeant concentration in the donor solution (mol cm^{-3}). The concentration at the underside of the skin is usually assumed to be zero, because the permeant is diluted in a large aqueous reservoir. For this reason, $\Delta C_d \approx C_d$.

At the end of the experiment, the diffusion data can be plotted as cumulative amount transported vs. time, as shown in Figure 2. The slope of the linear portion of the plot divided by the skin area (0.64 cm^2) equals the steady-state flux of the drug across skin, which allows calculation of the permeability coefficient using Equation 1. The lag time can also be estimated as the imaginary x-intercept of the linear portion of the plot.

Representative permeability and lag time data are shown in Table 1. Permeability coefficients and lag times determined from the same skin samples were analyzed using ratio paired t-tests[19] because the results were not independent ($p < 0.05$ was considered significant). Skin permeability to sulforhodamine B was significantly larger than FITC-dextran in both full-thickness and tape-stripped skin, which is expected given the much smaller molecular weight of sulforhodamine B. Skin permeability in tape-stripped skin was two orders of magnitude larger than full-thickness skin for both sulforhodamine B and FITC-dextran, which is expected given that tape stripping removes the stratum corneum, which is known to be a significant barrier to diffusion. Lag times were not significantly different from each other, except for comparison of the two dyes in tape-stripped skin, although this is an irrelevant difference of 0.1 h made significant as a result of the paired statistical analysis. The similarity of lag times for sulforhodamine B and FITC-dextran, despite large molecular weight differences, can be explained by significant binding of sulforhodamine B to tissue.[20-21]

We can also compare these data to literature. For example, the ratio of the FITC-dextran permeability and the sulforhodamine B permeability in tape-stripped skin was 2.4 : 1 which matches well with the theoretical estimate of 2.6 : 1 described above. The permeability value in full-thickness mouse skin

TABLE 1
Representative Permeability and Lag-Time Data

Model Permeant	Skin	Permeability coefficient (cm/h)[1]	Lag time (h)[1]
Sulforhodamine-B	Full-thickness	$(1.5 \pm 0.6) \times 10^{-4}$	1.9 ± 1.0
FITC-dextran	Full-thickness	$(2.1 \pm 0.5) \times 10^{-5}$	3.1 ± 0.9
Sulforhodamine-B	Tape-stripped	$(1.5 \pm 0.4) \times 10^{-2}$	3.4 ± 0.2
FITC-dextran	Tape-stripped	$(6.0 \pm 1.3) \times 10^{-3}$	3.3 ± 0.2

[1] Data show the mean ± standard deviation of n= 3 separate skin samples.

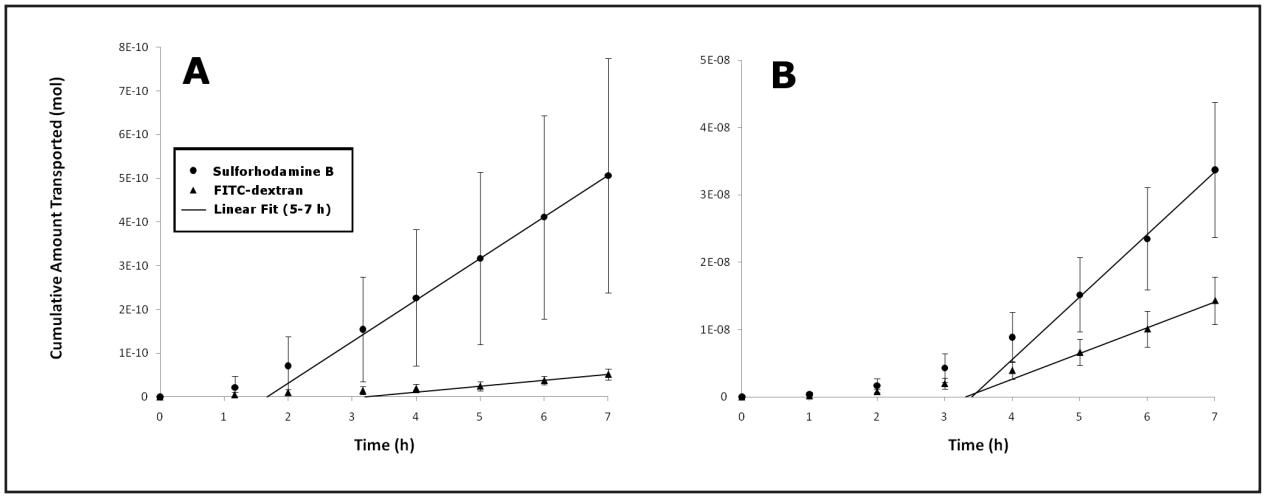

Figure 2. *Representative plots of cumulative transport of sulforhodamine B and FITC-dextran across mouse cadaver skin vs. time. (A) Cumulative transdermal transport across full-thickness skin. (B) Cumulative transdermal transport across tape-stripped skin with stratum corneum removed. Data points are the mean ± standard deviation of n = 3 separate skin samples. The lines shown on the graph are best fits through the data from hours 5 through 7 (*i.e.*, at steady state). The permeability coefficient can be determined from the slope of these lines using Equation 1. The lag time can be determined as the x-intercept. These data were taken during laboratory development and therefore have data points every hour. Guided by the kinetics determined from these graphs, student time was used more efficiently by requiring them to take data only at 0, 4, 5, 6, and 7 h into the experiment in order to capture the steady state region of the graph.*

for sulforhodamine B is six times greater than the model-based estimate for human epidermis. This can be explained by the significantly thinner skin found in the mouse, which has greater permeability.[9]

SUMMARY OF EXPERIENCES AND DISCUSSION

For its first year of implementation, 26 students in a total of 10 groups performed these experiments in 2010. After completing the lab, the students were surveyed about their satisfaction with the lab and their opinion about whether the objectives for the lab were met. The survey contained 16 items measured on a 5-point Likert scale with possible responses ranging from "Strongly Disagree" to "Strongly Agree," and two items about overall satisfaction and recommending the lab to other students. Fifteen out of the 26 students responded, and the data are provided in Table 2. The students' average overall satisfaction with the lab was 8.5 on a 10-point scale. All of the students would recommend the lab to other students in the biotechnology track in chemical and biomolecular engineering.

With respect to the main goals of the lab, the students agreed that they learned about key diffusion concepts (4.3/5.0), appreciated having a lab where they handled animal tissues (4.4 /5.0), and learned about the skin barrier and its importance to health (4.3/5.0). Overall, 10 of the 16 statements received scores of at least 4.0/5.0 and all but one of the statements received scores greater than 3.5/5.0.

The five scores between 3.5 and 4.0 were related to shaving of the mouse skin, time required for the lab, requiring assistance from the TA, experiencing difficulty with data analysis, and learning about fluorescence. The lowest score concerned variability of the data, which is common to experiments using biological tissue with variable properties. One way the variability concern was ameliorated was combining dyes for each skin condition and using paired statistical analyses. This improves statistical power in situations where one dye has a proportionately higher flux than the other independent of skin sample variability, as expected in our experiment.

It appears the educational goals for the lab were well met, but based on the students' comments, we learned that we could improve upon the actual laboratory experience. In the future,

TABLE 2
Student Survey Results

Statement[1]	Response[2,3]
The instructions for the lab were clearly written.	4.5 ± 0.5
My group was able to obtain good calibration curves.	4.2 ± 0.9
My group had no difficulties obtaining mouse skin samples with shaving defects.	3.9 ± 1.1
My group had no difficulties taking measurements from the diffusion cells.	4.2 ± 0.7
My group was able to finish the experiment in the allotted time.	3.6 ± 1.5
My group required minimal assistance from the TA[4].	3.9 ± 1.0
The variability of the results was low enough to allow my group to see clear distinctions between experimental groups.	2.8 ± 1.4
My group was able to complete the calculations required for the reports.	4.0 ± 0.8
My group had no difficulty analyzing our results.	3.7 ± 1.0
Overall the lab was designed well.	4.3 ± 0.6
I learned about diffusion, permeability coefficients, and lag times.	4.3 ± 0.7
I learned about the skin barrier and its importance to health.	4.3 ± 0.9
I learned about the physics and applications for fluorescence	3.9 ± 0.9
I knew how to apply the appropriate statistical tests to analyze my results.	4.3 ± 0.9
I appreciated having a lab where I handled animal tissue.	4.4 ± 1.0
I can use what I learned in this lab if I need to handle biological specimens again.	4.4 ± 0.6
Would you recommend this lab to future students on the biotech track in ChBE5? (Yes or No)	100% yes
On a 1-10 scale, what was your overall satisfaction with this lab as part of your undergraduate education?	8.5 ± 1.1

1 Twenty-six students were surveyed anonymously by e-mail at the end of the semester. Fifteen students responded (response rate: 58%)
2 Responses are the mean ± standard deviation.
3 The second-to-last statement had a yes/no response. The last statement was measured on a 1-10 scale. All other statements were measured on a 5-point Likert scale.
4 Teaching assistant.
5 Chemical and Biomolecular Engineering

we plan to use chemical depilatory creams (*e.g.*, Nair®) or use hairless rodent skin instead of using shaved skin. Also, to see larger differences in the lag times between compounds, we could replace sulforhodamine B with a small, non-green fluorescent dye that does not bind to tissue.

Some students felt the basic premise of the lab design was too elementary. To make the conclusions less obvious, we could ask students to investigate the effect of treating the skin with burns, punctures, UV light, or chemical enhancers, such as ethanol or dimethyl sulfoxide. Additionally, we could provide an "unknown" molecule and ask students to estimate its molecular weight based on its permeability and the Stokes-Einstein equation. Concepts related to the lipid bilayer-based anisotropic diffusivity in skin and the role of phase partitioning and binding could also be introduced.

CONCLUSIONS

We developed and implemented a unit operations laboratory module to educate students about transport across skin. The experiment was designed to teach students about diffusion, biological tissue, fluorescence, and applications in health. To meet the needs of Georgia Tech and possible implementation at other universities, the equipment and procedures were selected to fit within the constraints of a typical chemical engineering laboratory class. Student feedback during the first year of implementation was generally positive and indicated that the laboratory objectives were largely met.

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REFERENCES

1. Mosto, P., M. Savelski, S. Farrell, and G.B. Hecht, "The Future of Chemical Engineering: Integrating Biology into the Undergraduate Curriculum," *Chem. Eng. Ed.*, **41**(1), 43 (2007)
2. O'Connor, K.C., "An Introductory Course in Bioengineering and Biotechnology for Chemical Engineering Sophomores," *Chem. Eng. Ed.*, **41**(4), 247 (2007)
3. Shaeiwitz, J.A., and R. Turton, "Design Projects of the Future," *Chem. Eng. Ed.*, **40**(2), 88 (2006)
4. Aronson, M.T, R.W. Deitcher, Y. Xi, and R.J. Davis, "New Laboratory Course for Senior-Level Chemical Engineering Students," *Chem. Eng. Ed.*, **43**(2), 104 (2009)
5. Forciniti, D., "Teaching a Bioseparations Laboratory: From Training to Applied Research," *Chem Eng. Ed.*, **43**(4), 279 (2009)
6. Badio, A.C., Jr. and C.O. Hokka, "Laboratory Experiment in Biochemical Engineering: Ethanol Fermentation," *Chem. Eng. Ed.*, **33**(1), 4 (1999)
7. Ostafin, A.E., D. LaClair, and H.T. Schmidt, "A Course in Bioprocess Engineering: Engaging the Imagination of Students Using Experiences Outside the Classroom," *Chem. Eng. Ed.*, **37**(3), 180 (2003)
8. Carson, S., J.R. Chisnell, and R.M. Kelly, "Integrating Modern Biology into the ChE Biomolecular Engineering Concentration Through a Campuswide Core Laboratory Education Program," *Chem. Eng. Ed.*, **43**(4), 257 (2009)
9. Prausnitz, M.R., and R. Langer, "Transdermal Drug Delivery," *Nature Biotechnology*, **26**(11), 1261 (2008)
10. Skin exposures & effects <http://www.cdc.gov/niosh/topics/skin/> Accessed on Jan. 13, 2011
11. Williams, A.C., *Transdermal and Topical Drug Delivery: From Theory to Practice*, Pharmaceutical Press, London, p. 40 (2003)
12. Potts, R.O., and R.H. Guy, "Predicting Skin Permeability," *Pharm. Res.*, **9**(5), 663 (1992)
13. Sulforhodamine B compound summary <http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=65191&loc=ec_rcs> Accessed on Jan. 13, 2011
14. Edwards, D.A., M.R. Prausnitz, R. Langer, and J.C. Weaver, "Analysis of Enhanced Transdermal Transport by Skin Electroporation," *J. Control. Release*, **34**(3), 211 (1995)
15. Joback, K.G., and R.C. Reid, "Estimation of Pure-Component Properties from Group-Contributions," *Chem. Eng. Commun.*, **57**(1-6), 233 (1987)
16. Oliver, J.D. 3rd, S. Anderson, J.L. Troy, B.M. Brenner, and W.H. Deen, "Determination of Glomerular Size-Selectivity in the Normal Rat with Ficoll," *J. Am. Soc. Nephrol.*, **3**(2), 214 (1992)
17. Marttin, E., M.T. Neelissen-Subnel, F.H. De Hann, and H.E. Boddé, "A Critical Comparison of Methods to Quantify Stratum Corneum Removed by Tape Stripping," *Skin Pharmacol.*, **9**(1), 69 (1996)
18. Chilcott, R.P., N. Barai, A.E. Beezer, S.I. Brain, M.B. Brown, A.L. Bunge, S.E. Burgess, S. Cross, C.H. Dalton, M. Dias, A. Farinha, B.C. Finnin, S.J. Gallagher, D.M. Green, H. Gunt, R.L. Gwyther, C.M. Heard, C.A. Jarvis, F. Kamivama, G.B. Kasting, E.E. Ley, S.T. Lim, G.S. McNaughton, A. Morris, M.H. Nazemi, M.A. Pellett, J. Du Plessis, Y.S. Quan, S.L. Raghavan, M. Roberts, W. Romonchuk, C.S. Roper, D. Schenk, L. Simonsen, A. Simpson, B.D. Traversa, L. Trottet, A. Watkinson, S.C. Wilkinson, F.M. Williams, A. Yamamoto, and J. Hadgraft, "Inter- and Intralaboratory Variation of *In Vitro* Diffusion Cell Measurements: An International Multicenter Study Using Quasi-standardized Methods and Materials," *J. Pharm. Sci.*, **94**(3), 632 (2005)
19. Motulsky, H., *Intuitive Biostatistics*, 2nd Ed., Oxford University Press U.S., New York, p. 237 (2010)
20. Jiang, J., D.H. Geroski, H.F. Edelhauser, and M.R. Prausnitz, "Measurement and Prediction of Lateral Diffusion Within Human Sclera," *Invest. Ophthalmol. Vis. Sci.*, **47**(7), 3011 (2006)
21. Siegel, R.A., "Characterization of Relaxation to Steady State in Membranes With Binding and Reaction," *J. Membrane Sci.*, **251**(1-2), 91 (2005)

APPENDIX: CALCULATING HYDRODYNAMIC RADII

To calculate the hydrodynamic radius of sulforhodamine B, we used the following equation[14]:

$$R(\text{nm}) = 0.00683 \cdot V_c^{0.6288} \qquad (2)$$

where R is hydrodynamic radius and V_c is critical volume in units of cm^3/mol.

We applied the Joback method[15] to determine V_c, approximating the functional groups of the sulfonic acids with the best fits available in the method.

$$V_c\left(\frac{cm^3}{mol}\right) = 17.5 + \sum G_i \qquad (3)$$

where G_i is the contribution of each functional group (see Table A1). The total molecular weight of 559 Da shown in Table A1 matches the molecular weight of sulforhodamine B. The critical volume was determined to be 1529.5 cm^3/mol, resulting in a hydrodynamic radius of 0.69 nm.

To calculate the hydrodynamic radius of dextran, we used the following equation[16]:

$$R(Å) = 0.488 \cdot MW^{0.437} \qquad (4)$$

where MW is the molecular weight of the dextran, *i.e.*, 4000 Da. This equation predicted a radius of 1.83 nm for the dextran used in this study. ❐

TABLE A1
Group Contributions for Critical Volume of Sulforhodamine B Using the Joback Method

Functional Group	Molecular Weight (Da)	Group Contribution (cm^3/mol)	Group Count in Sulforhodamine B	Total Molecular Weight Contribution	Total Group Contribution
-O- ring	16	13	1	16	13
-OH non-phenol	17	28	2	34	56
=O other	16	36	4	64	144
CH3	15	65	4	60	260
CH2	14	56	4	56	224
>N-	14	9	2	28	18
-S- non-ring	32	54	2	64	108
=CH- ring	13	41	9	117	369
=C< ring	12	32	10	120	320